Review Article



# Contribution of heterogeneity of cancer associated fibroblasts to organ-specific metastasis of pancreatic cancer: will it change the paradigm of treating metastatic pancreatic cancer?

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**Abstract:** Metastasis occurs in the majority of pancreatic ductal adenocarcinoma (PDAC) patients at diagnosis or following resection. PDAC patients develop distinct patterns of metastasis at the time of recurrence following curative resection of primary tumors. Liver metastasis is associated with the poorest survival, whereas lung metastasis is associated with the best survival among all types of recurrence. The mechanism underlying organ-specific metastasis and subsequent outcomes remains unknown. Pan *et al.* sought to understand how cancer-associated fibroblasts (CAFs) play roles in the development of organ-specific metastasis. By using a genetically engineered mouse model of PDAC, they demonstrated the role of PDAC cells with different organ-specific metastasis potential in modulating metabolic gene methylation and heterogeneous phenotypes of CAFs at different metastatic sites and subsequently resulting in different levels of heterogeneity and different methylation and mRNA expression levels of metabolism genes in different metastatic sites. Furthermore, this work shed light on the relationship between CAF heterogeneity and organ-specific metastasis. More specifically, they demonstrated that mesenchymal stem cells (MSCs) could be reprogrammed by PDAC tumor cells with liver metastasis potential to acquire inflammatory CAF and myofibroblastic CAF transcriptomic signatures. In contrast, PDAC tumor cells with lung metastasis potential cannot reprogram MSCs which indicate a distinct mechanism between PDAC tumor cells and CAFs.

**Keywords:** Pancreatic ductal adenocarcinoma (PDAC); organ-specific metastasis; cancer-associated fibroblast (CAF); methylation

Received: 16 April 2023; Accepted: 24 August 2023; Published online: 20 November 2023.
doi: 10.21037/apc-23-4
**View this article at:** https://dx.doi.org/10.21037/apc-23-4

## Introduction

Pancreatic ductal adenocarcinoma (PDAC), with a high metastatic rate at diagnosis and poor prognosis, is the 3rd leading cause of cancer-related deaths in the United States and is projected to be the 2nd leading cause of cancer-related deaths by 2030 (1). The only chance of cure is surgical resection, which is eligible for patients with localized tumors without metastasis. Only 30% of total PDAC patients are eligible for surgical resections, including those

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whose diseases are resectable at the time of diagnosis or become resectable following neoadjuvant therapy (2). However, most of the resected PDACs would recur with metastasis, the overall recurrence rates vary from 69.6–74.3% according to available literatures (3-5). Treatment options for metastatic PDAC are limited and largely ineffective (6,7). Thus, there is an unmet need to better understand of the mechanism of PDAC metastasis. Clinical observations revealed a disease heterogeneity among patients with different sites of distant metastases, resulting in distinct clinical outcomes (8). Although liver metastasis is the most common site at the time of recurrence, it is also not uncommon to develop a recurrence of lung metastasis. Interestingly, the recurrence of lung metastasis is associated with better survival outcomes than any other recurrent PDAC (8). For instance, patients whose first site of recurrence is liver have a significantly worse median time from recurrence to death (9 months) than those with lung recurrence as the first site of recurrence (15 months) (9). Despite these clinical observations, little is known about the mechanisms of metastasis at different organ sites and what contributes to their different outcomes. Based on these findings, recent study has demonstrated the role of the tumor microenvironment (TME) in metastasis by promoting tumor invasion and angiogenesis (10). In PDAC, the TME consists of a large number of stroma cells and a small number of immune cells. Fibroblasts usually comprise 60–90% of the total tumor, which is an ideal model for studying the interaction between tumor cells and stromal cells. Here, the work by Pan and colleagues helps us better understand the role of cancer-associated fibroblasts (CAF) heterogeneity in the development of organ-specific PDAC metastasis.

## Heterogeneity of CAFs

The exact functions of CAFs in cancer progression continue to be debated. In some PDAC models, depletion of specific CAF subsets slowed PDAC progression and improved antitumor immunity (11), whereas in other models, depletion of CAFs accelerated PDAC progression (12). CAFs are a key contributor to tumor stroma that supports PDAC progression through the generation of dense desmoplasia, which is also a hallmark of PDAC and a major obstacle to the development of PDAC therapeutics (10). It has also been demonstrated that PDAC tumor cells can program CAFs through tumor-induced DNA methylation to promote the malignant growth and progression of PDAC (13). Recent studies revealed heterogeneity within CAFs comprising multiple subtypes, which function by interacting with tumor cells through direct contact or in a paracrine fashion through the secretion of cytokines (14-17). Typically, CAFs are classified as either myofibroblastic CAFs (myCAFs) that secrete extracellular matrix components and have high expression of α smooth muscle actin (α-SMA); or inflammatory CAFs (iCAFs) that secrete protumoral cytokines including IL-6, IL-11, and LIF1 (16,18). These three CAF subtypes represent distinct transcriptomics based on the single-cell RNA sequencing analysis (18). More recently, other novel CAF subtypes have also been identified, including antigen-presenting CAFs (apCAFs) which process and cross-present tumor antigens to $CD8^+$ T cells (19). $PLA2G2A^+$ CAFs with highly activated metabolic state (meCAFs) that can attenuate $CD8^+$ T cell antitumor activities (20); and complement-secreting CAFs (csCAFs) (21,22). The identification of these novel subsets adds further intricacy to the already complex PDAC TME.

CAFs heterogeneity has been mostly studied in the primary tumor setting. However, little is known about their role in metastatic niches and their contribution to metastasis. CAFs have been shown to mediate metastasis-promoting communications among other components of tumor stroma by activating the transforming growth factor β (TGFβ) signaling pathway or through focal adhesion kinase (FAK) activities (23-25). In prostate cancer, chemokine CCL2 produced by CAFs leads to significant increase in myeloid cell recruitment to the TME, and prostate cancer patients with elevated CCL2 levels are at higher risk of metastasis and poor prognosis (26).

Yet, the characteristics and functional features of CAFs present in metastatic niches may be different from CAFs in the primary tumor (23). Gao *et al.* demonstrated that heterogeneity of CAFs is associated with tumor invasion in ovarian cancer, and high-grade serous ovarian cancer has more CAFs and resultant metastatic units than low-grade serous ovarian cancer (27). Past studies also show CAF differentiation in the pre-metastatic niche can drive cancer invasion, and CAF subtypes define a metastatic matrisome in breast cancer (28,29). More recently, Li *et al.* found that PDAC patients with lung metastasis expressed higher levels of aortic carboxypeptidase-like protein (ACLP) compared to those without lung metastasis, and ACLP is mainly expressed by CAFs in the PDAC TME, suggesting that ACLP may play an important role in lung metastasis in PDAC (30). These findings further highlight the importance of elucidating the role of CAFs in promoting

PDAC metastasis.

## Epigenetic and metabolic reprogramming of CAFs

Epigenetic reprogramming in CAFs in response to cancer cell signaling has been shown to promote tumor progression and metastasis in various cancer types, including hepatic cellular carcinoma and breast cancer (31-35). In non-small cell lung cancer, CAFs can undergo epigenetic modifications upon stimulation by the proinflammatory cytokine leukemia inhibitory factor (LIF), leading to the activation and sustainment of the JAK1/STAT3 signaling pathway, which ultimately reprograms CAFs into a proinvasive phenotype (36,37).

It was previously demonstrated that PDAC tumor cells could induce DNA methylation at a whole genome level in CAFs through direct contact, downregulating a wide range of metabolic genes (13). Another study by Shakya *et al.* compared the gene expression profile between DNA hypomethylating agent-treated CAFs and untreated CAFs from the PDAC tumors of KPC-Brca1 mouse, a genetically engineered PDAC mouse model (38). They found that the expression of metabolic genes such as NAD(P)H:quinone oxidoreductase 1 (NQO-1) and aldehyde dehydrogenases 1 (ALDH1) were rescued following DNA hypomethylating agent. Based on these discoveries, Pan *et al.* hypothesized that PDAC tumor cells might induce unique DNA methylation patterns on metabolic genes in CAFs at the liver metastatic site distinct from the CAFs at the lung metastatic site (39). Pan *et al.* identified that CAFs in liver metastasis have hypermethylation epigenetic patterns on a number of metabolic genes, including NQO-1 and ALDH1a3 that possibly lead to a unique metabolism gene expression profile. This epigenetic reprogramming of metabolic genes was not observed in CAFs at the lung metastatic site (39). However, it remains to be investigated if DNA methylation is responsible for the observed downregulation of other metabolic genes.

## Molecular mechanism for CAF epigenetic alteration

Pan *et al.* found that the downregulation of NQO-1 was regulated by DNA methylation in CAFs at the liver metastatic site. This epigenetic change was also observed in mouse mesenchymal stem cells (MSC) after being co-cultured with PDAC tumor cells that possess liver metastasis potential (39). The molecular mechanism of the methylation of metabolic genes has been studied. Xiao *et al.* found that tumor-induced DNA methylation in CAFs and tumor-associated macrophages (TAMs) are mediated by direct cell-to-cell contact between tumor cells (13). Thus, cell surface receptors glycoprotein-A repetitions predominant (GARP) and integrin could potentially mediate the direct cell to cell contact between tumor cells and stromal cells in general. Furthermore, both integrin and adherence pathways were significantly upregulated at the mRNA level in CAFs and mouse MSCs upon co-culturing with human PDAC tumor cells (13). These findings support the investigation of both integrin and adherence families of cell surface molecules as candidates that mediate the direct contact between tumor cells and CAFs. Such a mechanism for the direct contact between PDAC tumor cells and CAFs may be specifically present in PDAC metastasized to the liver and mediate the interactions between PDAC tumor cells with liver metastatic potentials and CAFs but may be lacking in PDAC that metastasized to the lung. Thus, identifying such a mechanism is anticipated to uncover the underlying mechanisms of organ-specific metastasis.

## CAF heterogeneity and organ-specific metastases

CAFs in liver metastasis are more homogeneous, likely due to myCAF reprogramming into iCAF by PDAC tumor cells that metastasize to the liver. In contrast, as expected from the result showing that PDAC tumor cells with lung metastatic potential did not reprogram CAFs, Pan *et al.* showed that CAFs in lung metastasis remain heterogeneous (39). This finding suggests that a change in the heterogeneity of CAFs may be responsible for the aggressive feature of liver metastasis. iCAFs are known to release a number of immunosuppressive cytokines such as IL-6, IL-11, and LIF1 to suppress the antitumor immune response and subsequently promote tumor growth (16,18,19). Thus, they are likely contributing to the poorer prognosis in PDAC patients with liver metastasis as the first site of recurrence compared to PDAC patients with lung metastasis as the first site of recurrence. Therefore, shifting iCAFs to a more myofibroblastic state, which can restrain tumor progression (13), may reduce the secretion of tumor-promoting cytokines and chemokines and sensitize PDAC with liver metastasis to immunotherapy. Nevertheless, whether a more homogenous CAF with iCAF signatures confers a more aggressive biology and/or resistance to the treatments remains to be investigated.

## Future prospective

Findings from Pan *et al.* in human PDACs are promising yet still need to be validated (39). The link between DNA methylation in metabolism genes and distinct CAF subtype transcriptomic signatures remained to be explored in both mouse and human PDACs. Ideally, a merged analysis of whole genome methylation microarray and RNA sequencing of CAFs isolated from liver metastasis and lung metastasis from the same PDAC patients shall be performed. However, resected tumor specimens of metastases, particularly liver metastases, essentially do not exist as patients with metastatic diseases do not undergo surgical resection. The multi-omics single-cell technologies may make it possible to perform a merged analysis of DNA methylation and RNA sequencing with fine-needle biopsy specimens. If the findings in the mouse model hold true in human patients, targeting pathways that are involved in programming CAF heterogeneity phenotypes may help improve the prognosis of PDAC patients with liver metastasis and provide a novel treatment venue to prevent liver metastasis by targeting DNA methylation.

## Conclusions

CAF heterogeneity has been extensively studied in the TME of primary tumors of PDAC, little is known about CAF heterogeneity at different metastatic sites. Pan *et al.* was the first one to uncover CAF heterogeneity at metastatic niches and their association with organ-specific metastasis. In addition, MSCs can be reprogrammed by tumor cells with liver metastasis potential to acquire iCAF and myCAF transcriptomic signatures whereas MSCs cannot be reprogrammed by tumor cells with lung metastasis potential. The pattern of iCAFs presented by tumor-associated fibroblasts may contribute to a poor prognosis in PDAC with liver metastases, which was then found to be reversed by DNA demethylating agents *in vivo*. By studying the mechanism, potential targets may be identified and treatment of PDAC with liver metastasis is likely to be improved. These findings are thrilling but still need validation. There is a long way to go from mouse models to real applications in humans.

## Acknowledgments

*Funding:* None.

## Footnote

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at https://apc.amegroups.com/article/view/10.21037/apc-23-4/coif). T.Z. was supported by the Sidney Kimmel Comprehensive Cancer Center Support Grant: NCI P30 CA006973. The other authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.



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doi: 10.21037/apc-23-4
**Cite this article as:** Gong X, Zhang T, Zheng S. Contribution of heterogeneity of cancer associated fibroblasts to organ-specific metastasis of pancreatic cancer: will it change the paradigm of treating metastatic pancreatic cancer? Ann Pancreat Cancer 2023;6:11.