Spatially resolved transcriptomics identified distinct tumor-stroma crosstalk networks associated with chemoresistance in ovarian cancer

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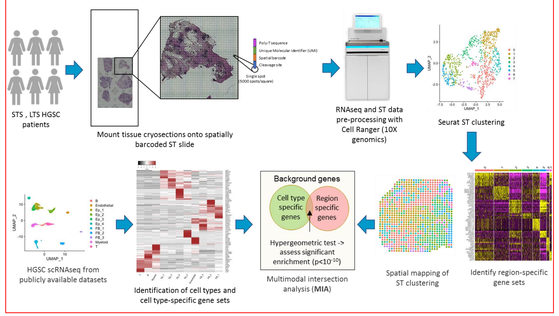
Introduction

More than 14,000 patients in the United States die of ovarian cancer each year, making this cancer the fifth leading, and therefore most lethal, cause of cancer death among women in the United States. High grade serous ovarian carcinoma (HGSC), which accounts for 70% of all cases, is the most aggressive form of ovarian cancer. It characteristically arises from fallopian epithelial tissue and spreads to multiple locations within the abdomen before it is diagnosed at stage 3 or 4. At this point, clinical intervention is limited, resulting in a 10-year survival rate of a devastating less than 20%. Treatment options currently consist of cytoreductive surgery before or followed by platinum- and taxane-based combination chemotherapy. A majority of patients respond to this first line treatment and thus are in possession of chemosensitive tumors; however, in a subset of patients, tumors recur within 6 months after the last cycle of treatment developing chemoresistance.

Additionally, ovarian cancer progression is often dictated by strategically located activity of cells and proteins in the tumor microenvironment (TME). Consisting of fibroblasts, extracellular matrix proteins, endothelial cells, lymphocytic infiltrates, and cancer cells, the TME allows for sustenance of the tumor through crosstalk signaling networks established between cancer and stromal cells. The TME directly affects chemoresistance and patient survival by reducing the positive effects of chemotherapeutic drugs by hindering drug absorption.

Recent discoveries suggest the existence of biological differences in advanced stage HGSC chemoresistant and chemosenstive tumors. Substantial efforts have been made to develop gene expression-based molecular signatures and biomarkers from these cells to predict chemoresistance in HGSC. Spatial transcriptomics (ST) is a cutting-edge technology capable of providing a rich spatial context to gene expression by generating thousands of spatially resolved transcriptomes on a single tissue section with a resolution of 10-50 cells. Recently, ST has been used to provide meaningful biological insights based on spatial proximity in complicated cancers such as pancreatic cancer. However, a transcriptome-based signature that can predict chemoresistance in HGSC is still lacking.

Pipeline



Results

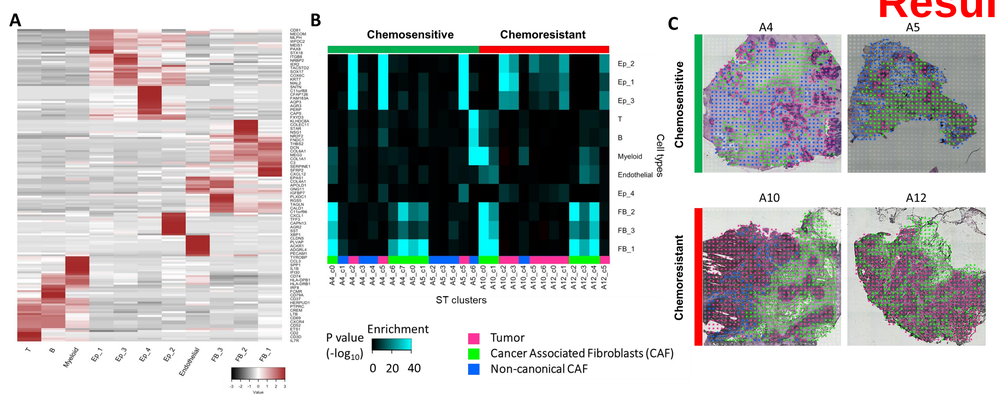


Figure 1. Tumor ST clusters assigned by the Multimodal intersection analysis (MIA) matched histology images. After alignment of fastq file using Cell Ranger (10x Genomics), an average of 3500 distinct genes were detected in each spot. Each sample was then loaded into R Seurat package and normalized by SCTransform. Phenograph clustering was then performed by using the first 10 dimensions of PCA. Genes with significantly higher expression in each ST cluster relative to the others were identified (P<0.01, Wilcoxon Rank Sum test and log_FoldChange>0). **A.** scRNAseq data from *Shih et al. PLoS One. 2018* were analyzed and used to define gene sets: for each cell type, genes whose expression was statistically higher in the cells annotated to that cell type in comparison with expression in the remaining cells were identified (P<10^{-5}, Wilcoxon Rank Sum test). **B.** With the gene sets extracted across the scRNA-seq and ST modalities, the overlap between each pair of cell type-specific and region-specific gene sets was computed by MIA. A hypergeometric test was performed to assess significant enrichment (Enrichment threshold P<10^{-10}). e.g. Tumor clusters were assigned if the enrichment p-value of any of the epithelial cell types Ep_1, Ep_2, and Ep_3 in that cluster was lower than 10^{-10}. Fibroblast clusters were assigned similarly. Clusters other than tumor and fibroblast were assigned as non-fibroblastic stroma. **C.** Assigned clusters overlaid on H&E images. Colors indicated the clustering assignments. Tumor clusters matched the morphology of tumor regions on H&E images.

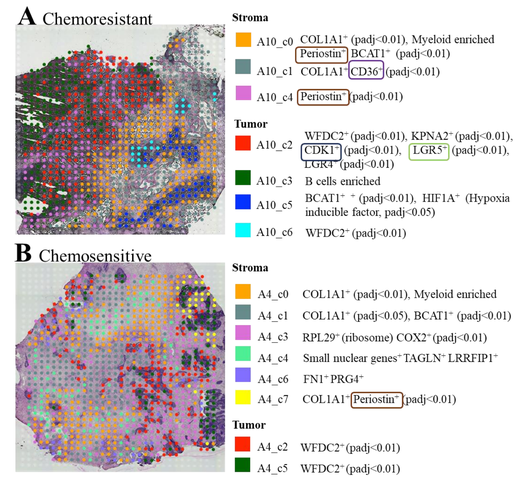


Figure 2: Characterization of tumor and stroma subclusters helped to elucidate the heterogeneity of these cellular compartments and validation of stroma subclusters by multiplex immunofluorescence (mIF). A-B. Multiple rounds of subclustering highlighted the heterogenicity in the HGSC tumors and TME. Differential gene expression analysis for each subcluster revealed markers useful to discriminate chemoresistant from chemosensitive tumors. **Periostin** (POSTN) is a matricellular protein that is expressed by fibroblasts. It has a key function in organizing the extracellular matrix, and high expression in the stroma has been shown to correlate with lower overall survival and greater treatment resistance. *Gonzalez L. et al. Frontiers in Oncology (2018).* POSTN recruits Wnt ligands. *Malanchi I. et al. Nature (2011).* **CD36** is a membrane glycoprotein receptor that is associated with tumor biology by playing a roll in antigen presentation, inflammation, angiogenesis and cell adhesion. *Ladanyi A. et al. Oncogene (2018).* Furthermore, it enhances lipid uptake and FA oxidation and is associated with tumor immune tolerance. By binding to TSP-1, CD36 induces apoptosis and blocks the VEGFR-2 pathway. *Wang J. et al. Theragnostics (2019).* **CDK1** is a commonly known cell cycle regulator whose abnormal activation results in high proliferation and apoptosis prevention of ovarian cancer cells. *Zhang R. et al. Journal of Ovarian Research (2017).* **LGR5** modulates Wnt/beta-catenin signaling, which regulates proliferation and differentiation of adult stem cells. It is able to induce EMT, thereby the expression of this molecule is associated with distant metastasis. *Liu W. et al. Cancer Medicine (2018).* **C.** mIF was used to validate the expression of Periostin and CD36 in stroma subclusters.

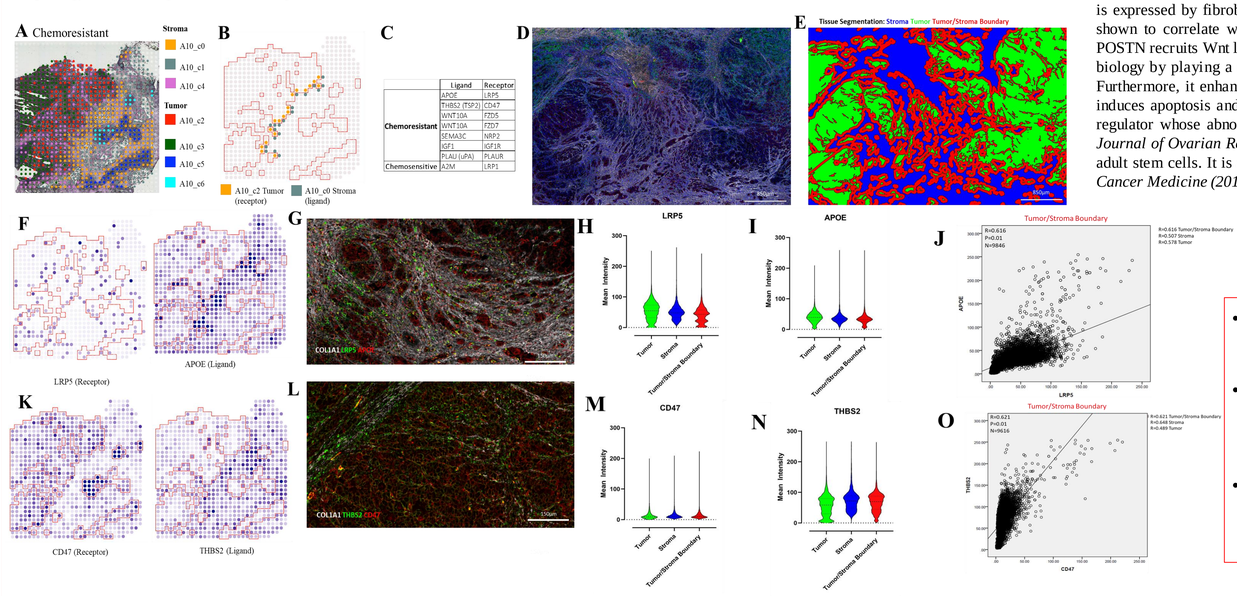


Figure 3: Crosstalk signaling network analysis and validation of ligand-receptor pairs associated with chemoresistance by mIF. A. H&E image of representative refractory sample with overlaid cluster assignment. **B.** Expression map of selected spots from tumor cluster c2 (orange) and stroma cluster c0 (grey) and used for cross-talk signaling network analysis (receptor in tumor, ligand in stroma). Red lines indicates tumor areas. **C.** Top 6 ligand-receptor pair selected after cross-talk signaling network analysis in refractory and chemosensitive samples. **D.** Multiplex IF image of a representative chemoresistant sample . **E.** Tissue segmentation of representative sample using Visiopharm analysis software to identify tumor (green), stroma (blue) and tumor/stroma boundary areas. **F.** ST RNA expression maps of LRP5 receptor and APOE ligand to validate their higher expression in tumor and stroma, respectively. **G.** Multiplex IF image highlighting presence of LRP5 receptor in the tumor and APOE ligand in the stroma. COL1A1 was used to define stroma areas. **H-I.** Graph depicting the mean intensity of LRP5 (**H**) and APOE (**I**) in the tumor, stroma, and tumor/stroma boundary. **J.** Graph correlating the mean intensity of LRP5 and APOE at the tumor stroma boundary resulting in a Pearson correlation value of 0.616 (p=0.01). The lower Pearson correlation coefficients for the tumor and stroma regions emphasize a significant association between ligand and receptor at the tumor stroma interface. **K.** ST RNA expression maps with CD47 receptor and THBS2 ligand to validate their higher expression in tumor and stroma, respectively. **L.** Multiplex IF image highlighting presence of CD47 receptor in the tumor and THBS2 ligand in the stroma. COL1A1 was used to define stroma areas. **M-N.** Graph depicting the mean intensity of CD47 (**M**) and THBS2 (**N**) in the tumor, stroma, and tumor/stroma boundary. **O.** Graph correlating the mean intensity of THBS2 and CD47 at the tumor stroma boundary resulting in a Pearson correlation value of 0.621 (p=0.01).

Conclusions

- New class of biomarkers based on spatial intratumoral heterogeneity and spatially resolved transcriptomic analyses can be used to develop predictive models for chemoresistance.
- Novel therapeutic strategies can be developed to target not only tumor and stromal cells, but also the ligand-receptor crosstalk networks established between cancer and stromal cells, which may result in improved survival rates for HGSC patients with chemoresistant disease.
- Validation of optimized mIF antibody panels including Periostin-CD36-COL1A1, APOE-LRP5-COL1A1 and THBS2-CD47-COL1A1 in a larger cohort of chemosensitive and chemoresistant tumor samples is ongoing.