Role of Inflammation in Pancreatic Carcinogenesis and the Implications for Future Therapy

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\textbf{Abstract}

\textbf{Background:} The link between inflammation and pancreatic cancer has been observed for a number of gastrointestinal neoplasms. This review examines the role of inflammation in pancreatic carcinogenesis and how it can be utilised to develop new therapies against pancreatic cancer.

\textbf{Methods:} A literature review of Pubmed, Medline and Web of Science databases was undertaken using the key words, pancreatic cancer, inflammation, inducible nitric oxide, interleukins, pro-inflammatory cytokines, cyclooxygenase-2, NF-kappa B, reactive oxygen species, DNA adducts, lipoxygenases, chemoprevention.

\textbf{Results:} Epidemiological evidence and molecular studies both in vitro and in vivo all support the hypothesis that inflammation plays an important in the initiation and progression of pancreatic tumours.

\textbf{Conclusion:} Sustained damage caused by chronic inflammation may precede the onset of frank malignancy by a significant interval. As such, suppression of inflammatory changes and oxidative damage, may help delay or even prevent the inception of pancreatic neoplasia.

\textbf{Introduction}

Pancreatic cancer is one of the most lethal cancers of the gastrointestinal tract, with a death to incidence ratio of 0.99 [1]. The poor prognosis of pancreatic cancer is attributable to its tendency for late presentation, aggressive local invasion, early metastases and poor response to chemotherapy [2]. The only curative treatment for pancreatic cancer is surgical resection, however, only 10–15% of patients will have localised disease at presentation [3]. Retrospective studies have revealed a global increase in the mortality rate from pancreatic cancer, reflecting a rising incidence of pancreatic tumours [4–7]. This trend of increasing incidence, coupled with the poor prognosis of pancreatic cancer, emphasises the need to elucidate the mechanisms underlying pancreatic carcinogenesis, in order to find new treatments against the disease.

Smoking remains one of the most important risk factors for pancreatic cancer. Smokers have a 2- to 3-fold increased risk compared to non-smokers [8]. The main carcinogens implicated in tobacco smoke are the tobacco-
specific nitrosamines (TSNA). There is some evidence that exposure to occupational carcinogens such as chromium [9], DDT [10] and halogenated hydrocarbons [11] may increase the risk of pancreatic cancer. Furthermore, heterocyclic amines and polycyclic aromatic hydrocarbons, formed during the cooking of red meat, may also contribute to pancreatic cancer risk [12]. Other risk factors include familial inheritance [13, 14], with a 50-fold increase in the risk of pancreatic cancer if three family members are affected [15], and Helicobacter pylori sero-positivity [16].

The possible causal relationship between inflammation and cancer has been observed in a number of gastrointestinal malignancies, such as inflammatory bowel disease with colorectal cancer, viral hepatitis with hepatocellular carcinoma and reflux oesophagitis with oesophageal adenocarcinoma. The exact link between chronic inflammation and carcinogenesis is unclear. It is possible that the drive for continued replication, due to continuous damage and subsequent repair, statistically increases the probability of cells accumulating enough ‘genetic hits’ for initiation to occur. The probability of cell initiation may be increased further by the formation of potential carcinogens produced during the inflammatory process, for example, reactive oxygen species, which can bind to DNA to form oxidative DNA adducts.

Once initiation has occurred, the microenvironment formed by inflammation in tissues, which has a high concentration of growth factors and cytokines, may enhance proliferation of initiated cells leading to promotion and eventually progression of a population of tumour cells (fig. 1). These changes may take many years to develop. Hence, interrupting the pro-carcinogenesis pathways associated with chronic inflammation could inhibit, retard or even prevent the onset of cancer. This review discusses the role of inflammation in pancreatic carcinogenesis, and how knowledge in this area could lead to new therapeutic possibilities in the treatment of pancreatic cancer.

**Chronic Pancreatitis and Pancreatic Cancer**

Several studies have observed an increased incidence of pancreatic cancer in patients with chronic pancreatitis, when compared to the average population [17–20]. The increase in incidence varies across different studies. The Standardized Incidence Ratio has previously been reported from 3.8 [21] to as high 18.5 [22]. Some studies have found the increase in risk to be as high 16-fold in patients with chronic pancreatitis [23]. Familial pancreatitis accounts for less than 1% of all cases of pancreatitis and is
also associated with an increased risk of pancreatic cancer [14, 24]. One study has found that among patients with a history of familial pancreatitis, the risk of developing pancreatic cancer can be as high as 53 times that of the normal population [25].

The theory that chronic pancreatitis is linked to the development of pancreatic cancer has been challenged. In addition to having an increased risk of pancreatic cancer, patients with chronic pancreatitis have been found to have an increased incidence of extrapancreatic cancer [22, 26]. It is, therefore, conceivable that other epidemiological risk factors associated with the development of chronic pancreatitis, such as smoking [27], are responsible for the increased pancreatic cancer incidence, rather than pancreatic inflammation itself. Furthermore, if a relationship between chronic pancreatic inflammation and cancer existed, a positive correlation with the length of pancreatitis and the risk of pancreatic cancer would be expected, such as is found with the risk of colorectal cancer in patients suffering from inflammatory bowel disease for 10 years or longer. One study, at least, has found that after 10 years the relative risk of pancreatic cancer in patients with chronic pancreatitis was identical with the rest of the normal population [21]. Finally, it is well documented that early pancreatic cancer can be difficult to distinguish from chronic pancreatitis, and so, patients misdiagnosed with chronic pancreatitis that are later found to have pancreatic cancer could be a major obfuscating factor in epidemiological studies of chronic pancreatitis and cancer risk.

These arguments disputing the link between chronic pancreatitis and pancreatic cancer can be countered by observations that after adjusting for factors such as smoking, and discounting pancreatic cancers which occur less than 5 years after the diagnosis of chronic pancreatitis (which most likely represent misdiagnosed pancreatic cancer) the risk of pancreatic cancer remains increased in patients with chronic pancreatitis [22]. In patients with familial chronic pancreatitis, the risk of cancer does appear to increase with the duration of the disease process. By 70 years of age, 40% of patients with familial chronic pancreatitis will develop pancreatic tumours [24]. In addition, molecular changes observed in pancreatic tissue of patients with chronic pancreatitis display similar features to pancreatic cancers. For example, K-ras mutations are almost universal in pancreatic cancer and are also found in up 42% of patients with chronic pancreatitis [28–30]. Furthermore, inflammatory mediators in chronic pancreatitis have also been linked to pancreatic carcinogenesis. The cyclooxygenase-2 (COX-2) enzyme, which has been found in a wide range of gastrointestinal malignancies, has been shown to be overexpressed in patients with chronic pancreatitis [31] and the mutagenic carbon-yl compound, malondialdehyde, has been found in high concentrations in chronic pancreatic tissue [32, 33]. These findings provide a plausible mechanism to the development of carcinogenesis in pancreatic tissue exposed to chronic inflammation and are discussed in further detail below.

**NF-KappaB (NF-κB)**

NF-κB comprises of a family of transcription factors which activate the expression of a wide array of genes involved in tumourgenesis, metastasis, differentiation, embryonic development, apoptosis and inflammation [34]. The family comprises of NF-κB1, NF-κB2, Rel A, c-Rel and Rel B. These proteins consist of hetero- or homodimers and share a 300-residue long Rel domain. In normal conditions NF-κB interacts with the protein IκBα, which covers the signal sequence responsible for DNA binding and nuclear translocation, on the NF-κB subunit [35]. This interaction results in the sequestration of NF-κB in the cytoplasm of cells. Stimulation can occur via a wide number of different pathogenic stimuli and results in phosphorylation of Iκ-Bα followed by degradation by the 26S proteasome. This uncovers the nuclear localisation signal, enabling nuclear translocation of the NF-κB complex with activation of a number of different genes, ultimately leading to increased expression of pro-inflammatory and pro-oncogenic messenger molecules, such as inducible nitric oxide (iNOS), cyclooxygenase-2 (COX-2) [36], cyclin D1 [36] and interleukin-8 (IL-8) [37] (fig. 2).

Constitutive activation of NF-κB has been observed in a number of different pancreatic cell lines including BxPC-3, PANC-1 [38] and MiaPaCa2 [39], but not in immortalised, non-tumourigenic pancreatic epithelial cells [40]. Activation of NF-κB has been observed in animal models of pancreatic cancer [41] and in human pancreatic tissue [39]. The main mechanism by which NF-κB appears to promote pancreatic cell growth is via inhibition of apoptosis [39, 42–46].

An alternate mechanism, by which NF-κB promotes pancreatic cancer growth, may involve activation of the mitogenic cyclin D1 gene [36, 47] which has been shown to be overexpressed in human pancreatic cancer tissue and to have an inverse correlation with patient survival [48]. In addition to promoting cell survival and growth, NF-κB has been implicated in increasing the angiogenic potential of pancreatic cancer cells via increased expression of the proangiogenic factors, vascular endothelial
growth factor (VEGF) and interleukin-8 (IL-8) [37, 49, 50]. IL-8 is produced by cells in response to hypoxia and can directly stimulate tumour cell growth [51, 52] as well as encouraging angiogenesis [53]. In pancreatic cell lines, IL-8 has been found to enhance invasiveness of tumour cells [54]. In vitro work has shown that NF-κB-dependent gene transcription can induce pancreatic cancer cell migration [55] and hence, NF-κB may be implicated in influencing the aggressiveness and metastatic potential of pancreatic cancer cells.

**Therapeutic Targeting of NF-κB**

NF-κB activation may contribute to the characteristic resistance of pancreatic tumour cells to the apoptotic effect of chemotherapeutic agents [56, 57]; hence, inhibiting the activation of NF-κB could be a useful adjunct in the medical management of the disease. In vitro work has shown that blocking NF-κB with the natural inhibitor IκBα suppresses tumourigenesis and increases the effect of chemotherapeutic agents such as gemcitabine, etoposide and doxorubicin [57–59]. In addition, NF-κB suppression using IκBα inhibits the angiogenic potential of pancreatic cancer cells [50] and so may have potential in reducing pancreatic cancer metastases. Alternatively, direct blockers of NF-κB, such as the NEMO-binding domain (NBD) peptide [60], have been used. Other methods of targeting the NF-κB pathway include the proteasome inhibitor, MG132, which prevents proteasome-dependent degradation of the NF-κB inhibitor protein, IκBα [57], and the protein LDCO1 which blocks upstream activation of the NF-κB cascade [61]. All these agents have shown potential in reducing the proliferation of pancreatic cancer cell lines, and in increasing the apoptotic influence of chemotherapy agents.

Sulphasalazine blocks phosphorylation of the inhibitor protein IκBα and has been used to inhibit NF-κB activity in pancreatic cell lines [59]. In murine models inoculated with Capan-1 pancreatic cells, treatment with sulphasalazine increased tumour cell sensitivity to the chemotherapeutic agent etoposide, and reduced microvessel density in the inoculated tumours [62]. Other anti-inflammatory compounds such as sodium salicylate have been employed to increase TNF-α-induced pancreatic cell apoptosis [63].

The formation of reactive oxygen species, comprise one of the many pathogenic stimuli which activate NF-κB and this has led to attempts to use anti-oxidants in pancreatic cancer cell lines [64]. Polyphenols are a huge and poorly defined class of substances which range from simple phenolic acids to highly polymerised condensed tannins. Polyphenols comprise a major constituent of human food and have been shown to exhibit a number of poly-mechanistic anti-cancer activity predominately through their anti-oxidant and anti-inflammatory effects. The
Polyphenols quercetin and resveratrol have shown potential to inhibit NF-κB activation in pancreatic cancer cell lines [65]. There is limited evidence that polyphenols may, at least in part, exert this effect by inhibiting phosphorylation of IkBα [66]. The possibility that changes in diet, to include a higher intake of polyphenols, may reduce pancreatic cancer risk is an attractive and simple idea, but further work is needed.

**Cyclooxygenase-2 (COX-2)**

Cyclooxygenase (COX) is the rate-limiting enzyme responsible for converting arachidonic acid to prostaglandin H2, which is the precursor module of a number of proinflammatory cytokines including prostaglandins, prostacyclins and thromboxanes [67] (fig. 3). At least two isoforms of COX have been identified which are labelled COX-1 and COX-2. COX-1 is constitutively expressed and is thought to be involved in ‘house-keeping’ roles in tissue homeostasis. In contrast, COX-2 is an inducible isoform which has been shown to have a key role in pathological processes, such as inflammation and carcinogenesis [68]. The first reported evidence of COX-2 upregulation in gastrointestinal malignancies was in 1994 [69], and since then the role of COX-2 in a wide range of malignancies has been reported.

As well being overexpressed in chronic pancreatitis [31], COX-2 has been found to be upregulated in human pancreatic cancer tissue using immunohistochemical staining, Western blotting, in situ hybridization and quantitative RT-PCR [70–79]. Increased COX-2 mRNA has also been observed in pancreatic cancer tissue, by up to 60-fold in some studies [74, 77]. COX-2 expression has been associated with a lower apoptotic index [73], increased perineural invasion [78] and a greater Ki-67 labelling proliferation index [73]. As yet, clinical studies have not shown a relationship with COX-2 expression and prognosis, apart from in endocrine tumours of the pancreas, where a greater COX-2 expression correlated with increased risk of metastases [80, 81].

COX-2 expression has been observed to be elevated in intraductal papillary mucinous tumours (IPMT) in a number of studies [72, 75], and its expression to correlate with the proangiogenic factor VEGF [82]. IPMTs are pre-malignant lesions of the pancreas and these findings of COX-2 upregulation offer the possibility that early suppression of COX-2 using nonsteroidal anti-inflammatory agents (NSAIDS) could, in theory, prevent the progression of these lesions into frankly malignant disease.

The exact mechanism by which COX-2 promotes pancreatic tumour growth is unclear. COX-2 inhibits apoptosis [83] and increases cell proliferation [73], as well as increasing VEGF production [84] and inducing angiogenesis.
COX-2 blockade induces apoptosis [83], inhibits inflammatory and Pancreatic Cancer 2-positive pancreatic cancer cell lines (BxPC-3) [70, 71, 86] and also COX-2-negative cell lines, such as PaCA-2 [71].

Aspirin has also been found to inhibit cell proliferation in pancreatic cancer cell lines and this inhibitory effect correlates with the degree of COX-2 expression [72]. COX-2 blockade induces apoptosis [83], inhibits endothelial cell migration, neovascularisation [85] and the invasive potential of pancreatic cancer cells [87]. Combination therapy of NSAIDs combined with conventional chemotherapy agents, such as gemcitabine, have been evaluated in pancreatic cancer cell lines with a resultant growth inhibition greater than could be achieved with each compound alone [86].

In carcinogen-treated animal models, COX-2 inhibitors have been used to inhibit the development of N-nitrosobis(2-oxopropyl)amine-induced pancreatic cancers [88] and BOP-induced pancreatic cancers [89]. In orthoptic mouse models, COX-2 inhibition with rofecoxib has been shown to inhibit tumour growth and induce expression of cell-cycle arrest genes [90]. Celecoxib, another COX-2 selective inhibitor, inhibits VEGF expression and reduces vascular proliferation and metastatic potential in pancreatic cancer cells injected into nude mice [91]. COX-2 inhibition also inhibits liver metastases in models of pancreatic adenocarcinoma using Syrian hamsters [92].

Clinical and preclinical studies of NSAIDs have evaluated COX-2 inhibitors as radiation sensitizers and combination treatments with chemoradiation for pancreatic tumours [93, 94]. However, few studies have examined the association between NSAID use and the subsequent risk of developing pancreatic cancer. These studies, both prospective and observational, have all concentrated on the ubiquitous NSAID, aspirin. A study of 28,283 participants, over a 7-year period, found that women who took regular aspirin had a 43% lower risk of pancreatic cancer than women who did not use aspirin [95]. Furthermore, the association was related to the dose of aspirin received. Women who took aspirin 2–5 times a week had a 53% lower risk of pancreatic cancer, and women who took aspirin 6 or more times a week had 60% lower risk of pancreatic cancer, when compared to women who took no aspirin at all. However, since that report, other studies have shown no relationship between aspirin use and reduced pancreatic cancer risk [96, 97], and a recently published paper has found that regular aspirin use may actually increase the risk of pancreatic cancer, with a dose-dependent escalation of risk observed in 88,378 women [98]. Despite the overwhelming evidence that COX-2 is overexpressed in pancreatic cancer and that inhibition of COX-2 can inhibit pancreatic tumour growth, the preclinical work has yet to be definitively confirmed in a clinical setting.

**Lipoxygenases**

The lipoxygenases (LOX) consist of four enzymes, 5-LOX, 8-LOX, 12-LOX and 15-LOX, whose nomenclature is dependent on their activity to insert oxygen at carbon 5, 8, 12 and 15, respectively, of the arachidonic acid molecule [99]. The products of this conversion are the hydroperoxyeicosatetraenoic acids (HPETEs) (fig. 3). These acids are further metabolised by 5-LOX to form LTA₄, LTA₄ is subsequently converted to 5(S)-hydroxy-6-trans8,11,14-cis-eicosatetraenoic acid (5-HETE) or the leukotrienes, leukotriene A₃, LTC₄, LTD₄ and LTE₄. The latter three leukotrienes were previously known as the ‘slow-reacting substance of anaphylaxis’.

Evidence is emerging that the lipoxygenases play an important role in carcinogenesis. Blockade of 5-LOX has been shown to inhibit lung and prostate carcinogenesis [100, 101]. 12-LOX has been found to be overexpressed in a number of tumours including melanomas, prostate cancer and epidermal cancers [102], and to relate to the metastatic potential of prostate cancer cells [103], whilst blockade of 12-LOX results in an increase in the apoptotic index of sarcoma cells [104]. The exact role of 15-LOX in carcinogenesis is unclear as yet, although limited data suggest that 15-LOX inhibition induces apoptosis in colon cancer cells [105]. 8-LOX is a relatively new discovery and the function of 8-LOX in carcinogenesis has not been fully evaluated.

Reverse transcriptase-polymerase chain reaction and Western blotting has revealed expression of 5-LOX mRNA and 5-LOX protein in all pancreatic cancer cell lines, but not in normal pancreatic ductal cells [106]. Inhibition of lipoxygenases has been shown to result in an increase in the pro-apoptotic protein, Bax, and a reduction in the levels of the anti-apoptotic proteins, Bcl-2 and Mcl-1, in mouse xenograft models of pancreatic cancer [107]. In vitro studies have shown that inhibition of 12-LOX and 5-LOX resulted in induction of apoptosis in PANC-1, MiaPaca2, Capan2, and HPAF cell lines [108, 109].
As yet there are no clinical trials of LOX inhibitors and pancreatic cancer; however, early data suggest that lipoxygenases may have as important a role to play as the cyclooxygenases in pancreatic cancer tumorigenesis.

**Inducible Nitric Oxide**

Nitric oxide (NO) is an important mediator in inflammation and carcinogenesis [110, 111]. Nitric oxide is a free radical and is produced from the conversion of L-arginine and molecular oxygen to L-citrulline by the enzyme nitric oxide synthase (NOS) [111] (fig. 4). There are three isoforms of the NOS enzyme. Endothelial NOS and neuronal NOS are expressed constitutively and produce low amounts of NO, involved in house-keeping roles such as neurotransmission and maintaining vascular tone. The third isoenzyme, inducible NOS (iNOS), is expressed by certain types of cancer cells and by activated macrophages [112].

The exact role of iNOS on carcinogenesis is unclear. Nitric oxide regulates prostaglandin production, activates the cyclooxygenase enzymes [113, 114] and regulates expression of the proinflammatory interleukin 8 (IL-8) [115], and by these mechanisms may promote cancer cell growth. However, high levels of NO have been demonstrated to have a pro-apoptotic role on tumour cells [116, 117] and induces G1 arrest [118], whereas low levels of iNOS and NO may enhance tumour progression and metastasis [119]. Western blot analysis and immunohistochemistry have shown an increased expression of iNOS in human pancreatic cancer tissue when compared to surrounding normal tissue [120, 121]. Immunohistochemical analysis has also shown increased iNOS expression in human pancreatic tissue, which correlated to an increased apoptotic index but not to prognosis [73, 122]. Pancreatic tumour cells do not seem to intrinsically express the L-arginine/NOS pathway, as shown by in vitro work where NOS activity and iNOS transcription were related to macrophage number [123].

These observations supporting the pro-apoptotic role of macrophage-released NO in pancreatic cancer have raised the possibility of blocking both the COX-2 pathway and donating NO, as means of retarding cancer cell growth. In theory, inhibiting the pro-proliferative COX system, in addition to inducing apoptosis, should achieve a greater effect than COX-2 inhibition alone. NO-donating NSAIDs (NO-NSAIDs) consist of a traditional NSAID to which a NO-donating group has been covalently attached [124]. One such NSAID is the NO-donating aspirin preparation, which has been found to be up to 5,000 times more effective than traditional aspirin in suppressing colon cancer cell growth [125]. In pancreatic cancer cell lines the use of NO-aspirin was more potent at inhib-

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Fig. 4. The iNOS pathway.
iting cell growth than NO-sulindac, NO-indomethacin and NO-ibuprofen [124]. Furthermore, all the NO-donating NSAIDs were more potent than their traditional counterparts at inhibiting growth and proliferation.

However, the long-term benefits of NO induction may be potentially detrimental. Recently, it has been shown that co-culturing fibroblasts with pancreatic cancer cell lines increases resistance to chemotherapeutic agents such as etoposide [126]. A possible mechanism for this has been shown to be attributable to NO release by fibroblasts leading to release of IL-1β. When the NO inhibitor aminoguanidine was added, the resistance conferred by NO-releasing fibroblasts was abolished.

**Proinflammatory Cytokines**

Several studies have shown that secretion of proinflammatory cytokines is increased in cancers, such as tumour necrosis factor α (TNF-α), interleukin-1 (IL-1), interleukin 8 (IL-8) and interleukin 6 (IL-6). TNF-α has classically been associated with ability to induce apoptosis in cancer cells [127–129]. Hence, by activating expression of NF-κB, TNF-α may act to increase survival of pancreatic cancer cells [63]. Other inflammatory cytokine products of NF-κB activation have been shown to positively feedback and stimulate NF-κB upregulation in an autoregulatory loop. These cytokines include IL-1α and IL-1β [34, 130].

In addition, to their role in promoting tumour growth, many of these cytokines contribute to the acute-phase response of advanced pancreatic cancer. Up to 85% of patients with pancreatic cancer exhibit severe weight loss, which continues until their death [131]. Cachexia can be very marked in advanced pancreatic cancer, and for many patients, may be the primary cause of death. These distressing symptoms dramatically reduce the quality of life in patients with end-stage pancreatic cancer, and their cause may be secondary to an acute-phase response mediated by inflammatory cytokines. Administration of cytokines such as TNF-α in animal models will result in many of the signs of cancer cachexia [132–134]. In vitro work has shown increased levels of IL-6 and TNF-α in pancreatic cancer cell lines, and raised serum levels in patients with pancreatic cancer when compared to controls [135]. Furthermore, blood samples from 13 patients with advanced pancreatic cancer revealed a positive association between serum levels of IL-6 and levels of acute-phase protein response, although this association was not found with the IL-6 soluble receptor [136]. Patients with a polymorphism of the interleukin-1β gene, which results in higher expression of IL-1β, have been shown to exhibit higher C-reactive protein levels and experience a shorter survival than patients who were heterozygous for allele 2 of the IL-1β gene [137].

**Interleukin-8**

IL-8 belongs to the CXC superfamily of chemokines which affect a number of different neutrophil functions such as chemotaxis, enzyme release and expression of surface adhesion molecules [138, 139]. IL-8 is released by a wide range of different cells, such as lymphocytes, monocytes, endothelial cells, fibroblasts and Kupffer cells, in response to a wide range of noxious stimuli, such as lipopolysaccharides, IL-1, and TNF-α [140] which induce IL-8 production via the NF-κB pathway [141, 142] (fig. 5). It is a key component of the inflammatory response.

In addition to its inflammatory role, IL-8 has been found to have an autocrine growth factor action on a number of different tumour cells [143, 144], including colorectal cancer [51, 52], breast cancer [145] and ovarian cancer [146]. In addition, IL-8 is an important proangiogenic factor for a number of different solid gastrointestinal tumours and their metastases [53]. IL-8 levels in tumour tissue and sera of patients with colorectal tumours have been reported to correlate with microvessel density and to be significantly higher in patients with hepatic metastases of their primary tumours, when compared to patients without metastases [147]. In gastric carcinoma cell lines, transfection with IL-8 resulted in rapidly growing, highly vascular tumours when compared to controls [148, 149]. In clinical studies of patients with gastric tumours, patients with the lowest survival rates had the highest expression of IL-8 in their tumour specimens [150].

IL-8 has been shown to be constitutively expressed by pancreatic cancer cells, driven by NF-κB activation [151] and in Capan-1 and HuP-T4 pancreatic cancer cell lines, IL-8 acts as an autocrine growth factor [152, 153]. IL-8 levels in pancreatic cancer cell lines are regulated by a number of factors other than NF-κB, including IL-1α, hypoxia and acidosis [49, 154, 155]. IL-8 also renders human pancreatic cancer more invasive, in Matrigel in vitro models [49, 54, 154, 155], and increases metastatic potential in animal models using implanted pancreatic cancer cells [155].

**Cytokine-Targeted Therapy**

Treating HuP-T4 pancreatic cancer cell lines with IL-8 antisense oligonucleotides has resulted in inhibition of cell growth [153]. Other methods tested have been using
NF-κB blockade and antioxidants in order to reduce up-regulation of the IL-8 gene [64]. Compounds assessed thus far include the polyphenol and anti-NF-κB agent, curcumin, which not only inhibited IL-8 expression, but also signal transduction via the IL-8 receptors [156]. Cytokines with anti-inflammatory activity, such as interleukin 12 and interleukin 15, have also been shown to inhibit pancreatic cancer cell growth in vitro [157]. Animal models of pancreatic cancer have demonstrated that IL-6 vaccines can reduce tumour growth [158].

**Reactive Oxygen Species and DNA Damage**

Reactive oxygen species (ROS) are highly reactive oxygen metabolites which include the superoxide radical $\text{O}_2^-$, hydrogen peroxide $\text{H}_2\text{O}_2$ and hydroxyl radical $\text{OH}^-$ [159]. ROS are produced as part of the inflammatory, by phagocytes, as well as being produced continually as part of normal cellular metabolism. ROS can cause lipid peroxidation, leading to mutagenic compounds such as the carbonyl compound malondialdehyde (MDA) or, alternatively, they can directly damage DNA bases (fig. 6). A huge variation of oxidised DNA adducts can be formed, examples include 3-(2-deoxy-beta-dierythropentafuranosyl)pyr[1,2-alpha]-purin-10(3H)one (M$_1$G) and 8-oxo-deoxyguanosine [160–162]. M$_1$G is formed from malondialdehyde (MDA) which is a by-product of lipid peroxidation and prostaglandin synthesis. MDA reacts with DNA to form up to six different adducts, of which M$_1$G is the predominant species and the most carcinogenic molecule [160]. 8-Oxo-deoxyguanosine (8-OH-dg) is formed by the reaction of oxygen radical or singlet oxygen with DNA. In DNA it induces G $\rightarrow$ T tranversions, which have oncogenic potential [163]. DNA adducts can also be formed via the MDA pathway from the manufacture of prostaglandins, catalysed by the enzyme COX-2 [160] and by the influence of iNOS which produces ROS.

In addition to their role in damaging DNA, evidence is emerging that ROS can directly stimulate cancer growth. Fibroblasts transfected with the viral $\text{ras}$ oncogene have increased superoxide production, and the generated superoxide may act as a second messenger molecule to promote cell proliferation [164]. Although traditionally ROS were thought to promote cell death, more recent data suggests that ROS may inhibit apoptosis in colorectal and pancreatic cancer cells [165, 166]. Up to $10^4$ DNA lesions can occur each day from different contributory sources including oxidative damage [167]. Cellular defence against this damage comprises of three mechanisms. The first mechanism is from enzymatic inactivation of superoxide by superoxide dismutase, and inactivation of hydrogen peroxide with catalysis. The second line of defence is hydrolysis of oxidised bases and thirdly, complex...
DNA repair mechanisms to, including base excision repair (BER), transcription-coupled repair (TCR), global genome repair (GGR) and mismatch repair (MMR) [168] (fig. 6). Disturbances in the balance between DNA damage, and the protective prevention and repair mechanisms can occur through heavy sustained oxidative damage, such as in chronic inflammation, or by inherited or acquired defects in the intricate defence systems.

**ROS and Pancreatic Cancer**

In vitro work in MiaPaCa2 and PANC-1 pancreatic cancer cell lines have shown that ROS produced by NAD(P)H oxidase inhibit apoptosis and may act as downstream messengers for growth factors such as serum insulin-1 growth factor and fibroblast growth factor-2 [159]. However, the exact role of ROS in pancreatic cancer is unclear, as conflicting reports exist suggesting that accumulation of ROS, via cytokines such as TNF-α and TGF-β1, act to enhance apoptosis in pancreatic cells [169, 170]. Oxidative DNA adducts and MDA have been found to be elevated in patients with chronic pancreatitis [32, 33, 171] and in normal pancreatic tissue [172]. Levels of the oxidative DNA adduct, 8-OH-dg, and malondialdehyde have been reported to be higher in human pancreatic tumour tissue [173, 174]. Oxidative DNA adducts have been found to be higher in normal pancreatic tissue from patients with pancreatic cancer, when compared to pancreatic tumour tissue and pancreatic tissue from control patients. This observation lend support to the precursor nature of oxidative stress in development of pancreatic cancer [175].

Impaired resistance to oxidative stress could also contribute to pancreatic cancer risk in susceptible individuals. Three species of superoxide dismutase (SOD) have been identified, SOD1 (CuZn-SOD), SOD2 (or Mn-SOD) and SOD3 (or EC-SOD). SOD1 is found in cell cytoplasm whilst SOD2 is mitochondrial and SOD3 is extracellular. Overexpression of manganese superoxide dismutase (Mn-SOD or SOD2) has been found to inhibit pancreatic cell growth both in vitro [176] and following implantation of pancreatic tumour cells into nude mice [176, 177]. These observations are further supported by immunohistochemical staining of human pancreatic tissue which revealed reduced levels of Mn-SOD in chronic pancreatitis specimens and in pancreatic cancer tissue [178].

Inability to repair oxidative DNA damage has also been implicated in pancreatic carcinogenesis. NO has been found to inhibit the repair of 8-OH-dg adducts [179], and this may be an important mechanism in the promotion of pancreatic cancer cell growth by iNOS. BRCA1 and BRCA2 have been shown to participate in the repair of 8-OH-dg adducts in pancreatic cell lines [180] and in Capan-1 pancreatic cell lines, expression of wild-type BRCA2 inhibited cell proliferation in culture and follow-
Targeting Oxidative Stress

Reducing oxidative stress and lipid peroxidation presents an attractive therapeutic target in the prevention of pancreatic cancer. The antioxidant vitamins, A, C and E, have been shown to reduce the rate of neoplastic growth in BOP-induced animal models of pancreatic cancer [186]. Octreotide has been found to increase the activity of SOD in BOP-induced pancreatic tumours and to reduce the number and size of hepatic metastases from carcinogen-induced primary pancreatic tumours in Syrian hamsters [187, 188]. Other compounds assessed have included β-carotene and selenium, which have been shown to inhibit carcinogen-induced pancreatic cancers in animal models [189, 190]. Green tea extracts are rich in the polyphenol epigallocatechin-3-gallate. Reports suggest that epigallocatechin-3-gallate can suppress pancreatic cell growth [65, 191] and invasiveness in a Matrigel model [192]. The question remains, however, whether these preclinical results will be borne out in clinical studies. A large randomised controlled trial of β-carotene and α-tocopherol found no statistically significant reduction in the incidence of pancreatic adenocarcinoma or mortality from the disease [193].

The exact genetic abnormalities underlying familial pancreatic cancer are unknown in up to 80% of cases [194]. It is possible that in certain individuals, inherited defects in DNA repair enzymes may contribute to their development of pancreatic cancer. In these individuals, even normal levels of oxidative stress, outside of the environment found in pancreatic cancer, may lead to the accumulation of enough DNA damage, leading to pancreatic cancer. Further work in this area may help identify high-risk subjects who could be suitably monitored or counselled, to reduce their long-term risk.

Conclusion

Pancreatic cancer frequently presents late, is refractory to traditional chemotherapeutic agents and can cause distressing symptoms in end-stage disease. Despite advances in oncology and surgery, the mortality from pancreatic cancer appears to be rising. Evidence is emerging that inflammation may be an important precursor in pancreatic carcinogenesis. In turn, however, molecular events associated with uncontrolled cell division, also results in further inflammation. These findings suggest that sustained inflammation may precede the onset of frank malignancy by a significant interval, and that once malignancy is established, the resulting inflammation occurred may act as a continued driving force in accelerating further malignant change. As such, suppression of inflammatory changes may be a useful adjunct in established pancreatic cancer treatment and may help delay or even completely prevent the inception of pancreatic neoplasia in premalignant states.

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