The advancement of tumor microenvironment in pancreatic carcinoma

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Pancreatic cancer is the known kind of tumor biologically featured as high malignancy, lack of effective methods for diagnosis and treatment. Tumor microenvironment is generally considered as an important promotor in tumor invasion and metastasis. This review aims to deeply understand the mechanism of the tumor progression and validly provide the evidence for diagnosis and treatment by means of focusing on the advances in the research of pancreatic cancer in the fields of stromal cells, inflammation and hypoxia in the tumor microenvironment of pancreatic carcinoma.

Pancreatic cancer is a kind of malignant tumor of digestive tract, which is highly malignant and difficult in diagnosis or treatment, approximately 90% originated from glandular epithelium of ductal adenocarcinoma. It is difficult to make early diagnosis of pancreatic tumor, mostly belonging to be locally advanced or late when definitely diagnosed. Patients would lose the opportunity of radical resection, with an overall five year survival rate less than 8% far below the penultimate liver cancer and lung cancer (both 18%), which is one of the worst prognosis of malignant tumors. From 1975 to 2010 in the USA, the overall 5-year survival rate of cancer patients increased from 49% to 69%, but pancreatic cancer only increased to 8% from 3% with the minimum improved prognosis. Although the incidence of pancreatic cancer in men beyond the top 10 and stay ninth in women cancer (53070 new cases expected in 2016), but it is the fourth leading cause of death (41780 cases) [1]. The causes of the high mortality of pancreatic cancer are the difficulty of early diagnosis and lack of effective clinical treatments. What’s more, it is still vague about the mechanism of carcinogenesis and progression, while the revelation of which is expected to become the key to a fundamental change in poor prognosis [2-4].

Previous studies on the mechanism of carcinogenesis and progression mainly focused on the tumor cells themselves. However, in recent years, researches have increasingly concerned by the non-tumor cells of the microenvironment, focusing on the role of microenvironment in the carcinogenesis and progression. The so-called tumor microenvironment is a local internal environment, which consists of tumor infiltrating immune cells, stromal cells,
active factors and tumor cells. The studies found that the tumor microenvironment would have an indispensable role in tumorigenesis, progression and metastasis [5-8]. This review is discussing the progress in the studies of microenvironment of pancreatic tumor from three aspects as the stromal cells, inflammation and hypoxia.

**Stromal Cell Microenvironment**

One of the most remarkable features of microenvironment in pancreatic cancer is that it contains amounts of dense stromal cells. The interaction between stromal cells and tumor cells is bidirectional, dynamic and complicated. Stromal cells alter the oncological characteristics of tumor cells through producing a particular microenvironment that promote progression by secreting growth factors, pro-angiogenic factors, proteases, adhesion molecules, etc. It facilitates tumor cells to proliferation, angiogenesis, invasion and metastasis [9,10].

**Pancreatic stellate cells, PSCs**

As early as 2004, researchers had found that PSCs not only promote fibrosis in the development progress of chronic pancreatitis, but also excrete extracellular matrix proteins that constitute the stroma of pancreatic tumor. Studies have confirmed the presence of activated PSCs in tumor stroma by immunohistochemical analysis with selectable marked stellate cells [11]. PSCs are a special kind of fibroblasts around the pancreatic lobules and pancreatic acinar. Pancreatic tumor cells can not only promote the proliferation and metastasis of PSCs, but also facilitate the formation of extracellular matrix components. Meanwhile, PSCs promote the synthesis of the extracellular matrix by the mediation of TGF-β and FGF. Furthermore, the secretion of platelet-derived growth factor (PDGF) accordingly promotes the proliferation of PSCs [12]. Another study showed that COX-2 and TFF1 also contribute to the proliferation of PSCs. Pandol et al. [15] found that in the process of tumorigenesis, precancerous lesions might activate PSCs. Conversely, PSCs also promote tumor cell proliferation and inhibit apoptosis, thereby increasing the number of tumor cells [16,17]. PSCs secrete another factor called MMP. MMP is the main factor to promote tumor metastasis by degrading the basement membrane, prompting the tumor cells to spread into the lymphatic and blood vessels and promoting tumor invasion and metastasis, especially MMP-2 associated with metastasis,

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**Figure 1.** The interaction between MMPs from PSCs and endothelial cells with cancer cells. PSCs can secrete MMP-2 or MMP-3, which contributes to degrading ECM and mobiles growth factors, as well as endothelial cells. Furthermore, MMP-2 accelerates metastasis of cancer cells, in contrary, the cancer cells secrete some cytokines interacting with PSCs that promotes the production of u-PA.
invasion and poor prognosis as malignant phenotypes of pancreatic cancer \[18\] (Figure 1). PSCs can also promote metastasis and EMT, such as increasing the expression of E-cadherin and other mesenchymal cell markers in epithelial cells. Pancreatic cancer cells exhibit morphologic characteristics of fibroblasts. Then EMT mediated by PSCs, facilitates epithelial cells to decrease expression of epithelial markers, and increase expression of interstitial markers, thereby promoting tumor metastasis \[19\]. Moreover, insulin-like growth factor, epidermal growth factor, hepatocyte growth factor, TGF-β and other inflammatory factors secreted by PSCs play an important role in the progress of pancreatic cancer and need to be further studied.

**Cancer-associated fibroblasts, CAFs**

In the solid tumor microenvironment, fibroblasts are a class of cells with extremely rich content, especially in breast cancer, ovarian cancer, pancreatic cancer, and prostate cancer. In the tumor microenvironment, fibroblasts can be activated and to obtain an activated phenotype, thus losing the original morphology, expressing amounts of α-SMA, and getting collagen I, elastin C and MMP that enhance extracellular matrix deposition \[20\]. After changing from normal fibroblasts, CAFs promote the generation of paracrine growth factors, proteolytic enzymes and matrix components, thereby performing a matrix remodeling and promoting invasion and metastasis \[21\]. Mitra et al \[22\] found that, miRNA are small non-coding RNA that negatively regulates gene expression after transcription, which adapt normal fibroblasts for CAFs. Therefore, miRNA will also become a new target for treatment with tumor microenvironment. Pang et al \[23\] found that pancreatic cancer cells promote fibroblasts into CAFs, while CAFs also promote tumor invasion and metastasis. In addition, microbubbles with miR-155 play an important role in the conversion process, so therapy targeted circulating microRNA will become a potential direction. Zhuang et al \[24\] also find that CAFs may mediate EMT by TGF-β-ZEB2NAT-ZEB2 axis to promote tumorigenesis and development. Due to difficulties in early diagnosis of pancreatic cancer, the prognosis is still relatively poor. The recent discovery of a clinically relevant marker matrix in pancreatic cancer: CAFs produced COL11A1 protein, which is mediated by the overexpression of COL11A1. Studies have clearly expressed proCOL11A1 as a particular marker for CAFs, and anti-proCOL11A1 (antibody) will likely become a novel tool for clinical diagnosis of pancreatic cancer \[25\]. In addition, the mechanism of CAFs promoting invasion and metastasis is also not yet clear, where Cav-1, PDON, TIMP-1 and IL-22 secreted by CAFs, all play a significant role \[26-29\].

**Inflammatory Microenvironment**

**Macrophages**

Macrophages are the main components of the inflammatory cells infiltrating into tumor tissues. The regarding relationship between TAMs and pancreatic cancer cells are of great complexity \[30\]. Numerous studies showed that, TAMs is an important part of inflammatory pathways in promoting tumor development. Sainz et al \[31\] demonstrated that CSCs have a significant impact on both the progression and metastasis of pancreatic cancer, and the IFN-stimulator ISG15 secreted by TAMs can make the phenotype of CSCs enhanced. TAMs stimulate CSCs to release IFN-β through secreting ISG15, which can build up the self-renewal, invasive and tumorigenic capabilities in terms of the microenvironment. Pancreatic cancer cells own high expression of CD68 and CD163 positively associated to macrophages which can validly promote the migration of macrophages but decrease the mRNA transcription of macrophages polarization related genes express IL-10, IL-12P40, iNOS and CD163. In addition, TAMs upregulate the gene expression of the EMT-associated mRNA, but downregulate E-cadherin expression in epithelial cells \[30\]. Hermano et al \[32\] further concluded that the new features of heparanase in TAMs is that it can promote tumor progression directly, which implies heparanase expression will be beneficial for patients with the treatment pattern of TAM / IL-6 / STAT3.

The latest studies have demonstrated that CD68 expressed by TAMs can be used for the early detection and diagnosis of cancer, which can probably be a novel approach for the early diagnosis of pancreatic cancer \[33\]. Otherwise, tumor microenvironment staying in the immunosuppressive conditions can inhibit the antitumor immunity. The stimulation of CD40 can not only reverse the immunosuppression, but also activate the reaction of anti-tumor T cells, macrophages with activated CD40 can quickly infiltrate into the tumor tissues, destroy the tumor cells and facilitate the consumption of tumor stroma. The activation of TAMs promotes the secretion of IL-10, TNF-α and IL-6 but reduces the positive expression of MHC class II molecules. Therefore, not all of the tumor immune surveillance depends on the mediation of T cells, in contrast, the CD40-dependent treatment aiming at the tumor matrix will be new methods for treating pancreatic cancer \[34\].

**Mast cells, MCs**

MCs are widely distributed in the skin and around the microvascular below visceral mucosa. It secretes a variety of cytokines which involve in immune regulation. MCs are well
known as their primary effect in allergic reactions, however as a member of the tumor microenvironment has it been ignored. Studies showed, MCs also play an important role in the process of tumor genesis [35]. For example, tumor cells facilitate the migration of MCs, and stimulate MCs with PSCs. Accordingly, IL-13 and tryptase secreted by MCs promote the proliferation of PSCs, thus contributing to the extracellular matrix deposition and tumor progress [36]. MCs can secrete pro-tumorigenic molecules and a variety of signaling molecules, including EGF, which serve as the effectors of tumor-promoting actions [6, 37]. Therefore, the therapy targeted MCs to facilitate tumorigenesis is expected to become a new treatment for pancreatic cancer.

**Lymphocytes**

T, B cells are the two most important inflammatory cells, in particular CTL play an important role in pancreatic anti-tumor immune process. However, due to the role of CTL immunologically suppressed by tumor microenvironment, tumor cells cannot be cleared by the tumor-specific immune response. The latest study finds that immunosuppressive myeloid cells are able to secrete GM-CSF, which is associated with KRAS gene mutation to promote tumor development. Therefore, the applying of monoclonal antibody to block GM-CSF can promote T cell proliferation in order to inhibit the immune escape mediated by myeloid-derived suppressor cell [38]. Our previous study also exhibited that intratumoral Tregs have negative relation with postoperative DFS, while peritumoral CD8+ T cells show to be a positive indicator of OS in pancreatic ductal adenocarcinoma patients. In the patients with relatively poor prognosis, the number of its intratumoral Tregs are significantly increased and the number of peritumoral CD8+ T cells are constrastly reduced, thereby it suppress the immune surveillance and anti-tumor immunity, and enhance the ability of tumor cells to escape, which suggests that Tregs and CD8+ T cells will become a new index for prognosis of postoperative pancreatic cancer patient after CD4+ T cells [2]. In addition, galectin 1 and CD3 are also expressed higher in tumor cells, and it promotes a large number of T cells apoptosis at the same time. Pancreatic cancer cells activate caspase 9 and caspase 3 through the activation of the mitochondrial apoptotic pathway, stimulate Th2 to secrete

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**Figure 2. The important factor of ROS in realizing invasion.** Cytokines, such as EGF, TGF-β, activate Src or PI3K, as well as the process by hypoxia-reoxygenation activates PI3K. Then they can both activate downstream signaling protein, Rac1. Rac1 is the upstream signal protein for NADPH oxidase-dependent ROS generation. The rise of Rac1 will promote the metaplasia of acinar catheter, pancreatic intraepithelial neoplasia and tumor genesis. Then generated ROS activates NF-κB signal, leading to the synthesis of MMP-2 and u-PA acting on the basement membrane. Finally, it enhances tumor cell invasion, etc. Reprinted with permission [6].
IL-6 and IL-10, and inhibit Th1 in secreting TNF-β and IFN-γ. Galectin 1 derived from pancreatic cancer cell is conducive to tumor cell immune escape through enhancing T cell apoptosis and incompetence [39]. It is also confirmed both in the tumor microenvironment and peripheral blood, Th17 increase significantly, so that Th17 and its related cytokines, such as IL-17 and IL-23, may be important indicators for prognosis [40]. Narumi et al. [41] achieved the latest result that S100A8 / A9 protein can promote NK cells to release IFN-γ, directly enhance the anti-tumor immunity with the existence of pancreatic tumor cells. Further studies have found PAR-2 and TLRs are presented in pancreatic cancer cell surface with high expression, activate the complicated immune regulation system and enhance the ability of tumor progression and metastasis, which is expected to be a new target in cancer therapy [42, 43].

Hypoxic Microenvironment

Although hypoxia can inhibit the metabolism and growth of normal cells, it can increase the production of various growth factors, inducing factors, etc. to promote the growth and metastasis of tumor cells. HIF is upregulated in pancreatic cancer cells binding with corresponding promoter region sites of PFKFB-4 and PFKFB-3 genes, promoting the production of PFKFB-4 and PFKFB-3. Thereby, it facilitates tumor glycolysis and enhances proliferation and metastasis of cancer cells [44]. Additionally, hypoxia may produce many pro-inflammatory mediators IL-6 that induce antitumor immunity through JAK / STAT pathway. Meanwhile, it has certain relevance with the generation of pro-tumorigenic microenvironment, tumor angiogenesis and metastasis [45, 46]. Another important factor for realizing invasion is the generation of ROS. Cytokines, such as EGF, TGF-β, activate Src or PI3K, as well as the process by hypoxia-reoxygenation activates PI3K. Then they can both activate downstream signaling protein, Rac1. Rac1 is the upstream signal protein for NADPH oxidase-dependent ROS generation. The rise of Rac1 will promote ductal metaplasia of acinar, pancreatic intraepithelial neoplasia and tumor genesis. Then generated ROS activates NF-xB signal, leading to the synthesis of MMP-2 and u-PA acting on the basement membrane and finally enhances tumor cell invasion, etc. [6] (Figure 2).

Conclusions

Various cell phenotypes and functions in the tumor microenvironment have undergone corresponding changes that are determined together by the characteristics of the tumor cells and microenvironment. Interactions and connections between the various matrix and cell components in pancreatic cancer microenvironment contribute to form a complex bidirectional regulation network, which jointly supports the development of tumorigenesis and metastasis. With further researches for pancreatic cancer microenvironment, drugs for different targets in pancreatic cancer, such as CD40 agonists, TGF-β inhibitor, IL-6 antagonists, MMP antagonists, etc., have made certain experimental results. But we still don’t completely break the complexity of treatments for pancreatic tumor, which leads to the current clinical survival rate is still generally low. With the continuous improvements of experimental models and in vivo models, we will have a better understanding upon the mechanism of tumorigenesis and progression of pancreatic cancer. Therefore, it enables truly to understand the whole picture of pancreatic cancer and provide new ideas for treatments.

Conflicting interests

The authors have declared that no conflict of interests exist.

Abbreviations

PSC: pancreatic stellate cell; TGF-β: transforming growth factor β; FGF: fibroblast growth factor; PDGF: platelet-derived growth factor; COX-2: cyclooxygenase-2; TFF1: trefoil factor 1; MMP: matrix metalloproteinases; EMT: epithelial-mesenchymal transition; CAF: cancer-associated fibroblast; α-SMA: α- smooth muscle actin; miRNA: microRNAs; COL11A1: collagen11A1 gene; Cav-1: caveolin-1; PDON: podoplanin; TIMP-1: tissue inhibitor of matrix metalloproteinase-1; TAM: tumor-associated macrophage; CSC: cancer stem cells; MC: Mast cell; EGF: epidermal growth factor; CTL: cytotoxic T lymphocytes; GM-CSF: granulocyte-macrophage colony stimulating factor; Treg: regulatory T cell; DFS: disease-free survival; OS: overall survival; IFN-γ: interferon γ; PAR-2: protease-activated receptor 2; TLR: toll-like receptor; HIF: hypoxia-inducible factor; ROS: reactive oxygen species; Rac1: Ras-related C3 botulinum toxin substrate 1; NADPH: nicotinamide adenine dinucleotide phosphate.

References


