Oral Abstract 7: Epithelial intra-tumoral heterogeneity in ovarian cancer resistance to anti-angiogenic therapy

Presenting Author: Amma Asare, MD Anderson Cancer Center

Topic
Ovarian

Objectives
Despite the clinical benefit from anti-angiogenesis therapy, anti-VEGF antibodies (AVA) are limited in utility due to drug resistance and consequent malignant progression. While AVA therapy is aimed at the tumor vasculature, we hypothesized that distinct ovarian cancer cell identities may confer a selective advantage during the development of resistance. This study defines populations of epithelial ovarian cancer cells transcriptionally and characterizes their evolution during therapeutic resistance.

Methods
Animals with established SKOV3ip-1 luciferase labeled intraperitoneal mouse ovarian tumors were treated with AVA therapy. During the treatment period, the mice were imaged twice weekly to assess tumor growth via luciferase intensity. Tumors were harvested from resistant and control tumors and single-cell suspensions of the tumors were generated. The suspensions were sorted via flow cytometry to enrich for endothelial, fibroblast and epithelial ovarian cancer cells. These populations were subjected to single-cell RNA sequencing, genomic alignment, and clustering.

Results
Using luciferase intensity levels, we tracked the temporal emergence of resistance to anti-angiogenic therapy. 41% of tumors were classified as “sensitive” (tumor reduction) and 58% were classified as “resistant” (tumor growth) after ~ 7 days of treatment. Upon dissociation, most recovered cells were immune cells. Epithelial cancer cells (EPCAM+) comprised 9.5% of live population, endothelial cells 4.4% and fibroblasts 1.25%. To enrich for these cell types of interest, immune cells (CD45+) were excluded and single cell RNA sequencing was performed on the remaining cells from resistant (n = 8) and control (n = 8) tumors. Single-cell transcriptional profiling identified cells originating from the host (murine) or from the ovarian cancer cell line (human). Populations aligning to the human genome expressed markers consistent with ovarian cancer epithelium such as KRT5, KRT8 and EPCAM, supporting their identification as xenograft derived ovarian cancer cells. Within this ovarian cancer population, 15 subpopulations with discrete expression profiles were identified. Multiple biological replicates were represented in each sub-cluster, suggesting functional concordance within the cohort. Notably, markers of proliferation (MKI67, TOP2A) were most highly enriched in only 4/15 populations, indicating that features other than proliferation rate may be central to the development of resistance. Populations enriched in resistant versus control tumors were characterized by increased levels of mitochondrial-related transcripts (ND5, ND4) and cell adhesion molecules (CCN2). These data suggest a potential role for cell motility or dysregulated oxidative phosphorylation in resistance.

Conclusions
Epithelial ovarian cancer cell populations are dynamic in their behavior during tumor growth and the development of resistance. Alterations in energy metabolism, TNF alpha signaling and MYC activation may play important roles in the development resistance to anti-angiogenic therapy and warrant further exploration.