1. Pathology of pancreatic stroma in PDAC

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Abstract. Pancreatic adenocarcinoma is a highly lethal disease that is histologically characterized by a dense desmoplastic reaction (DR) surrounding malignant epithelial cells. The DR is composed of extracellular matrix (ECM) proteins, fibroblasts, stellate cells, endothelial cells, immune cells, and neurons. Accumulating evidence indicates that the epithelial and stromal compartments interact to enhance the aggressive nature of this disease. Pancreatic cancer cells release various factors that stimulate the stroma. Stromal cells, in turn, release mitogenic substances that stimulate tumor growth, invasion, and resistance to therapy. As we better understand the interactions between the stromal and epithelial cell compartments in pancreatic adenocarcinoma, it is becoming evident that anticancer therapies targeting the stroma, in addition to epithelial cells, may play a key role in improving clinical outcomes for patients with this deadly disease.

Introduction

Pancreatic adenocarcinoma has one of the highest mortality rates of any malignancy. Most patients are initially diagnosed with unresectable
(metastatic or locally advanced) disease and the median survival for those patients is only six to nine months. Even for patients who undergo surgery for localized disease, the five-year survival rate is only about 20% [1]. The poor prognosis for patients with pancreatic adenocarcinoma is largely due to its propensity to metastasize and its resistance to radiation and chemotherapy. Most research has focused on better understanding the genetics and biology of pancreatic adenocarcinoma in order to develop better therapeutic strategies to treat patients with this malignancy. In recent years, one area of research has focused on the stromal compartment in pancreatic adenocarcinoma, and studies indicate that it contributes to the poor prognosis of patients with this malignancy.

A hallmark of pancreatic adenocarcinoma is the presence of a dense desmoplastic reaction (DR) that consist largely of fibroblasts, pancreatic stellate cells (PSCs), and extracellular matrix (ECM) proteins, including collagens I and III and fibronectin [2]. Other cells in the stroma include endothelial cells, immune cells, pericytes, and nerve fibers. In a number of malignancies, the presence of a DR in primary tumors has been associated with worse clinical outcomes [3, 4]. The DR in pancreatic adenocarcinoma is thought to contribute to the aggressive nature of this tumor by fostering tumor growth and metastatic spread and enhancing drug resistance. In this chapter we will review the biological importance of interactions between the stromal compartment and malignant epithelial cells of pancreatic adenocarcinoma. Better understanding these interactions are important for developing stroma-targeting therapies that could lead to improved patient outcomes.

**Pancreatic cancer stellate cells and fibroblasts**

The major cellular constituents of the DR in pancreatic adenocarcinoma are PSCs and fibroblasts. The initial isolation and culture of PSCs from rats and humans was described in 1998 [5, 6]. The origin of PSCs is unclear, but they are believed to arise from mesenchymal, endodermal, and neuroectodermal origins. In normal tissue they can be identified based on the expression of glial fibrillary acidic protein (GFAP) and desmin. PSCs are thought to play an important role in the pathobiology of pancreatitis and pancreatic cancer, in which case they transform to an activated state and acquire characteristics of myofibroblasts and express $\alpha$-smooth muscle actin ($\alpha$SMA). In the diseased organ, activated PSCs are postulated to arise from quiescent PSCs, fibroblasts, bone marrow-derived cells, or epithelial-mesenchymal transition (EMT). Whatever their origins, PSCs are activated in
response to pancreatic injury and inflammation and play an active role in the progression of malignancy.

PSCs secrete and respond to a number of cytokines and have been found to actively proliferate, migrate, and produce ECM proteins, including type I collagen and fibronectin. A number of pathways have been implicated in this process including transforming growth factor beta (TGF-β), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). Lohr et al. showed that TGF-β expressing Panc1 cells induced the proliferation of co-cultured fibroblasts with a concomitant increase in type I collagen and fibronectin expression. Furthermore, orthotopic injection of TGF-β expressing Panc1 cells in nude mice resulted in the formation of tumors with more desmoplasia and increased amounts of type I collagen and fibronectin [7]. A similar observation was made when PSCs were incubated in conditioned media from pancreatic cancer cell lines; PSCs cultured in conditioned media or with TGF-β displayed increased [³H] thymidine uptake and collagen synthesis [8]. Recent studies examining Hedgehog signaling in pancreatic adenocarcinoma have found that sonic hedgehog ligand secreted from malignant epithelial cells acts on fibroblasts and PSCs, promoting desmoplasia and increased metastasis in an orthotopic model [9, 10]. Secreted protein acidic and rich in cysteine (SPARC) is secreted by fibroblasts and found to be involved in cell migration and proliferation [11]. Furthermore, Infante et al. found that intratumoral SPARC expression was associated with worse prognosis in patients with resected disease [12]. Another protein that is secreted by PSCs upon stimulation by epithelial cells is periostin which modulates tumor cell invasion via AKT signaling and EMT [13, 14]. Global gene expression analysis of genes differentially expressed in fibroblasts cultured in the absence or presence of pancreatic cancer cell lines revealed 43 up-regulated and 31 down-regulated transcripts [15]. Among the most highly up-regulated genes were members of the CXC/CC chemokine family including MCP-1 (CCL2), interleukin- (IL)-8 (CXCL8), GRO1 (CXCL1), and GRO2 (CXCL2); all of which have been implicated in tumor invasion and angiogenesis.

Just as secreted factors from pancreatic cancer cells leads to activation of stromal cells, growth factors and proteins elicited from stromal cells have profound effects on the epithelial cell compartment. A number of studies have found that co-culturing fibroblasts or PSCs with pancreatic cancer cell lines leads to an increase in their invasive properties [15]. To explore the molecular mechanism for this phenomenon, Sato et al. studied global gene expression in cell lines co-cultured with fibroblasts and found differential expression of 143 genes [15]. The expression of five genes was validated:
COX-2, hyaluronan synthase 2 (HAS2), and MMP-1 were up-regulated and Gravin was down-regulated [15]. All of the up-regulated genes have been linked with tumor cell invasion. In another study, Ohuchida found that co-culture of fibroblasts with cancer cell lines or culturing cell lines in conditioned media from fibroblasts promoted invasiveness. They found that HGF secreted by the fibroblasts lead to increased c-Met phosphorylation and mitogen-activated protein kinase activity in the epithelial cell compartment [16].

PSCs, fibroblasts, and epithelial cells all contribute to regulating the composition of the ECM via proteolytic enzymes, or matrix metalloproteinases (MMPs), that are frequently over expressed in pancreatic cancer cells and are involved in the dynamic remodeling and turnover of ECM proteins [17, 18]. Specifically, MMP-9, MMP-2, and MMP-1 have been found to be expressed when cells come in contact with certain ECM proteins, such as type I collagen, thereby regulating the invasive properties of these cells [19-21]. Other studies have also identified tissue inhibitors of metalloproteinases (TIMPs), inhibitors of the extracellular proteinases, which are commonly over expressed in pancreatic adenocarcinoma and other malignancies. As an example, the serine protease inhibitor nexin-2 (SERPINE2) is secreted by epithelial cells and activates PSCs resulting in greater deposition of ECM proteins, increased tumor growth, and more invasive growth [22, 23].

A significant amount of research has focused on the effect of the ECM on tumor invasion, but it is also apparent that the ECM contributes to resistance to chemotherapy and radiation therapy. Cells cultured in the presence of conditioned media from PSCs and xenografts formed by co-injection of PSCs and pancreatic cancer cells lead to resistance to gemcitabine and radiation therapy [24]. In another study, type I collagen led to an increase in cell proliferation and relative protection from apoptosis [8].

**Endothelial cells**

Though in vitro and animal studies have shown that pancreatic cancer cells induce angiogenesis by secreting molecules like VEGF and FGF, clinically, pancreatic tumors have been found to be hypovascular and hypoxic [25, 26]. Studies have linked hypoxia in patient tumors with worse clinical outcomes, including increased rates of tumor growth and metastasis [27-29]. Furthermore, clinical trials of the VEGF inhibitor, bevacizumab, in patients with pancreatic cancer have not shown clinical efficacy, suggesting that the microenvironment in pancreatic cancer is already hypoxic [30-32]. The hypoxic environment is largely thought to be secondary to the fibrotic
microenvironment produced by PSCs and the expression of a number of antiangiogenic substances, including endostatin and matrix metalloproteinase 12 [26, 33].

The hypoxic microenvironment in pancreatic cancer has been shown to induce the expression of hypoxia-inducible factor-1 (HIF1) in a number of cancers and has been shown to be overexpressed in pancreatic tumors from patients [34]. HIF1 expression has been associated with drug resistance and enhanced cell invasion in pancreatic cancer, which may be mediated through c-Met and Hedgehog signaling [35-38]. Recent work by Olive et al. also revealed that Hedgehog signaling might regulate intratumoral vascular density via effects on the tumor stroma [39].

**Inflammatory cells**

Inflammatory cells are part of the stromal reaction found in pancreatic cancer and are thought to contribute to the development and progression of this disease. Supporting a role for inflammation in the development of pancreatic cancer is the finding that pancreatitis, or chronic inflammation of the pancreas, is a risk factor for developing pancreatic adenocarcinoma [40]. Several studies have shown that leukocytic infiltrates in pancreatic adenocarcinoma are largely immunosuppressive and associated with worse survival in humans [41, 42]. Furthermore, proinflammatory markers, such as IL-6, IL-8, IL-10, and IL-1 receptor antagonist, are elevated in the serum of patients with pancreatic cancer, and IL-6 has been associated with worse survival [43]. IL-6 has been shown to signal via signal transducer and activator of transcription 3 (Stat3), which is activated in pancreatic cancer and involved in tumor growth [44].

**Pericytes and nerve cells**

The normal pancreas has an abundant nerve supply consisting of ganglia and myelinated and unmyelinated nerve cells. The degree of perineural invasion in the tumor has been associated with worse survival after resection and has been shown to be mediated by the chemokine receptor CX3CR1 [45, 46]. The mechanism for the association between perineural invasion and worse prognosis is not clear, but it may be a reflection of the degree of differentiation of the tumor [45]. In addition, the size and density of nerves have been shown to be increased in pancreatic cancers compared to normal tissue [47], but the impact of these cells on tumor progression and or resistance is not clear. It is possible that this process leads to significant patient morbidity caused by chronic pain.
Clinically targeting the pancreatic stroma

The stromal compartment and its interactions with malignant epithelial cells in pancreatic adenocarcinoma are clearly important in the pathogenesis of this deadly malignancy. Recent studies are beginning to show that targeting the stromal compartment in pancreatic cancer may have antitumor effects and may enhance sensitivity to radiation and chemotherapy. Several novel inhibitors of the Hedgehog pathway are being clinically tested in pancreatic adenocarcinoma (http://www.clinicaltrials.gov) and preclinical studies have shown that they are able to deplete the tumor of its stroma, resulting in increased intratumoral vascular density and a concomitant increase in intratumoral chemotherapy concentrations [39]. Furthermore, inhibition of the Hedgehog pathway has been shown to abrogate the formation of metastases in murine models [48, 49]. Preclinical studies of HIF inhibition in pancreatic cancer have shown sensitization of tumors to radiation with or without concurrent treatment with gemcitabine or 5-fluorouracil [50]. Recent analogs of the active component in curcumin have been developed that inhibit Stat3 phosphorylation and have anti-tumor activities against pancreatic cancer [51, 52]. Likewise, an inhibitor of Src, one of the activators of Stat3 signaling, also abrogates Stat3 phosphorylation and reduces tumor growth in a murine model [53]. In a mouse model and in humans, activation of the tumor necrosis factor receptor using an agonist CD40 monoclonal antibody facilitated the infiltration of tumors with tumoricidal macrophages, resulting in depletion of tumor stroma and tumor regression [54].

Significant improvements in the survival of patients with pancreatic cancer have not been realized in more than four decades despite advances in our understanding of pancreatic cancer biology and genetics. Increasing evidence indicates that the DR in pancreatic cancer, consisting of stellate cells, fibroblasts, and a number of ECM proteins, plays an important role in the progression and resistance to chemotherapy of this malignancy. Through better understanding the interactions between the stromal compartment and malignant epithelial cells we are beginning to develop therapeutic strategies that target these interactions. It is yet to be determined if these approaches to treating pancreatic cancer will change the aggressive course of this malignancy.

References


