The Biology of K-Ras Signaling Pathways in Pancreatic Cancer

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Abstract Activating mutations in the K-Ras oncogene occur in approximately 90 % of cases of pancreatic ductal adenocarcinoma, and tumors containing mutant K-Ras often acquire a dependency on the expression of the oncogene. Therapies that block the oncogenic functions of K-Ras could have clinical efficacy for a disease that is currently refractory to all forms of treatment. This chapter describes the evidence, from both *in vitro* studies and studies using genetic mouse models, of the importance of oncogenic K-Ras and its downstream signaling pathways in driving pancreatic tumor formation and cancer cell growth.

Introduction

The decades-old observation that pancreatic ductal adenocarcinoma (PDA) is almost always associated with an activating mutation in the *KRAS* gene has focused attention on this oncogene as a key therapeutic target for this lethal disease. Many tumor cells containing *KRAS* mutations are considered to be K-Ras "addicted," meaning that they depend on the oncogene in order to survive. Therapies that block K-Ras

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D.M. Simeone and A. Maitra (eds.), *Molecular Genetics of Pancreatic Cancer*,
DOI 10.1007/978-1-4614-6549-2_5,

signaling could therefore potentially benefit almost all patients with PDA. Thirty years of study of the cell biology of K-Ras has yielded a wealth of information but an effective treatment for PDA is still elusive. This chapter describes how oncogenic K-Ras signaling is involved in almost every aspect of the initiation and progression of PDA from precursor lesions to metastatic disease. Oncogenic K-Ras induces numerous alterations that drive normal pancreatic cells to become invasive cancer cells and this provides many opportunities for therapeutic intervention. Understanding as much as possible about K-Ras signaling should allow us to determine where such intervention would prove the most effective in treating this disease.

The K-Ras Oncogene

Ras oncogenes were first identified in the 1970s as the sequences responsible for the transforming properties of the Harvey (Ha-MSV) and Kirsten (Ki-MSV) rodent tumor viruses. It was discovered that these recombinant retroviruses contained DNA sequences derived from the rat genome that encoded the *Hras* (Ha-MSV) and *Kras* (Ki-MSV) genes. Later *HRAS* became the first oncogene isolated from human cancer cells by its ability to transform NIH3T3 mouse fibroblasts (Shih and Weinberg 1982; Goldfarb et al. 1982; Pulciani et al. 1982). The transforming sequences from human tumor cells were found to be homologs of the viral *v-h-ras* and *v-k-ras* genes (Parada et al. 1982; Der et al. 1982; Santos et al. 1982).

Molecular cloning and sequencing showed that the oncogenes derived from tumor cells and the normal cellular *HRAS* and *KRAS* genes differed by only a single point mutation, most commonly in codons 12, 13, or 61 (Reddy et al. 1982; Tabin et al. 1982; Taparowsky et al. 1982; Santos et al. 1984). This information was used to screen a wide variety of different human tumors for the presence of oncogenic *RAS* mutations (Bos 1989). It is estimated that up to 30 % of human tumors contain *RAS* mutations making it the most frequently mutated oncogene in human cancer (Barbacid 1987).

There are three main isoforms of Ras proteins that are highly homologous. In addition to H-Ras and K-Ras, the third isoform N-Ras was identified after the other two isoforms as the transforming gene present in a neuroblastoma cell line (Shimizu et al. 1983a, b; Hall et al. 1983). K-Ras in fact exists as two alternatively spliced isoforms, K-Ras4A and K-Ras4B, which differ only in the sequence encoded by the fourth exon. K-Ras4B is considered to be the more relevant isoform to human cancer due to its more ubiquitous expression in tissues in both mice and humans (Pells et al. 1997; Plowman et al. 2006a), and due to the fact that a *KRAS* knockout mouse has an embryonic lethal phenotype (Johnson et al. 1997; Koera et al. 1997) whereas a targeted knockout of exon 4A has no phenotype (Plowman et al. 2003). However, because the oncogenic mutations in *KRAS* occur in the shared first and second exons, these mutations results in the production of oncogenic versions of both splice variants. Data regarding the importance of the K-Ras4A isoform *in vivo* is somewhat contradictory and more research is required to determine what role this

isoform may play in oncogenesis (Patek et al. 2008a, b; To et al. 2008; Plowman et al. 2006b; Abubaker et al. 2009).

The Ras proteins are prototypical small GTPases (Scolnick et al. 1979; Shih et al. 1980; Tamanoi et al. 1984; Temeles et al. 1985) that act as molecular switches cycling between an active GTP-bound and an inactive GDP-bound state (Field et al. 1987; Satoh et al. 1987). When GTP-bound, Ras can interact with downstream effectors involved in numerous cellular pathways that control cell growth, differentiation, and survival. The GTP/GDP cycle is controlled by guanine nucleotide exchange factors (GEFs) that activate Ras by promoting the release of GDP allowing the more abundant GTP to bind (Wolfman and Macara 1990), and GTPase activating proteins (GAPs) that dramatically accelerate the intrinsic rate of GTP hydrolysis, thereby inactivating Ras and curtailing signaling (Trahey and McCormick 1987). Oncogenic mutations in the Ras protein render it locked constitutively in the active GTP-bound state by reducing the intrinsic GTP hydrolysis rate and rendering the protein insensitive to the action of GAPs (McGrath et al. 1984; Sweet et al. 1984; Gibbs et al. 1984; Manne et al. 1985; Trahey and McCormick 1987).

A defining feature of Ras proteins is that they are peripheral membrane proteins that associate with cellular membranes by virtue of a series of posttranslational modifications (Wright and Philips 2006). The extreme C terminus of Ras ends with a "CaaX motif" in which C is a cysteine, "a" is generally an aliphatic residue and X is one of a number of amino acids (Fu and Casey 1999). This CaaX motif renders Ras a substrate for modification by farnesyltransferase, which catalyzes the addition of a 15-carbon farnesyl lipid to the cysteine of the CaaX motif (Schafer et al. 1989, 1990). Subsequently the aaX amino acids following the farnesylcysteine are cleaved off by a protease, Ras converting enzyme 1 (Rce1) (Boyartchuk et al. 1997; Freije et al. 1999; Otto et al. 1999). The α-carboxyl-group on the farnesylcysteine is then methylated by isoprenylcysteine carboxyl methyltransferase (Icmt) (Clarke et al. 1988; Gutierrez et al. 1989; Hrycyna et al. 1991; Pillinger et al. 1994). This methyl esterification neutralizes the negative charge of the carboxyl group and is therefore thought to increase the affinity of the farnesylcysteine for the plasma membrane by reducing the repulsion of the carboxyl group by the negatively charged head groups of the inner leaflet of the phospholipid bilayer (Hancock et al. 1991). Correct membrane association has been shown to be essential for both the biological and oncogenic functions of Ras proteins (Hancock et al. 1989; Gutierrez et al. 1989; Willumsen et al. 1984). Therefore, disrupting the addition of the modifications that enable Ras to associate with membranes has been seen as an attractive way to inhibit the function of oncogenic Ras in cancer (Downward 2003).

Oncogenic K-Ras Effector Pathways and Pancreatic Cancer

The exchange of the nucleotide bound to Ras from GDP to GTP results in a conformational change in the Ras protein that affects the affinity of binding to effector molecules (Ito et al. 1997; Geyer et al. 1996). Conformational changes in Ras occur

in two areas of the protein within the highly conserved GTPase domain termed switch I and switch II (Milburn et al. 1990). A Ras effector is defined as a protein that preferentially binds to the GTP-bound form of Ras. Effectors interact with Ras via a Ras-binding domain (RBD). While no sequence homology exists between RBDs from different effectors, they all share an ubiquitin superfold topology (ββαββαβ) (Nassar et al. 1995; Geyer et al. 1997; Walker et al. 1999). Oncogenic mutations in Ras, such as the substitution of valine or aspartic acid for glycine at codon 12 (G12V or G12D), render the protein constitutively GTP-bound because residues with a side chain in this position sterically interfere with the geometry of the transition state of GTP hydrolysis in the presence of GAPs (Scheffzek et al. 1997; Krengel et al. 1990; Tong et al. 1991). Mutation of the glutamine at position 61 is also oncogenic because this residue forms a hydrogen bond with the arginine at position 789 in GAP p120 (Scheffzek et al. 1997) and positions a catalytic water molecule for nucleophilic attack on the γ-phosphate of GTP (Buhrman et al. 2010; Scheidig et al. 1999), which is essential for GTP hydrolysis. These mutations therefore enable Ras to constitutively interact and activate downstream effectors. Thus, the oncogenic nature of Ras results from its ability to promote unchecked signaling down a variety of pathways that induce cell growth, proliferation, and survival.

The importance of Ras signaling in pancreatic cancer is highlighted by the fact that mutations in K-Ras are found extremely frequently in patient tumors. Early analysis of tumors revealed a prevalence of oncogenic mutations of K-Ras in pancreatic ductal adenocarcinoma (PDA) in excess of 90 % (Almoguera et al. 1988). However, recent evidence from analysis of the catalogue of somatic mutations in cancer (COSMIC) database (Forbes et al. 2011) suggests that the percentage of mutations in pancreatic cancer is 60 % (Prior et al. 2012). K-Ras mutations have been found to be present in early PanIN lesions and in surrounding areas of acinar-ductal metaplasia (ADM) (Shi et al. 2009; Kanda et al. 2012) consistent with the hypothesis that this mutation is an initiating event in PanIN formation. PDA is believed to originate from somatic mutations in *KRAS* during adulthood rather than during embryonic development. Indeed, although germ line mutations in *HRAS* and *KRAS* as well as other components of the downstream MAPK cascade have been found to be responsible for Noonan, LEOPARD, cardio-facio-cutaneous and Costello syndromes, that share similar features including facial abnormalities, heart defects, impaired growth and development, and, in some cases, cancer predisposition (Schubbert et al. 2007a, b), none of these syndromes appear to predispose to the development of PDA.

While the requirement for K-Ras signaling in pancreatic cancer is clear, what is not fully understood is what effector pathways downstream of Ras are necessary and sufficient to transmit its oncogenic signals. There are at least ten distinct functional classes of putative Ras effectors (Fig. 1) (Repasky et al. 2004). Raf-1 kinase was the first Ras effector to be discovered and remains the best characterized (Moodie et al. 1993; Warne et al. 1993; Zhang et al. 1993; Vojtek et al. 1993). The canonical pathway of Raf-1 activation occurs downstream of receptor tyrosine kinases (RTKs), such as the epidermal growth factor receptor (EGFR). When growth factors (such as EGF) bind to their cognate RTK, this induces dimerization

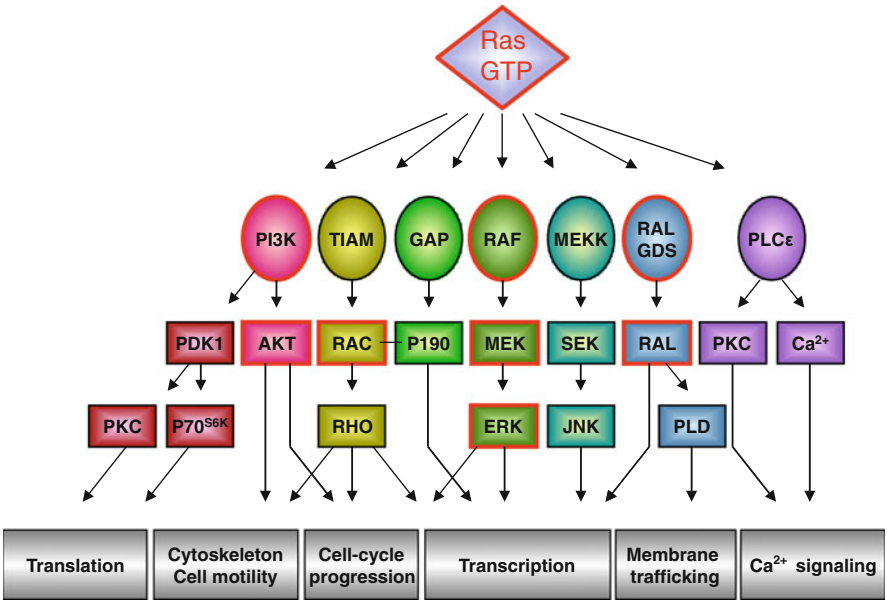

Fig. 1 Effector pathways downstream of oncogenic Ras stimulate many cellular processes. The signaling pathways shown have known or speculated roles in oncogenesis. Outlined in *red* are pathways involved in pancreatic cancer

and cross-phosphorylation of tyrosine residues in the cytosolic domain of the RTK (Schreiber et al. 1983; Ushiro and Cohen 1980; Yarden and Schlessinger 1987a, b; Zhang et al. 2006). The SH2 domain of the adapter protein Grb2 then binds to the phosphotyrosine residues in the RTK, and Grb2 in turn recruits the Ras GEF SOS to the plasma membrane via an SH3 domain in the Grb2 protein (Buday 1999). This recruitment enables SOS to interact with and activate Ras on the plasma membrane (Boriack-Sjodin et al. 1998). Ras-GTP is then able to bind and activate the effector Raf-1 by a mechanism that is not yet completely understood (Marais et al. 1995). Downstream of Raf-1 is the mitogen activated protein kinase (MAPK) cascade that includes MEK (MAPK/Erk kinase), Erk-1 and Erk-2. The Erk proteins are serine/threonine kinases with a variety of different substrates. Once phosphorylated, the Erk proteins form dimers that translocate into the nucleus where their substrates include proteins in the Ets family of transcription factors.

In addition to Raf-1, two other well-characterized effectors of Ras are phosphatidylinositol 3-kinase (PI3K) (Rodriguez-Viciana et al. 1994) and a group of exchange factors for the small GTPase Ral which includes RalGDS. PI3Ks are lipid kinases that phosphorylate the 3′ hydroxyl group of the inositol ring of phosphatidylinositol phosphates. Class 1A PI3Ks are activated downstream of RTKs and function primarily to generate the lipid second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP_3) by phosphorylating phosphatidylinositol-4,5-bisphosphate (PIP_2) in the plasma membrane. The presence of PIP_3 at the plasma membrane results

in the recruitment and activation of proteins containing plekstrin homology (PH) domains including serine/threonine kinases of the Akt family (Akt1, Akt2, and Akt3) and Pdk1 kinase (3-phosphoinositide-dependent kinase). Akt is activated by phosphorylation of two key residues, Thr308 by Pdk1 and Ser473 by the rapamycin-insensitive mammalian target of rapamycin complex 2 (mTORC2) (Sarbassov et al. 2005). Active Akt is able to phosphorylate a number of different downstream targets to control cell proliferation, survival, and metabolism. Notably, Akt activates the rapamycin-sensitive mTORC1 complex which results in the phosphorylation of p70 ribosomal protein S6 kinase 1 (S6K) and the eukaryotic initiation factor 4E binding protein 1 (4E-BP1), ultimately leading to an increase in protein synthesis (Inoki et al. 2002). PI3K signaling is antagonized by *PTEN*, a tumor suppressor gene encoding a phosphatase for PIP_3 (Li et al. 1997; Steck et al. 1997). RalGDS functions by acting as a GEF for the GTPases RalA and RalB. Effectors for Ral include components of the exocyst complex, which regulates vesicular trafficking and exocytosis (Moskalenko et al. 2002, 2003).

The Raf/MEK/Erk and PI3K pathways have the most well established roles in cancer development and progression. Mutations in the Raf isoform BRAF have been found to occur in 8 % of human cancers, most commonly in malignant melanomas (41 %), thyroid cancer (45 %) and colorectal cancer (14 %). A single base missense mutation that results in the replacement of valine for glutamic acid at codon 600 (V600E, previously described as V599E (Kumar et al. 2003)) in the activation segment of the kinase domain is responsible for at least 80 % of the BRAF mutations found in human cancer (Davies et al. 2002). The kinase activity of this mutant is greatly elevated; it is able to potently transform NIH3T3 cells and constitutively stimulates Erk activity *in vivo* independent of RAS. Gain-of-function mutations in the catalytic subunit of PI3K p110 (PI3KCA) also occur frequently in cancer. These mutations increase enzymatic function, enhance downstream signaling elements and promote oncogenic transformation (Kang et al. 2005; Samuels et al. 2005). However, mutations in effectors downstream of Ras are infrequent in pancreatic cancer, presumably because the pathways are sufficiently activated through oncogenic Ras signaling. *BRAF* mutations are rare in pancreatic cancer (Jones et al. 2008). They have been reported to occur in tumors that also had a K-Ras mutation with a frequency of around 10 % (Ishimura et al. 2003). Mutations in *PI3KCA* have been found to occur in 9 % of patients with PDA (Janku et al. 2011). Amplifications and overexpression of *AKT2* were found in 10-20 % of pancreatic cancer cell lines and tumors (Cheng et al. 1996; Ruggeri et al. 1998). *EGFR* mutations are also rare, occurring in less than 3 % of patients, but have also been found to coexist with K-Ras mutations (Oliveira-Cunha et al. 2012). Point mutations in the tumor suppressor *PTEN* are infrequently found in pancreatic cancer but functional inactivation of the gene occurs commonly by promoter methylation or inhibition of protein or mRNA synthesis (Ebert et al. 2002; Altomare et al. 2002; Asano et al. 2004).

EGFR genomic amplifications and overexpression are a common event in pancreatic cancer (Tzeng et al. 2007; Bloomston et al. 2006; Tobita et al. 2003; Fjallskog et al. 2003), as is expression of some of its ligands (Kobrin et al. 1994; Zhu et al. 2000). This observation is a little surprising as activating mutations in K-Ras, being

downstream, would be expected to a certain degree to circumvent the requirement for EGFR signaling. This appears to be the case in some other tumor types such as non small cell lung cancers where mutation in *KRAS* and *EGFR* are mutually exclusive (Shigematsu et al. 2005). It has been suggested that signaling through EGFR may still be necessary in the presence of oncogenic K-Ras to activate the other isoforms of Ras and also possibly any remaining wild type alleles of K-Ras (Ardito et al. 2012). In contrast, there are also studies that demonstrate a selective loss of the wild type allele of K-Ras in human tumors. Mutant allele specific imbalance, which can occur by either copy number gains or uniparental disomy, was found in 58 % of tumors including pancreatic cancers (Soh et al. 2009). Similar findings have also been found in mouse models (Qiu et al. 2011) and there is a growing body of evidence that suggests the wild type allele of K-Ras may function as a tumor suppressor (Zhang et al. 2001; Li et al. 2003, 2007; Hegi et al. 1994; Bremner and Balmain 1990).

Despite there being no known activating mutations found in the Ral pathway in cancer, it has been suggested that in human cells the Ral pathway may be the most important pathway downstream of Ras for cellular transformation (Hamad et al. 2002; Rangarajan et al. 2004). RalGDS appears to be required for the survival of Ras transformed cells in a mouse model (Gonzalez-Garcia et al. 2005). The two main substrates of RalGDS appear however to have different roles in oncogenesis. Ectopically expressed RalA is transforming and is required for K-Ras^{G12V} transformation, whereas RalB impedes transformation (Lim et al. 2005). However, RalB was found to be required for invasion and metastasis of two pancreatic cancer cell lines *in vivo* (Lim et al. 2006). In addition to this, RalA was found to be activated in a panel of pancreatic cancer cell lines (Lim et al. 2005) and both RalA and RalB were more frequently activated in pancreatic tumor samples than either Erk or Akt (Lim et al. 2006).

Mouse Models of Oncogenic K-Ras Driven Pancreatic Cancer

The importance of oncogenic K-Ras mutations in pancreatic cancer initiation and maintenance has now been verified with several mouse models. In 2003, David Tuveson utilized a mouse harboring a conditional oncogenic allele of K-Ras^{G12D} under the control of the endogenous K-Ras promoter (Jackson et al. 2001). Expression of the oncogene was blocked by a STOP element flanked by LoxP sites upstream of the gene. Crossing of the *Lox-STOP-Lox-KRAS*^{G12D} mouse (*LSL-KRAS*^{G12D}) to mice containing *Cre* recombinase under the control of pancreas specific promoters (*PDX-1-Cre* and *p48-Cre*) allowed for recombination of the STOP element and expression of the oncogene in a pancreas-specific manner (Hingorani et al. 2003). This was the first example of the expression of oncogenic K-Ras from its endogenous locus in a mouse model of pancreatic cancer. These animals showed a phenotype that recapitulated the progression of human pancreatic ductal adenocarcinoma from early stage PanIN lesions to invasive metastatic disease. This result was important because it helped to confirm the PanIN progression model that had

been put forward from examination of human specimens (Brat et al. 1998; Hruban et al. 1999; Maitra et al. 2003). In addition, the result was groundbreaking because previous attempts to develop mouse models that targeted K-Ras^{G12D} to the pancreas with a variety of different pancreas-specific promoters (e.g., cytokeratin-19, Elastase, Mist1) had failed to produce pancreatic lesions that resembled those seen in human PDA (Brembeck et al. 2003; Grippo et al. 2003; Tuveson et al. 2006).

The *LSL-KRAS^{G12D};PDX-1-Cre/p48-Cre* mouse models have been subsequently combined with a variety of different floxed, loss of function and dominant negative alleles of tumor supressors (Hingorani et al. 2005; Izeradjene et al. 2007; Bardeesy et al. 2006; Aguirre et al. 2003; Vincent et al. 2009). The rapidly accelerated disease progression in these models helps confirm the hypothesis that tumor suppressor genes such as p53, p16^{INK4A}, and Smad4 help keep oncogenic K-Ras-driven neoplasia in check.

These studies provided compelling evidence that K-Ras^{G12D} is required for PanIN formation; however, the requirement for PanIN progression and PDA maintenance had not been tested. To address this question, a mouse was created that contained an oncogenic allele of K-Ras that could be turned on or off by the administration or removal of doxycycline in the drinking water of adult mice (*p48-Cre;R26-rtTa-IRES-EGFP;TetO-Kras^{G12D}*, referred to as iKras) (Collins et al. 2012). Removal of doxycycline from these animals after 23 weeks of K-Ras^{G12D} expression resulted in an almost complete reversion of PanINs after 2 weeks and a regeneration of the acinar cell compartment. Similar results were also observed when K-Ras^{G12D} was expressed for 3 weeks with concomitant cerulein treatment to induce pancreatitis (see next section). PanIN reversion was associated with a down-regulation in phospho-Erk1/2 levels. Surprisingly, however, switching off oncogenic K-Ras expression did not cause an increase in apoptotic cells as shown by staining for cleaved caspase-3. Instead, loss of PanIN and acinar regeneration appeared to occur by a process of ductal-acinar metaplasia (DAM), as cells co-expressing the acinar cell marker amylase and the ductal maker cytokeratin-19 were frequently observed. However, if K-Ras^{G12D} expression was induced for 5 weeks with concomitant cerulein treatment, while removal of doxycycline resulted in PanIN regression there was an incomplete regeneration of the acinar cell compartment leaving a small fibrotic pancreas with fewer acini than expected. In these pancreata there was a dramatic increase in apoptotic cells upon doxycycline removal suggesting that either the regenerative capability of the pancreas decreases with the age of the mice or that more advanced stage PanIN lesions are not able to undergo DAM. Importantly, iKras mice crossed with p53 null mice produced disease that progressed to PDA and doxycycline removal resulted in complete regression of all tumors (Collins et al. 2012; Ying et al. 2012).

In some of these models, such as the *LSL-KRAS^{G12D};p48-Cre* model (Hingorani et al. 2003), K-Ras^{G12D} expression occurs in every cell of the pancreas raising the question of why some cells undergo neoplastic transformation while other cells remain normal. This observation led to speculation as to what is the precise cell of origin of the PanINs observed. Although PanINs have an obvious ductal morphology it is possible that they arise from another cell type by a process of transdifferentiation.

One study sought to address this question by targeting K-Ras^{G12D} expression to different cell types in the adult pancreas using *Cre* drivers with different expression patterns (Gidekel Friedlander et al. 2009). Expression in *Pdx1*$^{+}$ cells, which includes adult endocrine β cells, some ductal cells, acinar cells, and possibly adult progenitor/stem cells induced transformation resulting in PanIN formation. However, *proCPA1*$^{+}$ cells were not efficiently transformed by K-Ras^{G12D}. *ProCPA1* encodes for the pancreas specific pro-carboxypeptidase A expressed mostly in acinar cells and possibly some centroacinar cells. The same result was observed for *insulin*$^{+}$ cells. These results suggest that a *Pdx1*$^{+}$ cell is the most likely cell of origin for PDA. However, *insulin*$^{+}$ cells of the endocrine lineage of the adult pancreas were able transdifferentiate and give rise to PDA under certain conditions, highlighting the plasticity of the pancreas and complicating the question of the cell of origin in human PDA.

The difference in the efficiency of transformation of different cell types in the pancreas could occur because the threshold of Ras signaling required to transform is higher in some cells relative to others. One study showed that expression of a K-Ras^{G12D} transgene in adult acinar cells at higher levels than from the endogenous promoter was sufficient to induce PanINs that progressed to PDA whereas endogenous levels of expression was not. This study found higher levels of active Ras in pancreatic tumor samples than in untransformed areas of pancreas expressing K-Ras^{G12D} from the endogenous promoter suggesting that upregulation of Ras activity is necessary to bypass a transformation barrier in the pancreas (Ji et al. 2009). However, interpretation of these results is hindered by the in vitro assay used to determine the amount of active Ras that may not fully reflect the level of Ras signaling in intact cells. Two recent studies highlighting the importance of EGFR in the development of K-Ras driven pancreatic cancer lend some credence to this hypothesis (Navas et al. 2012; Ardito et al. 2012). EGFR was found to be required for pancreatitis-dependent acinar cell-derived tumorigenesis and ADM following cerulein treatment both *in vivo* and *in vitro*. One of these studies implicated Erk activation downstream of EGFR signaling in this process, implying that the signaling downstream of K-Ras^{G12D} alone was insufficient to transform cells whereas in combination with signaling through EGFR, a critical threshold could be reached to promote neoplasia (Ardito et al. 2012). However, a second study instead implicated signaling through Akt and Stat3 downstream of EGFR (Navas et al. 2012). Both studies agreed that mutations in p53 bypassed the requirement for EGFR signaling in tumor development, which may explain why the EGFR inhibitor erlotinib has shown poor efficacy when combined with gemcitabine in clinical trials (Moore et al. 2007). A third study showed that concomitant expression of TGFα, a ligand for EGFR, and K-Ras^{G12D} accelerates the progression of PanIN lesions in a *p48-Cre;LSL-KRAS*^{G12D} mouse model (Siveke et al. 2007), suggesting that signaling through EGFR in combination with oncogenic K-Ras signaling may indeed help to bypass a transformation barrier in the pancreas.

Mouse models have also been used to address the question of which pathways downstream of Ras are the most important for malignancy. Upregulation of nuclear phospho-Erk (pErk) staining downstream of K-Ras^{G12D} expression is an early feature of mouse PanIN lesions, whereas normal pancreatic tissue is negative for pErk

staining (Ijichi et al. 2006; Guerra et al. 2007). However, paradoxically some cell lines and tumor samples have low levels of pErk due to a negative feedback mechanism involving MAPK phosphatase 2 (Yip-Schneider et al. 1999, 2001). Activation of Akt has been found in up to 59 % of tumor samples (Altomare et al. 2002; Schlieman et al. 2003; Yamamoto et al. 2004). High levels of both pErk and phospho-Akt (pAkt) have been associated with reduced survival in patients following surgical resection (Chadha et al. 2006). Recently it has been shown that expression of $BRAF^{V600E}$, but not $PI3KCA^{H1047R}$, in the adult mouse pancreas can induce PanIN formation (Collisson et al. 2012), and when combined with gain-of-function $p53^{R270H}$ the PanINs progress to PDA. However, a pancreatic specific deletion of *PTEN* during embryogenesis in mice did result in the formation of some PanINs and papillary ductal adenocarcinomas in a subset of animals (Stanger et al. 2005), and was able to synergize with $K\text{-}Ras^{G12D}$ to accelerate the development of PDA (Hill et al. 2010). Rac1 is another small GTPase that is activated downstream of oncogenic Ras, either via PI3K signaling or via the Ras effector Tiam, and is a key component in the reorganization of the actin cytoskeleton induced by Ras oncogenes (Bar-Sagi and Feramisco 1986; Ridley et al. 1992; Qiu et al. 1995; Nimnual et al. 1998; Rodriguez-Viciana et al. 1997; Lambert et al. 2002). Active Rac1 functions to induce actin polymerization, and its overexpression has been detected in human patient samples of pancreatic cancer (Crnogorac-Jurcevic et al. 2001). Rac1 has long been found to be to be required for Ras transformation and recently conditional loss of *Rac1* in the pancreas was found to impair PanIN formation, early metaplastic changes and neoplasia-associated actin rearrangements in the *LSL-$KRAS^{G12D}$;p48-Cre* mouse model (Heid et al. 2011). It was suggested that Rac1 may be required for F-actin rearrangements that take place during the ADM that precedes PanIN formation in this mouse model (Bi et al. 2005), and the PanINs that form in the absence of Rac1 may develop from an alternative cell type that does not require ADM (Heid et al. 2011).

Oncogenic K-Ras and Pancreatitis

Chronic pancreatitis is a significant risk factor for PDA in humans (Lowenfels et al. 1999), which suggests that inflammation plays a role in the progression of the disease. Mouse models have been used to show that inflammation can act synergistically with oncogenic $K\text{-}Ras^{G12D}$ in driving carcinogenesis. Cerulein is an analog of cholecystokinin which, when administered to rodents in supraphysiologic doses, stimulates the premature intracellular activation of pancreatic digestive enzymes, which causes tissue damage resulting in pancreatitis (Lampel and Kern 1977; Watanabe et al. 1984; Ohshio et al. 1989; Silverman et al. 1989; Niederau et al. 1985). Cerulein-induced acute pancreatitis is a well-studied animal model that has been used to examine the effect of acute pancreatitis on PanIN progression in the *LSL-$KRAS^{G12D}$;PDX-1-Cre* mouse model (Carriere et al. 2009). Two brief episodes of acute pancreatitis were sufficient to accelerate pancreatic cancer development.

Thus, a brief inflammatory insult to the pancreas, when occurring in the context of oncogenic K-Ras^{G12D}, can enhance pancreatic malignant transformation.

Interestingly it has been shown that turning on K-Ras^{G12D} expression in adult pancreatic cells of mice or rats fails to induce the development of PanINs or PDA without concomitant or previous treatment with cerulein to induce pancreatitis (Guerra et al. 2007, 2011; Tanaka et al. 2010; Habbe et al. 2008; De La et al. 2008), whereas K-Ras^{G12D} expression during embryogenesis or early adulthood alone is sufficient to induce PanINs that are able to progress to PDA (Guerra et al. 2007). Thus, these studies in mouse models suggest that adult cells of the exocrine pancreas may be refractory to transformation by oncogenic K-Ras and that pancreatitis produces a permissive environment that enhances transformation.

Although the molecular mechanism underlying the cooperation between oncogenic K-Ras and pancreatitis remains to be established, one hypothesis is that pancreatic injury may induce a trans-differentiation or de-differentiation of cells to a less mature differentiated state similar to an embryonic progenitor cell that is more permissive to transformation. Cerulein treatment strongly induces ADM in the regenerating pancreas and could represent such a trans-differentiation event (Willemer et al. 1987). Pancreatitis and pancreatic regeneration have been found to induce expression of genes normally associated with undifferentiated pancreatic progenitor cells such as Sox9, Pdx1, E-cadherin, β-catenin, Notch components and Hedgehog components (Jensen et al. 2005; Fendrich et al. 2008; Sharma et al. 1999; Yoshida et al. 2008; Siveke et al. 2008). However, in a wild type pancreas, this response and the ADM observed is transient and the acinar cells rapidly regenerate. Somehow oncogenic K-Ras signaling seems to alter the fate of the regenerating cells so that they form PanINs instead of acini. Consistent with this, many of these pathways associated with the progenitor cell population remain active in PanINs and PDA including Sox9 (Prevot et al. 2012) and Notch (Miyamoto et al. 2003; Hingorani et al. 2003). Despite overwhelming evidence that oncogenic K-Ras signaling and inflammation synergize to promote pancreatic cancer development, there is some controversy regarding the contribution that cellular senescence plays in this process. It has been suggested that K-Ras^{G12D} expression in early PanINs either promotes oncogene-induced senescence that can be relieved by limited episodes of pancreatitis (Guerra et al. 2011), or inhibits senescence induced in normal ductal cells by pancreatitis (Lee and Bar-Sagi 2010). It remains to be seen what is the reason for these differences but it is possible that the age of animals used or the stage of PanINs observed could account for such discrepancies.

Oncogenic K-Ras and Developmental Reprogramming

It is not uncommon for tumors to display a reactivation of embryonic signaling pathways that are essential for development, such as the Notch, Hedgehog, and Wnt pathways. Indeed, pancreatic cancer exhibits several examples of this. Upregulated expression of Notch receptors and ligands has been observed in human

pancreatic cancer samples as has expression of the Notch target gene Hes1, which is usually restricted to centroacinar cells in the normal pancreas (Miyamoto et al. 2003). Aberrant cytoplasmic and nuclear expression of β-Catenin has been observed in human PanIN and PDA (Al-Aynati et al. 2004; Lowy et al. 2003), and canonical Wnt signaling has been found to be active in pancreatic cancer cell lines (Pasca di Magliano et al. 2007). Additionally, sonic hedgehog is abnormally expressed in pancreatic adenocarcinoma and PanINs (Thayer et al. 2003). The functional relationships between oncogenic K-Ras and these pathways have therefore been a subject of great interest. Activation of the Notch pathway by expression of the Notch1 intracellular domain (NICD) in adult acinar cells has also been found to synergize with oncogenic K-Ras expression in the pancreas to accelerate PanIN progression (De La et al. 2008). In contrast, another study suggested that Notch1 functions as a tumor suppressor in the mouse pancreas (Hanlon et al. 2010). One explanation for these differing results could be due to the difference in timing of the Notch activation and loss in these models being either in adulthood or during embryonic development. These pathways are extremely complex and changes in the specific roles or activity level of individual components or alterations in the balance of activity of components could have unpredictable effects. Notch signaling inhibits progenitor cell differentiation in the embryonic pancreas (Hald et al. 2003), so reactivation of Notch signaling may function to induce a more embryonic-like state in the pancreas that can synergize with K-Ras to enhance transformation. However, it is as yet unclear the precise role Notch signaling plays in pancreatic cancer development and progression, be it oncogenic or tumor suppressive. Another developmentally important pathway that is reactivated in pancreatic cancer is the Wnt pathway. Despite this, stabilized β-catenin was found to impair K-Ras^{G12D} induced PanIN development following cerulein-induced pancreatitis in mice. In contrast β-catenin signaling was found to be important for acinar cell regeneration following cerulein-induced pancreatitis: a *p48-Cre;*β-*catenin*$^{flx/flx}$ mouse was found to have a significant decrease in the acinar cell area 3 and 5 days following cerulein treatment (Morris et al. 2010). This suggests that oncogenic K-Ras signaling may function to suppress a β-catenin-driven acinar cell regeneration program in favor of neoplastic transformation and PanIN formation and emphasizes how important the timing of pathway activation may be. Hedgehog ligands secreted from pancreatic cancer cells seem to have an important role in paracrine signaling to the adjacent stroma (Tian et al. 2009). Autocrine signaling which occurs via secreted sonic hedgehog binding to the 12 trans-membrane domain receptor Patched (Ptch), resulting in the activation of the Smoothened (Smo) seven trans-membrane domain protein, does not appear to be required for PDA development in mice. Despite this, expression of the downstream target Gli1 is required for survival of mouse and human pancreatic cancer cell lines (Nolan-Stevaux et al. 2009). In contrast to the stroma, Gli expression in mouse PDA cells may depend on K-Ras signaling in a Smo independent manner, as depleting 80 % of K-Ras expression with *Kras*-targeted siRNAs resulted in a significant down-regulation of the Gli1 and Ptch1 mRNAs in PDA lines.

Oncogenic K-Ras and the Tumor Microenvironment

The microenvironment surrounding tumor cells consists of other cell types, soluble factors, signaling molecules, extracellular matrix, and mechanical cues (Swartz et al. 2012). It is becoming increasingly apparent how specific interactions with the microenvironment affect all aspects of tumor biology. In pancreatic cancer there is increasing evidence that the inflammatory response to tissue damage following pancreatitis synergizes with oncogenic K-Ras and promotes cancer development (Fig. 2). An abundant desmoplastic stroma is one of the characteristic histological features of PDA (Chu et al. 2007; Neesse et al. 2011; Korc 2007; Mahadevan and

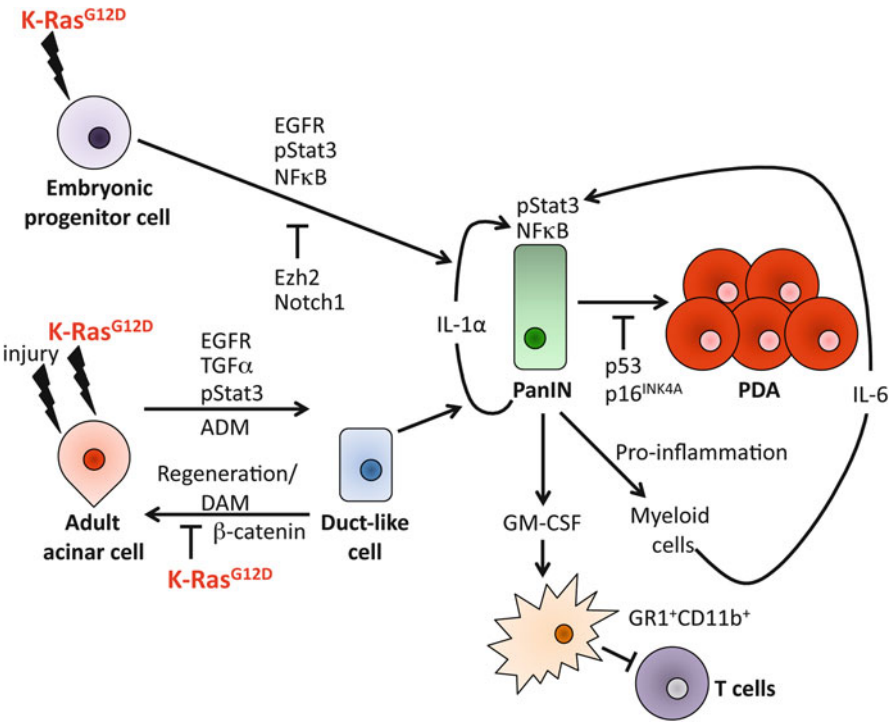

Fig. 2 Oncogenic K-Ras and injury in the form of pancreatitis synergize to induce development of PanINs that progress to PDA. If K-Ras^{G12D} is expressed during embryogenesis in an as yet unidentified progenitor cell, PanINs form that progress to PDA with a long latency but do not require pancreatic injury. This process may or may not proceed through ADM. However, K-Ras^{G12D} expression in adult acinar cells requires pancreatitis to develop into PDA. Injury induces ADM in the pancreas and K-Ras^{G12D} signaling diverts the metaplastic cells away from regenerative expansion of the acinar cell population in favor of PanIN formation. PanINs promote expression and activation of inflammatory mediators including GM-CSF, NFκB, Stat3, IL-6, IL-1α, and Cox2, which further synergize with K-Ras^{G12D} signaling and promote an immunosuppressive environment, which allows progression to PDA. Expression of tumor suppressors such as p53 and p16^{INK4A} is frequently lost during this progression

Von Hoff 2007). The desmoplastic stroma consists of extracellular matrix (ECM), activated fibroblasts, inflammatory cells and tumor vasculature. Importantly, K-Ras^{G12D} expression in the pancreas in mouse models also induces a desmoplastic response that is found in association with PanINs and areas of PDA (Hingorani et al. 2003, 2005). Cyclooxygenase-2 (Cox-2) promotes inflammation, and the expression of Cox-2 has been found to be upregulated in human PanINs and PDA (Maitra et al. 2002; Albazaz et al. 2005). Additionally, an anti-inflammatory selective Cox-2 inhibitor has been found to delay PanIN progression in the *PDX-1-Cre;LSL-KRAS^{G12D}* mouse model (Funahashi et al. 2007). Recently, the pro-inflammatory NF-κB pathway has been shown to be required for PDA development in the *PDX-1-Cre;LSL-KRAS^{G12D}* mouse model (Maniati et al. 2011; Ling et al. 2012) as conditional deletion of *IKK2* in the pancreas was found to inhibit both PanIN progression and K-Ras^{G12D} induced inflammatory responses. NF-κB is constitutively activated in human pancreatic adenocarcinoma and human pancreatic cancer cell lines but not in normal pancreatic tissues (Wang et al. 1999; Fujioka et al. 2003). Oncogenic K-Ras^{G12D} expression in the pancreas has been shown to induce expression of IL-1α, which in turn results in constitutive activation of NF-κB (Ling et al. 2012). There is also some evidence to suggest that an NF-κB-mediated positive feedback loop is able to further enhance oncogenic Ras signaling (Daniluk et al. 2012).

The protein signal transducer and activator of transcription 3 (Stat3) is another inflammatory mediator that is aberrantly activated in human PDA (Scholz et al. 2003). Activation and phosphorylation of Stat3 was found to be transiently induced by acute cerulein treatment in the mouse pancreas and this pStat3 persisted in PanINs following cerulein treatment in pancreata that expressed oncogenic K-Ras^{G12D} (Fukuda et al. 2011). The observed pattern of pStat3 staining by IHC was found to correlate with expression of IL-6, a known activator of Stat3 downstream of Ras signaling (Ancrile et al. 2007). An increase in IL-6 mRNA was found in pancreata expressing K-Ras^{G12D} and the source of IL-6 was found to be infiltrating macrophages (Lesina et al. 2011). Treatment of K-Ras^{G12D} expressing pancreatic acinar cells with an IL-6R/IL-6 complex but not IL-6 alone was able to induce phophorylation of Stat3 however, implying IL-6 transsignaling rather than classical IL-6 signaling (Lesina et al. 2011). Pancreatic Stat3 deletion in a *Stat3$^{flx/flx}$* mouse ameliorated both spontaneous and pancreatitis-induced PanIN formation in the *PDX-1-Cre;LSL-KRAS^{G12D}* mouse model and the PanIN formed in the absence of Stat3 displayed reduced inflammatory infiltrates (Corcoran et al. 2011; Fukuda et al. 2011). Similar results were seen in an IL6$^{-/-}$ mouse strain (Lesina et al. 2011). Consistent with this, Stat3 deficient acini were found to secrete less cytokines and inflammatory mediators that are known Stat3 target genes in response to cerulein in vitro. Knockdown of *Stat3* in mouse pancreatic cancer cells dramatically reduced PDAC formation compared with control shRNA following orthotopic injection into syngenic recipient mice (Corcoran et al. 2011). Stat3 signaling has also been implicated in controlling expression of matrix metalloproteinase 7 (MMP7), which has been found to be associated with metastatic disease in both humans and mouse models (Fukuda et al. 2011). This evidence all suggests that inflammation plays an important role in the progression from PanIN to PDA.

Recently oncogenic K-Ras signaling in the pancreas has been found to modulate the immune response in order to evade immune surveillance (Clark et al. 2007). The extensive stromal reaction surrounding PanINs and areas of PDA may provide an immunosuppressive environment that protects the transformed cells from T cells. Oncogenic K-Ras expressing PDECs, PanINs and PDA have been found to express GM-CSF (Pylayeva-Gupta et al. 2012; Bayne et al. 2012), which has been implicated in the regulation of proliferation and maturation of putative immunosuppressive $Gr1^{+}CD11b^{+}$ myeloid cells (Barreda et al. 2004) that have been implicated in tumor-induced immune tolerance (Dolcetti et al. 2010; Bronte et al. 1999; Gabrilovich and Nagaraj 2009; Marigo et al. 2010). $K\text{-}Ras^{G12D}$ expressing PDECs and cancer cells were found to induce the differentiation of progenitor $Gr1^{-}CD11b^{-}$ cells to $Gr1^{+}CD11b^{+}$ cells that were able to inhibit the proliferation of $CD3^{+}$ splenic T cells, and knockdown of GM-CSF in PDECs was found to both inhibit growth when engrafted into a wild type pancreas and increase the accumulation of $CD8^{+}$ cytotoxic T cells into the pancreas (Pylayeva-Gupta et al. 2012; Bayne et al. 2012).

To confirm that inflammation in the pancreas promotes PDA, conditional knockout animals that have impaired regeneration of the pancreas following cerulein-induced injury have been found to display accelerated PanIN progression. It has been shown that Ezh2, a polycomb group protein and a member of the polycomb repressor complex 2, is transiently upregulated during pancreatic regeneration, where it functions to suppress expression of $p16^{INK4A}$ and thereby promote cellular proliferation and regeneration. In the absence of pancreatic Ezh2, regeneration is impaired and the pancreas has a reduced ability to resolve cerulein-induced inflammation. The ability of Ezh2 to inhibit expression of $p16^{INK4A}$ makes it a good candidate for a tumor suppressor gene. However, loss of Ezh2 in the pancreas accelerated PanIN progression in the *p48Cre;LSL-KRAS*^{G12D} model (Mallen-St Clair et al. 2012). Thus, genetic alterations that enhance the inflamed state of the pancreas following damage are able to accelerate oncogenesis.

Oncogenic K-Ras and Pancreatic Cancer Cell Metabolism

One area of tumor biology that is receiving a lot of recent interest is alterations in metabolic pathways seen in cancer cells compared to normal cells. The Warburg effect was an observation made in the 1920s that under aerobic conditions, tumor tissues metabolize approximately tenfold more glucose to lactate in a given time than normal tissues (Warburg et al. 1924; Minami 1923). That is, the Pasteur effect, which is the inhibition of fermentation by oxygen, tends not to apply in tumor cells. Aerobic glycolysis is not an efficient method of producing ATP so there has been much confusion and debate regarding the advantages upregulating this pathway might have to cancer cells. It has been suggested that the Warburg effect occurs because proliferating cancer cells require not only ATP but also an abundant quantity of NADPH and macromolecular precursors needed to generate new cells such

as acetyl-CoA for fatty acids, glycolytic intermediates for nonessential amino acids, and ribose for nucleotides (Vander Heiden et al. 2009). Oncogenic Ras has been shown to promote glycolysis (Yun et al. 2009; Racker et al. 1985) and pancreatic cancer cells have been found by proteomic analysis to have increased expression of glycolytic enzymes (Zhou et al. 2011, 2012) compared to normal ductal cells. Recently the iKras p53 null mouse has been used to study the effects of oncogenic K-Ras on cancer cell metabolism in the pancreas (Ying et al. 2012). Withdrawal of K-Ras^{G12D} expression was found to significantly affect intermediates in glucose metabolism including glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), and fructose-1,6-bisphosphate (FBP), as determined by targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) metabolomic studies. This was accompanied by a decrease in glucose uptake and lactate production and down-regulation of expression of genes for glucose transporters and rate-limiting glycolytic enzymes. As expected, steady-state metabolite profiling and other methods showed that these changes in glycolytic flux were associated with a decrease in several intermediates of biosynthetic pathways such as hexosamine biosynthesis, protein glycosylation and ribose biogenesis through the nonoxidative arm of the pentose phosphate pathway. The effects observed of removal of K-Ras^{G12D} were recapitulated by treatment with the MEK inhibitor AZD8330, highlighting the importance of MAPK signaling downstream of K-Ras in this phenomenon.

Autophagy is a process that mediates the lysosomal degradation of cytoplasmic components such as damaged organelles and unused proteins. It is a vital contributor to cellular metabolism as it provides nutrients from internal sources when external sources are limited. Autophagy is considered to be a programmed pro-survival mechanism and therefore has a pro-tumor effect. However, there is some evidence to suggest that under certain conditions an "autophagic cell death" pathway may come into play to limit tumor growth (Levine and Yuan 2005; Hippert et al. 2006). It is known that pancreatic cancers have elevated levels of autophagy under basal conditions, despite the presence of abundant nutrients, and this has been correlated with poor outcome (Fujii et al. 2008; Yang et al. 2011). Also, genetic and chemical inhibition of autophagy was able to suppress the growth of pancreatic cancer cells *in vitro* and induce tumor regression in both pancreatic cancer xenografts and genetic mouse models (Yang et al. 2011). Data suggest that oncogenic Ras expression alters the requirement for autophagy within a cell and this may be attributable to an increase in the need for autophagic substrates for mitochondrial metabolism to preserve mitochondrial function (Guo et al. 2011). Another study suggested that the requirement for autophagy for the optimal growth and survival of K-Ras transformed cells was to impair mitochondrial respiration by mitophagy thereby facilitating the induction of the Warburg effect (Kim et al. 2011). This hypothesis is supported by studies which show a reduction in glucose metabolism in autophagy deficient MEFs (Lock et al. 2011) and that knockdown of K-Ras in a pancreatic cancer AsPC-1 cell line resulted in increased expression of mitochondrial genes (Ohnami et al. 1999).

K-Ras Signaling *In Vitro*

While the majority of insight into the role of K-Ras signaling in pancreatic cancer development and progression has been garnered from *in vivo* studies using mouse models, there is a significant contribution from *in vitro* experiments utilizing established pancreatic cancer cell lines and RNAi technology. The concept of oncogene addiction suggests that cancer cells become dependent on signaling from one particular oncogene in order to survive. Knocking down K-Ras has been found to induce apoptosis in pancreatic cancer cell lines in agreement with this model (Fleming et al. 2005). The extent of addiction to K-Ras signaling has been thoroughly tested in a panel of pancreatic cancer cell lines containing K-Ras mutations. Surprisingly the effect of knocking down K-Ras in these cell lines was found to vary significantly with some of the cell lines tested having very little dependency on K-Ras. Many of the K-Ras-dependent cells contained *KRAS* genomic amplifications, exhibited a classic epithelial morphology, and expressed E-Cadherin, whereas most K-Ras-independent cells appeared less uniformly epithelial and expressed little or no E-cadherin, suggesting that they may have undergone an epithelial to mesenchymal transition (EMT). From this study it was possible to identify a gene expression signature that can be used to accurately predict the K-Ras dependency of tumors in different tissue types (Singh et al. 2009). Such signature could prove useful in the future to predict what patients would benefit from therapies that target the Ras signaling pathway.

In one recent study a high-throughput loss-of-function RNAi screen was carried out to find genes with synthetic lethal interactions with oncogenic K-Ras, where knockdown of the gene would affect the viability of cell lines with oncogenic K-Ras mutations but not those without (Scholl et al. 2009). The screen was carried out with a panel of cell lines both with and without K-Ras mutations including the pancreatic cell lines Panc-1 that contains a $K\text{-}Ras^{G12D}$ mutation and BxPC3 that is wild type for K-Ras. The screen identified *STK33*, a putative member of the calcium/calmodulin-dependent protein kinase subfamily of serine/threonine protein kinases. Knockdown of *STK33* in Panc-1 cells impaired colony formation in semisolid medium and decreased their ability to form tumors in immunocompromised mice but had no effect on BxPc3 cells. Despite the apparent importance of *STK33* in these cancer cell lines, no amplifications of the gene or significant increases in gene expression were observed in cell lines with oncogenic K-Ras mutations. Knockdown of *STK33* was also found to decrease the phosphorylation of S6K1 serine/threonine protein kinase and its downstream substrate RPS6 in an oncogenic K-Ras dependent manner. There is evidence to suggest that this pathway may be involved in controlling apoptosis via the proapoptotic BH3-only protein BAD which is known to be phosphorylated and inactivated by S6K1 resulting in an inhibition of mitochondrial apoptosis (Scholl et al. 2009; Azoitei et al. 2012). Subsequent studies targeting STK33 both by siRNA and inhibitors in K-Ras mutant cancer cells were unable however to confirm the observed synthetic

lethality (Babij et al. 2011; Luo et al. 2012). These discrepancies highlight the drawback to using siRNAs, where the risks for off-target effects and false positive results are high and the need for these studies to be carefully controlled.

Conclusions

Oncogenic K-Ras and several of its downstream effector pathways have been shown to have essential roles in all aspects of pancreatic cancer initiation, progression, invasion, and metastasis. The evidence suggests that any pharmacological agents able to completely block K-Ras signaling in pancreatic cancer should result in significant tumor shrinkage and cell death and therefore have a significant clinical impact on a disease that is so refractory to all currently available treatments. Despite substantial effort, all attempts to therapeutically target the mutated Ras protein directly with small molecules that could promote the hydrolysis of GTP have been unsuccessful. Therefore, the focus of drug discovery has concentrated on either downstream components of the Ras signaling pathway or the upstream pathway involved in the posttranslational modification of the Ras protein. Effective inhibitors specific for many of the key components of the Ras/Raf/MEK/Erk and Ras/PI3K/PTEN/mTOR pathways have been developed. Some, such as the orally available MEK1 inhibitor Selumetinib, have been tested in phase I and phase II clinical trials (Chappell et al. 2011). However, there are many more pathways downstream of Ras than just these two, and it is as yet unclear the specific importance of these individual pathways in tumorigenesis. We do not know how many of these pathways will need to be inhibited to completely block oncogenic K-Ras signaling, and it seems likely that mutiple inhibitors would produce intolerable significant side effects. The failure of inhibitors to farnesyl transferase (FTIs), the enzyme that catalyzes the addition of a 15-carbon prenyl group to Ras, to show any efficacy in clinical trials serves as a cautionary tale to rational drug design. These FTIs, despite being very effective inhibitors of farnesyl transferase, failed because K-Ras was able to be alternatively prenylated by geranylgeranyltransferase (GGT), an enzyme that was not affected by FTIs (Whyte et al. 1997). Preclinical testing of FTIs was carried out using cells and tumors transformed with H-Ras, an isoform that is not a substrate for GGT (Appels et al. 2005; Brunner et al. 2003). The other enzymes in the posttranslational modification pathway of Ras, Rce1, and Icmt are now of interest as potential drug targets and have shown some promise in preclinical studies (Wahlstrom et al. 2008). Due to the potential difficulties of targeting K-Ras itself, another approach has been to look for other signaling pathways specifically required for cell survival only in the presence of oncogenic K-Ras. Screens for such synthetic lethal interactions have identified a number of potential drug targets (Scholl et al. 2009; Barbie et al. 2009), so there is hope that in the future these studies can generate effective therapies for K-Ras driven cancers.

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