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Review article

Pancreatic cancer: The microenvironment needs attention too!

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ABSTRACT

The abundant stromal/desmoplastic reaction, a characteristic feature of a majority of pancreatic adenocarcinomas (PDAC), has only recently been receiving some attention regarding its possible role in the pathobiology of pancreatic cancer. It is now well established that the cells predominantly responsible for producing the collagenous stroma are pancreatic stellate cells (PSCs). In addition to extracellular matrix proteins, the stroma also exhibits cellular elements including, immune cells, endothelial cells and neural cells. Evidence is accumulating to indicate the presence of significant interactions between PSCs and cancer cells as well as between PSCs and other cell types in the stroma. The majority of research reports to date, using in vitro and in vivo approaches, suggest that these interactions facilitate local growth as well as distant metastasis of pancreatic cancer, although a recent study using animals depleted of myofibroblasts has raised some questions regarding the central role of myofibroblasts in cancer progression. Nonetheless, novel therapeutic strategies have been assessed, mainly in the pre-clinical setting, in a bid to interrupt stromal–tumour interactions and inhibit disease progression. The next important challenge is for the translation of such pre-clinical strategies to the clinical situation so as to improve the outcome of patients with pancreatic cancer.

Introduction

Pancreatic cancer remains a disease with an unacceptably high mortality to incidence ratio [1], mostly because therapeutic approaches predominantly targeting pancreatic cancer cells (chemotherapy, radiotherapy with or without surgery) have failed to provide significant clinical benefit. The need for an improved understanding of the pathogenesis of pancreatic cancer so as to identify novel therapeutic targets has served as the impetus for the attention that has been focussed in recent years on the prominent desmoplastic/stromal reaction that is a characteristic histological feature of human pancreatic cancer. Evidence is now accumulating to indicate a close but complex interaction between cancer cells and their surrounding microenvironment, which may facilitate the rapid progression of the disease [2].

H&E stained sections of pancreatic ductal adenocarcinoma (PDAC) commonly reveal an expanse of dense stromal reaction in which pancreatic cancer cells are embedded [3] (Fig. 1). This stromal tissue can often constitute 50–80% of the tumour tissue and is composed of an extracellular matrix (ECM) comprising fibrous proteins such as collagens, fibronectin and laminin, as well as other non-collagenous proteins such as glycoproteins, proteoglycans and glycosaminoglycans. The ECM also contains factors that are thought to influence the interaction of cancer cells with the matrix including growth factors, osteopontin, periostin and serine protein acidic and rich in cysteine (SPARC). Blood vessels and neurons form an integral part of the stroma, as do immune cell infiltrates comprising lymphocytes, macrophages, mast cells and myeloid derived suppressor cells (MDSCs). Importantly, it is now well established that the cells responsible for the production of the collagenous stroma in pancreatic cancer are activated pancreatic stellate cells (PSCs) [4]. In the normal pancreas, PSCs are resident vitamin A storing cells in a quiescent state, comprising 4–7% of all parenchymal cells [5]. In pancreatic cancer, PSCs become activated, with the transformation resulting in a change of phenotype to myofibroblast-like cells which express the cytoskeletal protein

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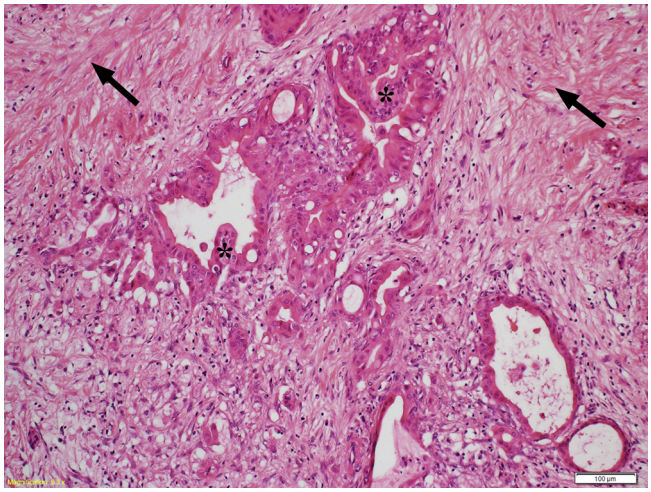

Fig. 1. Photomicrograph of a haematoxylin and eosin (H&E) stained pancreatic section from a patient with pancreatic cancer, showing a prominent stromal/fibrotic reaction (black arrows) surrounding tumour elements (asterisks).

alpha smooth muscle actin (αSMA), and exhibit increased proliferation, migration and ECM protein synthesis.

Whether the extent of the stromal reaction predicts clinical outcome has been a vexed question to which there is, as yet, no clear answer. In an immunohistochemical study of pancreatic sections from 233 PDAC patients who underwent surgery, Erkan et al. [6] determined an activated stroma index (calculated as the ratio of the αSMA positive area to the collagen stained area in the sections). They reported that a high activated stroma index (high stromal activity and low collagen deposition) was associated with a worse prognosis than patients with a low activated stromal index (low stromal activity and high collagen deposition). These findings supported the concept that activated PSCs facilitated disease progression. Subsequently, a smaller retrospective study with 63 patients reported that a high stromal density (calculated as the ratio of the area of collagen positive stroma to the area of the tumour mass in pancreatic sections) was associated with a significantly longer overall survival, while the stromal activity (percentage of stromal cells exhibiting strong αSMA positivity among all cells staining positive for αSMA) did not correlate with clinical outcome and therefore did not predict prognosis [7]. Most recently, Ozdemir et al. [8] assessed αSMA expression in pancreatic sections from 53 patients and reported that low αSMA scores correlated with significantly worse survival. Thus, the latter two studies proposed that the stromal/collagenous reaction was a protective reaction against cancer progression. However, larger studies with well stratified patient cohorts and well defined methods of assessing stromal density and PSC activation are required before any firm conclusions can be drawn.

PSCs and pancreatic cancer

A possible role for PSCs in the pathobiology of the collagenous stroma in pancreatic cancer was initially proposed based on the well documented central role for those cells in the fibrosis of chronic pancreatitis [9]. Several studies had established that during pancreatic injury (necroinflammmation), PSCs are activated by a range of factors including proinflammatory cytokines, growth factors, oxidant stress, toxic compounds such as alcohol and its metabolites, endotoxin, hypoxia, increased interstitial pressure and hyperglycaemia. Signalling pathways mediating the above changes in PSC functions include the MAPK pathway (mediating cell proliferation and migration), PI3K (migration); Hedgehog (migration, proliferation) transforming growth factor beta TGFβ related pathways, Rho kinase (ECM production) etc (see review [10]). Interestingly, Binkley et al. [11] also described a significant overlap in gene expression of the stromal compartments of chronic pancreatitis and pancreatic cancer. Given the above and the known increased risk of pancreatic cancer in patients with chronic pancreatitis [12], it was reasonable to interrogate the role of PSCs in the fibrosis of pancreatic cancer.

The presence of PSCs in stromal areas of human pancreatic cancer has been examined by immunostaining of serial sections of human pancreatic cancer tissue for PSC selective markers such as desmin, glial fibrillary acidic protein (GFAP), nestin, nerve growth factor (NGF) and neural cell adhesion molecule (NCAM) [4]. Dual staining techniques have also been applied to assess expression of αSMA and collagen mRNA on the same sections. The presence of activated PSCs in the stroma of pancreatic cancer has now been conclusively demonstrated and importantly, these activated PSCs have been identified as the predominant source of collagen in the stroma [4]. Intriguingly, recent studies have reported the presence of activated PSCs around pancreatic intraepithelial neoplasms (PANINs, early, premalignant lesions) both in human pancreas and the pancreas of genetically engineered mouse models (GEMMs) of the disease [2]. Furthermore, periostin (a cell adhesion protein known to be expressed by PSCs) has been found to be present in human intraductal papillary mucinous neoplasm (IPMN), again indicating the presence of PSCs around early neoplastic areas in the pancreas [13].

In view of the juxtaposition of activated PSCs and cancer cells, cross-talk between the two cell types in pancreatic cancer was postulated, and more recently, interactions between PSCs and other cell types in the cancer stroma (immune cells, endothelial cells and neural cells) have also been examined using in vitro and in vivo approaches. As outlined in the brief overview below, the weight of evidence available to date is on the side of a critical role for PSCs in cancer progression, although a very recent study (discussed later) using transgenic mice has reported that conditional knockout of myofibroblasts resulted in a worse outcome, suggesting a protective role for these cells.

In vitro studies

The experimental set-up for these studies usually involves co-culture of the cell of interest with PSCs or exposure of a cell type to conditioned medium from the other (and vice versa).

PSC – cancer cell interactions

Several studies (see review [2]) have now clearly demonstrated that in response to pancreatic cancer cells, PSCs are activated and exhibit increased proliferation, ECM synthesis and migration. In turn, PSCs can induce cancer cell proliferation, while at the same time, decreasing cancer cell apoptosis, thus facilitating cell survival. PSCs also stimulate cancer cell migration, an effect that is associated with increased epithelial–mesenchymal transition (EMT) of cancer cells (as indicated by an increase in expression of mesenchymal markers such as vimentin associated with a decrease in epithelial markers such as E-cadherin). Notably, interactions between PSCs and pre-neoplastic cells (PanIN cells) have also now been reported, with PSCs exhibiting increased proliferation, matrix metalloproteinase (MMP) expression and fibronectin synthesis, when exposed to PanIN secretions [14]. While direct evidence of reciprocal effects of PSCs on PanIN cells is not yet available, indirect evidence for such an interaction may be inferred from the observation that pharmacological inhibition of cyclooxygenase 2 (COX2, an inducible form of the enzyme expressed by PSCs), reduces the progression of pre-neoplastic to neoplastic lesions in a GEMM of pancreatic cancer [15].

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