Roles of CAFs in the TME of PDAC

Subjects: Surgery

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant digestive tumors, characterized by a low rate of early diagnosis, strong invasiveness, and early metastasis. The abundant stromal cells, dense extracellular matrix, and lack of blood supply in PDAC limit the penetration of chemotherapeutic drugs, resulting in poor efficacy of the current treatment regimens. Cancer-associated fibroblasts (CAFs) are the major stromal cells in the tumor microenvironment. Tumor cells can secrete exosomes to promote the generation of activated CAFs, meanwhile exosomes secreted by CAFs help promote tumor progression.

Keywords: PDAC ; exosomal miRNAs ; cancer-associated fibroblasts

1. Identification, Origin, and Classification of CAFs in PDAC

In PDAC, 60–70% of the tumor tissue is composed of CAF-dominant stromal cells and the deposition of extracellular matrix (ECM) components such as collagen [1]. CAFs are characterized by the expression of mesenchymal markers, morphological features (spindle shape), and by the absence of non-mesenchymal markers (markers of epithelial, endothelial, immune, and neuronal cells). The latest consensus states that CAFs are collections of cells in the tumors that are negative for epithelial, endothelial, and leukocyte markers; have an elongated morphology; and lack the genetic mutations found in cancer cells [2]. CAFs can be derived from normal fibroblasts, stellate cells, bone marrow-derived mesenchymal stem cells, adipocytes, or endothelial cells [3][4][5][6]. Quiescent PSCs are the main precursors of CAFs in the TME of PDAC. When injury or inflammation occurs, quiescent PSCs will be activated, accompanied by changes in cell morphology and the disappearance of lipid droplets. Subsequently, quiescent PSCs transform into α-smooth muscle actin (α-SMA)-positive myofibroblast-like cells, secreting a large amount of extracellular matrix components and remodeling the matrix of pancreatic tissue [7]. Matthew et al. [8] reported that the fibroblast subpopulations exist in both normal pancreas and pancreatic tumors in KC (Ptf1a-Cre; LSL-Kras G12D) mice, and these fibroblast subpopulations display characteristics of mesenchymal stem cells (MSCs), which can differentiate into chondrocytes, adipocytes, and osteoblasts. This would promote tumor growth by inducing the polarization of macrophages to pro-cancer subtypes. Huang et al. [9] found mesothelial cells could transform into antigen-presenting CAFs (apCAFs) by downregulating mesothelial characteristics and acquiring fibroblast characteristics under the induction of interleukin-1 and transforming growth factor β (TGF-β). The apCAFs can directly ligate and convert the naive CD4+ T cells into regulatory T cells in an antigen-specific way. Miyazaki et al. [10] found that the adipose-derived mesenchymal stem cells (AD-MSCs) could transform into variant CAF subtypes under different co-culture conditions in vitro. When co-cultured with PDAC Capan-1 cells directly, AD-MSCs could transform into myofibroblast CAFs (myCAFs) and inflammatory CAFs (iCAFs), while indirect co-culture induced differentiation into only iCAFs. Waghray et al. [11] identified and characterized cancer-associated MSC (CA-MSC) subpopulations from human PDAC samples and CAFs, highlighting the heterogeneity of fibroblast subpopulations in PDAC. CA-MSCs can significantly enhance the proliferation, invasion, and metastatic potential of PDAC cells by secreting granulocyte-macrophage colony-stimulating factor (GM-CSF). CAFs will then differentiate into different subtypes with similar characteristics to their origin cells, according to the various cell origins and culture conditions. Hence, CAFs in human PDAC show great heterogeneity and exert different functions.

2. Roles of Activated CAFs in the PDAC Microenvironment

The activated CAFs can express various proteins including α-SMA, fibroblast activation protein (FAP), fibroblast-specific protein 1 (FSP-1), podoplanin (PDPN), and PDGFR [5][12]. Some of these proteins were used as typing markers to classify CAFs into different subtypes, which might exert anti-cancer or pro-cancer roles in PDAC. CAFs with high expression of α-SMA were named myCAFs, whereas CAFs with low α-SMA levels that secrete inflammatory cytokines such as IL-6 and leukemia inhibitory factor (LIF) were defined as iCAF [13]. McAndrews et al. [14] found that depletion of FAP+ CAFs resulted in a survival benefit, while depletion of α-SMA+ CAFs caused increased mortality in mouse models of PDAC. The oncogenic FAP+ CAFs and anti-tumor α-SMA+ CAFs were proved to regulate cancer-related pathways and regulatory T

cells via different mechanisms. Elyada et al. [15] identified a subtype of apCAFs with MHCII expression and antigen-presenting capability in KPC PDAC mice, which could present the model antigen to the CD4+ T cells, decrease the CD8+T/Treg ratio, and contribute to immune suppression in the TME. The apCAFs could also transform into myCAFs under appropriate culture conditions, which implied that CAFs were in a dynamic cell state. Lin et al. [16] confirmed the presence of three CAF subtypes (myCAF, iCAF, and apCAF) in human PDAC tissues by single-cell transcriptomic technology. The subtype of CAFs with high Meflin expression was reported to correlate with a favorable outcome in both mouse models or patients with PDAC, and deficiency or low expression of Meflin resulted in straightened stromal collagen fibers, indicating that Meflin was a marker of cancer-restraining CAFs that suppressed the progression of PDAC [17]. Chen et al. [18] identified a subset of complement-secreting CAFs (csCAFs) which were located in the tissue stroma adjacent to tumor cells only in early PDAC and specifically expressed complement system components.

PSCs were found to produce myeloid-derived suppressor cell (MDSC)-promoting cytokines (interleukins, IL-6; VEGF; macrophage colony-stimulating factor, M-CSF) and chemokines (SDF-1, MCP-1) to promote differentiation of immune cells into the functional MDSC phenotypes to prevent innate or adaptive immune responses against cancer cells, among which IL-6 was the key factor contributing to STAT3 signaling and MDSC differentiation [19]. CAFs were also reported to upregulate the expression of immune checkpoints PD-1, cytotoxic lymphocyte-associated antigen-4 (CTLA-4), T-cell immunoglobulin, mucin-domain containing-3 (TIM-3), and lymphocyte-activation gene-3 (LAG-3) in both CD4+ and CD8+ T cells, thereby inhibiting the proliferation of T cells and diminishing immune function in PDAC [20].

The specific mechanisms by which CAFs regulate tumor progression have not yet been fully clarified. More and more evidence has shown that the activation mechanisms of different CAF subtypes varied widely, and the dominant subtype of CAFs could dynamically change in response to different biological signals in the TME of PDAC or different in vitro culture conditions [10][21]. Along with the understanding of the differentiation and regulational mechanism of CAFs and their effect on tumor cells in the TME of PDAC, novel therapeutic drugs targeting CAFs modulation may be developed to transform the tumor-promoting microenvironment into a tumor-suppressing microenvironment, thereby increasing the sensitivity of immunotherapy.

3. Regulatory Mechanism of CAFs Differentiation and Activation in PDAC

In a healthy pancreas, fibroblasts exist mainly in a quiescent state. During the progress of carcinogenesis, quiescent fibroblasts transform into active CAFs through the activation of distinct signaling pathways with the participation of miRNAs, cytokines, paracrine lactate, and growth factors secreted by tumor cells.

3.1. Tumor Cells Promote the Differentiation and Activation of CAFs

Tumor cells can secrete connective tissue growth factor (CTGF) to promote the activation, proliferation, migration, and adhesion of PSCs and fibroproliferation [22]. Biffi et al. [23] found that PDAC cells could secrete IL1α to activate PSCs and promote the formation of iCAFs by activating the IL1/JAK/STAT signaling pathway, and tumor cells could also increase the expression of inflammatory cytokines and chemokines like IL1a, IL6, and LIF in iCAFs, which promoted cancer progression and formed an immunosuppressive microenvironment. Moreover, PDAC cells secreted TGFβ to upregulate the expression of α-SMA in PSCs, and thus transformed the quiescent PSCs to myCAF, promoting the formation of a dense extracellular matrix [23]. The presence of oncogenic KRAS mutation was also reported to be involved in the activation of CAFs and to regulate tumor cell signaling via stromal cells [24]. Awaji [25] found that KRAS mutation could promote the secretion of paracrine factors such as IL4, IL10, and IL13 in tumor cells and simultaneously activate CXCR2 signaling in CAFs, resulting in the formation of a secreted CAF phenotype by activating NF-κB signaling and promoting tumor progression. The paracrine lactate secreted by PDAC cells could increase alpha-ketoglutarate (aKG) production in MSCs, which decreased cytosine methylation and increased hydroxymethylation during the differentiation of MSCs into CAFs by mediating the activation of the demethylase enzyme ten-eleven translocation (TET) [26]. Additionally, the reactive oxygen species (ROS) associated with the hypoxic TME of PDAC could also promote the activation of PSCs by stabilizing HIF-1α and increasing the expression of GLI1 [27].

3.2. Aberrant Expression of miRNAs Was Involved in the Activation of CAFs

Studies have revealed that the aberrant expression of miRNAs in PDAC played important roles in the activation of CAFs in the TME. Xu et al. [28] reported that miR-200a could attenuate the activation of PSC induced by TGF-β1 and inhibit ECM formation through the PTEN/Akt/mTOR pathway. Inhibition of miR-199a-3p and miR-214-3p was found to be involved in the de-differentiation of CAFs which could reduce the differentiation of PSCs into myofibroblasts [29]. Chu et al. indicated that the overexpression of miR-224 in pancreatic fibroblasts can significantly increase their proliferation, migration, and invasion ability, thus promoting the activation of CAFs [30]. Overexpression of miR-21 contributed to the

activation of CAFs by regulating the PDCD4 gene and increased the invasion ability of PDAC cells by stimulating the secretion of MMP-3, MMP-9, CCL-7, and PDGF in CAFs [31]. In a mouse model of alcoholic chronic pancreatitis (CP), Charrier et al. [32] reported that the expression of miR-21 was significantly elevated in activated PSCs, which promoted the expression of connective tissue growth factor (CCN2), and a positive feedback loop between miR-21 and CCN2 was found to increase the expression of collagen and the activation of quiescent PSCs. MiR-301a was also involved in the activation of PSCs by inhibiting Gadd45g expression and promoting STAT3 activation during the pancreatic intraepithelial neoplasia lesion formation [33].

3.3. Exosomal miRNAs Promote the Activation of CAFs in TME of PDAC

The specifically enriched miRNAs in the exosomes play vital roles as messengers of cell-to-cell interaction in the TME. Pang et al. [34] reported that PDAC cells delivered miR-155 into fibroblasts via exosomes, which could inhibit the expression of the target gene TP53INP1 and reprogram the quiescent fibroblasts into CAFs. MiR-1246 and miR-1290 were highly enriched in PDAC cell-derived exosomes, which could be transferred into PSCs to induce the activation of PSCs and the occurrence of pro-fibrosis, including proliferation, migration, collagen production, and expression of α-SMA [35].

In summary, the activation of CAFs is essential to form the immunosuppressive TME in PDAC. In the process of carcinogenesis, the differentiation and activation of CAFs was modulated by complex mechanisms, among which exosomal miRNAs derived from tumor cells play important roles, although the specific mechanism remains to be further studied.

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