Genes & Diseases (2023) 10, 1513–1524

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

REVIEW ARTICLE

The role of TGF-β in the tumor microenvironment of pancreatic cancer

Qiang Luo [1], Ziquan Hu [1], Hongcheng Zhao, Yanmei Fan, Xiancong Tu, Yueqing Wang **, Xiaowen Liu*

Hubei Key Laboratory of Tumor Microenvironment and Immunotherapy, College of Basic Medical Science, China Three Gorges University, Yichang, Hubei 443003, China

Received 6 April 2022; received in revised form 1 October 2022; accepted 20 October 2022
Available online 19 November 2022

KEYWORDS
Cancer therapy;
Pancreatic cancer;
Smad 4;
Transforming growth factor-β;
Tumor microenvironment

Abstract Pancreatic cancer (PC) is an aggressive malignant tumor with low rate of surgical resection and poor prognosis. Transforming growth factor-β (TGF-β) is a cytokine that has both protumor and antitumor activities, depending on tumor microenvironment. The interaction between TGF-β signaling and the tumor microenvironment in PC is complex. Here, we reviewed the role of TGF-β in the tumor microenvironment of PC, highlighting producers of TGF-β and TGF-β responders in the tumor microenvironment of PC.

Introduction

Pancreatic cancer (PC) is a deadly malignant tumor, which is called the king of cancers.[1] According to the American Cancer Society, PC is the fourth leading cause of cancer-related death worldwide[2] and the sixth leading cause of cancer-related death in China,[3] and it is expected to become the second leading cause of cancer death worldwide by 2030 due to its rising incidence and survival rates that have barely improved.[4] The tumor microenvironment (TME) of PC is dynamically evolving during its progression, which is of great significance for the progression and metastasis of PC. Previous studies have shown that transforming growth factor-β (TGF-β) and its signals play an important role in the changes in the pancreatic TME, but most of them did not clarify the specific mechanism. In this paper, we reviewed the progress of TGF-β in pancreatic TME in recent years, and discussed the role of TGF-β in the TME of PC in order to provide some ideas for the discovery of therapeutic targets of PC and the design of related drugs.

* Corresponding author.
** Corresponding author. Hubei Key Laboratory of Tumor Microenvironment and Immunotherapy, College of Basic Medical Science, China Three Gorges University, No.8 Daxue Road, Yichang, Hubei 443003, China.
E-mail addresses: wangyueqing@ctgu.edu.cn (Y. Wang), lxw@ctgu.edu.cn (X. Liu).
Peer review under responsibility of Chongqing Medical University.
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.gendis.2022.10.019

TGF-β and TGF-β family

The mammalian genome encodes 32 TGF-β-related polypeptides, and current research indicates that these TGF-β family members are found in human tissue cells, including bone morphogenetic proteins, growth and differentiation factors, anti-Müllerian tubular hormone proteins, inhibins, activins, nodal and TGF-βs.[5] Although most studies have referred to them as homodimers, various combinations of their heterodimers have also been identified as biologically active proteins. The mammalian genome encodes three different TGF-β isoforms, TGF-β1, -β2, and -β3, which act as disulfide-linked dimers.[6] The three TGF-βs are synthesized as pro-hormones that include a signal sequence, a large N-terminal portion called the latency-associated peptide (LAP), and a short C-terminal segment, which corresponds to the mature active cytokine monomer.[7–9] There are three TGF-β receptors (TGF-βRI, TGF-βRII, and TGF-βRIII). Both TGF-βRI and TGF-βRII contain serine/threonine protein kinases in their cytoplasmic domains while TGF-βRIII has no kinase activity. The binding of TGF-β to TGF-βRII and hetero-tetramerization with TGF-βRI initiate the intracellular signaling via SMADs.[10]

The three TGF-β isoforms are involved in many physiological processes, including cell growth, differentiation, apoptosis, migration, invasion, as well as tissue homeostasis and regeneration.[11–13] They also play the major roles in tumorigenesis, fibrotic disorders, immune malfunctions, and various congenital diseases.[14,15] Particularly, TGF-β1 is the most important TGF-β in human cancer development, which is most relevant to cancer initiation and progression.[11,16]

Relationship between TGF-β and pancreatic cancer

TGF-β induces anti-proliferative responses in many cell types, including normal epithelial cells and transformed cells. It has been suggested that disruption of TGF-β signaling is involved in the pathogenesis of tumorigenesis. According to this hypothesis, the inactivation of mutations and deletions of the TGF-βRII and Smad 4 motifs have been identified in cancer and are thought to be one of the drivers of tumorigenesis.[17,18] The function of TGF-β as an inhibitor of epithelial tumorigenesis may need to be alleviated at the early stages of tumor development, which does not exclude a role for TGF-β signaling at late stages. On the other hand, tumor cells display increased TGF-β expression, most commonly TGF-β1, and secrete TGF-β ligands compared to normal surrounding tissue. In addition, elevated TGF-β expression is associated with tumor progression and poor prognosis, suggesting a pro-cancer role for TGF-β in advanced stages.[19] Therefore, TGF-β signaling both controls the initial development of tumors and promotes the progression of advanced cancers.[20]

The expression of TGF-β is regulated by various factors, and we have found that some genes, proteins, and miRNAs can affect the signaling of TGF-β. They affect the signaling pathway by activating or inhibiting the synthesis of TGF-β protein and thus affecting the occurrence of PC.

TGF-β signaling pathways in pancreatic cancer

There are two TGF-β signaling pathways in PC, the canonical Smad signaling pathway and noncanonical non-Smad signaling pathways. The initiation of TGF-β signaling pathway transmission is the synthesis and release of TGF-β ligands. In classical Smad signaling pathway, TGF-β binds to TGF-βRII on the cell membrane surface, and then phosphorylates TGF-βRI. The Phosphorylated TGF-βRI/TGF-βRII phosphorylates the Smad 2 and Smad 3 proteins, which form a complex with Smad 4. The activated Smad complex moves to the nucleus and binds to specific DNA sequence motifs called Smad-binding elements (SBEs). Then, it interacts with other transcription factors to regulate the expression of TGF-β target genes.[5,21]

In addition, TGF-β can also activate many non-Smad pathways, including PI3K/AKT, JNK/P38, ERK/MAPK signaling pathways, etc.[5,21] Usually these non-Smad pathways also contribute to the progression of PC. PI3K/AKT signaling pathway contributes to tumorigenesis by regulating multiple transcription factors such as nuclear transcription factor-κB (NF-κB) and mammalian target of rapamycin (mTOR) via phosphorylation.[22,23] JNK/P38 signaling pathway leads to increased expression of c-Myc, β-linked protein, and PD-L1, which promotes tumor metastasis.[24] The JNK/P38 signaling pathway also promotes angiogenesis and controls the fate of mesenchymal cells, which promotes tumor progression.[25] ERK activation plays an important role in epithelial-mesenchymal transition (EMT), which is one of the major biological functions of TGF-β.[26] In TGF-β-mediated EMT, ERK/MAPK signaling pathway is considered as one of the most important non-Smad signaling pathways to disrupt intracellular wall junctions and induce cell motility, which promotes invasion and metastasis of PC.[27]

Producers of TGF-β

Most nucleated cells in the body are capable of secreting TGF-β. When cancer occurs, TGF-β is mainly secreted by tumor cells, stromal cells, and macrophages in the tumor infiltration zone in TME.[28–30] In PC, Treg cells are the key source of TGF-β,[31] and Schwann cells are also an important source of TGF-β.[32] TGF-β has a bi-directional role in PC. In the early stage of PC, TGF-β is an important tumor suppressor that inhibits cell proliferation, induces apoptosis, activates autophagy, inhibits growth factor signaling through stromal fibroblasts, suppresses inflammation, and inhibits angiogenesis to maintain the dynamic balance of normal tissues. Meanwhile, in the late stage of PC, it evades apoptosis by inducing EMT, thus promoting the formation of the TME, and facilitating the progression of advanced tumors.[33,34] Related studies show that the interaction between Smad 4, a downstream mediator of TGF-β, and Sox 4, a key transcription factor in embryonic development, partially determines whether TGF-β acts as a tumor suppressor or a tumor initiator.[35] Typically, overexpression of Sox4 plays a tumor-promoting role and Sox4 acts as an independent poor prognostic factor.[35–37] TGF-β may promote aggressiveness in Smad4$^-$ PCs. However, when

cells undergo TGF-β/Smad4-induced EMT in Smad4^{+} PCs, the function of Sox4 changes from pro-tumor to pro-apoptotic due to Snail-mediated repression of Klf5, thus triggering apoptosis of tumor cells and achieving tumor growth inhibition.[35]

Regulation of TGF-β expression

Transmembrane proteins regulate the TGF-β signaling pathway

TGF-β has been widely proved to be an effective inducer and a key control factor of EMT process in PC.[38] Transmembrane protein TMEM158 can mediate TGF-β1 signaling to affect the carcinogenesis of pancreatic cells. TGF-β1 protein plays an important role in the TGF-β signaling pathway. Fu et al reported that the mRNA and protein expression of TGF-β1 is decreased in PC cells with TMEM158 knock-down, and significantly increases in PC cells with TMEM158 overexpression.[22] After TMEM158 knock-down, the activity of the TGF-β1 promoter in PC cells is weakened, and the activity of the TGF-β1 promoter is significantly increased in PC cells with TMEM158. The TGF-β signaling pathway is inhibited by the up-regulation of the dominant-negative TGF-β II receptor (DNR), which significantly blocks the migration and invasion enhancement potential induced by ectopic TMEM158. In short, mechanism-related investigations disclosed that activation of TGF-β might be responsible for TMEM158-triggered PC aggressiveness.[22]

Regulation of TGF-β by other genes

According to the research of Pinho et al,[39] Epithelial Robo 2 loss is associated with the expansion of myofibroblasts and the induction of TGF-β signal transduction (significantly phosphorylated Smad 2 in stromal cells), which also proves the dependence on TGF-β signal transduction. This pathway provides a known stimulus for the matrix remodeling, wound healing, and the fibroproliferative response of acute pancreatitis, chronic pancreatitis, and PDAC, but how the stroma itself affects the development and progression of cancer is a strong debate.[40] TGF-β itself can also have dual effects here, which depend on the cell type (epithelial and matrix) and time (early or late stage of tumor development) of signal transduction; it can act as a tumor suppressor when activated in preconcerting epithelial cells, or as a tumor promoter when activated in advanced cancer cells or TME. In addition, through the Robo 2 deficient mouse model, they found that Wnt and TGF-β pathways were not autonomously activated, and their common target Robo 1 was significantly activated in stromal cells. TGF-β-mediated fibrosis actually requires activation of Wnt signaling.[41] In PDAC patients, high Robo 1 mRNA expression was negatively correlated with the structure of tumor epithelial cells and positively correlated with the markers of activated matrix and Wnt and TGF-β pathways. High-level Robo1 in the tumor matrix can in turn regulate Wnt pathway activation in response to TGF-β activation. This particular mechanism of interaction may form the subject of future research. On the other hand, Robo1 was found not only in myofibroblasts but also in pancreatic tumor epithelium, which may play a unique role in migration and invasion.[42]

Based on the study of Tu et al, it was found that the inactivation of CDKN2B was a necessary condition for the induction of PC. It activated the expression of TGF-β signaling and CDKN2B through carcinogenic overexpression and led to cell senescence together with CDKN2A, and protected cells from Kras-mediated transformation by inhibiting the phosphorylation of retinoblastoma. The expression of carcinogenic Kras in human pancreatic duct cells HPDE6-C7. Kras^{G12D} overexpression up-regulated the expression of CDKN2B and TGF-β1 at mRNA and protein levels. Reporter gene assay showed that CDKN2B promoter activity was up-regulated. This effect was eliminated in the presence of mitogen-activated protein kinase (MEK) inhibitor U0126 or TGF-β inhibitor SB431542, which mediated the Kras signaling pathway. The Smad binding elements in the CDKN2B promoter and TGF-β signaling pathway are necessary to increase CDKN2B expression. These results suggest that Kras upregulates CDKN2B gene transcription through TGF-β signaling.[43]

MiRNAs stimulate the proliferation and metastasis of pancreatic cancer cells by regulating TGF-β2/TGF-βRIII signaling miRNA

Fang et al further explored the potential mechanism of miR-193a on the proliferation and regeneration of pancreatic cancer cells[44]; according to the miR-193a binding site, it indicated that miR-193a might mediate cell regeneration after radiation by regulating the TGF-β2/TGF-βRIII signaling pathway. Notably, E2F6 is also predicted to be the target of miR-193a. It is well known that E2F6 can act as a dominant-negative suppressor to inhibit E2F- and Myc-responding genes. It was found that the ectopic expression of miR-193a decreased the protein expression of TGF-β2, TGF-βRIII, and E2F6, while in AsPC-1 cells transfected with miR-193a antagomir, inhibiting miR-193a could increase the expression of these proteins compared with the control group. TGF-β2, TGF-βRIII, and E2F6 were the target genes of miR-193a. In addition, the repair of TGF-β2 in PC cells with high miR-193a expression can eliminate the inhibitory effect of p-Smad 2, p-Smad 3, and E2F6, and inhibit the increase of c-Myc expression caused by ectopic expression of miR-193a.

Through TGF-β2/TGF-βRIII/Smads/E2F6/c-Myc signaling pathway, Fang et al showed that miR-193a promoted the regeneration of PC after radiation, and they explored whether miR-193a would affect intercellular connection and further promote metastasis. It was found that ectopic expression of miR-193 significantly reduced the expression of E-cadherin and N-cadherin and may promote tumor metastasis by inhibiting TGF-β2/TGF-βRIII/ARHGEF15/ABL2 signal transduction.

In addition, the expression of TGF-β2, TGF-βRIII, ARHGEF15, and ABL2 is lower than that in PC cells without miR-193a treatment, suggesting that miR-193a may destroy the connection between PC cells and promote tumor metastasis by inhibiting TGF-β2/TGF-βRIII/ARHGEF15/ABL2 signal transduction. In addition, according to the comparison of tumor growth after radiation between the experimental group and the control group, TGF-β2 can offset the effect of miR-193a overexpression on the proliferation and

regeneration of PC cells, while inhibit miR-193a or restore the TGF-β2/TGF-βRIII signaling pathway that can inhibit the proliferation and metastasis of PC cells after radiotherapy and prolong the survival time of patients (Fig. 1).

TGF-β responders in pancreatic TME

TGF-β impacts TME of pancreatic cancer

The major histological subtype of PC is pancreatic ductal adenocarcinoma (PDAC), which comprises approximately 90% of all PCs. Oncogenesis is rooted in genetic mutations. In PDAC, there are four common driving mutations (Kras, CDKN2A, TP53 and Smad 4).[45] TGF-β signaling pathway is one of the 12 core signaling pathways in PC, and is also closely associated with driver gene mutations in PC.[46] PC develops in the microenvironment, the stroma enriched with extracellular matrix proteins, mainly produced by stellate cells known as cancer-associated fibroblasts (CAFs), inflammatory cells such as mast cells, and small blood vessels that recent evidence suggests are a dynamic compartment rather than a mechanical barrier, strongly involved in the process of tumor formation, progression, invasion, and metastasis.[47,48] TGF-β plays an important role in the formation of such a microenvironment. TGF-β has a bidirectional role in regulating the development of PC at different stages, but TGF-β and its signaling pathway only contribute to generating a favorable microenvironment for tumor growth and metastasis during all steps of carcinogenesis at the microenvironmental level.[49,50] TGF-β promotes the secretion of pro-angiogenic factors such as matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase 9 (MMP-9) in the TME of PC, and downregulates the expression of anti-angiogenic factors, such as tissue inhibitor of MMP (TIMP), by recruiting inflammatory cells in the TME, thus keeping the blood vessels in a state of growth and remodeling, which provides good conditions for tumor growth and metastasis.[51–53] Among them, vascular endothelial growth factor (VEGF) is the main response factor for TGF-β-induced angiogenesis through JNK/P38 signaling pathway and others.[25,53] Another major alteration in the TME of pancreatic cancer is due to EMT, and TGF-β is one of the major inducers of EMT, which promotes tumor cell invasion and metastasis.[54] Induced by TGF-β, the level of E-cadherin is decreased and the vimentin level is increased in the pancreatic TME, which leads to the development of EMT.[55] In addition, TGF-β modifies the liver to initiate pre-metastatic niche formation via activated hepatic stellate cells and remodeling ECM in the liver microenvironment.[56,57]

Tumor cells and many components in the pancreatic TME respond to the TGF-β signaling; therefore, TGF-β plays an important and complex regulatory role in the development of PC and changes in the TME of PC (Fig. 2 and Table 1).[58]

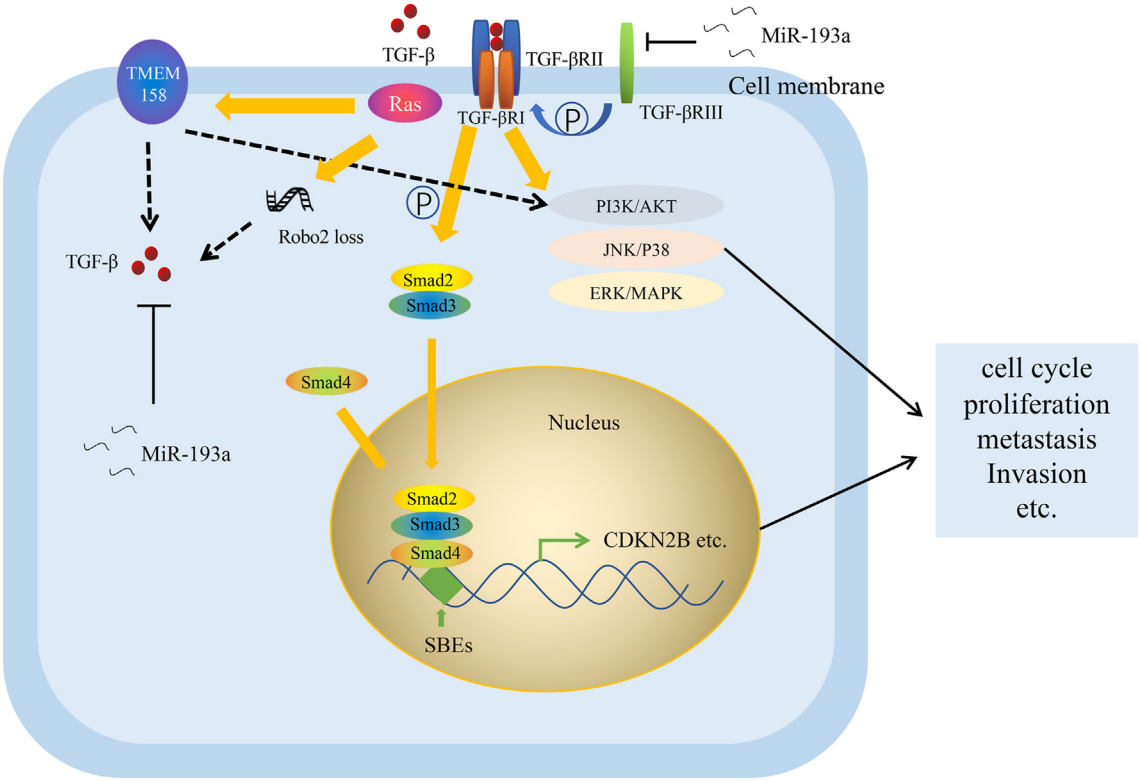

Figure 1 Regulation of TGF-β signaling in pancreatic cancer. There are two TGF-β signaling pathways that regulate the cellular effects of PC at different stages of the tumor. These two signaling pathways can also be regulated from protein and gene levels. Expression of TGF-β is increased in pancreatic cancer cells with TMEM158 overexpression. Robo 2 gene expression is lost under different Ras activation conditions, thereby promoting TGF-β expression. In contrast, miR-193a induces the opposite effect. miR-193a reduces TGF-β expression by regulating transcriptional and translational processes, and also directly targets TGF-βRIII so that TGF-β cannot bind to it.

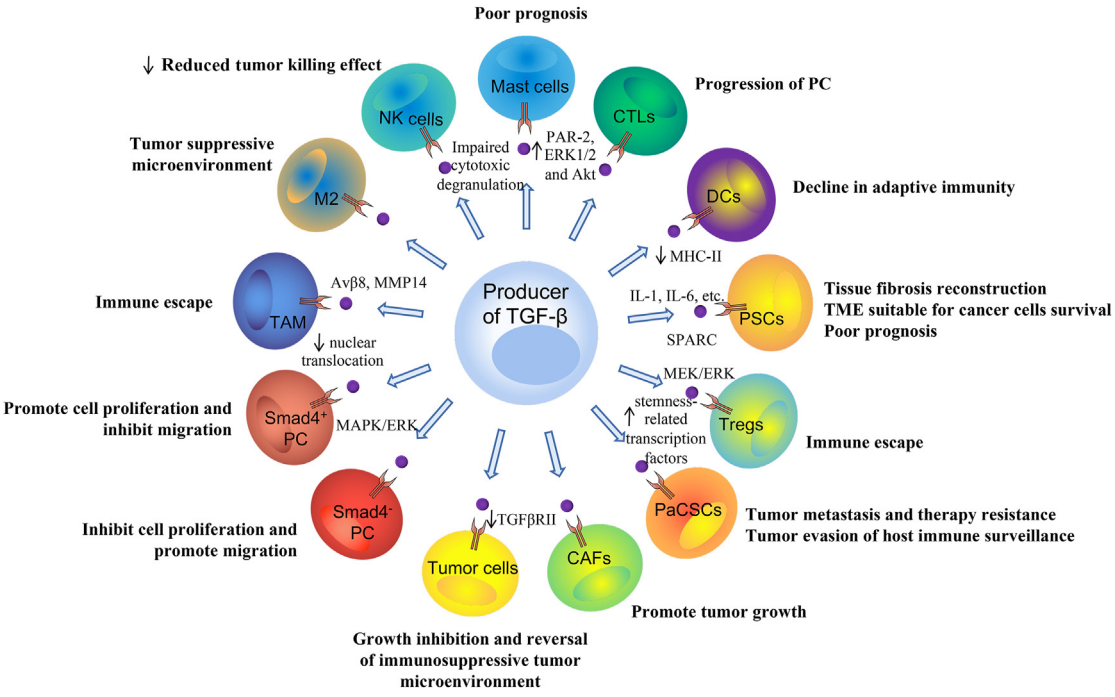

Figure 2 The effect of TGF-β in the tumor microenvironment of pancreatic cancer. Tumor cells and many components in the pancreatic tumor microenvironment can respond to TGF-β signaling. TGF-β has an important regulatory role in the pancreatic tumor microenvironment. The response effects of various cells in the pancreatic tumor microenvironment to TGF-β are summarized in the box and the specific response mechanism is summarized in Table 1.

Table 1 TGF-β responders in tumor microenvironment and their roles.

Responder	Effect	Mechanism
Tumor cells	Growth inhibition	Deletion or Downgrade of TGF-βRII
	Reversal of immunosuppressive tumor microenvironment	Inhibition of mesenchymal TGF-βRII
Smad 4^{+} PDAC cells	Promote cell proliferation and inhibit migration	Reduction of nuclear translocation of Smad 4
Smad 4^{-} PDAC cells	Inhibit cell proliferation and promote migration	Activation of MAPK/ERK signaling pathway
Pancreatic stellate cells	Tissue fibrosis reconstruction	Secretion of large amounts of extracellular matrix
	Tumor microenvironment suitable for cancer cells survival	Secretion of cytokines, such as IL-1, IL-6, etc.
	Poor prognosis	Synthesis of SPARC
Cancer-associated fibroblasts	Promote tumor growth	Differentiation of pancreatic stellate cells to myCAFs
Pancreatic cancer stem cells	Tumor metastasis and therapy resistance	Overexpression of stemness-related transcription factors
	Tumor evasion of host immune surveillance	
Tregs	Immune escape	Activation of MEK/ERK signaling pathway
Dendritic cells	Decline in adaptive immunity	Decreased expression of MHC-II gene
	Growth and metastasis of pancreatic cancer	Immune escape
CD8^{+} cytotoxic T cells	Progression of pancreatic cancer	Inhibiting the clonal proliferation of CD8^{+} T cells
		Inhibiting the cytotoxicity of CD8^{+} T cells
		Decreased antigen presentation by dendritic cells
Mast cells	Poor prognosis	Increased activation of PAR-2, ERK1/2 and Akt
Natural killer cells	Reduced tumor killing effect	Impaired cytotoxic degranulation of natural killer cells
Macrophages in the early stage	Reduction of tumorigenic tumor microenvironment	Conversion of monocytes to M2 macrophage phenotype
Macrophages in the advanced stage	Immune escape	Degradation of MYD88

Tumor cells

Tumor cells are the main responders to TGF-β signaling and the main secretors of TGF-β in the pancreatic TME. Plenty of evidence supports the notion that increased TGF-β expression by tumor cells leads to ECM deposition and tissue fibrosis, which disrupts immune and inflammatory functions, enhances tumor cell migration, invasion, and survival in later stages of tumorigenesis, thereby promoting tumor progression, stimulating angiogenesis, promoting EMT, increasing migration and invasion, and maintaining cancer stem cells.[59] TGF-β signaling through the type II receptor mediates growth inhibition of carcinoma cells. When TGF-βRII is deleted or downregulated, chemokine/chemokine-receptor signaling is increased, such as CXCL1-CXCL5/CXCR2 and SDF-1-CXCR4 leading to tumor invasion.[60] Inhibition of mesenchymal TGF-βRII reduces IL-6 production by cancer-associated fibroblasts, thereby reducing STAT3 activation in tumor cells and reversing the immunosuppressive TME.[61] In Smad 4^{+} PDAC cells, TGF-β1-induced autophagy promotes proliferation and inhibits migration by reducing the nuclear translocation of Smad4. In contrast, in $Smad4^{-}$ PDAC cells, TGF-β1-induced autophagy inhibits $Smad4^{-}$ cell proliferation and promotes migration by regulating MAPK/ERK activation.[62–64] In addition, TGF-β1 may create a favorable environment for tumor growth by inhibiting anti-tumor immunity, including NK cell function, in addition to its direct anti-tumor effects.[65]

Pancreatic stellate cells

Pancreatic stellate cells (PSCs) are the precursors of CAFs, which are one of the major components of the pancreatic TME, and the major mesenchymal cells in PC are also derived from PSCs.[66–68] Under normal conditions, PSCs are in a resting state. When inflammation or cancer occurs in the pancreas, PSCs change from a resting state to an activated state, promoting the growth and progression of pancreatic tumors.[69] TGF-β plays a major role in the transition from resting state to activated state in PSCs.[70] When PSCs are activated, on the one hand, they secrete a large amount of extracellular matrix to promote pancreatic tissue fibrosis reconstruction; on the other hand, they secrete a large number of cytokines, such as TGF-β, IL-1, and IL-6, to promote EMT, thus promoting the formation of a TME suitable for tumor cell survival and making PC more aggressive.[71–73]

Connective tissue hyperplasia is a hallmark of PC and consists of fibrotic cells and secretory extracellular matrix (ECM).[74] Tanaka et al used PSCs, a major cellular component of PC connective tissue, to generate a three-dimensional fibrosis model with a thickness up to the range of clinical observation. Using this model, they found that dependent on TGF-β/Rho-Associated Kinase (ROCK) signaling and matrix metalloproteinase activity, collagen fibril deposition was increased and fibronectin linkage direction was substantially reshaped by PSCs; abnormal ECM remodeling was important for the progression of PC.[75] It has also been shown that PSCs can synthetically express Secreted Protein, Acidic and Rich in Cysteine (SPARC), that there is a complex regulatory network between SPARC and TGF-β, and that the expression of SPARC in the mesenchyme of PC is a signal of poor prognosis.[75–77]

Cancer-associated fibroblasts

CAFs develop from PSCs, which are also a component of the TME of PC, and are one of the major responders to TGF-β signaling in pancreatic TME. TGF-β plays an integral role in promoting the activation, proliferation and differentiation of CAFs, and it also regulates the shape, stiffness and invasion of CAFs.[78–81] Biffi et al identified two subtypes of CAFs: one expresses inflammatory markers such as interleukin-6 (IL-6) and leukemia inhibitory factor (LIF) and is named inflammatory CAFs (iCAFs); the other expresses markers of myofibroblastic cells such as αSMA and is named myofibroblastic CAFs (myCAFs). Under the induction of interleukin-1 (IL-1), PSCs activate the downstream JAK/STAT signaling pathway in the autocrine form of LIF to differentiate and develop toward the iCAFs phenotype; TGF-β antagonizes this process by downregulating IL1R1 expression and promoting myofibroblast differentiation to induce PSCs development into the myCAFs phenotype, thus selectively promoting PSC differentiation into the CAF phenotype that promotes tumor cell growth.[82,83]

Pancreatic cancer stem cells

Stem cells play an important role in the initiation and development of cancer.[84,85] Evidence suggests that pancreatic cancer stem cells (PaCSCs) contribute to tumor metastasis and therapy resistance and the TGF-β signaling pathway may coordinate the acquisition of stem cell properties by PC cells.[86–90] TGF-β increases the expression of stemness-related transcription factors in PC through the Smad signaling pathway and promotes the acquisition of stemness characteristics by PC cells.[91] In addition, TGF-β forms a positive feedback loop with stem cell marker "nestin", driving the EMT that promotes tumor progression, maintains PDAC cells with a high metastatic ability, and escapes host immune surveillance.[92]

Tumorigenic immune cells

TGF-β mainly plays an immunosuppressive role in PC. It helps pancreatic cancer cells evade immune surveillance by suppressing host immune detection, thus accelerating the growth and metastasis of pancreatic cancer cells, and some scholars even advocate that TGF-β is the main immune checkpoint in PC patients.[60,93]

Macrophages

Macrophages play a key role in PDAC growth and metastasis. In the early stage of cancer, TGF-β reduces the tumorigenic TME by regulating macrophages.[94] Monocytes in the TME usually differentiate toward an M2 macrophage phenotype with anti-inflammatory, immune-suppressive, and pro-angiogenic functions.[8] In the advanced stage of cancer, tumor-associated macrophages (TAMs) mobilize activated TGF-β via integrin αvβ8 and matrix metalloproteinase 14 (MMP14).[95] At this time, TGF-β is one of the main immunosuppressive cytokines produced by TAMs. TGF-β signaling in macrophages also inhibits the anti-inflammatory response mediated by transcription factor NF-κB by promoting the degradation of MYD88, a key protein that

activates the NF-κB pathway.[96] Based on the key role of macrophages in PDAC, macrophage-targeted therapy offers a new option for PDAC patients.[91]

Tregs

Zhang et al reported that regulatory T cells (Tregs) are one of the key sources of TGF-β ligands,[31] and also Tregs are one of the important responder cells in the TME of PC. $Kras^{G12D}$ mutation activates the MEK/ERK pathway in PC, which upregulates the levels of interleukin-10 (IL-10) and TGF-β, and induces Foxp3 expression, thereby inducing Tregs transformation. This process contributes to the immunosuppressive TME and promotes immune evasion.[97,98]

Dendritic cells

Dendritic cells (DCs) are the main professional antigen-presenting cells (APCs).[99] DCs in the TME are regulated by TGF-β signaling: on the one hand, the downregulated MHC-II gene, whose antigen-presenting ability is greatly inhibited, leads to a decrease in the adaptive immune capacity of the body; on the other hand, they are induced to differentiate into an immature myeloid cell phenotype with potent immune suppressor functions, leading to immunosuppression, thus promoting the growth and metastasis of PC.[100]

$CD8^+$ cytotoxic T cells

The response of $CD8^+$ cytotoxic T cells (CTLs) to TGF-β signaling is divided into two parts: TGF-β inhibits the clonal expansion of $CD8^+$ T cells *in vivo* and suppresses the cytotoxicity of $CD8^+$ T cells. Together, they will eventually promote tumor progression.[101] The specific mechanisms are that inhibition of perforin, granzyme B and A, interferon-gamma (IFN-γ), and FAS ligand (FASL) expression, generation of Treg cells, and promotion of M2-macrophages (which secrete other immunosuppressive cytokines such as IL-10, and EMT induction).[101–103] In addition, the decreased function of CTLs is also partly due to the decreased antigen-presenting role of DCs.[104]

Mast cells

In pancreatic TME, mast cells promote angiogenesis, formation of the desmoplastic microenvironment and invasion of the tumor, thus leading to poor prognosis.[105,106] It has been experimentally demonstrated that the malignant role of mast cells in PC can be mediated by TGF-β signaling.[107] However, the exact mechanism of how they receive TGF-β signaling in PC remains to be explored. Recently, Otsuru et al found that mast cells induce the release of TGF-β1 and increase the activation of PAR-2, ERK1/2, and Akt expression, thus promoting the malignant process of PC. This process antagonizes the chemotherapeutic effect of gemcitabine/nab-paclitaxel (GEM/NAB) and leads to chemoresistance.[108]

Natural killer cells

Natural killer (NK) cells are the key effectors in cancer immunosurveillance and can be used as a prognostic biomarker in diverse cancers. During PC progression, the function of NK cells is reduced and the killing effect on tumor cells is diminished. Jun et al found that high levels of TGF-β in PC led to impaired cytotoxic degranulation of NK cells, resulting in impaired NK cells function and reduced natural killer effect on tumor cells. Therefore, NK cells degranulation can be used to predict the prognosis of PC patients.[65]

TGF-β based anti-cancer therapy

Based on the dual role of the TGF-β signaling pathway in PC genesis and its regulatory role in PC progression, many clinical treatment attempts have been made and many research advances have been made for the TGF-β signaling pathway in PC.

Immunotherapy

Here, following the finding that TGF-β suppresses T helper 2 (TH2)-cell-mediated cancer immunity, Li et al shows that blocking TGF-β signaling in $CD4^+$ T cells remodels the TME and restrains cancer progression.[109]

Galunisertib is a TGF-β blockade which is being investigated as a potential tumor immunotherapy candidate drug in clinical trials. However, primary or acquired resistance is often found in the recruited cancer patients, which limits its clinical application.[110] However, it has demonstrated better efficacy when used in combination with other drugs.

Melisi et al demonstrated through clinical trials that the Galunisertib-Gemcitabine combination improves overall survival with minimal toxicity.[111] Gueorguieva et al concluded by meta-analysis in agreement with Melisi et al and their pharmacokinetic analysis led to the conclusion that 300 mg/day galunisertib administered as 150 mg twice daily for 14 days on/14 days off treatment is an appropriate dosing regimen for patients with PC.[112]

Gal can also be used in combination with immune-targeted therapy.[113] In immunocompetent murine models, Liu et al improved chemotherapy efficacy through combined blockade of TGF-β1 and GM-CSF by inhibiting M2-polarized TAM and inducing $CD8^+$ T cells, which is considered the best potential target for improving chemotherapy efficacy.[114] Wang et al significantly increased tumor $CD8^+$ T cell infiltration and cytotoxicity by inhibiting both the TGF-β pathway and the PD-1/PD-L1 checkpoint and stimulated anti-tumor immunity to synergistically inhibit tumor growth.[115]

Other therapies

In addition to the above-mentioned small molecule drugs that specifically target TGF-β signaling, other seemingly tumor-unrelated drugs are also closely associated with TGF-β in PC and are closely related to the subsequent progression of PC, promising to be applied in the adjuvant treatment of PC with sufficient clinical evidence. Shi et al confirmed that combination therapy of TGF-β blockade and commensal-derived probiotics provides enhanced anti-tumor immune response and tumor suppression.[110] Magnolol inhibits cell proliferation, migration, and invasion *in vitro* and *in vivo* by inhibiting the TGF-β signaling pathway and EMT, therefore, it may be a potential drug for the treatment of PC.[116] Vitamin D has an inhibitory effect on EMT, while partially reversing PDAC cell-induced changes in intracellular calcium in immune cells and preventing PC-induced apoptotic signaling in inflammatory cells.[117]

Together with the ability to induce small amounts of TGF-β release, which may lead to an overall anti-tumor response, the combination of vitamin D and TGF-β inhibition could be an option for PC treatment.[118]

Relationship between TGF-β and the mechanism of chemotherapy resistance in pancreatic tumor microenvironment

For microenvironment in PC, conventional chemotherapy regimens will produce drug resistance, mainly through CAFs, myofibroblasts, mast cells, and other pathways.

Drug resistance caused by CAFs

There may be several reasons for the drug resistance of CAFs. The existing chemotherapy regimens, such as gemcitabine or albumin-taxel, are highly toxic, and patients are susceptible to drug resistance.[119] Therefore, finding ways to overcome the tumor interstitial barrier is the key to the success of PDAC chemotherapy.

The dominant cell group of the tumor matrix in PDAC is activated PSCs or CAFs, which can secrete a large number of ECM to compress intertumoral blood vessels and hinder effective chemotherapeutic drug delivery. In addition, CAFs, as "criminal partners", support and promote tumor progression and metastasis by producing active exosomes while creating immunosuppressive microenvironments.[120]

Feng et al found that to regulate the tumor microenvironment, CREKA peptide-targeted biodegradable polymer nanoparticles were constructed and loaded with TCMα-M. CRE-NP (α-M) not only has inactive CAFs but also effectively reduces ECM production *in vitro* and *in vivo* by blocking the TGF-β signaling pathway. In addition, the use of CRE-NP (α-M) to initiate a matrix-rich tumor environment can promote the normalization of tumor blood vessels and enhance blood perfusion, which is beneficial to the subsequent infiltration and distribution of chemotherapeutic drugs or nano-preparations in the tumor site. Therefore, non-toxic nano-Chinese medicine as a target, combined with chemotherapy, is expected to improve the chemotherapy effect of PC and provide new therapeutic ideas for the treatment of connective tissue proliferative tumors.[80]

Drug resistance in myofibroblasts

In Dominguez's study,[121] the TGF-β-driven cell population was identified as the most common CAFs in advanced tumors. In addition, they found that the LRRC15$^+$CAFs signal of myofibroblasts was related to the adverse reactions of several different types of human tumors to immune checkpoint blockade. These cells have myofibroblast characteristics and dominant ECM gene characteristics, which constitute the majority of CAFs in PDAC patients, mainly immune rejection phenotype. This indicates that these cells have immunoregulatory effects. It will be valuable to further explore whether early CAFs can avoid the tumor-causing fate of LRRC15$^+$ CAFs, or whether the phenotype of LRRC15$^+$ CAFs can be reversed to improve the efficacy of immunotherapy.

The team chose to focus on LRRC15$^+$ CAFs because they are ubiquitous in PDAC, but IL-1 CAFs have a transcription process that suggests the immune regulation of TME. The inhibition of the JAK signaling in PDAC has been proved to be related to the reduction of IL-1 CAFs and tumor burden.[82] Although it is difficult to distinguish between the direct effect of these inhibitors on tumor cells and the effect of IL-1 CAFs loss in TME, it should be noted that despite many transcriptional differences, both CAF types also express genes associated with myofibroblast characteristics, and both express various immunomodulatory and even inflammatory mediators. For TGF-β-CAFs, we have identified LRRC15$^+$ CAFs expression as a good proxy for multiple cancer indications[122,123]

Mast cell-generated gemcitabine resistance

A large amount of evidence has shown that chemotherapeutic drugs reshape the tumor microenvironment and promote tumor regrowth and drug resistance by promoting the release of pro-inflammatory and immunosuppressive cytokines.[124] In Porcelli's study,[108] by simulating mast cell crosstalk, GEM/NAB was found to promote the release of TGF-β1 from tumor cells, so that GEM/NAB becomes resistant in cells where TGF-β1 and PAR-2 signaling interact functionally. By dividing patients included in the prospective study into responding and non-responding groups, it was found that non-response to GEM/NAB was associated with significantly elevated TGF-β1, IL-6, IL-8, MIF, and CXCL11 in patients' blood, as well as elevated trypsin. It is well known that the release of TGF-β1 and IL-6 is triggered by the control of down-rod and TGF-β1 signaling while increasing MIF and CXCL11 accounts for the development of an immunosuppressive microenvironment, which is a characteristic of tumors with strong deregulation of activated ERK1/2 and TGF-β1 signaling.[125] The levels of trypsin did not reach significant *P*-values, but the above results strongly suggest a clinical impact of mast cells beyond their recognized role in tumor prognosis, as mast cells may play a crucial role in anti-GEM/NAB.

Conclusions

TGF-β plays a dual role during the development of PC. In the early stage of PC, TGF-β is an important tumor suppressor that inhibits cell proliferation. Meanwhile, in the late stage of PC, TGF-β promotes the formation of the tumor microenvironment and facilitates the progression of advanced tumors. Most nucleated cells in the body are capable of secreting TGF-β, and most cells in the tumor microenvironment of PC can respond to TGF-β signaling. Therefore, TGF-β has an important and complex regulatory role in the development of PC and changes in the tumor microenvironment of PC. With the clarification of its regulatory mechanism, many therapeutic approaches targeting TGF-β signaling have been put into clinical trials, which will help improve the survival rate and prognosis of patients with PC. In addition, chemotherapy resistance induced by TGF-β signaling in the tumor microenvironment of PC increases the difficulties in the treatment of PC patients. In order to overcome the tumor interstitial barrier, we need to

thoroughly elucidate the mechanism behind the chemotherapy resistance. Besides, we need to explore better treatments.

Conflict of interests

The authors declare no conflict of interests.

Funding

This work was supported by The National Natural Science Foundation of China (No. 81402568); Open Fund of Hubei Key Laboratory of Tumor Microenvironment and Immunotherapy (Three Gorges University, China) (No. 2019KZL02, 2020KZL03).

References

1. Kamisawa T, Wood LD, Itoi T, et al. Pancreatic cancer. *Lancet*. 2016;388(10039):73—85.
2. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018:GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394—424.
3. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. *CA Cancer J Clin*. 2016;66(2):115—132.
4. Rahib L, Smith BD, Aizenberg R, et al. Projecting cancer incidence and deaths to 2030:the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res*. 2014;74(11):2913—2921.
5. David CJ, Massagué J. Contextual determinants of TGFβ action in development, immunity and cancer. *Nat Rev Mol Cell Biol*. 2018;19(7):419—435.
6. Daopin S, Piez KA, Ogawa Y, et al. Crystal structure of transforming growth factor-beta 2:an unusual fold for the superfamily. *Science*. 1992;257(5068):369—373.
7. Derynck R, Turley SJ, Akhurst RJ. TGFβ biology in cancer progression and immunotherapy. *Nat Rev Clin Oncol*. 2021;18(1):9—34.
8. Batlle E, Massagué J. Transforming growth factor-β signaling in immunity and cancer. *Immunity*. 2019;50(4):924—940.
9. Travis MA, Sheppard D. TGF-β activation and function in immunity. *Annu Rev Immunol*. 2014;32:51—82.
10. Massagué J. TGFβ signalling in context. *Nat Rev Mol Cell Biol*. 2012;13(10):616—630.
11. de Streel G, Lucas S. Targeting immunosuppression by TGF-β1 for cancer immunotherapy. *Biochem Pharmacol*. 2021;192:114697.
12. Zhao M, Mishra L, Deng CX. The role of TGF-β/SMAD4 signaling in cancer. *Int J Biol Sci*. 2018;14(2):111—123.
13. Morikawa M, Derynck R, Miyazono K. TGF-β and the TGF-β family: context-dependent roles in cell and tissue physiology. *Cold Spring Harbor Perspect Biol*. 2016;8(5):a021873.
14. Meng XM, Nikolic-Paterson DJ, Lan HY. TGF-β: the master regulator of fibrosis. *Nat Rev Nephrol*. 2016;12(6):325—338.
15. Tauriello DVF, Batlle E. Targeting the microenvironment in advanced colorectal cancer. *Trends Cancer*. 2016;2(9):495—504.
16. Seoane J, Gomis RR. TGF-β family signaling in tumor suppression and cancer progression. *Cold Spring Harbor Perspect Biol*. 2017;9(12):a022277.
17. Markowitz S, Wang J, Myeroff L, et al. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science*. 1995;268(5215):1336—1338.
18. Hahn SA, Schutte M, Hoque AT, et al. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science*. 1996;271(5247):350—353.
19. Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. *Cancer Cell*. 2011;20(5):576—590.
20. Pickup M, Novitskiy S, Moses HL. The roles of TGFβ in the tumour microenvironment. *Nat Rev Cancer*. 2013;13(11):788—799.
21. Robertson IB, Rifkin DB. Regulation of the bioavailability of TGF-β and TGF-β-related proteins. *Cold Spring Harb Perspect Biol*. 2016;8(6):a021907.
22. Fu Y, Yao N, Ding D, et al. TMEM158 promotes pancreatic cancer aggressiveness by activation of TGFβ1 and PI3K/AKT signaling pathway. *J Cell Physiol*. 2020;235(3):2761—2775.
23. Wu B, Guo B, Kang J, et al. Downregulation of Smurf2 ubiquitin ligase in pancreatic cancer cells reversed TGF-β-induced tumor formation. *Tumour Biol*. 2016;37:16077—16091.
24. Hussain SM, Kansal RG, Alvarez MA, et al. Role of TGF-β in pancreatic ductal adenocarcinoma progression and PD-L1 expression. *Cell Oncol*. 2021;44(3):673—687.
25. Batlle R, Andrés E, Gonzalez L, et al. Regulation of tumor angiogenesis and mesenchymal—endothelial transition by p38α through TGF-β and JNK signaling. *Nat Commun*. 2019;10:3071.
26. Lee JM, Dedhar S, Kalluri R, et al. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. *J Cell Biol*. 2006;172(7):973—981.
27. Davies M, Robinson M, Smith E, et al. Induction of an epithelial to mesenchymal transition in human immortal and malignant keratinocytes by TGF-beta1 involves MAPK, Smad and AP-1 signalling pathways. *J Cell Biochem*. 2005;95(5):918—931.
28. Sanjabi S, Oh SA, Li MO. Regulation of the immune response by TGF-β: from conception to autoimmunity and infection. *Cold Spring Harbor Perspect Biol*. 2017;9(6):a022236.
29. Liénart S, Merceron R, Vanderaa C, et al. Structural basis of latent TGF-β1 presentation and activation by GARP on human regulatory T cells. *Science*. 2018;362(6417):952—956.
30. Qin Y, Garrison BS, Ma W, et al. A milieu molecule for TGF-β required for microglia function in the nervous system. *Cell*. 2018;174(1):156—171.
31. Zhang Y, Lazarus J, Steele NG, et al. Regulatory T-cell depletion alters the tumor microenvironment and accelerates pancreatic carcinogenesis. *Cancer Discov*. 2020;10(3):422—439.
32. Roger E, Martel S, Bertrand-Chapel A, et al. Schwann cells support oncogenic potential of pancreatic cancer cells through TGFβ signaling. *Cell Death Dis*. 2019;10(12):886.
33. Drubay V, Skrypek N, Cordiez L, et al. TGF-βRII knock-down in pancreatic cancer cells promotes tumor growth and gemcitabine resistance. importance of STAT3 phosphorylation on S727. *Cancers*. 2018;10(8):254.
34. Principe DR, Doll JA, Bauer J, et al. TGF-β: duality of function between tumor prevention and carcinogenesis. *J Natl Cancer Inst*. 2014;106(2):djt369.
35. David CJ, Huang YH, Chen M, et al. TGF-β tumor suppression through a lethal EMT. *Cell*. 2016;164(5):1015—1030.
36. Xu X, Zong K, Wang X, et al. miR-30d suppresses proliferation and invasiveness of pancreatic cancer by targeting the SOX4/PI3K-AKT axis and predicts poor outcome. *Cell Death Dis*. 2021;12(4):350.
37. Hasegawa S, Nagano H, Konno M, et al. A crucial epithelial to mesenchymal transition regulator, Sox4/Ezh2 axis is closely related to the clinical outcome in pancreatic cancer patients. *Int J Oncol*. 2016;48(1):145—152.
38. Giannelli G, Koudelkova P, Dituri F, et al. Role of epithelial to mesenchymal transition in hepatocellular carcinoma. *J Hepatol*. 2016;65(4):798—808.

39. Pinho AV, van Bulck M, Chantrill L, et al. ROBO2 is a stroma suppressor gene in the pancreas and acts via TGF-β signalling. *Nat Commun*. 2018;9(1):5083.
40. Neesse A, Algül H, Tuveson DA, et al. Stromal biology and therapy in pancreatic cancer: a changing paradigm. *Gut*. 2015;64(9):1476—1484.
41. Akhmetshina A, Palumbo K, Dees C, et al. Activation of canonical Wnt signalling is required for TGF-β-mediated fibrosis. *Nat Commun*. 2012;3:735.
42. Zhang QQ, Zhou DL, Lei Y, et al. Slit2/Robo1 signaling promotes intestinal tumorigenesis through Src-mediated activation of the Wnt/β-catenin pathway. *Oncotarget*. 2015;6(5):3123—3135.
43. Tu Q, Hao J, Zhou X, et al. CDKN2B deletion is essential for pancreatic cancer development instead of unmeaningful co-deletion due to juxtaposition to CDKN2A. *Oncogene*. 2018;37(1):128—138.
44. Fang C, Dai CY, Mei Z, et al. microRNA-193a stimulates pancreatic cancer cell repopulation and metastasis through modulating TGF-β2/TGF-βRIII signalings. *J Exp Clin Cancer Res*. 2018;37(1):25.
45. Carr RM, Fernandez-Zapico ME. Toward personalized TGFβ inhibition for pancreatic cancer. *EMBO Mol Med*. 2019;11(11):e11414.
46. Jones S, Hruban RH, Kamiyama M, et al. Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. *Science*. 2009;324(5924):217.
47. Nielsen MFB, Mortensen MB, Detlefsen S. Key players in pancreatic cancer-stroma interaction: cancer-associated fibroblasts, endothelial and inflammatory cells. *World J Gastroenterol*. 2016;22(9):2678—2700.
48. Gore J, Craven KE, Wilson JL, et al. TCGA data and patient-derived orthotopic xenografts highlight pancreatic cancer-associated angiogenesis. *Oncotarget*. 2015;6(10):7504—7521.
49. Neuzillet C, Tijeras-Raballand A, Cohen R, et al. Targeting the TGFβ pathway for cancer therapy. *Pharmacol Ther*. 2015;147:22—31.
50. Karamitopoulou E. Tumour microenvironment of pancreatic cancer: immune landscape is dictated by molecular and histopathological features. *Br J Cancer*. 2019;121(1):5—14.
51. Binker MG, Binker-Cosen AA, Gaisano HY, et al. TGF-β1 increases invasiveness of SW1990 cells through Rac1/ROS/NF-κB/IL-6/MMP-2. *Biochem Biophys Res Commun*. 2011;405(1):140—145.
52. Sui H, Zhao J, Zhou L, et al. Tanshinone IIA inhibits β-catenin/VEGF-mediated angiogenesis by targeting TGF-β1 in normoxic and HIF-1α in hypoxic microenvironments in human colorectal cancer. *Cancer Lett*. 2017;403:86—97.
53. Ahmed S, Bradshaw AD, Gera S, et al. The TGF-β/Smad4 signaling pathway in pancreatic carcinogenesis and its clinical significance. *J Clin Med*. 2017;6:5.
54. Heldin CH, Vanlandewijck M, Moustakas A. Regulation of EMT by TGFβ in cancer. *FEBS Lett*. 2012;586(14):1959—1970.
55. Wang W, Dong L, Zhao B, et al. E-cadherin is downregulated by microenvironmental changes in pancreatic cancer and induces EMT. *Oncol Rep*. 2018;40(3):1641—1649.
56. Teraoka H, Sawada T, Nishihara T, et al. Enhanced VEGF production and decreased immunogenicity induced by TGF-beta 1 promote liver metastasis of pancreatic cancer. *Br J Cancer*. 2001;85(4):612—617.
57. Costa-Silva B, Aiello NM, Ocean AJ, et al. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat Cell Biol*. 2015;17(6):816—826.
58. Melzer C, Hass R, von der Ohe J, et al. The role of TGF-β and its crosstalk with RAC1/RAC1b signaling in breast and pancreas carcinoma. *Cell Commun Signal*. 2017;15(1):19.
59. Neuzillet C, de Gramont A, Tijeras-Raballand A, et al. Perspectives of TGF-β inhibition in pancreatic and hepatocellular carcinomas. *Oncotarget*. 2014;5(1):78—94.
60. Yang L, Pang Y, Moses HL. TGF-β and immune cells: an important regulatory axis in the tumor microenvironment and progression. *Trends Immunol*. 2010;31(6):220—227.
61. Huang H, Zhang Y, Gallegos V, et al. Targeting TGFβR2-mutant tumors exposes vulnerabilities to stromal TGFβ blockade in pancreatic cancer. *EMBO Mol Med*. 2019;11(11):e10515.
62. Liang C, Shi S, Qin Y, et al. Localisation of PGK1 determines metabolic phenotype to balance metastasis and proliferation in patients with SMAD4-negative pancreatic cancer. *Gut*. 2020;69(5):888—900.
63. He R, Wang M, Zhao C, et al. TFEB-driven autophagy potentiates TGF-β induced migration in pancreatic cancer cells. *J Exp Clin Cancer Res*. 2019;38(1):340.
64. Liang C, Xu J, Meng Q, et al. TGFB1-induced autophagy affects the pattern of pancreatic cancer progression in distinct ways depending on SMAD4 status. *Autophagy*. 2020;16(3):486—500.
65. Jun E, Song AY, Choi JW, et al. Progressive impairment of NK cell cytotoxic degranulation is associated with TGF-β1 deregulation and disease progression in pancreatic cancer. *Front Immunol*. 2019;10:1354.
66. Masamune A, Watanabe T, Kikuta K, et al. Roles of pancreatic stellate cells in pancreatic inflammation and fibrosis. *Clin Gastroenterol Hepatol*. 2009;7(11):S48—S54.
67. Erkan M, Adler G, Apte MV, et al. StellaTUM: current consensus and discussion on pancreatic stellate cell research. *Gut*. 2012;61(2):172—178.
68. Apte MV, Wilson JS, Lugea A, et al. A starring role for stellate cells in the pancreatic cancer microenvironment. *Gastroenterology*. 2013;144(6):1210—1219.
69. Schnittert J, Heinrich MA, Kuninty PR, et al. Reprogramming tumor stroma using an endogenous lipid lipoxin A4 to treat pancreatic cancer. *Cancer Lett*. 2018;420:247—258.
70. Pines M. Targeting TGFβ signaling to inhibit fibroblast activation as a therapy for fibrosis and cancer: effect of halofuginone. *Expet Opin Drug Discov*. 2008;3(1):11—20.
71. Cave DD, Di Guida M, Costa V, et al. TGF-β1 secreted by pancreatic stellate cells promotes stemness and tumorigenicity in pancreatic cancer cells through L1CAM downregulation. *Oncogene*. 2020;39(21):4271—4285.
72. Yuan Y, Jiang JY, Wang JM, et al. BAG3-positive pancreatic stellate cells promote migration and invasion of pancreatic ductal adenocarcinoma. *J Cell Mol Med*. 2019;23(8):5006—5016.
73. Chen YT, Chen FW, Chang TH, et al. Hepatoma-derived growth factor supports the antiapoptosis and profibrosis of pancreatic stellate cells. *Cancer Lett*. 2019;457:180—190.
74. Schober M, Jesenofsky R, Faissner R, et al. Desmoplasia and chemoresistance in pancreatic cancer. *Cancers*. 2014;6(4):2137—2154.
75. Tanaka HY, Kitahara K, Sasaki N, et al. Pancreatic stellate cells derived from human pancreatic cancer demonstrate aberrant SPARC-dependent ECM remodeling in 3D engineered fibrotic tissue of clinically relevant thickness. *Biomaterials*. 2019;192:355—367.
76. Chen G, Tian X, Liu Z, et al. Inhibition of endogenous SPARC enhances pancreatic cancer cell growth: modulation by FGFR1-III isoform expression. *Br J Cancer*. 2010;102(1):188—195.
77. Infante JR, Matsubayashi H, Sato N, et al. Peritumoral fibroblast SPARC expression and patient outcome with resectable pancreatic adenocarcinoma. *J Clin Oncol*. 2007;25(3):319—325.
78. Cavaco ACM, Rezaei M, Caliandro MF, et al. The interaction between laminin-332 and α3β1 integrin determines differentiation and maintenance of CAFs, and supports invasion of pancreatic duct adenocarcinoma cells. *Cancers*. 2018;11(1):14.

79. Vennin C, Murphy KJ, Morton JP, et al. Reshaping the tumor stroma for treatment of pancreatic cancer. *Gastroenterology*. 2018;154(4):820—838.
80. Feng J, Xu M, Wang J, et al. Sequential delivery of nanoformulated α-mangostin and triptolide overcomes permeation obstacles and improves therapeutic effects in pancreatic cancer. *Biomaterials*. 2020;241:119907.
81. Stylianou A, Gkretsi V, Stylianopoulos T. Transforming growth factor-β modulates pancreatic cancer associated fibroblasts cell shape, stiffness and invasion. *Biochim Biophys Acta Gen Subj*. 2018;1862(7):1537—1546.
82. Biffi G, Oni TE, Spielman B, et al. IL1-induced JAK/STAT signaling is antagonized by TGFβ to shape CAF heterogeneity in pancreatic ductal adenocarcinoma. *Cancer Discov*. 2019;9(2):282—301.
83. Ling J, Chiao PJ. Two birds with one stone: therapeutic targeting of IL1α signaling pathways in pancreatic ductal adenocarcinoma and the cancer-associated fibroblasts. *Cancer Discov*. 2019;9(2):173—175.
84. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer*. 2008;8(10):755—768.
85. Das PK, Pillai S, Rakib MA, et al. Plasticity of cancer stem cell: origin and role in disease progression and therapy resistance. *Stem Cell Rev Rep*. 2020;16(2):397—412.
86. Li C, Heidt DG, Dalerba P, et al. Identification of pancreatic cancer stem cells. *Cancer Res*. 2007;67(3):1030—1037.
87. Chen S, Huang J, Liu Z, et al. FAM83A is amplified and promotes cancer stem cell-like traits and chemoresistance in pancreatic cancer. *Oncogenesis*. 2017;6(3):e300.
88. Kali A, Ostapchuk YO, Belyaev NN. TNFα and TGFβ-1 synergistically increase the cancer stem cell properties of MiaPaCa-2 cells. *Oncol Lett*. 2017;14(4):4647—4658.
89. Wang H, Wu J, Zhang Y, et al. Transforming growth factor β-induced epithelial-mesenchymal transition increases cancer stem-like cells in the PANC-1 cell line. *Oncol Lett*. 2012;3(1):229—233.
90. Zhou M, Gao Y, Wang M, et al. MiR-146b-3p regulates proliferation of pancreatic cancer cells with stem cell-like properties by targeting MAP3K10. *J Cancer*. 2021;12(12):3726—3740.
91. Zhang B, Ye H, Ren X, et al. Macrophage-expressed CD51 promotes cancer stem cell properties via the TGF-β1/smad2/3 axis in pancreatic cancer. *Cancer Lett*. 2019;459:204—215.
92. Su HT, Weng CC, Hsiao PJ, et al. Stem cell marker nestin is critical for TGF-β1-mediated tumor progression in pancreatic cancer. *Mol Cancer Res*. 2013;11(7):768—779.
93. Larson C, Oronsky B, Carter CA, et al. TGF-beta: a master immune regulator. *Expert Opin Ther Targets*. 2020;24(5):427—438.
94. Hussain SM, Reed LF, Krasnick BA, et al. IL23 and TGF-ß diminish macrophage associated metastasis in pancreatic carcinoma. *Sci Rep*. 2018;8(1):5808.
95. Kelly A, Gunaltay S, McEntee CP, et al. Human monocytes and macrophages regulate immune tolerance via integrin αvβ8-mediated TGFβ activation. *J Exp Med*. 2018;215(11):2725—2736.
96. Lee YS, Park JS, Kim JH, et al. Smad6-specific recruitment of Smurf E3 ligases mediates TGF-β1-induced degradation of MyD88 in TLR4 signalling. *Nat Commun*. 2011;2:460.
97. Cheng H, Fan K, Luo G, et al. $Kras^{G12D}$ mutation contributes to regulatory T cell conversion through activation of the MEK/ERK pathway in pancreatic cancer. *Cancer Lett*. 2019;446:103—111.
98. Shevach EM. Mechanisms of $Foxp3^{+}$ T regulatory cell-mediated suppression. *Immunity*. 2009;30(5):636—645.
99. Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. *Annu Rev Immunol*. 2000;18:767—811.
100. Papaspyridonos M, Matei I, Huang Y, et al. Id1 suppresses anti-tumour immune responses and promotes tumour progression by impairing myeloid cell maturation. *Nat Commun*. 2015;6:6840.
101. Thomas DA, Massagué J. TGF-β directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Cancer Cell*. 2005;8(5):369—380.
102. Zhang N, Bevan MJ. TGF-β signaling to T cells inhibits autoimmunity during lymphopenia-driven proliferation. *Nat Immunol*. 2012;13(7):667—673.
103. Dahmani A, Delisle JS. TGF-β in T cell biology: implications for cancer immunotherapy. *Cancers*. 2018;10(6):194.
104. Thepmalee C, Panya A, Junking M, et al. Inhibition of IL-10 and TGF-β receptors on dendritic cells enhances activation of effector T-cells to kill cholangiocarcinoma cells. *Hum Vaccines Immunother*. 2018;14(6):1423—1431.
105. Strouch MJ, Cheon EC, Salabat MR, et al. Crosstalk between mast cells and pancreatic cancer cells contributes to pancreatic tumor progression. *Clin Cancer Res*. 2010;16(8):2257—2265.
106. Guo X, Zhai L, Xue R, et al. Mast cell tryptase contributes to pancreatic cancer growth through promoting angiogenesis via activation of angiopoietin-1. *Int J Mol Sci*. 2016;17(6):834.
107. Principe DR, Diaz AM, Torres C, et al. TGFβ engages MEK/ERK to differentially regulate benign and malignant pancreas cell function. *Oncogene*. 2017;36(30):4336—4348.
108. Porcelli L, Iacobazzi RM, Di Fonte R, et al. CAFs and TGF-β signaling activation by mast cells contribute to resistance to gemcitabine/nabpaclitaxel in pancreatic cancer. *Cancers*. 2019;11(3):330.
109. Li S, Liu M, Do MH, et al. Cancer immunotherapy via targeted TGF-β signalling blockade in T_H cells. *Nature*. 2020;587(7832):121—125.
110. Shi L, Sheng J, Wang M, et al. Combination therapy of TGF-β blockade and commensal-derived probiotics provides enhanced antitumor immune response and tumor suppression. *Theranostics*. 2019;9(14):4115—4129.
111. Melisi D, Garcia-Carbonero R, Macarulla T, et al. Galunisertib plus gemcitabine *vs*. gemcitabine for first-line treatment of patients with unresectable pancreatic cancer. *Br J Cancer*. 2018;119(10):1208—1214.
112. Gueorguieva I, Tabernero J, Melisi D, et al. Population pharmacokinetics and exposure—overall survival analysis of the transforming growth factor-β inhibitor galunisertib in patients with pancreatic cancer. *Cancer Chemother Pharmacol*. 2019;84(5):1003—1015.
113. Majidpoor J, Mortezaee K. The efficacy of PD-1/PD-L1 blockade in cold cancers and future perspectives. *Clin Immunol*. 2021;226:108707.
114. Liu Q, Wu H, Li Y, et al. Combined blockade of TGf-β1 and GM-CSF improves chemotherapeutic effects for pancreatic cancer by modulating tumor microenvironment. *Cancer Immunol Immunother*. 2020;69(8):1477—1492.
115. Wang Y, Gao Z, Du X, et al. Co-inhibition of the TGF-β pathway and the PD-L1 checkpoint by pH-responsive clustered nanoparticles for pancreatic cancer microenvironment regulation and anti-tumor immunotherapy. *Biomater Sci*. 2020;8(18):5121—5132.
116. Chen S, Shen J, Zhao J, et al. Magnolol suppresses pancreatic cancer development *in vivo* and *in vitro via* negatively regulating TGF-β/smad signaling. *Front Oncol*. 2020;10:597672.
117. Fiz C, Apprato G, Ricca C, et al. TGF beta induces vitamin D receptor and modulates mitochondrial activity of human pancreatic cancer cells. *Cancers*. 2021;13(12):2932.
118. Moz S, Contran N, Facco M, et al. Vitamin D prevents pancreatic cancer-induced apoptosis signaling of inflammatory cells. *Biomolecules*. 2020;10(7):1055.
119. Péron J, Giai J, Maucort-Boulch D, et al. The benefit-risk balance of nab-paclitaxel in metastatic pancreatic adenocarcinoma. *Pancreas*. 2019;48(2):275—280.

120. Pothula SP, Xu Z, Goldstein D, et al. Key role of pancreatic stellate cells in pancreatic cancer. *Cancer Lett*. 2016;381(1):194—200.
121. Dominguez CX, Müller S, Keerthivasan S, et al. Single-cell RNA sequencing reveals stromal evolution into LRRC15^{+} myofibroblasts as a determinant of patient response to cancer immunotherapy. *Cancer Discov*. 2020;10(2):232—253.
122. Shi Y, Gao W, Lytle NK, et al. Targeting LIF-mediated paracrine interaction for pancreatic cancer therapy and monitoring. *Nature*. 2019;569(7754):131—135.
123. Shien K, Papadimitrakopoulou VA, Ruder D, et al. JAK1/STAT3 activation through a proinflammatory cytokine pathway leads to resistance to molecularly targeted therapy in non-small cell lung cancer. *Mol Cancer Therapeut*. 2017;16(10):2234—2245.
124. Liu Q, Liao Q, Zhao Y. Chemotherapy and tumor microenvironment of pancreatic cancer. *Cancer Cell Int*. 2017;17:68.
125. Hirota Y, Osuga Y, Koga K, et al. The expression and possible roles of chemokine CXCL11 and its receptor CXCR3 in the human endometrium. *J Immunol*. 2006;177(12):8813—8821.