frontiers | Frontiers in Oncology

REVIEW
published: 19 May 2022
doi: 10.3389/fonc.2022.881871

# Using Single Cell Transcriptomics to Elucidate the Myeloid Compartment in Pancreatic Cancer

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Pancreatic ductal adenocarcinoma (PDAC) is a dismal disease with a 5-year survival rate of 10%. A hallmark feature of this disease is its abundant microenvironment which creates a highly immunosuppressive milieu. This is, in large part, mediated by an abundant infiltration of myeloid cells in the PDAC tumor microenvironment. Consequently, therapies that modulate myeloid function may augment the efficacy of standard of care for PDAC. Unfortunately, there is limited understanding about the various subsets of myeloid cells in PDAC, particularly in human studies. This review highlights the application of single-cell RNA sequencing to define the myeloid compartment in human PDAC and elucidate the crosstalk between myeloid cells and the other components of the tumor immune microenvironment.

**Keywords: PDAC, single cell, tumor microenvironment, MDSC, myeloid, TAM**

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***Edited by:***
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***Specialty section:***
*This article was submitted to Gastrointestinal Cancers: Hepato Pancreatic Biliary Cancers, a section of the journal Frontiers in Oncology*

***Received:*** *23 February 2022*
***Accepted:*** *08 April 2022*
***Published:*** *19 May 2022*

***Citation:***
*Kadiyala P, Elhossiny AM and Carpenter ES (2022) Using Single Cell Transcriptomics to Elucidate the Myeloid Compartment in Pancreatic Cancer. Front. Oncol. 12:881871. doi: 10.3389/fonc.2022.881871*

## INTRODUCTION

Pancreatic cancer (PDAC) remains a deadly disease and is notoriously challenging to treat with a 5-year survival rate of 10% (1). While the only curative treatment is complete surgical resection, fewer than 20% of patients are eligible due to advanced stages of disease, making medical therapies the mainstay for most PDAC patients. Unfortunately, while PDAC initially responds well to the standard combination therapies of FOLFIRINOX or gemcitabine/nab-paclitaxel, most patients progress due to chemoresistance, leading to poor outcomes (2). As the mechanisms of tumor progression and chemoresistance are multifactorial and poorly understood, there is an unmet need for the development of better treatment strategies. Growing evidence demonstrates that the tumor microenvironment (TME) is a vital component in the pathogenesis of PDAC and plays an essential role in tumor progression, invasion, and therapeutic resistance (3). Desmoplastic stroma comprises up to 80% of total tumor volume and largely consists of immune cells, fibroblasts, and acellular collagens (4). In particular, the accumulation of myeloid cells in the TME drives immune suppression (5–7). Previous studies have shown that targeting the myeloid compartment within PDAC tumors in murine models led to increased cytotoxic T cell activity, decreased regulatory T cell activity, shrinkage of tumors, and improved survival (5–7); however, clinical trials targeting myeloid cells have failed or only partially recapitulate results from preclinical models in a subset of

patients (8, 9). This highlights the lack of fidelity in using preclinical murine models for human PDAC.

Strategies to molecularly profile human pancreatic tumors are thus crucial to help unravel the complexities of human disease. Recently, single-cell RNA sequencing (scRNA-seq) has been shown to provide the analytical power to define cell-specific molecular signatures and map out the interactions of cell types within the TME (10–15) (**Figure 1**). This analytical strategy is capable of characterizing cell types and states in an unbiased manner, and is key to elucidating the behavior of myeloid cells within the PDAC TME. Ultimately, harnessing the power of scRNA-seq technology can help unravel the intricacies of these cell-cell interactions in human PDAC and lead to the development of novel therapies targeting the microenvironment to improve outcomes for this dismal disease. In this review we highlight the latest progress made in classifying myeloid cell compartment in PDAC by unbiased single-cell analysis (**Figure 2**).

## THE ROLE OF MYELOID CELLS IN PDAC

Within the immune compartment of the PDAC microenvironment, myeloid cells have been shown to be key regulators of immunosuppression and strong correlators to poor clinical outcomes (16, 17).They are abundant in the TME by way of myeloid-promoting cytokines such as CSF-1 and CCL-2 (18, 19). Perhaps their most well-known role in PDAC is their ability to mitigate anti-tumor effector T cell function through the release of cytokines that are immunosuppressive and in turn recruit other cells known to further dampen cytotoxic immune responses, such as T regulatory cells (20). Myeloid cells also mediate the expression of immune checkpoint ligands on tumor cells as another mechanism of immune evasion (21).

Myeloid cells are also known to play other roles that are independent of T cell responses. Tumor-infiltration of these cells is critical for PDAC initiation, as they directly promote the formation and maintenance of preneoplastic lesions through factors including EGF ligand and PDGF in murine models of PDAC (22, 23).

Interestingly, myeloid cells have been shown to directly enhance chemoresistance in PDAC tumor cells *in vitro* using indirect co-culture assays, implicating soluble factors as mediators (24, 25). It has been previously shown that conditioned media from tumor-educated bone marrow-derived macrophages confers chemoresistance to gemcitabine *in vitro*, specifically through pyrimidine release in myeloid cells (25).

Studies have also suggested that myeloid cells play a vital role in the pre-metastatic niche as a precursor colonizer to metastatic sites that allow for a favorable environment for tumor cell seeding and growth (26, 27). Recently, a new role of myeloid cells was uncovered in mouse models linking myeloid cell invasion into the central nervous system leading to cachexia symptoms in PDAC (28).

While these studies provide information about the behavior of myeloid cells and their response to numerous environmental stimuli to promote PDAC pathogenesis, the bulk of these studies were performed in preclinical murine models, with limited correlation in human studies. Recent single cell studies on human tumor tissue have allowed for a better understanding of the transcriptional diversity and putative function of myeloid cells in human disease.

## PDAC MYELOID CELL SUBTYPES IN SINGLE CELL TRANSCRIPTOMICS

Previously, characterization of the myeloid compartment within TME in human studies was limited to immunostaining and flow cytometric techniques, while more in-depth study of transcriptional networks involved in the myeloid compartment of PDAC tumors could only be determined through deconvolution methods (29) (See **Table 1**). However, with the advent of multidimensional single-cell and spatial techniques, we now know that the myeloid compartment in PDAC has a complex heterogeneity. It is important to note that there is a diverse array of different transcriptomic patterns of myeloid cells across different cancer types, and care should be taken before generalizations are made regarding the myeloid transcriptome of PDAC, which varies from other solid tumor types (30). **Table 2** summarizes significant contribution to human single cell sequencing on PDAC to date.

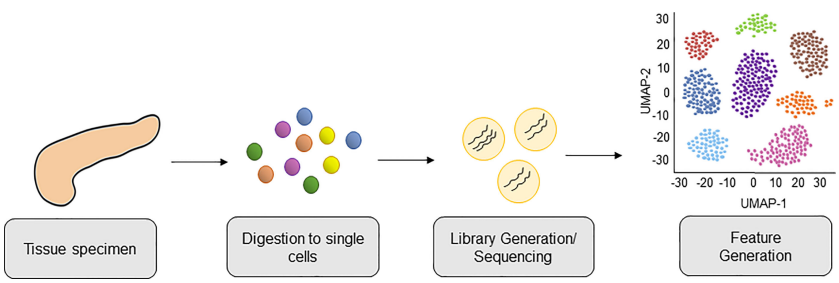


**FIGURE 1** | Workflow of Single-Cell RNA-sequencing PDAC tissue from patients. PDAC tissue is collected from patient donors and digested into a single cell suspension of live cells. Cells are lysed, cellular mRNA captured, and cDNA libraries are generated and subjected to high-throughput sequencing. This is followed by bioinformatics analysis, including downstream feature generation and visualization of cells clusters by Uniform Manifold Approximation and Projection (UMAP).

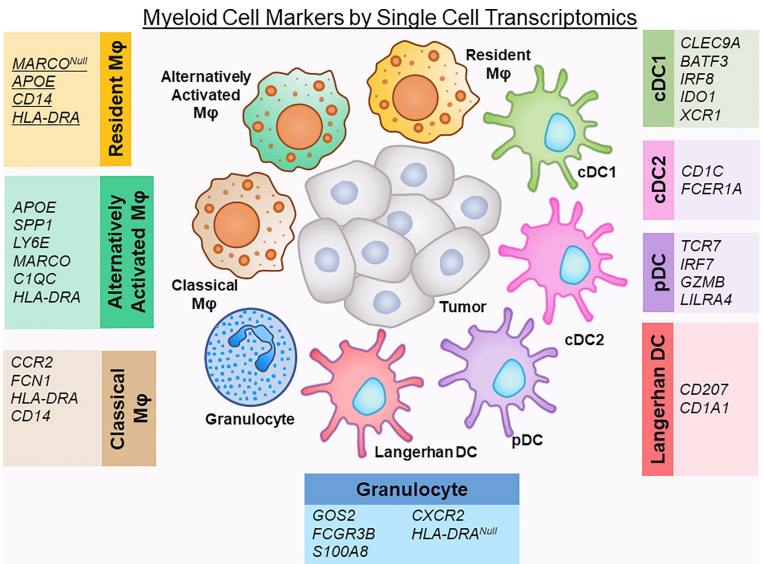


**FIGURE 2** | Defining myeloid cell markers by single cell transcriptomics.

## Myeloid-Derived Suppressor Cells (MDSCs)

MDSCs are a heterogeneous population of immature myeloid cells that have the ability to suppress adaptive T-cell immunity, resulting in mitigation of cytotoxic anti-tumor activity (32). In PDAC patients, levels of MDSCs in the peripheral blood correlate with stage of disease progression (33, 34). Their primary role in inhibiting anti-tumor immunity of effector T Cells is accomplished *via* direct and indirect mechanisms, including crosstalk with other immunosuppressive cell types. MDSCs have been shown to influence regulatory T cells, dendritic cells, and TAMs (tumor associated macrophages), thereby promoting tumor immunotolerance (20, 33, 35, 36). In a subset of cancers, temporal decline in MDSC levels with treatment has correlated with better survival (37–40). Further studies are needed to determine whether changes in MDSC levels over time bear clinical relevance in PDAC.

It is important to note that because there is no consensus set of protein markers for MDSCs, and an even more poorly-defined transcriptomic signature, MDSCs have yet to be identified within PDAC single cell datasets. MDSCs are comprised largely of monocytic MDSCs and granulocytic MDSCs in a nomenclature to mimic their normal counterparts. In murine model of breast cancer, one group arrived at a transcriptomic signature for monocyte MDSCs and granulocytic MDSCs; the signature for monocytic MDSCs did not translate to any population in a human dataset for breast cancer, but the granulocytic signature was enriched in breast cancer-associated neutrophils (41). More studies need to be done in PDAC to identify whether the MDSCs transcriptionally

**TABLE 1** | Advantages and disadvantages of technologies used for classifying myeloid compartments in pancreatic cancer.

| Technique | Advantages | Disadvantages |
|---|---|---|
| Single Cell RNA Sequencing | • Distinguish cell types at high-resolution in an unbiased manner<br>• Identify states of cells in different development, differentiation, and cell cycle states in tissues<br>• Gene expression profiles could be used to computationally map the cell trajectory | • Requires processing of fresh tissue<br>• Determining spatial distribution of the cell type is not possible.<br>• Read dropout and false discoveries |
| Flow Cytometry | • Identity frequency and activate state of the cells<br>• Characterize heterogenous cell populations.<br>• Cell populations can be sorted<br>• Results can be obtained in a short time. | • Cannot classify new cell types and their states in an unbiased manner<br>• Cell morphology cannot be visualized<br>• Cell populations with similar marker expression cannot be differentiated<br>• Fluorophore signal spillover |
| Immunohistochemistry | • Identify localization of the protein in tissue<br>• Acquire information about tissue architecture, size, and shape of the cells<br>• Results can be obtained in days | • Restricted to limited number of markers<br>• Immunolabelling depends on the specificity of primary antibodies<br>• Semi-quantitative approach |

**TABLE 2 |** All significant published studies that have provided new single cell RNA sequencing datasets in pancreatic cancer.

| Year | Study | Reference |
|---|---|---|
| 2019 | Elyada, E. et al.-Single cell sequencing of 6 treatment-naive PDAC tumors and 2 adjacent normal pancreas tissue. | (10) |
| 2019 | Peng, J. et al.-Single cell sequencing of 24 treatment-naive PDAC tumors and 11 normal pancreas tissue. | (11) |
| 2019 | Bernard, V. et al.-Single cell sequencing of 2 PDAC and 4 IPMN specimens. | (14) |
| 2020 | Steele, N.G. et al.-Single cell sequencing of 16 treatment-naïve PDAC tumors from surgical resections and fine needle biopsies as well as 3 adjacent normal pancreas tissue. | (13) |
| 2020 | Hwang, W.L. et al.-Single nucleus sequencing of frozen archival surgically resected tumors from 26 patients, 11 treated and 15 treatment naïve | (15) |
| 2021 | Raghavan, S. et al.-Single cell sequencing of core needle biopsies from 17 untreated and 6 treated liver metastasis | (12) |
| 2021 | Kemp, S.B. et al.- Single cell sequencing of 2 treated and 3 treatment-naive liver metastasis | (26) |
| 2021 | Cheng, S. et al.-Single cell sequencing of 6 treatment-naïve PDAC tumors and 3 adjacent normal pancreas tissue | (30) |
| 2021 | Zhou, D.C. et al.-Single cell sequencing of 7 treatment-naïve, 14 treated PDAC tumors and 4 adjacent normal pancreas tissue. | (31) |

represent a subpopulation unique from their normal myeloid counterparts.

## Tumor Associated Macrophages (TAMs)

Traditionally, the macrophages in PDAC have been oversimplified into a proinflammatory/antitumorigenic phenotype (M1) and an anti-inflammatory/protumorigenic phenotype (M2) (42), which does not accurately reflect the *in vivo* heterogeneity seen in human tumors. Indeed, traditional M1 and M2 markers do not dichotomize macrophage populations within single cell datasets and often canonical markers for both are found within the same cell (43).

Recent single cells studies on human PDAC tumor tissue have reclassified these macrophages into subtypes that more accurately represent their *in vivo* state, namely resident, classical, alternatively-activated TAMs (10, 13). Alternatively activated macrophages express *APOE*, *SPP1, LY6E*, and the macrophage scavenger receptor *MARCO*, while resident TAMs lack *MARCO* expression. Of note, in other solid tumor cancers, *MARCO* expression has been associated with a pro-tumor, immunosuppressive phenotype of macrophage activation (44, 45). *APOE* has recently been found in mouse models to promote an immunosuppressive microenvironment in PDAC through NF-kB signaling (46). Classical TAMs express less of a committed macrophage transcriptomic phenotype (lower expression of CD68 and HLA-DR) and suggest an intermediary state of monocytes migrating from blood to tissue and maturing into macrophages (30, 43).

Another classification system has also emerged whereby TAMs are subdivided into FCN1+ TAMs (monocyte-like, and akin to classical TAMs), SPP1+ TAMs, or C1QC+ TAMs (12, 30). Together, SPP1+ and C1QC+ TAMs overlap with resident and alternatively-activated TAMs in the previous classification system. Of note, complement-high macrophages (C1QA, C1QB, and TREM2) may play an important role is establishing the premetastatic niche, as these particular macrophages have been found to be further enriched in liver metastatic lesions compared to primary tumors in human PDAC (26). C1QC+ TAMs have been found to be associated with basal-like tumors where T cells are notably sparse (12).

## Neutrophils

Neutrophils are abundant in the TME of PDAC, and have been shown to have dual tumor-promoting and anti-tumorigenic functions (47, 48). Despite this, most single cell transcriptomic studies do not identify a neutrophil population, with possible causes for this underrepresentation including the techniques used to process and purify cells and the difficulty in capturing adequate RNA reads for this particular cell type (11, 12, 14, 30). Of note, in the dataset by Elyada, et al., it was noted that neutrophil markers were present within the myeloid dataset, but these particular genes were intermixed within the monocyte/macrophage populations (10). Steele and colleagues were able to identify a separate granulocytic population in their dataset that was defined by expression of *FCGR3B (CD16), S100A8, CXCR2*, and absence of *HLA-DRA* (13). Further studies need to be performed to dissect whether heterogeneity in the neutrophil population can be captured with single-cell transcriptomics.

## Dendritic Cells

Dendritic Cells are a specialized group of antigen-presenting cells that play a key role in initiating both innate and adaptive immune responses (49). The relative absence of dendritic cells in the PDAC TME has been linked to dysfunctional immune surveillance in PDAC, with poor T cell responses to tumor neoantigens (50). Single cell transcriptomic studies have identified several subsets of dendritic cells: conventional (cDC), plasmacytoid (pDC), and Langerhans-like. The cDCs can be further subdivided into cDC1 (Type 1), which cross-present antigens *via* MHC class I to activate $CD8^+$ T cells, and cDC2 (type 2), which produce high levels of IL-12 and are potent activators of CD4 T helper responses (51). By single cell sequencing, dendritic cells have been named using different defining markers, likely due to technical differences in specimen processing and read depth. cDC1s have been identified previously by expression of *CLEC9A, BATF3, IRF8, IDO1* (10). cDC2 have been characterized by expression of CD1C, FCER1A (14). Additionally, XCR1, a chemokine receptor, is selectively expressed on cDC1s and also has been used to subset cDC1 cells (12). Plasmacytoid dendritic cells (pDCs), in comparison to cDCs have poor antigen-presenting function, but are potent producers of type 1 interferons (51). They have been defined in single cell transcriptomics by *TCR7, IRF7* and *GZMB* positivity, as well as *LILRA4* positivity (10, 12, 14). Langerhans-like DC, which are immature dendritic cells that mediate immune tolerance, are defined by *CD207* and *CD1A* expression (10).

# LEVERAGING SINGLE CELL STUDIES FOR THE MYELOID COMPARTMENT IN PDAC

## Myeloid Expression of Checkpoints

Immunotherapy has notoriously been unsuccessful in improving outcomes in PDAC (52, 53). The reason for this has, in part, been elucidated through single cell studies showing abundant and varied expression of immune checkpoints across the myeloid compartments. For example, TAMs have upregulated *LGALS* (ligand for TIM3) as well as its binding counterpart *TIM3*, *PVR* (ligand for *TIGIT*), *and HLA-DRA* (13, 31). Certain subsets of dendritic cells also had elevated expression of immune checkpoint ligands, suggesting a potential immunosuppressive role (13). Of note, wide heterogeneity of immune checkpoint expression within the myeloid compartment was observed between patients, suggesting the need for a precision pipeline in identifying appropriate immunotherapeutic regimens for each patient (13).

## Myeloid Crosstalk Within the Tumor Microenvironment

Prior to single cell studies, several mediators of crosstalk involving the myeloid compartment of the TME have been identified, including the CSF1/CSF1R axis, the CCL2/CCR2 axis, and the ELR+ chemokine/CXCR2 axis (7, 18, 19). With the advent of singe cell signaling, one useful tool to identify putative cross-talk interactions in single cell datasets is the use of mapping algorithms of known ligand-receptor interactions across different cell types (54). Mapping these interactions are a boon in the study of the TME, which relies on the complex interplay between tumor and non-tumor cells. Using this technique, new putative ligand-receptor interactions between myeloid/epithelial cells and myeloid/lymphocytes have been identified. In the dataset published by Lee et al., myeloid populations were the most well-connected to epithelial cells, with notable interactions including MIF/CD74 (HLA-DR allele), and APP/CD74 (14). Steele and colleagues also reported multiple interactions between the myeloid and T cell compartment, including ICOS/ICOSLG, SIRPA/CD47, and TIGIT/PVR (13).

These data are in concordance with previous studies showing that myeloid cells are major drivers of the immunosuppressive TME, and provide insights for potential new combination immunotherapy trials in PDAC.

Another recent area of interest in TME crosstalk is the myeloid/fibroblast axis. Using a combination of functional studies and single cell analysis, a recent murine PDAC study demonstrated that hypoxia inducible factor signaling in cancer-associated fibroblasts drives CD86 and PDL1 expression on tumor associated macrophages (55) to dampen anti-tumor immune responses. Similarly, while the TGFβ signaling axis has also been implicated as a key modulator of regulatory T cells and fibroblast crosstalk in the microenvironment (56–58), its axis has also been shown recently to influence myeloid cell activity in PDAC. Both functional studies with patient-derived organoids and human single cells studies confirm that TGFβ ligand is produced by tumor epithelial cells and is associated with the more aggressive basal subtype of PDAC (10, 12, 57). In murine studies, TGFβ was found to decrease the proportion of MDSCs in liver metastasis and increase the expression of PD-L1$^{High}$ TAMs. Additionally, in correlative human bulk tumor sequencing studies TGFβ was found to be associated with an increased TAM signature (59). As TGFβ signaling has gained recent traction in cancer-associated fibroblast polarization (57), further studies are needed to determine if the role of TGFβ in myeloid cells is direct or involves the fibroblast compartment as an intermediary.

# DISCUSSION

Single cell transcriptomic technology has shed much-needed light on the heterogeneity and function of the myeloid compartment in human PDAC. While pre-clinical murine models have dominated the field in the study of the tumor immune microenvironment, results from these studies have led to an oversimplification of the myeloid cell types and have resulted in identifying targets that thus far have had mixed patient outcomes (8, 9, 53). The patient heterogeneity in drug response of these clinical trials is supported by single-cell studies, which highlight the inter-patient heterogeneity of the myeloid compartment. In many of the trials reviewed above, a small subset of patients had some response to the given immunotherapeutic strategy, suggesting that a precision medicine-based platform is needed which can match therapy to each tumor's microenvironmental characteristics. While there is no such tool in place to tailor these therapies, single-cell transcriptomics bring a promising avenue for both biomarker and therapeutic discovery.

One caveat to note is that the technique of single cell transcriptomics is not without its own flaws, which include variation in tissue acquisition and processing, read "dropout", and, unfortunately, false discoveries (60, 61). Therefore, validation of gene expression through complementary techniques such as multiplex immunofluorescence or mass cytometry is necessary (**Table 1**). Furthermore, putative interactions and identified signaling networks should be investigated with further functional studies using *in vitro* or *in vivo* systems.

Another limitation to single cell RNA sequencing is that spatial data is not preserved, and validating targets via immunostaining can be laborious. Indeed, work using multiplex immunofluoresence has shown that immune cell localization of myeloid cells within the tumor had important clinical significance in PDAC patients (62). Recent developments in spatial transcriptomics and multiplex staining can add a crucial dimension to identifying cell subtypes in the TME and validating putative crosstalk between cells (16, 63). Alternatively, machine learning has been leveraged with multiplexed immunofluorescence and whole-slide imaging for tissue segmentation and classification (64, 65). Recent bioinformatics pipelines are actively working to integrate these multi-dimensional datasets for a seamless approach and yield new insights on myeloid cells in the TME (66–68).

In conclusion, PDAC remains a deadly disease with an urgent need to find new and better therapies. Targeting the myeloid

compartment of the TME is a promising avenue to pursue; although given the complexities of these cells shown by single cell studies, single-agent immunotherapy is likely not sufficient and combinatorial approaches may be required. One exciting avenue to apply single-cell transcriptomics is through the study of tumor tissue longitudinally throughout the course of disease and therapy treatment, as myeloid cells have been shown in preclinical studies to play a major role in the development of chemoresistance (24, 25). Leveraging this technique to comprehensively study the immune microenvironment in the treatment-naïve and post-treatment states may provide new insights to the role of the TME in the development of chemoresistance and ultimately identify new pathways to target in this dismal disease.

## AUTHOR CONTRIBUTIONS

All authors participated in writing the manuscript, with EC taking the lead in organizing and compiling the final document. PK and EC illustrated **Figures 1**, **2**. All authors contributed to the article and approved the submitted version.

## FUNDING

This work was supported by the American College of Gastroenterology Career Development Award and the VA BLR & D Career Development Award to EC; T32-AI007413 to PK.

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