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Mini-review

The stromal compartments in pancreatic cancer: Are there any therapeutic targets?

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is characterised by an abundant stromal response also known as a desmoplastic reaction. Pancreatic Stellate Cells have been identified as playing a key role in pancreatic cancer desmoplasia. There is accumulating evidence that the stroma contributes to tumour progression and to the low therapeutic response of PDAC patients. In this review we described the main actors of the desmoplastic reaction within PDAC and novel therapeutic approaches that are being tested to block the detrimental function of the stroma.

1. Introduction

Pancreatic cancer is an aggressive malignancy with a very poor prognosis. The 5-year survival rate for pancreatic ductal adenocarcinoma (PDAC) patients is less than 5%, which is an indicator of the failure of current therapies. Pancreatic cancer is characterised by a dense desmoplastic reaction in which the stroma can expand to more than 50% of the tumour tissue [1]. This stroma surrounding the epithelial cells of the tumour is subject to modification. Pancreatic cancer cells (PCCs) signal to stroma cells changing the composition of extracellular matrix (ECM), increasing the recruitment of inflammatory cells and promoting the proliferation of fibroblasts, including a particular fibroblast cell type called the pancreatic stellate cell (PSC). PSCs are star-shaped cells that store vitamin A and are located in the periacinar and periductal regions of the exocrine pancreas. In their quiescent state, PSCs constitute about 4% of the whole pancreas [2]. However, after PSCs become activated, for example in response to injury, inflammatory stimuli and cancer, their morphology changes. In PDAC, the relation between the staining of α-smooth muscle actin (SMA), a marker for activated PSCs, and the expression of collagen was used to calculate an index for the activation of the stroma in each tumour [3]. The higher this index, the worse was the prognosis for patients with PDAC [3].

In this review we summarise the current state of research regarding the role of the main stromal components in PDCA initiation and progression, with a particular focus on the interaction between tumour cells and PSCs. In addition, we discuss the new therapeutic strategies that arise from the current discoveries in this field.

2. ECM components of the pancreatic tumour stroma

The fibrotic tissue present in PDAC tumours is characterised by an ECM to which both tumour and stroma cells contribute [4]. The pancreatic cancer ECM is composed of collagens, noncollagen glycoproteins, glycosamminoglycans, growth factors and proteoglycans as well as modulators of the cell matrix interaction such as periostin, tenascin C, SPARC (secreted protein acidic and rich

Abbreviations: COX-2, cyclooxygenase-2; CTLs, Cytotoxic T Lymphocytes; DCs, dendritic cells; ECM, extracellular matrix; EGF, epidermal growth factor; FAK, focal adhesion kinase; FAP-α, fibroblast activation protein-α; FGF, fibroblast growth factor; HSCs, hepatic stellate cell; IGF-1, Insulin Growth Factor-1; MDSCs, myeloid-derived suppressor cells; MMP, matrix metalloproteinases; PCCs, pancreatic cancer cells; PDAC, pancreatic ductal adenocarcinoma; PDGF, platelet-derived growth factor; PSCs, Pancreatic Stellate Cells; SERBIPINE2, serine protease inhibitor nexin 2; SHH, sonic hedgehog; SPARC, secreted protein acidic and rich in cysteine; TAMs, tumour associated macrophages; TGF-β, transforming growth factor-β; TIMP, tissue inhibitors of metalloproteinases; tPA, tissue plasminogen activator; Tregs, regulatory T cells (Tregs); uPA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor; α-SMA, α-smooth muscle actin.

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in cysteine) and thrombospondin. The changes in the level of ECM proteins play a major role in invasion and migration and correlate with increased invasive potential of pancreatic cancer cells (PCCs). As summarised in Table 1, pronounced expression of ECM proteins including thrombospondin, periostin, tenascin C, vitronectin, versican, biglycan, collagens and fibronectin is known to promote pancreatic cancer invasion and migration.

For example, although periostin suppresses the malignant phenotype of tumour cells at low concentrations, it increases migration when expressed at high levels [5]. Osteopontin-c is a sialoprotein that was shown to promote invasion in several cancer cell lines. Its expression was elevated in the majority of invasive PDACs, and most frequently in tumours from smokers [6]. Smoking is a risk factor for pancreatic cancer and nicotine was shown to stimulate the production of osteopontin-c in PCCs *in vitro* [7,8]. Importantly, SPARC is an ECM glycoprotein predominantly produced by the tumour-associated fibroblasts and PSCs in PDAC. The high expression of SPARC by stroma cells was shown to be a predictor of poor prognosis and PSCs-derived SPARC increased invasion of PCCs *in vitro* [9,10]. The drug nab-paclitaxel was developed to exploit the ability of SPARC to bind to albumin as a mean of increasing drug delivery to the tumour [11]. Very recently, a randomised phase III trial testing gemcitabine versus gemcitabine in combination with nab-paclitaxel showed a prolongation of overall survival after one year from 22% to 35% ($p = 0.0002$) [12,13]. In this trial high expression of the stromal protein was used to specifically enrich the concentration of a cytotoxic agent in the tumour. Additionally, Alvarez and colleagues demonstrated that nab-paclitaxel reduces the stiffness and the number of cancer associated fibroblasts in the human tumours treated with nab-paclitaxel [14].

The structural organisation of ECM proteins can also influence pancreatic cancer invasion. Expression of fibroblast activation protein (FAP)-α was shown to change the orientation of fibronectin fibres leading to increased scattering and motility of PCCs [15]. Decorin and lumican are small leucine-rich proteoglycans while versican is a large proteoglycan. These proteins are involved in matrix assembly and in invasion processes [16]. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMP) are other important regulator of matrix remodelling and are reported to regulate tumour cell invasion [4,17–20].

In addition, these EMC proteins are known to affect cell mechanisms other than migration. Tenascin C affects motility as well as tumour cell growth by activating the integrin signalling [21]. Proteases present in the interstitial space such as urokinase-type plasminogen activator (uPA) and tissue plasminogen activator (tPA) are produced by tumour cells and promote proliferation, angiogenesis and invasion [22,23]. Collagens I, III and fibronectin are major components of the pancreatic cancer tissue stroma [4,24]. Altered deposition or degradation of collagens can promote cell-survival signals and migration of tumour cells [25,26]. Also, attachment of PCCs to fibronectin, collagen types I, IV, and laminin was shown to decrease cytotoxicity of anticancer drugs and high expression of these ECM components correlates with PDAC chemoresistance [27].

Furthermore, ECM proteins such as hyaluronic acid, a matrix glycosaminoglycan found in PDAC stroma, can act as a barrier to perfusion causing high interstitial fluid pressure and vessel collapse [28]. The employment of hyaluronidase, an enzyme which degrades hyaluronic acid, in combination with gemcitabine further increases survival in mice bearing PDAC. This happens in correspondence to a re-expansion of the microvasculature and an increase of vascular permeability, which leads to greater drug delivery to the tumour [28,29]. Thus, stroma mediates chemoresistance by limiting the amount of drug that reaches tumour cells.

3. Inflammatory cells

The immune response is an important element in the progression of pancreatic cancer. Tumour cells exploit several immunosuppressive mechanisms to block the anti-tumoural response. Some evidence of the immunosuppressive regulation during PDAC development were collected using PDAC mouse models bearing an oncogenic KRAS mutation (Pdx1-Cre; LSL-$Kras^{G12D}$) or the double mutant $Kras^{G12D}$/$TP53^{R172H}$ [30]. In these mice, an intense inflammatory reaction was observed during the progression from normal histology to PDAC characterised by the progressive infiltration of $CD45^{+}$ leukocytic cells. Of these leukocytes, a few were effector T cells, while the majority were immunosuppressive cells, including tumour associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs). Natural killer cells were not detected.

Table 1
ECM composition in PDAC tumours.

ECM protein	Functional role	Secreted by	References
Biglycan	Binds to collagen, transfer TGF-β1	NA	[121]
Collagens I, III, IV	Angiogenesis, invasion, metastasis, chemoresistance	PSCs and PCCs	[16,81,89]
Decorin	Matrix assembly	PSCs/stroma	[16,122]
FAP-α	ECM remodelling, increase PCCs motility	PSCs	[15]
Fibronectin	Cell survival, migration, chemoresistance	PSCs	[15,27,81,97,123]
Hyaluronic acid	Chemoresistance	PCCs, Stroma	[28,29,124,125]
Laminin	Binds to integrin, angiogenesis	NA	[4,126,127]
Lumican	Cell growth and invasion	PSCs/stroma	[122,128]
MMP-11	Binds to integrin αvβ5, ECM degradation	NA	[129]
MMP-2	Binds to integrin αvβ5, ECM degradation	PSCs and stroma (mostly), PCCs	[19,99,129]
MMP-9	Matrix degradation, invasion	PCCs (mostly), PSCs	[20,130]
Osteopontin-c	Adhesion, migration, survival	TAMs and PCCs	[6,8]
Periostin	Tumour cells invasion, proliferation, chemoresistance	PSCs/fibroblasts	[5,92,131]
SPARC/osteonectin	Invasion	PSCs/fibroblasts (mostly) and PCCs	[9,10]
Tenascin C	Tissue remodelling, tumour growth, invasion	PSCs	[21,132,133]
Thrombospondin-1/2	Invasion, angiogenesis	PCCs, endothelium, PSCs/fibroblasts	[17,20]
TIMP-1/2	Matrix degradation, cell proliferation	PCCs, stroma and PSCs	[4,16,18]
tPA	Proliferation, angiogenesis, invasion	PCCs	[23]
uPA	Angiogenesis, invasion	PCCs	[22]
Versican	Matrix remodelling	PCCs	[16,134]
Vitronectin	Binds to integrin αvβ complex, motility	PCCs and PSCs/fibroblasts	[132,135,136]

NA, information not available; PSCs, pancreatic stellate cells; PCCs, pancreatic cancer cells; and TAMs, tumour associated macrophages.