735 IMPACT OF REVERSING AN EPITHELIAL-TO-MESENCHYMAL TRANSITION PROGRAM ON TUMOR METABOLISM AND IMMUNE SUPPRESSION

Michelle Williams*, Jessica Christenson, Kathleen O'Neill, Sabrina Hafeez, Nicole Spoelstra, Jill Slansky, Jennifer Richer. *University of Colorado Anschutz Medical Campus, Aurora, CO, USA*

Background To identify novel molecular mechanisms used by triple negative breast cancer (TNBC) to facilitate metastasis, we manipulated oncogenic epithelial-to-mesenchymal transition (EMT) by restoring the microRNA-200c (miR-200c), termed 'the guardian of the epithelial phenotype.' We identified several tumor cell catabolizing enzymes, including tryptophan 2,3-dioxygenase (TDO2) and heme oxygenase-1 (HO-1). The Richer lab has published that TDO2 promotes anchorage independent cell survival during TNBC metastasis via its catabolite kynurenine, which also induces $CD8^+$ T cell death. Similarly, published studies have demonstrated that HO-1 supports BC anchorage independent survival. However, effects of the HO-1 catabolite bilirubin on the tumor microenvironment had not been studied. We postulated that TNBC utilize targetable catabolizing enzymes, like HO-1, to simultaneously support tumor cell survival and dampen the anti-tumor immune response.

Methods To test our hypothesis in an immune competent mouse model, Met-1 mammary carcinoma cells from a late stage MMTV-PyMT tumor were engineered to inducibly express miR-200c. Tumor cell infiltrates were analyzed by immunohistochemistry (IHC), flow cytometry and multispectral fluorescence. RAW264.7 mouse macrophages were cultured with conditioned medium from carcinoma cells ± miR-200c or the HO-1 competitive inhibitor tin mesoporphyrin (SnMP). RAW264.7 macrophages were also treated with 0–20 μM bilirubin and macrophage polarization and efferocytic capacity, the ability to engulf dead tumor cells, were assessed using qRT-PCR and IncuCyte assays.

Results MiR-200c restoration to Met-1 orthotopic tumors decreased growth by 45% and increased infiltration of $CD11c^+$ dendritic cells and activation, determined by CD44 expression, of $CD4^+$ and $CD8^+$ T cells. While the number of $F4/80^+$ macrophages was unchanged by miR-200c, the percent of M1 anti-tumor macrophages ($F4/80^+iNOS^+$/total cells) increased by >6-fold in miR-200c$^+$tumors. RAW264.7 macrophages cultured with conditioned medium from miR-200c-restored mammary carcinoma cells had a 25–95% decrease in M2 pro-tumor genes (Arg1, Il4 and Il13) and a 15–55% increase in M1 genes (Nos2, Tnfa and Cxcl10). A similar decrease in M2 (30–50%) and increase M1 (35–160%) genes was seen in macrophages cultured with conditioned medium from SnMP treated mammary carcinoma cells. Conversely, bilirubin treatment alone enhanced M2 macrophage polarization and inhibited efferocytosis in a dose-dependent manner.

Conclusions Use of miR-200c to reverse EMT revealed that HO-1 promotes simultaneous TNBC cell survival and immune suppression. These studies are the first to show that tumor cell-HO-1 activity and subsequent bilirubin production may alter macrophage function in the tumor microenvironment. This finding could be clinically relevant since HO-1 inhibitors like SnMP are already FDA approved for treatment of other diseases.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0735

736 MICRORNA EXPRESSION PATTERNS IN MELANOMAS ORIGINATING FROM GYNECOLOGIC SITES

[1]Mallory DiVincenzo*, [1]Lorena Suarez-Kelly, [1]Maribelle Moufawad, [1]Casey Ren, [1]Zoe Barricklow, [1]Paolo Fadda, [1]Lianbo Yu, [1]Sarah Peters, [2]Alejandro Gru, [1]William Carson. *[1]The Ohio State University, Columbus, OH, USA; [2]University of Virginia, Charlottesville, VA, USA*

Background Melanomas originating from gynecologic sites (MOGS) are rare mucosal melanomas originating from the vulva, vagina, and cervix. MOGS are associated with a poor survival rate and limited therapeutic options, as patients often present an advanced disease stage. MiRNAs (miRs) are a class of small, non-coding RNA molecules composed of 21–23 nucleotides that control expression of target genes via post-transcriptional regulation and can exhibit dysregulated expression in cancer. Patterns of miR expression and their effects on disease progression have not yet been explored in the setting of MOGS. We hypothesize a unique miR expression profile exists in MOGS that can mediate disease progression via interaction with target genes.

Methods RNA was isolated from formalin fixed, paraffin embedded tissue samples of human vaginal and vulvar melanoma for comparison to normal adjacent vaginal mucosal tissue (NAT) and primary cutaneous melanoma (PCM), respectively. miR expression was then quantified using the NanoString human miRNA assay. Common experimentally validated gene targets of differentially expressed (DE) miRs were identified using miRNet, and pathway analysis was completed to examine potential downstream effects of dysregulated miR expression.

Results Comparison of miR expression in vaginal melanoma to NAT revealed 25 DE miRs (fold change > 1.5, $p < 0.05$), with 10 demonstrating a significant decrease in expression in vaginal melanoma tissue relative to NAT, including hsa-miR-145-5p, hsa-miR-99a-5p, and hsa-miR-1972, and 15 exhibiting a significant increase in expression including hsa-miR-17-5p, hsa-miR-19b-3p, hsa-miR-20a-5p, and hsa-miR-20b-5p. 45 DE miRs were identified between vulvar melanoma and PCM, among which 3 demonstrated a significant decrease in expression in vulvar melanoma including miR-200b-3p, miR-494-3p, and miR-200a-3p, and 44 demonstrated a significant increase in expression including miR-17-5p, miR-146a-5p, and miR-19b-3p (fold change > 2, $p < 0.01$). Among these DE miRs, both miR-17-5p and miR-146a-5p have recently been experimentally validated as direct or indirect regulators of PD-L1 expression in melanoma. Pathway analysis for DE miRs in vaginal and vulvar melanoma revealed significant enrichment of 35 and 30 pathways, respectively, each including TGF-β signaling, for which 57 genes in the pathway are validated targets of 13 DE miRs in vaginal melanoma ($p = 1.5e-12$), and 59 genes in the pathway are validated targets of 17 DE miRs in vulvar melanoma ($p = 2.4e-13$).

Conclusions The results of this study support miRNAs as important potential regulators of gene expression in vaginal and vulvar melanomas that can contribute to tumor progression, tumor immunogenicity, and response to current immunotherapies.

Ethics Approval This study was approved by the Ohio State University Institutional Review Board, approval #2007C0015.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0736