Project ID: 181

SR - Cellular and Molecular Biology

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Proteomic Analysis of Epithelial Cell Polarity Complexes Using TurboID, g:Profiler, and Cytoscape

Background: A majority of human carcinomas show loss of epithelial apical-basal polarity during the progression from benign to invasive carcinoma. Apical-basal polarity is shown to inhibit cell invasion and suppress tumor metastasis. However, how the apical-basal complex regulates tumor cell invasion is not fully understood. In this research, TurboID-based proximity labeling technology was used to identify proteins associated with the aPKC-PAR6B epithelial polarity complex.

Procedure: Caco2 cell lines expressing NES-TurboID, PAR6B-TurboID, or aPKC-TurboID were constructed for proximity labeling. NES-TurboID was used as the control background. Immunostaining and Immunoblot were performed to verify location and biotinylation labeling efficiency by PAR6B-TurboID and aPKC-TurboID. Mass spectrometry was performed to identify the proteomes associated with the PAR6-aPKC complex.

Result: Compared to the negative control TurboID-NES, 286 proteins were identified with a significant ≥ 2-fold enrichment associated with PAR6B-TurboID and 307 proteins associated with PKCz-TurboID. Among these enriched proteins, 252 proteins were shown to interact with both PAR6B and PKCz. Gene Ontology (GO) analysis of the 252 enriched proteins revealed enrichment of apical/basal polarity proteins, supporting the labeling specificity of our TurboID approach. GO analysis also showed that enriched proteins were involved in multiple biological functions, including regulation of Hippo signaling, TGF receptor signaling, and Notch signaling pathway.

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Exploring the Contribution of Innate Immune Cells to Breast Cancer Immunotherapy

Breast cancer is the leading type of cancer in women. About 10-15% of breast cancers are triple-negative breast cancer (TNBC), a subtype with the worst prognosis. Due to the lack of estrogen, progesterone and HER2 receptor expression, chemotherapies have been the standard of care for decades. Immunotherapy has emerged as promising for TNBC treatment. In 2020, the Food and Drug Administration (FDA) granted approval to Pembrolizumab in combination with chemotherapy for patients with advanced triple-negative breast cancer. However, only a subgroup of advanced TNBC patients live longer whose tumors have a PD-L1 Combined Positive Score of at least 10 (CPS>=10). There is still an unmet medical need to provide alternative treatment for the rest of patients. Interestingly, a few of patients at UCSD Moores Cancer Center were found to have had excellent responses to pembrolizumab despite low CPS score (termed Elite Responders). The hypothesis of this project is that there may be an alternative immune response mechanism and/or crosstalk happening between the innate and adaptive immune systems, especially in Natural Killer Cells and Macrophages, that contributed to this unexpected excellent response. Our procedure used ACDBio RNAscope Multiplex Fluorescence v2 method to spatially analyze innate immune cells (Natural Killer cells and macrophages) and adaptive immune cells (T-cells) in the Tumor MicroEnvironment. Our data demonstrated increased tumor infiltration of innate immune cells (macrophage and Natural Killer cells) in the Elite Responders. This conclusion indicated the joint effort of two immune systems (innate and adaptive) which eventually led to increased survival.

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Analysis of p53 Target Genes in Senescent Cells

Cellular senescence occurs when a cell permanently stops dividing but doesnâ€™t undergo apoptosis. Senescence is characterized by damaged DNA, altered gene expression and many other atypical features. Senescence is a hallmark of aging, and senescent cell buildup has been linked to a range of age-related diseases. Therefore, targeting senescence is a valuable approach to slowing progression of these diseases. Senescence is a complex process with various regulators, with one of the most prominent being p53. The p53 gene activates in response to DNA damage, by directly sensing DNA damage points or by acting as a transcription factor. When p53 acts as a transcription factor it can activate or repress the production of p53 target genes. P53 target genes have been well studied in cancer, but not in senescent cells. Here I compiled a list of six well-known p53 target genes: CD82, DUSP14, SUSD6, PRDM1, PPM1D, and XPC. Using quantitative PCR, I studied the relative gene expression of these genes in senescent cells with and without a knockdown of p53. Knocking down p53 reveals how much the gene of interest is affected by p53. Strikingly, I found expression of target genes PRDM1 and CD82 increased while expression of the rest did not change significantly. This evidence means that p53 is not required for expression of certain genes in senescent cells and may even act as a negative regulator for certain genes in senescence. This suggests p53 transcriptional function is different in senescent cells in a previously unthought of way. P53 and senescence are important aging targets, so these results may have implications in future anti-aging therapeutics.