167 COMPREHENSIVE PROFILING OF CANCER-ASSOCIATED FIBROBLASTS IN CD8+T CELL-EXCLUSIVE NON-SMALL CELL LUNG CANCER TUMOR MICROENVIRONMENTS USING THE NANOSTRING GEOMX® DIGITAL SPATIAL PROFILER

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Background Cancer-associated fibroblasts (CAFs) are a major component of the non-small cell lung cancer (NSCLC) tumor microenvironment (TME).[1–4] Recent studies indicate that CAFs play a role in generating a CD8+T cell (CTL)-exclusive TME.[5–9] Given that immune-checkpoint inhibitors (ICIs) rely on CTLs, an abundance of CAFs in the TME may result in reduced ICI efficacy. Although CAFs are considered potential targets for therapy, attempts to deplete CAFs have largely failed.[10] This is due to the fact that CAFs are a heterogenous population of cells and depletion of the wrong subpopulation could worsen disease.[1] Thus, to enhance ICI efficacy and improve patient outcome, we must identify and target CAF subtypes that promote CTL exclusion. Single-cell RNA sequencing (scRNAseq) has improved our understanding of CAF heterogeneity[11–13]; yet this technology cannot provide a comprehensive profile of CAF subtypes within the TME. For scRNAseq, tumor tissues are digested, causing cell death, and the resultant transcriptomic profile is incomplete. To generate a holistic profile of CAF subtypes within NSCLC, and identify the subpopulation that promotes CTL exclusion, we have utilized the GeoMX® Digital Spatial Profiler (DSP)[14–17], a state-of-the-art platform that allows for spatially resolved, high-plexed molecular profiling of intact tumor tissues.

Methods We performed digital spatial profiling on a tissue microarray (TMA) slide containing fifty-five cores of human lung tumors. We performed in-situ hybridization (ISH) on the slide with the GeoMx Whole Transcriptome Atlas (WTA), a mixture of photocleavable oligo-linked RNA probes that covers 18,000+ protein-coding human genes. Next, we stained the slide with fluorescent antibodies against Vimentin (VIM, a CAF marker), CD8, and SYTO (nuclear dye). Each core was a region of interest (ROI), and UV-light was masked to focus on CD8+ and VIM+ areas within each ROI to generate distinct areas of illumination (AOI) that were read out separately. NSG data was analyzed with DESeq2 to identify genes that were differentially expressed in CAFs living in CTL-exclusive tumors.

Results We identified 441 genes that were differentially expressed in CAFs residing in CTL-exclusive tumors compared to CAFs in CTL-inclusive tumors. Ingenuity Pathway Analysis (IPA) of those genes revealed several pathways which were inactivated in CTL-exclusive CAFs, including Leukocyte Extravasation Signaling and Ephrin Receptor Signaling, both of which could contribute to reduced CTL migration and infiltration.

Conclusions The GeoMX DSP can be used to identify CAF subpopulations that contribute to the formation of immune-exclusive TMEs and reveal novel molecular targets for immunotherapy.

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