PROGRAM AND ABSTRACTS FOR 2014 JOINT MEETING OF THE SOCIETY FOR GLYCOBIOLOGY (SFG) AND THE JAPANESE SOCIETY OF CARBOHYDRATE RESEARCH (JSCR)

November 16–19, 2014
Honolulu, Hawaii

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2014 SFG JSCR
JOINT MEETING SCHEDULE AT A GLANCE

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November 16–19, 2014

PROGRAM OVERVIEW

Sunday, November 16	8:40 am – 5:00 pm	**Satellite I:** Chemical Aspects of Glycobiology
	8:30 am – 5:00 pm	**Satellite II:** Glycans in Neuroscience
	9:00 am – 5:00 pm	**Satellite III:** Consortium for Functional Glycomics (CFG) - Microbiome Glycomics
	9:00 am – 5:00 pm	**Satellite IV:** New Vistas in Glycoscience; Challenges for Junior Scientists
	9:00 am – 5:00 pm	**Satellite V:** Glycoprotein Technologies
	6:00 pm – 6:10 pm	**Conference Opening Remarks**
	6:10 pm – 7:50 pm	**Session I:** Systems Glycobiology from Chemistry to Biology
	7:50 pm – 9:30 pm	**Welcome Reception & Exhibits**
Monday, November 17	8:00 am – 8:30 am	**Sunrise Session I**
	8:30 am – 10:00 am	**Session II:** Virus / Inflammation Glycobiology
	10:30 am – 12:30 pm	**Session III:** Glycan Binding Proteins / Immunology
	2:00 pm – 4:00 pm	**Poster Session I and Exhibits**
	4:00 pm – 6:00 pm	**Session IV:** Regulation and Signaling
	6:00 pm – 6:45 pm	**Rosalind Kornfeld Award for Lifetime Achievement in Glycobiology Award Presentation**
Tuesday, November 18	8:00 am – 8:30 am	**Sunrise Session II**
	8:30 am – 10:00 am	**Session V:** Prokaryotic Glycan Assembly
	10:30 am – 12:00 pm	**Session VI:** Stem Cells / iPS
	1:30 pm – 2:15 pm	**Karl Meyer Award Lecture**
	2:15 pm – 2:45 pm	**Business Meeting**
	2:45 pm – 3:45 pm	**Ngly1 Disease Session**
	3:45 pm – 5:45 pm	**Poster Session II and Exhibits**
	6:00 pm – 10:00 pm	**Banquet**
Wednesday, November 19	7:45 am – 8:15 am	**Sunrise Session III**
	8:15 am – 10:15 am	**Session VII:** Glycans, Metabolism and Functions
	10:45 am – 12:45 pm	**Session VIII:** Disease: Mechanism, Biomarker and Therapeutics
	1:30 pm – 3:30 pm	**Poster Session III and Exhibits**
	3:30 pm – 4:30 pm	**Session IX:** Glycan Structures and Bioinformatics
	4:30 pm – 4:45 pm	**Closing remarks**

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2014 SFG JSCR JOINT MEETING PROGRAM

SUNDAY, NOVEMBER 16, 2014

8:00 am – 6:00 pm
Registration
Coral Lounge

8:40 am – 5:00 pm
Satellite I: Chemical Aspects of Glycobiology (Sponsored by RIKEN and TOKYO CHEMICAL INDUSTRY CO., LTD., Japan)
**Break sponsored by Corden Pharma*
Coral 1
Chair: Yukishige Ito, RIKEN

8:30 am – 5:00 pm
Satellite II: Glycans in Neuroscience (Co-sponsored by RIKEN and Glyco-neuroscience project, Japan)
Coral 2
Chair: Kenji Kadomatsu, Nagoya University

9:00 am – 5:00 pm
Satellite III: Consortium for Functional Glycomics (CFG) - Microbiome Glycomics
**Break sponsored by Corden Pharma*
Coral 3
Chair: Michael Tiemeyer, CCRC

9:00 am – 5:00 pm
Satellite IV: New Vistas in Glycoscience; Challenges for Junior Scientists (Co-sponsored by JCGG, SFG Education Committee and RIKEN)
Hibiscus Suite
Chair: Yoshiki Yamaguchi, RIKEN

9:00 am – 5:00 pm
Satellite V: Glycoprotein Technologies
Kahili Suite
Chairs: Sam Tep, ProZyme and Wesley Wang, Amgen

6:00 pm – 6:10 pm
Conference Opening Remarks
Coral 3
Naoyuki Taniguchi, *RIKEN*
President, Society for Glycobiology
Yukishige Ito, *RIKEN*
President, Japanese Society of Carbohydrate Research

6:10 pm – 7:50 pm
Session I: Systems Glycobiology from Chemistry to Biology
Coral 3
Chair: Yasuhiro Kajihara, Osaka University

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7:50 pm – 9:30 pm
Welcome Reception & Exhibits
Coral 4 & 5

MONDAY, NOVEMBER 17, 2014

7:30 am – 4:00 pm
Registration
Coral Lounge

7:30 am – 8:30 am
Continental Breakfast
**Sponsored by Galen Laboratory Supplies*
Coral 4 & 5

8:00 am – 8:30 am
Sunrise Session I
Coral 3
Chair: Akemi Suzuki, Tokai University
Biological Roles of Glycans: Two Decades Later; Ajit Varki, *UCSD*

8:30 am – 10:00 am
Session II: Virus / Inflammation Glycobiology
Coral 3
Chair: Hideharu Ishida, Gifu University

Time		*Abstract Number*
8:00	Biological Roles of Glycans: Two Decades Later; Ajit Varki, *UCSD*	6
8:30	Natural immune programming of antibody glycosylation; Galit Alter, *Ragon Institute of MGH, MIT, and Harvard*	7

MCP/ ASBMB Plenary Lectureship Award

8:50	Immune functions of glycans in infectious disease; Richard Cummings, *Emory University*	8
9:10	Glycan targets of the antibody response in natural and experimental schistosome infections; Cornelis H. Hokke, *Leiden University Medical Center*	9
9:30	Development of highly sensitive diagnostic system for dengue viruses using the interaction between sulfated sugar-chain and viral particle; Yasuo Suda, *Kagoshima University*	10 **short talk*
9:40	Molecular regulation of antigen-specific antibody glycosylation following B cell activation; Wen-Han Yu, *MIT*	11 **short talk*
9:50	In vivo blockade of sialylation with a global sialyltransferase inhibitor causes irreversible kidney dysfunction; Matthew Macauley, *The Scripps Research Institute*	12 **short talk*

10:30 am – 12:30 pm
Session III: Glycan Binding Proteins / Immunology
Coral 3
Chair: Sachiko Sato, Laval University

Time		*Abstract Number*
10:30	Carbohydrate-binding lipoproteins enhance glycan sensing and acquisition by human gut bacteria; Nicole Koropatkin, *University of Michigan*	13
10:50	O-glycoprotein podoplanin is critical for vascular integrity during inflammation; Lijun Xia, *Oklahoma Medical Research Foundation*	14
11:10	Sialylated glycans on airway mucins, such as Muc5b, bind mouse eosinophils via Siglec-F and induce their apoptosis in vitro and in vivo; Bruce Bochner, *Northwestern University Feinberg School of Medicine*	15

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11:30 Role of CD22/Siglec2 and its ligand in B lymphocyte activation; Takeshi Tsubata, *Tokyo Medical and Dental University*. 16
11:50 Glycan-mediated control of immune homeostasis; Juan J. Garcia Vallejo, *VU University Medical Center*.....17
12:10 Galectin-3 protects intracellular Listeria monocytogenes by suppressing autophagy activation via inhibition of nitric oxide production and bacterial ubiquitination; I-Chun Weng, *Academia Sinica* 18
.....*short talk*
12:20 Functional evaluation of sialoglycans expressed in activated T cells; Hiromu Takematsu, *Kyoto University* 19
.....*short talk*

12:30 pm – 2:00 pm
Glycobiology Editorial Board Meeting Lunch (Invitees Only)
Kahili Suite

2:00 pm – 4:00 pm
Poster Session I and Exhibits
**Sponsored by Caisson Biotech*
Coffee break provided
Coral 4 & 5

4:00 pm – 6:00 pm
Session IV: Regulation and Signaling
Coral 3
Chair: Kelly Ten Hagen, NIH

Time *Abstract Number*
4:00 Regulation of neuronal development by glycosaminoglycans; Hiroshi Kitagawa, *Kobe Pharmaceutical University*..... 20
4:20 Roles of glycosaminoglycans in neurophysiology and neurological disorders; Yu Yamaguchi, *Sanford-Burnham Medical Research Institute* 21
4:40 Crystal structures of an archaeal oligosaccharyltransferase provide insights into the catalytic cycle of N-linked protein glycosylation; Daisuke Kohda, *Kyushu University*.....22
5:00 Protein O-mannosylation in mammals: news and views; Sabine Strahl, *Centre for Organismal Studies Heidelberg*..... 23
5:20 Molecular mechanism of O-GlcNAc transferase translocation into nucleus; Jin Won Cho, *Yonsei University* 24
5:40 The Molecular Mechanisms of Fringe Modification on Drosophila Notch: Examining the Structure and Function of Notch EGF Repeats; Beth M. Harvey, *Stony Brook University* 25
.....*short talk*
5:50 An E3 ubiquitin ligase regulates neural-specific glycosylation in the Drosophila embryo; Nickita Mehta, *CCRC*.....26
.....*short talk*

6:00 pm – 6:45 pm
Rosalind Kornfeld Award for Lifetime Achievement in Glycobiology Award Presentation
Coral 3
Chair: Michael Pierce, CCRC
2014 recipient: Ten Feizi
Imperial College London

TUESDAY, NOVEMBER 18, 2014

7:30 am – 4:00 pm
Registration
Coral Lounge

7:30 am – 8:30 am
Continental Breakfast
**Sponsored by Galen Laboratory Supplies*
Coral 4 & 5

8:00 am – 8:30 am
Sunrise Session II
Coral 3
Chair: Inka Brockhausen, Queen's University
A Serendipic Career; Harry Schachter, *Hospital for Sick Children*

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8:30 am – 10:00 am
Session V: Prokaryotic Glycan Assembly
Coral 3
Chair: Christopher West, OUHSC

Time		*Abstract Number*
8:00	A Serendipic Career; Harry Schachter, *Hospital for Sick Children*	27
8:30	Bacterial sialyltransferases: structure, function and engineering for glycoconjugate synthesis; Warren Wakarchuk, *Ryerson University/ University of Guelph*	28
8:50	Some like it hot and sweet: Structure and role of *N*-glycans in the thermoacidophilic archaeon Sulfolobus acidocaldarius; Sonja Alber, *MPI for Terrestrial Microbiology*	29
9:10	Tannerella forsythia - A sweet periodontal pathogen; Christina Schäffer, *Universität für Bodenkultur Wien*	30
		**short talk*
9:20	Insights into rare Ser/Thr O-glycosylation of heterologous proteins expressed in plants; Patricia Bubner, *University of California Berkeley*	31
		**short talk*
9:30	Synthesis of biotinylated keratan sulfate oligosaccharides; Naoko Takeda, *Tottori University*	32
		**short talk*
9:40	Sweet Neurobiology: New insights into the role of alterations in protein glycosylation in Alzheimer's disease pathology; Moran Frenkel-Pinter, *Tel- Aviv University*	33
		**short talk*
9:50	EDEM1/2/3 are α1,2-mannosidases essential for endoplasmic reticulum-associated degradation of glycoproteins; Satoshi Ninagawa, *Kyoto University*	34
		**short talk*

10:30 am – 12:00 pm
Session VI: Stem Cells / iPS
Coral 3
Chair: Rita Gerardy-Schahn, Hannover Medical School

Time		
10:30	New Insights Into the Pathophysiology of Glycolipid Biosynthetic Disorders Using iPS-Derived Cell Lines; Richard Steet, *CCRC*	35
10:50	Glycan structures regulate the states of pluripotent stem cells; Shoko Nishihara, *Soka University*	36
11:10	Sugar-coated webs to drive stem cell differentiation; Catherine Merry, *University of Manchester*	37
11:30	Finding of a Novel Lectin Probe for Pluripotent Stem Cells and its Installation to Regenerative Medicine; Jun Hirabayashi, *AIST*	38
		**short talk*
11:40	Glycocalyx Remodeling with Synthetic Proteoglycan Mimetics Promotes Neural Specification in Embryonic Stem Cells; Kamil Godula, *University of California*	39
		**short talk*
11:50	Golgi-resident polysialic acid defines distinct brain cell populations; Sebastian Werneburg, *Hannover Medical School*	40
		**short talk*

12:00 pm– 1:30 pm
SFG Board of Director's Lunch (Invitees Only)
Kahili Suite

1:30 pm – 2:15 pm
Karl Meyer Award Lecture
Coral 3
Chair: Michael Pierce, CCRC
2014 Recipient: Ronald L Schnaar
Johns Hopkins University School of Medicine

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2:15 pm – 2:45 pm
Business Meeting (open to all attendees)
Coral 3

2:45 pm – 3:45 pm
Ngly1 Disease Session
Coral 3
Chairs: Hudson Freeze, Sanford-Burnham Medical Research Institute and Tadashi Suzuki, RIKEN

Time

2:45 The cytoplasmic PNGase (NGLY1): a 20-plus year scientific journey encounters a human genetic disorder; Tadashi Suzuki, *RIKEN*
3:00 Analysis of NGLY1-deficient human cells; Hudson Freeze, *Sanford-Burnham Medical Research Institute*
3:15 What I learned about NGLY1 from the little fly; Hamed Jafar-Nejad, *Baylor College of Medicine*
3:30 Stories from Patients & Families
NGLY1: Going from bench to bedside, Matt Might, *University of Utah*
The dawn of a new research model: the case for parent-clinician-researcher collaboration; Matt Wilsey, *Grace Wilsey Foundation*

3:45 pm – 5:45 pm
Poster Session II and Exhibits
Coffee break provided
Coral 4 & 5

6:00 pm – 10:00 pm
Banquet
Coral 3
Nominal fee. Extra tickets for guests may be ordered.

WEDNESDAY, NOVEMBER 19, 2014

7:15 am – 1:00 pm
Registration
Coral Lounge

7:15 am – 8:15 am
Continental Breakfast
Coral 4 & 5

7:45 am – 8:15 am
Sunrise Session III
Coral 3
Chair: James Paulson, The Scripps Research Institute
Nutrient Regulation of Signaling & Transcription by O-GlcNAcylation; Gerald Hart, *John Hopkins University*

8:15 am – 10:15 am
Session VII: Glycans, Metabolism and Functions
Coral 3
Chair: Koichi Furukawa, Nagoya University

Time *Abstract Number*

7:45 Nutrient Regulation of Signaling & Transcription by O-GlcNAcylation; Gerald Hart, *Johns Hopkins University*.....41
8:15 Biosynthesis and Biogenetics of Lipopolysaccharide; Peng George Wang, *Georgia State University* 42

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8:35 Biosynthesis and mutant studies indicate roles for pectin and pectin-containing proteoglycans in plant cell wall architectural integrity and plant growth; Debra Mohnen, *CCRC* 43
8:55 Probing glycan functions by precise gene editing; Henrik Clausen, *University of Copenhagen* 44
9:15 Functional characterization of PMM2-CDG patient-derived iPSCs; Falk F. Buettner, *MH-Hannover* 45
9:35 Manipulating glycan processing in the baculovirus-insect cell system for diverse biomedical applications; Don Jarvis, *University of Wyoming* 46
9:55 Role of glycans in gastric cancer development and progression; Celso Reis, *IPATIMAP* 47

10:45 am – 12:45 pm
Session VIII: Disease: Mechanism, Biomarker and Therapeutics
Coral 3
Chair: Tamao Endo, Tokyo Metropolitan Institute of Gerontology

Time *Abstract Number*
10:45 Shedding of GPI-anchored proteins by a novel GPI cleaving enzyme; Morihisa Fujita, *Jiangnan University* 48
11:05 Genetic and epigenetic regulation of IgG glycosylation; Gordan Lauc, *University of Zagreb* 49
11:25 A heterozygous mutation of glycosyltransferase-like gene causes asthenozoospermia; Hisashi Narimatsu, *AIST* 50
11:45 Glycomimetic Antagonists of the Selectins Applied to Diseases in Hematology and Oncology; John L. Magnani, *GlycoMimetics Inc.* 51
12:05 Anti-adhesion therapy for urinary tract infections: A Study on lead optimization of FimH antagonists; Lijuan Pang, *University of Basel* 52
....*short talk
12:15 Targeted drug delivery to brain tumor vasculature by carbohydrate mimetic peptide in mouse glioma model; Misa Suzuki-Anekoji, *Sanford- Burnham Medical Research Institute* 53
....*short talk
12:25 Transcriptional factor Snail controls neuraminidase-1 and matrix metalloproteinase-9 signaling platform in regulating epidermal growth factor receptor, tumor neovascularization, growth and invasiveness in mouse model of human ovarian carcinoma; Myron Szewczuk, *Queen's University* 54
....*short talk
12:35 MAN1B1-CDG: how stressed-out can the Golgi be?; Romain Péanne, *KU Leuven* 55
....*short talk

1:30 pm – 3:30 pm
Poster Session III and Exhibits
Coffee break provided
Coral 4 & 5

3:30 pm – 4:30 pm
Session IX: Glycan Structures and Bioinformatics
Coral 3
Chair: Stuart Haslam, Imperial College London

Time *Abstract Number*
3:30 Development of an International Glycan Structure Repository; Kiyoko F. Aoki Kinoshita, *Soka University* 56
3:50 A systems glycobiology approach to study leukocyte-endothelial cell interactions; Sriram Neelamagham, *SUNY Buffalo* 57
4:10 From glycostructures to glycoknowledge; Nicki Packer, *Macquarie University* 58

4:30 pm – 4:45 pm
Closing remarks
Coral 3
Naoyuki Taniguchi, *RIKEN*
President, Society for Glycobiology
Yukishige Ito, *RIKEN*
President, Japanese Society of Carbohydrate Research

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POSTER PROGRAM
MONDAY, NOVEMBER 17, 2014

Poster Session I: 2:00 pm – 4:00 pm
Coral 4 & 5
Session I: Systems Glycobiology from Chemistry to Biology

Poster Number *Abstract Number*

#B1 **"Synthetic study and structural analysis of highly glycosylated hydrophilic motif of plant extensins";** Akihiro Ishiwata[1], Sophon Kaeothip[2], Yoichi Takeda[2], Yukishige Ito[2] *[1]RIKEN; [2]ERATO Glycotrilogy Project, Japan Science and Technology Agency*.....59

#B2 **"Metabolic production of photocrosslinking O-GlcNAc: method improvement and application";** Andrea Rodriguez, Seok-Ho Yu, Bin Li, Jennifer Kohler *UT Southwestern*.....60

#B3 **"Noninvasive Molecular Imaging and Targeting Studies of Neo-N-glycoproteins: Significant Effects of N-Glycan Clusterization on Albumin";** Katsunori Tanaka, Akihiro Ogura *RIKEN, Biofunctional Synthetic Chemistry Laboratory*.....61

#B4 **"Fucosyl chondroitin sulfate from sea cucumber - chemical synthesis and structural analysis -";** Jun-ichi Tamura[1], Haruna Tanaka[1], Ayumi Nakamura[1], Ryosuke Toita[1], Yoshinao Z. Hosaka[2], Ken-ichi Nagamine[3], Ayaka Himono[3], Naoko Takeda[4] *[1]Department of Regional Environment, Tottori University, Tottori, 680-8551 Japan; [2]Joint Department of Veterinary Medicine, Tottori University, Tottori, 680-8553 Japan; [3]Research & Development Center, Nichirei Biosciences Inc., Higashimurayama, Tokyo, 189-0003 Japan; [4]JSPS Research Fellow, Tottori University, Tottori, 680-8551 Japan*.....62

#B5 **"Glycoform imaging: use of transmembrane FRET to investigate the internalization of glycosylated proteins";** Yoshimi Haga[1], Kumiko Ishii[1], Kayo Hibino[2], Yasushi Sako[2], Yukishige Ito[3], Naoyuki Taniguchi[4], Tadashi Suzuki[1] *[1]Glycometabolome Team, RIKEN Global Research Cluster; [2]Cellular Informatics Laboratory, RIKEN; [3]Synthetic Cellular Chemistry Laboratory, RIKEN; [4]Disease Glycomics Team, RIKEN Global Research Cluster*.....63

#B6 **"Catalytic Domain of UDP-Glc: Glycoprotein Glucosyltransferase; Functional Analysis using Synthetic Substrates";** Yoichi Takeda[1], Akira Seko[1], Keiichiro Ohara[1], Masakazu Hachisu[1], Kohki Fujikawa[1], Ning Wang[1], Yukishige Ito[2] *[1]ERATO, Japan Science and Technology Agency (JST), Ito Glycotrilogy Project; [2]Synthetic Cellular Chemistry Laboratory, RIKEN / ERATO, Japan Science and Technology Agency (JST), Ito Glycotrilogy Project*.....64

#B7 **"Cell interaction and physicochemical characterization of hyaluronic acid nanoparticles.";** Akari Hisada, Keisuke Koizumi, Toshinori Sato *Keio Univ.*.....65

#B8 **"Analysis of the interaction between peptide aptamers and hemagglutinin.";** Rabi Shibata, Teruhiko Matsubara, Toshinori Sato *Keio Univ.*.....66

#B9 **"Critical importance of the surface expression of α2,6-sialosides during early development of medaka fish";** Ken Kitajima[1], Di Wu[1], Katsue Tajima[1], Emi Maruyama[1], Yuko Yasukawa[1], Lan-Yi Chang[2], Yoshihito Taniguchi[3], Yasuhiro Kamei[4], Tomoko Adachi[2], Hisashi Hashimot *[1]Biosci. Biotech. Center, Nagoya Univ; Grad. Sch. Bioagr. Sci, Nagoya Univ; [2]Biosci. Biotech. Center, Nagoya Univ; [3]Sch. Med., Keio Univ.; [4]Dept Appl. Life Sci., Nat. Inst. Basic Biol.*.....67

#B10 **"Polyploidization triggered by psychosine is modulated by cellular glycosphingolipids and sphingomyelin";** Hiroshi Watanabe[1], Kyohei Okahara[2], Yasunori Kozutsumi[2], Shogo Oka[1], Hiromu Takematsu[1] *[1]Lab Biol Chem, Human Health Sci, Grad Sch of Medicine, Kyoto Univ; [2]Lab Membrane Biochem and Biophys, Grad sch of Biostudies, Kyoto Univ*.....68

#B11 **"Protecting-group-free Synthesis and Binding with Influenza Virus of Glycopolymers Bearing Sialyloligosaccharides";** Tomonari Tanaka[1], Hideki Ishitani[1], Yoshiko Miura[2], Kenta Oishi[3], Tadanobu Takahashi[3], Takashi Suzuki[3] *[1]Graduate School of Science and Technology, Kyoto Institute of Technology; [2]Graduate School of Engineering, Kyushu University; [3]School of Pharmaceutical Sciences, University of Shizuoka*.....69

#B12 **"Immobilization of Endoglycosidases for Analysis of Glycoproteins";** Aihua Zhang, Inca Ghosh, Guillaume Vinet, John Buswell, James McFarland, Ivan R. Correa Jr., Christopher H. Taron, Christopher J. Noren, Ming-Qun Xu *New England Biolabs, Inc.*.....70

#B13 **"Analytical Services And Trainings At The Complex Carbohydrates Research Center";** Roberto Sonon, Stephanie Archer-Hartmann, Mayumi Ishihara, Zhirui Wang, Christian Heiss, Radnaa Naran, Ian Black, Dandan Zhou, Artur Muszynski, Scott Forsberg, Parastoo Azadi *University of Georgia*.....71

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#B33 **"Biological activities of the homogeneous glycosylated chemokines CCL1 and Ser-CCL1 prepared by total chemical synthesis";** Ryo Okamoto[1], Kalyanswer Mandal[2], Morris Ling[3], Andrew Luster[3], Michael Sawaya[4], Todd Yeates[4], Yasuhiro Kajihara[1], Stephen Kent[1] *[1]Osaka University; [2]University of Chicago; [3]Massachusetts General Hospital, Harvard Medical School; [4]University of California* 91

#B34 **"A synthetic study of a homogeneous hematopoietic glycoprotein bearing three biantennary sialyloligosaccharides";** Masumi Murakami, Ryo Okamoto, Masayuki Izumi, Yasuhiro Kajihara *Graduate School of Science, Osaka University* 92

#B35 **"Synthetic Study of Erythropoietin Having High Mannose-type Oligosaccharide by Chemical Methodology";** Tatsuto Kiuchi[1], Ryo Okamoto[1], Masayuki Izumi[1], Akira Seko[2], Yukishige Ito[3], Yasuhiro Kajihara[1] *[1]Osaka University; [2]JST ERATO; [3]RIKEN* 93

#B36 **"The Synthesis of Sialo-containing Glycopolymers by π-Allyl Nickel Catalyzed Coordination Polymerization.";** Shuichi Ohira[1], Yu Yasuda[2], Chihiro Sato[2], Ken KItajima[2], Ikuyoshi Tomita[3], Takashi Takahashi[4], Hiroshi Tanaka[1] *[1]Department of Applied Chemistry, Tokyo Institute of Technology; [2]Graduate School of Bioagricultural Sciences and Bioscience and Biotechnology Center, Nagoya University; [3]Department of Electronic Chemistry, Tokyo Institute of Technology; [4]Yokohama College of Pharmacy* 94

#B37 **"Analysis of glycan processing in the endoplasmic reticulum based on selective inhibition of mannosidases";** Taiki Kuribara, Makoto Hirano, Kiichiro Totani *Seikei University* 95

#B38 **"Synergistic improvement in chemical synthesis of high-mannose glycans.";** Masaaki Shiba, Kodai Iwata, Yuki Shinoda, Makoto Hirano, Kiichiro Totani *Seikei University* 96

#B39 **"Integrated Proteomic and Glycoproteomic Analyses of Prostate Cancer Cells Reveals Glycoprotein Changes in Protein Expression, Glycosylation Occupancy and Glycosite Heterogeneity";** Punit Shah, Xiangchun Wang, Weiming Yang, Shadi Toghi Eshghi, Shuang Yang, Shisheng Sun, Hui Zhan *Johns Hopkins University* 97

#B40 **"Synthesis of glucosylated *N*-glycans using transglycosylation activity of Golgi endo-α-mannosidase";** Shogo Iwamoto[1], Yoichi Takeda[2], Akira Seko[2], Yukishige Ito[3], Ichiro Matsuo[1] *[1]Gunma University, Division of Molecular Science, Faculty of Science and Technology; [2]ERATO-JST; [3]ERATO-JST, RIKEN, Synthetic Cellular Chemistry Laboratory* 98

Session II: Virus / Inflammation Glycobiology

Time *Abstract Number*

#B41 **"Development of highly sensitive diagnostic system for dengue viruses using the interaction between sulfated sugar-chain and viral particle";** Yasuo SUDA[1], Budi SAKSONO[2], Beti Ernawati DEWI[3], Leonardo NAINGGOLAN[4] *[1]Kagoshima University and SUDx-Biotec Corporation; [2]Kagoshima University and Indonesian Institute of Sciences; [3]Universitas Indonesia; [4]Universitas Indonesia and Cipto Mangun Kusumo Hospital* 10

#B42 **"Molecular regulation of antigen-specific antibody glycosylation following B cell activation";** Wen-Han Yu[1], Doug Lauffenburger[2], Galit Alter[3] *[1]Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard University Department of Biological Engineering, Massachusetts Institute of Technology; [2]Department of Biological Engineering, Massachusetts Institute of Technology; [3]Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard University* 11

#B43 **"In vivo blockade of sialylation with a global sialyltransferase inhibitor causes irreversible kidney dysfunction";** Matthew Macauley[1], Britni Arlian[1], Cory Rillahan[1], Poh-Choo Pang[2], Nikki Bortell[1], Maria Cecilia Marcondes[1], Stuart Haslam[2], Anne Dell[2], James Paulson[1] *[1]The Scripps Research Institute; [2]Imperial College London* 12

#B44 **"Nonencapsulated group A *Streptococcus* associated with human invasive disease";** Anna Henningham[1], Masaya Yamaguchi[1], Ramy Aziz[1], Kirsten Kuipers[1], Samira Dahesh[1], Yuka Yamaguchi[1], Lisa Seymour[2], Nouri Ben Zakour[2], Lingjun He[3], Helen Smi *[1]University of California San Diego; [2]University of Queensland; [3]San Diego State University; [4]Queensland Health Forensic and Scientific Services* 99

#B45 **"Beyond Sweet Attractions: The role of glycans in Rotavirus Infection";** Thomas Haselhorst[1], Raphael Böhm[1], Fiona Fleming[2], Vi Dang[2], Mark von Itzstein[1], Barbara Coulson[2] *[1]Institute for Glycomics, Griffith University Gold Coast Campus; [2]Department of Microbiology and Immunology, The University of Melbourne* 100

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#B46 **"Antigenic Potential of a Highly Conserved Lipopolysaccharide Inner Core Structure Defined by Synthetic Approach";** Anika Reinhardt[1], You Yang[2], Heike Claus[3], Claney L. Pereira[2], Andrew D. Cox[4], Ulrich Vogel[3], Chakkumkal Anish[2], Peter H. Seeberger[1] *[1]Department of Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany and Institute for Chemistry and Biochemistry Freie Universität Berlin, Arnimallee 22, 14195 Berlin, Germany; [2]Department of Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany; [3]Institute for Hygiene and Microbiology, University of Würzburg, 97080 Würzburg, Germany; [4]Vaccine Program, Human Health Therapeutics Portfolio, National Research Council, 100, Sussex Drive, Ottawa ON K1A 0R6, Canada*.....101

#B47 **"Enzymatically active extracellular hyaluronidase (HylA) of group A *Streptococcus* promotes intracellular survival and virulence";** Masaya Yamaguchi[1], Yuka Yamaguchi[2], Masanobu Nakata[3], Anna Henningham[4], Joshua Olson[2], Samira Dahesh[2], Jason Cole[4], Shigetada Kawabata[3], Ajit Varki[5], Victor *[1]Graduate School of Dentistry, Osaka University; Department of Pediatrics, School of Medicine, University of California, San Diego; [2]Department of Pediatrics, School of Medicine, University of California San Diego; [3]Graduate School of Dentistry, Osaka University; [4]Department of Pediatrics, School of Medicine, University of California San Diego; The School of Chemistry and Molecular Biosciences, and The Australian Infectious Diseases Research Centre, The University of Queensland; [5]Departments of Medicine, and Cellular and Molecular Medicine, University of California San Diego; [6]Department of Pediatrics, School of Medicine, and Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego* 102

#B48 **"TREX1 (DNase III) Prevents Dysregulation Of Oligosaccharyltransferase";** Mark Lehrman, Maroof Hasan, Ningguo Gao, Charles Fermaintt, Nan Yan *UT Southwestern Medical Center*.....103

#B49 **"Superficially-Located Enlarged Lymphoid Follicles Characterize Nodular Gastritis";** Takuma Okamura[1], Yasuhiro Sakai[2], Hitomi Hoshino[2], Yugo Iwaya[3], Eiji Tanaka[3], Motohiro Kobayashi[2] *[1]Department of Molecular Pathology, Shinshu University Graduate School of Medicine, Matsumoto, Japan; [2]Division of Tumor Pathology, Department of Pathological Sciences, Faculty of Medical Sciences, University of Fukui, Eiheiji, Japan; [3]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Shinshu University School of Medicine, Matsumoto, Japan* 104

#B50 **"Agalactosylated antibodies with enhanced binding to MUC16 may contribute to mucosal protection against HIV transmission";** Bronwyn Gunn[1], Maryam Shansab[1], Jeffrey Schneider[2], Anna Licht[1], Ivan Zvonar[1], Marcus Karim[1], Alison Mahan[1], Jacqueline Tedesco[1], Thomas Hope[2], Galit Alter < *[1]Ragon Institute of MGH, MIT, and Harvard; [2]Dept of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University* 105

#B51 **"Mechanism of up-regulation of a glycoprotein: Angpt-1 in Kaposi's sarcoma-associated herpesvirus infected primary effusion lymphoma cell lines";** Xin Zheng, Eriko Ohsaki, Keiji Ueda *Division of Virology, Department of Microbiology and Immunology, Osaka University Graduate School of Medicine* 106

#B52 **"*N*-glycosylation of plasma proteins during systemic inflammatory response provoked by surgery";** Olga Gornik[1], Mislav Novokmet[2], Frano Vučković[2], Toma Keser[1], Manuela De Gregori [3], Massimo Allegri[3], Gordan Lauc[1] *[1]University of Zagreb Faculty of Pharmacy and Biochemistry; [2]Genos Ltd. Glycobiology Division; [3]University of Pavia, Italy*.....107

#B53 **"Fucosyltranferase deficiency enhances during acute neutrophil infiltration into the lung airway inflammation";** Alexander Buffone, Jr.[1], Amit Lugade[1], Mehrab Nasirikenari[1], Yasmin Thanavala[1], Sriram Neelamegham[2], Joseph Lau[1] *[1]Roswell Park Cancer Institute; [2]State University of New York at Buffalo* 108

#B54 **"Galectin-8 in the experimental Trypanosoma cruzi infection";** Carla Pascuale[1], Adriano Bertelli[1], Hernan Garcia Rivello[2], Virginia Tribulati[1], Gabriela Levy[1], Oscar Campetella[1], Maria Susana Leguizamon[1] *[1]Instituto de Investigaciones Biotecnológicas -Universidad Nacional de San Martín. Buenos Aires. Argentina; [2]Servicio de Patologia. Hospital Italiano. Buenos Aires, Argentina* 109

#B55 **"Structural and functional insights into Group A *Streptococcus* gacA: An essential dTDP-4-dehydrorhamnose reductase (RmlD)";** Samantha L. van der Beek[1], Yoann Le Breton[2], Andrew T. Ferenbach[3], Daan M.F. van Aalten[3], Iva Navratilova[4], Kevin McIver[2], Nina M. van Sorge[1], Helge C. Dorfmueller[5] *[1]University Medical Center Utrecht, Medical Microbiology, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands; [2]Department of Cell Biology and Molecular Genetics, University of Maryland, 3124 Biosciences Research Building, College Park, MD 20742, United States of America; [3]Division of Molecular Microbiology, University of Dundee, College of Life Sciences, Dow Street, DD1 5EH, Dundee, United Kingdom; [4]Division of Biological Chemistry and Drug Discovery, University of Dundee, College of Life Sciences, Dow Street, DD1 5EH, Dundee, United Kingdom; [5]Rutherford Appleton Laboratory, Research Complex at Harwell, Didcot OX11 0FA, United Kingdom / Division of Molecular*

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Session III: Glycan Binding Proteins / Immunology

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TUESDAY, NOVEMBER 18, 2014

Poster Session II: 3:45 pm – 5:45 pm
Coral 4 & 5
Session IV: Regulation and Signaling

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#B149 **"The role of CMP-Sialic acid synthetase in *Drosophila* neural transmission";** Hilary Scott[1], Courtney Caster[1], Ilya Mertsalov[1], Michelle Alfert[1], Brooke Howell[1], Mark J. Zoran[2], Vladislav Panin[1] *[1]Department of Biochemistry and Biophysics Texas A&M University, College Station, Texas 77843; [2]Department of Biology, Texas A&M University, College Station, Texas 77843* 200

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#B150 **"*Tannerella forsythia* - A sweet periodontal pathogen";** Markus B. Tomek[1], Valentin Friedrich[1], Zoe A. Megson[1], Gerald Posch[2], Andrea Koerdt[1], Irene Nimeth[1], Philipp Andesner[1], Friedrich Altmann[3], Paul Messner[1], Christina Schäffer[1] *[1]Universität für Bodenkultur Wien, Department of NanoBiotechnology, NanoGlycobiology unit, Muthgasse 11, 1190 Vienna, Austria; [2]Universität für Bodenkultur Wien, Department of NanoBiotechnology, NanoGlycobiology unit, Muthgasse 11, 1190 Vienna, Austria. Present address: Alberta Glycomics Centre and Department of Biological Sciences, University of Alberta, Edmonton, A; [3]Universität für Bodenkultur Wien, Department of Chemistry, Division of Glycobiology, Muthgasse 18, 1190 Vienna, Austria* 30

#B151 **"Insights into rare Ser/Thr O-glycosylation of heterologous proteins expressed in plants";** Patricia Bubner[1], Heidi Szemenyei[1], Shu-Lun Tang[1], Anthony Iavarone[2], Stefan Bauer[1], Douglas Clark[1], Chris Somerville[1] *[1]Energy Biosciences Institute, University of California, Berkeley, CA, USA; [2]California Institute for Quantitative Biosciences, University of California, Berkeley, CA USA.* 31

#B152 **"Synthesis of biotinylated keratan sulfate oligosaccharides";** Naoko Takeda[1], Jun-ichi Tamura[2] *[1]JSPS Research Fellow, Tottori University, Tottori, 680-8551 Japan; [2]Department of Regional Environment, Tottori University, Tottori, 680-8551 Japan* 32

#B153 **"Sweet neurobiology: New insights into the role of alterations in protein glycosylation in Alzheimer's disease pathology";** Moran Frenkel-Pinter[1], Sharon Tal[1], Yelena Solovey[1], Avnika Singh-Anand[1], Daniela Escobar[1], Shiri Stempler[2], Yedael Waldman[2], Eytan Rupin[2], Ehud Gazit[1] *[1]Dept. Molecular Microbiology & Biotechnology, The Interdisciplinary Characterisation Sagol School of Neurosciences, George S. Wise Faculty of Life Sciences, Tel-Aviv University. Tel-Aviv 69978, Israel; [2]The Blavatnik School of Computer Science, Raymond and Baverly Sackler Faculty of Exact Sciences, Tel-Aviv University. Tel-Aviv 69978, Israel* 33

#B154 **"EDEM1/2/3 are α 1,2-mannosidases essential for endoplasmic reticulum-associated degradation of glycoproteins";** Satoshi Ninagawa[1], Tetsuya Okada[1], Yoshiki Sumitomo[1], Yukiko Kamiya[2], Satoshi Horimoto[1], Tokiro Ishikawa[1], Shunichi Takeda[3], Tetsushi Sakuma[4], Takashi Yamamoto[4], Koichi Kato[2], Kazutoshi Mori[1] *[1]Department of Biophysics, Graduate School of Science, Kyoto University; [2]Institute for Molecular Science and Okazaki Institute for Integrative Bioscience, National Institute of Natural Sciences; [3]Department of Radiation Genetics, Graduate School of Medicine, Kyoto University; [4]Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University* 34

#B155 **"Sequence Determinants of Linkage Specificity and Polymer Length in Neisserial Polysialyltransferases";** Hazel L. S. Fuchs[1], Timothy G. Keys[1], Sebastian P. Galuska[2], Jörg Ehrit[1], Friedrich Freiberger[1], Rita Gerardy-Schahn[1] *[1]Institute for Cellular Chemistry, Medical School Hannover, Carl-Neuberg-Str. 1, 30625 Hannover, Germany; [2]Institute of Biochemistry, Justus-Liebig-University, Friedrichstr. 24, 35392 Giessen, Germany* 227

#B156 **"Characterisation and exploitation of the capsule biosynthesis machineries of *Neisseria meningitidis* serogroups A and X: Towards in vitro vaccine production";** Timm Fiebig[1], Maria Rosaria Romano[2], Friedrich Freiberger[1], Vittoria Pinto[2], Daniela Proietti[2], Barbara Brogioni[2], Christa Litschko[1], Andrea Bethe[1], Monika Berger[1], Paolo Costantino[2], Roberto Adamo[2], Francesco Berti[2], Rita Gerardy-Schahn[1] *[1]Institute for Cellular Chemistry, Hannover Medical School, 30625 Hannover, Germany; [2]Novartis Vaccines, Research, Via Fiorentina 1, 53100 Siena, Italy* 228

#B157 **"Biosynthesis of the Sialyl-T antigen: Specificity of human ST3Gal1 and comparison to a novel sialyltransferase WbwA from *Escherichia coli* O104.";** Diana Czuchry[1], Paul Desormeaux[1], Melissa Stuart[2], Donald Jarvis[2], Khushi L. Matta[3], Walter A. Szarek[4], Inka Brockhausen[1] *[1]Queen's University, Department of Biomedical and Molecular Sciences, Kingston, ON, Canada; [2]University of Wyoming, Department of Molecular Biology, Laramie, Wyoming, USA; [3]TumorEnd, Baton Rouge, Louisiana, USA; [4]Queen's University, Department of Chemistry, Kingston, ON, Canada* 229

#B158 **"Structural and molecular characterization of the S-layer anchoring system of *Lactobacillus buchneri*";** Eva Smolar[1], Paul Kosma[2], Christina Schäffer[3], Paul Messner[1] *[1]Universität für Bodenkultur*

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Session VI: Stem Cells / iPS

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Session VII: Glycans, Metabolism and Functions

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#B200 **"N-glycan sialylation in silkworm protein expression system and application";** Yukiko Kataoka[1], Hironobu Okazaki[1], Tsuyoshi Nomura[1], Masatoshi Suganuma[1], Yukiko Higa[1], Shyunsuke Hunaguma[1], Takeo Suzuki[1], Akihiro Usami[1], Kazuhito Fujiyama[2], Hideki Sezutsu[3], Ken-ichiro Tatematsu[3], Toshiki Tamura[3] *[1]Sysmex Corp.; [2]ICBiotech., Osaka Univ.; [3]Gen. Mod. Org. Res. Cent., Natl. Inst. Agrobiol. Sci.* 269

#B201 **"Enzymatic properties of Golgi α-1,2 mannosidases toward denatured glycoproteins";** Jun-ichi Aikawa[1], Yoichi Takeda[2], Akira Seko[2], Ichiro Matsuo[3], Yukishige Ito[4] *[1]RIKEN; [2]JST ERATO; [3]Grad. Sch. Eng., Gunma Univ.; [4]RIKEN & JST ERATO*..... 270

#B202 **"Pancreatic α-amylase controls glucose assimilation in duodenum through N-glycan-specific binding, followed by endocytosis and degradation";** Kimie Date[1], Ayano Satoh[2], Haruko Ogawa[3] *[1]Grad. Sch. Human. Sci., Ochanomizu Univ.; [2]Grad. Sch. Natural Sci. & Tech., Okayama Univ.; [3]Grad. Sch. Human. Sci., and Glycosci. Inst., Ochanomizu Univ.*..... 271

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#B204 **"The mechanism underlying anti-cancer bioactivity of glycan";** Lei Zhang, Peipei Wang, Kan Ding *Shanghai Institute of Materia Medica, Chinese Academy of Sciences* 273

#B205 **"Discovery of novel monosaccharides in animal glycans: Natural occurrence of N-glycolylhexosamines";** Sandra Diaz, Anne K. Bergfeld, Roger Lawrence, Oliver Pearce, Jeremy Van Vleet, Jeffrey Esko, Biswa Choudhury, Ajit Varki *Glycobiology Research and Training Center, Departments of Medicine and Cellular & Molecular Medicine, University of California, San Diego*..... 274

#B206 **"Hemicellulose synthesis and function in land plants";** William York, Breeanna Urbanowicz, Maria Peña, Kelley Moremen, Malcolm O'Neill, Heather Moniz, Ameya Kulkarni, Shuo Wang *Complex Carbohydrate Center, University of Georgia*..... 275

#B207 **"Novel CE system for high throughput N-glycan screening";** Zoltan Szabo, Samnang Tep, Ted Haxo, Michael Kimzey, Sybil Lockhart, Justin Hyche, Aled Jones, Jo Wegstein *ProZyme, Inc.* 276

#B208 **"O-GlcNAc acts as a glucose sensor to epigenetically regulate the insulin gene in pancreatic beta cells.";** Sean Durning, Heather Flanagan-Steet, Lance Wells *CCRC at UGA* 277

#B209 **"Mitochondrial O-GlcNAc transferase and its role in the glycosylation of mitochondrial proteins";** Juliana Lessa Sacoman[1], Amanda Burnham-Marusich[1], Raul Dagda[2], Ruben Dagda[2], Patricia Berninsone[1] *[1]Department of Biology, University of Nevada at Reno, 1664 North Virginia Street, Reno, NV, 89557; [2]Department of Pharmacology, University of Nevada at Reno, 1664 North Virginia Street, Reno, NV, 89557*..... 278

#B210 **"Amino acid sequence and site-specific glycosylation of windmill palm peroxidase";** Margaret Baker, Qing Li *University of Hawaii*..... 279

#B211 **"Structural basis for glycoprotein quality control mediated by glucose tagging in the endoplasmic reticulum";** Tadashi Satoh[1], Takayasu Toshimori[2], Takumi Yamaguchi[3], Zhu Tong[3], Koichi Kato[3] *[1]Grad. Sch. of Pharm. Sci., Nagoya City Univ.; [2]Human Health Sci., Grad.Sch. of Med., Kyoto Univ.; [3]Okazaki Inst. for Integra. Biosci., Nat. Insti. of Nat. Sci.*..... 280

#B212 **"Functional studies of cytosolic deglycosylating enzymes in mammalian cells";** Chengcheng Huang[1], Yoichiro Harada[1], Akira Hosomi[1], Yuki Masahara-Negishi[1], Junichi Seino[1], Haruhiko Fujihira[1], Yoko Funakoshi[1], Takehiro Suzuki[2], Naoshi Dohmae[2], Tadashi Suzuki[1] *[1]Glycometabolome Team, Systems Glycobiology Research Group, RIKEN-Max Planck Joint Research Center for Systems Chemical Biology, RIKEN Global Research Cluster; [2]Collaboration Promotion Unit, RIKEN Global Research Cluster*..... 281

#B213 **"Endosomes-to-TGN retrograde transport mediated by GARP is required for post-Golgi anterograde transport and glycosylation";** Tetsuya Hirata[1], Morihisa Fujita[2], Shota Nakamura[3], Kazuyoshi Gotoh[3], Daisuke Motooka[3], Yoshiko Murakami[4], Yusuke Maeda[4], Taroh Kinoshita[4] *[1]Immunology Frontier Research Center, Osaka Univ.; JSPS Research Fellow; [2]Sch. Biotech., Jiangnan Univ.; [3]Research Institute for Microbial Diseases, Osaka Univ.; [4]Immunology Frontier Research Center, Osaka Univ.; Research Institute for Microbial Diseases, Osaka Univ.*..... 282

#B214 **"Perturbation of autophagic flux is involved in the dystrophic endball formation induced by a proteoglycan gradient";** Tomoya Ozaki, Kazuma Sakamoto, Yuanhao Gong, Kenji Uchimura, Kenji Kadomatsu *Department of Biochemistry, Nagoya University Graduate School of Medicine* 283

#B215 **"Identification of minimum essential structure in chondroitin sulfate which is responsible for their interaction with their receptor, PTP sigma and LAR";** Yuanhao Gong[1], Kazuma Sakamoto[2], Naoko Takeda[3], Tomoya Ozaki[2], Nao Tsutsumishita-Nakai[4], Manami Kawano[4], Jun-ichi Tamura[4],

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WEDNESDAY, NOVEMBER 19, 2014

Poster Session III: 1:30 pm - 3:30 pm
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Session VIII: Disease: Mechanism Biomarker and Therapeutics

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#B285 **"Identification and characterization of a missense mutation in O-GlcNAc transferase that segregates with disease in a family with X-linked intellectual disability";** D. Brent Weatherly[1], Krithika Vaidyanathan[1], Peng Zhao[1], Melanie May[2], Charles Schwartz[2], Lance Wells[1] *[1]CCRC at UGA; [2]Greenwood Genetic Center, SC* 350

#B286 **"B4GAT1 is the priming enzyme for the LARGE-dependent functional glycosylation of α-dystroglycan";** Jeremy Praissman[1], David Live[1], Shuo Wang[2], Annapoorani Ramiah[2], Kelley Moremen[2], Lance Wells[2] *[1]UGA at CCRC; [2]CCRC at UGA* 351

#B287 **"Ex vivo gene therapy improves the accumulation of glycosaminoglycans in brain from murine model of mucopolysaccharidosis type II";** Taichi Wakabayashi[1], Yohta Shimada[2], Takashi Higuchi[2], Hiroshi Kobayashi[2], Hiroyuki Ida[1], Toya Ohashi[2] *[1]Department of Pediatrics, Jikei University School of Medicine; [2]Division of Gene Therapy, Research Center for Medical Sciences, Jikei University School of Medicine* 352

#B288 **"Potential for using lectin sugar chains as diagnostic markers in oral precancerous lesions";** Michiko Ehara[1], Juna Nakao[1], Motohiko Nagayama[1], Masaaki Shiota[2], Kiyoko F. Aoki-Kinoshita[2], Jun-ichi Tanuma[1] *[1]Department of Oral Pathology Asahi University School of Dentistry; [2]Graduate School of Engineering, Soka University* 353

#B289 **"Correlating glycosylation and immunoreactivity of HIV GP120";** Peng Zhao[1], Nickita Mehta[1], Galit Alter[2], Michael Tiemeyer[1], Lance Wells[1] *[1]CCRC at UGA; [2]Ragon Institute, Harvard* 354

#B290 **"Oral N-acetylmannosamine reverses glomerular hyposialyation and ameliorates proteinuria in a mouse model of nephrotic syndrome";** May Christine V. Malicdan[1], Steven Bodine[2], Veeraya Tanawattanacharoen[3], Tadafumi Yokoyama[2], Petcharat Leoyklang[2], Patricia M Zerfas[4], Avi Rosenberg[2], Shashi Shrivastav[3], Taichi Murakami[3], Koji Okamoto[3], Jeffrey B. Kopp[3], William A. Gahl[5], Marjan Huizing[2] *[1]Medical Genetics Branch, National Human Genome Research Institute, NIH; 5NIH Undiagnosed Diseases Program, Common Fund, Office of the Director, NIH; [2]Medical Genetics Branch, National Human Genome Research Institute, NIH; [3]Kidney Disease Section, National Institute of Diabetes and Digestive and Kidney Diseases, NIH; [4]Office of Research Services, Office of the Director, NIH; [5]NIH Undiagnosed Diseases Program, Common Fund, Office of the Director, NIH; and Office of the Clinical Director, NHGRI, NIH* 355

#B291 **"A genome-scale systems biology approach to modeling glycosylation";** Nathan Lewis[1], Anders Bruntse[2], Philipp Spahn[1], Hooman Hefzi[1] *[1]University of California, San Diego; [2]Technical University of Denmark* 356

#B292 **"Structural analyses of plasma glycoproteins in exacerbation and emphysema model mice as biomarker candidates for chronic obstructive lung disease (COPD)";** Katsunori Shirai[1], Reiko Fujinawa[2], Satoshi Kobayashi[2], Fumi Ota[2], Shinobu Kitazume[2], Naoyuki Taniguchi[2], Miyako Nakano[1] *[1]Graduate School of Advanced Sciences of Matter, Hiroshima University, Hiroshima, Japan; [2]Disease Glycomics Team, RIKEN Global Research Cluster, RIKEN-Max Planck Joint Research Center, Saitama, Japan* 357

#B293 **"GNE defects in zebrafish lead to impairment of sialylation and myopathy";** Dino Maglic[1], Leoyklang Petcharat[1], Pongsathorn Chaiyasap[1], Kevin Bishop[2], Raman Sood[2], Patricia M. Zerfas[3], William A. Gahl[4], Marjan Huizing[1], May Christine Malicdan[5] *[1]Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA; [2]Zebrafish Core Facility, Genetics and Molecular Biology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA; [3]Office of Research Services, Office of the Director, National Institutes of Health, Bethesda, Maryland, USA; [4]NIH Undiagnosed Diseases Program, Common Fund, Office of the Director, National Institutes of Health, Bethesda, Maryland, USA; Office of the Clinical Director, NHGRI, National Institutes of Health, Bethesda, MD USA; [5]Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA; NIH Undiagnosed Diseases Program, National Institutes of Health, Bethesda, Maryland, USA* 358

#B294 **"Acquired drug resistance by decrease of sialylated glycans on acute lymphoblastic leukemia cell-membrane glycoproteins";** Miyako Nakano[1], Ryohei Shirai[1], Jun Ito[1], Maria Kavallaris[2], Nicolle Packer[3] *[1]Graduate School of Advanced Sciences of Matter, Hiroshima University, Hiroshima, Japan; [2]Children's Cancer Institute Australia, Lowy Cancer Research Centre, NSW, Australia; [3]Biomolecular Frontiers Research Centre, Macquarie University, NSW, Australia* 359

#B295 **"Sialylation of Thomsen-Friedenreich antigen is a noninvasive blood-based biomarker for GNE myopathy";** Marjan Huizing[1], Petcharat Leoyklang[1], Tal Yardeni[2], Frank Celeste[3], Carla Ciccone[1], Xueli Li[4], Rong Jian[4], Nuria Carrillo-Carrasco[3], Miao He[4], William A. Gahl[5], May Christine V. Malicdan[1] *[1]Medical Genetics Branch, National Human Genome Research Institute, NIH; [2]Medical Genetics Branch, National Human Genome Research Institute, NIH; Sackler Faculty of Medicine, Tel Aviv University; [3]Therapeutics for Rare and Neglected Diseases, National Center for Advancing Translational Sciences, NIH; [4]Department*

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Session IX: Glycan structures and Bioinformatics

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#B304 **"Chemical characterization of acidic milk oligosaccharides of the platypus (*Ornithorhynchus anatinus*)";** Tadasu Urashima[1], Hiroaki Inamori[1], Kenji Fukuda[1], Tadao Saito[2], Michael Messer[3] *[1]Obihiro University of Agriculture & Veterinary Medicine; [2]Tohoku University; [3]The University of Sydney* 201

#B305 **"Comparative performance of four methods for high-throughput glycosylation analysis of immunoglobulin G in genetic and epidemiological research";** Maja Pucic-Bakovic[1], Jennifer E. Huffman[2], Lucija Klaric[1], René Hennig[3], Maurice H. J. Selman[4], Yurii S. Aulchenko[5], Erdmann Rapp[6], Manfred Wuhrer[7], Gordan Lauc[8] *[1]Genos Glycoscience Laboratory, Zagreb; [2]MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh; [3]Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg; glyXera GmbH, Magdeburg; [4]Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden; [5]Institute of Cytology & Genetics SB RAS, Novosibirsk; "Yurii Aulchenko" consulting, Groningen; [6]Dynamics of Complex Technical Systems, Magdeburg; glyXera GmbH, Magdeburg; [7]Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden; Division of BioAnalytical Chemistry, VU University Amsterdam; [8]Genos Glycoscience Laboratory, Zagreb; University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb* 202

#B306 **"High throughput glycomics investigation of the breast cancer progression";** Zengqi Tan[1], Wei Lu[1], Ganglong Yang[1], Zheng Li[2], Feng Guan[1] *[1]The Key Lab of Carbohydrate Chem & Biotech; School of Biotechnology, Jiangnan University, 1800 Lihu Avenue, Wuxi, 214122, China.; [2]Laboratory for Functional Glycomics, College of Life Sciences, Northwest University, 229 Taibai Beilu, Xi'an 710069, China* 203

#B307 **"Normalization and batch correction methods for high-throughput glycomics";** Frano Vučković[1], Gordan Lauc[1], Yurii Aulchenko[2] *[1]Genos Glycobiology Laboratory, Zagreb, Croatia; [2]Institute of Cytology and Genetics SD RAS, Novosibirsk, Russia* 204

#B308 **"Development of novel methods for glycan characterization";** Renpeng Liu, Paula Magnelli, Elizabeth McLeod, Ellen Guthrie *New England Biolabs Inc.* 205

#B309 **"A MALDI-TOF MS approach for the quantitive analysis of total N-glycans";** Kyoung-Jin Kim[1], Yoon-Woo Kim[1], Jangmi Jin[2], Young Hwan Kim[2], Yun-Gon Kim[1] *[1]Department of Chemical Engineering, Soongsil University, South Korea; [2]Division of Mass Spectrometry Research, Korea Basic Science Institute, South Korea* 206

#B310 **"GRITS Toolbox - A freely available software suite for the interpretation of glycomics high-throughput MS/MS data";** Rene Ranzinger, Brent Weatherly, Shahnawaz Khan, Khalifeh AlJadda, Mindy Porterfield, Michael Tiemeyer, William York *Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA* 207

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(1) Chemistry and Biology of Glycosylation: a Search for Universal Cancer Vaccine

Chi-Huey Wong
Academia Sinica

Biological glycosylation is one of the most complex biological processes and is known to modulate protein folding, stabilization, trafficking, signaling and many intercellular recognition events associated with bacterial and viral infection, cancer progression and immune response. Development of new tools and methods for use to understand the roles of carbohydrates in biology may provide new solutions to many of the unsolved disease problems. This lecture will describe our efforts in this regard with focus on the development of universal vaccines to combat the problems of influenza and cancer.

(2) Bacterial glycoconjugates; chemical synthesis and functional analysis

Yukari Fujimoto
Keio University

Many of Bacterial glycoconjugates from the cell surface are known as immunomodulators. We have been interested in the precise chemical syntheses and the functional analyses of these compounds. One of the major components is bacterial cell wall peptidoglycan (PGN), which has common structural motives including glycan chains of alternating GlcNAc-MurNAc with peptide linkage as the unit structure. Presumably because of the structural commonality, macrocellular organisms recognize the PGN with various proteins of their immune system, and bacteria have many kinds of proteins to recognize the PGN, as their biosynthetic target or for anchoring to the cell wall. We have synthesized various PGN fragment structures to make the compound library for applying to the functional analysis of the molecule. One of the applications is the array of PGN fragment structures; It enabled to analyze the recognition site of PGN by the various proteins.

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(3) Challenges in Glycan Synthesis with Applications to Immunology

Nicola L.B. Pohl
Department of Chemistry, 212 S. Hawthorne Dr., Indiana University, Bloomington, IN 47405 USA

Many advances in understanding the role of glycans in a range of biological systems are stalled by the lack of chemically well-defined carbohydrate structures. For automation to be as important in the synthesis of oligosaccharides as it currently does in peptide and nucleic acid production, the major bottlenecks of building block access and product analyses must be overcome. This talk will outline steps to surmounting these two problems- specifically new glycosyl activation protocols and mass-spectrometry-based analysis protocols- along with applications of the resulting molecules to vaccine and vaccine adjuvant design.

(4) Using chemistry to understand the mechanisms and biological roles of endo-acting α-mannosidases

Spencer Williams
University of Melbourne

α-Mannosidases are important enzymes in glycoprotein maturation and play key roles in protein quality control, as well as influencing the cellular targeting and immunological properties of the resulting glycoprotein. Within fungi there is emerging evidence for their role as transglycosylases in the construction of the cell wall, and there is evidence that certain members of the gut microbiota have the unique capacity to utilize yeast cell wall α-mannan. While significant attention has focused on the study of exo-acting α-mannosidases such as the endoplasmic reticulum- and Golgi-located enzymes, less attention has been directed at the study of endo-acting α-mannosidases/α-mannanases. In this presentation I will discuss recent progress in understanding the mechanisms and roles of endo-acting α-mannosidases/α-mannanases from mammalian and bacterial sources that act on N-linked glycans. In particular I will highlight the development of substrates and inhibitors and their use as chemical tools to provide new insights into enzyme mechanism, and as reagents to facilitate cell biology investigations.

(5) Discovery and improvement of novel glycosidases through metagenomics and directed evolution

Stephen G. Withers
Centre for High-throughput Biology (CHiBi) and Dept. of Chemistry University of British Columbia, Vancouver, B.C., Canada

Mother Nature has already supplied an enormous diversity of biocatalysts, each of which could serve as a starting point for directed evolution studies. The problem can be in accessing this diversity in a reasonably efficient manner. Here we shall describe our use of activity-based, or functional metagenomics to generate a library of over 300 expressed glycosidases. We shall also describe the high-throughput characterisation of these enzymes for substrate specificity, thermal stability, pH profile and mechanism. Finally we shall describe the use of this library to identify preferred catalysts for cleavage of specific unnaturally modified sugars (e.g. azido sugars) and the generation of glycosynthase" versions that can be used to "tag" glycans. Such libraries can be used to select the optimal candidate for further improvements through directed evolution.

(6) Biological Roles of Glycans: Two Decades Later

Ajit Varki
University of California, San Diego

In keeping with the goals of Sunrise Sessions this talk will focus on a subject of broad general interest. Glycans have long been known to play many major metabolic, structural and biophysical roles in biology. Pathogen recognition of host glycans has also been studied for decades. But such roles cannot explain the remarkable complexity and organismal diversity of glycans in nature. Reviewing the subject twenty years ago (*Glycobiology* 3:97-130,1993), one could find very few clear-cut instances of other specific biological roles of glycans that were of intrinsic value to the organism synthesizing

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them. Some general principles emerged. First, the consequences of experimental modification of glycosylation seemed highly variable, making it difficult to predict a priori the functions that a given glycan might be mediating, or its relative importance. Second, the same glycan might mediate different functions at different locations within an organism, or at different times in its ontogeny. Third, the more specific intrinsic biological roles known at the time were mediated by unusual sequences, unusual presentations of common sequences, or further modifications. However, such sequences were also more likely to be targets for pathogenic toxins and microorganisms. It was therefore posited that host-pathogen interactions might contribute to aspects of intra- and inter-species glycan variation. Two decades later, there are innumerable examples of biological roles. Thus, a current review cannot be comprehensive. Instead, a historical overview will be presented, broad principles outlined and a few examples cited, representing diverse types of roles. What remains unchanged is that while all theories regarding biological roles of glycans are supported, exceptions to every one can be found. In retrospect this is unsurprising. Complex glycans are ubiquitous to all living cells in nature, and essential to all life forms. Thus, >3 billion years of evolution generated organisms that use these molecules for key biological roles, even while coopting them for relatively unimportant roles, or sometimes for no obvious roles at all. In this respect, glycans are no different from other major macromolecular building blocks of life. It is time for the diverse roles of glycans to be incorporated into the mainstream of biological studies.

(7) **Natural immune programming of antibody glycosylation**

Galit Alter
Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA, 02139

In addition to neutralization, antibodies (Abs) represent a critical bridge between the adaptive and innate immune system, as they mediate their activity by harnessing and instructing the innate immune system on how to clear the antigen to which they are bound. The ability of Abs to provide specificity to the innate immune system is tightly regulated by: a) the isotype of the antibody (Ab), and b) the glycan structure attached at the asparagine 297 within the CH2-domain of the Ab heavy chain. Importantly, Ab engineering has revolutionized the clinical efficacy of monoclonal Abs through the optimization of Ab glycan structures for the treatment of malignancies and autoimmune disorders, yet little is known about how Ab glycosylation is naturally tuned *in vivo* nor how it may be harnessed actively through vaccination. However, accumulating evidence suggests that B cells actively alter Ab glycosylation under inflammatory conditions to dramatically tune the activity of an Ab. While significant shifts have been observed in antibody galactosylaton and sialyation, how the addition of these sugars is actively regulated in B cells is poorly understood. Here we observed that glycosyltransferase expression is rapidly altered by distinct adjuvant signals, in such a way that bacterial and viral inflammatory signals induced distinct non-overlapping functional antibody profiles via the expression of distinct glycosyltransferase signatures. Moreover, *in vivo*, glycosyltransferase profiles selected during the priming of a B cell response were durably programmed and persisted upon antigen-re-exposure, linked to unique transcription factor profiles that regulate the expression of these glycosyltransfersases for the life of the B cell. These data argue that the selection of glycosyltransferase genes, aimed at regulating antibody glycosylation (and likely other B cell functions), are a vital component of the epigenetic program selected during antigen-specific adaptive immune programming aimed at controlling and clearing pathogens as effectively as possible. Together, knowledge gained from these studies provide critical insights into the mechanism by which Ab-effector functions are regulated for vaccine design, but also lay the groundwork to begin to understand how glycosylation is regulated across the immune system.

(8) **Immune functions of glycans in infectious disease**

Richard Cummings
Department of Biochemistry, Emory University School of Medicine, Atlanta, Georgia 30322

Viruses, bacteria, and parasites, such as parasitic protozoans and helminths, express a wide variety of glycans structures and these are typically recognized by host glycan-binding proteins (GBPs) and antibodies. In addition, many pathogens express multiple GBPs used for cellular adhesion and entry into hosts. The complex pathogen-derived glycans are also immunostimulating and can induce both adaptive and innate immune responses in infected hosts. To explore the immunoglycobiology of these interactions we have studied the glycan structures of pathogens and their interactions with host cells, as well as exploring the interactions of pathogen-derived GBPs with host glycans using glycan microarray technologies. This presentation focus on studies arising from these approaches and novel information about host-pathogen interactions involving glycan recognition for multiple viruses, bacteria, and parasites, including immune responses to parasitic helminths, including trematodes, e.g. Schistosoma mansoni, and nematodes, e.g. Trichinella spiralis, where immunity is primarily directed against glycan, rather than protein antigens. To explore host glycans responsible for recognition by pathogens we have developed "shotgun glycomics" approaches, in which host-derived glycans are released, fluorescently tagged, separated, and covalently printed to generate glycan microarrays. Interrogation of such microarrays with specific GBPs leads to the identification of candidate glycan ligands, which can then be sequenced and identified by multiple approaches, including metadata-assisted glycan sequencing (MAGS) that also incorporates structural information from mass spectrometry approaches.

(9) **Glycan targets of the antibody response in natural and experimental schistosome infections**

Cornelis Hokke[1], Cornelis Smit[1], David Dunne[2], Alan Wilson[3], Angela van Diepen[1]
[1]Leiden University Medical Center, NL; [2]Cambridge University, UK; [3]University of York, UK

Schistosomiasis is a chronic and potentially deadly parasitic disease that affects millions of people in (sub)tropical areas. Immunity to Schistosoma can be acquired in humans, but this takes many years

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of exposure, multiple infections and treatments, and maturation of the immune system. Most antibodies generated are directed against the numerous schistosome glycans, but the precise structure of the glycan antigens and the relation to immunity are poorly understood. To be able to efficiently study anti-glycan antibodies in schistosomiasis we have generated a microarray containing hundreds of naturally occurring N-, O-, and lipid-glycans isolated from different life stages of S. mansoni. During this process we have first mapped the overall glycomes of the different developmental stages of the schistosome, identifying a number of striking shifts and switches in the expression of antigenic glycan motifs on proteins and lipids during the maturation of the worm and of its eggs. The larval cercariae and the mature eggs abundantly express LeX and multifucosylated LDN motifs as well as unique stage-specific O-glycan core structures, but in the worm these patterns strongly change. The microarray printed of these glycans was applied to the analysis of anti-glycan IgG and IgM in sera from an S. mansoni-endemic community in Uganda. We observed age-dependent differences, especially when looking at changes before and after treatment with PZQ, and between groups with high and low infection intensities. In addition we have used sera from baboons vaccinated with irradiated S. mansoni cercariae to study longitudinally the development of anti-glycan responses. Intense anti-glycan antibodies responses are observed within 2 weeks after the 2nd vaccination, in particular against cercarial lipid-derived glycans as well as cercarial O-glycans carrying unique Fucα1-2Fucα1-3GlcNAc motifs, raising the possibility that such glycans could be useful as vaccine target. These glycan microarray studies shed light on the development of anti-glycan responses in schistosomiasis and may correlate antibodies to specific glycan motifs to parameters such as exposure, infection intensity, history of previous infection and resistance to re-infection in different natural and experimental cohorts and settings.

(10) Development of highly sensitive diagnostic system for dengue viruses using the interaction between sulfated sugar-chain and viral particle

Yasuo SUDA[1], Budi SAKSONO[2], Beti Ernawati DEWI[3], Leonardo NAINGGOLAN[4]

[1]Kagoshima University and SUDx-Biotec Corporation; [2]Kagoshima University and Indonesian Institute of Sciences; [3]Universitas Indonesia; [4]Universitas Indonesia and Cipto Mangun Kusumo Hospital

Dengue viruses (DENVs), which have four phylogenetically and antigenically distinct types, are emerging arthropod-borne flaviviruses that cause dengue fever (DF), dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS) mostly in tropical and subtropical countries. More than 200 million cases of DF and of 1 ~ 5% become more severe into DHF and/or DSS. DHF/DSS often occurs in patients with second, heterotypic DNEV infection. All DENV subtypes spread in the world and the co-circulation of multiple DENVs had been reported, increasing the number of DHF/DSS. Unfortunately, neither licensed vaccines nor effective antiviral drugs against DENV are available on the market. Thus, a highly sensitive, accurate, and convenient diagnosis of DENV infection should be performed in order to initiate patient's treatment in specific preventive health conditions. A novel method to diagnose DENVs in trace amount of sera is proposed. The method is based on the interaction between sulfated sugar-chain and surface glycoproteins of DENVs. By capturing DENV with sugar-chain immobilized gold-nanoparticles (SGNPs), the formed complex was precipitated, followed by measuring viral RNA with the rivers-transcriptase quantitative polymerase chain reaction SYGR Green I method. Sugar-chains to which DENVs bind were evaluated using the array type Sugar Chip and the SPR imaging, and heparin and low-molecular-weight dextran sulfate were selected and immobilized on gold-nanoparticles to prepare three kinds of SGNPs. The ability for capturing and concentration of low abundant DENVs of these SGNPs were evaluated in vitro. Then, the best SGNP was verified in the clinical research performed in Indonesia in 2013-2014. As a result, our method was able to detect low abundant of DENVs from 6 μL of sera and showed a similar detection percentage to that with RNA extraction method by Qiagen kit using 140 μL of sera. In addition, our method gave a multiplex-like detection for diagnosing the serotype of DENV.

(11) Molecular regulation of antigen-specific antibody glycosylation following B cell activation

Wen-Han Yu[1], Doug Lauffenburger[2], Galit Alter[3]

[1]Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard University Department of Biological Engineering, Massachusetts Institute of Technology; [2]Department of Biological Engineering, Massachusetts Institute of Technology; [3]Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard University

Antibodies represent one of the most potent secreted antiviral proteins in nature, as they act as rapidly evolving antigen-specific effector molecules that direct the containment and killing of any binding object. The mechanism behind is to directly recruit the innate immune system, through alterations in the constant region of the antibody's affinity for different innate immune receptors (Fc- and lectin-like receptors). These changes include: 1) differential selection of antibody subclass (4 isotypes + 4 IgG subclasses) coupled to 2) differential glycosylation of the constant domain of the antibody. Importantly, accumulating data suggests that antibody glycosylation changes rapidly during an inflammatory response and can be actively and directly tuned by B cell stimulation. However, very little is known about the mechanism that underlies B cell tuning of glycosylation, which requires elaborate coordination of a repertoire of glycoenzymes. Here we focused on defining transcription factor regulatory network, in B cells, which potentially regulated glycosyltransferase expression. To do so, we used a systems biology approach to reverse-engineer almost complete TF regulatory networks in B cells. We first analyzed genome-wide data on chromatin accessibility (DNase-seq) of B cells to identify TFs that can physically interact with the targets, and verified TF-target regulatory interactions (activation/repression) based on a correlation in their co-expression behavior from a compendium of transcriptomic profilings. The network showed that each glyco-enzyme was regulated by multiple potential TFs, and some of which have been identified as the regulators of interferon expression as well as antibody class

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switching, suggesting that antibody glycosylation is co-modulated with a variety of B cell responses. Next we identified the impact of distinct adjuvants in tuning TF sub-networks, associating to glycosyltransferase expression, using RNA-sequencing where naive B cells were stimulated with agonists for TLRs2-9, in the presence or absence of BCR cross-linking and/or CD40-ligation (CD4help). We observed that several core TFs that regulated glycosyltransferase expression were conserved in all stimuli, as well as stimulus-dependent TFs. Together all suggested that combinatorial regulation may play a critical role in fine-tuning stimulus-dependent glycosylation, providing first in class insights into the mechanism by which glycosylation is regulated in antigen-specific B cells.

(12) *In vivo* blockade of sialylation with a global sialyltransferase inhibitor causes irreversible kidney dysfunction

Matthew Macauley[1], Britni Arlian[1], Cory Rillahan[1], Poh-Choo Pang[2], Nikki Bortell[1], Maria Cecilia Marcondes[1], Stuart Haslam[2], Anne Dell[2], James Paulson[1]
[1]*The Scripps Research Institute;* [2]*Imperial College London*

Sialic acid terminates glycans of glycoproteins and glycolipids that play numerous biological roles in health and disease. While genetic tools are available for interrogating the effects of decreased or abolished sialoside expression in mice, pharmacological inhibition of the sialyltransferase family has to date not been possible. We have recently shown that a sialic acid analog, 3F-NeuAc, added to the media of cultured cells shuts down sialylation by a mechanism involving its intracellular conversion to CMP-3F-NeuAc, a competitive inhibitor of all sialyltransferases. Here we show that administering 3F-NeuAc to mice dramatically decreases sialylated glycans in cells of all tissues tested, including: blood, spleen, liver, brain, lung, heart, kidney, and testes. A single dose results in greatly decreased sialoside expression for over 7 weeks in some tissues. While blockade of sialylation with 3F-NeuAc does not affect viability of cultured cells, its use *in vivo* has a deleterious 'on-target' effect on liver and kidney function. After administration of 3F-NeuAc, liver enzymes in the blood are dramatically altered, and mice develop proteinuria concomitant with dramatic loss of sialic acid in the glomeruli within 4 days, leading to irreversible kidney dysfunction and failure to thrive. These results confirm a critical role for sialosides in liver and kidney function and document the feasibility of pharmacological inhibition of sialyltransferases for *in vivo* modulation of sialoside expression.

(13) Carbohydrate-binding lipoproteins enhance glycan sensing and acquisition by human gut bacteria

Nicole Koropatkin
University of Michigan Medical School

The mammalian intestinal tract is home to a diverse array of glycan-degrading bacteria that harbor several times more glycoside hydrolases than present in the human genome. Because this is a densely packed and competitive ecosystem, many prominent gut species have evolved ways to effectively sense and scavenge carbohydrate nutrition. The *Bacteroidetes*, a prominent Gram-negative phylum of bacteria in the human gut, display a greatly expanded capacity for glycan degradation via the expression of multiple outermembrane protein complexes, termed Sus-like systems. These multiprotein complexes are comprised of several proteins that sequentially bind, degrade and import a specific carbohydrate. One functionally conserved class of proteins, the carbohydrate-binding lipoproteins (CBLPs), within the Sus-like systems display a wide range of sequence length and glycan-binding diversity. Our previous investigation of the CBLPs SusE and SusF, from the starch utilization system (Sus) of *Bacteroides thetaiotaomicron* revealed a tandem array of starch-specific carbohydrate binding modules (CBMs) that aid in the acquisition of starch through the thick polysaccharide capsule. SusE and SusF possess two and three CBMs respectively, which can recognize different forms of the alpha-glucan structure. Most recently we have solved the crystal structure of *Bacova_02650*, a functional homolog of SusE/F that targets the plant cell wall hemicellulose xyloglucan to the surface of the human gut symbiont *Bacteroides* ovatus. This protein displays four beta-sandwich (Ig-like) folds, with a single xyloglucan-binding site that resides at the "top" of the most membrane-distal domain. A bound dimer of the repeated xyloglucan tetrasaccharide motif reveals that glycan binding is mediated via hydrophobic stacking of several aromatic residues along four consecutive glucose residues. Although the protein makes few interactions with glycan side chains, it does not bind cellohexaose, suggesting that binding is xyloglucan-specific. While deletion mutants of *Bacova_02650* grow on xyloglucan in vitro, we hypothesize that the CBLPs confer a competitive advantage to the organism in the densely populated and competitive mammalian gut. We are currently characterizing CBLPs from other gut *Bacteroides* sp. in order to understand the structural diversity and specificity of this functionally conserved class of proteins.

(14) O-glycoprotein podoplanin is essential for vascular integrity during inflammation

Jianxin Fu[1], Brett H. Herzog[2], Lijun Xia[1]
[1]*Oklahoma Medical Research Foundation;* [2]*Oklahoma Medical Research Foundation, University of Oklahoma Health Sciences Center*

We previously discovered that mice lacking endothelial mucin-type O-glycans have impaired expression of the O-glycoprotein podoplanin in lymphatic endothelium and mixed blood and lymphatic vessels. We found that mice lacking podoplanin display a similar phenotype, uncovering the requirement for podoplanin in the separation of lymphatic vessels from blood vessels during development (Fu *et al.*, JCI, 2008). Podoplanin controls the separation of blood and lymphatic vessels by activating platelets through interactions with platelet C-type lectin-like 2 (CLEC-2) receptors. Podoplanin and platelet CLEC-2 are also required to maintain the separated blood and lymphatic vascular systems after birth (Hess *et al.*, JCI 2014). Furthermore, our recent work reveals that podoplanin activation of platelet CLEC-2 increases the high endothelial venules integrity by promoting VE-cadherin expression at the endothelial adherens junctions effectively sealing the functional leakage caused by

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transmigrating lymphocytes (Brett *et al.*, Nature 2013). These findings suggest that podoplanin function to protect vascular integrity during leukocyte trafficking in inflammation. Inflammation is a fundamental pathophysiological process in nearly all diseases, including bacterial infections, autoimmune reactions, cancers, and ischemic injuries. The hallmark of inflammation is the infiltration of leukocytes into inflamed tissues, which is accompanied by a significant increase in vascular permeability. Platelet immunoreceptor tyrosine-based activation motif (ITAM) receptors, which are platelet glycoprotein VI (GPVI) and CLEC-2 in mice, are required in the prevention of inflammation-induced vascular leakage and hemorrhage. However, the activating ligand for CLEC-2 that functions in the protection of vascular integrity during inflammation remains unclear. In addition, the mechanism by which ITAM signaling protects vascular integrity during inflammation is unknown. Our recent studies demonstrate that blocking platelet CLEC-2 function exacerbates vascular permeability. We found that podoplanin and CLEC-2-activated platelets is critical for protecting vascular integrity of venules during inflammation. These results not only provide new insights into vascular dysfunction in inflammatory conditions such as ischemic injuries, but will also allow us to determine whether promoting podoplanin-CLEC-2 signaling can be used as a new therapy.

(15) **Sialylated glycans on airway mucins bind mouse eosinophils via Siglec-F and induce their apoptosis *in vitro* and *in vivo***

Bruce Bochner

Northwestern University Feinberg School of Medicine, Division of Allergy-Immunology

Asthma is a chronic inflammatory disease characterized by bronchial hyper-reactivity and reversible obstruction of the airway. Current approved asthma therapies, such as corticosteroids, target these inflammatory processes either directly or indirectly by reducing airway eosinophils, a leukocyte linked to asthma pathogenesis and exacerbations in a sizable subset of asthmatics. Therefore, novel therapeutic opportunities may arise from understanding the cellular and molecular processes that limit lung eosinophilia in asthma.

Toward this goal, we have investigated the functions of the Siglec family of receptors in lung inflammatory diseases. Among these, human Siglec-8 is selectively expressed on eosinophils and mast cells, and its closest functional paralog in the mouse is Siglec-F, which is specifically expressed by eosinophils and alveolar macrophages, but is absent from mast cells in the mouse. Glycan array screening demonstrated that both Siglec-8 and Siglec-F preferentially bind the sialoside glycans 6'-sulfo-sialyl-Lewis X (6'-S-Sialyl-Le^x) and 6'-sulfo-sialyl-N-acetyl-D-lactosamine (6'-S-Sialyl-LacNAc). Engagement of Siglec-8 or Siglec-F with stimulatory antibodies or with synthetic glycan ligands that carry binding epitopes causes eosinophil death in vitro. Administration of Siglec-F antibodies in mouse models of allergic asthma normalizes eosinophil numbers and abrogates lung remodeling. Siglec-F deficient mice, as well as mice deficient in a key enzyme required to synthesize its α3-sialylated glycan ligands, namely the α2-3 sialyltransferase ST3Gal-III, display a selective enhancement of allergic eosinophilic inflammation, but the identities of endogenous sialoside ligands and their glycoprotein carriers in vivo remained unknown.

Our latest studies have demonstrated the existence of inflammation and cytokine-inducible, protease-sensitive, high molecular weight material in the mouse airway that bind Siglec-F in a sialic acid-dependent manner. By applying a series of cellular, molecular, glycoproteomic and whole animal approaches, we report that endogenous airway mucins carry glycans that bind Siglec-F and induce eosinophil apoptosis in vitro and in vivo. These data support the concept that airway mucins, via their sialylated glycan ligands for Siglec-F, represent an important innate pathway for controlling lung eosinophilia.

Supported in part by grants AI72265 and HL107151.

(16) **Role of CD22/Siglec-2 and its ligand in B lymphocyte activation**

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CD22/Siglec-2 is a member of the Siglec family specifically recognizes α2,6 sialic acid. CD22 contains immunoreceptor tyrosine-based inhibition motifs (ITIMs) at the cytoplasmic tail and negatively regulates B cell receptor (BCR) signaling. α2,6 sialic acid is abundantly expressed in B cells, and the ligand binding site of CD22 on B cells is mostly occupied by the ligand expressed on the same cell (cis ligand). How the interaction of CD22 with the cis ligand regulates CD22 is controversial. There are pieces of evidence suggesting that the cis ligand positively or negatively regulates CD22, respectively. We have developed synthetic sialosides that bind to CD22 with sub-micromolar affinity whereas α2,6 sialic acid binds to CD22 with millimolar affinities. Among these synthetic sialosides, GSC718 contains a biphenyl group at the C9 position and a benzyl group at the C2 position. In vitro treatment with GSC718 enhanced activation and proliferation of mouse spleen B cells probably by disrupting the interaction of CD22 with the cis ligand. Interestingly, GSC718 failed to enhance Ca^{2+} signaling induced by BCR ligation. These results suggest that the cis ligand of CD22 regulates B cell activation independently of BCR ligation-induced signaling. We are currently investigating how the cis ligand regulates signaling and activation of B cells.

(17) **Glycan-mediated control of immune homeostasis**

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Myelin oligodendrocyte glycoprotein (MOG), a constituent of central nervous system myelin, is an important autoantigen in the neuroinflammatory disease multiple sclerosis (MS). We have demonstrated that in healthy human myelin, MOG is decorated with fucosylated N-glycans that support recognition, binding and internalization (as demonstrated by imaging flow cytometry) by the C-type lectin

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receptor (CLR) dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) expressed on microglia and dendritic cells (DCs). The interaction of MOG with DC-SIGN in the context of simultaneous Toll-like receptor 4 activation resulted in enhanced IL-10 secretion and decreased T cell proliferation in a DC-SIGN-, glycosylation-, and Raf1-dependent manner. Interestingly, the exposure of oligodendrocytes to proinflammatory factors resulted in the down-regulation of fucosyltransferase expression, reflected by altered glycosylation at the MS lesion site. In parallel, the removal of fucose on myelin resulted in a reduced DC-SIGN-dependent homeostatic control, and translated in inflammasome activation, increased T cell proliferation, and differentiation toward a Th17-prone phenotype. These data demonstrate a new role for myelin glycosylation in the control of immune homeostasis in the healthy human brain through the MOG-DC-SIGN homeostatic regulatory axis, which is comprised by inflammatory insults that affect glycosylation. This phenomenon should be considered as a basis to restore immune tolerance in MS. But also the properties of DC-SIGN as an antigen-uptake receptor with signaling properties make this receptor an excellent target for antigen delivery to DCs in the context of anticancer immunotherapy. We have used imaging flow cytometry to investigate the intracellular and endocytic routing of DC-SIGN to better understand its Biology and help in the design of new glycan-based antigen delivery systems with strong cross-presentation capacity.

(18) Galectin-3 protects intracellular Listeria monocytogenes by suppressing autophagy activation via inhibition of nitric oxide production and bacterial ubiquitination

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Galectin-3 is an evolutionarily conserved β-galactoside-binding animal lectin expressed in various immune cell types. In macrophages, galectin-3 has been reported to regulate multiple functions such as phagocytosis, LPS-induced inflammation, and alternative macrophage activation. Recently, galectin-3 was suggested as a marker for vacuole lysis by invasive intracellular bacteria and co-distributed with proteins of the ubiquitin-autophagy pathway. Interestingly, galectin-8 was also reported to serve as a danger receptor that detects host glycans exposed on damaged bacteria-containing vacuoles and activates antibacterial autophagy. Autophagy is a fundamental eukaryotic pathway that maintains cellular homeostasis, and it is also an important cellular defense mechanism for elimination of intracellular microorganisms through lysosomal degradation. Here we show in mouse bone marrow-derived macrophages (BMMs) that galectin-3 protects intracellular *Listeriamonocytogenes* by suppressing the activation of selective autophagy through down-regulating nitric oxide (NO) production and inhibiting bacterial ubiquitination. We found that in gal3$^{-/-}$ BMMs, elevated autophagy activation was responsible for reduced *Listeria* survival, since inhibition of autophagy by 3-methyladenine (3-MA) diminished the differential bacteria load between gal3$^{+/+}$ and gal3$^{-/-}$ BMMs. Moreover, immunofluorescence staining of *Listeria*-infected macrophages revealed more efficient recruitment of ubiquitin, adaptor protein p62, and LC3 to the bacteria in the absence of galectin-3. We observed that rapid recruitment of galectin-3 to the vicinity of intracellular *Listeria* is dependent on the presence of host N-glycans, but the difference in autophagy activation and bacteria survival between gal3$^{+/+}$ and gal3$^{-/-}$ BMMs was still present when host N-glycan is depleted, suggesting that galectin-3's anti-autophagic effect can be independent of its binding to host N-glycans. In addition, we found that NO production was enhanced in gal3$^{-/-}$ BMMs compared to that in gal3$^{+/+}$ BMMs during *Listeria* infection, and inhibition of NO synthesis also eliminated the differential bacterial load between gal3$^{+/+}$ and gal3$^{-/-}$ BMMs. Importantly, NO has recently been reported to act as a signaling molecule for autophagy induction via promotion of target ubiquitination. Together, our data demonstrated a previously unrecognized anti-autophagic function of endogenous galectin-3 that confers protection on vacuole-breaking intracellular *Listeria* in macrophages.

(19) Functional evaluation of sialoglycans expressed in activated T cells

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T cells are regulatory cells controlling overall immunity. Different subpopulation of T cells expresses different glycans and difference in the glycan expression is useful for identification of particular cell types as subpopulation. However, underlying functional aspects of such glycan changes/differences are still elusive. We tried to understand glycosylation change in the activated T cells.

Cell surface glycans are often terminated with 9-carbon acidic sugar called sialic acids (Sias). Expression of Sia varies in its underlying sugar and linkage. Sia molecule also could be modified to give rise various molecular species. In activated T cells, N-glycolylneuraminic acid (Neu5Gc) expression was suppressed and subsequently N-acetylneuraminic acid (Neu5Ac), precursor form of Sia was induced; indicating that Neu5Gc biosynthesis by CMP-Neu5Ac hydroxylase (Cmah) is altered. Change in the glycans can be translated into biological context as recognition events from glycan-binding proteins, thus activated T cells were analyzed with glycan-binding probes. Among siglecs, activated T cells suppressed sialylated ligands for CD22 (prefers α2-6 linked Neu5Gc) and induced ligand for sialoadhesin/CD169 (prefers α2-3 linked Neu5Ac). Activated T cells also induced the epitope of GL7, a monoclonal antibody recognizes α2-6 linked Neu5Ac on N-acetyllactosamine. Therefore, T cell activation seemed to alter Sia species Neu5Gc to Neu5Ac and linkage α2-6 to α2-3, respectively.

We further studied the functionalities of this altered glycosylation using Neu5Gc-deficient *Cmah* KO mice in combination of *CD22* KO mouse in the context of T cell-B cell interaction in germinal center reaction. Neu5Gc expression in T cells caused CD22-mediated non-cognate *trans* recognition from CD22 ligand unmasked *Cmah*-deficient B cells.

Antigen presentation event(s) involving CD169/sialoadhesin is in active foci of research in the field. Since sialoadhesin/CD169 ligand

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was preferentially induced on activated CD8+ T cells, we examined cytotoxic T cell activation event in the sialoadhesin ligand-deficient *Cmah* transgenic mice. When *Cmah* expression is forced in T cells to biosynthesize Neu5Gc leading to suppress CD169/sialoadhesin ligand, cytotoxic T cell activation in OVA-latex immunized was reduced. Overall, change in the major Sia species could affect various cell-cell recognition events in mouse immune cells.

(20) **Regulation of neuronal development by glycosaminoglycans**

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Extracellular environment that surrounds almost all cell surfaces has been known to play essential roles in a wide spectrum of neurobiological functions including neuronal development and subsequent plasticity. Because of the ubiquitous emergence of glycans within extra/pericellular spaces, their potentials as microenvironmental cues for such neuronal regulations have been studied. Recent advances in "Glyco-Neuroscience" have provided strong evidence for a functional relevance of distinct glycans, especially sulfated glycosaminoglycan (GAG) chains, in neuronal plasticity. GAGs are linear polysaccharides that consist of repetitive disaccharide units comprised of an amino sugar, either *N*-acetylgalactosamine or *N*-acetylglucosamine, and a glucuronic acid [1]. Despite their simplified polysaccharide backbones, GAGs acquire remarkable structural variability via distinct enzymatic modifications, which is the primary reason for their functional diversity [1]. During brain development, sulfation profiles, defined as the 4-sulfation/6-sulfation (4S/6S) ratio, of chondroitin sulfate (CS), a representative sulfated GAG, change dramatically. The developmental increase in 4S/6S ratio is tightly coupled to the termination of the critical period for ocular dominance plasticity [2]. Notably, systemic overexpression of human C6ST-1, a sulfotransferase responsible for the 6-sulfation of CS chains, in mice shows a substantially lower 4S/6S ratio and retains juvenile levels of the cortical plasticity even in adulthood [2]. In addition to the variable sulfation status of GAG chains, the chain length and/or the number of GAG chains per core protein are also finely tuned under the control of GAG biosynthetic machineries. Here, we will present recent data regarding the importance of GAG chains in neural development.

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(21) **Roles of glycosaminoglycans in neurophysiology and neurological disorders**

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Glycosaminoglycans (GAGs) are a family of linear acidic polysaccharides, including heparan sulfate, chondroitin sulfate, keratan sulfate, and hyaluronan. Physiological roles of GAGs in various tissues can be effectively studied by using targeted ablation of glycosyltransferase genes. We have been using this approach to determine the physiological roles of GAGs in the nervous and skeletal systems. These studies have revealed the essential role of these GAGs in development of these tissues. More recently, our research focus has shifted toward the understanding of how GAGs are involved in the physiology and pathophysiology of adult tissues, and found that GAGs play important roles in the regulation of neurophysiology, cognition, and behavior. For instance, removal of heparan sulfate from excitatory neurons results in striking recapitulation of autism-like behavioral deficits. On the other hand, knocking out of hyaluronan synthase genes increases the susceptibility of mice to epileptic seizures. The molecular mechanisms underlying these phenotypes will be discussed.

(22) **Crystal structures of an archaeal oligosaccharyltransferase provide insights into the catalytic cycle of N-linked protein glycosylation**

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Asparagine-linked glycosylation (N-glycosylation) of proteins is the most ubiquitous protein modification. The oligosaccharyl transfer from the donor substrate, the lipid-linked oligosaccharide (LLO), to the asparagine residues in the N-glycosylation sequon (Asn-X-Ser/Thr, X ≠ Pro) is catalyzed by a membrane-bound enzyme, oligosaccharyltransferase (OST). In eukaryotes, the reaction occurs on the luminal side of ER, whereas it occurs on the exterior surface of the plasma membranes in archaea and eubacteria. The catalytic subunit of the OST enzyme is a polypeptide chain referred to as STT3 in eukaryotes, AglB in archaea, and PglB in eubacteria, although they originated from a common ancestor. The eukaryotic OST is a multisubunit protein complex containing STT3, but the archaeal and eubacterial OSTs, are single subunit enzymes consisting only of the AglB and PglB proteins. We determined the crystal structures of the full-length AglB from a hyperthermophilic archaeon, *Archaeoglobus fulgidus*. This is the second solved catalytic subunit structure of OST, after the eubacterial PglB. The AglB and PglB proteins share the overall molecular architecture: an N-terminal transmembrane region, which contains the catalytic center consisting of conserved acidic residues and a divalent metal ion, and a C-terminal globular domain, which contains a binding site for the Ser and Thr residues in the N-glycosylation sequon. In addition to the crystallographic data, NMR and biochemical studies suggested the essential flexibility of one long loop in the TM region and the Ser/Thr pocket in the C-terminal globular domain for the enzymatic activity. The flexibility enables the conversion between the structured and unstructured states of the plastic loop in the transmembrane region and the formation and collapse of the Ser/Thr-binding pocket in the C-terminal globular domain. It is likely that their dynamic nature facilitates the efficient scanning of a nascent polypeptide chain for the N-glycosylation sequons when coupled with ribosomal protein synthesis. Finally, the overall structural similarity between the distantly related AglB and PglB proteins strongly

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indicates the conserved protein architecture and catalytic mechanism in the eukaryotic counterpart, the STT3 protein.

(23) Protein O-mannosylation in mammals: news and views

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Protein *O*-mannosylation has become a focus of attention as a pathomechanism underlying severe congenital muscular dystrophies associated with neuronal migration defects. Very recently, we demonstrated a functional link between *O*-mannosyl glycans and cadherin-mediated cell-cell adhesion [1]. In the course of this study, we established antibodies (α-Oman) that are specifically directed towards Thr-α1-mannose [1], and set up a mass spectrometry (MS)-based approach leading to the identification of cadherins as novel O-mannosylated proteins [2].

Using these novel tools, we characterized the distribution of *O*-mannosyl glycans in mouse tissues and screened for additional proteins carrying this modification. Our results show that single O-linked mannose residues are far more abundant than currently assumed.

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(24) Molecular mechanism of O-GlcNAc transferase translocation into nucleus

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It has been reported that one of the downstream molecules generated from glucose via the hexoamine biopsynthetic pathway (HBP) is uridine diphosphate-N-acetly glucosamine(UDP-GlcNAc). The dynamic cycle of addition and removal of O-linked-N-acetylglucosamine (O-GlcNAc) to Ser/Thr residues is involved in regulating nuclear and cytoplasmic proteins. Nucleocytoplasmic O-GlcNAc transferase (ncOGT) adds a single GlcNAc onto hydroxyl groups of serine and threonine residues. Interestingly, ncOGT dynamically modifies its target proteins in both cytoplasm and nucleus. For this reason, ncOGT has to be existed in these two compartments at the same time. The localization of ncOGT is important because it is related to the its substrate specificity, however, the mechanism of how this enzyme is sequestered in the cytoplasm and imported to the nucleus is not clear. Another interesting factor regarding ncOGT is that it is also modified by O-GlcNAc. Although it has been reported before, it is not known yet where the modification sites are and what their exact functions are. In this research, our aims are to answer these questions. First, we identified specific nuclear localization signal (NLS) in O-GlcNAc transferase that is required for nuclear transport. Also, we show that ncOGT binds importin α protein. Using ESI-Q-TOF mass spectrometry and site-direst mutagenesis we found two O-GlcNAc modification sites.

(25) The Molecular Mechanisms of Fringe Modification on *Drosophila* Notch: Examining the Structure and Function of Notch EGF Repeats

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The Notch signaling pathway is an evolutionarily conserved signaling pathway that plays critical roles in both development and disease. The Notch receptor, a large cell-surface single-pass transmembrane protein, is canonically activated upon binding to ligand expressed on an adjacent cell. In *Drosophila*, Notch can bind to one of two ligands, Delta or Serrate, to activate downstream signaling events. A marked characteristic of the Notch protein is that there are thirty-six tandem epidermal growth factor-like (EGF) repeats in the extracellular domain (ECD). EGF repeats 11-12 are designated the ligand binding domain, and are necessary and sufficient for ligand binding, although recently other EGF repeats have also been implicated in ligand binding. Interestingly, glycosylation of EGF repeats is not only essential for Notch activity, but also modulates Notch activity. Extension of the *O*-fucose by Fringe with a β3-linked GlcNAc affects how Notch responds to ligands expressed on adjacent cells, specifically increasing Notch-Delta binding while decreasing Notch-Serrate binding. However, the exact mechanism by which Fringe modification alters ligand binding is unknown. We predict that Fringe modification changes the conformation of the EGF repeats in the ECD, therefore changing the ability of the EGF repeats to interact with either ligand. First, we performed mass spectral studies on EGF repeats 1-36 from*Drosophila* Notch and found that Fringe modifies some EGF repeats more efficiently than others. We will be using site-directed mutagenesis and cell-based binding assays to study which *O*-fucose sites are important for mediating the ligand binding effect by Fringe. In addition, using electron microscopy we have seen that Fringe modification causes slight conformational changes in the Notch ECD. Additional studies are in progress to better understand how Fringe modifications regulate Notch-ligand binding. Supported by NIH grant R01GM061126 and T32GM008468.

(26) An E3 ubiquitin ligase regulates neural-specific glycosylation in the *Drosophila* embryo

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Precise glycoprotein glycosylation is essential for normal development. However, the pathways and mechanisms that control tissue-

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specific glycan expression are not well understood. A previously described mutation in the *Drosophila* tollo/toll-8 gene (a member of the Toll-like receptor family) abolishes expression of a set of neural-specific N-linked glycans known as HRP-epitopes. Tollo/Toll-8 is part of a transcellular signaling pathway that induces neural-specific glycosylation in the embryonic nervous system. In order to further characterize this pathway, a random EMS mutagenesis screen was performed to find additional genes that alter neural glycan expression using HRP-epitopes as a marker. This screen produced a mutation, designated ms16, which exhibits significant reduction of HRP-epitope expression, shares other phenotypic characteristics and genetically interacts with tollo/toll-8 as well as with one other mutant generated from the screen. Genetic mapping strategies placed the ms16 mutation in the roc2 gene, an E3 ubiquitin ligase previously demonstrated to be essential for neural development. The ms16 mutation affects transcript levels and results in decreased amount of roc2 protein in mutants. Glycomic analysis verified the reduction of HRP-epitope glycans in ms16 embryos. Additionally, the total N-linked glycomic profile was shifted toward greater glycan complexity, including increased sialylation. Altered Golgi compartmentation was detected by confocal imaging, indicating a cellular basis for altered glycosylation in the mutant. Analysis of global protein ubiquitination detected decreased polyubiquitin accompanied by increased monoubiquitin in mutant embryos. Since protein ubiquitination modulates secretory pathway trafficking by modifying actin and other proteins, altered ubiquitination in ms16 embryos results in altered protein glycosylation.

(27) **A Serendipic Career**

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Early life in Vienna and Trinidad. The early work of Saul Roseman and Charles LeBlond played a major role in the contributions of my laboratory proving that glycosyltransferases are located in the Golgi apparatus. A productive collaboration with Lou Siminovitch and a young post-doctoral fellow in his laboratory (Pamela Stanley) led to our discovery of N-acetylglucosaminyltransferases I and II (GlcNAcTI and GlcNAcTII). The major tool in this work was the use of Pamela's Chinese Hamster Ovary (CHO) mutant cell lines. These early discoveries led to several years of work on what we called "The Yellow Brick Road", the cloning of the genes encoding GlcNAcTI and II and the crystal structure of GlcNAcTI by Jim Rini's group in Toronto. In a most productive collaboration with Jaak Jaeken in Belgium, we showed in 1994 that one of the Congenital Disorders of Glycosylation (CDGs) was due to a mutation in a gene (*MGAT2*) encoding a glycosyltransferase (GlcNAcTII). This was the first CDG gene to be discovered. Jamey Marth and colleagues subsequently produced a mutant mouse that lacked the *MGAT2* gene thereby providing an animal model for this human disease. In the final years of my career I worked on the effects of mutations in GlcNAcTI deletions in invertebrates: *C. elegans* (with the help of Andrew Spence) and*Drosophila melanogaster* (with the help of Gabrielle Boulianne).

(28) **Bacterial polysialyltransferases: structure, function and engineering for glycoconjugate synthesis**

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The capsular polysaccharides of *Neisseria meningitidis* serotype B, *Escherichia coli* K1, and *Mannheimia haemolytica* A2 bacteria are composed of polysialic acid (PSA) chains containing α2,8 linked N-acetyl neuraminic acid. Being chemically and structurally identical to those found in mammals, these PSA structures provide a cloaking mechanism for the pathogenic bacteria to evade destruction by the host's immune system. The bacterial polysialyltransferases (PST) that synthesize these capsular PSAs belong to the glycosyltransferase GT-38 family and are predicted to be structurally distinct from the mammalian PSTs that belong to the GT-29 family. The bacterial PSTs are of interest as they may be exploited for synthesis of therapeutics where PSA modifications are beneficial. Such modifications have been shown to increase the circulation half-life of therapeutic proteins and, more recently, to assist in neural tissue repair.

We have indentified a new PST from the bovine/ovine pathogen *Mannheimia haemolytica* A2 (PST-Mh). The enzyme was compared with PSTs from *E. coli* K1 and*N. meningitidis* group B. We have been able to produce high levels of soluble recombinant PST-Mh that has limited stability and activity at 37°C. To develop a more robust enzyme that can be used effectively at physiological temperatures for therapeutic applications, we are engineering PST variants using directed evolution. A high-throughput plate-based assay that has been developed to measure PST activity will be used to screen for mutants with improved properties.

(29) **Some like it hot and sweet: Structure and role of N-glycans in the thermoacidophilic archaeon Sulfolobus acidocaldarius**

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The archaeon *Halobacterium salinarum* was the first prokaryotic organims, which was shown to use N-glycosylation as a posttranslational modification. Nowadays it is known that almost all archaea N-glycosylate many of their extracellular proteins and employ a mechanism that bears similarities partly with bacterial and also the eukaryotic N-glycosylation pathways. Although this process has only been studied in a few archaea in detail, the already observed diversity of different N-glycan structures is impressive.

Sulfolobus acidocaldarius grows optimally at temperatures around 75C and pH values between 2-3. We showed that in contrast to other studied archaea in *S. acidocaldarius* N-glycosylation is essential and studied its role in different processes. The surface protein (S-layer) is glycosylated every 30 amino acids with a tribranched hexasaccharide ($Man_2Glc_1GlcNAc_2$and a sulfated sugar called sulfoquinovose). Although reduction of the N-glycan size led to a decrease of motility, the mutational analysis of the subunits of the archaeal motility structure, the archaellum, showed that N-glycosylation seems to be important for archaellum assembly, but not for its filament function per se.

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Moroever, N-glycosylation plays a major role during the exchange of DNA after *S. acidocaldarius* experienced DNA damage. Before the cells can exchange DNA they aggregate species specifically and thereby ensure that the exchanged DNA can be integrated into the genome via homologous recombination. We showed that the N-glycan composition on the S-layer of the cells ensures the self-self recognition. These different aspects demonstrate that N-glycosylation is a very important posttranslational modification in the archaeon *S. acidocaldarius.*

(30) ***Tannerella forsythia* - A sweet periodontal pathogen**

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Tannerella forsythia is a Gram-negative, anaerobic periodontal pathogen inhabiting subgingival plaque biofilms. Despite being considered as a major etiological agent of periodontitis [1], the virulence mechanisms of this bacterium are still poorly understood. We provided evidence that the glycobiology of *T. forsythia* is crucial to the pathology of the disease [2]. *T. forsythia* is covered by a 2-D crystalline cell surface (S-) layer composed of two glycoproteins which are modified within the D(S/T)(A/I/L/M/T/V) motif with a unique, pseudaminic acid-containing O-linked oligosaccharide [3, 4]. Also several other Tannerella proteins are modified with this oligosaccharide. The identification of a general protein O-glycosylation gene locus allowed the construction of defined *T. forsythia* glycosylation mutants. Protein O-glycosylation impacts the life-style of *T. forsythia* as demonstrated by defined mutants with truncated glycosylation favoring biofilm formation and an O-glycan trisaccharide branch acting to modulate dendritic cell effector functions to suppress Th17 responses [5]. Further, the S-layer is a virulence factor and delays the bacterium's recognition by the innate immune system of the host [6]. Recently, we have shown that *T. forsythia* employs a novel type 9 secretion system (T9SS) during glycoprotein biosynthesis [7]. There, specific C-terminal domain sequences mediate protein translocation across the outer membrane [8]. The finding that a *T. forsythia* T9SS-deficient mutant has the complete O-glycan attached to the proteins supports the general assumption that glycan biosynthesis and transfer to the protein take place in the cytoplasm and the periplasm, respectively, and that these processes are decoupled from outer membrane translocation.This work was supported by the FWF projects P20605-B12, P24317-B22 (to CS) and the FWF-PhD Programme W1224.1. Socransky SS et al (1998) J Clin Periodontol 25, 134-144; 2. Posch G et al (2012) *Biomolecules* 2, 467-482; 3. Sekot G et al (2012) Arch Microbiol 194, 525-539; 4. Posch G et al (2011) *J Biol Chem* 286, 38714-38724; 5. Settem RP et al (2013) *Mucosal Immunol* 6, 415-426; 6. Sekot G et al (2011) J Dent Res 90, 109-114; 7. Tomek MB et al (2014) Mol Oral Microbiol, doi 10.1111/omi.12062; 8. Saiki K & Konishi K (2014) Mol Oral Microbiol 29, 32-44.

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(31) **Insights into rare Ser/Thr O-glycosylation of heterologous proteins expressed in plants**

Patricia Bubner[1], Heidi Szemenyei[1], Shu-Lun Tang[1], Anthony Iavarone[2], Stefan Bauer[1], Douglas Clark[1], Chris Somerville[1]

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In order to tap the potential of plants as a platform for the production of therapeutic proteins, detailed understanding of glycosylation of

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secreted proteins is key. While *N*-glycosylation pathways are well studied and highly conserved among eukaryotes, there is a general lack of knowledge about *O*-glycosylation, both in terms of the enzymes that catalyze glycosylation and glycan synthesis as well as its biological function. This is especially true for proteins secreted by plants. In most organisms capable of *O*-glycosylation, sugars are added to a serine or threonine. In plants, by contrast, *O*-glycosylation is thought to occur mainly on hydroxyprolines in the hydroxyproline-rich glycoproteins present as structural proteins in cell walls [1]. These *O*-glycan structures consist almost exclusively of arabinose. Little is known about *O*-glycosylation of secreted proteins in plants, and only rare reports of glycosylation of Ser/Thr exist [2].

Surprisingly, besides *N*-glycosylation sites, which were identified using PNGaseF digestion in $H2O^{18}$ and MS, we also found several *O*-glycosylation sites in plant expressed proteins of archaeal and eukaryotic origin. We here report identification of untypical glycosylation sites on serines and threonines in various proteins heterologously expressed in *Nicotiana benthamiana* and *Zea mays*. Transient expression of the proteins was targeted to the apoplast. The *O*-glycosylation sites were identified using ammonia-based β-elimination (ABBE) and LC/MS/MS analysis. We were able to identify several glycopeptides with single hexoses on serine and threonine in complementary experiments using tryptic digestion only or previous treatment with glycosidases. Further glycan analysis with LC/MS/MS, enzymatic linkage analysis, and monosaccharide analysis of hydrolyzed glycans using HPAEC-PAD allowed for detailed elucidation of the glycan structures. We also investigate the presence of a possible *O*-glycosylation motif using MS and *in silico* analysis. Therefore, it appears that plants may have significantly more *O*-glycosylation of serine and threonine residues than previously thought.

Acknowledgments

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(32) Synthesis of biotinylated keratan sulfate oligosaccharides

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Damaged neurons in the adult mammalian central nervous system can not regenerate spontaneously. It is known that the inhibitory factors associated with proteoglycans especially with keratan sulfate (KS), an important family of the proteoglycans, in glial scar and myelin inhibit the axonal regrowth. The mechanisms of the interaction between regiospecifically sulfated KS oligosaccharide and the inhibitor of axonal regrowth should be clarified. KS oligosaccharide has a linear glycan chain composed of a repeating disaccharide unit, -4Galβ1-4GlcNAcβ1- which are often sulfated at *O*-6 of Gal and GlcNAc.

Here, we report the synthesis of different types of KS di- and tetrasaccharides attached to biotin via a hydrophilic linker at the reducing terminal for biological use. We synthesized the sulfated KS oligosaccharides by using common disaccharide unit (Gal-GlcN) suitably protected at its amino and hydroxyl groups. The common disaccharide unit is designed to be alternatively used as both disaccharide donor and acceptor for glycan elongation. Disaccharide donor and acceptor were equipped with chemoselectively removable NAP (2-naphthylmethyl) and TBDPS (*tert*-butyldiphenylsilyl) groups at the 6 positions of the Gal and GlcNAc residues, respectively, for regioselective sulfation. The disaccharide donor was stereoselectively coupled with the disaccharide acceptor. The protected oligosaccharides were sulfated at the primary positions of Gal and / or GlcN. These oligosaccharides were suitably deprotected and biotinylated at the reducing terminals [1].

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(33) SWEET NEUROBIOLOGY: NEW INSIGHTS INTO THE ROLE OF ALTERATIONS IN PROTEIN GLYCOSYLATION IN ALZHEIMER'S DISEASE PATHOLOGY

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Human neurodegenerative diseases, such as Alzheimer's disease (AD) which is the primary cause of dementia, Creutzfeldt-Jakob disease and Parkinson's disease, are devastating illnesses that predominantly affect elderly people. The hallmark of these diseases are pathogenic oligomers and fibrils of misfolded amyloidogenic proteins (e.g. Aβ and hyper-phosphorylated tau in AD), which cause progressive loss of neurons in the brain and nervous system. Interestingly, several reports indicated vast deviations from normal protein glycosylation in the brain of AD patients, while others demonstrated alterations in glycosylation of specific proteins related to AD pathology in the disease state, such as tau and APP, the Aβ precursor. Yet, a causal link between alterations in protein glycosylation and AD-related neurodegeneration remains to be demonstrated. This is the goal of our work. Using an in silico approach we found that many glycosylation-related enzymes exhibit different expression profile in brains of human AD patients as compared with healthy subjects. Utilizing a more direct approach, we experimentally intervene in global protein glycosylation and study the effect of enhancing or reducing expression of the glycosylation-related genes

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in transgenic *Drosophila* over-expressing human tau, which serve as an established AD model. We were able to identify leading glycosylation enzymes, both augementing and ameliorating tauopathy symptoms using the fly as a model. We will next verify these effects of alterations in global protein glycosylation on AD pathology using human cultured cells over-expressing Aβ or tau and AD model mice.

(34) EDEM1/2/3 are α 1,2-mannosidases essential for endoplasmic reticulum-associated degradation of glycoproteins

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Proteins misfolded in the endoplasmic reticulum (ER) are degraded by the cytosolic proteasome *via* a series of molecular events collectively termed ER-associated degradation (ERAD). Among the various pathways utilized, the best characterized is N-glycan-dependent glycoprotein degradation (gpERAD), in which the structures of high-mannose-type oligosaccharides play crucial roles, particularly the position of trimmed mannose residues. In yeast, α1,2-mannosidase Mns1 converts $Man_9GlcNAc_2$ (M9) to M8B, and α1,2-mannosidase Htm1 conducts to oligosaccharides with the α1,6-mannose exposed for subsequent disposal. The mammalian ER expresses ER mannosidase I (ERmanI) as the sole homologue of Mns1, but expresses multiple homologues of Htm1, namely EDEM1/2/3. A similar mannose trimming is thought to operate in mammals, because ERmanI converts M9 to M8B *in vitro*, and because overexpression of EDEM1 or EDEM3 but not EDEM2 stimulates mannose trimming. Nevertheless, the finding that EDEM1 recognizes and delivers misfolded proteins to downstream components has raised controversy about whether EDEMs indeed function as α1,2-mannosidases or as lectins.

Here, using transcription activator-like effector nuclease (TALEN) technique, we have conducted gene disruption to analyze on the role of ERmanI and EDEMs in mannose trimming and gpERAD. Total cellular N-glycan composition of each EDEM KO cells clearly showed that the conversion from M8B was conducted mainly by EDEM3 and to a lesser extent by EDEM1. Surprisingly, conversion from M9 to M8B was conducted slightly by ERmanI and mostly by EDEM2, which was thought to lack enzymatic activity. Importantly, degradation of the gpERAD substrate ATF6 was most effectively delayed in EDEM2-deficient cells, indicating the importance of EDEM2 in gpERAD.

We propose that mammalian cells double-check gpERAD substrates by evolving a novel-type Htm1 homologue which catalyzes the first mannose trimming step from M9 and thereby expressing three EDEMs with different specificity, for glycoprotein folding/misfolding, first by EDEM2 and then by EDEM1/3, prior to recognition by lectin OS-9. This is more sophisticated than single check by Htm1 in yeast cells, as it avoids the unnecessary destruction of proteins, whose synthesis is energy-expensive, and allows the escape of folded proteins or unfolded but yet foldable proteins from ERAD. (Ninagawa et al., J. Cell Biol. in press).

(35) New Insights into the Pathophysiology of Glycolipid Biosynthetic Disorders Using iPS-Derived Cell Lines

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Mutations in the ST3Gal5 gene, which encodes for the enzyme responsible for GM3 biosynthesis, result in developmental disorders with neurocutaneous manifestations that include severe seizures, hearing/vision loss, and dermal hypo- and hyperpigmentation. ST3Gal5 deficiency, originally referred to as Salt and Pepper (S&P) Syndrome due to the unique pigmentation phenotype, arises from homozygosity for one of two mutations identified in Old Amish communities in the US and France and in an African-American cohort in South Carolina. GM3 serves as the precursor for most complex glycosphingolipids - molecules highly enriched in neural tissue and proposed to play essential roles in cell signaling, neuronal stability, and axonal integrity. How loss of GM3 impacts the relative abundance/complexity of different glycan classes, and the molecular mechanisms whereby altered glycan expression affects the development and function of neuronal cell types, is incompletely understood. Our initial efforts to address these issues focused on in-depth glycomic analysis on S&P fibroblasts. While GM3 was missing in S&P fibroblasts, other glycosphingolipids were increased as a result of alternative utilization of the GM3 precursor, LacCer. Collateral alterations in N-linked and O-linked glycans such as increased sialylation were observed in S&P fibroblasts, stressing the importance of comprehensive glycomic analyses. Parallel changes in protein and lipid glycosylation were detected in *st3gal5*-deficient zebrafish embryos, and these morphant embryos also exhibited increased cell death in multiple brain regions, emphasizing the need for proper glycan expression during neural development. To further investigate cell-specific phenotypes, iPS cells and iPS-derived neural crest (NC) cells were generated from S&P fibroblasts and characterized on a glycomic and biochemical level. Importantly, the S&P NC cells exhibited glycosphingolipid and O-linked glycosylation changes similar to those seen in the fibroblasts. However, an unexpected alteration in the abundance of high mannose N-glycans - and in the balance of lipid-linked oligosaccharides and free oligosaccharides - was detected within the S&P NC population. We further identified potential changes in the differentiation program of S&P NC cells that correlate with altered receptor tyrosine kinase expression. Collectively, these findings demonstrate the ability of our approach to identify emergent phenotypes in disease-relevant cell types associated with glycolipid biosynthetic disorders.

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(36) Glycan structures regulate the states of pluripotent stem cells

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Mouse and human ES/iPS cells are considered to be in different developmental stages, "naïve" and "primed" states, respectively. Mouse ES cells are defined as naïve pluripotent stem cells because of their functional similarity to the preimplantation epiblast. Their self-renewal ability is maintained by LIF/STAT3 signaling. On the other hand, mouse epiblast stem (EpiS) cells are derived from post-implantation blastocysts. The pluripotent state of mouse EpiS cells has been described as primed based on their post-implantation epiblast-like transcriptional and epigenetic properties. Mouse EpiS cells are maintained by Nodal/Activin/Smad2 and FGF2/ERK signals. Human ES/iPS cells share defining features with mouse EpiS cells and, therefore, human ES/iPS cells are considered to be primed pluripotent stem cells. These facts raise the question of how these two states are distinguished and regulated.

To answer these questions from a glycobiology standpoint, we screened for glycosyltransferases (Sasaki et al. Stem Cells 29,641 (2011)) and sulfotransferases (Hirano et al. PLoS One 7,e43440 (2012); BBRC 430,1175(2013)) using RNAi and overexpression systems, respectively. Then we found that two kinds of glycans, LacdiNAc and 3-*O*-sulfated heparan sulfate (HS), involved in the transition between naïve and primed states ofmouse pluripotent stem cells.

LacdiNAc carbohydrate structures on LIFR and gp130 were required for a sufficiently strong LIFR/STAT3 signal to maintain self-renewal in naïve state cells and the transition from primed state to naïve state. The expression of these structures act by controlling the localization of the receptors to the cell membrane lipid rafts.

The 3-*O*-sulfated HS was required for the transition from naïve state to primed state. The 3-*O*-sulfated HS associates with Fas and translocates intracellular Fas into lipid rafts on ES cell surface. The Fas/caspase-8/caspase-3 signaling cascade is then activated by the accumulation of Fas in the lipid rafts and subsequently induces the degradation of Nanog protein by activated caspase-3.

In both cases, the signals were regulated through controlled localization of receptors on lipid rafts by glycans. The characterization and functional analysis of cell surface glycans on pluripotent stem cells at each state will shed new light on the characteristics of stage-specific pluripotent stem cells.

(37) Sugar-coated webs to drive stem cell differentiation

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Glycosaminoglycans (GAGs) are essential cofactors for many signalling molecules regulating stem cell expansion and differentiation. Whereas many groups are investigating the protein components of these signalling complexes, the carbohydrate fraction is less well understood and remains an under-appreciated factor in strategies for stem cell expansion or directed differentiation. Several key issues need to be addressed before pluripotent human stem cells can fulfil their potential for therapy and improved understanding of disease. Foremost amongst these is the development of defined cell culture systems for the high-efficiency differentiation of stem cells to the target cell type. These systems must be scalable, fully-defined and xeno-free.

Our work [1, 2], and that of others, has defined specific GAG epitopes in mouse ES cells and has demonstrated that selected GAG saccharides can be used to influence specific signalling pathways during neural [3] and mesodermal [4] differentiation. Importantly we were also able to show lineage-specific requirement for sulphation pattern and size of saccharides. This suggests that GAG saccharides could be used in addition to, or possibly in place of, protein additives in differentiation protocols.

Improvements in the chemoenzymatic production of xeno-free structurally-defined GAG oligosaccharides now allow exploitation of these compounds in the determination of specific sulphation pattern requirements for the control of cell signalling. We are combining this work with the design of artificial 3D cell environments, generated by electrospinning or by the formation of hydrogels [5]. By permeating these scaffolds with defined GAG oligosaccharides, we control the mechanical environment of the cells (via the scaffold architecture) as well as their biological signalling environment (using the oligosaccharides). This enables control of ES cell pluripotency and differentiation in a 3D setting, allowing the generation of differentiated cell types for use in drug discovery/testing or in therapeutics.

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(38) Finding of a Novel Lectin Probe for Pluripotent Stem Cells and its Installation to Regenerative Medicine

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AIST

The author's group realized a lectin microarray technology with an evanescent-wave activated fluorescent detection principle (Kuno et al., *Nature Methods*, 2005). This principle enables rapid and reproducible glycan profiling analysis without washing procedures, which will eliminate relatively weak (e.g., $K_d > 10^{-4}$ M) binding between fluorescently labelled glycoproteins and immobilized lectins. So far, many applications have been successfully reported (Hirabayashi et al., *Chem Soc Rev*, 2013), which include investigation of a new probe for pluripotent stem cells including iPS cells (Tateno et al. *JBC*, 2011). Recently, this probe lectin designated rBC2LCN (recombinant N-terminal domain of the lectin BC2L-C from Burkholderia cenocepacia) was found to specifically recognize *O*-glycans containing H type 3 structure (Fucα1-2Galβ1-3GalNAc) (Hasehira et al., *Mol Cell Proteomics*, 2012). Further, the structure is prominently expressed on podocalyxin, a mucinous glycoprotein with apparent molecular mass of >240 kDa, in a

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pluripotent cell-specific manner (Tateno et al. *Stem Cells Trans Med*, 2013). Fluorescently labelled rBC2LCN immediately stained pluripotent stem cells without a fixation procedure (Onuma et al., *BBRC*, 2013). Such ability of rBC2LCN is expected to be applicable to both "detect" and "remove" tumorigenic undifferentiated cells, which should remain even after differentiation procedures, and thus, must be quantified upon transplantation. For this purpose, we recently established a unique lectin-lectin sandwich assay system, named the GlycoStem test, targeting the soluble hyperglycosylated podocalyxin using rBC2LCN (Tateno et al., *Cell Rep*, 2014). These findings along with practical installation protocols should contribute to realization of regenerative medicine using pluripotent stem cells.

(39) **Glycocalyx Remodeling with Synthetic Proteoglycan Mimetics Promotes Neural Specification in Embryonic Stem Cells**

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Proteoglycans are large, glycosylated proteins populating membranes of cells. One of their roles is to serve as mediators of growth factor signaling during embryonic development. Proteoglycans are an enticing target for exploration as mediators of stem cell differentiation; however, the carbohydrate components of proteoglycans responsible for growth factor recognition and binding are structurally complex and compositionally heterogeneous and, thus, are unwieldy synthetic targets. We have developed synthetic materials that emulate the architecture and function of these biomolecules but afford control over glycan structure, valency and presentation. We have now generated a number of proteoglycan mimetics endowed with a range of useful functionalities for their integration with synthetic materials as well as living cells. In my presentation, I will describe the synthesis of our proteoglycan mimetics and a microarray platform designed for high-throughput evaluation of their interactions with growth factors. We identified proteoglycan mimetics that engage the fibroblast growth factor 2 (FGF2) and can be integrated within the glycocalyx of embryonic stem cells, where they promote specific signaling pathways leading to neural differentiation.

(40) **Golgi-resident polysialic acid defines distinct brain cell populations**

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NG2 cells are a heterogeneous population of precursors. They serve as the primary source for myelinating oligodendrocytes in development and during myelin repair, but are also able to give rise to astrocytes and neurons under certain conditions (Trotter *et al.* 2010, Brain Res Rev 63:72, Franklin, Ffrench-Constant 2008, Nature Rev Neurosci 9:839). Distinguishing features of the assumed subpopulations of NG2 cells remain to be characterized at the molecular level. In 2010, we described that a fraction of the synaptic cell adhesion molecule SynCAM 1 in murine NG2 cells can be modified by the polymeric glycan polysialic acid (polySia; Galuska *et al.* 2010, PNAS 107:10250). As for the major carrier of polySia, the neural cell adhesion molecule NCAM, the adhesive properties of SynCAM 1 can be modulated by posttranslational modification with polySia but the cellular and subcellular distribution of polySia-SynCAM 1 and its functional implications are unresolved. Here we characterize polySia-SynCAM 1 expression by NG2 cells in mixed glial cultures derived from wildtype and *Ncam*$^{-/-}$ mice and demonstrate that polySia-SynCAM 1 defines a subfraction of the heterogeneous pool of oligodendrocyte precursor cells (OPCs). Notably, the expression of polySia-SynCAM 1 in these OPCs was confined to the Golgi compartment. Synthesis of polySia in NCAM-negative OPCs was restricted to *N*-glycans and exclusively mediated by ST8SIA2, one of the two mammalian polysialyltransferases (polySTs). In addition, further cells with Golgi-localized polySia were detected in cultures derived from *Ncam*- and *St8sia2*-double knockout mice, indicating that ST8SIA4, the second mammalian polysialyltransferase, mediates Golgi-confined polysialylation in another cell type. Our current research focuses on the characterization of these polySia-expressing cells and the identification of possible novel polySia protein carriers. Additionally, a first analysis of polySia expression in human embryonic stem cell-derived OPCs will be presented.

(41) **Nutrient Regulation of Signaling & Transcription by O-GlcNAcylation**

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O-GlcNAcylation, which is the cycling of the monosaccharide, β-N-acetylglucosamine, on Ser/Thr residues of most nuclear and cytoplasmic proteins, serves as a major sensor of cellular nutrient status, and has extensive crosstalk with phosphorylation. Extensive crosstalk with other PTMs regulates transcription, signaling and cellular metabolism in response to nutrient status. O-GlcNAcylation plays a fundamental role in gene transcription at many levels, including regulation of the basal machinery, as well as modifying the interactions and localizations of transcription factors. O-GlcNAc is part of the histone code. O-GlcNAcylation regulates the binding of the TATA-binding protein (TBP) to DNA. O-GlcNAcylation also regulates circadian clocks by controlling the turnover of transcription factors and DNA methylation by regulating the TET proteins. Prolonged excess O-GlcNAcylation, as occurs in diabetes, underlies fundamental mechanisms of glucose toxicity. Abnormal O-GlcNAcylation is associated with neurodegenerative disease, and is elevated in most types of cancer. Recent glycomic analyses have found that over one-half of all kinases are both modified and many are regulated by O-GlcNAcylation. For example, O-GlcNAcylation either inhibits or activates, depending upon the kinase, and can alter substrate specificity. Hyper-O-GlcNAcylation of mitochondrial proteins in diabetic tissues contributes directly to mitochondrial

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malfunction. Mitochondrial O-GlcNAc transferase (OGT) is strikingly elevated and mis-localized in cardiac mitochondria from diabetic rats. O-GlcNAcylation is very abundant at nerve terminals, where it is highly enriched on myriad synaptic vesicle proteins, and appears to play a direct role in learning and memory. Focused, inducible knock out of OGT in adult brain neurons produces a satiety defect and a morbidly obese mouse within only two to three weeks. Supported by NIH R01CA42486, R01DK61671; N01-HV-00240; P01HL107153 and the Patrick C. Walsh Prostate Cancer Research Fund. Dr. Hart receives a share of royalty received by the university on sales of the CTD 110.6 antibody, which are managed by JHU.

(42) Biosynthesis and Biogenetics of Lipopolysaccharide

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Lipopolysaccharides (LPS) are characteristic components of cell walls of Gram negative bacteria, localize in the outer leaflet of the asymmetric outer membrane (OM) and expose on the cell surface. LPS contribute to the integrity of OM and protect the cells against various environmental stress including lipophilic antibiotics and host immune system. LPS typically consist of a hydrophobic domain known as Lipid A (or endotoxin), a nonrepeating core oligosaccharide and a distal polysaccharide (O-antigen or O-PS).

We have elucidated the wzy-dependent O-PS biosynthetic pathway by reconstituting the total biosynthetic pathway of O-PS *in vitro*. Starting with chemically prepared N-Acetyl-D-galactosamine-diphospho-undecaprenyl, the *E. coli* O86 oligosaccharide repeating unit was assembled via sequential enzymatic glycosylation. Successful expression of the putative polymerase Wzy via a chaperone co-expression system then allowed demonstration of polymerization *in vitro* using this substrate. Analysis of additional substrates revealed a defined mode of recognition for Wzy towards the lipid moiety. Specific polysaccharide chain length modality was furthermore demonstrated to result from the action of Wzz. Therefore, such a work provides a well-defined system for further underpinning molecular details of the O-PS biosynthetic pathway.

We also defined the function of WaaL, the O-repeat unit ligase, which catalyze the last step in LPS biosynthesis. This membrane protein (typically over ten transmembrane helix) has been overexpressed and purified (~ 5 mg/L LB) in our lab. We then evaluated its donor substrate specificity. The results show that WaaL has a highly relaxed specificity towards both the lipid and the glycan moieties of the donor substrates. This finding provides the strong experimental evidence to many of previous observations. For example, E. coli K12 can ligate structurally diverse O-antigens from cloned O antigen gene cluster; and the fact there are over 180 O-serotype of E. coli but only 5 core type indicates that each core type may correspond to more than one O-type (O-PS). These properties would allow us to synthesize an array of LPS analogues for immune analysis.

To further understand the biosynthesis of LPS, we have studies the mechanism of LPS chain length regulation, a process which is controlled primarily by Wzz and is associated with bacterial pathogenicity. Our preliminary results from Far UV-CD spectroscopy and small-angle X-ray scattering (SAXS) studies supported an interaction between Wzz and O-PS. Recently, we have obtained solid evidences to confirm this interaction by pull-down and Surface Plasmon Resonance (SPR) experiments. We further observed the interaction between Wzz and its natural substrate Und-PP linked O-PS, and demonstrated that structural changes in repeat unit of O-PS affect LPS chain length regulation. In addition, Wzz homologues exhibit different binding specificity towards O-PS in a chain length-dependent manner. Molecular docking reveals that O-PS binding sites are contributed by two adjacent subunits and predominantly located inside the bell-shaped Wzz oligomer. Based on these observations, a new model is proposed in which the specific interaction of Wzz and O-PS plays a key role in O-PS chain length modulation.

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(43) Biosynthesis and Mutant Studies Indicate Roles for Pectin and Pectin-containing Proteoglycans in Plant Cell Wall Architectural Integrity and Plant Growth

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Each year 100 billion tons of CO_2 are fixed via photosynthesis into glycopolymer-rich biomass, the bulk being plant cell walls, highly complex multi-functional polysaccharide and proteoglycan-rich extracellular matrices that function in plant morphology, growth, development, strength, and disease resistance. Plant walls also serve as nutritional fibers metabolized by human gut microbiota that yield beneficial effects on digestive physiology, as well as pharmaceuticals, drug delivery materials, and biomedical implantation aids. Walls are also used for food enhancement and products including wood, paper, textiles, fine chemicals, and gelling and thickening agents. Plant cell walls are comprised of the polysaccharides cellulose (synthesized at the plasma membrane) and the matrix polysaccharides hemicelluloses and pectins (synthesized in the Golgi apparatus and transported via vesicles to the wall). Pectins, the most structurally complex plant wall glycans, are defined as a family of polysaccharides containing galacturonic acid linked at the 1- and 4-position. They include homogalacturonan (HG), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II). The recent identification of RG-I and HG as glycan domains in the

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cell wall proteoglycan arabinoxylan pectin arabinogalactan protein1 (APAP1), in which RG-I is covalently linked to the arabinogalactan moiety of an arabinogalactan protein (AGP) and HG linked to RG-I, along with the covalent attachment of hemicellulosic xylan domains, reveals that some pectins and hemicelluloses exist as cell wall proteoglycans. The presence of pectin as a glycan domain in APAP1 indicates that current wall models depicting pectin as a free polysaccharide do not fully account for the diverse roles of pectin in cell wall architecture. New data on the enzyme activity and biological function of proven and putative HG:galacturonosyltransferases (GAUTs), including studies of the GAUT1:GAUT7 complex and characteristics of the wall in *gaut1* knockout mutants will be presented. The hypothesis that plant walls contain multiple and diverse pectin-containing cell wall structures critical for wall integrity will be presented. This work was supported by USDA AFRI 2010-65115-20396, NSF-MCB 0646109, and BioEnergy Science Center grant DE-AC05-00OR22725. The BioEnergy Science Center is a US Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the Department of Energy's Office of Science.

(44) **Probing Glycan Functions by Precise Gene Editing - A Small Revolution for Glycomics**

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Glycosylation is the most abundant and diverse posttranslational modification of proteins, and glycosylation directs important biological functions for proteins. Several types of protein glycosylation can be predicted with reasonable reliability by the protein sequence context (e.g. N-linked, O-Fuc, O-Glc) and substantial knowledge of these glycoproteomes is available. In contrast, several types of O-glycosylation (e.g. O-GalNAc, O-Man, O-GlcNAc) remain more elusive and simple sequence context motifs are not as useful for prediction of glycosites. This in combination with great heterogeneity of O-glycan structures at individual glycosites has hampered our knowledge of these types of glycosylation as well as the diverse biological functions they serve. We have started employing precise gene editing technologies (ZFNs, TALENs, CRISPR/Cas9) to engineer cell systems with simpler and more homogenous glycosylation by knocking out or knocking in key glycosyltransferase genes, which enables lectin enrichment and proteome-wide discovery of O-glycosites using "bottom-up" mass spectrometric analysis. We have implemented this on O-GalNAc and O-Man glycoproteomes of human cell lines from different organs, and we are now probing the contribution of individual isoenzymes involved in initiation of O-glycosylation using comparative analysis of glycoproteomes. These studies are generating surprising new insight into the abundance of O-glycosylation and new biological functions. The presentation will present an update on our glycoproteome studies and highlight examples of new important biological functions of site-specific O-glycosylation.

(45) **Functional characterization of PMM2-CDG patient-derived iPSCs**

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With the establishment of cell reprogramming technologies, new options have become available to study the pathophysiology of human genetic disorders by use of induced pluripotent stem cells (iPSCs). We set out to apply iPSC technology to the study of congenital disorders of glycosylation (CDGs), a complex of rare diseases following gene defects that cause deficient N-glycosylation. A mutation in the phosphomannomutase 2 gene (*PMM2*) causes the most common CDG, called PMM2-CDG. PMM2 converts mannose-6-phosphate to mannose-1-phosphate which is the precursor of GDP-mannose, an essential metabolite in the assembly of the lipid linked oligosaccharide precursor used in the N-glycosylation pathway. The shortage of activated mannose in PMM2-CDG causes incomplete N-glycosylation of glycoproteins. The clinical presentation of PMM2-CDG is variable and can affect multiple organs; typically individuals suffer from neurologic disorders. We reprogrammed fibroblasts from a PMM2-CDG patient that was affected from the most common mutation and could derive patient-specific iPSCs (PMM2-iPSCs). These cells displayed all features of pluripotency including high expression of pluripotency markers and very low expression of lineage markers. Genomic integrity was proven and our PMM2-iPSCs revealed low events of vector integrations. PMM2-iPSCs could be successfully differentiated into derivatives of the three germ layers and no obvious differences were detectable when compared to human embryonic stem cells (hESCs). The transcriptomes of PMM2-iPSCs and hESCs did not differ substantially. Analysis of N-glycans by capillary gel electrophoresis with laser induced fluorescence detection (CGE-LIF) revealed a comparable repertoire of N-glycans when compared to hESCs. Probing of blotted proteins with the mannose-specific lectin GNA suggested a slight reduction of protein mannosylation in PMM2-iPSCs compared to hESCs. Supplementation of PMM2-iPSC and hESC cultures with mannose substantially increased mannose incorporation into surface associated glycoconjugates in both cell types. Because our results showed that N-glycosylation of PMM2-iPSCs differed only marginally from hESCs, we assume that the glucose-rich culture conditions needed in stem cell culture compensated for the reduction in PMM2 activity. To further reduce PMM2 enzyme activity we therefore did a stable RNAi knockdown of *PMM2* in PMM2-iPSCs. Initial results show that the obtained knockdown models much better approximate the glycosylation defects observed in patient materials.

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(46) **Manipulating glycan processing in the baculovirus-insect cell system for diverse biomedical applications**

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It is widely held that most human proteins are glycosylated and it is well known that the covalently linked carbohydrates on these molecules are far more than inert, decorative side chains. In fact, these sugars typically have a direct and/or indirect influence on glycoprotein structure and function that can dramatically impact the overall behavior of the macromolecule. Thus, in choosing a system to produce recombinant glycoproteins for a specific biomedical application, it is important to consider which protein glycosylation patterns are supported by the system, in context of the application.

In the baculovirus-insect cell system, protein-linked glycans are less extensively processed than in mammalian systems and the resulting products have relatively simpler, truncated carbohydrate side-chains. However, our group and others have demonstrated that the baculovirus-insect cell system can be glycoengineered in various ways, with an initial focus on enabling human-type N-glycan elongation.

This presentation will include a brief review of those previous efforts, with an update on our recent efforts to use glycoenzyme engineering and alternative promoters to increase the efficiencies of human-type N-glycan elongation in the baculovirus-insect cell system. We also will describe new baculovirus vectors that can be used to produce non-fucosylated recombinant glycoproteins, which solves a major problem associated with the production of immunogenic core alpha 1,3-fucosylated N-glycans by some insect cells and extends the utility of the baculovirus-insect cell system for recombinant antibody production. Finally, we will describe our ongoing efforts to explore the utility of these new vectors for biomedical applications in structural biology. Our preliminary data indicate that, in combination with other functions, these new vectors can be used to produce recombinant glycoproteins with nearly homogeneous, insect-type, paucimannosidic N-glycans. We will present this as a simpler way to enhance the crystallizability of recombinant glycoproteins. Specifically, this approach eliminates the inefficiencies and other problems associated with target de-glycosylation and re-purification, which is a common approach in contemporary structural biology. Perhaps more importantly, the products of this modified baculovirus-insect cell system still have core N-linked glycans, which can potentially preserve at least some of the contributions of the native carbohydrate side chains to overall glycoprotein structure.

(47) **Role of glycans in gastric cancer development and progression**

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Glycosylation alterations are frequent during gastric carcinogenesis and gastric cancer progression. *Helicobacter pylori* attachment to human gastric tissue is mediated by bacterial adhesins. The bacterial adhesin BabA recognizes neutral histo-blood group antigens expressed by gastric epithelial cells. Persistent *H. pylori* colonization promotes gastric mucosa expression of sialylated glycan structures. These include receptors for the SabA adhesin that recognizes sialylated-Lewis antigens [Expert Rev Proteomics. 2010 7:307]. We have demonstrated in human gastric tissues and in cell models that *H. pylori* infection induces the expression of glycosyltranferases involved in the biosynthesis of sialyl-Lewis antigens, leading to an increased *H. pylori* adhesion [J Clin Invest. 2008; 118(6):2325]. Noteworthy, we demonstrated that the increased sialylation levels result in higher SabA-mediated *H. pylori* adhesion to inflamed gastric mucosa.

Furthermore, major glycosylation alterations are observed in gastric carcinoma. Specific modifications on N-glycosylation of E-cadherin have been shown to be crucial in gastric cancer cell invasion [Trends in Mol Medicine. 2013; 19:664]. In addition, increased expression of sialyl-Lewis X in gastric cancer cells activates c-Met and downstream signaling pathways [PLoS One. 2013; 8(6): e66737.]. Moreover, the expression of simple O-glycans, specifically sialyl-Tn, induces remarkable modifications in gastric cancer cells. The increased sialylation underlies the aggressive features observed in gastric carcinoma.

These findings show that activation and modulation of the glycosylation pathways in gastric cancer cells play key roles in the process of carcinogenesis and in the biology of the cancer cell, and constitute a source of cancer biomarkers [J Proteome Res. 2013; 12:1454] with clinical applications [Trends in Mol Medicine. 2013; 19:664].

(48) **Shedding of GPI-anchored proteins by a novel GPI cleaving enzyme**

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Glycosylphosphatidylinositol (GPI) anchoring of proteins is a conserved post-translational modification in eukaryotes. It has been suggested that GPI-anchors act as functional molecules regulating trafficking and membrane localization of the modified proteins. One of the characteristic features of GPI-anchored proteins (GPI-APs) is that these proteins are releasable from the cell membrane by cleaving GPI moieties. It has been reported that several GPI-APs are released from the cell membrane by GPI cleaving enzymes, such as GPI-phospholipase D and glycerophosphodiesterases. Here, we report a novel GPI cleaving enzyme termed PGAP6, which belongs to a putative transmembrane hydrolase superfamily and shows weak homology to PGAP3 that is involved in GPI fatty acid remodeling at the Golgi apparatus. PGAP6 is a transmembrane protein conserved among metazoa and was localized at the cell surface and the intracellular organelles. Overexpression of PGAP6 significantly decreased the surface expression of GPI-APs and enhanced secretion of GPI-APs, whereas knockdown of PGAP6 decreased release

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of GPI-APs to the medium. Mutations in putative catalytic sites conserved in the hydrolase superfamily caused loss of the release activity. These results suggest that PGAP6 is a GPI cleaving enzyme. The GPI-APs released to the medium were fractionated into aqueous phase by TritonX-114 partitioning and structural analysis by mass spectrometry indicated that released GPI-APs were cleaved between inositol and phosphate by phospholipase D-like activity. In contrast, we observed that a GPI-AP purified from the surface of PGAP6 expressing cells is a lyso-form, which harbors an acyl-chain in the lipid moiety. Based on these results, we proposed a model for two steps cleavage of GPI-APs on the cell surface; First, PGAP6 hydrolyzes an acyl-chain at sn-2 position of GPI with a phospholipase A2 activity, forming lyso-GPI-APs. Second, lyso-GPI-APs were then cleaved by a phospholipase D-like enzyme.

(49) **Epigenetic regulation of IgG glycosylation in hybridoma clones**

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Glycosylation of Asn297 of the Immunoglobulin G (IgG) Fc region is important for the regulation of effector functions of IgG and therapeutic efficacy of monoclonal antibodies. However, the mechanisms that regulate IgG glycosylation are still unknown. It has been shown that different monoclonal antibodies have different glycosylation patterns and altered glycosylation of antigen-specific antibodies was demonstrated in several diseases. This indicates that some aspects of IgG glycosylation are defined by heritable elements, which can preserve IgG glycome composition through cell generations. Epigenetic mechanisms are involved in cell memory, therefore we hypothesized that at least some features of IgG glycosylation have to be determined by specific DNA methylation patterns. Recent genome-wide association study (GWAS) of the IgG glycome identified 16 genetic loci that associate with the composition of the IgG glycome. Only four of these 16 genes are "classical" glyco-genes, while other 12 genes have not been associated with protein glycosylation until now. We investigated the importance of DNA methylation by comparing CpG methylation and gene expression level of these genes and IgG glycome composition in secretome of 20 different hybridoma clones. CpG methylation was analyzed by pyrosequencing of bisulfite converted DNA, gene expression by qPCR and glycome composition by UPLC analysis of 2-AB-labelled glycans. Specific associations between CpG methylation, gene expression and composition of the IgG glycome were observed for some of GWAS hits, i.e. for the transcription factor BACH2 and glycosyltransferase MGAT3, indicating that CpG methylation could be one of the mechanisms that enable clonal fixation of some aspects of IgG glycosylation.

(50) **A heterozygous mutation of glycosyltransferase-like gene causes asthenozoospermia**

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We have performed comprehensive identification of the mammalian glycosyltransferase genes using various approaches. During these studies, we identified the glycosyltransferase-like gene, human O-16 (*GALNTL5*), belongs to the polypeptide GalNAc-transferase (pp-GalNAc-T) gene family because of its conserved glycosyltransferase domains, but it uniquely truncates the C-terminal domain, and is expressed exclusively in human testis. However, we could not confirm the glycosyltransferase activity of *O-16* including whether it is a functional molecule in spermatogenesis. Therefore, using the mouse *O-16* gene, we attempted to elucidate the biological role of *O-16* in spermatogenesis and found that the heterozygous mutation of *O-16* causes male infertility by reducing sperm motility, which highly resembles human asthenozoospermia. In reference to the aberrant protein compositions of sperm from the *O-16* heterozygous mutant mice, we found two asthenozoospermic patients carrying the mutations of the human *O-16* gene. One was a point mutation at the splicing acceptor site in the asthenozoospermic sperm, but not in the blood cells of the same patient. Interestingly, this mutation in sperm disappeared spontaneously two years later. This case strongly suggests that the mutation of human*O-16* in spermatogonial stem cells results in asthenozoospermia. The other case was a hereditary heterozygous nucleotide deletion at the sixth exon of the human *O-16* gene. We succeeded to separate motile sperm not containing the mutation with the swim-up method for the treatment of intracytoplasmic sperm injection using sperm (ICSI). Together with these data, we speculate that the function of *O-16* is indispensable for mature sperm formation and that *O-16* might have a unique role in mammalian spermiogenesis.

(51) **Glycomimetic Antagonists of the Selectins Applied to Diseases in Hematology and Oncology**

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Historically, the carbohydrate ligands that bind E-selectin (sialyl $Le^{a/x}$) were originally discovered as tumor markers defined by monoclonal antibodies. By facilitating extravasation from the bloodstream, they function in inflammatory disorders and cancer metastasis. The identification of a common trisaccharide domain shared among these ligands allowed for the rational design of potent small molecule glycomimetic antagonists. Rivipansel (GMI-1070) is a pan-selectin antagonist with potent pre-clinical effects in a sickle cell disease mouse model and recently completed a Phase II clinical trial for the treatment of vaso-occlusive crisis (VOC) in sickle cell patients at 22 hospital sites in the United States and Canada. Treatment of this crisis with Rivipansel resulted in consistent reduction of multiple endpoints such as the need for narcotic pain relief,

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the duration of VOC, and the length of hospital stay. Recently, we designed a new glycomimetic antagonist, GMI-1271, having higher specificity and affinity for E-selectin. Applications in multiple preclinical animal models for both solid tumors and hematological malignancies demonstrate efficacy in significantly reducing metastasis and in targeting the interactions of cancer stem cells with the tumor microenvironment. E-selectin is constitutively expressed in microdomains within the vasculature of the bone marrow where bound leukemic cells are resistant to the effects of standard chemotherapy. Binding of AML blasts to immobilized E-selectin *in vitro* confers resistance to cytarabine and daunorubicin and GMI-1271 is able to overcome this resistance. To explore this mechanism, AML blasts bound to E-selectin were screened in quantitative RT-PCR arrays which revealed E-selectin-mediated upregulation of the cancer survival pathways, Wnt and sonic hedgehog. GMI-1271 significantly reduced this gene activation. Xenograft models of mice fully engrafted with human AML blasts were then treated by standard chemotherapy drugs, cytarabine and daunorubicin. Combining GMI-1271 significantly reduced tumor burden over treatment with chemotherapy alone. The use of GMI-1271 with standard chemotherapy regimens for AML patients is now being advanced into Phase 1 clinical studies. The success of Rivipansel in Phase 2 clinical trials and the initiation of GMI-1271 in Phase 1 trials supports the use of glycomimetic compounds as innovative therapeutic strategies to address unmet medical needs.

(52) **Anti-adhesion therapy for urinary tract infections: A Study on lead optimization of FimH antagonists**

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Urinary tract infections (UTIs), primarily caused by uropathogenic *E. coli* (UPEC), are among the most frequent infectious diseases worldwide. The key step in the pathogenesis of UTIs is the bacterial adhesion to urothelial cells, which is mediated by a bacterial lectin, FimH, located on their fimbriae. Blocking FimH with FimH antagonists and therefore the adhesion of bacteria to urothelial cells offers a new therapeutic approach for the prevention and treatment of UTIs. However, the antagonists developed so far have hardly met the requirements for clinical applications due to poor pharmacokinetic (PK) properties. [1, 2] In a preliminary study, [2b] oral availability of the biphenyl FimH antagonists could be established by an ester prodrug approach, although for the price of a dramatically reduced solubility. To address the solubility problem as well as to study the binding mode of FimH antagonists, we chemically modified the lead structures by disrupting the molecular planarity and symmetry of the biphenyl aglycone by means of diverse substitution patterns and replacing the terminal aromatic by heteroaromatic rings. The pharmacodynamic (PD) profile of these new FimH antagonists was evaluated by a cell-free competitive binding assay as well as a competitive fluorescence polarization assay. Furthermore, with the parallel artificial membrane permeability assay (PAMPA) and the Caco-2 cells assay the compound with an optimal PK/PD profile was identified. As a result, the antagonist bearing a phenylpyrrolyl aglycone represents a promising candidate for oral application in UTI treatment.

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(53) **Targeted drug delivery to brain tumor vasculature by carbohydrate mimetic peptide in mouse glioma model**

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Although glioma cells cultured *in vitro* are responsive to several anti-cancer drugs, it is very difficult to treat glioma *in vivo* due to blood-brain barrier (BBB). Chemotherapeutics injected to blood circulation do not pass from blood circulation to brain stroma due to tight junctions between endothelial cells. In the previous study, we identified a carbohydrate mimetic peptide, designated as IF7, that homes tumor vasculature [1]. IF7 binds to annexin A1, which is expressed specifically on the endothelial cell surface of malignant tumors. We showed that IF7 conjugated anti-cancer drug SN38 (IF7-SN38) suppressed growth of colon, melanoma, breast, prostate and lung tumor models in the mouse. Since IF7 was thought to be transported through transcytotic pathway, we hypothesized that IF7 can deliver anti-cancer drug to brain stroma overcoming BBB.

In this study, we produced glioma tumors in mouse brains by intracranial injection of C6 cells [2]. To determine targeting of IF7 peptide to C6 glioma tumors, Alexa 488-tagged IF7 (IF7-A488) was injected intravenously. IF7-A488 targeted tumor vasculature, penetrated through endothelial cell wall and spread to the glioma sphere. We injected luciferase-expressing C6 (C6-Luc) cells intracranially into C57BL/6 mouse brains, it formed large solid tumor in striatum. Tumor growth was monitored by Xenogen IVIS imager. Daily intravenous injection of IF7-SN38 reduced size of C6-Luc glioma with minimum effective dose at 5.8 mg/kg, whereas as much as 95 mg/kg of non-targeted SN-38 was required to suppress subcutaneous tumors [3]. To compare targeting efficacy of IF7-SN38 between brain tumor and subcutaneous tumor, two C6-Luc tumors, one in the brain and another under the skin, were generated in a NOD-SCID mouse. C6 cells formed diffused infiltrating glioma in NOD-SCID mouse brain. Intravenous injection of IF7-SN38 suppressed growth of these tumors with comparable efficiency regardless of their locations. We also observed reduction of mouse 005 glioma in NOD-SCID mice. These results demonstrated that IF7-SN38 is effective regardless of type of glioma or locations overcoming BBB, thusstrongly encourage clinical application of IF7-SN38 to glioma tumors.

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(54) Transcriptional factor Snail controls neuraminidase-1 and matrix metalloproteinase-9 signaling platform in regulating epidermal growth factor receptor, tumor neovascularization, growth and invasiveness in mouse model of human ovarian carcinoma

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Background: Snail, a transcriptional factor and repressor of E-cadherin is well known for its role in cellular invasion. It can regulate epithelial to mesenchymal transition (EMT) during embryonic development and in epithelial cells. Snail also mediates tumor progression and metastases. Silencing of Snail and its associate member Slug in human A2780 ovarian epithelial carcinoma cell line was investigated to identify its role in tumor neovascularization. Methods: Live cell sialidase, WST-1 and immunohistochemistry assays were used to evaluate sialidase activity, cell survival and the expression levels of tumor E-, N- and VE-cadherins, and host endothelial CD31 + (PECAM-1) cells in archived paraffin-embedded ovarian A2780, A2780 Snail shRNA GIPZ lentiviral knockdown (KD) and A2780 SlugKD tumors grown in RAGxCγ double mutant mice. Results: Oseltamivir phosphate (OP), anti-Neu1 antibodies and MMP-9 specific inhibitor blocked Neu1 activity associated with epidermal growth factor (EGF) stimulated A2780 ovarian epithelial carcinoma cells. Snail KD and not Slug KD A2780 cells abrogated the Neu1 activity following EGF stimulation of the cells compared to A2780 cells. OP treatment of A2780 and cisplatin-resistant A2780cis cells reproducibly and dose-dependently abated the cell viability with a LD_{50} of 7 and 4 μM, respectively. Heterotopic xenografts of A2780 ovarian tumors developed abnormally robust and bloody tumor vascularization in RAGxCγ double mutant mice. Preclinical in vivo anti-tumor activity of OP monotherapy at 50 mg/kg daily intraperitoneally did not significantly impede A2780 tumor growth rate in a time-to-progression but did cause a significant reduction of lung metastases compared with the untreated and OP 30 mg/kg cohorts. A2780 Snail KD cells, but not the Slug KD member completely abrogated the tumor vascularization, growth and spread to the lungs in these mice. A2780 and A2780 Slug KD xenograft tumors expressed high levels of human N- and VE-cadherins, and host CD31+ endothelial cells, while one A2780 Snail KD tumor expressed higher levels of E-cadherin and host CD31+ cells. OP 50mg/kg cohort tumors had reduced numbers of host CD31+ cells compared to those from the untreated control and OP 30mg/kg cohorts. Conclusion: Transcriptional factor Snail is an important intermediate player in human ovarian tumor neovascularization.

(55) MAN1B1-CDG: how stressed-out can the Golgi be?

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Congenital Disorders of Glycosylation (CDG) are a group of genetic diseases, due to deficient protein and lipid glycosylation. Our group recently identified MAN1B1-deficiency as a frequent type of CDG associated with impaired Golgi glycosylation, intellectual disability and obesity [1]. The ER-mannosidase MAN1B1 was for long assumed to contribute to ER-associated protein degradation by initiating the formation of degradation signals on misfolded N-linked glycoproteins. However, it was recently demonstrated that MAN1B1 is Golgi-localized, and may have a gatekeeping role to retrieve escaped misfolded glycoproteins back to the ER for degradation [2].

The Golgi apparatus has a central role in the trafficking and processing of membrane and secretory proteins. Still, how its various functions are integrated to ensure appropriate membrane assembly and distribution of cargo effectors is poorly understood. While perturbations in ER homeostasis are known to create a condition termed ER stress and leading to activation of a complex signaling cascade, the mechanism(s) regulating Golgi capacity still remain unclear. We hypothesized that MAN1B1-deficiency would lead to an accumulation of escaped misfolded proteins in the Golgi, overwhelming its capacity.

We showed that Golgi trafficking and morphology are impaired in MAN1B1-depleted cells. We further monitored an altered transcription of Golgi-related genes, including structural proteins such as the peripheral Golgi protein GRASP55 and the phosphatidylinositol-4-phosphate (PtdIns(4)P) effector GOLPH3. Interestingly, PtdIns(4)P have been described to be required to coordinate Golgi functions [3]. The Golgi phosphatidylinositol-4-kinases PI4KA and PI4KB also presented decreased transcription levels. We propose that MAN1B1-deficiency is associated with an altered transcription of a specific set of genes, likely triggered by stimuli compromising Golgi capacity. On-going RNA sequencing experiments should allow us to identify the transcription factor(s) involved in the regulation Golgi secretory capacity, and the nature of the sensing system monitoring Golgi homeostasis. In conclusion, MAN1B1-deficiency appears as an ideal model to study what might be part of a Golgi stress response involving PtdIns(4)P signaling.

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(56) Development of an International Glycan Structure Repository

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The 5th ACGG-DB meeting was held in Dalian, China in June 2013 with representatives of glycomics centers and databases worldwide to obtain a consensus on the framework for an international glycan structure repository, similar to GenBank and PDB. In January, 2014, representatives of several NIGMS-supported P41 Glycoscience Resource Centers began formal discussions to facilitate the development of a glycan repository that will have the capability to integrate the major glycomics databases. In April, 2014, the Japanese Ministry of Science, Technology, Sports and Education granted a group of glycoscientists in Japan a three-year grant to develop an international glycan structure repository using Semantic Web technologies which would enable the integration of various informatics resources. Due to these parallel developments, an international collaboration began between these groups with the aim to implement a single International Glycan Structure Repository.

The glycan repository will consist of two modular components that together form the backend and user interfaces that allow the information in the repository to be accessed. GlySpace.org is the *backend server* with the database storing the glycan structures and user information. The server provides a RESTful web service interface allowing software to search, insert and update the information in the database. Third-party software applications can freely use the web services directly to access the data content and to modify or extend it. GlyTOUcan.org is the *web frontend server* providing human readable and usable web pages to display the information in the repository. The web pages allow users with no programming skills to visually search, inspect, insert or update the data in the repository using web browsers. The *frontend server* uses the web services provided by the *backend server* to interact with the database.

This repository will be a freely available, uncurated registry for glycan structures that will assign globally unique accession numbers to any glycan independent of the level of information provided by the experimental method used to identify the structure(s). Any glycan structure, ranging in resolution from monosaccharide composition to fully defined structures including glycosidic linkage configuration, can be registered as long as there are no inconsistencies in the structure.

(57) A systems glycobiology approach to study leukocyte-endothelial cell interactions

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The talk will present an overview of some of our efforts to develop computational tools and complementary experimental methodologies to examine cellular glycosylation processes from a systems level. The computational tools to be discussed include two open source software: 1. Glycosylation Network Analysis Toolbox (GNAT): This program can be applied to analyze cellular glycosylation by mathematically describing the biochemical processes occurring within the Golgi as a network of chemical reactions. Facilities are available here to create machine readable definitions of enzymes (glycosidase, glycosyltransferases etc.), and to integrate glycan structure data with the Systems Biology Markup Language. As an example, we will illustrate how this toolbox can be applied to quantitatively analyze Glycomics based experimental data. 2. GlycoProteomics Analysis Toolbox (GlycoPAT): This is an user extensible package that can be applied to analyze glycoproteomics based LC-MS/MS data. An example will be provided to demonstrate how this program can rapidly score N- and O-glycans in mixtures of glycoproteins. In order to provide experimental data for the computational tools, we have developed a series of single, dual and triple knockout cell systems using recently developed genomic editing tools. Functional studies performed with these cells, which lack a variety of glycosyltransferases, along with computational analyses provides key quantitative and qualitative insight regarding the glycosyltransferases that uniquely regulate human leukocyte adhesion to the vascular endothelium.

(58) From glycostructures to glycoknowledge

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As every glycobiologist knows, the analysis of glycans suffers; compared to proteomics, from a lack of a genomic template This has resulted in a reluctance of the non-glycobiologist researcher to enter

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the field. However, we can now determine most eukaryotic glycostructures in detail and can associate these structures with specific changes correlated with many diseases. This capability allows not only the discovery of new biomarkers but also gives us the opportunity to 'reverse engineer' and deduce the enzymes and genes that have been regulated in the perturbed cellular states that produce the aberrant glycosylation. In addition, the expansion of systems biology that brings together genomics, proteomics, epigenetics and metabolomics is slowly but surely integrating glycomics data. Bioinformatics databases and tools are being co-operatively designed to connect with other -omics knowledgebases to enhance the prediction of protein and glycan function and interactions. Information relevant to the glycostructures expressed in different tissues and on different proteins can be obtained experimentally, but importantly is starting to be integrated into whole cell systems biology by the creation of glyco-related databases that are structured to bridge to the proteomics, genomics and interactomics knowledgebases. This presentation will illustrate how the development of the UniCarbKB glycoknowledgebase addresses these needs, and will give examples of exploring and correlating glycomics structural data obtained from the global protein glycosylation analysis of cancer and infectious disease, through a combination of experimental and bioinformatics approaches.

(59) Synthetic study and structural analysis of highly glycosylated hydrophilic motif of plant extensins

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Hydroxyproline-rich glycoprotein (HRGP) [1] is the structural motif of plant extracellular matrix proteins, such as extensin. Extensin possesses unique highly glycosylated, hydrophilic and repeating Ser_1Hyp_4 pentapeptide unit which has been proposed to include post-translational hydroxylation at proline residue and subsequent oligo-L-arabinosylations at all of the resultant hydroxyprolines as well as galactosylation at serin residue. Here, we wish to report synthetic study of the hydrophilic pentapeptide motif, Ser(Galp_1)-Hyp(Araf_4)-Hyp(Araf_4)-Hyp(Araf_3)-Hyp(Araf_1). For the achievement of the synthesis of the target molecule, 2-(naphthyl)methyl (NAP) ether-mediated intramolecular aglycon delivery (IAD) [2] and Fmoc-solid phase peptide synthesis (SPPS) have been applied to the stereoselective constructions of the Ser(Galp_1) and Hyp(Araf_n) (n = 1, 3, 4) fragments and to the backbone peptapeptide using their fragments, respectively [3].

Application of our NAP-IAD to the construction of Hyp(L-Araf_n) (n = 1, 3, 4) and Ser(Galp_1) derivatives has been achieved successfully with complete stereoselectivity which had been already applied to the synthesis of the substrate [4] for β-L-arabinofuranosidase [5]. Subsequently Fmoc-SPPS was carried out using Fmoc- deprotection with pipridine and coupling in the presence of COMU used for the synthesis of glycopeptide hormone CLAVATA3 [6] with an exception with piperidine in the presence of HOBt and using DIC-HOBt for the coupling of the dipeptide derivative in order to avoid the diketopiperazine formation. Structural analysis of the hydrophilic motif of extensin has been carried out by using NMR and Mass techniques in detail, leading the glycopeptide into polyPro II helix-like structure.

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(60) Metabolic production of photocrosslinking O-GlcNAc: method improvement and application

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O-linked N-acetyl-D-glucosamine (O-GlcNAc) is an abundant and highly dynamic single sugar post-translational modification regulated by two known enzymes, O-GlcNAc transferase (OGT) and O-GlcNAc hydrolase (OGA). Altered O-GlcNAcylation is associated with human diseases, such as cancer and Alzheimer's. While more than 1000 O-GlcNAc-modified proteins have been identified, much less is known about how O-GlcNAcylation alters protein function. To gain insight into the functional consequences of O-GlcNAcylation, we reported a method to metabolically incorporate the diazirine photocrosslinking group onto O-GlcNAc residues in cellular proteins. Photocrosslinking O-GlcNAc, which we call O-GlcNDAz, can be activated by UV irradiation, yielding covalent crosslinks between O-GlcNAcylated proteins and their binding partners. Further analysis of the crosslinked complexes can reveal protein-protein interactions that are promoted by O-GlcNAcylation. However, the metabolic labeling technology comes with limitations: O-GlcNDAz is a static modification, and OGT prefers to produce O-GlcNAc, rather than O-GlcNDAz. I will describe how I mutated OGA to accommodate the diazirine of O-GlcNDAz, and how we mutated OGT to favor the production of O-GlcNDAz to improve our technology. Furthermore, I will describe the application of O-GlcNDAz crosslinking to discover binding partners of NUP98 fusion proteins produced due to chromosomal translocation in leukemia. NUP98 fusion proteins encode the phenylalanine-glycine (FG) repeat domain of nucleoporin NUP98 fused to homeodomain transcription factors or other proteins that interact with nucleic acids. FG domains of NUPs are natively unfolded and highly modified by O-GlcNAc. Cell-based O-GlcNDAz crosslinking experiments demonstrate that NUP98 fusion proteins (NUP98-HOXA9 and NUP98-DDX10) each interact with discrete sets of proteins in cell lines derived from both leukemia and cervical carcinoma. Ongoing work includes efforts to identify the binding partners of NUP98 fusions and determine the role of O-GlcNAc modification of NUP98 fusions in leukemogenesis.

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(61) **Noninvasive Molecular Imaging and Targeting Studies of Neo-N-glycoproteins: Significant Effects of N-Glycan Clusterization on Albumin**

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In vivo kinetics analysis and targeting studies have been reported using the neoglycoproteins, which were prepared by chemically introducing monosaccharides on proteins. A few molecules of oligosaccharides, e.g., N-glycans, has also been introduced on albumin, but any significant *in vivo* effects could not be observed.

We have succeeded in introducing more than 10 molecules of the complex type N-glycans on human serum albumin (HSA) using the newly developed 6pai-azaelectrocyclization [1] to generate the N-glycoclusters on protein surface. HiLyte Fluor™ 750-labeled neo-N-glycoproteins were intravenously injected to the nude mice and noninvasive fluorescence imaging was systematically performed. Depending on the N-glycan structures, i.e., by each monosaccharide constituent in N-glycans or by their linkage formation, the rate of clearance, the clearance pathways, and the organ-specific accumulation were significantly altered. These in vivo properties were totally different from those observed using the N-glycodendrimers [2]. Furthermore, the dissection experiments showed the cell-selective distribution by the N-glycan structures; thus we achieved the selective targeting at both organ- and cell-levels. Some of the neo-N-glycoproteins also targeted the tumor implanted in nude mice with high imaging contrasts.

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(62) **Fucosyl chondroitin sulfate from sea cucumber - chemical synthesis and structural analysis -**

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Fucosyl chondroitin sulfate (FCS) isolated from sea cucumber is a highly sulfated polysaccharide consisted of chondroitin sulfate backbone and fucose branch. However, the detail structure is still obscure due to the complicated sulfation patterns. Nevertheless, FCS has many remarkable biological properties such as anticoagulant activities like heparin. We started to find the pivotal oligosaccharide as a functional domain for the biological activities by two different approaches: chemical synthesis of some plausible oligosaccharides and identification of the structure of FCSs from different kind of sea cucumbers.

We have synthesized a representative repeating trisaccharide composed of a chondroitin sulfate E backbone having a disulfated fucose branch at *O*-3 of glucuronic acid residue, β-D-GalNAc (4,6-diS)(1-4)[α-L-Fuc(2,4-diS)(1-3)]-β-D-GlcA, in a stereocontrolled manner [1]. The glycosylation reaction of the GalN3 donor with the Fuc-Glc acceptor failed, while the coupling of Fuc donor with the GalN3-Glc acceptor was successful. We finally obtained the target trisaccharide via oxidation at C-6 of Glc, sulfation at the four hydroxyl groups, and deprotection procedures.

We also isolated FCSs from several sea cucumbers by adopting the extraction procedures as already reported [2]. Sea cucumbers were freeze-dried and digested with protease. Glycans (FCS) were obtained after dialysis and ion-exchange purification, which resisted chondroitinase ABC digestion. However, mild hydrolysis enabled us to remove the fucose branches from FCS. We found that the unsaturated disaccharides from the residual polysaccharide by chondroitinase ABC digestion mainly contained highly sulfated chondroitin sulfate E [β-D-GalNAc(4,6-diS)(1-4)-β-D-GlcA].

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(63) **Glycoform imaging: use of transmembrane FRET to investigate the internalization of glycosylated proteins**

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Numerous studies have shown the importance of glycans in various cellular events, especially in intracellular and intercellular trafficking of proteins. Recently, analyses of glycosyltransferase knockout mice have suggested that their pathological phenotypes are not always attributable to overall changes in sugar modifications, but rather the result of changes in glycan structures on a specific "target" glycoprotein. Therefore, establishment of a novel technique to analyze the role of glycan structure on glycoprotein function in living cells is important. Recent advances in metabolic sugar engineering have proven to be a powerful tool for glycoproteomic analysis of a specific sugar modification. In this technique, chemically modified sugars such as azide- or alkyne-modified sugars are incorporated into cellular glycans by means of the normal biosynthetic pathway, and subsequently detected using click chemistry with a detectable probe such as fluorescent molecules. However, this method results in the labeling of all glycoproteins bearing the modified sugars; therefore, unless the protein of interest is isolated through laborious biochemical purification, analysis of a specific glycoprotein with the labeled sugar is not possible. To overcome the current problems, we established a novel glycan imaging tool to detect or monitor the different glycoforms of a protein of interest. The rationale behind the idea is that if sugars can be labeled with a fluorescent probe which can be designed to be an acceptor for emission fluorescence released by GFP, the specific glycoform-bearing target protein may be visualized

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in a specific manner as intramolecular fluorescence resonance energy transfer (FRET) signals. Using model proteins, we detect characteristic FRET signals from the specific glycoform-bearing target glycoprotein. We also show that, upon insulin removal, sialylated glycoforms of green fluorescent protein-tagged GLUT4 seem to be internalized more slowly than non-sialylated GLUT4. This novel glycan imaging tool allows for clarification of the functional significance of specific glycan modifications on a protein of interest.

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(64) Catalytic Domain of UDP-Glc: Glycoprotein Glucosyltransferase; Functional Analysis using Synthetic Substrates

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N-linked high mannose-type oligosaccharides transferred to newly synthesized polypeptides in the endoplasmic reticulum (ER) function as key signals that reflect folding status of client polypeptides during the folding process. UDP-glucose:glycoprotein glucosyltransferase 1 (UGGT1) recognizes incompletely folded states of non-glucosylated glycoproteins and re-generates the mono-glucosylated glycoforms, which associate with ER-resident lectin chaperones, calnexin (CNX) and calreticulin (CRT). CNX and CRT facilitate the folding of glycoproteins in collaboration with an ER oxidoreductase, ERp57, which forms complexes with them. As such, UGGT1 can be seen as a folding sensor. Efforts have been made to reveal its properties by *in vitro* experiments using denatured glycoproteins as substrates such as 8 M urea-treated thyroglobulin (Tg) or ribonuclease B [1]. In our study, we aimed to investigate function of each domain using various truncates of recombinant UGGT1. It was reported that truncated recombinant UGGT1s are inactive against the denatured Tg, even though they comprise the catalytic region [2]. Recently, our group has established the assay system for UGGT1 using synthetic non-proteinous substrates [3], which has unequivocally revealed the glucosyltransferase activity of human UGGT2, an isoform of UGGT1 which has been believed to be enzymatically inactive [4]. Our analysis clarified that only the C-terminal region of UGGT1 accounting for approximately 20% of the full-length, which shares a similarity in amino acid sequence with members of glycosyltransferase family 8, is enzymatically active and has similar glycan specificity to that in cases of full-length UGGT1. Our findings indicate that the C-terminal region of UGGT1 per se has essential function of the glucosyltransferase and raise the possibility that N-terminal regions of UGGT1 remaining 80% of the full length play a role in discriminating folding states of glycoproteins.

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(65) Cell interaction and physicochemical characterization of hyaluronic acid nanoparticles

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Hyaluronic acid (HA) is an anionic polysaccharide and one of the abundant polysaccharides in human and animals. Chitosan is a cationic polysaccharide and is currently a prominent candidate for the development of drug delivery system. Because of the biocompatibility and low cost, they are widely used in biomedical fields such as drug delivery system. In this study, we aimed at application to transdermal nanocarriers. Therefore, we aimed at the development of safe HA nanoparticles with hyaluronic acid, chitosan and chondroitin sulfate (CS). In order to increase the permeability of the skin, we prepared HA/chitosan complexes and HA/chitosan/CS complexes of the size to be 50-100 nm by electrostatic interactions. First, we examined the composition ratio of each component using various molecular weights HA. As a result, the composition ratio of most stable chitosan/HA was 1:0.5, the size was 65 nm and zeta potential was -35 mV. Chitosan/HA/CS was 1:0.5:0.5, the size was 57 nm and zeta potential was -50 mV. Second, we examined the effects of solution concentration of preparing nanoparticles. As a result, when we produced in high concentration, particle size of chitosan/HA was about 1 μm. On the other hand, particle size of chitosah/HA/CS was about 150 nm. Third, to evaluate the stability of the nanoparticles, we examined the physicohemical characterization of nanoparticles untile one month after preparation. The particle size of chitosan/HA nanoparticles increased with time. On the other hand, the particle size of chitosan/HA/CS nanoparticles was about 100 nm in one month later preparing. So, the stability of nanoparticles was improved by the addition of CS. In the end, we examined cell interaction analysis of nanoparticles by using normal human dermal fibroblast (NHDF) cells. To evaluate if nanoparticles uptake into the cell, we synthesized fluorescently (FA)-labeled HA, and observed by confocal laser scanning microscopy. After incubation for 4h, the HA alone was not incorporated into cells. On the other hand, cellular uptake was enhanced by the nanoparticles of chitosan/HA and chitosan/HA/CS.

(66) Analysis of the interaction between peptide aptamers and hemagglutinin

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Influenza Hemagglutinin (HA) has important role of the first step in influenza virus (IFV) infection that initiate the binding of the virus to the alpha 2,3- or alpha 2,6-sialylgalactose linkages of the receptors on the host cells. For detection of IFV, saccharides are often immobilized on solid support. However, saccharides are difficult to synthesize. So we attempt to develop the detection method for influenza HA by using HA-binding peptide aptamers which can be synthesized easily. In previous study, we obtained HA-binding peptide s2 (1-5)(ARLPR) from peptide phase display library [1].

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The peptide aptamers mimic saccharides and bind to influenza HA. We synthesized peptide-conjugated lipids (pep-DPPE) by conjugating the peptide aptamers and phospholipids DPPE (dipalmitoyl phosphatidylethanolamine). We immobilized peptide-conjugated lipids on the mica plate by hydrophobic interaction, and observed the interaction between peptide aptamers and influenza HA by an atomic force microscope (AFM). As a result, we revealed that HA can be detected by using peptide-conjugated lipids immobilized on solid support. In this study, first, we mixed pep-DPPE and other phospholipids (DOPC, dioleoyl phosphatidylcholine) and made raft structure (lipids cluster). Then we observed the interaction between peptide aptamers and H1HA in dependence of the peptide aptamers' aggregation states. Consequently, pep-DPPE/DOPC (50:50) mixed raft structured lipids membrane had higher affinity with H1HA than non-raft structured peptide-conjugated lipids membrane (pep-DPPE only). Second, we prepared ganglioside GM3 immobilized solid support and compared the interaction between GM3 and H1HA with the interaction between peptide-conjugated lipids and H1HA. In consequence of the observation, we revealed that peptide-conjugated lipids had higher affinity with H1HA than GM3. Third, we revealed that peptide aptamers specifically recognized influenza HA by observing the interaction between peptide-conjugated lipids and Bovine Serum Albumins (BSA), Human Serum Albumins (HSA).

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(67) Critical importance of the surface expression of α2,6-sialosides during early development of medaka fish

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Sialic acid (Sia) commonly exists in vertebrate glycoconjugates, and is involved in a myriad of biological phenomena. In mouse, gene deficiency of the UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE), a key enzyme for *de novo* synthesis of Sia, causes embryonic lethality at animal level(Schwarzkopf *et al.*, 2002), while no apparent abnormality occurs at cell level. Thus, Sia is critically important for mouse embryogenesis. However, why Sia is required for early development still remains unknown. To answer this question, we used medaka, *Oryzias latipes*, as a model vertebrate. In this study, we performed the following lines of experiment using medaka: (i) Medaka mutants expressing impaired CSS activity were reverse-genetically established using TILLING (targeting induced local lesions in genomes) method. Significant abnormality was observed in a mutant medaka with low-activity CSS; (ii) To gain an insight into the significance of Sia residues, effects of microinjection of SSA and MAA lectins into the perivitelline space of fertilized eggs were examined. SSA-injected embryos were mostly lethal prior to embryo body formation, while MAM-injected embryos normally developed. Heat-inactivated SSA had no effect on the embryos. These results suggest the importance of Siaα2,6Gal in early development; (iii) ST6Gal I and II, which catalyze the synthesis of Siaα2,6Gal, were cloned from medaka embryos and suppressed in their embryonic expression by injecting the ST6Gal I- and/or II-specific morpholino-oligonucleotides into one- or two-cell embryos. In the single or double morphants with these genes, embryonicdevelopment stopped at the gastrulation stage. The impaired phenotypes were partially rescued by co-injection of the corresponding RNAs; (iv)SSA lectin-staining of the isolated blastodisc was performed. The SSA epitope was observed on the cell surface and the interstitial space. This staining was reduced in the morphants with ST6Gal I, ST6Gal II, and ST6Gal I/ST6Gal II, and recovered by the co-injection of the rescue RNAs. Taken all together, it is concluded that the expression of Siaα2,6Gal residues driven by ST6Gal I- and II on the blastodisc is important for the progression of gastrulation.

(68) Polyploidization triggered by psychosine is modulated by cellular glycosphingolipids and sphingomyelin

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Glycosphingolipid (GSL) is one of constituents of cell membrane and has various functional roles in neuronal cells. Psychosine, a lyso-form of galactosylceramide, is characteristic GSL species in globoid cell leukodystrophy (GLD). Psychosine is regarded as a causal factor of GLD resulting in severe neurological defects. Pathologically, formation of polyploid globoid cells in patients' brain is utilized for GLD diagnosis. Psychosine triggers multiploid cell formation due to inhibition of cytokinesis, the final step of cell division. Understanding of psychosine's action may give us new insights into still elusive cytokinesis.

Psychosine susceptibility to induce polpolidization varied among different cell lines. This indicated the presence of cellular factor which affect susceptibility to psychosine. Our screening suggested that cellular GSLs affects polyploidization triggered by psychosine. In this study, to confirm this hypothesis, we evaluated the psychosine-triggered polyploidization in Namalwa cells with perturbed cellular biosynthetic flow of GSLs. Namalwa cells were chosen because this lymphoma cell line exhibited the strongest susceptibility to psychosine. Glucosylceramide synthase (GlcCerSyn) reaction determines total GSL level and Namalwa cells treated with PDMP, an inhibitor of GlcCerSyn, suppressed polyploidization. In contrast to PDMP treatment, when genes for GlcCerSyn and neutral sphingomyelinase 2 were introduced, multiploidization was enhanced.

Alteration of the major GSL species, GM3 to Gb3 by Gb3 synthase gene transfection, did not affect psychosine susceptibility. Consistently with PDMP treatment, reduction of overall GSL level, both GM3 and lactosylceramide, by means of dominant-negative Gb3 synthase expression suppressed psychosine-triggered polyploidization.

These data indicated that cellular level of GSL is important to alter susceptibility to psychosine. GlcCer is biosynthesized from ceramide thus could affect sphingomyelin (SM) biosynthesis. Cellular SM level

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negatively influenced psychosine-triggered polyploidization because gene overexpression of SM synthases attenuated psychosine susceptibility. SM is recruited to outer membrane of cleavage furrow and could play important role in cytokinesis. Curiously, cell surface SM level was decreased by psychosine although its localization was not affected.

We concluded that inhibition of cytokinesis by psychosine is controlled by membrane sphingolipids, more specifically, balance between GSL and SM, in which GSL enhances and SM suppresses psychosine-triggered multuploidy, respectively.

(69) Protecting-group-free Synthesis and Binding with Influenza Virus of Glycopolymers Bearing Sialyloligosaccharides

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Glycopolymers having pendant saccharides received attention in the fields of polymer chemistry, materials science, and biomedicine. Although saccharide-protein interactions are generally weak, multivalent forms of saccharides such as glycopolymers amplify these interactions due to the "glycocluster effect". However, the synthesis of glycopolymers is laborious, requiring multistep processes, including the protection and deprotection steps. In this study, we synthesized glycopolymers from free sialyloligosaccharides without any protection of the hydroxy and carboxy groups on the saccharides. In addition, we demonstrated the detection of protein-saccharide interactions on gold-coated quartz crystal microbalance (QCM) sensors, and the interaction of glycopolymers with influenza viruses using the hemagglutination inhibition (HI) test. The glycomonomers having pendant triazole-linked sialyloligosaccharides were synthesized by the direct azidation of free saccharides using 2-chloro-1,3-dimethylimidazolinium chloride and sodium azide followed by copper catalyzed azide-alkyne cycloaddition with N-propargyl acrylamide. The glycopolymers were synthesized from the glycomonomers by RAFT co-polymerization with acrylamide. The glycopolymers were immobilized on gold-coated QCM sensors and shown to strongly bind their corresponding lectins without non-specific adsorption in aqueous solution. A glycopolymer bearing complex-type sialyl N-linked oligosaccharide strongly bound with both human and avian influenza A virus. This strong binding with influenza virus was attributed to the glycocluster effect of the glycopolymer and the biantennary structure of the N-linked oligosaccharide.

(70) Immobilization of Endoglycosidases for Analysis of Glycoproteins

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New England Biolabs, Inc

Endoglycosidases including PNGase F, Endo H and Endo S are commonly used for identifying sites of N-linked glycosylation and characterizing N-linked glycan structures, particularly on therapeutic proteins. We have expressed and purified these recombinant glycosidases fused to a self-labeling SNAP-tag and shown that they are highly active against a wide panel of substrates including purified glycoproteins and complex serum glycoproteins. These SNAP-tagged endoglycosidase fusion proteins have been employed to generate immobilized forms via stable covalent linkage to benzylguanine-functionalized beads. We show that immobilized endoglycosidases are effective in processing various glycoprotein substrates in on-column digestion format and in MS-compatible workflows. We demonstrate the utilization of magnetic beads in easy separation of enzyme from processed protein/glycan mixture. In addition, use of pre-packed enzyme columns permits scale-up processing of a large amount of glycoproteins including IgG.

(71) ANALYTICAL SERVICES AND TRAININGS AT THE COMPLEX CARBOHYDRATES RESEARCH CENTER

Roberto Sonon, Stephanie Archer-Hartmann, Mayumi Ishihara, Zhirui Wang, Christian Heiss, Radnaa Naran, Ian Black, Dandan Zhou, Artur Muszynski, Scott Forsberg, Parastoo Azadi

University of Georgia

The CCRC Analytical Services Laboratory at The University of Georgia offers services for structural characterization of glycoconjuagtes derived from animal, plant or microbial origin. Our scientists have many years of experience on designing/conducting a wide array of experiments in the area of Glycobiology. The service laboratory is complimented with state-of-the-art instruments such as Velos Orbitrap-Elite MS, LTQ-Orbitrap MS, AB 5800 MALDI-TOF/TOF MS, Bruker MALDI-TOF MS, Capillary Electrophoresis MS, High Field NMR, HPLC, HPAEC, GC-MS, Robotic ELISA instrument, Pyrolysis Molecular Beam Mass Spectrometer, and Pyrolysis GC-MS. We present examples of glycoconjugate analyses that utilize complimentary techniques and a combination of analytical instruments. Briefly described below are some analyses for each class of glycoconjugate.

Glycoproteins: release of N-and O-linked glycans from purified, gel-prepared, cell or crude tissue glycoproteins; mapping N-and O-linked glycosylation sites on peptides, identification of type of N-linked glycans, i.e. biantennary, triantennary, tetraantennary, high mannose, hybrid or complex; determination of residue and linkage composition and their sequence in oligosaccharides, ring size, anomeric configuration, and determination of points of attachment, and linkage of non-carbohydrate constituents such as phosphate and sulfate.

Glycosaminoglycans (GAGs): isolation of GAGs from cells or tissues, depolymerization, analysis and quantification of disaccharide building blocks (HS/CS/HA) by HPLC, capillary electrophoresis, and mass spectrometry, CTA-SAX, molecular weight determination, degree of sulfation, isolation and sequencing of individual oligosaccharides by MS and NMR.

Polysaccharides: determination of glycosyl and linkage composition, ring size, and anomeric configuration; and purification to homogeneity by SEC and/or ion exchange chromatography.

Lipopolysaccharides (LPS): extraction and purification of LPS directly from bacterial cells, isolation of Lipid A, core oligosaccharide

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and O-antigen. Composition analysis of intact LPS and characterization of Lipid A and carbohydrate moiety by mass spectrometry and NMR spectroscopy and other techniques used for analysis of polysaccharides and glycolipids.

Lipids and glycolipids: determination of fatty acid composition of ceramide, diacylglycerol or triacylglycerol, and phospholipids.

We also offer annual hands-on training courses for structural characterization of both glycoproteins and polysaccharides.

(72) Mammalian cell-surface lectins can be detected with fluorescent magnetic

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Yonsei University

Recognition of glycans, which are present in the form of glycoconjugates in cells, by proteins is crucial for a variety of physiological and pathological processes. Accordingly, understanding the role of glycan-mediated binding events and blocking glycan-protein interactions associated with diseases are of great importance for both basic biological research and biomedical applications. To investigate binding of glycans by lectins on the mammalian cell surface, we prepared Lewis antigen conjugated fluorescent magnetic nanoparticles and applied them to detect human DC-SIGN and mouse SIGN-R1 on mammalian cell-surfaces. To our knowledge, glycan-modified fluorescent magnetic nanoparticles have not been used to study glycan-mediated recognition events. When DCEK, DCEK-DC-SIGN, and DCEK-SIGN-R1 cells were incubated with glyconanoparticles, Lea and Leb but not H1 conjugated nanoparticles bind to DC-SIGN and SIGN-R1 expressing cells. Subsequently, glyconanoparticles were internalized by the DC-SIGN and SIGN-R1-expressing cells. However, these events were greatly suppressed by pre-treatment of cells with mannan which is a tight ligand for these lectins, indicating that glyconanoparticles mostly enter cells via lectin-mediated endocytosis. Finally, we found that glyconanoparticles lead to induction of immune responses by examining the production of reactive oxygen species and p-c-Raf-1. This study will provide opportunities for developing finely tuned multifunctional nanoparticle-based drug and diagnostic nanoplatforms.

(73) Detection of Helicobacter pylori with fluorescent magnetic glyconanoparticles

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Yonsei University

Helicobacter pylori is a Gram-negative, microaerophilic bacterium found in the stomach and causes chronic gastritis and gastric ulcers. It is also associated with the development of duodenal ulcers and stomach cancer. Therefore, sensitive detection of H. pylori is crucial for diagnosis and treatment of H. pylori associated diseases. It has been known that H. pylori expresses a sialic acid-binding adhesin (SabA) and a Leb-binding adhesin (BabA) that adhere, through glycan-adhesin interactions, to the human gastric mucosa for infection. To detect H. pyroli expressing BabA, we prepared fluorescent magnetic glyconanoparticles by conjugating aminoethylated Lea, Leb and H1 oligosaccharides to carboxy-containing fluorescent magnetic nanoparticles. The successful preparation of glyconanoparticles was examined by zeta potential and transmission electron microscopy (TEM). When H. pyroli J99 strain which expresses BabA was incubated with three kinds of glyconanoparticles, fluorescence microscopy analysis showed that Leb and H1 conjugated but not Lea conjugated nanoparticles bound to this strain. However, these glyconanoparticles did not recognize H. pyroli strains lacking BabA. These glyconanoparticles were further used to examine what kind of Korean H. pylori strains express BabA. Finally, they were employed to enrich BabA expressing H. pyroli by using a magnet. It is expected that fluorescent magnetic glyconanoparticles will be powerful tools to sensitively detect pathogens including H. pylori.

(74) α-Selective glycosidation of using N-acetyl sialyl donors possessing a free hydroxyl group at the C4 position

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Sialic acids are frequently located at the non-reducing end of the glycocojugates such as glycoproteins and glycolipids and play important roles in many biological events on the cell surface. Structurally defined and chemically synthesized sialic acid derivatives served as effective chemical probes for elucidation of their biological activity. We have investigated glycosidation of sialyl donors varying at the 5-N substituents. We found that 5,4-*N*, *O*-carbonyl protected sialyl donor underwent α-selective sialylation in dichloromethane. Dichloromethane is a weaker coordinating solvent to oxonium cation compared to acetonitrile solvent used normally in α-sialylation and could not make the reactivity of the donor towards glycosidation to be reduced. However, use of the 5-N modified sialyl donors requires modification to the N-acthyl group after the glycosidation. Therefore, an effective method for the synthesis of α-sialoside from a N-acetyl sialyl donor is still required.

Herein we report α-selective glycosidation of the N-acetyl sialyl donor possessing the C4 free hydroxyl group. The acyl-type protecting group at the O4 and O7 hydroxyl groups might block glcosyl acceptor to be attached at the α-face. We found that a N-acethyl sialyl donor possessing the C4 free hydroxyl group underwent α-sialylation of primary alcohols in dichlolomethane. We also report on the synthesis of α(2,4) disialic acid by using the sialylated product as glycosyl acceptor.

(75) Convergent synthesis of biantennary complex type nonasaccharide containing LacdiNAc structure based on the regioselective glycosylation and inversion reactions

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Recent advances in the synthesis of glycoproteins involve glycoprotein remodeling using transglycosylation, native chemical

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ligation, and biosynthetic pathway engineering to effectively produce homogeneous glycoproteins with well defined glycans for structural and functional studies. However, chemical syntheses of the glycoproteins, naturally occurring glycoprotein glycan which is the simple biantennary sialo-complex type N-glycan was used after isolation from egg yolk. Therefore, to conduct synthetic studies of glycoprotein containing more complex and rare structure of glycans is provocative and troubling issue currently organic chemistry. To solve this, the efficient routes for the chemical synthesis of glycan are still requested. In this report, we chose the biantennary complex type nonasaccharide containing LacdiNAc (GalNAcβ1-4GlcNAc) structure at nonreducing end as a synthetic target. Because of this moiety is one of the rare natural occurrences structure and its biological importance. In order to reduce the synthetic steps, we employed a convergent strategy, a regioselective glycosylation, and using a common oligosaccharide parts. Furthermore, we easily constructed the difficult to synthesize β-mannose residue and two galactosamine residues by using simultaneously inversion reactions of the stereochemistry of four hydroxyl groups including the C-2 and C-4 hydroxyl groups at galactose residue and C-4 hydroxyl group at non-reducing end of glucosamine residues, to obtain the complex type nonasaccharide containing LacdiNAc structure.

(76) A study toward understanding cellular dynamics of glycosphingolipid based on chemical engineering approach

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Glycolipids are deeply involved in a wide range of biological phenomena, and are also expected to become important biological markers for diagnosis. Despite their importance, very little is known about the synthetic mechanism of the class of molecules. To understand the underlying mechanism of glycolipid synthesis taking place inside the cell, various analysis methods allowing us to obtain information about location and about the molecular structures and their quantities of the glycans being synthesized have to be employed. In such analyses, reproducibility has been one of the issues in quantitative investigations. Microfluidic device that has micro-channels in a fabricated material has focused in biotechnology and chemical engineering fields. We envisaged applying the technique for the analysis in glycolipid research would solve reproducibility problems. Thus, we were able to stimulate a part of cultured cells (CHO-K1) by applying laminar flow conditions in a single channel. Trypsin was used as an example of "stimulants" in this first experiment resulting in peeling off of the attached cells at the half of the channel. The area that a stimulant is introduced could be controlled by changing individual flow rates. We are further investigating live-cell imaging of PC-12D cells using BODIPY-labeled lactosylsphingosine, which has been suggested to undergo different glycan transformations, in the presence and absence of NGF in a single microfluidic channel.

(77) Glycoprotein folding influences on its association with lectin-like molecular chaperone calreticulin

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Calreticulin (CRT) has been known to be a lectin-like molecular chaperone, which captures $Glc_1Man_9GlcNAc_2$ (G1M9)-glycoproteins in the endoplasmic reticulum. Although association of CRT with glycoprotein has known to be based on its lectin function [1, 2], we found the binding ability was expected to change with folding status of glycoproteins [3]. In this study, interaction analysis of CRT using a series of synthetic glyco-probes with different aglycon structure and glycoproteins with various denaturing status were carried out.

For the interaction experiment, we used thermal shift assay [4]: the denaturing temperature (T_d) of protein-ligand complex increases depending on the strength of binding. We first confirmed that the thermal shift assay was available for detection of interaction between recombinant CRT and synthetic G1M9. Then we synthesized a series of G1M9 derivatives with various aglycon structures. With these synthetic ligands, we next investigated aglycon specificity of CRT binding, resulted in higher hydrophobicity of the aglycon showed higher T_d values for the CRT-glycan complex. Thus, CRT might more strongly recognizes misfolded G1M9-protein than mature glycoprotein.

To verify this hypothesis, we next investigated interaction of CRT with IgY having various folding status. IgY is an antibody-glycoprotein including 25% of G1M9-glycan. With thermal treatment under various temperatures, we obtained several IgY with different folding status. Interaction analysis of CRT with the resulting IgYs showed this lectin tightly binds with a glucosylated glycoprotein having higher misfolded state.

These results indicated that glycoprotein is likely to release from calreticulin with its folding maturation, supporting our previous findings as follows: further glycan-processing from CRT-G1M9 complex by glucosidase II is available only in the case of G1M9 with hydrophilic aglycan as mature glycoprotein [3]. The aglycon specificity of CRT being identical to folding sensor enzyme UGGT will be also discussed.

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(78) Chemical synthesis of misfolded glycoproteins as substrates of folding sensor enzyme UGGT

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Glycoprotein quality control is a very important process in order to produce native glycoprotein efficiently and to prevent accumulation of aberrant glycoproteins that are often toxic to the cell. Folding sensor enzyme UDP-glucose:glycoprotein glucosyltransferae (UGGT) is one of the key player is this process that can distinguish misfolded glycoprotein from native one. It transfers one glucose

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residue to the high-mannose type N-glycan (M9: $Man_9GlcNAc_2$) on the glycoprotein only when the protein part is not properly folded. UGGT consists of about 1500 amino acid residues and the C-terminal 300 residues are reported to form a glucosyltransferase domain. However, how UGGT recognize the folding status of the glycoprotein is not well understood. Therefore, we synthesized glycoproteins bearing a M9 N-glycan in both native form and misfolded forms and used them as substrates of UGGT to analyze its substrate recognition mechanism at the molecular level.

We selected interleukin-8 (IL-8) as a protein scaffold that consists of 72 amino acids with two disulfide bonds. We reported the chemical synthesis of IL-8 bearing a M9 N-glycan in native and misfolded forms through the formation of native or nonnative disulfide bond patterns. [1] Assay toward these synthetic glycosyl-IL8 derivatives using recombinant UGGT and analysis of glucose transfer by LC-MS clearly showed that all misfolded glycoproteins and glycopeptides analyzed were substrates of UGGT but the IL-8 with native 3D-structure was barely glucosylated. Next we analyzed an interaction of synthetic IL-8 derivative with UGGT using NMR. Chemical shift change of one hydrophobic amino acid was observed in the titration study of the glycopeptide and UGGT. These results suggested that UGGT recognizes specific hydrophobic surfaces on the misfolded glycoproteins.

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(79) Synthesis of 3-deoxy-GlcNAc and its application to transglycosylation using Endo-M

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Endo-β-*N*-acetylglucosaminidase from *Mucor hiemalis* (Endo-M) is used for catalyzing the hydrolysis of the β-(1,4)-glycosidic linkages between the *N,N′*-diacetylchitobiose moiety of N-glycans. Endo-M is unique because it can act on N-glycans by catalyzing the hydrolysis and transglycosylation of appropriate acceptors containing *N*-acetylglucosamine (GlcNAc) residues. Endo-M is an effective tool for the reconstruction and remodeling of glycopeptides and glycoproteins. However, a problem with this reaction is that the yield of the transglycosylation product decreases on hydrolysis. We have studied the acceptor recognition site of Endo-M and observed that the narrow region of the 1,3-diol structure from 4-OH to 6-OH of GlcNAc was essential for transglycosylation using Endo-M. This suggested that we should focus on the study of the 3-OH function of GlcNAc for the transglycoslation reaction using Endo-M.

Here to elucidate the effect of the 3-OH function of GlcNAc in transglycosylation using Endo-M, 3-deoxy-GlcNAc was synthesized. Although the synthesis of 3-deoxy-GlcNAc via a 3-Cl derivative was reported, there are several problems with this reaction, such as low yield of the precursor. Therefore, we examined the development of the new method that was easier than the conventional method. We studied 3-deoxy-GlcNAc under several conditions. 3-Deoxy-GlcNAc was synthesized from 4,6-*O*-benzylidene-β-allyl glycoside via a 3-bromo derivative, which was derived by the Appel reaction. This method is superior to the conventional method. The synthesis and transglycosylation reaction of Fmoc-Asn (3-deoxy-GlcNAc)-OH was also studied. These results will be discussed.

(80) High-throughput determination of non-human epitope Gal-alpha-(1 → 3)-Gal on glycoproteins using a specific antibody fragment in a microarray format

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The non-human epitope Gal-α-(1, 3)-Gal found on glycoprotein therapeutics produced inmurine derived cell lines (SP2/0 and NS0) or Chinese hamster ovary cells has been demonstrated to produce a hyperacute immune response reaction in a number of patients, leading to fatality in severe cases. Thus it is of importance to be able to detect and measure the presence of this epitope in glycoproteins.

We have generated a panel of single chain antibody fragments (scFv) against this glycan epitope. Recognition profiling of these scFvs using neoglycoconjugate (NGC) microarrays demonstrated that they were highly specific and selective for the Gal-α-(1, 3)-Gal target. The scFvs were immobilised on a surface in microarray format and were tested for their specificity using NGCs and inhibitory sugars, including the monosaccharide galactose, the Gal-α-(1, 3)-Gal disaccharide, the trisaccharide Gal-α-(1, 3)-Gal-β-(1, 4)-Gal and the Gal-α-(1, 3)-Gal-β-(1, 4)-Gal-α-(1, 3)-Gal tetrasaccharide. The scFvs were found to maintain their unique specificity in this format.

Binding constants from these data were also ascertained and compared to the same assay in an ELISA format. In addition, inhibition was carried out with Gal-α-(1, 3)-Gal NGC analogues, which differed in their linker lengths, and the scFvs recognised the epitopes in both analogues. The scFv microarrays showed increased sensitivity compared to the reported suspension assays. Importantly, IC_{50} values of microarray bound glycan recognition molecules were generated for the first time using a panel of glycans and neoglyconjugates. Furthermore, protein bound Gal-α-(1,3)-Gal motif from the natural glycoprotein, bovine thyroglobulin, was subsequently determined. This sensitive and specific scFv microarray approach lends itself to a high throughput format and surpasses the current profiling application of plant lectin and anti-glycan antibody microarrays as it can be extended to specific identification and quantification of glycans on natural glycoproteins.

(81) Biological function of endomannosidase activity found in the endoplasmic reticulum

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Newly synthesized glycoproteins with Glc1Man9GlcNAc2 (G1M9) glycan are folded to form functional structures through calnexin/carleticulin (CNX/CRT) cycle. Even though about 30% of newly

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synthesized glycoproteins are introduced into degradation pathway, the mechanisms that release these proteins from CNX/CRT cycle remain unclear.

Recently, we established an *in vitro* quantitative analysis system to evaluate*N*-glycan processing in the endoplasmic reticulum (ER) using synthetic fluorescent G1M9 glycan that includes BODIPY on the reducing terminus (G1M9-BODIPY) [1]. Using this system with an ER fraction from rat liver in the presence of both inhibitors for known glucosidases and mannosidases, we found a production of M8A-BODIPY that was trimmed glucosylmannose (Glc-Man) from G1M9-BODIPY so called endomannosidase (EM) activity. An EM with the same activity is known to localize in Golgi apparatus (Golgi-EM). Then, we thought the possibility that the EM activity found in the ER fraction was derived from Golgi-EM. Therefore, to investigate the possibility, we prepared a specific substrate for Golgi-EM, Glc-Man-4-methylumbelliferone, and analyzed the activity in the ER fraction using the substrate. The substrate was digested in a fraction that included Golgi apparatus, while not in the ER fraction. In the clear contrast to this, the substrate was digested in the ER fraction in the presence of a hydrophobic molecule, Fmoc-glycine. These results indicate that EM apart from that in Golgi apparatus exists in the ER (ER-EM), and that ER-EM is regulated allosterically with hydrophobic molecules such as unfolded glycoprotein that exposes hydrophobic regions. In addition, glycoprotein with M8A glycan generated by ER-EM is neither re-glucosylated by UGGT (UDP-Glc: glycoprotein glucosyltransferase) nor recognized by CNX/CRT in the ER. Taken together, our findings suggest that ER-EM plays important roles for triaging unfolded glycoproteins, for releasing unfolded glycoproteins from CNX/CRT cycle, and for introducing the glycoproteins into degradation pathway.

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(82) Novel Strategy to Release and Tag Glycans from Glycoproteins and Glycosphingolipids for Functional Glycomics

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Functional glycomics has been impeded by the lack of inexpensive enzymatic and mild chemical methods to acquire natural glycans in significant amounts. We have developed a new strategy we term “threshing and trimming” (TaT) to quickly release glycans from glycoproteins and glycosphingolipids through specific degradation of aglycon moieties. For glycoproteins, we employ low-cost Pronase to degrade peptides and N-bromosuccinimide (NBS) to effect oxidative decarboxylation under very mild reaction conditions to generate homogeneous aglycon moieties such as nitriles or aldehydes. These aglycons can be readily conjugated with fluorescent tags for profiling and functional study. For glycosphingolipids, a simple chemical treatment with NBS oxidatively degrades the lipid moiety to yield cyanomethyl glycosides. The resulting glycan derivatives all retain the natural reducing end linkage. The cyano(nitrile) group can be activated by specific chemical reactions for installation of various fluorescent tags/linkers. The new methods provide an easy way to analyze and/or prepare large amounts glycans from natural sources, and will significantly facilitate further advances in functional glycomics.

(83) Proteomics analysis of sialylated glycoproteins identifies substrates for sialyltransferases and sialidases

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The presence or absence of sialic acid can dramatically alter the activity of glycoproteins, affecting both extracellular binding and intracellular signaling events. The sialylation status of glycoproteins is regulated by two key classes of enzymes: sialyltransferases, which add sialic acid, and sialidases, which remove sialic acid. Glycoproteomics methods are beginning to inventory the glycoproteins that may be sialylated, but these efforts have not yet linked sialylation to the activity of specific sialyltransferases and sialidases. We report the integration of the periodate oxidation and aniline- catalyzed oxime ligation (PAL) reaction for specific biotinylation of sialylated molecules with quantitative proteomics methods, and show that this type of glycoproteomics analysis can be used to identify sialyltransferase and sialidase substrates. First, we used PAL in combination with stable isotope labeling by amino acids in cell culture (SILAC) to identify substrates for the human sialyltransferase ST6GAL1 in a breast cancer cell line. We confirmed that the pro-metastatic protein CDCP1 (also known as Trask) is an ST6GAL1 substrate and showed that signaling through CDCP1 is activated by ST6GAL1 expression. In addition, we show that both ST6GAL1 and CDCP1 are involved in promoting a mesenchymal phenotype in a breast cancer cell line. Thus, the PAL-SILAC approach provides new mechanistic insight into the role of sialylation in cancer cell behavior. In a second application, we used PAL in combination with label-free SINQ "normalized spectral index quantitation to identify glycoprotein substrates for three pneumococcal sialidases. Sialidase substrates were identified from a cell line that models the human endothelial blood-brain barrier, yielding insights into possible mechanisms by which pneumococcus crosses the blood-brain barrier in meningitis."

(84) Proteomics and Genomics Provide Novel Insights Into Streptomyces Lectin Biochemistry

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SHA, a ~11 kDa blood group-B specific lectin produced by *Streptomyces* sp. 27S5 was previously purified by affinity chromatography and characterized. SHA is rich in tryptophan residues and was shown to have two carbohydrate-binding sites specific for L-rhamnose or D-galactose (*Biochemistry*, 14, 4465, 1975; *Biochim. Biophys. Acta*., 701, 86, 1982). While strain 27S5 has been lost forever, the purified SHA (approximately 50 mg) survived frozen since 1975 and appeared to be intact by SDS PAGE analysis. We analyzed SHA using Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometry (MS), which revealed an average molecular mass of 13,314.67 daltons and the presence of a

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covalently attached hexose in ~25% of the SHA molecules. SHA peptides were obtained in several separate enzymatic digestions with trypsin, chymotrypsin, LysC, ArgC, V8 protease, and pepsin, and then analyzed by liquid chromatography quadrupole time-of-flight MS as well as MALDI MS. MS/MS collision induced fragmentation data from these peptides was analyzed with PEAKS software. The result was a strikingly close match to the C-terminal portion of a hypothetical protein encoded by the genome of *Streptomyces* sp. Mg1 (*Genome Announc.* 1, 1, 2013). This C-terminal portion has 88% homology to a polysaccharide deacetylase of *Streptomyces lavendulae*. Both proteins were deduced from genome sequences and can be considered SHA homologues. Each of these homologues contains five conserved tryptophan residues, four of which are located in three ChW domains. ChW stands for *Clostridial* hydrophobic with conserved W. The ChW family is almost exclusively limited to the *C. acetobutylicum* species. The fact that three ChW domains have been found in the SHA homologues strongly suggests a unique opportunity for studying the role of ChW domains in the context of carbohydrate binding. Isolation and purification of these SHA homologues is currently in progress.

In conclusion, the finding of SHA lectin-like proteins in the genomes of several strains of *Streptomyces* is novel and significant. It should permit us to further characterize their expected carbohydrate binding activity, and to eventually design recombinant lectins which could be used for the advancement of glycobiology-based medical applications, such as diagnostics and drug delivery approaches.

(85) Multiple modes of (glyco)peptide substrate recognition/ binding by the ppGalNAc-T's

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UDP-GalNAc:polypeptide GalNAc transferases (ppGalNAc-T's) are responsible for initiating and defining sites of mucin type O-glycosylation. Structurally, this large family of ~20 isoforms contains an N-terminal catalytic domain linked to a C-terminal ricin-like lectin domain. The roles of the catalytic and lectin domains on modulating ppGalNAc-T peptide and glycopeptide specificity are largely unknown. We have systematically analyzed ppGalNAc-T (glyco)peptide substrate specificity, by exploiting a series of novel random (glyco)peptide substrates designed to probe the functions of the catalytic and lectin domains (Gerken *et al., J.Biol.Chem*., 286, (2011)),(Gerken *et al., J.Biol.Chem*., 288, (2013)). We have shown that the ppGalNAc-T's possess preferences that vary among isoforms for peptide and Ser/Thr-O-GalNAc glycopeptide substrates. The placement of Ser/Thr-O-GalNAc residues near a glycosylation site produces a range of transferase specific effects: relative inhibition of glycosylation, alteration/shift in glycosylation site, or large rate enhancement (requirement for glycosylated substrate). Interestingly, alteration/shifts in glycosylation sites are mediated by the lectin domain where large differences in glycosylation rates are observed for different isoforms between substrates with N- or C-terminal placed Thr-O-GalNAc residues. We also found for some isoforms, an absolute glycopeptide requirement which resides in their catalytic domain and not their lectin domain. These studies show that the functions of the catalytic and lectin domains differ between peptide and glycopeptide preferring isoforms and these isoforms have specific modes of glycopeptide recognition/binding which serve to provide exquisite control of mucin type O-glycosylation. We are now extending our studies to transferases that require multiple glycosylated substrates (i.e. *Drosophila* GANT4) using a novel series of di-glycosylated random glycopeptides. PGANT4 shows preferential transfer to a di-glycosylated MUC5AC-3,13 substrate (Ten Hagen *et.al. J.Biol. Chem*.,278, (2003)). These new di-glycopeptide substrates will allow us to characterize simultaneously the lectin and catalytic domain requirements and their N- or C- directionality for these unusual transferases. In summary we have identified catalytic domain specific, lectin domain assisted and combined catalytic and lectin domain assisted modes of substrate recognition. With this work, an additional level of control of mucin type O-glycosylation has been identified that will further advance the field and our understanding of the regulation of this type of modification. (NIH-R01CA078834).

(86) The chemical synthesis of various N-linked oligosaccharides as the substrates for endo-β-N-acetylglucosaminidase Endo-M or its mutated enzyme (Endo-M-N-175Q) toward production of glycoproteins having homogenous N-glycans

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Since N-glycans are attached to proteins as post-translational modification in protein biosynthesis, and the heterogeneity of the glycan is discussed in quality control of the glycoproteins such as biopharmaceuticals. TCI produces Endo-M which is one kind of endo-β-*N*-acetylglucosaminidase, or its mutated enzyme (Endo-M-N175Q, Glycosynthase), and these enzymes can transfer intact N-linked oligosaccharides to GlcNAc residues of proteins, peptides and other materials [1, 2], so that these enzymes can be used to produce glycoproteins having homogeneous N-glycans. In order to produce the variety of glycoproteins which include the structure-defined N-linked oligosaccharides or the standard compounds for oligosaccharide analysis of glycoproteins, we will present herein the large scaled chemical synthesis of N-linked oligosaccharides and their oxazoline derivatives as the substrates for Endo-M or Endo-M-N175Q toward the production of glycoproteins in industrial level.

For the synthesis of variety of N-linked oligosaccharides, 4-methoxyphenyl (MP) glycoside of core tetrasaccharide (Manα(1-3)[Manα(1-6)]Manβ(1-4)GlcN) derivative was selected as a key intermediate compound, and we have achieved the preparation of the core tetrasaccharide in hundred grams scale with minimum use of column chromatography. Glycosidation of GlcN derivatives onto Man residues of non-reduced terminal of the core tetrasaccharide gave G0-type hexasaccharide, and consequent coupling with various non-reduced terminal blocks such as Gal, Neu5Acα(2-3/6)Gal, Neu5Gcα(2-3/6)Gal or Galα(1-3)Gal can give the human-type

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or non-human-type N-liked oligosaccharides. On the other hand, direct conversion of MP glycoside in reduced terminal of oligosaccharides into oxazoline and followed by removal of protective groups gave the oxazoline derivatives of N-liked oligosaccharides as the substrates for the enzymes. Moreover, the MP glycoside of oligosaccharide derivatives can be converted into the corresponding glycosyl donors such as trichloroacetimidate, which can be coupled with another GlcN derivative to get natural type N-linked oligosaccharides having *N,N'*-diacetylchitobiose moiety. These oligosaccharides will be used not only as the standard N-glycans but also as the substrates for the enzymes.

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(87) NMR and MS Based Analysis of Glycans of Glycoproteins

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Analysis of glycoproteins is still challenging. Many protocols have been developed and are being used to elucidate the structure of the glycans by MS or NMR spectroscopy. We have recently developed new analytical protocols to elucidate the structure of glycans and other post-translational modifications.

Recently, it was found that nonhuman glycosylation of recombinant antibodies can cause tremendous problems for some patients. Therefore, unambiguous assignment of the glycosylation pattern of therapeutic antibodies is of high importance for assessment of human compatibility. We present results from a broad and detailed N-glycan analysis of the therapeutic antibody cetuximab by LC-MS/MS analyses tightly integrated with 1H NMR to obtain unambiguous structures. Thirty-seven N-glycan compositions were identified by LC-MS(/MS). Subsequently, ten abundant structures were structurally characterized by applying the recently introduced method called three-dimensional cross correlation (3DCC). Even mass isobaric structures that differ only in the branching position of one monosaccharide unit were distinguished and characterized. 24% of all detected glycans, possess the immunogenic α-1,3-Gal epitope and/or N-glycolyl-neuraminic acid. These results illustrate the importance of integrated use of LC-MS(/MS) and 1H NMR for the glycome analysis of biopharmaceuticals in research, development, and quality control.

In another example we analyzed sequence polymorphisms and post translational modification (PTM) status of fibrinogen. We aim for the simultaneous analysis of fibrinogen subunits for sequence polymorphisms (SNPs), phosphorylation and glycosylation by top-down mass spectrometry. A statistically significant anti-correlation between the amount of O glycans and the amount of mono-phosphorylation of the Aα subunit is found. Interestingly, phosphorylation status of the Aα-subunit seems to also correlate with the age of the individuals. Two coding single nucleotide polymorphisms on the Aα- and Bβ-subunit could be identified on the basis of their mass shifts. Isolation and analysis of fibrinogen can be achieved in a few hours, and thus the method presented here should assist in a quick assessment and prevention of stroke and infarction.

(88) *N*-Glycan Replacement of a Therapeutic Antibody with Structure-defined Glycan by Chemoenzymatic Glycoengineering using Endo-M and Glycosynthase

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Concerning up-regulation of physiological function derived from a glycan structure of glycoproteins for biopharmaceuticals, some effects have been reported as described; a half-life of erythropoietin during blood circulation relating to frequency of a sialylated non-reducing terminal of its glycans [1], ADCC activity of an antibody correlating with lack of core fucosylation of its *N*-glycans [2], and anti-inflammatory effect participating sialylated *N*-glycans on IgG [3]. Nevertheless, it is generally difficult to evaluate bio-function caused by a definite glycan structure owing to heterogeneity of glycosylation biosynthesized on a manufacture scale of protein pharmaceuticals secreted into culture media from mammal or insect cells. Therefore, comparative analysis using a standard glycoprotein possessing a homogenous glycoform for investigation of relationship between a glycan structure of biopharmaceuticals and its physiological activity is important. In this study, we show that heterogeneous glycans of a therapeutic antibody are rearranged by a structure-defined glycan as a typical procedure of chemoenzymatic glycoengeneering. Endo-M cleaved heterogeneous *N*-glycans on antibody by hydrolysis reaction, and Glycosynthase introduced the homogenous *N*-glycan to the glycan-trimmed antibody by transglycosylation reaction. In future, it is anticipated that various standard glycoproteins would be generated by using Endo-M and Glycosynthase to explore how glycosylation regulates physiological function in organisms.

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(89) Synthetic study of branched inner-core oligosaccharides of LPS/LOS

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Lipopolysaccharides (LPSs) and lipooligosaccharides (LOSs) are important surface antigens for a wide variety of gram-negative bacteria. LPS and LOS consist of a lipid A and a polysaccharide (PS) or an

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oligosaccharide (OS). The core OS moiety of LPS and LOS consists of a structurally variable region and a conserved inner-core OS. 3-Deoxy-d-*manno*-oct-2-ulosonic acid (Kdo) is a component of the inner-core OS. Recently, human IgG2 antibodies, which were isolated by using 15253 LOS from serum-resistant gonococcal strain 15253 as an affinity ligand, recognized the 3,4- branched and 2,3:3,4-dibranched neisserial LOSs and *Salmonella minnesota* Rb and Re mutant LPSs. To understand these epitopes, we are synthesizing Kdo containing oligosaccharides of various sizes. In this presentation, we will describe the syntheses of Kdo containing di-, tri-, and branched oligosaccharides from a common Kdo intermediate.

A common Kdo intermediate, methyl 7,8-di-*O*-benzoyl-4,5-*O*-isopropyliden-D-*manno*- oct-2-ulosonate was prepared D-mannose by using our reported procedure. It was easily converted to the corresponding glycosyl imidate, phosphate, fluoride, however, long reaction time was needed. Chemoselective deprotection of the 2-*O*-allyl Kdo intermediate gave corresponding the 4,5- or 7,8-diol in good yield. In the glycosylation of the 4,5-diol acceptor with Kdo donors, it was found that the use of glycosyl fluoride was suitable donor for the synthesis of 2-4 linked Kdo disaccharide. Installation of mannose, L-*grycero*-D-mannoheptose, and galactosamine derivatives at 5-OH position of Kdo(α2-4)Kdo was successively given 4,5-branched trisaccharides in good yield with high stereoselectivity. By the treatment with aqueous trifluoroacetic acid, followed with 0.1 M sodium hydroxide gave fully deprotected oligosaccharides in moderate to good yield. The deprotected allyl glycosides were attached with methyl thioglycolate under UV irradiation and resulted methyl ester was converted into hydrazine amide. Coupling of the spacer compounds with biotin-sulfo-OSu afforded the corresponding biotin-OS conjugates.

(90) **Analysis of substrate specificity for endo-M and its mutants using synthetic glycans**

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Endo-β-*N*-acetylglucosaminidase from Mucor hiemalis (endo-M) catalyzes hydrolyses of β-1,4-glycosyl linkages in *N*, *N*'-diacetylchitobiose moieties of the asparagine (*N*)-linked glycans in glycoproteins. This enzyme also has transglycosylation activities toward a variety of acceptor substrates having GlcNAc residues (ex. *p*NP-GlcNAc). However, the transglycosylated products also could be substrates and hydrolyzed. Recently, endo-M mutants (N175Q and N175A) which possess much enhanced transglycosylation activities and less hydrolytic activities have been developed by replacement of the amino acid residue in the catalytic domain, and used remodeling of glycoprotein glycans. Applied researches have been reported, but the detailed substrate specificity comparative studies of these enzymes have not been performed. For this purpose, we have focused on configuration of hydroxyl groups on donor substrates, and designed tetrasacchride derivatives exhibited different hydroxyl configuration, namely tetrasaccharide ($Man_3GlcNAc$) and its stereoisomers which replaced β-mannoside by β-glucoside, β-galactoside or β-taloside residues. To construct of the tetrasaccharide structures, we carried regioselective glycosylations on for hydroxyl groups on C-3 and C-6 of lactosamine derivative to give β-galactoside type tetrasaccharide ($Man_2GalGlcNAc$). Inversion reactions of stereo chemistries for hydroxyl groups on C-2, C-4, both of C-2 and C-4 led β-taloside (protected $Man_2TalGlcNAc$), β-glucoside (protected $Man_2GlcGlcNAc$) and β-mannoside (protected $Man_3GlcNAc$) type tetrasaccaride derivatives, successively. After deprotections, reducing ends were converted into oxazoline structure using 2-chloro-1,3-dimethylimidazolinium chloride, then all glycosyl donors were in hand. We performed transglycosylation reactions to*p*NP-GlcNAc acceptor using endo-M and its mutants. In the case of endo-M, $Man_3GlcNAc$, $Man_2TalGlcNAc$ and $Man_2GlcGlcNAc$ became substrates, and products from β-mannosde and β-taloside type donors were hydrolyzed but from β-glucoside type was not. On the other hand, endo-M mutants, N175Q and N175A, both of $Man_3GlcNAc$ and $Man_2TalGlcNAc$ were introduced to *p*NP-GlcNAc and these resulting products were not hydrolyzed properly. On the presentation, we will discuss the more details about the difference of the substrate specificity of these enzymes.

(91) **Biological activities of the homogeneous glycosylated chemokines CCL1 and Ser-CCL1 prepared by total chemical synthesis**

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CCL1 is a naturally glycosylated chemokine protein that is secreted by activated T-cells and acts as a chemoattractant for monocytes. Originally, CCL1 was identified as a 73 amino acid protein having one N-glycosylation site, and a variant 74 residue non-glycosylated form, Ser-CCL1, has also been described. There are no systematic studies of the effect of glycosylation on the biological activities of either CCL1 or Ser-CCL1.

In order to elucidate the roll of the glycosylation on the biological activities of the glycoproteins, we carried out the chemical synthesis of homogeneous *N*-glycosylated and non-glycosylated forms of (Ser-)CCL1. Chemotaxis assays of these structurally-defined glycoproteins and the corresponding non-glycosylated proteins were conducted. The results were correlated with the chemical structures of the (glyco)protein molecules; Non-glycosylated form exhibited stronger activity than that of the glycosylated forms. We also succeeded in the X-ray structural analysis of the synthetic CCL1 derivatives by a unique protein crystallization using quasi-racemic protein solution consisting of glycosylated L-protein and non-glycosylated-D-protein. As a result, it revealed that the tertiary structures of the protein moieties are identical between glycosylated and non-glycosylated forms despite the difference of their biological activities. To the best of our knowledge, these are the first investigations of the effect of glycosylation on the chemotactic activity of the chemokine (Ser-)CCL1 using N-glycosylated protein molecules of defined covalent structure.

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(92) **A synthetic study of a homogeneous hematopoietic glycoprotein bearing three biantennary sialyloligosaccharides**

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Erythropoietin (EPO) is a 166-amino acid glycoprotein hormone that regulates the production of red blood cells. EPO possesses three complex-type oligosaccharides linked to side-chain of asparagine residues at positions 24, 38 and 83. EPO analogues used as biopharmaceuticals are a mixture of glycoforms because they are produced by eukaryotic cell expression system. Because of the inherent difficulty in preparing the single glycoform of EPO by biological procedure, the functions of oligosaccharides have not been well understood. Therefore, we decided to develop a synthetic strategy of EPO by chemical approach.

Chemical synthesis is a powerful approach to investigate how glycosylation of the protein can affect the protein functions.[1] In modern procedures for chemical glycoprotein synthesis, target glycosylated polypeptide chains are assembled by chemical ligation of peptide and glycopeptide segments. Recently many efficient methods for the preparation of glycopeptide and peptide-peptide ligation methods have been reported [2] , which make glycoprotein synthesis more accessible. We have developed an efficient method to obtain sialylglycopeptide thioester, which is an activated form of glycopeptide essential for native chemical ligation, by Boc solid-phase peptide synthesis. [3] Using this method, we thought EPO bearing three *N*-linked sialyloligosaccharides could be chemically synthetized. Not only peptide thioesters but also peptide hydrazides [4] were used in order to employ the convergent ligation strategy to access the full-length glycosylated polypeptide. This synthetic route is also suitable for chemical synthesis of EPO analogues having one or two oligosaccharides. Here we discuss this efficient synthetic strategy of EPO glycoforms in detail.

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(93) **Synthetic Study of Erythropoietin Having High Mannose-type Oligosaccharide by Chemical Methodology**

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Glycoprotein folding is rigorously controlled by the endoplasmic reticulum (ER) quality control (QC) system. The folding sensor enzyme UDP-glucose:glycoprotein glucosyltransferase (UGGT) monitors the folding state of glycoproteins bearing high mannose-type (M9) oligosaccharides, and transfers one glucose residue specifically to the misfolded ones to form a monoglucosylated glycoform (G1M9). In order to elucidate the substrate recognition mechanism of UGGT in detail and its role in the glycoprotein QC system, we prepared the cytokine, called erythropoietin (EPO), which has a homogeneous M9 oligosaccharide as chemical probe.

EPO is a cytokine involved in the maturation of red blood cells and has three *N*-linked oligosaccharides at *N*24, *N*38, and *N*83. To investigate how the number of oligosaccharides and their positions affect the UGGT recognition process, we synthesized several EPO glycopolypeptides varying the number of glycosylation and their positions. Our synthetic route employed a peptide segment coupling strategy using peptide-thioester and its glycosylated form under the native chemical ligation condition. In this synthesis, the glycopeptide-a-thioester bearing the M9-oligosaccharide is essential. These key building blocks were prepared by Boc solid phase peptide synthesis (SPPS) with Asn-(M9 oligosaccharide) isolated from egg yolk. Other peptide-a-thioesters and the C-terminal peptide were obtained by using Boc or Fmoc SPPS. The repetitive native chemical ligation with these building blocks yielded the full-length glycosylpolypeptides. In this presentation, we discuss the assembly of whole glycosylpolypeptide chains of the target EPO in detail.

(94) **The Synthesis of Sialo-containing Glycopolymers by π-Allyl Nickel Catalyzed Coordination Polymerization**

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Carbohydrate cluster on cell surface play important roles of many biological processes. The multiple carbohydrates amplify the observed carbohydrate-receptors interaction to initiate various biological responses. Structurally well-defined glycopolymers served as mimics of the assembly of the carbohydrates on cell surface to modulate the carbohydrate-mediated biological process. Direct polymerization of glycosylated monomers is an effective for assembling well-defined multiple monomers bearing carbohydrates in terms of molecular weights with narrow polydisparities and random carbohydrate density. However, the glycomonomers possessing a complex and highly functionalized carbohydrates with biologically importance are still difficult to prepare and assemble due to their multiple functional groups and their low reactivity towards polymerization.Herein we report on the synthesis of sialo-containing glycopolymers by pai-allyl nickel catalyzed coordination polymerization. A pai-allyl nickel catalyzed coordination polymerization of allenes is an effective method for living polymerization, in which π-allyl nickel complex as a polymerization growth terminal exhibits highly chemoselectively reactivity to the allene monomers in the presence of hydroxyl groups, carboxylic acids and amide groups in protic solvents. The glycomonomers bearing glucoside, galactoside and

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mannoside smoothly underwent polymerization without protecting groups. Sialic acid reduced reactivity of the pai-allyl nickel catalyst towards polymerization. We also reported on the synthesis of glycopolymers bearing an alfa(2,8) sialic acid and their biological evaluation.

(95) Analysis of glycan processing in the endoplasmic reticulum based on selective inhibition of mannosidases

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Seikei University

Structural isomers of $Man_8GlcNAc_2$ (M8) glycans function as signals that mediate secretion and degradation of glycoprotein in the endoplasmic reticulum (ER), respectively. Accumulation of the misfolded protein was known to increase ER stress that was caused by failure of glycoprotein quality control. Thus, balance of secretion and degradation of glycoproteins is important for protein biosynthesis. However, precise functions of mannosidases that produce M8 isomers have not been understood. In the ER, M8 isomers were known to be generated from $Man_9GlcNAc_2$ (M9) with α1,2-mannosidases. Namely, ER α1,2-mannosidase I (MI) mainly produce $Man_{8B}GlcNAc_2$(M8B) for secretion signal of glycoproteins, whereas ER degradation enhancing α1,2-mannosidase-like protein (EDEM) believed to produce $Man_{8A/8C}GlcNAc_2$ (M8A or M8C) for degradation signals of glycoproteins. In spite of whole the terminal mannose linkages of M9 are identical to α1,2-manner, these enzymes regio selectively hydrolyze the terminal mannose. To obtain molecular basis of selectivity of MI and EDEM, recombinant enzymes should be useful. However, enzyme activity of recombinant EDEM has not been detected *in vitro*. Therefore, selective inhibition of MI or EDEM in the ER fraction is necessary for analysis of secretion and degradation pathway, respectively. In this study, we explored selective inhibitor for MI and EDEM to elucidate glycan processing during the secretion and degradation pathway.

We carried out comparison of percent production of the M8 isomers with the ER fraction in the presence or absence of various inhibitors. As a result, we found that 1-deoxymannojirimycin (dMJ) showed 23-fold selective inhibition toward EDEM, and Kifunensine (Kif) showed 6.8-fold selective inhibition toward MI. Moreover we happened to observe selective inhibition of MI by high concentration of 1-deoxynojirimycin (dNJ). Moreover we found glycan processing difference under selective inhibition of MI or EDEM. With these selective inhibitors, we also investigated influence of cellular state and substrate difference on MI and EDEM mediated glycan processings.

(96) Synergistic improvement in chemical synthesis of high-mannose glycans

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For facile access to obtain structurally defined high-mannose glycans, optimization of chemical synthetic approach should be an important subject. In particular, complicated process for various monosaccharide segments are time-consuming task. In glycosylation steps, some considerations are needed for efficient formation of 1,2-cis glycosides. Diversity oriented approach is necessary to prepare various branched structures. Although several pioneer works for chemical synthesis of high-mannose glycans have been successful [1, 2], some improvements for the facile access seem to be left. In this study, we investigated synergistic improvement of chemical synthesis of Glc3Man9GlcNAc2, Man9GlcNAc2, Man8(B)GlcNAc2 and Man8(C)GlcNAc2 as follows:

(1) Reducing the synthetic steps using common intermediates.

Various specialized monosaccharides are generally required for efficient stereoselective formation of glycosidic bond. However, Glc3Man9GlcNAc2 is only constructed from three sugar components. Here, we examined efficiency of positive use of common intermediates on reducing the synthetic process. As compared with previous reported study [2], 19 steps were reduced for even in the segment synthesis of Glc3Man9GlcNAc2.

(2) Development of 1,2-cis-α-glycosylation assisted with a silyl-protecting groups as an electron-donating elements.

In previous study [2], the second glycosylation step for synthesis of Glc1-2Glcα1-3Glc segment of Glc3Man9GlcNAc2 provided 45% yield. Therefore, we attempted to improve the 1,2-cis-α-glycosylation by our original approach. Namely, we examined enhancing anomeric effect with powerful electron-donating of TBDMS group for protecting C-2 hydroxy group, resulted in high yield and α-selectivity.

(3) Regioselective one-pot glycosylation toward diverse branched oligosaccharide.

Synthesis of both structural isomer Man8(B)GlcNAc2 and Man8(C)GlcNAc2 has been reported with independent strategy [2], causing increase of total synthetic steps. Here we examined one-pot glycosylation with common glycosyl donors and acceptor for each of branched tetra saccharide of Man8(B)GlcNAc2 and Man8(C)GlcNAc2. Consequently, both of their one-pot glycosylations were successfully proceeded with high regio- and stereoselectivity.

We demonstrated synergistic improvement for synthesis of high-mannose glycans. We synthesized all of the segments required for chemical synthesis of Glc3Man9GlcNAc2. Coupling of the each segment for total synthesis of the target glycans will be also reported.

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(97) Integrated Proteomic and Glycoproteomic Analyses of Prostate Cancer Cells Reveals Glycoprotein Changes in Protein Expression, Glycosylation Occupancy and Glycosite Heterogeneity

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LNCap and PC3 are prostate cancer cell lines that have been used as cell models for prostate cancer research. LNCap is an androgen-dependent cancer cell line and is considered a model for the

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relatively early stages of prostate cancer; whereas PC3 is an androgen-independent highly aggressive cell line. These cell lines have been utilized in various genomic and biological approaches for understanding the mechanism underlying androgen dependency as well as the aggressive behavior of prostate cancer cells. However, a complete analysis of protein glycosylation, known to play critical roles in aggressive cell behavior, has not been reported. Herein, we report the glycoproteomic analysis between these two prostate cancer cell lines using integrated proteomics and glycoproteomics. Glycoproteins were isolated from the two cell lines using solid phase extraction of glycosite-containing peptide (SPEG) followed by liquid chromatography and tandem mass spectrometry analysis. Among the 1794 unique N-linked glycosylation site-containing peptides identified, 174 glycoproteins were observed to be differentially expressed between the two cell lines. Strikingly, the N-Glycan degradation pathway was enriched in differentially expressed glycoproteins. To further understand the regulation of the glycoproteins at the protein level, global proteomes of the cell lines were analyzed using iTRAQ labeling and 2D liquid chromatography tandem mass spectrometry, and 8063 proteins were identified and quantified. A majority of the differentially expressed glycoproteins were the result of protein expression changes. However, 23 differentially expressed glycoproteins showed no change at the protein levels indicating glycosylation site occupancy was different between the two cell lines. To determine the glycan heterogeneity at specific glycosylation sites of glycoproteins, we identified and quantified 1145 N-linked glycopeptides with attached glycans. These intact glycopeptides accommodated 67 glycan compositions. Our data showed that N-glycoproteins with no change in total protein level or glycosylation occupancy contained site-specific changes in specific glycans. The altered glycosylation occupancy and site-specific glycosylation forms have great potential in aiding our understanding of aggressive prostate cancer.

(98) Synthesis of glucosylated *N*-glycans using transglycosylation activity of Golgi endo-α-mannosidase

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Background: An efficient method for the synthesis of *N*-glycan is essential for the elucidation of the structural and functional roles of glycans in glycoproteins. Golgi endo-α-mannosidase (G-EM) belongs to GH99 family and hydrolyzes the glucose-substituted mannose residue in *N*-glycan A-arm structure. We have reported that a tetrasaccharide derivative (Glcα1-3Manα1-2Manα1-2Man-Dansyl) is a good substrate for G-EM [1]. In this presentation, we focused on the transglycosylation activity of G-EM as a tool for the synthesis of *N*-glycans and will introduce the transglycosylation reactions using Golgi endo-α-mannosidase. Methods and Results: To analyze the transglycosylation activity of G-EM, we incubated wild-type G-EM with fluoride derivative (Glcα1-3Man-F) and mannobiose acceptor (Manα1-2Man-Dansyl). As a result, 0.6% transglycosylated product was observed in HPLC chromatogram. However, the product was hydrolyzed immediately. To overcome this problem, we prepared G-EM glycosynthase that catalyze transglycosylation reactions without hydrolysis-activity by the mutantion of the catalytic site. This enzyme showed high transglycosylation activity without hydrolysis activity in comparison with the wild-type enzyme. Using this mutant, we optimized the transglycosylation reaction conditions of pH, the ratio of donor/acceptor and buffer. Finally, we succeeded to obtain transglycosylation product over 45% yields. In addition, we analyzed the substrate specificity of G-EM mutant towards several mannobiose acceptors such as Manα1-2Man, Manα1-3Man or Manα1-6Man. As a result, a transglycosylation product was obtained when used Manα1-2Man as an acceptor. Moreover, we performed transglycosylation toward high-mannose-type glycan (M8A), and glycosylated high-mannose-type dodecasaccharide was obtained in 40% yield. In addition, we studied substrate specificity of the transglycosylation reaction using donors such as Glcα1-3Man-F, Glcα1-2Glcα1-3Man-F and Glcα1-2Glcα1-2Glcα1-3Man-F towards Manα1-2Man-Dansyl acceptor. Tr3ansglycosylation reaction was smoothly proceeded, we obtained tetra, penta and hexa saccharides ($Glc_{1\text{-}3}α1\text{-}3Man_3$) derivatives, respectively. Conclusion: Our results have shown that the G-EM mutant could efficiently transglycosylate fluoride derivative (Glcα1-3Man-F, Glcα1-2Glcα1-3Man-F and Glcα1-2Glcα1-2Glcα1-3Man-F) as a donor to the α1-2 linked mannobiose acceptor (Manα1-2Man). In this study, we have established a chemo-enzymatic method using a Golgi endo-α-mannoisidase as a tool for glycosylation.

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(99) Nonencapsulated group A *Streptococcus* associated with human invasive disease

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Group A *Streptococcus* (GAS) is a leading human pathogen, causing 650,000 severe invasive diseases annually with 25% mortality. M1 GAS is the most frequently isolated serotype from invasive disease worldwide; however, a recent occurrence of invasive infections in Australia was attributed to M4 GAS. Here, we use a combined molecular genetics and bioinformatics approach to characterize 17 clinical M4 isolates associated with this invasive disease episode. The M4 isolates were classified into 3 pulsed-field gel electrophoresis groups, and 2 multilocus sequence types. All isolates lacked hyaluronic acid (HA) capsule, a major antiphagocytic virulence factor, and whole genome sequence analysis of 2 isolates revealed the complete absence of the *hasABC* capsule biosynthesis operon. Most isolates contained mutations within the *covRS* two-component regulator, a hallmark of hyperinvasive GAS. Transformation of a plasmid expressing *hasABC* into M4 GAS restored capsule production. However, encapsulation neither promoted survival in whole human blood, nor enhanced virulence of

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wild-type M4 GAS in a mouse model of systemic infection. Bioinformatic analysis found no *hasABC* homologs in closely related species, suggesting that this operon was a recent acquisition by ancestral GAS. Together, these data demonstrate that nonencapsulated GAS is capable of causing life-threatening invasive human infections.

(100) Beyond Sweet Attractions: The role of glycans in Rotavirus Infection

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Rotavirus is a global pathogen that causes acute gastroenteritis resulting in dehydration and high levels of infant mortality. The ability of viruses to infect host cells and cause disease is substantially determined by cellular expression of receptors bound by virus, thus facilitating virus penetration of the cell membrane and delivery into the cell. Rotavirus association with the host cell is achieved by interaction of the virion outer capsid and spike proteins with several important receptors including sialic acid-containing glycoconjugates (sialylglycoconjugates) and integrins. We have structurally investigated the interaction of rotavirus spike proteins (VP8*) of a number of important human and animal rotavirus strains with selected glycans at an atomic level to determine a VP8*-glycan interaction map (VP8* glycointeractome). This important structural information will not only advance our understanding of rotavirus host-cell tropism but also provides a unique structural basis for the development of novel anti-viral drugs and vaccines.

(101) Antigenic Potential of a Highly Conserved Lipopolysaccharide Inner Core Structure Defined by Synthetic Approach

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The cell surface glycocalyx of pathogens contains a variety of complex carbohydrates presenting promising targets for vaccine development. The human pathogen *Neisseria meningitidis* causes meningitis and/or sepsis worldwide. Vaccinations based on capsular polysaccharides are available against invasive meningococcal serogroups, but not for serogroup B due to an autoantigenic [α2-8] polysialic acid capsule. Thelipopolysaccharide (LPS), a sub-capsular antigen of *N. meningitidis*, isanchored via lipid A in the outer membrane. Meningococcal LPS does not contain an *O*-antigen polysaccharide; it only consists of a core oligosaccharide that can be subdivided further into inner and outer core. Diverse outer core compositions determine the twelve distinct LPS immunotypes whereas the inner core is highly conserved. Antibodies specific to LPS inner core of *N. meningitidis* are protective by promotingopsonophagocytosis and complement mediated killing. Building on earlier studies of another group using isolated LPS [1–3] we explored synthetic oligosaccharides based on the conserved LPS inner core as potential antigen candidates for *N. meningitidis*. A series of syntheticLPS inner core oligosaccharides were covalently immobilized on glycan arrays. By employing these arrays, naturally occurring LPS core-specific antibodies in human sera of convalescent patients and asymptomatic carriers of *N. meningitidis* were detected. A promising synthetic LPS core epitope of *N. meningitidis*, the tetrasaccharide, α-D-GlcNAc-(1->2)-L-α-D-Hep-(1->3)-L-α-D-Hep-(1->5)-α-Kdo, was selected based on the array results and conjugated to the immunogenic carrier protein CRM_{197}. The resulting tetrasaccharide glycoconjugate was immunogenic in a mouse model and revealed a robust IgG response in a formulation- and dose-dependent manner. Binding of mouse serum antibodies to a broad collection of wild type *N. meningitidis* strains expressing twelve diverse LPS immunotypes and LPS mutants demonstrated the accessibility of the LPS inner core on viable bacteria. We identified the mono-Kdo motif as the immunodominant part of the tetrasaccharide antigen. High immunogenicity of Kdo resulted in the induction of a significant portion of non-protective antibodies. In contrast, antibodies directed against the distal trisaccharide α-D-GlcNAc-(1->2)-L-α-D-Hep-(1->3)-L-α-D-Hep are crucial for binding to the inner core of *N. meningitidis* LPS. This study illustrates how glycan array-based epitope refinement using synthetic oligosaccharides could help to design improved antigens for a broadly protective immunization against *N. meningitidis*.

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(102) Enzymatically active extracellular hyaluronidase (HylA) of group A *Streptococcus* promotes intracellular survival and virulence

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Group A *Streptococcus* (GAS) is a human-specific Gram-positive bacterial pathogen associated with various diseases including pharyngitis, rheumatic fever and toxic shock syndrome. GAS strains are distinguished serologically on the basis of the immunovariable surface-anchored M protein. M1 serotype is the most frequently isolated serotype from invasive GAS infections, while outbreaks of M4 serotype GAS infections are often reported throughout the world. Most GAS serotypes express a surface hyaluronan (HA) capsule, identical in structure to host HA, which is a major virulence factor promoting opsonophagocytosis resistance and survival in the host. However, M4 GAS lacks the HA biosynthesis genes (*hasABC*) and expresses an active extracellular hyaluronidase enzyme (HylA). In this study, we analyzed the role of HylA in M4 GAS pathogenesis. Using a spectrophotometric assay to measure the release of dissacharide units from the HA polymer, wild-type (WT) M4 GAS strain 4063-05 and recombinant M4-HylA protein showed hylauronidase activity, whereas M1 GAS strain 5448 and recombinant M1-HylA protein did not. Recombinant M4-HylA and M1-HylA proteins did not degrade other glycosaminoglycans including chondroitin sulfate, heparan sulfate and dermatan sulfate. WT M4 GAS and the isogenic mutant strain lacking the *hylA* gene (*ΔhylA*) did not express HA capsule. Introduction of the HylA-expression vector pM4-HylA, but not pM1-HylA, into M1 *ΔhylA* led to a marked reduction (90%) in HA expression on the bacterial surface. Next, we performed bacterial intracellular survival assays using M4 GAS strains and human epithelial cell lines, HeLa and HaCaT. WT M4 showed significantly higher intracellular survival rates compared to M4 *ΔhylA*. In addition, heterologous expression of M4-HylA in *Lactococcus lactis*, a Gram-positive bacterium traditionally used for manufacturing dairy products, showed significantly higher intracellular survival compared to the vector only control. Finally, in a mouse model of systemic infection, WT M4 was significantly more virulent compared to M4 *ΔhylA*; however, there was no difference between WT M1 and M1 *ΔhylA*. These results indicate that enzymatically active M4 HylA contributes to intracellular bacterial survival and *in vivo* pathogenesis.

(103) **TREX1 (DNase III) Prevents Dysregulation Of Oligosaccharyltransferase**

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N-linked glycosylation in the ER involves a) entry of nascent polypeptide bearing the Asn-X-Ser/Thr sequon into the lumen, b) synthesis of the lipid-linked oligosaccharide (LLO) $Glc_3Man_9GlcNAc_2$-P-P-dolichol (G3M9-LLO), and c) transfer of the G3M9 glycan to the Asn residue by oligosaccharyltransferase (OST), a multisubunit complex. This process is highly specific, but is not known to be regulated in a biologically significant way. In addition, a fraction of LLO is hydrolyzed at a slow rate by OST to produce free G3M9 and dolichol-P-P, but the role (if any) of this hydrolysis has been enigmatic.

With an N-terminal catalytic domain, TREX1 (DNase III) removes potentially immunogenic self-DNA from the cytosol. Curiously, TREX1 is bound to the ER membrane (facing the cytosol) by a C-terminal anchor. TREX1 defects in humans lead to either of two clinical phenotypes. DNase domain mutations cause early onset recessive autoimmunity, while stem-anchor defects cause late onset dominant autoimmunity reminiscent of lupus. The DNase domain is active in the latter group of mutations, suggesting a novel etiology.

We find that TREX1 functionally interacts with the OST complex. In the absence of TREX1, LLO hydrolysis by OST is accelerated *in vitro* and *in vivo* although N-glycosylation continues. Suppression of LLO hydrolysis requires anchoring of TREX1 to the ER membrane, but not its DNase function. DNase-active/anchor deficient TREX1 mutant cells (as well as TREX1 knockouts) have a characteristic "interferon stimulated gene" (ISG) transcriptional signature associated with autoimmunity. ISGs can be induced by treatment of normal cells with neutral aqueous extracts of TREX1 knockouts, containing free saccharides resulting from LLO hydrolysis. Based upon these and other results, we speculate that ISG induction in TREX1 anchor-defect mouse cells and autoimmune patients may involve immunogenic activities of free saccharides released from dolichol by dysregulated OST. This also suggests that LLO hydrolysis may have a role in normal cells, by modulation of TREX1.

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(104) **Superficially-Located Enlarged Lymphoid Follicles Characterize Nodular Gastritis**

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Nodular gastritis is a form of chronic *Helicobacter pylori* gastritis affecting predominantly gastric antrum and is characterized endoscopically by the presence of small nodular or granular lesions resembling gooseflesh. It has been accepted that hyperplasia of lymphoid follicles histologically characterizes nodular gastritis; however, quantitative analyses of these lymphoid follicles to serve as the basis for this theory was not found in the literature. Our goal was to determine whether nodular gastritis is characterized histologically by hyperplasia of lymphoid follicles. In the present study, we quantified the number, size, and location of lymphoid follicles in nodular gastritis, and compared to those in atrophic gastritis and normal gastric mucosa. The percentages of mucosal addressin cell adhesion molecule 1 $(MAdCAM\text{-}1)^+$ and $MECA\text{-}79^+$ high endothelial venule (HEV)-like vessels were also quantitatively analyzed. We also carried out an integrin

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alpha4beta7•IgG heterodimeric chimera *in situ* binding assay to probe functional MAdCAM-1 on HEV-like vessels in nodular gastritis. We found that superficially-located hyperplastic lymphoid follicles histologically characterize nodular gastritis, and these lymphoid follicles correspond to endoscopically observed nodular and/or granular lesions resembling gooseflesh. We also found that the percentage of MAdCAM-1^{+}HEV-like vessels, which were bound by an integrin alpha4beta7•IgG heterodimeric chimera in a divalent cation-dependent manner, in both nodular gastritis and atrophic gastritis were significantly greater than that seen in normal gastric mucosa, while the difference between the two types of gastritis was not statistically significant, suggesting that MAdCAM-1 protein does not specifically contribute to the pathogenesis of nodular gastritis. The percentage of MECA-79^{+} HEV-like vessels in nodular gastritis is greater than that in atrophic gastritis with high statistical significance at the biopsy sites A1, A2 (lesser and greater curvature of the antrum, respectively) and IA (incisura angularis), where endoscopic gooseflesh appearance is evident. Taken together, these results indicate that superficially-located hyperplastic lymphoid follicles characterize nodular gastritis, and these follicles correspond to endoscopically observed gooseflesh-like nodular lesions.

(105) Agalactosylated antibodies with enhanced binding to MUC16 may contribute to mucosal protection against HIV transmission

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Mucosal transmission of HIV accounts for a significant proportion of new infections each year, and women are at particular risk of infection with HIV. Thus, there is a great need to enhance protection within the female reproductive tract in order to aid in prevention of HIV transmission. Mucus at the mucosal barriers protects the underlying epithelium, and the heavily glycosylated mucin proteins play a critical role in mucosal protection through capturing and trapping pathogens in mucus. We hypothesized that antibodies could induce more robust trapping of virus, and particularly, fix these trapped viruses in mucus. Here, we demonstrate that antibodies isolated from chronically infected HIV patients show elevated and specific binding to mucin 16 (MUC16), a large transmembrane mucin that extends up to 500 nm from the epithelial surface, compared to healthy HIV negative controls. Importantly, MUC16-bound antibodies can trap HIV particles, suggesting that induction of antibodies that bind MUC16 may be a desirable feature of prophylactic or vaccine-elicited antibodies to aid in prevention of HIV transmission. To define the features of the antibodies that mediate enhanced binding to MUC16, we evaluated IgG subclass and glycosylation profiles of MUC16-binding antibodies from HIV patients and healthy controls. While we did not observe significant correlations with any IgG subclass, we observed significant correlations with distinct antibody glycosylation profiles. Interestingly, antibodies containing the inflammatory agalactosylated (G0) glycan structure showed a significant positive correlation with MUC16 binding, and conversely galactosylated (G1 and G2) antibodies showed a significant negative correlation with MUC16 binding. Further, antibody pull-down assays with MUC16 demonstrated an enrichment of antibodies containing G0F structures, indicating that fucosylated and agalactosylated antibodies bind to MUC16 with high affinity. In addition, deglycosylation of MUC16 eliminated antibody binding to MUC16, suggesting the interaction may be glycan-glycan dependent. Taken together, these data suggest that glycan variation on the antibody Fc-domain alters the capacity of antibodies to bind to MUC16, highlighting a novel mechanism of antimicrobial control regulated by antibody Fc-glycosylation, thus providing new avenues through which HIV vaccines may boost immunity to prevent infection more effectively on a global level.

(106) Mechanism of up-regulation of a glycoprotein: Angpt-1 in Kaposi's sarcoma-associated herpesvirus infected primary effusion lymphoma cell lines

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Angiopoietin-1 (Angpt-1), a secreted glycoprotein firstly characterized as a ligand of TIE2 receptor, is primarily expressed in growing vascular endothelial cells. Angpt-1 could promote the adhesion of endothelial cells to fibronectin and stabilize blood vessels. Recent researches suggested that Angpt-1 could also induce lymphatic vessel formation and contribute to tumor angiogenesis in vivo.

Kaposi's sarcoma-associated herpesvirus (KSHV) infection could up-regulate both*ANGPT-1* and *ANGPT-2* at mRNA level in Kaposi's sarcoma, a malignant tumor occurring in AIDS patients. In another KSHV infected tumor, primary effusion lymphoma (PEL), we found that *ANGPT-1* appeared to be expressed significantly highly, comparing with that of EBV infected cell lines or the other T-cell leukemia cell lines.

In this study, we confirmed Angpt-1 expression in PEL cell lines at protein level, and tried to reveal the mechanism of *ANGPT-1* up-regulation in KSHV infected PEL cell lines. Secreted Angpt-1 was detected in the supernatant with ELISA and the cellular Angpt-1 with immunofluorescent assay (IFA). With immunoprecipitation followed by western blot, Angpt-1 was detected as a polymerized glycoprotein in the culture medium of KSHV infected PEL cell lines.

Luciferase reporter plasmids with *ANGPT-1* regulatory region up to about 1kb confirmed that *ANGPT-1* was significantly active in the PEL cell lines. Finally we found a 19-bp fragment (termed D1A) in the *ANGPT-1* regulatory sequence was responsible for the up-regulation and some nuclear proteins in the KSHV infected cells could contribute to this enhancement by EMSA.

Future works should be done to characterize the factors binding with D1A, and to reveal the function of Angpt-1 to determine the pathophysiology in KSHV infected patients under AIDS setting.

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(107) *N*-glycosylation of plasma proteins during systemic inflammatory response provoked by surgery

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Systemic inflammation is still a poorly understood process and its control represents major clinical problem. Lack of a good study model which involves humans also contributes to this fact. Protein glycosylation is essential in many steps of the inflammatory cascade, but the role of individual variation in glycosylation has not been addressed until now. Recently, we analyzed the composition of total plasma glycome in patients with systemic inflammatory response provoked by major surgery. *N*-glycosylation of plasma proteins has been followed through 72 hours after the operation in 107 patients that underwent cardiac surgery and the glycosylation changes have been identified. To confirm these findings, the same phenomenon was now studied in another cohort comprising 188 patients that underwent abdominal surgery (upper abdominal, lower abdominal, XIFO-PUBIC and bilateral subcostal surgery). *N*-glycans released from plasma proteins were analyzed using HILIC-UPLC. In nearly all individuals, in both cohorts, plasma N-glycome underwent the same pattern of changes indicating the real biological phenomenon. These included the decrease in almost all glycan structures, except the most abundant disialylated forms and highly branched tetra-antennary structures. The notable exceptions from this pattern of changes were triantennary trisialylated structures with fucose on the outer arm, which decreased in the first 24 hours, but then significantly increased in the following 48 hours. This structure has often been associated with inflammation and its structural analogue present on leukocytes is responsible for leukocytes' interaction with selectin ligands on endothelial walls and the initiation of inflammation. The increase in this structure on plasma proteins after a rapid decline in the first 24 hours seems to be a reflection of a molecular mechanism that controls inflammation by competitive inhibition of selectin adhesion.

(108) Fucosyltranferase deficiency enhances during acute neutrophil infiltration into the lung airway inflammation

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Acute airway inflammation is characterized by the influx of inflammatory granulocytes and macrophages into the lung in response to various insults. Long-term exposure to these inflammation causing agents can exacerbate Chronic Obstructive Pulmonary Disease (COPD), which is characterized by cough, shortness of breath, and thickening of the airways. COPD is the 4th leading cause of death and affects 5% of the global population. Minimizing infiltration of immune cells into the airway, particularly the neutrophils (PMNs) that comprise most of the infiltrating cells, would be desirable in managing acute airway inflammation and retarding COPD pathogenesis.

Glycan-based therapeutic strategies currently under investigation to minimize inflammation target selectin-selectin ligand interactions to disrupt immune cell recruitment. To assess whether we could minimize immune cell recruitment to the acute airway upon exposure to Non-Typable Haemophilus Influenzae (NTHi), we used two glycosyltransferase deficient mouse models (fut7$^{-/-}$ and fut4$^{-/-}$fut7$^{-/-}$) that are unable to express selectin ligands and are known to have impaired neutrophil trafficking. Suprisingly, the number of total immune cells, particularly the neutrophils was increased more than two-fold in the fut4$^{-/-}$fut7$^{-/-}$ mice as compared to wild-type (WT). Similarly, blocking with an anti-selectin antagonist TBC1269 lowered the total amount of cells in the BALF by >50% in all subtypes, but once again threre were two-fold more PMN in the fut4$^{-/-}$fut7$^{-/-}$ BALF as compared to WT.

Differentially labeled bone marrow neutrophils from WT and fucosyltransferase-deficient animals were used to confirm the enhanced emigration into NTHi-induced acute airway. In 24 hrs after NTHi exposure, 5- to 7-fold more fucosyltransferase-deficient PMNs, compared to WT PMNs were recovered in the BALF of recipient WT animals. In contrast, when peritonitis was elicited by thioglycollate in the WT recipient animals, >10-fold more WT PMN, compared to the fucosyltransferase-deficient PMNs were recovered in the peritoneum. Taken together, these data show the mechanism of inflammatory cell recruitment into the airway may be significantly different than that controlling recruitment into the peritoneum. Disruption of the selectin-selectin ligand axis, while effective in attenuating recruitment in peritonitis, had the opposite effect in NTHi-induced acute airway.

(109) Galectin-8 in the experimental Trypanosoma cruzi infection

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Galectin-8 is distributed in different tissues and in the endothelium, which links it to various processes such as migration, apoptosis induction, and platelets activation, and to the role as pro-inflammation molecule. No information is available on Gal-8 in infections by *Trypanosoma cruzi*, the agent of Chagas disease. This protozoan induces megaviscera and/or cardiomegaly on 30% of infected patients, during the chronic phase of the disease. We started said analysis assaying various murine infection models combined with *T. cruzi* strains to induce different evolution. We studied Gal-8 expression in different organs during the acute period of the infection in BALB/cJ mice (60 day old males), using the RA strain (DTU TcVI). Assays were conducted at 17 days post-infection (dpi) with parasitemia levels ranging 7x10^5-1.5x 10^6 trypomastigotes/ml. qRT-PCR showed a significant decrease on Gal-8 expression in infected mice hearts. Organs were

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analyzed in triplicate and normalized to host β-actin expression. C57BL/6J (WT) and Gal-8KO four month old males were infected with the Ac strain, (DTU TcI), which induces chronic infection (survival 90%). Parasitemia were similar between groups (followed up to 80 dpi). At four months pi, Gal-8KO mice showed exacerbated splenomegaly than that developed by infected WT mice. The weight of infected Gal8-KO mice's spleen was significantly larger than the WT counterpart, while the heart's and liver's weight were similar between groups. HE-stained spleen tissue samples showed greater degree of follicular hyperplasia in Gal-8KO along with numerous secondary follicles, a higher number of centerblasts and a pronounced amount of plasmocytes, when compared to WT both infected and normal. In Gal-8KO, while follicle structure is maintained, there was important lymphocyte movement towards the red pulp.These preliminary results show, for the first time, that *T. cruzi* modulates Gal-8 expression. The splenomegaly seen in Gal-8KO mice could suggest the involvement of this galectin in the development of polyclonal activity, a hallmark in *T. cruzi* infection.

(110) Structural and functional insights into Group A *Streptococcus* gacA: An essential dTDP-4-dehydrorhamnose reductase (RmlD)

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The sugar nucleotide dTDP-rhamnose is essential for the biosynthesis of Group A Carbohydrate (GAC), the molecular signature and virulence determinant of the human pathogen Group A *Streptococcus* (GAS). dTDP-L-rhamnose is synthesized by four enzymes from glucose-1-phosphate and dTTP. The final step, the conversion of dTDP-4-keto-L-rhamnose to dTDP-L-rhamnose, is catalyzed by dTDP-4-dehydrorhamnose reductase (RmlD) in an NAD(P)H-dependent manner. The first gene of the recently identified GAC gene cluster, *gacA*, potentially encodes an RmlD homologue. We will present structural, functional and genetic data that confirm the function that GacA catalyzes the production of dTDP-rhamnose. We furthermore present data revealing that *gacA is e*ssential for GAS and that GacA is an attractive drug target against invasive streptococcal infections in humans.

(111) Enhanced expression of Siglec-8 and Siglec-9 counter-receptors in inflamed human airways

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The sialic-acid-binding immunoglobulin-like lectins Siglec-8 (on eosinophils and mast cells) and Siglec-9 (on neutrophils) down-regulate inflammation by inducing apoptosis or inhibiting inflammatory responses of the leukocytes on which they are expressed. These immune inhibitory effects are induced when siglecs bind to their endogenous glycan counter-receptors on tissues. The current study investigated the cellular distribution and expression levels of Siglec-8 and Siglec-9 counter-receptors on normal and inflamed human airways and human airway cells. The tissue distributions and relative densities of siglec counter-receptors were determined by overlaying sections of human upper airway (resected inferior turbinate) with the expressed Fc-tagged siglec chimeras Siglec-8-Fc and Siglec-9-Fc, followed by anti-Fc immunohistochemistry. Siglec-8 counter-receptors were found predominantly on sub-mucosal serous cells, while Siglec-9 counter-receptors were found on mucous and serous cells, connective tissue and on the surface of the airway epithelium. Counter-receptor densities were significantly increased (2.5-fold for Siglec-8 and 1.6-fold for Siglec-9, $p < 0.01$) in tissues from patients suffering from chronic rhinosinusitis, a chronic inflammatory condition, compared with unaffected control patients. To investigate the mechanisms responsible for regulated counter-receptor expression we used Calu-3 lung adenocarcinoma cells, a model for human gland cells that express Siglec-9 counter-receptors as measured by detergent-extraction, gel electrophoresis, and lectin overlay. Siglec-9 counter receptors migrated as very large (>1 million dalton) proteins on mixed agarose-acrylamide gels. The concentration of counter-receptors increased 4-fold in response to inflammatory stimuli, which included bacterial lipopolysaccharides (LPS), TNF-α and IL-13. Inhibiting the inflammatory pathway molecule NF-κB (with BAY11-7082) blocked the increase in Siglec-9 counter-receptors in response to inflammatory stimuli and decreased their constitutive levels as well. Siglec-9 counter-receptor expression was diminished by treatment with benzyl-α-GalNAc and 3F(ax)-Neu5Ac, indicating that the Siglec-9 counter-receptor on Calu-3 cells is a sialic acid terminated, O-linked glycan. Lectin capture and Western blotting indicates that MUC5B may be a major carrier of Siglec-9 counter receptors in Calu-3 cells. These studies demonstrate the tissue distribution of Siglec-8 and Siglec-9 counter-receptors in human upper airways, their over-expression in chronic inflammation and infer that activation of the NF-κB inflammatory signaling pathway up-regulates Siglec-9 counter-receptors. Supp. by HL107151, HL068546 and AI106683.

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(112) **Structure-guided discovery of potent, dual acting human parainfluenza virus haemagglutinin-neuraminidase inhibitors**

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Human parainfluenza virus (hPIV) type 3 is the second leading cause of respiratory disease in infants and young children after the respiratory syncytial virus and is a particular concern for the immunocompromised, chronically ill and elderly. To date, there are neither vaccines nor specific antiviral therapy available to prevent or treat hPIV infections.

The multifunctional hemagglutinin-neuraminidase (HN) protein presents an ideal target for antiviral drug discovery. Firstly, HN protein recognizes and binds sialic acids exposed on the host cell surface. Moreover, HN binding is necessary for the activation of the hPIV fusion protein to allow the fusion of cell/virus membranes. HN protein has also an important action during the viral budding process because it cleaves sialic acids from sialoglycoconjugates to prevent the accumulation, through virion auto-agglutination, at the cell surface. Our recent advances in evaluating structure-based anti-parainfluenza inhibitors have revealed highly active compounds. Moreover, our recently reported flexibility of the hPIV-3 HN 216-loop suggests that accommodation of larger compounds is possible [1].

Herein, we present a multidisciplinary approach to investigate how this class of inhibitor targets a novel structural feature in the hPIV-3 HN binding pocket. For the first time, we show that 'designer' sialic acid based inhibitors can exploit this highly flexible protein. By saturation difference transfer (STD) Nuclear Magnetic Resonance spectroscopy we have determined that these inhibitors are well accommodated in the hPIV-3 HN binding pocket, in good agreement with our molecular modeling prediction. Taken together these novel designer inhibitors are able to efficiently lock open the flexible hPIV-3 HN 216 loop and block both virus cell entry and virus progeny release. Our study provides not only future directions towards anti-parainfluenza drug design, but also advances our understanding of hPIV-3 HN receptor recognition and catalytic mechanism.

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(113) **3-Substituted-Neu5Ac2en derivatives: novel influenza A virus sialidase inhibitors and probes of 150-loop flexibility**

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The influenza virus surface glycoprotein sialidase (neuraminidase, NA) has proven to be a successful target for anti-influenza drug design, with potent enzyme inhibitors developed in the 1990s based on knowledge of the structure of influenza A virus N2 sialidase [1]. Influenza A viruses causing the vast majority of human influenza infection carries sialidase of either the N2 or N1 subtype. Interestingly, structural data for N1 sialidase, first revealed in 2006, showed a large cavity adjacent to the enzyme active site, which was not apparent in the N2 sialidase structures. This cavity is generated by opening of the '150-loop' [2]. Subsequently, computational studies on the protein's dynamics, as well as more recent X-ray crystallographic studies, have suggested some flexibility of the N2 sialidase 150-loop as well.

A new direction for influenza virus sialidase inhibitor design exists through targeting the cavity revealed upon opening of the 150-loop. To explore this opportunity we have developed novel inhibitors based on the unsaturated *N*-acetylneuraminic acid template (2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid, Neu5Ac2en), which incorporates a substituent at the normally unsubstituted C-3 position. On binding of the inhibitor in the sialidase active site, the C-3 substituent is directed towards the '150-cavity'. Prototype inhibitors give low micromolar IC_{50} values against influenza A virus N1 sialidase. Pre-incubation studies of the C-3-substituted Neu5Ac2en derivatives with enzyme indicate slow binding of the inhibitors to the protein. This is in contrast to the C-3 unsubstituted parent template, which shows no apparent slow binding kinetics, and supports the concept that the 150-cavity, formed by opening of the 150-loop, is accessed by the C-3 substituent. Aspects of our latest work in the design, synthesis and evaluation of these new influenza virus sialidase inhibitors will be presented.

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(114) **GALECTIN-1 INHIBITS DENGUE VIRUS TYPE 1 INFECTIVITY**

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Dengue is an important arthropod-borne viral disease in tropical and sub-tropical countries. Infection with any DENV serotypes (DENV-1, -2, -3, and -4) can result in a range of sub-clinical to

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severe disease. Dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) is the most severe disorder diagnosed by hemorrhaging and plasma leakage. Despite efforts to provide vector control and development of vaccines against the virus, there are currently no available antiviral treatments or effective vaccines for Dengue disease. Accordingly, we investigated Galectin-1, a mammalian β-galactoside-binding lectin, as a novel mechanism to control Dengue virus type 1 (DENV-1) infection. Herein, we report that macrophages obtained from galectin-1-deficient mice (*Lgals1*$^{-/-}$) are more susceptible to *in vitro*-DENV-1 infection compared to wild-type macrophages. Moreover, we found that newborn *Lgals1*$^{-/-}$ mice succumb to DENV-1 intra-cerebral infection earlier than wild-type mice; suggesting that Galectin-1 expression is important for resistance to DENV-1 infection. Furthermore, resistance to infection was shown to be independent of cell specificity, as Galectin-1 inhibited DENV-1 infection in three distinct cell types. Conversely, this inhibitory effect was found to be dependent on Galectin-1 dimerization and lectin activity. Combined, these findings highlight a novel immune mechanism to block DENV infectivity and viral particle production.

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(115) Rational Design and Development of Novel Multi-target Inhibitors for Influenza Virus

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The furan derivatives from Japanese apricot fruit-juice concentrate effectively inhibit influenza virus. [1] These furan derivatives have inhibitory activities against not only the Neuraminidase (NA) but also the Hemagglutinin (HA) of pandemic influenza virus. These furan derivatives are expected as a good lead compound based on new inhibitory mechanism. But the detail of the molecular mechanism of the furan derivative, Mumefural to the influenza virus has not clear. Mumefural has R- and S- optical isomers because a chiral center exists. So, we performed interaction analysis between R/S-Mumefurals and surface glycoproteins of the influenza virus by using the FMO method at the correlated MP2/cc-pVDZ levels. With the FMO-interfragment interaction analysis, we can reliably estimate the electrostatic and van der Waals dispersion interactions between the inhibitors and hydrophic/hydrophobic amino acid residues composed of the NA and the HA of influenza virus. Recently, flexible structure change of 150-loop in the NA was reported. [2] We, therefore, traced dynamical structure change of the NA/HA with the derivatives including changing of the loop by using molecular dynamics simulations. The theoretical binding affinities as Gibb's free energies were evaluated by the MM-PBSA method for sampling structures. Theoretical binding analysis shows S-Mumefural more effectively can bind than R-Mumefural, because S-Mumefural forms a stable hydrogen bond with an amino acid residue in 150-loop in the NA, even though the difference of binding structure is not enough large.

Acknowledgement

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(116) Implication of a galectin switch in the regulation of luteal function in women

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Galectins are β-galactoside-binding animal lectins, and 15 members are distributed throughout mammalian body with subtype-specific localization. The corpus luteum (CL) in the ovary is a temporal endocrine organ that produces large amount of progesterone, which is essential for the establishment and maintenance of pregnancy. Although the CL is crucial to human reproduction, we still do not understand how it is regulated at a molecular level. We previously reported that galectin-1 and galectin-3 are expressed in the regressing CL and regulated by luteolytic prostaglandin $F_{2\alpha}$ in the mouse ovary. However, exact function of galectins in the regulation of luteal function is still unclear. This study examined the expression and regulation of galectins in the CL of women with special reference to α2,6-sialylation of glycoconjugates. Galectin-1 was intensely expressed in the CL of women throughout the menstrual cycle and localized to steroidogenic granulosa lutein cells. Galectin-3 mRNA was elevated in the CL at the regressing stage with increased number of galectin-3-positive infiltrating macrophages. Healthy steroidogenic luteal cells did not express galectin-3, however, degenerating luteal cells in the regressing CL, which cease progesterone production, contained abundant cytoplasmic galectin-3. Luteotrophic hormones (human chorionic gonadotrophin and prostaglandin E_2) acutely increased galectin-1 expression while they suppressed galectin-3 expression in cultured human steroidogenic luteal cells. In addition, ST6GAL1, which catalyzes α2,6-sialylation on terminal galactose to interfere galectin-1 binding, was significantly suppressed by luteotrophic hormones. Histochemical analysis using α2,6-sialic acids-recognizing plant lectin (*Sambucus*

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sieboldiana: SNA) revealed that α2,6-sialic acids were specifically localized to degenerating luteal cells in the regressing CL. The expression of ST6GAL1 in the CL was increased in the regressing CL like galectin-3, and there was a contrasting relationship between the expression of ST6GAL1 and HSD3B1, a key enzyme for progesterone production, with an inverse correlation in level of expression ($r = -0.5428$, $P < 0.01$). Galectin-1 is a luteotrophic factor whereas galectin-3/α2,6-sialylation may play some roles in luteolysis in the human CL. Luteotrophic hormones inversely regulate galectin-1 and galectin-3/α2,6-sialylation in steroidogenic luteal cells, suggesting an existence of galectin switch regulated by luteotrophic stimuli during luteolysis and luteal rescue.

(117) **The role of *N*-glycosylation on *Paracoccidioides brasiliensis* biological process and on the biological activities of underglycosylated fungal components**

Fausto Dos Reis Almeida, Maria Cristina Roque-Barreira
University of Sao Paulo

The fungus *Paracoccidioides brasiliensis* is a human pathogen that causes paracoccidioidomycosis, the most prevalent systemic mycosis in Latin America. The cell wall of *P. brasiliensis* is a network of glycoproteins and polysaccharides, such as chitin and glucan, performing several functions. N-linked glycans are involved in glycoprotein folding, intracellular transport, secretion, and protection from proteolytic degradation. We investigated the influence of tunicamycin (TM)-mediated inhibition of N-linked glycosylation on N-acetyl-β-D-glucosaminidase (NAGase), α- and β-(1,3)-glucanases and on α-(1,4)-amylase in *P. brasiliensis* yeast and mycelium cells. The underglycosylated yeasts were smaller than their fully glycosylated counterparts and exhibited a drastic reduction of cell budding, reflecting impairment of growth and morphogenesis by TM treatment. The intracellular distribution in TM-treated yeasts of the *P. brasiliensis* glycoprotein paracoccin was investigated using highly specific antibodies. Paracoccin was observed to accumulate at intracellular locations, far from the yeast wall. Paracoccin demonstrated lower NAGase activity when underglycosylated, although no difference was detected between the pH and temperature optimums of the two forms. Murine macrophages stimulated with underglycosylated yeast proteins produced significantly lower levels of TNF-α and NO. Moreover, incubation with TM did not alter α- and β-(1,3)-glucanase activity in yeast and mycelium cell extracts. In contrast, NAGase and α-(1,4)-amylase activity was significantly reduced in underglycosylated yeast and mycelium extracts after exposure to TM. In spite of its importance for fungal growth and morphogenesis, N-glycosylation was not required for glucanase activities. This is surprising because these activities are directed to wall components that are crucial for fungal morphogenesis. On the other hand, N-glycans were essential for α-(1,4)-amylase activity involved in the production of maltooligosaccharides that act as primer molecules for the biosynthesis of α-(1,3)-glucan. Our results suggest that reduced fungal NAGase and α-(1,4)-amylase activity affects cell wall composition and may account for the impaired growth of underglycosylated yeast and mycelium cells. Taken together, the impaired growth and morphogenesis of tunicamycin-treated yeasts and the decreased biological activities of underglycosylated fungal components suggest that N-glycans play important roles in *P. brasiliensis* yeast biology.

(118) **Deciphering Glycan-Antibody Interactions: towards a Carbohydrate-based Vaccine against Clostridium difficile**

Felix Broecker, Christopher Martin, Jonas Hanske, Christoph Rademacher, Claney Pereira, Chakkumkal Anish, Peter Seeberger
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We employ synthetic glycan antigens of *Clostridium difficile* to evaluate carbohydrate-based immunotherapeutic approaches and to characterize antibody-glycan interactions. We have recently demonstrated the potential of synthetic *C. difficile* glycans, named PS-I, PS-II and PS-III, for active vaccination approaches, by showing their ability to induce glycan-specific T cell-dependent antibody responses in preclinical mouse studies. Using a combination of glycan microarray-based screenings of clinical samples from *C. difficile* patients and immunization studies in mice we have identified a minimal glycan epitope (glycotope) of the PS-I antigen. The minimal glycotope is the disaccharide Rha-(1à3)-Glc found twice in the biological repeating unit of PS-I, which is a branched pentasaccharide composed of rhamnose and glucose moieties. This raised the question of what structural determinants within the PS-I antigen mediate interactions with antibodies. Such knowledge may be helpful for the rational design of epitope-focused synthetic carbohydrate vaccines against *C. difficile*.

Here, we identified the structural features involved in the interaction of anti-PS-I monoclonal antibodies with the minimal glycotope using a combination of different biophysical methods. Surface plasmon resonance measurements revealed nanomolar and micromolar affinities towards the pentasaccharide repeating unit and the disaccharide minimal glycotope, respectively. Saturation transfer difference-NMR showed that the antibodies almost exclusively interact with terminal rhamnoside residues of PS-I, especially with their methyl residues. Isothermal titration calorimetry revealed that higher antibody affinity towards the pentasaccharide compared to the disaccharide can be explained by an entropically favored binding process, while enthalpic contributions were similar. Therefore, the increase of affinity towards the pentasaccharide is likely due to higher affinity, not avidity, which was also confirmed by molecular interaction studies showing higher association but similar dissociation rates towards the pentasaccharide.

In conclusion, our data suggests that high-affinity antibody binding to PS-I is achieved through immunogenic rhamnose residues that are presented on a non-immunogenic glucoside scaffold. In a broader sense, this study highlights the potential of identifying and connecting minimal glycotope units for designing epitope-focused synthetic carbohydrate vaccines and may also explain how strong antibody binding against natural repetitive polysaccharides is achieved, despite their usually low affinities against individual glycotopes.

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(119) Novel Interactions of Human Milk Glycans with Human Galectins

Alexander Noll, Ying Yu, Yi Lasanajak, Jean-Phillipe Gourdine, David Smith, Richard Cummings
Emory University School of Medicine

Human milk contains over 200 unique free lactose-derived glycans (HMGs), many of which contain potential determinants recognized by galectins, a family of animal glycan-binding proteins. Although some simple HMGs are known to bind galectins, no systematic study on galectin-HMG interactions has been performed. In this study, the recognition of HMGs by human galectins was explored using a recently developed HMG microarray consisting of over 200 natural and defined HMG structures. Each recombinant human galectin tested, including galectin-1, -3, -4, -7, -8, and -9, but not galectin-2, bound specific HMGs and exhibited a relatively unique HMG binding motif. Furthermore, a novel binding motif for human galectin-7 was identified that eluded detection on a non-HMG glycan microarray. In many cases, the reducing end glucose ring conformation was also important for HMG binding. These results demonstrate that human galectins bind selective HMGs, which may have numerous implications for understanding the roles of HMG-galectin interactions in development, immunity, and/or the gut microbiota of infants.

(120) CD44 is a major E-selectin ligand on human activated T-cells

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Endothelial selectins play a critical role in mediating the migration of activated T-cells to infection and inflammatory sites during adaptive immunity. Although P-selectin is critical for early migration of the T-cells, E-selectin plays a more prominent role at later stages and is responsible for significantly enhancing T-cell recruitment. Though it is known that PSGL-1 and CD43 function as P- and/or E-selectin ligands on human T cells, *in vivo* studies using mouse inflammatory models have shown that the concomitant deficiency of both ligands is not sufficient to completely abrogate E-selectin dependent migration, suggesting the availability of other ligand(s). We thus used a mass-spectrometry approach to define and characterize potential E-selectin ligands on membrane proteins from human activated T cells and identified a repertoire of glycoproteins. Our data show that human activated T cells express a number of previously unrecognized E-selectin ligands in addition to the previously identified ligands. Of particular interest was CD44 due to its major functional role in migration, activation and regulation of T-cells. The E-selectin ligand activity of CD44 is supported by sialylated, fucosylated binding determinants on N-glycans and can mediate E-selectin-dependent rolling interactions of both human activated CD4 and CD8 T-cells over a broad shear range on E-selectin expressing cells (CHO-E). These findings demonstrate that human activated T-cell recruitment may be further controlled by CD44 binding to E-selectin offering new insights into the importance of CD44 in T-cell function and physiology.

(121) Immunotherapy by targeting pathogens-surface glycans–induced immunosuppressions

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Many pathogens including RNA viruses and intracellular bacteria usually cause chronic or latent infection in humans. These pathogens-surface glycans have been implicated as immunosuppressive epitopes of pathogens and might contribute to chronic or latent infection. In this study, we found that human serum lectin-ficolin-2 binds to Hepatits C Virus (HCV) and Mycobacterium tuberculosis (M. tb) -surface glycans and mediates anti-infection response *in vitro* and *in vivo*. Our data provide a new immunotherapeutic strategy against pathogens based on the innate immune molecule ficolin-2. Apolipoprotein E3 (ApoE3), which is enriched in the low-density fractions of HCV-RNA-containing particles, promotes HCV infection, blocks the effect of ficolin-2 and mediates an immune escape mechanism during chronic HCV infection. Both pathogens-glycans and ApoE3 may be as new targets to combat infection. Our data demonstrate that both ficolin-2 and aptamer against pathogen-surface glycans can be used as new anti-pathogens drugs without cross-resistance with known anti-microbe agents.

(122) Role of endothelial sialic acid expression on xenogenic neutrophil adhesion

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University of Maryland Baltimore

Objective: Transplantation of organs across species, xenotransplantation, is limited by the sequestration of platelets and neutrophils in injured organs. We hypothesized that modulating sialic acid expression at the plasma membrane of human and/or pig cells may attenuate coagulation and inflammation dysregulation that are connected with and may cause lung xenograft injury.

Methods: An adhesion assay was developed with freshly isolated human neutrophils and porcine aortic endothelial cells (pAECs). Neutrophils labeled with 0.5 uM of Calcein were quantified using a Spectramax fluorescent reader. Neutrophil adhesion was expressed as arbitrary fluorescence units. Neuraminidase (*Clostridium Perfringens, 25 mU/ml*) was added to remove sialic acids from pAECs 30 minutes before neutrophil addition. Sialic acid expression was assessed by flow cytometry using lectins (Elderberry lectin, (SNA), Peanut agglutinin (PNA) and Maackia amurensis hemoagglutinin (MAC)). Some pAECs wells were incubated with human TNF (25ng/ml) for 3 hrs at 37°C before addition of neuraminidase and neutrophils. Results: Human neutrophil adhesion was detectable on resting porcine endothelial cells (52 +/−15) and was increased by TNF activation (72 +/−4.7). Neuraminidase treatment increased neutrophil adhesion on resting pAECs to a level similar to TNF activation (71 +/−8.8).

Conclusion: These pilot data show that removal of sialic acid with neuraminidase dramatically increases the adhesion of human

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neutrophils to porcine endothelial cells. Thus, targeting levels of sialic acid expression may be a novel therapy to limit neutrophil adhesion during xenotransplantation. Future studies will examine the role of endothelial sialic acid on platelet adhesion as well as the role of neutrophils and platelet sialic acid expression.

(123) Atomic details of the glycosylation-dependent and independent ligand interactions of C-type lectin-like receptor-2 (CLEC-2)

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C-type lectin-like receptor-2 (CLEC-2) is expressed on platelet and induces platelet aggregation upon ligand binding via its extracellular lectin domain. Two ligands of CLEC-2 have been identified: podoplanin and rhodocytin. Podoplanin is a transmembrane *O*-glycoprotein expressed in the cardinal vein and lymphatic endothelial cells. Podoplanin works as physiological ligand for CLEC-2 and is essential for the differentiation of lymphatic vessels. The binding of podoplanin to CLEC-2 is known to be highly dependent on the sialylation of *O*-glycan on podoplanin. The other ligand, rhodocytin, is a snake venom protein produced by the Malayan pit viper *Calloselasma rhodostoma* and composed of one alpha and one beta subunit. Although rhodocytin seems to have no glycans, it does bind to CLEC-2 and induces platelet aggregation as the *O*-glycosylated podoplanin does. To elucidate the structural bases of the glycan-dependent and independent ligand interactions, we performed crystallographic studies of podoplanin and rhodocytin in complex with CLEC-2. We found two novel interaction modes of CLEC-2, in sharp contrast to the modes of other C-type lectin receptors reported to date. Firstly, CLEC-2 lectin domain utilizes its non-canonical "side" face for binding to both podoplanin and rhodocytin in a Ca^{2+} independent manner. Secondly, both ligands interact with CLEC-2 at two separate loci. One locus is common, which is attained by the interaction between consecutive acidic residues on the ligands and conserved arginine residues on CLEC-2. In contrast, the other locus is ligand-specific: Carboxyl groups from the sialic acid residue on podoplanin and from the C-terminus of rhodocytin alpha subunit utilizes each "second" binding site on CLEC-2. The unique and versatile binding modes open a way to understand the functional consequences of CLEC-2-ligand interactions.

(124) The use of RNA-Seq to identify a transcript encoding an N-acetylglucosamine-binding lectin from the edible Kurokawa (Boletopsis) mushroom

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Lectins are a ubiquitous class of proteins that bind to specific sugar moieties in a non-catalytic and reversible manner. They play important roles in both biology and biotechnology. Lectins are involved in a diverse array of important biological processes such as cell adhesion, viral infection, glycoprotein synthesis and trafficking, and immune response to pathogens. Because of their ability to bind specific epitopes within oligosaccharides, lectins have been adopted for use in techniques such as glycoprotein affinity purification, glycan microarrays and imaging. Most lectins used in molecular techniques are derived from plants where they are abundantly produced and readily purified. However, there is a need for identification of novel lectin specificities to improve the sensitivity and selectivity of these methods. In recent years, more attention has been paid to identifying and characterizing lectins from yeasts and fungi. One interesting fungal lectin (BLL) derives from the Japanese edible mushroom "Kurokawa" (*Boletopsis leucomelaena*). BLL is small (~15 kDa), aglycosylated, and selectively binds terminal N-acetylglucosamine (GlcNAc) on partially or fully agalactosylated biantennary complex-type N-glycans [1, 2]. However, despite its useful specificity, the gene encoding BLL has not been previously identified and *B. leucomelaena* is difficult to source for the isolation of native BLL. In the present study, we have utilized RNA-Seq to identify a transcript encoding an ortholog of BLL from *B. grisae* (termed BGL), a close North American relative of *B. leucomelaena*. A strand specific transcript library was constructed, sequenced on an Illumina platform, and raw sequence data was assembled using Trinity software. Over 43,000 transcript sequences were obtained and queried via blastx against signature peptides derived from the purified BLL and BGL proteins. A transcript harboring a putative BGL-encoding sequence was identified and verified through heterologous protein expression and determination of the ability of recombinant BGL to bind GlcNAc using isothermal titration calorimetry.

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(125) Intracellular trafficking of MytiLec, a Gb3-specific R-type mussel lectin with cytotoxic activity

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A 17 kDa MytiLec isolated from the Mediterranean mussel was found to have a novel triple tandem primary structure and affinity to an alpha-galactoside, Gb3 (Gala1-4Galb1-4Glc-Cer). The lectin specifically killed Gb3-expressing Burkitt lymphoma Raji cells dose- and glycan-dependently [1]. A crystallographic study showed that MytiLec had beta-trefoil conformation which is a characteristic of Ricin B-chain type (R-type) family lectins, however, the amino acid sequence was not similar.

Analogous to Ricin, which killed cultured cells expressing Galb1-4GlcNAc as the ligand, we administered MytiLec to four

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cultured cell lines that expressed Gb3 glycans and each cell line showed different degrees of dose-sensitivity against MytiLec. FITC-labeled MytiLec was administrated to these cells and its incorporation was observed by confocal laser scanning microscopy. Despite expression of Gb3 on the cell surface, some cell lines incorporated the lectin into the cytosol whereas others did not.

Our results clarified that MytiLec has an R-type lectin conformation and was incorporated into cancer cells in a similar manner to Ricin, however, unlike the toxin, the lectin killed the cells directly by N-glycosyl hydrolase activity without having any toxic subunit. Our next focus will be to determine the mechanism of activation triggered by MytiLec for inducing cellular death.

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(126) **Influence of SIGLEC9 polymorphisms on the phenotypes of chronic obstructive pulmonary disease (COPD)**

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Phenotype-based patient stratification provides opportunity to optimize therapeutic approaches. Exacerbation-prone phenotype is important in COPD, since exacerbations lead to both temporary decline in QOL and long-term progression of COPD. However, the factors that determine this phenotype remain obscure. We previously found that the COPD patients who lack Siglec-14, a lectin that recognizes some bacteria and triggers myeloid cell activation, are less prone to exacerbation. Assuming other Siglecs may also play some roles in exacerbation, we investigated the possible association of Siglec-9 with exacerbation-prone phenotype of COPD. Siglec-9 is expressed on myeloid cells as Siglec-14 is, while it suppresses inflammatory responses in contrast to Siglec-14.

We genotyped two non-synonymous single nucleotide polymorphisms (nsSNPs) of *SIGLEC9* gene in 361 COPD patients, of which 135 patients were monitored for exacerbation episodes over one-year period. We tested whether these nsSNPs are associated with the frequency of exacerbations (in 135 patients) and the severity of airflow obstruction (defined by FEV1%predicted, in all 361 patients). We found that the two nsSNPs (rs2075803 and rs2258983) were associated with frequency of exacerbations ($P = 0.0097$ and 0.0371, respectively) and also with airway obstruction ($P = 0.0388$ and 0.0427, respectively). Ancestral alleles of both nsSNPs were found protective against exacerbation and airway obstruction.

In quest of mechanistic explanation for these associations, we prepared the four variants of recombinant Siglec-9 protein (corresponding to the combination of the two nsSNPs) and tested their glycan binding. The ancestral form of Siglec-9 protein showed the strongest binding to the sialylated glycans. We also expressed the four variants of Siglec-9 protein on THP-1 human monoblastoid cell line, and tested their effect on Fcγ receptor-mediated cell activation. The cells expressing the ancestral form of Siglec-9 protein showed the weakest IL-8 response, suggesting that this form has the strongest suppressive function. Taken together, we concluded that the ancestral form of Siglec-9 protects COPD patients against exacerbation by suppressing excessive pro-inflammatory reactions of myeloid cells more efficiently, which in turn prevent the progression of the disease. These results imply that over-activation of innate immune system may be a good target to treat COPD exacerbations.

(127) **Synthetic β-1,3-Oligoglucans as Probes to Study for Hydrolysis and Recognition of Endo-β-1,3-Glucanase**

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β-1,3-Glucanases are widely distributed among bacteria, fungi, and higher plants. In plants, β-1,3-glucanases play an important role to protect against fungal invasion by hydrolyzing β-(1,3)-glucans, which are fungal cell wall components [1]. The hydrolyzed β-(1,3)-glucans have phytoalexin elicitor activity in higher plants, thus the hydrolysate is interested in the structure and the activity especially [2]. In fungi, β-1,3-glucanases are related to morphogenetic processes and supply of energy source. The role of β-1,3-glucanases have been elucidated by biological methods. However, the substrate specificity of β-1,3-glucanase for β-glucans still remain unclear, because natural β-1,3-glucans having β-1,6-branched chain irregularly are complex structures.

In this research, β-1,6-branched β-1,3-oligoglucans were prepared as synthetic substrates to elucidate the substrate specificity of endo-β-1,3-glucanase from *Cellulosimicrobium cellulans*. The oligoglucans were synthesized from D-glucose. Firstly the main chain and the branch units were prepared divergent synthetically. The synthesized units were converged to construct the oligoglucans. Their β-1,3-linked main chains were three glucose residues and their β-1,6-linked branch unit were zero, one, or two glucose residues. The synthesized oligoglucans were introduced fluorescent group to aglycone and converted into probes. The probes were applied to evaluation for hydrolysis activity of endo-β-1,3-glucanase. The time course of hydrolysis was analyzed by HPLC and the structures of the hydrolysates were determined using MALDI-TOF-MS. Moreover, the probes gave thermodynamic parameters of interaction with endo-β-1,3-glucanase by ITC instrument. The results suggested the binding site of endo-β-1,3-glucanase and the binding mode for the probes.

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(128) **A new role for *N*-glycans: allosteric regulation of protein activity through an intramolecular interaction between immunoglobulin G1 polypeptide and Asn297-linked glycan residues**

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Immunoglobulin G1(IgG1)-based therapies are widespread and many initiate cell-mediated pro-inflammatory cascades by binding low-affinity Fc gamma receptors(FcgR). N-glycosylation of the IgG1 Fc domain is required for FcgR binding, though it is unclear why. Structures of the FcgR:Fc complex fail to explain this because the FcgR polypeptide does not bind the Fc N-glycan. Here we use NMR spectroscopy to identify a link between motion of the N-glycan and Fc:FcgRIIIa affinity that explains the N-glycan requirement. Fc F241 and F243 mutations decreased the N-glycan/polypeptide interaction and increased N-glycan mobility as measured by NMR spectroscopy. The affinity of these Fc mutants, once remodeled to identical galactose-terminated core fucosylated complex-type biantennary N-glycans, for FcgRIIIa was directly proportional to the degree of glycan restriction (R2 = 0.82). This result indicates that restricting N-glycan motion relative to the wild-type Fc should increase affinity for FcgRIIIa. One Fc mutation, K246F, stabilized the N-glycan and enhanced affinity for FcgRIIIa (Kd = 0.34 ± 0.06 micromolar versus 0.55 ±0.05 for wild type).

It was a surprise when we found Fc, trimmed to display an N-glycan consisting only of a single N-linked N-acetylglucosamine residue, bound FcgRIIIa with an affinity only 5-fold less wt (Kd = 2.4 micromolar compared to 0.55 micromolar for a complex-type biantennary N-glycan). This allowed us to determine that the F241 and F243 mutations only affected FcgRIIIa binding when a complex-type biantennary N-glycan was present on Fc. Furthermore, these data confirmed the presence of an allosteric mechanism regulating Fc affinity which represents a previously undescribed role for an N-glycan in biology. Interactions between Fc N-glycan and polypeptide residues lead to stabilization of the protein loop that contains the glycan linkage residue Asn297 and also forms part of a binding interface with FcgRIIIa. Related immunoglobulins contain N-glycosylation and surface aromatic residues at identical locations and suggest the same allosteric regulatory mechanisms may be utilized in IgD, G, E and M, but not A.

(129) **Selective cross-reactivity and functionality displayed by antibodies against the inner core of Neisseria meningitidis lipooligosaccharide**

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Neisseria meningitidis continues to cause disease in both developed and developing countries and remains especially dangerous to unvaccinated children. Targeting of capsular polysaccharide for vaccines is ineffective for *N. meningitidis* serogroup B as the capsular polysaccharide mimics structures found in the host. The conserved inner core of lipooligosaccharide (LOS)/ lipopolysaccharide (LPS) has been a target of vaccine development for *N. meningitidis*, with a focus on serogroup B strains. A panel of six antibodies that differ in both functionality and recognition of the inner core variants of*N. meningitidis* has provided insight into the immune response to the inner core. The crystal structure of one of these antibodies, the partially protective antibody LPT3-1, has been solved to 2.69 Å resolution in complex with an eight-sugar inner core fragment from *N. meningitidis* corresponding to one of the three inner core variants recognized by LPT3-1. The epitope is centered about a trisaccharide containing an *N*-acetylglucosamine moiety specific to *N. meningitidis*, and with no recognition of the lipid A moeity. Mutations from the antibody germline allow antibody LPT3-1 to cross-react with the inner core variant expressed in 70% of all strains, but the mechanism underlying the ability of LPT3-1 to cross-react with three different variants remains unresolved. Current work to characterize the binding of LPT3-1 to the other two inner core variants is under way and will provide a more complete picture of the immune response to the inner core of *N. meningitidis*.

(130) **NMR interaction analysis of intestinal soluble lectin ZG16p with mycobacterium phosphatidylinositol mannosides**

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Mucosal immunity in human intestine plays a vital role to avoid invasion of infectious microorganisms. Lectins in intestine are among key players on the defense by interacting with the glycans of virulent organisms. ZG16p is a soluble mammalian lectin especially expressing in the pancreas and intestine. Although ZG16p is involved with storage of digestive enzymes in zymogen granules of the pancreas, the function especially in intestinal tract remains unclear. The reported interaction of ZG16p with mannosides and glycosaminoglycan could be a clue to its biological function. To identify the target mannosylated glycans, we investigated its glycan preference using glycan microarray. The profile clearly exhibited that short phosphatidyl inositol mannoses (PIMs) are potent glycan ligands. We will describe NMR analysis of atomic level interactions between ZG16p and the ligands, PIM1 and PIM2 glycans and glycosaminoglycans. NMR spectra of the PIM glycan revealed binding epitopes and bound conformations. The binding epitope of PIM1 and 2 were analyzed by saturation transferred difference NMR, which indicated C4-C6 of the PIM1/2 mannose. Further, protein bound conformations were analyzed using transferred NOE spectra. Structural calculation of PIM1 and 2, constrained with the NOE distances, revealed the protein bound conformations. The protein NMR spectra showed specific binding residues to the PIM glycans. Uniformly ^{15}N-labeled human ZG16p was prepared by *E. coli* expression system, and ^{1}H-^{15}N HSQC spectra of^{15}N-ZG16p were collected under various PIM concentrations. The signal perturbations of the backbone amides suggested interactions at the GG-loop (Gly31 to Lys36) and the binding

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loop (Arg145-Ala152). In contrast, the heparin oligomer (dp4) perturbed widely distributed amino acid residues at one protein face. A model of the atomic interaction between PIM1 and ZG16p has been constructed with a docking simulation with our NMR data. The model indicates that PIMs interact with ZG16p using shallow binding site consisting with the GG- and binding loops.

(131) Integrating glycan array data and computer modeling: extending the Influenza species specificity paradigm

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Recently, a new source for carbohydrate specificity data has emerged through the extensive development of glycan microarrays. Glycan array screening provides rapid insight into binding specificities, limited only by the number of elements in the array, the largest of which currently contain on the order of 600 members. Although rich in information, array data do not provide direct insight into the 3D structural properties of the glycan-protein complexes responsible for the observed specificities.

Here we employ a new technology [1,2], Computational Carbohydrate Grafting (CCG), to generate 3D models of glycan-hemagglutinin complexes that provide a structural rationalization for the sometimes perplexing data from glycan array screening. Given a structure for the protein in complex with a minimal binding determinant, CCG can be employed to generate a 3D model of any related glycan bound to the complex, by splicing the additional branches of the glycan into the bound fragment. This virtual approach overcomes many of the experimental challenges associated with generating structures for these complexes. CCG is a high-throughput screening method that can be readily validated by comparison with specificity data from glycan array screening.

We show that CCG predictions explain differences in specificity for a range of sialylated N-linked glycans interacting with human and avian influenza hemagglutinins. The analysis suggests that glycans with poly-LacNAc extensions can bind in a bidentate fashion to the trimeric HA, consistent with affinity enhancements seen for some hemagglutinins in glycan array data. CGG analysis may prove valuable in elucidating the preferred receptors for influenza adhesion. (Supported by the National Institutes for Health (GM094919 (EUREKA), and P41 GM103390), as well as the Science Foundation of Ireland (08/IN.1/B2070) (RJW), the Netherlands Organization for Scientific Research (NWO) with Rubican and VENI grants to RPdV, and AI050143 & AI099141 (JCP).

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(132) Structural basis for antibody recognition of lipid A carbohydrate backbone

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Enterobacterial Gram-negative infections are responsible for an estimated 150,000 annual cases of sepsis in the United States (Martin GS *et al*, 2000). Considerable research effort has been devoted to antibodies specific to lipid A, the endotoxic principle of lipopolysaccharide (LPS), aimed towards treatment of sepsis. However, none led to successful clinical implementation. Findings by Brade *et al.* (1993, 1997) suggested that such antibodies bind exclusively to the bisphosphorylated glucosamine disaccharide backbone due to their inability to recognize lipid A lacking a free primary hydroxyl at C6 on the β-glucosamine. At the time there were no reports of crystal structures of any such antibody unliganded or in complex with lipid A. To investigate antibody recognition of lipid A we determined high resolution crystal structures of antigen binding fragments for 4 monoclonal antibodies: S1-15, A6, S55-5, and S55-3, both unliganded and in complex with the lipid A carbohydrate backbone. The lipid A epitopes recognized by these antibodies include the disaccharide backbone phosphate groups, but are independent of the acyl chains. Comparison between the unliganded and liganded structures show a range of recognition mechanisms, where 'lock and key' type binding is used by S1-15, modest induced-fit of the variable domain regions for S55-5 and S55-3, and significant induced-fit for monoclonal antibody A6. Interestingly, while A6, S55-5, and S55-3 bind with both variable light and heavy chain residues, S1-15 forms a pocket exclusively on the variable heavy chain. This is the only structural example to date of an antibody forming a specific pocket outside of the variable heavy and light chain interface to a carbohydrate moiety larger than a monosaccharide. Despite different germ-line origins, each antibody forms a pocket highly complementary to lipid A. These structures demonstrate how these antibodies cannot accommodate lipid A while it is attached to the inner core residues of the LPS, and provide a structural explanation why antibodies against lipid A generally have failed in clinical trials.

(133) Recombinant Human Intelectin-1 expressed in a heart capillary endothelial cell line displays specific pathogen binding

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Xenopus laevis oocyte cortical granule lectin (XL35), members of this family have been reported from many animals down to the

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ascidians; in higher animals the term, intelectin, is in common use. In addition to its function in formation of the fertilization membrane, which serves as a block to sperm and pathogens, the expression of intelectins has been studied in the innate immune response with possible function in pathogen surveillance. The infection by several types of pathogens induces a dramatic increase in the expression of Intelectin transcripts in intestinal and bronchial epithelial cells, mediated by IL-13. Identifying the functions of these lectins has been hampered by the inability to express sufficient quantities of recombinant intelectins in mammalian cells. Since we showed that Int-1 is constitutively expressed in human heart endothelial cells (and not those found in most tissues), we stably expressed the cDNA of human Int-1 in H5V cells, a mouse heart capillary endothelial cultured line, and were able to produce mg levels of secreted int-1. MS analysis determined that Int-1 was not a GPI-anchored protein in H5V cells. There have been reports that Int-1 binds specifically to galactofuranose coupled to agarose; however, H5V-secreted, recombinant Int-1 bound to native agarose resin (as does XL35) and was eluted by EDTA, but also by 100 mM ribose. The eluted protein fraction showed a single band in silver staining after SDS-PAGE resulting in a yield of 20 µg of purified Int-1/ml of starting culture media. Although Int-1 binds tightly to agarose in a calcium-dependent manner, a high affinity glycan ligand has not yet been identified. Int-1 does not bind glycans on the CFG Glycan Microarray; by contrast, XL35 binds to a single glycan with the structure Gal alpha 1,3 GalNAc. Recently, however, using the new Microbial Glycan Array, Int-1 bound to several pathogen outer polysaccharides with high affinity, confirming its innate immune function in pathogen surveillance.

(134) Crystal structure of Surface Layer Homology domains from Paenibacillus alvei S-layer protein SpaA provides insight to secondary cell wall polymer recognition

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Two-dimensional crystalline surface layers (S-layers) composed of self-assembling (glyco-)protein subunits are common structures of prokaryotic cell envelopes. Their functions can include maintenance of structural integrity, environmental protection, cell-cell or exo-enzyme adhesion, and selective ion/molecular filtering. S-layers have gained attention in nanobiotechnology as well-defined scaffolds for high-density display of biofunctional epitopes (Zarschler *et al.*, 2010, *Carbohydr. Res.*).

S-layer proteins of Gram-positive bacteria non-covalently attach to peptidoglycan (PG) via a species- and strain-specific non-classical secondary cell wall polymer (SCWP) that is linked to muramic acid residues of PG. The most common mode of anchorage involves the recognition of SCWP ketal-pyruvate modifications by S-layer homology (SLH) domains (Schäffer and Messner, 2005. *Microbiology*). These domains often are present in triplicate repeats within S-layer proteins, and each possess a conserved motif TRAE (or variant) that has been implicated in SCWP binding.

Paenibacillus alvei CCM 2051^T is a Gram-positive bacterium with an S-layer composed of the protein SpaA in oblique two-fold symmetry. Interestingly, SpaA possesses three SLH domains with variants of the conserved motif: TRAE, TVEE and TRAQ. These variants have displayed unequal contributions to SCWP binding, and any two of the three motifs are sufficient to maintain anchorage (Janesch *et al.*, 2013. *J. bacteriology*).

The crystal structure of *P. alvei* CCM 2051^T $SpaA_{SLH}$ reveals a largely α-helical threefold pseudo-symmetric structure with the TRAE, TVEE, and TRAQ motifs presented in three putative binding grooves. The synthetic SCWP fragment 4,6-Pyr-β-D-ManNAc was co-crystallized with $SpaA_{SLH}$ and surprisingly was only observed in one of three grooves. The lack of binding at the other two grooves indicates either their requirement for larger SCWP fragments, or a loss or change of specificity. The latter is an intriguing possibility as SpaA has also displayed direct binding to PG. These studies will benefit the fundamental understanding of SLH domain and S-layer structure and function, with potential applications in nanobiotechnology surface display.

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(135) Developing unique glycan binding reagents using an ancient immune system

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The jawless vertebrates (lamprey and hagfish) have evolved a novel adaptive immune system (AIS), with many similarities to the jawed vertebrate AIS, including the production of antigen-specific circulating antibodies in response to immunization. However, instead of immunoglobulin (Ig)-based receptors, the jawless vertebrates use leucine-rich repeat (LRR)-based antigen receptors termed variable lymphocyte receptors (VLRs) for immune recognition. VLRs are generated in developing lymphocytes through a gene conversion process, in which random assembly of hundreds of LRR gene segments results in a potential repertoire of >1014 distinct antigen receptors. Due to the divergence of lampreys from our jawed vertebrate ancestors >500 million years ago, we hypothesize that lampreys will respond to conserved glycans that are "invisible" to the mammalian AIS due to self-tolerance. To test this hypothesis, we have immunized lampreys with human erythrocytes and a B-cell line (Tn4) and tested the immune stimulated plasma on the Consortium for Functional Glycomics microarray (CFG version 5.1). The results of these experiments suggested that lampreys generate a wide variety of glycan-specific VLRs in response to antigenic stimulation. To isolate these VLRs, the Cooper laboratory has developed a high-throughput VLR antibody display platform in which VLR antibodies from immunized lampreys are expressed on

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the surface of yeast cells. VLR expressing yeast are then bound to the glycan microarrays directly, thus enabling us to test the specificity of individual yeast clones against hundreds of glycans simultaneously, and develop an enrichment strategy in which a broad array of glycan-specific VLRs can be identified from the yeast surface display library itself. Subsequently, the bound yeast cells are directly removed from the microarray, the VLR antibody clone is sequenced, and this molecule is expressed as a VLR-IgG-Fc fusion protein that can be used for ELISA, western blotting, flow cytometry, and immunomicroscopy. Thus, by combining yeast surface display with glycan microarray technology, we have developed a rapid, efficient and novel method for generating chimeric VLR-IgG-Fc proteins that recognize a broad array of unique glycan structures with exquisite specificity.

(136) Connecting the microbiota to peripheral immune quiescence by T cell cooperativity

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The ability to mount a rapid and effective response upon re-exposure to an antigen is mediated through Memory T cells (Tmem). Conversely, regulatory T cells (Treg) play key roles in suppressing immune responses, preventing autoimmunity, and maintaining immune homeostasis, generally through the secretion of immunosuppressive cytokines like IL-10. Moreover, commensal microbiota may have forged an elaborate evolutionary relationship with the gut mucosal immune system, where certain bacterial products elicit potent inflammatory responses, and others educate the immune system resulting in immune suppression. Our preliminary data show that gastrointestinal exposure to the *Bacteroides fragilis* glycoantigen (GlyAg) polysaccharide A (PSA) increases a population of antigen experienced $CD4^{+}CD45Rb^{lo}T_{EM}$ cells which are capable of amplifying IL-10 production in peripheral tissues upon inflammatory stimulus. Additional data demonstrates that these $CD45Rb^{lo}T_{EM}$ cells synergistically induce IL-10 production with Tregs via a soluble mediator when co-cultured in vitro. We hypothesize that commensal microflora are linked to peripheral immunomodulation through T-cell cooperation between commensal-specific $CD45Rb^{lo}T_{EM}$ cells and tissue-resident Tregs. Further investigation on dissecting the mechanism of this synergy and defining the key secreted factor(s) could provide not only a novel regulatory pathway by which the $CD45Rb^{lo}$ population of T cells is able to dampen potentially harmful immune reactivation, but also provide new therapeutic targets for the treatment of inflammatory disease and autoimmunity.

(137) Galectin-7 regulates keratinocyte proliferation and differentiation through JNK-miR-203-p63 signaling

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Galectin-7 is a β-galactoside-binding protein primarily expressed by stratified epithelial cells, including keratinocytes. This protein has been shown to promote apoptosis in keratinocytes, as well as a number of other cell types, but the pattern of its expression in the epidermis suggests it might also be involved in the regulation of keratinocyte differentiation and proliferation. In this study, we investigated the function of galectin-7 in keratinocytes by examining its regulation of microRNA expression. By microarray and deep-sequencing analyses, we found that galectin-7 positively and negatively regulates miR-203 and miR-146a expression, respectively. We demonstrate that galectin-7 regulates keratinocyte proliferation and differentiation through miR-203, but not miR-146a. Both galectin-7 and miR-203 knockdown in keratinocytes cause increased expression of p63, an essential transcription factor involved in skin development through the regulation of epidermal stem cell proliferation. Rescue of miR-203 levels in galectin-7 knockdown cells reduces the expression of p63 to that seen at baseline. Increased galectin-7 expression leads to increased levels of JNK, which is required for miR-203 expression. Galectin-7 is associated with JNK and protects it from ubiquitination and degradation. This study identifies a novel intracellular function of galectin-7 in the regulation of keratinocyte proliferation and differentiation through JNK1-miR-203-p63 signaling.

(138) Hyaluronan as a regulator of macrophage function: implications for inflammation and angiogenesis

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Growing evidence has suggested that as a dynamic entity, cleaved and native hyaluronan (HA) may provide intrinsic signals that coordinate and modify interdependent inflammation and angiogenesis in homeostasis and disease. Macrophages are highly receptive to these endogenous signals and are exceptionally plastic in their activation, yielding a unique role in regulating both processes. We tested the hypothesis that the dynamic nature of HA regulates macrophage inflammatory and angiogenic function. Specifically, we assessed the role of low (LMWHA) and high molecular weight (HMWHA) HAs in modulating inflammation and angiogenesis. We first examined the effect of HMWHA or LMWHA treatment on macrophage function in three different macrophage populations: resting, classically activated, and alternatively activated. By measuring transcription of genes and quantifying production of cytokines and chemical mediators involved in both pro-resolving and pro-inflammatory roles of macrophages, we were able to conclude that regardless of initial macrophage state, LMWHA induced a classically activated state by promoting pro-inflammatory function. Conversely, HMWHA promoted the transcription and production of pro-resolving and anti-inflammatory mediators associated with alternative activation. Next, we analyzed how the macrophage polarization states induced by hyaluronan contributed to or inhibited angiogenesis. Angiogenesis was quantified by transcription of pro- and anti-angiogenic mediators in macrophages, proliferation and migration of endothelial cells in response to conditioned media

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from macrophage populations treated with HAs, and vessel branching, density, and diameter in response to conditioned media using the chick chorioallantoic membrane. Interestingly, we observed that unlike phenotype, angiogenesis was highly dependent on both hyaluronan treatment as well as initial polarization state of the macrophage. LMWHA was found to induce angiogenesis in resting macrophages, but inhibit angiogenesis in polarized macrophages. The level of inhibition in the activated states was greatest in classically activated macrophages, where visible vessel regression was observed. Conversely, conditioned media from macrophage populations treated with HMWHA promoted sustained or increased angiogenic responses. We conclude that HMWHA and LMWHA promote functional differences in resting and polarized macrophages.

(139) **Investigation of organic framework based on carbohydrate-protein interaction**

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Investigation of new functional materials has been focused recently. Especially, metal organic framework is paid much attention because of its potentials as catalyst, gas-storage material, conductive- and semiconductive materials, etc. We are interested in the formation of ordered structures composed of certain combination of a couple of elemental entities. We set our program to create "organic framework" based on carbohydrate-protein interactions. The type of interaction is of prime importance in biological systems, which can be found in intercellular interactions through glycan-lectin bindings. Furthermore, such interactions often rely on multivalent bindings. In the first step towards creating ordered organic framework, we prepared a series of valence-controlled lectin molecules. Such molecules consist of HaloTag fused Galectin 3 (Gal-3) as an example and a series of valence-controlled core molecules carrying 1-4 chloro-functions that is used to bind HaloTag-Gal-3 covalently. Another important molecule in this investigation is divalent carbohydrate molecule, which interconnects valence controlled lectin molecules together through specific non-covalent binding. For this, we synthesized "di-LacNAc" molecule linked together by hexaethyleneglycol linker. Having these elements in hands, we incubated di-LacNAc and divalent Gal-3. The mixture was directly sprayed on a mica surface, and used for the atomic force microscope (AFM) observations. In this experiment, formation of linear-open and closed geo-structures is expected. We were able to observe double-helix-linear-open structures. This first result showed successful formation of a highly ordered structure based on carbohydrate-protein interactions. We are going to examine other combinations as well as studies of influence of concentrations. We expect that the new organic framework finds utility in viral and bacterial sensors.

(140) **Sulfoglycomics of human and murine T lymphocytes reveals additional sulfation common to both despite differences in terminal glycosylation**

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Glycan remodeling between naïve T lymphocytes and activated effector T lymphocytes remains an unsolved issue. Previous work by others have found that the expression of α1-3-galactosyltransferase increases in activated murine effector T lymphocytes, leading to a switch of the terminal glycotope from predominantly NeuGc-sialylation to α1-3-galatosylation. It has also been shown that the level of NeuGc-sialylation on the surface of activated effector T lymphocytes is significantly reduced in favor of NeuAc-sialylation. However, neither the observed changes associated with α1-3-galactosylation nor NeuAc-hydroxylation is applicable to human, for which any glycan remodeling upon naïve T lymphocyte activation is unclear. The same phenomenon has also been noted for murine B lymphocyte activation, in which NeuGc to NeuAc-sialylation leads to weakened binding to mCD22, which prefers NeuGcα2-6LacNAc, and is paralleled in human by a decrease in GlcNAc-6-O-sulfation, thereby attenuating binding to human CD22, which prefers the sulfated ligand. To determine if human T lymphocytes carry any sulfated N- and O-glycan and whether the degree or the exact sulfation pattern may be altered upon activation, we have initiated a concerted sulfoglycomic analysis of both murine and human T lymphocytes. By using our established mass spectrometry (MS)-based analytical pipeline, we successfully demonstrated that sulfated N-glycans are constitutively expressed in both human CD4+ and CD8+ T lymphocytes, carrying either 6-sulfo-sialyl LacNAc or 6-sulfo-sialyl Lewis X glycotopes while further developing highly sensitive LC-MS/MS-based methods to quantify the relative amount of sulfated versus non-sulfated glycotopes. Interestingly, we not only confirmed previous findings with respect to changes in the terminal glycotopes of mouse CD4+ T lymphocytes from NeuGc- to NeuAc- and α-Gal after treatment with activating cytokines, but also identified the occurrence of sulfated N-linked glycans in both treated and non-treated cells. This is in contrast to murine B lymphocytes in which very little sulfated glycans could be detected.

(141) **Endogenous airway mucins carry glycans that bind Siglec-F and induce eosinophil apoptosis**

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Background: Siglec-F is a glycan binding protein selectively expressed on mouse eosinophils. Its engagement induces apoptosis,

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suggesting a pathway for ameliorating eosinophilia in asthma and other eosinophil-associated diseases. Siglec-F recognizes sialylated, sulfated glycans in glycan binding assays, but the identities of endogenous sialoside ligands and their glycoprotein carriers *in vivo* are unknown. Methods: Lungs from normal and mucin-deficient mice, as well as mouse tracheal epithelial cells (mTEC) from mice, were interrogated *in vitro* and *in vivo* for the expression of Siglec-F ligands. Western blotting and immunocytochemistry using Siglec-F-Fc as a probe for directed purification, and liquid chromatography-tandem mass spectrometric analysis of recognized glycoproteins. Purified components were tested in mouse eosinophil binding assays and flow cytometry-based cell death assays. Results: We detected mouse lung glycoproteins that bound to Siglec-F; binding was sialic-acid dependent. Binding of Siglec-F-Fc to mTEC was sialidase-sensitive and was increased after treatment with IL-4 or IL-13 *in vitro*. Sialidase-sensitive, PNGaseF-resistant binding of Siglec-F-Fc to glycoproteins of apparent MW ≈500 kDa and 200 kDa was detected by western blotting of mTEC lysates and culture supernatants, indicating the importance of sialylated O-linked glycoprotein glycans for Siglec-F binding. The expression of these glycoprotein ligands was increased during mouse allergic airways inflammation. Liquid chromatography-tandem mass spectrometry-based proteomic analysis of Siglec-F binding material identified Muc5b and Muc4. Cross-immunoprecipitation and histochemical analysis of lungs from mucin-deficient mice assigned and validated the identity of Muc5b as one glycoprotein ligand for Siglec-F. Purified Muc5b/Muc4 preparations carried sialylated and sulfated glycans, bound to eosinophils and induced their death *in vitro*. Mice conditionally deficient in Muc5b displayed exaggerated eosinophilic inflammation in response to intratracheal installation of IL-13. Conclusion: These data identify a previously unrecognized endogenous anti-inflammatory property of mucins by which their sialylated glycans can control lung eosinophilia through Siglec-F engagement. Funded in part by NIH grant P01 HL107151.

(142) Induction of proinflammatory responses by galectin-3, a soluble lectin that could link the pathogen pattern-associated and damage-associated innate immune responses

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Galectin-3 belongs to a family of soluble lectins that are defined by an evolutionarily conserved carbohydrate binding domain, which has preferential affinity for β-galactoside. Galectins lack signal peptide sequence to be escorted to the secretory pathway, and thereby are synthesized and stored in the cytosol. In other words, galectins can exert their functions only when released from the cytosol, such as an insult-introduced cell damages, suggesting the hypothesis that galectins could act as damage-associated molecule patterns (DAMPs) although the hypothesis waits for further investigations. Our previous studies suggest that galectin-3 is critically involved in the recruitment of neutrophils to the affected areas when invading pathogens trigger galectin-3 release in the extracellular space, suggesting that galectin-3 plays a role in the initial neutrophil migration in a pathogen-specific manner. Since galectin-3 itself is not a chemoattractant for neutrophils, it remains elusive the molecular mechanism by which galectin-3 facilitates neutrophil recruitment. Our previous findings suggest that galectin-3 mediates initial adhesion of neutrophils to the endothelium. In this study, we further investigate whether galectin-3 could directly induce proinflammatory responses. Injection of galectin-3 to air pouches induced neutrophil recruitment, which peaked at 6 h after the injection. Accumulation of IL-1β, TNF-α, IL-6, KC, MIP-2, MIP-1α and MCP-1 in the air pouches was also observed. This lectin induced the secretion of those chemokines and cytokines except IL-1β by air pouch lining fibroblasts in vitro. Interestingly, galectin-3 also induced the recruitment of small number of monocytes ($Ly6G^{-}CX3CR1^{+}F4/80^{+}$), which appears to precede neutrophil recruitment, implying the role of patrolling monocytes for this galectin-3-induced neutrophil migration. The present study suggests that galectin-3 is a DAMP that could induce the initial neutrophil migration by modulating several processes involved in the recruitment. Galectin-3 is shown to bind to self-glycans as well as glycans presented by various pathogens. Thus, ongoing studies are aimed at providing a better understanding on the role of galectin-3 as a molecule linking the microbial pattern-associated and damage-associated innate immune response to parasites and fungi.

(143) Purification and characterization of the novel lectin from Ceroplastes ceriferusi

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To isolate novel lectin, we have investigated various organisms. As a result, it was found that high hemagglutination activity from the extract of Wax scale insect, *Ceroplastes ceriferus*. Wax scale insects have been distributed more than 7,800 species in the world. Most of the Wax scale insects are parasitic onJapanese and Chinese hollies, euonymus, and boxwood. Wax scale insects are induced reduce growth of host plants. Therefore, it becomes a big problem, especially citrus tree and fruits cultivation.

In this study, we predicted that the hemagglutination activity substance was the cause component of the decline of host plants, we tried to purification of the hemagglutination activity substance. The hemagglutination activity substance was purified by anion exchange chromatography and gel filtration chromatography. It was confirmed that the hemagglutination activity substance were at least 2 types of proteins, “CC-1” and “CC-2”. The molecular weight of CC-1 and CC-2 were estimated to be 50 kDa and 10 kDa, respectively. Interestingly, CC-1’ showed both of the hemaggultination activity and hemolysis activity whereas CC-2 showed only hemaggultination activity. The hemaggultination activity of CC-1 was strongly inhibited by asialofetuin and mucin, however, it was not inhibited by monosaccharides. On the other hand, CC-2 was not inhibited all

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monosaccharides and glycoproteins. These results suggest that CC-1 recognize higher-order complex structure of glycan, and it is also suggested that CC-2 may have the recognition system at completely different ways.

CC-2 was analyzed cytotoxicity for human myeloid leukemia (U937) cell line andgreen monkey continuous (Vero) cell line. CC-2 inhibited the growth of U937 cells and Vero cells. The cytotoxic effect of CC-2 for both cell lines was concentration-dependent, and the viable cell count of U-937 cells and Vero cells decreased to less than 10% of the control even at 4 μg/ml CC-2 and 40 μg/ml CC-2, respectively. These results suggest that the difference of the cytotoxic effect of two cell lines was due to the difference of cell surface, and we also suggested that the cytotoxic phenomenon might be caused apoptotic cell death because of morphological change of cells.

(144) **Trypanosoma cruzi trans-sialidase, a virulence factor that modifies immune cells sialylation pattern**

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Trypanosoma cruzi, the protozoan agent of Chagas disease, is unable to synthesize sialic acids *de novo* but requires the sugar to interact with the host cell and to avoid destruction by serologic factors. The parasite acquires the sialyl residue from the mammalian glycoconjugates through a modified sialidase known as *trans*-sialidase (TS). TS readily sialylates acceptor mucins on the parasite surface, allowing the evasion of lysis by seric components and the interaction with the host cell during the invasion process. The use of azido-modified sialyl donors, allowed us to analyze the sialylation kinetics, turnover, shedding and membrane distribution of these mucins. However TS is also shed, being systemically distributed by the bloodstream inducing strong alterations on the immune system and hematological abnormalities, mainly during the acute phase previously to the elicitation of TS-neutralizing antibodies. TS modify the sialylation pattern of several lymphocyte molecules such as CD45, as determined with the unnatural sugars approach. TS affects the entire T cell lineage. It induces apoptosis of $CD4^+CD8^+$ thymocytes, delineates CD4 T lymphocytes response by leading them to acquire the unprotective Th2 phenotype, and other authors found it also inhibits the effector CD8 T cells. On B lymphocytes, sialyl residue mobilization induces a CD45-mediated pathway that leads to IL17 production. By learning how the parasite manipulates the immune system in its benefits by altering the glycosylation pattern, a therapy for inflammatory or autoimmune diseases might be eventually explored.

(145) **Construction and use of a mammalian lectin microarray for deciphering microbial-host and disease processes**

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Glycosylation has important roles in the immune system and lectin-carbohydrate interactions are critical in immune regulation and response. Lectin and glycan microarrays are increasingly utilised to study important biological interactions involving carbohydrates. Low sample and probe consumption combined with potential for high data yield makes these platforms highly desirable. Although mammalian lectins are of great importance in developmental, immune, inflammation and disease processes, their use as probes on a microarray platform has been slow due to their natural instability, scarcity and narrow functional parameters.

To address this gap and to facilitate profiling innate immune interactions, we immobilised a panel of selectins, siglecs and other C-type lectin receptors on a microarray platform. A number of technical considerations were tested and optimised to maintain the function of the mammalian lectins, including print buffers, incubation buffers and storage conditions. Specific carbohydrate binding was assessed using relevant glycoproteins and neoglycoconjugates and binding constants for the lectins in this platform were determined through inhibition with mono- and oligosaccharides.

The interactions of clinical and laboratory strains of pathogenic and commensal bacteria were profiled to examine potential pathogen-associated molecular patterns (PAMPs) and associate these with particular types of pathogenic infections and host infection responses. In addition, the interactions of the innate immune system in cancer remain poorly understood. Patient serum from breast cancer and multiple myeloma was also profiled to provide insights in to potential avenues for immunotherapeutic targeting.

This platform is a valuable development towards investigations of relevant host-specific interactions in disease and microbial pathogenesis and commensalism.

(146) **Galectin-8 induces dendritic cells activation**

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Galectins (Gals), a family of mammalian lectins, have emerged as key regulators of the immune response. We found that Gal-1 and Gal-8 costimulated antigen (Ag)-specific T cell responses, as evaluated in the DO11.10 TCR_{OVA}-transgenic mouse model, by acting simultaneously on antigen presenting cells (APCs) and target CD4 T cells (Tribulatti et al. 2012). Remarkably, a single dose of recombinant Gal-1 or Gal-8 administered together with a suboptimal Ag dose to DO11.10 mice strengthened weak responses *in vivo*. These results raised the hypothesis that APCs could be activated after galectin binding. In fact, other groups have previously shown that Gal-1-treatment promoted dendritic cell (DC) maturation. Whether Gal-8 exerts a similar effect on these cells is still undetermined. After Gal-8 treatment, an increment of coestimulatory molecules expression (MHCII, CD80 and CD86) was observed by flow cytometry analysis, in primary bone marrow derived-DCs. Gal-8 also induced a mature/activated phenotype in these cells, as showed by F-actin labeling with conjugated phalloidin and fluorescence microscopy analysis. Moreover, the analysis of supernatans from Gal-8-treated DCs showed a marked increase of the pro-inflammatory cytokine IL-6. Expression of endogenous Gal-8 in DCs was confirmed by western blot assays, and interestingly, an increased expression level of Gal-8 protein was found after

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activation with LPS. Participation of endogenous Gal-8 was also evidenced using bone marrow-derived DCs from Gal-8 KO mice. DCs from KO mice were less responsive to LPS stimulation than those derived from control mice. Taken together, our results suggest that Gal-8 could be involved in DC maturation/activation process, which argues in favor of the participation of Gals in the initiation of adaptive immune response.

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(147) **Comprehensive syntheses of sialyl galactoside regioisomers**

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Sialic acid-containing glycans have attracted attention in the fields of immunology and medicinal chemistry. In naturally occurring sialosides, sialic acids link to galactosides through the α(2-3) or a (2-6) bonds. The difference in the linkage position modulates its interaction with receptor proteins. However, syntheses of sialyl galactose derivatives with (2-2) and (2-4) linkages have not been investigated to date. The artificial gangliosides with non-natural linkages would lead to the discovery of novel biological functions and new aspects of the enzymatic mechanism related to gangliosides.

In this study, sialylation with each of the hydroxyl groups on galactose were investigated for comprehensive syntheses of sialyl galactoside isomers. Generally, the outcomes of glycosylation are known to depend on electronic and steric properties of the protecting groups introduced on the glycosyl donor and acceptor. Thus, in regioselective sialylations, the protective groups of the galactosyl acceptor were selected the benzyl group having an electron donating property. On the sialyl donor, a 5-*N*-protecting group have an effect on yield and stereoselectivity of the sialylation. Therefore *N*-acetyl-5-*N*,4-*O*-oxazolidinone-protected donor was employed here and all the natural and non-natural sialyl galactoside isomers were synthesized using these acceptors and the donor. In addition, the aglycone of the galactosyl acceptors was chosen an allyl group that can be removed selectively and converted easily into a wide variety of functionalized groups after sialylation.

The sialylations proceeded by treating the sialyl donor and the glycosyl accepters with the presence of Lewis acid in a mixture solvent of CH_3CN and CH_2Cl_2 to give corresponding disaccharides in moderate to high yield. The desired sialosides were afforded in 99% yield (2-2); (α:β = 2.4:1), 77% yield (2-3); (α:β = 1:1), 57% yield (2-4); (α:β= 0:1), and 98% yield (2-6); (α:β = 10:1), respectively. The structures of all sialylation products were identified by NMR and high-resolutional MS analyses. Moreover, the terminal olefin moiety of sialyl galactosides was converted easily into various functional groups for applications.

(148) **Functional and biochemical characterization of a modified Galectin-8 protein**

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Galectins (Gals) constitute a family of mammalian lectins with affinity for ß-galactosides, characterized by the presence of conserved carbohydrate-recognition domains (CRDs). Gal-8, from tandem-repeat group, contains two CRDs joined by a linker peptide. We found that Gal-8 exerts two different actions on CD4+ T cells: antigen-independent proliferation and, at lower concentration, antigen-specific T cell costimulation. To study the relevance of glycan-lectin interaction on these activities, we generated a double-mutated protein (Gal-8mut) by replacing canonical arginine residues on each CRD, as to abolish sugar-binding capacity. Mutant protein was properly folded as determined by comparative circular dicroism assays. As expected, the absence of lactose competition observed in pull down and immunoprecipitation tests confirmed that recognition of this sugar was precluded. However, preservation of lectin activity was suspected since Gal-8mut still displayed binding capacity to T cell surface and hemoagglutination effect. To test this hypothesis, glycan affinity analysis using glycochips from the Consortium For Functional Glycomics was conducted. Interestingly, the screening revealed that Gal-8mut lost low and intermediate, but maintained higher affinity interactions. Regarding biological activity, Gal-8mut was unable to induce T cell proliferation, but it retained costimulation of antigen-specific response efficiently. Therefore, Gal-8mut dissects the Gal-8 activities on T cells and represents a useful tool to identify the specificity of lectin-glycan interactions underlying the lectin activities on T cell biology.

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(149) **Alteration of the carbohydrate-binding specificity of the C-type lectin CEL-I by site-directed mutagenesis**

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CEL-I is a Gal/GalNAc-specific C-type lectin isolated from the marine invertebrate*Cucumaria echinata* (sea cucumber). This lectin has the QPD (Gln-Pro-Asp) motif in its carbohydrate-binding site, and shows Gal/GalNAc specificity. We have previously reported that the mutation of this QPD motif to EPN (Glu-Pro-Asn) sequence lead to an increase in Man-specificity of CEL-I. However, affinity for mannose of this mutant (EPN-CEL-I) was relatively low, suggesting that the specificities of C-type lectins for galactose and mannose are not exclusively determined by the QPD and EPN

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motifs. In this study, we have prepared another CEL-I mutant EPNH-CEL-I, in which an additional mutation in the binding site (Trp105 to His105) had been introduced, and examined its carbohydrate-binding specificity.

The gene for EPNH-CEL-I was prepared by PCR, and the recombinant protein was expressed in *Escherichia coli* BL21(DE3)pLysS cells using the pET-3a vector. Since induced protein was obtained in inclusion bodies, they were solubilized with guanidine hydrochloride, and refolded in the presence of arginine. The solubilized EPNH-CEL-I showed binding affinity for mannose-immobilized column. Association constant for the binding of EPNH-CEL-I and mannose was determined to be 3.17 x 10^3 M^{-1} by isothermal titration calorimetry. Binding assay using polyamidoamine dendrimer (PD) conjugated with sugars (sugar-PD) also indicated that EPNH-CEL-I preferably binds mannose rather than galactose, although *N*-acetylgalactosamine still showed relatively high affinity, probably because of the interaction of its acetamide group with the binding site of the protein. To elucidate mannose-recognition mechanism of EPNH-CEL-I, EPNH-CEL-I/mannose complex was crystallized and three-dimensional structure was determined by X-ray structural analysis at a resolution of 1.7 Å. In the crystal structure, mannose molecule is recognized through coodination bonds with Ca^{2+} ion as well as hydrogen bond network with nearby residues in the binding site. The binding of mannose was also stabilized by stacking with His105, which had been introduced in place of Trp105, suggesting that difference in the nature of the stacking residues greatly influences Gal- or Man-specificity in addition to the QPD and EPN motifs.

(150) **Characterization of sparsely labeled glycosylated proteins by NMR**

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Many mammalian proteins require glycosylation for proper function. Therefore, biophysical characterization, including structure, is best done on properly glycosylated forms produced by expression in mammalian cell culture. This presents a challenge for NMR characterization by typical methodology, because uniform isotopic labeling with ^{13}C and ^{15}N becomes extraordinarily expensive and perdeuteration needed for larger proteins usually leads to cell death. We demonstrate an alternative methodology based on sparse labeling with single (or small numbers of) isotopically enriched amino acids, combined with the high structural content experiments that generate long range paramagnetic and orientational constraints. Characterization of domain orientation and glycosaminoglycan binding of two domain constructs from Robo1 and structurally related proteins will be used to illustrate the methodology. Robo1 is a cell surface signaling molecule important in axon guidance during development. Its interaction with members of the Slit family is believed to be regulated by interaction with segments of heparan sulfate chains. Binding site identification and structural characterization of Robo1 : heparan sulfate complexes are key objectives of the project.

(151) **Expression and characterization of the recombinant lectin SUL-1 derived from the venom of the sea urchin Toxopneustes pileolus**

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The venom derived from the globiferous pedicellarie of the sea urchin *Toxopneustes pileolus* contains several biologically active proteins. Among them, galactose specific lectin SUL-1 (32 kDa) isolated from the venom of the large globiferous pedicellarie shows chemotactic activity on guinea-pig neutrophils and mitogenic activity on murine splenocytes through binding to the carbohydrate chains on target cells. N-terminal sequence analysis of SUL-1 suggested that this lectin shows some similarity with rhamnose-binding lectins, many of which have been isolated from fish eggs. To elucidate the nature of SUL-1 in connection with the role in the venom, we have cloned cDNA of SUL-1 by isolating mRNA from the large globiferous pedicellarie of *T. pleolus*, and expressed the recombinant protein in *Escherichia coli* cells. Carbohydrate-binding specificity of SUL-1 was examined by sugar-linked polyamidoamine dendrimer (sugar-PD).

The cDNA coding for SUL-1 was cloned by reverse transcription PCR using the degenerate primers designed based on the N-terminal sequence, which had been determined from the purified protein, and the recombinant SUL-1 was expressed in *E. coli* cells. SUL-1 gene contained an open reading frame of 927 nucleotides corresponding to 308 amino acid residues, including 24 residues of a putative signal sequence. The mature protein with 284 residues is composed of three homologous regions, each showing similarity with the carbohydrate-recognition domains of the rhamnose-binding lectins, which have been found mostly in fish eggs. Recombinant SUL-1 expressed in *E. coli* cells exhibited binding activity for galactose-related sugars as well as L-rhamnose with the highest affinity among the sugars tested, confirming that SUL-1 belongs to the rhamnose-binding lectin family. Rhamnose-binding activity of SUL-I may play an important role in toxic action of the venom of *T. pileolus*.

(152) **Biophysical characterization of immunoglobulin G1 Fc bearing an N-glycan consisting of a single (13C)-GlcNAc residue**

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The N-glycan attached to the Fragment crystallizable (Fc) region of immungolobulin G1 (IgG1) is essential for interactions between Fc and various “low-affinity” Fc gamma Receptors (FcγRs). Furthermore, the heterogeneous composition of the N-glycan impacts receptor affinity and correlates with immunological disorders such as rheumatoid arthritis and inflammation. It is thought that a complete, complex-type N-glycan is required for high binding affinity. The N-glycans have been shown to make intramolecular

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glycan/polypeptide interactions and intermolecular carbohydrate-carbohydrate interactions. To date, complex-type biantennary N-glycans structures, often terminated with sialic acid or galactose, have been extensively studied to identify a correlation between Fc N-glycan structure and rheumatoid arthritis. We recently discovered that Fc with a truncated N-glycan, trimmed back to a single N-acetylglucosamine residue (GlcNAc), still binds FcγRIIIa. Here, we will present a characterization of this previously unstudied IgG1-Fc N-glycoform containing a single N-linked GlcNAc and also discuss the structure of the polypeptide loop that contains the N-glycan linkage site. We are able to incorporate ^{13}C in the Fc N-glycans by adapting an in-vivo labeling strategy (Yamaguchi, et. al., (2000) J Biomol. NMR. 18(4):357) and enzymatically trim these back to a single ^{13}C-labeled GlcNAc. We will also present a functional characterization of this novel IgG1-Fc glycoform based on amino acid mutations designed to disrupt the N-glycan/polypeptide interface. NMR spectroscopy of selective ^{15}N amino acid labeling of these wild type and mutant glycoforms will also be discussed.

(153) **Maintenance of circulatory ST6Gal-1 levels requires B cells**

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Glycans occupy the interface between a cell and its external environment through which critical signaling cues are communicated. Hematopoiesis, the process through which all blood cells are generated, is regulated in response to such signals relaying the necessity for stimulation or suppression of blood cell production. Hematopoietic progenitor cell proliferation and differentiation are profoundly suppressed by the sialyltransferase ST6Gal-1 present in circulation. This extracellular ST6Gal-1 acts presumably by attaching α2,6-linked sialic acids and thereby extrinsically remodeling progenitor cell surface glycans. Therefore, factors contributing to circulatory ST6Gal-1 are anticipated to be important in maintaining hematopoiesis. It is commonly accepted that much of the circulating ST6Gal-1 originates from the liver. Deletion of the hepatic P1 promoter of the *St6gal1* gene in a mouse model (*St6gal1*-dP1) reduced circulating ST6Gal-1 to 35% at baseline, and furthermore abolished the 3 to 4-fold increase in blood ST6Gal-1 during acute systemic inflammation. The *St6gal1*-dP1 mouse also displays excessively robust acute peritonitis and airway inflammation due to increased production of inflammatory cells.

Here, we report that B-lineage cells participate intimately in maintaining circulatory ST6Gal-1 levels. Mature B cells and plasma cells also express high levels of ST6Gal-1. Circulatory ST6Gal-1 level is reduced to 30% in the B cell deficient μMT mouse. The μMT mouse has also more severe acute airway inflammation consistent with increased inflammatory cell production. In the globally ST6Gal-1 null mouse (*St6gal1*-KO), adoptive bone marrow transfer with wild-type bone marrow cells showed emergence of circulatory ST6Gal-1 to 15% and 30% of normal wild-type level within 4 and 8 weeks, respectively, post transplantation in the otherwise totally ST6Gal-1 deficient background, in a time course consistent with repopulation of wild-type B cells in the *St6gal1*-KO recipients. Furthermore, transplantation of μMT marrow into lethally irradiated *St6gal1*-dP1 recipients resulted in a further 50% decrease in the already suppressed circulatory ST6Gal-1 levels in the *St6gal1*-dP1 mouse. Together, our data implicate B-lineage cells, in addition to the liver, as major suppliers of circulatory ST6Gal-1, through which new blood cell production and severity of inflammatory responses can be modulated.

(154) **Galectin-7 Displays Specific Antimicrobial Activity Toward Microbes Expressing Self-like Antigens**

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Emory University

The adaptive immune response continually protects against an infinite number of potential pathogens that are recognized by their non-self markers. However, since adaptive immunity maintains strict tolerance towards cells that possess "self" structures, pathogens that display self-like antigens on their surface may escape protective measures of adaptive immunity. Recent studies demonstrate that innate immune lectins, galectin-4 (Gal-4) and galectin-8 (Gal-8), are able to provide protective immunity against potential pathogens that display self-like antigens within the gastro-intestinal tract. However, as other locations of host-pathogen interaction exist, it remains unclear whether similar protective mechanisms reside at other key interfaces of host-pathogen interactions. Our studies demonstrate that Gal-7, a prototypical galectin specifically expressed in keratinized epithelia, provides similar antimicrobial activity against pathogens bearing self-like antigens as Gal-4 and Gal-8. Gal-7 binds directly to bacteria bearing self-like carbohydrate antigens and binding results in disruption of membrane integrity and death of these bacteria. These results suggest a mechanism of protection from self-antigen expressing pathogens upon disruption of keratinized epithelia. In addition, these studies provide the first example of direct bacterial killing by a prototypical galectin, indicating that galectin-antimicrobial activity is a common trait among unique members of the galectin family.

(155) **Aging-related effects of galectins on neuronal viability and excitability**

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Galectins are glycan binding proteins expressed in the periphery and in the CNS. Galectins bind polysaccharides whose expression patterns can change during aging or in neurodegenerative conditions, therefore galectin expression or galectin-mediated effects in the CNS also likely change with aging or disease. Moreover, galectin secretion by nearly all types of brain cancer exposes peritumoral neurons to sustained and relatively high levels of galectins. We are examining the effect of prolonged exposure to galectin-1 (gal-1) or gal-8 on neuronal viability and physiology, as well as aging-related changes in their effect on neuronal function. Our preliminary data show that galectins can potentially modulate neuronal function in a

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variety of ways. 24 hour exposure to gal-1 or gal-8 in neuronal cultures leads to cell death and changes in morphology, but exposure of neocortical pyramidal neurons to gal-1 or gal-8 in acute slices for 13 hours doesn't affect morphology. In addition to altering cellular morphology and affecting viability, exposure to gal-8 leads to changes in cellular excitability in neocortical pyramidal neurons. In young animals, exposure to gal-8 causes irregular spiking in neocortical pyramidal neurons, potentially affecting synaptic efficacy at downstream cortical targets. In contrast, gal-8 exposure in neurons from aged mice leads to more regular spiking patterns. Hence prolonged exposure to galectins can alter cellular viability and morphology as well as neuronal excitability, and these galectin-mediated effects can change with aging. These experiments will shed light on the role of galectins in the heathy CNS as well as on their potential role in pathological conditions.

(156) **Gal-1 modulation of ROS production in neutrophils**

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Introduction: Galectin-1 (Gal-1) is a mammalian β-galactoside-binding lectin that participates in the pathophysiology of inflammatory responses, infectious diseases, autoimmunity, and cancer. Numerous studies have shown that Gal-1 can modulate the functions of immune cells, in particular neutrophil migration, phagocytic recognition and reactive oxygen species (ROS) production. However, the mechanisms that control ROS production in Gal-1 treated neutrophils are not well defined. Thus, this study evaluated how Gal-1 affects the dynamics of ROS generation in naïve, fMLP (formyl-Met-Leu-Phe) activated human neutrophils and peritoneal neutrophils from Gal-1$^{-/-}$ mice. Methods and Results: Using a chemiluminescence (CL) assay it was possible to analyze the effect of recombinant human Gal-1 to induce or inhibit ROS production in naïve and activated human neutrophils. As previously observed, Gal-1 induced ROS production in a dose-dependent way only in fMLP pre-activated cells (10^{-7}M fMLP + 10 μM Gal-1 CL area: $3x10^7 \pm 1.9x10^7$). Interestingly, the oxidative stress induced by fMLP or PMA on naïve neutrophils was inhibited by pretreatment of the cells with 10 μM Gal-1 (55% and 40%, respectively). In both non-activated and activated cells, these effects were partially dependent on lectin activity. Moreover, peritoneal neutrophils from Gal-1$^{-/-}$ mice (n = 3) released a greater amount of ROS in response to 10^{-7}M fMLP (CL area: $1.9x10^7 \pm 5.4x10^6$) and exogenous 10 μM Gal-1 (CL area: $8.4x10^5 \pm 1.0x10^5$) compared to neutrophils from wild-type animals (10^{-7}M fMLP CL area: $1.3x10^7 \pm 5x10^6$ and 10 μM Gal-1 CL area: $5x10^5 \pm 2.1x10^5$). Conclusion: Combined, these results suggest that Gal-1 can negatively or positively modulate neutrophil ROS production. Moreover, the production of ROS by neutrophils seems to be associated with the level of cell activation and the presence or absence of endogenous Gal-1. Furthermore, these findings help build the foundation to elucidate the participation of Gal-1 in the regulation of inflammatory disorders and infectious diseases. Financial Support: CNPq n° 9320/13-0, Núcleo de Apoio à Pesquisa em Doenças Inflamatórias [NAPDIN] under grant agreement n°11.1.21625.01.0.

(157) **Binding activity and specificity of Trans-sialidase lectin domain from Trypanosoma congolense**

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Trypanosoma congolense is the most prevalent causative agent of Animal African Trypanosomiasis (AAT) also known as Nagana or sleeping sickness in cattle. The parasite expresses trans-sialidases (TS), which transfer sialic acids from host cell glycoconjugates to the parasite's surface molecules. It is known that TS play an important role in anaemia in animals and survival of the parasite [1,2]. The enzyme comprises a N-terminal catalytic domain (TS-CD), which is already well characterised and a C-terminal lectin-like domain (TS-LD), whose binding specificity and function in catalysis is still not known. We applied glycan array, SPR and STD NMR experiments to identify potential binding partners of TS-LD. To further characterise the affinity and selectivity of TS-LD we established microtitre plate based binding and inhibition assays, which can be used to determine kinetic data and IC50 values.

Recombinant glycoproteins expressed in CHO-LecI cells and therefore containing only mannosylated N-glycans, showed different binding activities to TS-LD. On the glycan arrays and in SPR experiments several mannose-containing oligosaccharides, such as mannobiose, mannotriose or higher mannosylated glycans showed relatively strong binding, whereas only weak interactions were observed for GalNAc and LacNAc. Interestingly, terminal mannose residues are not acceptor substrates for TS-CD, indicating a different, yet unknown biological function for TS-LD. Testing more natural potential substrates is part of the current research and may indicate a possible biological function of TS-LD.

(158) **The tandem-repeat galectin-8 is widely expressed in the central and peripheral nervous system**

Yomayra Guzman, Tristan Hedrick, Sonia Bhangoo, Geoffrey Swanson

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Galectins, a family of galactoside-binding proteins, impact a variety of physiological functions. In the periphery, galectins have important roles in angiogenesis, metastasis, inflammation, and immunity. In recent years, a role for galectins on central nervous system function has also emerged. Specifically, different members of the galectin family have been implicated in neurogenesis, glioblastoma

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metastasis, neurodegeneration, and epilepsy. Despite their impact on CNS physiology and pathology, the expression profile of a number of these lectins in the brain has not been studied. The purpose of this research is to determine the localization of one such galectin, the tandem-repeat galectin-8, in the mouse central and peripheral nervous systems.We found that galectin-8 is widely expressed by neurons in the CNS and PNS. Localization of the protein was determined using confocal microscopy following immunolabeling of the protein with a selective antibody in thin slices of the juvenile mouse brain. Galectin-8 immunoreactivity was especially pronounced in cerebellar Purkinje cells and neurons of the deep nuclei, in hippocampal granule cells of the dentate gyrus, hilar neurons and distributed neurons in the CA1 and CA3 regions, and piriform cortical neurons. This lectin was also present in a large proportion of neurons in lumbar dorsal root ganglia. No detectable co-localization was observed with a marker for astrocytes in the CNS. These initial studies reveal that galectin-8 expression is likely to be the most widely distributed of all galectins examined in mouse brain to date and is suggestive of a role in CNS function.

(159) Investigation into molecular mechanism of synapse elimination mediated by complement C1q and C3

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In the last decade, many cell adhesion molecules and secreted factors regulating synapse formation have been identified. Recently, several molecules that belong to the immune system have been reported to regulate certain aspects of synapse formation. We have previously shown that Cbln1, which belongs to the C1q complement family, plays crucial roles in formation and maintenance of synapses in developing and mature cerebellum. On the other hand, C1q and C3, the latter of which is activated by C1q, are implicated in non-inflammatory synapse pruning during normal brain development of synapses between retinal ganglion cells and dorsal lateral geniculate nucleus. In addition, as an innate immune system protein, C1q activates the classical pathway of the complement system to induce inflammation in infection and in neurodegenerative diseases, such as Alzheimer's disease. These findings indicate that C1q family proteins regulate various aspects of synapse formation, elimination and maintenance throughout development and adulthood. However, it remains unclear how synapses are differentially maintained or eliminated in an activity dependent manner by C1q family proteins. To obtain clues to mechanisms by which C1q mediates the pruning of inactive synapses, we first examined protein localization of C1q and C3 by immunohistochemical analyses during normal development when synaptic pruning occurs. We compared the immnolocalization of C1q and other related proteins at several developmental stages among wild-type, C3 knockout (KO) and C1q KO mice. Interestingly, certain C1q-immunopositive regions in wild-type mice disappeared in C3 KO mice. Recently, sialic acid on the surface of active axons was reported to prevent C1 binding and CR3-mediated removal by microglia. To test the hypothesis that sialic acid on the cell surface of neurons may regulate synaptic pruning, we treated cultured hippocampal neurons and brain slices with exo- and endo-sialidase. Finally, synapse elimination at developmental stage occurs in several brain regions including lateral geniculate nucleus, barrel cortex and cerebellum. Thus, we investigated whether multiple input fibers to these regions were normally eliminated in C1q and C3 KO mice. Based on these preliminary data, we would like to discuss and exchange ideas regarding the mechanisms of complement-mediated synapse pruning.

(160) Characterization and interaction analyses of multispecific Pleurocybella porrigens lectins

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Several years ago, patients of acute encephalopathy appeared to be associated with ingestion of *Pleurocybella porrigens* mushroom. As to *P. porrigens*, one lectin, PPL1 which was purified by acid-treated Sepharose has been reported in the mushroom [1]. We purified other lectins, PPL2 and PPL3, by using LNnT-Sepharose or melibiamyl Sepharose column as an affinity absorbent.

PPL2 was consistent with PPL1 in the following properties; molecular size of 14.2 kDa, amino acid composition lacking detectable amounts of Lys and Cys, and that its hemagglutination was inhibited best with $_D$ - GalNAc. However, unlike PPL1, PPL2 is blocked at the N-terminus, unglycosylated and preferentially binds to α -Gal by ELISA using various biotinylated sugar-polymers. PPL2 was purified by melibiamyl Sepharose column with lactose elution, furthermore, another lectin (PPL3) was obtained by using the same column with melibiose elution. PPL3 has molecular size of 18.2 kDa and it seemed to have complexed multispecificity by ELISA, possessing Jacalin-related putative lectin domain.

We further studied the carbohydrate binding activity of PPL2 by using glycosylated Fmoc-amino acids as a ligand for surface plasmon resonance (SPR). This method was devised in our laboratory [2] and easily be applied to proteins like PPL2 that lacks an accessible functional group: unsubstituted NH_2- or SH-group which is necessary to immobilize a protein to the CM5 sensor chip by ordinary coupling methods. PPL2 exhibited high affinity toward both linear and biantennary diLacNAcs ((Galβ1-4GlcNAc)$_2$), which identified it as a unique Gal / GalNAc-detection probe.

The effects of PPL2 and PPL3 on the cells are under investigation.

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(161) **Homogeneous Heparan Sulfate Oligomers for NMR Studies of VACV B18 GAG Binding**

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The extraordinary structural diversity of glycosaminoglycans (GAGs) enables them to interact with a wide variety of biological molecules to modulate processes, including immune response and regulation of cell growth. They are also involved in microbial pathogenesis, but this normally harmful process can be turned to advantage, as in the use of oncolytic viruses in cancer treatment. For these applications there is obvious interest in improving targeting to surface receptors up-regulated in cancer, including GAGs. While pox viruses do not use GAGs for attachment or invasion, they do produce GAG-binding proteins for other purposes. The particular target of this study, VACV B18, is the type I interferon (IFN) binding protein (IFNα/βBP) secreted by the vaccinia virus (a member of the poxvirus family), that suppresses immune response through binding to IFN. This protein interacts with cell surface GAGs in order to extend the range of this suppressive effect over multiple cell surfaces. In particular, B18 has been shown to bind strongly to both heparin (HP) and heparan sulfate (HS); however, the structural basis for this recognition and binding remains unknown. Understanding intermolecular interactions involved in B18-GAG binding can lay the basis for manipulating GAG-protein interactions for therapeutic purposes.

NMR methods provide means of structurally characterizing both GAG and protein components. In order to determine the structural basis of GAG-B18 binding, it is necessary to use homogeneous HS oligomers, and it is helpful to be able to incorporate isotopic labeling of HS. Towards this goal, we have developed expression protocols for glypican (a glycoprotein with linked HS chains) in order to yield ^{15}N-HS with specific sulfation patterns. Subsequent heparinase digestion and size-exclusion chromatography yields oligomers homogeneous in both sulfation pattern and length. These HS oligomers are screened for binding to the VACV B18 protein using STD NMR. Several oligomers are identified for future NMR studies.

(162) **GALECTIN-1 PARTICIPATES ON ACUTE EXPERIMENTAL Trypanosoma cruzi INFECTION**

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Galectin-1 (Gal-1) is a protein that recognizes β-galactosides and participates in many biological processes, including the modulation of immune responses. Several reports show the potential therapeutic use of Gal-1 to treat inflammatory disorders, autoimmune and infectious diseases. However, there are few reports on the involvement of Gal-1 on infection caused by *Trypanosoma cruzi* parasites. Here, we evaluated the involvement of endogenous Gal-1, as well as its exogenous administration (recombinant human Gal-1: rhGal-1), in the development of acute experimental infection by *T. cruzi* in mice. A number of parameters were determined in these animals: *i*) parasitemia and survival; *ii*) histopathology of cardiac tissue; *iii*) leucocyte immunophenotyping; *iv*) cytokine assay;*v*) determination of invasion and release rates of parasites from infected cells; *vi*) nitric oxide production by peritoneal macrophages. Accordingly, Gal-1 binds to glycans on the surface of the parasites preventing their invasion on fibroblasts and their phagocytosis by macrophages. rhGal-1 treated macrophages released a lower amount of parasites and had increase production of nitric oxide (NO). The NO production is apparently independent of TLR4 signaling pathway. Both rhGal-1 treated WT mice and untreated Gal-1 KO mice showed decreased parasitemia when compared to untreated WT mice. On the other hand, Gal-1KO mice were naturally more resistant to *T. cruzi*infection than WT mice. In addition, the absence of endogenous Gal-1 reduced inflammatory infiltrate and the parasite load in the cardiac tissue, elevated serum levels of cytokines (IL-2, IL-4, IL-6, IL-10 and IL- 17A), and increased influx of neutrophils into the peritoneal cavity and heart tissue. Based on this set of results we suggest that either the absence of endogenous Gal-1 or the treatment of animals with exogenous rhGal-1 promoted immunological phenotypes that led to the resolution of acute experimental *T. cruzi* infection.

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(163) **Modulation of malignant properties of cancer cells by binding of a sialic acid-recognizing lectin Siglec-9 via calpain-mediated degradation of focal adhesion kinase and related proteins**

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Majority of Siglecs, sialic acid-recognizing endogenous lectins, are expressed in hematopoietic immune cells. Although regulatory mechanisms for immune cells with inhibitory signals via immunoreceptor tyrosine-based inhibitory motifs (ITIM) have been well analyzed, effects of interaction between Siglecs and sialylated

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carbohydrates on the counter receptors on target cells are scarcely known. In this study, we found that AS, an astrocytoma cell line, showed detachment from culture plates when co-cultured with Siglec-9-expressing U937 cells or soluble Siglec-9-Fc proteins. Although detached AS cells appeared to undergo apoptosis, they re-grew and showed increased motility and invasiveness upon Siglec-9 binding. In immunoblotting, Siglec-9-expressing U937 cells showed phosphorylation of SHP2 during the co-culture with AS as expected. As for AS cells, degradation of focal adhesion kinase (FAK) and related signaling molecules such as Akt, p130Cas and paxillin was observed immediately after the co-culture. Despite of degradation of these molecules, phosphorylated forms of them were sustained during the co-culture. In particular, increased p-Akt was found at the front region of AS cells, probably representing increased cell motility. The protein degradation was completely suppressed by treating cells with a Calpain inhibitor, MDL-28170. These results suggest that protein degradation of FAK and related molecules was induced by Siglec-9 binding to its counter receptors, leading to the modulation of tumor cell properties. Differential effects triggered by Siglec-9/ligand interaction on the intracellular signaling pathways were suspected. This could be a mechanism by which cancer cells utilize signals derived from Siglec-9/sialylated cancer antigen interaction for their survival.

(164) **Core Fucosylation: A Key To The Activity of 1918 H1N1 Neuraminidase**

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Introduction: The recent Ebola attention and fundamental concerns for our precarious coexistence with viral pathogens should spotlight research for virion binding and cell entry mediated by spike glycoproteins which mediate the primary stimulus and initial human response. Influenza, one of the most deadly pandemics in human history killed up to 20% of those infected, as opposed to usual 0.1% mortality rate. The 1918 flu pandemic was unusually deadly, the first of the two pandemics involving H1N1 influenza virus by infecting 500 million people across the world, including remote Pacific islands and the Arctic, thereby killing 50 to 100 million representing three to five percent of the world's population. This was one of the deadliest natural disasters in human history. The RNA-protein complexes are packed in a lipoprotein envelope lined from the inside with a matrix protein, with hemagglutinin, neuraminidase, and M2 proteins exposed on the outer surface of the viral particle. The virus attaches through its spike cell surface glycoprotein called hemagglutinin (HA) and specifically to a receptor sialic acid, a common epitope terminating many cell surface glycoproteins. The fundamental question is the uniqueness of this HA binding pocket and detailed receptor structure. Approach and Results: To search for these features on the neuraminidase of the 1918 virus that might have contributed to its unusual virulence, we expressed and characterized this enzyme. The purified HA protein appears to exist as an active tetramer, and inactive monomer and dimer. Only the tetramer was active and under varying conditions the inactive form could not be converted to the active form, possibly due to different glycosylation. This was approached using mass spectrometry which provided a detailed picture showing that the active form contained significantly lower levels of core fucose than the inactive form. These preliminary findings suggest that the level of core-fucosylation could be a key factor in the formation of the active form of 1918 H1N1 neuraminidase. These and further details will be discussed.

(165) **Laminin binding glycan depletion on α-dystroglycan in prostate cancer cells promotes epithelial-mesenchymal transition and enhances tumor formation**

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α-dystroglycan (α-DG) represents a highly glycosylated cell surface molecule that is expressed in the epithelial cell-basement membrane (BM) interface and plays an essential role in epithelial cell-BM interaction. α-DG mediated epithelial cell-BM interaction is often impaired in aggressive prostate cancer (PCa), yet roles and underlying mechanisms in PCa progression remain unclear. We report here a suppressor function of laminin binding (LB) glycan on α-DG in PCa progression.

PC3 cells express varying amounts of LB glycans attached to α-DG. After 2 consecutive cell sorting, PC3 cells were separated into LB glycan expressing substantial (PC3-H) and minimal (PC3-L) amount of LB glycans. Epithelial-mesenchymal transition (EMT) related gene and LB glycan related gene expression profiles of PC3-H and PC3-L were analyzed by Affymetrix GeneChip human Gene1.0 ST Array. Expression of EMT related proteins were also analyzed immunoblotting. To evaluate LB glycan function in PCa progression, we performed cell motility, matrigel invasion assay and orthotopic tumor formation assay.

We found that LB glycan depletion caused cell shape change. PC3-L has a mesenchymal shape (spindle-like) and downregulated E-cadherin while upregulated N-cadherin and vimentin. PC3-H cells reverted to an epithelial shape (rounded) and expressed epithelial cell markers. PC3-H cells increased transcription levels of glycosyltransferases, including β3-*N*-acetylglucosaminyltransferase 1 (β3GnT1), and like-acetylglucosaminyltransferase 1B (LARGE2) and Fukutin, which are required to synthesize LB glycans. Matrigel invasion assay showed PC3-L cells invaded much faster than PC3-H cells. Strikingly, PC3-L cells produced much bigger tumor after being inoculated in the orthotopic prostate of SCID mice and much metastasis to the draining lymph nodes compared with PC3-H and parental PC3 cells. ERK/AKT phosphorylation is also increased PC3-L tumor compare with PC3-H.

These results indicate that the LB glycan depletion on prostate cancer cells promotes EMT and correlated to the progression of PCa.

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(166) **HNK-1 carbohydrate regulates the cell surface expression level of AMPA-type glutamate receptors**

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A unique trisaccharide (HSO_3-3GlcAβ1-3Galβ1-4GlcNAc), named human natural killer-1 (HNK-1) carbohydrate, is highly expressed in the nervous system. So far, we succeeded in generating gene-deficient mice of glucuronyltransferase (GlcAT-P), one of the key enzymes responsible for HNK-1 carbohydrate biosynthesis. The GlcAT-P-deficient mice showed an almost complete disappearance of HNK-1 expression and exhibited reduced long term potentiation (LTP) at hippocampal CA1 synapses, indicating that HNK-1 carbohydrate plays essential roles in synaptic plasticity. Moreover, we identified an HNK-1-carrying glycoprotein accumulating in postsynaptic region as a subunit of AMPA-type glutamate receptor (AMPAR), GluA2. AMPAR, one of the ionotropic receptors, is a hetero or homotetrameric complex composed of various combinations of four subunits (GluA1-4). Since AMPARs mediate most of the fast excitatory synaptic transmission in the nervous system, the abundance of AMPARs in postsynaptic region modulates synaptic plasticity. Together, we speculated that theimpairment of synaptic plasticity seen in GlcAT-P-deficient mice was due to the change in the cell surface expression level of AMPAR by the disappearance of HNK-1 expression on GluA2.

While GluA2 has four potential *N*-glycosylation sites (N256, N370, N406, and N413) in their extracellular domains, it is unclear which *N*-glycosylation sites possess HNK-1 carbohydrate and how those *N*-glycans regulate the cell surface expression level of AMPAR. In this study, to elucidate the role of *N*-linked HNK-1 carbohydrate on GluA2, we generated a series of GluA2 mutants of which Asn residue in the consensus sequence (N-X-S/T) was mutated to Ser and investigated cell surface expression levels of these mutants by using a cell surface biotinylation assay.

As a result, the cell surface expression level of N370S was decreased compared with that of wild-type and mainly distributed in ER, indicating that the *N*-glycan at N370 is important for intracellular trafficking of GluA2. Moreover, we revealed that GluA2 possessed HNK-1 carbohydrate mainly on the *N*-glycan at N413 and that promoted the interaction with N-cadherin, resulting in enhancing the cell surface expression level of GluA2. Our results show that *N*-linked glycans including HNK-1 carbohydrate on GluA2 strictly regulate the cell surface expression level of AMPARs and synaptic plasticity.

(167) **aCaMKII-positive neurons of the PVN regulates feeding via intrinsic nutrient sensing by the O-GlcNAc transferase**

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aCaMKII-positive neurons of the PVN regulate feeding via intrinsic nutrient sensing by the O-GlcNAc transferase.

It is essential for the survival and well-being of organisms to preserve energy homeostasis. The paraventricular nucleus (PVN) in the hypothalamus of the brain contains neuronal circuitry critical for normal feeding behavior. While it is known that subpopulations of neurons in the PVN regulate food intake by responding to extrinsic neuronal and hormonal input, it is unclear to what extent the activity of PVN neurons is modulated by intrinsic nutrient sensing. We have identified a novel molecular nutrient sensor in aCaMKII-positive neurons of the PVN that is essential for feeding-induced gene transcription and meal termination. When glucose enters cells, it can be derivatized to UDP-GlcNAc via the hexosamine biosynthesis pathway. The O-GlcNAc transferase (OGT) cleaves UDP-GlcNAc and transfers GlcNAc to the hydroxyl group of serine and threonine in nuclear and cytoplasmic proteins (O-GlcNAc), thereby regulating protein function in a nutrient-dependent manner. We developed a mouse model where OGT was acutely deleted from aCaMKII-positive neurons in adult mice. Remarkably, this led to extreme obesity where body weight was doubled in three weeks due to voracious overeating. The hyperphagia was related to longer meal length rather than higher meal frequency or disturbed circadian rhythm. Using immunohistochemistry we mapped where OGT is knocked out in the feeding circuit. The activity of the feeding circuitry was analyzed by screening the expression of neuropeptides known to modulate food intake. Interestingly, where OGT was deleted in many areas underlying feeding, functionally a subpopulation of thyroid-releasing hormone (Trh) positive neurons in the PVN was most strikingly affected. Removing OGT from aCaMKII-neurons in the PVN locally by stereotaxic virus-injection recapitulated the weight increase and hyperphagia. In the PVN, OGT is shown to be necessary for activity-dependent gene expression in aCaMKII-neurons upon re-feeding after starvation. In summary, we describe a novel molecular mechanism in aCaMKII-positive neurons of the PVN whereby intrinsic nutrient sensing via OGT regulates feeding-induced gene transcription and feeding behavior.

(168) **Functional interaction of POMT and DPM synthase in protein O-mannosylation**

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A defect of protein *O*-mannosylation causes some forms of congenital muscular dystrophies with neuronal migration disorder. These muscular dystrophies are termed α-dystroglycanopathies, since the aberrant *O*-mannosylation on *α*-dystroglycan (α-DG) is detected in common. The complex of protein *O*-mannosyltransferase1 (POMT1) and POMT2 catalyzes the initial step of *O*-mannosyl glycan biosynthesis, the transfer of a mannosyl residue from dolichol-phosphate-mannose (Dol-P-Man) to Ser/Thr residues on certain proteins in the endoplasmic reticulum. Dol-P-Man is an essential mannose donor that is required not only for *O*-mannosylation, but also for *N*-glycosylation, C-mannosylation and the formation of glycosyl-phosphatidylinositol anchors. Dol-P-Man is synthesized from dolichol-phosphate and GDP-Man by the DPM synthase, which consists of three subunits, DPM1, DPM2, and DPM3. MPDU1 (Man-P-dolichol utilization defect 1) was identified to be related to Dol-P-Man synthesis. Mutations in

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MPDU1 cause congenital disorder of glycosylation due to defects in *N*-glycosylation, whereas a defect in DPM3 has been shown to cause abnormal *O*-mannosylation but only mildly abnormal *N*-glycosylation. Here, we examined complex formation between POMT1-POMT2 and DPM3 and/or MPDU1, and found that complex formation between POMT1-POMT2 and DPM3, and between POMT1-POMT2 and MPDU1. Furthermore, complex formation between DPM3 and MPDU1 was also observed. These results suggest that the amount of available Dol-P-Man affects the *O*-mannosylation of α-DG, and that DPM3 and MPDU1 affect the efficient *O*-mannosylation of α-DG by POMT1-POMT2.

(169) C-Mannosylated TSR-derived peptides modulate TGF-beta signaling in cultured lung epithelial-derived cells

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C-Mannosylation, a unique protein glycosylation, occurs at the first Trp in the Trp-x-x-Trp motif, which is found in various target proteins including thrombospondin type 1 repeat (TSR) superfamily proteins (e.g., thrombospondin-1 (TSP-1), F-spondin, and mindin) and the type I cytokine receptor superfamily proteins (e.g., EPO receptor, IL-12, and IL-21 receptor). In case of TSP-1, the Trp-x-x-Trp motif plays a functional role in the maturation process of TGF-beta through the binding with latent form of TGF-beta. C-Mannosylation in the motif may have a regulatory role in TGF-beta signaling, but it has not been fully explored. TGF-beta signaling plays important roles in a variety of cellular processes, such as tumor suppression and epithelial-mesenchymal transition (EMT). In this study, we investigated the effect ofsynthesized C-mannosylated TSR-derived peptides (e.g., C-Man-Trp-Ser-Pro-Trp) on EMT-inducing TGF-beta signaling in lung epithelial-derived A549 cells. In control cells, the expression level of E-cadherin was significantly decreased with TGF-beta, indicating a typical change in EMT-related molecules. In the presence of C-mannosylated TSR-derived peptides, the TGF-beta-induced downregulation of E-cadherin was apparently enhanced, compared to control peptides, suggesting that C-mannosylated peptides exert an enhancing effect on TGF-beta-induced signaling leading to EMT. To investigate whether C-mannosylated peptides modulate the TGF-beta signaling, we examined the effect of C-mannosylated peptides on Smad2/3, downstream molecules of TGF-beta signals in the cells treated with TGF-beta. However, TGF-beta-induced phosphorylation of Smad2/3 was suppressed in the cells with C-mannosylated peptides, indicating that TGF-beta-induced signaling is suppressed with the C-mannosylated peptides. Then we also found that, even in the absence of TGF-beta, the expression level of E-cadherin was suppressed in the cells solely treated with C-mannosylated peptides, compared with control peptides. Taken together, these results demonstrate that C-mannosylated peptides exert enhancing effects on TGF-beta-induced downregulation of E-cadherin expression, in spite of suppression of TGF-beta/Smad signaling by the peptides, suggesting contradictory or multifactorial effects of C-mannosylated peptides on TGF-beta-induced signaling and events in the lung epithelial-derived cells.

(170) Deciphering the distribution of O-mannosylated proteins in murine brain

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Protein O-mannosylation is a conserved and essential posttranslational modification of proteins entering the secretory pathway. Mutations in genes associated with protein O-mannosylation result in severe congenital muscular dystrophies and neuronal migration defects of varying severity in humans [1]. A hallmark of the gravest of those diseases is an abnormal brain development (cobblestone lissencephaly), including cerebellar hypoplasia and distorted cell layering in the hippocampus and the cerebral cortex. Although most of the observed phenotypes can be assigned to hypoglycosylation of α-dystroglycan, many proteins expressing O-mannosyl glycans remain to be identified in the mammalian brain.

In order to decipher the diversity of protein O-mannosylation in the mammalian brain, we established an antibody (α-Oman) that is specifically reactive to Thr-α1-mannose. In wild-type murine brains, the α-Oman antibody revealed distinct staining in the Purkinje and granular cell layers of the cerebellum, in the pyramidal and granular cell layer of the hippocampus, as well as in the different cell layers of the cerebral cortex. The α-Oman signal showed complete co-localization with Neurocan, a member of the lectican family, that is a known target of protein O-mannosylation [2]. Staining patterns of further known O-mannosylated proteins such as Cadherins [3] were also highly comparable to the obtained α-Oman staining. In addition, signal intensity of theα-Oman antibody was considerably reduced in conditional *POMT2* knockout mice. Taken together, this novel tool provides new insights into the tissue-specific expression of O-mannosyl glycans.

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(171) A novel HNK-1 epitope in perineuronal nets

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HNK-1 (human natural killer-1) carbohydrate epitope, comprising a unique trisaccharide HSO_3-3GlcAβ1-3Galβ1-4GlcNAc-R, is a highly

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expressed on the *N*-linked glycans in the developing brain. We generated the gene deficient mice of GlcAT-P, a major glucuronyltransferase involved in the HNK-1 biosynthesis. Using the GlcAT-P deficient mice (PKO), we demonstrated that the HNK-1 epitope has important roles in higher-order brain functions such as synaptic plasticity and spatial learning. The HNK-1 epitope were mostly abolished in the PKO mouse brain but still remained at the specific regions so called perineuronal nets (PNNs). PNNs surrounding the parvalbumin-expressing interneuron are composed of extracellular matrix, such as hyaluronan, chondroitin sulfate proteoglycan (CSPG), tenascin-R and link protein. PNNs are generally considered to regulate neural plasticity such as ocular dominant plasticity.

To reveal the role of HNK-1 epitope in PNNs, we examined HNK-1 carrier protein using PKO mouse brain. As a result, HNK-1 epitope in PNNs was expressed on CSPG and the carrier protein was identified as aggrecan. To investigate whether the HNK-1 epitope in PNNs is biosynthesized by GlcAT-S, another glucuronyltransferase in the HNK-1 biosynthesis, we generated the double (GlcAT-P and GlcAT-S) knockout mice (DKO). The HNK-1 epitope, however, was still remained in the DKO brain, indicating that a novel glucuronyltransferase is responsible for the synthesis. We previously reported that HNK-1 sulfotransferase (HNK-1ST) transferred a sulfate group to the reducing terminal GlcA of linkage tetrasaccharide (GlcAβ1-3Galβ1-3Galβ1-4Xyl-R) on thrombomodulin and suppressed chondroitin sulfate biosynthesis. Therefore we hypothesized that GlcAT-I, a homologous glucronyltransferase involved in the biosynthesis of the linkage tetrasaccharide on CSPG, is responsible for the HNK-1 epitope in PNNs. To confirm this, recombinant aggrecan tagged Fc (aggrecan-Fc) was transiently expressed with or without HNK-1ST in COS-1 cells endogenously expressing GlcAT-I. HNK-1 epitope was detected on aggrecan-Fc in an HNK-1ST dependent manner. These results suggest that HNK-1 epitope in PNNs is a unique structure (HSO_3-3GalAβ1-3Galβ1-3Galβ1-4Xyl-R) biosynthesized by GlcAT-I.

(172) Signaling-inhibitory effects of sErbB3 is enhanced by single N-glycan deletion

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The ErbB receptors are implicated in various tumor formations and are thus targets of cancer therapy. The family consists of four members: epidermal growth factor receptor (EGFR), ErbB2, ErbB3, and ErbB4. They are type I transmembrane receptors, consisting of an extracellular domain, a transmembrane domain, an intracellular tyrosine kinase domain, and a C-terminal regulatory domain. Binding of ligands to the extracellular domain induces a conformational change that leads to homo- or heterodimerization of receptors, which activates downstream signaling such as the Ras/Erk pathway or PI3K/Akt pathway.

EGFR, ErbB2, ErbB3, and ErbB4 contain 12, 8, 10, and 11 glycosylation sites, respectively. The functional regulation of ErbB receptors by N-glycans has been investigated. We previously reported that N-glycan on Asn418 of ErbB3 is involved in ligand-induced receptor dimerization; deletion of the N-glycan on Asn418 of ErbB3 leads to spontaneous heterodimerization with ErbB2 and exerts downstream signaling. In the present study, we examined suppressing effects of extracellular domains of ErbB receptors (soluble ErbB, sErbB) on heregulin signaling in human breast cancer cell line MCF7. It was found that sErbB3 suppresses ligand-induced signaling most effectively, and additionally, the deletion of N-glycan on Asn418 of sErbB by introducing N418Q mutation increased the signaling-inhibitory effects by 2 to 3-fold. Moreover, the sErbB3 N418Q mutant enhanced anticancer effects of lapatinib more effectively than the wild type. It was revealed that sErbB3 does not decrease the effective ligands but decreases the effective receptors, which suggested that sErbB3 acts on ErbB2/ErbB3 heterodimers on the cell surface. These results suggested that the N-glycan deletion mutant of sErbB3 binds to ErbB2 or ErbB3 on the cell surface at a higher frequency, and suppresses heregulin signaling more than the wild type. Thus, we demonstrated that the sErbB3 N418Q mutant is a potent inhibitor for heregulin signaling.

(173) Cytokeratin 1 interacts with the cytoplasmic tail of Core 2 N-acetylglucosaminyltransferase 2/M to retain the enzyme in the Golgi

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One major Golgi retention mechanism of glycosyltransferases is through a direct interaction of their cytoplasmic tail (CT) with a cytosolic protein. Such proteins have been identified for yeast mannosyltransferases (*Schmitz Dev Cell 14:523, 2008*), core 2 N-acetylglucosaminyltransferase 1/L (C2GnT-L) (*Ali J Biol Chem 287:39564, 2012*), and O-mannose β2GlcNAc transferase (*Pereira J Biol Chem 289:14672, 2014*). The goal of this study was to identify the protein that retains C2GnT-M, an enzyme responsible for the synthesis of core 2, core 4 and "I" antigen found in secreted mucins, in the Golgi. By proteomics analysis, we identified keratin type II cytoskeletal 1 (Cyt 1) as one of the proteins pulled down with anti-c-Myc antibody (Ab) from the lysate of Panc1-C2GnT-M-c-Myc cells. The result was confirmed by C2GnT-M pulldown with Cyt 1 Ab. The direct interaction of Cyt 1 with C2GnT-M was demonstrated by Cyt 1 pulldown from cell lysate and recombinant Cyt 1 with C2GnT-M CT. Yeast-two-hybrid analysis shows that the rod domain of Cyt 1 molecule interacts directly and tightly with the WKR^6 motif in the C2GnT-M CT. Knockdown of Cyt 1 did not affect Golgi morphology but resulted in ER relocalization of C2GnT-M, which is mediated by the interaction of C2GnT-M with NMIIA followed by C2GnT-M ubiquitination and degradation as we previously described (*Petrosyan Int J Biochem Cell Biol 44:1153, 2012*). We also observed that knockdown of Cyt 1 in HeLa cells transiently expressing C2GnT-M-GFP did not block the recovery of Golgi morphology after Brefeldin A treatment, but left C2GnT-M outside of the Golgi. During Golgi biogenesis,

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C2GnT-M formed complex with giantin, which is the C2GnT-M Golgi docking partner (*Petrosyan J Biol Chem 287:37621, 2012*), followed by Golgi retention of C2GnT-M via association with Cyt 1. Finally, in cells lacking Cyt 1, the failure of C2GnT-M to localize to the Golgi was accompanied by an increase in α2-3sialylated glycans in the cytosol. We conclude that Cyt 1 is the C2GnT-M Golgi retention protein and through this interaction modulates the O-glycosylation pattern of the secreted mucins. (The work is supported by VA Merit Award 1I1BX000985 and Nebraska LB506 grant).

(174) Studies in physiological roles of asialoglycoprotein receptors (ASGPRs) variants and application of hepatic-targeted delivery via ASGPRs

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The asialoglycoprotein receptor (ASGPR) is a high-capacity C-type lectin receptor mainly expressed on mammalian hepatic cells. The human ASGPR is comprised of two homologous polypeptides, H1 and H2. ASGPR H1 has two splice variants (H1a and H1b) and ASGPR H2 has three splice variants (H2a, H2b, and H2c). Variant H1b, which has an in-frame deletion of exon 2 resulting in the loss of the transmembrane domain and is secreted as a soluble protein, encodes functional soluble ASGPR (s-ASGPR). Based on our results, we proposed the possible physiological function of s-ASGPR, which is well interpreted in the Galactosyl Homeostasis Hypothesis proposed by Weigel. ASGPR is one of the most promising targets for hepatic delivery. A novel cyclodextrin-modified mesoporous silica nanoparticles will be modified with Gal- or GalNAc- residues for the drug delivery targeting ASGPR. Two types of ASGPR-ligand-based delivery systems: carbohydrate-conjugated polymeric nanoparticles and multifunctional carbohydrate-based liposomes will be also designed and synthesized for siRNA delivery.

(175) Epigenetic regulation of colon cancer stem cells by O-GlcNAc protein modification

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The post-translational modification by O-linked β-N-acetylglucosamine (O-GlcNAc) is a unique glycosylation of serine and/or threonine residues of a broad range of cytosolic and nuclear proteins, and catalyzed by a single enzyme, the O-linked N-acetylglucosamine transferase (OGT). Studies have implicated O-GlcNAc in the regulation of pathways that links to oncogenic phenotypes, as well as the roles of O-GlcNAcylation in development and epigenetic regulation of gene expression. In the present study, the expression levels of O-GlcNAc was investigated. We found that when colon tumor cells with OGT-shRNA expression were injected into NOD/SCID mice as xenografts, tumors grew significantly slower than the tumors formed by injection of control cells, suggesting a reduced proliferation of tumor cells due to inhibition of OGT expression. Significant reductions in the percentage of CCSC were observed in tumor cells populations with OGT knockdown, compared to control cells, while tumor cells treated with TMG, a specific O-GlcNAcase (OGA) inhibitor showed increased levels of O-GlcNAc and an increased CCSC population, indicating that O-GlcNAc levels regulated the CCSC compartment. When grown in suspension, tumor cells with OGT knockdown showed a reduced ability to form tumorspheres (both in size and number), indicating a reduced self-renewal of CCSC due to reduced levels of O-GlcNAc. Combining microarray and RNA-seq analyses, the transcription factor MYBL1 was identified as showing increased expression levels in tumor cells with OGT knockdown. Overexpression of MYBL1 in the tumor cells led to a reduced population of CCSC, similar to the effects of OGT silencing. Furthermore, ChIP-seq using an anti-O-GlcNAc antibody illustrated there is significant chromatin enrichment of O-GlcNAc modified protein at promoter region of MYBL1, which is overlapped with H3K27me3, a histone repression maker. This result strongly argues that O-GlcNAc epigenetically regulates MYBL1 expression, functioning similarly to H3K27me3. Therefore, the aberrant CCSC compartment observed after modulating O-GlcNAc levels is likely to result, at least in part, from the epigenetic regulation of MYBL1 expression by O-GlcNAc, thereby significantly affecting tumor progression.*Keywords:* O-GlcNAc / Colon cancer stem cells / Epigenetic regulation / MYBL1

(176) Tissue-specific expression of the short O-linked N-acetylglucosamine transferase isoform modulates UDP-GlcNAc levels by balancing hydrolysis and utilization

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The balance between cell proliferation and programmed death is a tightly regulated process essential for normal development, differentiation and immunity. A cell must interpret extracellular energy availability and determine if conditions support proper growth or if it is advantageous to undergo apoptosis. Tight regulation of this balance is essential as disruptions often contribute to tumorigenesis. One such nutrient sensor is *O*-linked N-acetylglucosamine transferase (OGT), which adds a single*O*-GlcNAc modification onto a variety of intracellular proteins. Importantly, levels of *O*-GlcNAc correspond with glucose availability, as OGT utilizes the end product of the hexosamine biosynthetic pathway, UDP-GlcNAc, as

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a sugar donor. This modification is a key regulator of diverse cellular processes including, signal transduction, transcription, apoptosis and protein stability. Thus, how OGT is regulated in order to interpret nutrient signals remains an important question with implications for human health. The single mammalian OGT gene encodes three isoforms of OGT that have been shown to differ in localization, substrate recognition and expression patterns. Whereas research has focused on the larger nucleocytoplasmic OGT isoform (ncOGT) and the mitochondrial isoform (mOGT), little is known about the short nucleocytoplasmic isoform (sOGT). sOGT shares the same catalytic domain and cellular localization as ncOGT, however, it only has two out of the twelve TPR repeats known to be involved in substrate recognition. Moreover, sOGT exhibits tissue specific expression patterns whereas ncOGT is ubiquitously expressed. We hypothesized that sOGT acts to inhibit ncOGT activity and that deregulation of sOGT has physiological consequences contributing to disease. Using *in vitro* assays that measure both glycosyltransferase activity as well as UDP-GlcNAc hydrolysis we have shown that sOGT hydrolyzed UDP-GlcNAc, but was unable to glycosylate a well-characterized OGT substrate, Nucleoporin 62kDa. Moreover, ncOGT had decreased glycosyltransferase activity in the presence of sOGT. These data suggest that sOGT acts to inhibit ncOGT by decreasing local pools of UDP-GlcNAc. Additionally, we have demonstrated tissue-specific protein expression of sOGT in adult mice. Thus, sOGT is poised to endogenously regulate ncOGT activity, allowing for cell-type specific responses to glucose flux.

(177) **TLR4-Mediated Innate Inflammatory Response is Modulated by Cell Surface Sialic Acid Composition**

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In mammals, the major sialic acids are N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc). Human like inactivation of the Cmah gene and loss of expression of Neu5Gc has been shown to increase the disease severity of dystrophin-associated muscular dystrophies in mouse models of the human condition. The major mechanisms responsible for such a profound change in life expectancy in Cmah-/-/mdx mice remain largely unknown. One major hallmark of muscular dystrophy is chronic inflammation at sites of damaged muscle. Thus, steroid therapies have been utilized to mildly delay the pathological progression of muscular dystrophies in patients. Previously, we reported that the absence of Neu5Gc on the cell surface of human and Cmah null mouse CD4 T cells enhances the TCR-mediated proliferative response. Here, we report that the absence of Neu5Gc on the cell surface of human and mouse innate immune cells enhances TLR-4 mediated inflammatory responses. Specifically, we stimulated granulocytes, monocytes, and lymphocytes from the bone marrow of Cmah -/-, wild-type, and Cmah overexpressing mice with LPS. We observed a suppressed inflammatory response in all major cell types (measured by intracellular TNFa expression) in Cmah overexpressing mice. Conversely, cells isolated from Cmah -/- mice expressed the highest levels of intracellular TNFa. We also stimulated human monocyte derived macrophages pre-fed with Neu5Gc or Neu5Ac for 5 days. We found that Neu5Ac fed cells secreted higher TNFa levels than Neu5Gc fed cells. Conversely, we found that Neu5Gc fed cells secreted higher IL-10 levels, supporting the evidence in our mouse models that surface Neu5Gc can enhance the anti-inflammatory response. This difference in the inflammatory response may have implications in the human specific pathology of the dystrophin-associated muscular dystrophies and in other inflammatory processes affecting humans.

(178) **Caveolin-1 up-regulates core-fucosylation and alpha1,6-fucosyltransferase (FUT8) expression level in hepatocarcinoma cells via Wnt/beta-catenin signaling**

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Caveolin-1 (Cav-1) is a major structural protein of caveolae and is implicated in lipid transport, signal transduction and tumor progression. Previous results indicate that Cav-1 expression up-regulates *N*-glycosylation and alpha2,6-sialylation on mouse hepatocarcinoma cell surface. Herein, we reported that core-fucosylation and alpha1,6-fucosyltransferase (Fut8) expression level reduced in heart, kindey and liver tissues of Cav-1 gene knockout mice. Cav-1 expression enhenced beta-catenin protein enriched in the nuclei of mouse hepatocarcinoma cell hepa1-6. mRNA level, ChIP and promoter activity assay indicated FUT8 gene expression at transcriptional level is significantly increased by Wnt-1, beta-catenin and Cav-1 expression in human hepatocarcinoma cell SMMC-7721. These results suggest that Caveolin-1 may up-regulate core-fucosylation and alpha1,6-fucosyltransferase expression level in hepatoma carcinoma cells via Wnt/beta-catenin signaling. This work was supported by grants from the Major State Basic Research Development Program of China (2012CB822103) and National Natural Science Foundation of China (31170774).

(179) **O-GlcNAc proteome revealed proteins important for B cell activation and apoptosis**

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O-GlcNAcylation is a post-translational modification that adds O-linked β-N-acetylactosamine (O-GlcNAc) to serine/threonine residues on proteins. It has been proposed that the crosstalk between O-GlcNAcylation and phosphorylation may regulate cellular processes including cell signaling, differentiation, metabolism, etc. Given that ligation of the B cell receptor (BCR) on B cells results in the phosphorylation and activation of several signaling molecules, we here aim to study the functional significance of protein O-GlcNAcylation in B cell activation and to identify the crucial O-GlcNAcylated proteins

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involved in the regulation of this process using a global proteomic approach. By treating anti-IgM stimulated mouse splenic B cells with Thiamet G, an O-GlcNAcase (OGA) inhibitor, we observed that B cell activation and apoptosis were enhanced, suggesting that accumulation of protein O-GlcNAcylation may promote BCR-mediated B cell activation and apoptosis. O-GlcNAc proteome of activated B cells treated with or without Thiamet G was next profiled by using Click-iT O-GlcNAc enzymatic labeling coupled with mass spectrometric analysis. More than 600 proteins were identified. One O-GlcNAcylated protein relevant to BCR-induced apoptosis was validated and its site-specific O-GlcNAcylation is a prerequisite for phosphorylation. Furthermore, BCR-mediated apoptosis was attenuated after site-specific disruption of O-GlcNAcylation or phosphorylation of this identified protein. Thus, we showed that the conjunction of protein O-GlcNAcylation and phosphorylation plays an important role in B cell activation and apoptosis.

(180) Super-Resolution Microscopy Mapping Sites of O-GlcNAc modification in the Native Nuclear Pore Complex

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O-Linked beta-N-acetylglucosamine (O-GlcNAc) post-translational modification occurs for many nucleoporins (Nups) in the nuclear pore complexes (NPCs) of mammalian cells, of which the majority is intrinsically disordered and rich in phenylalanine-glycine (FG) repeats. These FG Nups, playing critical role in mediating selective trafficking of macromolecules between the cytoplasm and the nucleus, are commonly O-GlcNAc-modified at their FG regions. However, identifying either FG repeats or sites of O-GlcNAc-ylation on FG Nups in the native NPC is still a major challenge. We developed a super-resolution microscopy-based method for the identification of these sites within the NPCs of semi-intact cells in three dimensions. New findings obtained with this new technique revealed that the spatial distributions of FG repeats and sites of O-GlcNAc-ylation on FG Nups have fundamental similarities but also distinct features in native NPCs.

(181) Control of oxygen sensing in protists by glycosylation-dependent changes in Skp1 protein conformation

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One mechanism of O_2-sensing adopted by *Dictyostelium*, and probably many other protists, resembles human O_2-sensing by involving a HIFα PHD-like prolyl 4-hydroxylase, PhyA. PhyA modifies Skp1, an adapter protein in the Skp1/Cullin1/F-box protein (SCF) family of E3 ubiquitin ligase complexes, but its full effect depends on addition, on the resulting hydroxyproline-143, of a novel pentasaccharide (Galα1,3Galα1,3Fucα1,2Galβ1,3GlcNAc-, or GGFGGn-) by five cytoplasmic glycosyltransferase activities. Since $E3^{SCF}$Ub ligases are regulated in part by assembly of the complex, we examined the role of O_2-dependent Skp1 modification by interactome analysis. Proteomic analysis of co-immunoprecipitates from mutants that abrogated Skp1 processing at discrete steps showed that glycosylation promotes binding to the F-box proteins FbxA and FbxD in the following order: GGFGGn-Skp1 > FGGn-Skp1 > Gn-Skp1 = HO-Skp1 = Skp1. To investigate how the glycan contributes to this difference, the two terminal Gal moieties of the pentasaccharide were uniformly labeled with [^{13}C]-Gal on the recombinant ^{15}N-labeled protein. Comparison of unmodified and fully glycosylated Skp1 ^{1}H-^{15}N-HSQC NMR spectra indicate increased polypeptide backbone ordering and rearrangement when Skp1 is glycosylated, correlating with previous circular dichroism and small-angle X-ray scattering experiments. ^{13}C-NMR relaxation measurements and rotational correlation time estimates indicate that the outer Gal residues have additional motion with respect to the polypeptide. While further studies are being conducted to determine the full extent of their freedom, the terminal Gal residues evidently do not of themselves mediate the conformational changes in Skp1. The ^{1}H-^{13}C-HSQC-NOESY and -TOCSY experiments revealed the previously unknown linkage of the final sugar to be Galα1,3Gal, which now enables chemical synthesis of the pentasaccharide to assess its direct interaction with FbxD and FbxA as an explanation for improved binding. Our results show that the terminal Gal-Gal disaccharide, whose O_2-dependent addition is mediated by the novel glycosyltransferase AgtA, contributes improved binding to two native F-box proteins, which we anticipate promotes the activity of their respective $E3^{SCF}$Ub ligase complexes and, in turn, the targeting of specific proteins for polyubiquitination and subsequent degradation by the 26S proteasome.

(182) Identification of single O-mannosylated proteins in murine brain

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Protein glycosylation is an extremely complex and abundant post-translational modification with a diverse spectrum of biological functions. To analyze such modifications by high throughput approaches like liquid chromatography coupled mass spectrometry (LC-MS) a variety of methods have been developed in the recent decades. Nevertheless, the high diversity especially of O-linked glycan structures hampered the identification of O-glycosylated proteins in LC-MS high throughput approaches.

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Very recently we developed a method to identify O-mannosylated proteins and their site of modification by combining the specificity of enzymatic treatment with the high accuracy of quantitative proteomics. We identified T-cadherin from rabbit muscle as a new O-mannosylated protein and demonstrated that T-cadherin carries just single mannose residues attached to threonine [1]. Now, we used our developed method to screen for additional proteins carrying this modification. Results and implications for biological functions will be discussed.

Reference

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(183) Glypican and the heparan sulfate fine structure at the *Drosophila* neuromuscular junction

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Heparan sulfate proteoglycan (HSPG), a glycoprotein bearing heparan sulfate (HS) as a side chain, is a major component of cell surface and extracellular matrix. HSPGs interact with various ligand proteins, possibly through the myriad of distinct HS fine structures, which were generated by various HS modifying enzymes. In this study, we attempted to clarify the roles of HSPGs and the HS fine structures in synaptic plasticity using the fruit fly *Drosophila* neuromuscular junction (NMJ), a widely used model system of excitatory glutamatergic synapses. In larval *Drosophila*, starvation induces higher locomotor activity, which leads to an increase in the number of synaptic boutons at NMJ. We found that starvation affected postsynaptic expression levels of Dally-like (Dlp), a *Drosophila* homolog of glypican, suggesting that locomotor activity regulates Dlp localization. RNAi-mediated knockdown of *dlp* prevented such an activity-dependent bouton proliferation. We also found that activity-dependent bouton proliferation was prevented by RNAi for *brother of tout-velu* (*botv*) and *Sulf1*, which encode Ext-like and 6-*O*-endosulfatase, respectively. Several studies have shown that neuronal activity affects the subunit composition of glutamate receptors at postsynaptic sites. We found that alteration of Dlp level affects the localization of glutamate receptor IIA subunit at postsynaptic sites. Thus, our study shows that glypican and the HS fine structure play critical roles in activity-dependent bouton proliferation and glutamate receptor localization.

(184) Sulfation patterns of chondroitin sulfate regulate neural development and plasticity

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Chondroitin sulfate (CS) is a class of sulfated glycosaminoglycan chains that covalently linked to core proteins in the form of proteoglycans. CS chains have been thought to present a physical barrier that limits neural plasticity by inhibiting axonal sprouting and synapse formation. However, it is unlikely that all CS chains inherently inhibit plasticity, as CS chains are very abundant in the highly plastic juvenile brain. CS chain is a linear polysaccharide consisting of repeating disaccharide units that can be substituted with sulfate groups at various positions, thereby producing characteristic sulfation patterns. Using transgenic mouse model overexpressing chondroitin 6-sulfotransferase-1, we found that a developmental increase in the 4-sulfation/6-sulfation (4S/6S) ratio of CS chains restrict plasticity in the mouse visual cortex. Condensation of CS-proteoglycans into perineuronal nets that enwrapped parvalbumin-expressing interneurons (PV-cells) was prevented by cell-autonomous overexpression of chondroitin 6-sulfation. Mechanistically, the increase in the 4S/6S ratio was required for accumulation of Otx2, a homeoprotein that activates development of PV-cells, and for functional maturation of electrophysiological properties of these cells. Our study reveals a novel mechanism for visual cortical plasticity, in which specific sulfation patterns of CS chains regulate the maturation of PV-cells through the incorporation of Otx2. Furthermore, we also investigated the roles of CS chains in neuronal development in the embryonic mouse cerebral cortex. A morphological transition from multipolar to bipolar neurons was markedly impaired by knockdown of chondroitin 4-sulfotransferase-1. Consequently these neurons failed to migrate to their correct position, and were arrested in the intermediate zone. We propose that a neuronal surface CS proteoglycan that contains chondroitin 4-sulfation regulates neuronal polarity and migration during early development.

Reference

1. Miyata S., *et al*. (2012) *Nat. Neurosci*. 15, 414–422.

(185) Highly sulfated chondroitin sulfate chains regulate neuronal polarity formation

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Chondroitin sulfate (CS) proteoglycans are major component of extra/pericellular matrix in central nervous system, and therefore their neuronal functions are highly divergent. Although inhibitory effects of CS chains on axon growth are widely accepted, CS chains does not always impede neurite outgrowth. In fact, several CS preparations, such as CS-D and CS-E, serve as stimulatory substrates for neurite outgrowth of cultured primary neurons in a cell type-dependent manner. Such apparently contradictory functions are supposed to be attributable to the structural diversity of CS chains, and also indicate that neuronal cells have distinct CS-recognition mechanisms. In support of this notion, we previously identified contactin-1 (CNTN-1), a glycosylphosphatidylinositol-anchored cell adhesion molecule of the immunoglobulin superfamily, as a cell surface CS receptor for CS-E-mediated neurite outgrowth [1, 2].

Here, based on this novel mechanism of action of CS chains, we further examined the effects of highly sulfated CS on neuronal polarization processes. Interestingly, *in vitro* neuronal polarity

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formation was controllable by varying the ratio of CS-D to CS-E in defined adhesive substrata for neuronal cells. In addition, our recent findings suggest that several CS receptor molecules including CNTN-1 are required for CS-mediated neuronal polarity formation through the regulation of distinct intracellular signaling pathways.

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(186) Protein O-GlcNAcylation Regulates Cardiac Mitochondrial Function

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O-linked β-D-N-acetylglucosamine addition (O-GlcNAcylation) plays fundamental roles in many important biological processes, including signal transduction, transcriptional control, cell cycle regulation, protein degradation, and stress responses. As a highly dynamic process, O-GlcNAcylation cycling is mainly regulated by two enzymes: O-GlcNAc transferase (OGT, which transfers O-GlcNAc onto target proteins) and O-GlcNAcase (which removes O-GlcNAc). Abnormally regulated O-GlcNAcylation is involved in numerous chronic diseases including diabetes and diabetic cardiomyopathy. Although extensive studies have been performed on nuclear/cytoplasmic protein O-GlcNAcylation, the mitochondrial protein O-GlcNAcylation and its potential biological functions are still largely unexplored.

In our recent study, by using refined O-GlcNAc enrichment and quantification approach as well as liquid chromatography coupled with tandem mass spectrometry, a number of proteins have been found to be O-GlcNAc modified, including mitochondrial electron transport chain proteins, enzymes in the citric acid cycle and beta-oxidation. Moreover, differential O-GlcNAcylation between control and diabetic heart mitochondria has been observed. To elucidate the roles of O-GlcNAcylation on cardiac mitochondrial function, another animal model was developed by using Thiamet-G, a specific and potent inhibitor of GlcNAcase. After Thiamet-G treatment, cardiac mitochondrial O-GlcNAcylation is elevated without an increase in glucose levels. Concomitantly, Thiamet-G treatment induces enhanced oxygen consumption rate, ATP production rate, and improved Ca^{2+}capacity before mitochondrial transition permeability pore opening. In contrast, the diabetic heart mitochondria have decreased oxygen consumption rate and ATP production rate as well as impaired Ca^{2+} capacity. Different mitochondrial O-GlcNAcylation targets between Thiamet treatment and hyperglycemia-initiated diabetic status might be the major reason for the mitochondrial functional divergence observed. These data suggest that mitochondrial proteins are differentially O-GlcNAcylated by chronic hyperglycemia as opposed to O-GlcNAcase inhibition, and that acute O-GlcNAcylation directly regulates cardiac mitochondrial function. Our findings provide novel insights into mitochondrial physiology and, more importantly, to the etiology and progression of hyperglycemia-induced diabetic cardiomyopathy.

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(187) Glycosphinglipid CD77 specifically attenuates the CD19-PI3K-Akt pathway of the B cell receptor signaling in model germinal center B cells

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Germinal center (GC) is a microenvironment arose in secondary lymphoid organs upon immunization with T-cell dependent antigen. In GC, activated B cells undergo various responses such as clonal expansion, somatic hyper-mutation, antigen-affinity driven selection and class-switching recombination. GC B cells subsequently differentiate into plasma cells or memory B cells. Antigen ligation onto cell surface B cell antigen receptor (BCR) triggers B cell activation, thus subsequent various B cell responses. Glycosphingolipid CD77 (Gb3Cer) is specifically induced on human GC B cell and presence of this glycolipid is utilized to mark GC B cells, more specifically, centroblasts. However, immunological significance of CD77 expression remains unclear. In this study, we tried to elucidate the function of CD77, especially in the context of BCR signaling upon BCR ligation.

Downstream of BCR, various signaling pathways are activated and orchestration of these pathways determines the fate of GC B cells. To functionally analyze CD77, we first tried to develop model B cells whose difference is limited to CD77 expression. We utilized Namalwa cells because this lymphoma cells appropriately elicit BCR-mediated signal transduction events known in mature B cells. CD77-negative Namalwa cells could be converted to CD77-positive cells upon CD77 synthase (*A4GALT*) expression, due to its genetic dominance in the biosynthetic branching at lactosylceramide utilization. We analyzed CD77 function in various signaling pathways downstream of BCR. We especially focused onto the post-translational modification events of adaptor molecules, BLNK and CD19, which control cellular signaling to PLCγ-Ca^{2+}pathway and PI3K-Akt pathway, respectively. We found that CD19 glycosylation in the extracellular domain was enhanced and CD19 phosphorylation in the intracellular domain was attenuated in the CD77-positive cells. Consistently, attenuation was found for phosphorylations of both Akt and Akt substrates, representing the signaling events downstream of CD19. In contrast, no effect was found on Ca^{2+} influx downstream of BLNK-PLCγ pathway in the CD77-positive cells. Moreover, MAP kinase phospholylation of Erk and p38 was indifferent in the CD77-positive cells. These results suggest that CD77 expression in GC B cells specifically attenuates the CD19-PI3K-Akt pathway thought post-translational modification of CD19 in the context of BCR signaling.

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(188) **X-inactivation normalizes O-GlcNAc Transferase levels and generates an O-GlcNAc-depleted Barr body**

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GlcNAc Transferase (OGT) catalyzes protein *O*-GlcNAcylation, an abundant and dynamic nuclear and cytosolic modification linked to epigenetic regulation of gene expression. The steady-state levels of *O*-GlcNAc are influenced by extracellular glucose concentrations suggesting that *O*-GlcNAcylation may serve as a metabolic sensor. Intriguingly, human *OGT* is located on the X-chromosome (Xq13) close to the X-inactivation center (XIC), suggesting that *OGT* levels may be controlled by dosage compensation. In human female cells, dosage compensation is accomplished by X-inactivation. Long non-coding RNAs and polycomb repression act together to produce an inactive X chromosome, or Barr body. Given that OGT has an established role in polycomb repression, it is uniquely poised to autoregulate its own expression through X-inactivation. In this study, we examined *OGT* expression in male, female and triple-X female human fibroblasts, which differ in the number of inactive X chromosomes (Xi). We demonstrate that *OGT* is subjected to random X-inactivation in normal female and triple X cells to regulate *OGT* RNA levels. In addition, we used Chromosome isolation by RNA purification (ChIRP) and immunolocalization to examine *O*-GlcNAc levels in the Xi/Barr body. Despite the established role of *O*-GlcNAc in polycomb repression, OGT and target proteins bearing *O*-GlcNAc are largely depleted from the highly condensed Barr body. Thus, while *O*-GlcNAc is abundantly present elsewhere in the nucleus, its absence from the Barr body suggests that the transcriptional quiescence of the Xi does not require OGT or *O*-GlcNAc.

(189) **Expression of the Tn and STn Antigens on Tumor Cells Attenuates their Sensitivity to TRAIL-Induced Apoptosis**

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Emory University

The TNF-related apoptosis-inducing ligand (TRAIL) triggers apoptosis in some tumor cells by signaling through the O-glycosylated death receptor 5 (DR5), but many human tumors are TRAIL-resistant through poorly understood mechanisms. Here we show that expression of the tumor-associated O-glycan antigen termed Tn and its sialylated derivative termed STn on tumor cell surface glycoproteins is associated with resistance to apoptotic killing by TRAIL. Expression of the Tn and STn antigens in some tumor cells arises from acquired mutations of the X-linked *Cosmc* gene that encodes a molecular chaperone required for normal O-glycan biosynthesis. Such tumor cells transfected to express wild-type *Cosmc*, and hence express normal O-glycans, are more sensitive to TRAIL-induced apoptosis. In tumor cells expressing Tn/STn, DR5 shows reduced homo-oligomerization and has an enhanced tendency to non-functionally heterooligomerize with the decoy receptor 2 (DcR2). These results represent the first mechanistic insight as to how Tn/STn expression alters cell signaling to promote tumor cell survival through altering DR5 function.

(190) **Epigenetic and Transcriptional Regulation of a Core 2 Branching Enzyme during T Cell Activation**

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A defining property of adaptive immunity is the activation of naïve T cells into effector cells armed with enhanced navigation potential towards inflammatory sites. Sialyl Lewis-X/A ($sLe^{x/a}$) glycan structures on the activated T cells are believed to mediate such recruitment through the initiation of a binding cascade with endothelial cells inducibly expressing selectins. Glycan structures are not directly encoded by the genome, but determined by the expression and activity of glycosyltransferases (GT) that are responsible for their biosynthesis. Focused on the GTs responsible for forming $sLe^{x/a}$ glycans, our aim was to study how transcription factors (TFs) control GT expression during naïve T cell activation towards T helper 1 (Th1) and T helper 2 (Th2) subsets. Transcript-specific expression analysis of 50 GTs involved directly in $sLe^{x/a}$biosynthesis or indirectly by competing over intermediate substrates revealed that the core 2 branching enzyme, core 2 β1,6-N-acetylglucosaminyltransferase I (GCNT1), is one of the differentially expressed GTs. GCNT1 is therefore one of the key regulators of the activation-dependent switch from $sLe^{x/a}$-negative to -positive state. Cross correlation analysis and area under the curve (AUC) of DNAse hypersensitivity profiles propose a concerting role for chromatin remodeling, epigenetic and transcriptional factors in the control of GCNT1 expression. TFs responsible for such regulation were identified using transcription factor binding site prediction methods. A polycomb group (PcG) protein is exclusively recruited to GCNT1 promoter in CD4 naïve T cells, but not Th subsets, leading to repressive chromatin remodeling through H3K9me and H3K27me modifications. Furthermore, CD4 naïve T cells show exclusive binding of ETS2 to the SWI/SNF complex and in turn acting as a repressor of chromatin remodeling. Our data also show the exclusive binding of multiple transcriptional activator complexes in the Th subsets and multiple repressor complexes with higher binding rate/expression in naïve T cells.

We suggest for the first time a model for regulation of GCNT1 expression and $sLe^{x/a}$biosynthesis at the epigenetic and transcriptional level. These data will initiate further studies to help identify target receptors, signaling pathways and transcription complexes important in modulating $sLe^{x/a}$-mediated inflammation, immune diseases and cancer metastasis.

(191) **Molecular Characterization and Expression Analyses of ST8Sia II, III and IV in Piglets During Postnatal Development: Lack of Correlation Between Transcription and Posttranslational Levels**

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The two mammalian a2,8-polysialyltransferases (polyST's), ST8Sia II (STX) and ST8Sia IV (PST), catalyze synthesis of the α2,8-linked polysialic acid (polySia) glycans on neural cell adhesion molecules (NCAMs), the major carrier protein of polySia. ST8Sia III catalyzes synthesis of the α2,8-triSia glycans expressed on gangliosides and several neural glycoproteins. The objective of this study was to clone the coding sequence of the piglet ST8Sia II and determine the mRNA expression levels of ST8Sia II, III and IV during postnatal development. The amino acid sequence deduced from the coding sequence of ST8Sia II was compared with seven other mammalian species. Piglet ST8Sia II was highly conserved and shared 67.8% sequence identity with ST8Sia IV. Genes coding for ST8Sia II-IV were differentially expressed and distinctly different in tissues at postnatal days 3 and 38. Unexpectedly, the cellular levels of mRNA coding for ST8Sia II, III and IV showed no correlation with the posttranslational level of the cognate α2,8-triSia and polySia glycans in different tissues. In contrast, mRNA abundance coding for the NCAM protein correlated with expression of tri- and polySia glycans. Message level coding for neuropilin-2 (NRP-2), also a carrier protein of polySia, correlated with expression of ST8Sia II and IV but not ST8Sia III. These new findings show that the cellular abundance of ST8Sia II, III and IV in postnatal piglets is regulated at the level of translation/post translation, and not at the level of transcription, a finding that has not been previously reported. These studies also highlight differences in the molecular mechanisms controlling polysialylation in adult rodents and neonatal piglets.

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(192) Detection of Glycosyltransferase activities with homogenous bioluminescent UDP and GDP detection assays

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Glycosyltransferases (GTs) play a pivotal role in many biological processes. These enzymes are widely studied and because of their likely involvement in disease states some are becoming potential drug targets. Thus, assays that monitor glycosyltransferase activities are desirable in order to study and understand their mode of regulation, and to search for their selective and potent inhibitors. Traditional assays for GT activity are not easily configured for rapid GT activity detection nor for high throughput screening because they rely on detection of radiolabeled substrate which requires product isolation, the use of non-homogenous antibody-based assays or mass spectrometry. Glycosylating enzymes use nucleotide-sugars as substrates, and in a glycosyltransferase reaction, the nucleotide moiety is released as a product. Therefore, an assay that detects nucleotide as the universal product of these reactions would be suitable for monitoring most glycosyltransferases activity. We developed homogenous, bioluminescent UDP and GDP detection assays for measuring glycosyltransferase activity. These assays are performed in one-step detection that relies on converting simultaneously the UDP or GDP product to ATP, then to light in a robust luciferase reaction. The light output is proportional to the nucleotide concentration ranging from low nM to 25 μM. These assays are highly sensitive and robust, two features that are highly desirable and essential for measuring the activity of the majority of GT classes. Therefore, the nucleotide detection assays allow significant savings of enzyme usage in GT reactions. The assays are simple, and do not require antibodies, nor modified substrates. These assays can be used with GTs that are tagged, native, free or bound to beads. Examples of various applications of these nucleotide detection assays (UDP-Glo and GDP-Glo) will be presented, including studies on specificity of transfer of different sugars to different acceptors by diverse GTs. We will show their utility in screening for specific GT (OGT) inhibitors and the study of their mode of action. The development of the UDP and GDP detection assays and the future development of CMP detection for Sialyltransferases will make it possible to investigate a large number of GTs and will have significant impact on diverse areas of glycobiology research.

(193) Exploring consequences of *N*-glycolylneuraminic acid overexpression in the brain

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The brain is rich in sialic acids, which are acidic sugars that often occupy the non-reducing termini of glycans. In mammals, the major sialic acids are *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc). The ratio of these two major sialic acids markedly varies among different tissues in different animal species due to the regulation of expression of CMP-Neu5Ac hydroxylase (Cmah), which catalyzes the conversion of nucleotide donors from CMP-Neu5Ac to CMP-Neu5Gc. However, the brain shows extremely low Neu5Gc expression (<3%) in all mammalian species tested to date. We have found that even this small amount is mostly expressed in the endothelium lining blood vessels. This unusual evolutionary pattern suggests possible negative effects of Neu5Gc on neural development and/or function.

In this study, we generated Neu5Gc-overexpressing *Cmah* transgenic mice to address the physiological significance of Neu5Gc

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suppression in the brain. In initial attempts using cytomegalovirus (CMV) or neuron-specific enolase (NSE) promoters to express a Cmah transgene, no live mice overexpressing Neu5Gc were obtained, suggesting embryonic toxicity. Next we applied a Cre-loxP system to allow controlled expression of transgene-derived Cmah by Cre recombinase. In contrast to the earlier results, such forced *Cmah* expression did not show lethality or gross developmental defects with several different Cre mouse lines. The transgenic mice showed remarkably high and widely distributed Neu5Gc expression in the whole brain. Thus, it was revealed that Neu5Gc expression in the brain itself is not lethal and the embryonic lethality seen initially seems to be due to some other effect of very early Neu5Gc overexpression. Nevertheless, the success in the establishment of transgenic mice with high Neu5Gc expression allows us to examine Neu5Gc function in the brain. In initial behavioral analysis Neu5Gc-overexpressing transgenic mice showed less locomotor activity and impaired object recognition memory. These results indicate that Neu5Gc expression in the brain can have a negative effect on function. We are currently focusing on molecular recognition events specific to sialic acid species to further understand the detrimental effects of Neu5Gc expression in the brain.

(194) *N*-glycosylation of the Reactive Centre Loop of Corticosteroid-Binding Globulin Regulate Neutrophil Elastase-Based Cleavage and Cortisol Release

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Human corticosteroid-binding globulin (CBG) is a heavily *N*-glycosylated protein which binds and transports corticosteroids i.e. cortisol in blood circulation. We recently mapped the site-specific *N*-glycosylation of human blood-derived CBG and showed that the high degree of CBG sialylation is regulating the CBG receptor interaction on cell surfaces [1]. Herein, we describe a site-specific function of CBG *N*-glycosylation located at Asn347 on the reactive centre loop (RCL) of CBG. It is known that the RCL undergoes human neutrophil elastase (NE)-based proteolytical cleavage at the proximal Val344-Thr345, which induces a conformational change of CBG that, in turn, favours the release of corticosteroids [2, 3]. NE-based digestion assays of blood-derived CBG isolated from healthy individuals and gel electrophoresis showed that NE preferentially cleaves non-occupied Asn347 glycoforms in a concentration- and time-dependent manner. LC-MS/MS based glycopeptide profiling of the Asn347 *N*-glycans indicates a regulatory role of volume-enhancing glycan features including α1,6-core fucosylation, β1,4-branching and α2,3-sialylation in NE digestion efficiency. The macro- (85% *N*-glycan occupancy) and micro-heterogeneity (>19 *N*-glycoforms) of Asn347 of blood-derived CBG may be a mechanism to regulate the amount/rate of NE-induced RCL cleavage and, thus, indirectly regulate the release and availability of anti-inflammatory corticosteroids. In conclusion, we propose that the heterogeneous *N*-glycosylation of human CBG has multiple regulatory functions in hormone signalling.

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(195) Global O-GlcNAc levels modulate adipocytokine transcription during chronic insulin resistance

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Increased flux through the hexosamine biosynthetic pathway and the corresponding increase in intracellular glycosylation of proteins via O-linked beta-N-acetylglucosamine (O-GlcNAc) is sufficient to induce insulin resistance (IR) in multiple systems. Previously, our group used shotgun proteomics to identify multiple rodent adipocytokines whose levels are modulated upon the induction of IR by indirectly and directly modulating O-GlcNAc levels. We have validated the relative levels of several of these adipocytokines using immunoblotting. Since adipocytokines levels are regulated primarily at the level of transcription and O-GlcNAc alters the function of many transcription factors, we hypothesized that elevated O-GlcNAc levels on key transcription factors are modulating adipocytokine expression. Here, we show that upon the elevation of O-GlcNAc levels and the induction of IR in mature 3T3-F442a adipocytes, the transcript levels of multiple adipocytokines reflect the modulation observed at the protein level. We validate the adipocytokine transcript levels in male mouse models of diabetes. Using inguinal fat pads from the severely IR db/db mouse model and the mildly IR diet-induced mouse model, we have confirmed that the adipocytokines regulated by O-GlcNAc modulation in cell culture are likewise modulated in the whole animal upon a shift to IR. By comparing the promoters of similarly regulated adipocytokines, we determine that Sp1 is a common cis-acting element. Furthermore, we show that the LPL and SPARC promoters are enriched for Sp1 and O-GlcNAc modified proteins during insulin resistance in adipocytes. Thus, the O-GlcNAc modification of proteins bound to promoters, including Sp1, is linked to adipocytokine transcription during insulin resistance.

(196) Non-enzymatic regulation of Skp1 function through a development-dependent association with its α-galactosyltransferase (AgtA)

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Skp1, a subunit of the Skp1-Cullin1-Fbox (SCF) E3 Ubiquitin ligase, plays a role in directing protein turnover as an adaptor between Cullin1 and members of the F-box family of proteins, the variable substrate recognition modules of the SCF complex. In the model organism *Dictyostelium*, Skp1 is modified by a novel pentasaccharide. This modification, which promotes association of Skp1 with F-box proteins, is mediated by three sequentially acting cytoplasmic glycosyltransferases. These enzymes regulate the O_2-dependent development of *Dictyostelium*, the starvation-induced process in which single cellular amoebae aggregate to form

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multicellular fruiting bodies. The final enzyme in the pathway, AgtA, transfers two galactose molecules from UDP-galactose to Skp1.

In addition to its catalytic domain, AgtA possesses a WD40-repeat domain predicted to form a β-propeller. A tight AgtA/Skp1 interaction, mediated in part by the WD40 domain, was previously characterized *in vitro*, and was shown to activate the α-galactosyltransferase activity with an apparent K_D of 60 nM. Surface plasmon resonance studies confirm the interaction but indicate a K_D of 430 nM that is not affected by Skp1 glycosylation. The discrepancy might be partially explained by Skp1 oligomerization negatively affecting AgtA activation. Tight association of AgtA with its product raises the possibility of a non-enzymatic function. In line with this, constitutive overexpression of catalytically inactive AgtA(D132A) partially rescues a delayed developmental phenotype seen in an AgtA-KO strain. This hypothesis is further supported by previous findings that knock-out of AgtA causes a developmental phenotype in a mutant strain in which no Skp1 glycosylation occurs.

This interaction has now been demonstrated *in vivo* through coimmunoprecipitation of overexpressed FLAG-AgtA and Skp1 from *Dictyostelium*growth phase cell extracts. Surprisingly, association diminishes dramatically during development. These data, taken with previous observations that AgtA competes with a model F-box protein for Skp1 binding, support a model in which AgtA sequesters Skp1 during growth. *Dictyostelium* development inhibits the interaction by an unknown mechanism, freeing up Skp1 to bind F-box proteins forming new SCF complexes which promote development. This mechanism does not appear to be affected by Skp1 glycosylation, which therefore activates Skp1 binding to the F-box proteins by an alternative mechanism.

(197) Lactosylceramide mediates innate immune responses depending on PAMPs in human neutrophils

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Host-pathogen interactions are critical for the innate immunity. A diverse array of pattern-recognition receptors (PRRs), such as integrins, CD14, and glycosphingolipids (GSLs), cooperate with each other to trigger pathogen-specific innate immune responses. These molecules express on professional phagocytes including neutrophils and macrophages, and can bind to highly conserved pathogen-associated molecular patterns (PAMPs) expressed on microorganisms. Several types of pathogens, such as*Mycobacteria* and *Salmonella*, target membrane microdomains (lipid rafts) to enter into phagocytes, suggesting that the membrane microdomains are pivotal for host-pathogen interactions. For instance, pathogenic mycobacteria utilize both CD11b/CD18 (αM/β2-integrin) and membrane microdomains to enter into host cells. Lipopolysaccharide (LPS), which is the outer membrane component of Gram-negative bacteria and is a potent stimulator of innate immune responses, has been demonstrated to interact with membrane microdomains. GSLs are known to be central components of membrane microdomains. We recently found that the phagocytosis of non-opsonized zymosans is dependent on CD11b/CD18 and lactosylceramide (LacCer)-enriched membrane microdomains in human neutrophils. Here, we investigated the role of LacCer-enriched membrane microdomains in lipoarabinomannnan (LAM)- and LPS-induced neutrophil activation.

Binding assays showed both *M. smegmatis*-derived phospho-*myo*-inositol-capped LAM (PILAM) and *M. tuberculosis*-derived ManLAM bind to LacCer-coated plate but not gangliosides. Not only PILAM-coated but also ManLAM-coated polystyrene beads were phagocytosed by neutrophils. Lactose inhibited the phagocytosis of both PILAM- and ManLAM-coated beads. Moreover, regardless of mycobacterial pathogenicity, LAM activated the Src family kinase Lyn-coupled LacCer-enriched microdomains. Therefore, it seems that LacCer binds to monomannose chains of LAM, which are common structures in LAMs derived from non-pathogenic and pathogenic mycobacteria, and mediates the LAM-dependent activation of neutrophils. Interestingly, we also found that LPS activated Lyn-coupled LacCer-enriched microdomains, and the activation was suppressed by pre-blocking with anti-CD14 monoclonal antibody. Confocal microscopic analysis showed the co-localization of LacCer with CD14 on plasma membranes of resting neutrophils. These findings indicate that LacCer-enriched membrane microdomains play a pivotal role in both LAM-mediated CD11b/CD18-dependent mycobacterial phagocytosis and LPS-mediated CD14-dependent activation in neutrophils.

(198) Regulatory mechanism of chondroitin sulfate-mediated axon guidance

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Chondroitin sulfate (CS) proteoglycans are a family of molecules consisting of a core protein and one or more sulfated CS polysaccharides. CS is divided into several subtypes (CS-A, C, D, and E) based on its sulfation patterns. Although CS acts as a major axon growth-inhibitory molecule in the injured central nervous system, it can also promote axon growth in different experimental contexts. These facts raise a possibility that CS acts as an axon guidance cue in the developing nervous system. Recent studies have identified several CS receptors. However, intracellular signaling downstream of the receptors remains largely unknown.

In the present study, we examined the role of second messengers (cAMP and Ca^{2+}) in CS-induced axonal growth cone guidance of cultured embryonic chicken dorsal root ganglion neurons. Application of CS gradients through a glass micropipette attracted or repelled the growth cone depending on culture substrates and intracellular cAMP levels. Both the CS-induced growth cone attraction and repulsion were abolished by treatment with BAPTA-AM, a cytosolic Ca^{2+} chelator. In addition, CS application caused Ca^{2+} elevations in growth cones.

Taken together, these results indicate that cAMP and Ca^{2+} control bidirectional growth cone guidance.

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(199) **Consecutive detection of sialylation changes of plasma vitronectin by isoelectric points during liver regeneration**

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Vitronectin (VN) is a multifunctional glycoprotein present in plasma and in extracellular matrix. Linking cellular adhesion and tissue lysis, VN plays an essential role during tissue remodeling. Previously, we discovered that carbohydrate concentration of VN decreased to 1/3 of non operated (NO) rats at 24 h after rat partial hepatectomy (PH-24 h). Because of decreased sialylation, PH-24 h-VN exhibited enhanced binding to collagen, and induced the decreased spreading of hepatic stellate cells [1]. On considering the importance of sialylation in controlling the activities of VN, we aimed to detect the changes in sialylation of plasma VN consecutively throughout the regeneration process between 24 h to 168 h after 70% partial hepatectomy of rats.

VN is present in two forms in plasma, inactive monomer with small amount of active multimer. Utilizing the property of VN to acquire ligand-binding activity by denaturation treatment, VNs have been purified by 2-step heparin-affinity chromatography (Hep-AFC) [2]: the 1st Hep-AFC under non-denaturing condition, the heparin-binding proteins in native plasma which contains active VN are removed, and the 2nd step, the pass-through fraction which contains inactive VN was treated with 8 M urea, then the activated VN is purified by the Hep-AFC under denaturing condition.

By chromatofocusing and following immunodetection of sample rat plasma, pI of PH-VNs were found to increase to more than pH 5 until 72 h from pH 4.5 of NO-VN. Sialic acids of purified VN by 2-step Hep-AFC were increased after PH-48h. However, the purified PH-VN at 48 h showed pI of 4.3 on 2D-gel electrophoresis, while the heparin-binding VN in the non-denatured plasma of PH-48h, which was obtained by the 1st Hep-AFC, was found to have higher pI of pH 4.8. These results indicate that heparin-binding VN in native plasma having higher pI was missed by the purification. Because it has been considered that the active VN in plasma tends to transfer into matrix from blood, it is necessary from now on that both active and inactive VNs in plasma must be analyzed in the viewpoint of glycosylation-biological functions.

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(200) **The role of CMP-Sialic acid synthetase in *Drosophila* neural transmission**

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Sialic acids are large negatively charged sugars that occupy terminal positions on glycoconjugates affecting such important biological processes in vertebrates as cell signaling, immune functions, and development, while alterations in cell surface sialic acids are linked to cancer and other pathological conditions. Sialylation is mediated by sialyltransferases that attach sialic acids onto glycoconjugate acceptors, glycoproteins or glycolipids. Sialylation has been extensively studied in mammals, however the understanding of cellular and molecular mechanisms underlying sialylation is still limited. Revealing these mechanisms has been difficult because of the complexity of glycosylation in vertebrate organisms, as well as the redundancy of sialyltransferase activity due to a large family of sialyltransferase enzymes present in mammals. *Drosophila* has emerged as a promising model because it possesses a single sialyltransferase and has significantly simplified sialylation. We recently demonstrated that *DSiaT* and *CSAS*, the sialylation pathway genes encoding sialyltransferase and CMP-sialic acid synthetase, respectively, regulate neural excitability in the CNS and affect neural transmission at neuromuscular junctions [1]. Here we investigated the role of sialylation at different developmental stages by rescue approach using *DSiaT* and *CSAS* mutants and transgenic expression constructs. We found a critical developmental period when neural development requires the activity of sialylation pathway. We further focused on the activity of *CSAS* and investigated its structure-function relationship using in vivo and in vitro approaches. Additionally, we studied interplay between sialylation genes, which revealed a complex regulation of sialylation in the nervous system. Our results provided further insight into regulatory mechanisms and functions of sialylation in Drosophila and suggesting some are potentially conserved in mammals. This project was supported by NIH/ NS075534 to VP.

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(201) **Chemical characterization of acidic milk oligosaccharides of the platypus (Ornithorhynchus anatinus)**

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In the milk of eutherian mammals the predominant saccharide is usually the disaccharide lactose rather than oligosaccharides, which are generally found at much lower concentrations. Exceptions include the milk of monotremes (echidna and platypus), in which oligosaccharides predominate over lactose. Early studies showed that the chief saccharide in a sample of echidna milk was an acidic trisaccharide Neu4,5Ac(α2-3)Gal(β1-4)Glc (4-O-acetyl 3'-sialyllactose), while that in a sample of platypus milk was a neutral tetrasaccharide Fuc(α1-2)Gal(β1-4)[Fuc(α1-3)]Glc (difucosyllactose). Subsequent studies found that, in addition to this tetrasaccharide, platypus milk contains several neutral fucosyl oligosaccharides whose core structures are lacto-N-neotetraose, lacto-N-neohexaose or lacto-N-tetraose. To date, however, acidic oligosaccharides of platypus milk have not been investigated. In this study, acidic oligosaccharides were purified from the carbohydrate fraction of platypus milk and characterized

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by [1]H-nuclear magnetic resonance spectroscopy and matrix assisted laser desorption/ionization time-of-flight mass spectrometry. 4-O-acetyl 3'-sialyllactose and Neu5Ac(α2-3)Gal(β1-4)Glc (3'-sialyllactose) were found in addition to oligosaccharides which had lacto-N-neotetraose or lacto-N-neohexaose as core units with α (2-3) or α(2-6) linked Neu4,5Ac at their non-reducing ends. Some oligosaccharides contained Lewis x or Lewis y units. The presence of Neu4,5Ac, i.e. oligosaccharides in which the N-acetylneuraminic acid residues have an O-acetyl group attached at position C-4, appears to be a unique species specific feature of the milk of monotremes.

(202) Comparative performance of four methods for high-throughput glycosylation analysis of immunoglobulin G in genetic and epidemiological research

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The biological and clinical relevance of glycosylation is becoming increasingly recognised, leading to a growing interest in large-scale clinical and population-based studies. In the past few years several methods for high-throughput analysis of glycans have been developed, but thorough validation and standardization of these methods is required before significant resources are invested in large-scale studies. In this study we compared liquid chromatography, capillary gel electrophoresis (CGE), and two mass spectrometric (MS) methods for quantitative profiling of *N*-glycosylation of IgG in the same dataset of 1201 individuals. To evaluate the accuracy of the four methods we then performed analysis of association with genetic polymorphisms and age. Chromatographic methods with either fluorescent or MS-detection yielded slightly stronger associations than MS-only and multiplexed CGE, but at the expense of lower levels of throughput. Advantages and disadvantages of each method were identified, which should inform the selection of the most appropriate method in future studies.

(203) High throughput glycomics investigation of the breast cancer progression

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Epithelial-to-mesenchymal transition (EMT) is an essential biological process that occurs in embryonic development, metastatic diseases, and cancer progression. Altered expression of glycans is known to be associated with cancer progression. No studies to date have presented global analysis of the precise variation of N-glycans in EMT. We describe here the profile of N-glycans and glycogene expression in the EMT process induced by transforming growth factor-β1 (TGFβ1) in a normal mouse mammary gland epithelial (NMuMG) cell model. An integrated strategy with a combination of mass spectrometry, glycogene microarray analysis, and lectin microarray analysis was applied, and results were confirmed by lectin histochemistry and quantitative real-time PCR. In TGFβ-induced EMT, levels of high-mannose-type N-glycans were enhanced, antennary N-glycans and fucosylation were suppressed, and bisecting GlcNAc N-glycans were greatly suppressed. The expression of 7 N-glycan-related genes was significantly changed. The products of glycogenes *ALG9*, *MGAT3*, and *MGAT4B* appeared to contribute to the observed alteration of N-glycans. The findings indicate that dysregulation of N-glycan synthesis plays a role in the EMT process.

To further investigate the aberrant N-glycosylation during breast cancer progression, the N-glycans of mouse and human mammary gland epithelial cell lines, NMuMG and MCF10A, mouse and human mammary gland tumor cell lines, 4T1 and MDA-MB-231 were profiled and compared. Bisecting GlcNAc N-glycans was suppressed detected by MALDI-TOF-MS and lectin histochemistry. The N-acetylglucosaminyltransferase-III (GnT-III), which is responsible for synthesis of bisecting GlcNAc glycans, was decreased at protein level. The suppression of the bisecting GlcNAc N-glycans was also confirmed in malignant breast tissues. The identification and function of the glycoproteins, which bisecting GlcNAc N-glycans were attached on, will be further explored.

(204) Normalization and batch correction methods for high-throughput glycomics

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Glycomics is rapidly emerging field in high-throughput biology that aims to systematically study glycan structures of a given protein, cell type or organic system. As it is characteristic for any other high-throughput method in biology (microarrays, next-generation sequencing, mass spectrometry), accuracy of high-throughput glycomics methods is highly affected by complicated experimental procedure. Standard experiment requires highly trained personnel, complicated sample collection and preparation procedure, large set of chemicals and calibrated machines. Standard study includes 1000 to 2000 samples, experiment can take several months and during that time many experimental conditions can vary. As a consequence, differences in experimental procedure represent huge source of variation and need for normalization and batch correction arises naturally. We

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compared most popular normalization and batch correction methods, from microarray and metabolomics field, on several glycomics datasets. We evaluated them based on variation of standards and correlation of replicates. According to standard variation and replicate correlation measures, every normalization and batch correction method performs relatively well, showing that use of any preprocessing method decreases experimental variation and increases the statistical power of the analysis.

(205) Development of novel methods for glycan characterization

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Glycan profiling and characterization are essential for the characterization of a glycoprotein drug product as well as discovery, characterization and QC for endo- and exo-glycosidases. Since they lack a chromophore, glycans are difficult to detect when analyzed by chromatography and it is normally to form a derivative at the reducing end to enable detection by fluorescence and mass spectrometry. While 2-aminobenzamide (2-AB) is the most widely used fluorescent tag, poor glycan ionization efficiency limits its identification and characterization of minor glycan species. Here a novel fluorescent tag, procainamide was investigated for analysis of N- and O-glycans isolated from different glycoproteins. The procainamide derivatives produced fluorescent glycan profiles comparable to the 2-AB derivatives but showed much improved ionization efficiency. Also, a tag removal method was investigated to further expand our ability to fully characterize glycans. Meanwhile, in order to improve glycan characterization especially for minor glycan species, a new HPLC column was also tested for high resolution glycan characterization and glycan quantitation. Altogether, our methods greatly improve our ability for glycan characterization and help to identify and characterize novel endo- and exo-glycosidases.

(206) A MALDI-TOF MS Approach for the Quantitive Analysis of Total *N*-Glycans

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Glycosylation, whereby glycans are attached to organic compounds, such as proteins and lipids, is one of the most important PTMs. Mass spectrometry analysis combined with stable isotopic labeling is a promising method for the relative quantification of aberrant glycosylation in diseases and disorders. Although many isotopic labeling techniques have been used in recent years, reductive amination-based isotopic labels result in the loss of labile sialic acids during oligosaccharide preparation or MS analysis, and permethylation methods with isotopically labeled reagents generate complex mass spectra and difficulties in MS data interpretation. In this study, we developed a novel strategy for the MS analysis of glycans using isotope labeling in combination with neutralization of the sialic acid in oligosaccharides for reliable comparative quantitation. This method was applied to compare N-glycan profiles between normal human serum and prostate cancer patient serum. In this way, we could quantify N-glycans derived from normal person and cancer patient serum at the same time, and hence monitor disease through the direct difference in quantitative changes in N-glycans.

(207) GRITS Toolbox - A freely available software suite for the interpretation of glycomics high-throughput MS/MS data

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Ongoing technical advancements have made mass spectrometry (MS) the dominant experimental technique used for the analysis and identification of glycan structures, whether as purified glycans or as complex mixtures of glycans extracted from biological samples. Currently, most interpretation and annotation of the high throughput MS/MS data generated in this context is done manually, due to lack of software support. The freely available tools (e.g. GlycoWorkbench, GlycoPeakfinder, GlycoMOD) and commercial systems (e.g. SimGlycan®) that have been developed over the last decades are not suited for analysis of datasets that include hundreds or thousands of MS^n spectra. Thus, the development of new software tools capable of processing such high throughput datasets and assisting in their interpretation and annotation is crucial to keep up with the rapid pace of technological development and data generation.

GRITS Toolbox is a modular software suite for the processing, interpretation and storage of glycomics data with a focus on MS data. After loading MS data into the software (as RAW and/or mzXML files), users can invoke various integrated data processing modules, including the Glycomics Elucidation and Annotation Tool (GELATO), which associates spectral features in the data sets with structures supplied by customizable databases. Alternatively, annotations generated by SimGlycan® can be imported into GRITS. An extensive graphical user interface allows the annotated data to be browsed, visualized, manually modified and exported to Excel for further processing. The results of several different experiments can also be merged and displayed side by side to identify differences in the glycosylation patterns of analyzed samples.

The validity of the data annotation depends critically on the quality of the databases that provide structures that are associated with spectral features. Therefore, GRITS provides several of its own N-glycan, O-glycan and glycolipid databases that have been generated using knowledge from a highly curated glycan structure ontology. This ontology was populated by experts at the Complex Carbohydrate Research Center using a web based curation system, called Qrator (http://glycomics.ccrc.uga.edu/qrator/).

The current version of the software suite is freely available via the project website: http://www.grits-toolbox.org

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(208) **Purification of Human Skin N-Deacetylase**

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Purdue University Calumet

Hyaluronic acid (HA), a polymer of [4)-ß-D-GlcA-(]n, is ubiquitous in mammalian connective tissue. It decreases in white, female skin around age 47 years, and becomes undetectable in the 7th decade of life. The CH3C = O's bonded to D-GlcNAc become undetectable around 76.5 years.

The enzyme that removes CH3C = O bonded to D-GlcNAc is N-deacetylase. One control was 14 g of young, female skin that was sheared with a knife and homogenized at 0 ± 2^{o}C in 0.05M $(NH_4)SO_4$, pH 7.86. HA-Sepharose Cl-4β (200 mL) was stirred 30 min with 1.405 mL precipitate 7 known to contain *N*-deacetylase. This mixture was packed in a 147 mL column and eluted with 0.1M NaCl - 1.0M NaCl gradient. Fractions (84, 2 mL/each) were collected at room temperature and stored at -60^{o}C. Enzyme sandwich assay used 5% (w/v) HA-Acrylamide, 24-well plates with 0.05M Tris-HCl, pH 6.8, additional chromatography ingredients, and was polymerized at room temperature for 45 min. Gels were cut into 1 cm x 1 cm slides. All fractions (84) dialyzed in enzyme buffer, were placed into their respective chambers in buffer and incubated at 37^{o}C for 16 hr. A portion of each gel was removed for gel electrophoresis. After washing with 0.5M NaCl, gels were stained 10 min with 0.1 M Calcofluor, which was removed with 0.5M NaCl and Distain-1. Gels were visualized under long wavelength UV light to test for *N*-deacetylase.

HA *N*-deacetylase containing fractions eluted in fractions 68-70 with 0.848 M NaCl, and a molecular wt. around 66,500 g/mol.

(209) **Mass spectrometry-based glycomics of human bone marrow mesenchymal stem cells and their differentiated progenies**

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Human mesenchymal stem cells (MSCs) are adult multipotent progenitor cells that have therapeutic potential for regenerative medicine and are easy to induce into several differentiated types of cells, including adipocytes, chondrocytes, osteoblasts and fibroblast reticular cells. To date, there is little knowledge on the chemical properties of glycosylated surface markers characterizing MSCs and their various differentiated stages, particularly those of sulfated N- and O-glycans. In this study, we aimed to identify several development stage-specific glycans by a mass spectrometry (MS)-based glycomic mapping of the N- and O-glycans of undifferentiated and 14 days adipogenically/osteogenically differentiated human MSCs, as well as gp38+ fibroblast reticular cells (FRC) derived from human MSCs after 7 days of differentiation. We first obtained an overall MS profile by MALDI-MS analysis in both positive and negative ion modes of permethylated glycans derived from hMSCs and their differentiated stages, followed by advanced nanoLC-MS2/MS3 analysis in conjunction with exo-glycosidase digestions to identify the glycotopes. We found that the complex type N-glycans were mostly core-fucosylated when carrying a single fucose but Lewis X, sialyl Lewis X could be identified. Almost all of the N-glycans including those with mono- to tetra-sialylated and those carrying poly-*N*-acetyllactosamine structures were found to have mono- and disulfated counterparts carrying sulfates on the GlcNAc or Gal of the LaNAc unit. Likewise, the major O-glycans of hMSCs and their differentiated progenies were mostly based on mono- or di-sialylated extended core 1 and 2 structures, some of which additionally fucosylated and most could also be mono- and disulfated. Induced differentiation to different progenies, however, did not lead to obvious changes in the overall glycomic repertoire expressed although more subtle alterations in relative amounts were noted.

(210) **Development of an advanced glycotope centric LC-MS/MS acquisition method coupled with glycoinformatics tool for high-throughput glycomics**

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Glycans attached on lipid and proteins mediate a variety of structural and functional roles in cell-cell recognition, adhesion and host-pathogen interactions. While the overall branching and extension from the core determine the binding valency and accessibility, it is the specific terminal glyco-epitope (glycotope) that constitutes the distinguishing recognition code presented by the glycoconjugates. Taking advantages of latest advances in mass spectrometry (MS), we have continued to develop ever more efficient MS-based glycomic approach to globally profile the glycome of a cell or tissue and to identify its unique glycotopes. We present here our recent efforts in optimizing a reversed-phase nanoLC-MS/MS workflow for analysis of permethylated glycans on a new Orbitrap Fusion Tribrid instrument. The collision energy was systematically evaluated along different scan functions. Higher collision energy dissociation (HCD)-MS2 and product dependent ion trap collision (pd-CID)-MS3 were used for stepped fragmentation and target glycotope analysis, respectively. For automated assignment of glycan compositions and associated data mining of MS2/MS3-identified glycotopes, we have developed an in-house informatics tool. In essence, the LC-MS/MS data can be filtered by pre-defined sets of target fragment ions at MS^2 level, followed by annotating the glycan compositions of the corresponding MS^1 parents after mono-isotopic peak fitting, and assignment of glycotopes carried as deduced from acquired MS^3 spectra. To validate this workflow, we performed a comparative glycomic mapping of human gastric and colonic carcinoma cell lines and delineated myriad fucosylated glycotopes as H type 2 antigen and Lewis $^{x/y}$ epitopes, along with other sulfated and sialylated glycotopes.

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(211) WURCS: Web3 Unique Representation of Carbohydrate Structures for Semantic Web

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In recent years, the Semantic Web, otherwise known as Web 3.0, which functions on top of the World Wide Web, has been rapidly growing in importance as a means to link life science data. To support this new trend, research on applying Semantic Web technologies to glycan data has progressed. In order to support this trend, we have developed the Web3 Unique Representation of Carbohydrate Structures (WURCS) as a new unique linear notation for representing carbohydrates for the Semantic Web. WURCS has the following features.

- Linear notation so that it can be used as a Universal Resource Identifier (URI).
- A unique notation such that any published glycan structure can be represented distinctively.
- A glycan structure needs the following normalization processings at the beginning of the WURCS method.
- A representation of glycan structure, excluding aglycons.
- Removing isotopes.
- Converting the ionic form to a neutral form.
- For the uniqueness of the glycan structure notation, the generation method of WURCS contains the following algorithm to ensure a unique notation.
- Represent monosaccharides by stereochemistry and modification.
- A unique string generation method for substituent moieties.
- Various rules for sorting, etc.

WURCS can be used to search for the same carbohydrate moiety in databases implementing this format. Furthermore, we developed a tool that generates WURCS using a structure-drawing editor. We also organized a WURCS Working Group for the promotion of WURCS, which currently involves glycoscientists from around the world.

Acknowledgement:
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(212) Comprehensive analysis of the *N*-glycan biosynthetic pathway using bioinformatics

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Various databases related to glycan structures have been developed during the last decade. UniCarbKB [1] allows users to search glycan structures and their related information from glycomics experiments and publications. UniCarb-DB stores experimental data using mass spectrometry. RINGS (Resource for INformatics of Glycomes at Soka) provides algorithmic and data mining tools [2] for glycomics analysis. Although over 2,000 glycans associated with human cells are stored in databases, about half of these are missing structural details such as anomeric configurations and linkages. The Glycan Pathway Predictor Tool, a free tool developed in RINGS, was implemented based on the literature [3] to dynamically compute the N-glycan biosynthetic pathway. In this research, we have modified this algorithm and calculated the theoretical N-glycan pathways. We gathered reaction pattern data from 63 glycosyltransferases and applied the algorithm to understand the association between these glycosyltransferases and glycan structures. We used Man3GlcNAc2 as an initiation structure for the calculation. The calculation covers all possible glycosyltransferase reaction patterns for glycans having less than 18 monosaccharides. We were able to calculate six million glycosyltransferases reaction patterns and three million theoretical glycan structures. The majority of these structures are not registered in databases due to unknown substrate specificity, but it may also be due to limitations in current glycomics technologies. Therefore, it is suggested that unknown mechanisms work for regulating glycan expressions in vivo. We consider that our comprehensive glycan pathway will be able to act as a key role to fill the gaps between glycobiological analysis in vivo and in silico.

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(213) Variability analysis of N-linked glycans in each growth stage of rice

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Amount of production of rice is globally increased because of good nutritive value, palatable taste, handiness in processing and cultivation. The rice is evaluated according to the yield, appearance quality, and taste, etc. Among these evaluation items, “ripening” is one of the most important for rice quality. The next generation is strongly influenced by previous generation’s ripening. To understand the good ripening, it is important to obtain the information of the rice germ.

On the other hand, *N*-linked glycans are involved in all life phenomena. Rice is no exception, *N*-glycans are involved in all life stages. It is expected to clarify about influence of internal factors (nutritive conditions, germination rate, and ripening) and external factors (climates, mineral, and chemical) by analyzing *N*-linked glycans. However, there is hardly information about relationship between the transition of *N*-glycan structures and each growth stages.

In this study, we performed the structure analysis of the *N*-linked glycans of rice germ, and also the correlation analysis between growing and *N*-linked glycans. The *N*-linked glycans were prepared from *Oryza sativa* by hydrazinolysis. The *N*-linked glycans released from rice germ

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glycoproteins were tagged with a fluorophore, 2-aminopyridine. The pyridylaminated *N*-linked glycans were continuously separated by gel filtration, normal-phase HPLC, and reverse-phase HPLC. The mass numbers of purified glycans were confirmed by using MALDI-TOF mass spectrometry. Moreover, the sugar composition and these linkage types were determined by using various glycosidase digestions.

As the results, we confirmed that rice germ (no germination) has only some major *N*-linked glycans. On the other words, the result implies that rice germ has only little diversity at no germination stage. Furthermore, we also analyzed the *N*-linked glycans at two different growing stages (seedlings at 2 and 5 days after germination). As a result, it is confirmed that a fundamental structure expressed in rice germ, and the *N*-glycan structures drastically changed in each growing stages. These results supported to the proposed plant N-glycosylation pathway.

(214) Lectin recognition pattern analysis using MCAW

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In order to understand glycan-recognition patterns of glycan-binding proteins, we have been developing the MCAW (Multiple Carbohydrate Alignment with Weights) [1] tool in RINGS (Resource for INformatics of Glycomes at Soka) [2], which is a web resource providing a variety of data mining and analytical tools for glycoscience research. The MCAW tool calculates a multiple glycan alignment based on ClustalW [3] and visualizes the results graphically. Percentages of matched monosaccharides and glycosidic-bonds, and gaps are clearly described in the result. In order to illustrate the utility of MCAW, we used the data in the Lectin Frontier DataBase (LfDB) [4] which stores glycan-binding data experimentally observed by automated frontal affinity chromatography with fluorescence detection (FAC-FD). We extracted data related to CRLL, a Jacalin-related lectin which has specific binding with mannose [5]. We used 16 types of N-glycan structures as input data. The calculation results show that core structures and mannoses at non-reducing terminal sites are highly aligned. Especially, Man (a1-2)Man is a clearly featured pattern (around 50% matched) compared to other monosaccharides. Other interesting patterns were also found for other lectins as well.

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(215) Characterization of oligosialic acids in cancer

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Sialic acid (NeuAc) is well known to be of fundamental importance for the biology of mammals in health and disease. These acidic nine-carbon sugars cap glycans found in cell surface glycolipids and glycoproteins, where they are ideally placed to interact with the cell environs. The structure and context of sialic acid is crucial for its biological activity. For example, there are lectins which bind NeuAc-α2,3-Gal, but not NeuAc-α2,6-Gal, and those that recognize α2,8-linked disialic acid but not trisialic acid. In addition to interactions with specific binding partners, the negative charge carried by NeuAc at neutral pH means that the length of NeuAc chains effects the physical properties of the cell surface. While much progress has been made into understanding the effects of polysialic acid (from 10 to 400 NeuAc residues), there is little information on the shorter oligosialic acids. Research in this area has been hampered by challenging analytical techniques, hydrolysis of α2,8-linked NeuAc in acidic conditions and poor sensitivity of oligosialic acid-specific antibodies. In order for the biology of oligosialic acid to be investigated, it is imperative to know more about its structure and distribution. Using a combination of engineered lectin, sialic acid transferases and mass cytometry, new methods of identifying and monitoring oligosialic acids are being developed.

(216) Deep Sequencing Using an Ultra High Resolution Column and Mass Spectrometer for Isomer Separation and Structural Identification of Glycans

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Understanding, measuring, and controlling glycosylation in glycoprotein-based drugs and thorough characterization of biosimilars has become increasingly important. Liquid chromatography (LC) coupled to mass spectrometry (MS) has emerged as one of the most powerful tools for the structural elucidation of glycans. Here we present a novel stationary phase that provides superior selectivity and ultra fast resolution, with isomeric separation, as compared to commercially available columns. The column is based on novel mixed-mode column chemistry, combining both weak-anion exchange (WAX) and reversed-phase (RP) retention mechanisms. The WAX functionality provides retention and selectivity for negatively charged glycans, while RP mode facilitates the separation of the same charge according to their isomeric structure, polarity, and size. As a result this column provides resolution with more than 4 times the glycan structures identified compared to existing commercial columns, for bovine fetuin N- glycans. The ability to separate isomers reveals a greater complexity of the glycan population from a given glycoprotein. Namely, far more MS/MS spectra need to be triggered in a single analysis. Additionally, wider dynamic range and sensitivity are needed to detect and generate good quality MS2 spectra not only for the most abundant glycans but the low abundant species as well. Orbitrap Fusion with it's wide dynamic range and ultrahigh mass resolution was selected for looking deeper into the glycome and confidently identifying low-abundance glycans. Overall, 135 unique glycan structures were identified using the mixed-mode column and Orbitrap Fusion. To our knowledge this is

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the largest number of glycans identified for bovine fetuin in a single analysis. Similar column performance was demonstrated for 2-AA labeled N-glycans from antibodies.

(217) Carbonyl-Reactive Tandem Mass Tag (TMT) Reagents for Mass Spectrometry-Based Quantitative Glycomics

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Introduction: Despite the rapid growth in innovation related to mass MS-based glycomics, accurate quantitative analysis of glycans is still challenging. Stable isotope labeling of glycans is one method which has been used for MS-based glycan quantitation. Recently, we have introduced a set of isobaric carbonyl-reactive Tandem Mass Tag (TMT) reagents, aminoxy TMT Reagents, which enable efficient relative quantitation of carbohydrates, improved labeled-glycan ionization efficiency and increased analytical throughput. In this work we demonstrate the utility of these reagents for quantitative analysis of native *N*-glycans by direct infusion ESI-MS, as well as online HILIC LC-MS, to improve the quantitation of labeled glycans in complex mixtures. Methods: Tryptic digests of several standard glycoproteins, including bovine RNase B, bovine lactoferrin, bovine thyroglobulin and several monoclonal antibodies, were treated with PNGase F glycosidase to release *N*-glycans. Following reversed-phase extraction/desalting steps, the glycans were labeled with aminoxyTMT reagents according to our optimized protocol. Labeled glycans were analysed using Velos Pro dual-pressure linear ion trap and Orbitrap Fusion Tribrid (Thermo Scientific) mass spectrometers in the positive ion mode to assess MS/MS fragmentation patterns of glycans at different charge states with different cation adducts by direct infusion or in combination with hydrophilic interaction (HILIC). Combination of multiple-stage trap-CID/HCD fragmentation was used to obtain both quantitative and structural information for sample glycans. Preliminary Data: We have established a protocol for complete derivatization of *N*-glycan mixtures (up to 100 µg total sample), including all necessary clean-up steps and storage conditions. In our preliminary studies, ionization efficiency and fragmentation patterns of labeled and untreated forms of native glycans were compared. At least 20-fold improvement in signal intensity at the MS-level was observed for native labeled glycans ions with at least one sodium adduct. The TMT reporter ions were observed in the HCD spectra for all precursors. HCD fragmentation of fully protonated precursors produced abundant oxonium ions and Y-ions (with intact TMT tag), with the Y1 ion usually being the most intense peak. For fully protonated precursors, which are were also the dominant species in the MS spectra in LC-MS experiments using formic acid-containing mobile phases, targeting Y1 ion for MS3 (HCD3) appears to be the best approach for quantitation, as reporter ions are produced in very high yields at the MS3 step. At present, we are focusing on defining a practical dynamic range for the relative quantitation of glycans using this approach and further developing LC-MS methods for simultaneous quantitation and composition/structural analysis of sample glycoforms. Novel Aspect: Application of novel carbonyl-reactive tandem mass tag (TMT) reagents in MS-based quantitative analysis of *N*-linked glycans coupled with liquid chromatography.

(218) Ion-pairing online LC-ESIMS method for profiling mixture of sulfated oligosaccharides

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Low-molecular-weight heparins (LMWHs) are used as anticoagulants to prevent and treat deep vein thrombosis or pulmonary embolism. A common strategy for the structural analysis of LMWHs involves either complete or partial depolymerization by either enzymatic or chemical means to obtain constituent disaccharides for disaccharide analysis. Modern separation techniques, including high-performance liquid chromatography (HPLC), size exclusion chromatography (SEC), polyacrylamide gel electrophoresis (PAGE), and capillary electrophoresis (CE), coupled with UV and fluorescence detection have been used to analyze the constituent disaccharides and oligosaccharides in order to help solve complex structures. However, these methods require disaccharide standards and even chemical derivatization. Furthermore, disaccharide analysis provides only compositional, but not molecular weight or sequence information. Here we provides a simple and efficient reversed-phase ion pair high-performance liquid chromatography mass spectrometry (RPIP-HPLC-MS) method to evaluate and separate of various commercially available intact low-molecular-weight heparins (LMWHs). The methods can be used to analyze samples without pre-enzymatic or chemical treatment or to analyze samples that have been partially or exhaustively depolymerized and, optionally, reduced. Specific saccharides can be detected including 1,6-anhydro form and saturated non-reducing end oligosaccharides. The oligosaccharides with DP less than 18 are well resolved by this method. The oligosaccharides with up to DP24 were observed with this method. This method can be applied for the separation, identification, characterization, and pharmaceutical stability analysis of various LMWHs.

(219) Strategies for Glycosaminoglycan Analysis in Human Blood Platelets

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The Analytical Services Laboratory of the Complex Carbohydrates Research Center (CCRC) at The University of Georgia is a non-profit entity that offers services for structural characterization of glycoconjugates derived from animal, plant, or microbial origin. The Analytical Services Group routinely analyzes samples from a wide variety of institutions including universities, federal agencies, and industry groups from the US and other countries. Here we will show examples of projects in our laboratory that highlight a combination of analytical techniques for the structural elucidation of proteoglycans. The CCRC service laboratory is well complemented with instruments such as LTQ-Orbi-MS, MALDI-TOF, and high field

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NMRs in addition to SAX-HPLC, CE, HPAEC-PAD, and GC-MS. Finally, this poster highlights on a recent collaboration project between the CCRC and the Blood Research Institute at the Blood Center of Wisconsin.

Blood platelets are irregular, disc-shaped fragments of bone marrow cells that, during normal blood clotting, aggregate to stop bleeding. Blood platelet cells are typical examples of cells that exhibit cell-cell aggregation following specific stimulation through a number of agents, including heparan and chondroitin sulfates. Because abnormal platelet function results in a range disorders resulting in irregular aggregation or adhesion, it is vital to better understand the roles glycosaminoglycans play in the process of platelet aggregation.

Collaboration between the Blood Research Institute and the CCRC Analytical Services is underway to identify GAGs in whole platelet lysates, preparations of human platelet membranes, as well as platelet releasates. Interestingly, in addition to the characterization of heparan sulfate found in these samples, chondroitin sulfate appears in these samples as a homopolymer. In addition to identification and characterization of these GAGs, whole GAG was isolated for further studies of GAG-platelet interactions.

(220) GlycoPAT: An open-source MATLAB based toolbox for glycoproteomics analysis

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Glycosylation represents the most abundant and diverse protein post-translational modification (PTM) identified to date. The structural analysis of this PTM is challenging, in part, due to the chemical structure of individual monosaccharides which are not conserved among organisms, the branched nature of glycans that result in non-linear structures and site-specific variations in the glycosylating pattern. Glycoproteomics experiments have adopted the traditional proteomics work flow to perform high-throughput LC tandem-MS runs using proteolytically digested products. Comprehensive computational platforms and glycopeptide-specific scoring schemes for the analyses of such experiments are scarce.

The paper describes a MATLAB based toolbox, GlycoPAT (GlycoProteomics Analysis Toolbox) for the analysis of LC-MS/MS experimental data. It enables the identification of N- and O-linked glycopepetides following tandem LC-MS experiments using a three-step workflow. This computational workflow starts with digestion of a library of anticipated glycopeptides, followed by tandem MS analysis using a novel scoring scheme, and ends with result browsing with annotation. In the workflow, three new concepts are introduced including: i) 'SmallGlyPep', a minimal linear glycopeptide representation of glycopeptides used in the data analysis; ii) 'Connection Inference', a method to dynamically generate glycan search libraries using enzyme definitions and limited Glycomics profiling data [1, 2]; and iii) 'Ensemble Score', a scheme to score glycopeptide hits based on precursor ion match. Four parameters are used to derive the ensemble score including MS/MS spectra peak intensity cross-correlation analysis, percentage of top-10 peak matched, p-value based on decoy glycopeptide generation and percentage of ion fragments matched. Parallel computing facilities are available in order to enable usage of high performance computing resources. A number of user-friendly GUIs (Graphical User Interface) are also provided. Scoring validation results will be presented for single protein and complex mixtures. The manuscript describes one of the first open-source comprehensive software for glycoproteomics data analysis.

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(221) A Streamlined Workflow for Characterizing Low-Abundance Glycans on Therapeutic Proteins

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ProZyme, Inc

A strategy is presented for characterizing low-abundance N-glycans using single exoglycosidase digestions with LC-MS and LC-MS/MS. N-glycans from Rituxan® (rituximab) and Enbrel® (etanercept) were released and labeled with Procainamide (PCA), treated with a single exoglycosidase, and the products analyzed. Using a threshold of 0.01%, forty-one (41) N-glycans were identified on Rituxan and seventy-one (71) on Enbrel. Minor species included N-Glycolylneuraminic acid (NGNA), hybrid glycoforms and triantennary N-glycans (Enbrel only).

(222) Next JCGGDB Plan for Semantic Web

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The second JST/NBDC integrated database project has just kicked off. JCGGDB was selected as one of the nine database integration projects, aiming to RDFize the glycoscience databases to make it available on the Semantic Web. In this project, we focus on the

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following three priority tasks:

1. To develop and manage the glycan structure repository (described in detail in the oral presentation by Prof. Aoki-Kinoshita titled "Development of an International Glycan Structure Repository").

2. To establish new databaseσ for glycoproteins and other related glycomics data generated by the AIST.

3. To make the current JCGGDB applicable to Semantic Web.

The AIST members mainly pursue 2 and 3. Specifically, we expanded Glycoprotein DB to cover lectin array profiles of mouse tissue specimens. Wehave also developed a new technology and combined it with IGOT-LC/MS, which enables detection of glycoforms in glycopeptides as well as their glycosylation sites. The data collected from the same samples by these technologies are integrated on the database. This enables us to understand "tissue-specific and glycosite-specific glycoforms of a certain glycoprotein" in a comprehensive manner.

We have established multiple glycoscience-related databases such as the Glycan Database, GlycosideDB, Lectin Frontier Database, and GlycoGene Database. To transfer these databases into the Semantic Web, we have developed the necessary ontologies and RDFized them. Users will be able tosearch the data from our website using Faceted Navigation.

In the future, it may become possible to connect the data of other scientific fields for more flexible integrated search, which would yield in-depth understanding of glycan function.

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JCGGDB web site: http://jcggdb.jp

(223) 2-Amino Benzamide Labeling of Oligosaccharides: How Much Sialic Acid is Lost?

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Determination of a glycoprotein's asparagine-linked (*N*-linked) oligosaccharide content is one of the important assays in the characterization of a glycoprotein biotherapeutic where the manufacturer needs to produce a product with a consistent state of glycosylation. Due to the recognized importance of sialylation, and the terminal positions of sialic acids, oligosaccharide sialylation state is especially important. A commonly used *N*-linked oligosaccharide assay method labels the released oligosaccharides with a fluorophore by reductive amination prior to separation by liquid chromatography. The conditions used for labeling can potentially lead to oligosaccharide desialylation. 2-Amino benzamide (2-AB) is a popular label for this purpose. This presentation evaluates the extent of sialic acid loss during 2-AB labeling of *N*-linked oligosaccharides released from three common glycoproteins as well as of sialylated oligosaccharide reference standards. HPLC with fluorescence and/or mass spectrometric detection, and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) of both labeled and unlabeled oligosaccharides were used to evaluate oligosaccharide desialylation. For more highly sialylated oligosaccharides the loss of sialic acids is greater than the <2% value commonly cited. We discuss the experimental reasons for the discrepancy between our findings and the <2% value.

(224) Development and Application of an Intelligent Consecutive Reaction Monitoring (iCRM) Method for the Analysis of O-Glycans

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With glycosylation being arguably the most common post-translational modification found on mammalian proteins and essential for a variety cellular processes, it is crucial to have a streamlined, quantitative method that facilitates a comprehensive glycomic analysis. Previous methods applied for the analysis of O-glycans, such as total ion mapping (TIM), has been shown to be inadequate for distinguishing between isobaric O-glycan structures and for quantification of those structures. To circumvent these pitfalls, we have developed a new mass spectrometry based workflow using intelligent Consecutive Reaction Monitoring (iCRM) to improve O-glycomics analysis. Using this method we were able to select for specific m/z values of permethylated O-glycan species across the entire m/z range for O-glycan analysis. Following fragmentation of the targeted m/z values, fragment ions unique to different isobaric glycan structures were trapped via consecutive reaction monitoring and their intensities were extracted from the collected CRM scans. The intensity of the trapped fragment ion was used to ascertain whether a particular glycan structure was present within a given sample and used for quantification relative to a spiked-in standard. Through application of the iCRM method we were able to identify and quantify changes in isobaric glycan structures between healthy and disease samples and perform a comparison of O-glycan structures across species. The limit of detection for the iCRM method was determined by recording signal intensities of 14 samples with decreasing amounts of spiked-in standard, and linearity of response with regards to quantification was examined using serial dilutions of O-glycans recovered from mouse and rat brains over three orders of magnitude. Due to the architecture of the iCRM method, we have been able to couple our front-end data acquisition with automated data analysis software developed in-house to facilitate quantitative throughput. Our goal in developing the iCRM method was to apply this method as a diagnostic and invasive tool for identifying diseases caused by abnormalities in glycosylation, specifically applying iCRM to identify cases of Congenital Muscular Dystrophy. To demonstrate the clinical relevance of this method, we have successfully used iCRM to detect and quantify relative amounts of O-mannosyl glycans from control and POMGnT1 -/- mice (a model of human Muscle-Eye-Brain disease) brain and blood samples. In conclusion, we have developed an automated method for O-Glycan analysis with improved quantitative sensitivity compared to Full MS methods of quantification that is also able to identify and individually quantify isobaric glycan structures.

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(225) **UniCarbKB: a glycobioinformatics infrastructure for data discovery using semantics**

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Glycobioinformatics databases and tools are now co-operatively adopting semantic technologies for managing data content. Recently, an international team of developers have published standard/controlled ontology for representing glycan structure, annotation information and analytical data collections using RDF (Resource Description Framework). The availability of GlycoRDF and access to RDFized databases is opening new and exciting avenues for connecting and interrogating large volumes of publicly accessible data.

UniCarbKB has implemented this standard prototype and generated RDF versions for all curated glycan structures and glycoprotein (site-specific and global) information. Here, we will give examples of the capability of UniCarbKB-RDF for exploring and correlating glycomics structural and experimental data. Furthermore, the utility of semantic technologies to connect glycan-related knowledge bases with other omics resources (UniProtKB and NeXtProt) to enhance data discovery and inference of protein and glycan biological function will be highlighted. In addition, we will introduce innovative semantic concepts for describing glycan structures (fully defined, linkage ambiguity and residue substitutions) and strategies for efficiently data mining GlycoRDF affiliated databases.

To achieve this ambitious goal a coordinated biocuration program focuses on extracting glycan structure and glycoprotein (site-specific and global) information from the literature. This is supported by the development of workflows designed to improve data capture whilst ensuring consistency and accuracy. An overview of the strategy will be presented including efforts to enhance community-driven curation partnered with UniProtKB to increase content and value of data provided by UniCarbKB. Especially, our efforts to align data capture with the GlycoRDF, MIRAGE and international glycan repository initiatives.

(226) **Analysis of glycosphingolipids using LC-MS and a GSL MS library**

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Recent advances in mass spectrometry provide an excellent analytical stool for studies on the functions of glycosphingolipids (GSLs). LC-MS is applicable to GSL mixtures even in a small amount, and provides structural information of ceramides as well as carbohydrate chains. Although problems still remain to be solved, LC-MS becomes an indispensable method for functional analysis of GSLs especially in the microdomains, because ceramide structure is critical for their formation and sufficient analytical methods providing ceramide structures have not been available particularly for GSL mixtures in crude preparations. One of the problems is that supporting tools for characterization of MS spectra, like proteomics, are not well developed yet. Therefore, we aimed to compile MS spectra and establish a GSL MS library, and then construct a search program for selecting comparable MS spectra from the GSL MS library to the MS spectra we actually obtained from biological samples in routine analyses.

We have compiled MS, MS/MS, and MS/MS/MS spectra of 256 GSLs including gangliosides and ceramides. MS/MS/MS analysis in negative ion mode is able to characterize sphingosine and fatty acid structures of both gangliosides and neutral GSLs. Characterization of GSLs using the search program for MS spectra obtained from biological samples, like major neutral GSLs of human red blood cells and major gangliosides of mouse brain, is demonstrated to be successful. Depending on ceramide structures, the analysis in positive ion mode provides informative fragment ion signals. Therefore, we are compiling MS spectra of positive ion mode in addition to increasing the number of covering GSLs. One major drawback at present is adduct-ion formation in the case of neutral GSLs. When we use HPLC solvents containing matrices like formic acid or acetic acid together with ammonia, $[M- H]^-$ ions, which provide structure information, are about one fourth of total molecular related ions. We report here our resent progress in LC-MS analysis of GSLs including the establishment of the GSL MS library and the search program.

(227) **Sequence Determinants of Linkage Specificity and Polymer Length in Neisserial Polysialyltransferases**

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Many bacteria express a thick layer of surface-associated polysaccharides known as capsular polysaccharides (CPSs). In some neuroinvasive bacteria (e.g. *Neisseria meningitidis*) the CPSs are homopolymers of the negatively charged nona-sugar sialic acid (Sia). Chemical, structural and immunological properties of these polySia chains are defined by the type of glycosidic linkage and polymer chain length. These properties are determined by the polysialyltransferases (polySTs; CAZy family GT38) which catalyse biosynthesis of the CPSs. Our goal is to understand sequence features of the polySTs which control polymer regiospecificity and length. We have previously shown that amino acid 52 is a molecular switch that toggles the linkage specificity of $polyST_{E.coliK1}$ and $polyST_{E.coliK92}$ [1]. In a separate study, we demonstrated that residue 69 of $polyST_{NmB}$ controls the mechanism of chain elongation and thus enables tailoring of polymer length [2]. In a recent study on the $polyST_{NmC}$, exchanging the first 107 N-terminal amino acids from $polyST_{NmC}$ with those from polySTNmB appeared to switch the

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enzyme's regiospecificity from α2,9- to α2,8-linkages, based on α2,8-neuraminidase sensitivity [3]. However, we were unable to observe this specificity switch using an alternative immunoreactivity-based method.

Employing detailed bioinformatics studies on published protein structures we identified target regions in the polyST$_{NmC}$ which we hypothesise to impact linkage specificity. These targets were then assessed by immunological screening of combinatorial site-directed mutagenesis libraries and subsequently characterised using a highly sensitive chemical method for unequivocal linkage analysis [4]. Thus, we have succeeded in identifying amino acids which are involved in determining the linkage specificity of the neisserial polySTs. In addition, we observed interplay between linkage specificity and length of the synthesised polymer which may provide a means to exert length control.

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(228) Characterisation and exploitation of the capsule biosynthesis machineries of *Neisseria meningitidis* serogroups A and X: Towards in vitro vaccine production

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Neisseria meningitidis (Nm) is one of the leading causes of bacterial meningitis globally. Especially serogroup A (*NmA*), but recently also serogroup X (*NmX*), have been and still are a huge burden for the population of the African meningitis belt. Available vaccines against *NmA* and a potential vaccine candidate against *NmX* are based on the capsular polysaccharide (CPS) of the pathogen, which induces the production of bactericidal antibodies in the human host, and even T-cell dependent immune answers if administered as glycoconjugate vaccine (CPS coupled to a protein carrier). The manufacturing of these vaccines involves the purification of CPS from large batches of pathogenic bacteria, a step that is cost intensive and associated with considerable biohazard. To develop alternative ways of glycoconjugate vaccine production, which omit the large scale fermentation of Neisseria, we concentrated in this study on the cloning and functional expression of the minimal number of enzymes needed for the *in vitro* production of immunologically active CPSs. In the case of *NmX* the CPS consist of a α1,4-linked N-acetylglucosamine(GlcNAc)-1-phosphate and only one enzyme, the capsule polymerase CsxA, was needed to produce the polymer from the donor substrate UDP-GlcNAc. The CPS of *NmA* consists of α1,6-linked N-acetylmannosamine(ManNAc)-1-phosphate, whereby ~80% of the O-3 positions in the polymer are acetylated. Since O-acetylation is crucial for immunogenicity and UDP-ManAc is commercially not available, the synthesis of the *NmA* polymer required, besides the capsule polymerase CsaB, two additional enzymes: the epimerase CsaA to convert commercially available UDP-GlcNAc to UDP-ManNAc and the O-acetyltransferase CsaC to acetylate the O-3 position of ManNAc in the assembled polymer. Using the minimal number of enzymes, both capsular polymers could be synthesized *in vitro* in milligram amounts. Identity to the natural polysaccharides was confirmed by physicochemical and immunological means.

(229) Biosynthesis of the Sialyl-T antigen: Specificity of human ST3Gal1 and comparison to a novel sialyltransferase WbwA from *Escherichia coli* O104

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The sialyl-T antigen, sialylα2-3Galβ1-3GalNAc-R, is a core 1 based structure commonly found in O-glycans of glycoproteins and mucins. The sialyltransferase ST3Gal1 is responsible for the synthesis of the sialyl-T antigen. The enzyme is overexpressed in breast cancer and has been shown to play a role in the survival of cancer cells *in vivo*. We have expanded the characterization of purified human ST3Gal1 and showed that it can act on acceptor substrates containing core 1, Galβ1-3GalNAc-R, with a requirement for specific features of the core 1 structure and the aglycone group. The enterohemorrhagic *Escherichia coli* bacteria, serotype O104, have the unique ability to synthesize a sialyl-T antigen mimic which is an internal and modified structure in the O104 antigen. We show here that the *wbwA* gene of the O104 antigen gene cluster encodes a novel sialyltransferase that transfers sialic acid from CMP-sialic acid to the core 1-containing Galβ1-3GalNAcα-diphosphate acceptor. WbwA was inactive with ST3Gal1 acceptor substrates and has only 13.6% sequence identity with human ST3Gal1. However, other properties of the two enzymes were found to be similar. For example, both enzymes were inhibited by bis-imidazolium salts with aliphatic chains of 20 or more carbons. This work shows that human and bacterial sialyltransferases have common features in spite of low sequence identity, and that the bacterial enzyme can be used to synthesize an important human-like glycan structure. This work was supported by the Natural Sciences and Engineering Council of Canada, the Canadian Institutes of Health Research and the Glycorepository Project NIH P41GM103390 and P41RR005351.

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(230) Structural and molecular characterization of the S-layer anchoring system of *Lactobacillus buchneri*

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Lactobacillus buchneri CD034 is a Gram-positive lactic acid bacterium with GRAS status, that is completely covered with a 2D crystalline S-layer that is modified by glucosylation [1]. In Gram-positive bacteria, including LABs, the cell wall usually possesses a rich glyc (oprote)ome. Typical components are secondary cell wall polymers (SCWPs) such as teichoic acids (TA), lipoteichoic and teichuronic acids and also polysaccharides that are linked to the multilayered peptidoglycan [2]. There can be, however, differences regarding architecture, composition and function of specific cell wall constituents between S-layer-carrying and S-layer-deficient bacteria.

In S-layer-carrying bacilli, so far, no "classical" teichoic acids, otherwise typical components of Gram-positive bacteria, have been found. Instead, SCWPs anchor the S-layer (glyco)protein noncovalently to the peptidoglycan [3]. In some of these organisms, the SCWP exclusively consists of several repeats of the linkage unit of "classical" TAs, but with additional chemical modifications [3]. In LABs including *L. buchneri* CD034, SCWPs are only poorly characterized. Preliminary SCWP analyses indicate that the *L. buchneri* CD034 SCWP structure is different from that of known bacilli and resembles to some extent typical TA glycans, but with additional modifications. While the backbone consists of a glycerol phosphate-type TA, additional components such as pyruvic acid and galactose have also been found.

Currently, the SCWP structure is being elucidated in detail for studying of the anchoring mechanism of the S-layer to the cell wall in this bacterium. This knowledge might significantly impact the application of this bacterium as an *in vivo* cell surface display system for biologically active components.

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(231) Next generation approaches to polysaccharide preparation for *Burkholderia pseudomallei* vaccine development

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Burkholderia pseudomallei is a Gram-negative bacterial pathogen found in tropical and sub-tropical environments throughout the globe. It is the causative agent of melioidosis, a potentially fatal disease, in humans. *B. pseudomallei* has been classified a Tier 1 select agent by the Centres for Disease Control due to its natural resistance to many antibiotics and low infectious dose via aerosol. A prophylactic vaccine could therefore provide significant health benefits for both civilian and military populations.

One promising strategy is to develop a conjugate vaccine against the *B. pseudomallei* capsular polysaccharide (CPS). Similar vaccines have already proven successful against a number of bacteria, including *Neisseria meningitidis* and *Haemophilus influenzae*. The *B. pseudomallei* CPS is an ideal target antigen as it has been demonstrated an essential virulence factor [1] and is able to elicit a cellular immune response in mice [2]. However, yields of CPS recovered from the host organism are prohibitively low, and large-scale culture of pathogenic *B. pseudomallei* for vaccine production is unfeasible.

The CPS is a simple unbranched polymer of a repeated monomer with a single acetylation modification. It is therefore an ideal candidate for *in vitro* biosynthesis, as an alternative to challenging and costly chemical synthesis.

This project aims to biologically engineer synthetic CPS coupled with a carrier protein in *Escherichia coli*. A six-gene operon on a single plasmid for the precursor sugar-nucleotide of the polymer has been constructed. The plasmid has been successfully expressed in *E. coli* and synthesis of the sugar-nucleotide has been confirmed by mass spectrometry. Production and purification of this molecule is currently being scaled-up for NMR analysis and for use in kinetic assays for determining glycosyltransferase activity.

Future challenges will include assembly of the full polysaccharide from nucleotide sugars in vitro, chain length determination and the biological conjugation of the CPS to a suitable carrier. For this, *E. coli* will be transformed with several combinations of plasmids and screened for the optimal synthesis of *B. pseudomallei* CPS.

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(232) Engineering bacterial polysialyltransferases by directed evolution

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Polysialic acid (polySia) is a polyanionic polysaccharide naturally occurring in humans and on the surface of a few bacterial pathogens. The key enzyme for polySia synthesis is a polymerizing glycosyltransferase known as a polysialyltransferase (polyST). Bacterial polySTs have demonstrated potential in a range of biomedical applications, for example, in the synthesis of polySia on protein therapeutics, cell surfaces, and tissues *in vivo*. The addition of polySia to drug molecules improves pharmacodynamic properties, whereas the addition of polySia to cells and tissues can promote plastic processes necessary for repair of the nervous system. However, limitations of

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the bacterial polySTs include low solubility, low operational stability, poor protein expression levels and the tendency to synthesize polymers of widely varying lengths. In our work, we address these limitations of the bacterial polySTs.

We describe how two simple methods for high-throughput screening and detailed characterization of polyST activity have opened the door to comprehensive enzyme engineering. Using these methods we have begun to explore polyST sequence-function space focusing on key catalytic and physical properties of the enzymes. We have succeeded in identifying mutations which switch the linkage specificity of the *E. coli* polySTs. Further, we have engineered variants of the polyST from *N. meningitidis* serogroup B with increased thermal stability (>10°C increased T_m), improved expression characteristics (>25 fold increased yield), and with greatly improved chain elongation characteristics yielding products with a narrow distribution of chain lengths. Furthermore, using the new polyST variants we have investigated the molecular basis of improved product distributions. We have tested the enzyme's preference for different chain length acceptors, and we demonstrate that the wild type polyST preferentially uses longer polySia acceptor substrates, which leads to broadening of the product distribution. In contrast, new polyST variants with narrow product distributions exhibit little preference for different chain lengths.

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(233) Structure and biosynthesis of bacterial polysialic acid capsules reveals novel retaining Kdo-transferases

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Bacterial capsules protect many bacterial pathogens from complement-mediated killing and phagocytosis. Isolates of *Escherichia coli*, *Neisseria meningitidis*, *Haemophilus influenzae*, and *Pasteurella multocida* share a conserved structural format in their capsular polysaccharides (CPSs), consisting of long polysaccharide chains attached to the cell surface via a phospholipid. These CPSs are assembled via similar mechanisms, where biosynthesis is completed on the cytoplasmic face of the inner membrane, before transport to the cell surface using a process involving a pathway defining ATP-binding cassette (ABC) transporter. While the enzymes that synthesize the polysaccharide components have been studied in detail, little is known about the structure and biosynthesis of the phospholipid terminus. To determine the structure of the non-reducing terminal phospholipid, purified CPSs from *E. coli* K1, K5, and *N. meningitidis* group B were examined. All three polysaccharides possess terminal lyso-phosphatidylglycerol, which is connected to the CPS repeat unit by a poly-3-deoxy-D-*manno*-octulosonic acid (Kdo) linker. NMR spectroscopic analysis established that the Kdo residues are β-linked. In addition to describing the structure of the glycolipid terminus, we also investigated its biosynthesis. Two previously unassigned proteins (designated KpsC and KpsS) encoded by the capsule-assembly locus were identified as the Kdo transferases responsible for synthesis of the poly-Kdo linker. This research has contributed significantly to the understanding of the structure and biosynthesis of capsular polysaccharides and has revealed a new class of Kdo transferases.

(234) Characterization of the UDP-GlcNAc biosynthetic pathway in Archaea by experimental confirmation of each enzymatic activity

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UDP-GlcNAc, an activated form of GlcNAc and an important substrate for polysaccharide, is synthesized by the four-step reaction from Frc-6-P. However, this biosynthetic pathway was not assigned in most archaea including a hyperthermophilic archaeon *Sulfolobus tokodaii*, even though the entire genomic data was determined. To identify *S. tokodaii* biosynthetic pathway, enzymatic activity on each candidate protein was analyzed.

For clue to analyze the entire UDP-GlcNAc biosynthetic pathway in *S. tokodaii*, the ST0452 protein, which was originally detected as Glc-1-P thymidylyltransferase (TTase) homologue by similarity, showed the GlcNAc-1-P uridylyltransferase and GlcN-1-P acetyltransferase activities as well as Glc-1-P TTase activity. This observation indicated that the protein catalyzed the final two reactions of the bacteria-type UDP-GlcNAc biosynthetic pathway. It proposed presence of the bacteria-type UDP-GlcNAc biosynthetic pathway in this archaeon. Therefore, candidate proteins catalyzing the second (phospho-glucosamine mutase; P-GlcNM) and the first (glutamine: fructose-6-phosphate aminotransferase; GF6P AmTase) reaction in this pathway were analyzed.

The activity on the sole phospho-sugar mutase (PSM) homologue in this archaeon, detected as phosphomannnomutase/phosphoglucomutase (PMM/PGM) by similarity, was analyzed. After confirmation of the PMM and PGM activities, its P-GlcNM activity was analyzed under presence of the ST0452 protein as the coupling enzyme. When GlcN-6-P, the ST0452 protein and acetyl-CoA were added into the reaction mixture as substrate, coupling enzyme and co-factor, UDP-GlcNAc, the expected final product of this coupling reaction, was detected. This observation indicated that the PSM protein can convert GlcN-6-P to GlcN-1-P, the second reaction in the bacteria-type UDP-GlcNAc biosynthetic pathway.

Full-sized GF6P AmTase candidate protein was produced but did not show any expected activity. Conversely, the expected activity was detected, when expressed using the third codon as the start codon. The short-form protein did not catalyze the reverse reaction and this activity was not inhibited by any product in this pathway. These results proposed a regulation system, that in this archaeal cell inactive full-sized form is stored, and required GF6P AmTase activity may be supplied by removal of the N-terminal three residues from the stored full-size protein.

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The unpredicted UDP-GlcNAc biosynthetic pathway in archaea was experimentally confirmed, revealing the effectiveness of experimental analyses of the genomic data.

(235) Enzymatic Synthesis of Lipid II and Analogues

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The emergence of antibiotic resistance has prompted active research in the development of antibiotics with new modes of action. Among all essential bacterial proteins, transglycosylase polymerizes lipid II into peptidoglycan and is one of the most favorable targets because of its vital role in cell wall synthesis. Described in this study is a practical enzymatic method for the synthesis of lipid II, coupled with cofactor regenerations, to give the product in a 50-70% yield. This development depends on two key steps: the overexpression of MraY for the synthesis of lipid I and the use of undecaprenol kinase for the preparation of polyprenol phosphates. This method was further applied to the synthesis of lipid II analogues. It was found that MraY and undecaprenol kinase can accept a wide range of lipids containing various lengths and configurations. The activity of lipid II analogues for bacterial transglycolase was also evaluated.

(236) Protein N-glycosylation in the thermoacidophilic archaeon *Sulfolobus acidocaldarius* is essential for cell survival, cell motility, cell-cell interaction, and cellular defense

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Protein N-glycosylation is conserved across all three domains of life. In contrast to Bacteria, N-glycosylation is distributed across all Archaea with just two known exceptions. Archaeal N-glycans display a degree of diversity in terms of composition and architecture not seen elsewhere, which indicates a specific adaptation of this posttranslational modification in Archaea in order to thrive in extremely harsh (110°C, extremely low pH) as well as in more moderate environmental conditions.

Here we will report the first results elucidating the N-glycosylation pathway in the thermoacidophilic crenarchaeon *Sulfolobus acidocaldarius*, which growth at 75°C and pH of 2. So far all analysed secreted proteins, including the (S)-layer protein SlaA and SlaB, Cytochrome b558/566, or the archaellin FlaB, have been shown to be heavily N-glycosylated with a heterogeneous family of glycans, with the largest composed of a tribranched hexasaccharide (Glc-QuiS)-(Man)-(Man)-GlcNAc2-Asp. N-glycosylation is essential in *S. acidocaldarius* as deletion of the oligosaccharyltransferase (AglB) was only possible when a second *aglB* copy was integrated in the genome. Defects in the N-glycosylation process resulted in a severe reduction of growth at elevated salinities as well as in none or reduced motile cells. However, strains lacking one to all six conserved N-glycosylation sites within the archaeallin FlaB still remained motile. Transmission electron microscopy analyses of non glycosylated and glycosylated archaellum filament revealed no structural change in length of the archaellum. Therefore N-glycosylation does not appear to be important for the stability and assembly of the archaellum filament itself, but plays a role in other parts of the archaellum assembly.

(237) Deciphering the Role of N-Glycan Modifications on Bacterial Proteins

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Pathways for the biosynthesis of N-linked glycoproteins are present in many proteobacteria. We have recently demonstrated that all 29 species of the *Campylobacter* genus are capable of modifying their proteins and releasing free glycans during stress. In the foodborne pathogen *Campylobacter jejuni*, inactivation of the pathway results in deglycosylation of >60 different proteins and several altered biological phenotypes including host colonization, natural competence, antimicrobial susceptibility and changes in innate and adaptive immune recognition. However, it is still unclear why campylobacters maintain this biosynthetic pathway while related organisms such as *Escherichia coli* express functionally orthologous proteins without sugar modifications. This report demonstrates that N-glycosylation in *C. jejuni* assists in protein complex stability and protection against proteolysis.

Mutants defective in N-glycosylation through inactivation of the *C. jejuni* oligosaccharyltransferase enzyme, PglB, exhibited reduced growth in chicken cecal contents compared to the wild-type (WT). Inactivation of the cecal proteases completely restored bacterial viability and partially rescued bacterial growth demonstrating that protein modification enhances *C. jejuni* fitness in the chicken gut. Interestingly, *Campylobacter* species that predominately live in the oral cavity, a milieu also rich in proteolytic activity, have acquired a gene encoding a conserved serine protease inhibitor (ecotin) within their glycosylation loci. We demonstrate that purified ecotins from 5 different *Campylobacter* species protect proteins from proteolytic degradation. Therefore this protein might provide an additional level of protection for those campylobacters that reside in this environment.

In addition, glycosylation is key to the function of the major *C. jejuni* efflux pump complex, CmeABC. A *cmeA* mutant is highly susceptible to erythromycin, ciprofloxacin and bile salts when compared to the WT. Complementation of the *cmeA* mutant with WT *cmeA* restores the WT phenotype whereas expression of a *cmeA* allele with point mutations in both CmeA glycosylation sites was comparable to the *cmeA* mutant strain. Moreover, we could show that loss of CmeA glycosylation led to reduced chicken colonization levels similar to the *cmeA* mutant, whereas the mutant complemented with the wild-type *cmeA* allele restored colonization suggesting that the N-glycosylation pathway of *C. jejuni* is maintained due to environmental pressures.

(238) Hyaluronan synthase assembles activated chitin oligomers with -GlcNAc(α1→)UDP at the reducing end

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Class I hyaluronan synthases (HASs) assemble a polysaccharide containing the repeating disaccharide $[GlcNAc(\beta1,4)GlcUA(\beta1,3)]_n$ and

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vertebrate HASs can also assemble (GlcNAc-β1,4)$_n$ homo-oligomers (chitin) in the absence of UDP-GlcUA. This multi-membrane domain CAZy GT2 family glycosyltransferase, which couples HA synthesis and translocation across the cell membrane, is atypical in that monosaccharides are incrementally assembled at the reducing, rather than the non-reducing, end of the growing polymer. Thus, the growing HA chain is always attached to UDP (*i.e.* it is HA-UDP, not HA) and is the donor; the hyaluronyl chain is added to the next sugar-UDP acceptor to extend the chain. Using *E. coli* membranes containing recombinant *Streptococcus equisimilis* HAS (SeHAS), we demonstrate that this prokaryotic Class I HAS also synthesizes chitin oligomers. Furthermore, chitin oligomers were found attached at their reducing end to -4GlcNAc(α1 →)UDP. These oligomers, which contained up to 7 HexNAc residues based on *m/z* values observed in a subpopulation of permethylated products, consisted of β4-linked GlcNAc residues, based on sensitivity of the native products to jack bean β-N-acetylhexosaminidase. None of these products are made by empty vector control membranes lacking SeHAS. Interestingly, shorter unmodified oligomers and a subpopulation of permethylated oligomers exhibited mass defects of -2 (or -4 or -6 for longer oligomers) that strictly depended on conjugation to UDP. However, MS/MS analyses indicate that these species result from chemical dehydrogenations occurring in the gas phase. Identification of (GlcNAc-β1,4)$_n$-GlcNAc (α1 →)UDP as HAS reaction products, made in the presence of UDP-GlcNAc only, provides strong independent confirmation for the reducing terminal addition mechanism of Class I HAS. We conclude that chitin oligomer products made by prokaryotic and vertebrate HASs are derived indirectly from the cleavage of these novel activated chitosyl-UDP oligomers. Furthermore, these UDP-activated chitin oligomers could serve as self-assembled primers for initiating HA synthesis, in which case they would ultimately modify and mark the distal non-reducing terminus of HA with a chitin oligosaccharide cap. (Supported by NIH grant GM35978).

(239) **Analysis of E-cadherin mediated cell-cell interactions in human pluripotent stem cells**

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The cell surface glycoprotein E-cadherin is an important element of adherens junctions by building homophilic interactions with E-cadherin proteins from neighboring cells. E-cadherin is expressed in various cell types including human embryonic stem cells (hESCs) where it is down-regulated during differentiation [1]. Several proteases have been reported to cleave E-cadherin at various sites that can be located in the cytosol or extracellular. The calcium dependent cysteine protease calpain cleaves E-cadherin in the cytosol between the binding sites for p120 catenin and β-catenin and this calpain-dependent cleavage of E-cadherin has been shown to appear in spheroids of various cancer cell lines [2]. We recently reported that culture of hESCs as suspension culture spheroids also caused activation of calpain and we detected beside the full length protein of 120 kDa a fragment of 100 kDa which could be attributed by antibody mapping to be the cleavage product of calpain. Furthermore, spheroid formation of hESCs was prevented by calpain inhibitors and their addition to preformed spheroids caused disassembly [3]. These observations led to the hypothesis that the 100 kDa fragment of E-cadherin might be required for the formation 3-dimensional spheroids in hESCs. To further study the role of the 100 kDa E-cadherin fragment in cell-cell interaction, cell-surface exposed glycoproteins were biotinylated by periodate oxidation and aniline-catalyzed oxime ligation (PAL, [4]). Upon capture of biotinylated proteins we could show by western blot analysis that the 100 kDa fragment is - like the full length protein - localized at the plasma membrane. A crosslinking experiment of cell-surface proteins with the cleavable crosslinker DTSSP shows that E-cadherin could be cross-linked into a complex displaying a molecular weight above 250 kDa. Upon reversion of crosslinking by reduction with DTT we could show that the complex was composed of two E-cadherin forms of about 120 kDa and 100 kDa. Taken together our results show that both, the full length E-cadherin as well as the calpain cleavage product are embedded in the plasma membrane and can interact with each other. These observations suggest a functional role of the 100 kDa E-cadherin fragment in cell-cell-interaction.

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(240) **O-GlcNAcase is a critical epigenetic regulator of nutrient-responsive *Drosophila* oogenesis**

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Nutrient-responsive oogenesis in *Drosophila* is a complex and dynamic process regulated, in part, by members of the Pc and Trx complexes. Because cellular levels of glucose, glutamine, Acetyl co-A and UDP are required for its synthesis, UDP-GlcNAc may function as a nutrient sensor. The O-GlcNAc modification of nucleocytoplasmic proteins is sensitive to UDP-GlcNAc levels. Two enzymes, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), catalyze the reversible protein modification by O-GlcNAc. OGT adds the O-GlcNAc modification onto substrates and is known to participate in Pc repression (Ogt = Sxc). Here we produced a null allele of OGA (O-GlcNAcase) in *Drosophila* to examine its in vivo function. We found that Oga mutant females displayed greatly reduced fecundity. The ovaries from the female OGA knockout flies exhibited a starvation-like phenotype, even under well-fed conditions. Germline stem cell division was slowed in the germarium of OGA knockout fly ovarioles. The Trithorax family members catalyzing H3K4 trimethylation, Ash1, Set1 and Trx are all O-GlcNAc

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modified in Oga mutant ovaries. Highlighting the importance of OGA in Trithorax function, ovaries from the Oga mutants displayed significantly decreased H3K4 trimethylation. Our results suggest that the loss of OGA disrupts oogenesis by interfering with the Trithorax complex in germ cells in the ovary. The findings also suggest that O-GlcNAc cycling is an essential part of the nutrient-responsive epigenetic machinery regulating *Drosophila* oogenesis in response to a changing nutrient supply.

(241) Whole Transcriptome Analysis of Human Embryonic Stem Cells and Differentiated Cell Populations

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We previously employed a high-throughput qRT-PCR approach to analyze changes in transcript abundance for a specific set of ~900 glycan-related genes in undifferentiated human embryonic stem cells (hESCs) and several ESC-derived differentiated cell populations. The goal of these studies were to correlate changes in transcript expression with alterations in cellular glycan structures in an effort to determine whether transcriptional regulation of the glycosylation machinery plays a role in controlling glycan structures during development. We have now expanded our investigation of these cell types by performing whole-transcriptome sequencing (RNA-Seq) analysis of undifferentiated H9 hESCs, ESC-derived cardiac progenitors (WT-1), and smooth muscle (SM) differentiated cell typeσ. RNA-Seq data sets potentially provide an unbiased view of transcript profiles of all genes in an RNA sample, but require extensive sequencing depth to quantify and analyze the low abundance transcripts for glycan-related genes. In addition, the large RNA-Seq data sets must be effectively aligned to the respective genome for appropriate quantitation of gene expression and currently no standard approach exist for performing the downstream analysis. We performed RNA-Seq analysis of hESC-derived cell populations and are testing and optimizing workflows for data analysis using a variety of sequence alignment tools. The results from the RNA-Seq analysis are being compared with data from our qRT-PCR platform as a validation tool and comparative data analysis from both approaches will be presented. Potential benefitσ of broad-based analyses like RNA-Seq include the identification of genes involved in hESC differentiation that had previously been overlooked using our prior glycogene-centric qRT-PCR approach. The long-term goalσ of the project are to identify changes in transcript abundance for glycan biosynthetic and catabolic genes, assignment of these transcript changes to discrete metabolic pathway steps, and correlation of these data with glycan structural abundance data derived from mass spectrometry analysis. Correlations of these disparate data types in the context of biosynthetic pathways should allow us to determine the regulatory nodes and mechanisms that control the abundance of glycan structures as well as predicting where glycan structures are influenced by mechanisms other than transcriptional control (supported by NIH grant P41GM103490 to JMP).

(242) Interactions of Disialyl Gangliosides GD2/GD3 with Growth Factor Receptors Maintain Phenotypic Properties of Breast Cancer Stem Cells

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Our previous studies (in PNAS 110 (13) 4968-4973, 2013) demonstrated a causal relationship between GD2/GD3 gangliosides and maintenance of breast cancer stem cells (BCSCs) properties, based on reversal of stem cells properties following knockdown of ST8SIA1 or B4GALNT1, the enzymes that control GD2/GD3 biosynthesis. Recently, we analyzed growth factor receptors (GFRs) associated with GD2/GD3 and their related signaling pathways to clarify how interactions with GD2/GD3 maintain stem cell properties and signaling in BCSCs. Immunofluorescence staining studies indicated that most GD3 is co-localized with epidermal growth factor receptor (EGFR). In contrast, GD2 and cMet showed only partial colocalization in the ER-Golgi intermediate compartment. Interactions between GD2/GD3 and GFRs were also detected by co-immunoprecipitation of BCSC lysates using specific anti-GD2 and anti-GD3 antibodies. We observed that there was strong interaction between GD3 and EGFR but weak GD2/ cMet association. On the other hand, β1-integrin, which modulates functions of various GFRs, was strongly associated with GD2. Immunogold labeling and transmission electron microscopy confirmed the localization of GD2/GD3 and GFRs in membrane microdomains. GD3 was clustered with EGFR in electron-dense membrane patches, whereas GD2 and cMet showed little colocalization on BCSC membrane sheets. EGFR expression and ERK signaling were increased in BCSC populations showing high GD2/GD3 expression. Proteomic analysis of the "enzyme-mediated activation of radical source (EMARS)" reaction was performed to identify associated molecules possibly involved in co-clustering with GD2/GD3 on cell surface of BCSCs. Co-immunoprecipitation with a chemical cross-linker in living cells was used to identify protein complexes associated with GD2/GD3. A combination of this approach with nano liquid chromatography coupled to tandem mass spectrometry (nano-LC-MS/MS) showed there was a total of 143 GD2-associated and 320 GD3-associated proteins. Thus, our findings from proteomics analysis and nano-LC-MS/MS provide an important basis for further studies of functional molecular interactions that maintain stem cell properties in BCSCs.

(243) Novel Carbohydrate-Recognizing Antibodies for Human iPS/ES Cells

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For medical and industrial applications of pluripotent stem cells, to reduce the risk of teratoma formation in regenerative tissues and to maintain homogeneous pluripotent cell populations in culture, both positive and negative selections of pluripotent stem cells are necessary. For this purpose, marker antibodies, which recognize specifically human iPS/ES cells, would be valuable. Recently, we generated two monoclonal antibodies (R-10G and R-17F) by injecting human iPS (Tic) cells into mice. These antibodies recognize carbohydrates on the surface of human iPS/ES cells but have little or no binding to most of human adult and embryonal tissues.

R-10G antibody recognizes a type of keratan sulfate lacking oversulfated structures on human iPS/ES cells. The binding property of R-10G is clearly distinct from that of a most frequently used anti-keratan sulfate antibody, 5D4, which recognizes a high-sulfated keratan sulfate. In this respect, R-10G is similar to TRA-1-60 and TRA-1-81, conventional marker antibodies frequently used to characterize human iPS/ES cells, which have been shown to recognize keratan sulfate. However, R-10G binding to human iPS cells, as visualized on laser confocal microscopy, does not overlap completely either with TRA-1-60 binding or TRA-1-81 binding. These results indicate that a single colony of undifferentiated human iPS cells does not mean a homogenous population of cells but they consist of different cell subtypes containing keratan sulfate with slightly different structures [1].

R-17F antibody recognizes and binds to almost all over the cell membranes. These profiles were similar to those of SSEA-3 and SSEA-4, other conventional marker antibodies frequently used to characterize human iPS/hES cells, which recognize globoseries glycolipids. However, R-17F binding to human iPS cells does not overlap completely either with SSEA-3 binding or SSEA-4 binding. A major R-17F positive component was isolated from the total lipids of human iPS cells by TLC and subsequently characterized to be a lactoseries glycolipid by MALDI-TOF MS and others. Interestingly, R-17F antibody had a strong cytotoxic activity to human iPS/ES cells, not only in free-cell suspension cultures but also in colony-forming cultures.

These novel antibodies may be practically valuable in the regenerative medicine.

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(244) **Interaction of ganglioside GD3 and EGF-receptor sustains neurogenesis in adult mouse brain by regulating EGF-induced neural stem cell proliferation**

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Gangliosides are abundant in the nervous system and are known to play important roles in regulating neurogenesis [1]. Most of the glycosyltransferase knockout mice, with deficiency in certain gangliosides, exhibit neural dysfunction and degenerative changes with aging. These mutant animals provide powerful tools for investigating the biological function of specific gangliosides. We previously showed that ganglioside GD3 is a major species in embryonic mouse brain and it plays a crucial role in maintaining the self-renewal capacity of neural stem cells (NSCs) [2]. We further demonstrated that GD3 regulates EGF-induced NSC proliferation *in vitro* by interaction with EGFR in the lipid raft region. The interaction enabled EGFR to go through the recycling pathway for reutilization rather than to the lysosomal pathway for degradation [3]. Those findings provide evidence that the interaction of GD3 with EGFR is responsible for sustaining the expression of EGFR and its downstream signaling pathway to maintain the self-renewal capability of NSCs [3]. Recently, we studied neurogenesis in adult GD3-synthase knockout (GD3S-KO) mice and their wild-type littermates and found impaired neurogenesis in the subventricular zone (SVZ) and dentate gyrus (DG) of the hippocampus of GD3S-KO mice. This deficiency was characterized by a reduced number of bromodeoxyuridine (BrdU) incorporated cells in the SVZ and DG of GD3S-KO mice, and decreased nestin and GFAP in GD3S-KO brain sections. These differences are more pronounced in older mice (>6-months old) than in young mice (1-month old). Most interestingly, the KO animals exhibit a depression-like phenotype, as revealed by forced swimming and tail-suspension tests. We conclude that: 1) the brain of GD3S-KO mice exhibited defects in long-term neurogenesis *in vivo;* 2) the of EGFR in the NSCs, regulated by GD3, played a role in maintaining their self-renewal capacity, and 3) defects in long-term neurogenesis resulted in behavioral deficits in the GD3S-KO animals. (Supported by grants from VA Merit Award and NIH).

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(245) **Perturbing circulatory ST6Gal-I alters granulopoiesis in-vivo**

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Recent studies in our lab have begun to extend the traditional paradigm of glycosylation by describing an “extrinsic” mechanism, where extracellular glycosyltransferases modify existing cell surface glycans. The sialyltransferase ST6Gal-I, which is present in systemic circulation, can alter how hematopoietic cells interact with the extracellular environment by conferring an α2,6 sialic acid linkage extracellularly. Hematopoietic progenitors are dependent on cytokine interactions to instruct specific lineage differentiation, and G-CSF stimulates granulocyte production in bi-potent granulocyte-monocyte progenitors (GMPs). Mice with diminished circulatory ST6Gal-I levels have a more severe inflammatory response due at least in part to an elevated production of inflammatory cells in the marrow.

In order to elucidate the effects on hematopoiesis from a range of circulatory ST6Gal-I levels, we utilized 3 mouse models: Wild-type

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(Native), *St6gal1*-dP1 (Low) and a genetically modified, implantable B16-F10 skin tumor overexpressing circulatory ST6Gal-I into the wild-type mouse (High). We then utilized our multi-color flow panel to track the various hematopoietic lineages and correlated these changes to SNA (α2,6 specific lectin) reactivity in all subsets. Our results show that overall SNA reactivity in the bone marrow was reduced in the order of $B16^{ST6Gal\text{-}I} > WT > \Delta P1$ and that specific subsets in the marrow were either expanded or reduced based on extracellular ST6Gal-I levels and cell surface SNA reactivity. To that end, the amount of ST6Gal-I in circulation regulates a specific proliferative checkpoint in hematopoietic development such that high circulatory ST6Gal-I reduces the affected populations while low ST6Gal-I environments expand them. Furthermore, we found that serum ST6Gal-I correlated inversely with granulocyte production and lipopolysaccharide induced lung inflammation, and that GMPs treated with ST6Gal-I showed poor $CD11b^{+}Gr\text{-}1^{+}$ granulocyte differentiation in response to G-CSF *in vitro*.

Together, our data demonstrate that extrinsic ST6Gal-1 in serum is a systemic factor in regulating hematopoiesis. Manipulation of circulatory ST6Gal-1 levels may be leveraged for clinical benefit in instances where either recovery or attenuation of granulopoiesis is desired.

(246) **A crucial role of polysialic acid in developmental migration of cortical interneurons**

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The neural cell adhesion molecule NCAM and its modification with polysialic acid (polySia) are major determinants of brain development. Polysialylation of NCAM is implemented by the polysialyltransferases ST8SIA2 and ST8SIA4. Polysialyltransferase-negative mice are characterized by defects of major brain axon tracts and impaired migration of olfactory interneurons (Weinhold et al. 2005, JBC 280:42971; Hildebrandt et al. 2009, Brain 132:2831). Here we demonstrate reduced densities of parvalbumin-positive interneurons in the prefrontal cortex of ST8SIA2- or ST8SIA4-negative mice. Lower densities of genetically labelled interneurons were detected at postnatal day 1 indicating developmental defects. Acute enzymatic removal of polySia by endosialidase treatment of organotypic slice cultures attenuated the entry of interneurons into the embryonic cortex, caused slower migration and shorter leading processes. Moreover, in ST8SIA2- as well as in ST8SIA4-negative embryos accumulations of interneuron precursors and increased apoptosis were found in the medial ganglionic eminence, where cortical interneurons originate. Data obtained by live cell imaging support the assumption that altered interneuron migration within the cortex of ST8SIA4-negative mice is caused by a preselection of specific cell populations prior to their entry into the cortex. In conclusion, the attenuation of polySia interferes with developmental migration and causes pathological changes of cortical interneurons. Ongoing analyses of mice with interneuron-specific deletion of ST8SIA2 will shed light on a potentially cell-autonomous mechanism of polySia-dependent interneuron migration.

(247) **Genetic ablation of CMP-sialic acid synthetase results in an asialo phenotype and early embryonic lethality**

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Cellular Chemistry

The properties of cell surface glycans are commonly modulated by their terminal sugar, which in the majority of cases is the negatively charged sugar sialic acid (Sia) with Neu5Ac, Neu5Gc and KDN as the most prominent Sia members in vertebrates. The synthesis of sialoglycoconjugates requires the metabolical activation of Sia to CMP-Sia by the nuclear localized CMP-sialic acid synthetase (CMAS). CMP Sia is subsequently transported into the Golgi where sialyltransferases add Sia to nascent glycoconjugates. Sialoglycoconjugates are involved in a broad spectrum of cellular processes such as receptor-ligand interactions, cell adhesion, migration and proliferation. To analyze the role of Sia during mammalian development, we generated a Cmas knock-out mouse (Cmas-/-) and could show that a complete loss of CMAS results in an asialo phenotype and lethality around embryonic day 8.5. Cmas-/- mice show augmented Collagen IV deposit in the outermost basal membrane of the embryo, the Reichert's membrane, whereby the function of this extraembryonic membrane as filtration barrier of fetal-maternal waste and nutrient exchange is impaired. Although retarded in development, the embryo proper is able to undergo complex developmental processes like gastrulation and neurulation. Thus, malnutrition due to increased accumulation of extraembryonic basal membrane material most likely accounts for the lethal phenotype of Cmas-/- mice.

(248) **Molecular characterization of hiPSCs from a PMM2-CDG patient revealed aberrant glycosylation**

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PMM2-CDG, former known as Congenital Disorder of Glycosylation-Ia, is the most abundant form of the genetically heterogeneous CDG-diseases mainly affecting N-glycan synthesis [1, 2]. N-glycosylation is crucial for protein folding, stability and localization of proteins. Located at the cell surface, N-glycans are ideally positioned to contribute to inter- and intracellular signaling. A total loss of N-glycans is lethal [3]. PMM2-CDG affects diverse organs including brain, liver, blood or heart and can cause clinical symptoms like psychomotor retardation, coagulation abnormalities and cardiomyopathy for example. Currently no treatment is available and only little is known about the relationship of the defective PMM2 enzyme and the disease phenotype [3].

In order to better understand the impact of a reduced PMM2 activity on early human development, induced pluripotent stem cells

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(iPSC) from PMM2-CDG-patient's fibroblasts were generated by lentiviral gene transfer of the four transcription factors OCT-4, SOX2, KLF4 and c-Myc (OSKM) initially applied by Takahashi and Yamanaka [4].

These cells were positively tested for their genetic stability, their expression of classical pluripotency associated factors and differentiation capacity. On the transcriptomic level, PMM2-CDG-iPSCs are closely related to common pluripotent stem cell lines. N-glycomic analyses showed a "normal" N-glycan repertoire in PMM2-CDG-iPSCs with high-mannose type N-glycans as the predominant species. GNA-lectin staining of PMM2-CDG-iPSCs indicated a slight hypoglycosylation of proteins compared to control cell lines. Of note, mannosylation could be increased in PMM2-CDG-iPSCs by supplementation of 10 mM mannose to culture media. This observation coincides with the recent finding that dietary mannose supplementation in mice during pregnancy is of therapeutic importance for PMM2-CDG phenotypic outcome in offspring [5].

The PMM2-CDG-iPSC model has the potential to gain a deeper understanding about the role of glycosylation on stem cell level and during early human development.

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(249) Functional analysis of the expression of N-glycans in epithelial-mesenchymal transition: Importance of α2,6 sialylation

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Epithelial-mesenchymal transition (EMT) is a cellular transdifferentiation process that has been considered as a central step during carcinogenesis. In this process, a vast amount of knowledge has been obtained from the research examining EMT at the mRNA and protein levels. The target genes, as recently evidenced in several types of cancer cell lines by mass spectrometry and microarray analysis, at least include the glyco-genes involved in *N*-glycosylation, *O*-glycosylation and sialylation, indicating the importance of cellular glycosylation pattern in both the transition to and the maintenance of the mesenchymal state.

We have previously reported that TGF-β-induced EMT down-regulates the expression of *N*-acetylglucosaminyltransferase III (GnT-III) that is responsible for the synthesis of a bisecting GlcNAc in *N*-glycan, and up-regulates the expression *N*-acetylglucosaminyltransferase V (GnT-V), which catalyzes the β1,6 GlcNAc branching in *N*-glycan in EMT (Xu Q., et al., JBC. 2012). In fact, introduction of a bisecting GlcNAc suppresses β1,6 GlcNAc branching formation since GnT-V cannot utilize the bisected oligosaccharide as an acceptor, indicating the coordinated regulation of gene expression during EMT.

In the present study, we investigated the role of sialylation in EMT (Lu J., et al., submitted). In particular, we found that ST6GAL1 and α2,6 sialylation were specifically up-regulated during the TGF-β-induced EMT process. Knockdown of ST6GAL1 strongly suppressed TGF-β-induced EMT. Conversely, overexpression of ST6GAL1 increased the turnover of cell surface E-cadherin and promoted TGF-β-induced EMT. Furthermore, silencing ST6GAL1 partially reversed the basal mesenchymal phenotype of MDA-MB-231 human breast cancer cells. The expression of α2,6 sialylated *N*-glycans was also specifically induced by the oncogenic gene GOLPH3 (Isaji T., et al., JBC. 2014). Taken together, our study clearly demonstrates the requirement of ST6GAL1 for the sufficient induction of TGF-β-mediated EMT and maintenance of mesenchymal state, which may provide a plausible explanation for the up-regulated ST6GAL1 during malignant progression of multiple cancers.

(250) Effect of a polysaccharides from Crassostrea gigas against ethanol induced liver injury

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A water-soluble polysaccharide was isolated from Crassostrea gigas (CGPS-1) by hot-water extraction. Based on the calibration with Dextran, the CGPS-1 had a molecular weight of about 3.93-10.84x106 Da. CGPS-1 was analyzed by high-performance liquid chromatography, Fourier-transform infrared and 13C nuclear magnetic resonance spectroscopy. Based on the data obtained, CGPS-1 was found to be a uniform glucose polymer. CGPS-1 was subjected to investigate the protective effect on ethanol induced liver injury in Kunming mice. The CGPS-1 could decrease serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), and malondialdehyde aldehydes (MDA) leves, increase the superoxide dismutase (SOD) activity, and improve hepatic injury in the ethanol induced liver injury in mice. The work was supported by grant from the Marine Public Welfare Research Project (201405017-03).

(251) The crystal structure of human UDP-glucose pyrophosphorylase*UDP-glucose complex gives new insight into substrate binding and enzymatic mechanism

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In all kingdoms of life, UDP-glucose pyrophosphorylase (UGP; EC 2.7.7.9) interconverts glucose-1-phosphate (Glc-1-P) and uridine triphosphate (UTP) into UDP-glucose (UDP-Glc) and pyrophosphate (PPi). UDP-Glc, constituting the activated form of glucose, is of central importance in carbohydrate metabolism since it is involved in a multitude of cellular pathways: as the precursor of the activated sugars UDP-galactose and UDP-glucuronic acid, it is essentially involved in lactose synthesis and in glucuronidation, respectively. In addition, these two nucleotide sugars - together with UDP-Glc itself - are also utilized for the synthesis of cell surface glyoconjugates, which play crucial roles in the interaction of cells with their surroundings. Furthermore, UDP-Glc serves as a substrate for glycoprotein folding control, and is the building block of glycogen, the main storage form of glucose in human muscle and liver. Despite its central position in human carbohydrate metabolism, the human UGP (hUGP) has not been extensively characterized so far. Importantly, the functional relevance of the octameric assembly of hUGP is not yet understood, and the active site has not fully been explored due to the lack of structural data that include substrates or products. We therefore crystallized hUGP isoform 1 in complex with UDP-Glc, allowing us to identify the active site residues responsible for product binding, whose functional importance was then confirmed by site-directed mutagenesis. Analysis of the octameric structure of hUGP in complex with UDP-Glc revealed previously unknown allosteric intermolecular interactions, formed by amino acid residues that are only conserved in octameric UGPs from the animal and fungal kingdoms. We could demonstrate in a mutational approach that these residues are essential for hUGP activity, although not involved in oligomerization. Furthermore we could demonstrate that the octameric assembly provides increased protein stability in the wild-type enzyme, compared to hUGP variants displaying lower oligomerization states induced by mutation. We propose that octamerization is essential for hUGP function because it increases protein stability and enables an allosteric intermolecular interaction between neighboring hUGP subunits, which may be a common concept in oligomeric nucleotidyltransferases.

(252) **A proteomic approach to investigate CSL effects in yeast cells**

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A Ca^{2+}-dependent GalNAc/Man-specific lectin (CSL) from *Cyclina sinensis* was isolated, with data on its stimulating action on yeast. CSL shows potent effect on ethanol production by *Saccharomyces cerevisiae*. In this work, the changes in protein expression profiles after 24 h of incubation *S. cerevisiae* with CSL were analyzed using Label-free quantitative proteomics. 1410 proteins were identified, only 110 proteins were significantly different in the normalized volume ($P < 0.05$). Among the preselected proteins there were 26 up-regulated and 84 down-regulated proteins. Analysis of the proteome revealed that CSL triggered the concentration changes of some enzymes, including hexokinase, glyceraldehyde 3-phosphate dehydrogenase, enolase, dihydrolipoamide dehydrogenase and aldehyde dehydrogenase. The western blotting analysis of the differentially-expressed enzymes are under progress in our laboratory. The work was supported by grant from the Marine Public Welfare Research Project (201205022-7).

(253) **Expression Analysis of Hyaluronidases in the Mouse Brain during Development**

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Chondroitin sulfate (CS) chains have been demonstrated to play important roles in brains including neural network formation, promotion of neurite outgrowth, and cortical plasticity during a critical period in early life. The cellular degradation of CS occurs predominantly in lysosomes. Hyaluronidases, hyaluronan (HA)-degrading enzymes, are considered to act at the initial stage of the degradation process of CS, because HA is similar in structure to nonsulfated CS, chondroitin. The preferred substrate of hyaluronidase-1 (Hyal1) had been expected to be HA rather than CS. However, we demonstrated that HYAL1 hydrolyzed CS at a higher velocity than HA at pH 4.0 - 4.5 [Honda *et al.*, Biomolecules, 2012]. Furthermore, we identified hyaluronidase-4 (Hyal4) to be a CS-specific endo-β-*N*-acetylgalactosaminidase that hardly degraded HA at all [Kaneiwa *et al.*, Glycobiology, 2010]. Although CS distributes in brains relatively in a large amount, no hyaluronidase family members are expressed in the brain of adult mice. The catabolic mechanism of CS in brains has not been clarified and endoglycosidase(s) responsible for the CS catabolism in brains has not been identified. In this study, the expression of hyaluronidase family members in the mouse brain during development has been investigated in detail. Total RNAs were extracted from the brain samples at embryonic day 15 (E15), E17, postnatal day 0 (P0), P7, P14, week 3 (3W), and 7W, and the cDNAs were synthesized. Using specific primers for *Hyal1*, *hyaluronidase-2* (*Hyal2*), *hyaluronidase-3* (*Hyal3*), or *Hyal4*, quantitative real-time PCR was performed. The expression level of each mRNA during development was normalized to that of the transcript of *glyceraldehyde 3-phosphate dehydrogenase*. *Hyal2* as well as *Hyal3* showed higher expression levels among hyaluronidase family members. Although the degradation activity of Hyal2 and Hyal3 toward CS has not been detected, Hyal2 and/or Hyal3 may play the indispensable role on degradation of CS in the mouse brain. The possibilities cannot be excluded that other protein(s) without sequence similarity to hyaluronidases may

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mainly hydrolyze CS in brains. Investigation of the enzyme responsible for CS catabolism in brains is in progress.

(254) Metabolically programmed quality control system for dolichol-linked oligosaccharides

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The fully assembled dolichol-linked oligosaccharide (DLO; $Glc_3Man_9GlcNAc_2$-PP-dolichol) serves as the glycosyl donor substrate for asparagine (*N*)-linked glycosylation in mammals. We found that low-glucose environment causes premature degradation of DLO during the early stage of DLO biosynthesis by a putative pyrophosphatase, releasing phosphorylated oligosaccharides (POSs) into the cytosol [1]. We identified that low-glucose supply resulted in a substantial reduction of the level of GDP-Man, which serves as an essential sugar donor substrate for DLO biosynthesis. We further showed that the selective shutdown of GDP-Man biosynthetic pathway is sufficient to induce POS release. Our results thus indicate that low-glucose environment causes the biosynthetic arrest of DLO by downregulating GDP-Man biosynthetic pathway and facilitates the degradation of aberrant DLO intermediates by the pyrophosphatase. We propose that this degradation process functions as a quality control system to avoid abnormal *N*-glycosylation under conditions where efficient DLO biosynthesis is impaired.

Reference

1. Harada, *et al*. (2013) Metabolically programmed quality control system for dolichol-linked oligosaccharides. *Proc. Natl. Acad. Sci. U. S. A*. 110: 19366–19371.

(255) Seminolipid is required for transfer of MCT4 from Sertoli cells to the spermatocyte plasma membrane where MCT4 forms a functional lactate transporter assembly with basigin

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Seminolipid is a testis-specific sulfoglycolipid and biosynthesized by galactosylceramide sulfotransferase (CST). Disruption of either of the CST gene (*Cst*) or the basigin gene (*Bsg*) in mice results in male infertility due to arrest of spermatogenesis at a late stage of the prophase of the first meiosis. The similarity in the phenotypes of both mutant mice prompted us to investigate association between seminolipid and basigin in spermatocytes. Seminolipid was co-localized and associated with basigin on the plasma membrane in spermatocytes. We further found that monocarboxylate transporter 4 (MCT4) was associated with basigin in spermatocytes. Unexpectedly, mRNA of MCT4 was not expressed in the germ cells but in Sertoli cells. On the other hand, MCT4 protein was robustly observed in the wild-type germ cells, implying that it is transferred from Sertoli cells. The MCT4 protein level was significantly reduced in the *Cst*-null spermatocytes, whereas it was preserved in the *Bsg*-null spermatocytes. In terms of MCT4 function, uptake of lactate, the primary energy substrate for spermatocytes, was significantly deteriorated in the *Cst*-null and the Bsg-null spermatocytes. The *Cst*-null and the *Bsg*-null spermatocytes were vulnerable to cell death under a lactate-dependent condition, to which condition spermatocytes are exposed *in vivo*. These results indicate that a shortage of energy source supply causes death of spermatocytes in the *Cst*-null and the *Bsg*-null mice, and suggest that seminolipid is required for transfer of MCT4 to the spermatocyte plasma membrane where MCT4 forms a functional complex with basigin.

(256) Fidelity in developmental patterning requires O-GlcNAc transferase

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NIDDK, NIH

Cell signaling, protein turnover, and gene expression are, in part, controlled by the dynamic posttranslational modification O-linked N-acetylglucosamine (O-GlcNAc). Addition and removal of O-GlcNAc to and from serine and threonine residues is controlled by the enzymes O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively. Data from our lab and others suggests that proper O-GlcNAcylation is critical for appropriate organismal development. Indeed, OGT is required for Polycomb group (PcG) homeotic gene repression and C. *elegans ogt-1* is known to genetically interact with players in Hox gene regulation including *bar-1* and *pal-1*, the homologs of beta-catenin and Drosophila caudal, respectively. Furthermore, in *C. elegans*, ogt-1resides on chromosome III directly upstream of the Hox gene cluster, suggesting an evolutionarily conserved role linked to development.

Given these findings, we hypothesize that O-GlcNAc "fine-tunes" the expression of genes required for the fidelity of body plan development. Utilizing the *C. elegans* model organism, we explored several phenotypes to determine whether loss of *ogt-1* and *oga-1* influence development of characteristic hermaphrodite and male body segments. Importantly, loss of solely OGT-1 or OGA-1 activity yielded mutants that lacked gross physiological phenotypes. In contrast, hermaphrodites lacking *bar-1* display a protruding vulva phenotype linking Wnt signaling to vulva precursor cell fate specification. Interestingly, animals lacking both OGT-1 and BAR-1 activities have an everted gonad, or spew phenotype, suggesting an important intersection between the nutrient-responsive OGT-1 and Wnt signaling. Moreover, while male *C. elegans* lacking either *bar-1* or *ogt-1* alone have negligible V6 male ray development defects, *bar-1;ogt-1* double mutants display up to 43% deviant V6 rays.

Our data highlight that O-GlcNAc plays a novel, indispensable role in *C. elegans* developmental patterning. We suggest that the

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addition of O-GlcNAc may play a key role in regulating PcG chromatin proteins required for early patterning of Hox gene repression in *C. elegans*.

(257) Heavy metals removal of crab shell powder from scallop byproducts hydrolyzate

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Crab shell powder after acid treatment could significantly reduce the content of Cr and Pb, but it slightly impacted on the content of Cd. Acid treated crab shell powder had better removal efficiency to heavy metals on the scallop byproducts hydrolyzate than untreated sample. Under the conditions as dosage of 3g/L, pH of 5 ~ 6, adsorption time of 90 min and temperature of 25°C, the removal rate of Cr, Cd, Pb of the hydrolyzate was 49.7%, 77.6% and 78.1%, respectively.The adsorption capacity increased with the temperature increased between 10°C to 50°C, which indicated that chemical adsorption dominated. The adsorption isotherm was conformed to the Langmuir equation. The pattern of adsorption were single molecular adsorption and the adsorption kinetic process conformed the quasi second order kinetic model. Under the conditions of 1bv/h flow velocity and 2 h, the adsorption capacity of crab shell powder to Cr, Cd, Pb was 0.378 mg, 0.323 mg/g and 0.464 mg/g respectively. And the removal rate of Cr, Cd and Pb reached 83.1%, 99.3% and 99.5% respectively.Crab shell powder could perfectly remove the hydrolyzate of Cr, Cd and Pb by quantitative column tests, which removal rates of them were almost 100%. And the retention rate of protein and amino acid nitrogen was 94.70% and 96.53%, respectively. But the adsorption performance was poor after rebirth.

Key words: Crab shell powder, heavy metal, removal

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(258) The hypolipidemic effect of fucoidan extracted from sargassum fusiforme with comparison with those from other brown seaweed

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Sargassum fusiforme Fucoidan extracted by composite enzyme method was taken as raw material, its hypolipidemic effect in mice with comparing with those of fucoidan from Laminaria Jjaponica, Undaria pinnitafida, Japanese Kjellmaniella crassifolia and Chinese Kjellmaniella crassifolia was studied. The contents of total polysaccharide and $SO4^{2-}$ were determined for five brown seaweed which showed that the total polysaccharide were 60.74%, 61.56%, 54.91%, 55.27% and 69.66% for Sargassum fusiforme, Laminaria Japonica, Undaria pinnitafida, Japanese Kjellmaniella crassifolia and Chinese Kjellmaniella crassifolia respectively. The contents of $SO4^{2-}$ were 27.03%, 20.43%, 19.52%, 31.58% and 25.17% for five corresponding seaweed.

The results obtained from animal test indicated that serum TC contents of mice were decreased for all the fucoidan groups with different doses from five brown seaweed, while high dose groups of Sargassum fusiforme, Laminaria Japonica and Japanese Kjellmaniella crassifolia significantly reduced the mice serum TC levels. The serum TG contents of mice were significantly decreased for the fucoidan extracted from Sargassum and Undaria pinnitafida. The levels of LDL for all the fucoidan samples with different doses from five brown seaweed were reduced significantly. All the MDA contents were inhibited significantly for the fucoidan groups with different doses except those from Undaria pinnitafida, while all the hepatic GSH-Px activities were increased except the low dose group of the fucoidan from Sargassum fusiforme. Fucoidan from Chinese Kjellmaniella crassifolia with high and low doses improved the mice hepatic SOD activities, while only low doses of fucoidan from Sargassum fusiforme and Undaria pinnitafida significantly improved the activities of mice SOD activities. Significant elevating effect on hepatic SOD activities were also obtained for the high fucoidan dose groups fuocoidan from Laminaria Japonica and Japanese Kjellmaniella crassifolia. Therefore, the results indicated that these fucoidan from five kinds of brown seaweeds had antioxidation and hypolipidemic effects.

Key words: brown seaweed, fucoidan, hypolipidemic effect

(259) Evaluation and implementation of iAB-N-glycan analysis for characterization of therapeutic proteins

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Merck

N-linked glycan profile is an important attribute for therapeutic proteins. Variation in glycosylation can greatly impact the function of the protein drugs. Analytical tools that can accurately assess the glycan profile are very important for characterization during the drug development. This study showcases the development and implementation of a high-throughput UPLC-based method for N-glycan characterization using Instant AB™ technology (Prozyme) across multiple testing groups and sites. The Instant AB (iAB) UPLC is a rapid and cost-effective method, which speeds up the sample preparation to less than one day compared to the 3-4 days required for the traditional 2AB-labeling method. Two methods (iAB-labeled UPLC and 2AB-labeled UPLC) were compared using several different therapeutic proteins. The study showed that iAB labeling minimizes sialic acid hydrolysis from N-glycans relative to 2AB labeling. iAB-UPLC has comparable glycan profile, sensitivity, and robustness as 2AB-UPLC method, tested across 2 laboratories and different analysts. In addition, we show that iAB-labeled glycans can be characterized by mass spectrometry. The comprehensive comparison indicate that iAB-UPLC glycan assay is an excellent platform that can be used to support upstream process development (e.g., clone selection, variation of bioreactor control) through late-stage drug substance/drug product characterization or GMP/QC release testing (e.g., comparability studies, batch analysis).

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(260) **Alcohol-induced impairment of asialoglycoprotein receptors in hepatocytes is triggered by non-muscle Myosin IIA-mediated Golgi fragmentation**

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Chronic alcohol abuse and alcoholism are associated with alcoholic liver disease and high morbidity. Altered protein trafficking and glycosylation, and increased apoptosis have been reported in ethanol-exposed liver cells in vitro and in vivo. But the mechanism remains unresolved. Recently, we discovered that interaction of non-muscle myosin IIA (NMIIA), a motor protein, with the cytoplasmic tail of Golgi glycosyltransferases (GTs) is responsible for the stress-induced Golgi fragmentation (Petrosyan & Cheng, Cell Stress Chaperones 19:241, 2014). Also, the efficiency of this phenomenon is dependent on the levels of Golgi GTs (Petrosyan & Cheng, Glycobiology 23:690, 2013). Here, we report that ethanol treatment (25 mM) induces Golgi fragmentation in VA-13 cells, which are HepG2 cells expressing alcohol dehydrogenase (ADH), but not VA-13 cells treated with the ADH inhibitor, pyrazole, or wild-type HepG2 cells (Wu et al. Toxicol Appl Pharmacol 216:238, 2006). Ethanol treatment reduces giantin, a Golgi matrix protein serving as a Golgi targeting site for most GTs, but increases NMIIA and Golgi-specific GTPase (Rab6a) and interaction of NMIIA with Golgi GTs. Further, after 72 h treatment of VA-13 with ethanol, the levels of GTs are reduced by elevated proteasomal degradation. Also, alcohol treatment of VA-13 cells and rat hepatocytes changes the distribution of asialoglycoprotein receptors (ASGP-R) from Golgi and endoplasmic reticulum (ER) to exclusively ER. This observation is supported by the switch of ASGP-R-associated N-glycans from α2,6-sialylated in control cells to high-mannose type in alcohol-treated cells. Interestingly, the same results are obtained in VA-13 cells after siRNA silencing of giantin. These data indicate that Golgi targeting of ASGP-R and its subsequent transport to the plasma membrane require functioning giantin and intact Golgi. Significantly, ethanol-induced Golgi fragmentation is characterized by apoptotic chromosomal DNA fragmentation and elevated caspase-3, but inhibition or knockdown of NMIIA prevents ethanol-induced Golgi disorganization and ASGP-R mislocalization. Given that ASGP-R-deficient hepatocytes are more susceptible to ethanol-induced apoptosis (Dalton et al. Biochem Pharmacol 65:535, 2003), we conclude that alcohol exposure impairs trafficking of ASGP-R by induction of Golgi fragmentation, thereby facilitating apoptosis of hepatocytes. (The work is supported by VA Merit Award 1I1BX000985).

(261) **Altered N–Glycan Expression Profile between Two Functionally Distinct Human Marrow Stromal Cell Lines Revealed by an Integrated Strategy Using Mass Spectrometry and Glycogene and Lectin Microarray Analysis**

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Two bone marrow stromal cell lines HS-5 and HS-27a presented significant functional differences when co-cultured with myeloma cells (KG1a) in studying the molecular mechanism of Myelodysplastic syndrome (MDS). An integrated strategy with the combination techniques of genomics, proteomics and glycomics were applied to analyze the differences of N-glycan expression between two cell lines. N-glycan profiles were analyzed by Mass spectrometry-based quantitative techniques and lectin microarray, and verified by lectin staining. Expression of N-glycan related genes were analyzed with glycogene microarray, and validated by quantitative PCR. Stable isotope labeling (SILAC) method combined with LC-MS/MS for whole cell protein levels was analyzed, and validated by Western Blot. The findings presented that levels of bisecting GlcNAc N-glycans were higher in HS-5 cells, whereas bi, tri-antennary, tetra- antennary N-glycans, and core fucosylation were higher in HS-27a. Low expression of MGAT3, β-1,4-mannosyl-glycoprotein 4-β-N-acetylglucosaminyltransferase 3, may result in low expression of bisecting GlcNAc N-glycan in HS-27a. High level of β4GalT1? β-1,4-galactosyltransferase 1, may cause the more complex-type glycan expression in HS5a. The results provided essential information for further study of functional role of glycogene, glycosyltransferase and N-glycan expression in bone marrow microenvironment on cell proliferation and apoptosis signaling pathways.

(262) **Endogenous glucuronyltransferase activity of LARGE or LARGE2 required for functional modification of alpha-dystroglycan in cells and tissues**

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LARGE gene have been identified in congenital muscular dystrophy (CMD) patients with brain abnormalities. Both LARGE and its paralog, LARGE2 are bifunctional glycosyltransferases with xylosyltransferase (Xyl-T) and glucuronyltransferase (GlcA-T)

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activities, and are capable of forming polymers consisting of [-3Xyl-α1,3GlcAβ1-] repeats. LARGE-dependent modification ofα-dystroglycan (α-DG) with these polysaccharides is essential for the ability of α-DG to act as a receptor for ligands in the extracellular matrix. Here we report on the endogenous enzymatic activities of LARGE and LARGE2 in mice and humans, using a newly developed assay for GlcA-T activity. We show that normal mouse and human cultured cells have endogenous LARGE GlcA-T, and that this activity is absent in cells from the *Large*^{myd} (*Large*-deficient) mouse model of muscular dystrophy, as well as in cells from CMD patients with mutations in the *LARGE* gene. We also demonstrate that GlcA-T activity is significant in the brain, heart, and skeletal muscle of wild-type and *Large2* null mice, but negligible in the corresponding tissues of the *Large*^{myd} mice. Notably, GlcA-T activity is substantial, though reduced, in the kidneys of both the *Large*^{myd} and *Large2* null mice, consistent with the observation of α-DG/laminin binding in these contexts. Finally, our assay can be applied to cryosections, of both mouse and human skeletal muscle biopsies, and thus may have useful clinical applications. Our results provide the first direct evidence that not only LARGE, but also LARGE2, is vital to effective functional modification of α-DG *in vivo*.

(263) Mutational studies of an endo-beta-N-acetylglucosaminidase from Ogataea minuta (Endo-Om)

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Endo-β-N-acetylglucosaminidase (ENGase) digests the *N,N′*-diacetylchitobiose core of asparagine-linked oligosaccharides on glycopeptides and glycoproteins. The gene encoding the ENGase from *Ogataea minuta* (Endo-Om) was cloned previously (Murakami et al., Glycobiology, 2013). In this study, we constructed a new Endo-Om expression system by *Escherichia coli* due to rapid growth and rapid expression of proteins. The recombinant protein was overexpressed in *E. coli* BL21(DE3) with pLysS, and the ENGase activity against fluorescent-labeled oligosaccharides was detected by using the soluble fraction of the cells expressing Endo-Om. However, the majority of the recombinant proteins remained in the insoluble fraction. The recombinant Endo-Om digested high-mannose and biantennary *N*-linked glycans, but did not hydrolyzed tetraantennary *N*-glycans. Endo-Om was able to hydrolyze *N*-glycans attached to RNase B and human transferrin under both denaturing and non-denaturing conditions.

We also isolated another ENGase in *Candida parapolymorpha*, and termed it Endo-Cp. Endo-Om and Endo-Cp shared the conserved catalytic domain among GH family 85 ENGases in the N-terminal region and no consensus sequence was observed in the C-terminal region. Endo-Om showed higher specific activity than Endo-Cp, whereas Endo-Cp was more soluble than Endo-Om. To improve the solubility of Endo-Om, we made a chimeric protein that consists of the N-terminal region from Endo-Om and the C-terminal region from Endo-Cp. The His-tagged chimeric protein (named Endo-OC) was expressed in *O. minuta* or *E. coli*, and purified by the Ni-affinity column chromatography. Although Endo-Om required a high salt buffer containing 0.5 M NaCl for its solubility, Endo-OC was still active even in the low salt buffer. The optimal pH, optimal temperature and substrate preferences of Endo-OC were almost same as those of Endo-Om. A truncated Endo-Om, in which 92 C-terminal amino acids were deleted, showed no enzymatic activity.

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(264) Epigenetic regulation of glycosyltransferase

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Glycans are secondary gene products, and their expression largely depends on specific primary gene products (polypeptides) such as glycosyltransferases, glycosidases and sugar-nucleotide transporters. Although most of the glycogenes encoding these proteins have been identified, it is still poorly understood how these glycogenes are regulated in mammals.

Epigenetics, which is a gene regulation system without any change in DNA sequence, is one of the clues to understand the mechanisms by which glycogenes are regulated. Epigenetics is now vigorously studied in various fields, but little is known about epigenetic regulation of glycans or glycogenes. We recently revealed that a brain-specific glycosyltransferase, *GnT-IX(Mgat5b)*, is under control of epigenetic histone modifications (Kizuka et al., 2011, *J. Biol. Chem.*, 286, 31875-84). In addition, we identified two specific chromatin modifiers, HDAC11 and OGT-TET3 complex, that specifically modify histones around *GnT-IX* gene (Kizuka et al., 2014, *J. Biol. Chem.*, 289, 11253-61). In this study, we extended such epigenetic analysis to a large number of glycosyltransferases. Also, by using epigenetic drugs we tried to clarify epigenetic mechanisms of glycogene regulation in primary cells but not in cancer cell lines, and explored the mechanisms involving novel epigenetic factors for glycan regulation such as microRNAs. This work may provide a new concept to understand the cellular mechanisms of glycan expression.

(265) The Enzymes of the O-GlcNAc Cycling: Writers AND Readers of the Histone Code?

Katryn Harwood, John Hanover
NIH/NIDDK

Epigenetic regulation of gene expression is essential for a multitude of processes in an organism and aberrant transcription can result in disease development. Better understanding of how transcriptional profiles are regulated, particularly in response to external signals such as nutrient supply, could aid in the development of methods to detect, treat, or prevent disease. One way cells regulate gene expression is through posttranslational modification (PTM) of histones. An often-overlooked PTM is the *O*-GlcNAcylation of serine/threonine residues. *O*-GlcNAc is added and removed by a single pair of enzymes, *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA),

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respectively. *O*-GlcNAc addition is the final step of the nutrient driven hexosamine-signaling pathway, and as such it is likely that *O*-GlcNAc addition/removal informs upon an organism's nutritive state. Interplay between *O*-GlcNAcylation and other PTMs on histones may allow cells to fine-tune transcriptional activity in response to cellular cues. To examine this possibility, we are focusing on the interaction of OGA and OGT with both known modifiers of histone PTMs and modified histones themselves. Previous studies suggest that OGT plays a role in down regulating gene expression by binding and *O*-GlcNAcylating the SIN-3 histone deacetylase complex. We recently demonstrated through transcriptional analysis in *Caenorhabditis elegans* that about half of the genes that are deregulated when OGA or OGT are absent are also deregulated when SIN-3 is absent. We are taking a genetic approach using these knockout *Caenorhabditis elegans* strains to further characterize the relationship between the enzymes of *O*-GlcNAc cycling, SIN-3 and histone acetylation. These studies will provide insight into how the nutritive state is translated into changes in transcriptional profiles on the level of an entire organism. In addition, OGA is known to possess a putative histone acetyltransferase (HAT) domain, prompting us to question what role it might play in OGA/histone interactions. Using recombinant OGA protein and differentially modified histone tail peptides, we have identified specific modifications with well known roles in transcriptional regulation to which OGA exhibits binding selectivity. Further characterization of theses binding interactions will allow for the us to begin "decoding" the intricate relationship between PTMs, transcriptional regulation, and intermediary metabolism.

(266) Generation of Immortalized MEF cell lines to study O-GlcNAc Metabolism and Neurodegeneration

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O-GlcNAcylation is an abundant post-translational modification in which the monosaccharide β-*N*-acetyl-D-glucosamine (O-GlcNAc) is added to and removed from Ser/Thr residues by O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively. Signaling and metabolism are affected dramatically in null alleles of OGT and OGA in *C. elegans* and *D. melanogaster*, thus suggesting O-GlcNAc metabolism serves an essential function. Further, O-GlcNAc cycling has been linked to a variety of diseases including insulin resistance, tumorigensies, cancer, and neurodegeneration. However, the implications of O-GlcNAc metabolism are difficult to study in vertebrates as conditional knockout mutants of the enzymes of O-GlcNAc cycling result in low viability of embryos or embryonic lethality. OGA and OGT knockout mutants have been generated in mice, and primary MEFs have been used to study O-GlcNAc cycling. However, use of primary MEFs is time consuming and results can be difficult to replicate. Here we generated immortalized wild type, OGA null allele, and OGT floxed allele MEF cell lines to study O-GlcNAc metabolism. We demonstrate the utility of these cell lines by using the transformed cell lines to study a known tauopathy (P301L) and the effects of O-GlcNAc metabolism on proteostasis.

(267) Identification of novel nucleotide sugar transporters in plants and animals

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Glycans are synthesized by glycosyltransferases most of which are located in the lumen of the Golgi and ER, and which use nucleotide sugars largely produced in the cytoplasm as substrates. Therefore, the transport of nucleotide sugars into the lumen of Golgi and ER is critical for glycan biosynthesis, and it is mediated by a family of specific nucleotide sugar transporters (NSTs). The human genome encodes 31 NSTs, most of which have unknown function. We have investigated the 44 NSTs encoded in the Arabidopsis genome in vitro and in vivo. The transporters were expressed in yeast and incorporated into proteoliposomes for activity studies. Uptake of nucleotide sugars by the proteoliposomes was dependent on counter transport of UMP or GMP and was quantified by LC-MS/MS. With this approach we identified the activity of more than 20 different transporters including six bifunctional UDP-L-rhamnose / UDP-D-galactose transporters (URGTs), three bifunctional UDP-D-xylose / UDP-L-arabinopyranose transporters, six UDP-L-arabinofuranose transporters, two UDP-D-apiose transporters, and two GDP-D-mannose transporters. Most of these NSTs were shown to be located in the Golgi apparatus. In addition we identified a novel human UDP-xylose transporter, which is unrelated to the previously identified mammalian UDP-xylose transporters. Plants with mutation in or overexpression of the main UDP-Rha / UDP-Gal transporter, URGT1, show changes in galactan content, but not in galactosylation of xyloglucan. Likewise, plants with altered expression of UDP-xylose transporters showed changes in xylan content but not in xyloglucan. These observations indicate that the transporters appear to have a more limited specificity in vivo than in vitro. This could be due to substrate channeling or kinetic differences between the glycosyltransferases.

(268) Give up sugar with this one weird trick!

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Asparagine-linked glycosylation is a common post-translational modification of proteins that promotes productive protein folding and increases protein stability. Although N-glycosylation is important for glycoprotein folding, the precise sites of glycosylation are often not conserved between protein homologues. We have shown that, in *Saccharomyces cerevisiae*, proteins upregulated during

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sporulation under nutrient deprivation have few N-glycosylation sequons and in their place tend to contain clusters of like-charged amino-acid residues. Incorporation of such sequences complemented loss of *in vivo* protein function in the absence of glycosylation at particular sites in yeast. Targeted point mutation to create such sequence stretches at glycosylation sequons in several model glycoproteins increased *in vitro* protein stability and activity. A dependence on glycosylation for protein stability or activity can therefore be rescued with a small number of local point mutations, providing evolutionary flexibility in the precise location of N-glycans, allowing protein expression under nutrient-limiting conditions, and improving recombinant protein production.

(269) N-glycan sialylation in silkworm protein expression system and application

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Background and purpose: Most human proteins (>50%) contains covalently linked sugar chains, which controls to functions, stability, activity and solubility of the proteins. Recently, it is known that alterations in the glycosylation pattern are a promising biomarker which contributes to clinical diagnosis. On the other hand, the difference of the sugar chain in the protein drugs influences the drug controls, such as the point of ADCC (Antibody-dependent cell mediated cytotoxicity), half-life time in blood, and the targeting in DDS (Drug delivery system). Therefore, glycosylation control of proteins is needed in diagnostics and drug development.

Baculovirus-silkworm recombinant protein expression system has post-translational modification pathway and can produce bioactive recombinant proteins. Normally, *N*-linked glycans added in silkworm recombinant proteins are mostly pauci mannose type, not sialylated complex type which is often added to human proteins.

As the first step for the regulation of glycosylation, we tried to develop *N*-glycan sialylation system in silkworm protein expression system, and evaluated the functions of the sialylated recombinant protein produced by this system. Methods: Several recombinant baculoviruses and transgenic silkworms introduced *N*-glycan-related genes containing sialyltransferase gene were constructed. ALP (alkaline phosphatase) as target protein was co-expressed with these baculoviruses or transgenic silkworms. Sialic acid substrate was injected directly to silkworm from the outside. The fluid was collected and the purified recombinant ALP was analyzed the glycan structures by lectin-blot and mass spectrometry, and evaluated the activity and stability. Results: » Sialylated complex type sugar chain was confirmed by lectin-blot and mass spectrometry.

» The activity of the sialylated recombinant ALP (Sia-ALP) was twice higher than the non-sialylated recombinant ALP (Normal-ALP). When Sia-ALP was purified by SNA lectin (specific to terminal silaic acid), the activity increased to the same level as native ALP (calf small intestine origin). And then pH and temperature stability of the SNA-lectin purified Sia-ALP was almost equivalent to native ALP. Conclusion: » We could add sialylated complex type sugar chain to recombinant proteins expressed in silkworm.

» The improvement of the recombinant ALP in activity and stability are expected by adding sialylated complex type sugar chain to recombinant proteins.

(270) Enzymatic properties of Golgi α-1,2 mannosidases toward denatured glycoproteins

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Alpha-1,2 linked mannoses in *N*-glycans of newly synthesized glycoproteins were sequentially removed by concerted actions of α-1,2 mannosidases in endoplasmic reticulum (ER) and Golgi apparatus of living cells. Those enzymes belong to glycosyl hydratase family 47 which includes, in mammals, ER a-1,2 mannosidase I (ERManI), Golgi α-1,2 mannosidase IA, IB & IC (GolgiManIA, IB & IC), and three EDEM proteins. Catalytic properties of ERManI and the Golgi enzymes have been studied using oligosaccharides to prove preferred utilization of B-chain and the other chains of high-mannose type *N*-glycans, respectively. The properties, however, had scarcely been reported using glycoproteins as substrates.

We had chemically synthesized *N*-glycans by our convergent methods and utilized them to study biochemical properties of proteins which engaged in ER quality control, *e.g.* calreticulin [1] and UDP-glucose:glycoprotein glucosyltransferase [2]. We further extend our interests to α-1,2 mannosidases which have some and still debating roles in the quality control. For this purpose, natural glycoproteins possessing high-mannose type *N*-glycans were first used to investigate properties of those enzymes.

We have prepared human ERManI [3] and GolgiManIA & IB as recombinant forms using *Escherichia coli*. First, $Man_9GlcNAc_2$-posseing soybean agglutinin (SBA) and bovine thyroglobulin (bTg) were used as substrates for de-mannosylation reaction of one hour at 37°C. GolgiManIA & IB could remove only a small fraction of mannoses in SBA even when they were forced to be denatured, while ERManI had shown to more efficiently remove mannose moieties from denatured SBA and bTg [3]. Second, $GlcMan_9GlcNAc_2$-posseing chicken immunoglobulin Y (IgY) and *Aspergillus oryzae* β-galactosidase (Aoβgal) were used as substrates. ERManI had shown to remove more mannose moieties from heat-treated IgY and Aoβgal to yield $GlcMan_{8B}GlcNAc_2$ [4]. Similarly, GolgiManIA & IB removed a mannose in $GlcMan_9GlcNAc_2$ to give $GlcMan_{8C}GlcNAc_2$ in denatured IgY. Collectively, GolgiManIA & IB as well as ERManI preferentially removed mannoses from denatured glycoproteins. These properties of α-1,2 mannosidases might contribute to selecting denatured glycoproteins for disposal. Catalytic properties of mannosidases using oligosaccharides would also be presented.

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(271) Pancreatic α-amylase controls glucose assimilation in duodenum through N-glycan-specific binding, followed by endocytosis and degradation

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α-Amylase (EC 3.2.1.1), a major pancreatic protein, is a key enzyme in energy acquisition because it catalyzes the initial step in hydrolysis of starch for glucose production. In the previous study, we showed that pancreatic α-amylase binds specifically to glycoprotein *N*-glycans [1] in the brush-border membrane (BBM) to activate glucose production with sucrase-isomaltase, while the bound α-amylase significantly inhibits glucose uptake by Na^+/glucose cotransporter 1 (SGLT1) at high concentrations equal to that in the intestine after eating [2]. It suggested that α-amylase plays a key role in regulating glucose assimilation for blood homeostasis. In this study, we show where α-amylase is destined and how a new control mechanism of the postprandial blood glucose homeostasis is achieved by *N*-glycan-specific interaction of α-amylase.

Immunohistochemistry revealed the α-amylase in the enterocytes of duodenums of non-fasted pigs, but little in fasted ones, α-amylase had been internalized from the pancreatic fluid and was immunostained. Time-lapse confocal laser microscopy revealed that after *N*-glycan binding in BBM, pancreatic α-amylase underwent internalization into lysosomes in a process that was inhibited by α-mannoside. The internalized α-amylase was degraded, showing low enzymatic activity and molecular weight at the basolateral membrane. In a human intestinal Caco-2 cell line, the AlexaFluor488-labeled pancreatic α-amylase bound to the cytomembrane was transported to lysosomes through the endocytic pathway, and then disappeared, suggesting degradation. This result was the same at pig and human.

Our findings indicate that *N*-glycan recognition by α-amylase protects enterocytes against a sudden increase in glucose concentration and restores glucose uptake by gradual internalization, which homeostatically controls the postprandial blood glucose level. The internalization of α-amylase may also enhance the supply of amino acids required for the high turnover of small intestinal epithelial cells. This study provides novel and significant insights into the control of blood sugar at the absorption stage in the intestine.

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(272) Mapping interactions between the soluble domains of glycosylphosphatidylinositol transamidase: A step towards a miniaturized, soluble, active enzyme complex

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Glycosylphosphatidylinositol transamidase (GPI-T) catalyzes the attachment of GPI anchors to the C-termini of approximately 0.5% of encoded eukaryotic proteins. These proteins enter the secretory pathway and are ultimately presented on the outside of cells via association with the plasma membrane. Despite the abundance of this modification, GPI-T remains one of the most poorly understood post-translational protein modification systems, largely because of the complexity of GPI-T, which is composed of five different subunits, all of which are membrane-associated. We are interested in simplifying GPI-T into a more tractable enzyme for study. We have chosen to approach this challenge by focusing on the soluble domains of the three GPI-T subunits that can be isolated as a heterotrimeric complex from *Saccharomyces cerevisiae*: Gpi8 (the active site subunit), Gaa1 and Gpi16. [1] We have overexpressed each of these soluble domains and they are currently being characterized. The soluble domain of Gpi8 has weak sequence similarity to caspases and assembles into a caspase-like homodimer that is catalytically inert. [2] All three soluble domains separately interact with each other as stable heterodimeric complexes. Additionally, these three soluble domains can be isolated as an intact heterotrimeric complex. Efforts are underway to determine the stoichiometry of this complex and whether or not it maintains GPI-T-like catalytic activity. These results are providing an important glimpse into the organization and function of this complicated enzyme.

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(273) The mechanism underlying anti-cancer bioactivity of glycan

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Although nature glycans from plant or microbe are relative less toxic but with broad spectrum of therapeutic properties. However, which functional target molecules they bind to, how these macromolecules function in cancer cells, whether glycans could be absorbed after oral administration remains a controversial scientific question and the underlying mechanism was largely unknown. By using quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) techniques, we show that bioactive glycans may target galectin-3 or epidermal growth factor (EGF) to inhibit tumor cells growth, bind to bone morphogenetic protein-2 (BMP2) and its receptors to block tumor angiogenesis, or target detectin-1 on cell membrane to stimulate immune system to impede tumor cells growth in vivo. Interestingly, these glycans can be taken in by intestine mucosa through clathrin/ dynamin/ Eps15/rab5 complex function, and cell membrane receptor mediating way. In addition, wnt/β-catenin, NF-κB and EGFR signaling also play important roles in the process. These findings provide novel insight into the mechanism underlying glycans against cancer and pave the way to support the development of new oral glycan-based drugs.

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(274) Discovery of Novel Monosaccharides in Animal Glycans: Natural Occurrence of N-glycolylhexosamines

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Cytosolic metabolism of the non-human sialic acid N-glycolylneuraminic acid (Neu5Gc) generates *N*-glycolylglucosamine (GlcNGc) as a byproduct of its degradative pathway [1]. Mammalian cells cultured with chemically synthesized GlcNGc were found to synthesize UDP-GlcNGc [2]. Chemically synthesized *N*-glycolylgalactosamine (GalNGc) was also utilized by mammalian cells to synthesize UDP-GalNGc and UDP-GlcNGc, and these could serve as donors for assembly of most major glycan classes [3]. We asked if these novel reactions could happen naturally. Comparing samples from wild-type and *Cmah* null Neu5Gc-deficient mice, we demonstrate that Neu5Gc metabolism is indeed a source of naturally-occurring UDP-GalNGc and UDP-GlcNGc. These endogenously derived *N*-glycolylated aminosugars are also naturally incorporated into animal glycans such as chondroitin sulfate (CS). As humans lack Neu5Gc biosynthesis they should therefore be devoid of GalNGc and GlcNGc in glycans, and also be deficient in any as yet unknown functions these glycans may mediate. Comparisons of human and chimpanzee samples indeed show the predicted difference. However, there is detectable trace amounts of GalNGc in human chondroitin sulfate, likely derived from Neu5Gc-containing animal-derived foods, particularly of mammalian origin. As CS is significantly more stable than sialic acids, we were also able to detect GalNGc in CS isolated from over 3 million year old animal fossils (courtesy of Meave Leakey in Kenya). This raises the possibility that analysis of glycosaminoglycans in fossil samples may allow dating of the loss of Neu5Gc in the human ancestral lineage. A search for proteins that selectively recognize GalNGc-containing CS is also underway.

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(275) Hemicellulose Synthesis and Function in Land Plants

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A major goal of plant glycobiology is to understand the roles of plant cell walls in growth and development, disease resistance, and response to environmental stimuli. To this end we are studying the mechanisms leading to the biosynthesis of hemicelluloses and the relationships between the structures of these polysaccharides and their biological roles. Hemicelluloses, which include xylans and xyloglucans, are major components of the cell walls of land plants. They are synthesized in the Golgi, most likely by multi-enzyme complexes, and exported to the apoplast where they are then incorporated into the cell wall. Many of the structural features of hemicelluloses are conserved across diverse taxa, suggesting that they have important functions in plant growth and development. We have developed an integrated approach that involves characterizing the changes in hemicellulose structure introduced by mutation or deletion of specific genes that have been implicated in hemicellulose biosynthesis and identifying the effects of these changes on plant growth. These studies are extended by the heterologous expression of some of these genes and the functional characterization of the expressed proteins. As a result, we have obtained the first biochemical evidence establishing the enzymatic activities of three key enzymes involved in xylan biosynthesis. These are the xylan synthase (XYS1) that catalyzes the elongation of the nascent xylan backbone, the xylan O-acetyl transferase (XOAT1) that catalyzes the regiospecific acetylation of O-2 of the xylosyl residues in this backbone, and the O-methyl transferase (GXMT1) that catalyzes the regiospecific methylation of O-4 of the glucuronosyl sidechains. We are now characterizing heterologously expressed enzymes involved in xyloglucan biosynthesis and have identified specific structural features of xyloglucan that contribute to its biological functions. Additional insight into the relationship between hemicellulose structure and function has been obtained by structurally characterizing xylans and xyloglucans from diverse monocots and dicots and from non-flowering plants. The results of our most current research into the biochemical basis for the regiospecificity of hemicellulose biosynthesis enzymes, the mechanisms by which plants control hemicellulose chain length and the evolution of the cellular machinery for hemicellulose biosynthesis will be presented.

(276) Novel CE System for High Throughput N-Glycan Screening

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A novel CE system for high throughput N-glycan screening has been developed. The system incorporates a streamlined workflow that allows for the preparation and analysis of two 96-well plates in one day. High data quality is achieved with an average %CV of ~5.0% (peaks ≥ 1.0%). The system provides an easy-to-use, cost-effective, rapid solution to upstream glycan analysis in early stage processes. With regulatory agencies expressing a greater concern for glycosylation in recent years, the earlier determination of quality cell-line or clonal candidates could allow for better process control, as well as, provide a critical edge toward achieving faster-to-market therapeutics.

(277) O-GlcNAc acts as a glucose sensor to epigenetically regulate the insulin gene in pancreatic beta cells

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The post-translational protein modification O-linked beta-N-acetylglucosamine (O-GlcNAc) is a proposed nutrient

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sensor that has been shown to regulate multiple biological pathways. This dynamic and inducible enzymatic modification to intracellular proteins utilizes the end product of the nutrient sensing hexosamine biosynthetic pathway (HBP), UDP-GlcNAc, as its substrate-donor. Type II diabetic patients have elevated O-GlcNAc modified proteins within pancreatic beta-cells due to chronic hyperglycemia-induced glucose overload, but a molecular role for O-GlcNAc within beta cells remains unclear. Using directed pharmacological approaches in the mouse insulinoma-6 (Min6) cell line, we demonstrate that elevating nuclear O-GlcNAc preserves glucose stimulated insulin secretion during chronic hyperglycemia. The molecular mechanism for these observed changes appears to be, at least in part, due to elevated O-GlcNAc-dependent increases in Ins1 and Ins2 mRNA levels via elevations in histone H3 transcriptional activation marks. Further, RNA-sequencing reveals that this mechanism of altered gene transcription is restricted and that the majority of genes regulated by elevated O-GlcNAc levels are similarly regulated by a shift from euglycemic to hyperglycemic conditions. These findings implicate the O-GlcNAc modification as an intracellular sensor for hyperglycemic-regulated gene expression in the beta cell.

(278) **Mitochondrial O-GlcNAc Transferase and its Role in the Glycosylation of Mitochondrial Proteins**

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Mitochondria are crucial organelles that are responsible for regulating physiological processes such as oxidative respiration and apoptosis. Our previous work has shown that some mitochondrial proteins, such as ATP synthase α-subunit and pyruvate dehydrogenase E1-α, have glycosylated isoforms, suggesting that glycosylation may play a regulatory role in its functions. Here we report 67 novel mitochondrial proteins that are glycosylated in mitochondrial-enriched samples as detect by at least one of our three independent approaches (lectin capture and purification, GalT1 enzymatic labeling or metabolic labeling followed by Click Chemistry and immunoblotting). Among the 67, eight proteins were detected by more than one method. Interestingly, some of them, such as several subunits of NADH-dehydrogenase complex (complex I of electron transport chain) are mitochondrially-encoded (mtDNA) and synthesized within the organelle, raising the question of where and which enzyme is responsible for these glycosylation events. Based on this evidence, we hypothesize that the mitochondrial localized O-GlcNAc transferase (mtOGT), rather than the nucleocytoplasmic isoform (ncOGT), is the enzyme that glycosylates mitochondrially-encoded proteins, affecting mitochondrial function and structure. In order to study the contribution of each isoform to mitochondrial glycosylation, we developed a small-interference RNA (siRNA) approach to specifically knockdown each isoform in HeLa cells. We performed an analysis of unique regions in each isoform, including the mitochondrial targeting sequence in mtOGT, and designed several siRNAs that target each isoform. One mtOGT targeted siRNA showed 65-93% reduction in mtOGT isoform with only marginal reduction in ncOGT. RNAi-mediated knockdown of mtOGT caused significant mitochondrial fragmentation as evidenced by the reduced area/ perimeter ratio of the organelle. Ongoing efforts include metabolically labeling mOGT knockdown cell lines with azido-sugars followed by Click Chemistry, avidin purification and immunoblotting against known mitochondrial glycosylated proteins. In addition, we are evaluating the effect of mtOGT knockdown on mitochondrial respiratory capacity, oxygen consumption rate and ATP production. This study will open new avenues in our understanding of how glycosylation can affect mitochondrial function and, consequently, cell physiology.

(279) **Amino acid sequence and site-specific glycosylation of windmill palm peroxidase**

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Plant secretory (Class III) peroxidases have been indispensable models for studying enzyme structures and functions. They are widely used in bioassays and other applications. A newly discovered group of peroxidases from palm trees may owe their exceptional stability to their glycosylation. Recently, the 3-D structure of a highly glycosylated palm peroxidase was solved. This and related palm peroxidases may be good models for increasing our understanding of the roles of complex *N*-glycans in plant glycoproteins.

A unique peroxidase was isolated from the leaves of a cold tolerant palm, *Trachycarpus fortunei*, (*i.e.*, windmill palm tree). Like other palm peroxidases, windmill palm peroxidase (WPP) is stable at high temperatures and acidic pH and in the presence of denaturants. It is distinct from other palm peroxidases in its substrate specificity and it uniquely has 6.5 Ca^{2+} ions per protein.

Here, we report the site-specific glycosylation of WPP. Until now, only the first 20 amino acids of the *N*-terminus were known. We have filled in the gaps including the complete amino acid sequence and the glycosylation profile using cDNA sequencing and biological mass spectrometry. The native WPP material was analyzed at the glycopeptide level to give a qualitative and quantitative assessment of glycosylation at each site. The intact protein was analyzed to learn the molecular mass and the *C*-terminal processing of the mature enzyme. The mature amino acid sequence is 306 residues in length. The presence of a *C*-terminal signal peptide predicts vacuolar targeting of the enzyme. WPP has 13 sites for *N*-linked glycosylation, 2 of which are unique to this enzyme. Each site is at least partially occupied by a glycan. Major glycans are paucimannosidic-type, which corroborates the assignment of WPP as a vacuolar peroxidase. This is the first detailed description of palm peroxidase glycosylation. This knowledge will be used to study the roles of glycosylation in this exceptionally stable and unique palm peroxidase. Additionally, vacuolar peroxidases may be linked to cold acclimation, so WPP may be a key player in *T. fortunei*'s ability to withstand the cold.

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(280) **Structural basis for glycoprotein quality control mediated by glucose tagging in the endoplasmic reticulum**

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The endoplasmic reticulum (ER) is equipped with a sophisticated quality control system of glycoproteins. A variety of *N*-linked oligosaccharide intermediates displayed on the nascent proteins serves as signals recognized by a series of intracellular lectins operating as ER chaperones, cargo receptors, and degradation mediators. In this system, glucosidase II cleaves the non-reducing terminal glucose residue of the *N*-glycan intermediates as quality tag of glycoprotein. This enzyme consists of approximately 110-kDa catalytic α subunit (GIIα) and 60-kDa non-catalytic regulatory β subunit (GIIβ). By contrast, UDP-glucose:glycoprotein glucosyltransferase (UGGT) catalyzes re-glucosylation of the glycoproteins yet to be completely folded. It has been shown that GIIα alone can hydrolyze a small α-glycosidase model substrate such as *p*NP-glucose, while it cannot catalyze deglucosylation of the *N*-linked oligosaccharide substrates unless it makes a complex with GIIβ.

In this study, we determined the first crystal structure of GIIα in the absence and presence of its inhibitor 1-deoxynojirimycin at 1.6-Å resolution. The crystal structure revealed that GIIα has a characteristic segment at the N-terminus as compared with the cognate glycoside hydrolases (GH31). Interestingly, the N-terminal segment was accommodated on the substrate-binding pocket. Based on our data, structural basis for the functional interplay between glucosidase II and UGGT will be discussed.

(281) **Functional studies of cytosolic deglycosylating enzymes in mammalian cells**

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The ER-associated degradation (ERAD) constitutes one of the quality control machineries for newly synthesized proteins in the ER, which eliminates terminally misfolded proteins for degradation. The cytoplasmic peptide:*N*-glycanase (PNGase; Ngly1 in mammals) occurs ubiquitously in eukaryotes and is known to be involved in deglycosylating misfolded glycoproteins during the ERAD process [1]. While the Ngly1-deglycosylation process is believed to be critical for efficient degradation of misfolded *N*-glycoproteins, the detailed functional importance of this process still remains unknown in mammalian cells. Recent reports of human patients bearing mutations in *NGLY1* gene [2, 3], however, clearly indicate the critical function of this protein in mammalian cells. In this symposium, we present our experimental evidence for the functional importance of Ngly1 in ERAD of mammalian cells. Using mouse embryonic fibroblast (MEF) cells from various mice and a model glycoprotein ERAD substrate, our analyses clearly suggest the dysregulation of ERAD in *Ngly1*-KO cells. Our results underscore the functional importance of Ngly1 in the ERAD process and may provide insight into the pathological mechanism of the *NGLY1* genetic disorder.

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(282) **Endosomes-to-TGN retrograde transport mediated by GARP is required for post-Golgi anterograde transport and glycosylation**

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Vesicular transport is divided into two pathways: anterograde transport, which is the route from the endoplasmic reticulum (ER) to the plasma membrane, and the opposite route, retrograde transport. Whereas retrograde transport from the Golgi to the ER is essential for the anterograde transport, the requirement of the endosomes-to-the *trans*-Golgi network (TGN) retrograde transport for the anterograde transport is still debated. Using genetic screening of molecules required for anterograde protein transport, we identified subunits of GARP complex as the candidate factors. GARP complex is composed of 4 subunits, VPS51, VPS52, VPS53 and VPS54, and acts as a tethering factor for the retrograde carriers from the endosome to the TGN. To analyze the involvement of GARP complex with anterograde transport, VPS54 KO cells were generated with CRISPR-Cas9 system. VPS54 KO cells showed severely delayed anterograde transport of both glycosylphosphatidylinositol (GPI)-anchored and transmembrane proteins. Microscopic observation revealed post-Golgi anterograde transport was defective in VPS54 KO cells. Lectin staining assay suggesting that protein *O*-glycosylation, but not *N*-glycosylation was also impaired in VPS54 KO cells. These results indicate that protein recycling from endosomes to the TGN is required for anterograde transport and glycosylation. To identify the molecules recycled via GARP-dependent pathway and related to the anterograde transport, we further screened genes whose overexpression can restore the phenotype of VPS54 KO cells and identified a gene encoding a multipass-transmembrane protein whose function is unknown. Overexpression of this gene partially restored the defect of the anterograde transport

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of both GPI-anchored and transmembrane proteins in VPS54 KO cells. Further functional analysis of this gene is in progress.

(283) Perturbation of autophagic flux is involved in the dystrophic endball formation induced by a proteoglycan gradient

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In the adult central nervous system, axons are incapable of spontaneously regenerating through the lesion site after injury. Regenerating axons display growth cones at their tips and elongate under the normal condition. At injured sites, growth cones are forced to swell and stop. These abnormal growth cones are known as dystrophic endballs. The formation of dystrophic endballs is a hallmark of regeneration failure of axons. It has been well established that an extracellular gradient of proteoglycans at the glial scar is responsible for the dystrophic endball formation. *In vitro*, the dystrophic endball formation is induced in adult mouse dorsal root ganglion (DRG) neurons cultured on a gradient of increasing concentration of proteoglycans. Interestingly, dystrophic endballs contain numerous vacuoles, of which Ramon Y Cajal have already reported about dystrophic endballs over 80 years ago. However, molecular mechanisms and intracellular events underlying the dystrophic endball formation remain elusive. Here, we show that autophagy is associated with the dystrophic endball formation by employing an *in vitro* model of a proteoglycan gradient. Using electron microscopy, we have identified a significant number of vacuoles in dystrophic endballs as autophagosomes, which contain cytosolic fractions and mitochondria. It has been also verified that a number of vacuoles are positive for LC3 (a marker of autophagosomes), not monodansylcadaverine (a marker of autophagolysosomes). Furthermore, a treatment of adult mouse DRG neurons with choloroquine or bafilomycin A1, which blocks the fusion of autophagosomes and lysosomes, induced the formation of dystrophic endball-like structures. These data suggested that perturbation in autophagic flux induced by a proteoglycan gradient leads to the dystrophic endball formation.

(284) Identification of Minimum Essential Structure in Chondroitin Sulfate Which Is Responsible for Their Interaction with Their Receptor, PTP sigma and LAR

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Proteoglycans (PGs) are glycoproteins in which glycosaminoglycans (GAGs) such as chondroitin sulfate (CS) and keratan sulfate (KS) covalently attach to their core proteins. PGs are major component of extracellular matrix (ECM) in our central nervous system (CNS) and thought to be involved in axonal guidance as repulsive cues during CNS development. In mature CNS, PGs form perineuronal net (PNN) to regulate synaptic activities. However, once the CNS is damaged, PGs are overproduced in reactive astro-glial scar and acts as major inhibitors for axonal regeneration. For a long time, CS on PGs had been regarded to be responsible for inhibitory activities for axonal regeneration. In addition to CS, we have reported that KS also plays a pivotal role in axonal regeneration failure. Recently, PTPσ and LAR, both of which belong to Type IIa receptor type tyrosine phosphatase (RPTP), were identified as neuronal receptors for CS. However, so far, underlying molecular mechanisms of KS or its interacting proteins have been poorly understood.

Here, we found that Phosphacan is a chimeric PG with both KS and CS on its core protein. Phosphacan is unregulated in reactive astrocytes and strongly inhibits neurite outgorowth from primary cultured cerebellar granule neuron. Enzymatic digestion of KS and CS, with Keratanase-II and Chondroitinase ABC, respectively, successfully rescued neurite elongation, suggesting that both KS and CS are essential for biological activity of Phosphacan. Furthermore, based on surface plasmon resonance (SPR) assay, we confirmed that phosphacan interacts with PTPσ and LAR. Surprisingly, not only CS but also KS are involved in this interaction at high affinity. In addition, we revealed that KS binds to PTPσ at a different site from CS does.

Our findings will provide new insight how RPTP works as negative regulator for axonal regeneration and new therapeutic strategy to traumatic CNS injury.

(285) Keratan sulfate is another ligand for PTPσ & LAR, which are involved in axonal regeneration failure after injury

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Proteoglycans (PGs) are glycoproteins in which glycosaminoglycans (GAGs) such as chondroitin sulfate (CS) and keratan sulfate (KS) covalently attach to their core proteins. PGs are major component of extracellular matrix (ECM) in our central nervous system (CNS) and thought to be involved in axonal guidance as repulsive cues during CNS development. In mature CNS, PGs form perineuronal net (PNN) to regulate synaptic activities. However, once the CNS is damaged, PGs are overproduced in reactive astro-glial scar and acts as major inhibitors for axonal regeneration. For a long time, CS on PGs had been regarded to be responsible for inhibitory activities for axonal regeneration. In addition to CS, we have reported that KS also plays a pivotal role in axonal regeneration failure. Recently, PTPσ and LAR, both of which belong to Type IIa receptor type tyrosine phosphatase (RPTP), were identified as neuronal receptors for CS. However, so far, underlying molecular mechanisms of KS or its interacting proteins have been

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poorly understood.Here, we found that Phosphacan is a chimeric PG with both KS and CS on its core protein. Phosphacan is unregulated in reactive astrocytes and strongly inhibits neurite outgorowth from primary cultured cerebellar granule neuron. Enzymatic digestion of KS and CS, with Keratanase-II and Chondroitinase ABC, respectively, successfully rescued neurite elongation, suggesting that both KS and CS are essential for biological activity of Phosphacan. Furthermore, based on surface plasmon resonance (SPR) assay, we confirmed that phosphacan interacts with PTPσ and LAR. Surprisingly, not only CS but also KS are involved in this interaction at high affinity. In addition, we revealed that KS binds to PTPσ at a different site from CS does. Our findings will provide new insight how RPTP works as negative regulator for axonal regeneration and new therapeutic strategy to traumatic CNS injury.

(286) Developmental roles and pathogenic mechanisms associated with protein O-mannosylation in Drosophila

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O-mannosylation of proteins is a crucial modification for healthy development. It is mediated by the Protein O-mannosyl Transferase (POMT) enzyme complex that consists of two known subunits, POMT1 and POMT2. Mutations in either subunit can cause failure to O-mannosylate, which can in turn lead to severe diseases including muscular dystrophy. We have analyzed various manifestations of POMT mutant phenotypes at cellular, tissue and organismal levels. We employed a *Drosophila* model to reveal functional mechanisms of O-mannosylation and its roles in animal development, with the focus on *in vivo* defects associated with mutations of the *rotated abdomen* (*rt*) and *twisted* (*tw*) genes encoding *Drosophila* homologues of POMT1 and POMT2, respectively. The mutants have been studied at several developmental stages, revealing defects in muscle contractions in embryos, staggering of dentical belts in larvae, and a clockwise twist in the abdominal segment of adults.

We investigated the spatial and temporal requirements for POMT activity by ectopic expression of *rt* and *tw* in POMT mutant backgrounds. We found that the rotation phenotype can be fully rescued in adults by ubiquitous expression of POMT during late larval and early pupal stages. To determine which tissues require POMT activity, we expressed these proteins in mutant backgrounds using UAS-GAL4 system with a panel of expression drivers that are specific to certain tissues. We found several drivers that were sufficient to fully rescue rotation. While these drivers are normally associated with muscle-specific expression, other muscle drivers were unable to produce complete rescue. More detailed analyses of driver expression patterns suggested that POMT activity is also required outside of muscles, including epidermal cells and neurons. Further investigation using expression of POMTs in these cells types revealed their relative importance in rescuing the phenotype. These novel data on the developmental manifestations, tissue-specific and spatio-temporal requirements for POMT activity has shed light on the pathogenic mechanism associated with defects in O-mannosylation and may lead us to identifying the molecular basis for these phenotypes. This project was supported in part by CONACYT 2012-037(S) grant to AGH and VP, and by NIH/NS075534 to VP.

(287) The Role of Toxoplasma Skp1 Prolyl Hydroxylation and Glycosylation in Oxygen Sensing

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oxoplasma gondii is an obligate intracellular protozoan parasite that can proliferate in warm-blooded animal cells residing at 0.5-21% O_2, but the mechanism of adaptation is unknown. Previous studies showed that *T. gondii* depends on a cytoplasmic prolyl 4-hydroxylase (Tg*phyA*), which hydroxylates Skp1, an adaptor subunit of SCF (Skp1/Cullin-1/F-box protein) class of E3 ubiquitin ligases, for optimal proliferation especially at low O_2. Disruption of Skp1 hydroxylation reduces its apparent M_r by approximately 1 kDa based on Western blotting. We now show by tandem mass spectrometry that TgSkp1 hydroxyproline is modified by a branched pentasaccharide. Contributions of the predicted glycosyltransferases (TgGnt1 and TgPgtA) were confirmed by gel shift analysis and mass spectrometry of TgSkp1 from the disruption strains. Furthermore, the glycosyltransferase knockout strains exhibited reduced growth in fibroblast monolayers similar to those of Tg*phyA*-KO strains, suggesting that glycosylation normally collaborates with prolyl hydroxylation for optimal growth. Proteomic interactome studies revealed that the SCF complex exists in *Toxoplasma* and suggest that the abundance of assembled complexes are regulated by TgSkp1 hydroxylation and glycosylation. Detection of substantial levels of the unmodified TgSkp1 peptide in Skp1 proteomic analyses of all strains implicate TgPhyA activity as rate limiting for TgSkp1 modification. Finally, the Skp1 modification pathway is conserved with that of the social amoeba *Dictyostelium discoideum* (but not in metazoans), and expression of Tg*PhyA* complements a DdPhyA knockout mutant. Thus, as documented in *Dictyostelium*, hydroxylation-dependent glycosylation of TgSkp1 may be important for $E3^{SCF}$ubiquitin-ligase assembly and activity as the parasite senses changes in O_2-availability. Moreover, our data indicate that TgPhyA O_2-sensing is a protist-specific mechanism suggesting that it may be a novel drug target.

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(288) **Galactosyloligosaccharides of early human milk attenuate inflammation in human intestine**

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The ratio of individual oligosaccharides in colostrum relative to later milk is highest for galactosyloligosaccharides (GOS), but their biological function remains undefined. Immature intestinal mucosa tends to hyperrespond to exogenous and endogenous inflammatory insults, but human milk protects infants from intestinal inflammation, and human milk oligosaccharides (HMOS) modulate immune signaling. The expression of galactosyllactose from colostrum to mature human milk was measured, its ability to directly attenuate inflammation was tested, and their mechanisms of immunomodulation were identified. Qualitative and quantitative GOS composition of sequential human milk samples from three mothers was measured. The ability of a native mixture of HMOS or synthetic GOS to attenuate inflammation induced by pro-inflammatory signaling molecules, infection by *Salmonella*, and infection by *Listeria* was investigated using models of immature and mature human enterocyte cell lines. These results were confirmed ex vivo in immature human intestinal organ culture. The three galactosyllactoses expressed in the colostrum of all three mothers tested rapidly declined over 21 days of lactation. Galactosyllactose interacts with intestinal epithelial cells. GOS, like the HMOS mixture, significantly attenuate both TNFα and pathogen induced expression of pro-inflammatory IL-8, MIP-3α and MCP1 protein and mRNA levels. This GOS and HMOS mitigation of inflammatory signaling is mediated through curbing TNFα-induced nuclear translocation of NF-κB p65. HMOS and GOS suppress NF-κB activation, thereby directly attenuating TNFα- and pathogen-induced inflammatory signaling in human intestine. This indicates a novel mechanism whereby human milk oligosaccharides, and especially colostrum oligosaccharides, quenches the inflammatory state of the immature intestine of neonates. Specific galactosyloligosaccharides found in human milk may prove to be novel therapeutic agents against gut inflammation.

(289) **N-glycan characterization of Colorectal Cancer tissue reveals the role of cancer and EGFR expression in regulating N-glycosylation phenotype**

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Colorectal cancer (CRC) is the third most common malignancy, worldwide. CRC follows a gradual progression from small benign polyps to early and late metastatic stages. Unfortunately, 60% of CRC cases are detected at late or metastatic stage when prognosis is poor with a five-year survival < 10%. Current CRC tests have a low compliance rate due to their low specificity and invasive nature. Therefore, there is an urgent need for improved detection methods. Eventhough, aberrant glycosylation is a known hallmark event during carcinogenesis, the glycoproteome has not yet been extensively mined for potential disease biomarkers. Cell surface glycans have been shown to influence various biological processes including, cell adhesion, proliferation and immune response and changes in these glycans may play crucial role in malignant diseases, including CRC, and therefore hold considerable promise as potential disease markers.

In this study, we used tumor tissues (n = 5) and their adjacent normal tissues (n = 5). All tissue samples were adenocarcinomas obtained from male patients varying in age (62.6 ±13.1) and tissue site (rectum, transverse, cecum). The samples were grouped according to their EGFR expression, based on western blot and immunohistochemical analyses [$EGFR^{+} = 2$; $EGFR^{-} = 3$]. Membrane proteins were extracted by Triton-X114 phase partitioning, *N*-glycans were released enzymatically using PNGase F enzyme and analyzed on PGC-LC-ESI-MS/MS. Qualitative analysis of the global *N*-glycan profiles revealed close similarities between normal and tumor samples. However, relative quantitation of the glycan data identified significant differences between tumor and normal samples. An increase in high mannose ($P = 0.03$) and a decrease in complex type ($P = 0.01$) *N*-glycans were observed for the tumors, when compared to normal tissues. Further analysis of complex *N*-glycans indicated no significant changes in fucosylation but sialylation was found to be significantly higher ($P = 0.01$) in tumor samples, with an upregulation of α-2,6 linked sialic acid ($P = 0.003$) and downregulation of α-2,3 linked sialic acid ($P = 0.03$). Interstingly, comparison of EGFR + /- tissues revealed significantly higher biseting GlcNAc ($P = 0.05$) and lower α-2,3 linked sialic acid ($p = 0.04$) structures in EGFR+ tissues. Overall, this study presents a detailed structural *N*-glycomic analysis of CRC tumor tissues as well as the role for EGFR in regulating *N*-glycosylation in cancer. This provides valuable insights into *N*-glycosylation changes during cancer, advancing our mechanistic understanding of the disease.

(290) **Suppression of inflammatory response by Keratan sulfate disaccharide in the development of COPD model mice**

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The pathology of chronic obstructive pulmonary disease (COPD) includes the obstruction of the small airways (bronchiolitis) and destruction of the lung parenchyma (emphysema). COPD is considered

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as a progressive and not fully reversible disease. Cigarette smoking is the major risk factor for the development of COPD. Currently, the available therapies for COPD are relatively ineffective, as there are no drugs available that are capable of reducing the progression of the disease or mortality or the incidence of exacerbations.

On the other hand, glycosaminoglycans (GAG) are important components of the lung extracellular matrix and are considered to be essential for normal lung function and in responding to an injury by regulating signal transduction events. Keratan sulfate (KS) is reported to be the most abundant GAG in airway secretions, and our previous report indicated that KS disaccharide, [SO_3–6]Galβ1-4[SO_3–6]GlcNAc, specifically blocks the interaction of flagellin with TLR5 and subsequently suppresses IL-8 production in normal human bronchial epithelial cells [1]. In this study, KS disaccharide were administered intratracheally or intravenously to elastase-induced emphysema model mice.

We found significantly reduced neutrophil influx and, MMP activity in bronchial alveolar (BAL), and the alveolar destruction was decreased as judged by both histological analysis and micro CT assessment. The underlying mechanism by which KS suppresses inflammation is undergoing.

Acknowledge

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(291) Combination of two glyco-biomarkers could make a noninvasive diagnosis for nonalcoholic steatohepatitis

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Objectives: Nonalcoholic fatty liver disease (NAFLD) is a growing medical problem around the world. NAFLD patients with nonalcoholic steatohepatitis (NASH) can develop cirrhosis and hepatocellular carcinoma. Liver biopsy remains the gold standard for diagnosing NASH. However, invasive liver biopsy is poorly suited as a diagnostic test for such a prevalent condition. Matteoni's classification is one of the universal methods for the histological NASH diagnosis. In Matteoni's classification, ballooning hepatocytes, characteristic of typical pathological NASH, and liver fibrosis are quite important in distinguishing NASH from NAFLD. Recently, we found two glyco-biomarkers, Mac-2 binding protein (Mac2bp) and fucosylated haptoglobin (Fuc-Hpt), are useful for the diagnosis of NASH. Mac2bp and Fuc-Hpt are especially useful for the diagnosis of liver fibrosis progression and prediction of the ballooning hepatocyte presence, respectively. In this study, we tried to diagnose NASH noninvasively using these two biomarkers. Methods: We evaluated serum fucosylated haptoglobin (Fuc-Hpt) levels in biopsy-proven NAFLD patients (n = 127). Mac2bp was measured using ELISA kit, and Fuc-Hpt was measured using lectin-antibody ELISA kit. Results: Serum Mac2bp was increased as the liver fibrosis progression (F0; 1.18 ± 0.53, F1; 1.49 ± 0.70, F2; 2.16 ± 1.55, F3&F4; 2.47 ± 1.21 μg/mL, P<0.01), and serum Fuc-Hpt was increased as the ballooning hepatocyte score elevation (0-2) (119.8 ± 226.0, 363.3 ± 539.7, 792.7 ± 1157.0 U/mL, Π<0.01). Multivariate analysis demonstrated that Mac2bp was an independent determinant for the diagnosis of advanced liver fibrosis (>F2), and Fuc-Hpt was an independent determinant of the presence of ballooning hepatocytes. Using receiver operating characteristic analyses, we set cutoff values for serum Mac2bp (1.224 μg/mL; for advanced liver fibrosis) and Fuc-Hpt (36.1 U/mL; for the presence of ballooning hepatocyte). Using these cutoff values, we could classify our NAFLD patients noninvasively according to the Matteoni's classification. Conclusions & Discussions: Serum Mac2bp and Fuc-Hpt levels could predict liver fibrosis progression and ballooning hepatocyte, respectively. Combination of Mac2bp and Fuc-Hpt could predict NASH diagnosis noninvasively according to the Matteoni's classification.

(292) Spondyloepimetaphyseal dysplasia and Ehlers-Danlos syndrome caused by mutations of glycosaminoglycan biosynthetic enzymes, GalT-II and DS-epimerase

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Glycosaminoglycans (GAGs) including chondroitin sulfate, dermatan sulfate (DS) and heparan sulfate are synthesized on the core proteins through the common linkage tetrasaccharide, Xyl-Gal-Gal-GlcA, forming proteoglycans, which are ubiquitously distributed in extracellular matrix and on the cell surface. Human genetic disorders including bone and skin diseases caused by mutations in the genes encoding the biosynthetic enzymes for GAGs have recently been reviewed [1]. We have identified mutations in the *B3GALT6* encoding GalT-II, responsible for spondyloepimetaphyseal dysplasia with joint laxity type I (SEMD-JL1) and Ehlers-Danlos syndrome (EDS) progeroid type 2 [2]. SEMD-JL1 is characterized by hip dislocation, elbow contracture, clubfeet, platyspondyly, hypoplastic ilia, kyphoscoliosis, metaphyseal flaring, and craniofacial dysmorphisms. EDS-progeroid type 2, characterized by an aged appearance, hypermobile joints, lax skin, muscle hypotonia, joint dislocation, and spinal deformity, was also caused by mutations in *B3GALT6* [2]. Galactosyltransferase activity was reduced in the mutant enzymes; S65G, P67L, D156N, E174A, R232C, C300S, and S309T, compared to the wild-type enzyme [2].

EDS musculocontractural type 2 characterized by a wide range of involvements of connective tissues, including lax skin, muscle hypotonia and skeletal dysplasias, is caused by a mutation in *DSE*

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(S268L) encoding DS-epimerase [3]. DS-epimerase activity was markedly decreased not only in the recombinant mutant DSE-S268L, but also in the cell lysate from the patient [3]. Furthermore, a reduction in the amount of DS has been observed in the patient fibroblasts. These results suggest that the deficiencies associated with GalT-II and DS-epimerase affect the biosynthesis of GAGs, which is essential for the development of skin and bone in addition to regulation of their extracellular matrices.

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(293) An anti-schizophrenic drug affects the surface expression of polySia-NCAM in IMR-32 human neuroblastoma cells

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Polysialic acid (polySia) is a homopolymer of sialic acid with the degree of polymerization (DP) 8-400 Sia residues. PolySia is highly expressed on the neural cell adhesion molecule (NACM) in embryonic brains and the adult brains where neural plasticity, remodeling of neural connections or neural generation is ongoing. PolySia on NCAM is known to have anti-adhesive effects on the cell-cell interactions due to its bulky polyanionic nature and is involved in the regulation of neurogenesis and neuronal functions. Recently, we have demonstrated that polySia functions not only as an anti-cell adhesion molecule, but also as a reservoir scaffold for various neurological active molecules, such as the brain-derived neurotrophic factor (BDNF), dopamine, and the fibroblast growth factor 2 (FGF2) [1]. The reservoir function is a newly proposed function, and is deeply related to the polySia-glycan structure, which appears to be strictly regulated by the two biosynthetic enzymes, ST8SIA2/STX and ST8SIA4/PST. Recently, we demonstrated that polySia-NCAM biosynthesized by the mutated polysialyltransferases (ST8SIA2/STX) derived from schizophrenic patients was impaired not only in the structure, but also in the reservoir scaffold function [1–3]. It is thus suggested that the quantity and quality of polySia are important for normal neuronal functions. Therefore, it can be hypothesized that anti-schizophrenic drugs might improve the impaired properties of polySia. To demonstrate this hypothesis, effects of chlorpromazine, an anti-schizophrenic drug, on the surface expression of polySia were examined by Western blotting, FACS and fluorometric anion-exchange chromatography analyses using human neuroblastoma IMR-32 cells. Interestingly, the surface expression of polySia was increased in the chlorpromazine-treated cells in a degree of polymerization-dependent manner, while the mRNA expression of ST8SIA2 and 4 remained unchanged. All these data suggest that the level of cell surface expression of polySia is critical and chlorpromazine might improve the symptoms through controlling the surface polySia level.

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(294) Reverted expression of the β4-galactosyltransferase 2 or 5 gene in cancer cells impairs tumor growth

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Our previous study showed that the expression of the β4-galactosyltransferase (β4GalT) 2 gene is down-regulated and that of β4GalT 5 gene is up-regulated upon malignant transformation of NIH3T3 cells [1]. This was also observed in several types of tumors. To examine the significance of the changes in the β4GalT gene expression levels, we isolated several clones from B16-F10 mouse melanoma cells with the enhanced expression of the β4GalT2 gene or with the reduced expression of the β4GalT5 gene by transfection of the respective sense or antisense cDNA. Both clones showed no significant change in the growth rate of the cells in culture. However, when they were transplanted subcutaneously into C57BL/6 mice, their tumor growth activities were suppressed markedly. Immunohistochemical and biochemical studies revealed that apoptosis induction and angiogenesis inhibition are observed in the tumors formed with the clone showing the enhanced expression of the β4GalT2 gene [2], and the activation level of the MAPK pathway is lowered in the tumors formed with the clone showing the reduced expression of the β4GalT5 gene [3], when compared with the respective controls. These results indicate that the galactosylation of N-glycans by β4GalT2 and the amount of lactosylceramide synthesized by β4GalT5 are critical to malignant transformation of cells. Currently, the molecular mechanisms underlain the above results are investigated.

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(295) Catalytic Mechanism and Allosteric Regulation of UDP-Glucose Pyrophosphorylase from Leishmania major

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UDP-glucose pyrophosphorylase (UGP) is a nucleotidyltransferase of central importance in all organisms and considered an attractive drug target in the human pathogens *Leishmania* and *Trypanosoma*. We used wild-type and mutant *Leishmania major* UGP to solve the crystal structures of its key reactive states and performed kinetic and theoretical chemistry analysis of the enzymatic reaction. The new

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data filled critical gaps in the knowledge of the UGP mechanism and allowed reconstructing the complete enzymatic cycle on three levels: global (movements of molecular functional blocks), local (behavior of separate residues), and chemical (quantum mechanical description of enzymatic reaction). Results were integrated into a model of UGP activity describing structural changes along the cycle, the mechanisms of substrate binding, UGP catalysis, and product release. Our study revealed the mechanisms of allosteric regulation common for nucleotidyltransferases and, in particular, the mechanical control of the chemical reaction in the active site.

(296) A Drosophila Model of CDG-Ia

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The congenital disorders of glycosylation (CDGs) are a class of glycan-related disease states characterized by prominent neurological impairments. The most common CDG, CDG-Ia, is caused by phosphomannomutase type 2 (PMM2) gene mutations. This work aims to generate a new and improved CDG-Ia disease model in the malleable *Drosophila* genetic model system. Using the new CRISPR DNA editing technology, we have made multiple PMM2 null and targeted mutations, and are currently making PMM2 transgenic rescue constructs for directed expression under Gal4 control. Our work shows that targeted neural knockdown of PMM2 causes severe impairments in coordinated movement, negative geotaxis recovery, survival and overall lifespan. Strong ubiquitous knockdown of PMM2 causes early developmental lethality, whereas weaker ranges of ubiquitous knockdown allow further development and reveal later occurring phenotypes. We hypothesize that movement and survival defects are cause by synaptic dysfunction. We will present a thorough analysis of PMM2 requirements in the nervous system, with a particular focus on the neuromuscular synapse in both larval and adult stages. Future work will include use of CRISPR to knock-in labeled and mutant PMM2 variants in the native genomic locus, studies to rescue *Drosophila* neural phenotypes by expression of the human PMM2 gene (hPMM2), and analyses of the common human PMM2 mutations in this new *Drosophila* disease model. This work will provide the highest level of genetic control to test PMM2 requirements in the causation of the CDG-Ia disease state.

(297) The glycosylation-dependent interaction of perlecan core protein with LDL: implications for atherosclerosis

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Perlecan is the major proteoglycan in the arterial wall. Various studies have linked it to atherosclerosis. Perlecan is highly expressed in atherogenic lesions and its expression correlates with the lesion progression in mice. It is widely believed that the retention of atherogenic lipoproteins by the proteoglycan is an early step for the development of atherosclerosis and the retention is largely based on the ionic interaction between the basic amino acids of apolipoprotein (Apo) B-100 and the negatively charged heparan sulfate (HS). Perlecan contains a core protein and three HS side chains. Its core protein has five domains (I-V) with disparate structures and domain II is highly homologous to the ligand-binding portion of low-density lipoprotein receptor (LDLR), but its binding activity has never been investigated. Here, we show that the domain is functional in LDL binding. Surprisingly, the binding is largely mediated by O-linked glycans that are only present in the secreted domain II. Among the five repeat units of domain II, most of the glycosylation comes from the second unit, which is highly divergent and rich in serine/threonine but no cysteine residues. Interestingly, most of the glycans contain the terminal sialic acid that is well known for its negative charge, and we provided evidence that the sialic acid moiety is critical for the LDL binding. We also show an additive effect of HS and domain II on binding with LDL/ApoB-100. Unlike LDLR, which directs LDL uptake through endocytosis, this study uncovers a novel feature of the perlecan LDLR-like domain II in receptor-mediated lipoprotein retention, which depends on its glycosylation. Consistent with this hypothesis, we further demonstrate that perlecan and its sialic acid-containing glycosylation are highly expressed in the human atherosclerotic arteries. Thus, the perlecan glycosylation may play a role in the early LDL retention during the development of atherosclerosis.

(298) Sialyl-Tn antigen is a potential target for the development of metastasis specific anti-tumor drugs

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Sialyl-Tn (sTn) antigen is a small carbohydrate epitope clinically used as a prognostic marker for various cancers, which synthesized by a glycosyltransferase, ST6GalNAc-I. We have previously demonstrated that intratumoral hypoxic conditions induce the transcriptional activation of *ST6GalNAc-I* gene and consequently up-regulate the production of sTn antigen that confer metastatic characteristics encompassing the remodeling of extracellular matrix (ECM), the enhancement of cell-ECM interactions, the elevation of the autonomous cellular motility, and the alteration of intratumoral immunological conditions. Elimination of sTn antigen by genetic inactivation of *ST6GalNAc-I* or functional inhibition of sTn antigen by masking the epitope with monoclonal antibodies abolished the enhanced collagen bindings and collagen-mediated cellular invasions that were coincident with the attenuation of intratumoral invasion of tumor cells exposed to hypoxic conditions, and with the significant reduction of tumor intravasation. These results indicated

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that sTn antigen is a functional molecule facilitating tumor metastasis, and further suggested that it can be a target for the development of metastasis specific anti-tumor drugs. We would like to discuss the strategy for exploring the drugs for novel chemotherapy.

(299) **(Dys)regulation of T cell receptor by N-glycosylation in Inflammatory Bowel Disease pathogenesis**

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Inflammatory Bowel Disease (IBD) is a chronic immune-mediated disorder of the gastrointestinal tract that comprises Ulcerative Colitis (UC) and Crohńs disease (CD). The incidence of IBD is increasing worldwide and the disease remains incurable, having an increased risk to evolve to colorectal cancer. Although progress has been made in understanding the disease, the etiopathogenesis of IBD is far from being fully elucidated. One of the major clinical gaps in IBD medical practice is to predict the severity of flares and their response to treatment. In addition, prognosis of patients with IBD is very difficult to determine. It is therefore of paramount importance to characterize the underlying molecular mechanism of IBD pathogenesis in order to improve the development of novel biomarkers that may help the determination of prognosis and also improve the patientś stratification for appropriate treatment. Several reports support that T cell activity is tightly regulated by glycosylation, particularly GnT-V-mediated glycosylation.

In this study we address whether the (dys)regulation of N-glycosylation in T cell activity and function is a major contributory factor in UC. Our results showed that UC patients exhibit a dysregulation of GnT-V-mediated glycosylation of the T Cell Receptor (TCR) from intestinal lamina propria T cells (*Dias AM et al., Human Molecular Genetics 2014*). Patients with severe UC showed the most pronounced defect on N-glycan branching in mucosal T cells. Interestingly, we further demonstrated both *ex vivo* (in intestinal T cells from IBD patients) and in IBD-induced mouse models, that dysfunction of GnT-V-mediated TCR branched glycosylation affects T cell function and signaling, being associated with increased T cell proliferation; increased TH1 differentiation and hyperimmune response. We also showed that UC patients (with active disease) exhibit, in T lymphocytes, a reduced MGAT5 gene expression, which underlies the observed dysregulation of T cells N-glycosylation. This deficiency in T cells branched N-glycosylation accompany disease severity (*Dias AM et al., Human Molecular Genetics 2014*).Overall, this study shows a new molecular mechanism in IBD pathogenesis through T cells regulation by N-glycosylation, opening new windows of opportunity to further explore the potential applicability of this mechanism in predicting disease course and/or susceptibility.

(300) **Clinching the elusive prostate cancer antigen F77**

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Monoclonal antibody (mAb) F77, IgG3, was raised against human prostate cancer cell line PC3 and shown to bind both to androgen-dependent and androgen-independent prostate cancer cell lines, and to stain strongly prostate cancer tissues. Glycolipids were found to be the main carriers of F77 antigen in PC3 cells, but the antigenic determinant remained elusive.

The antigen-positive glycolipids being extremely minor components in PC3 cells, an abundant source of F77 antigens was sought. Using a microarray of epithelial mucin glycoproteins, F77 antigen was, intriguingly, found to be strongly expressed on porcine stomach mucin (PSM). This opened the way to the designer array approach, whereby neoglycolipid (NGL) arrays were derived from the PSM *O*-glycome and probed with F77. A F77 antigen-active *O*-glycan was thus isolated, and in conjunction with mass spectrometry assigned as a blood group H-related structure, **a**, based on a branched backbone of I-antigen type and originating from the C6 position of the core GalNAcol[1]. OY is an ion in the mass spectrum diagnostic of a 6-linked branch at core GalNAc arsing after periodate oxidation.

a

Microarray analyses with a series of glycolipid analogs of the A, B and H series showed that mAb 77 can accommodate also the blood group A and B analogs [1], thus accounting for the expression of the antigen in prostate cancer tissues irrespective of blood group of patients.

With a newly synthesised nona-saccharide (structure **b**), we performed sequential enzymatic treatments and obtained two additional oligosaccharides (**c** and **d**). These were converted to NGLs and probed with F77. The results indicate that F77 antigen resides on the 6-linked outer arm.

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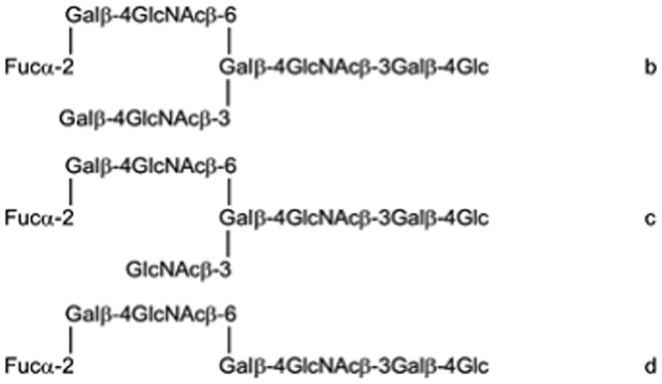

We will discuss the biosynthetic basis of the expression of F77 antigen [2] and account for its prevalence in prostate cancer rather than other epithelial cancers. With knowledge of the structure and prevalence, the F77 antigen can now explore rationally as a cancer biomarker.

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(301) Glycomic consequences of TLR4 deficiency in mouse lung

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Lung inflammatory diseases such as Asthma and Chronic Obstructive Pulmonary Disease (COPD) affect large numbers of individuals and dramatically impact quality of life. The interactions between cell surface glycans and complementary glycan binding proteins in lung tissues contribute to the pathophysiology of these inflammatory diseases. Unfortunately, the mechanisms that regulate the expression of inflammatory and anti-inflammatory glycans in lung tissues are poorly understood, but studies in model systems indicate that Toll-like receptor (TLR) signaling can modulate protein glycosylation. Furthermore, TLR4 deficiency induces COPD-like changes in mouse lung. Therefore, in order to identify endogenous lung glycans whose expression is regulated by TLR signaling, we have undertaken the structural characterization of glycosphingolipid as well as N- and O-linked glycoprotein glycans in TLR4 deficient mouse lung tissue. We observe a significant decrease in N-linked glycoprotein glycosylation, that affects all classes of N-linked structures (high-mannose, hybrid, complex), in TLR4-deficient lungs. Tie2-driven expression of TLR4 in endothelial cells and leukocytes of TLR4-deficient mice prevented the lung phenotype and rescued some, but not all, N-linked glycans, indicating specific glycan deficiencies related to loss of TLR4 expression. Glycosphingolipid expression is less dramatically impacted by loss of TLR4 and characterization of O-linked glycosylation is still in progress. Our results confirm that innate immune signaling mechanisms can modulate the glycome by cell autonomous and non-autonomous pathways.

(302) Quantification of plasma and red blood cell glycosphingolipids in Amish epilepsy syndrome by NSI mass spectrometry

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A mutation in the ganglioside biosynthetic enzyme ST3GAL5 causes Amish epilepsy syndrome and results in a complete lack of ganglioside GM3 with collateral changes in the biosynthesis of other glycosphingolipids (GSLs). We have developed a quantitative and robust method to comprehensively profile intact GSLs, including both their ceramide and glycan moieties, in human plasma and red blood cells (RBCs). Our glycolipidomics approach discriminates patient genotypes based on quantitative analysis of GSL content in plasma or RBCs. This method will be a useful tool for the diagnosis of Amish epilepsy syndrome and for monitoring the clinical efficacy of therapeutic strategies aimed at replacing the missing GSLs. Three cohorts (Old Order North American Amish, African-American, and French) have been reported that each carry one of two identified mutations in the ST3GAL5 gene. We previously demonstrated that loss of GM3 and more complex gangliosides in 'Salt & Pepper' syndrome, caused by a mutation in ST3GAL5 in an African-American family, profoundly affects neural cell viability. Despite being caused by different molecular lesions, both S&P syndrome and ST3GAL5 deficiency in the Amish present with profound psychomotor disability, severe epilepsy, multiple sensory disruptions (hearing and vision), and aberrant neural crest cell migration and function (facial dysmorphogenesis and dermal pigmentation, respectively). To date, seizure disorders have not been reported in the ST3GAL5 deficient mouse model and neither lymphocyte function nor insulin homeostasis have been investigated in human patients. To further elucidate the functions and biomedical importance of ST3GAL5 activity, we developed a method for structural analysis of intact GSLs using neutral loss scanning and data-dependent acquisition by NSI mass spectrometry. Complete GSL expression profiles, including ceramide heterogeneity and glycan structural diversity, were quantified in plasma and RBCs by reference to appropriate external standards. This method was applied to clinical samples derived from a dietary supplementation trial to monitor GSL levels in Amish patients.

(303) Molecular mechanisms underlying the formation of laminin-binding glycans displayed on α-dystroglycan

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Dystroglycanopathy is a major class of congenital muscular dystrophy that is caused by a deficiency of functional glycans on α-dystroglycan (α-DG) with laminin-binding activity. Although several causative genes for dystroglycanopathy were recently identified, their functions

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as well as the precise structure of laminin-binding glycans remain to be fully elucidated. A product of a recently identified causative gene for dystroglycanopathy, AGO61, acted *in vitro* as a protein *O*-mannose β-1-4-*N*-acetylglucosaminyltransferase, although it was not functionally characterized. Here we show the phenotypes of AGO61-knockout mice and demonstrate the function of AGO61 for the formation of laminin-binding glycans on α-DG.

AGO61-knockout mouse brain exhibited abnormal basal lamina formation and a neuronal migration defect due to a lack of laminin-binding glycans. These neurodevelopmental abnormalities are commonly seen in dystroglycanopathy mouse models, which indicated an essential role for AGO61 in the functional maturation of α-DG. Furthermore, our results indicate that functional α-DG glycosylation was primed by AGO61-dependent GlcNAc modifications of specific threonine-linked mannosyl moieties of α-DG, that display the laminin-binding glycans produced by LARGE. These findings provide a key missing link for understanding how the physiologically critical glycan motif is displayed on α-DG and provides new insights on the pathological mechanisms of dystroglycanopathy.

In addition, we report our latest study on the formation of the laminin-binding glycans.

We thanks for collaborators, Drs. Sz-Wei Wu, Kay-Hooi Khoo (Acadeia Sinica, Taiwan), Satoshi Ninagawa (Okazaki Inst. for Integra., Japan), Motoi Kanagawa, and Tatsushi Toda (Kobe Univ., Japan).

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(304) **Mannose-functionalized mesoporous nanocarriers for targeted drug delivery and tumor imaging**

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A major challenge to a successful cancer chemotherapy is how to achieve specific drug accumulation at tumor sites. The incorporation of a specific targeting moiety to drug carrier may result in active drug uptake by cancer cells. Carbohydrates are important mediators of cell-cell recognition events and have been implicated in related processes such as cell signaling regulation, cellular differentiation, and immune response. The biocompatibility of carbohydrates and their ability to be specifically recognized by cell-surface receptors indicate their potential utility as targeting ligands in imaging, disease diagnosis, and drug delivery. Here, we describe the development of multifunctional nanocarriers that are designed for cancer cell-specific delivery of anticancer drugs and tumor imaging. Mesoporous silica nanoparticles were labeled with fluorescent molecules followed by modification with targeting ligands (mannose). An anticancer drug, doxorubicin, was loaded into the mesopores of nanocarriers, and controlled drug release triggered by glutathione in the cytoplasm was achieved. The current research has demonstrated the capability of mannose-functionalized nanocarriers as a targeted and controlled drug delivery system, and the potential of employing this system to deliver drugs for enhanced chemotherapy.

(305) **Galnt1 is required for normal heart valve development and cardiac function**

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Congenital heart valve defects in humans occur in approximately 2% of live births and are a major source of compromised cardiac function. In this study we demonstrate that normal heart valve development and cardiac function are dependent upon *Galnt1*, the gene that encodes a member of the family of glycosyltransferases (GalNAc-Ts) responsible for the initiation of mucin-type O-glycosylation. In the adult mouse, compromised cardiac function that mimics human congenital heart disease, including aortic and pulmonary valve stenosis and regurgitation; altered ejection fraction; and cardiac dilation, was observed in *Galnt1* null animals. The underlying phenotype is aberrant valve formation caused by increased cell proliferation within the outflow tract cushion of developing hearts, which is first detected at developmental stage E11.5. We surveyed mouse models that phenocopy our observations and focused on ADAMTS5 and one of its substrates, the proteoglycan versican. We found that loss of *Galnt1* resulted in diminished levels of ADAMTS5, decreased versican cleavage and increased BMP and MAPK signaling. We also observed aberrations in the accumulation of extracellular matrix proteins. Taken together, the ablation of *Galnt1* appears to disrupt the formation/remodeling of the extracellular matrix and conserved signaling pathways that regulate cell proliferation. Our study provides insight into the role of this conserved protein modification in cardiac valve development and may represent a new model for idiopathic valve disease.

(306) **The molecular morphology of the cytotoxic retrotranslocation by an N-acetylhexosamine-binding lectin isolated from slipper lobster in breast and ovarian cancer cells**

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An *N*-acetylhexosamine-binding lectin, iNL was newly isolated from the blood of slipper lobster, *Ibacus novemdentatus*. It had strong hemagglutinating activity against human and rabbit erythrocytes and was inhibited by the co-presence of *N*-acetyl group

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containing monosaccharides such as *N*-acetyl-D-mannosamine, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine and *N*-acetyl-D-neuramic acid. iNL showed the anticancer activity against breast (MCF7, T47D) and ovarian cancer (Hela) cells dose-dependently. Both biochemical and ultra-microstructure analysis found that iNL exists as a polygonal ringed shape of super molecular structure with 500 kDa consisted of the plural subunits having 70, 40 and 30 kDa polypeptides linked by disulfide-bond.

In spite of the lectin possessed giantic molecular mass, confocal laser scanning analysis showed that iNL was incorporated into the target cells after binding the lectin to cell surface glycan ligands. The lectin co-localized with the marker proteins of endosomes and the cytotoicity and incorporation of iNL into the cells were cancelled by the co-presence of its haptenic sugars.

Our results imply that iNL is incorporated into organella by the manner with the emitting cytotoxic signals through the binding to the specific glycoconjugates ligands expressed on the cell surface.

(307) **Fast Immunoglobulin deglycosylation for accurate N-glycan analysis**

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New England Biolabs

A growing number of monoclonal antibodies and antibody chimeras are currently being developed as therapeutic agents. A conserved *N*-glycan at Asn297 of the Fc region of IgG is critical for biological activity. Also, some IgGs and IgG chimeras have additional *N*-glycans that, together with the conserved Asn297 *N*-glycan, affect recognition, half life, and inflammatory reactions.

Variables in cell culture systems can greatly influence glycan heterogeneity. Therefore, it has become increasingly important to monitor the IgG glycosylation profiles during production to ensure the right active glycoforms are obtained, while keeping undesired glycans below minimum acceptable levels.

For effective monitoring, it is essential to obtain a complete and accurate *N*-glycan profile in the shortest time possible. Enzymatic release of *N*-glycans with PNGase F typically requires incubation times of (at least) a few hours, which is only the first step in a process involving glycan derivatization and analysis by liquid chromatography and/or mass spectrometry.

We present here an improved reagent allowing complete and rapid deglycosylation of therapeutic monoclonal antibodies. The total sample preparation time is cut down dramatically (including the time for fluorescent labeling), leaving the glycans ready for analysis by LC-MS.

A variety of theraputic monoclonals were used to validate this technique: different subclasses (IgG 1 to 4), isotypes (IgA, IgM, IgE), organisms (mouse, human, and humanized), sources (CHO, murine myeloma), and structures (IgG, IgG-chimeras). We here demonstrate the complete delgycosylation of all these proteins in a very short time. For glycoproteins with more than one *N*-glycan site, we show the efficient removal of glycans from each one (a requisite to obtain a complete *N*-glycan profile).

Results are in accordance with published data, showing that sensitivity or accuracy are not compromised by a faster and more convenient glycoprotein characterization workflow.

(308) **Physiological function of deglycosylating enzymes in mice**

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Peptide:*N*-glycanase (Ngly1) is an enzyme which cleave *N*-glycans from glycoproteins. This enzyme is highly conserved among eukaryotes and known to be involved in endoplasmic reticulum-associated degradation (ERAD) [1]. Moreover, patients who harboring Ngly1 mutation have been found recently [2, 3] and they showed severe systemic symptoms such as hypotonia, developmental delay and involuntary movement [3]. These facts indicate the importance of the physiological function of Ngly1, however, it still remains unclear. To reveal the precise physiological function of Ngly1, we generated Ngly1 deficient mice and performed phenotypic analyses. As a result of the analyses, we found that Ngly1 deficient mice show a very severe phenotype. This result strongly supports the importance of its physiological function in mice. Surprisingly, it was also found that an additional deletion of a gene, while itself exhibit no significant phenotype, suppresses the effect of the Ngly1-KO phenotype. In this presentation, we will describe the results of phenotypic analyses of Ngly1-KO mice, as well as the rescue phenotypes shown by the additional gene KO mice.

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(309) **MANNITOL - A BBB DISRUPTER IS ALSO A POTENT ALPHA-SYNUCLEIN AGGREGATION INHIBITOR FOR TREATING PARKINSON'S DISEASE**

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Misfolding and aggregation of α-synuclein is the hallmark of Parkinson's Disease. Osmolytes, e.g. polyols, are small molecules

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which accumulate under stress conditions and stabilize protein structure, acting as 'chemical chaperones'. They may reduce protein misfolding and aggregation in neurodegenerative diseases. The sugar alcohol sweetener Mannitol is a non-metablolized FDA-approved osmotic diuretic agent that also has BBB disrupting properties. We examined its ability to interfere with the aggregation of α-synuclein *in vitro* and *in vivo*. Low concentrations of Mannitol (450 and 225 mM) inhibited the *in vitro* formation of α-synuclein fibrils. High concentrations (900 mM) significantly decreased formation of tetramers and high molecular weight oligomers, and shifted the secondary structure from α-helical to a different structure, suggesting alternative potential pathways for aggregation. Feeding α-synuclein expressing Drosophila, which serve as an established model for PD, with 75 mM Mannitol dramatically corrected their behavioral defects and reduced the amount of α-synuclein aggregates in their brains. Daily injection (IP) of 1 g/kg Mannitol to mThy1-human α-synuclein transgenic mice caused a significant decrease of α-synuclein accumulation in several brain regions, suggesting that Mannitol promotes α-synuclein clearance from the cell bodies. Mannitol appears to have a general neuroprotective effect in the transgenic treated mice, which includes the dopaminergic system. No adverse effects were observed in control Mannitol-treated flies or mice. We suggest that Mannitol has a dual therapeutic mechanism for the treatment of PD - a BBB disruptor that also serves by itself as a chemical chaperone correcting the pathogenic misfolding of α-synuclein. Preliminary experiments indicate that Mannitol is also effective in inhibiting the aggregation, *in vitro*, of the Alzheimer's disease associate amyloids tau and Aβ.

(310) Functional analyses of a novel type of CA19-9 carrier molecules in micro lipid membrane

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Carbohydrate antigen 19-9 (CA19-9) is a well-known tumor marker for pancreatic cancer. While the CA19-9 level is measured using anti sialyl Lewis A antibodies, it was unknown which molecules carry CA19-9 other than mucins. We have reported the identification and characterization of a novel type of CA19-9 carrier, BGM (bile globular membrane), which is thought to exist in normal bile and to be secreted into sera of patients with pancreatic cancer because of polarity failure. BGM might be another CA19-9 carrier (lipid membrane complex) other than mucins and could be applied to the diagnosis of pancreatic cancer. We identified characteristic molecules for BGM such as a novel golgin family member, CABIN (CAsein Binding domain integral protein with golgIN motif) by mass spectmetry analysis. So CABIN was thought to be involved in secreted CA19-9 membrane lipid complex. To know biological functions of CABIN, we made 5 kinds of deletion mutants of N-terminal region of CABIN (N-CAB). When these mutants were transfected into pancreatic cancer cell lines, down-regulation of CA19-9 secretion and accumulation of small vesicles were observed in the mutants, which contained ATP(GTP)-binding region. Moreover when full length of CABIN was transfected into the cells, CABIN was co-localized with CA19-9 lipid membrane comlex labeled with coumarin-ceramide. These results suggest that CABIN regulates traffic of lipid membrane carrying CA19-9. Since coumarin-ceramide is internalized into Panc1 cells in diffusion manner, glycosylated ceramide was transported into ER at1 hour. At 3 days later, the internalized ceramide was further glycosylated and localized in certain micro-organelles. When halo tag-labeled CABIN was transfected, in Panc1 cells lines, numbers of coumarin-labeled micro-organelles were increased and they were co-localized with CABIN-Halo Tag. These organelles stained with coumarin were co-localized with neither KDEL nor GM130. These results suggest that overexpression of CABIN Halo-Tag could stop the traffic of lipid membrane carrying CA19-9 after the ER and Golgi complex in membrane traffic. Next, we purified the coumarin-labeled micro-organelles, which were increased by CABIN Halo-tag transfection. Mass spectrometry analyses revealed certain kinds of proteins consisted of the micro-organelles, and functional analysis on these proteins is underway.

(311) Genetic and epigenetic regulation of IgG glycosylation

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Immunoglobulin G (IgG) is one of the key elements of our immune system that reliably recognizes non-self molecules, microorganisms and cells. Variations in the amino acid sequence of the hypervariable region (Fab) enables high-affinity binding to foreign epitopes, but equally important are variations in structure of the Fc part that activate different effector pathways of the immune system. *N*-glycans attached to the conserved Asn297 are essential component of this variability since it was reliably demonstrated that they promote binding of IgG to different Fc receptors and other elements of the immune system. IgG glycosylation changes have been reported to associate with a number of different diseases, but also with chronological and biological age of an individual. Individual variations in IgG glycosylation are very large, but our understanding of the mechanisms that regulate this very important biological process is limited. Our recent genome-wide association studies identified a number of genetic loci that associate with variations in IgG glycosylation. The majority of these loci were previously not associated with protein glycosylation, but are known risk factors for different autoimmune and inflammatory diseases and cancer, indicating that altered IgG glycosylation could be a molecular mechanism that explain their association with a disease. Some elements of IgG glycosylation were shown to be clone-specific, indicating the existence of molecular mechanisms that can pre-define a clone of B cells for a specific pattern of IgG glycosylation. As the most stable epigenetic modification, CpG methylation is the most probable candidate for this type of cellular memory and we were able to confirm this hypothesis by analyzing CpG methylation in promoter regions of genes that associate with the IgG glycome.

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(312) **Identification of sialylated glycoproteins in doxorubicin-treated hepatoma cells with glycoproteomic analyses**

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We previously reported that sialylated glycans are useful markers for cancer stem cell (CSC)-like cells in hepatoma cell lines (Moriwaki K *et al*, Cancer Science 2011). Glycomic analysis using a lectin microarray showed dramatic increases in binding of *Sambucus sieboldoana* agglutinin (SSA) to a $CD133^{+}CD13^{+}$ CSC-like cell subpopulation. It is well known that sialic acid is associated with many physiological and pathological events, especially tumor malignancy. For example, increases in sialic acid moieties are engaged with cancer cell behavior, such as invasiveness, metastasis, and anti-cancer drug resistance. However, it remains unknown which sialylated proteins are involved in cancer stemness of hepatoma cells.

In the present study, we identified specifically sialylating glycoproteins in doxorubicin (DXR)-treated hepatocarcinoma cell line Huh7 using glycoproteomic analyses. Since CSCs constitute a small subset of cells within carcinoma cell lines, the identification of sialylated proteins using general glycoproteomic strategies seemed difficult. Since it is well known that short-term treatment with anti-cancer drug can condense CSCs, we used DXR to concentrate CSCs of hapatoma cells. Among emerging proteomic technologies, isobaric tags for relative and absolute quantitation (iTRAQ) is a shotgun-based technique which allows the concurrent identification and relative quantification of hundreds of proteins from different biological samples in a single experiment. Then we used iTRAQ technique for the analysis of DXR-treated Huh7 cells, and identified 17 sialylated glycoproteins. Most of the identified glycoproteins were cancer-associated proteins. Further we analyzed and two proteins of approximately 70 kDa were detected using SSA blot analyses. Whereas iTRAQ analysis leads to a more comprehensive analysis of sialylated proteins, the SSA lectin precipitation technique followed by liquid chromatography-tandem mass spectrometry analysis allows for protein enrichment, which aids in the identification of specific target proteins. Finally we have identified as beta-galactosidase and alpha-2-HS-glycoprotein (fetuin-A) by using this technique. Sialylation levels of fetuin-A were increased in DXR-treated Huh7 cells, and small amounts of fetuin A were changed in molecular weight as well as intracellular localization. These changes in sialylation of glycoproteins might be involved in the establishment of cancer stemness of hepatoma cells.

(313) ***N*-glycan deletion mutant of soluble ErbB3 protein attenuates heregulin-induced tumor progression by blockade of HIF-1 pathway**

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It has been well documented that activation of the PI3K-Akt pathway implicates tumor survival and progression. Additionally, the active PI3K-Akt pathway augments the nuclear accumulation of hypoxia inducible factor (HIF)-1, which activates the transcription of many target genes and drives cancer progression. Therefore, the PI3K-Akt signaling pathway has been considered as a target for cancer therapy. Moreover, we have previously indicated that single *N*-glycan deletion mutant of the soluble ErbB3 protein (sErbB3 N418Q) attenuates heregulin -induced ErbB3-PI3K-Akt signaling (Takahashi M, *J Biol Chem*, 2013). To further elucidate the physiological relevance of sErbB3 N418Q to cancer progression, first, we checked whether sErbB3 N418Q attenuated heregulin-induced HIF-1 activation. The results indicated that after 6 h incubation, heregulin induced the nuclear expression of HIF-1 in human breast carcinoma cell line, MCF-7 cells. Pretreatment of MCF-7 cells with sErbB3 N418Q suppressed HRG-induced HIF-1 activation. Similar results were also obtained in other breast cancer cell lines, T-47D and BT474. Interestingly, these suppressive effects were not observed sErbB3 WT. Incubation with heregulin also induced the nuclear expression of Nrf2, which is master transcriptional activator of cytoprotective genes leading to resistance to chemotherapy, and this inductive effect was also reduced by sErbB3 N418Q. Next, to examine whether sErbB3 N418Q suppressed heregulin-induced cell migration and invasion, we used wound healing assay to assess motility of the cells. Pretreatment of sErbB3 N418Q suppressed the cell migration of MCF-7 cells in the presence of heregulin. These findings indicated that sErbB3 N418Q suppressed malignant formation of cancer cells by imposing a blockade of the HIF-1 and Nrf2 pathways, and further suggested that sErbB3 N418Q might be a new therapeutic application in cancer.

(314) **Characterizing Molecular Mechanisms of Cosmc/T-synthase Interactions**

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O-glycosylation is prominent throughout all domains of life, and the biological roles of O-glycoproteins are similarly diverse, and include signaling and communication, cell adhesion, immune surveillance and host-pathogen interactions, inflammation, and endocytosis. As a consequence, abnormal O-glycosylation is implicated in a number of human diseases, including cancers. Notably, the abnormal O-glycans Tn antigen (GalNAca1-Ser/Thr) and its derivative sialyl Tn (STn) are tumor biomarkers whose expression level correlates with metastatic potential and poor prognosis in a number of cancers including breast, colon, lung, bladder, cervix, ovary, stomach, and prostate cancers. Tn expression is often associated with a reduction in T-synthase activity, which requires a specific protein chaperone, termed Cosmc, which directly interacts with its client to facilitate the active form of the enzyme. Therefore, Cosmc has emerged as a key regulator of Tn antigen expression, and in fact, loss of Cosmc activity, through epigenetic silencing and gene mutations, has been identified as the cause of Tn expression in several human and murine tumor cell lines and in Tn syndrome patients. To define the molecular mechanisms underlying Tn antigen expression, we need a better understanding of Cosmc and T-synthase interactions. Here we utilized mutagenesis, biochemical and biophysical

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tools to characterize recombinantly produced Cosmc protein and its activity. We find that Cosmc chaperone function does not require the full length protein, as a C-terminal truncation mutant is functional, and thus we are defining the residues required for in vitro activity. Furthermore, we demonstrate that Cosmc binds specifically to a peptide sequence containing the recognition sequence of its client, T-synthase, in a fluorescence polarization experiment. Additionally, we utilize biochemical assays with artificial substrates to further define the chaperone function of Cosmc. Our biochemical and biophysical studies of Cosmc have informed mechanisms of T-synthase activation which lead to a better understanding of Tn antigen expression.

(315) Development of IgG Antibodies with Stage-Dependent Glycans on the Fc of ALS Tg mice

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We recently revealed IgG antibodies of ALS patients comprise Fc with a unique glycan of bi-antennary structure missing core fucose and sialic acid but having bisecting GlcNAc (A2BG2) and mediate antibody-dependent cell cytotoxicity (ADCC). Little is known, however, about the incidence of A2BG2 expression and IgG cytotoxicity under ALS conditions with well-defined clinical stages. Here, we characterize IgG antibodies produced in ALS Tg mice detecting intra- and extra-cellular antigens of motor neuron and expressing different glycan pattern during the disease. Increased number of innate immune cells found at disease onset was insufficient to induce optimal systemic T-cell responses. Nevertheless, antibodies were produced at the pre-symptomatic stage in secondary lymphoid organs under the conditions of poor systemic immune responses. Moreover, glycosyltransferases of plasma B-cells synthesizing the Fc-glycans were regulated by IL-2 or IL-4; however the observed glycosyltransferase pattern did not match to that found in ALS Tg mice. We further found that A2BG2 glycan quantity increased with disease progression and IgG antibodies identifying extracellular motor neuron antigens are produced at the final disease stage. Thus, the most effective ADCC of motor neuron cells observed at the end disease stage. We conclude that in ALS the frequency of A2BG2 glycan expression on the Fc domain and quantity depends on the clinical stage, thus is a potential biomarker to predict ALS progression. Indeed, A2BG2 glycan of IgG antibodies enriched from additional 70 ALS patient sera are correlated with disease score.

(316) Optogenetic control of the function of the Golgi apparatus in neurons

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The Golgi apparatus (GA) is a major organelle found in most eukaryotic cells. It mediates transporting, modifying, and packaging proteins and lipids into vesicles for delivery to targeted destination. In neurons, because of the enormous distances involved, GA is thought to play crucial roles in trafficking of proteins involved in synaptic transmission and plasticity, as well as lipids required for forming and remodeling of elaborated dendrites and axons. Indeed, in addition to perinuclear GA found in most non-neuronal cells, neurons have dendritic Golgi compartments, termed Golgi outposts. Furthermore, dysfunction of GA has been implicated in certain neuropsychiatric and neurodevelopmental disorders. Previous studies have shown that the E3 ubiquitin ligase Ube3a, which is the responsible gene for a neurodevelopmental disorder Angelman syndrome (AS), is required for normal GA functions by maintaining acidic environment inside GA. However, whether and how functions of GA are modulated by intraluminal pH of GA and lead to neuronal dysfunctions have remained unclear.

In the present study, to establish causal relationships between GA and neural functions in a spatially and temporally controlled manner, we aimed to develop a new tool to modify GA functions. We designed a genetically engineered light-sensitive proton pump and fused it to sequences found in GA resident proteins. When expressed in HEK293 cells and in hippocampal neurons, this chimeric pump successfully localized to the GA. Brief light application to the cells rapidly disorganized the structural integrity of GA. We are currently characterizing and optimizing this light-sensitive GA pH modulator. We are also investigating whether the secretory trafficking pathway and protein glycosylation levels in neurons could be regulated by light-induced changes in GA or Golgi outpost functions. This light-sensitive GA pH modulator will become a powerful and useful tool to better understand how GA mediates normal neuronal functions and how its dysfunctions lead to certain brain disorders, such as AS.

(317) Pathophysiological roles for dystroglycan glycosylation in skeletal muscle and gene therapy challenge using glycosylation-deficient muscular dystrophy models

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Dystroglycan is a highly glycosylated peripheral membrane protein that functions as a cell surface receptor for proteins in the extracellular matrices and synapses. Abnormal glycosylation of dystroglycan causes several forms of muscular dystrophy, collectively called dystroglycanopathy. To understand the pathophysiological roles of dystroglycan glycosylation and to establish effective treatment, we generated 2 distinct conditional knock-out (cKO) mice for fukutin, the first dystroglycanopathy gene identified for Fukuyama congenital muscular dystrophy. Myofiber-selective fukutin-cKO (MCK-fukutin-cKO) mice showed mild muscular dystrophy, whereas muscle precursor cell (MPC)-selective cKO (Myf5-fukutin-cKO) mice exhibited more severe phenotypes of muscular dystrophy. Using an isolated MPC culture system, we demonstrated that defects in the fukutin-dependent glycosylation of dystroglycan lead to impairment of MPC proliferation, differentiation, and muscle

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regeneration. These results indicate that dystroglycan plays important roles in the maintenance of muscle cell membrane integrity and MPC viability, and their impairments contribute to the pathology of dystroglycanopathy. Since our data suggested that frequent cycles of myofiber degeneration/regeneration accelerate substantial and/or functional loss of MPC, we expected that protection from disease-triggering myofiber degeneration provides therapeutic effects even in mouse models with MPC defects; therefore, we restored fukutin expression in myofibers. Adeno-associated virus (AAV)-mediated rescue of fukutin expression that was limited in myofibers successfully ameliorated the severe pathology even after disease progression. Our findings indicate that dystroglycanopathy is a regeneration-defective disorder, and gene therapy is a feasible treatment for the wide range of dystroglycanopathy.

(318) **Regulatory function of b-series gangliosides in adipose tissues leptin secretion and in central nervous system which controls the lipid metabolism**

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The expression patterns and levels of acidic glycosphingolipids, gangliosides are different among tissues and organs, and high expression in the central nervous system has been observed. In murine serum samples, extremely low levels of leptin were observed in GD3S KO mice compared with WT mice. In GD3S KO mice, gene expression of leptin exhibited the similar levels to WT mice, although the protein levels were significantly high. In addition, immunohistochemistry of leptin showed strong staining both in white and brown adipose tissues, suggesting that the leptin secretion from adipocytes was impaired. Further, primary adipose-derived stromal vascular cells also showed high leptin levels, which could be alleviated by addition of b-series gangliosides. On the other hand, leptin secretion was impaired by MβCD treatment in dose and time dependent manner in 3T3-L1. Furthermore, markers of lipid-rafts shifted to non-rafts in adipose tissues of GD3S KO mice, suggesting leptin secretion was regulated by lipid-rafts.

Although blood levels of leptin were significantly low, body weights of GD3S KO mice were normal. Furthermore, when they were fed with high fat high sucrose diet, WT and GD3S KO mice showed similar obesity and no significant differences in body weights. In addition, the cell numbers of brown adipose tissues were significantly increased. Examination of expression levels of UCP-1 and PGC1α gene revealed that they were increased, and higher body temperature was also observed. These results suggested that brown adipocytes of GD3S KO mice are highly activated.

In the brain of GD3S KO mice, ObRb gene and protein expression were up-regulated in hypothalamus. In situ hybridization also showed increased expression of ObRb in hypothalamic arcuate nucleus. Moreover, strong staining of STAT3 was detected in nucleus of GD3S KO mice in contrast with cytoplasmic staining in WT mice. Expression of p-STAT3 examined by western blotting and immunohistochemistry were increased in hypothalamus of GD3S KO mice. Higher levels of p-STAT3 and c-Fos expression were observed after leptin stimulation. These results suggested that b-series gangliosides are involved both in the leptin secretion from adipocytes and in leptin/ObRb signaling in hypothalamus.

(319) **A sialic acid-binding lectin(SBL)dependent apoptosis is triggered by sialylated-glycoconjugates in GEM of P388 cells**

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SBL was originally isolated from frog (*Rana catesbeiana*) oocytes as a novel sialic acid-binding lectin (SBL) that displayed strong anti-cancer activity. SBL was later shown to be identical to a ribonuclease from oocytes of the same species. The administration of SBL induced apoptosis in mouse leukemia P388 cells but did not kill umbilical vein endothelial or fibroblast cells derived from normal tissues. The cytotoxicity of SBL was inhibited by desialylation of P388 cells and the co-presence of free bovine submaxillary mucin and heparan sulphate. SBL was observed to be incorporated into cells after attachment to cholesterol-rich microdomains and SBL induced apoptosis through the caspase-3 pathway following activation of caspase-8. Addition of the cholesterol remover, methyl-β-cyclodextrin reduced SBL-induced apoptosis with caspase-3 activity. Mass spectrometric and flow cytometric analyses appeared that both a heat shock cognate protein (Hsc70) and a heat shock protein (Hsp70) on the cell membrane bound to SBL and their inhibitor, quercetin significantly reduced SBL-induced apoptosis. Taken together, these findings suggest that sialyl-glycoconjugates present in cholesterol-rich microdomains form complexes with Hsc70 and/or Hsp70 that act as triggers for SBL to induce apoptosis through a pathway involving the activation of caspase-3 and caspase-8.

(320) **OGT Isoform expression is an X-linked trait predictive of Disease susceptibility**

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Turner Syndrome (or monosomy X, 45,X) occurs in 1 of 2500 live female births and is the most common genetic disorder effecting

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women. Some Turner patients display a complete loss of either the paternally - or maternally-derived X chromosome. Women monosomic for a maternally derived X (Xm) have android patterns of fat deposition, atherogenic metabolic profile and increased susceptibility to heart disease compared to women monosomic for a paternally transmitted X (Xp). Our hypothesis is that the parent-of-origin of the monosomic X in Turner Syndrome patients may be diagnostic of their risk for developing metabolic, neurodevelopmental, and cardiovascular disease. We have identified changes in gene expression associated with inheritance of Xp or Xm and have validated a number of biomarkers including the genes *XIST* and *OGT* as candidates which may influence metabolic programming. OGT splice variants appear to be differentially expressed in Turner patients inheriting the maternal rather than the paternal X chromosome. *OGT* is of interest because it has been implicated in nutrient-dependent chromosome remodeling and genomic imprinting. Recently, OGT has been shown to interact with the Tet family of enzymes that catalyse the conversion of 5-methylcytosine of DNA to 5-hydroxyl-methylcytosine. We have recently found that the parent of origin of the X-chromosome is also diagnostic for aortic stiffness in Turner Syndrome. Our findings enable a dissection of the role of the human X-chromosome in metabolic and cardiovascular disease in the absence of confounding sex hormonal influences and identified OGT as an X-imprinted therapeutic target.

(321) Initiation of Clustered O-glycosylation of IgA1 by GalNAc-transferases in IgA Nephropathy: New Methods for Complex Product Analysis

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Classical enzyme assays measure either the depletion of a substrate or the formation of a product. Polypeptide-*N*-acetylgalactosaminyltransferases (GalNAc-Ts) initiate glycosylation of polypeptides with clustered *O*-glycans, multiple GalNAc moieties per acceptor. GalNAc-Ts have preferred glycosylation sites, attributed to amino-acid sequence recognition by the catalytic domain. Follow-up glycosylation, responsible for high-density clustered glycans, is affected by carboxy-terminal lectin domain. To tease out the influences of both domains of GalNAc-Ts on clustered *O*-glycosylation, classical enzyme assays fall short. We developed an alternative approach that allows a more detailed assessment of the reaction kinetics in hopes of understanding the implications of initiation of IgA1 *O*-glycosylation in the disease IgA nephropathy. Recombinant soluble forms of GalNAc-T2 and -T14 were expressed, secreted, and purified from the media of 293 FreeStyle cells. Enzymes were reacted with synthetic IgA1 hinge-region peptide (sHR) with nine potential sites of *O*-glycosylation under various conditions and the products were analyzed by high-resolution mass spectrometry. Up to eight sites of sHR were readily glycosylated by GalNAc-T2. In time-course experiments, the relative rate of glycosylation was determined and compared for various average glycoforms in reaction mixture. The first three glycoforms appeared in quick order with similar rates of GalNAc incorporation. The addition of the second GalNAc quickly followed the first, presumably due to the existence of a second glycosylation motif in addition to lectin domain-dependent follow-up glycosylation. After the addition of four GalNAc moieties, the rate of addition drastically decreased through the 8th addition. GalNAc-T14 glycosylated sHR peptide to a lesser extent. Notably, GalNAc-T14 behaved differently at various buffer pH conditions. At pH 7.3, higher activity was observed, whereas at pH 6.6 glycans were added in a more clustered nature. Specifically, at pH 7.3, only two GalNAc residues were added whereas at pH 6.6 up to four residues were added. This phenomenon may be due to inhibition of lectin-domain binding at the higher pH, resulting in only high-affinity catalytic domain-driven glycosylation-motif glycosylation. These new methods for analysis of complex clustered *O*-glycosylation GalNAc-T reactions may help to determine the role of initiation of IgA1 *O*-glycosylation in IgA nephropathy and other diseases involving aberrant clustered *O*-glycosylation.

(322) Dietary intake of non-human sialic acid Neu5Gc promotes tumor growth in human-like mouse models of colorectal cancer

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Colorectal cancer (CRC) is the leading cause of cancer-related death in United States and the most common malignancy in the western world. Epidemiological data have shown that the human susceptibility to CRC is strongly impacted by diet and especially by consumption of "red meat" of mammalian origin. Although the presence of heme iron or various mutagens in red meat is proposed as explanation for increased risk, the data is equivocal, and a more conclusive mechanism that links red meat intake to CRC development or progression remains to be established. Previous studies from our group demonstrated that the non-human isoform of sialic acid, *N*-glycolylneuraminc acid (Neu5Gc) is enriched in red meat, and can be incorporated by human tissues, becoming exposed on cell surface glycoconjugates. Such Neu5Gc-containing glycans act as xeno-autoantigens that can be targeted by naturally occurring anti-Neu5Gc antibodies, leading to an inflammatory response. In this study, we used two different mouse models in a Cmah-/- background (mimicking the human lack of Neu5Gc) based on mutation of adenomatous polyposis coli (APC) protein, aiming to model the role of dietary Neu5Gc and anti-Neu5Gc antibodies as a risk factor for CRC. To induce the production of anti-Neu5Gc antibodies, 6-8 week old ApcMin/$^{+}$ and CDX2P-NLSCre Apc flox/$^{+}$ (APC-CPC) mice were immunized with Neu5Gc expressing chimpanzee red blood cell membranes or with *N*-acetylneuraminic acid (Neu5Ac) expressing human red blood cell membranes as a control. Both groups were fed with Neu5Gc or Neu5Ac containing soy chow for 120 days. The dietary intake of Neu5Gc induced a

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significant increase in the number of polyps in the ApcMin/+ Neu5Gc immunized mice. Interestingly, no alterations were observed in intestine of the groups fed with Neu5Ac containing chow. In addition, the Neu5Gc dietary intake induced an increase in tumor appearance and growth in the colon of the APC-CPC mice expressing anti-Neu5Gc antibodies. Further studies are being conducted in order to elucidate this mechanism, but the data so far point to Neu5Gc presence in red meat as an explanation for CRC risk.

(323) **Assessment of O-glycosylation of different molecular forms of IgA1 in sera of patients with IgA nephropathy, an autoimmune renal disease**

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IgA nephropathy (IgAN), the commonest glomerulonephritis worldwide and a frequent cause of end-stage renal disease, is an autoimmune disease in which kidneys are damaged as innocent bystanders. We have proposed a multi-hit mechanism for its pathogenesis, wherein circulating immune complexes consisting of IgA1 with some *O*-glycans deficient in galactose (Gd-IgA1; autoantigen) and anti-glycan autoantibodies deposit in the glomeruli and induce renal injury.

IgA1 has 3-6 clustered *O*-glycans in the hinge region. Normally, IgA1 has core 1 *O*-glycans, some of which may be sialylated on galactose and/or *N*-acetylgalactosamine. Patients with IgAN usually have increased serum levels of Gd-IgA1, as determined by lectin-ELISA. Using this assay, we determined that elevated levels of Gd-IgA1 in sera of IgAN patients (n = 275) collected at the time of diagnosis by renal biopsy are associated with a faster decline in kidney clearance function. Thus, serum levels of Gd-IgA1 may have prognostic value.

IgA1 may be in monomeric or polymeric forms, and some can be bound in immune complexes. About 90% of serum IgA1 is monomeric. To obtain better insight into the distribution of Gd-IgA1 among these different molecular forms in IgAN, we purified monomeric, polymeric, and immune-complex-bound IgA1 from sera of IgAN patients and analyzed the degree of galactose deficiency by lectin-ELISA. Most of the lectin-reactive IgA1 was in immune complexes, predominantly as polymers. In contrast, monomeric IgA1, the most abundant form, was the least galactose-deficient (reactive in the lectin-ELISA). Thus, polymeric IgA1 is affected by galactose deficiency more than is monomeric IgA1.

These findings are consistent with our earlier observations that IgA1-producing cells from patients with IgAN secrete Gd-IgA1 predominantly in polymeric form. It is not clear whether polymeric Gd-IgA1 is produced by a specialized subset of IgA1-secreting cells or whether the same cells secrete polymeric and monomeric IgA1 but the glycosylation abnormality affects only polymers. Future studies will reveal the molecular details of this glycosylation aberrancy and provide leads for developing disease-specific biomarkers and therapeutic targets for IgAN.

(324) **AFM Observation of Beta-Amyloid Aggregates Induced by Ganglioside GM1-containing Lipid Membrane**

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Alzheimer's disease is the most common and devastating dementia that causes decreasing memories and changing personalities. The number of its patients is increasing all over the world. However, there is no way to treat and/or prevent the disease because the mechanism of onset of the disease has not been clear yet. The early and invariable neuropathological hallmark of Alzheimer's disease is the deposition of amyloid β (Aβ) as fibrils. Besides its direct toxicities, the amyloid deposition can also act as potential reservoir and generator of Aβ oligomers, which are prime synaptotoxic agents in the patients' brains. It is widely accepted that amyloid deposition is a consequence chronic imbalance between Aβ production and Aβ clearance. However the mechanism of the initation of Aβ assembly into Amyloid in the brain remains to be clarified.

We have investigated that the interaction of Aβ with the lipid membrane of neurons. Neuronal cell membrane is abundant in gangliosides and monomeric Aβ is able to bind to gangliosides. In this study, we constructed lipid bilayers containing monosialo ganglioside GM1 (GM1/cholesterol/sphyngomyelin = 10:45:45) on mica substrate and observed the surface topography by an atomic force microscope (AFM) to investigate the interaction with Aβ. Aβ aggregation was found at more than 1 μM and its height was about 20 nm. The amount of Aβ aggregation increased with incubation time, and finally we found that Aβ formed fibrillar structure after 24 h incubation. Our result indicated that GM1 is essential to Aβ deposition and the structure of Aβ depends on GM1 and cholesterol contents, concentration of Ab, and incubation time.

Furthermore, to prevent the aggregation of Aβ, the interaction of GM1 cluster-binding peptide (GCBP) with GM1-containing membrane was investigated. GCBP was found to bind to the GM1-containing membrane and prevent the aggregation of Aβ.

(325) **Impaired O-GlcNAc Modification in the Endoplasmic Reticulum by Mutated EOGT Associated with Adams–Oliver Syndrome**

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EGF domain-specific O-linked N-acetylglucosaminyl transferase (EOGT) is an endoplasmic reticulum (ER)-resident glycosyltransferase that acts on EGF domain-containing proteins such as Notch receptors. Recently, mutations in *EOGT* have been reported in patients with Adams-Oliver syndrome (AOS). Here we show the enzymatic properties of mouse EOGT and EOGT mutants associated with AOS. In HEK293T cells, simultaneous expression of wild-type

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EOGT with Notch1 EGF repeats led to be immunoreactivity with the anti-O-GlcNAc antibody (CTD110.6) in the ER. The pH optimum of EOGT ranges from 7.0 to 7.5, and the K_m value for UDP-N-acetylglucosamine (UDP-GlcNAc) is 25 μM, indicating the enzymatic properties of EOGT are distinct from Golgi-resident GlcNAc transferases. Despite the relatively low K_m value for UDP-GlcNAc, EOGT-catalyzed GlcNAcylation depends on the hexosamine pathway, as revealed by the increased O-GlcNAcylation of Notch1-EGF repeats upon supplementation with hexosamine. As compared with wild-type EOGT, O-GlcNAcylation in the ER is nearly abolished in HEK293T cells exogenously expressing EOGT variants associated with AOS. EOGT W207S mutation resulted in degradation of the protein via the ubiquitin-proteasome pathway, while the stability and ER localization of the R377Q mutant were not affected. Importantly, the interaction between UDP-GlcNAc and the R377Q mutant was impaired. Our results suggest that impaired glycosyltransferase activity in mutant EOGT proteins and the consequent defective O-GlcNAcylation in the ER constitute the molecular basis for AOS.

(326) Development of Reverse Transfection Method Using pDNA/polysaccharide Complexes

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Chitosan is low toxic, low immunogenic and biocompatible polysaccharide. For this reason, chitosan has been widely investigated as a useful gene carrier. In conventional transfection method, so called forward transfection (FTF) method, cells are cultured in advance, gene complexes which are freshly prepared are added into cell culture medium. Recently, reverse transfection (RTF) method has received increased attention because it resolves complicated process of FTF method. For RTF method, gene complexes were immobilized on the plastic plate in advance, followed by the addition of cells. Therefore, it is necessary to maintain the gene expression activity of gene complexes after immobilization in RTF method. In this study, we employed pDNA/polysaccharide complexes for RTF method.

pDNA/chitosan complexes and pDNA/chitosan/anionic polysaccharide complexes were prepared by mixing the solutions of pDNA, chitosan and anionic polysaccharide such as hyaluronic acid. The composition ratio of complexes were determined on the basis of P:N:(-) ratio (molar ratio of pDNA's phosphate groups to chitosan's amino groups to anionic polysaccharide's carboxy and sulfate groups). Particle size was measured by a dynamic light scattering method and zeta potential measurement were performed using laser doppler velocimetry. pDNA/polysaccharide complexes were immobilized using cast method and layer-by-layer self assembly technique. COS7 cells were seeded on the immobilized pDNA complexes, and gene expression activity was evaluated by luciferase gene expression. RTF method using cast films of pDNA/chitosan complexes and pDNA/chitosan/anionic polysaccharide complexes showed higher cell adhesion and cell transfection efficiency than multilayer of pDNA/chitosan complexes and anionic polysaccharide. Moreover, we found that pDNA/chitosan/hyaluronic acid complexes showed high gene expression efficiency for RTF method.

Therefore it was indicated that immobilization of pDNA/chitosan/hyaluronic acid complexes were useful for RTF method.

(327) Deficiency in C-6 sulfation of GlcNAc within keratan sulfate mitigates Alzheimer's pathology and memory impairment in mice

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Keratan sulfate (KS) is a glycosaminoglycan present in the extracellular space. KS is connected to a protein core through an *N*- or *O*-linked glycan. KS comprises repeating disaccharides of galactose and *N*-acetylglucosamine (GlcNAc) that are modified with C-6 sulfation. We have previously shown that GlcNAc 6-*O*-sulfotransferase-1 (GlcNAc6ST-1) is an enzyme responsible for KS synthesis in the central nervous system and that KS is a molecular barrier to nerve regeneration/sprouting after injury in the brain and spinal cord. Pathological roles of KS in neurodegenerative diseases such as Alzheimer's disease and related disorders are remained unknown. We analyzed expression of a KS epitope and KS synthesis enzymes in the brains of J20 and Tg2576 Alzheimer's disease model mice by Western blotting and immunohistochemical staining. We have found that expression levels of KS recognized by the 5D4 antibody and GlcNAc6ST-1 were greatly increased in brains of 18- to 20-m-old J20 and Tg2576 mice. The 5D4 staining signals were colocalized with amyloid beta plaques and Iba1-positive cells surrounding plaques. The 5D4 staining signals were abolished by disrupting the GlcNAc6ST-1 gene in J20 mice (J20/GlcNAc6ST-1 KO). Reduced levels of cerebral amyloid beta deposition and attenuation of spontaneous alternation behavior deficits were observed in J20/GlcNAc6ST-1 KO mice. These results indicated that KS and GlcNAc6ST-1 are involved in cerebral amyloid plaque formation and that inhibition of GlcNAc6ST-1 could show beneficial effects in modulating Alzheimer's disease pathogenesis.

(328) Fucosylation is a common type of glycosylation in the cancer stem cell-like phenotype of pancreatic cancer under various conditions

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Cancer stem cells (CSCs) are an emerging topic in cancer biology for the last decades. However, their definition varies and various kinds of cell surface markers have been used to evaluate/isolate CSCs from cancer tissue or cell lines. Especially, in the case of

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pancreatic cancer, the existence of CSC is concerned with poor prognosis including early recurrence and anti-cancer drug resistance. While certain kinds of cell surface markers are used for CSC markers of pancreatic cancer, their availability is limited. Since oligosaccharide structure on the cell surface is dissimilar in each cell type, it could be applied to variety of cell markers including CSC markers. Among many glycans, fucosylation is one of the most important glycosylation in carcinogenesis. In this study, we found that fucosylation is a common oligosaccharide modification in pancreatic cancer CSCs, which are established one of three strategies; anti-cancer drug treatment for long time, sphere formation, and cell sorting using antibodies against typical CSC markers (CD24 and CD44) recognizing antibodies by flow cytometer. Many linkages of fucosylation were increased under these conditions and expression of fucosylation-regulatory genes such as fucosyltransferases, GDP-fucose synthetic enzymes and GDP-fucose transporters were dramatically enhanced in CSCs. These changes were especially significant in gemcitabine-resistant cells and sphere cells of a human pancreatic cancer cell line, Panc1. However, down-regulation of cellular fucosylation by knockdown of the GDP-fucose transporter did not alter gemcitabine resistance, suggesting that increased cellular fucosylation is not the cause but a result of of CSC-like transformation. Thus, fucosylation might be a biomarker of CSCs in pancreatic cancer.

(329) **Heparan Sulfate Containing Unsubstituted Glucosamine Residues: Biosynthesis and Heparanase Inhibitory Activity**

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Degradation of heparan sulfate (HS) in the extracellular matrix by heparanase is linked to the processes of tumor invasion and metastasis. Thus, a heparanase inhibitor can be a potential anticancer drug. As HS with unsubstituted glucosamine residues accumulates in heparanase-expressing breast cancer cells, we assumed that these HS structures are resistant to heparanase and can therefore be utilized as a heparanase inhibitor. As expected, chemically synthetic HS-tetrasaccharides containing unsubstituted glucosamine residues, GlcAβ1-4GlcNH$_3^+$(6-*O*-sulfate)α1-4GlcAβ1-4GlcNH$_3^+$(6-*O*-sulfate), inhibited heparanase activity and suppressed invasion of breast cancer cells *in vitro*. Bifunctional *N*-deacetylase/*N*-sulfotransferase-1 (NDST-1) catalyzes the modification of *N*-acetylglucosamine residues within HS chains and the balance of *N*-deacetylase and *N*-sulfotransferase activities of NDST-1 is thought to be a determinant of the generation of unsubstituted glucosamine. We also report here that exostosin-like 3 (EXTL3) controls *N*-sulfotransferase activity of NDST-1 by forming a complex with NDST-1 and contributes to generation of unsubstituted glucosamine residues.

(330) **Insight into single nucleotide polymorphisms (SNPs) of the polysialyltransferase ST8SIA2/STX in psychiatric disorders**

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Polysialic acid (polySia) is a homopolymer of sialic acid with a degree of polymerization of 8-400. PolySia is expressed not only in embryonic brains, but also in distinct regions of adult brains where neurogenesis is ongoing, such as hippocampus and the olfactory system. When present on neural cell adhesion molecule (NCAM), polySia is known to exhibit anti-adhesive effects on cell-cell interactions due to its bulky polyanionic nature, to regulate the neurogenesis. We have recently demonstrated that polySia also functions as a reservoir scaffold for brain-derived neurotrophic factor (BDNF) and fibroblast growth factor 2 (FGF2), which are biologically active molecules in neurogenesis and neural functions. PolySia is known to be biosynthesized by two polysialyltransferases *ST8SIA2/STX* and ST8SIA4/PST. Interestingly, the genome-wide screenings of psychiatric disorders have suggested that there are associations between those disorders and polymorphisms in the *ST8SIA2/STX*. The objective of this study is to understand underlying mechanisms for these associations, and we have sought to see how the *ST8SIA2/STX* genes containing single nucleotide polymorphisms (SNPs) found in psychiatric disorder patients affect the structure of their final product polySia. In this study, we focused on several SNPs of the *ST8SIA2/STX* that were identified in schizophrenia, autism spectrum disorder and bipolar spectrum disorder patients. The results not only showed that missense mutations (coding SNP) impaired the polySia structure through some structural changes of *ST8SIA2/STX*, but also showed that even silent mutations (regulatory SNP, silent SNP and intronic SNP) affected the amount of *ST8SIA2/STX* by changing transcriptional and translational efficiencies of *ST8SIA2/STX*. These quantity alterations of the enzyme may also affect the quantity and quality of polySia and subsequent impairments of polySia functions.

(331) **Enhanced expression of polysialic acid is correlated with malignant phenotype in breast cancer cell lines and clinical tissue samples**

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Sialic acids, a group of monosaccharides with 9-carbon backbones, are abundantly present in many organisms and have been shown to mediate a variety of cellular processes in bacteria and mammals. PSA, a linear homopolymer composed of α2,8-linked sialic acid residues, plays an important role in brain development, and is involved in certain psychiatric disorders, including schizophrenia,

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autism, and bipolar disorder. PSA level is reduced in most tissues during the course of development, but is aberrantly re-expressed in many tumors (e.g., lung cancer, pancreatic cancer, neuroblastoma, glioma), where it modulates cell adhesion, migration, and invasion. In mammals, PSA is added to the terminal moieties of glycoproteins, where it helps modulate cell-cell adhesion and repulsion because of its highly negative charge. Biosynthesis of PSA is catalyzed by two Golgi-resident polysialyltransferases, ST8SiaIV (PST) and ST8SiaII (STX). In current study, we evaluated PSA expression and found its expression was significantly higher in malignant breast cancer (BC) cells and in normal mouse mammary epithelial cells that undergo EMT (epithelial-mesenchymal transition). Meanwhile, we demonstrated that increased PSA expression was correlated with disease stage in BC patients using 24 BC specimens. The polysialyltransferase ST8Sia IV (PST), which is responsible for PSA synthesis, was re-expressed in clinical BC samples. Our findings of higher PST expression in advanced-stage BC may reflect the presence of more highly polysialylated N-glycans in these patients and indicate the pathophysiology of BC involves aberrant regulation of PSA level and PST gene expression.

(332) Analysis of Glycans Related to Metastasis of Human Cancer Cells by Saccharide Primer Method

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It is known that profile of glycan expressions in cells changes drastically during the process of malignant transformation. For example, sialyl Lewis^x and sialyl Lewis^y are useful markers of cancer, and are involved in malignancy of cancer. Glycans related to metastasis are dependent on cell lines.

In this study, we developed the convenient method to evaluate glycans expressed in metastatic tumor cells. Glycan related to metastasis of human cancer cells were analyzed by saccharide primer method. Saccharide primer method is useful for elucidating the kinds of glycans expressed in living cells. Saccharide primers are mimicking of intermediates in the biosynthetic pathway of glycans and act as acceptors for glycosyltransferase in cells. By comparing the glycosylated products derived from saccharide primers between the cells showing different metastasis, it was indicated that sulfated glycans largely expressed in metastatic human cells. The sulfate groups were conjugated to C3-position of galactose. Next, the expression of genes involves in the synthesis of sulfated glycan (galactose-3-O-sulfotransferase1 ～4 (GAL3ST1 ～4)) was detected by real time RT-PCR. GAL3ST1 and GAL3ST3 were largely expressed in highly metastatic cells. To identify the genes involved in metastasis, knockdown of GAL3ST1 ～4 was carried out and cell migration was investigated by wound healing assay and transwell migration assay. Gene knockdown of GAL3ST1 ～4 in metastatic cells with siRNA indicated that GAL3ST3 was related to cell migration. Furthermore, over expression of GAL3ST3 in pooring metastatic cells induced cell migration.

Thus, We concluded that GAL3ST3 contribute to metastasis of lung adenocarcinoma cells.

(333) Study of protein O-GlcNAcylation in the brain tissue in Huntington's disease

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Introduction: Recent studies hypothesize a role of O-GlcNacylation in the pathophysiology of neurodegenerative diseases [1]. Additionaly, altered O-GlcNacylation has been linked to impaired mitochondrial function [2]. In a *Caenorhabditis elegans* model of Huntington's disease (HD), it was found that genetic manipulation of the O-GlcNAc cycling enzymes OGT and OGA affected proteotoxicity and disease progression [3]. However, no study has been published yet examining the changes of O-GlcNAc signaling in HD patients. Our aim: was to analyze protein O-GlcNAcylation in the brain tissue of an HD patient and transgenic (TgHD) minipigs for the N-terminal part of human mutated htt. Material and methods: The analyses were performed in the postmortem brain tissue (basal ganglia - BG, frontal cortex - FC) of an HD patient and TgHD minipigs (24 months old), plus in corresponding samples from age-matched controls. The global extent of O-GlcNAcylation and levels of O-GlcNAc cycling enzymes were detected by Western blot using the antibodies RL2, anti-OGT and anti-OGA. Results: Elevated levels of global protein O-GlcNAcylation were found both in BG (2.9-fold) and FC (1.8-fold) of the studied HD patient. Moreover, markedly increased level (2.4-fold) of OGT was observed in BG. Also in BG, noteworthy was the increase of an additional, lower band detected by OGA antibody, which possibly represents a smaller OGA isoform. No significant changes have been found in the asymptomatic 24-month-old TgHD minipigs. Conclusion: Our preliminary results show that in the postmortem HD brain, global O-GlcNAcylation is elevated, accompanied by a disturbed balance of the (de)glycosylating enzymes. We believe that O-GlcNAcylation dysregulation could contribute to HD pathophysiology, including mitochondrial dysfunction observed in this disease. Supported by Czech-Norwegian research programme 7F14308, GAUK 638512, ExAM-CZ.1.05./2.1.00/03.0124

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(334) **Globo-series glycans as therapeutic targets for cancers**

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The aberrant expression of glycan epitopes is highly associated with tumorigenesis. Finding cancer-specific glycan biomarkers and further evaluation of their therapeutic applications are important for treating cancers. In this study, we screened the expression of globo-series glycosphingolipids, including Globo H, SSEA3 and SSEA-4, in the cell lines derived from 14 kinds of cancers. We found that these globo-series glycosphingolipids were highly expressed in almost all cancer cell lines. Among malignant tumors, brain tumor, especially glioblastoma multiforme, is the most difficult to treat and lead to poor survival rate even with surgery, chemotherapy and radiotherapy. With the result that SSEA-4 abundantly expressed in most glioblastoma multiforme cells, we further examined and observed a positive correlation between the expression of SSEA-4 and disease progression in glioblastoma multiforme specimens. Moreover, treatment with anti-SSEA-4 mAb significantly induced complement-dependent cytotoxicity *in vitro* and suppressed tumor formation in mice. Therefore, our finding indicates that SSEA-4 is an important target for treatment of glioblastoma multiforme and other cancers.

(335) **Development of recombinant Aleuria aurantia lectins for the detection of cancer specific changes in hepatocellular carcinoma**

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Changes in protein glycosylation have long been associated with many diseases. Specifically, we have reported that increases in core alpha-1,6-linked fucosylation of serum proteins correlates with the development of hepatocellular carcinoma (HCC) and this finding has been extended by others both in liver cancer and in other cancers such as lung, ovarian and colon cancer. Sugar binding proteins, called lectins, are often used in plate based assays to measure changes in glycosylation. The *Aleuria aurantia* lectin (AAL) derived from orange peel mushroom contains five fucose-binding sites that recognizes fucose bound in α-1,2, α-1,3, α-1,4 and α-1,6 linkages to N-acetylglucosamine (GlcNAc). Unfortunately, while fucose in an α-1,6 linkages is associated with cancer, fucose in α-1,2, α-1,3 or α-1,4 linkages are often associated with inflammation and not cancer. In an effort to develop better reagents for the detection of cancer specific changes, we have recently created several recombinant AAL (rAAL) proteins that have high affinity specific to core-linked (α-1,6) fucose. Driven by the sensitivity and specificity of these rAAL, we further study the capabilities these recombinant lections using molecular modeling and used them to analyze the level of core fucosylation in HCC liver tissues using western blot analysis, ELISA and lectin peroxidase immunohistochemistry (IHC). We observed increased specific core fucose staining resulting from the high affinity binding of rAAL to fucosylated HCC tissues suggesting an increase in fucosylation directly in HCC tissues. The need, development and use of these recombinant lectins in the development of biomarkers of HCC and to further understand the changes in glycosylation with the development of HCC are discussed.

(336) **Depletion of (neo-) lacto series glycosphingolipids by genome editing of B3GNT5**

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Glycosphingolipids (GSL) of the (neo-) lacto series are involved in cell development and determine various blood group antigens. Precursor of this series is lactotriaosylceramide, which is processed from lactosylceramide by β1,3 *N*-acetylglucosaminyltransferase (B3GNT5). Its gene is associated with malignant diseases, shown by gene amplifications in TCGA data sets from lung (42.1%), ovary (27.0%), and several other cancers. In addition, our recent studies suggest P1 (neolacto series), as an ovarian cancer associated-carbohydrate that is recognized by anti-glycan antibodies [1, 2]. We aim to deplete (neo-) lacto series GSL by genome editing *B3GNT5*.

The human embryonic kidney cell line HEK293T and the P1-positive ovarian cancer cell line IGROV1 were *B3GNT5* engineered using the CRISPR-Cas9 system. Co-transfected single guided RNA and single- strand oligonucleotides were designed to either partly or fully delete the protein coding sequence, a deletion of 899bp and 2448bp, respectively. Candidate clones were screened by genotyping PCR, sequenced and investigated on transcript, protein and GSL levels.

Transfected cells showed Cas9 activity to different extents in respective amplicon sizes (Δ899bp *B3GNT5* for IGROV1; Δ2448bp *B3GNT5* for HEK293T). Corresponding transcripts and proteins were analyzed using RT-qPCR and immunoblot, respectively, confirming successful editing of *B3GNT5*. The expression of the P1 in *B3GNT5* edited IGROV1 cells was clearly reduced. These results on P1 were confirmed by confocal fluorescence microscopy.

We have introduced CRISPR-Cas9 system to deplete (neo-) lacto series GSL *via* targeting the key enzyme B3GNT5. Moreover, we could demonstrate that one candidate of this series namely P1 was affected by this depletion. We are currently investigating more GSL to further characterize the effect of *B3GNT5* deletion and verifying the allelic status in those two cell lines. The knockout cells will serve as model for further studies to elucidate the cellular function of (neo-) lacto series GSL in general and in particular in ovarian cancer.

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(337) Identification of fucosylated Fetuin-A as a serum biomarker for cholangiocarcinoma

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Cholangiocarcinoma is a malignancy of the bile ducts that carry bile from the liver to the small intestine. No effective serum marker exists for surveillance of cholangiocarcinoma. Using a glycomic method, we determined that patients with cholangiocarcinoma have increased levels of fucosylated glycan in their serum. Proteomic analysis of the fucosylated proteome identified proteins such as alpha-2-macroglobulin, kininogen, hemopexin, fetuin-A, alpha-1 anti-trypsin, and ceruloplasmin as being hyperfucosylated in HCC. To determine if the identified hyper-fucosylated proteins could act as biomarkers of cholangiocarcinoma, we examined the levels of these glycoproteins in 109 patients with cholangiocarcinoma, primary sclerosing cholangitis (PSC), a disease that often precedes CCA, and control patients and compared them to the performance of CA-19-9, the current "gold standard" serum assay for cholangiocarcinoma. Of all the identified proteins, fucosylated Fetuin-A (fc-Fetuin-A) had the best ability to differentiate CCA from PSC, with an AUROC of 0.812. CA-19-9, which has been used by others to detect cholangiocarcinoma in the background of PSC, had poor ability to differentiate PSC from cholangiocarcinoma (AUROC of 0.625). A combination of two markers, fc-Fetuin-A and fucosylated kininogen, improved the AUROC to 0.867 in the differentiation of CCA from PSC. In conclusion, fucosylated Fetuin-A may have value either alone or in combination in the surveillance of people at risk for the development of cholangiocarcinoma.

(338) Overexpression of individual subunits of the glycosylphosphatidylinositol transamidase (GPI-T) induce subunit- and C-terminal signal sequence-specific changes in GPI anchoring of proteins

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Glycosylphosphatidylinositol (GPI) anchoring of proteins is catalyzed by a multi-subunit transamidase (GPI-T) that has garnered attention for its connections to cancer. Expression of the five GPI-T subunits varies widely among different cancers and between subunits; these changes have been proposed to disrupt normal cell function. [1] We developed a system in *Saccharomyces cerevisiae* for measuring GPI anchoring of proteins. Invertase was expressed with different C-terminal GPI-T signal sequences based on the GPI-T signal sequences from the human campath-1 antigen (CD52), the human urokinase plasminogen activated receptor (uPAR), and the yeast yapsin 2 protease (Y2P); the extent of cell surface presentation of GPI anchored invertase was assessed for each construct *in vivo*. We discovered that the nature of the C-terminal signal sequence impacts the extent of GPI anchored invertase presented at the plasma membrane. The Y2P sequence was the most effective; uPAR produced intermediate levels of GPI anchored invertase; and the CD52 sequence was only a weak substrate for GPI-T. [2] Here, we demonstrate that overexpression of individual GPI-T subunits further influences the variable impact of these sequences (compared to basal subunit expression). Overexpression of Gpi8, the active site of GPI-T, had little effect on invertase anchoring with either the Y2P or uPAR sequences, but nearly doubled the effectiveness of the CD52 sequence. Overexpression of either Gpi16 or Gaa1, two other GPI-T subunits, reduced the extent of GPI anchoring in all three cases but to different extents based on the GPI-T signal sequence used. These results offer clear indications that GPI-T's role in cancer is not to uniformly upregulate GPI anchoring of proteins. Instead, our results suggest a much more intricate and complex series of modulations wherein alterations in the expression level of one GPI-T subunit produces a pattern of changes in GPI anchoring of substrate proteins that is specific to that subunit. Consequently, with respect to tumor progression, we propose that mechanistically critical inequalities exist among the different subunits of GPI-T and its substrates.

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(339) O-GlcNAc Transferase Directs Cell Proliferation in Idiopathic Pulmonary Arterial Hypertension

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Idiopathic Pulmonary arterial Hypertension (IPAH) is a cardiopulmonary disease characterized by cellular proliferation and vascular remodeling. A more recently recognized characteristic of the disease is dysregulation of glucose metabolism. The primary link between altered glucose metabolism and cell proliferation in IPAH has not been elucidated. Using human IPAH and control patient lung tissues and pulmonary artery smooth muscle cells (PASMCs), we analyzed the role of the hexosamine biosynthetic pathway (HBP) in initiating and/or perpetuating smooth muscle cell proliferation in IPAH. In addition, we measured the levels of O-linked N-acetylglucosamine modification (O-GlcNAc), O-GlcNAc transferase (OGT), and O-GlcNAc hydrolase (OGA) in control and IPAH cells and tissues. Our data suggests that the activation of the HBP directly increased OGT levels and activity triggering changes in glycosylation and PASMC proliferation. Partial knockdown of OGT in IPAH PASMCs resulted in reduced global O-GlcNAc levels and abrogated PASMC proliferation. The increased proliferation observed in IPAH PASMCs was directly associated with enhanced proteolytic activation of the cell cycle regulator, host cell factor-1 (HCF-1). Our that

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HBP flux is increased in IPAH and drives OGT-facilitated PASMC proliferation through specific proteolysis and direct activation of HCF-1. These findings establish a novel regulatory role for OGT in IPAH, shed a new light on our understanding of the disease pathobiology, and provide opportunities to design novel therapeutic strategies for IPAH.

(340) Development of novel method for analysis of disease-specific glycosaminoglycans in mucopolysaccharidosis type II

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Mucopolysaccharidosis type II (MPS II) is an X-linked lysosomal storage disorder caused by the deficiency of iduronate-2-sulfatase (IDS), leading to progressive accumulation of glycosaminoglycans (GAGs) in multiple tissues. The accumulated GAGs are used as "classic" biomarker for diagnosis, prognosis, and therapeutic evaluation of MPS II. However, measurement of total GAGs has insufficient specificity and sensitivity for analysis of MPS II abnormality, because variable amounts of GAGs are present in not only patients with MPS II but also healthy individuals. To resolve above problem, we developed the pathological iduronic acid assay based on the analysis of 2-sulfoiduronic acid in non-reducing end of the GAGs accumulated in MPS II. The GAGs were purified from tissue homogenates derived from MPS II mice, digested with recombinant human IDS and recombinant human iduronidase to generate a desulfated iduronic acid from non-reducing terminal of them. Analysis by HPLC revealed that the levels of iduronic acid were significantly increased in liver and cerebrum derived from MPS II mice. In contrast, iduronic acid was not detected in the samples from wild-type mice. These results indicate that 2-sulfoiduronic acid in non-reducing end of GAGs is a disease specific biomarker for MPS II, and our method may contribute to a better evaluation of GAG accumulation in the disease.

(341) Ligand-mediated Siglec-8 internalization in eosinophils is influenced by the actin cytoskeleton, tyrosine kinases, and sialylated cis ligands

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Sialic acid-binding immunoglobulin-like lectin (Siglec)-8 is a human cell surface protein expressed on eosinophils, mast cells, and basophils that induces eosinophil apoptosis and inhibits mast cell mediator release. This makes Siglec-8 an ideal target for monoclonal antibody- and glycan ligand-based therapies for diseases involving these cell types.

However, the dynamics of Siglec-8 surface expression, which are crucial to these targeted therapies, are poorly understood. Using monoclonal antibodies against Siglec-8 as well as a biotinylated synthetic ligand (6′-O-sulfo-3′-sialyl-LacNAc-decorated 1-MDa polyacrylamide), we examined the internalization and trafficking of Siglec-8 in eosinophils by flow cytometry and confocal microscopy. Inhibitors of components of various endocytic pathways were used to identify their roles in Siglec-8 dynamics. Finally, sialidase was used to establish specificity of binding and internalization of the receptor with the synthetic ligand and determine whether sialylated *cis* ligands may influence Siglec-8 internalization pathways or alter its surface trafficking.

We observed that Siglec-8 internalization in human eosinophils proceeds slowly in response to ligation with either antibody or the polyvalent synthetic ligand; about half of the surface pool of Siglec-8 is internalized in 90 minutes. This process is prevented by treatment with disruptors of actin cytoskeletal dynamics (latrunculin B and jasplakinolide) and an inhibitor of tyrosine kinases (genistein), but not by an inhibitor of microtubule assembly (nocodazole) or a Src family kinase inhibitor at a concentration appropriate for these kinases (PP1, 100 nM). Interestingly, the same agents that prevent receptor internalization were also found to inhibit apoptosis due to Siglec-8 engagement. Internalized Siglec-8 forms a punctate pattern within the cell confined to an intracellular compartment that has yet to be identified. Sialidase treatment of eosinophils prior to Siglec-8 ligation enhances ligand binding, suggesting the presence of masking *cis* sialylated ligand on the eosinophil surface, and accelerates Siglec-8 internalization. The dynamics of Siglec-8 surface expression therefore appear to be suitable for sustained targeting and may deliver drugs effectively to intracellular compartments to treat diseases including malignancies of these cell types. While the identity of the *cis* sialylated ligand is currently unknown, modulation of this interaction may improve the efficacy of Siglec-8-targeted therapeutics.

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(342) Heparan sulfate storage alters nervous system development in Sanfilippo syndrome, MPSIIIA

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Glycosaminoglycans (GAGs) are abundantly expressed on the surface of nearly all cell types and in the extracellular matrix. In the developing central nervous system, GAGs play important roles in developmental processes and alterations in their expression alter brain structure and function. GAGs undergo degradation in the lysosome by the sequential action of a series of lysosomal hydrolases.

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Deficiencies in any of these enzymes cause accumulation of GAGs and a class of lysosomal storage disorders (LSDs) called mucopolysaccharidoses (MPSs). The Sanfilippo syndromes (MPSIIIA-E) result from mutations in specific enzymes that degrade heparan sulfate (HS). The Sanfilippo syndromes are unusual in that, neuropathology and pathophysiology presents typically in young children. At disease onset, MPSIII patients become severely hyperactive, behavioral symptoms that are unique to LSDs wherein HS is the primary storage metabolite. As the disease progresses, neurodegeneration takes place causing loss of the extreme behaviors at disease onset in addition to loss of acquired skills and cognitive function. The mechanism by which HS storage leads to early behavioral changes is unknown. Primary storage of HS causes a "snow ball effect" wherein deficits in lysosomal function cause storage of secondary metabolites. To determine how primary and secondary storage may alter brain function we have performed a comprehensive natural history study in a murine model of MPSIIIA. Our results are surprising and suggest that there are likely two distinct stages in MPSIIIA pathophysiology, encompassing both developmental and degenerative mechanisms. Further investigations into the cellular source of pathophysiological changes at disease onset suggest that HS storage in glia may have a major role in altering the developing brain. Our findings provide valuable information regarding how HS storage leads to changes in brain function and provide potential targets for therapeutic strategies to treat MPSIII.

(343) **Targeting the Tn antigen of Muc1 in Cancer with a Chimeric Antigen Receptor**

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University of Pennsylvania

The Mucin-1 (Muc1) protein is normally localized to the basal membrane of normal epithelial cells, and is highly glycosylated with complex O-linked polysaccharide chains. In contrast, Muc1 is often overexpressed and no longer restricted to the basolateral surface of many cancer cells. Moreover, Muc1 protein in transformed cells is decorated with only O-linked GalNac (Tn antigen), due to mutation or epigenetic silencing of the gene encoding the T synthase chaperone Cosmc. As the Tn antigen contains a unique glycoepitope recognized by the antibody 5E5, we have developed a chimeric antigen receptor (CAR) using an scFv sequence derived from the 5E5 mAb and the intracellular signaling domains of CD3zeta and 4-1BB, which promote T cell activation and co-stimulation, respectively. Human T cells redirected with this novel 5E5-CAR exhibit cytotoxicity and cytokine secretion when exposed to a broad array of tumor cell lines, including leukemia, pancreatic, and breast cancer cell lines. Overexpression of normal Cosmc in Jurkat clone E6.1, which contain a truncated Cosmc, or other tumor cell lines with cosmc promoter methylation, corrects the T synthase defect and protects the tumor cells from cytolysis by 5E5-CAR T cells. These results demonstrate the specificity of the 5E5-CAR for the Tn antigen. We have also demonstrated the safety of the 5E5-CAR against a panel of normal human primary cells. Finally, human T cells expressing the 5E5-CAR demonstrate rapid clearance of established Jurkat tumors in NSG mouse xenograft models, as assessed by serial bioluminescence imaging and prolonged survival compared to mice expressing a control CD19-specific CAR T cells ($P < 0.005$). Taken together, these results suggest that the 5E5-CAR targeting the Tn-antigen of Muc1 would provide a safe, multi-tissue cancer-specific adoptive immunotherapy. Furthermore, these results suggest that the targeting of tumor specifc glycoepitopes may enable the development of universal CARs, creating T cells that are specific for many cancer histotypes that do not have shared expression on normal tissues.

(344) **Mitigation of Non-Typeable Haemophilus Influenzae induced acute airway inflammation by manipulating circulatory ST6Gal-1 levels**

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Our earlier work demonstrated that circulatory deficiency of the sialyltransferase, ST6Gal-1, resulted in an overly robust hematopoiesis, especially in the over-production of inflammatory cells. Mutant mice with reduced STGal-1 in circulation have strikingly more robust Th1 and Th2 inflammation with elevated infiltration of effector cells. These data strongly suggest that manipulation of blood STGal-1 levels may have clinical benefit in the management of a dysregulated inflammatory response. We hypothesize that raising the blood ST6Gal-1 level will suppress inflammation and decrease de novo production of inflammatory effector cells.

Non-typeable *Haemophilus influenzae* (NTHI) bacteria contribute to the progression and exacerbation of Chronic Obstructive Pulmonary Disease (COPD) at least in part by inducing neutrophilic influx into the airway. Intravenous administration of purified recombinant ST6Gal-1 into mice with NTHI-induced acute pulmonary inflammation resulted in significant reduction in the proliferation of granulocyte progenitors and neutrophil production in bone marrow, reduced numbers of inflammatory cells in the bronchoalveolar lavage fluid (BALF) and less severe pulmonary inflammation by histopathological evaluation. ST6Gal-1 infusion also results in reduced release of pro-inflammatory mediators, TNFα and IL-6, in the airways of animals challenged with NTHI.

During acute lung inflammation, macrophages are the major source of inflammatory mediators. Primary alveolar macrophage cultured *ex vivo* and pretreated with recombinant ST6Gal-1, downregulated the TNFα and IL-6 production in response to lipopolysaccharide (LPS) exposure. Taken together we demonstrate that elevation of circulatory ST6Gal-1 can mitigate inflammation by at least two distinct pathways: a) by reducing the production of inflammatory effector cells and b) by down-regulating the release of inflammatory mediators. Our data point to a novel approach in the management of inflammation by manipulating circulatory ST6Gal-1 levels.

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(345) **The sialyltransferase ST6Gal-I is upregulated in ovarian and pancreatic cancer and promotes tumor survival by activating a cancer stem cell phenotype**

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Elevated sialylation in tumor compared to normal tissue has been noted for decades, although the role of individual sialyltransferase expression and activity in cancer is still unclear. The ST6Gal-I sialyltransferase catalyzes the addition of sialic acid in an α2-6 linkage to critical receptors involved in tumorigenesis, which has been shown to alter receptor function. We report that ST6Gal-I protein is expressed in 32/33 serous ovarian tumors, whereas ST6Gal-I expression is minimal in the normal ovary. Comparing a set of 10 metastatic ovarian tumors to 10 primary tumors reveals a higher percentage of ST6Gal-I expressing tumor cells in the metastatic tumors, suggesting that ST6Gal-I is enriched during the metastatic process. Additionally, 11/11 pancreatic tumors express the enzyme, while ST6Gal-I is undetectable in normal pancreatic epithelium. Previous reports have correlated ST6Gal-I with a less differentiated cellular state, which led us to investigate whether ST6Gal-I expression could promote a cancer stem cell (CSC) phenotype characterized by chemotherapy resistance, tumor cell spheroid forming potential, and tumor initiating potential in mice. In addition to our previous findings that ST6Gal-I promotes resistance to cisplatin therapy in ovarian cells, we report ST6Gal-I expression promotes resistance to gemcitabine in pancreatic cells demonstrating chemoresistance across cell types and therapeutic agents. Ovarian and pancreatic cells with high ST6Gal-I expression form spheroids when grown in serum-free media in low attachment dishes, however, knockdown of ST6Gal-I reduces the size and proliferation of spheroids formed. Furthermore, culturing a cell population with heterogenous ST6Gal-I expression in spheroid conditions known to enrich for CSCs, selects for high ST6Gal-I-expressing clones, implicating ST6Gal-I in survival. We next determined the tumor initiating potential of pancreatic and ovarian cancer cells by injecting increasing dilutions of tumor cells into mice. These experiments reveal that knockdown of ST6Gal-I significantly decreases tumor incidence and size. Similarly, we show in an orthotopic model of ovarian cancer that knockdown of ST6Gal-I reduces the size of tumors in the peritoneal cavity. These findings together highlight the role of ST6Gal-I in endowing tumor cells with a phenotype consistent with a CSC, a tumor subpopulation that drives tumor metastasis and recurrence after chemotherapy.

(346) **Detailed characterization of glycans from Erbitux, Rituxan, and Enbrel using Recombinant PNGase F and a panel of Exoglycosidases**

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New England Biolabs

Detailed characterization of glycans on therapeutic proteins is critical as the type and degree of glycosylation can have a profound impact on the stability, activity and effector function of the drug. The structure of IgG glycans has been reported to affect biological functions such as complement dependent cytotoxicity (CDC), antibody-dependent cytotoxicity (ADCC), binding to various Fc receptors, and binding to C1q protein. Since IgG glycans are typically structurally heterogeneous and some glycoforms are much less abundant than others, it is critical that analytical workflows that rely on enzymatic release of N-glycans as a first step be efficient and unbiased so that all species are represented. Recombinant PNGase F, an amidase that cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides of N-linked glycoproteins, is used here in conjunction with downstream LC/MS analysis to characterize the glycans of three therapeutic glycoproteins: Erbitux, Rituxan and Enbrel. Erbitux, a recombinant human-mouse chimeric monoclonal antibody that is produced in murine myeloma cell culture, is unique in that it has two glycosylation sites on its heavy chain. One site is located in the conserved CH2 domain (Asn 299), and the second in the variable portion of the heavy chain at Asn88. Glycans on the Fab portion of IgG are particularly resistant to endoglycosidase digestion, often requiring a longer denaturation step at higher temperatures in conjunction with extended digestion time with PNGase F. Separate Erbitux Fab and Fc fragments pools were created using Ide S protease and protein A columns. Using a panel of well characterized exoglycosidases, we performed sequential digests of the intact Fab fragment and then electrospray ionization and time of flight (ESI-TOF) detection to elucidate sequence information. This "middle up" approach combined with exoglycosidase digestion exemplifies the value of using enzymatic manipulation of glycoproteins, as the protein structure remains intact after the release of glycans and allows for downstream analysis.

(347) **The Glycosylation Profile of Metastatic Melanoma Lymph Node Tumours**

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Metastasis accounts for the majority of mortality associated with melanoma, as limited treatment options exist for advanced stages of the disease. Alterations in cell surface glycosylation, in particular, increases in highly branched sialylated tetra-antennary *N*-glycan structures contribute to the invasive and metastatic potential of melanoma cells.

Despite a growing understanding of the role of *N*-linked oligosaccharides in melanoma biology, there has been little progress in using

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glycans as a screening tool for the early diagnosis of metastasis and predictor of patient prognosis.

Here, we demonstrate a targeted method combining PGC-LC-MS with exoglycosidase digestion for the complete structural characterisation and relative quantitation of *N*-glycans released from metastatic melanoma lymph node tumours. Released glycans were treated with a full array of exoglycosidase enzymes to assign monosaccharide linkage and confirm terminal epitopes from pools of good and poor prognosis patient samples.

The global membrane *N*-glycosylation profile of tumour tissue from individual patient samples have been compared. Structures were quantitated before and after selected exoglycosidase combinations to investigate differences in structural features including the degree of branching, sialylation and fucosylation. Over 80 glycan structures were identified, including high mannose, pauci mannose, hybrid and complex type glycans.

This study contributes to our understanding of glycosylation alterations in melanoma metastasis towards using specific glycosylation changes as prognostic markers and the identification of specific targets for therapeutic intervention.

(348) High-throughput screening of GnT-III inhibitors using UDP-Glo system to develop a novel drug candidate for Alzheimer's disease

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Glycans play key roles in development of several diseases, indicating that inhibiting a glycosyltransferase could be a new drug target for a particular disease. However, conventional method of high-throughput screening is lacking to discover chemical inhibitors for a glycosyltransferase.

Very recently, we found that GnT-III, a GlcNAc-transferase responsible for the biosynthesis of bisecting GlcNAc structure, plays a pathological role in Alzheimer's disease (AD) development in vivo. GnT-III knockout mice show a drastic decrease in amyloid- (Aβ), a hallmark of AD, resulting in improvement of cognitive function. We demonstrated that BACE1, an essential protease for Aβ generation, is a novel target for bisecting GlcNAc and is highly modified with bisecting GlcNAc in AD patients, suggesting that bisecting GlcNAc promotes AD development by regulating BACE1 function. Indeed, the reduction of Aβ in GnT-III-knockout mice is caused by a shift in intracellular distribution of BACE1 from early endosomes to lysosomes. These results suggest that bisecting GlcNAc is a novel regulator for BACE1 trafficking and that GnT-III inhibitor could be a promising drug target for AD.

In this study, we established a new high-throughput GnT-III assay method using UDP-Glo system for screening GnT-III inhibitors, as novel AD drug candidates. This system can measure glycosyltransferase activity by quantifying the UDP levels generated by glycosylation reaction. First, we confirmed that the Km and Vmax values of GnT-III in UDP-Glo assay were comparable to those in conventional HPLC assay. By using UDP-Glo system, we tested over 100,000 synthetic compounds within 6 months and found 40 chemicals that inhibited GnT-III activity with IC50 less than 20 μM. These candidate inhibitors are now being further validated in vitro and in vivo. This UDP-Glo-based glycosylatransferase assay system provides a simple method for the large-scale screening of new glycosylation inhibitors as glycan-targeted therapeutics.

(349) Effects of Sesamin on the Biosynthesis of Chondroitin Sulfate Proteoglycans In Human Articular Chondrocytes in Primary Culture

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Osteoarthritis (OA) is a degenerative joint disease that progressively causes a loss of joint functions and the impaired quality of life. The most significant event in OA is a high degree of degradation of articular cartilage accompanied by the loss of chondroitin sulfate-proteoglycans (CS-PGs). Recently, the chondroprotective effects of sesamin, the naturally occurring substance found in sesame seeds, have been proved in a rat model of papain-induced osteoarthritis. We hypothesized that sesamin may be associated with possible promotion of the biosynthesis of CS-PGs in human articular chondrocytes. The aim of the study was to investigate the effects of sesamin on the major CS-PG biosynthesis in primary human chondrocyte. The effects of sesamin on the gene expression of the PG core and the CS biosynthetic enzymes as well as on the secretion of glycosaminoglycans (GAGs) in a pellet culture system of articular chondrocytes. Sesamin significantly increased the content of GAGs both in culture medium and pellet matrix. Real-time-quantitative PCR showed that sesamin promoted the expression of the genes encoding the core protein (*ACAN*) of the major CS-PG aggrecan and the biosynthetic enzymes (*XYLT1, XYLT2*, *CHSY1* and *CHPF*) required for the synthesis of CS-GAG side chains. Safranin-O staining of sesamin treated chondrocyte pellet section confirmed the high degree of accumulation of GAGs. These results were correlated with an increased level of secreted GAGs in the media of cultured articular chondrocytes. Thus, sesamin would provide a potential therapeutic strategy for treating OA patients.

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(350) Identification And Characterization Of A Missense Mutation In O-GlcNAc Transferase That Segregates With Disease In A Family With X-Linked Intellectual Disability

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O-GlcNAc is a regulatory post-translational modification of serine and threonine residues of nuclear and cytoplasmic proteins that has been implicated in epigenetics. In humans, single genes encode enzymes for the attachment (O-GlcNAc transferase (OGT)) and removal [O-GlcNAcase (OGA)] of the O-GlcNAc modification. An X-chromosome exome screen identified a missense mutation in OGT (762G > T (p.L254F)) that segregates with X-linked intellectual disability (XLID) in an affected family. A decrease in steady-state OGT protein levels, in part due to a shorter half-life of L254-OGT, was observed, as predicted by in-silico modeling, in isolated lymphoblastoid cell lines from affected individuals compared to controls. Recombinant expression of L254-OGT demonstrated that the enzyme is active against in cell protein substrates and in vitro peptide substrates. Surprisingly, steady-state global O-GlcNAc levels and the O-GlcNAcome of the lymphoblasts remain grossly unaltered. Affected lymphoblastoids display a marked decrease in steady-state OGA protein and mRNA levels, implying that a compensation mechanism exists, albeit imperfect given the phenotype of the patients, for maintaining global O-GlcNAc levels. L254F-OGT samples also display decreased luciferase reporter expression driven from the proximal promoter of OGA, suggesting that OGT regulates the gene expression of OGA. We observed an enrichment of the OGT containing transcriptional repressor complex mSin3A-HDAC1 at the proximal promoter region of OGA in affected individuals, which would explain the decrease in OGA gene expression observed. Global transcriptome analysis of L254F-OGT lymphoblastoids compared to controls revealed a small subset of genes, many involved in chromatin remodeling, that are differentially expressed in affected patients. Here, we have elucidated the molecular consequence of the L254F mutation in OGT that uncovered a compensation mechanism, which appears to be at the level of chromatin remodeling, to maintain steady-state O-GlcNAc levels. Further investigation has revealed other XLID families with SNPs in OGT segregating with the condition and thus we have uncovered the first human disorder associated with mutations in OGT.

(351) B4GAT1 is the Priming Enzyme for the LARGE-dependent Functional Glycosylation of a-Dystroglycan

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Recent studies demonstrated that mutations in B3GNT1, an enzyme proposed to be involved in poly-N-acetyllactosamine synthesis, were causal for congenital muscular dystrophy with hypoglycosylation of alpha-dystroglycan (secondary dystroglycanopathies). Since defects in the O-mannosylation protein glycosylation pathway are primarily responsible for dystroglycanopathies and with no established O-mannose initiated structures that contain a beta 3 linked GlcNAc known, we biochemically interrogated this human enzyme. Here we report this enzyme is not a beta-1,3-N-acetylglucosaminyltransferase with catalytic activity towards beta-galactose but rather a beta-1,4-glucuronyltransferase, designated B4GAT1, towards both alpha- and beta-anomers of xylose. The dual-activity LARGE enzyme is capable of extending products of B4GAT1 and we provide experimental evidence that B4GAT1 is the priming enzyme for LARGE. Our results further define the functional O-mannosylated glycan structure and indicate that B4GAT1 is involved in the initiation of the LARGE-dependent repeating disaccharide that is necessary for extracellular matrix protein binding to O-mannosylated alpha-dystroglycan that is lacking in secondary dystroglycanopathies.

(352) Ex vivo gene therapy improves the accumulation of glycosaminoglycans in brain from murine model of mucopolysaccharidosis type II

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Mucopolysaccharidosis type II (MPS II) is an X-linked lysosomal storage disorder characterized by a deficiency of iduronate-2-sulfatase (IDS) which leads to progressive intralysosomal accumulation of glycosaminoglycans (GAGs). Accumulated GAGs in MPS II induce diverse clinical manifestations such as skeletal deformity, cardiac hypertrophy, and neurological symptoms. Presently, two strategies, which consisted of enzyme replacement therapy and bone marrow transplantation, are available to treat MPS II, and have been shown to improve the pathology in visceral organs. However, because the enzyme does not across the blood-brain barrier, the correction of central nervous system (CNS) lesions remains on-going challenge. In this study, we showed the effect of *ex vivo* gene therapy by using lentiviral vector on CNS disease in MPS II mice. IDS-transduced hematopoietic stem cells derived from MPS II donor mice increased the levels of enzyme activity in serum of MPS II recipient mice at 24 weeks after treatment (1422 fold-increase). Increased activity of IDS was also observed in cerebrum derived from MPS II mice after *ex vivo* gene therapy (35.9 fold-increase). Furthermore, *ex vivo* gene therapy improved the accumulation of disease-specific GAGs and autophagic marker proteins in cerebrum from MPS II mice. These results indicate that *ex vivo* gene therapy is a promising approach for treating CNS involvement in MPS II mice.

(353) Potential for using lectin sugar chains as diagnostic markers in oral precancerous lesions

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It is known that histological lectin binding patterns in normal human tissue vary from those in carcinoma. However, it is unclear

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whether these differences are due to the modification of carbohydrate structures in oral precancerous lesions. To delineate these characters, we performed experiments on samples of oral mucosa surgically removed from normal (N), epithelial dysplasia (ED) and intraepithelial squamous cell carcinoma (CIS) of the tongue. Lectin-peroxidase-diaminobenzidine binding assays using 14 different lectins revealed that PNA, UEA-1 and LCA were negative in N but positive in ED and CIS. In contrast, DBA, GSL-I and WGA were positive in N but negative in ED and CIS. Lectin microarray analysis against these six lectins demonstrated that the relative order of reactivity of DBA, GSL-I and WGA was N > ED > CIS. We found that lectin binding patterns could be classified into three categories showing (1) binding predominantly in the precancerous lesions and carcinoma, (2) equivalent binding in all three tissues, and (3) binding predominantly in normal tissue. The combination of lectin histochemistry and lectin microarray analysis has enabled us to show that carbohydrate chain modification can be indicative of precancerous transformation. Thus, lectins may represent a novel class of disease-associated glycological markers.

(354) Correlating Glycosylation and Immunoreactivity of HIV GP120

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The HIV virus mutates at a high rate. For example, more than 60 different sequences of GP120, a key determinant for broadly neutralizing antibodies, have been clinically isolated. Here we examined purified sequences of GP120 from different clades by mass spectrometry approaches. We performed glycomics of released N-linked glycans demonstrating complex glycans in addition to the predominating high mannose N-glycans. Further, we used a heavy isotope approach to identify sites being utilized for N-linked glycosylation. Finally, using cutting edge mass spectrometry approaches including HCD-triggered ETD, we mapped specific glycan structures to specific asparagine residues in the protein. We also examined the binding properties of the various GP120 sequences using multiple antibodies. Finally, we have correlated glycosylation patterns with immunoreactivity of the various antibodies available for HIV-1 GP120.

(355) Oral N-acetylmannosamine reverses glomerular hyposialyation and ameliorates proteinuria in a mouse model of nephrotic syndrome

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Glomerular diseases are important causes of end-stage renal disease, and are becoming increasingly prevalent. Current treatments for glomerular diseases have limited efficacy and many have side effects . Recent research on glomerular diseases and kidney response to injury have focused on the podocyte because of its critical role in maintaining filtration barrier integrity and excluding proteins from the glomerular filtrate. We and others have shown that alteration in the sialylation status of glomerular proteins are associated with podocyte injury and severe glomerular disease.

We explored the consequences of podocyte injury in mice and test the therapeutic effect of *N*-acetylmannosamine (ManNAc), an uncharged monosaccharide that is the biologic precursor of sialic acid. To establish proof of principle that desialylation results in podocyte injury, we administered various agents known to cause injury in conditionally immortalized (Saleem) podocytes and measured cell-surface sialylation by lectin staining. Treatment with sialic acid-cleaving enzyme (neuraminidase), pro-oxidants (hydrogen peroxide and menadione), and known agents that cause podocyte injury (puromycin aminoglycosidase, doxurubicin [Adriamycin]) all led to decreased *Sambucus nigra* (SNA) staining, indicating hyposialylation. We also induced podocyte injury in Balb/c mice by a single intravenous injection of Adriamycin, an anthracycline antibiotic known to induce a nephropathy that mimics human nephrotic syndrome and focal segmental glomerulosclerosis (FSGS). We measured serum albumin and proteinuria and performed morphologic (histochemistry, SEM and TEM), proteomic, and biochemical changes. Adriamycin injection induced proteinuria that peaked 8-12 days after injury induction, renal failure, flattening of podocyes, and hyposialylation of glomerular glycoproteins; treatment with ManNAc significantly reduced proteinuria and podocyte injury.

We propose that human glomerular disease may be associated with in sialylation of glycoproteins and glycolipids that can be mitigated by restoring sialylation. Since ManNAc is currently in a phase 1 clinical trial for the treatment of a hyposialylation disorder *GNE* myopathy, plans are underway for a phase 1 study to repurpose ManNAC for glomerular disease.

(356) A genome-scale systems biology approach to modeling glycosylation

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Glycosylation serves essential functions on many proteins produced in biopharmaceutical manufacturing, making it mandatory to thoroughly consider its biogenesis during the production process. Thus, glycoengineering efforts have aimed to rationally design glycosylation by adjusting culturing conditions or genetically modifying host cell lines. Computational models have been developed to aid this process, aiming to offer cheaper and faster

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alternatives to costly screening strategies. However, it has been difficult to account for environmental factors in the prediction of glycan synthesis. Here we have developed a framework in which we integrate genome-scale models of metabolism with glycosylation. Using this framework, we are able to couple all media components that have direct or indirect influences on glycan synthesis. We leverage this framework to analyze the effects of various factors on glycan synthesis in Chinese hamster ovary cells, including variations in media composition, detected mutations in different cell lines, and potential gene deletion mutants. Through these analyses we demonstrate that detailed insights can be gained on how processes external to glycosyltransferase activity ultimately may influence glycan diversity, and can be leveraged for cell line development and glycoengineering.

(357) Structural analyses of plasma glycoproteins in exacerbation and emphysema model mice as biomarker candidates for chronic obstructive lung disease (COPD)

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Introduction and Objectives: Chronic bronchitis and pulmonary emphysema are features of chronic obstructive pulmonary disease (COPD). Biomarker discovery is essential for the diagnosis, especially at the stage of exacerbation, and treatment for COPD. Methods: We analyzed glycan structures by mass spectrometry on plasma glycoproteins in model mice which mimics COPD and its exacerbation. We created four groups of model mice. Group 1 was the emphysema group: we administered pancreatic elastase via mice trachea to break down elastin in the extracellular matrix. Group 2 was the inflammation model group: we administered lipopolysaccharide (LPS). Group 3 was the exacerbation model group: after administering elastase, we gave LPS to stimulate inflammation. Group 4 was the emphysema treatment group: before administering elastase, we gave the glycosaminoglycan (GAG). Plasma from each mouse was subjected to SDS-PAGE, and the transferrin (TF) band and the alpha1-antitrypsin (A1AT) band were collected. TF and A1AT were in-gel digested with trypsin after reduction and alkylation. Tryptic peptides and glycopeptides were separated on an ODS column, and the eluate was introduced into an electrospray ionization-mass spectrometry system to characterize the glycan structures of TF and A1AT in each mouse. Results and Discussion: In the inflammation model group and the exacerbation model group, increases in trisialo-biantennary glycans and decreases in fucosylated glycans were observed as compared to those in the non-treated control mice. In the emphysema treatment group, decreases in trisialo-biantennary glycans were observed compared with those in non GAG-treated mice. The changes of these glycan structures were observed on TF and A1AT, and it was suggested that LPS-induced inflammation might influence the structures of glycans in the two glycoproteins. These glycan changes were well correlated with the number of inflammatory cells (neutrophils, macrophages and lymphocytes) in the bronchoalveolar lavage fluid of each model mice. Changes of trisialo-biantennary glycans and fucosylated glycans of TF and A1AT would be biomarker candidates for the exacerbation of chronic bronchitis/COPD. (Supported by the grants from NIBIO (NT) and MEXST (NM), Japan)

(358) Gne defects in zebrafish lead to impairment of sialylation and myopathy

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GNE myopathy is a rare adult onset myopathy due to defects in an essential enzyme required for sialic acid biosynthesis. That enzyme, UDP-GlcNAc 2-epimerase/ManNAc kinase, is encoded by the *GNE* gene. Previous work has demonstrated that sialylation is critical for normal muscle function. Our studies are focused on characterizing GNE myopathy using different zebrafish models. A *Gne* germline homozygous splice site mutation (IVS10-261T > A) results in fish that generally do not survive into adulthood, whereas a germline homozygous missense mutation (C312T) generally results in fish surviving into adulthood. Morpholino knockdown of *Gne* results in embryos with small body size, eyes, and head, curled tail, edema, and abnormal muscle banding structure staining. Treatment of these fish with a nontoxic sialic acid precursor, ManNAc (*N*-acetylmannosamine), caused slight recovery of the muscle banding pattern. Currently, we are using AC4-ManNAz (Peracetylated *N*-azidoacetylmannosamine), a nontoxic sialic acid precursor with an azido group, so that we can visualize sialic acid that has been incorporated into the live zebrafish embryos. Using this approach, we are able to elucidate the underlying cellular pathology of human GNE myopathy in zebrafish with different *Gne* aberrations. Specifically, administration of Ac4-ManNAz may identify (muscle-) specific glycoproteins or glycolipids that are severely hyposialylated in *Gne* deficient fish and respond to therapy. This may help identify biomarkers and further develop therapy for this devastating human disease. Importantly, this approach allows us to demonstrate the effectiveness of using zebrafish to model human genetic diseases related to defects in glycosylation.

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(359) **Acquired drug resistance by decrease of sialylated glycans on acute lymphoblastic leukemia cell-membrane glycoproteins**

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The main treatment of leukemia is chemotherapy using antitumor drugs, but the acquisition of drug resistance often makes treatment continuation impossible. The mechanism of acquired drug resistance must be elucidated so that clinical treatment can be optimized. We focused on glycan structures on cell-membrane glycoproteins to find some clues for the elucidation of mechanism. The human acute lymphoblastic leukemia cell line (CEM cell line) was selected for acquired drug resistance against desoxyepothilone B (dEpoB) and vincristine (VCR), which are major antitumor drugs targeting microtubules, and glycan structures on the cell-membrane glycoproteins of these resistant cell lines were analyzed by LC-ESI MS.

We found that CEM/dEpoB and CEM/VCR cell lines showed a significant decrease of α2-6 sialylated *N*-glycans and of all sialylated glycans, respectively. The amounts of mRNA of the sialyltransferases in the CEM/dEpoB cell line were measured by qRT-PCR, and the expression of β-galactosamide α2-6 sialyltransferase 1 (ST6GAL1) gene was decreased significantly. To ascertain whether reduction in α2-6 sialylated *N*-glycans on the CEM/dEpoB cell membranes was a cause or a result of acquired drug resistance, we knock-downed and transfected the ST6GAL1 gene in the CEM and CEM/dEpoB cell lines, and compared the effect on cell-growth inhibition by addition of dEpoB drug to these cell lines. The CEM cell line with the knock-down ST6GAL1 gene showed significantly less cell-growth inhibition with dEpoB than the parent CEM cell line. Moreover, we transfected the ST6GAL1 gene into CHO cells (which usually lack epression of α2-6 sialylated glycans) and subjected them to the challenge with dEpoB. The transfected CHO cells showed significantly greater cell-growth inhibition than the parent CHO cells. Both these results suggest that reduction in the α2-6 sialylation of the *N*-glycans on cell membrane could be the cause of dEpoB-resistance acquisition.

Currently, we are investigating the relationship between α2-6 sialylated *N*-glycans on cell surface and the uptake/ pump out of drugs. There was no difference in pump out efficiency, although the CEM/dEpoB cell line showed a slower uptake of dEpoB than the CEM cell line.

(360) **Sialylation of Thomsen-Friedenreich antigen is a noninvasive blood-based biomarker for GNE myopathy**

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GNE myopathy is an adult-onset progressive myopathy resulting from mutations in GNE, the key enzyme of sialic acid synthesis. The pathomechanism of GNE myopathy likely involves aberrant sialylation, since administration of sialic acid itself, or its precursor, N-acetylmannosamine (ManNAc), rescued hyposialylation of GNE myopathy mice. Recently, clinical trials for GNE myopathy patients were initiated. A robust, noninvasive biomarker is highly desirable for diagnosis of GNE myopathy and evaluating response to therapy. Since GNE myopathy muscle biopsies demonstrated hyposialylation of predominantly *O*-linked glycans, we analyzed the *O*-linked glycome of patients' plasma proteins using mass spectrometry. Most patients showed increased plasma levels of the core 1 *O*-linked glycan species Thomsen-Friedenreich (T)-antigen and/or decreased amounts of its sialylated form, ST-antigen. And, consistently, all analyzed patients had an increased ratio of T-antigen versus ST-antigen compared to unaffected individuals. Importantly, the T/ST ratios were in the normal range in a GNE myopathy patient treated with immunoglobulins (highly sialylated), indicating response to therapy. Natural history study and clinical trial data will reveal whether T/ST ratios can be correlated to muscle function. These findings not only highlight plasma T/ST ratios as a robust blood-based biomarker for GNE myopathy, but may also help explain the pathology and adult onset of the disease.

(361) **Quantitative glycomes analysis of N-glycan patterns in bladder cancer vs. normal bladder cells using an integrated strategy**

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Bladder cancer is one of the most common types of human cancer, and its incidence has steadily increased during the past decade. Diagnosis of bladder cancer at an early (nonmuscle-invasive) stage is the best way to reduce the mortality rate. Tumor malignancy in general is closely associated with the types of glycans expressed. Glycosylation status, particularly global glycomes, in bladder cancer are not well known. We integrated lectin microarrays and mass spectrometry (MS) methods to quantitatively analyze and compare glycan expression levels in superficial bladder cancer KK47 and normal bladder mucosa HCV29 cells. Glycopattern alterations were analyzed using lectin microarrays and confirmed by

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lectin staining and lectin blotting. N-glycans were derivatized by amidation of sialylated glycans with acetohydrazide and reductive amination with the stable isotope tags [$^{12}C_6$]- and [$^{13}C_6$]-aniline, and were quantitatively analyzed by MALDI-TOF/TOF-MS. Twenty-seven derivatized N-glycans were identified from the two cell lines, and their relative abundance ratios were calculated. N-glycan biosynthesis associated proteins were quantitatively analyzed by a SILAC proteomics method, which revealed significant differential expression of 13 glycosyltransferases and 4 glycosidases. Our findings indicate that sLe^x, terminal GalNAc and Gal, and high mannose-type N-glycans were more highly expressed in KK47 than in HCV29, that patterns of fucosylated complex-type N-glycans differed significantly between the two cell lines, and that the glycome changes were directly related to bladder cancer progression.

(362) **Sweet Role of Platelet Endothelial Cell Adhesion Molecule (PECAM) in Understanding Angiogenesis**

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The vascular endothelial cells are covered with glycocalyx, but the precise role of the endothelial glycocalyx remains unclear. Our previous study showed that N-glycan α2,6-sialylation regulates the cell surface residency of an anti-apoptotic molecule, platelet endothelial cell adhesion molecule (PECAM), as well as the sensitivity of endothelial cells toward apoptotic stimuli [1]. We speculated that PECAM would possess lectin-like activity toward α2,6-sialic acid to ensure its homophilic interaction. We first found that a longer α2,6-sialylated oligosaccharide exhibited strong inhibitory effect on the homophilic PECAM interaction in vitro. Furthermore, we found that a cluster-type α2,6-sialyl N-glycan probe specifically bound to PECAM-immobilized beads. Glycan structure analysis of the PECAM purified from mouse lung tissues revealed a presence of biantennary N-glycans having α2,6-sialic acid. Several unique glycans were also identified. Moreover, addition of the α2,6-sialylated oligosaccharide to endothelial cells enhanced the internalization of PECAM as well as the sensitivity to apoptotic stimuli. Taken together, these findings suggest that PECAM is a sialic acid-binding lectin and that this binding property supports endothelial cell survival. Notably, our findings that α2,6-sialylated glycans influenced the susceptibility to endothelial cell apoptosis shed light on the possibility of using a glycan-based method to modulate angiogenesis.

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(363) **Binding of langerin/CD207 to keratan sulfate disaccharide, Gal (6SO3) β1, 4-GlcNAc (6SO3) and its triangle derivative in vitro and in vivo: possible drug targets for COPD (chronic obstructive pulmonary disease)**

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COPD is the fourth leading cause of death worldwide and progressive obstruction of the airways due to the acute and/or chronic inflammation is the drug target, since no effective treatment currently exists. Our group found that a keratan sulfate disaccharide (Gal ($6SO_3$) β1,4-GlcNAc ($6SO_3$)), designated L4, attenuated elastase-induced emphysema in mice by inhibiting the influx of neutrophils in the bronchoalveolar lavage fluid (BALF) and deceasing alveolar destruction as evidenced by both histological analysis and micro CT assessment. Binding assay of L4 to various C-type lectins indicated only langerin/CD207 was positive but other lectins such as MGL-1, Lox-1, CLEC2, MICL, Dectin-1, DCIR, DCAR, Mincle and SIGNR3 were all negative. The binding of L4 (purified from Shark fin) to recombinant langerin (E. coli) was confirmed by NMR spectroscopy. Chemical shift changes in signals corresponding to langerin were observed upon titration with L4. In contrast, the addition of an L4-triangle derivative (chemically synthesized) to langerin resulted in the broadening of the langerin signals.compounds can be considered to be promising glycomimetics for use in the treatment of the inflammatory aspects of COPD. Supported by the Program for Promotion of Fundamental Studies in Health Science of the National Institute of Biomedical Innovation (NIBIO) and RIKEN Strategic Project.

(364) **Development of a sensitive assay method of keratan sulfate disaccharide levels in mice plasma and bronchoalveolar lavage fluid**

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Our group found that a keratin sulfate disaccharide, designated as L4, Gal(6SO3)beta-1,4-GlcNAc(6SO3), attenuated the elastase-induced

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emphysema as well as cigarette smoke-induced emphysema in mice. The L4 and its derivatives are thus promising compounds for drug development for treatment of COPD (chronic obstructive pulmonary disease) in human, which is the fourth leading cause of death worldwide. COPD is caused by long-term cigarette smoking and/or environmental pollutants, and its appropriate therapeutics are still limited.

In order to perform pharmacokinetic studies of L4, we have developed a sensitive post-column fluorescence-derivatization high performance liquid chromatography (HPLC) system to assess L4 levels. In the developed system, L4 was first separated by using an Imtakt Unison UK-Amino column and then followed by fluorescence-derivatization of the reducing end with 2-cyanoacetamide to assess L4 levels with a fluorescence detector. The system was found to show a good performance in quantitatively measuring L4 levels in plasma or bronchoalveolar lavage fluid (BALF) at least in the range of 66-530 ng/ml. As a result, the developed system was judged to be sufficient for monitoring L4 levels in vivo.

Finally, we assessed time-course changes in L4 levels in elastase-induced emphysema model mice following tracheal inhalation of 1 mg L4/body. The administered L4 was found to rapidly decrease in levels in BALF, and its half-life was determined around 45-47 min. Additionally, we found that 30-60 min after tracheal inhalation of L4, some of the L4 appeared in plasma where the L4 might exhibit pharmacological effects that are different from those of L4 in BALF.

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(365) **Sialyl Lewis X conjugated nanodiamonds for vascular targeting**

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Arteriovenous malformations (AVMs) of the brain are congenital lesions that are the major causes of haemorrhagic stroke in young adults. Most small AVMs are curable while a majority of the large lesions are difficult to cure using currently available techniques. Novel therapeutic strategies such as vascular targeting are attractive options for the treatment of these large lesions by selective occlusion of the AVM vessels. This targeted approach requires specific endothelial surface markers that are highly discriminatory between normal and AVM vessels. Although AVM vessels differ from normal vessels morphologically, there is a lack of specific markers that could be used as targeting agents.

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This model system has potential for vascular targeting based therapy of AVM's. Sialyl Lewis X conjugated nanodiamonds could be eventually used as drug delivery vehicles for prothrombotic agents that can block the AVM vessels and eliminate the risk of haemorrhagic stroke. Targeted delivery of drugs and imaging probes to the selectins on irradiated endothelium, or to inflammation sites, holds promise to improve management and treatment of many diseases.

(366) **High throughput detection of an alpha2,6-sialylated glycoform of transferrin in cerebrospinal fluid: Application for dementia diagnosis**

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We previously reported that cerebrospinal fluid (CSF) contains two glycoforms of transferrin (Tf): one had alpha2,6-sialylated N-glycans whereas the other had non-sialylated N-glycans. The ratio of non-sialylated/alpha2,6-sialylated one (Tf index) was elevated in idiopathic normal pressure hydrocephalus (iNPH), an elderly dementia caused by abnormal metabolism of CSF (Futakawa et al., Neurobiol Aging, 2012, 33; 1807-15). The ratio was not elevated in other dementia such as Alzheimer's disease and front temporal dementia, suggesting that the glycoform analysis is useful for differential diagnosis of dementia (Futakawa et al., Neurobiol Aging, 2012; 33: 1807-15).

In the previous study, glycoforms were analyzed by immunoblotting, but the method is time- and labor-consuming. We recently found that SSA lectin inhibited anti-transferrin antibody binding to alpha2,6-sialylated Tf in a sandwich ELISA (SSA inhibition). The inhibition was not observed with other glycoforms such as periodate-treated, sialidase-treated, and sialidase/galactosidase-treated Tf, suggesting that the inhibition was glycoform specific. SSA inhibition was applied to an automated latex-agglutination immunoassay (ALI), which is a rapid and simple method for measuring antigen concentration by using antibody-coated latex beads. SSA-ALI was applied to measurement of alpha2,6-sialylated Tf in CSF in a high throughput manner (1 assay/2 min). SSA-ALI analysis revealed that diagnostic transferrin index was significantly higher that of controls, suggesting that SSA-ALI would be a simple and rapid method for measuring Tf glycoform to diagnose iNPH (Hoshi et al., J Biochem, 2013; 154: 229-32).

We observed SSA inhibition with three Tf polyclonal antibodies and several monoclonal antibodies. In addition, SSA inhibits binding of anti-alpha2macroglobulin antibodies to the antigen.

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These results suggest that SSA inhibition is applicable to assays for various alpha2,6-sialylated glycoproteins.

We also applied SSA inhibition to immunohistochemistry. In conventional immunohistochemistry, glycoforms of Tfs are hard to be distinguish becasuse most anti-Tf antibodies recognize protein epitopes regardless of the glycoform. Conversely, lectins that recognize glycan epitopes cross-react with glycoproteins that share those glycan epitopes. Here we successfully visualize hepatic alpha2,6-sialylated transferrin in a glycoform-specific manner by using a combination of anti-transferrin antibody and SSA lectin. The result is the first demonstration of localizing a specific glycoform by conventional light microscope.

(367) Proteomic analysis of ganglioside-associated microdomain in malignant melanomas

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Melanoma is difficult to be cured because of its malignant properties. Therefore, it has been urgent issue to be solved to identify target molecules for novel therapeutics. Ganglioside GD3 is highly expressed in melanomas. It has been shown that GD3 enhances cell proliferation and invasion using GD3 synthase cDNA-transfectant cells of a GD3-negative (GD3-) mutant line SK-MEL-28 N1. p130Cas, paxillin and FAK were identified as highly tyrosine-phosphorylated molecules involved in the increased cell proliferation and invasion with GD3 expression. However, remaining issue to be clarified is how GD3 interacts with known/unknown molecules in the vicinity of cell membrane. To clarify these mechanisms, we isolated the glycosphingolipid-enriched microdomain (GEM)/rafts with sucrose density-gradient ultra-centrifugation of Triton X-100 extracts from GD3+ and GD3- cells. We also labeled cell surface molecules present in the vicinity of a target molecule in living cells with EMARS reaction (Kotani and Honke et al. 2008). Isolated molecules as components in GEM/rafts and EMARS products were comprehensively analyzed with LC/MS (LTQ-Orbitrap XL). In the GEM/rafts, we identified 60 and 63 membrane proteins in GD3+ and GD3- cells, respectively. Among them, 50 membrane proteins were common. In the EMARS with GD3, 9 molecules such as neogenin, integrin α3, β1 and MCAM were identified as GD3-neighboring molecules. They were also identified in the GEM/raft fraction. To confirm the results of LC/MS, we performed immuno-blotting and imaging analysis with confocal microscopy. Neogenin and MCAM were found in GEM/raft fraction of GD3+ cells in immuno-blotting, and were labeled with EMARS reaction. They were co-localized with GD3 in immunocytostaining. Differences in the molecular profiles identified in GEM/rafts and these defined as EMARS products suggest the presence of heterogeneity in GEM/rafts.

(368) Comprehensive N-glycome profiling of cultured human epithelial breast cells identifies unique secretome N-glycosylation signatures enabling tumorigenic sub-type classification

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The secreted cellular sub-proteome is a rich source of biologically active glycoproteins. *N*-glycan profiling of secretomes of cultured cancer cells provides an opportunity to investigate the link between protein *N*-glycosylation and tumorigenesis. Utilizing carbon-LC-ESI-CID-MS/MS of protein released native *N*-glycans, we accurately profiled the secretome *N*-glycosylation of six human epithelial breast cells including normal mammary epithelial cells (HMEC) and breast cancer cells belonging to luminal A subtype (MCF7), HER2-overexpressed subtype (SKBR3) and basal B subtype (MDA-MB157, MDA-MB231, HS578T). Based on intact molecular mass, LC retention time and fragmentation pattern, a total of 74 *N*-glycans were confidently identified and quantified. The secretomes comprised significant levels of highly sialylated and fucosylated complex type *N*-glycans, which were elevated in all cancer cells relative to HMEC (57.7-87.2% vs 24.9%, $p < 0.0001$ and 57.1-78.0% vs 38.4%, $p < 0.0001$-0.001 respectively). Similarly, other glycan features were found to be altered in breast cancer secretomes including expression of paucimannose *N*-glycans, complex type *N*-glycans containing bisecting β1,4-GlcNAc and LacdiNAc determinants. Subtype-specific glycosylation patterns were observed. Prominently, the basal B breast cancer cells expressed preferentially *N*-glycan α2,3-sialylation. Pathway analysis indicated that the regulated *N*-glycans were closely related in the biosynthetic machinery. Tight clustering of the breast cancer sub-types based on the obtained *N*-glycome profiles supported the involvement of *N*-glycosylation in cancer. Complementing proteome and lipid profiling, *N*-glycome mapping yields important pieces of structural information to understand the biomolecular de-regulation in breast cancer development and progression - knowledge which may facilitate the discovery of candidate cancer markers and potential drug targets.

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