

A TRANSLATIONAL APPROACH TO CATALOG PANCREATIC CANCER HETEROGENEITY USING SPATIAL GENOMICS IN LARGE PATIENT COHORTS FOR TARGET VALIDATION AND RATIONAL COMBINATION SELECTION

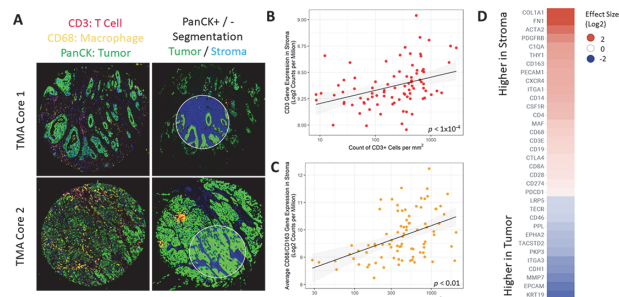
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Background Pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer with short overall survival; the standard of care (SoC) is chemotherapy. Immunotherapies in development aim to remodel the stroma by depleting immunosuppressive cell types or using T-cell redirection to kill tumor cells. To date, none of these methods have improved overall survival beyond SoC. Next generation immunotherapies that employ histopathology and molecular subtyping¹ for target and patient selection may succeed. Here we leverage a spatial transcriptomics platform (Nanostring Digital Spatial Profiling, DSP) to reveal molecular signaling in tumoral and stromal cells in 57 PDAC patients using tumor microarrays (TMAs). This approach is rapid and clinically relevant as molecular and histology data can be easily bridged.

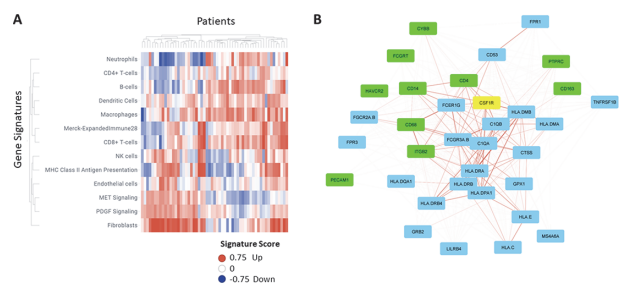
Methods TMAs generated from surgical resection tissue were commercially sourced. DSP was performed using the CTA RNA panel (1,800 target genes) using PanCK fluorescence for tumor/stroma segmentation. In parallel, slides were chromogenically stained for T-cells (CD3) and macrophages (CD68/CD163). Differential gene expression, gene signature and gene co-expression network analysis was performed using linear models in R.^{2,3}

Results Differential gene expression analysis and correlation to IHC confirmed the DSP platform successfully profiled tumor and stromal compartments (figure 1). Immune cell signatures⁴ and pathway analysis revealed a heterogeneous stromal environment. Using a fibroblast gene signature derived from single-cell RNAseq⁵ we found fibroblast density was positively correlated to PDGFR signaling and MHC-II expression but negatively correlated to B, CD4+ T and neutrophil cell levels (figure 2a). This finding supports the idea that atypical antigen presentation in cancer associated fibroblasts (CAFs) may be exploitable for immunotherapies.⁶ We constructed a co-expression network from in-situ stromal gene expression and used it to identify receptors coordinately expressed with the immunosuppressive macrophage marker CSF1R as a bispecific antibody partner (figure 2b).⁷ Classical macrophage markers were identified but also receptors with lesser-known functions in macrophages (TIM3/HAVCR2, FPR3, MS4A6A, LILRB4). Surveying target pairs in this method allows rapid, patient-specific confirmation in serial TMA sections with singleplex IHC or RNAscope.

Conclusions In this study we were able to recapitulate known PDAC biology using very small samples of primary tumors. The combination of TMAs and DSP enables a rapid validation of targets and hypothesis generation for bispecific pairings. Further analysis of untreated (n=14) and post-adjuvant chemotherapy (n=26) patients using RNA DSP, IHC and bulk RNA-seq is under way. Results from this cohort will enable an integrated histopathology and molecular approach to developing next-generation immunotherapies.



Abstract 928 Figure 1 Segmentation strategy and validation of DSP (A) PanCK, CD68 and CD3 staining from two representative tumor cores; (B, C) correlation of gene transcripts in stroma to cell counts from chromogenic staining; (D) heatmap of selected genes differentially expressed in tumor and stroma (n=57 patients).



Abstract 928 Figure 2 Exploration of the stromal compartment in PDAC TMAs. (A) Heatmap of selected cell type and gene signatures from gene expression in the stroma, color represents single sample enrichment score using GSVA method; (B) a gene co-expression subnetwork in the stroma centered on CSF1R, edge thickness represents strength of correlation, green nodes have evidence for cell surface expression based on proteomic profiling.⁷

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Ethics Approval Specimens were harvested from unused tissue after a surgical tumor resection procedure. A discrete legal consent form from both hospital and individuals was obtained by the commercial tissue vendor BioMax US for all samples analyzed in this abstract. All human tissues are collected under HIPPA approved protocols.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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