75 THE MUTATIONAL LANDSCAPE DEFINES THE PROTEOME AND SPATIAL ORGANIZATION OF TUMOR, STROMA, AND IMMUNE CELLS IN OVARIAN CANCER

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Background High-grade serous ovarian cancer (HGSOC) is highly aggressive and lethal, with clinical challenges to both diagnosis and treatment. The genomic instability of HGSOC, further complicated by homologous recombination deficiency (HRD), leads to heterogeneity in the HGSOC tumors and patient response to treatment. We have previously profiled the complete proteome of tumors from 32 HGSOC patients before and after multiple rounds of chemotherapy, and we found that tumors with germline or somatic loss of BRCA1 or BRCA2 had increased expression of proteins related to immune pathways, more shared TCR CDR3 repertoires of tumor-infiltrating T cells, increased neoantigen counts, and enriched immune clusters after chemotherapy. However, our present knowledge regarding the abundance of the tissue-infiltrating immune cell populations and their spatial organization relative to the tumor remains elusive. To gain a deeper understanding, further research employing spatial biology solutions is necessary in order to elucidate this aspect.

Methods Single-cell spatial profiling of 24 paired primary and recurrent tumors was performed with PhenoCycler-Fusion on whole tissue sections using a 27-plex antibody panel including markers for various T and B cell subtypes, macrophages, granulocytes, dendritic cells, natural killer cells, as well as stromal, tumor, and epithelial cells. Large high-resolution multiplex images encompassing the entire tumor sections were processed and analyzed using a customized bioinformatics pipeline. The first step of the analysis workflow consists of nuclear and cell segmentation. Subsequently, protein expressions were calculated from the respective cell compartments, and batch effect correction was applied to mitigate potential discrepancies arising from technical variability among the samples. Unsupervised clustering using Leiden algorithm was then performed on the batch-corrected data, and clusters were manually annotated into 17 cell phenotypes based on their protein expression patterns displayed on a hierarchical clustering heatmap. Furthermore, the tissues were categorized into three distinct compartments: the tumor, tumor front, and stroma. Then, cell percentages within each compartment were calculated and subsequently compared between different patient groups.

Results We observed elevated levels of tumor-infiltrating cytotoxic T cells and M1 macrophages in recurrent tumors when compared to primary tumors. Additionally, there were higher percentages of helper T cells detected in the 'tumor front' compartment. We also defined specific cellular neighborhood differences based on HRD status that correlated with survival.

Conclusions Through proteo-genomic and spatial analysis, this work has shown that the immune landscape of HGSOC tumors is influenced by HRD status and identified candidate drivers of HGSOC biology.

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