Editorial

FAPα in pancreatic stellate cells upregulated by TGFβ1: a novel insight into pancreatic cancer progress

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Pancreatic cancer is one of the most deadly tumors, with extremely low 5-year survival rates (1). The majority of pancreatic cancer patients are diagnosed with locally advanced stage or distant metastases, rendering the disease incurable by surgical resection. While chemotherapy and radiotherapy serve as alternative options for the treatment of pancreatic cancer, the outcomes of these strategies remain largely disappointing. Recently, growing attention has been paid to the role of the tumor microenvironment in the development and progression of malignant diseases. Previous publications suggest that tumor microenvironment is linked to proliferation, invasion, metastasis, angiogenesis, immunosuppression and drug resistance of tumors (2). Therefore, deeper understanding of various components in tumor microenvironment is expected to provide benefits to treat cancer patients. In pancreatic cancer, stromal cells and stromal elements in tumor microenvironment can support and promote cancer cell growth, invasion and metastasis (3). Moreover, a former study by Mahajan *et al.* shows that the infiltration of immune cells and the composition of the pancreatic stroma are associated with the progression-free survival of pancreatic cancer patients after resection (4). It is now generally acknowledged that the abundant stroma of pancreatic cancer is produced by cancer-associated fibroblasts (CAFs), better known as activated pancreatic stellate cells (PSCs) in the context of cancer. Increasing number of studies demonstrate that the interactions between PSCs and cancer cells are bi-directional. Proximity to cancer cell activates PSCs and leads to increased cell proliferation, migration and production of extracellular matrix (ECM). In turn, activated PSCs facilitate local tumor proliferation, invasion, cancer stem cell maintenance as well as distant metastasis through several growth factors and immune modulatory signals (5).

In this issue of *Annals of Translational Medicine*, Wen *et al.* (6) report that FAPα+ PSCs in tumor microenvironment play a critical role in pancreatic cancer progression. In their study, pathological specimens obtained from 56 pancreatic cancer patients who underwent pancreatic resection for adenocarcinomas were examined for FAPα expression in both cancer tissue and cancer-adjacent tissue by immunohistochemistry. Strong FAPα expression is present in 60.7% (34/56) of the cancer tissue, which is considerably higher than in adjacent normal tissue with merely 0.71% (4/56) of strong FAPα expression. Moreover, FAPα expression is significantly higher in the interstitial region adjacent to pancreatic cancer cells as compared with the interstitial region away from pancreatic cancer cells. Most importantly, the levels of FAPα expression are positively correlated with the possibility of lymph node metastases and overall survival of the patients with pancreatic cancer. This highlights that FAPα could be a potential biomarker for predicting pancreatic cancer prognosis after resection. In order to detect the source of FAPα, human primary PSCs in adjacent tumor tissues were isolated by using an outgrowth method. In those, FAPα expression was confirmed by flow cytometry and immunofluorescence.

The authors then suggested that the expression of FAPα

in PSCs is induced by TGFβ1 which is in turn released by pancreatic cancer cells, since TGFβ1 expression in pancreatic cancer cells is significantly higher than that in human primary PSCs. FAPα+ PSCs function as a tumor promoter to effectively enhance pancreatic cancer cell migration, invasion and metastasis, which are illustrated by a number of transwell experiments and a mouse model of metastasis, where a suspension of cancer cells and FAPα+ PSCs were injected into the spleen. The FAPα-dependent tumor promoting effects can be disrupted effectively by utilizing talabostat (PT100), a FAP inhibitor. Asking how FAPα+ PSCs act on pancreatic cancer progression, the authors describe that FAPα+ PSCs secret significantly higher levels of CXCL1 compared with FAPα– PSCs and identify CXCR2, a receptor of CXCL1, expressed on multiple pancreatic cancer cell lines. In addition, co-cultured with FAPα+ PSCs, pancreatic cancer cell lines, such as BxPC3 and PANC1, highly express phosphorylated tyrosine kinase receptors, like EphB1 and EphB3, which interact with CXCR2 to promote Akt phosphorylation, enhancing metastatic abilities of pancreatic cancer cells. Taken together, Wen *et al.* provide new insights into the interaction of pancreatic cancer cells and activated stroma cells.

Fibroblast Activation Protein, also referred to as FAPα, is a member of the prolyl-specific serine proteases family. FAPα is involved in a variety of biological interactions through exopeptidase and endopeptidase activities. Traditionally, FAPα has been associated with non-malignant events, such as wound healing and fibrosis. However, recent studies show that FAPα also participates in cancer cell invasion, migration and metastasis. Due to its strong expression in stromal fibroblasts, FAPα has been viewed as a marker of activated fibroblasts in multiple types of cancer and was implicated in a number of tumor-promoting functions (7,8). A previous study shows that stromal FAPα derived from CAFs facilitate gastric cancer proliferation, migration and invasion by promoting epithelial mesenchymal transition (EMT) through Wnt/β-catenin signal pathway (9). In a breast cancer mouse model, depletion of FAP-expressing CAFs by small-molecule immunotoxin, namely αFAP-PE38, effectively reduces tumor growth by influencing the levels of various growth factors, cytokines, chemokines and matrix metalloproteinases in the tumor microenvironment. Combining αFAP-PE38 and paclitaxel therapy considerably prolongs the overall survival of cancer bearing mice (10). As a tumor promotor, FAPα expression in stromal cells also associates with worse prognosis in hepatocellular carcinoma (11), esophageal squamous cell carcinoma (12), and non-small cell lung cancer (13). Targeting FAPα by either genetic deletion or pharmacological approaches effectively inhibits tumor stroma development and progression in both a mouse model of lung cancer and a mouse model of colon cancer, suggesting that FAPα could be a therapeutic target to tackle multiple tumor types (14). Previous studies already described that FAPα expressed in stromal PSCs significantly associate with the clinical outcome of patients with pancreatic cancer. Strong FAPα expression in stromal cells is linked to significantly reduced overall survival rates, increased positive lymph nodes and poor differentiation of pancreatic cancer (15,16). In this present study, Wen *et al.* confirm that FAPα is highly expressed in pancreatic cancer stroma, predominantly in the interstitial region adjacent to pancreatic cancer cells and negatively correlated to survival (6). These findings highlight that FAPα could be a potential biomarker for pancreatic cancer progression and therapeutic options against this disease. Recently, preclinical studies demonstrate that targeting FAPα enzymatic activity through pharmacological inhibition sufficiently suppress tumor progression. A previous phase II clinical trial suggests that inhibition of FAPα enzymatic activity by talabostat (PT100), a small molecule inhibitor of FAPα, attenuates tumor growth of the patients with metastatic colorectal cancer (17). This translates back into a murine xenograft colon cancer model, in which the pharmacological inhibition of FAPα-expressing CAFs by PT100 improves the response of the tumor to chemotherapy by regulating tumor associated immune cells and tumor angiogenesis in tumor microenvironment (18). Wen *et al.* now demonstrate in PDAC that FAPα+ expression in PSCs promotes the invasion and migration of pancreatic cancer cells by utilizing transwell assays (6). Moreover, co-injection of FAPα+ PSCs and pancreatic cancer cells PANC1 through spleen considerably increases the formation of liver metastases in nude mice. Those effects of FAPα+ PSCs on pancreatic cancer cell invasion, migration and metastasis can be sufficiently disrupted by using PT100, suggesting a similar role of FAPα in PDAC. TGFβ signaling pathway participates in malignant progression by promoting cancer proliferation, invasion, metastasis, immune evasion and EMT (19). In pancreatic cancer, PSCs activated by TGFβ signaling pathway exerts tumor-promoting functions by stimulating ECM synthesis in tumor microenvironment (20). The study by Wen *et al.* now for the first time shows that FAPα+ PSCs regulated

by TGFβ1 support pancreatic cancer progression through releasing CXCL1 which combined with its receptor CXCR2 expressed pancreatic cancer cells, leads to increased invasiveness (6). Previous studies show that the Eph and ephrin are pro-tumorigenic and involved in a variety of biological processes, including tumor progression (21). However, the role of the Eph/ephrin system signaling pathway in pancreatic cancer remains largely unknown. The study by Wen *et al.* dissects one pathway in the Eph/ephrin system in pancreatic cancer progression. CXCR2 interacts with EphB1 or EphB3 by CXCL1 stimulation, leading to phosphoinositide 3-kinase (PI3K)/AKT signaling pathway activation which facilitates pancreatic cancer cell migration and invasion. The authors used siRNA to inhibit EphB1 and EphB3 expression, leading to significantly suppressed migration and invasion of pancreatic cancer cells (6). Phosphoinositide 3-kinase (PI3K)/AKT, a key cellular signaling pathway, participated in tumor cells survival and metastasis (22,23). This pathway is regulated by a series of chemokines and their receptors, including CXCL1 and its receptor CXCR2, and promotes tumor progression, which is further illustrated in the present study by Wen *et al.* considering pancreatic cancer progression.

Taken together, the encouraging findings of the study by Wen *et al.* offer a novel insight into the mystery of pancreatic cancer progression, suggesting a potential way to deal with this lethal disease. However, before those findings in the study by Wen *et al.* are translated to a clinical application, other vital experiments and investigations should be performed. The first step is to establish a genetic deletion of FAPα in an endogenous mouse model of pancreatic cancer to better validate its role in malignant progression. Additionally, it would be worth to include large numbers of patients with pancreatic cancer from multiple-centers or existing clinical trials and include more details of clinic-pathological features of individual patients to confirm the predictive and prognostic role of FAP expression in pancreatic cancer. Finally, since tumor metastasis is due to a wide range of biological events, the combination of therapeutic strategies is a promising way to slow down metastatic spread. For instance, combining inhibition of TGFβ signaling with targeting immune checkpoints is expected to be an intriguing study to be involved in treatment of pancreatic cancer (24). In summary, the study by Wen *et al.* clearly describe the critical role of FAPα in PSCs in pancreatic cancer progression and point out a promising approach for treating patients with pancreatic cancer.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-20-2559). The 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.

References

1. Moffitt RA, Marayati R, Flate EL, et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. Nat Genet 2015;47:1168-78.
2. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell 2012;21:309-22.
3. Hwang RF, Moore T, Arumugam T, et al. Cancer-Associated Stromal Fibroblasts Promote Pancreatic Tumor Progression. Cancer Res 2008;68:918-26.
4. Mahajan UM, Langhoff E, Goni E, et al. Immune Cell and Stromal Signature Associated With Progression-Free Survival of Patients With Resected Pancreatic Ductal Adenocarcinoma. Gastroenterology 2018;155:1625-39.e1622.
5. Apte MV, Wilson JS, Lugea A, et al. A starring role for stellate cells in the pancreatic cancer microenvironment. Gastroenterology 2013;144:1210-9.

6. Wen Z, Liu Q, Wu J, et al. Fibroblast activation protein alpha-positive pancreatic stellate cells promote the migration and invasion of pancreatic cancer by CXCL1-mediated Akt phosphorylation. Ann Transl Med 2019;7:532.
7. Isella C, Terrasi A, Bellomo SE, et al. Stromal contribution to the colorectal cancer transcriptome. Nature Genetics 2015;47:312-9.
8. Berdiel-Acer M, Sanz-Pamplona R, Calon A, et al. Differences between CAFs and their paired NCF from adjacent colonic mucosa reveal functional heterogeneity of CAFs, providing prognostic information. Mol Oncol 2014;8:1290-305.
9. Liu J, Huang C, Peng C, et al. Stromal fibroblast activation protein alpha promotes gastric cancer progression via epithelial-mesenchymal transition through Wnt/ beta-catenin pathway. BMC Cancer 2018;18:1099.
10. Fang J, Xiao L, Joo KI, et al. A potent immunotoxin targeting fibroblast activation protein for treatment of breast cancer in mice. Int J Cancer 2016;138:1013-23.
11. Zou B, Liu X, Zhang B, et al. The Expression of FAP in Hepatocellular Carcinoma Cells is Induced by Hypoxia and Correlates with Poor Clinical Outcomes. J Cancer 2018;9:3278-86.
12. Kashima H, Noma K, Ohara T, et al. Cancer-associated fibroblasts (CAFs) promote the lymph node metastasis of esophageal squamous cell carcinoma. Int J Cancer 2019;144:828-40.
13. Liao Y, Ni Y, He R, et al. Clinical implications of fibroblast activation protein-alpha in non-small cell lung cancer after curative resection: a new predictor for prognosis. J Cancer Res Clin Oncol 2013;139:1523-28.
14. Santos AM, Jung J, Aziz N, et al. Targeting fibroblast activation protein inhibits tumor stroma genesis and growth in mice. J Clin Invest 2009;119:3613-25.
15. Kawase T, Yasui Y, Nishina S, et al. Fibroblast activation protein-alpha-expressing fibroblasts promote the progression of pancreatic ductal adenocarcinoma. BMC Gastroenterol 2015;15:109.
16. Cohen SJ, Alpaugh RK, Palazzo I, et al. Fibroblast Activation Protein and Its Relationship to Clinical Outcome in Pancreatic Adenocarcinoma. Pancreas 2008;37:154-8.
17. Narra K, Mullins SR, Lee HO, et al. Phase II trial of single agent Val-boroPro (talabostat) inhibiting fibroblast activation protein in patients with metastatic colorectal cancer. Cancer Biol Ther 2007;6:1691-9.
18. Li M, Li M, Yin T, et al. Targeting of cancer associated fibroblasts enhances the efficacy of cancer chemotherapy by regulating the tumor microenvironment. Mol Med Rep 2016;13:2476-84.
19. Massagué J. TGFbeta in Cancer. Cell 2008;134:215-30.
20. Bachem MG, Schunemann M, Ramadani M, et al. Pancreatic carcinoma cells induce fibrosis by stimulating proliferation and matrix synthesis of stellate cells. Gastroenterology 2005;128:907-21.
21. Xi HQ, Wu XS, Wei B, et al. Eph receptors and ephrins as targets for cancer therapy. J Cell Mol Med 2012;16:2894-909.
22. Shaw RJ, Cantley LC. Ras, PI(3)K and mTOR signalling controls tumour cell growth. Nature 2006;441:424-30.
23. Qiao M, Iglehart JD, Pardee AB. Metastatic potential of 21T human breast cancer cells depends on Akt/protein kinase B activation. Cancer Res 2007;67:5293-9.
24. Miyazono K, Katsuno Y, Koinuma D, et al. Intracellular and extracellular TGF-beta signaling in cancer: some recent topics. Front Med 2018;12:387-411.

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