Inflammation and Epithelial-Mesenchymal Transition in Pancreatic Ductal Adenocarcinoma: Fighting Against Multiple Opponents

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ABSTRACT: Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer and one of the most lethal human cancers. Inflammation is a critical component in PDAC initiation and progression. Inflammation also contributes to the aggressiveness of PDAC indirectly via induction of epithelial-mesenchymal transition (EMT), altogether leading to enhanced resistance to chemotherapy and poor survival rates. This review gives an overview of the key pro-inflammatory signaling pathways involved in PDAC pathogenesis and discusses the role of inflammation in induction of EMT and development of chemoresistance in patients with PDAC.

KEYWORDS: Inflammation, PDAC, EMT, chemoresistance

Introduction
The year 2015 witnessed a surge in the estimated new cases of pancreatic cancer to 48,960 and the associated 40,560 deaths in the United States, making it the third leading cause of cancer deaths.1 Moreover, 1 in 67 American is at a risk of pancreatic cancer. Pancreatic ductal adenocarcinoma (PDAC), a predominant histologic subtype making 90% of all pancreatic cancers, exhibits local invasion and distant metastasis during early disease stages that directly correlate with an extremely poor prognosis and an overall survival rate of only 5%.2 At the time of diagnosis, 80% of patients are considered inoperable, and surgery is the only hope for the remaining 20%. Pancreatic ductal adenocarcinoma postsurgical 5-year survival rates are significantly low spanning from 15% to 20% with most of the patients dying due to local recurrence or metastasis.3 Nonsurgical approaches have been attempted in advanced-stage PDAC via targeting tumor growth using adjuvant chemotherapies or chemoradiotherapy (CRT) in combination with gemcitabine, 5-fluorouracil (5-FU), cisplatin, erlotinib, or interferon alfa-2b. In vitro studies and murine animal models highlighted the critical role of STAT3 in driving cancer progression at different stages.4-8

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Inflammation and PDAC: The Underlying Mechanisms
Interleukin-6 (IL-6) signaling pathway
Under inflammatory conditions, the nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) induce secretion of interleukin 6 (IL-6) in myeloid cells, a process known as trans-signaling where IL-6 forms a complex with soluble IL-6 receptors, which mediates the effects of secreted IL-6. Interleukin 6 induces phosphorylation of signal transducer and activator of transcription 3 (STAT3) and promotes synthesis of the neutrophil attractant CXCL1 in pancreatic acinar cells.9 In addition to IL-6, multiple growth factors and pro-inflammatory cytokines are involved in mediating STAT3 phosphorylation.10 As opposed to normal pancreatic microenvironment, in PDAC, tyrosine phosphorylation triggers STAT3 activation and nuclear translocation leading to the transcription of numerous target genes involved in inflammation as well as stem cell renewal.11,12 STAT3 plays a vital role in the development of acinar-to-ductal metaplasia (ADM) lesions; in some instances, these ADM lesions may develop into pancreatic cancer.13 The role of STAT3 as an inflammatory mediator of the development of pancreatic precursor lesion formation was observed in vitro, and in vivo studies confirmed its role in the development of preneoplastic lesions.14-18 Moreover, several studies using pancreatic cell lines and murine animal models highlighted the critical role of STAT3 in driving cancer progression at different stages.19 Corcoran et al18 demonstrated that STAT3 is vital both for the formation of precursor lesions (ie, ADM, pancreatic intraepithelial neoplasia [PanIN]) and progression to PDAC. Another study showed that STAT3 contributes to PDAC initiation by enhancing the development of preneoplastic cancer lesions, cell proliferation, and inflammatory responses associated with metaplasia.19 Fukuda et al19 validated that STAT3 overexpressed in the epithelial cells after cerulein-induced inflammation in a KrasG12D mouse model assists in the initialization of tumor development and progression. However,
STAT3 inhibition attenuates precursor lesion formation, cell proliferation and enhances apoptosis. In addition, the loss of STAT3 in the epithelial tissue reduces inflammatory cell infiltration and expression of inflammatory cytokines, indicating that STAT3 does not only influence the proliferative and dedifferentiated state of epithelial cells but also regulate inflammatory processes associated with metaplasia. In an extension of this study, Lesina et al. identified the myeloid origin cells as a source of pro-inflammatory cytokine IL-6 that activates STAT3 in the pancreas and nourishes the formation and progression of PanIN lesions. The recognition of this mechanism endorses the role of the inflammatory microenvironment in the development of PDAC in mouse models and stands true for human PDAC based on the analysis of human PDAC specimen and patient data. Increased levels of mitochondrial pSTAT3 enhance the pool of available adenosine triphosphate and increase cellular proliferation.

**NF-κB signaling pathway**

Nuclear factor κB is a key transcription factor that regulates inflammation and thus plays a critical role in the development of pancreatitis and pancreatic carcinogenesis. Under normal physiological conditions in pancreas, the IκB family of inhibitory proteins (IκB-α, IκB-β, IκB-γ, IκB-ε, Bcl-3, p105/ NF-κB1, and p100/NF-κB2) keeps the NF-κB signaling pathway in an inactive state by sequestering the regulatory subunits of NF-κB in the cytoplasm. However, under the influence of microbial or viral infections or pro-inflammatory cytokines, the IκB kinase (IKK) complex is activated and phosphorylates the IκB proteins leading to its ubiquitination and subsequent degradation by the 26S proteasomal system. This allows the regulatory subunits of NF-κB to translocate to the nucleus and regulate the transcription of various genes responsible for survival and inflammation. The activation of NF-κB pathway is one of the early events in pancreatitis where it promotes the pro-inflammatory response through the upregulation of inflammatory genes in addition to boosting anti-apoptotic genes assisting pancreatic cancer cells in evading apoptosis. Nuclear factor κB delivers its antiapoptotic effects on pancreatic cancer cells by upregulation of the anti-apoptotic gene B-cell lymphoma extra large (Bcl-xL) and the cell cycle gene cyclin D1. Another report demonstrates that low expression of the NF-κB subunit p65 in pancreatic cancer cells leads to downregulation of the anti-apoptotic gene B-cell lymphoma 2 (Bcl-2), cyclin D1, vascular endothelial growth factor (VEGF) in addition to activation of caspase-3 leading to growth attenuation in the pancreatic cancer cell line BxPC-3. Nuclear factor κB seems to act downstream of the epidermal growth factor receptor (EGFR) because EGFR pathway inhibition in the pancreatic cancer cell line MDA Panc-28 results in lesser NF-κB binding activity and downregulation of the antiapoptotic genes Bcl-xL and Bfl-1.

Recently, it was reported that persistent activation of NF-κB in pancreatic acinar cells leads to the development of chronic pancreatitis characterized by severe pancreatic damage, immune cell infiltration, and fibrosis. Another study showed that the deletion of IKK, IKK2, in all pancreatic epithelial cells averts the development of PanIN lesions in PdxCre/+; LSL-KrasG12D/+ mice. IκB protein is a substrate of β-TrCP that encodes a member of the F-box protein family and plays an important role in regulating cell cycle checkpoints. High levels of β-TrCP1 and constitutive activation of NF-κB are hallmarks of chemoresistant PDAC cell lines compared with chemosensitive PDAC cell lines. Overexpression of β-TrCP1 in chemosensitive PDAC cell lines results in enhanced NF-κB activity and reduced sensitivity to chemotherapy drugs, whereas small interfering RNA–dependent knockdown of β-TrCP1 in chemoresistant PDAC cell lines attenuates NF-κB activation and chemoresistance. Nuclear factor κB seems to enhance the development of chronic pancreatitis, pancreatic precursor lesions, and their transformation to invasive PDAC at least in part through mediating the interplay between oncogenic Kras signaling and inflammatory responses.

Pancreatic ductal adenocarcinoma is believed to be mainly originated from the pancreatic duct cells. Nevertheless, under the activation mutation of KRasG12D, during pancreatitis, acinar cells can go through ADM and form duct cells and eventually PanIN and PDAC. Hence, PDAC can also originate from acinar cells by means of ADM. Mitogen-activated protein kinase (MAPK), Wnt, Notch, and PI3K/Akt signaling are involved in this acinar transdifferentiation process. Moreover, during this transdifferentiation to ADM, acinar cells lose their grape-like phenotype and alter the transcriptome from acinar-like (carboxypeptidase, amylase, elastase, and Mist expression) to duct-like (expressing cytokeratin-19, 20, and carbonic anhydrase II). Also, in vivo studies have demonstrated that both acute and chronic pancreatitis can lead to ADM. These findings complement that chronic pancreatitis may be one of the etiologic factors of pancreatic cancer.

In addition, mutant Kras mouse model system supports the idea that ADM might be a prerequisite for PanIN and PDAC development. Furthermore, inflammation is critical in mediating tumorigenesis was demonstrated in an in vivo study where the acceleration to PDAC lesions was seen with the chronic administration of cerulein in mutant Kras mice. Fascinatingly, in mice, direct targeting of acinar cells with KrasG12D is sufficient for spontaneous transformation of acinar cells to PanIN lesions even in the absence of injury or inflammation. However, the development of PanIN lesions in vivo may involve the emergence of a progenitor population that is either an indirect or direct precursor to cells that will contribute to a PanIN. This progenitor population expresses Pdx1, which is normally low or absent in ducal cells. It is possible that a resident progenitor population exists among ductal cells or centroacinar cells, which undergoes neoplastic transformation.
without ADM. The existence of such a population is suggested by the finding that a subset of adult mouse centroacinar cells/terminal duct cells harbor high aldehyde dehydrogenase isoenzyme 1 (ALDH1) enzymatic activity, which is important in retinoic acid metabolism and has been associated with stem and progenitor cells in a variety of tissue types.54,55

Inhibition of the MAPK and NF-κB survival pathways with U0126 and caffeic acid phenethyl ester (CAPE), respectively, potently blocks pancreatic tumor growth without inducing apoptotic death. Interestingly, apoptosis was induced by U0126 and CAPE after inhibition of autophagy in a caspase-independent manner in Panc-1 cells and in a caspase-dependent manner in MiaPaCa-2 cells.56

**Transforming growth factor β signaling pathway**

Transforming growth factor β (TGF-β), a secreted anti-inflammatory cytokine that regulates apoptosis, cell growth, and differentiation, has been associated with advanced tumor stages57–59 where TGF-β plays an antitumorigenic role via restricting cell growth and enhancing apoptosis. On ligand binding, TGF-β receptors type I (TGF-βR1) and type II (TGF-βR2) undergo heterodimerization. The TGF-βR2 phosphorylates TGF-βR1 kinase domain triggering phosphorylation and activation of various isoforms of SMAD proteins.60 Phosphorylated SMAD shuttles to the nucleus and stimulates transcription of target genes responsible for tumor suppression.61

Similar to its overall tumor suppressive roles under homeostatic conditions, TGF-β signaling inhibits cell growth in early stages of pancreatic cancer and in a number of pancreatic cancer cell lines such as Colo-357.62 However, during late stages of pancreatic cancer, TGF-β signaling is dysregulated on multiple levels. Defects in TGF-β receptors and mutations in SMADs have been observed in numerous pancreatic cancer cell lines.63 These defects result in the emergence of an opposite role of TGF-β signaling where it promotes tumorigenesis through enhancing cancer cell growth, survival, invasion, and metastasis leading to reduced survival of patients with pancreatic cancer.61,64–66 The defective response of TGF-β signaling following TGF-β stimulation has been confirmed in several pancreatic cancer cell lines including Panc-1, MiaPaCa, and BxPC3 by β3[H] thymidine incorporation, and TGF-β–sensitive reporter assays. Along the same line, treatment of Panc-1 and IMIM-PC1 cells with recombinant TGF-β enhances their invasiveness, an effect that is completely blocked in the presence of TGF-β–neutralizing antibody. Transforming growth factor β–induced invasiveness could be attributed at least in part to the enhanced expression of matrix metalloproteinase 2 (MMP2) and the urokinase plasminogen activator (uPA) system in Panc-1 and IMIM-PC1 cell lines.67 Although SMAD2 and SMAD3 do not seem to be part of the dysregulated TGF-β system in pancreatic cancer, SMAD4 seems to be directly involved in the malfunctional response of TGF-β. Introducing SMAD4 into the SMAD4 homozygous-deficient pancreatic cell line, BxPC3 restores responsiveness to TGF-β.68 Similarly, inhibition of NF-κB pathway impairs invasiveness of BxPC3 and Capan-1 cells only on restoration of SMAD4 expression, indicating the downstream role of SMAD4 in NF-κB signaling.69 Kindlin-2, a target protein that is upregulated by TGF-β1 in PDAC cells, is another mediator of TGF-β1–induced tumorigenic effects, where it enhances PDAC cell growth, migration, and invasion and promotes overall PDAC progression via downregulation of HOXB9 and E-cadherin.70 In addition, SMAD371 and SMAD472 together contribute to TGF-β1–induced invasiveness in PDAC cells by inducing expression of EMT–associated transcription factors and subsequent phenotypic changes.

Various tumor-stroma interactions have been reported of having the capability to foster pancreatic cancer cell invasion and metastasis. Growth factors that have been derived from cancer cells, mainly TGF-β, along with fibroblast growth factors (FGFs), platelet-derived growth factor BB (PDGF-BB), and insulin-like growth factor 1 (IGF-1).73 These growth factors gets encompassed within the stromal areas and thus acts as a site of storage for these growth factors.74 The invading cancer cells release MMPs that cause the release of these growth factors.75–77 The stroma itself is a very complex structure consisting of various cell types including mesenchymal cells (cancer-associated fibroblasts [CAF]), endothelial cells, extra-cellular matrix (ECM) proteins (mainly, type I collagen), nerve cells, endothelial cells and pericytes, bone marrow–derived stem cells, and immune cells.76 Transforming growth factor β receptors are expressed by all these cell types, and the TGF-β pathway can thus influence tumor microenvironment by affecting fibrosis, angiogenesis, and immune cell infiltration.77 Both the generation of cancer from a nontumoral environment and the maintenance of a favorable tumoral microenvironment are governed by the TGF-β pathway activation.76 The activated TGF-β pathway enhances production and lowers the degradation of ECM components, mainly type I collagen, as well as mesenchymal cell proliferation.78–80 Furthermore, TGF-β promotes reactive oxygen species production via several mechanisms (such as activation of nicotinamide adenine dinucleotide phosphate oxidases family members), leading to targeting downstream signaling pathways such as Src, EGFR, SMADs, and MAPK family, thus promoting profibrotic gene expression (eg, TGF-β1, angiotensinogen, PAI-1, and connective tissue growth factor).81 Overproduction of TGF-β not only drives the fibrotic process/chronic phases of inflammatory diseases but also precedes tumor formation and thus creates a favorable microenvironment for cancer cells’ growth.76,78,82

In addition, TGF-β activates surrounding CAFs and stellate cells. These activated CAFs and stellate cells are responsible for the secretion of several factors (such as PDGF, FGF, MMP, EG, type I collagen, and IGF-1) that enhance tumor proliferation, growth, invasion, metastasis, and above all chemoresistance.83 Furthermore, they take part in the creation of hypoxic
microenvironment, thus applying a selection pressure leading to an invasive cancer cell phenotype. In conclusion, the stroma, depending on collagen I structure, can behave as a barrier or a promoter to metastatic dissemination of cancer.

**IL-1α and IL-1β signaling pathways**

Numerous pro-inflammatory molecules have been recognized as key players in PDAC invasion and metastasis. Interleukin 1α (IL-1α) is a major inflammatory cytokine that promotes adhesion, proliferation, and migration of the pancreatic cancer cell lines SW1990, BxPC3, and Capan-2 by upregulating the expression of the urokinase receptor and integrin subunits αv and β1. These effects are linked with the activation of RAS-ERK (extracellular signal-regulated kinase) signaling pathway. Inhibition of αv and β1 integrins and uPA leads to downstream inhibition of ERK signaling and subsequent impairment of proliferative, migratory, and adhesive responses of pancreatic cells. Xu et al. showed that IL-1α synthesized by pancreatic cancer cells induces expression of hepatocyte growth factor (HGF) in fibroblasts. Coculture experiments demonstrated a paracrine effect of IL-1α-dependent fibroblast-driven HGF on neighboring cells where fibroblast-secreted HGF promotes invasive and proliferative behavior of pancreatic cancer cells and human umbilical vein endothelial cells. In another study, forced expression of IL-1α in the pancreatic cancer cell line MiaPaCa-2 results in activation of NF-κB signaling pathway leading to an increase in the invasive phenotype of pancreatic cancer cells. Along the same line, blocking NF-κB pathway by the expression of a dominant-negative IκB protein impairs the metastatic behavior of pancreatic cancer cells. Similar responses were observed when IL-1α was silenced in the metastatic pancreatic cancer cell line L3.6pl, supporting the notion that IL-1α-induced NF-κB expression promotes the invasive and metastatic behavior of pancreatic cancer cells.

The pro-inflammatory cytokine interleukin 1β (IL-1β) is another member of the IL-1 family that influences metastasis and tumor growth in various types of cancers. Interleukin 1β along with IL-1α induces the expression of pro-inflammatory genes including inducible nitric oxide synthase, cyclooxygenase 2 (COX-2), and IL-6. Pancreatic cancer cell lines treated with recombinant IL-1β show a strong invasive behavior with no influence on ECM adhesion.

**CXC chemokine signaling**

Recent studies have suggested the dysregulation of CXC chemokines in late-stage PDAC. Expression of CXCL5, a ligand for CXCR2, is enhanced in human PDAC and has been linked to increased tumor size, advanced tumor stage, and poor outcome. Genetic mutations that dysregulate chemokine signaling, such as TP53 mutation, have been attributed to promoting invasion and metastasis in PDAC. Another example is SMAD4 mutations that are found in 50% of PDAC cases and known to dysregulate chemokine signaling. In a mutant Kras mouse model-based study, TGF-βR2 knockout leads to aggressive PDAC that histologically resembles human disease. Mutant Kras mice are also characterized by enhanced secretion of CXCR2-specific chemokines, including CXCL1 and CXCL5, which are regulated by TGF-β and NF-κB signaling. Interestingly, stromal fibroblasts express markedly higher levels of CXCR2 than epithelial cells, and acute inhibition of CXCR2 improves survival and reduces microvessel density, further validating the involvement of CXCR2 ligands in driving PDAC progression.

Current reports suggest that metastasis may occur in PDAC even before primary tumor formation, a behavior associated with early epidermal mesenchymal transformation. This is accelerated in the presence of pancreatic inflammation where the most invasive areas of tumor are located at the foci of inflammation. This phenomenon is quenched by dexamethasone indicating the integral role played by tumor-associated inflammation. Hence, identification of inflammatory signaling pathways involved in PDAC metastasis is critical for developing combinatorial antimetastatic therapies in the near future.

**The Role of EMT in PDAC Development and Drug Resistance**

Epithelial-mesenchymal transition is a process by which epithelial cells undergo numerous genotypic and phenotypic changes to attain mesenchymal phenotype. The mesenchymal phenotype is characterized by enhanced migratory capacity, invasiveness, resistance to apoptosis, and production of ECM. Newly transformed mesenchymal cells typically show poor cell adhesion parallel to loss of E-cadherin. This phenomenon also features the gain of mesenchymal markers, including vimentin, N-cadherin, and fibronectin. Epithelial-mesenchymal transition plays a crucial role during development and in adult tissue repair following injury. Epithelial-mesenchymal transition initiated by genetic and epigenetic changes in the tumor microenvironment represents a pivotal event during cancer progression and metastasis.

Transition to mesenchymal phenotype is regulated at the cellular level by certain key zinc finger transcription factors, such as Snail, Slug, Zeb-1, and Twist, which perturb the regulation of genes driving epithelial phenotype. Tumor-budding cells in the tumor microenvironment of aggressive PDAC express EMT markers at both messenger RNA (mRNA) and protein levels. These budding cells display classical EMT phenotypic changes and are surrounded by a heterogeneous population of stromal cells that express high levels of the E-cadherin repressors ZEB1, ZEB2, and SNAIL1.

There is a close association between chemoresistance and the gain of the EMT phenotype in various carcinoma cells including PDAC. Pancreatic ductal adenocarcinoma cell lines BxPC3, L3.6pl, CFPAC-1, and SU86.86 with enhanced E-cadherin expression and reduced expression of the mesenchymal marker...
Zeb-1 display sensitivity to the chemotherapeutic agents 5-FU, gemcitabine, and cisplatin, whereas other cell lines Hs766T, Panc-1, MiaPaCa-2, AsPC-1, and MPanc96 express low E-cadherin, high Zeb-1 levels and display EMT as well as exhibit resistance to the aforementioned chemotherapeutic drugs. Zeb-1 downregulation in PDAC cells with EMT phenotype enhances the expression of epithelial markers and retrieve drug sensitivity, indicating the involvement of Zeb-1 and other EMT regulators in enhancing the resistance of PDAC cells to chemotherapy. This notion was further validated in an in vivo mouse model. In this report, although EMT suppression enhanced cancer cell proliferation, it also increased expression of nucleoside transporters leading to enhanced sensitivity to gemcitabine treatment and prolonged survival in mice. Furthermore, this study highlights the need of a combination of EMT inhibitors to efficiently blunt chemotherapeutic resistance during treatment of pancreatic cancer.

A small population of permanent proliferating cells and a large population of differentiated cells (with limited proliferation potential) exist in the carcinoma tissue. Within the permanently proliferating cells, cancer stem cells (CSCs) are believed to be culpable for the initiation, chemoresistance, metastases, and tumor recurrence. Cancer stem cells are self-renewing cells that bear the potential to differentiate into other cell types, as well as initiate tumors in the immunodeficient mice. It is known from the recent studies that CSC and EMT-type cells not only show similarities such as higher metastatic potential and chemoresistance but also have the molecular pathways such as Notch and Wnt in common, indicating the direct correlation among CSC property and EMT program. Moreover, constant Notch–1 overexpression is known to induce self-renewal potential, expression of CSC markers CD44 and epithelial surface antigen, as well as EMT properties via upregulation of Zeb-1 in the PDAC cell line AsPC-1. Similarly, forced expression of forkhead box protein M1 (FoxM1) induced EMT state by enhancing expression of vimentin, Zeb-1, and Snail2, as well as promoted the gain of the CSC phenotype in PDAC cells. Furthermore, reduced expression of stem cell–related transcription factors Sox2 and Oct4, reversal of the EMT phenotype, decreased sphere formations, and the in vivo tumorigenicity in PDAC cells were seen after silencing of Snail with small hairpin RNA introduction.

One of the most critical property of CSCs is to gain the EMT-induced stemness phenotype that leads them to resistance to several chemotherapeutic agents. Pancreatic ductal adenocarcinoma cells with the CSC phenotype under the influence of hypoxia gain EMT and enhanced migration ability. In addition, it was reported that only the CSC-like cells acquire high migratory potential and thus may be responsible for invasion and metastasis.

Also, human pancreatic cancers have a cell subset known as side population. These side population cells are highly resistant to gemcitabine, a very routine chemotherapeutic agent in used in the therapy of pancreatic cancer. In addition, these cells exhibit enhanced gene expression profiles associated with multidrug resistance (ABCG2 and ABCA9), EMT (SNAI2, LEF1), and regulation of apoptosis (ETS1, FASLG). Also, it is reported that in pancreatic CSCs, microRNAs (miRNAs) such as miR99a, miR100, miR-125b, miR-192, and miR-429 are differentially expressed. These miRNA clusters are related to the stem cell–associated mRNAs in pancreatic CSCs. These findings indicate that stem cell–like properties imparted during EMT could attribute to chemoresistance in pancreatic cancer.

**Tumor-Infiltrating Inflammatory Cells and EMT: Crosstalk in Cancer Pathogenesis and Progression**

An important question that needs further investigation is “How do tumor-infiltrating inflammatory cells and EMT impact one another toward cancer progression?” Many mechanisms have been described in literature, including autocrine/paracrine extracellular signals as well as genetic and epigenetic modifications.

Epithelial-mesenchymal transition–inducing signals are released through a process where a reactive stroma is formed after the recruitment of variety of inflammatory cells, such as myofibroblasts, fibroblasts, macrophages, granulocytes, myeloid cell–derived suppressor cells, lymphocytes, and mesenchymal stem cells, under the influence of certain factors synthesized by islands of cancer cells in advanced primary carcinomas. Using human PDAC primary tumors and Kras(G12D)/Snail mice, it was shown that SNAIL overexpression is associated with enhanced infiltration of mast cells via stem cell factor. Enhanced recruitment of Gr-1+ and F4/80+ cells was also reported in Kras(G12D)/Snail mice compared with control Kras (G12D) mice. Interaction between inflammatory and EMT pathways toward cancer progression is observed in multiple types of cancers and not restricted to PDAC. Coculture of tumor–associated macrophages (TAMs) and ovarian cancer cells demonstrated that TAMs promote the invasive phenotype of cancer cells in tumor necrosis factor α (TNF-α) and NF-κB–dependent manner. In PDAC, macrophage infiltration is seen at a significantly higher numbers than in normal pancreatic tissue, and their infiltration does not match with chronic pancreatitis–like features in the neighboring tissue. The TAM M2 subtype has been associated with a poor prognosis. It was shown in an in vivo mouse model that when human tumor cells were co-engrafted with high numbers of human monocytes, enhanced tumor growth was seen. But, when they co-engrafted tumor cells with a low ratio of human monocytes, they noticed inhibition of tumor growth. Continuous and regular contact of monocytes with tumor cells downregulates the production of cytotoxic molecules (such as reactive oxygen intermediates, TNF-α, and IL-12) and upregulates the levels of immunosuppressive cytokine IL-10. This indicates that...
there could be a threshold ratio of the tumor cells to the number of monocytes and a set-limit of the immune mediators/molecules they make, which when exceeded antitumor effects are not seen and more protumor phenotype is displayed. Furthermore, it was reported in an in vitro study that TNF-α made by TAMs increased with macrophage motility as well as pancreatic tumor cell numbers and ultimately transforming the phenotype of tumor cells to EMT phenotype.124 These findings support the notion that the increment in the number of TAMs and their products such as TNF-α in PDAC could overpower a definite threshold and transform from an antitumor to a protumor response.139 However, further studies are required to better understand the importance and impact of the number and type of TAMs that play a critical role in PDAC.

Cancer-associated fibroblasts represent another major cell type present in chronic inflammatory microenvironment and express growth factors such as FGF and HGF in addition to matrix-degrading enzymes, which are known inducers of EMT.125-127 Pancratic ductal adenocarcinoma cells and CAFs reciprocally enhance each other’s proliferation and differentiation. Cell culture supernatants from PDAC cells trigger the production of ECM proteins and proliferation of pancreatic stellate cells (PSCs).128,129 Similarly, coculture of PDAC cells with CAF cell culture supernatant enhances the proliferation and migration of PDAC cells, as well as the rate of growth of PDAC cells when PSCs are co-injected into nude mice.129-131 It was demonstrated that coculture of PSCs with PDAC cells leads to downregulation of epithelial markers, E-cadherin, cytokeratin 19, and β-catenin, and upregulation of mesenchymal markers, vimentin and Snail, subsequently leading to enhanced cancer cell migration.132 Furthermore, in an in vivo study, male human CAFs were orthotopically co-injected along with female PDAC cells as a xenograft into the pancreas of female mice. It was observed that CAFs followed the pancreatic cancer cells to the metastatic sites, indicating that CAFs could play a potential role in the colonization of metastatic PDAC cells.133

Furthermore, it has been reported that CAFs protect pancreatic cancer cells from CRT.130 In an in vitro study, it was shown that when pancreatic cancer cells were cultured in the presence of culture supernatant (conditioned medium) from PSCs, the components in the PSC-conditioned media blocked the apoptosis of the gemcitabine-treated (100 µmol/L) or radiation therapy (100 Gy)-treated pancreatic cancer cells.130 Moreover, pancreatic cancer cell survival during radiation was enhanced in the presence of PSCs in both the monocultures or direct coculture-based conditions.131,134 However, in another study, contact between CAFs and the PDAC cells was necessary for PDAC cells to gain radioprotective, but when β1-integrin signaling was blocked using blocking antibodies, this radioprotective effect of CAFs was significantly attenuated.131 Furthermore, in an in vivo xenograft model system, it was shown that CAFs provide radioprotection to the implanted tumor cells when co-injected with pancreatic cancer cells, indicating critical role of CAFs in pancreatic cancer.128,131,134

Inflammation and EMT: A Vicious Cycle in PDAC Progression
Inflammation, EMT and cancer are closely interconnected (Figure 1).78,135-137 In this section, we will discuss the molecular mechanisms involved in the regulation of inflammation and EMT in cancer pathogenesis and progression with a focus on the interplay between NF-κB, TGF-β, TNF-α, and STAT3 signaling pathways.

Nuclear factor κB is not only a direct and powerful inducer of EMT but also promotes mobilization of innate immunity and inflammation, thus representing a molecular bridge between inflammation, EMT, and cancer.78,138-147 Akt-mediated activation of NF-κB leads to enhanced SNAIL expression and induction of EMT.143,148 Subsequently, upregulated SNAIL inhibits expression of the metastasis suppressor gene products Raf kinase inhibitor protein (RKIP) and phospatase and tensin homology (PTEN) leading to blocking of NF-κB/MAPK and PI3K/AKT pathways, respectively.149-151 Nuclear factor κB has been shown to regulate a number of miRNAs. Nuclear factor κB upregulates expression of miR-9,152 a miRNA whose overexpression in breast cancer cells directly targets CDH1 (the E-cadherin-encoding messenger RNA) leading to enhanced cell motility and invasiveness.153 Nuclear factor κB also directly binds to miR-448 promoter and downregulates miR-448 transcription leading to EMT induction. miR-448 suppression induces EMT via targeting specific AT-rich sequence-binding protein-1 (SATB1) mRNA, enhancing EGFR-mediated TWIST1 expression and NF-κB activation. Moreover, patients who were subject to combinatorial chemotherapy exhibited lower miR-448 levels and higher SATB1 and TWIST1 levels. Thus, a feedback loop between miR-448 and NF-κB seems to play a critical role in the regulation of chemotherapy-induced EMT.154 Nuclear factor κB activation in myeloid cells has also been associated with EMT and tumor progression in inflammation-associated cancer models.155

Transforming growth factor β is another major regulator of EMT through canonical SMAD-dependent156 and noncanonical SMAD-independent pathways. Transforming growth factor β also modulates the expression of other EMT regulators, such as SLUG157 and SNAIL,158,159 through SMAD and MAPK activation in both normal and malignant mammary epithelial cells (MECs).160-161 In addition, TGF-β-TGF-βR-SMAD2 signaling axis controls maintenance of epigenetic silencing of crucial EMT genes in breast cancer progression.164 Along with canonical SMAD-dependent pathways, several reports demonstrate that TGF-β can also regulate MECs behavior and induce EMT independently of SMADs. Noncanonical SMAD-independent effectors include phospatidylinositol-4,5-bisphosphate 3-kinase (PI3K), MAPKs,
guanine triphosphate–binding proteins, and NF-κB. In addition, TGF-β targets include Na and K-ATPase, IGFBP3, SKIP, TGF-βR1, Dab2, ROCK and LIMK, PIAS1, as well as multiple nuclear transcription factors, including members of SNAIL, SIP1, TWIST, and the family of homeobox (Six1). Transforming growth factor β regulation of EMT does take place at the miRNA level as well in both normal and cancerous cells. In normal MECs, TGF-β stimulation enhances miR-155 expression through a SMAD4-dependent pathway. Transforming growth factor β also mediates miR-21 and miR-29a expression leading to EMT induction. miR-200 is another miRNA that falls under the umbrella of TGF-β–regulated small RNAs. Transforming growth factor β downregulates miR-200 expression, thus enhancing expression of E-cadherin repressors ZEB1 and ZEB2, which in turn results in E-cadherin downregulation and EMT induction. Moreover, TGF-β signaling induces hypermethylation of E-cadherin promoter leading to differentiation of Ras–transformed MECs that have undergone a serum–induced stable EMT. Overall, a long list of targets have been identified downstream of TGF-β in the regulation of EMT. Nevertheless, the relative importance of these downstream targets and the crosstalk among them in TGF-β–mediated EMT is not yet fully understood. However, TGF-β signaling in EMT has been shown to be regulated by a number of miRNAs, such as miR-30 and/or miR-200 family members, in cells derived from anaplastic thyroid carcinoma cells.

Similar to NF-κB and TGF-β, TNF-α is a potent stimulator of EMT. Transforming growth factor α induces SNAIL1 promoter activity and stabilizes SNAIL1 protein. Transforming growth factor α–induced EMT is partly mediated by TGF-β1 activation. Transforming growth factor α and TGF-β act in a synergistic manner expediting EMT via a p38 MAPK–dependent pathway. Transforming growth factor α also promotes CD44 expression and moesin phosphorylation via TGF-β and protein kinase C activation along with actin remodeling. This leads to the dissociation of cell–cell contacts and increase in cellular motility. In addition to TGF-β–mediated EMT induction, TNF-α induces EMT via NF-κB activation or IKK2 constitutive upregulation and activation. As previously discussed, the downstream targets of TNF-α, TGF-β, and NF-κB are also interconnected. Transforming growth factor β–mediated EMT induction, TNF-α induces EMT via NF-κB activation or IKK2 constitutive upregulation and activation. As previously discussed, the downstream targets of TNF-α, TGF-β, and NF-κB are also interconnected. Transforming growth factor β–mediated NF-κB activation induces EMT and metastasis by upregulation of an autocrine cascade of Cox-2/prostaglandin E2 (PGE2) receptor 2 (EP2) signaling. Altogether, these findings elucidate the regulation of EMT induction via a triad system of NF-κB, TGF-β, and TNF-α pro-inflammatory signaling pathways.

Another pro-inflammatory mechanism that primarily contributes to EMT induction is STAT3-mediated expression of TWIST. However, STAT3 has been reported to be a negative regulator of adenoma–carcinoma transition in colon cancer in contrast to the general dogma where pro-inflammatory signals induce EMT and promote tumor progression.

**Current Treatment Options and Therapeutic Approach**

For patients diagnosed with PDAC, at the moment, only surgical resection is the hope. But, about 80% of the patients with PDAC at the time of diagnosis already have a locally
advanced or metastatic disease, thus rendering surgical intervention ineffective.\textsuperscript{199,200} For the past 2 decades, the standard therapeutic strategy for these patients has been a combinatorial strategy of chemotherapy along with the nucleoside analogue gemcitabine.\textsuperscript{199} Despite this, only a meager 5-week increase in median survival of these patients has been observed using gemcitabine.\textsuperscript{201} Moreover, therapeutic strategy to combine either thymidylate synthetase inhibitor (capecitabine) or platin-based agents (cisplatin and oxaliplatin) along with gemcitabine has been unsuccessful in enhancing the therapeutic efficacy.\textsuperscript{202–204} A limited increase in the median survival (6.24 vs 5.91 months) for the patients with unresectable PDAC was seen in a phase 3 study with the combinatorial treatment of erlotinib, an EGFR inhibitor along with gemcitabine in comparison with gemcitabine alone.\textsuperscript{205} Recent advances show that the use of FOLFIRINOX (irinotecan, oxaliplatin, leucovorin, and FU) has shown a significant increase in the median survival of these patients by more than 4 months in comparison with gemcitabine alone (11.1 vs 6.8 months).\textsuperscript{206}

Precision medicine in oncology has been critical in understanding diverse molecular mechanisms of PDAC oncogenesis.\textsuperscript{207} Nevertheless, transforming this knowledge toward the development of targeted therapy has been a daunting task due to the complex biology of PDAC.\textsuperscript{207} Axitinib, an oral inhibitor of VEGF receptors (VEGFR), was investigated in a randomized, placebo-controlled phase 2 study enrolling 103 patients with unresectable or metastatic PDAC as supplement to gemcitabine. Median overall survival for gemcitabine with axitinib was 6.9 months, whereas for gemcitabine alone was 5.6 months.\textsuperscript{208,209} Although the study was extended with a phase 3 trial including 632 patients,\textsuperscript{210} an interim analysis suggested that the study was a failure and hence was terminated.\textsuperscript{210}

Germline mutations in the \textit{BRCA1} or \textit{BRCA2} genes render PDAC tumors highly sensitive to poly (ADP-ribose) polymerase (PARP) inhibitors.\textsuperscript{211} To this effect, several PARP inhibitors, such as olaparib, are being tested in clinical trials. In a recent multicenter phase 2 study, olaparib (400 mg twice per day) was given to the enrolled 298 patients, including a subgroup of patients with pancreatic cancer with a germline BRCA1/2 mutation.\textsuperscript{212} The overall response rate for patients with PDAC (treated previously with gemcitabine) was 21.7% (5 of 23).\textsuperscript{212} In another study, treatment of Marimastat 25 mg, an oral MMP inhibitor did not change the survival rate for patients in a randomized study enrolling 414 patients with unresectable pancreatic cancer in comparison with patients receiving gemcitabine alone.\textsuperscript{213} In a mouse model–based study, a combination of gemcitabine along with saridegib, an inhibitor of the Hh pathway, depleted desmoplastic stroma, enhanced delivery of gemcitabine to tumor cells, and thus displayed a significant improvement in the survival of tumor-bearing mice.\textsuperscript{214} Conversely, a randomized, double-blind, placebo-controlled phase 2 trial with gemcitabine plus saridegib resulted in worse median survival in comparison with gemcitabine plus placebo arm; this study was discontinued.\textsuperscript{208}

In patients with solid tumors, targeting ERBB family members (eg, EGFR and VEGF) and VEGFR using monoclonal antibodies has been most effective.\textsuperscript{208} But some of these antibodies have not been successful in the trials with patients with advanced PDAC. Monoclonal antibodies targeting PD-1, PD-L1, and CTLA-4 (so-called checkpoint blockade, reviewed by Postow et al\textsuperscript{215}) have been shown in recent clinical trials to promote endogenous antitumor immune activity.\textsuperscript{216–218} Various phase 1 and 2 trials are going on to study the effect of antibodies against PD-1, PD-L1, and CTLA-4 in the solid tumors including advanced or metastatic pancreatic adenocarcinoma.\textsuperscript{208} Furthermore, new studies have been started to test monoclonal antibodies against tissue factor (CD142), Notch, human growth factor receptor, and tumor endothelial marker 1 (TEM1, endosialin) in patients with PDAC.\textsuperscript{208}

In addition, vaccines and immunotherapies are being used to target PDAC. Algenpantucel-L is a vaccine derived of 2 irradiated allogeneic pancreatic cancer cell lines (HAPa-1 and HAPa-2) transfected to express murine α-1,3-galactosyltransferase has reached phase 3. It was successfully tested in a phase 2 trial (multicenter, open label) with 70 resected (R0–1) patients with PDAC along with the combination of gemcitabine chemotherapy and chemoradiation.\textsuperscript{208} In this study, the median overall survival was 86% and disease-free survival was 62% for the first year during a follow-up of 21 months.\textsuperscript{208} The GVAX, a granulocyte-macrophage colony-stimulating factor–secreting allogenic pancreatic tumor cell vaccine was investigated recently in 90 patients with metastatic PDAC along with low-dose cyclophosphamide (Cy/GVAX) to block regulatory T cells, and with or without CRS-207, a live-attenuated \textit{Listeria monocytogenes} expressing mesothelin. This was performed in a prime/boost vaccination manner, ie, Cy/GVAX followed by CRS-207 (arm A) in comparison with Cy/GVAX alone (arm B),\textsuperscript{219} where overall survival of 6.1 months in arm A and 3.9 months in arm B (\textit{P} = .02) was seen. Higher levels of mesothelin-specific CD8\textsuperscript{+} T-cell responses were linked to the longer overall survival.\textsuperscript{208}

For adoptive immunotherapy, ex vivo genetic engineered T cells collected from patients are used to generate chimeric antigen receptors (CAR), efficient in detecting mesothelin expressed on PDAC cells.\textsuperscript{220,221} The CAR-T cell infused back into the patient immediately detects tumor cells and thus avoids antigen processing and HLA expression. In preclinical studies, CAR-T cells displayed strong antitumor activity.\textsuperscript{222} Also, CAR-T cell therapy is now a discipline of active research in PDAC and there are ongoing studies in this field. (ClinicalTrials.gov identifiers: NCT01897415 and NCT01583686).\textsuperscript{207}

In context to this article, although targeting signaling pathways downstream from \textit{KRAS} has been unsuccessful so far,\textsuperscript{207}
but there is a renewed fascination for targeting the outcome of an activated Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway in PDAC due to the association of PDAC and cachexia.\textsuperscript{20,22} Furthermore, addition of ruxolitinib, the JAK inhibitor to capcetibine for refractory metastatic patients with PDAC in a phase 2 trial exhibited overall benefit for a patient subgroup with increased levels of C-reactive protein.\textsuperscript{20,22} and thus has created the rationale for phase 3 trials for the evaluation of ruxolitinib in patients with metastatic PDAC (ClinicalTrials.gov identifiers: NCT02117479 and NCT02119663). Analysis of data from global genomic studies also disclosed alterations in the gene expression patterns of the Wnt/Notch and TGF-\(\beta\) signaling pathways in all PDACs.\textsuperscript{225} To this effect, at present, there are ongoing clinical studies to trial the potency of specific inhibitors of these pathways (ClinicalTrials.gov identifiers: Wnt inhibitors: NCT02050178, NCT01764477; mAb against Notch: NCT01647828; Oral anti TGF-\(\beta\) receptor type 1: NCT01373164).\textsuperscript{207}

**Conclusions**

Low survival rates of patients with PDAC have been primarily attributed to the resistance to chemotherapy. Inflammation does not only contribute to PDAC initiation but also promote cell survival, inhibit apoptosis, and induce EMT eventually leading to chemoresistance and enhanced invasiveness and metastasis of PDAC. Hence, simultaneous targeting of inflammation and EMT is crucial to overcome chemoresistance and improve survival in the battle against PDAC.

**Author Contributions**

MWK conceived and designed the experiments. MWK and FGK analyzed the data. MWK wrote the first draft of the manuscript. FGK and MWK contributed to the writing of the manuscript, agree with manuscript results and conclusions, jointly developed the structure and arguments for the paper, made critical revisions, and approved the final version. All authors reviewed and approved the final manuscript.

**Disclosures and Ethics**

As a requirement of publication, author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality, and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

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