Abstract

Chronic pancreatitis, an inflammatory disease of the exocrine pancreas, has been reported to be a major risk factor for the development of pancreatic ductal adenocarcinoma. Evidence from pre-clinical mouse models has shown that both diseases share a common origin in the digestive enzyme-producing acinar cells, through acinar to ductal metaplasia. Moreover, both diseases are characterised by the presence of an abundant stroma, the components of which include activated pancreatic stellate cells and immune cell infiltrates, which signal to epithelial cells through the production of cytokines and chemokines. In this review we explore the links between chronic pancreatitis and pancreatic ductal adenocarcinoma, with particular reference to the role of the microenvironment in both diseases. A better understanding of the nature of the epithelial and stromal changes, as well as their interactions, has led to trialling novel therapeutic strategies for the prevention and/or treatment of pancreatic cancer.

Pancreatitis and pancreatic cancer - diseases of the exocrine pancreas

The pancreas is a glandular organ composed of two distinct compartments, exocrine and endocrine. The exocrine compartment constitutes the majority of pancreatic tissue, in which the endocrine islets of Langerhans are embedded. While the endocrine islets regulate glucose homeostasis, the exocrine acinar cells secrete enzymes essential for digestion of food. The exocrine duct cells secrete mucins and a bicarbonate-rich fluid, that is transported to the duodenum via a branched network of intra and inter-lobular ducts that drain into the main pancreatic duct.2

Chronic pancreatitis incidence in industrialised countries ranges from 3.5 to 10 per 100,000 population.2 It is a progressive inflammatory disorder that arises from repeated overt or silent episodes of acute pancreatitis, where deregulated secretion and premature activation of acinar enzymes results in increasing residual damage to the pancreas (the necrosis-fibrosis sequence). The resulting damage eventually results in chronic pain, maldigestion and diabetes. The histopathologic features of this disease include acinar atrophy, fibrosis, fatty replacement, chronic inflammation and abnormal, distorted ducts.2,3 In the majority of patients, the disease results from a combination of genetic and environmental factors, with alcohol consumption being the best-defined risk factor.3 Smoking is a risk factor for disease progression.4,5

Pancreatic ductal adenocarcinoma (PDAC) is the most common neoplasm of the pancreas, accounting for more than 85% of pancreatic cancer cases.6 Despite the relatively low incidence of about 6-12 per 100,000 per year in western countries,7 PDAC is the fifth cause of cancer related death in Australia and the fourth in the United States, but is predicted to become the second leading cause of cancer-related death by 2030.8,9 The astonishing mortality (median survival of <6 months and a 5-year survival rate of <5%)9 is attributed to late diagnosis and to the tumour being often refractory to existing therapies such as gemcitabine. Novel therapies (Abraxane, 5-Fluorouracil/Irinotecan/Oxaliplatin) have sparked some hope, but often only add a few weeks to the median survival of six months. The heterogeneity of PDAC may be the cause of failure of most drugs in clinical trials that have comprised biologically unselected cases.10 To apply drugs in a more targeted fashion, pancreatic tumour biology needs to be unravelled.

In recent years, progress has been made in our understanding of the origin of PDAC. The most widely accepted model of PDAC progression is that the tumour originates in histologically well-defined precursor lesions through the accumulation of multi-step genetic alterations. These non-invasive preneoplastic lesions are named pancreatic intraepithelial neoplasias (PanINs) and have been found to harbour many of the genetic alterations that are found in PDAC.11 Mutations in the KRAS oncogene are thought to be the initiating event
during PDAC progression, being found in 93% of cancer cases, and in approximately 36-44% of early PanIN lesions and 87% of advanced PanIN lesions. During the PanIN-PDAC progression, KRAS mutations are followed by loss of tumour suppressor genes such as INK4A/CDKN2A, TP53 and SMAD4.

**Chronic pancreatitis predisposes to pancreatic cancer**

**Evidence from epidemiologic studies**

Pancreatitis is a risk factor for pancreatic cancer. A meta-analysis of 22 studies found a 5.1 fold increased relative risk of developing pancreatic cancer in patients with unspecified pancreatitis, a 13.3 fold increase in relative risk in patients with chronic pancreatitis and a 69 fold increase for hereditary pancreatitis. Despite the increased risk, only around 5% of patients diagnosed with chronic pancreatitis will develop carcinoma over a period of 20 years. Hereditary pancreatitis has been associated with mutations in several genes including PRSS1, PRSS2, SPINK1 and CTRC. These individuals have a cumulative risk of developing pancreatic cancer of 40-55%.

**Evidence from experimental mouse models**

Chronic pancreatitis and PDAC were historically regarded as unrelated diseases that arose from different cells in the pancreas, i.e. acinar and ductal cells, respectively. Evidence has now accumulated for a common origin of both diseases in acinar cells. Pancreatic acinar cells can lose their differentiated state and re-acquire characteristics very similar to embryonic and adult duct cells, a process called ‘acinar to ductal metaplasia’. This metaplasia has been observed in clinical samples and has been well documented in experimental rodent models of pancreatitis. The most widely used model involves treatment with the cholecystokinin agonist caerulein, which induces local oxidative stress, inflammation, oedema and loss of the acinar parenchyma that is transiently replaced by a duct-like epithelium, reminiscent of human pancreatitis. Genetic lineage tracing experiments in mice have shown that the intermediate ductal metaplastic epithelium present in this model can arise from acinar cells. We have further documented how during pancreatitis, acinar cells can dedifferentiate and acquire features of pancreatic progenitor duct-like cells.

Mouse models, where oncogenic Kras was activated specifically in acinar cells early in embryonic development, developed neoplastic lesions and invasive ductal carcinoma, supporting the idea that acinar cells can be the cell of origin of PDAC. Adult acinar cells are more refractory to Kras-driven neoplastic transformation. Even if the cells dedifferentiate in pancreatitis, they undergo growth arrest through activation of a p53-dependent senescence program, which constitutes a barrier to malignant transformation. Nevertheless, in the presence of mutant Kras, chronic pancreatitis renders acinar cells susceptible to transformation by the oncogene, leading to the development of the full spectrum of PanINs and PDAC. More recently, the ductal transcription factor Sox9 has been shown to be required for the occurrence of acinar to ductal metaplasia and consequent initiation of PDAC, acting through the activation of the EGFR/ERBB signalling pathway.

In summary, PDAC is most commonly preceded by PanIN lesions that can originate from acinar cells through acinar to ductal metaplasia. This results from the combination of genetic alterations in epithelial cells (KRAS oncogenic mutation and loss of tumour suppressors) and tumour-promoting signalling derived from the surrounding stromal cells, including activated stellate cells and inflammatory components (figure 1), as detailed below.

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**Figure 1**: Acinar to ductal metaplasia, as it occurs in chronic pancreatitis, is a recognised precursor of pancreatic ductal adenocarcinoma. Signalling from activated stellate cells and immune cell infiltrates contribute to the development of pancreatitis and cooperate with oncogenic Ras signalling and loss of tumour suppressor barriers in the subsequent progression to pancreatic intraepithelial neoplasias (PanINs) and, ultimately, to invasive ductal adenocarcinoma. Adapted from Pinho et al. Cancer Letters 2015.
Microenvironment in chronic pancreatitis and pancreatic cancer

Chronic pancreatitis and pancreatic cancer are both characterised by the presence of a dense stroma, composed of extracellular matrix (ECM) proteins, including collagen, and other cell types such as pancreatic stellate cells, endothelial cells, neurons and immune cell infiltrates.\(^3\)

Pancreatic stellate cells

Pancreatic stellate cells (PSCs) are resident cells of the normal pancreas, constituting 4-7% of all parenchymal cells.\(^3\) In response to pancreatic injury or inflammation, quiescent PSCs undergo activation to become myofibroblast-like cells, which express-SMA (alpha smooth muscle actin). Upon activation, PSCs lose their vitamin A-containing lipid droplets, proliferate, migrate, produce ECM components and secrete cytokines and chemokines.\(^3\) Cytokines and growth factors produced by acinar cells, inflammatory cells, platelets, ductal cells, endothelial cells and by PSCs themselves can activate PSCs, and induce cellular responses through paracrine and autocrine mechanisms. Chemokines produced by PSCs contribute to the recruitment of inflammatory cells to the inflamed pancreas. PSCs also produce matrix metalloproteinases and their inhibitors, being involved in the maintenance of normal tissue architecture by regulating ECM turnover. Additionally, PSCs play a ‘macrophage-like’ role in the pancreas, contributing to organ restitution and homeostasis by phagocytising necrotic acinar cells.\(^3,34\)

Activated PSCs can have two fates. If the inflammation and injury are limited, as in an acute episode of pancreatitis, PSCs might undergo apoptosis or revert to quiescence. If the inflammation and injury are sustained or repeated, PSC activation is perpetuated, leading to development of pancreatic fibrosis, as observed in chronic pancreatitis.\(^2,33,34\)

Activated PSCs are also responsible for the production of the ECM proteins that constitute the abundant stroma around pancreatic tumours.\(^35\) For many years, data acquired from both in vitro and in vivo models reinforced the notion that PSCs contribute to cancer progression.\(^36\) In this regard, it has been shown that pancreatic cancer cells recruit PSCs to their vicinity and promote their activation with consequent increases in proliferation and ECM synthesis. In turn, PSCs stimulate tumour cell proliferation, inhibit cancer cell apoptosis, promote cancer cell migration and epithelial-mesenchymal transition.\(^36,37\) Moreover, several studies have shown that PSCs not only stimulate fibrosis, local tumour growth and metastasis, but also lead to chemoresistance.\(^39\)

Two recent studies have generated controversy by proposing that pancreatic cancer stroma may protect against cancer progression.\(^39,40\) In these studies, depletion of PSCs in mouse models of the disease was achieved either by genetic targeting or drug based inhibition.\(^39,40\) resulting in the development of more aggressive and undifferentiated tumours. These apparent contradictory findings highlight the possibility that the role of PSCs may be context-dependent and emphasise the need for further studies on the mechanisms mediating stromal-tumour interactions in PDAC.

A central role for inflammation

Immune cells and endothelial cells in the pancreas also produce inflammatory cytokines and chemokines that, together with reactive oxygen species, cause epithelial cell damage and increased proliferation. Inflammatory mediators, such as cyclooxygenase-2 (Cox2), NF-kB and STAT3, play key roles with respect to inflammation. In turn, inflammation can generate sustained and exacerbated secondary oxidative injury and, as such, mediate the promotion of inflammatory infiltration and acinar cell injury.\(^41,42\)

Various studies using genetically engineered mouse models have shown that genes involved in inflammatory pathways have a role in pancreatic cancer development. Cox2 is activated by inflammatory cytokines and its expression is upregulated in pancreatitis and pancreatic cancer.\(^42\) Interestingly, transgenic overexpression of Cox2 induces chronic pancreatitis and the formation of PanINs.\(^43,44\)

In a KRAS mutant background, inflammation overcomes barriers that prevent tumour development. A well-defined tumour suppressive barrier inhibited by pancreatitis is senescence.\(^22,28,46\) In animals bearing a KRAS oncogenic mutation, a mild inflammatory stimulus in the pancreas triggers an NF-kB mediated positive feedback mechanism, which amplifies Ras activity to pathological levels, causing the development of chronic inflammation and neoplastic lesions.\(^46\) Another study showed that, also in a KRAS mutant context, TNF-α–induced activation of the NF-kB pathway in pre-malignant epithelial cells creates a feed forward loop that retains the transformed cells in an inflammatory state.\(^47\)

STAT3 activation has also been shown to be essential for initiation and progression of pancreatic cancer. STAT3 contributes to cancer initiation by promoting the de-differentiation of the acinar cells during pancreatitis, which consequently become more vulnerable to Kras-mediated transformation. The STAT3 pathway can be activated in pancreatic epithelial cells by both paracrine and autocrine mechanisms.\(^46,49\)

Inflammatory signalling coming from the epithelium can also exert paracrine effects on stromal components. GM-CSF is one of the inflammatory cues from the tumour cells that modulate the microenvironment.\(^50\) Chemokine production by the tumour epithelium also promotes connective tissue growth factor secretion from the stromal cells.\(^51\) Interestingly, loss of mutant KRAS in the tumour epithelium results in involution of the stroma and its inflammatory components.\(^52\)

In summary, the pancreatic microenvironment, including stellate and immune cells, have an important role both in pancreatitis and in pancreatic tumour development.
The epithelial cells themselves also produce inflammatory molecules, both at precursor stages and in established tumours, leading to the remodelling of the microenvironment. A better understanding of this tumour-stroma crosstalk could provide the platform for the development of novel therapeutic strategies for prevention and treatment of pancreatic cancer.

**Improved detection, chemoprevention and treatment of pancreatic cancer**

Novel discoveries that improve our understanding of the mechanisms mediating initiation of pancreatic tumours will be critical for the development of better detection strategies, together with major advances in sophisticated imaging techniques that can detect early neoplastic lesions. In addition, new ways are being devised to target the pro-tumourigenic effects of the stromal stellate and immune cells.

**Approaches targeting stellate cells**

The hedgehog signalling pathway has been shown to mediate interactions between PSCs and PDAC cancer cells. Inhibition of this pathway using IPI-926 in combination with gemcitabine in a pre-clinical model of PDAC had an inhibitory effect on tumour growth, attributed to the consequent increased concentration of intra-tumoural gemcitabine. However, a phase 2 clinical trial with IPI-926 had to be prematurely stopped due to significantly reduced survival of patients. Concordantly, a more recent study reported that genetic inactivation of the hedgehog pathway in a mouse model decreased tumour stroma, but increased tumour vascularity, resulting in increased aggressiveness. These results underscore a need for better understanding of the mechanistic complexities of targeted pathways, as well the importance of confirming therapeutic effects in a range of pre-clinical models before using them in the clinic.

Enzymatic degradation of the ECM component hyaluronan using PEGPH20 has been shown to deplete the stroma in an animal model of PDAC, increasing the delivery of gemcitabine and improving survival. A randomised clinical trial is now ongoing to evaluate PEGPH20 as a first-line therapy for patients with metastatic pancreatic cancer, but the results have been variable. Blockade of the angiotensin II receptor using olmasartan or losartan have also shown promising effects in reducing stroma and reducing tumour growth in pre-clinical models of PDAC. A phase 2 trial is currently ongoing to evaluate the efficacy of the use of losartan in combination with FOLFRINOX and proton beam radiation.

A very recent study has also shown promising results for the vitamin D receptor ligand calcipotriol. In an orthotopic model of pancreatic cancer, calcipotriol was shown to induce quiescence of PSCs leading to stromal remodelling, suppression of pancreatitis, reduced tumour volume and increased survival. Further studies are now needed to evaluate the safety and efficacy of calcipotriol in the clinical setting.

**Anti-inflammatory agents**

The established link between inflammatory pathways and cancer development suggests a potential prophylactic and/or therapeutic use of anti-inflammatory agents for pancreatitis and pancreatic cancer.

Numerous nonsteroidal anti-inflammatory drugs (NSAIDs) have shown an effect in prevention and/or treatment of pancreatic cancer in experimental studies. These include Cox2 specific inhibitors such as celecoxib, apricoxib or NS-398, as well as non-specific NSAIDs such as aspirin, nimesulide or sulindac.

A recent epidemiological study found that aspirin significantly reduced deaths due to pancreatic cancer after five years of follow up. Accordingly, recent case-control studies suggest a reduction in risk of pancreatic cancer for long-term users of NSAIDs.

Regarding treatment for pancreatic cancer with anti-inflammatory agents, several early phase trials support the feasibility of Cox2 inhibitors for therapeutic use. Although the study of Cox2 inhibitor apricoxib in combination with gemcitabine and erlotinib did not reach its endpoint, it showed a trend towards benefit with the anti-inflammatory compound, but at the cost of increased incidence of gastrointestinal haemorrhage.

Several clinical trials are now testing the addition of anti-inflammatory agents to chemotherapy for pancreatic cancer, most in a palliative setting, but also as an adjunct to surgery and adjuvant chemotherapy. Results from these clinical studies will be essential to inform the potential of these agents as valuable chemopreventive and/or therapeutic approaches for pancreatic cancer.

**Conclusion**

Evidence from the study of mouse models in combination with epidemiological and patient-derived data have challenged prevailing dogmas and established a connection between chronic pancreatitis and pancreatic cancer. These diseases not only have mechanistic pathways in common, but also share the presence of an abundant stroma, including stellate and immune cells, which through the production of cytokines, chemokines and ECM components, establish a microenvironment that influences pancreatic epithelial cell differentiation and growth. The study of the interaction between pancreatic epithelial cells and the microenvironment has generated more questions for further research, but has also provided clues for the development of novel preventive and therapeutic approaches to tackle pancreatic cancer.

**References**


