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# Pancreatic Ductal Adenocarcinoma Progression Is Associated With Reduced Stromal Organization and Adaptive Tumor Signaling at Single-Cell Resolution

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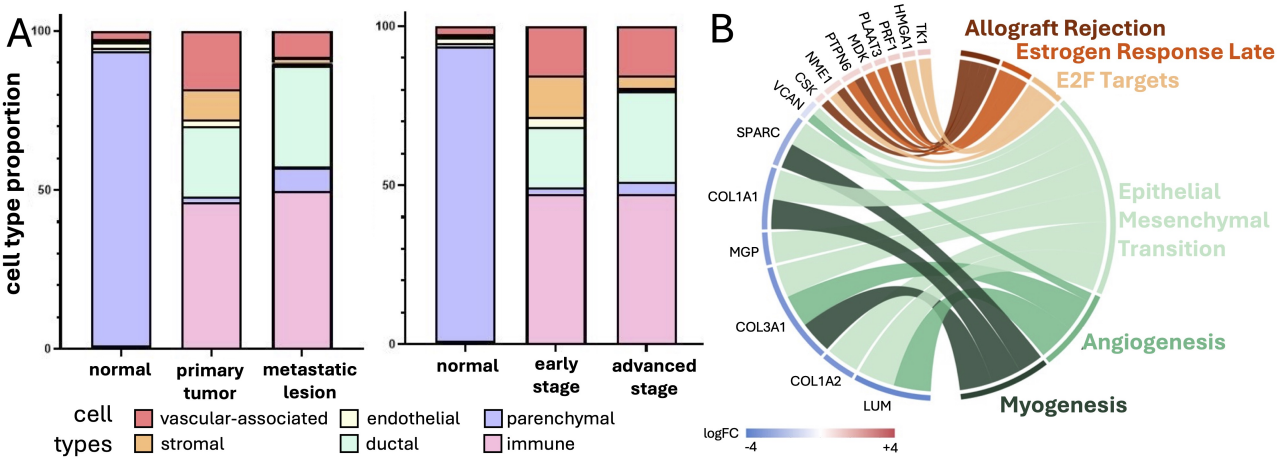
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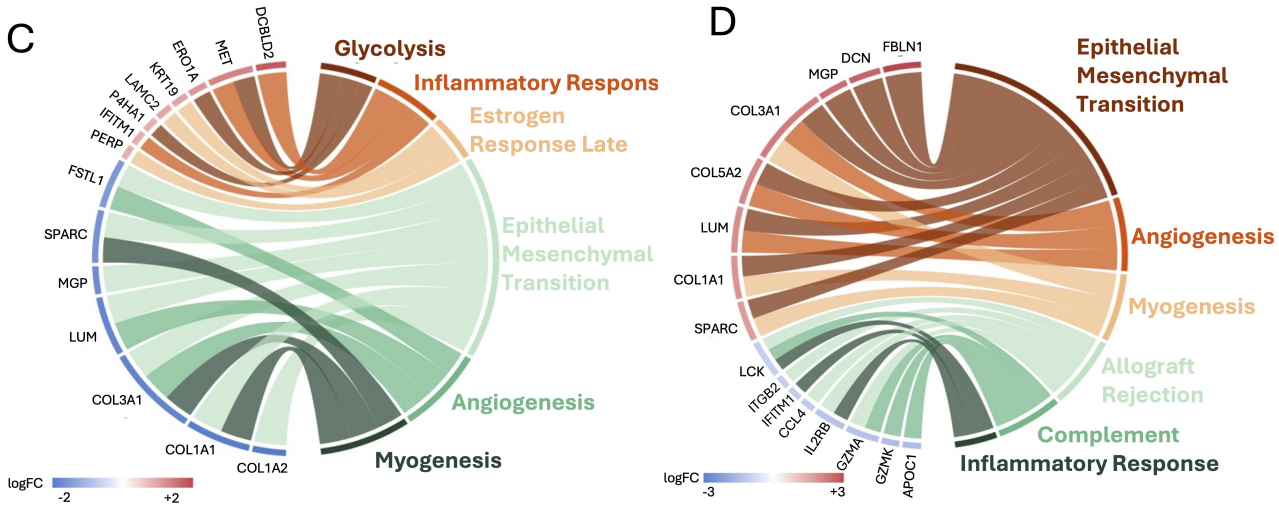
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## Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer with a poor survival rate. Publicly available single-cell RNA sequencing datasets were analyzed to characterize transcriptional changes across 33 PDAC and three normal pancreas samples. Early-stage samples exhibited greater stromal abundance and were enriched for microenvironmental remodeling, whereas advanced-stage samples showed increased proliferation and cellular plasticity. Chemotherapy-treated tumors demonstrated remodeling enrichment alongside reduced immune activity, suggesting adaptive changes. COMET analysis identified SNCG and LY6D as candidate basal-like tumor markers, supporting molecular subtype as a source of transcriptional heterogeneity. These findings highlight distinct tumor microenvironment characteristics between PDAC subtypes and progression.




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| Gene | TN | TP |
|---|---|---|
| SNCG | 0.978267 | 0.223467 |
| IGKC | 0.9396 | 0.358933 |
| LY6D | 0.9788 | 0.175867 |
| TPM2 | 0.915733 | 0.330667 |
| IGFBP3 | 0.944 | 0.2268 |
| ADIRF | 0.960933 | 0.1856 |
| PCSK1N | 0.976267 | 0.1576 |
| RBP1 | 0.957333 | 0.188133 |
| PLAT | 0.959867 | 0.1784 |
| CAVIN3 | 0.946267 | 0.209867 |

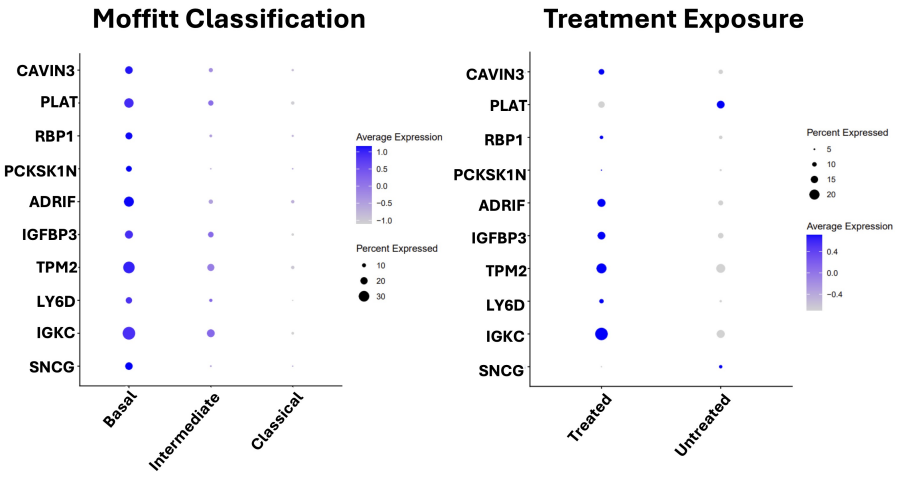



**Figure 1. Single-cell profiling reveals that PDAC progression and treatment converge on aggressive, immune-evasive transcriptional and cellular states:**

(A) Stacked bar plots showing proportion of all annotated cell types across tissue states and progression groups, comparing normal samples (n=3), and primary tumor samples (n=20) vs. metastatic site samples (n=13), and early-stage samples (n=12) vs. advanced staged samples (n=21). Tumor samples display increased representation of malignant and immune cell populations compared to normal tissue. Immune representation remains relatively consistent across advanced-stage (47%) and metastatic (49%) samples compared to early-stage (47%) and primary tumor (46%) samples. However, substantial sample-to-sample variability was observed, with immune cell proportions ranging from 11%-83% in advanced-stage samples, 14%-84% in metastatic samples, 2%-80% in early-stage samples, and 2%-80% in primary tumors. Primary tumor (10%) and early-stage (12%) samples showed greater stromal (fibroblasts and stellate population) cell abundance compared to advanced-stage (4%) and metastatic (2%) samples, consistent with a more structured tumor

microenvironment. Stromal representation ranged up to 10% in advanced-stage samples and up to 9% in metastatic samples, compared with 4%-20% in early-stage samples and up to 20% in primary tumors. (B-D) Chord diagrams show the relationships between differentially expressed genes and enriched hallmark pathways across tumor conditions. Orange pathways indicate an upregulation, and green pathways indicate a downregulation. Primary tumors versus metastatic samples (B) and early-stage versus advanced-stage (C) samples show enrichment of pathways associated with increased proliferation, metabolic activity, and cellular plasticity in the metastatic and advanced-stage samples, consistent with their more aggressive tumor states. In contrast, primary tumor and early-stage samples were characterized by pathways related to structural organization and microenvironment remodeling. (D) Treatment naive (n = 15) vs. chemotherapy-treated (n = 7) had enrichment of remodeling and colonization pathways and reduced immune-related pathways in the treated samples, suggesting increased tumor adaptability and suppression of anti-tumor immune responses. (E) Table showing identified top ten genes from COMET analysis associated with Moffitt molecular subtypes, which are prioritized by cluster specificity and expression magnitude. The table summarizes the top-ranked discriminatory genes enriched in basal-like populations based on COMET-derived true positive (TP) and true negative (TN) classification metrics. Dot plots show expression of top 10 genes across Moffitt subtype assignments and treatment exposure, with dot size representing percent of cells expressing the gene and color representing average scaled expression. All ten genes show highest expression in the Basal subtype, decreasing through Intermediate and largely absent in Classical phenotypes. Most genes trended higher in treated samples, though this was not consistent across all markers.

## Description

Pancreatic cancer is a highly aggressive malignancy with a five-year survival rate of approximately 13%, largely due to its diagnosis at advanced stages and resistance to therapy (Siegel et al., 2026). Early-stage pancreatic cancer is more likely to be curable and is typically characterized by resectable, well differentiated tumors, often corresponding to Stage I disease, whereas advanced stage disease (Stages III-IV) is associated with local invasion and metastasis (Chari et al., 2007). For the purposes of this study, tumor progression was defined as early-stage (Stages I-II) versus advanced-stage (Stages III-IV), consistent with clinical distinctions between resectable and unresectable pancreatic cancer. Diagnosing pancreatic cancer at early stages remains challenging, highlighting the importance of improved early detection strategies.

Advances in single-cell RNA sequencing (scRNA-seq) have enabled high resolution characterization of tumor heterogeneity and the tumor microenvironment in pancreatic ductal adenocarcinoma (PDAC). Prior studies have demonstrated that PDAC exhibits substantial intra-tumoral heterogeneity, with cell populations contributing to disease progression (Peng et al., 2019). Interactions between tumor cells and the surrounding microenvironment play a critical role in shaping tumor behavior and treatment response. PDAC tumors can also be classified according to the Moffitt molecular subtype framework, which stratifies tumors into classical and basal-like transcriptional states. This classification has important prognostic significance, as basal-like tumors are characterized by a less differentiated transcriptional state and are associated with increased aggressiveness, therapeutic resistance, and poorer overall survival compared to classical tumors (Moffitt et al., 2015). As a result, molecular subtyping has emerged as an important determinant of tumor behavior and a potential source of transcriptional heterogeneity within PDAC.

A sampling of single-cell RNA sequencing from 33 pancreatic cancer samples was compiled to explore the differences in cellular compositions in pancreatic cancer among primary tumors vs. metastatic site samples, early-stage vs. advanced-stage samples, and treatment naive vs. chemotherapy treated samples. Using marker-based references, cells were assigned to major cell type populations. This included immune cells, stromal cells (including fibroblasts and stellate cells), ductal epithelial cells, endothelial cells, parenchymal cells, and vascular-associated cells (Figure 1A). Immune cell abundance remained relatively consistent across disease states, with metastatic samples exhibiting 49.75% immune cell proportions (range: 13% to 83%, n =13) compared to 46.30% in primary tumor samples (range: 2% to 80%, n=20). A similar overlap was observed between advanced-stage (average: 47.18%, range: 11% to 83%, n=21) and early-stage (average: 47.38%, range: 2% to 80%, n=12) samples. Despite the variable immune cell representation observed across samples, pancreatic tumors are known to exhibit immunosuppressive microenvironments, suggesting that immune cell abundance may reflect altered composition or suppressed functionality (Sivakumar et al., 2025).

In contrast, stromal representation differed substantially across conditions. Stromal cells, which included fibroblasts and stellate cell populations, were more abundant in primary tumors (average: 9.48%, range: 0% to 20%, n=20) and early-stage (average: 12.99%, range: 4% to 20%, n=12) samples compared to metastatic (average 1.80%, range: 0% to 9%, n=13) and advanced-stage (average: 4.01%, range: 0% to 10%, n=21) samples. This is consistent with a more structured tumor microenvironment in earlier disease states. These findings align with prior work demonstrating the role of cancer associated fibroblasts in shaping the tumor microenvironment (Elyada et al., 2019; Loveless et al., 2025).

Differential gene expression and pathway enrichment analysis further highlighted consistent transcriptional and cellular patterns. Early-stage (Figure 1B) and primary tumors (Figure 1C) were characterized by pathways associated with structural organization and microenvironmental remodeling, whereas advanced-stage and metastatic samples exhibited signatures consistent with increased proliferation, metabolic stress, and cellular plasticity. Together, these findings suggest

a shift from a more organized stromal-dependent tumor state to a more aggressive and adaptive phenotype during progression, which is consistent with known alterations in pancreatic cancer signaling pathways (Javadrashid et al., 2021).

When exploring the effect of treatment in comparing chemotherapy-treated versus treatment naive samples, there was up-regulation of pathways associated with extracellular remodeling and colonization and down-regulation of immune related activity (Figure 1D). These findings suggest that treatment is associated with increased cellular plasticity and environmental adaptation. Such changes may reflect mechanisms by which tumor cells persist following therapy and contribute to treatment resistance.

To identify biomarkers associated with molecular subtype, COMET differential gene expression analysis was performed to identify discriminatory genes enriched in basal-like and classical tumor populations defined by the Moffitt classification system (Figure 1E). Because PDAC molecular subtype is an established determinant of tumor aggressiveness independent of clinical stage, COMET analysis was performed to discriminate between basal-like and classical-like tumor populations. The Moffitt framework stratifies PDAC into classical and basal-like subtypes, with basal-like tumors associated with more aggressive disease and poorer clinical outcomes. COMET analysis identified SNCG and LY6D among the top genes enriched in basal-like populations. SNCG and LY6D were identified for further characterization because of their established associations with basal-like differentiation and aggressive tumor behavior, though other top-ranked genes identified by COMET may also warrant further investigation. SNCG encodes gamma-synuclein, a protein previously associated with tumor invasion, metastasis, and poor prognosis across gastrointestinal malignancies (Takemura et al., 2021). Similarly, LY6D has been identified as a marker of basal and squamous-like cellular states and has been linked to therapy-resistant tumor subpopulations (Haensel et al 2022). Notably, expression of these genes localized preferentially to basal-like populations, and did show a slight, inconsistent correspondence with treatment status, consistent with previous findings demonstrating that chemotherapy does not induce a widespread transcriptional shift toward a basal phenotype (Werba et al, 2023). Together, these findings support molecular subtype as a major source of transcriptional heterogeneity in PDAC, and while SNCG and LY6D represent genes of particular interest given prior associations, the broader panel of top-ranked COMET markers also warrants further investigating as candidate subtype-specific markers.

One limitation of this study is the difference in mean age between non-diseased (36.3 years) and diseased (64.6 years) samples, with the non-diseased cohort being younger. This age disparity may introduce confounding effects, as age-related biological changes that could contribute to observed differences between the cohorts independently of disease status. Specifically, these normal samples (Fasolino et al., 2022) came from individuals in the Human Pancreas Analysis Program (HPAP) focused on comprehensive molecular profiling of various stages of diabetes, which can present at a younger age and thus captures samples from a larger age range.

Overall, these results support a model in which pancreatic cancer progression is accompanied by coordinated changes in tumor cell behavior and the tumor microenvironment. Early-stage and primary tumor samples exhibited a more organized and stromal cell-rich microenvironment, whereas advanced-stage and metastatic samples were characterized by transcriptional programs associated with increased proliferation, metabolic activity, and cellular plasticity. Although immune cell representation remained relatively consistent across disease states, treatment was associated with enrichment of extracellular remodeling and colonization pathways and reduced immune-related activity, suggesting adaptive changes within the tumor microenvironment that may contribute to disease progression and therapeutic resistance. Furthermore, identification of the basal-associated markers, such as SNCG and LY6D, highlights molecular subtype as an important source of transcriptional heterogeneity in PDAC and suggests candidate biomarkers for stratifying aggressive tumor states.

## Methods

Single-cell RNA sequencing datasets were obtained from publicly available Gene Expression Omnibus (GEO) studies (Werba et al., 2023; Lin et al., 2020; Fasolino et al., 2022) and processed using the Seurat package (v5.4.0) (Hao et al., 2024) in R (v4.5.3). Datasets included both disease-specific and non-diseased pancreatic samples to allow for comparative analysis. Raw count matrices generated from Cell Ranger (10X Genomics, v8.0.0) were imported, and individual samples were converted into Seurat objects with associated clinical metadata, including stage, tissue type, age**,** and treatment status. Diseased samples had a mean age of 64.6 years (SD = 12.1 years, n=33) with 18 male and 15 female patients. Non-diseased samples had a mean age of 36.3 years (SD = 2.31 years, n=3) with one male and two female donors. Data normalization and variance stabilization were performed. To account for batch effects across datasets, integration was conducted using Harmony (Korsunsky et al., 2019). Cells were clustered using graph-based clustering (Louvain algorithm, resolution 0.075) and visualized by UMAP projections.

Marker genes used for manual cell type annotation were derived from published single-cell studies of pancreatic cancer (Peng et al., 2019; Elyada et al., 2019; Loveless et al., 2025). Differential gene expression analysis between defined groups was conducted using Seurat to identify significantly upregulated and downregulated genes. Pathway enrichment analysis was performed using MSigDB Hallmark gene sets to identify biological pathways associated with differentially expressed genes. Additionally, combinatorial marker analysis was performed using COMET (Delaney et al., 2019) to identify gene signatures that distinguish cell populations.

Artificial intelligence (NaviGator Chat, University of Florida) was used as a programming aid for debugging R scripts and troubleshooting computational workflows.

Data and Code Availability

The datasets analyzed in this study are publicly available in the Gene Expression Omnibus (GEO) under accession numbers GSE205013, GSE154778, and GSE148073. Code for this analysis is publicly available on GitHub at https://github.com/mcfefa/Pancreatic-Cancer-scRNAseq-.

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