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Mini-review

Crosstalk between stromal cells and cancer cells in pancreatic cancer: New insights into stromal biology

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ABSTRACT

Pancreatic cancer (PC) remains one of the most lethal malignancies worldwide. Increasing evidence has confirmed the pivotal role of stromal components in the regulation of carcinogenesis, invasion, metastasis, and therapeutic resistance in PC. Interaction between neoplastic cells and stromal cells builds a specific microenvironment, which further modulates the malignant properties of cancer cells. Instead of being a "passive bystander", stroma may play a role as a "partner in crime" in PC. However, the role of stromal components in PC is complex and requires further investigation. In this article, we review recent advances regarding the regulatory roles and mechanisms of stroma biology, especially the cellular components such as pancreatic stellate cells, macrophages, neutrophils, adipocytes, epithelial cells, pericytes, mast cells, and lymphocytes, in PC. Crosstalk between stromal cells and cancer cells is thoroughly investigated. We also review the prognostic value and molecular therapeutic targets of stroma in PC. This review may help us further understand the molecular mechanisms of stromal biology and its role in PC development and therapeutic resistance. Moreover, targeting stroma components may provide new therapeutic strategies for this stubborn disease.

Introduction

Pancreatic ductal adenocarcinoma (PDAC, hereafter referred to as pancreatic cancer [PC]) remains the most lethal malignant tumor worldwide; an estimated 53,070 patients will be diagnosed with PC in 2016, while an estimated 41,780 patients died at the same time, and this malignant disease is predicted to be the second leading cause of cancer death by 2030 [1,2]. Despite inspiring advancements in our understanding of the biology of PC, improvements in surgical skills, and new chemotherapeutic agents for neoadjuvant and adjuvant therapy in patients with PC, the long-term survival did not show significant improvement during the last two decades. Radical resection with clean resection margin (R0 resection) offers the best and the only curative treatment; however, when first diagnosed 80–85% of patients present with metastatic or locally advanced disease that is unresectable [3,4]. Even for patients who undergo resection the prognosis remains poor because of the high rate of local recurrence and/or distant metastasis. Resistance to chemotherapy and radiation is another important reason for the poor prognosis of PC [5]. There are few effective treatments that can extend the overall survival of patients with PC. New strategies to explore the mechanisms of carcinogenesis, proliferation, metastasis, and therapeutic resistance of PC are urgently needed [6,7].

An intense stromal desmoplastic reaction surrounding the cancer cells is the typical histological hallmark feature in PC [8–10]. This desmoplastic stromal tissue occupies approximately 80% of the total cancer nodule [10]. The stromal components consist of pancreatic stellate cells (PSCs), activated fibroblasts, macrophages, infiltrating immune cells, endothelial cells, and extracellular matrix (ECM), as well as a variety of enzymes and growth factors [9,10]. Interaction between pancreatic cancer cells and stromal cells builds a specific microenvironment that further influences the malignant properties of cancer cells. The role of the pronounced stroma reaction in PC carcinogenesis, metastasis, and therapeutic resistance has been thoroughly investigated in previous studies [8,10]. Although the stroma was thought to be a passive bystander for many years, several studies indicate that it is actually a "partner in

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crime" in PC [10–12]. However, some recent studies have demonstrated that targeting the stroma results in undifferentiated, aggressive pancreatic cancer, revealing a protective role of stroma in PC. Is stroma a "friend or foe" in PC [13]? This issue remains controversial and unclear, and the complex role of stroma in PC must be further investigated. In this article, we review recent advances regarding the regulatory roles and mechanisms of stromal components in PC, paying particular attention to the crosstalk between stromal cells and cancer cells. The prognostic value and molecular therapeutic targeting of stroma in PC are also reviewed.

Tumor microenvironment in PC

Tumor microenvironment (TME) is a complex and well-organized physical and biochemical system and the complicated biological interactions between tumor cells and the stroma play a pivotal role in tumor carcinogenesis, progression, metastasis, and therapeutic resistance [14–16]. Compared with normal tissue, the TME is characterized by altered functions of molecules in the extracellular matrix (ECM), the vascular and lymphatic networks, and abnormal phenotypes of stromal cells [17]. The special conditions of hypoxia, acidic extracellular pH, and increased interstitial fluid pressure in TME orchestrate tumorigenesis and tumor progression. Furthermore, TME has profound effects on therapeutic efficacy through intrinsic or acquired response systems, which include regulation of drug delivery, vascular remodeling, metabolic activities, and signal pathways involved in DNA repair and apoptosis [14,17,18]. TME-targeted therapy provides a novel therapeutic strategy for malignant tumors.

Major components of TME include vasculature, cancer-associated fibroblasts (CAFs), inflammatory and immune cells, and ECM [10]. As shown in Fig. 1 and mentioned previously, an excessive amount of desmoplastic tissue (up to 80–90%) is a marked feature of PC compared with other malignant tumors [8,10]. Besides excessive ECM tissue, CAFs are another important component of PC stroma. These cells can produce a wide variety of ECM molecules and cytokines, which play important roles in tumor progression and drug resistance. CAFs can originate from different sources, including pancreatic stellate cells (PSCs), resident fibroblasts, and bone marrow-derived cells (BMDCs) [19,20]. Infiltrating immune cells, including myeloid-derived suppressor cells (MDSCs), regulatory T (Treg) cells, and tumor-associated macrophages (TAMs), have a highly immunosuppressive composition and further contribute to immune evasion. Together, these stromal cells and acellular components such as collagen, cytokines, and soluble growth factors constitute a complex stromal microenvironment. Crosstalk between stromal cells and cancer cells in TME contributes to tumor progression and therapeutic resistance of PC [14,21,22]. To date, stromal-targeted therapies have proved frustrating. Understanding the complexity and complicity of the tumor–stroma interaction may elucidate the molecular mechanisms of PC carcinogenesis and progression and, moreover, provide strategies for future therapy and clinical trial design.

Crosstalk between stromal cells and cancer cells in PC

Stromal cells in PC are a group of heterogeneous connective tissue cells that fulfill distinct but complementary functions. They form the supportive structure in which the PC cells reside.

Cancer-associated fibroblasts

Fibroblasts associated with cancer have been termed cancer-associated fibroblasts (CAFs), tumor-associated fibroblasts (TAFs), activated fibroblasts, or activated myofibroblasts, and might include cancer-associated mesenchymal stem cells (MSCs) [23]. CAFs are defined as all the fibroblastic, non-neoplastic, nonvascular, nonepithelial, and noninflammatory cells found in a tumor [24,25] and can originate from various precursor cells including PSCs, normal resident fibroblasts, BMDCs, endothelial cells (ECs), and adipocytes [24,26–29]. The desmoplastic stroma of PC contains high numbers of CAFs. There is currently no specific marker that completely and exclusively defines CAFs, although fibroblast-specific protein 1 (FSP-1) seems to be the most useful marker [24,25,30]. In fact, some researchers have proposed that CAFs should be defined as a cell state like epithelial-to-mesenchymal transition (EMT) rather than a cell type.

Tumor cells secrete many soluble factors including transforming growth factor (TGF)-β, PDGF, chemokine (C-X-C motif) ligand 2 (CXCL2) and endothelin [31] or lipid-based particles such as phospholipids that recruit and activate CAFs [25]. Upon direct contact, PC cells can induce *SOCS1* methylation in CAFs resulting in activation of STAT3 and induction of insulin-like growth factor-1 (IGF1) expression to support PC cell growth [32]. The conversional CAFs produce signaling factors or extracellular vesicles (EVs, also called exosomes) that mediate CAF-tumor cell crosstalk and enhance ECM remodeling and promote PC growth, invasion, and chemoresistance [33]. Hirakawa et al. suggested that pancreas CAFs can stimulate the invasion activity of PC cells through paracrine IGF1/IGF1R signaling, especially under a hypoxic microenvironment [34]. Leca et al. reported that increased PC aggressiveness was dependent on tumor cell-mediated uptake of CAF-derived ANXA6^{+} EVs carrying the ANXA6/LRP1/TSP1 complex [35]. CAF-derived exosomes increase expression of the chemoresistance-inducing factor Snail in recipient epithelial cells and promote proliferation and drug resistance [36].

Resident quiescent fibroblasts

Quiescent, or resting, fibroblasts are fibroblast-like cells that are waiting to become activated when the need arises [23]. These cells are inert and are identified as spindle-shaped single cells in the connective tissue that are embedded in physiological ECM. In response to stimuli such as stress, growth factors (e.g., TGF-β), chemokines, hypoxia, reactive oxygen species (ROS), and cytokines, the quiescent fibroblasts are reversibly activated to normal activated fibroblasts (NAFs) that express α smooth muscle actin (α-SMA) and vimentin and become stellate in shape. These fibroblasts are also called myofibroblasts because they express α-SMA [37]. In some studies, patients with PC with high stromal activity, measured by increased levels of α-SMA expressing myofibroblast cells within their tumors, showed worse overall survival [38,39].

When the repair process is complete, NAFs can be reverted to quiescent fibroblasts through reprogramming or apoptosis. However, activated fibroblasts may gain persisting and unrepaired injurious stimuli, such as the development of cancer lesions. Such FAFs and CAFs gain enhanced proliferative properties and lead to a fibrotic tumor microenvironment. Based on the notion that CAFs stimulate tumorigenesis and drug resistance, multiple strategies have been suggested to achieve therapeutic benefits by targeting the stromal barrier [40,41], CAF-secreted factors [42,43], ECM interactions [44], and the CAFs themselves [45]. Although these approaches increase drug delivery and decrease the number of stromal cells, the tumor cells were found to be more aggressive [46,47]. Stromal deletion in the pancreatic epithelium by Sonic Hedgehog (Shh) pathway inhibitors or via genetic depletion of proliferating α-SMA-expressing myofibroblasts using a thymidine kinase-mediated strategy led to more aggressive and undifferentiated tumors with enhanced metastatic capacity in genetically engineered mice [46,47]. These studies suggested that the stroma may play a protective role in "restraining" tumor cells.