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 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 chemosensitive 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 transcriptomics on a single tissue section with a resolution of 10-50 μm. 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.

Results

Figure 1. Tumor ST clusters assigned by the Multimodal intersection analysis (MIA) matched histology images. After alignment of auIq tissue using Cell Ranger (10x Genomics), an average of 7500 distinct genes were detected in each spot. Each sample was then loaded into R Seurat package and normalized by SC3stdev. Photograph 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>4). A, sRNAseq data from Shi et al. PLoS One 2018 were analyzed and used to define gene clusters for each cell type, genes whose expression was statistically higher in the cells assigned to that cell type in comparison with expression in the remaining cells were identified (P<0.01, Wilcoxon Rank Sum test). B, With the gene sets extracted across the sRNA-seq and FF analyses, the clustering between each pair of cell types was calculated and a representative gene set was compared by MIA. A hypergeometric test was performed to assess significant enrichment (Enrichment threshold P<0.01). e.g. Tumor clusters were assigned if the enrichment p-value of any of the cell types. Figures 2 and 3 in this paper are lower than 10 −7. Fibroblast clusters were assigned similarly. Clusters other than tumor and fibroblast were assigned as non-fibroblastic STs. 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.

Conclusions

• New class of biomarkers based on spatial intratissue heterogeneity and spatial resolved transcriptomics 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-CDS-COL1A1-APOE-LRPS-COL1A1 and THBS2-CDS-COL1A1 in a larger cohort of chemosensitive and chemoresistant tumor samples is ongoing.

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 d2 (grey) and used for cross-talk signaling network analysis (receptor in tumor, ligand in stroma). Red lines indicate tumor areas. C, Top 6 ligand-receptor pair selected after cross-talk signaling network analysis in refractory and chemosensitive samples. D, Multiple IF image of a representative chemoresistant sample. E, tissue segmentation of representative sample using Visiopharm and Biocut software to identify tissue (green), stroma (blue) and tissue/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, Multiple IF image highlighting presence of LRP5 receptor in the tumor and APOE ligand in the stroma. COLL1A1 was used to define stroma areas. H-L, Graph depicting the mean intensity of LRP5 (H) and APOE (I) in the tumor, stroma, and tumor-stroma boundary area. J, Graph correlating the mean intensity of LRP5 and APOE at the tumor-stroma boundary resulting in a Pearson correlation coefficient of 0.016 (p<0.01). The lowest Pearson correlation coefficient in the tumor and stroma regions emphasizes a significant association between ligand and receptor at the tumor-stroma interface. K, ST RNA expression maps with CD44 receptor and THBS3 ligand to validate their higher expression in tumor and stroma, respectively. L, Multiple IF image highlighting presence of CD44 receptor in the tumor and THBS3 ligand in the stroma. COLL1A1 was used to define stroma areas. M-N, Graph depicting the mean intensity of CD44 (M) and THBS3 (N) in the tumor, stroma, and tumor-stroma boundary. O, Graph correlating the mean intensity of THBS3 and CD44 at the tumor-stroma boundary resulting in a Pearson correlation value of 0.621 (p<0.01).