2. Zheng K, Yoda N, Chen J, Liao Z, Zhong J, Wu C, Wan B, Koyama S, Sasaki K, Peck C, Swain M, Li Q. Bone remodeling following mandibular reconstruction using fibula free flap. J Biomech. 2022 Mar;133:110968. https://doi.org/10.1016/j.jbiomech.2022.110968. Epub 2022 Jan 22. PMID: 35139441.

POSTER 13

Developing Novel Therapeutics for Oral Cancer Leveraging Drosophila Models

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Abstract: The prevalence of oral squamous cell carcinoma (OSCC) has been increasing worldwide. Surgical resection and chemoradiotherapy are the main OSCC treatments currently, but their effectiveness is limited. One of their major problems is the decline in patients' quality of life due to oral dysfunction caused by the extensive resection and oral mucositis. Although targeted therapies have emerged for treating OSCC and have improved the patients' survival rate, these therapies also cause severe side effects that hamper completion of the regimens. Hence, the 5-year overall survival rate of OSCC patients remains to be approximately 50% for past decades. Therefore, developing novel, effective OSCC therapeutics with decreased toxicity is highly demanded. Thus far, mammalian models such as cultured cells obtained from human OSCC and their xenografts in mice have provided critical insights into the molecular mechanisms in OSCC development. However, these models are difficult to execute comprehensive analysis due to their expensive breeding and time-consuming aspects. Recently, the fruit fly Drosophila melanogaster has made contributions to cancer research. Our previous works based on a novel platform combining flies and mammalian models have successfully developed therapeutic seeds and leads for various types of cancers.[1,2] Here, we employed flies to generate the novel animal model reproducing OSCC patients' genotype and to discover novel therapeutic targets and candidates for OSCC treatment. To generate flies modeling OSCC patients' genotype, we employed the binary GAL4/UAS system obtained from yeast which enables targeted expression of exogenous genes in a desired tissue in flies. We first obtained GAL4 driver strains which express a transcription factor GAL4 specifically in the compound eye (for confirming the functions of each transgene) or in squamous cells in wing discs (for modeling OSCC patients' complex genotype and for evaluating phenotypes). In parallel, we generated UAS transgenic strains which carry a mutated 'A', 'B' or 'C' gene that are frequently found in OSCC patients downstream of UAS, the GAL4 target DNA sequence. Expression of wild-type A in fly eyes using eye-specific driver Glass Multimer Reporter-GAL4 caused 'rough eye' phenotype by promoting apoptosis, as previously reported. Moreover, we found that additional expression of missense-mutated form of A suppressed the rough eye phenotype, confirming that this form works as a dominant negative mutant against wild-type as expected. Next, we found that the expression of missense-mutated B or overexpression of C using the same driver increased the fly eye area. These results indicated that both genes induced hyperplasia in fly eyes as previously reported. After confirming function of each gene, we induced all of these 3 genes in fly squamous cells by employing squamous cells-specific driver Ultrabithorax-GAL4 to reproduce OSCC patients' genotype. Resulting flies showed decreased viability (60%). Additionally, expression of these 3 genes under patched-GAL4 that expresses GAL4 in a limited area of squamous cells induced statistically significant enlargement of squamous cell area compared with non-transgenic control (Dunnett's test). In this study, we confirmed that the eye phenotypes induced by each transgene were consistent with their reported functions and our expectation. Furthermore, we identified that the flies displayed hyperplastic phenotype in the squamous tissue. These findings prove our strategy effective to validate transgene functions and to model at least a portion of OSCC phenotypes in flies. We will perform whole-body assays such as comprehensive genetic and drug screenings in these flies to obtain novel insights into the OSCC mechanisms and novel therapeutic targets and drug candidates in OSCC, finally validating them in the mouse model. We expect that the flies modeling OSCC genotypes accelerate development of novel therapeutics for OSCC.

References:

1. Sonoshita et al. Nat Chem Biol 2018
2. Jiang & Kimura et al. Front Oncol 2022

POSTER 14

Tumor Microenvironment Modulation by Cetuximab and its Contribution to Subsequent Immune-Checkpoint Inhibition Therapy in Recurrent/Metastatic Head and Neck Squamous Cell Carcinoma

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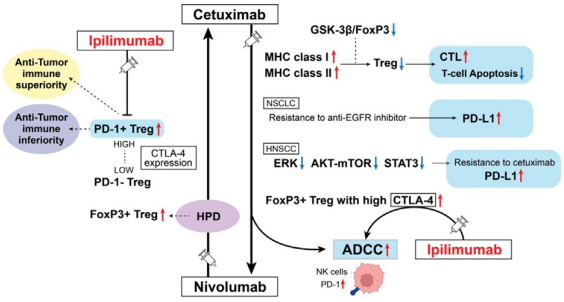
Abstract: Current clinical and observational evidence supports the EXTREME regimen as 1 of the standards of

care for patients with recurrent or metastatic head and neck squamous cell carcinoma (R/M HNSCC) followed by the administration of immune checkpoint inhibitors (ICIs). In addition to the inhibition of the endothelial growth factor receptor (EGFR) pathway, cetuximab-mediated EGFR blockade has been shown to modulate tumor microenvironment (TME) characteristics, such as antibody-dependent cellular cytotoxicity (ADCC) activity, cytotoxic T-lymphocyte (CTL) infiltration into the tumor, antiangiogenesis activity, and cytokine secretion via associated natural killer (NK) cells. On the other hand, there are reports that nivolumab affects the TME via PD-1 inhibition, IL-10 upregulation via T-cells, MDSC-mediated immune escape induction, and tumor vessel perfusion by promoting CD8+ T-cell accumulation and IFN-γ production in treatment-sensitive tumor cells. Based on the above evidence, the aim of this study is to find a novel treatment strategy for R/M HNSCC focusing on TME modulation by the administration of those recent representative therapeutic agents, cetuximab and nivolumab. We obtained background evidence regarding the biological effects of cetuximab on response to nivolumab and nivolumab on response to cetuximab in the TME from PubMed database and summarized those findings. HNSCC treatment using cetuximab increases the frequency of FoxP3+ intratumoral effector Tregs expressing CTLA-4 and targeting CTLA-4+ Tregs using ipilimumab restores the cytolytic function of NK cells, which mediate ADCC activity.[1] Treg-mediated immune suppression also contributes to clinical response to cetuximab treatment, suggesting the possibility of adding ipilimumab or the use of other Treg ablation strategies to promote antitumor immunity. Moreover, in hyper progression disease (HPD), intratumoral frequency of FoxP3+ effector Tregs expressing CTLA-4 increases. Therefore, combination treatment involving cetuximab and an anti-CTLA-4 antibody for R/M HNSCC and HPD after nivolumab administration may be expected to result in a higher tumor-control response. Another strategy by which TME can be modified using nivolumab is by interfering with MDSC function. Specifically, MDSCs exert their immunomodulatory effects via diverse mechanisms, including Arginase-1-mediated depletion of l-arginine and NO production via nitric oxide synthase 2 (NOS2). It is reported that Arginase-1 starves L-arginine limiting T-cell proliferation. In addition to L-arginine depletion, NO production transforms the TME to promote immune escape. This MDSC-related driven-immune escape cascade contributes to developing HPD, implying that the appropriate control of MDSCs may improve the response rate of ICI treatment. Previous evidence reported that the inhibition of the Notch signaling pathway is associated with reduced MDSC, tumor-associated macrophage, and Treg counts within emerging mouse tumor tissues. Its inhibition significantly inhibits the mRNA and protein expression levels of the most relevant immune checkpoint molecules, PD-1, CTLA4, TIM3, and LAG3, all of which represent targets for approved or developmental ICIs, implying as 1 of the therapeutic targets.[2] Moreover, recent



TME modulation by cetuximab and nivolumab and potential breakthrough treatment strategy using ipilimumab for HPD

studies also have demonstrated the potential of combining anti-EGFR with anti-PD-1/PD-L1 therapies to treat tumors and overcome drug resistance. Biologically, this may be partly due to the activation of EGFR signaling, which also modulates antitumor immunity via mechanisms, such as PD-L1 upregulation via the ERK, AKT-mTOR, and STAT3 pathways. These pieces of evidence may explain the biological efficacy of cetuximab followed by ICI treatment. Here, we suggest the efficacy of using these therapeutic combined strategies for patients with R/M HNSCC and patients who do not respond well to nivolumab administration.

References:

1. Jie HB, et al. CTLA-4+ regulatory T cells increased in cetuximab-treated head and neck cancer patients suppress NK cell cytotoxicity and correlate with poor prognosis. Cancer Res (2015) 75:2200–10.
2. Mao L, et al. γ-Secretase inhibitor reduces immunosuppressive cells and enhances tumour immunity in head and neck squamous cell carcinoma. Int J Cancer (2018) 142:999–1009.

POSTER 15

Identifying Great Auricular Nerve via Bony Landmarks: A Cadaver Study

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Abstract: Great Auricular Nerve (GAN) is the most commonly sacrificed nerve during rhydectomies due to anatomical variation and lack of a reliable landmark.[1] Even though quality of life does not decrease, surgeons should strive to avoid iatrogenic injury to the nerve. The aim of this study is to utilize 2 bony landmarks for the GAN to determine which if there is a reliable, bony anatomical landmark during surgery, specifically calculating the ratio at which the angle of the mandible (gonion) and tip of the mastoid process bisect GAN. Forty-six cadavers were dissected to expose the GAN along the angle of mandible (AOM), Erb's point, and mastoid process. Via 2 independent observers, the ratio was measured as the distance between AOM and mastoid process divided by the AOM to