[Frontiers in Bioscience 9, 1889-1898, May 1, 2004]

ALTERATIONS OF CELL SIGNALING PATHWAYS IN PANCREATIC CANCER

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1. ABSTRACT

Pancreatic ductal adenocarcinomas continue to have the worst prognosis of any adult malignancy with a five-year survival rate of less than 4%. One approach to improve patient survival from pancreatic cancer is to identify new biological targets that contribute to the aggressive pathogenecity of this disease and to develop reagents that will interfere with the function of these targets. Apart from the identification of the genetic profile of pancreatic cancer, a number of studies have focused on aberrant cell signaling pathways and their role in pancreatic cancer biology and response to therapy. This review, although not comprehensive, will discuss the salient features of several of these pathways. These include the roles of TGFβ signaling in both tumor suppression and tumor promotion and the effects of deregulation of phosphotyrosine kinase receptor signaling pathways in pancreatic cancer.

2. INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer deaths of adults in the United States with a one-year survival rate of less than 20% and a 5-year survival rate of a mere 4%, (1). There are several histological sub-types; however, about 90% of all pancreatic neoplasms are ductal adenocarcinomas (PDAC), (2). Although not comprehensive, this review will discuss some of the salient features by which alterations in signaling pathways contribute to the progression of PDAC. PDAC generally occur without appreciable symptoms and thus can go undetected until tumor development is in an advanced stage (3). Surgical resection has been considered the only curative modality for this disease, but only 10% of patients are candidates for surgery and even then the 5-year survival rate does not increase much above 4% (1, 2). In addition, these tumors are highly resistant to conventional chemotherapy and radiation treatments (4, 5). Although the use of combined modality therapy has produced modest increases in survival, significant advances in the treatment of pancreatic cancer have not been made (4, 5). Thus, further understanding the molecular events involved in the resistance of these tumors to chemotherapy and radiation is needed to establish a basis for development of more effective treatments of pancreatic cancer.

Studies over the past several years have identified a number of genetic alterations that occur in PDAC and these have been extensively reviewed elsewhere (2, 4). A list of the most common mutations and their frequency of occurrence in PDAC compared to colon cancers is shown in table 1. Epigenetic silencing has also been demonstrated as a mechanism for inactivation of genes in PDAC that contribute to tumor progression. Ueki *et al.* (6) recently showed that hypermethylation of promoter regions led to silencing of a number of genes in PDAC including *PENK, RARB, CDKN2A, CACNAIG, TIMP3, CDH1* and *MLH1*. Our laboratory has recently shown that histone deacetylase (HDAC) represses the expression of the TGFβ type II receptor genes in some PDAC cell lines (7). It seems likely that HDAC may play a role in the transcriptional silencing

Table 1. Predominant genetic alterations in pancreatic ductal adenocarcinomas

	Pancreas	Colon
Ink4A/ARF	98	0
APC/β Catenin	0	95
c-Ki-ras	95	50
P53	75	60
DPC4	55	15
BRCA2	7	?
RER+TGFβRII	3	10

other tumor suppressor genes in PDAC although this area of research has not been thoroughly explored.

PDAC exhibit a growth factor independent (GFI) phenotype, indicating that they produce their own autocrine growth factors and growth factor receptors enabling them to grow in the absence of serum (8, 9). PDAC cells are also relatively resistant to undergoing apoptosis (8). These two features are hallmarks of tumor progression and give cells selective growth and survival advantages (9-11). Inappropriate expression of autocrine growth factors, overexpression or constitutive activation of growth factor receptors, and/or constitutive activation of growth-regulatory signaling pathways have all been linked to the development of growth factor independence and resistance to apoptosis (12, 13). Additionally, mutations or inactivation of tumor suppressor genes contributes to deregulation of growth control and resistance to apoptosis (14, 15).

In respect to cell signaling alterations, PDAC cells generally show changes in TGFβ and phosphotyrosine receptor kinase signaling pathways (8, 16-18). In PDAC, the tumor suppressive functions attributed to TGFβ signaling including growth inhibition and sensitivity to apoptosis are commonly lost and there is a switch of TGFβ mediated tumor suppressive activity to one where TGFβ signaling promotes tumor progression. Signaling pathways initiated through phosphotyrosine kinase receptors including the erbB family members and the IGF-1 receptor are commonly up regulated in PDAC.

3. TGFβ SIGNALING PATHWAY

3.1. Overview of TGFβ signaling

TGFβ molecules are multifunctional cytokines involved in a variety of cellular functions including organogenesis, differentiation and cell proliferation (19, 20). TGFβ signaling is associated with tumor suppression since this pathway induces the expression of cyclin dependent kinase inhibitors (21) i.e. $p21^{cip1/waf1}$, $p27^{kip1}$ and down-regulates mitogen-induced c-Myc expression. Thus, interference of the TGFβ signaling often contributes to a loss of growth control (21). TGFβs signal through both receptor-mediated activation of Smads (22-24) and through Smad-independent pathways. It is becoming clear that during tumor progression, TGFβ signaling switches from tumor suppressive to tumor promoting activity and that this alteration of TGFβ signaling pathway involves a different set of target genes and may be at least partially independent of the conventional Smad pathway (24). Several pathways induced by Smad-independent signaling have been identified and the mechanisms by which TGFβ induces these pathways are under extensive investigation.

TGFβs signals by binding to transmembrane serine-threonine kinases termed receptor I (RI) and receptor II (RII) (22-24). Genetic evidence shows that both receptors are required for signaling (24). A third TGFβ receptor (RIII) is not believed to be involved directly in TGFβ signaling but acts to present TGFβs to RII (20). TGFβligand first binds RII, which then recruits RI to form a functional receptor complex. After this complex is formed, RI is phosphorylated by the constitutively active and autophosphorylated RII (20). For Smad-dependent signaling, RI directly interacts with and phosphorylates Smads 2 and 3 and these activated Smads bind Smad 4, and subsequently the complex is translocated to the nucleus. This Smad complex associates with other co-factors to bind efficiently to specific DNA sequences and the activated complex promotes transcription of TGFβ responsive genes (25-27).

The mechanism by which TGFβ inhibits growth in epithelial cells has been studied in some detail (22). This effect is caused, at least in part, by the ability of TGFβ to block the activation of G1 cyclin-dependent kinases leading to suppression of Rb phosphorylation and by down-regulation of c-Myc expression. TGFβ signaling and signaling by phosphotyrosine kinase growth factor receptors (PTKRs) are known to have opposing effects. An imbalance between these pathways likely contributes to the tumorigenic phenotype. In epithelial cells, TGFβ is reported to inhibit the mitogenic effect of ras-activating phosphotyrosine kinase growth factors (28). However, cells transformed with oncogenic ras and/or where ras is activated by growth factor signaling pathways can override the growth- inhibitory effects of TGFβ (29. One potential intermediary between TGFβsignaling and PTKR pathways is c-Myc. These two pathways reciprocally regulate the c-Myc protein. Growth factors stimulated the expression of c-Myc, in part, by promoting stability of the Myc protein. TGFβ is able to rapidly down-regulate transcription of *c-Myc* in most cell types, provided that they are TGFβ responsive (30, 31). However, during tumor progression, alterations in TGFβ signaling may lead to co-operation with PTKR signaling as described below.

3.2. Mechanisms causing loss of TGFβ/Smad signaling in PDAC

Several different mechanisms account for loss of TGFβ signaling. These include mutations or transcriptional repression of TGFβ receptors and mutations of DPC4. A region of 18q was found to be deleted in about a third of all PDAC. (32). This finding led to the discovery of a gene termed *DPC4* indicating deleted in pancreatic cancer. This gene was found to have homology to the drosophila MAD family and is the homolog for Smad 4. About half of PDACs show biallelic inactivation of *Smad 4* (*DPC4*). Of these, 30% show homozygous deletion of the gene and another 20% exhibit intragenic mutations (2, 3).

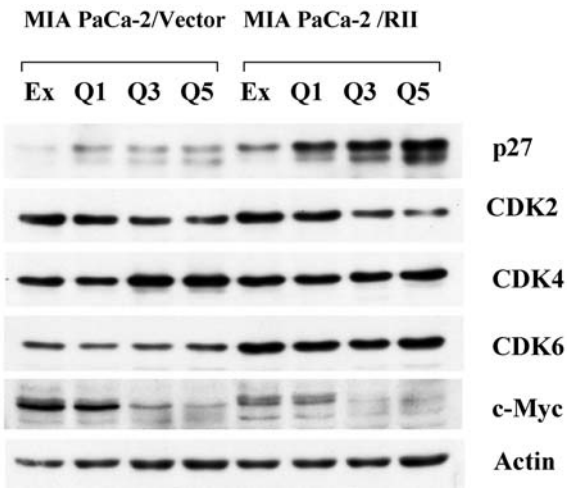

Figure 1. Restoration of TGFβ signaling in MIA PaCa-2 cells alters the expression of p27kip1 and c-Myc. Equal amounts of protein (30μg) lysates from MIA PaCa-2/vector or MIA PaCa-2/RII transfected cells were resolved by SDS-PAGE and analyzed by Western blotting with indicated antibodies. Cells were expontentially growing or deprived of serum for various times; i.e. Q1, 1 day; Q3, 3 days or Q5, for 5 days. The basal level of the cyclin dependent kinase inhibitor was higher in cells where TGFβ signaling was restored and the level increased further as cells were subjected to serum deprivation. Conversely, the basal level of c-Myc was lower in cells where TGFβ signaling was restored. β-actin was used as loading control. Vector represents empty vector control; RII represents vector with RII cDNA insert.

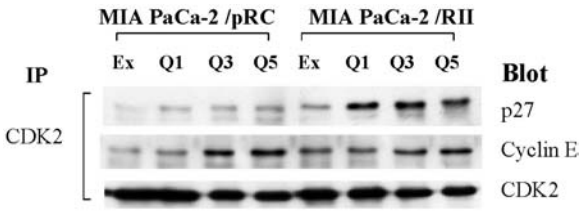

Figure 2. Restoration of TGFβ signaling in MIA PaCa-2 cells alters the interaction of $p27^{kip1}$/cdk/cyclins. CDK2 was immunoprecipitated from whole cell lystates (150 ug). Samples were then subjected to western blotting with antibodies against p27 and cydlin E and CDK2.

We (33, 34) and others (35, 36) have reported that transcriptional repression may also account for down-regulation of TGFβ receptors. We found that mutations of the RII gene that are common in HNPCC type of colon cancer are relatively uncommon in PDAC (37) but that loss of RII expression occurs in a sub-population of PDAC and is caused by transcriptional repression (33). In some instances transcriptional repression of RII may occur indirectly through the methylation-specific down-regulation of Sp1, a critical transcriptional activator of the RII gene (33). There is also convincing evidence that histone deacetylases (HDACs) contribute to transcriptional repression of the RII gene in PDAC cell lines (34). Treatment of PDAC cells with a HDAC inhibitor readily up-regulate RII promoter activity and RII expression in PDAC cells (38). Interesting studies by Fields and his colleagues using a rat intestinal epithelial cell line (34) show that the TGFβ type II receptor gene is transcriptionally repressed by PKCβII signaling by a mechanism involving Cox-2. This study further indicates that inhibiting either PKCβII signaling or Cox-2 function restores TGFβsignaling in these cells. Investigations are now underway to assess whether these pathways play any role in transcriptional repression of TGFβ receptor genes in PDAC. If this proves to be the case, it is intriguing to speculate that inhibitors of PKCβII, Cox-2 and HDACs may provide and effective strategy for enhancing TGFβ signaling in cancer cells that show down regulation of TGFβ type II receptor.

3.3. Loss of autocrine TGFβ signaling in PDAC contributes to resistance to apoptosis and deregulation of cell cycle progression

The human PDAC cell line MIA PaCA-2 expresses a functional DPC4 molecule; however, it is not responsive to growth inhibition by TGFβ since the RII gene is transcriptionally repressed (8, 33, 34). MIA PaCa-2 cells overexpress PTKRs including IGF-1R and erbB family members and are highly tumorigenic (8). We have used the MIA PaCa-2 cell line as a model to determine the role of TGFβ signaling in the growth and tumorigenic properties of PDAC. Restoring TGFβ/Smad signaling pathway by re-expressing RII in these cells renders them more sensitive to radiation through a TGFβ dependent mechanism involving the up regulation of the pro-apoptotic molecule BAX (39).

It was unclear however, whether restoring TGFβ/Smad signaling in this cell line can overcome the deregulation of cell cycle progression caused by overexpression and signaling by PTKRs. Analysis using the MIA PaCa-2 cell model showed that restoration of autocrine TGFβ signaling caused a reduction of the steady-state levels of c-Myc (Figure 1). A reduction of c-Myc by TGFβ signaling has been previously reported (40-43) suggesting that this represents a mechanism by which TGFβ inhibits cell growth. Restoration of TGFβ signaling dramatically induced the expression of $p27^{Kip1}$ without altering the expression of cyclin dependent kinases (cdks), (Figure 1). The increase in over-all levels of $p27^{Kip1}$ was associated with an increase in $p27^{Kip1}$ bound to cdk2 (Figure 2). Unexpectedly, the association of cdk2 with cyclin E increased as cells progressed towards quiescence. It is possible that this event relates to the aberrant IGF-1R signaling that we reported occurs in quiescent MIA PaCa-2 cells (18); however, consistent with the idea that TGFβ signaling inhibits cell cycle progression, the level of association of cdk2 with cyclin E decreased in MIA PaCa-2/RII where TGFβ signaling was restored (Figure 2). Furthermore, the association of $p27^{Kip1}$ with these complexes was increased in MIA PaCa-2 cells that have restored RII expression.

Thus, TGFβ/Smad signaling likely contributes to the establishment of quiescence and regulates the progression of cells through the cell cycle. In cells where TGF β signaling is lost (i.e. MIA PaCa-2 cells) cells are more

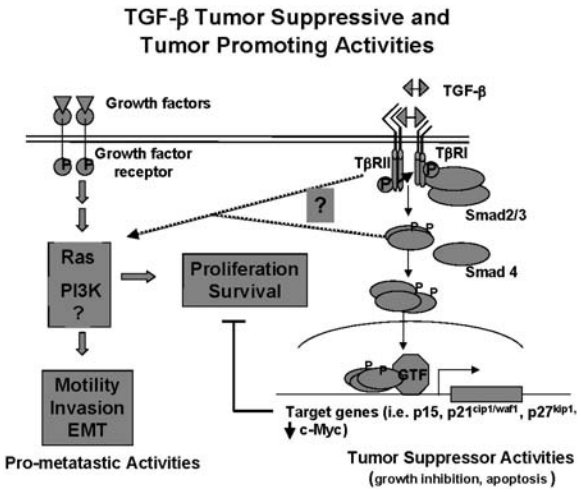

Figure 3. Illustration showing the potential mechanism by which TGFβ signaling pathway is altered as it switches from tumor suppression to a tumor promotion.

resistant to entering a quiescent state and upon entering quiescence they remained primed to more rapidly re-enter the cell cycle. Using this cell model, exponentially growing cells lacking TGFβ/Smad signaling show an increase in the activation of cyclin complexes and phosphorylation of Rb (not shown) resulting in a selective growth advantage. Thus, decreasing TGFβ/Smad signaling may contribute to tumor progression by altering sensitivity to apoptosis and by deregulating cell cycle controls. This notion is supported by similar findings in other tumor models. For example the Wakefield group (44) recently demonstrated in breast cell lines that down-regulating the RII receptor in premalignant or weakly tumorigenic cells made these cells histologically and proliferatively more aggressive. However, in highly tumorigenic cell lines decreasing TGFβ responsiveness by down-regulating RII made these cells less metastatic.

3.4. Role of TGFβ signaling in metastasis

The tumor suppressive effects of TGFβ signaling are well documented and hinge on the role of autocrine and paracrine TGFβ signaling to control growth by regulating the expression of genes that code for molecules involved in cell cycle progression and apoptosis. However, it is becoming apparent that TGFβ signaling also plays critical roles in metastasis (45-47). These seemingly paradoxical properties of the TGFβ signaling pathway are likely attributed to the multifunctional aspects of TGFβ signaling. The current paradigm implies that TGFβ signaling is tumor suppressive in normal and at early stages of carcinogenesis but a switch towards tumor promoting activities occurs during tumor progression. The precise molecular aspects of this switch are not totally delineated. However, a number of recent studies have begin to shed light on this issue; several of these are described below (47, 48). Although, these studies were not done in PDAC, similar effects are likely to occur in PDAC based on preliminary studies in our laboratory.

Studies by both the Massaque and Arteaga groups (47, 48) show that TGFβ signaling pathways cooperate with ErbB2 signaling to promote metastasis. This raises the important question as to how alterations in the TGFβ signaling pathway result in the switch from TGFβ-mediated tumor suppressive to oncogenic effects. The preliminary results in PDAC cells suggest that TGFβ signaling is altered during tumor progression. Loss of DPC4 function by mutation occurs as a middle to late stage event in about 55% of PDAC tumors (1). A study by the Markowitz and his colleagues (49) show that Smads2 and 3 can be activated and undergo nuclear translocation in a PDAC cell line that is null for DPC4. Our studies show that TGFβ induces the activation of both PI3K and ERKs in DPC4 null PDAC cells causing an increase in motility and invasive properties (unpublished). It is clear that these metastatic inducing properties of TGFβ signaling are Smad4 independent. However, it has not been determined whether Smads2/3 can induce gene expression independent of Smad4. It is also possible that TGFβ receptors mediate signals downstream and independent of all Smads. The switch from tumor suppressive to tumor promoting effects of TGFβ may therefore relate to TGFβ receptor levels, levels of ligand and the presence or absence of Smad4. An theoretical overview of TGFβ related tumor suppressive and tumor-promoting pathways is shown in Figure 3.

4. PHOSPHOTYROSINE KINASE SIGNALING PATHWAYS

Inappropriate expression of autocrine growth factors and/or constitutive activation of growth factor receptors are linked to the development and progression of PDAC (1). Recent evidence made possible the description of a genetic progression model of this disease (1, 4). According to this model, increased expression of phosphotyrosine kinase receptors and their ligands occur as an early event in the development of PDAC. Human PDAC are reported to produce autocrine growth factors including IGF-1, EGF, TGFα. and to over express receptors for EGF, ErbB2, IGF-1, VEGF, FGF and NGF (50-54). This review will not attempt to describe all of these in detail but will provide an overview of the role of the erbB family and IGF-1R in the pathogenesis of PDAC.

4.1. ErbB family of receptors

The ErbB family of receptor tyrosine kinases includes four members, EGFR (ErbB1), ErbB2 (HER2/neu), ErbB3, and ErbB4, which share a similar primary structure and are widely expressed in human tissues. ErbB4 is however expressed at low levels or lacking in PDAC (unpublished result). A number of ErbB ligands have also been identified that bear homology to EGF but differ in their affinity for ErbB family members. The precise nature of ErbB ligand/receptor interactions is a subject of ongoing investigation but, in general, ErbB ligand binding is thought to induce the dimerization or oligomerization of ErbB receptors, both in hetero-oligomers and homo-oligomers of varying ErbB receptor combinations. ErbB2 differs from the other ErbB receptors in that to date it is without an identified, soluble ligand, yet appears to be the preferred dimerization patner for the other

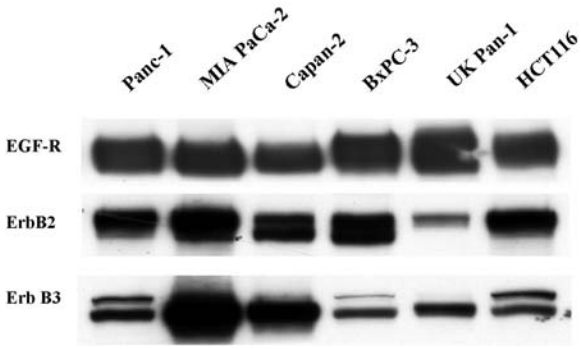

Figure 4. Expression of erbB family of receptors in pancreatic cancer cells. Western blot analysis of lysates from five PDAC cell lines (Panc-1, MIA PaCa-2, Capan2, BxPc-3, and UK Pan-1) showing the expression of EGFR, ErbB2 and erbB3. HCT116 is a colon cancer cell line and was used as a control since it is known to over express ErbB2.

ErbB receptors in their ligand-bound state and acts generally to potentiate and prolong ErbB signaling (55, 56). The receptors then undergo phosphorylation on tyrosine residues due to their intrinsic tyrosine kinase activity. The phosphorylation of specific tyrosine residues on ErbB receptors establishes docking sites for signaling molecules harboring either Src homology 2 (SH2) or phosphotyrosine binding/interacting (PTB/PI) domains. The activation of these intracellular signaling pathways regulates a host of cellular functions. ErbB2 possesses phosphorylation sites within the carboxy-terminus at 1023, 1139, 1196, 1221/2 and 1248, (57, 58). The role of each of these individual docking sites and the components that bind these sites are not fully understood. However, phoshorylation site 1248 has been implicated in transformation and Shc has been shown to bind sites 1196 and 1248, whereas Grb2 is reported to bind site 1139, (57, 58).

4.2. Expression of erbB family of receptors in PDAC

Family members of erbB are overexpressed in numerous human cancers and their overexpression is associated with multiple drug resistance, higher metastatic potential, and decreased patient survival times (55, 57). The cellular mechanisms of ErbB-associated tumorigenicity remain to be elucidated in their full scope but seem to involve at least in part the maintenance of aberrantly activated intracellular mitogenic signaling pathways. The overexpression of ErbB2 alongside the overexpression of other ErbB family members, particularly EGFR, leads to greatly augmented proliferative and cell-survival signaling and synergistic transforming effects (55). ErbB2 over expression alone, upon reaching a certain threshold ($>10^6$ receptors/cell), may also independently lead to cellular transformation by allowing for the spontaneous formation of receptor dimers/oligomers (57, 58). Aberrant ErbB2 over-expression in PDAC has been reported in a number of studies (59, 60). The ErbB2 overexpression observed in PDAC, though associated with higher ErbB2 mRNA levels, is rarely linked to ErbB2 gene amplification, unlike breast, colon, and ovarian cancers (61). ErbB2 over expression in PDAC, similarly to breast and gastric cancer, has been correlated with more glandular, well-differentiated tumor histology (59). A high percentage of intraductal pancreatic lesions demonstrate ErbB2 expression, particularly intraductal mucin-hypersecreting neoplasms, which, like comedo-type ductal carcinoma *in situ* (DCIS) in the breast, may represent an early-stage or precursor lesion to invasive cancers (60, 61). Over expression of EGFR, EGF, and TGFα has also been reported in PDAC (59, 62-65) and in the widely accepted genetic progression model of Hurban and his colleagues (1). The up regulation of EGFR and ErbB2, and EGFR ligands occurs as an early event in the initiation and progression of PDAC. Western blot analyses of several PDAC cell lines show that EGFR is highly expressed whereas, both ErbB2 and ErbB3 show variable expression levels (Figure 4). A study from our lab suggests that even if EGFR is more highly expressed, ErbB2 remains critical for mediating the full tumorigenic potential from EGFR signaling (9).

4.3. Role of ErbB2 signaling in cell cycle progression

ErbB2-associated proliferative signaling contributes to cellular transformation in human cancers through the aberrant regulation of cell cycling (56, 65). D-type cyclins, which serve the dual functions of pRb phosphorylation in early G1 and facilitation of cyclin E kinase activity through the sequestering of cyclin-dependent kinase inhibitors (CDKIs), are induced by a number of mitogenic stimuli including that associated with ErbB2 (66). Breast tumors in which ErbB2 is overexpressed display concomitant cyclin D1 overexpression in the vast majority of cases (67, 68). Additionally, breast cell lines transfected with oncogenic ErbB2 showed a marked increase in the level of cyclin D1 protein (69). ErbB2 has been shown to up-regulate the expression of D-type cyclins both at the transcriptional level and by post-translational stabilization (70-72). Similar to that reported for breast cancers (67-70) ErbB2 signaling regulates cyclin D expression in PDAC (9). The end result of D-type cyclin overexpression is to lessen tumor cell dependency on mitogenic stimuli for cell-cycle entrance and thereby to enhance cellular proliferation (66). Blockade of ErbB2 signaling in a PDAC cell line causes a rapid induction of the cyclin dependent kinase inhibitors (CDKIs), $p21^{Cip1}$ and $p27^{Kip1}$ (9).

In the case of the CDKIs, both induction and repression have been reported in conjunction with ErbB2 signaling, most likely owing to the observed stoichiometric effects of these proteins on cyclin dependent kinase (CDK) activity. Low levels of CDKIs have been shown to activate cyclin D/CDK4 complexes (71), and the association of cyclin D1 with CDK4 is inhibited in the absence of CDKIs (72-74). However, high levels of CDKIs may overwhelm the sequestering capacity of D-type cyclins, allowing CDKIs to bind to cyclin E/CDK2 complexes and resulting in G1 arrest (75). Thus, the modulation of CDKI levels by ErbB2 involves a balancing act that leads ultimately to optimizing cell proliferation. ErbB- associated

proliferative signaling is classically linked to the Ras/MAPK and AKT pathways, both of which have been shown to promote increased cyclin D1 levels and cell cycle progression (56).

4.4. A novel ErbB2-STAT3 pathway in PDAC

Recent studies (76-78) suggest a role of STAT3 in ErbB mitogenic signaling has been explored in greater depth. STATs are a family of transcription factors that were initially investigated in the setting of cytokine signaling, where the mechanism of their activation has been well characterized (76-79). STATs were shown recently to undergo activation in response to a host of non-cytokine growth factors including TGFα, EGF, and neu differentiation factor (9, 77-79). However, under these conditions, the mechanism of STAT tyrosine phosphorylation is less well defined. A variety of receptor and non-receptor tyrosine kinases, including EGFR, ErbB2, and *src*, have been shown to bind and activate STATs, in some cases directly and in others indirectly through intermediaries such as JAKs (79). EGFR activation of STATs, for example, may occur through JAK-dependent or independent mechanisms (80), or EGFR may act in conjunction with *src*. (81) In human malignant epithelial cells, both EGFR and ErbB2 were shown to be required for STAT3 activation, presumably though the recruitment of *src* (81). We found in pancreatic ductal adencarcinoma cells (PDAC) that ErbB2 kinase was complexed with STAT3 and that ErbB2 kinase was required for activation of STAT3. Blockade of ErbB2 kinase activity or inhibiting the function of STAT3 prevented autocrine-induced growth of PDAC cells. Moreover, an examination of PDAC tumor specimens showed that STAT3 was constitutively activated in these tumors and that these tumors all express ErbB2. Thus, STAT3 mediates, at least in part, ErbB2 signaling in PDAC and this pathway may provide novel therapeutic targets.

4.5. IGF-1R signaling

IGF-1R is a transmembrane receptor tyrosine kinase that is involved in growth, transformation, development, and resistance to apoptosis (82-84). IGF-1R exists as an $\alpha_2\beta_2$ heterodimer linked by disulfide bonds and shows the highest binding affinity for IGF-1, although IGF-2 and insulin can also bind and activate the receptor (83). The anti-apoptotic and growth-promoting effects of IGF-1R signaling have been studied extensively and differ among various cell types (85). Over expression of IGF-1R has been implicated in cell proliferation, tumorigenesis, and protection of cells from apoptosis induced by several agents (86). Activation of the IGF-1R results in the initiation of a signaling cascade that involves IRS-1, IRS-2, Shc and Grb2, and activtion of kinases such as phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK), ultimately resulting in a cellular response (87, 89). Recently, additional pathways, one involving the mitochondrial translocation of Raf and its interaction with the 14.3.3 proteins and the other involving the Janus kinase (JAK)/signal transducer and activator of transcription-3 (STAT3) signaling, were identified in IGF-1R-mediated protection from apoptosis (89, 90).

4.6. Alteration of IGF-1R receptor signaling in PDAC

In comparison to normal pancreas, PDAC are reported to over express the IGF-1R and some pancreatic cancer cells also over express insulin receptor substrates, IRS-1 and IRS-2, which are docking proteins for insulin receptor (IR) and the IGF-1R (87, 89). The role of these growth factor receptors and/or autocrine growth factors and docking proteins in maintaining malignant phenotypes in pancreatic cancer cells is not well understood. We have previously shown that the pancreatic cancer cells possess an autocrine IGF-1 loop (8). Furthermore, not only is IGF-1R over expressed in many PDAC cell lines, IGF-1R appears to show aberrant activation that facilitates rapid entry into the cell cycle and promotes cell survival. (18) In the PDAC cell line MIA PaCa-2, we unexpectedly found an increase in the expression and activation of the IGF-1R during the transition of growth states from exponential to quiescent. This increase in expression and activation during serum deprivation contributed to resistance of these cell to apoptosis. The increase in IGF-1R expression was attributed to both an increase in IGF-1R promoter activity and an increase in IGF-1R mRNA stability. These studies support the notion that IGF-1R may be a target for therapy in PDAC. Indeed, treatment of cells with neutralizing antibodies to IGF-1R blocked IGF-1R signaling and induced rapid apoptosis in the MIA PaCa-2 cell line (18).

5. PERSPECTIVES

Aside from the genetic alterations ascribed to PDAC, there is deregulation of cell signaling mediated by modulation of TGFβ and phosphotyrosine kinase pathways. Alteration of TGFβ signaling in PDAC and other cancers involves a switch from tumor suppression to one that promotes tumor progression. The exact mechanism for this switch is under intensive investigation and likely involves Smad 4 independent signaling and probably occurs as a late event during tumor progression. In a normal setting, TGFβ signaling counters many of the signaling events induced by phosphotyrosine kinase receptors including induction of c-myc and activation of the cyclin/Rb/E2F pathway. However, Smad 4 independent signaling by TGFβ contributes to tumor promotion and may involve cross talk and co-operativity with phosphotyrosine kinase signaling pathways. There is some evidence that these pathways may converge at erks, p38 and PI3K pathways. Deregulation of phosphotyrosine kinase receptor signaling occurs at multiple levels in PDAC. These include up regulation of growth factors, their receptors as well as in some cases down-stream adaptor molecules. In addition, there is emerging evidence for inappropriate activation of receptors including the activation of the IGF-1R during serum deprivation and quiescence. Cells showing this inappropriate activation of phosphotyrosine kinase receptor activation are more resistant to apoptosis and are primed to rapidly re-enter the cell cycle as conditions become favorable. Blocking aberrant signaling from these pathways may provide targets for therapeutic intervention of PDAC

and/or may sensitize PDAC cells to radiation or conventional therapies.

6. ACKNOWLEDGEMENTS

This work was supported by NIH Grant CA96122 and the Pancreatic Cancer Research Network (to J.W.F.).

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Key Words: Pancreatic cancer, Cell signaling, Growth factors, Growth factor receptors, TGFβ, Review

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