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Aberrant signaling pathways in pancreatic cancer: A two compartment view

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Abstract

Pancreatic cancer is a devastating disease with historically limited success in treatment and a poor prognosis. Pancreatic cancer appears to have a progressive pathway of development, initiating from well-described pancreatic intraepithelial neoplasia lesions and concluding with invasive carcinoma. These early lesions have been shown to harbor specific alterations in signaling pathways that remain throughout this tumorigenesis process. Meanwhile, new alterations occur during this process of disease progression to have a cumulative effect. This series of events not only impacts the epithelial cells comprising the tumor, but they may also affect the surrounding stromal cells. The result is the formation of complex signaling networks of communication between the tumor epithelial cell and the stromal cell compartments to promote a permissive and cooperative environment. This article highlights some of the most common pathway aberrations involved with this disease, and how these may subsequently affect one or both cellular compartments. Consequently, furthering our understanding of these pathways in terms of their function on the tumoral epithelial and stromal compartments may prove to be crucial to the development of targeted and more successful therapies in the future.

Pancreatic ductal adenocarcinoma from a histology perspective

The pancreas is a small but complex organ vital for normal digestion as well as glucose homeostasis. To accomplish these functions, the pancreas is composed of both exocrine and endocrine compartments. The exocrine cells are responsible for producing digestive enzymes. The endocrine compartments function in glucose regulation mainly through the production of insulin and glucagon. Structurally, the pancreatic tissue is composed of functional epithelial cells arranged in acini or islet formations [1,2]. The acini are responsible for the exocrine function, with digestive enzymes produced within these cells secreted into the acinar lumen and transported to the duodenum through the pancreatic ducts. The islets are responsible for the endocrine activity of the tissue. In order to maintain this cellular architecture, a variety of stromal and supporting cells are present. The stromal components include fibroblasts, pancreatic stellate cells (PSC), endothelial cells, nerves, and inflammatory cells. The crosstalk between the stromal cells and the other cellular compartments is complex and crucial for function of the organ. Much research is being directed to understand this crosstalk between these two cellular compartments and its impact in pancreatic physiology and disease.

As the normal cell composition of the pancreas is varied, so too are the tumors seen [1,2]. Endocrine tumors, such as insulinomas and glucagonomas are well-described in both their pathology and in their profound effects on glucose regulation resulting in potentially severe clinical manifestations. Although there are many types of pancreatic tumors, the focus of this extensive review is on the epithelial tumors arising from exocrine compartment. Pancreatic ductal adenocarcinoma (PDAC), often simply referred to as pancreatic carcinoma or pancreatic cancer, is the most well-known exocrine-based pancreatic tumor, and it is in desperate need of development of early detection methods as well as effective therapies. Most importantly, as described throughout the remainder of this article, it has become clear that the progression of this tumor is not solely dictated by the tumor cells themselves, but rather there is an elegant interplay with the surrounding tumor stromal cells mediated by multiple signaling pathways. Knowledge of the crosstalk between these compartments will help us define the molecular mechanisms underlying the pathogenesis of PDAC. As this topic is of current interest and importance, the remainder of this article will describe the most common and well-studied signaling pathways involved in PDAC development and discuss the cellular processes influenced by these signaling cascades in a dual compartment view: the tumor epithelial compartment and the surrounding tumor stromal compartment.

PDACs are thought to most commonly arise from the ductal epithelium [3–5]. Interestingly, almost all of these tumors are found to progress from pre-neoplastic lesions to the invasive carcinoma category. These precursor lesions include intraductal papillary mucinous neoplasms (IPMNs), mucinous cystic neoplasms (MCNs), and pancreatic intraepithelial neoplasia (PanIN) [6,7]. Of these precursor lesions, PanINs are the most studied and well-characterized. It is from these lesions that an established progression model of pancreatic carcinogenesis has been suggested in which the normal ductal epithelium undergoes a series of alterations that allows its progression through well-defined precursor lesions to invasive carcinomas [8,9]. Until recently, the PanIN lesions were often reported by pathologists using a wide range of descriptors without a defined consensus as to how these lesions were classified. However, undertakings within the last decade have led to more defined descriptions of PanINs, with an initial proposal for the classification and description of pancreatic duct lesions put forth in 2001 [4]. More minimal dysplastic epithelium is classified as PanIN grade 1A or 1B, and more severely dysplastic lesions are classified as PanIN grade 2 or 3. The grade 3 PanIN lesions have characteristics similar to carcinoma, but they have not yet invaded into the basement membrane [4,8]. Once invasion into the basement membrane is seen, the lesion is termed a carcinoma. Of note is that as these histologic changes are seen, so too are changes seen in the surrounding stroma.

A striking characteristic of pancreatic ductal adenocarcinoma is the profound desmoplastic reaction associated with the tumor. This reaction is characterized by small vasculature, inflammatory and immune cells, the presence proliferating fibroblasts, PSC, and increased extracellular matrix (ECM) deposition [10,11]. Similar to other tumors, angiogenesis is an important part of tumor development and progression. Microvessel density of PDAC is fairly low, but evidence of angiogenesis in PanIN lesions is apparent as well [12,13]. Inflammatory cells may play a role in this process. The presence of mast cells and macrophages in PDAC correlated with an increase in microvessel density [14]. T-cells may also be found in the tumor stroma. Interestingly, cytokines secreted (*e.g.*, TGF- β) by tumor cells, PSC, and regulatory T cells can lead to a reduction in the activity of helper T cells, particularly TH1 cells, thus lessening the functional T-cell surveillance toward the tumor [15,16]. Evidence of altered T-cell function has been shown where antigen-specific T-cells are found in the bone marrow of patients with PDAC but fewer of these cells are concurrently observed in circulation, perhaps suggesting the immunosuppressive cytokines generated by the tumoral and stromal compartments may play a functional role in this compartmentalization [15,16]. Furthermore, growth factors and cytokines produced by

inflammatory and immune cells may function as mitogenic stimuli for the stromal fibroblasts and PSC along with an increase in collagen expression [17,18]. It appears that the proliferating fibroblasts as well as the more specialized PSC, are mostly responsible for the production of extracellular matrix proteins seen in this response [17,19]. Activation of the PSC can occur following the release of growth factors and cytokines from inflammatory cells, the neighboring acinar cells, pancreatic adenocarcinoma cells, or even by the PSC themselves, thus demonstrating an activation resulting from both paracrine and autocrine signaling [17,19–23]. Proof that activated PSC are present in and are the source of the profound collagen accumulation contributing to the desmoplastic reaction in pancreatic carcinomas been demonstrated [19,22]. PSC can promote tumor growth with a greater number of metastases and an increased desmoplastic reaction demonstrated [24]. In a more recent study, it was found that PSC are able to traverse blood vessel endothelium, stimulate angiogenesis, and move with cancer cells to metastatic sites [25]. The changes in the stroma and development of the desmoplastic response can be seen throughout the progression of PanIN lesions to invasive carcinoma as well, with early PanIN lesions having fairly normal appearing stroma and late PanIN lesions having dense and proliferative stroma with or without inflammatory infiltrates [reviewed in [26,27]].

This well-characterized progression of pancreatic adenocarcinoma integrated with progressive changes in the surrounding stroma strongly suggests the interactive basis of a dual compartmental model of pancreatic cancer. Alterations in specific signaling pathways may drive the tumor compartment, the stromal compartment, or both. Understanding the complex interplays of these pathways within the tumor epithelium and stroma may help provide further insight in molecular targeting of this tumor.

Molecular basis of pancreatic cancer: pathway alterations at a compartmental level

As alluded to in the above histologic description of pancreatic ductal adenocarcinoma, a variety of alterations in signaling pathways are known to play pivotal roles in the pathogenesis of this disease. These alterations may not only affect the tumor epithelial cell compartment but also the tumor stromal compartment. Presented in this section is a review of the predominant signaling pathways deregulated in pancreatic cancer and associated with the tumor compartment and/or the stromal compartment (Fig. 1). Due to the quantity of research that has been done on this topic, this cannot be a comprehensive overview of all of the signaling pathways involved. As such, important works by research groups may be omitted. This article focuses on the following: *KRAS*, *CDKN2A*, *TGF- β* , *TP53*, *HEDGEHOG*, *NOTCH*, *WNT*, *EGFR family*, *NF- κ B*, and *MYC*.

KRAS

The *RAS* family of oncogenes includes *NRAS*, *HRAS*, and *KRAS*, with the latter being a key player in pancreatic adenocarcinoma development. Specifically, mutation of *KRAS* appears to be a very early occurrence in tumorigenesis that takes place within the tumor epithelium itself [8]. While this inciting incident is a key driver of PDAC pathogenesis of the epithelial compartment, other alterations that are permissible under *KRAS* mutation in the epithelial compartment may have secondary effects on the stromal compartment, as will be mentioned elsewhere (Fig. 1). The *KRAS* oncogene located on human chromosome 12p encodes for a membrane-bound protein with GTP-ase activity. The activity of this protein is tightly regulated, and normally it is found in the inactive GDP-bound form. Exogenous signals, such as growth factor stimulation, activate RAS GTP-ase to subsequently bind and further activate downstream effectors. RAS activity in PDAC affects multiple signaling pathways, including PI3K, RAF, and MEK signaling, each of which has a role in promoting

cell cycle progression, cytoskeletal alterations, and inhibition of apoptosis [28]. Early studies identified three major point mutations in codons 12, 13, and 61 that result in abnormal *KRAS* protein products and are associated with transformation, with mutations in codon 12 being the most prevalent of the mutations in PDAC[29,30]. Furthermore, with the idea of the progression model of pancreatic adenocarcinoma, additional insights have been made in which mutation of *KRAS* appears to play a fundamental role early in tumorigenesis. *KRAS* mutations can be seen in the earliest of lesions in which there is minimal atypia, and these mutations only increase in frequency as the lesions progress through the PanIN lesions to invasive carcinomas [31,32]. As with other diseases, attempts to generate accurate mouse models have been undertaken using a variety of methods and genetic alterations. However, it appears only using *Kras* mutants give a more relevant histological model [33–36]. One of the most clinically relevant mouse models mimicking the human progression model developed has been the pancreas targeted and endogenous expression of the *Kras*^{G12D} oncogenic allele described by Hingorani *et al.* In this model, the mutant form of *KRas* contains a modified exon 1 (*LSL-Kras*^{G12D}) that results in a glycine to aspartic acid transition in the protein product, one of the most common *KRAS* mutations seen in human PDAC[35]. This *LSL-Kras*^{G12D} allele remains nonfunctional until it is activated by Cre recombinase. This is achieved by crossing the *LSL-Kras*^{G12D/+} mice to *pdx1-Cre* or *p48-Cre* mice [35,36]. The *Pdx1-Cre;Kras*^{G12D/+} and *p48Cre;Kras*^{G12D/+} mice show PanIN formation with a concomitant increase in the number and grade of PanIN lesions with age as seen in humans [35]. Hence, further gains in knowledge of the role of oncogenic *KRAS* in pancreatic cancer may better identify new ways to target therapy or provide earlier diagnoses. As direct targeting of *KRAS* itself or the use of its mutations to diagnose this disease have been unsuccessful to date, its effects in other aspects of the biology of pancreatic adenocarcinoma development may lend to new strategies to overcome these problems.

CDKN2A

The *p16-Ink4A* locus is encoded by the *CDKN2A* tumor suppressor gene. Mutations of *CDKN2A* typically affect the tumor epithelial compartment of PDACs with no direct effect on the stromal compartment. With greater than 95% of PDACs demonstrating a loss of *CDKN2A* activity, it is the most frequently inactivated tumor suppressor gene in this cancer [37]. The p16 tumor suppressor protein acts to inhibit the binding of the D-family cyclins to their respective cyclin-dependent kinase (CDK) partners. Loss of functional p16 protein can result in increased phosphorylation of the Retinoblastoma (Rb) protein, and therefore lead to increased cell cycle progression through G1 phase into S phase. Loss of function of *CDKN2A* may be due to mutation, deletion, or by methylation of its promoter at the 5'-CpG island [37–39]. The loss of p16 also appears with progression of lesions. Identification of an increasing loss of p16 was detected from early PanIN lesions through invasive carcinomas [40]. These findings suggest that p16 inactivation occurs later in tumor progression and may be confounding to *KRAS* mutations. Generation of a mouse with homozygous loss of *p16* on the previously described *Kras*^{G12D} mouse model resulted in formation pancreatic adenocarcinoma lesions in less time than the *Kras*^{G12D} mouse model alone [41]. Taken together, these findings suggest an early and cooperative function of p16 loss in PDAC development.

Additionally, other members of the p16 tumor suppressor pathway are altered in human pancreatic adenocarcinomas. An example of these molecules is tumor suppressor gene *RB1* that encodes the RB protein. Alteration of the normal tumor suppressive activity of this gene is predominantly confined to the tumor component. This protein has an integral role in cell cycle regulation by binding to the E2F-family of transcription factors to prevent their nuclear translocation and activation of transcription of genes involved with cell cycle

progression from G1 to S phase. Upon the binding of D-family cyclins to their respective CDKs, the complex is then able to phosphorylate RB. The hyperphosphorylated RB status results in the release of E2F and subsequent transcription of cell cycle progression genes. This represents one arm of a complicated pathway of cell cycle regulation. Very early studies in a small number of tissue samples and cell lines of PDACs found alterations in expression of *RB1* [42]. However, on a larger evaluation of the *RB1* gene from primary pancreatic carcinomas, less than 10% had *RB1* mutations [43]. Thus, loss of functional *RB1* results in the potential deregulation of cell cycle control that can contribute to tumorigenesis, but it is unlikely a primary driver.

TGF- β

The transforming growth factor- β (*TGF- β*) signaling pathway is another important and commonly deregulated signaling pathway in pancreatic carcinomas. Alteration of this pathway has a prominent function in both the tumor cell and stromal cell compartments, and it is a hallmark example of having effects in a dual compartment manner (Fig. 1). Activation of this signaling pathway is initiated when a ligand binds to both a type I and type II serine/threonine kinase receptor. The binding to both receptors allows their association and the subsequent phosphorylation of receptor I by receptor II. This then leads to phosphorylation of the receptor-mediated SMADS (R-SMADs), SMAD2 and SMAD3, and association with the co-SMAD, SMAD4. The R-SMAD/SMAD4 complex then translocates to the nucleus [44]. Once in the nucleus, this complex can associate with cofactors to transcriptionally regulate their target genes, specifically genes mitigating cell cycle progression.

Within the tumor cell compartment, the *TGF- β* pathway is unique in that it has a tumor suppressor function in normal cells and early in tumor cells by inhibiting cell growth, but later into tumorigenesis, it mediates an oncogenic effect. This difference in signaling outcome is likely due to SMAD-dependent and SMAD-independent signaling. Although multiple gene mutations in the *TGF- β* pathway have been identified, the most common mutation is that of *SMAD4* [45]. The *SMAD4* gene, also known as *DPC4*, is located on chromosome 18q21 [46]. *SMAD4* inactivation is present in more than half of all pancreatic tumors, and this phenomenon appears to be specific for pancreatic adenocarcinoma [31,46–48]. *SMAD4* inactivation in these tumors is due to homozygous deletions or mutation in one allele with the loss of the second allele [31,47,48]. Additionally, the loss of *SMAD4* appears to be a late event in the progression of pancreatic adenocarcinoma, as normal *SMAD4* expression was seen in all early PanIN lesions evaluated but a loss of normal *SMAD4* expression occurred in one-third of later lesions [31]. A prognostic association with the loss of *SMAD4* has been shown, with one study demonstrating *SMAD4* gene mutations are associated with a poor prognosis [49]. Through a functional *TGF- β* signaling pathway and the presence of activating *KRAS* mutations, *TGF- β* ligand stimulation promotes tumor cells to undergo epithelial-to-mesenchymal transition (EMT) and thus develop an aggressive and invasive phenotype [50]. Evaluation of *Elastase-Kras^{G12D};Tgfr1^{+/-}* mice against *Elastase-Kras^{G12D}* revealed fewer precursor lesions in the *Tgfr1* haploinsufficient mice compared to wild-type, but those lesions that did form in the haploinsufficient mice were significantly greater in size [51]. However, the loss of *SMAD4* appears to lead to a more malignant phenotype, and it is here where the switch from SMAD-dependent to SMAD-independent signaling becomes important. Deletion of *Smad4* on the background of an activated *Kras* (*Kras^{G12D}*) mouse showed loss of *Smad4* resulted in the faster formation of PanIN-type lesions and greater fibrosis with some examples of invasive tumors compared to the activated *Kras* mouse alone [52]. However, no pathology was seen in the *Smad4* deletion mutants alone [52]. Further evidence for the role of *Smad4* activation was seen when a mouse model of *Smad4* haploinsufficiency on the previously described *Kras^{G12D}* background resulted in the development of invasive PDAC lesions from MCNs [53].

The activated TGF- β receptors can activate the non-SMAD pathways through MAPK, RhoA, and PI3K pathways [54]. Further evidence for the role of SMAD-independent signaling was shown whereby NFAT activation and NFAT release of SMAD3 repressing complexes from the TGF- β inhibitory element of the *C-MYC* promoter to induce *C-MYC* expression [55]. Despite all of these TGF- β mechanisms acting at the level of the tumor compartment, the effects of TGF- β signaling also extend to the stromal compartment in these tumors.

The PSC and fibroblasts comprising the stromal compartment appear to have functional TGF- β pathways that are sensitive to the secreted ligands produced by the tumor cells. This was demonstrated in experiments using a transgenic mouse model of Tgfb overexpression where it was observed there was an increase in PSC coupled with an increase in type I and type III collagen [56]. Interestingly, increased expression of TGF- β produced by local granulocytes over PDAC cells resulted in collagen synthesis by pancreatic stromal cells [18]. In a study by Bardeesy and colleagues, the impact of *SMAD4* loss alone or in combination with other mutations was further evaluated. A mouse model was produced to have deleted *Smad4* alone or an inactive *Smad4* on the activated *Kras*^{G12D} mutation or on a *Kras*^{G12D}*Ink4a/arf*^{+/-} background. The tumors that resulted in the mice with *Smad4* loss alone showed no overt propensity for tumor formation. However, those with *Smad4* loss on the *Kras*^{G12D} or the *Kras*^{G12D}*Ink4a/arf*^{+/-} background demonstrated and accelerated formation differentiated tumors with activated PSC and considerable deposition of ECM proteins [57]. Further support of the role of TGF- β signaling in the activation of PSC and their subsequent production of collagens has been studied in the pancreas during chronic injury and fibrosis under experimental conditions both *in vitro* and *in vivo* [58,59]. Thus, the paracrine activity of TGF- β , along with other ligands of the pathway, helps to activate and maintain productivity by the stromal compartment, particularly through the PSC.

TP53

Tumor suppressor 53 (*TP53*) is a well-known and characterized tumor suppressor gene that is located on chromosome 17 that encodes the protein p53. Alterations in this tumor suppressor occur in the tumor cells and therefore solely impact the tumor epithelial compartment. *TP53* is an integral component of cell regulation and DNA damage response mechanisms. DNA damage specifically causes activation of the p53 protein that results in a cascade of events leading to DNA damage repair mechanisms and stoppage of the cell cycle to allow this repair. If the damage can be repaired, the cell is released from cycle inhibition and is allowed to continue. If the damage cannot be repaired, then apoptosis is initiated in order to keep the cell from propagating under damaged conditions that may allow the formation of a tumorigenic state. In an early study evaluating *TP53* mutations from pancreatic adenocarcinoma tissues, *TP53* mutations were identified in more than half of samples evaluated with the mutations most commonly being missense point mutations [60–62]. Given these findings, *TP53* appears to have a role in tumorigenesis for this particular cancer, but with the prevalence of other mutations found in these tumors, whether *TP53* mutations are a driving abnormality or are a consequence to other cellular mutations remained unclear and studies utilizing mouse models were investigated. With the knowledge that mutant p53 proteins can cooperate with *KRAS* mutations using *in vivo* mouse models to cause tumorigenesis, it was suggested that p53 protein mutations generally work in concert with the activation of oncogenes to promote tumor formation [41,63,64]. One of the first mouse models of human PDAC involved evaluation of p53-null animals. It was found by Wagner and colleagues that mice overexpressing Tgf- α developed pancreatic ductal lesions through associated RAS overactivation, but induction of p53 in these cells lengthens progression time and the degree of progression [64]. When these mice were crossed with p53-null mice, the incidence of tumor formation and the time of onset of tumor development

as well as progression and subsequent survival was significantly shortened [64]. Indeed this has been demonstrated by through the development of another mouse model by Hingorani and colleagues, whereby a conditionally expressed endogenous mutant of *Trp53*, *Trp53^{R172H}*, was made in combination with the *Kras^{G12D}* mutant [63]. Activation of both of these mutant alleles by breeding with *Pdx-1-Cre* transgenic mice occurs in a tissue specific manner, with only the pancreatic tissues harboring these mutations. They showed this addition of the mutant *Trp53^{R172H}* to the *Kras^{G12D}* resulted in the development of invasive and metastatic pancreatic carcinomas from the early, pre-invasive lesions that were demonstrated with the *Kras^{G12D}* [63]. Mutations in *TP53* are predominately seen in some *in situ* lesions as well as invasive carcinomas, indicating this mutation may occur mid-to-late stage in the pathogenesis of this disease [65].

HEDGEHOG

Components of the HEDGEHOG (Hh) signaling pathway engage in essential roles in pancreatic cancer pathogenesis. This pathway serves as another example of having importance in both the epithelial and stromal compartments of PDAC (Fig. 1). Canonical signaling of this pathway is regulated by the 12-pass transmembrane receptor PATCHED (PTCH) that normally has a suppressive function over the 7-pass transmembrane receptor SMOOTHENED (SMO) in the absence of its ligand. Upon binding of the Hh ligands (such as Sonic Hh or SHH), PTCH releases its inhibitory effects on SMO and thus positively signals the downstream cascade to continue. Although there are numerous proteins involved in this signaling cascade, the final result of the loss of repressive activity of SMO causes the release and activation of the GLI family of transcription factors (GLI1, GLI2, or GLI3) with their subsequent nuclear localization. Ultimately, the Hh pathway is responsible for activating genes involved with cell cycle progression and apoptosis.

Multiple alterations in Hh signaling have been demonstrated in the pancreatic tumor cell compartment. In a global genomic analysis of pancreatic cancer, all tumors tested had alterations in at least one of the Hedgehog signaling genes [45]. The SHH ligand has been found to be highly expressed in the majority of patient PDAC evaluated [66,67]. At the same time, it has been found that the greater the atypia present in ductal epithelial cells, the greater the expression of SHH [66]. Upon evaluation of pancreatic tissue from *Pdx-Shh* mice that overexpress *Shh*, significant abnormalities that resembled human early PanIN lesions were discovered [66]. These lesions were also found to have activating *Kras* mutants and Her2 overexpression [66]. However, GLI can be regulated independently of PTCH and SMO activity and thereby represent ligand-independent functions. Other pathways, such as *TGF-β*, can regulate GLI-mediated transcription in pancreatic cancer cell lines [68]. Through treatment of cell lines with *TGF-β* ligand, researchers found an induction in the expression of GLI1 and GLI2 mRNA. Furthermore, they found that this induction of GLI expression secondary to *TGF-β* stimulation was occurring in a SMAD3-dependent and SMO-independent manner [69]. The idea of a SMO-independent Hh signal regulation was further evaluated by researchers using a *Smo* loss of function allele in mice of a *p48-Cre;Kras^{G12D/+};Trp^{F/+}* background in which there was pancreatic-specific loss of *Smo* but overexpression of *Shh*. These researchers found that there was no difference in the ability to form tumors compared to animals without a loss of *Smo* [68]. However, there was still expression of Gli target genes, suggesting that this may be Hh pathway independent [68]. They further found that the Gli transcription was driven by *TGF-β* and *Kras* [68].

A fascinating aspect of Hh signaling is its ability to directly affect the surrounding stroma in PDAC. Hh signaling is activated in stromal cells associated with PDAC cells that are overexpressing Hh ligands, indicating a paracrine function [70,71]. Evaluation of tumors from an orthotopic model from PDAC cells overexpressing SHH showed increased desmoplasia compared to those treated with a SHH-specific antibody [72]. Furthermore,

cancer-associated fibroblasts appear to overexpress SMO and subsequently have a hyperactivated Hh pathway resulting in GLI1 overexpression [73]. Using the previously described *Pdx-1-Cre;Trp^{R172H/+};Kras^{G12D/+}* mouse model, researchers found a decrease in the amount of reactive stroma and collagen type I deposition when the mice were treated with a Hh inhibitor [74]. Furthermore, it was found that when this inhibitor was administered in combination with gemcitabine, there was a temporary control over tumor growth, an increase in overall survival time, and a decrease in the number of metastatic lesions when compared to those animals that were administered only the vehicle or gemcitabine alone [74]. Taken together, these findings suggest the targeting of some of these Hh pathway components would be effective therapeutic options by affecting both the tumor and the stromal compartments.

NOTCH

Similar to the Hh pathway, NOTCH is primarily active during embryogenesis, but is commonly seen to be reactivated in many types of cancers, including pancreatic cancer. In the case of pancreatic adenocarcinoma, the effects of NOTCH signaling primarily involve the tumor compartment with little known impact on the stromal compartment. NOTCH functions in development by preventing terminal differentiation of cells until appropriate [75]. Likewise, it functions at low levels to maintain a population of undifferentiated cells to serve as progenitor cells in normal tissues. The binding of transmembrane ligands from neighboring cells to NOTCH receptors (NOTCH-1 and NOTCH-2) results in the cleavage of the intracellular domain of the receptor by γ -SECRETASE. This cleaved portion of the receptor can then freely move through the cytoplasm and translocated to the nucleus, where it can interact with other transcription factors to promote transcription of its target genes. Again referring to the established mode of pancreatic adenocarcinoma progression, it is reasonable to believe re-activation of NOTCH is one of the factors involved with tumorigenesis. In fact, researchers have shown increased expression of NOTCH pathway genes, including NOTCH receptors and ligands in early PanIN lesions over the normal pancreas [3]. Up-regulation of NOTCH receptors and ligands is seen in the presence of damaged exocrine cells, integrating the role for NOTCH in chronic inflammation and in cancer [76]. Additionally, it has been found that overexpression of NOTCH ligands constitutes a common cause of NOTCH activation in pancreatic cancer, and NOTCH activation is required for not only tumor initiation but also maintenance [77].

The NOTCH pathway can also interact with other pathways, such as EGFR and nuclear factor-kappa B (NF- κ B). *In vitro* EGFR signaling activates NOTCH[3]. Down-regulation of NOTCH-1 has been shown to decrease NF- κ B DNA binding and MMP-9 expression, a target gene of NF- κ B[78]. The same group also demonstrated that down-regulation of NOTCH-1 resulted in inhibition of invasion of pancreatic cancer cells *in vitro*, suggesting that overexpression of NOTCH-1 is involved in the evolution of an invasive phenotype [78]. Further investigation into the role of Notch in pancreatic cancer found differences in function between NOTCH-1 and NOTCH-2. NOTCH-1 has been found to be present in normal acinar cells but rarely in PanIN lesions, while NOTCH-2 is found in ductal cells [79]. Moreover, it was found that loss of NOTCH-2 in a *Kras^{G12D}* driven mouse model of PDAC tumorigenesis resulted in inhibition of PanIN progression and is a factor in regulating *MYC* activity in tumorigenesis [79]. With these findings that NOTCH may be involved with the progression of pancreatic adenocarcinoma, Notch appears to be an attractive therapeutic target. The NOTCH pathway has been inhibited through the use of a γ -SECRETASE inhibitor (GSI)[80]. Using the *Pdx1-Cre;Kras^{G12D};p53^{lox/+}* mouse model, the NOTCH pathway was found to be upregulated in the resulting mouse lesions, and treatment of these animals with a GSI inhibited the progression of the early, PanIN equivalent lesions to invasive carcinoma [80]. These findings culminate to suggest that overexpression of

NOTCH pathway components functions to promote pancreatic tumorigenesis in when combined with deregulation of other critical pathways.

WNT

The WNT signaling pathway is yet another pathway that has a major role in cell growth and differentiation during development, and similar to the NOTCH pathway, alterations in WNT signaling primarily affect the PDAC tumor cell compartment. The pathway is activated when ligands bind to the cell membrane WNT receptor, resulting in the release of β -Catenin into the cytoplasm. Normally, β -Catenin is kept inactivated by its phosphorylation by GSK3- β kinase and binding to axin and APC. This phosphorylation and complexing triggers β -Catenin ubiquitination and its subsequent degradation by the proteasome. Once free, β -Catenin can translocate into the nucleus where it associates with the transcription factors TCF-LEF to activate its target genes. Increased β -Catenin levels and function has been found in a majority of pancreatic carcinomas but not in the normal pancreas [81,82]. Loss of β -Catenin expression was found to correlate to loss of differentiation in pancreatic carcinoma tumors examined, while nuclear expression was seen in cases of pancreatic carcinoma *in situ*, suggesting differences associated with its function in the progression of disease [83]. A decrease in carcinoma proliferation via accelerated apoptosis has been demonstrated with siRNA knockdown of β -Catenin [82]. Morris and colleagues further investigated the role of β -catenin in the context of mutant *Kras* in a mouse model. They evaluated the effect of mutant *Kras* following caerulein-induced injury in transgenic *Pdx1-Cre;Kras^{G12D}* mice, and found this mutation results in acinar-to-ductal transformation and the development of PanIN lesions [84]. In addition, they evaluated alterations with caerulein-induced injury in the context of continuous Wnt signaling through a *p48Cre; β -catenin^{exon3/+};Kras^{G12D}* transgenic mouse. Without caerulein treatment, the development of PanIN lesions were not seen as with the *Kras* only mutant mice [84]. With pancreatic injury, these mice did not show acinar-to-ductal transformation as the mice harboring mutant *Kras* did but that PanIN formation was possible with lower levels of β -catenin present [84]. These results support the idea that deregulation of WNT signaling occurs later in lesion development as upregulation of WNT signaling through β -catenin is somewhat inhibitory early in activated *Kras*-driven tumorigenesis. Other extracellular regulatory elements of the WNT pathway have been implicated in pancreatic tumorigenesis. For example, extracellular proteins of the heparin sulfate proteoglycan family, as well as the related sulfatase Hsulf-1, that are positive regulators of WNT activity have been shown to be overexpressed in tumors as opposed to normal pancreatic tissue [85–88]. These findings support the idea of WNT pathway contribution to pancreatic adenocarcinoma formation by actions not only on the epithelial tumor compartment, but also on the stromal compartment through its contributions to extracellular matrix protein formation.

Epidermal Growth Factor Receptor

The epidermal growth factor receptor (EGFR) family encompasses a group of cytoplasmic receptor tyrosine kinases that includes the human EGF receptor or Her1 (EGFR or ErbB-1), Her2 (EGFR2 or ErbB-2), Her3 (EGFR3 or ErbB-3) and Her4 (EGFR4 or ErbB-4). While aberrant EGFR signaling often implies a specificity for the epithelial tumor compartment, as described later in this section, it can also have effects in the stromal compartment. Generally, binding of a ligand results in receptor dimerization and activation to allow initiation of downstream signaling pathways. The extracellular N-terminal regions of the receptors are available for specific ligand binding while the intracytoplasmic portion of the receptor contains the region of kinase function. Many ligands have been found that bind with these receptors and include epidermal growth factor (EGF) and transforming growth factor- α (TGF- α) among others. Binding of the ligand to receptor induces dimerization that results in activation of the catalytic domain and autophosphorylation of tyrosine residues.

Downstream signaling proteins that recognize these phosphorylated tyrosines dock to the receptor, thereby initiating the particular signaling cascade. EGFR is able to induce downstream signaling through multiple pathways, such as Ras, phosphoinositol-3 kinase, and phospholipase C pathways. Most of these pathways have overlapping players that function to stimulate transcription of target genes that stimulate cell growth and proliferation as well as cell survival. This implies signaling diversity by the cell through EGFR. Deregulation of this system can contribute to tumorigenesis in pancreatic cancer through overexpression of both the receptors and the ligands.

Overexpression of ErbB-2 has been shown to be present in certain pancreatic carcinoma cell lines secondary to ErbB-2 gene amplification [89]. Evaluation of some subsets of invasive human PDAC tissues also has shown overexpression of ErbB-2 [89,90]. To lend further support to the role of ErbB-2 overexpression in pancreatic cancer pathogenesis, the level of ErbB-2 in pancreatic ductal lesions has been evaluated. Little expression in normal pancreatic ducts was observed while significantly increasing overexpression was observed in PanIN lesions and carcinoma *in situ* lesions [91]. However, these increases are also often seen with concurrent increases in the ligands. EGFR and two of its more commonly recognized ligands, EGF and TGF- α , were found to be overexpressed in pancreatic carcinoma tissues [92]. In studies evaluating the effect of transgenic mice overexpressing Tgfa solely in the pancreas through an *Ela-Tgfa* transgenic mouse, pancreatic adenocarcinoma lesions were found along with a significant increase in fibrotic proliferation [93]. Questions regarding the role of TGF- α remained, particularly in the differences in progression of lesions compared to the human pathology. Another model in which a Tgfa transgenic was crossed with the *p48^{Cre/+};Kras^{G12D/+}* mouse was developed [94]. In this model, it was found that invasive PDAC lesions developed faster than either the *Kras^{G12D/+}* and *Ela-Tgfa* single transgenic mice [94]. Additionally, while these mice had the expected progression of lesions from PanIn to invasive PDAC, they developed cystic papillary lesions not before seen in the Kras model, therefore discovering a new and important understanding of the role of Tgfa in PDAC pathology [94]. Notch signaling has been found to be activated downstream of EGFR signaling through TGF- α , further demonstrating the intimate role EGF signaling has with other pathways [3]. Association studies have found significance between these overexpressed receptors and ligands and overall outcome in patients with PDAC. In approximately one-half of PDAC tissues examined, ErbB-3 mRNA levels were found to be increased over levels found in normal tissues, and this was associated with a more advanced tumor stage and shorter survival time [95]. This indicates that adjuvant targeting of the EGFR receptor may be a beneficial therapeutic target as both the tumor and the stromal compartments can be targeted.

Aberrations in this pathway primarily affect the tumor epithelial compartment, but the production of EGFR family ligands may affect the cells of the stromal compartment. Interestingly, these ligands are produced not only by the neoplastic epithelial cells themselves, but also by the desmoplastic cells in the surrounding tumor environment [92]. In a mouse expressing an EGFR hypomorphic allele with overexpression of heparin-binding epidermal growth factor-like growth factor (HB-EGF), a decrease in pancreatic fibrosis was observed in animals with reduced EGFR activity compared to those without the hypomorphic allele [96]. The summation of all of these findings suggests that both autocrine and paracrine EGFR signaling may be important components to PDAC pathogenesis.

NF- κ B

The nuclear factor- κ B (NF- κ B) proteins constitute a family of transcription factors most commonly associated with mediating inflammatory responses. However, these transcription factors also control other genes involved with development, apoptosis, and cell proliferation. Specifically, the activity of these transcription factors in both the tumor and stromal

compartments of PDAC progression is becoming increasingly more apparent and highlights their important role in facilitating tumor-stromal crosstalk (Fig. 1). These factors include p65 (RelA), RelB, c-Rel, p105/p50, and p100/p52. They are present in the cytoplasm of most cells in a homo- or heterodimeric form. Normally, NF- κ B resides in the cytoplasm in an inactive form through its association with I κ B- α , an inhibitor of NF- κ B that covers its nuclear localization sequence. When an appropriate stimulatory signal is received by the cell, I κ B- α is phosphorylated and labeled for degradation. This enables NF- κ B movement into the nucleus to regulate transcription of its target genes [reviewed in [97]]. Given these roles for NF- κ B, one may deduce that activation of this complex of proteins may be playing important roles in both the tumor and stromal compartments. Support for such conclusions come from the findings that chronic pancreatitis greatly increases the risk to the development of PDAC[98]. One study found the incidence of PDAC is significantly increased in people affected by chronic pancreatitis compared to those unaffected[99]. In a mouse model of pancreatitis, rapid elevations of NF- κ B activation was seen following cerulein administration[100]. These findings suggest changes to the stromal environment in terms of inflammatory stimuli play an important role in PDAC development. For example, infiltration of immune cells in the tumor microenvironment may be associated with the production of cytokines that promote NF- κ B activity within the neighboring tumor cells. TNF- α is one such example of a cytokine produced by immune cells in the stroma that stimulates NF- κ B activity in tumor cells [reviewed in[97]]. The influence of inflammatory cells present in the stroma demonstrates again how the stroma may mediate influential signals on the tumor cells. Clear evidence has been demonstrated for the role of NF- κ B in pancreatic tumorigenesis.

The NF- κ B subunit RelA has been shown to be constitutively activated in PDAC cells but not in normal pancreatic tissues[101]. Other NF- κ B subunits, including p105, p100, and c-Rel have been found to have increased expression in certain PDAC cell lines[102]. By inhibiting NF- κ B expression, researchers were able to evaluate in an orthotopic mouse model the effects on tumorigenicity[103,104]. Those cells not expressing a phosphorylation active NF- κ B were found to induce VEGF expression and thereby angiogenesis[103]. However, those cells expressing the mutant NF- κ B showed decreased tumorigenicity through reduced expression of Bcl- χ_L and Bcl-2 as well as VEGF[104]. In cancer cells, NF- κ B activity may be influenced by other signaling, such as through glycogen synthase kinase-3 β (GSK-3 β). Evaluation of multiple pancreatic cancer cell lines showed active GSK-3 β with concomitant activation of NF- κ B[105]. Upon inhibition of GSK-3 β in these cell lines, a decrease in the NF- κ B activity was observed with subsequent decrease in cell proliferation and survival as a result of down regulation of its target genes[105]. This example suggests the potential for NF- κ B to be targeted through its regulators in a clinical setting. Taken together, these results suggest the role of NF- κ B in early tumorigenesis of pancreatic adenocarcinoma pathogenesis as well as potentially serving a role in both the tumor and the stromal compartments.

C-MYC

C-MYC is an important regulator of many facets of cell growth. The *C-MYC* oncogene encodes for a DNA binding protein that functions as a downstream transcriptional regulator of many distinct pathways, ultimately facilitating cell growth and even cell transformation[reviewed in[106]]. MYC is present in proliferating cells with its binding partner, MAX, as a dimer. MYC can both activate and repress transcription of its target genes depending upon other cellular influences[reviewed in[107]]. C-MYC induces transcription of genes involved in cell cycle progression, inhibition of checkpoint responses, and cellular metabolism[reviewed in[107]]. Downstream activation of transcription of *C-MYC* itself can be accomplished through different signaling pathways, such as WNT or

TGF- β [reviewed in [107]]. In general, MYC deregulation alone does not result in transformation, but its cooperation with other oncogenic pathways, such as RAS, is sufficient to promote tumorigenesis[108]. As such, aberrations in *C-MYC* oncogene expression are primarily confined to the tumor cell compartment. Overexpression of c-myc in pancreatic acinar cells using *Ela-1-myc* transgenic mice resulted in the development of acinar cell lesions early in the tumorigenic process and the addition of ductal cell lesions in the more progressed lesions[109]. Another study by the same group developed a transgenic mouse with TGF- α overexpression alone or with the *Ela-1-myc* transgenic mouse they previously utilized. They found that TGF- α overexpression alone was not as oncogenic as the overexpression of myc alone[110]. However, the combination of the two resulted in more rapid development of early lesions and advanced tumors with a more malignant phenotype in the pancreas[110]. Another study evaluating the pancreatic tumors of both ductal and acinar origin from these *Ela-1-myc* transgenic mice found no evidence of *Kras* mutations in any of the samples [111]. This suggests that this mouse model of *c-myc* overexpression may not parallel human PDAC pathogenesis as well as others. Although *C-MYC* mutations can be seen in human tissues, these alterations are not as common as some of the other mutations described, and few large-scale studies have evaluated the presence of *C-MYC* gene alterations. A more recent study evaluated a larger number of human PDAC samples along with a few PanIN lesions found amplification of *C-MYC* in 30% of the tumors, with most of these having very low-number amplification[112]. Most interesting from this study was the finding that less than one-third of the tumor samples overexpressing the C-MYC protein had concurrent gene amplification[112]. Rather, other modes of C-MYC protein overexpression are likely at work. One of these mechanisms may be through altered transcription. NFAT has been implicated in *C-MYC*-dependent growth of pancreatic cancer. The NFAT family has been found to be overexpressed in pancreatic carcinomas and promotes tumorigenic activity through activation of *C-MYC* gene expression[113]. Increased NFAT nuclear translocation and binding to the *C-MYC* promoter results in increased p300-dependent histone acetylase activity and binding of other factors to promote fully active *C-MYC* transcription[114]. Thus, these works suggest the influential role of alternative gene regulation in cancer. This leads to the possibility of new targeted therapies related to various modes of gene regulation yet to be elucidated in pancreatic cancer.

Translational opportunities targeting pancreatic cancer compartments

Understanding of the biological basis for PDAC development, progression, and metastasis has the potential to guide logical therapeutic strategies. Indeed, knowledge of the role of the pathways mentioned above in this phenomenon has led to the identification of potential therapeutic targets that are currently under study in the clinical setting. The above discussion of the signaling cascades involved in pancreatic carcinogenesis highlights the importance of targeting both the tumor and stromal compartments. Unfortunately, not all of these targets affect both compartments, nor can they all be discussed here. We will discuss a few examples illustrating potential pathways of interest for targeting in PDAC.

Targeting of the common activating mutation of *KRAS* has not yet been successful in pancreatic cancer therapy. Antisense suppression of KRAS appears to impede growth of mutated pancreatic tumor *in vitro*[115]. However, a phase III clinical trial of tipifarnib, a farnesyl transferase inhibitor that blocks KRAS activity[116], demonstrated no survival benefit over gemcitabine alone[117]. The high frequency of activating KRAS mutations and the apparent alteration in the biology after inhibition of this activity[118] provide the basis for continued searching for ways to effectively target RAS and its related pathways in PDAC. Additionally, many of the pathways previously described above require KRAS mutations in order to fully show effect. For example, erlotinib, a small molecule EGFR inhibitor, has been FDA approved for treatment of advanced pancreatic cancer after

demonstration of a modest survival benefit in combination with gemcitabine over gemcitabine alone[119]. In a subset of 117 patients in the trial from whom surgical tissue was available for KRAS analysis, there were suggestions of a benefit of erlotinib in KRAS wildtype patients (HR 0.66 vs. 1.07 in KRAS mutant patients), but due to low numbers, statistical significance was not reached. This would be in alignment with the now well-established absence of benefit with EGFR inhibition in KRAS mutated colorectal cancer[120,121]. Development of RAF and MEK inhibitors, downstream kinases activated by KRAS, may provide some targeting of constitutive activation of this pathway. At least one MEK1/2 inhibitor (AS703026) is being studied in a phase II clinical trial in PDAC, in combination with gemcitabine (NCT01016483 www.cancer.gov/clinicaltrials). Sorafenib, a multitargeted kinase with inhibitory properties against the RAF/MEK/ERK pathway[122], in addition to its well known VEGF pathway targeting, is being studied in a phase III clinical trial in combination with gemcitabine (Goncalves, A *et al*; ASCO Meeting Abstracts June 2011). Although KRAS activation affects the PDAC tumoral cell compartment, it can affect the activities of other pathways that do contribute to alterations in the stromal compartment. Clinical evaluations of these other pathways are ongoing.

As has been emphasized throughout this review, the importance stromal compartment in the resistance of PDAC to therapy is becoming increasingly clear. Pathologists have long well known the intense desmoplastic reaction associated with primary PDAC, and likewise the radiologist well knows the associated decreased contrast signal in these hypovascular tumors. Because of the changes associated with the stromal compartment, whether chemotherapy reaches tumor cells in PDAC is of debate. Therefore, targeting the stromal compartment so that more active chemotherapy can reach the tumor cells remains a logical approach. One candidate pathway chosen to target this compartment is the Hh cascade. In addition, the Hh pathway appears to be aberrantly activated by KRAS signaling[123], so targeting this pathway provides further opportunity for impeding the effects of mutant KRAS activity. As such, there are several clinical trials investigating the use of Hh pathway inhibitors in PDAC (Table 1). This logic allows a strategy to target both the tumor and stromal compartments. At least six clinical trials targeting this cascade are currently underway.

As noted above, there are numerous potential targets for therapeutic development in pancreatic cancer. Because of this and the differences in tumorigenesis provided by the tumor or stromal compartments, the complex interactions of pathways may require future rational combinations of agents. Some of the most prominent pathways include the TGF- β pathway[124,125] and the Notch[126] signaling pathway. The TGF- β pathway can be targeted through agents inhibiting SRC-kinase, a key regulator enzyme of the pathway[127]. The proliferative effects of Notch signaling have been targeted most often at the level of γ -SECRETASE, but this pathway's complexity may lend itself to multiple potential targets or combinations (Conroy, T; ASCO Meeting Abstracts; May 2010). Both Notch and SRC-kinase inhibitors are being used in clinical trials targeting pancreatic cancer, but these have shown so far little if any success.

Other investigators have suggested to block the action of pathways (e.g. TGF- β and HEDGEHOG) involved in the regulation of the stromal compartment through the targeting proteins intimately involved in the function these pathways of cellular compartment. One of such molecules is secreted protein acidic and rich in cysteine (SPARC). SPARC is a protein that mediates cell-matrix interactions, binds specific collagens in the ECM, and thereby alters cell adhesion properties [reviewed in [128]] SPARC is upregulated in both chronic pancreatitis as well as in the peritumoral stroma of PDAC[129–131]. Clinical trials focused on inhibition of SPARC through the use of albumin-bound paclitaxel in PDAC are ongoing (NCT00844649 www.cancer.gov/clinicaltrials).

In summary, the complexity of PDAC biology requires a rational targeting of novel therapeutic combinations may be necessary to have a clinically meaningful effect. Thus a more complex trial design, with predetermined biologic endpoints rather than the traditional clinical outcome measures of overall and progression-free survival alone will be needed for the treatment of this dismal disease.

Conclusions

Given the number of signaling pathway aberrations contributing to PDAC tumorigenesis, it becomes evident that the complicated pathogenesis of this disease appears to correlate with the less than successful treatments that have been evaluated to date. Likewise, the active and functional cross-talk between PDAC epithelial cells and the surrounding tumor stromal cells demonstrates the importance of gaining further understanding into the role of the microenvironment and its contribution to tumor maintenance and progression. It has become increasingly apparent that focusing research and treatment efforts on both the tumor compartment as well as the stromal compartment will likely provide the greatest breakthrough in our understanding as well as patient outcomes. This is reflected in the number and scope of clinical trials currently underway as highlighted in this article. Furthermore, combining some of these therapies may prove to be more successful in addressing the positive feedback interactions between the tumor and stromal compartments than the use of conventional chemotherapy alone. As this research continues to move forward, likely so will the ability to treat this dismal disease.

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References

1. Gomori G. The Histology of the Normal and Diseased Pancreas. Bull N Y Acad Med. 1945; 21(2): 99–111. [PubMed: 19312426]
2. Wharton GK. The Clinical Aspects of the Histology and Pathology of the Pancreas. Can Med Assoc J. 1934; 30(2):148–152. [PubMed: 20319391]
3. Miyamoto Y, Maitra A, Ghosh B, et al. Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. Cancer Cell. 2003; 3(6):565–576. [PubMed: 12842085]
4. Hruban RH, Adsay NV, Albores-Saavedra J, et al. Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. Am J Surg Pathol. 2001; 25(5): 579–586. [PubMed: 11342768]
5. Zhu L, Shi G, Schmidt CM, Hruban RH, Konieczny SF. Acinar cells contribute to the molecular heterogeneity of pancreatic intraepithelial neoplasia. Am J Pathol. 2007; 171(1):263–273. [PubMed: 17591971]
6. Hruban RH, Maitra A, Kern SE, Goggins M. Precursors to pancreatic cancer. Gastroenterol Clin North Am. 2007; 36(4):831–849. vi. [PubMed: 17996793]
7. Maitra A, Fukushima N, Takaori K, Hruban RH. Precursors to invasive pancreatic cancer. Adv Anat Pathol. 2005; 12(2):81–91. [PubMed: 15731576]
8. Hruban RH, Goggins M, Parsons J, Kern SE. Progression model for pancreatic cancer. Clin Cancer Res. 2000; 6(8):2969–2972. [PubMed: 10955772]
9. Hansel DE, Kern SE, Hruban RH. Molecular pathogenesis of pancreatic cancer. Annu Rev Genomics Hum Genet. 2003; 4:237–256. [PubMed: 14527303]
10. Omary MB, Lugea A, Lowe AW, Pandol SJ. The pancreatic stellate cell: a star on the rise in pancreatic diseases. J Clin Invest. 2007; 117(1):50–59. [PubMed: 17200706]

11. Pandol S, Edderkaoui M, Gukovsky I, Lugea A, Gukovskaya A. Desmoplasia of pancreatic ductal adenocarcinoma. *Clin Gastroenterol Hepatol*. 2009; 7(11 Suppl):S44–47. [PubMed: 19896098]
12. Esposito I, Kayed H, Keleg S, et al. Tumor-suppressor function of SPARC-like protein 1/Hevin in pancreatic cancer. *Neoplasia*. 2007; 9(1):8–17. [PubMed: 17325739]
13. Sharma S, Green KB. The pancreatic duct and its arteriovenous relationship: an underutilized aid in the diagnosis and distinction of pancreatic adenocarcinoma from pancreatic intraepithelial neoplasia. A study of 126 pancreatectomy specimens. *Am J Surg Pathol*. 2004; 28(5):613–620. [PubMed: 15105649]
14. Esposito I, Menicagli M, Funel N, et al. Inflammatory cells contribute to the generation of an angiogenic phenotype in pancreatic ductal adenocarcinoma. *J Clin Pathol*. 2004; 57(6):630–636. [PubMed: 15166270]
15. Schmitz-Winnenthal FH, Volk C, Z'Graggen K, et al. High frequencies of functional tumor-reactive T cells in bone marrow and blood of pancreatic cancer patients. *Cancer Res*. 2005; 65(21):10079–10087. [PubMed: 16267034]
16. von Bernstorff W, Voss M, Freichel S, et al. Systemic and local immunosuppression in pancreatic cancer patients. *Clin Cancer Res*. 2001; 7(3 Suppl):925s–932s. [PubMed: 11300493]
17. Bachem MG, Schunemann M, Ramadani M, et al. Pancreatic carcinoma cells induce fibrosis by stimulating proliferation and matrix synthesis of stellate cells. *Gastroenterology*. 2005; 128(4):907–921. [PubMed: 15825074]
18. Aoyagi Y, Oda T, Kinoshita T, et al. Overexpression of TGF-beta by infiltrated granulocytes correlates with the expression of collagen mRNA in pancreatic cancer. *Br J Cancer*. 2004; 91(7):1316–1326. [PubMed: 15365564]
19. Apte MV, Park S, Phillips PA, et al. Desmoplastic reaction in pancreatic cancer: role of pancreatic stellate cells. *Pancreas*. 2004; 29(3):179–187. [PubMed: 15367883]
20. Masamune A, Watanabe T, Kikuta K, Shimosegawa T. Roles of pancreatic stellate cells in pancreatic inflammation and fibrosis. *Clin Gastroenterol Hepatol*. 2009; 7(11 Suppl):S48–54. [PubMed: 19896099]
21. Svegliati-Baroni G, De Minicis S, Marziani M. Hepatic fibrogenesis in response to chronic liver injury: novel insights on the role of cell-to-cell interaction and transition. *Liver Int*. 2008; 28(8):1052–1064. [PubMed: 18783548]
22. Apte MV, Wilson JS. Mechanisms of pancreatic fibrosis. *Dig Dis*. 2004; 22(3):273–279. [PubMed: 15753610]
23. Vonlaufen A, Phillips PA, Yang L, et al. Isolation of quiescent human pancreatic stellate cells: a promising in vitro tool for studies of human pancreatic stellate cell biology. *Pancreatol*. 2010; 10(4):434–443. [PubMed: 20733342]
24. Vonlaufen A, Joshi S, Qu C, et al. Pancreatic stellate cells: partners in crime with pancreatic cancer cells. *Cancer Res*. 2008; 68(7):2085–2093. [PubMed: 18381413]
25. Xu Z, Vonlaufen A, Phillips PA, et al. Role of pancreatic stellate cells in pancreatic cancer metastasis. *Am J Pathol*. 2010; 177(5):2585–2596. [PubMed: 20934972]
26. Kore M. Pancreatic cancer-associated stroma production. *Am J Surg*. 2007; 194(4 Suppl):S84–86. [PubMed: 17903452]
27. Chu GC, Kimmelman AC, Hezel AF, DePinho RA. Stromal biology of pancreatic cancer. *J Cell Biochem*. 2007; 101(4):887–907. [PubMed: 17266048]
28. Hruban RH, Maitra A, Schulick R, et al. Emerging molecular biology of pancreatic cancer. *Gastrointest Cancer Res*. 2008; 2(4 Suppl):S10–15. [PubMed: 19343140]
29. Hruban RH, van Mansfeld AD, Offerhaus GJ, et al. K-ras oncogene activation in adenocarcinoma of the human pancreas. A study of 82 carcinomas using a combination of mutant-enriched polymerase chain reaction analysis and allele-specific oligonucleotide hybridization. *Am J Pathol*. 1993; 143(2):545–554. [PubMed: 8342602]
30. Lemoine NR, Jain S, Hughes CM, et al. Ki-ras oncogene activation in preinvasive pancreatic cancer. *Gastroenterology*. 1992; 102(1):230–236. [PubMed: 1309358]
31. Wilentz RE, Iacobuzio-Donahue CA, Argani P, et al. Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. *Cancer Res*. 2000; 60(7):2002–2006. [PubMed: 10766191]

32. Lohr M, Kloppel G, Maisonneuve P, Lowenfels AB, Luttges J. Frequency of K-ras mutations in pancreatic intraductal neoplasias associated with pancreatic ductal adenocarcinoma and chronic pancreatitis: a meta-analysis. *Neoplasia*. 2005; 7(1):17–23. [PubMed: 15720814]
33. Leach SD. Mouse models of pancreatic cancer: the fur is finally flying! *Cancer Cell*. 2004; 5(1):7–11. [PubMed: 14749121]
34. Pasca di Magliano M, Sekine S, Ermilov A, Ferris J, Dlugosz AA, Hebrok M. Hedgehog/Ras interactions regulate early stages of pancreatic cancer. *Genes Dev*. 2006; 20(22):3161–3173. [PubMed: 17114586]
35. Hingorani SR, Petricoin EF, Maitra A, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell*. 2003; 4(6):437–450. [PubMed: 14706336]
36. Aguirre AJ, Bardeesy N, Sinha M, et al. Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. *Genes Dev*. 2003; 17(24):3112–3126. [PubMed: 14681207]
37. Schutte M, Hruban RH, Geradts J, et al. Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. *Cancer Res*. 1997; 57(15):3126–3130. [PubMed: 9242437]
38. Caldas C, Hahn SA, da Costa LT, et al. Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. *Nat Genet*. 1994; 8(1):27–32. [PubMed: 7726912]
39. Rozenblum E, Schutte M, Goggins M, et al. Tumor-suppressive pathways in pancreatic carcinoma. *Cancer Res*. 1997; 57(9):1731–1734. [PubMed: 9135016]
40. Wilentz RE, Geradts J, Maynard R, et al. Inactivation of the p16 (INK4A) tumor-suppressor gene in pancreatic duct lesions: loss of intranuclear expression. *Cancer Res*. 1998; 58(20):4740–4744. [PubMed: 9788631]
41. Bardeesy N, Aguirre AJ, Chu GC, et al. Both p16(Ink4a) and the p19(Arf)-p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse. *Proc Natl Acad Sci U S A*. 2006; 103(15):5947–5952. [PubMed: 16585505]
42. Ruggeri B, Zhang SY, Caamano J, DiRado M, Flynn SD, Klein-Szanto AJ. Human pancreatic carcinomas and cell lines reveal frequent and multiple alterations in the p53 and Rb-1 tumor-suppressor genes. *Oncogene*. 1992; 7(8):1503–1511. [PubMed: 1630814]
43. Huang L, Lang D, Geradts J, et al. Molecular and immunochemical analyses of RB1 and cyclin D1 in human ductal pancreatic carcinomas and cell lines. *Mol Carcinog*. 1996; 15(2):85–95. [PubMed: 8599583]
44. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell*. 2003; 113(6):685–700. [PubMed: 12809600]
45. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science*. 2008; 321(5897):1801–1806. [PubMed: 18772397]
46. Hahn SA, Schutte M, Hoque AT, et al. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science*. 1996; 271(5247):350–353. [PubMed: 8553070]
47. Hahn SA, Hoque AT, Moskaluk CA, et al. Homozygous deletion map at 18q21.1 in pancreatic cancer. *Cancer Res*. 1996; 56(3):490–494. [PubMed: 8564959]
48. Schutte M, Hruban RH, Hedrick L, et al. DPC4 gene in various tumor types. *Cancer Res*. 1996; 56(11):2527–2530. [PubMed: 8653691]
49. Blackford A, Serrano OK, Wolfgang CL, et al. SMAD4 gene mutations are associated with poor prognosis in pancreatic cancer. *Clin Cancer Res*. 2009; 15(14):4674–4679. [PubMed: 19584151]
50. Ellenrieder V, Hendler SF, Boeck W, et al. Transforming growth factor beta1 treatment leads to an epithelial-mesenchymal transdifferentiation of pancreatic cancer cells requiring extracellular signal-regulated kinase 2 activation. *Cancer Res*. 2001; 61(10):4222–4228. [PubMed: 11358848]
51. Adrian K, Strouch MJ, Zeng Q, et al. Tgfbr1 haploinsufficiency inhibits the development of murine mutant Kras-induced pancreatic precancer. *Cancer Res*. 2009; 69(24):9169–9174. [PubMed: 19951995]
52. Kojima K, Vickers SM, Adsay NV, et al. Inactivation of Smad4 accelerates Kras(G12D)-mediated pancreatic neoplasia. *Cancer Res*. 2007; 67(17):8121–8130. [PubMed: 17804724]

53. Izeradjene K, Combs C, Best M, et al. Kras(G12D) and Smad4/Dpc4 haploinsufficiency cooperate to induce mucinous cystic neoplasms and invasive adenocarcinoma of the pancreas. *Cancer Cell*. 2007; 11(3):229–243. [PubMed: 17349581]
54. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature*. 2003; 425(6958):577–584. [PubMed: 14534577]
55. Singh G, Singh SK, Konig A, et al. Sequential activation of NFAT and c-Myc transcription factors mediates the TGF-beta switch from a suppressor to a promoter of cancer cell proliferation. *J Biol Chem*. 2010; 285(35):27241–27250. [PubMed: 20516082]
56. Vogelmann R, Ruf D, Wagner M, Adler G, Menke A. Effects of fibrogenic mediators on the development of pancreatic fibrosis in a TGF-beta1 transgenic mouse model. *Am J Physiol Gastrointest Liver Physiol*. 2001; 280(1):G164–172. [PubMed: 11123210]
57. Bardeesy N, Cheng KH, Berger JH, et al. Smad4 is dispensable for normal pancreas development yet critical in progression and tumor biology of pancreas cancer. *Genes Dev*. 2006; 20(22):3130–3146. [PubMed: 17114584]
58. Apte MV, Haber PS, Darby SJ, et al. Pancreatic stellate cells are activated by proinflammatory cytokines: implications for pancreatic fibrogenesis. *Gut*. 1999; 44(4):534–541. [PubMed: 10075961]
59. Haber PS, Keogh GW, Apte MV, et al. Activation of pancreatic stellate cells in human and experimental pancreatic fibrosis. *Am J Pathol*. 1999; 155(4):1087–1095. [PubMed: 10514391]
60. Redston MS, Caldas C, Seymour AB, et al. p53 mutations in pancreatic carcinoma and evidence of common involvement of homocopolymer tracts in DNA microdeletions. *Cancer Res*. 1994; 54(11):3025–3033. [PubMed: 8187092]
61. Scarpa A, Capelli P, Mukai K, et al. Pancreatic adenocarcinomas frequently show p53 gene mutations. *Am J Pathol*. 1993; 142(5):1534–1543. [PubMed: 8494051]
62. Barton CM, Staddon SL, Hughes CM, et al. Abnormalities of the p53 tumour suppressor gene in human pancreatic cancer. *Br J Cancer*. 1991; 64(6):1076–1082. [PubMed: 1764370]
63. Hingorani SR, Wang L, Multani AS, et al. Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell*. 2005; 7(5):469–483. [PubMed: 15894267]
64. Wagner M, Greten FR, Weber CK, et al. A murine tumor progression model for pancreatic cancer recapitulating the genetic alterations of the human disease. *Genes Dev*. 2001; 15(3):286–293. [PubMed: 11159909]
65. DiGiuseppe JA, Hruban RH, Goodman SN, et al. Overexpression of p53 protein in adenocarcinoma of the pancreas. *Am J Clin Pathol*. 1994; 101(6):684–688. [PubMed: 8209852]
66. Thayer SP, di Magliano MP, Heiser PW, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature*. 2003; 425(6960):851–856. [PubMed: 14520413]
67. Berman DM, Karhadkar SS, Maitra A, et al. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. *Nature*. 2003; 425(6960):846–851. [PubMed: 14520411]
68. Nolan-Stevaux O, Lau J, Truitt ML, et al. GLI1 is regulated through Smoothened-independent mechanisms in neoplastic pancreatic ducts and mediates PDAC cell survival and transformation. *Genes Dev*. 2009; 23(1):24–36. [PubMed: 19136624]
69. Dennler S, Andre J, Alexaki I, et al. Induction of sonic hedgehog mediators by transforming growth factor-beta: Smad3-dependent activation of Gli2 and Gli1 expression in vitro and in vivo. *Cancer Res*. 2007; 67(14):6981–6986. [PubMed: 17638910]
70. Yauch RL, Gould SE, Scales SJ, et al. A paracrine requirement for hedgehog signalling in cancer. *Nature*. 2008; 455(7211):406–410. [PubMed: 18754008]
71. Tian H, Callahan CA, DuPree KJ, et al. Hedgehog signaling is restricted to the stromal compartment during pancreatic carcinogenesis. *Proc Natl Acad Sci U S A*. 2009; 106(11):4254–4259. [PubMed: 19246386]
72. Bailey JM, Swanson BJ, Hamada T, et al. Sonic hedgehog promotes desmoplasia in pancreatic cancer. *Clin Cancer Res*. 2008; 14(19):5995–6004. [PubMed: 18829478]

73. Walter K, Omura N, Hong SM, et al. Overexpression of smoothened activates the sonic hedgehog signaling pathway in pancreatic cancer-associated fibroblasts. *Clin Cancer Res.* 2010; 16(6):1781–1789. [PubMed: 20215540]
74. Olive KP, Jacobetz MA, Davidson CJ, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science.* 2009; 324(5933):1457–1461. [PubMed: 19460966]
75. Apelqvist A, Li H, Sommer L, et al. Notch signalling controls pancreatic cell differentiation. *Nature.* 1999; 400(6747):877–881. [PubMed: 10476967]
76. Su Y, Buchler P, Gazdhar A, et al. Pancreatic regeneration in chronic pancreatitis requires activation of the notch signaling pathway. *J Gastrointest Surg.* 2006; 10(9):1230–1241. discussion 1242. [PubMed: 17114010]
77. Mullendore ME, Koorstra JB, Li YM, et al. Ligand-dependent Notch signaling is involved in tumor initiation and tumor maintenance in pancreatic cancer. *Clin Cancer Res.* 2009; 15(7):2291–2301. [PubMed: 19258443]
78. Wang Z, Banerjee S, Li Y, Rahman KM, Zhang Y, Sarkar FH. Down-regulation of notch-1 inhibits invasion by inactivation of nuclear factor-kappaB, vascular endothelial growth factor, and matrix metalloproteinase-9 in pancreatic cancer cells. *Cancer Res.* 2006; 66(5):2778–2784. [PubMed: 16510599]
79. Mazur PK, Einwachter H, Lee M, et al. Notch2 is required for progression of pancreatic intraepithelial neoplasia and development of pancreatic ductal adenocarcinoma. *Proc Natl Acad Sci U S A.* 2010; 107(30):13438–13443. [PubMed: 20624967]
80. Plentz R, Park JS, Rhim AD, et al. Inhibition of gamma-secretase activity inhibits tumor progression in a mouse model of pancreatic ductal adenocarcinoma. *Gastroenterology.* 2009; 136(5):1741–1749. e1746. [PubMed: 19208345]
81. Zeng G, Germinaro M, Micsenyi A, et al. Aberrant Wnt/beta-catenin signaling in pancreatic adenocarcinoma. *Neoplasia.* 2006; 8(4):279–289. [PubMed: 16756720]
82. Pasca di Magliano M, Biankin AV, Heiser PW, et al. Common activation of canonical Wnt signaling in pancreatic adenocarcinoma. *PLoS One.* 2007; 2(11):e1155. [PubMed: 17982507]
83. Lowy AM, Fenoglio-Preiser C, Kim OJ, et al. Dysregulation of beta-catenin expression correlates with tumor differentiation in pancreatic duct adenocarcinoma. *Ann Surg Oncol.* 2003; 10(3):284–290. [PubMed: 12679314]
84. Morris, JPt; Cano, DA.; Sekine, S.; Wang, SC.; Hebrok, M. Beta-catenin blocks Kras-dependent reprogramming of acini into pancreatic cancer precursor lesions in mice. *J Clin Invest.* 2010; 120(2):508–520. [PubMed: 20071774]
85. Nawroth R, van Zante A, Cervantes S, McManus M, Hebrok M, Rosen SD. Extracellular sulfatases, elements of the Wnt signaling pathway, positively regulate growth and tumorigenicity of human pancreatic cancer cells. *PLoS One.* 2007; 2(4):e392. [PubMed: 17460759]
86. Iacobuzio-Donahue CA, Maitra A, Olsen M, et al. Exploration of global gene expression patterns in pancreatic adenocarcinoma using cDNA microarrays. *Am J Pathol.* 2003; 162(4):1151–1162. [PubMed: 12651607]
87. Su AI, Welsh JB, Sapinoso LM, et al. Molecular classification of human carcinomas by use of gene expression signatures. *Cancer Res.* 2001; 61(20):7388–7393. [PubMed: 11606367]
88. Li J, Kleeff J, Abiatari I, et al. Enhanced levels of Hsulf-1 interfere with heparin-binding growth factor signaling in pancreatic cancer. *Mol Cancer.* 2005; 4(1):14. [PubMed: 15817123]
89. Williams TM, Weiner DB, Greene MI, Maguire HC Jr. Expression of c-erbB-2 in human pancreatic adenocarcinomas. *Pathobiology.* 1991; 59(1):46–52. [PubMed: 1675057]
90. Satoh K, Sasano H, Shimosegawa T, et al. An immunohistochemical study of the c-erbB-2 oncogene product in intraductal mucin-hypersecreting neoplasms and in ductal cell carcinomas of the pancreas. *Cancer.* 1993; 72(1):51–56. [PubMed: 8099533]
91. Day JD, Diguseppe JA, Yeo C, et al. Immunohistochemical evaluation of HER-2/neu expression in pancreatic adenocarcinoma and pancreatic intraepithelial neoplasms. *Hum Pathol.* 1996; 27(2):119–124. [PubMed: 8617452]
92. Korc M, Chandrasekar B, Yamanaka Y, Friess H, Buchler M, Beger HG. Overexpression of the epidermal growth factor receptor in human pancreatic cancer is associated with concomitant

- increases in the levels of epidermal growth factor and transforming growth factor alpha. *J Clin Invest.* 1992; 90(4):1352–1360. [PubMed: 1401070]
93. Wagner M, Weber CK, Bressau F, et al. Transgenic overexpression of amphiregulin induces a mitogenic response selectively in pancreatic duct cells. *Gastroenterology.* 2002; 122(7):1898–1912. [PubMed: 12055597]
 94. Siveke JT, Einwachter H, Sipos B, Lubeseder-Martellato C, Kloppel G, Schmid RM. Concomitant pancreatic activation of Kras(G12D) and Tgfa results in cystic papillary neoplasms reminiscent of human IPMN. *Cancer Cell.* 2007; 12(3):266–279. [PubMed: 17785207]
 95. Friess H, Yamanaka Y, Kobrin MS, Do DA, Buchler MW, Korc M. Enhanced erbB-3 expression in human pancreatic cancer correlates with tumor progression. *Clin Cancer Res.* 1995; 1(11):1413–1420. [PubMed: 9815939]
 96. Blaine SA, Ray KC, Branch KM, Robinson PS, Whitehead RH, Means AL. Epidermal growth factor receptor regulates pancreatic fibrosis. *Am J Physiol Gastrointest Liver Physiol.* 2009; 297(3):G434–441. [PubMed: 19608732]
 97. Zhang Z, Rigas B. NF-kappaB, inflammation and pancreatic carcinogenesis: NF-kappaB as a chemoprevention target (review). *Int J Oncol.* 2006; 29(1):185–192. [PubMed: 16773199]
 98. Whitcomb DC. Inflammation and Cancer V. Chronic pancreatitis and pancreatic cancer. *Am J Physiol Gastrointest Liver Physiol.* 2004; 287(2):G315–319. [PubMed: 15246966]
 99. Malka D, Hammel P, Maire F, et al. Risk of pancreatic adenocarcinoma in chronic pancreatitis. *Gut.* 2002; 51(6):849–852. [PubMed: 12427788]
 100. Steinle AU, Weidenbach H, Wagner M, Adler G, Schmid RM. NF-kappaB/Rel activation in cerulein pancreatitis. *Gastroenterology.* 1999; 116(2):420–430. [PubMed: 9922324]
 101. Wang W, Abbruzzese JL, Evans DB, Larry L, Cleary KR, Chiao PJ. The nuclear factor-kappa B RelA transcription factor is constitutively activated in human pancreatic adenocarcinoma cells. *Clin Cancer Res.* 1999; 5(1):119–127.
 102. Chandler NM, Canete JJ, Callery MP. Increased expression of NF-kappa B subunits in human pancreatic cancer cells. *J Surg Res.* 2004; 118(1):9–14. [PubMed: 15093710]
 103. Fujioka S, Scialabba GM, Schmidt C, et al. Function of nuclear factor kappaB in pancreatic cancer metastasis. *Clin Cancer Res.* 2003; 9(1):346–354. [PubMed: 12538487]
 104. Fujioka S, Scialabba GM, Schmidt C, et al. Inhibition of constitutive NF-kappa B activity by I kappa B alpha M suppresses tumorigenesis. *Oncogene.* 2003; 22(9):1365–1370. [PubMed: 12618762]
 105. Ougolkov AV, Fernandez-Zapico ME, Savoy DN, Urrutia RA, Billadeau DD. Glycogen synthase kinase-3beta participates in nuclear factor kappaB-mediated gene transcription and cell survival in pancreatic cancer cells. *Cancer Res.* 2005; 65(6):2076–2081. [PubMed: 15781615]
 106. Evan GI, Littlewood TD. The role of c-myc in cell growth. *Curr Opin Genet Dev.* 1993; 3(1):44–49. [PubMed: 8453273]
 107. Adhikary S, Eilers M. Transcriptional regulation and transformation by Myc proteins. *Nat Rev Mol Cell Biol.* 2005; 6(8):635–645. [PubMed: 16064138]
 108. Land H, Parada LF, Weinberg RA. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature.* 1983; 304(5927):596–602. [PubMed: 6308472]
 109. Sandgren EP, Quaife CJ, Paulovich AG, Palmiter RD, Brinster RL. Pancreatic tumor pathogenesis reflects the causative genetic lesion. *Proc Natl Acad Sci U S A.* 1991; 88(1):93–97. [PubMed: 1986386]
 110. Sandgren EP, Luetke NC, Qiu TH, Palmiter RD, Brinster RL, Lee DC. Transforming growth factor alpha dramatically enhances oncogene-induced carcinogenesis in transgenic mouse pancreas and liver. *Mol Cell Biol.* 1993; 13(1):320–330. [PubMed: 8417334]
 111. Schaeffer BK, Terhune PG, Longnecker DS. Pancreatic carcinomas of acinar and mixed acinar/ductal phenotypes in Ela-1-myc transgenic mice do not contain c-K-ras mutations. *Am J Pathol.* 1994; 145(3):696–701. [PubMed: 8080049]
 112. Schlegel C, Verbeke C, Hildenbrand R, Zentgraf H, Bleyl U. c-MYC activation in primary and metastatic ductal adenocarcinoma of the pancreas: incidence, mechanisms, and clinical significance. *Mod Pathol.* 2002; 15(4):462–469. [PubMed: 11950922]

113. Buchholz M, Schatz A, Wagner M, et al. Overexpression of c-myc in pancreatic cancer caused by ectopic activation of NFATc1 and the Ca²⁺/calcineurin signaling pathway. *EMBO J*. 2006; 25(15):3714–3724. [PubMed: 16874304]
114. Koenig A, Linhart T, Schlengemann K, et al. NFAT-induced histone acetylation relay switch promotes c-Myc-dependent growth in pancreatic cancer cells. *Gastroenterology*. 2010; 138(3): 1189–1199. e1181–1182. [PubMed: 19900447]
115. Kita K, Saito S, Morioka CY, Watanabe A. Growth inhibition of human pancreatic cancer cell lines by anti-sense oligonucleotides specific to mutated K-ras genes. *Int J Cancer*. 1999; 80(4): 553–558. [PubMed: 9935156]
116. Rowinsky EK, Windle JJ, Von Hoff DD. Ras protein farnesyltransferase: A strategic target for anticancer therapeutic development. *J Clin Oncol*. 1999; 17(11):3631–3652. [PubMed: 10550163]
117. Van Cutsem E, vande Velde H, Karasek P, et al. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. *J Clin Oncol*. 2004; 22(8):1430–1438. [PubMed: 15084616]
118. Fleming JB, Shen GL, Holloway SE, Davis M, Brekken RA. Molecular consequences of silencing mutant K-ras in pancreatic cancer cells: justification for K-ras-directed therapy. *Mol Cancer Res*. 2005; 3(7):413–423. [PubMed: 16046552]
119. Moore MJ, Goldstein D, Hamm J, et al. Erlotinib Plus Gemcitabine Compared With Gemcitabine Alone in Patients With Advanced Pancreatic Cancer: A Phase III Trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol*. 2007 JCO.2006.2007.9525. 10.1200/JCO.2006.07.9525
120. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol*. 2008; 26(10):1626–1634. [PubMed: 18316791]
121. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol*. 2008; 26(35):5705–5712. [PubMed: 19001320]
122. Wilhelm SM, Carter C, Tang L, et al. BAY 43–9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res*. 2004; 64(19):7099–7109. [PubMed: 15466206]
123. Ji Z, Mei FC, Xie J, Cheng X. Oncogenic KRAS activates hedgehog signaling pathway in pancreatic cancer cells. *J Biol Chem*. 2007; 282(19):14048–14055. [PubMed: 17353198]
124. Truty MJ, Urrutia R. Basics of TGF-beta and pancreatic cancer. *Pancreatology*. 2007; 7(5–6): 423–435. [PubMed: 17898532]
125. Tascilar M, Skinner HG, Rosty C, et al. The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. *Clin Cancer Res*. 2001; 7(12):4115–4121. [PubMed: 11751510]
126. Rizzo P, Osipo C, Foreman K, Golde T, Osborne B, Miele L. Rational targeting of Notch signaling in cancer. *Oncogene*. 2008; 27(38):5124–5131. [PubMed: 18758481]
127. Park SS, Eom YW, Kim EH, et al. Involvement of c-Src kinase in the regulation of TGF-beta1-induced apoptosis. *Oncogene*. 2004; 23(37):6272–6281. [PubMed: 15208664]
128. Lane TF, Sage EH. The biology of SPARC, a protein that modulates cell-matrix interactions. *FASEB J*. 1994; 8(2):163–173. [PubMed: 8119487]
129. Infante JR, Matsubayashi H, Sato N, et al. Peritumoral fibroblast SPARC expression and patient outcome with resectable pancreatic adenocarcinoma. *J Clin Oncol*. 2007; 25(3):319–325. [PubMed: 17235047]
130. Mantoni TS, Schendel RR, Rodel F, et al. Stromal SPARC expression and patient survival after chemoradiation for non-resectable pancreatic adenocarcinoma. *Cancer Biol Ther*. 2008; 7(11): 1806–1815. [PubMed: 18787407]
131. Guweidhi A, Kleeff J, Adwan H, et al. Osteonectin influences growth and invasion of pancreatic cancer cells. *Ann Surg*. 2005; 242(2):224–234. [PubMed: 16041213]

Figure 1.

Schematic representation of prominent signaling pathways involved with PDAC tumoral compartment and stromal compartment crosstalk. Induction of some of these pathways by RAS activation is a contributing factor. The cells of the tumoral compartment can both respond to TGF- β pathway activation as well as produce ligand that can then act in a paracrine fashion to affect cells of the stromal compartment. Likewise, cells of the tumoral compartment can respond to Hh activation and also produced ligand to affect the surrounding stromal cells. Chemokines and growth factors produced by inflammatory cells, fibroblasts, and PSCs can stimulate NF- κ B signaling in cells of both compartments. Likewise, these cells can produce chemokines and growth factors to further stimulate NF- κ B signaling in a paracrine manner. This schematic greatly simplifies the complexity of this crosstalk but highlights its importance in function of both cellular compartments.

Table 1

Selected clinical trials utilizing targeted Hedgehog therapy to treat pancreatic adenocarcinomas

Setting	HH pathway inhibitor	Cytotoxic or other agent	Phase
Metastatic pancreatic cancer	IPI-926 (Infinity Pharmaceuticals [†])	Gemcitabine	IB/II
Metastatic pancreatic cancer	GDC-0449 (Genentech/Roche [‡])	Gemcitabine	Randomized phase II
Metastatic pancreatic cancer	GDC-0449 (Genentech/Roche)	Gemcitabine nab-paclitaxel	II
Neoadjuvant	GDC-0449 (Genentech/Roche)	None	II
Metastatic pancreatic cancer	GDC-0449 (Genentech/Roche)	Erlotinib +/- Gemcitabine	I/II
Metastatic pancreatic cancer	GDC-0449 (Genentech/Roche)	Gemcitabine	pilot

[†] Cambridge, MA.[‡] San Francisco, CA.