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

can extend ATC patient survival, drug resistance and tumor reoccurrence often develop (2). Therefore, there is a critical need for more effective targeted therapies for ATC. Although the cell signaling landscape of ATC tumors is well described, very little is known about tumorigenic adaptations in ATC cellular metabolism. Tumors exhibit an increased consumption of glucose compared to normal tissues to fuel tumor progression. Some cancers meet this high glucose requirement by storing and breaking down glycogen. In our studies here, we show for the first time that normal thyroid, PTC, FTC, and ATC cells express genes necessary for glycogen metabolism. We confirm these observations in patient samples in normal thyroid and thyroid cancer patient samples via immunofluorescence in tissue microarrays. Furthermore, we detect intracellular glycogen stores in cell lines representing normal thyroid, PTC, FTC, and ATC cells. Importantly, we demonstrate that glycogen phosphorylase inhibitors result in accumulation of intracellular glycogen and induce subsequent apoptosis in ATC cells. We further show that glycogen phosphorylase inhibitors synergize with kinase inhibitors such as sorafenib and buparlisib to decrease ATC cell viability. Our work establishes glycogen metabolism as a novel metabolic process in thyroid cells that is associated with thyroid cancer dedifferentiation and provides insight to the effectiveness of inhibiting glycogen metabolism as a therapeutic strategy in ATC.

**References:** 1. Siegel, R.L., Miller, K.D. and Jemal, A. (2017), Cancer statistics, 2017. CA: A Cancer Journal for Clinicians, 67: 7-30. doi:10.3322/caac.21387

2. Saini S, Tulla K, Maker AV, Burman KD, Prabhakar BS. Therapeutic Advances in Anaplastic Thyroid Cancer: A Current Perspective. Mol Cancer. 2018;17(1):154. doi:10.1186/s12943-018-0903-0

## Tumor Biology

### HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

***Triple Negative Breast Cancer is Dependent on the Lysosomal Cholesterol Transporter NPC1***

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**Background:** Triple Negative Breast Cancer (TNBC) is an aggressive subtype of breast cancer (BC) with peak rate of metastasis within the first few years post diagnosis and few targeted therapies. Normal epithelial cells and estrogen receptor alpha (ER) positive BC express the microRNA-200c (miR-200c), a potent suppressor of epithelial-to-mesenchymal transition (EMT). However, miR-200c is silenced or lost in TNBC, allowing aberrant expression of genes conferring a de-differentiated, non-epithelial phenotype that confers invasive and chemo-resistant properties. Recent literature demonstrated that EMT also promotes altered tumor cell metabolism. **Hypothesis:** We postulate that EMT reversal in TNBC will reveal selective advantages and identify novel therapeutic vulnerabilities. **Methods:** We used restoration of miR-200c as a tool to identify selective advantages conferred by EMT. In addition to driving global metabolic changes, miR-200c-repressed key cholesterol metabolism genes that support the uptake of dietary cholesterol, which is delivered via low-density-lipoproteins (LDL) and processed by the lysosomal cholesterol transporter, Niemann-Pick Type C1 (NPC1). Manipulation of NPC1 by genetic and pharmacological means was used to determine if and how TNBC are reliant on this pathway. **Results:** We determined that *NPC1* is overexpressed in TNBC relative to ER+BC (*Nature* Metabric P<0.0001). Restoration of miR-200c directly targets the *NPC1* 3'UTR and represses *NPC1* by two-fold (p=0.01). While silencing of *NPC1* in ER+ BC cells led to slowed proliferation, TNBC cell lines died within 48-72 hours. NPC1 is associated with mitochondrial dysfunction and mTOR suppression. Intracellular cholesterol homeostasis is critical for cell survival and is carefully regulated, but how these homeostatic mechanisms adapt during tumor progression is poorly understood. **Conclusions:** This study demonstrates that while mesenchymal-like TNBC cells do not require exogenous cholesterol from the microenvironment, this cancer type is sensitive to the loss of NPC1. Overall, this work identifies NPC1 as a novel target in TNBC and sheds light on how lysosomes and mitochondria interact to sense cholesterol and drive cell survival.

## Tumor Biology

### HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

***Using Multimodal Functional Imaging in the Management of SDHx-Related Pheochromocytoma and Paraganglioma***

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**Background:** Succinate Dehydrogenase *(SDH)* subunit pathogenic variants predispose to Pheochromocytoma and Paraganglioma (PPGL). Functional imaging harnesses the innate receptor expression and the aberrant cellular pathways in PPGLs to improve diagnostic accuracy & guide treatments, including nuclear medicine therapies. Currently commonly available functional imaging modalities include $^{18}$F-FDG PET, $^{123}$I-MIBG and $^{68}$Ga-DOTATATE. **Aims**: To analyze the use of $^{123}$I-MIBG, $^{18}$F-FDG PET and $^{68}$Ga-DOTATATE in patients harboring *SDHB* & *SDHD* pathogenic variants and determine the detection rates for both primary tumors and metastatic sites of disease. **Methods:** Retrospective review of patient records and imaging reports allowed tumor characteristics and imaging features of 21 patients with *SDH*-related PPGL to be recorded. Contrast enhanced CT/MRI were used as control to calculate the sensitivity of each functional imaging modality. Avidity of the primary lesion and metastatic deposits were used to calculate detection rates.