**The role of the androgen receptor in reciprocal activation of breast cancer metastases and the lung epithelium during metastatic outgrowth**

Jessica L. Christenson[1], Nicole S. Spoelstra[1], G. Devon Trahan[2], Kathleen I. O'Neill[1], Michelle M. Williams[1], Jennifer K. Richer[1]

[1]Department of Pathology, University of Colorado Anschutz Medical Campus
[2]Department of Pediatric Hematology, Oncology and Bone Marrow Transplantation, University of Colorado Anschutz Medical Campus

The lung is one of the most common sites of breast cancer (BC) metastasis. A major gap in our ability to effectively treat BC patients with established lung metastases lies in our lack of knowledge regarding how the lung microenvironment is remodeled during metastasis and how this contributes to disease progression. Our data suggest that the lung epithelium, in particular lung type II alveolar epithelial (AT2) cells, contribute to metastatic outgrowth. We **hypothesize** that BC lung micrometastases activate surrounding lung epithelial cells which, in turn, support the survival and outgrowth of BC metastases within the lung. The **overall goa**l of these studies is to identify factors secreted by resident lung cells that could be used as metastasis-specific therapeutic targets/agents. We utilized immunocompetent mouse metastasis models to study metastatic progression within the lung. Multispectral fluorescent imagining of metastases demonstrated that a wound repair-related phenotype, characterized by chronic inflammation, develops within the lung microenvironment during metastatic outgrowth. We observed an increase in the number and activation of AT2 cells surrounding metastases as they grow. To gain mechanistic insight into how growing metastases may impact AT2 cells, we conducted single-cell RNA-sequencing (scRNAseq) on mouse lungs with high vs. low metastatic burden. This investigation showed that metastatic outgrowth significantly changes AT2 gene expression resulting in a modified secretome. Cell culture experiments, using multiple AT2 cell models, indicated that no contact co-culture with TNBC also caused significant changes in AT2 gene expression. Upstream regulator analysis of these datasets predicted that the androgen receptor (AR) may contribute to AT2 activation during metastatic outgrowth. Compared to non-adjacent tumor-free MMTV-PyMT lung tissue, there was an increased percentage of AR+ cells in the lung adjacent to metastases, which was further associated with large metastases only. Co-immunofluorescence experiments demonstrated that these lung-specific AR+ cells were primarily AT2 cells and fibroblasts, while in human lungs the majority of AR+ cells were AT2 cells and macrophages. Interestingly, co-culturing of TNBC and AT2 cells enhanced BC proliferation, suggesting that following activation, AT2 secreted factors may reciprocally impact metastatic growth. In **conclusion**, these data indicate that a tumor supportive microenvironment develops as lung metastases grow and suggests that by the time patients present with established/detectable lung metastases, the lung microenvironment has already undergone fundamental alterations that promote metastatic progression. Future studies will elucidate how activated lung cells support metastatic progression and could lead to the development or repurposing of therapeutic strategies to prevent destructive metastatic outgrowth.