REVIEW ARTICLE

Microenvironmental Factors and Extracellular Matrix Degradation in Pancreatic Cancer

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ABSTRACT

Pancreatic cancer is a devastating malady with proclivity for early metastasis, accounting for its poor prognosis. Pancreatic ductal adenocarcinoma, the most common type of pancreatic malignancy, exhibits an over-expression of several growth factors such as epidermal growth factor and transforming growth factor beta, which correlate with a decrease in patient survival. These growth factors as well as hypoxia-reoxygenation conditions have been shown to increase pancreatic tumor cell invasiveness. This review will focus on the signaling pathways used by these distinct microenvironmental factors to promote extracellular matrix degradation and invasion by pancreatic tumor cells.

Introduction

Pancreatic cancer is a devastating malady associated with poor survival and ranks as the fourth most common cause of cancer mortality. Currently, surgical resection with negative margins offers the only hope for cure, but a mere 10-20% of patients are suitable for resection at the time of diagnosis, which often occurs at advanced stages due to: a) the indolent growth of the tumors within the pancreas, b) the non-existence of truly effective conventional radiological tests to identify the disease at an early phase, c) the absence of specific and sensitive diagnostic serum markers, and d) the proclivity for early metastasis [1-3].

Interactions between cells of the stromal tissue and cancer cells are bidirectional, dynamic and complex. The non-malignant cells of stromal tissue produce a particular microenvironment that can modify the neoplastic properties of tumor cells, promoting tumor progression by providing growth factors, pro-angiogenic factors, proteases, and adhesion molecules that facilitate tumor cell proliferation, angiogenesis, invasion, and metastasis [4].

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Abbreviations AP-1, activator protein-1; CSLC, cancer stem-like cells; ECM, extracellular matrix; EGF, epidermal growth factor; ERK, extracellular signal regulated kinase; GTPase, guanosine triphosphatase; HIF-1, hypoxia-inducible factor-1; IGF-I, insulin-like growth factor I; MMP, matrix metalloproteinase; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor-kappa beta; PDAC, pancreatic ductal adenocarcinoma; PSC, pancreatic stellate cells; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; TGF-β, transforming growth factor-beta; u-PA, urokinase-type plasminogen activator.
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Pancreatic ductal adenocarcinoma (PDAC), the most common type of pancreatic malignancy, is characterized by a desmoplastic reaction consisting of an extensive and dense fibrotic stroma that surrounds and infiltrates clusters of malignant epithelial cells, together with the loss of basement membrane integrity and an abnormal vasculature. This fibrotic stroma is composed of connective tissue rich in collagen (types I and III) and fibronectin produced mainly by activated pancreatic stellate cells (PSC). Pancreatic cancer cells have been shown to activate PSC by paracrine mechanisms involving transforming growth factor-beta (TGF-β). However, after recurrent injury, PSC can retain its activated state by autocrine mechanisms as they can secrete cytokines and growth factors that stimulate receptors on their own cell surfaces [5].

Infiltrating immune cells are regularly present in tumors. The tumor-promoting inflammatory cells include macrophages, mast cells and neutrophils, as well as T and B lymphocytes [6, 7]. Amongst these infiltrating cells, mast cells can secrete several signaling molecules, including epidermal growth factor (EGF), which serve as effectors of their tumor-promoting actions [8]. Interaction between EGF and its receptor (EGFR) stimulates intrinsic activity of tyrosine kinase [9]. The EGFR signaling plays important roles in human cancers, enhancing tumor growth, invasion, motility, tumor spreading and metastasis. Over-expression of both EGF and EGFR is observed in pancreatic cancer and is linked with its development and poor prognosis [10-15].

Other growth factors, such as TGF-βs, derive from tumor cells and become sequestered within the stroma, which thus acts as a storage site. The invading cancer cells produce

matrix metalloproteinases (MMPs) that release these growth factors [3]. The binding of TGF-β to its receptors activates many non-canonical signaling pathways, such as the phosphoinositol-3 kinase (PI3K), mitogen-activated protein kinase, and small guanosine triphosphatase (GTPase) pathways [16]. Remarkably, PDAC is a solid tumor that exhibits a notorious up-regulation of TGF-β isoforms, which correlates with a decrease in patient survival [17].

Solid tumors are complex structures presenting an abnormal vasculature that greatly contributes to the heterogeneous oxygenation of tumor cells. The irregular blood flow in these tumors during ischemia-reperfusion events is responsible for hypoxia and reoxygenation phases, also called intermittent hypoxia, which has been associated with both invasion and metastasis [18-21]. The periodicity in the detention of the blood flow is highly variable from one tumor to another as well as within the same tumor, depending on the architectural complexity and maturation level of the tumor vascular network [22, 23]. Studies of murine and human tumors have shown that the blood flow fluctuations observed in these tumors could vary from several minutes to more than one hour [22-24]. Hypoxia produces several changes in gene expression in normal and tumor cells [25]. This adaptive response to hypoxia is orchestrated by transcription factors such as hypoxia-inducible factor-1 (HIF-1), which induces the transcription of target genes involved in glycolysis and angiogenesis, thus allowing cells to adapt to hypoxia [26, 27]. Moreover, HIF-1alpha (HIF-1 α) also targets fascin, an actin-bundling protein that is overexpressed in PDAC and enhances motility, scattering, and invasiveness of pancreatic cancer cells [28, 29]. Interestingly, HIF-1 α has been shown to induce the conversion of non-stem pancreatic cancer cells into pancreatic cancer stem-like cells (CSLCs) [30]. The importance of CSLCs lies in their ability to self-renew, differentiate into heterogeneous types of tumor cells, and sustain tumor growth *in vivo* [31]. Consequently, CSLCs are responsible for tumor formation, progression, drug resistance, metastasis, and recurrence [31]. Patients, whose tumors express high levels of CSLCs markers such as CD44, have a significantly reduced median survival rate. Noteworthy, it was recently shown that the treatment of pancreatic cancer cell lines with an antibody anti-CD44 down-regulates the stem cell self-renewal genes Nanog, Sox-2, and Rex-1 and inhibits STAT-3-mediated cell proliferation and survival signaling [32]. Furthermore, anti-CD44 treatment also reduces growth, metastasis, and post-radiation recurrence of pancreatic xenograft tumors in mice [32]. Subpopulations of CSLCs have been identified from most tumors including pancreatic cancer, and targeting differentially expressed CSLC-related genes could result in better treatment outcomes of aggressive tumors. Indeed, FoxQ1 knock-down inhibits CSLCs aggressiveness and attenuates tumor formation and growth, and expression of CSLCs markers in the xenograft tumor derived from CSLCs of the moderately-to-poorly differentiated pancreatic adenocarcinoma cells MIA PaCa-2 [33].

The reactive oxygen species (ROS) generated during the reoxygenation periods are also important, since they modify gene expression through the regulation of the activity of some transcription factors, such as activator protein-1 (AP-1) or nuclear factor kappa B (NF-κB). While AP-1 is known to fulfill a pivotal role in tumorigenesis, regulating the expression and function of cell cycle regulators [34], NF- κB is a key player in tumor development through its ability to induce the transcription of genes coding for apoptosis inhibitor factors, proliferation molecules, pro-angiogenic factors, and extracellular matrix (ECM) degrading enzymes [35].

Cancer metastasis involves tumor cells invading the surrounding tissue. Cell invasion is a complex process that can be defined as the migration of cells within a tissue in response to chemical signals, physical cues, and physicochemical processes. Invasive cells must traverse tissue barriers such as the basement membrane, comprised largely of type IV collagen. Remodeling of these tissue barriers depends on the ability of tumor cells to degrade the surrounding collagen matrix and then migrate through the matrix defects. Several proteolytic enzymes, including MMPs, seem to be implicated in this process. Among the MMP family, MMP-2 (gelatinase A, 72kDa type-IV collagenase) is a main character in the proteolytic degradation of basement membranes [36], being secreted by cells as a pro-enzyme and subsequently activated in the extracellular milieu to execute its proteolytic activity [36]. Although *mmp*-2 deletion produces mice with a relatively mild phenotype, exhibiting minor defects in developmental angiogenesis, tumor angiogenesis and tumor growth are highly reduced [37]. In humans, high expression and activation levels of this ECM-degrading proteinase have been found in various cancer tissues [38-44]. In particular, high levels of activated MMP-2 were shown to correlate with tumor invasion and metastasis in pancreatic carcinoma [41, 42]. Although pre-clinical data supports the use of MMP inhibitors in cancer treatment, clinical trials involving these agents have rendered disappointing results, suggesting that the manipulation of MMPs to achieve tumor stasis may require altering the signal-transduction pathways that regulate the activity of MMPs rather than global inhibition [36]. Other proteolytic enzymes like urokinase-type plasminogen activator (u-PA), indirectly degrade the ECM. Secreted as an inactive pro-enzyme, u-PA is activated by proteolytic cleavage, and then converts plasminogen to plasmin, which degrades matrix proteins by itself or by activating, several MMPs [45, 46]. Activation of u-PA receptor (u-PAR) by u-PA binding results in activation of the Ras/extracellular signal regulated kinase (ERK) pathway, leading to cell proliferation, migration, and invasion [47, 48]. As for MMP-2, high levels of u-PA and u-PAR have been shown in pancreatic cancers [49], correlating with poor prognosis [50, 51].

A Common Signaling Pathway for Invasion

The small GTPases of the Rho family are molecular switches that control a broad variety of signal transduction pathways, regulating actin cytoskeleton, cell polarity,

microtubule dynamics, membrane transport pathways and transcription factor activity [52]. Activation of the Ras-related C3 botulinum toxin substrate 1 (Rac1), a member of this family, has been shown to be detrimental not only during the reoxygenation posterior to hypoxia in non-tumor cells [53, 54], but also during the reperfusion that follows ischemia of tissues [55-57]. However, 'beneficial' effects of Rac1 activation were found in various tumor cells [58]. In the well characterized *KrasG12D*-induced murine model of pancreatic ductal adenocarcinoma, the pancreatic epithelial metaplasia is accompanied by apical-basolateral redistribution of F-actin, along with basal expression of Rac1 [59]. Notably, deletion of *rac1* reduces formation of acinar-ductal metaplasia, pancreatic intraepithelial neoplasia and tumors [59].

The above mentioned growth factors EGF and TGF-β1, as well as the hypoxia-reoxygenation conditions, have been shown to increase tumor cell invasion through MMP-2 in various tumor cell types [60-66]. Additionally, our group established the requirement of Rac1 activation for these signals to stimulate secretion and activation of MMP-2, and cell invasion by the poorly and the well-to-moderately differentiated pancreatic adenocarcinoma cells PANC-1 and SW1990, respectively [62, 65, 66]. Mediators of Rac1 activation seem to be stimuli- and cell type-dependent. Accordingly, while EGF activates Rac1 through PI3K and Src [62], hypoxia-reperfusion conditions only stimulate PI3K in PANC-1 cells [66]. In concordance, the glial cell line-derived neurotrophic factor was shown to stimulate invasion via PI3K in PANC-1 cells [67]. In the well-to-moderately differentiated human pancreatic cancer cells CAPAN-2, TGF-β1 stimulates Rac1 activation also through PI3K (unpublished data). Other cell types such as MIA PaCa-2 cells and the moderately differentiated pancreatic adenocarcinoma cells BxPC-3 exhibit reduced invasiveness upon Src inhibition [68].

It is well established that Rac is the upstream signal protein for nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-dependent ROS generation [69]. Growth factors including EGF, TGF-β1, insulin-like growth factor I (IGF-I) and fibroblast growth factor-2 as well as hypoxia-reperfusion, are responsible for stimulating the production of ROS in PANC-1, SW1990 and MIA PaCa-2 cells through non-mitochondrial NADPH oxidase, the major source of ROS induced by growth factors in these tumor cells [62, 65, 66, 70]. In addition to being a pro-survival, anti-apoptotic factor in pancreatic cancer cells [70], ROS mediate invasion by various pancreatic cancer cells, including PANC-1, SW1990 and BxPC-3 [62, 65, 66, 71, 72]. Moreover, our studies show NADPH oxidase-generated ROS as the downstream signal for Rac1-mediated pancreatic cancer cells' invasion [62, 65, 66].

Pathways triggered by ROS in tumor invasion include MAPK signaling (JNK, ERK, p38) and regulation of transcription factors (AP-1, Ets-1, NF-κB) that lie upstream of MMPs and u-PA [28, 34, 35, 73, 74]. Transfection with an antibody against RelA (p65), a NF-κB subunit, not only decreases expression and bioactivity of MMP-9 and u-PA, but also suppresses invasion and angiogenesis in glioma cells [75]. SW1990 cells exhibit a similar behavior, since TGF-β1-induced MMP-2 secretion and activation, and cell invasion depend on NF-κB activity [65]. Interestingly, we

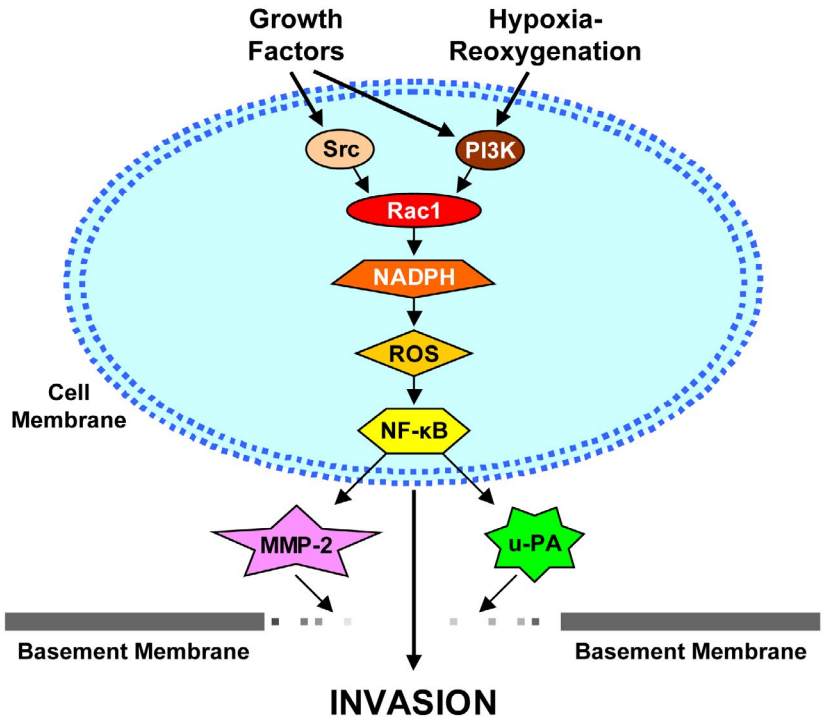

Figure 1. Model for Rac1/ROS/NF-κB-mediated pancreatic cancer invasion. Distinct signals such as growth factors and hypoxia-reoxygenation conditions activate PI3K and/or Src, which alone or combined switch on Rac1 in pancreatic cancer cells. Through NADPH oxidase, activated Rac1 induces formation of ROS. Once generated, these intermediate molecules activate NF-κB, which mediates secretion and activation of MMP-2 and/or u-PA in the extracellular milieu. These proteolytic enzymes are directly or indirectly responsible for degradation of the basement membrane, promoting invasion by pancreatic cancer cells. For this common signaling pathway, mediators of Rac1 activation as well as proteolytic enzyme effectors seem to be cell type dependent.

observed that TGF-β1-stimulated CAPAN-2 cells increased the expression of u-PA and cell invasiveness, which were mediated by Rac1/ROS activation of NF-κB (unpublished data). Furthermore, TGF-β1 was shown to increase both u-PA and invasiveness of responsive pancreatic cancer cell lines PANC-1, IMIM-PC1 and SW1990 [63]. However, TGF-β1 failed to augment invasiveness in non-responsive cell lines CAPAN-1, MIA Paca-2 and IMIM-PC2 [63]. Other growth factors positively regulating invasiveness through the induction of u-PA and u-PAR include IGF-I and hepatocyte growth factor [49, 76, 77].

In summary, the experimental data available support a sequential model that explains increased invasiveness by pancreatic cancer cells ranging from poorly to well-to-moderately differentiated types (Figure 1). In this hypothetical mechanistic model, different signals such as growth factors and hypoxia-reoxygenation conditions promote invasion by pancreatic cancer cells through a common Rac1/ROS/NF-κB dependent pathway. Mediators of Rac1 activation as well as proteolytic enzyme effectors seem to be cell type dependent. While PI3K and Src appear as the intermediate molecules for Rac1 activation, MMP-2 and u-PA emerge as the main proteolytic enzymes that directly or indirectly degrade the proteins of the ECM in pancreatic cancer invasion.

Future Directions

The exact cell type that gives origin to PDAC is not known. However, it is supposed to arise from a poorly differentiated ductal cell, a de-differentiated acinar or islet cell, a progenitor cell, or a stem cell [78]. While most of the studies on pancreatic cancer invasion are focused on single stimulus, the influence of concomitant multifactors on pancreatic cancer progression needs to be deciphered. Additionally, co-culture of tumor and non-tumor cells requires to be further explored in order to properly address microenvironment cell interactions.

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Conflicts of Interest

The authors hereby state that there is no conflict of interest to disclose.

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