Steroid Receptor Co-Activators Regulate Metabolic Kinases to Drive Therapy Resistant ER+ Breast Cancer

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Recurrence of metastatic breast cancer stemming from acquired endocrine and chemotherapy resistance remains a health burden for women with luminal (ER+) breast cancer. Disseminated ER+ tumor cells can remain viable but quiescent for years to decades. Contributing factors to metastatic spread include the maintenance and expansion of breast cancer stem cells (CSCs). Breast CSCs are poorly proliferative and frequently exist as a minority population in therapy resistant tumors. Our objective is to define novel signaling pathways that govern therapy resistance in ER+ breast cancer. In this study, we show that cytoplasmic complexes composed of steroid receptor (SR) co-activators, PELP1 and SRC-3, modulate breast CSC expansion through upregulation of the HIF-activated metabolic target genes *PFKFB3* and *PFKFB4*. Seahorse metabolic assays demonstrated that cytoplasmic PELP1 influences cellular metabolism by increasing both glycolysis and mitochondrial respiration. PELP1 interacts with PFKFB3 and PFKFB4 proteins, and inhibition of PFKFB3 and PFKFB4 kinase activity blocks PELP1-induced tumorspheres and protein-protein interactions with SRC-3. PFKFB4 knockdown inhibited *in vivo* emergence of circulating tumor cell (CTC) populations in ER+ mammary intraductal (MIND) xenografts. Application of PFKFB inhibitors in combination with ER targeted therapies blocked tumorsphere formation in multiple models of advanced breast cancer, including tamoxifen (TamR) and paclitaxel (TaxR) resistant models and ER+ patient-derived organoids (PDxO). Together, our data suggest that PELP1, SRC-3, and PFKFBs cooperate to drive ER+ tumor cells that include CSCs and CTCs. Identifying non-ER pharmacological targets offers a useful approach to blocking metastatic escape from standard of care ER/estrogen (E2)-targeted strategies to overcome endocrine and chemotherapy resistance in ER+ breast cancer.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Stress-Induced Differential miR-4633-5p Expression in Thyroid Cancer Health Disparities

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Filipino Americans (FA) are known to have higher rates of thyroid cancer incidence and disease recurrence compared to European Americans (EA). FA are also known to be two times more likely to die of thyroid cancer compared to EA. Epidemiological studies in California have shown that thyroid cancer is the second most common cancer among FA women. Currently, there are no studies that demonstrate the mechanism behind these discrepancies. Evidence shows a strong correlation between obesity and more aggressive forms of thyroid cancer; obesity has an increased frequency in FA populations. The exact connection between the mechanisms of obesity and cancer is poorly understood. This epigenetic phenomenon may be due to microRNAs (miRNAs), which post-transcriptionally regulate gene expression. Dysregulated miRNA profiles have been associated with various diseases including obesity and cancer. MiRNAs are linked to different types of cancer; tumor suppressor genes and oncogenes are subject to modulation by dysregulated miRNAs. No study elucidates the association of miRNAs to tumor staging or prognosis in thyroid cancer health disparities. In this study, we determined miRNA expression profiles and found significant differences in the miRNA profiles between FA and EA thyroid cancer patients. Our pilot study showed several dysregulated miRNAs, from which we chose to assay dysregulated miR-4633-5p segments that are known to be associated with thyroid cancer signaling. We used QIAGEN's miRNA extraction kit to obtain high-quality miRNA from paraffin-embedded thyroid tissues. We performed next-generation miRNA sequencing using equal number of FA and EA samples and identified the top ten significantly up- and down-regulated miRNAs from the pool of differentially expressed miRNAs by qPCR assays. Our investigation demonstrated a 1.5-2-fold higher expression of an upregulated miR-4633-5p in FA versus EA miRNA samples (n=70) after normalized to controls. In contrast, miR-323b-3p showed no difference between FA and EA after normalized to controls. For our future work, we plan to analyze multiple up- and down-regulated miRNAs by qPCR, determine whether the miRNA signatures are consistent between samples from FA versus EA, and explore the use of these miRNA signature differentials for affordable and rapid thyroid cancer screening and prognosis.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Systemic Immune Response in Murine Bilateral Pheochromocytoma Model During Immunotherapy Based on a Combination of Mannan-BAM, TLR Ligands and Anti-CD40 Antibodies (MBTA Therapy)

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Immunotherapy has become an essential component of cancer treatment, however, a majority of patients with solid metastatic cancers, such as pheochromocytoma (PHEO), do not respond to this type of therapy. Recently, we developed an intratumoral (i.t.) immunotherapy based on the unique combination of TLR ligands, anti-CD40 antibodies, and mannan, which is artificially bound to tumor cells via an anchor (MBTA therapy). This therapy resulted in the complete eradication of aggressive subcutaneous PHEO in 67% of mice and demonstrated a systemic antitumor immune response and regression of non-treated lesions in the metastatic model (1). To further evaluate this systemic effect generated during MBTA therapy, we established a murine bilateral PHEO model, where MBTA therapy was i.t. injected into one tumor, and the distant (non-treated) tumor was monitored for changes in size and immune cell infiltration. The growth of both MBTA-treated and distant tumors was reduced compared to that of the control. Interestingly, survival of the MBTA-treated mice was twice as long compared to the control mice. Moreover, we have made several unique observations during the experiments which were focused on the tumor microenvironment. Flow cytometry analysis revealed the ability of MBTA therapy to significantly increase the infiltration of innate immune cells (monocytes, DCs, macrophages, NK cells) not only in MBTA-treated tumors, but also in distal tumors, despite the fact that MBTA therapy was designed to elicit only local inflammation. An analysis of the macrophage phenotype revealed a switch from protumor M2 to antitumor M1 macrophages in both tumors during the entire MBTA therapy treatment. Analysis of splenic adaptive immune cells revealed that naïve CD4+ or CD8+ T cells differentiated into central memory cells and effector memory cells. CD4+ and CD8+ T cells were elevated in MBTA-treated and distant tumors with a significantly higher frequency of CD8+ effector memory T cells. Moreover, the adoptive transfer of CD4+ and CD8+ T cells revealed that immune memory, after tumor rechallenging, was driven by CD4+ T cells. Collectively, these results illustrate the ability of MBTA therapy to activate both parts of the immune system and render a systemic antitumor response against non-treated metastases. We believe that our results could lead to the use of MBTA therapy in patients with aggressive, metastatic lesions. **Reference:** Caisova et al., Cancers (Basel), 2019. 11(5).

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Taking Advantage of the TGFB1 Biology in Differentiated Thyroid Cancer to Stimulate Sodium Iodide Symporter (NIS)-Mediated Iodide Uptake in Engineered Mesenchymal Stem Cells

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The sodium iodide symporter (NIS) mediates the active transport of iodide into thyroid follicular cells, providing the basis for the use of radioiodide for diagnostic imaging and therapy of differentiated thyroid cancer and also non-thyroidal tumors after tumor-selective *NIS* gene transfer. Based on their excellent tumor-homing capacity, mesenchymal stem cells (MSCs) can be employed as tumor-selective *NIS* gene delivery vehicles. Transgenic expression of *NIS* in genetically engineered MSCs allows noninvasive imaging of functional *NIS* expression as well as therapeutic application of ^{131}I. The use of promoters activated by tumor micromilieu-derived signals to drive *NIS* expression enhances selectivity and effectiveness, while limiting potential off-target effects. In this study we aimed to exploit the central role of transforming growth factor B1 (TGFB1) in tumor milieu-associated signaling using a TGFB1-inducible synthetic SMAD-responsive promoter to selectively drive *NIS*-transgene expression in engineered MSCs (SMAD-NIS-MSC) in the context of differentiated thyroid cancer based on the critical role of TGFB1 in the pathogenesis of radioiodine refractory differentiated thyroid cancer. To evaluate the TGFB1 expression in thyroid cancer cell lines, the TGFB1 concentration in conditioned medium (CM) from an array of established human papillary thyroid cancer (PTC) cell lines (BCPAP and K1) was measured by ELISA. BCPAP-CM showed a higher concentration of TGFB1, while a lower concentration was measured in K1-CM. Stimulation of SMAD-NIS-MSCs with PTC-CM showed a significant increase of NIS-mediated radioiodide-125 uptake in these MSCs *in vitro*. In addition, iodide uptake in SMAD-NIS-MSCs was significantly stimulated by co-culture with thyroid cancer cells. Cell migration assay was performed to validate the effect of PTC-CM in MSC recruitment. MSCs subjected to a gradient between tumor CM and serum free medium showed a directed chemotaxis towards CM with increased forward migration index (FMI) and center of mass (CoM). In a next step, based on the *in vitro* studies, SMAD-NIS-MSCs will be systemically applied via the tail vein to mice harboring subcutaneous PTC tumors and tumoral iodide uptake will be monitored by ^{123}I-scintigraphy. Taken together, these data indicate the feasibility of commandeering TGF-β/SMAD signaling in the TGFB1-rich tumor environments of radioiodine refractory differentiated thyroid carcinomas to re-establish functional *NIS* expression using engineered mesenchymal stem cells as therapy vehicles.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Targeting Glycogen Metabolism as a Novel Therapeutic Approach in Anaplastic Thyroid Cancer

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Effective treatment options for well-differentiated papillary (PTC) and follicular (FTC) thyroid cancers afford positive patient prognoses. The absence of effective interventions for the stem-like, dedifferentiated anaplastic thyroid cancer (ATC) results in poor patient outcomes with a mortality rate higher than all other endocrine cancers combined (1). While receptor tyrosine kinase inhibitors such as sorafenib