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Review Article

Tumor microenvironment in chemoresistance, metastasis and immunotherapy of pancreatic cancer

Shunda Wang, Yatong Li, Cheng Xing, Cheng Ding, Hanyu Zhang, Lixin Chen, Lei You, Menghua Dai, Yupei Zhao

Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences/ Peking Union Medical College, Beijing 100730, China

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Abstract: Pancreatic cancer (PC) is a fatal disease with high malignancy and difficult for early diagnosis. PC causes more than 400,000 patient deaths world widely and becomes the severe health problems. The tumor microenvironment (TME) is comprised of acellular stroma, pancreatic stellate cells, immune cells, and soluble factors. TME is maintained by continuous cell-matrix and cell-cell interactions. TME induced by the interaction among pancreatic cancer cells, epithelial cells and stromal cells is essential for the progression of PC and leads to resistance to chemotherapy. Components in the microenvironment can also promote the formation of connective tissue in the primary or metastatic site, or promote the metastatic ability of PC by enhancing angiogenesis, epithelial-mesenchymal transformation, and lymph angiogenesis. In addition, the TME also leaves pancreatic cancer unsusceptible to different immunotherapeutic strategies. In this review, we summarized the current knowledge about TME in PC. And the focus was placed on the role of TME in chemotherapeutic resistance and metastasis in the field of PC. And we also paid attention to the immunological therapy targeting the TME, aiming to provide the novel therapy for pancreatic cancer.

Keywords: Pancreatic cancer, tumor microenvironment, chemoresistance, metastasis, immunotherapy

Introduction

Despite meaningful breakthrough in cancer research, pancreatic cancer (PC) remains a malignant disease with high mortality. According to the latest data from epidemiological survey, 55,440 patients in the United States were newly diagnosed with PC, of which 44,330 lost lives. The early diagnosis of pancreatic cancer is difficult compared with other cancer types and the 5-year survival rate is only 8% [1]. 90% of pancreatic cancer patients carry KRAS mutations, which are considered to be driving genes for pancreatic cancer progression, and approximalty 60-70% carry inactivating mutations of CDKN2A, TP53 and SMAD4 [2]. Most patients are in advanced stage at the time of treatment and the median survival is only about 6-10 months [3]. Pancreatic cancer is known to be insensitive to chemoterapy and radiotherapy. Although various chemotherapies have certain effects, they are not obvious in prolonging the survival time of patients and improving the quality of life, which is mainly related to the special microenvironment of PC.

The tumor microenvironment (TME) was proposed by Ioannides in 1993, specifically referred to the local environment where tumors occurred and developed [4]. The tumor microenvironment of pancreatic malignacy is made up from extracellular components, stromal cells and cancer cells. The cells contributing to the progression of PC are mainly regulatory T cells (Tregs), tumor-associated macrophages (TAM), myeloid derived suppressor cells (MDSCs) and pancreatic stellate cells (PSC). Pancreatic cancer produces an immunosuppressive microenvironment, leading to immune escape of the host anti-tumor immune system, which enhances tumor progression rapidly. These component could secrete extracellular molecules such as matrix metalloproteinase, extracellular matrix (ECM), growth factors and transforming growth factor β (TGF-β) to maintain the microenvironment [5]. Generally, the TME of PC is

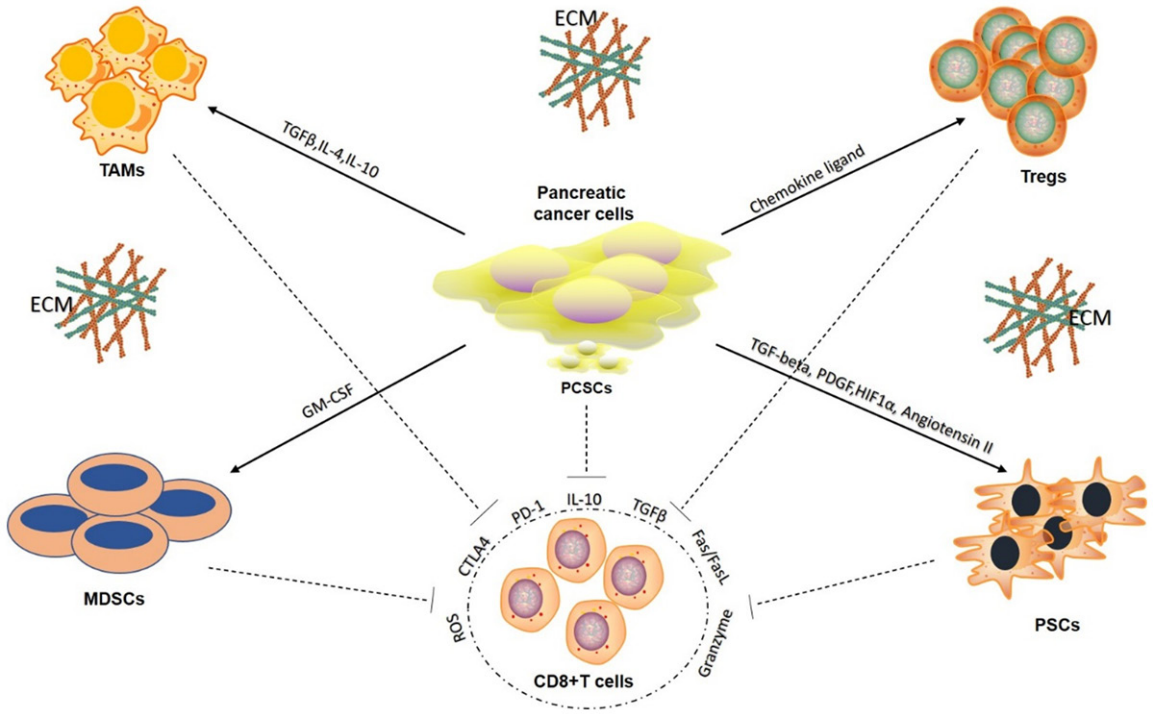

Figure 1. Characteristics and development of pancreatic cancer microenvironment. Pancreatic cancer microenvironment was composed by a variety of immunosuppressive cell types such as tumor-associated macrophages (TAMs), regulatory T cells (Tregs) and myeloid derived suppressor cells (MDSCs). Pancreatic stellate cells (PSC) and extracellular matrix (ECM) contributed to pancreatic matrix components. Cancer cells secreted cytokines and chemokines to recruit related cells for immune evasion. These different type of cells in microenvironment together with pancreatic cancer cells inhibited CD8+ T cells to escape the immune surveillance through many molecules.

characterized by abundant stroma, hypoxia, insufficient blood supply and high immunosuppression. Studies have shown that the tumor microenvironment, including cancer-related fibroblasts, such as extracellular matrix, stellate cells, varied immunity cells and cytokines released by them, are involved in the control of proliferation, metastasis, chemoresistance and immunotherapy of pancreatic cancer [6, 7]. Therefore, finding new therapeutic targets for the TME of PC is a potential research direction. Herein, we reviewed the recent research progress on the tumor microenvironment of pancreatic cancer, aiming to provide the novel approach for comprehensive treatment of pancreatic cancer.

Composition of TME

Tumor microenvironment commonly refers to the interaction between tumor cells and their surrounding tissues, forming a complicated but beneficial internal environment for the biological behavior of cancer cells. TME is generally composed of three parts: (1) matrix components: including extracellular matrix and stromal cells; (2) cell components: including tumor cells, endothelial cells, and immune cells; and (3) soluble factors: including cytokines and immune regulatory molecules [8] (**Figure 1**).

Components of matrix components in the TME of pancreatic cancer

Pancreatic stellate cells, one of the important cells in the microenvironment of pancreatic cancers, are located at the base side of pancreatic acinar cells and distributed around blood vessels. Under physiological conditions, pancreatic stellate cells are in a resting state, and transformed pancreatic stellate cells can rapidly proliferate and secrete a large amount of extracellular matrix and cytokines [9]. When pancreatic cells become cancerous, TGF-β, PDGF, Angiotensin II and other cytokines can be secreted. These cytokines bind to the receptors of PSCs and activate downstream signaling pathways, such as ERK, c-Jun, p38, MAPK,

JAK-STAT, to promote the activation and proliferation of PSCs [10]. What's more, activated PSCs can secrete a variety of growth factors through paracrine, activating EGFR, PI3K-AKT, and mTOR signaling pathways, and then promote the proliferation of PC cells. PSCs can also inhibit the apoptosis of PC cells and enhance their invasive ability [11, 12]. Besides, PSCs make it difficult for chemotherapeutics to enter into the tumor tissue through the bloodstream by occluding non-functional blood vessels, leading to the resistance to gemcitabine [13].

In addition, tumor-associated fibroblasts (TAFs) in the TME of pancreatic cancer, contribute to secreting extracellular matrix proteins and participate in tumor angiogenesis. In addition, TAFs can also affect the activity of IFN-γ and TNF-α by secreting fibroblast activating protein, thereby inhibiting the function of killing effect of T cells [14]. Besides, TAFs can secrete inflammatory factor IL-6, activating STAT3 signaling pathway to promote tumor growth [15]; and also secrete the CXCL12, hampering the infiltration of T cells into tumors [16]. Furthermore, TAFs can mediate the formation of chemoresistance through multiple signaling pathways, and the use of specific inhibitors can improve the efficacy of chemotherapeutic drugs [17].

What's more, there is a large amount of extracellular matrix around pancreatic cancer cells, which is mainly composed of collagen, fibronectin, hyaluronic acid, and other components. After deposition and accumulation, the dense fibrous tissue is formed, which creates a favorable tumor microenvironment for the proliferation of PC cells [18].

Immune cells in the TME of pancreatic cancer

Regulatory T cells: It is known to all that pancreatic cancer is considered to be immunological resting. In pancreatic cancer tissues, there are abundant Tregs and cytotoxic T lymphocyte-associated protein 4 (CTLA-4). CTLA-4, constantly expressing on Tregs, exerts an important function in inhibiting antigen presentation. Regulatory T cells mainly induce immune tolerance and play an immunoregulatory role in normal organisms, and play an immunosuppressive role in tumor patients. They promote immune escape by inhibiting the killing ability of effector cells [19]. In addition, low level of major histocompatibility complex-I (MHC-I) molecules in PC cells can inhibit the activation of T cell, thereby activating CD8+ T cells via interacting with antigens conveyed by MHC-I expressed on antigen-presenting cells (APC). The activating CD8+ T cells, which secrete perforin and granzymes and express Fas ligand, can decrease the ability of anti-tumor cells. CD8+ T cells can also secrete immune checkpoint-related signaling components, thereby inhibiting Tregs' function and inducing immune failure [7, 20]. In addition, regulatory T cells can also interact with APC to inhibit the expression of CD80 and CD86, leading to dysfunction of cytotoxic T cells [19].

Tumor-associated macrophages: Macrophages can differentiate into M1 and M2 phenotypes in different conditions. M1-type macrophages can promote local inflammatory response and participate in immune surveillance. M2-type macrophages can promote tumor angiogenesis and induce chemoresistance, thus accelerating the proliferation, infiltration, and metastasis of PC [21, 22]. IL-27, histidine-rich glycoprotein can make M2-type macrophages transform to M1-type and reproduce its anti-tumor efficacy [23]. TAM contributes to the immunosuppression of PC cells and tumor-related angiogenesis. Cytokines and vascular endothelial growth factors (VEGF) can attract TAMs to the TME, and TAMs support the proliferation of pancreatic cancer cells by secreting a series of growth factors [24]. Among these molecules, IL-10 and TGF-β can establish an immunosuppressive TME by preventing the dendritic cell-mediated anti-tumor immune response [25]. It was proved that TAM could also prevent CD8+ tumor-infiltrating lymphocytes, thereby reducing the anti-tumor effect. Additionally, researches also manifested that the mast cell also influenced the growth pattern of cancer cells through accelerating their angiogenesis and promoting cancer cell motility. If mast cell infiltration occurred in the tumor, it often indicated the dismal prognosis of PC patients [26].

Myeloid derived suppressor cells: Myeloid derived suppressor cells, defined as heterogeneous immature cells from bone marrow, exert a key function in the immunosuppression of PC. The surface markers of MDSCs are usually CD11b+CD33+HLA-DR-. Peripheral blood MD-

SCs and pro-MDSC cytokines were higher in pancreatic cancer patients, and some studies suggested that peripheral blood MDSCs might act as a sensitive marker of chemotherapy tolerance in patients diagnosed with pancreatic carcinoma. And MDSCs in bone marrow can be induced to proliferate and migrate to the TME by granulocytic macrophage colony stimulating factor (GM-CSF). Additionally, GM-CSF is correlated with differentiation from the bone marrow progenitor cell to MDSC and recruitment of MDSCs to the TME in PC [27]. The driver gene KRAS G12D, expressing in more than 90% PC patients, induces the overexpression of GM-CSF [28]. IL-10, IFN-γ, and TGF-β in the TME can activate MDSCs, enhancing the proliferation of Tregs and further mediate the immunosuppression [29]. Besides, MDSCs can release reactive oxygen radicals, leading to oxidation stress, thus promoting tumorigenesis and development.

Pancreatic cancer stem cells: Pancreatic cancer stem cells (PCSCs) are regarded as a minor part of pancreatic cancer cells, which could generate heterogeneous cancer cell in tumors. The basic driving force for the occurrence and development of pancreatic cancer is partly originated from PCSCs [30]. The latest studies have revealed that PCSCs could mimic the tumor microenvironment and contribute to the maintenance of their stemness, including self-renew, tumorigenesis and metastatsis. The important signaling pathway related to these processes includes the Wnt, NF-κB, PI3K/Akt and PTEN [31]. Although the literature reported is less than other components in TME, the property of stem cells such as enhancing tumor progression, promoting self-generation, and accelerating distant metastasis cannot be neglected.

TME and chemoresistance

Chemotherapy resistance is a huge challenge faced by lots of patients with malignant tumors, which may be mediated by the tumor microenvironment. Tumor heterogeneity contributes to the response of tumors to different types of chemotherapy, and the current literature on pancreatic tumors has confirmed that tumors has complex microenvironment containing the huge amount of independent components, each of which exert a unique role in conferring chemoresistance to pancreatic cancer [6, 32]. These components consist of the inflammatory stroma, the infiltrating immune population, the extracellular matrix, and the tumor initiating cell population [6]. Immunosuppressive cells such as TAMs, MDSCs, Tregs can not only directly inhibit killer cells such as cytotoxic T cells (CTL) and natural killer cells (NK), but also interactwith each other, enhance the effect of inhibitory factors, and recruit more immunosuppressive cells to aggregate, leading to the immune escape. Chemotherapeutic drugs can act on the tumor immune microenvironment, and immune cells in the microenvironment can also influence the pharmacological effect of chemotherapeutic drugs (**Figure 2**).

Matrix is a physical barrier for drug delivery

As is known to all, the role of stroma in TME of pancreatic tumors is still controversial and whether it exerts the crucial effect in the pancreatic chemoresistance has not been clarified [33, 34]. The main constitutions of the matrix are immunity cells, fibroblasts and their secreted extracellular matrix. It was reported that the function of stroma in PC might inhibit tumor growth, especially in the early stage of tumor development, and inhibiting its function could lead to extensive metastasis [35]. Subsequently, further studies proved that the myofibroblast depletion in the TME of pancreatic cancer led to excessive remodeling of tumor extracelluar matrix, resulting in a important reduction in tumor tissue stiffness and total collagen content. And gemcitabine treatment after myofibroblast depletion resulted in decreased survival. The most significant effect of the depletion of myofibroblasts was the alteration of immune infiltrating components through the TME in pancreatic cancer [36]. ECM was also identified to prompt the vascular constriction, thereby decreasing the level of oxygen and reducing the concentration of chemotherapeutic drugs [37, 38].

Hypoxic microenvironment contributing to chemical resistance

As tumors progress, the evolving stroma exerts pressure on the anigogenesis, causing them to contract and keep the tumors in a hypoxic state [39]. Pancreatic cancer is a hypoxic tumor with a distinct hypoxic microenvironment. Low oxygen levels in tumors lead to stabilization of hypoxia-inducible factor 1 (HIF1) [40]. At the

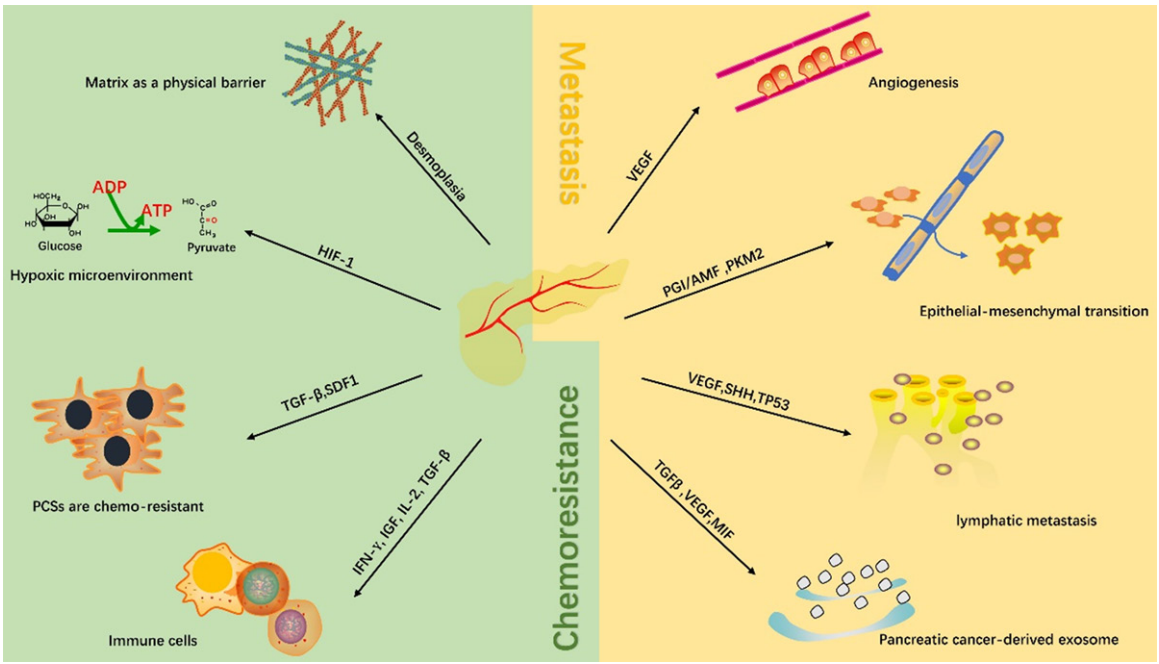

Figure 2. The tumor microenvironment in chemoresistance and metastasis of pancreatic cancer. In pancreatic cancer, matrix forms a physical barrier for chemotherapeutic drug delivery and the hypoxic microenvironment contributes to chemoresistance. Pancreatic stellate cells and other immune cells in tumor microenvironment get together to promote chemoresistance through different cytokines and signaling pathways. Additionally, angiogenesis, EMT, lymphatic metastasis and pancreatic cancer-derived exosome in the tumor microenvironment are widely involved in pancreatic cancer metastases.

same time, HIF1 acts as the central node of hypoxia signaling pathway, which changes the metabolic pathways of tumor cells, and then induces invasiveness, promotes chemoresistance, and leads to the poor prognosis of patients [41]. HIF-1 was reported to induce the carbonic anhydrases and alter the pH shift to reduce the absorption of anti-cancer drugs [42]. The hypoxic TME could change the glucose uptake and the glycolysis, which altered the metabolism from oxidative phosphorylation to aerobic glycolysis in PC cells [43, 44]. And cyclic hypoxia could increase the resistant ability to chemotherapy because of the acidification of the TME. In addition, hypoxia directly reduced the cytotoxicity of chemotherapy and radiotherapy, made the tumoral tissue more resistant to treatment and was also correlated with unfavorable clinical prognosis [45]. HIF-1 could induce the expression of drug resistance P-glycoprotein (P-gp) to improve the transport ability of pancreatic cancer, resulting in the reduction of the accumulation of chemotherapeutics in tumor cells. On the other hand, the anti-apoptotic ability of PC can also be improved by regulating Bcl-2 and Bax [31, 46].

Pancreatic stellate cells are resisting to chemotherapy

Pancreatic stellate cells are quiescent cell clusters in the pancreatic cancer and the indispensable part of the TME [47]. Under normal conditions, they express glial fibrillary acidic protein and conserve droplets containing cytoplasmic Vitamin A lipids [48, 49]. The droplets of Vitamin A make the unique cell types distinct from normal pancreatic fibroblasts. They can act through growth factors secreted by damaged cells, while activated pancreatic stellate cells stimulate them to evolve into fibroblasts [50, 51]. Studies revealed that PSCs promoted the expression of chemo-resistant cells and reduced the sensitivity of PC cells to radiotherapy through inhibiting apoptosis and increasing the cellular viability [52]. Activated PSCs can promote the secretion of TGF-β, which in turn activates PSCs and plays an important role in the formation of pancreatic cancer microenvironment [53]. TGF-β can also upregulate both protein kinase C-alpha and P-gp, increasing the tolerance of pancreatic cancer to sensitive drugs [53]. Activated PSCs can also activate

autophagy, increase the level of ECM, and hinder the delivery of chemotherapeutic drugs [17]. What's more, PSCs could secrete periostin, conferring resistance to gemcitabine in PC cells and promote the expression of Hes1, leading to chemotherapy resistance [54, 55]. In addition, it was reported that the SDF1 secreted by the PSCs could activate the FAK-AKT and ERK1/2 signaling pathways to regulate the tumor-stroma interaction, contributing to chemoresistance [56, 57]. Besides, PSCs also influence the radiotherapy resistance and protect the PC cells from radiological damage, leading to the radioprotection of cancer cells, however, the underlying mechanism related to this process need to be further investigated [58-60].

Role of immune cells in promoting chemoresistance

In addition to immunosuppressive cells, there are many anti-tumor phenotypic immune cells in the TME, which also play an important role. Dendritic cells act as antigen-presenting cells to present antigens to CD4+ or CD8+ T cells and initiate specific immunity. NK cells are nonspecific killer cells that proliferate continuously during tumor progression, and their number is positively correlated with survival [7]. High levels of IL-2 in the microenvironment promote NK cells to secrete IFN-γ and other immunoregulatory cytokines [61]. Immune cells also play a role in promoting chemoresistance in cancer treatment. TAMs and their exosome miR-365 upregulated in tumor cells enhance gemcitabine metabolism, inhibit the apoptotic pathway, and promote tumor resistance [62, 63]. TAMs secrete TGF-β to upregulate Gfi-1 expression in tumor cells, and Gfi-1 can bind to the promoter region to inhibit CTGF and HMGB1 expression and reduce the sensitivity of tumor cells to gemcitabine [64]. In addition, TAMs can express IGF acting on IGF1 receptors to promote chemoresistance to gemcitabine and albumin-binding paclitaxel [65]. And TAMs also enhance the chemoresistance via overexpressed CDA, an enzyme catabolizing the activation of gemcitabine [66]. All these immune cells contribute to the chemoresistance in pancreatic cancer.

TME and metastasis

Metastasis is the main reason of high mortality in PC patients, and the ways of metastasis include hematogenous metastasis, localized invasion, and lymphatic metastasis. Most patients with pancreatic cancer are already at locally advanced and unresectable stage when initially diagnosed. Most patients die of liver, lung, or peritoneal metastases [67]. The immune cells in our body, such as NK cells and CD8+ T cells and NK cells, can defeat or even kill the cancer cells during metastasis, even in the early stage of epithelial-mesenchymal transition (EMT). However, PC cells can escape from the immune surveillance and metastasize to distant sites owing to regulating the interactions among immunosuppressive components in TME [68]. Previous studies have focused on the inner mechanism of cancer cells, but in recent years, clinical studies have found that tumor stroma in TME, which was previously considered to only exert a supplemental role in the pathogenesis of PC, may play an important role in the maintenance of the malignant phenotype of PC, leading to metastasis. There are many studies focused on the mechanism of PC metastasis, and latest studies have shown that the relevant molecules and mechanisms in the immune microenvironment are widely involved in pancreatic cancer metastasis [69-71] (**Figure 2**).

Angiogenesis

Pancreatic cancer cells and various immunosuppressive cells in TME, such as TAM and MDSCs, induce angiogenesis and metastasis through secreting pre-angiogenic factors, growth factors and cytokines. Each of them exerts the specific role in caner metastasis and VEGF plays an important role in the angiogenesis of PC among them. According to the former studies, multiple signaling pathways regulate the secretion of VEGF. In pancreatic cancer cells, STAT3 can activate the expression of VEGF to promote angiogenesis [72]. MUC1 in pancreatic carcinoma can induce the hypoxia of VEGF and promote the production of PDGF, contributing to the formation of endothelial ducts [73]. In addition, NK-κB is another key molecule that regulates the expression of VEGF. Activated NK-κB in PC cells can activate the expression of VEGF, while xanthohumol is able to reduce the level of VEGF and inhibit angiogenesis of PC cells by inhibiting the NF-κB signaling pathway [74]. What's more, the metabolic alteration in TME is also verified to influence the angiogenesis in PC. Due to the hypoxic condition in TME,

the lactate can accumulate to a high level, leading to elevated glycolysis process [75, 76]. Glycolysis in turn promotes tumor angiogenesis by increasing VEGF and regulating stromal cells [77]. Moreover, enhanced glycolysis in TME can also accelerate vessel sprouting to promote angiogenesis [78].

Epithelial-mesenchymal transition

Epithelial-mesenchymal transition is a process in which cancer cells lost adhesive capacity during the initiation of metastasis and break through the basement membrane to invade the periphery. Experiments in mouse models of precancerous lesions and pancreatic cancer have shown that epithelial cells ran into the bloodstream and acted as the circulating epithelial cell (CEC). Besides, the pancreatic cancer also maintains a mesenchymal phenotype and enhances invasiveness and the spread of epithelial cells [79]. In addition, intraductal papillary mucinous neoplasm (IPMN) is a precursor lesion of pancreatic cancer, and its incidence has gradually increased in recent years. Studies have shown that in different stages of IPMN, markers of EMT process have been detected, ranging from low-grade dysplasia, moderate dysplasia, to high-grade dysplasia (carcinoma in situ) [80]. In addition, CEC was proved to be found in nearly 90% of IPMN patients, and the results of RNA-seq analysis revealed that EMT in IPMN might be driven by the mucin gene [81]. Moreover, PC cells exhibit a “mesenchymal phenotype” under enhanced glycolysis in TME [82]. Glycolytic enzymes can activate and maintain the EMT program by regulating the expression of transcription factors [83]. Key metabolic enzymes in glycolysis, such as PKM2, can interact with endothelial cells to promote cell proliferation and migration [84, 85]. Studies have also shown that PGI/AMF promoted PC metastasis by binding to gp78 [76]. Overexpression of PGI/AMF also resulted in the downregulation of E-cadherin, contributing to the aggressive phenotype of PC [86].

Lymph angiogenesis and lymphatic metastasis

Tumor-associated lymph angiogenesis is also a key factor in the progression of pancreatic cancer, especially in promoting lymph node metastasis of PC. The grow of lymphatic vessel can be mediated by cytokines in the TME of PC, such as M2-type TAMs [87]. MDSCs, Tregs and other immunosuppressive largely cytokines exist in tumoral infiltrating lymph nodes [88, 89]. And the density of Tregs in PC in lymph nodes of pancreatic cancer is associated with the potential of lymphatic metastasis [90]. Clinical observational studies also indicated that low level of lymphatic vessel density in PC was correlated with the high-differentiated grade, which decreased the risk of lymph node metastasis and increased the 5-year survival of PC patients [91-93]. Additionally, several factors including VEGF, pancreatic cancer-derived SHH and proline TP53 variant also participate in lymph angiogenesis and lymphatic metastasis [94-96].

Pancreatic cancer-derived exosome

Exosomes are extracellular vesicles ranging from 30 to 150 nm which actively shed by malignant cells. They act as carriers for the transportation of proteins, lipids, and nucleic acids [97, 98]. Exosomes not only mediate the intercellular signaling, but also participate in PC progression. Secretion of exosomes promotes cell activity in TME, enabling them to utilize distantly located cells to promote metastasis in the future [98]. Exosome was reported to enhance the development of the hepatic pre-metastatic niche in PC. Due to repertoires of integrins expressed on their surface, exosome can also control the organotropic metastasis [99]. Cytokines in TME such as TGFβ and VEGF can be obviously detected in PC-derived exosomes during the initial metastasis of PC, indicating a vital role in the formation of pre-metastatic niche [97, 100]. In addition, macrophage migration inhibitory factor (MIF) in TME, an important molecule in pancreatic cancer cell-derived exosomes, is indispensable for the construction of a permissive niche in the liver. MIF was found to be highly expressed in exosomes, and genetic ablation of MIF prevented pancreatic cancer metastasis in experimental mice [101].

TME and immunotherapy

Tumor immunotherapy is a novel treatment that uses the principle of immunology to improve the sensitivity of the body’s immune system to tumor cells, aiming to inhibit tumor growth and even eliminate tumors. With the in-depth understanding of the pathogenesis of PC, immunotherapy has become the hotspot in

recent years. Some immunotherapy methods have entered the clinical trial stage and showed preliminary effects. Pancreatic cancer highly expresses a variety of tumor-associated antigens, such as MUC-1, CEA, PSC, VEGF, MSLN and mutant KRAS. These antigens have become the important target for immunotherapy of pancreatic cancer [102]. In addition, the use of immunomodulators can also enhance the body's immune response and improve the therapeutic effect. For example, immune cytokines IL-2, which contain tumor-targeting antibodies, significantly enhance the antitumor activity through specifically targeting to CTLs [103].

Except for actively enhancing the immunity of the body, attenuating the immune escape of PC cells is another new therapeutic perspective, which provides a new idea for cancer treatment. The immune escape of pancreatic cancer cells is mainly attributed to its special tumor environment. There are a large number of dense matrix components in the TME of pancreatic cancer, forming a barrier around the tumor, preventing the infiltration of immune cells, providing a favorable environment for the growth of PC cells [103]. At the same time, there are immunosuppressive molecules such as TGF-β, IL-10 and Treg cells in the TME, which provide a basis for the immune escape of pancreatic cancer [104]. Recently, immune checkpoint inhibitors and chimeric antigen receptor (CAR) T cell therapy become popular immunotherapies related to the TME in PC.

Immune checkpoint blockade therapy

Immune checkpoint blockade therapy is an immunotherapy method to reverse the immunosuppressive signal by targeting the blockade of immunoregulatory molecules, and then enhance the anti-tumor immune response. Programmed cell death protein 1 (PD-1) is under normal physiological conditions and the expression level of PD-1 is significantly increased when T cells are stimulated and activated. At the same time, activated T cells secrete IFN-γ to induce the overexpression of programmed cell death protein 1-related ligand (PD-L1) in tumor cells. After PD-1 binding to PD-L1, tyrosine phosphatases SHP-1 and SHP-2 are recruited [105]. These phosphatases can inhibit multiple signaling pathways which promote cell proliferation in T cells, such as PI3K/AKT and RAS/MEK, thereby inhibiting T cell proliferation [106]. PD-1 expressed on the surface of lymphocytes can bind to PD-L1, which highly expressed on the surface of tumor cells, transmits co-inhibitory signals to regulate T cell activation, and finally leads to the immune escape of tumor cells [107].

At present, PD-1/PD-L1 inhibitors have been approved for the clinical treatment of a variety of tumors, among which PD-1 inhibitors mainly include Pembrolizumab (Keytruda) and Nivolumab (Opdivo). As for PD-L1 inhibitors, they mainly include Atezolizumab and Durvalumab [108-110]. In animal model, anti-PD-1 or PD-L1 blockade treatment promoted CD8+ T cell infiltration into the tumor and enhanced anti-tumor immune response. However in several clinical trials, no objective tumor remission was observed in patients with pancreatic cancer treated with anti-PD-1 or anti-PD-L1 blockade alone, suggesting that PD-1 or PD-L1 blockade alone did not produce a promising therapeutic effect on pancreatic cancer [111, 112]. At present, the failure of PD-1/PD-L1 monotherapy is mainly attributed to the lack of T cell infiltration in the TME of pancreatic cancer. Therefore, increasing the proportion of T cells in the TME can be effective theoretically. And it has been reported that the combination of IL-6 and PD-L1 could significantly inhibit the growth of pancreatic cancer tumors in mice and improve the effect of PD-1/PD-L1 monotherapy [113]. Because there are many immunosuppressive cells in the TME of pancreatic cancer, the effect of immune checkpoint inhibitors alone is not satisfactory. In addition, the combination of immune checkpoint inhibitors with tumor vaccines, anti-cytokine antibodies and other treatments can reverse the immunosuppressive state in the TME, which will become the potential treatment in future. Clinical trials of anti-PD-1/PD-L1 combined with chemotherapy or radiotherapy in pancreatic cancer are recruiting or planning to recruit patients (https://clinicaltrials.gov). The latest clinical trials of anti-PD-1/PD-L1 therapy in pancreatic cancer are shown in **Table 1**.

Chimeric antigen receptor T cell treatment

Chimeric antigen receptor (CAR) is composed of monoclonal antibodies including single chain variable fragment, hinge region, transmembrane region of TCR receptor and intracellular

Table 1. The latest clinical trials concerning anti-PD-1/PD-L1 therapy in pancreatic cancer

Identifier	Status	Phase	Tumors	Interventions	Combination
NCT03716596	Recruiting	Phase I	Late Stage or Recurrent PC	Stereotactic Body Radiotherapy and PD-1	Yes
NCT03989310	Recruiting	Phase I/II	Local Advanced/Metastatic PC	Anti-PD-1 antibody, nPG, chemotherapy	Yes
NCT04116073	Recruiting	Phase II	Unresectable or Metastatic Adenosquamous PC	INCMGA00012	No
NCT03331562	Active, not recruit	Phase II	Stage IV PC	Pembrolizumab with or without Paricalcitol	Yes
NCT02648282	Recruiting	Phase II	Locally Advanced PC	Cyclophosphamide, Pembrolizumab, GVAX, Radiotherapy	Yes
NCT03153410	Recruiting	Phase II	Borderline Resectable PC	Cyclophosphamide, GVAX, Pembrolizumab and IMC-CS4	Yes
NCT03816358	Recruiting	Phase I	Mesothelin-Positive Advanced PC	Anetumab Ravtansine, Anti-PD-1 Antibody, Anti-CTLA4, Gemcitabine	Yes
NCT03970252	Recruiting	Phase I/II	Borderline Resectable PC	Nivolumab in Combination with Chemotherapy	Yes
NCT03634332	Recruiting	Phase II	Metastatic PC	PEGPH20 plus Pembrolizumab	Yes
NCT04098432	Recruiting	Phase I/II	Locally Advanced Unresectable PC	Stereotactic radiotherapy followed by Nivolumab	Yes

Table 2. Summary of latest clinical trials using CAR T immunotherapies in pancreatic cancer

Identifier	Status	Phase	Tumors	Interventions	Outcome	Country
NCT03638193	Recruiting	I	Metastatic Pancreatic Cancer	CART-meso cells	Adverse events	China
NCT03323944	Active, not recruiting	I	Pancreatic Cancer	Hu CART-meso cells	Adverse events	USA
NCT03497819	Active, not recruiting	I	Pancreatic Cancer	CART meso/19 treatment	Adverse events	China
NCT03818165	Active, not recruiting	I	Pancreatic Carcinoma with Liver Metastases	CAR2 Anti-CEA CAR-T cell	Overall survival	USA
NCT04037241	Not yet recruiting	II	Patients with Liver Metastases	Anti-CEA CAR-T cells with chemotherapy	Overall survival	USA
NCT04404595	Not yet recruiting	I	Pancreatic Adenocarcinoma	Autologous Claudin 18.2 CAR T-cell	Adverse events	USA
NCT02850536	Active, not recruiting	I/II	Liver Metastases or Pancreas Cancer	Anti-CEA CAR-T cells	Adverse events	USA
NCT04348643	Recruiting	I	Pancreatic Cancer	CEA+ CAR-T	Adverse events	China

signal transduction region in series. CAR-T cells are usually constructed by viral infection or transduction [114]. For solid tumors, the heterogeneity of tumor cells and the low expression of solid tumor antigens in normal tissues reduce the specificity and targeting of CAR-T. Therefore, the design of specific CAR-T cells for pancreatic cancer can make the treatment more specific.

Commonly, MUC, CEA, MSLN and PSCA CAR-T have shown to exert therapeutic effects on pancreatic cancer model. For example, after treatment with Tn MUC-1 CAR-T, the survival time of pancreatic cancer xenograft mice was increased from 40 days to 113 days and the survival rate was 100% compared with the unmodified T cell treatment group. At the same time, a large amount of Tn MUC-1 CAR-T was observed in the tumor [115]. What's more, after PSCA targeted CAR-T was used to treat human pancreatic cancer xenografts in mice, 40% of the tumors were eliminated [116]. Besides, designed CD8 + CAR-T cells targeting MSLN was also verified through in vitro experiments. It was found that MSLN CAR-T cells could specifically kill PC cells and produce a large amount of IFN-γ. The metastasis rate decreased from 64% to 46%, and the overall survival increased from 54 days to 96 days [117]. At present, MSLN CAR-T therapy and MSLN CAR-T combined with chemotherapeutic drugs are in phase I clinical trials. Besides, the latest clinical trials of CAR-T therapy which are recruiting or planning to recruit patients in PC are shown in **Table 2**.

CAR-T has already made progress in the treatment of pancreatic cancer. Combining CAR-T with other cytokines can further improve the efficacy. Dual receptor CAR-T improves the safety of treatment, and switch-regulated CAR-T can safely inhibit the progress of tumors [114, 118, 119]. However, numerous soluble immunosuppressive factors inhibit the function of T cells in the TME of pancreatic cancer. Except for influenced by immunosuppressive factors in the TME, the fibrous stromal layer around pancreatic cancer cells can also hinder the infiltration of CAR-T into tumors and affect the therapeutic efficacy [119]. Taken together, deeply understand the characteristics of the immune microenvironment of pancreatic cancer and its influence on immunotherapy can accurately improve the efficacy of therapeutic drugs.

Conclusions and prospects

It is well known that the treatment of pancreatic cancer is a tuff challenge, and so far, little progress has been made in the early diagnosis and effective treatment of PC patients. Researches have revealed that components in the TME of PC are associated with poor prognosis of patients. Many molecules in the microenvironment and related signaling pathways can promote immunosuppression or promote metastasis. There are lots of dense matrix components in the TME of PC, which provide favorable conditions for cancer cell growth. At the same time, dense fibrous tissue prevents the infiltration of CD8+ T cells, NK cells and other immune cells in the tumor tissue, making the tumor tissue escape from the surveillance of the immune system. In addition, there are also a variety of soluble immunosuppressive molecules (TGF-beta, IL-10, IL-23, and VEGF) and immunosuppressive cells (Tregs, MDSCs and TAMs) that lead to the imbalance of immune effector cells, forming a unique immunosuppressive environment for pancreatic cancer.

Despite the use of multidrug chemotherapy regimens, PDAC is still one of the most lethal solid tumors. Strategies to deconstruct desmoplastic stroma and target immunosuppressive pathways have largely failed. Therapeutic approaches targeting stromal connective tissue formation focus on depleting stromal components, but the results are unsatisfactory due to the multifaceted nature of the tumor stroma. Strategies to overcome specific immune targets have also achieved limited success due to the existence of multiple immune regulatory pathways in the TME of PDAC. Combining matrix-targeted and immune-targeted therapies may be more rational [120]. In order to design effective immunotherapy strategies to improve the immunotherapeutic effect of pancreatic cancer, we should start from the following aspects: (1) increase T cell infiltration in pancreatic cancer tissues; (2) remove excessive Tregs, MDSCs and other immunosuppressive cells; reverse the immunosuppressive microenvironment; (3) design novel tumor vaccines and CAR-T cells to combine multiple modalities for comprehensive treatment of pancreatic cancer.

Recent advances in the fields of genetics, metabolism, biology, and immunology of pancreatic cancer have brought new hope for the

early diagnosis and treatment of pancreatic cancer. However, the problem that whether targeted therapy aiming different components in the tumor microenvironment can improve the prognosis of pancreatic cancer patients remains to be investigated. Understanding the changes of tumor microenvironment in the process of metastasis and chemoresistance of pancreatic cancer will make good contributions to breakthroughs in immunotherapeutic studies and provide new insights to the basic and clinical application.

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Disclosure of conflict of interest

None.

Address correspondence to: Menghua Dai and Yupei Zhao, Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 1 Shuai Fu Yuan, Dongcheng District, Beijing 100730, China. E-mail: Daimh@pumch.cn (MHD); zhao8028@263.net (YPZ)

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