Chapter 6
The Role of Inflammation in Pancreatic Cancer

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Abstract Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with an extremely poor prognosis. Inflammatory processes have emerged as key mediators of pancreatic cancer development and progression. In genetically engineered mouse models, induction of pancreatitis accelerates PDAC development, and patients with chronic pancreatitis are known to have a higher risk of developing pancreatic cancer. In recent years, much effort has been given to identify the underlying mechanisms that contribute to inflammation-induced tumorigenesis. Many inflammatory pathways have been identified and inhibitors have been developed in order to prevent cancer development and progression. In this chapter, we discuss the role of inflammatory pathways in the initiation and progression of pancreatic cancer as well as the role of inhibitors used in treatment and prevention of pancreatic cancer.

6.1 Introduction: Clinical Aspects and Current Therapy Options in Pancreatic Ductal Adenocarcinoma

A relationship between inflammation and cancer was hypothesized by Rudolph Virchow back in the 1850s (Balkwill and Mantovani 2001). Dvorak (1986) later described tumors as ''wounds that do not heal'', where microenvironment-derived growth-promoting factors sustain the survival and proliferation of initiated cells. It is known that chronic persistent inflammatory conditions are associated with cancer in many organs, such as ulcerative colitis and colon carcinoma, Barrett's

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esophagus and esophageal cancer, hepatitis and liver cancer. The fibroinflammatory stroma of chronic pancreatitis resembles that of pancreatic cancer, and patients with familial chronic pancreatitis have a 26-fold increased risk of developing pancreatic cancer compared with the normal population—probably owing to chronic inflammation (Lowenfels et al. 1993). In this chapter, key pathways involved in this multifaceted interaction between inflammatory cells and pancreatic cancer are summarized.

6.1.1 Incidence and Survival

Pancreatic ductal adenocarcinoma is the fourth deadly cancer worldwide with an age-adjusted incidence rate of 12.1 per 100,000 men and woman per year (Siegel et al. 2013). It is estimated that in the United States, 45,220 patients will be diagnosed with pancreatic cancer in 2013 (Siegel et al. 2013). With a five-year survival rate of only 6 % and 38,460 estimated deaths in 2013, its incidence and mortality rates are almost identical (Siegel et al. 2013). Despite intensive research to prolong the survival of pancreatic cancer patients, little has been achieved, and like thirty years ago, surgery remains to be the only therapy option to cure some and provide the best palliation for many patients (Winter et al. 2012). However, only 15–20 % of the patients are initially candidates for surgical resection (Winter et al. 2012). Unfortunately, with the conventional non-surgical therapy options such as radiotherapy and chemotherapy, the survival of pancreatic cancer patients can only be prolonged for a couple of weeks to a few months (Moore et al. 2007; Michl and Gress 2013).

6.1.2 Current Therapy Options

The poor prognosis of pancreatic cancer patients is mostly due to late diagnosis and the absence of effective therapy options. Considering that three quarters of patients are not candidates for surgery at the time of first diagnosis, the most commonly employed therapy consists of radio- and chemotherapy. In the last decade, gemcitabine-based chemotherapy became the reference treatment since studies comparing gemcitabine with 5-fluorouracil showed similar survival advantages but better quality of life with the former (Neoptolemos et al. 2010). Different combinations of gemcitabine with other cytotoxic drugs could not improve the survival of patients significantly compared to gemcitabine treatment alone. Even the highly toxic regimen Folfirinox, consisting of irinotecan, oxaliplatin, 5-fluorouracil and folinic acid, resulted in a median survival of less than one year in patients with advanced pancreatic cancer (Conroy et al. 2011). Many targeted agents, including angiogenesis inhibitors, which have shown success in the preclinical setting, failed to prolong survival of pancreatic cancer patients in the clinical setting (Erkan et al. 2012). The

only FDA approved targeted agent Erlotinib (a tyrosine kinase inhibitor that acts on human epidermal growth factor receptor type 1 [HER1/EGFR]) is no exception. This agent only increases median survival from 5.91 months (gemcitabine and placebo) to 6.24 months (gemcitabine and erlotinib) in patients with advanced pancreatic cancer (Moore et al. 2007).

Recently, the abundant fibrotic stroma of pancreatic cancer is shown to form a mechanical barrier for the effective delivery of chemotherapeutic agents. This observation has led to the emergence of anti-fibrotic therapies which appear to be effective in the preclinical setting. For example, the inhibition of the hedgehog signaling pathway in a genetically engineered mouse model of pancreatic cancer showed a better penetrance of the tumor with gemcitabine and a longer survival of the mice (Olive et al. 2009). However, the first phase II trial (IPI-926-03) has to be stopped after interim analysis due to increased mortality in the therapy arm (Erkan 2013a). These results hint that the problem in pancreatic cancer is multifaceted, and a successful therapy should aim at correcting several defects at genetic, epigenetic, and microenvironmental levels.

6.2 Inflammatory Signaling Pathways Associated with Pancreatic Cancer

6.2.1 The NFκB Signaling Pathway as Key Modulator of Inflammation-Induced Carcinogenesis

The transcription factor nuclear factor κB is a key regulator of inflammatory processes and therefore plays an important role in the development of pancreatitis and pancreatic carcinogenesis (DiDonato et al. 2012). NFκB belongs to a family of proteins sharing the Rel homology domain (RHD), which can bind to DNA either as hetero- or homodimers. There are 5 NFκB/Rel family members, p65 (RelA), c-Rel, Rel-B, and the precursor proteins NFκB1 (p105/p50) and NFκB2 (p100/p52), which form homo- or heterodimers (Karin et al. 2002). In the pancreas, the p65/p50 heterodimer is the predominant form of NFκB (Han et al. 2001). In the healthy pancreas, the NFκB signaling pathway is inactivated, and the above-mentioned regulatory subunits are kept in the cytoplasm by interaction to the IκB family of inhibitory proteins, which include IκB-α, IκB-β, IκB-γ, IκB-ε, Bcl-3, p105/NFκB1, and p100/NFκB2 (Beg and Baldwin 1993; Thompson et al. 1995; Baldwin 1996; Verma et al. 1995). Due to microbial and viral infections as well as pro-inflammatory cytokines, the IκB kinase (IKK) complex gets activated and phosphorylates the IκB proteins at two conserved serine residues (Ling et al. 1998). The IKK complex consists of the two catalytic active protein kinases IKKα and IKKβ and the regulatory subunit IKKγ which is also called NEMO (Israel 2010). By phosphorylation of the inhibitory proteins, IκB gets targeted for ubiquitination and subsequent degradation by the 26S proteasomal system (Chen et al. 1995).

After the separation from the inhibitory protein IκB, the subunits can translocate to the nucleus, bind to κB-sequences within promoter regions and regulate the transcription of different genes involved in survival, inflammation as well as its own inhibitor IκB-α (Pahl 1999; Hayden and Ghosh 2011).

In many studies, it could be shown that the NFκB pathway is activated in early stages of pancreatitis and enhances the pro-inflammatory response through the activation of anti-apoptotic and inflammatory genes (Gukovsky et al. 1998; Steinle et al. 1999; Karin 1998). In a recent paper published by Huang and colleagues, it was demonstrated that the level of NFκB correlates with the severity of acute pancreatitis. Furthermore, the group displayed that long periods of activated NFκB in pancreatic acinar cells lead to a chronic pancreatitis characterized by severe pancreatic damage, immune cell infiltration, and fibrosis (Huang et al. 2013). Another group showed that the deletion of the IκB kinase IKK2 in all pancreatic epithelial cells prevented the formation of PanIN lesions in $Pdx^{Cre/+}$; $LSL\text{-}Kras^{G12D/+}$ mice (Maniati et al. 2011) thus indicating that the NFκB pathway plays an important role in the carcinogenesis of pancreatic cancer.

6.2.2 TheIL-6–STAT3 Axis and its Importance in Development of Pancreatic Cancer

The signal transducer and activator of transcription 3 (STAT3) is known to be an important regulator of stem cell renewal, cancer cell survival as well as inflammation. In the normal pancreas, STAT3 is inactive and located in the cytoplasm (Lee and Hennighausen 2005). In inflammatory conditions as well as in PDAC, however, STAT3 gets activated by phosphorylation on a tyrosine residue. Subsequent dimerization and translocation to the nucleus lead to the transcription of many target genes involved in inflammation and stem cell renewal (Shuai et al. 1993, 1994; Frank 2007; Bromberg and Darnell 2000). In inflammatory conditions, growth factors and cytokines such as IL-6 activate the Janus-activated kinase (JAK) family of tyrosine kinases that in return phosphorylate STAT proteins on their tyrosine residue (Zhong et al. 1994). Other tyrosine kinases such as src have also been reported to activate STAT proteins (Cao et al. 1996). The importance of STAT3 in the process of pancreatic cancer was recently shown by Miyatsuka et al. (2006) who proved that STAT3 is essential for the development of acinar-to-ductal metaplasia (ADM), an early event in the pathogenesis of pancreatic cancer. Furthermore, STAT3 was shown to be important for fostering progression of pancreatic cancer at different stages in mouse models and pancreatic cancer cell lines (Corcoran et al. 2011). The group of Hebrok additionally showed that the inflammatory mediator STAT3 contributes to PDAC initiation by promoting pancreatic cancer precursor lesions and support of cell proliferation and metaplasia-associated inflammation (Fukuda et al. 2011). In line, Lesina et al. (2011) showed

that inhibition of IL-6 or STAT3 can reduce PanIN progression and diminishes the development of PDAC. These results from in vitro and in vivo studies emphasize the importance of the IL-6–STAT3 axis in the initiation as well as progression of pancreatic cancer.

6.2.3 The Role of Toll-like Receptors in Pancreatic Cancer

Toll-like receptors (TLRs) belong to the pattern recognition receptors and are mainly expressed on innate immune cells as well as on neoplastic tissues (Huang et al. 2008). Ligands for the Toll-like receptors include conserved patterns of bacterial and viral origin also referred to as pathogen-associated molecular patterns (PAMPs) as well as damage-associated molecular patterns (DAMPs). A recent paper published by Ochi et al. (2012) showed that the TLR7 is not only overexpressed in the epithelial compartment in pancreatic cancer but also in the tumor stroma in mice and humans. Using a mouse model of pancreatic cancer ($p48^{Cre/+}$; $Kras^{G12D/+}$), the group showed that TLR7 ligation accelerated the development of pancreatic cancer and inhibition of TLR7 was able to inhibit pancreatic tumorigenesis. The activation of TLR7 induced STAT3 activation and interacted with Notch, canonical NFκB, and MAP kinase pathways. Another group showed that the inflammatory substance lipopolysaccharide (LPS) which activates TLR4 increased the invasive behavior of pancreatic cancer cell lines Panc-1 and AsPC-1 through the activation of the NFκB signaling pathway. These results demonstrate the interplay between TLR4 and NFκB signaling may be one of the pathways linking inflammation and PDAC progression in vitro (Ikebe et al. 2009).

6.2.4 TGF-β Signaling Pathway

TGF-β is an anti-inflammatory cytokine which plays an important role in cell growth, apoptosis, and differentiation of cells and often correlates with advanced tumor stage (Patterson and Padgett 2000; Lu et al. 1997; Daroqui et al. 2012). Under normal conditions, TGF-β has suppressive effects on tumorigenesis through inhibition of cell growth and promotion of apoptosis. Upon ligand binding, the TGF-β type I and TGF-β type II receptors heterodimerize and the type II receptor phosphorylates the receptor I kinase domain. The signal cascade is further forwarded by phosphorylation of SMAD proteins which is performed only by the type I receptor (Massague et al. 2000). The activated SMAD proteins then translocate into the nucleus and activate the transcription of target genes that mediate the tumor-suppressive effects. In pancreatic cancer, the role of the TGF-β signaling pathway is well established (Friess et al. 1993a). Like

in many cancers, the TGF-β signaling is impaired in pancreatic cancer leading to tumor-promoting effects such as increased cell growth, survival of cancer cells, invasion and metastasis as well as decreased survival of pancreatic cancer patients (Friess et al. 1993a, b). For many pancreatic cancer cell lines, it has been shown that SMAD4 is deleted or that the cancer cells have defects in TGF-β receptors (Villanueva et al. 1998). TGF-β has been shown to play an important role in the development and progression of chronic pancreatitis. In a study in which TGF-β signaling in the mouse pancreas was inhibited, the mice showed a stronger response to cerulein-mediated pancreatitis which was characterized by severe pancreatic edema, immune cell infiltration, hyperactivation of B and T cells and antibodies against pancreatic acinar cells (Hahm et al. 2000). Due to the important role of TGF-β in development and progression of pancreatitis, it has become an interesting drug target. In the recent years, several TGF-β receptor kinase inhibitors have been developed and have shown promising results in in vitro and in vivo experiments.

6.3 Role of Inflammatory Molecules in the Development of Pancreatic Cancer: Evidence from In Vitro Studies

6.3.1 Role of Inflammatory Molecules in the Transformation of Pancreatic Cancer Cells

Disturbances of pancreatic tissue homeostasis through various mechanisms lead commonly to a fibroinflammatory response in the pancreas. If there is an imbalance of the inflammatory reaction, chronic pancreatitis can ensue, which in the long term may enable transformation of premalignant cells to a malignant state. Apart from loss of tumor suppressor genes and deregulation of genes controlling the cell cycle, cytokines have been shown to contribute to the malignant transformation of cells. Moreover, during the typical fibroinflammation seen in chronic pancreatitis, microenvironmental factors, specifically hypoxia, acidosis, and reactive oxygen species, are also shown to induce genetic instability in the epithelial cells (Gillies et al. 2012).

6.3.1.1 The Role of the Cytokine TNF-α and the EGFR Signaling in the Transformation of Pancreatic Cancer Cells

In response to acinar damage, the expression of the cytokine tumor necrosis factor alpha (TNF-α) is induced. In human pancreatic cancer cell lines, it could be shown that the treatment of these cell lines with TNF-α was able to induce the expression of epidermal growth factor receptor (EGFR) and its ligand, transforming growth factor α (TGF-α) (Schmiegel et al. 1993). In vitro studies by Means et al.

the further demonstrated the importance of the EGFR signaling pathway in the transformation of acinar cells toward a malignant phenotype. Treatment of wild-type acinar cells with TGF-α resulted in transformation of acinar cells into a ductal phenotype which was accompanied by loss of acinar markers and expression of ductal markers like cytokeratin 19 (Means et al. 2005).

6.3.1.2 The Influence of the Cytokine IL-1α on Transformation of Pancreatic Cancer Cells

Another cytokine that plays an important role in the malignant transformation of pancreatic cells is the pro-inflammatory cytokine interleukin-1α (IL-1α). In a study by Sawai and colleagues, it could be shown that IL-1α enhanced proliferation, adhesion, and migration of the pancreatic cancer cell lines BxPC3, Capan-2, and SW1990. These changes were explained by the upregulation of the integrin subunit α_6 as well as by alterations of the urokinase plasminogen activator (uPA) and urokinase plasminogen activator receptor (uPAR) expression, which are both known to be upregulated in pancreatic cancer and play a role in disease progression (Cantero et al. 1997). Furthermore, IL-1α induced the activation of Ras and the downstream ERK signaling pathway (Sawai et al. 2006). By using an integrin α_6 antibody, the IL-1α-mediated effects could be abolished indicating that IL-1α mediates its effects through the integrin signaling pathway. Another study demonstrated that forced expression of IL-1α in the pancreatic cancer cell line MiaPaCa-2 activated NFκB expression, uPA as well as vascular endothelial growth factor (VEGF) and IL-8. Due to these changes in the expression profile, the non-metastatic cell line MiaPaCa-2 showed an invasive behavior in in vitro as well as in an orthotopic mouse model (Melisi et al. 2009).

6.3.2 Role of Inflammatory Molecules in Survival of Pancreatic Cancer Cells

6.3.2.1 NFκB and IL-6 Induce Anti-Apoptotic Genes

Resistance to apoptosis is one of the hallmarks of cancer and promotes tumor growth and metastasis (Hanahan and Weinberg 2000). In pancreatic cancer, the key regulator of inflammatory processes NFκB contributes to apoptosis resistance of pancreatic cancer cells (Liptay et al. 2003; Greten et al. 2002). Different studies showed that NFκB has anti-apoptotic effects on pancreatic cancer cells by activating different downstream target genes. In several pancreatic cancer cell lines, NFκB- and STAT3-dependent upregulation of the anti-apoptotic gene BcL-xL was demonstrated. However, this is not the only mechanism by which

NFκB exerts its anti-apoptotic effects. Several studies were able to show that NFκB is also involved in the regulation of cyclin D1 expression (Yamamoto and Gaynor 2001). In a recent study, it was shown that downregulation of the NFκB subunit p65 in pancreatic cancer cells leads to a subsequent downregulation of the pro-apoptotic gene BcL-2 as well as to the cell cycle gene cyclin D1 leading to growth inhibition of the pancreatic cancer cell line BxPC-3 (Kong et al. 2010). Another study showed that blocking the EGFR pathway in the pancreatic cancer cell line MDA Panc-28 resulted in a decreased NFκB binding activity as well as a reduced expression of the pro-apoptotic gens BcL-xL and Bfl-1 (Sclabas et al. 2003). The pro-inflammatory cytokine IL-6 was also shown to contribute to survival of pancreatic cancer cells by upregulating BcL-2 and BcL-xL. This effect could be reverted by the use of an IL-6 antibody (Miyamoto et al. 2001).

6.3.3 Role of Inflammatory Molecules in the Proliferation of Pancreatic Cancer Cells

6.3.3.1 The Cytokines IL-4, IL-6, and IL-8 have Proliferative Effects on Pancreatic Cancer Cells

Cytokines are found abundantly in the fibroinflammatory microenvironment of pancreatic cancer. The pro-inflammatory cytokine IL-6 was shown to affect pancreatic cancer cell proliferation in vitro by activating the STAT3 signaling pathway (Friess et al. 1999; Huang et al. 2010). In a recent study, it could be demonstrated that IL-6 induces the release of Th2-type cytokines as well as activates the ERK2 signaling pathway in pancreatic cancer cells. These results indicate that IL-6 signaling creates a tumor environment which promotes the development of pancreatic cancer by Th2-driven events as well as by upregulating cell proliferation-related genes (Feurino et al. 2007). Another cytokine that has a major role in promoting proliferation of pancreatic cancer cells is IL-8. The pancreatic cancer cell line Capan-1 has been identified to secret IL-8 as well as its receptor CXCR2. When IL-8 was inhibited using an IL-8 antibody, growth of Capan-1 cells was inhibited (Takamori et al. 2000). Another study showed that IL-8 inhibition in the cell line Hup-T4 via IL-8 antisense oligonucleotides also reduced the cell growth (Miyamoto et al. 1998). Additionally, IL-4 was identified to influence pancreatic cancer cell growth since pancreatic cancer cells as well as pancreatic cancer tissue show a high upregulation of the IL-4 receptor (Kawakami et al. 2001). In vitro studies displayed that the anti-inflammatory cytokine IL-4 significantly enhances the tumor growth of different pancreatic cell lines (AsPC-1, Colo-357, Capan-1, Panc-1). Moreover, the ablation of IL-4 in cell culture showed a reduced tumor growth, confirming the proliferative effect of IL-4 on pancreatic tumor cells (Prokopchuk et al. 2005).

6.3.4 Role of Inflammatory Molecules in the Invasion, Metastasis, and Angiogenesis of Pancreatic Cancer Cells

6.3.4.1 The Pro-inflammatory Cytokine Interleukin-1α Plays an Important Role in Invasion and Metastasis of Pancreatic Cancer Cells

Many inflammatory molecules have been indicated to play a role in invasion, metastasis, and angiogenesis of PDAC. One of these is the pro-inflammatory cytokine IL-1α which is produced by pancreatic cancer cells. In recent studies, it could be demonstrated that IL-1α promotes proliferation, adhesion, and migration of the pancreatic cancer cell lines BxPC-3, SW1990, and Capan-2 through the upregulation of the integrin subunits α_6 and β_1 and the uPAR. The above-mentioned effects are associated with the activation of RAS and the downstream ERK signaling pathway. By using inhibitory antibodies against α6, β1, and uPA, the group showed that the activation of the ERK signaling as well as proliferation, adhesion, and migration of pancreatic cancer cell lines was prevented (Sawai et al. 2006). In an additional study, it was elucidated that IL-1α produced by pancreatic cancer cells is able to induce the expression of hepatocyte growth factor (HGF) by fibroblasts (Xu et al. 2010). In co-culture experiments with pancreatic cancer cells and fibroblasts, the group showed not only the IL-1α-dependent expression of HGF by fibroblasts but also an increased invasive and proliferative behavior of pancreatic cancer cells as well as of human umbilical vein endothelial cells (HUVECs). This can be explained by binding of HGF to its receptor c-met/HGF on the surface of pancreatic cancer cells and thus fostering the observed behavior of pancreatic cancer cells (Xu et al. 2010). Another study demonstrated that forced expression of IL-1α in the pancreatic cancer cell line MiaPaCa-2 activated the NFκB signaling pathway as assessed by an increase in NFκB downstream targets. As a result of the forced expression of IL-1α and subsequent NFκB activation, the cells gained an invasive phenotype. However, when the NFκB pathway was inactivated by the expression of a dominant negative IκB protein, the metastatic behavior was prevented. The same behavior of the cells was observed when IL-1α was silenced in the metastatic pancreatic cancer cell line L3.6pl, indicating that IL-1α-induced NFκB expression is contributing to the metastatic phenotype of pancreatic cancer cells (Melisi et al. 2009).

6.3.4.2 The Pro-Inflammatory Cytokines TNF-α, IL-6, and IL-1β are Important for the Survival, Metastasis of Cancer Cells, and Escape from Immune Surveillance

In many cancer types, the pro-inflammatory cytokine IL-1β has been indicated to influence metastasis and tumor growth (Apte et al. 2006). IL-1β together with IL-1α belongs to the IL-1 family and has been shown to induce the expression of

pro-inflammatory genes such as cyclooxygenase-2 (COX-2), inducible NO synthetase (iNOS), and IL-6. Pancreatic cancer cell lines treated with recombinant IL-1β revealed that the cancer cells stimulated with IL-1β showed a strong invasive behavior, whereas extracellular matrix adhesion was not influenced (Greco et al. 2005).

The NFκB pathway has been shown to have an important impact on survival of pancreatic cancer cells through the upregulation of anti-apoptotic genes such as BcL-XL. Several studies showed that inhibition of NFκB in human pancreatic cancer cells resulted in increased apoptosis of cancer cells. Different mechanisms contributing to the anti-apoptotic effects of NFκB in pancreatic cancer cells have been described. In a paper by Sclabas and colleagues, EGFR-dependent NFκB activation was analyzed (Sclabas et al. 2003). Therefore, the EGF receptor was blocked in the human pancreatic cancer cell line MDA Panc-28 using an anti-EGFR monoclonal antibody which resulted in decreased NFκB activity as well as a diminished expression of the apoptotic genes BcL-XL and BfL-1. Furthermore, the group was able to show a significant increase in apoptosis of MDA Panc-28 cells when they were treated with the EGFR monoclonal antibody and gemcitabine together (Sclabas et al. 2003). The results of this study indicate that signaling through the EGF receptor can induce NFκB signaling and subsequently influence apoptosis of pancreatic cancer cells in vitro. Another study showed that silencing of NFκB had an effect on gemcitabine-sensitive pancreatic cancer cell lines BxPC3, L3.6pl, and CFPAC-1 alone or in combination with gemcitabine. However, if NFκB was silenced in gemcitabine-resistant pancreatic cancer cell lines (MPanc-96, Panc-1, MiaPaCa-2), no effect on apoptosis could be detected (Pan et al. 2008). Therefore, inhibition of NFκB may only be a therapeutic advantage for a subset of pancreatic cancer patients.

6.3.4.3 TGF-β Mediates Invasiveness of Pancreatic Cancer Cells

In normal epithelial cells, TGF-β functions as inhibitor of cell growth (Logsdon et al. 1992). The same effects can be observed in early stages of cancer and in some pancreatic cancer cell lines such as Colo-357 (Kleeff and Korc 1998). However, at late stages of cancer, the cells are not responsive to the growth inhibitory effects due to mutations of downstream molecules such as SMADs or the expression of TGF-β signaling inhibitors (Kleeff et al. 1999a, b), and hence TGF-β functions as tumor-supporting factor. Treatment of the pancreatic cancer cell lines Panc-1 and IMIM-PC1 with recombinant TGF-β increased the invasiveness of these cells. The invasive behavior of Panc-1 and IMIM-PC1 could be completely abolished by using a neutralizing TGF-β antibody. Furthermore, the treatment of cells with TGF-β upregulated the matrix metalloproteinase 2 (MMP2) and the uPA system and thus mediated the invasive behavior of Panc-1 and IMIM-PC1 (Ellenrieder et al. 2001). Another study analyzed the pancreatic cancer cell lines Panc-1, BxPC3, and MiaPaCa in regard to stimulation of TGF-β and found out that the pancreatic cancer cell lines had a defective response to the TGF-β stimulation as determined by 3[H]thymidine incorporation and TGF-β-sensitive reporter assays.

Furthermore, no correlation of the unresponsiveness to TGF-β and TGF-β type I and II receptor or Smad2 and Smad3 was identified. However, when Smad4 was introduced into the cell line BxPC3 which has a homologous deletion of SMAD4, it restored the responsiveness to TGF-β, indicating that Smad4 plays a crucial role in the loss of TGF-β responsiveness at least in some pancreatic cancer cells (Simeone et al. 2000). The role of SMAD4 in mediating TGF-β effects was further confirmed by a study by Chow et al. The group showed that TGF-β facilitates motility and invasiveness of the pancreatic cancer cell lines BxPC3 and Capan-1 through inhibition of PTEN expression and activation of NFκB. However, when SMAD4 expression was restored in BxPC3 and Capan-1 cells, the invasive behavior was prevented due to the inhibited activation of NFκB pathway (Chow et al. 2010).

6.3.4.4 IL-6 and IL-8 Induce Angiogenesis by Activating Vascular Endothelial Growth Factor

In a variety of tumors, IL-8 is known to contribute to the regulation of tumor growth, invasion, and angiogenesis (Strieter et al. 1995). In head and neck cancer, IL-8 was identified as an autocrine growth factor and it could be shown that IL-8 expression leads to cancer cell survival and tumor growth. IL-8 expression was induced by IL-1α-dependent activation of the transcription factors NFκB and AP-1 which in turn promoted survival of head and neck squamous cell carcinoma cells in vitro (Wolf et al. 2001). Similarly, in pancreatitis and pancreatic cancer, an upregulation of IL-8 can be detected which correlates with an increase in angiogenesis and metastatic behavior of cells (Farrow et al. 2004). In fact, many pancreatic cancer cell lines produce a mixture of pro- and anti-angiogenic substances. Their dominant effect on angiogenesis remains mostly inhibitory (Erkan et al. 2009). IL-8 belongs to the pro-angiogenic factors produced by pancreatic cancer cell lines. In vitro, HUVEC co-cultured with some other pancreatic cancer cells show an increase in proliferation and angiogenesis (Matsuo et al. 2004). Moreover, the human pancreatic cancer cell line Panc-1 shows an increased metastatic behavior when stimulated with exogenous IL-8 (Kuwada et al. 2003). Recent studies revealed that the observed angiogenic effect mediated by IL-8 is in part due to induction of VEGF and neuropilin-2 produced by pancreatic cancer cells (Li et al. 2008).

Another important cytokine involved in mediating angiogenesis in pancreatic cancer cell lines is IL-6. A recent study showed that the IL-6 levels are increased in different pancreatic cancer cell lines such as BxPC-3, MiaPaCa-2, Panc-1, and PaCa-2 compared to human pancreatic ductal epithelium cells. Further investigations showed that similar to IL-8, IL-6 also induced expression of VEGF and neuropilin-1 supporting angiogenesis and metastasis of pancreatic cancer cells (Feurino et al. 2007). Both IL-8 and IL-6 activate the MAP kinase pathway. This could be demonstrated by an increase in ERK2 phosphorylation when Panc-1 cells were stimulated with IL-8 or IL-6. Through the activation of this signaling pathway, proliferation of pancreatic cancer cells is fostered and thus progression of pancreatic cancer in enhanced.

6.4 Role of Inflammatory Molecules in the Development of Pancreatic Cancer: Evidence from In Vivo Studies

6.4.1 STAT3 Contributes to Pancreatic Ductal Adenocarcinoma Initiation and Progression

Not only in vitro studies were able to show that the inflammatory mediator STAT3 is linked with pancreatic precursor lesion formation, but also in vivo studies demonstrated the role of STAT3 in the development of preneoplastic lesions (Corcoran et al. 2011; Fukuda et al. 2011; Lesina et al. 2011; Li et al. 2011). Corcoran et al. (2011) showed that STAT3 is necessary both for the development of precursor lesions [i.e., ADM, pancreatic intraepithelial neoplasia (PanIN)], and progression to PDAC. Fukuda et al. (2011) confirmed that STAT3, which is overexpressed in epithelial cells after cerulein-induced inflammation in a KrasG12D mouse model, helps to initiate tumor development and progression. Blocking of STAT3 has led to attenuation of precursor lesion formation and proliferation as well as increased apoptosis, proving the contribution of STAT3 to cancer initiation. Moreover, the group also identified that the loss of epithelial STAT3 leads to a reduced inflammatory cell infiltration as well as decreased expression of inflammatory cytokines. These results indicate that STAT3 not only has an influence on the proliferative, dedifferentiated state of the epithelial cells but also contributes to inflammatory processes associated with metaplasia (Fukuda et al. 2011). Lesina et al. (2011) observed the same events but additionally identified the myeloid compartment to secrete the pro-inflammatory cytokine IL-6 which leads to the activation of STAT3 in the pancreas and fosters the development and progression of PanIN lesions. The identification of this mechanism strengthens the role of the microenvironment in the development of PDAC and was also shown to be valid for human PDAC by analyzing human PDAC specimen and patient data. Therefore, the results of these studies indicate STAT3 as a potential therapeutic target for preventing inflammation-induced development of PDAC at an early stage.

6.5 Clinical Evidence on the Role of Fibroinflammation in Pancreatic Cancer

Inflammation has early been indicated to play a major role in pancreatic cancer development. Similarities between the fibroinflammatory stroma (Fig. 6.1) composition in chronic pancreatitis and pancreatic cancer emphasize the pathogenetic link between them (Chu et al. 2007). Inflammatory cells such as macrophages, mast cells, neutrophils, dendritic cells, B and T lymphocytes as well as activated PSC have all been described in the stroma of pancreatic cancer. However, only

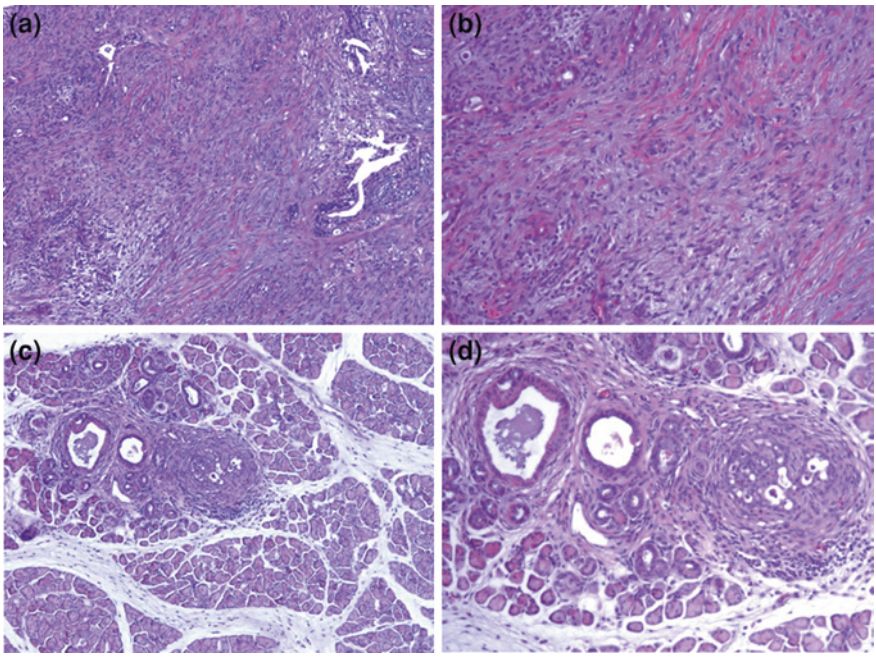

Fig. 6.1 Hematoxylin + Eosin staining of human and murine tissues depicting the fibroinflammatory stroma. **a** (50×) and **b** (100×) tissue from a pancreatic cancer patient showing the strong inflammatory stroma. **c** (50×) and **d** (100×) tissue of a mouse overexpressing the oncogene Kras under the control of the pancreas-specific promoter p48. Precancerous lesions develop which are surrounded by a strong stromal response with concomitant immune cell infiltration

a few experimental studies exploited the functional role of immune cells in the biology of PDAC. Most of these studies rely on correlation analysis which needs further confirmation using functional analysis as well as animal experiments. For example, studies on mast cells show that they foster neoangiogenesis (Esposito et al. 2002, 2004) and that there is a positive correlation between the number of mast cells in the pancreatic fibroinflammatory stroma and angiogenesis. To such extent that the number of mast cells correlates positively with the occurrence of metastasis and negatively with survival of patients with PDAC (Esposito et al. 2002, 2004). Epidemiologic studies show that inflammation significantly increases the risk of pancreatic cancer development. Importantly, these studies reveal that the elevated risk was independent of gender, ethnicity, and type of pancreatitis (Lowenfels et al. 1993; Malka et al. 2002). A recently conducted study identified inflammatory monocytes to play a role in survival of pancreatic cancer patients. The study revealed that monocytes in the peripheral blood are negatively correlated with the survival of pancreatic cancer patients, whereas a low amount of peripheral monocytes showed an increased survival of pancreatic cancer patients with a resected tumor (Sanford et al. 2013). It is also shown that

M2 macrophages which are characterized by a high expression of the cytokines IL-4 and IL-10 are associated with large tumor size and shortened survival time in pancreatic cancer patients indicating the important role of inflammatory cells in the survival of pancreatic cancer patients (Yoshikawa et al. 2012). Furthermore, elevated serum levels of the cytokine IL-6 have been found in pancreatic cancer patients compared to healthy individuals and chronic pancreatitis patients. These increased serum levels correlated with tumor size as well as liver metastases in pancreatic cancer patients (Talar-Wojnarowska et al. 2009; Okada et al. 1998). Lesina et al. (2011) highlighted the importance of IL-6 in pancreatic cancer development and progression by identifying myeloid cells to produce IL-6 which is responsible for activating the STAT3 signaling pathway that promotes the progression of precancerous lesions. Recently, it was also shown that IL-6 produced by pancreatic stellate cells enhances myeloid-derived suppressor cell (MDSC) differentiation and function from peripheral blood mononuclear cells, which promotes an immunosuppressive microenvironment in PDAC (Mace et al. 2013). PSC supernatants promoted peripheral blood mononuclear cells differentiation into an MDSC (CD11b+CD33+) phenotype and a subpopulation of polymorphonuclear CD11b+CD33+CD15+ cells. The resulting CD11b+CD33+ cells functionally suppressed autologous T lymphocyte proliferation. Culture of normal peripheral blood mononuclear cells with PSC supernatants led to STAT3 but not STAT1 or STAT5 phosphorylation. In these interactions, IL-6 was an important mediator as its neutralization inhibited PSC supernatant-mediated STAT3 phosphorylation and MDSC differentiation. Moreover, chemical inhibition of STAT3 abrogated PSC supernatant-mediated MDSC differentiation, PSC viability, and reduced autocrine IL-6 production, indicating these processes are STAT3 dependent. These results identify a novel role for PSC in driving immune escape in pancreatic cancer and extend the evidence that STAT3 acts as a driver of stromal immunosuppression to enhance its interest as a therapeutic target (Mace et al. 2013).

In the last decade, the fibroinflammatory stroma/desmoplasia, produced by the activated pancreatic stellate cells, has attracted attention as it forms a physical barrier for the effective delivery of therapeutic agents. There is also considerable amount of evidence stemming from in vitro and animal experiments that PSC and various ECM components support tumor growth by various mechanisms such as promoting tumor growth, creating apoptosis resistance, creating a niche for cancer stem cells, enabling immune escape of cancer cells, modulation of angiogenesis, facilitation of metastatic spread, and increasing therapy resistance (Erkan 2013b). Moreover, depletion of the desmoplastic stroma of the PDAC has led to better chemotherapy delivery and drug response in Kras-based genetic mouse models confirming the previous observations (Olive et al. 2009; Jacobetz et al. 2013; Provenzano et al. 2012). Although anti-fibrotic therapy appears as a new hope in the treatment of PDAC, it is not for certain that this fibrotic reaction is exclusively pro-tumorigenic as there is also evidence that various stromal components can as well be protective (see below).

6.6 Inhibitors of Inflammation for the Prevention and Treatment of Pancreatic Cancer

Currently, there is not enough clinical evidence to support the routine usage of anti-inflammatory drugs to improve outcome in pancreatic cancer patients. Some underpowered studies show partial benefit when anti-inflammatory therapy (i.e., COX-2 inhibition) is added to conventional chemotherapy (Lipton et al. 2010). Similarly, there is some circumstantial evidence that anti-inflammatory drugs reduce the risk of malignant pancreatic lesions. Below, some experimental data are reported.

6.6.1 TGF-β Receptor Kinase Inhibitors

As mentioned above, in normal cells, TGF-β exerts tumor-suppressive functions by inhibiting proliferation and inducing apoptosis. However, in many cancers including pancreatic cancer, TGF-β levels increase significantly and can support tumor growth, angiogenesis, invasion, and metastasis. Therefore, small molecular inhibitors have been developed to block TGF-β function and to inhibit these tumor-promoting effects. In a preclinical study, the TGF-β receptor kinase inhibitor SD-208 was investigated using the human pancreatic cell line Panc-1. The study demonstrated that the TGF-β receptor kinase inhibitor was able to inhibit invasion of Panc-1 cells in vitro. Moreover, the study revealed that the use of SD-208 in a xenograft mouse model reduced the size of the primary tumor and diminished the incidence of metastasis (Gaspar et al. 2007). Another study tested an inhibitor against the TGF-β receptors I and III (LY2109761). In cell culture experiments, the inhibitor was able to prevent migration, invasion, and induced anoikis in soft agar experiments. In further in vivo studies, LY2109761 in combination with gemcitabine was able to decrease the tumor burden in an orthotopic mouse model, prolonged the survival, and reduced liver metastases in these mice (Melisi et al. 2008). These promising results from in vitro and in vivo studies implicate that TGF-β receptor kinase inhibitors may serve as therapeutic agents in prevention of metastasis of pancreatic cancer.

6.6.2 Cyclooxygenase-2 Inhibitors

Cyclooxygenase (COX) and 5-lipoxygenase are the main regulators of the arachidonic acid metabolism and have been shown to be dysregulated in pancreatic cancer (Hennig et al. 2002, 2005; Ding et al. 2001). COX-2, which is a prostaglandin synthetase, catalyzes the conversion of arachidonic acid into prostaglandin G2. In the pancreas, COX-2 expression is induced by inflammatory cytokines, growth factors, and mitogenic stimuli and was shown to be overexpressed in pancreatic cancer

(Yip-Schneider et al. 2000). By supporting proliferation, invasion, and angiogenesis of pancreatic cancer cells, COX-2 contributes to the aggressive phenotype of PDAC (Chu et al. 2003; Ito et al. 2004; Eibl et al. 2003). Hermanova et al. (2008) found a different expression pattern in normal, premalignant, malignant pancreatic tissue indicating the important role of COX-2 in the progression of precancerous lesions. Treatment of pancreatic cancer cell lines displaying a high COX-2 expression such as BxPC-3 with gemcitabine and celecoxib (COX-2 inhibitor) showed a significantly inhibition of growth and enhanced apoptosis compared to gemcitabine treatment alone. However, in pancreatic cancer cell lines with low COX-2 expression, no such effect could be observed (El-Rayes et al. 2004). In vivo studies using COX-2 inhibitors in $Pdx^{Cre/+}$; $LSL\text{-}Kras^{G12D/+}$ mice which recapitulate all steps of pancreatic cancer development also demonstrated a decreased pancreatic tumor growth as well as a delay in the progression from precancerous lesions into pancreatic cancer (Funahashi et al. 2007; Eibl et al. 2005). In a study by Guerra and colleagues, mice were treated with the COX-1/2 inhibitor sulindac for a period of 3 months after induction of pancreatitis by the cholecystokinin analog cerulein for 3 months. Histology showed that the pancreata of these mice were almost normal with the exception of few areas displaying atrophy and immune cells (Guerra et al. 2011). Interestingly, sulindac hardly reduced low-grade lesions, but a significant reduction of high-grade lesions and PDAC could be observed. These results stress the importance of inflammation in the progression of early lesions to PDAC development.

Following these promising results in cell culture and mouse models, selective COX-2 inhibitors were developed for phase II studies. In a trial with patients suffering of advanced or metastatic pancreatic adenocarcinoma, treatment with gemcitabine and an additional daily oral dose of celecoxib twice a day was performed. However, the results of this study were disappointing since the additional administration of celecoxib did not improve the clinical outcome (Dragovich et al. 2008). Another phase II trial involving patients with unresectable pancreatic cancer radiotherapy combined with uracil/tegafur plus leucovorin and celecoxib did not show a response and moreover the patients showed gastrointestinal toxicity. Therefore, treatment of patients with a locally advanced pancreatic tumor cannot be advised as standard therapy (Morak et al. 2011). Despite the promising effects of COX-2 inhibitors in vitro and in mouse models, so far there is no promising treatment for pancreatic cancer patients with COX-2 inhibitors.

6.6.3 Inhibition of NFκB

The NFκB signaling pathway has been shown to play multitudes of roles in the development of pancreatic cancer as well as in metastatic spread due to its role in controlling proliferation, apoptosis, and angiogenesis. Therefore, inhibition of NFκB expression is a promising therapeutic target to reduce tumor growth and metastasis formation in pancreatic cancer patients. In vitro studies showed that inhibition of NFκB signaling in combination with gemcitabine resulted in

decreased angiogenesis, proliferation, and induction of apoptosis of BxCP-3 and Panc-1 cells (Kong et al. 2010). The same anti-tumor effects could be observed when NFκB activity was inhibited in a human pancreatic cancer cell line and subsequently implanted into the pancreas of nude mice (Xiong et al. 2004). Due to these promising in vitro and in vivo results, pharmacological NFκB inhibitors have been developed and investigated in clinical studies. One of them is the proteasome inhibitor bortezomib which was analyzed in a clinical trial with metastatic pancreatic cancer patients. In this trial, 44 enrolled patients received bortezomib alone, and 43 patients were treated with bortezomib and gemcitabine. However, the results of this study revealed that the treatment with bortezomib and the treatment with the combination of bortezomib and gemcitabine did not have a better outcome for metastatic pancreatic cancer patients compared to gemcitabine treatment alone (Alberts et al. 2005). Despite of the promising results of bortezomib in in vitro experiments, this NFκB inhibitor does not have an effect on metastatic pancreatic cancer patients. Therefore, further substances need to be developed to target pancreatic cancer development and chemoresistance.

6.6.4 Anti-Fibrotic Therapies

Recently, the abundant fibrotic stroma, produced by the activated pancreatic stellate cells, has attracted attention as it might form a physical barrier for the effective delivery of therapeutic agents. There is a considerable amount of evidence stemming from in vitro and animal experiment that PSC and various ECM components support tumor growth by various mechanisms such as promoting tumor growth, creating apoptosis resistance, creating a niche for cancer stem cells, enabling immune escape of cancer cells, modulation of angiogenesis, facilitation of metastatic spread, and increasing therapy resistance (Erkan 2013b). In line with these observations, depletion of the desmoplastic stroma of the PDAC has led to better chemotherapy delivery and drug response in Kras-based genetic mouse models (Conroy et al. 2011; Erkan et al. 2012; Olive et al. 2009). Taken together, anti-fibrotic therapy appears as a new hope in the treatment of PDAC. However, as of today, data from clinical studies are largely missing. However, as a proof of principle, Von Hoff et al. (2011) used in a phase I/II trial nanoparticle albumin-bound (nab) paclitaxel (to deplete the stroma in PDAC) alone and in combination with gemcitabine and showed that through depletion of the stroma, higher concentrations of gemcitabine can be delivered in the tumor.

Despite the initial hope mostly stemming from the success achieved in genetic mouse models of PDAC, the clinical reality seems to be more complex. As mentioned above, the first trial using an inhibitor of sonic hedgehog signaling to deplete the stroma of PDAC (IPI-926-03 trial, http://www.clinicaltrials.gov/) has been stopped due to increased mortality in the treatment arm. Currently, several other trials are recruiting patients where various forms of anti-fibrotic therapies are applied concomitantly with conventional therapies. The results of these trials

will help understanding whether nonselective anti-fibrotic therapy would improve the results of conventional therapies (Erkan 2013b). Nonetheless, judging by the results of the above-mentioned trial and previous failures, it is likely that nonselective anti-fibrotic therapy may also not be the solution to overcome therapy resistance in PDAC (Erkan 2013b).

Considering the normal function of stromal cells (forming a barrier between a noxious stimuli and the body), we have previously argued that PSC are initially activated around genetically defective cells (i.e., Kras mutated) in a preventive manner. This type of activation is also observed in chronic pancreatitis tissues. However, due to the fibrosis created by PSC, the microenvironment becomes gradually more fibrotic and hypoxic (Erkan 2013b). The ensuing stromal barriers specifically hypoxia, acidosis, and reactive oxygen species are not only highly selective but also able to induce genetic instability in the epithelial cells (Gillies et al. 2012). As a result, malignant cancers evolve through negative selection of the fitter clones under the selection pressure applied by their defensive microenvironment (Gillies et al. 2012). These evolved clones are also resistant to conventional therapies that induce apoptosis (Gillies et al. 2012). As mentioned above, the fibrotic stroma also forms a mechanical barrier preventing effective delivery of chemotherapeutics in advanced cancer. However, at this late stage, where selection of aggressive clones has already taken place, applying anti-fibrotic therapy can be a double-edged sword (Erkan 2013c). Since conventional chemotherapeutics are not powerful enough to eradicate all cancer cells (i.e., tumor-promoting cells) in the tumor, breaking down the stromal wall may also lead to the increased dissemination of cancer cells (Erkan 2013c).

6.7 Conclusions and Further Directions

Pancreatic cancer is the fourth deadly cancer worldwide and has a 5-year survival rate of only 6 %. The cellular mechanisms contributing to pancreatic cancer development and progression are still not completely identified. Inflammation has emerged to be a key mediator of pancreatic cancer development. In a paper by Guerra and colleagues, it could be shown that in adult mice the expression of the mutant Kras is not sufficient to induce pancreatic cancer. However, when additionally inflammation was induced, the mice developed pancreatic cancer stressing the importance of inflammation in the development of pancreatic cancer (Guerra et al. 2007). Furthermore, many studies showed the impact of inflammatory molecules on the development and progression of pancreatic cancer. So far, different approaches have been made to inhibit the main inflammatory signaling pathways in pancreatic cancer. Although, having shown promising results in vitro and in vivo experiments, inhibitors of inflammation have not been successful in cancer prevention or cancer progression in clinical trials. Therefore, further research is needed to elucidate the mechanisms through which inflammation contributes to tumor initiation and progression. It is very likely that that there are several altered mechanisms on various levels contributing to the aggressiveness of PDAC. Therefore, effective therapy of PDAC should aim at overcoming various obstacles at several levels.

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