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Alternative analytical approach for biomarker discovery: Chemical modifications on a target protein

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[Introduction] Recent strategies in biomarker discovery have relied on mass spectrometry-based proteomics. However, biomarker discovery from the entire proteome is analogous to "*finding a needle in a haystack*". Accordingly, we have attempted to establish a novel omics strategy to facilitate biomarker discovery. The strategy focuses on only an individual protein (abundant or closely related to the targeted disease) and screen the chemical modifications exhaustively-we call this approach *chemical modificomics*[1-3]. Proteins and peptides are exposed to higher chemical stresses during certain physiological events, i.e. increased oxidative stress in degenerative disease of aging and higher glucose stress in diabetes mellitus. Therefore, the resulting chemical modifications can provide meaningful information about biological events. In other words, the modifications can serve as dosimeters for stress exposure and as diagnostic/therapeutic markers. However, bioactive proteins associated with specific diseases have been quantified almost exclusively by immunoassay-based procedures, with no concern for possible chemical modifications. Hence, critical information could be overlooked, or considerable misunderstandings could occur, if the unidentified modifications alter protein functions. Here, I introduce a novel strategy based on mass spectrometric identification to screen chemical modifications of a specific target protein.

[Serum albumin: The most promising global stress marker] Human serum albumin (HSA), the most abundant plasma protein, has several important functions including maintaining osmotic pressure and serving as a carrier for hydrophobic compounds. Since HSA is circulated through the entire body, this protein is a potential target of many chemical stresses. We have optimized a robust method to obtain 100% sequence coverage, thereby enabling exhaustive screening of all the chemical modifications of HSA[4,5]. Through screening of drug-HSA and chemical-HSA adducts, the strategy is expected to contribute to toxicity and risk assessments of early-stage drug candidates[6] and environmental chemicals, respectively. We also found *N*-terminal epimerization of HSA by aldehyde stress[7].

[Keratins: Non-invasive assessment of skin damage] Keratins, the main constituents of skin, have recently been recognized as major target proteins of chemical modifications, since skin is a main barrier of the body against environmental stresses such as UV and chemicals. However, because of the difficulties associated with keratins' insolubility and handling, few studies have investigated chemical modification of keratins. We have developed a robust method to screen keratin modifications as a combination of non-invasive sampling (tape stripping), simple clean-up, and tryptic digestion (filter-aided sample preparation)[4]. Using the method, we have identified sites modified by oxidation with H_2O_2[8] and UV irradiation[9,10], and found that $Met^{259,\ 262,\ 296,\ and\ 469}$ in K1 were good candidate skin aging markers. We also found inflammation-related modifications, such as 4-hydroxy-2(*E*)-nonenal adduct of H^{255} in K1[11].

[Supporting techniques for chemical modificomics and other target] We have also developed supporting techniques to attain exhaustive screening of chemical modifications using negative ionization[12] and quantitative approach[13,14]. Also, chemical modifications on other proteins such as insulin[15], hemoglobin[16,17] amyloid β[18], and α-synuclein[19] have been also investigated.

[Summary] My recent projects on chemical modificomics were reviewed. More information will be provided in my talk.

[References] [1] *J. Mass Spectrom. Soc. Jpn.*, **57**, 167 (2009), [2] *Jpn. J. Clin. Chem.*, **38**, 177 (2009), [3] *Anal. Methods*, **4**, 1945 (2012), [4] *Anal. Bioanal. Chem.*, **405**, 7383 (2013), [5] *Bunseki Kagaku*, **64**, 653 (2015), [6] *Anal. Biochem.*, **449**, 59 (2014), [7] *Chem. Res. Toxicol.*, **26**, 1926 (2013), [8] *J. Proteomics*, **75**, 435 (2011), [9] *J. Proteomics*, **133**, 54 (2016), [10] *Data in Brief*, **7**, 100 (2016), [11] *J. Proteome Res.*, **19**, 3837 (2020), [12] *Anal. Methods*, **2**, 1144 (2010), [13] *Rapid Commun. Mass Spectrom.*, **24**, 173 (2010), [14] *Anal. Bioanal. Chem.*, **405**, 8001 (2013), [15] *Int. J. Mass Spectrom.*, **373**, 72 (2014), [16] *Anal. Bioanal. Chem.*, **408**, 5379 (2016), [17] *Int. J. Mass Spectrom.*, **468**, 116651 (2021), [18] *Rapid Commun. Mass Spectrom.*, **20**, 911 (2006), [19] *Biochem. J.*, **393**, 343 (2006).