

## REVIEW

# Genetics and biology of pancreatic ductal adenocarcinoma

Aram F. Hezel,<sup>1,8</sup> Alec C. Kimmelman,<sup>1,3,8</sup> Ben Z. Stanger,<sup>5</sup> Nabeel Bardeesy,<sup>6</sup> and Ronald A. DePinho<sup>1,2,4,7,9</sup>

<sup>1</sup>Department of Medical Oncology, Dana-Farber Cancer Institute; <sup>2</sup>Department of Medicine, Brigham and Women's Hospital; <sup>3</sup>Harvard Radiation Oncology Program; <sup>4</sup>Department of Genetics; <sup>5</sup>Gastrointestinal Unit, <sup>6</sup>Massachusetts General Hospital Cancer Center, Massachusetts General Hospital; <sup>7</sup>Center for Applied Cancer Science and Belfer Institute for Innovative Cancer Science, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115, USA

**Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer death in the United States with a median survival of <6 mo and a dismal 5-yr survival rate of 3%–5%. The cancer's lethal nature stems from its propensity to rapidly disseminate to the lymphatic system and distant organs. This aggressive biology and resistance to conventional and targeted therapeutic agents leads to a typical clinical presentation of incurable disease at the time of diagnosis. The well-defined serial histopathologic picture and accompanying molecular profiles of PDAC and its precursor lesions have provided the framework for emerging basic and translational research. Recent advances include insights into the cancer's cellular origins, high-resolution genomic profiles pointing to potential new therapeutic targets, and refined mouse models reflecting both the genetics and histopathologic evolution of human PDAC. This confluence of developments offers the opportunity for accelerated discovery and the future promise of improved treatment.**

## Pancreas anatomy and physiology

The pancreas, an organ of endodermal derivation, is the key regulator of protein and carbohydrate digestion and glucose homeostasis (Fig. 1). The exocrine pancreas (80% of the tissue mass of the organ) is composed of a branching network of acinar and duct cells that produce and deliver digestive zymogens into the gastrointestinal tract. The acinar cells, which are organized in functional units along the duct network, synthesize and secrete zymogens into the ductal lumen in response to cues from the stomach and duodenum. Within the acinar units near the ducts are centroacinar cells. The endocrine pancreas, which regulates metabolism and glucose homeo-

stasis through the secretion of hormones into the bloodstream, is composed of four specialized endocrine cell types gathered together into clusters called Islets of Langerhans.

Mirroring the physiologic and cellular diversity of the pancreas is a spectrum of distinct pancreatic malignancies that possess histological and molecular features that recall the characteristics of the various normal cellular constituents. These multiple tumor types and hallmark features are summarized in Table 1. Pancreatic ductal adenocarcinoma (PDAC), whose nomenclature derives from its histological resemblance to ductal cells, is the most common pancreatic neoplasm and accounts for >85% of pancreatic tumor cases (Warshaw and Fernandez-del Castillo 1992; D. Li et al. 2004). PDAC is the focus of this review, and the reader is directed to the following excellent review covering other pancreas cancer types (Hruban et al. 2006b).

## Epidemiology of PDAC

PDAC is associated with only a few known demographic and environmental risk factors and a handful of autosomal dominant genetic conditions. Multiple studies have established advanced age, smoking, and long-standing chronic pancreatitis as clear risk factors; diabetes and obesity also appear to confer increased risk (Everhart and Wright 1995; Fuchs et al. 1996; Gapstur et al. 2000; Michaud et al. 2001; Berrington de Gonzalez et al. 2003; Stolzenberg-Solomon et al. 2005). Increased risk has also been documented in relatives of PDAC patients, and it is estimated that 10% of PDAC cases are associated with an inherited predisposition based on familial clustering (Schenk et al. 2001; Petersen and Hruban 2003). Correspondingly, germline mutations have been linked to familial PDAC, including those targeting the tumor suppressor genes *INK4A*, *BRCA2*, and *LKB1*, the DNA mismatch repair gene *MLH1* and the cationic trypsinogen gene *PRSS1* (Whitcomb et al. 1996; Jaffee et al. 2002). *BRCA1* mutation appears to confer increased susceptibility to PDAC, albeit with a lower associated risk than *BRCA2* (Thompson and Easton 2002). Given the low

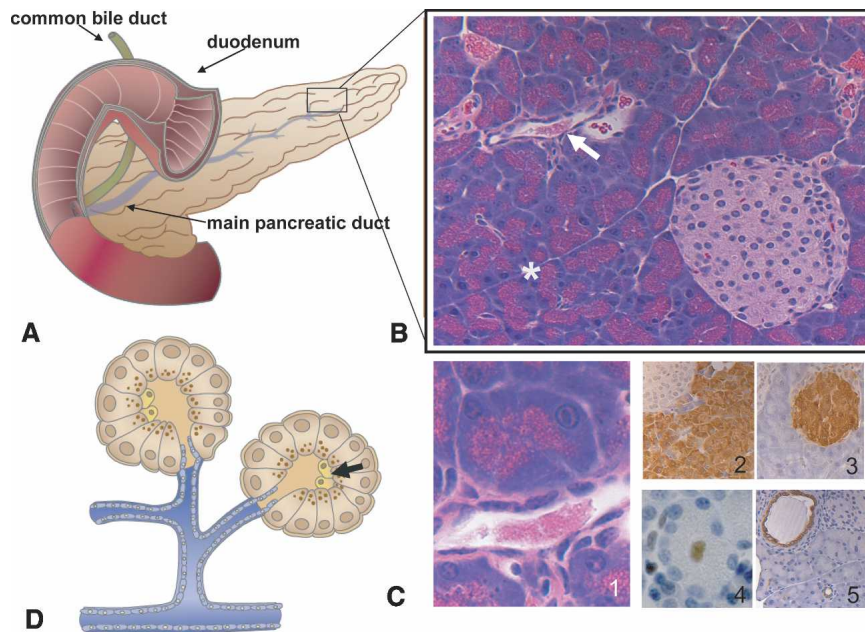
[Keywords: Pancreatic cancer; genetics; pancreas; mouse models; genomic stem cell]

<sup>8</sup>These authors contributed equally to this work.

<sup>9</sup>Corresponding author.

E-MAIL [ron\\_depinho@dfci.harvard.edu](mailto:ron_depinho@dfci.harvard.edu); FAX (617) 632-6069.

Article and publication are at <http://www.genesdev.org/cgi/doi/10.1101/gad.1415606>.



**Figure 1.** Anatomy of the pancreas. The pancreas is comprised of separate functional units that regulate two major physiological processes: digestion and glucose metabolism. (A) Gross anatomy of the pancreas demonstrating its close anatomical relationship with the duodenum and common bile duct. (B) The major components of the pancreatic parenchyma on a histological level. At lower right is an islet of Langerhans, the endocrine portion of the pancreas, which is principally involved in regulating glucose homeostasis. The asterisk is placed among acini, which are involved in secreting various digestive enzymes (zymogens) into the ducts (indicated by the solid arrow). (C) Photomicrographs of H&E- and immunohistochemical-stained sections of pancreatic tissue demonstrating the various cell types. (Panel 1) An acinar unit in relationship to the duct. (Panel 2) Acinar units visualized with an antibody to amylase are seen as brown due to Diaminobenzidine staining. (Panel 3) Islet of Langerhans shown

stained with an antibody to insulin. (Panel 4) A centroacinar cell showing robust Hes1 staining. (Panel 5) Ductal cells (seen here in cross-section) are stained with an antibody to cytokeratin-19. (D) Representation of an acinar unit showing the relationship to the pancreatic ducts. Also depicted are centroacinar cells (arrow), which sit at the junction of the ducts and acini.

penetrance of PDAC and the typical age of onset associated with the above germline mutations, these genetic lesions appear to impact malignant progression of precursor lesions rather than cancer initiation. Supporting this hypothesis, *INK4A* and *BRCA2* mutations are not detected in the earliest sporadic PDAC premalignant lesions but are only found in the later intermediate or advanced pancreatic intraepithelial neoplasm (PanIN) lesions (Wilentz et al. 1998; Goggins et al. 2000). Additionally, mice engineered with germline *INK4A* mutations do not develop PDAC unless combined with activated *K-RAS* mutations (see below).

The germline mutations listed above are estimated to account for <20% of PDAC-prone familial cases. It is clear that additional novel disease predisposition genes

exist as evidenced by rare families in which PDAC is inherited as an autosomal dominant trait with high penetrance (Lynch et al. 1996). In one family, the *4q32-34* locus has been linked to the development of diabetes, pancreatic exocrine insufficiency, and PDAC with a penetrance approaching 100% (Eberle et al. 2002). The gene associated with this syndrome has yet to be identified. Furthermore, genes that predispose to pancreatitis are associated with increased occurrence of PDAC. In patients with hereditary pancreatitis caused by germline mutations in the cationic trypsinogen gene *PRSS1*, there is a 53-fold increased incidence of PDAC (Lowenfels et al. 1997). Another link has also been forged between mutations in the cystic fibrosis gene (*CFTR*) and PDAC. Heterozygous *CFTR* mutations are associated with

**Table 1.** Pancreatic tumors and associated genetic alterations

Pancreatic neoplasm	Histological features	Common genetic alterations
Ductal adenocarcinoma <sup>a</sup>	Ductal morphology; desmoplasia	<i>K-RAS</i> , <i>p16<sup>INK4a</sup></i> , <i>TP53</i> , <i>SMAD4</i>
Variants of ductal adenocarcinoma		
a. Medullary carcinoma	Poorly differentiated; intratumoral lymphocytes	<i>hMLH1</i> , <i>hMSH2</i>
b. Colloid (mucinous noncystic) carcinoma	Mucin pools	<i>MUC2 overexpression</i>
Acinar cell carcinoma	Zymogen granules	<i>APC/β-catenin</i>
Pancreatoblastoma	Squamoid nests, multilineage differentiation	<i>APC/β-catenin</i>
Solid pseudopapillary neoplasm	"Pseudo" papillae, solid and cystic areas, hyaline globules	<i>APC/β-catenin</i> , CD10 expression
Serous cystadenoma	Multilocular cysts; glycogen-rich epithelium	<i>VHL</i>
Pancreatic endocrine tumors	Hormone production	<i>MEN1</i>

Table kindly provided by Anirban Maitra. Adapted from Hruban et al. (2006b).

<sup>a</sup>There are three recognized precursor lesions of invasive ductal adenocarcinomas: PanINs, IPMNs, and MCNs. Colloid carcinomas of the pancreas almost always arise in the backdrop of an IPMN.

Hezel et al.

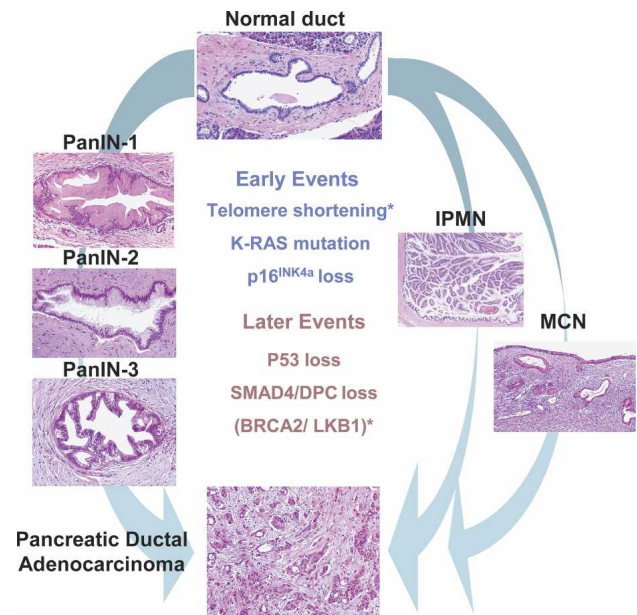
chronic pancreatitis, a known risk factor for pancreatic cancer. Recently, a direct link between CFTR mutation and cancer has been posited with the detection of a mutant allele in early-onset PDAC cases. Previous studies had not conclusively identified such a link, but may have been limited by smaller numbers of cases and more limited mutational analysis (Neglia et al. 1995; Sharer et al. 1998; Malats et al. 2001; Matsubayashi et al. 2003; McWilliams et al. 2005).

While the question as to how these separate genetic conditions lead to PDAC remains to be fully understood, the clinical observation of exocrine insufficiency and pancreatitis as a common patho-physiologic process leading to PDAC is compelling. Exocrine organ dysfunction and pancreatitis could promote tumorigenesis in part by promoting the local release of growth factors, cytokines, and reactive oxygen species (ROS), thereby inducing cell proliferation, disrupting cell differentiation states, and selecting for oncogenic mutations. The observation that activating *K-RAS* mutations are detectable in up to a third of patients with chronic pancreatitis is consistent with this hypothesis (Lohr et al. 2000). Further evidence from mouse models also suggests the presence of a ductal precursor cell population that undergoes expansion in response to organ damage (see below in section Origins of Pancreatic Cancer). In states of pancreatic inflammation or damage, an expanded “stem cell”-like compartment could represent a subpopulation of cells susceptible to oncogenic transformation upon somatic mutation of key proto-oncogenes and tumor suppressor genes (Beachy et al. 2004).

### Morphological characteristics of PDAC and evolving pancreatic neoplasms

PDAC commonly arises in the head of the pancreas with infiltration into surrounding tissues including lymphatics, spleen, and peritoneal cavity, and with metastasis to the liver and lungs. The disease is characterized by the presence of a dense stroma of fibroblasts and inflammatory cells, termed desmoplasia. Pancreatic stellate cells, a subpopulation of cells in the normal pancreas with fibroblast characteristics, have been observed in experimental models to respond to pancreatic injury and may contribute to the desmoplastic response in the setting of cancer (Jaster 2004). PDAC primarily exhibits a glandular pattern with duct-like structures and varying degrees of cellular atypia and differentiation (Fig. 2). Less common subtypes of PDAC include colloid, adenosquamous, or sarcomatoid histology. Often within an individual tumor, there are regional differences in histology, tumor grade, and degree of differentiation. Even the smallest primary lesions commonly exhibit perineural and lympho-vascular invasion, suggesting a propensity for early distant spread.

Clinical and histopathologic studies have identified three PDAC precursor lesions (Fig. 2): PanIN, mucinous cystic neoplasm (MCN), and intraductal papillary mucinous neoplasm (IPMN) (Brugge et al. 2004; Maitra et al. 2005). Of these precursor lesions, the most common and



**Figure 2.** Pancreatic precursor lesions and genetic events involved in pancreatic adenocarcinoma progression. Pictured are three known human PDAC precursor lesions: PanIN, MCN, and IPMN. The PanIN grading scheme is shown on the left; increasing grade (1–3) reflects increasing atypia, eventually leading to frank adenocarcinoma. The right side illustrates the potential progression of MCNs and IPMNs to PDAC. The genetic alterations documented in adenocarcinomas also occur in PanIN, and to a lesser extent MCNs and IPMNs, in an apparent temporal sequence, although these alterations have not been correlated with the acquisition of specific histopathologic features. The various genetic events are listed and divided into those that predominantly occur early or late in PDAC progression. Asterisks indicate events that are not known to be common to all precursors (telomere shortening and BRCA2 loss are documented in PanIN and LKB1 loss is documented in a subset of PDACs and IPMNs). Images were generously provided by Gregory Lauwers.

extensively studied is PanIN, which is found in the smaller-caliber pancreatic ducts. Surveys of pancreas specimens from autopsy studies and surgical resection cases have suggested that PanINs are a common finding in older adults, occurring in as many as 30% of specimens. A documented increased incidence of PanINs in patients with PDAC initially suggested their biologic relationship. PanINs show a spectrum of divergent morphological alterations relative to normal ducts that seem to represent graded stages of increasingly dysplastic growth (Fig. 2; Hruban et al. 2000a, 2001; Maitra et al. 2005). PanINs are graded from stages I to III, with the earliest stage characterized by the appearance of a columnar, mucinous epithelium and with increasing architectural disorganization and nuclear atypia through stages II and III (Figs. 2, 4 [below]). The high-grade PanINs ultimately transform into frank PDAC with evidence of areas of invasion beyond the basement membrane. Several molecular profiling studies have subsequently reinforced the PanIN-to-PDAC progression



model through documentation of an increasing number of gene alterations in higher grade PanINs (Fig. 2; Klimstra and Longnecker 1994; Moskaluk et al. 1997; Heinmoller et al. 2000; Hruban et al. 2000b, 2001; Wilentz et al. 2000; Yamano et al. 2000; Luttges et al. 2001; Maitra et al. 2003).

Less common precursor lesions are MCNs and IPMNs. MCNs are large mucin-producing epithelial cystic lesions that harbor a distinctive ovarian-type stroma with a variable degree of epithelial dysplasia and focal regions of invasion. IPMNs resemble PanINs at the cellular level but grow into larger cystic structures. Of interest, two subtypes of invasive cancer have been found in association with IPMNs; typical PDAC, and a colloid type characterized by copious mucin production. Among IPMNs and MCNs, both common and distinct molecular events in comparison with PanINs have been described, suggesting that each precursor lesion may reflect variations on a common theme of malignant transformation of the duct (Fig. 2; Sato et al. 2001b, 2004a; Adsay et al. 2004). Along these lines, expression profiling has revealed several up-regulated genes commonly associated with PanINs, IPMNs, and PDAC, pointing toward key shared molecular events (Crnogorac-Jurcevic et al. 2002; Sato et al. 2004b; Prasad et al. 2005). How these PDAC precursor lesions relate to each other, their common or separate cellular origins, and whether each type leads to distinct molecular and biological PDAC subtypes remain to be fully explored.

### Molecular genetics of PDAC

The molecular analysis of evolving PDAC has provided a compendium of genetic lesions, often implicating known cancer genes and classical cancer signaling cascades. In many cases, these molecular events have been linked with defined histopathologic stages of PDAC progression. While many of these genetic alterations have been validated in PDAC pathogenesis, major lingering questions center on how these mutations contribute to the tumor biological features of the neoplasms. In the following subsections, we provide a summary of the current state of knowledge surrounding the PDAC signature mutations, their linked pathways, and biological activities.

#### *The K-RAS oncogene and its signaling pathways*

*K-RAS* is a member of the RAS family of GTP-binding proteins that mediate a wide variety of cellular functions including proliferation, differentiation, and survival (Campbell et al. 1998; Malumbres and Barbacid 2003). Although RAS is a GTPase, its intrinsic activity is inefficient and requires GTPase activating proteins (GAPs) to promote GTP hydrolysis and attenuate downstream signaling. Activating *K-RAS* point mutations at codon 12 (from GGT to GAT or GTT, and more rarely CGT) results in substitution of glycine with aspartate, valine, or arginine. These mutations are the first known genetic alterations, occurring sporadically in normal pancreas tissue,

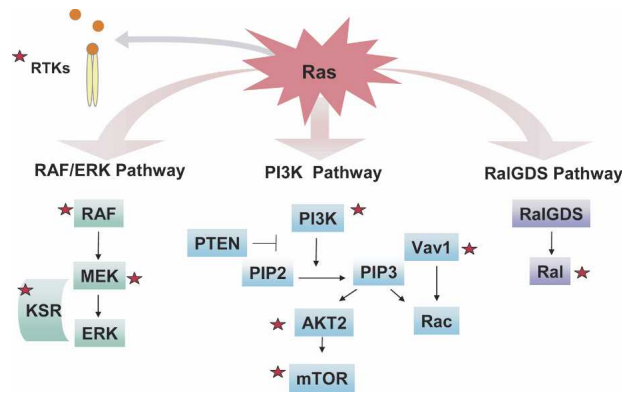
and are detected in ~30% of early neoplasms with the frequency rising to nearly 100% in advanced PDAC (Klimstra and Longnecker 1994; Rozenblum et al. 1997). Consistent with a central pathogenic role of the *K-RAS*<sup>G12D</sup> mutation, mice engineered with pancreas-specific expression of this activated *K-RAS* allele sustain classical PanIN lesions that can progress to PDAC in the appropriate tumor suppressor background (as discussed in depth below).

Although RAS is considered to be an attractive therapeutic target given its prominent role in the genesis of PDAC and many other human malignancies, specific biochemical properties of the protein have made this an elusive goal. Importantly, the hotspots of RAS mutations in human cancer are located near the bound nucleotide and decrease the intrinsic rate of GTP hydrolysis and make the molecule insensitive to GAPs (for review, see Wittinghofer et al. 1997; McCormick 1998). This results in a constitutively activated molecule that is essentially independent of growth factor stimulation. In contrast to the activating mutations of other oncogenes such as kinases, which increase their catalytic activity, the oncogenic mutations of RAS inhibit its enzymatic activity. Thus, rather than using the traditional paradigm of inhibiting an oncogene's enzymatic function (e.g., c-Kit, EGFR, HER2/Neu), an effective RAS antagonist would increase the GTPase activity of RAS or make it more sensitive to GAPs. There have been attempts to inhibit *K-RAS* in this malignancy, mainly through inhibition of essential post-translational modifications. Despite showing promise in vitro and in PDAC xenografts (Omer and Kohl 1997), farnesyltransferase inhibitors (FTIs), which inhibit a lipid modification of the C terminus of RAS proteins, have not been clinically effective (Van Cutsem et al. 2004). Among the explanations for the clinical failure of FTIs, compensatory geranyltransferase activity preserving RAS function has been suggested (Lebowitz et al. 1995; Lerner et al. 1997).

*K-RAS* is mutated in nearly all human PDAC specimens (Almoguera et al. 1988). Mouse models have convincingly shown that *K-RAS* mutations are an initiating step in PDAC pathogenesis (see below on mouse models), and detailed pathological studies have demonstrated that *K-RAS* mutation is one of the earliest genetic events seen in human PanIN progression (Moskaluk et al. 1997). The essentiality of *K-RAS* in the maintenance of advanced PDAC is suggested by dominant-negative mutant studies (Hirano et al. 2002). More recently, RNA interference (RNAi) knockdown studies have provided complementary evidence that *K-RAS* plays a vital role in PDAC maintenance (Brummelkamp et al. 2002; Fleming et al. 2005). Additional genetic evidence will be needed to address the specific biological role of *K-RAS* across the progressive stages of this cancer.

Activated *K-RAS* engages multiple effector pathways, notably the RAF-mitogen-activated kinase (MAPK), phosphoinositide-3-kinase, and Ral GDS pathways (Fig. 3; for review, see Campbell et al. 1998). Given the aforementioned difficulties in *K-RAS* inhibition, these downstream targets may provide alternative effective points of

Hezel et al.



**Figure 3.** RAS signaling network. Ras uses a multitude of downstream effectors. Depicted here are three major signaling cascades that have been implicated in PDAC progression and maintenance: the Raf/Map Kinase (ERK) pathway, the PI3K pathway, and the Ral GDS pathway. Inhibition of each of these cascades at various levels (indicated by the presence of a star) has been shown to inhibit PDAC tumorigenesis in a variety of in vitro and in vivo systems.

therapeutic intervention and thus are the focus of ongoing studies in pancreatic specific systems.

**Raf-Mapk.** The RAF family of serine/threonine kinases, which are bound by activated RAS, lead to MAPK/ERK kinase activation through a series of phosphorylation events resulting in proliferative phenotypes in a variety of cellular systems (for review, see Baccharini 2005). The importance of RAF in cancer has come to light with the identification of activating *B-RAF* mutations in many malignancies, including melanoma, papillary thyroid, colorectal, and serous ovarian cancers. Interestingly, *B-RAF* and *RAS* mutations appear to be mutually exclusive in these cancers (Davies et al. 2002; Garnett and Marais 2004). *B-RAF* mutations are rare in PDAC but are present in ~33% of the histologically distinct pancreatic medullary carcinomas, which are characterized by wild-type *KRAS* and DNA mismatch repair defects (Calhoun et al. 2003; Ishimura et al. 2003).

Inhibition of MAPK, either through the use of dominant negatives or pharmacological inhibition of the upstream activator MEK, results in decreased proliferation of PDAC cell lines and cell cycle arrest (Hirano et al. 2002; Gysin et al. 2005). This arrest may be mediated through increased expression of p27KIP1, as RNAi-mediated knockdown of p27KIP1 partially abrogates this growth arrest effect. Kinase suppressor of RAS (KSR) is a scaffolding molecule important in RAS signaling that mediates activation of the MAPK pathway (for review, see Ory and Morrison 2004). Antisense neutralization of KSR can inhibit proliferation, soft-agar growth, and invasion of cultured PDAC cells. Additionally, infusion of KSR antisense oligonucleotides causes PDAC xenograft regression and, in some cases, pretreatment with the oligos resulted in complete regression of tumors even after treatment was discontinued (Xing et al. 2003). These inhibitory effects were likely through the MAPK pathway,

as transduction of activated RAF was able to restore invasion and transformation.

**Phosphoinositide 3-kinase (PI3K) pathway.** The PI3K signaling pathway, which can be activated by RAS (Rodriguez-Viciano et al. 1996), regulates cell survival, size, and proliferation via several downstream effectors including AKT, p70-S6K, and the small GTPase, RAC (for review, see Cantley 2002; Vivanco and Sawyers 2002). Mutations in the PI3K pathway that are common in other cancer types, including activating mutations of the catalytic subunit of PI3K and loss-of-function mutants of the PTEN tumor suppressor, have not been commonly observed in human PDAC (Okami et al. 1998; Samuels and Velculescu 2004). Mounting evidence, however, has pointed to the general importance of this pathway and its downstream signaling elements in PDAC. There are reports of decreased PTEN expression in PDAC, possibly due to promoter hypermethylation (Asano et al. 2004), and conditional knockout studies of PTEN in the pancreas have produced PDAC in a small percentage of mice (Stanger et al. 2005). In addition, microinjection experiments of mutant *K-RAS* into cultured primary pancreatic ductal cells elicit an increase in proliferation and cell size that is mediated through PI3K and mTOR (Agbunag and Bar-Sagi 2004). Lastly, activation of the PI3K pathway appears necessary and sufficient to maintain oncogenic RAS-transformed xenograft tumors after the elimination of RAS expression (Lim and Counter 2005).

The PI3K downstream effector *AKT2* is amplified in 10%–20% of PDAC, providing genetic evidence supporting the pathway's importance in this tumor type (Cheng et al. 1996; Ruggeri et al. 1998; Altomare et al. 2003; Schlieman et al. 2003). Functionally, the significance of this amplification has been suggested in PDAC cell lines through the use of antisense oligonucleotides, which inhibit the growth of PDAC lines in xenograft assays (Cheng et al. 1996). Additionally, pharmacological inhibition of PI3K appears to increase the sensitivity of PDAC cell lines to chemotherapy as well as TNF- $\alpha$ -induced apoptosis, and diminishes serum-induced proliferation (Ng et al. 2000; Perugini et al. 2000; Shah et al. 2001). The mammalian target of rapamycin (mTOR), which acts downstream of AKT2, has been shown to be activated in PDAC. Treatment with an mTOR inhibitor impedes growth of several PDAC cell lines (Asano et al. 2005). Additionally, rapamycin inhibits PDAC xenograft growth and metastasis possibly through induction of endothelial cell death and tumor vessel thrombosis (Bruns et al. 2004).

**Nuclear factor  $\kappa$ B (NF $\kappa$ B).** The NF $\kappa$ B transcription factor may be another important downstream mediator of mutated K-RAS signaling in PDAC (Sclabas et al. 2003). Activation of this pathway occurs in response to a variety of cell stresses through stimulation by proinflammatory cytokines and growth factors, and is known to regulate the immune response, apoptosis, and many other processes (Ghosh et al. 1998; Karin and Ben-Neriah 2000; Hayden and Ghosh 2004). Constitutive NF $\kappa$ B activity is

observed in many cancers, where it is thought to contribute to cell survival, angiogenesis, and invasion (Orlowski and Baldwin 2002).

Most primary pancreatic cancers and cell lines, but not normal pancreas specimens, show constitutive NF $\kappa$ B activity (Wang et al. 1999; Chandler et al. 2004). Induction of NF $\kappa$ B may directly involve K-RAS signaling since expression of a dominant-negative RAS allele abrogates NF $\kappa$ B activity in PDAC cell lines (Liptay et al. 2003). While NF $\kappa$ B induction has been shown to be crucial for RAS transformation of several cell types (Mayo et al. 1997; Arsura et al. 2000), the NF $\kappa$ B subunits RelA/p65 and c-Rel appear to be dispensable for H-RAS-induced transformation of MEFs (Hanson et al. 2004). A possible mechanism for increased NF $\kappa$ B activation may be increased expression of components of the ubiquitin-mediated degradation pathway leading to increased degradation of I $\kappa$ B (Muerkoster et al. 2005). In vitro, NF $\kappa$ B appears to play a role in the regulation of cell survival genes, *VEGF* (vascular endothelial growth factor), urokinase, and other proinvasive or angiogenic factors (Fujioka et al. 2003; Xiong et al. 2004). The NF $\kappa$ B pathway may also contribute to the prominent chemoresistance of PDAC (Dong et al. 2002; Arlt et al. 2003), perhaps via its capacity to up-regulate BCL-2 and BCL-XL, as well as multiple other anti-apoptotic proteins (for review, see Bharti and Aggarwal 2002). These diverse roles of NF $\kappa$ B highlight the need to further explore this complex pathway specifically in PDAC.

**Other Ras superfamily GTPases.** The RAS superfamily of GTPases is comprised of at least 150 members and subdivided into five subfamilies—RAS, RHO, RAB, ARF, and RAN. The common and distinct functions as well as the biochemical inter-relationships among the members are complex and are the subject of considerable study (for review, see Mitin et al. 2005). The RHO family of GTPases is involved in several important cellular processes including actin cytoskeleton rearrangement, cell size, proliferation, survival, polarity, and membrane trafficking (for review, see Gomez del Pulgar et al. 2005; Wennerberg et al. 2005).

RHO proteins with GTPase-inactivating mutations transform mouse fibroblasts and studies using dominant-negative RHO family mutants have shown that they are necessary for RAS transformation of rodent fibroblasts. Activating mutations of RHO family members have not been demonstrated in human cancers; however, overexpression of RHO-C has been shown to correlate with PDAC tumor metastasis and prognosis (Suwa et al. 1998). Recent work has also implicated the RHO family in PDAC through the exchange factor VAV1 (Fernandez-Zapico et al. 2005). Although normally restricted to the hematopoietic system, VAV1 is expressed in PDAC specimens likely through promoter demethylation. Importantly, this ectopic expression correlates with decreased patient survival, and RNAi-mediated knockdown of VAV1 expression suppresses tumorigenicity in xenografts.

RAL GTPases are members of the RAS subfamily and

are thought to be downstream of RAS through RAS's capacity to activate RAL exchange factors (for review, see Feig 2003). Activated RAL and RAL exchange factors enhance RAS-induced cellular transformation, and the dominant-negative mutants inhibit it (Chien and White 2003). Recently, RAL A was shown to be activated in a variety of PDAC cell lines, and knockdown of RAL A suppressed tumorigenicity of RAS-transformed human cells (Lim et al. 2005). The role of RAL proteins in tumorigenesis may relate to the requirement of these factors for maintaining the polarity of epithelial cells through the regulated transport of basolateral membrane proteins (for review, see Camonis and White 2005).

A preponderance of evidence supports the role of K-RAS and many of its downstream effectors in both the initiation and likely the maintenance of PDAC. Given this important place, it is crucial to define precisely which K-RAS effector pathways are important to each aspect of tumorigenesis to guide future therapeutic strategies.

#### *The 9p21 locus and the INK4A and ARF tumor suppressors*

Loss of *INK4A* function—brought about by mutation, deletion, or promoter hypermethylation—occurs in 80%–95% of sporadic PDAC (Rozenblum et al. 1997; Hustinx et al. 2005). *INK4A* loss is generally seen in moderately advanced lesions that show features of dysplasia. Germline mutations in *INK4A* are associated with the Familial Atypical Mole–Malignant Melanoma (FAMMM) syndrome, which is characterized by a high incidence of melanoma, as well as a 13-fold increased risk of pancreatic cancer (Goldstein et al. 1995; Whelan et al. 1995). The appearance and age of onset of pancreatic adenocarcinoma are variable among FAMMM kindreds with *INK4A* mutations, indicating a modulating role for environmental factors in disease penetrance (Goldstein et al. 2000; Lynch et al. 2002). FAMMM kindreds that harbor mutant loci other than *INK4A*, such as cyclin-dependent kinase 4 (*CDK4*) alleles that abrogate *INK4A* binding or other, as yet uncharacterized loci, do not have an increased incidence of PDAC (Goldstein et al. 1995; Borg et al. 2000), suggesting that *INK4A* may participate in pancreatic carcinogenesis by additional mechanisms distinct from its regulation of G1 CDK activity.

The 9q21 locus encodes two overlapping tumor suppressors—*INK4A* and *ARF*, and their respective protein products p16<sup>INK4A</sup> and p19<sup>ARF</sup>—via distinct first exons and alternative reading frames in shared downstream exons (Sherr 2001). *INK4A* inhibits CDK4/6-mediated phosphorylation of RB, thereby blocking entry into the S (DNA synthesis) phase of the cell cycle; *ARF* stabilizes p53 by inhibiting its MDM2-dependent proteolysis. Given this physical juxtaposition and frequent homozygous deletion of 9p21 (in ~40% of tumors), many pancreatic cancers sustain loss of *INK4A* and *ARF* tumor suppression pathways. *INK4A* clearly plays a central role as a PDAC tumor suppressor, as germline and sporadic mutations have been identified that target *INK4A*, but spare



Hezel et al.

*ARF* (Rozenblum et al. 1997; Liu et al. 1999; Lal et al. 2000). Recent mouse modeling studies have begun to shed light on the specific roles of *INK4A* and *ARF* tumor suppression pathways in this disease. These studies reinforce the relevance of *INK4A* in the pathogenesis of PDAC, since *INK4A* mutations cooperate with *KRAS* in the development of PDAC and accelerate tumor progression in the setting of concurrent mutations in *p53* (Bardesy et al. 2006). Moreover, *ARF* was shown to have an independent, cooperative function in PDAC tumor suppression. These mouse studies and the functional interactions of *ARF* and *p53* are discussed in depth below.

*INK4A* displays a highly restricted expression pattern and is dispensable for normal development and tissue homeostasis (Zindy et al. 1997; Nielsen et al. 1999; Krimpenfort et al. 2001; Sharpless et al. 2001a). When primary cells are placed into culture, *INK4A* expression is induced via a presumed stress response to the inappropriate growth environment associated with in vitro culture (Sherr and DePinho 2000; Ramirez et al. 2001). Similar induction is observed in vivo in association with reactive processes and aging associated with senescence (Nielsen et al. 1999; Krishnamurthy et al. 2004). This regulation of *INK4A* by environmental stress, age, and aberrant proliferative signals provides a plausible basis for its tumor suppression function. A role for *INK4A* has also been suggested in response to telomere erosion, which is observed in PanINs (van Heek et al. 2002) and is an established stimulus of senescence, although other studies suggest that *p53* and *INK4A* are operative in separate senescent pathways (Beausejour et al. 2003; Herbig et al. 2004; Jacobs and de Lange 2004).

The genetic evidence of coincident mutations in PDAC and a wide range of other human malignancies (lung, colon, ovarian, melanocytic, etc.) have led to a significant effort in functionally defining the relationship between *RAS* and *INK4A/ARF*. In human fibroblasts, robust *RAS* activation can induce *INK4A*, which, in turn, results in premature senescence (Serrano et al. 1997; Zhu et al. 1998; Brookes et al. 2002; Drayton et al. 2003; for review, see Sharpless and DePinho 2005), a presumed defense mechanism against oncogene activation. The relevance of these findings has recently been demonstrated in vivo with mouse models in which oncogenic *Kras* has been expressed in the lung and pancreatic ducts. *Kras*-induced preneoplastic lung adenomas and PanINs exhibit markers of senescence, including *INK4A* expression among others, which are then lost in fully transformed invasive lesions (Collado et al. 2005). Cooperation between activated *RAS* alleles and the loss of *INK4A* has been observed in animal models, including PDAC (Chin et al. 1997; Fisher et al. 2001; Aguirre et al. 2003). This relationship had initially been thought to contribute to the coincident mutations of *RAS* and *INK4A* in cancer—namely, that *RAS* mutation leads to senescence directly through induction of *INK4A* expression. Thus, mutation in *RAS* leads to selective pressure for subsequent mutation in the *INK4A/ARF* locus.

Several lines of evidence suggest, however, that the *K-RAS*-induced senescent phenotype requires additional

cooperating stimuli, as *K-RAS* activation, in itself, seems to confer a survival advantage in certain cell systems. This is corroborated by studies in mouse cells in which an activated *K-RAS* allele, expressed at physiological levels, provokes immortalization of fibroblasts in vitro and preneoplastic hyperplasias of the lung and GI track when activated in vivo (Jackson et al. 2001; Guerra et al. 2003; Tuveson et al. 2004). The observation that *K-RAS* induces a proliferative phenotype without attendant senescence and *INK4A* induction is echoed in studies using very early passage human fibroblasts, which have not had time to experience culture-induced changes, and mouse fibroblasts grown under reduced oxygen tensions and in serum-free media. In both of these systems, which seek to minimize known prosenescent signals such as ROS, growth factors, and culture shock (for review, see Sherr and DePinho 2000), oncogenic *K-RAS* alone induced proliferation/immortalization phenotypes and senescence was not observed (Benanti and Galloway 2004; Woo and Poon 2004). Pathologic/molecular data from human pancreases are also supportive of a proliferative role for *K-RAS*. *K-RAS* mutations may be found in non-neoplastic states such as chronic pancreatitis and possibly in normal pancreas (Luttges et al. 1999). Furthermore, multiple different *K-RAS* mutations may be detected in individual PanIN lesions, suggesting that these mutants lead to the propagation of clonal populations (Moskaluk et al. 1997; Laghi et al. 2002). Finally, the loss of *INK4A* generally occurs in later stages of pancreatic neoplasia, subsequent to the acquisition of *K-RAS* mutations. Together, these observations point toward the possibility of intermediary events, such as disrupted contacts with the extracellular matrix, elevations in the level of activated *K-RAS*, concurrent growth factor signaling, or genomic damage from ROS, en route to *INK4A* loss and the development of frank PDAC.

#### *The p53 tumor suppressor*

The *p53* tumor suppressor gene is mutated, generally by missense alterations of the DNA-binding domain, in >50% of PDAC cases (Rozenblum et al. 1997). Consistent with a role in constraining malignant progression, *p53* mutation appears in later-stage PanINs that have acquired significant features of dysplasia (Boschman et al. 1994; Maitra et al. 2003). In these more advanced PanINs, the selective pressure to eliminate *p53* may stem in part from a collective accumulation of genetic damage, from telomere erosion and ROS, for example, resulting in the activation of *p53*-dependent DNA damage checkpoint responses. Thus, loss of *p53* function could serve to enable the growth and survival of cells harboring procarcinogenic chromosomal aberrations. Given that human PDAC is characterized by profound aneuploidy and complex chromosomal rearrangements, as well as significant intratumoral genomic heterogeneity, a clear understanding of how *p53* participates in genome stability mechanisms would provide important insights into disease pathogenesis and ultimately treatment. The rampant genomic instability in PDAC could

serve to both fuel the rise of advanced disease and provide a basis for its resistance to therapeutic modalities (Gorunova et al. 1998; Harada et al. 2002).

Finally, in many other cancer types, there exists a near reciprocal relationship in the loss of *ARF* and *p53* (Rosenblum et al. 1997; Pomerantz et al. 1998; Ruas and Peters 1998; Sharpless and DePinho 1999). As mentioned above, this relationship likely reflects the fact that *ARF* inhibits MDM2-mediated targeting of the *p53* protein for proteasomal degradation (Pomerantz et al. 1998; Zhang et al. 1998). Thus, *ARF* deficiency would result in marked reduction of *p53* protein levels and attenuation of *p53* pathway function in diverse cancer-relevant processes (Lowe and Sherr 2003). On the other hand, in human PDAC, *p53* mutations and *ARF* deletions coexist in ~40% of cases, potentially pointing to nonoverlapping functions for these factors in pancreatic cancer suppression (Heinmoller et al. 2000; Maitra et al. 2003; Hustinx et al. 2005). Mounting evidence suggests that *ARF* possesses *p53*-independent functions including the inhibition of ribosomal RNA processing (Rocha et al. 2003; Sugimoto et al. 2003; Qi et al. 2004; Paliwal et al. 2006). In addition, *ARF* does not appear to neutralize the DNA damage checkpoint that would be activated upon genetic damage (e.g., induced by telomere dysfunction), thereby necessitating the additional loss of *p53* function as such signals intensify during PDAC progression (Greenberg et al. 1999). Alternatively, *ARF* deletion in PDAC could represent a “bystander” effect associated with mutational events targeting *INK4A*. The resolution of these issues will require systematic genetic analysis of various mutant genotype combinations in murine PDAC mouse models as well a greater understanding of the molecular actions of *ARF* versus *p53* in PDAC tumor biology.

#### *The SMAD4/DPC4 tumor suppressor and complexities of transforming growth factor- $\beta$ (TGF- $\beta$ ) signaling*

Another frequent event associated with PDAC progression is loss of the *SMAD4* (*DPC4*) transcriptional regulator (Hahn et al. 1996), which serves as a central component in the TGF- $\beta$  signaling cascade (Massague et al. 2000). The *SMAD4* gene maps to chromosome 18q21 and is targeted for deletion or intragenic point mutations in ~50% of PDAC cases (Hahn et al. 1996). *SMAD4* has been designated a progression allele for PDAC on the basis of its loss in later-stage PanINs (Wilentz et al. 2000; Luttgies et al. 2001; Maitra et al. 2003). The impact of *SMAD4* loss on PDAC prognosis is not clearly established, and different studies have reached opposite conclusions regarding the relationship between *SMAD4* status and survival (Tascilar et al. 2001; Biankin et al. 2002). On the histo-pathologic level, tumors with an intact *SMAD4* may have a higher propensity for showing poorly differentiated features (Biankin et al. 2002). The mechanism by which *SMAD4* loss contributes to tumorigenesis is likely to involve its central role in TGF- $\beta$ -mediated growth inhibition.

**TGF- $\beta$ .** TGF- $\beta$  is the prototypic member of a superfamily of secreted proteins, whose other members include

the Bone Morphogenic Proteins (BMPs) and Activins (for review, see ten Dijke and Hill 2004). These growth factors signal through serine/threonine kinase receptor complexes that, upon ligand binding, phosphorylate receptor-regulated Smad proteins (*SMAD2*, *SMAD3*, and the obligate binding partner *SMAD4*) regulating a variety of cellular functions including proliferation, differentiation, migration, and apoptosis. The biological role of the TGF- $\beta$  pathway in human malignancy is complex, exerting both growth-inhibitory and growth-promoting effects depending on the cell type and cell context (for review, see Siegel and Massague 2003). In numerous epithelial cell lines and in epithelial tissue in vivo, TGF- $\beta$  exerts a growth inhibitory program that involves modulation of cell cycle regulators including induction of *p15<sup>INK4B</sup>* and *p21<sup>CIP1</sup>* expression and repression of *c-Myc* and *ID* family transcription factors, as well as induction of apoptotic machinery, and repression of telomerase (for review, see Elliott and Blobel 2005). Likewise, elevations in TGF- $\beta$  signaling inhibit epithelial cancer initiation in vivo, and lesions in this pathway promote intestinal, ovarian, and pancreatic tumorigenesis. On the other hand, TGF- $\beta$  promotes the proliferation and transformation of fibroblasts and the epithelial-to-mesenchymal transition (EMT) in breast cancer and skin cancer, a process by which advanced carcinomas lose their differentiated features and acquire a highly aggressive, invasive phenotype (Janda et al. 2002; Oft et al. 2002; Tang et al. 2003; for review, see Zavadil and Bottinger 2005). Therefore, in some carcinomas, TGF- $\beta$  signaling can have biphasic effects, inhibiting tumor initiation yet promoting the high-grade advancement of established tumors (Akhurst and Derynck 2001).

The importance of TGF- $\beta$  signaling in pancreatic cancer is illustrated by the fact that 90% of tumors show loss of heterozygosity (LOH) at the *SMAD4* locus, with 50% of PDAC having either homozygous deletion or mutational inactivation of the second allele, as discussed above. The loss of *SMAD4* in PDAC may have a primary role in modulating the interaction of the tumor with the microenvironment rather than in growth control of the tumor cells themselves. Along these lines, *SMAD4* restoration in some pancreatic cancer cell lines has a minimal impact on cell growth in vitro, although some inhibition of anchorage-independent growth has been observed in specific cell lines. Importantly, the prominent impact of *SMAD4* restoration has been observed in tumor formation in xenotransplants with documented repression of angiogenesis and extracellular matrix remodeling (Schwarte-Waldhoff et al. 2000; Peng et al. 2002; Duda et al. 2003).

There is recent evidence that *SMAD4* deficiency may inhibit TGF- $\beta$ -induced cell cycle arrest and cell migration, while not affecting EMT, thereby shifting the balance of TGF- $\beta$  signaling from tumor suppression to tumor promotion (Levy and Hill 2005). Consistent with these observations, it appears that elevated TGF- $\beta$  expression contributes to PDAC progression. TGF- $\beta$  family ligands are expressed at elevated levels in PDAC cells relative to normal pancreas (Friess et al. 1993) and may



Hezel et al.

help to promote the characteristic desmoplastic response of this malignancy as suggested from xenograft studies (Lohr et al. 2001). TGF- $\beta$  signaling may also contribute to tumorigenesis in an autocrine manner since PDACs often overexpress the type II TGF- $\beta$  receptor relative to normal pancreas (Wagner et al. 1999; for review, see Rane et al. 2006) while experimental blockade of TGF- $\beta$  signaling by expression of soluble type II TGF- $\beta$  receptor attenuates tumorigenicity and metastasis of xenografts (Rowland-Goldsmith et al. 2001, 2002). Furthermore, antibodies to TGF- $\beta$  inhibit the invasion of PDAC cell lines in vitro, while exogenous addition of this cytokine enhanced invasion and promotes the EMT (Ellenrieder et al. 2001a,b).

#### *The LKB1/STK11 tumor suppressor*

The Peutz-Jeghers syndrome (PJS), linked to *LKB1/STK11* mutations, is another familial cancer syndrome associated with an increased incidence of PDAC (Hemminki et al. 1998; Jenne et al. 1998; Giardiello et al. 2000). PJS patients are primarily afflicted with benign intestinal polyposis at a young age (Cooper 1998), although advancing age carries increased risk of gastrointestinal malignancies including a >40-fold increase in PDAC (Giardiello et al. 2000). At the same time, somatic mutation of *LKB1* in sporadic PDAC appears to be rare, detected in only 4%–6% of sporadic cases examined (Su et al. 1999), although there is some evidence that the rates of inactivation are higher in IPMNs (Sahin et al. 2003).

*LKB1* encodes a serine/threonine kinase that is involved in regulation of diverse processes such as cell polarity and metabolism, and has been linked to specific signaling pathways including mTOR, the latter via its capacity to regulate AMPK (Bardeesy et al. 2002b; Ossipova et al. 2003; Baas et al. 2004; Corradetti et al. 2004; Lizcano et al. 2004; Shaw et al. 2004a,b, 2005; Hardie 2005). Exactly how *LKB1* loss, and through deregulation of which of these pathways/processes, promotes tumorigenesis remains to be established. Control of mTOR signaling through AMPK links this gene to a common pathway harboring two other tumor suppressors, PTEN and TSC. The biochemical link to these well-characterized cancer signaling pathways may provide insights into the biological mechanisms through which *LKB1* suppresses tumor formation. At the same time, the role of *LKB1* in cell polarity, and likely regulation of several less-well-characterized kinases, leaves open several other plausible mechanisms of tumor suppression. Current efforts are now directed toward defining additional *LKB1* substrates and linked biological processes.

*The BRCA2 tumor suppressor.* Inherited *BRCA2* mutations are typically associated with familial breast and ovarian cancer syndrome, but also carry a significant risk for the development of pancreatic cancer. One study estimates that ~17% of pancreatic cancers occurring in a familial setting harbor mutations in this gene (Murphy et al. 2002); the del6174T founder mutation is particu-

larly common in familial pancreatic cancers that arise in the Ashkenazi Jewish population. As is the case for those with germline *INK4A* mutations, the penetrance of PDAC in *BRCA2* mutation carriers is relatively low, and the age of onset is similar to patients with the sporadic form of the disease. Loss of the wild-type *BRCA2* allele seems to be a late event in those inheriting germline heterozygous mutations of *BRCA2*, restricted to severely dysplastic PanINs and PDACs (Goggins et al. 2000). Together, these data are consistent with the model that loss of function of *BRCA2* promotes the malignant progression of pancreatic neoplasms.

*BRCA2* is known to play a critical role in the maintenance of genomic stability by regulating homologous recombination-based DNA repair processes. Consequently, *BRCA2* deficiency in normal cells results in the accumulation of procarcinogenic or lethal chromosomal aberrations (Venkitaraman 2002). The fact that *BRCA2* is selectively mutated late in tumorigenesis likely reflects the need for DNA damage response pathways (which in a normal cell would lead to senescence or apoptosis) to be inactivated first—for example, by *p53* mutation—so that the genetic damage incurred can be tolerated. Thus, as is the case for telomeres, the carcinogenic role of *BRCA2* deficiency may be manifest only in the appropriate genotypic and cell-type context. *BRCA2* mutational status may also have therapeutic implications, as it seems to confer susceptibility to DNA cross-linking agents such as Mitomycin C, as is seen in other related Fanconi anemia family genes, particularly FancG and FancC (Taniguchi et al. 2003; van der Heijden et al. 2004).

#### *Additional growth factor receptor signaling circuits in PDAC*

*Epidermal growth factor.* PDAC shows elevated expression of EGF receptors (EGFR and ERBB3) and their ligands (TGF- $\alpha$  and EGF), consistent with the presence of an autocrine loop (Barton et al. 1991; Korc et al. 1992; Lemoine et al. 1992; Friess et al. 1995, 1999). Importantly, EGFR inhibitors decrease PDAC cell growth and tumorigenesis in vitro (J. Li et al. 2004), as well as inhibit growth of orthotopic tumors in combination with cytotoxic chemotherapy (Bruns et al. 2000). This inhibition appears to be due to a decrease in tumor vasculature through inhibition of proangiogenic factors, resulting in endothelial apoptosis. In line with these antineoplastic activities, EGFR inhibitors have been approved for clinical use in PDAC patients.

*Insulin-like growth factor (IGF).* The IGF signaling pathway regulates survival, invasion, and angiogenesis of many human cancers. PDACs show elevated expression of IGF-I in both the tumor cells and the stroma and display aberrant activation of the IGF-I receptor (IGF-IR) in tumor cells (Bergmann et al. 1995; Ouban et al. 2003; Stoeltzing et al. 2003). In vitro, autocrine IGF-I signaling promotes cell proliferation and growth-factor-independent survival (Nair et al. 2001). Inhibition of the pathway

by anti-IGF-IR antibodies or expression of a dominant-negative form of IGF-IR inhibits the growth of xenografts and sensitizes tumor cells to chemotherapy (Maloney et al. 2003; Min et al. 2003).

*Met and hepatocyte growth factor (HGF).* The Met receptor tyrosine kinase and its ligand, HGF/scatter factor, regulate cell motility, invasion, and proliferation and the deregulation of this signaling pathway contributes to the progression of several malignancies (for review, see Corso et al. 2005). The Met receptor is expressed at low levels in the exocrine pancreas and shows marked up-regulation in PanIN lesions and in PDACs. Additionally, HGF is induced during PDAC progression, present in the epithelium of PanIN lesions and in the stromal cells of advanced tumors (Ebert et al. 1994; Di Renzo et al. 1995; Furukawa et al. 1995; Paciucci et al. 1998). HGF promotes motility of PDAC cells in vitro, and inhibition of this pathway through the administration of blocking antibodies or the truncated HGF fragment NK4 inhibits invasive growth and angiogenesis of xenografts (Tomioka et al. 2001; Saimura et al. 2002).

*Fibroblast growth factor (FGF).* FGF signaling (for review, see Cross and Claesson-Welsh 2001) appears to contribute to mitogenesis and angiogenesis of PDAC. Expression of numerous FGF receptors and glypican-1, a membrane heparin sulfate proteoglycan that facilitates FGF-FGFR interactions, have been detected in primary PDAC samples (Kobrin et al. 1993; Yamanaka et al. 1993a,b; Ohta et al. 1995; Kornmann et al. 1997; Ishiwata et al. 1998; Kleeff et al. 1998; Kornmann et al. 2002). Consistent with a role for FGF signaling in supporting PDAC growth, dominant-negative FGFR-1 mutants or antisense glypican-1 can inhibit the growth of pancreatic cancer cell lines in vitro and suppress their tumorigenic potential in xenografts (Wagner et al. 1998a; Ogawa et al. 2002; Kleeff et al. 2004). FGF signaling may also contribute to the desmoplasia associated with PDAC, since elevated bFGF levels are associated with this phenotype in primary tumors (Kuniyasu et al. 2001).

*VEGF.* VEGF promotes endothelial cell proliferation and survival by binding to the VEGFR-1 and VEGFR-2 endothelial cell transmembrane receptors (for review, see Ferrara et al. 2003). VEGF is overexpressed by PDAC cells (Itakura et al. 1997; Seo et al. 2000), whereas disruption of VEGF signaling by expression of soluble VEGF receptors, VEGF high-affinity binding chimeras, anti-VEGF antibodies, or ribozymes strongly suppresses the tumorigenic growth of pancreatic cancer xenografts (von Marschall et al. 2000; Hoshida et al. 2002; Tokunaga et al. 2002; Hotz et al. 2003; Fukasawa and Korc 2004). VEGF-C, a regulator of lymphoangiogenesis, is also overexpressed in PDAC and may contribute to lymphatic spread and the lymph node metastasis common in this malignancy (Tang et al. 2001; Kurahara et al. 2004). Further study will be required to validate VEGF-C as a drug development target.

### *Developmental signaling pathways in PDAC*

The roles of the Hedgehog and Notch signaling pathways in PDAC pathogenesis have recently been appreciated, further drawing attention to the connections between development and cancer. The relationship between these two pathways, normal pancreatic organogenesis, tissue homeostasis, disease, and the development of cancer are discussed in detail in subsequent sections. Below we briefly describe the biochemical and molecular circuitry of Hedgehog and Notch signaling and their links with PDAC.

*Hedgehog.* The mammalian Hedgehog family of secreted signaling proteins comprised of Sonic, Indian, and Desert Hedgehog (SHH, IHH, and DHH, respectively) regulates the growth and patterning of many organs, including the pancreas, during embryogenesis (Ingham and McMahon 2001). The Hedgehog pathway is negatively regulated by the Patched (PTC) tumor suppressor protein, which tonically inactivates the Smoothed protein (SMO). Hedgehog ligands engage the PTC transmembrane protein and disrupt inhibition of Smo, activating the Gli family of transcriptional regulators. Alterations that activate this pathway, including loss of *PTC*, activating mutations in *SMO*, and overexpression of *GLI* and *HH* proteins, have been implicated in a variety of cancers (for reviews on Hedgehog signaling and cancer, see Taipale and Beachy 2001; Pasca di Magliano and Hebrok 2003). Activation of the Hedgehog pathway has been implicated in both the initiation of pancreatic ductal neoplasia and in the maintenance of advanced cancers. SHH is absent from the normal adult pancreas, but is activated in PanINs, exhibiting a graded increase in progressively later-stage lesions and carcinomas, where signaling seems to be necessary for tumor maintenance (Berman et al. 2003; Thayer et al. 2003).

*Notch.* The Notch signaling pathway, which is important in directing cell fate and cell proliferation during embryonic development, has been shown to contribute to cell transformation in vitro and to the development of human cancers when aberrantly regulated (for review, see Radtke and Raj 2003; Kadesch 2004; Lai 2004; Sjölund et al. 2005). Notch pathway activation involves the binding of membrane-bound Notch receptors (Notch 1–4) to their ligands (Delta-like and Jagged). These receptor–ligand interactions induce proteolysis of the Notch receptor and subsequent nuclear translocation of the Notch intracellular domain (NICD), which mediates the transcriptional activation of a series of target genes. Notch and its ligands are expressed at low or undetectable levels in the normal adult pancreas. However, in PanIN lesions and in pancreatic adenocarcinomas, there are prominent elevations in expression of these factors and an associated induction of transcriptional target genes such as *HES-1*, consistent with activation of this pathway during malignant progression of this malignancy (Miyamoto et al. 2003). Although ectopic activation of Notch signaling within rodent pancreatic progenitor cells in vivo does not result in subsequent

Hezel et al.

carcinogenesis (Murtaugh et al. 2003), there is an accumulating body of literature demonstrating interactions of Notch with RAS, both in development as well as in tumorigenesis (for review, see Sundaram 2005). In particular, several different cell-based systems have shown that activated RAS cooperates with Notch to transform cells; however, others have demonstrated that in certain settings, Notch may suppress transformation. Given the critical role of K-RAS in PDAC and the context-dependent relationship with Notch signaling, it will be imperative to investigate these interactions on the genetic level using pancreatic ductal model systems.

#### *Telomere shortening and dysfunction in PDAC*

Telomere dynamics play a central role in shaping the genomes of many cancer types, particularly epithelial cancers (for review, see Maser and DePinho 2002). While telomerase-mediated preservation of telomere function has been shown to promote the development of advanced malignancies (Hahn et al. 1999), there is equally compelling experimental evidence in both mouse and human cancers that the lack of telomerase activity and a transient period of telomere shortening and dysfunction during early neoplasia drives cancer initiation. This telomere-based mechanism involves generating procarcinogenic chromosomal rearrangements via breakage–fusion–bridge BFB cycles (Artandi et al. 2000) that promote regional amplifications and deletions at the sites of chromosomal breakage (O'Hagan et al. 2002). Importantly, the survival of cells with critically short telomeres and ongoing BFB events is enhanced by deactivation of p53-dependent DNA damage responses; thus telomere dysfunction and p53 loss cooperate to promote the development of carcinomas in multiple tissues (Chin et al. 1999a).

On the basis of these data, telomere erosion might contribute to the high incidence of PDAC in the setting of advancing age or inflammatory conditions as occurs in hereditary pancreatitis as a function of epithelial turnover. Indeed, shortened telomeres and anaphase bridging have been detected in low-grade PanINs, marking telomere erosion as one of the earliest documented genetic events in the evolution of these ductal neoplasms (van Heek et al. 2002). Such observations are in line with previous findings in pancreatic cancer cell lines of the frequent absence of telomeres at chromosome ends and occurrence of anaphase bridging indicative of ongoing BFB cycles and persistent genomic instability (Gisselsø et al. 2001). Although reactivation of telomerase appears critical to the emergence of pancreatic cancer cells, it is a late event in PDAC progression and is preceded by a period of telomere shortening and dysfunction that would appear likely to promote carcinogenesis by leading to the formation of cancer-relevant chromosomal rearrangements. In the evolution of human PDAC, telomere shortening appears to precede the development of p53 mutations, which are found in ~50% of advanced lesions (Hruban et al. 2000a,b; Luttges et al. 2001; van Heek et al. 2002). Such observations raise the possibility

that other p53 pathway components involved in the telomere-induced checkpoint responses are neutralized in a subset of these neoplasms. Alternatively, the loss of p53-independent responses in some tumors could obviate the need to inactivate this pathway. These findings underscore the need to define the wiring of the telomere checkpoint response in evolving PanINs and established PDACs. To this end, it will be of interest to specifically correlate telomere length, p53 status, and the onset of genomic instability in PanINs, and to develop pancreatic cancer models with telomere dysfunction.

#### *Chromosome structural alterations, expression profiles, and other cancer loci in PDAC*

PDAC is characterized by genomic complexity and instability. Telomere shortening, loss of p53, K-RAS mutation, and defects in the mitotic spindle apparatus are all likely contributors to this phenotype. Centrosome abnormalities are detected in 85% of PDAC samples, and there is a correlation between levels of such abnormalities and the degree of chromosomal aberrations (Sato et al. 1999, 2001a). Overall, the pattern of p53 and BRCA2 mutations and the detection of abnormal mitosis and nuclear abnormalities in PanIN-2 and PanIN-3 lesions suggest that genomic instability is initiated in these stages of the tumor progression. The known stereotypical PDAC mutations described above are likely to represent only a small fraction of the genetic lesions resident in these cancers. This view is supported by the detection of recurrent chromosomal amplifications and deletions by karyotype analysis, comparative genomic hybridization (CGH), and LOH studies. Regions of consistent alteration include gains involving 3q, 5p, 7p, 8q, 11q, 12p, 17q, and 20q and losses targeting 3p, 4q, 6q, 8p, 9p, 10q, 12q, 13q, 17p, 18q, 21q, and 22q (Mahlamaki et al. 1997, 2002; Gorunova et al. 1998; Armengol et al. 2000; Schlegel et al. 2000; Sirivatanauskorn et al. 2001; Harada et al. 2002; Adsay et al. 2004; Gysin et al. 2005; Nowak et al. 2005).

Several groups have conducted expression profiling of PDAC cell lines as well as primary tumors, pointing to many novel markers and targets, some of which have been validated by IHC or RT-PCR, including s100P, mapsin, ADAM9, mesothelin, fascin, pleckstrin, 14–3–3, AGR2, IGFBP3 and IGFBP4, and FOXJ1 (Argani et al. 2001; Han et al. 2002; Iacobuzio-Donahue et al. 2002, 2003; Rosty et al. 2002; Grutzmann et al. 2003). Other studies have shown up-regulated expression of known cancer-relevant genes including ABL2, NOTCH4, and SOD1 or have also sought to determine a metastatic signature within evaluated primary PDACs (Crnogorac-Jurcevic et al. 2002; Missiaglia et al. 2004; Nakamura et al. 2004). These transcriptional studies have provided invaluable lists of variably regulated genes in PDAC cell lines, which offer several substrates for therapy, future modeling studies, and potential prognostic markers (Thomas et al. 2004).

Recent high-resolution array CGH analyses of the PDAC genome have uncovered a large number of recur-



rent and highly focal amplifications and deletions, both novel and previously described (Aguirre et al. 2004; Heidenblad et al. 2004; Holzmann et al. 2004; Bashyam et al. 2005). The identification of recurrent chromosomal amplifications and deletions indicate that the current compendium of known genetic lesions represents a very limited collection of molecular mechanisms driving this disease. In order to identify the target of copy number alterations intersecting these array-CGH data with expression profiles has proven useful in further delimiting the candidate cancer gene at each locus. Another filtering approach used to further refine genomic profiles has been the comparisons of copy number alterations across different cancer types (Signoretti et al. 2000; Adsay et al. 2004; Garraway et al. 2005; Tonon et al. 2005).

#### *Tumor biological implications and lessons from PDAC genetics and genomics*

These genetic and genomic observations have several implications for PDAC pathophysiology. Although *K-RAS* mutations are an early, and likely necessary, event in the development of PDAC, their absence in a proportion of the earliest lesions suggests that *K-RAS* activation alone may be insufficient for neoplastic initiation (Klimstra and Longnecker 1994). The onset of PanIN-like lesions in genetically engineered mouse models including *PTEN* loss and elevated Hedgehog and Notch signaling suggests the potential for multiple coinitiating events. One possibility is that the earliest lesions may be nonclonal areas of aberrant proliferation, representing a population of expanded ductal precursor cells and/or cells exhibiting altered states of differentiation that are associated with pancreatic damage or inflammation. Disruptions in tissue architecture and induction of cell proliferation could create conditions that select for cells that sustain activating *K-RAS* mutations. Along these lines, inflammatory stimuli promote the expression of both TGF- $\alpha$  and EGFR in the pancreatic ducts, pathways that are known to synergize with activated *K-RAS* (Barton et al. 1991; Wang et al. 1997).

In addition to the extreme aneuploidy of pancreatic adenocarcinomas, there is a high degree of genetic heterogeneity within these tumors. For instance, different *K-RAS* mutations and 9q, 17p, and 18q LOH patterns have been observed in adjacent PanINs, and multiple *K-RAS* mutations have been detected in the same adenocarcinomas (Moskaluk et al. 1997; Yamano et al. 2000; Luttges et al. 2001). Karyotypic analysis identifying multiple clones within early-passage PDAC cell lines (Gorunova et al. 1998) and distinct array-CGH profiles from separate regions within a single tumor have further demonstrated this heterogeneity (A.F. Hezel and R.A. DePinho, unpubl.) and suggested a spatial distribution of genetic heterogeneity. Neoplastic foci from adjacent regions tend to show similar mutation patterns, whereas increasing genetic divergence has been documented in more geographically distant foci (Yamano et al. 2000). It seems likely that PDAC can develop from the clonal progression of one of several related but divergent le-

sions. These features may indicate that a key event beyond the initiation of PanINs is the acquisition of a mutator state that allows initiated cells to acquire progression-associated genetic lesions. It is tempting to speculate that this tremendous degree of heterogeneity and ongoing instability are at the heart of the intense resistance of pancreatic tumors to chemotherapy and radiotherapy.

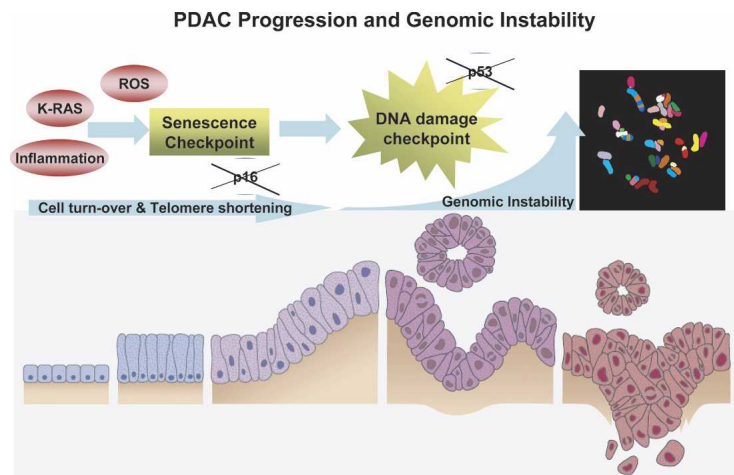
The observation across several tissue types, most notably colon and breast, of a histological evolution of normal epithelium, through preneoplastic stages, to cancer in a graded manner has proven to be both clinically and scientifically informative (Kinzler and Vogelstein 1996). These observations have formed the backbone of most genetic progression models that have sought to characterize molecular profiles at each stage of neoplastic development. While evidence is suggestive of a dominant pattern of serial mutational events in the evolution toward PDAC, this linear tumor progression model will draw continued scrutiny. Such a model must also take into account the altered states of differentiation of PanINs and other precursor lesions, a potential cell or cells of origin, the role of developmental signaling pathways, and an emerging knowledge of genomic and transcriptional alterations as they relate to each stage of disease. A paradigm of pancreatic carcinogenesis must accurately reflect this expanding body of direct evidence. In particular, the acquisition of the genetic mutations may be irregular, occurring in fits and starts, rather than a measured process with consecutive mutations occurring at intervals in time (Fig. 4). This episodic mutational activity could be prompted by key events undermining genomic integrity such as the loss of DNA damage repair and response checkpoints and the erosion of telomeres (Chin et al. 1999a, 2004; Maser and DePinho 2002). Understanding the relationship between the deregulation and/or loss of these lynchpin cellular processes governing genomic stability and the acquisition of a neoplastic genetic profile will also be crucial to the development of accurate disease progression models. Indeed, such an understanding of disease progression is vital for the rational and effective implementation of early detection strategies and preventive therapies.

#### **The cellular basis of PDAC**

Molecular pathology and cancer genetic studies have provided an outline of the cellular perturbations that are associated with PDAC; however, the current picture remains static, with only correlative links to underlying tumor biology. A more direct mechanistic view of how classical lesions influence pancreatic cancer biology is required, and some key questions need to be answered. An important attribute of the signaling pathways activated in pancreatic cancer is their specificity—a permissive context is required for the cell-biological impact of an activated oncogenic pathway to become manifest. A comprehensive appreciation of PDAC pathogenesis must include consideration of the cell type, developmental stage, the constellation of other genetic lesions, and

Hezel et al.

**Figure 4.** Senescence, genomic instability, and the INK4A/ARF and p53 tumor suppressor pathways in PDAC progression. A variety of stimuli including K-RAS oncogenic activation, inflammation, and ROS lead to increased cell proliferation and turnover as well as DNA damage. Various safeguards such as cell cycle checkpoints and oncogene-induced senescence are present to constrain these events. Loss of key tumor suppressor genes, such as p53 and p16, shown here, likely abrogate this constraint, and thus cells begin to accumulate genomic changes. The increasing grades of PanINs en route to invasive PDAC accumulate increasing genetic changes, ultimately resulting in the rampant genomic instability and aneuploidy seen in PDAC. Shown here is an example of spectral karyotyping from a PDAC showing numerous translocations and aneuploidy. The transition of the normal ductal epithelium (far left) through the graded PanIN stages (1–3) to invasive PDAC (far right) is illustrated at the bottom.



microenvironment. Recently, insights into the role of developmental pathways and a possible cell of origin have sharpened our view of the context required for PDAC-associated genetic lesions. Here, we describe the role these pathways play in normal pancreatic development and in malignant transformation, as well as the possible cell type(s) from which PDAC arises.

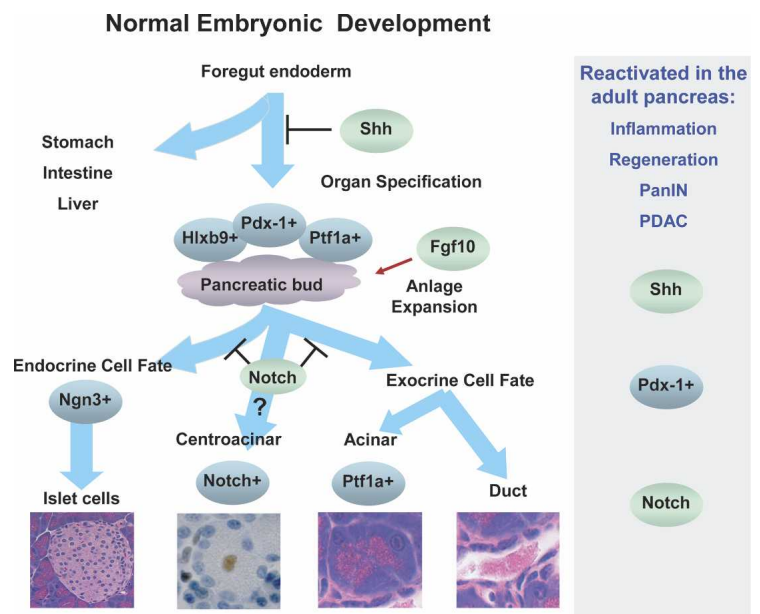
#### Development and cancer

An old concept in cancer biology is the idea that cancers represent an aberrant recapitulation of development. The idea that “oncology recapitulates ontogeny” has gained acceptance as more links between cancer and development have been discovered. Indeed, mounting evidence has supported such a link in PDAC, with critical new insights gleaned from the study of normal pancreas developmental pathways and mechanisms.

The pancreas arises from dorsal and ventral buds in the anterior endoderm that later fuse to become a single organ. A critical event in the specification of the pancreas is repression of sonic hedgehog (*Shh*) within the endoderm of the presumptive pancreatic domain (Fig. 5; Hebrok et al. 1998). *Shh* repression is mediated by signals from adjacent mesodermal structures and results in the expression of the pancreatic homeobox transcription factor *Pdx1* in the nascent pancreatic bud. *Pdx1* is required for further pancreatic development (Ohlsson et al. 1993; Offield et al. 1996), and its expression in all progenitor cells has made the *Pdx1* promoter a useful tool for directing transgene expression during the bud stage (Gu et al. 2002) (see mouse models below).

Prior to the differentiation of the three functional compartments of the pancreas—acinar, ductal, and endocrine—multipotent *Pdx1*<sup>+</sup> cells are maintained in an undifferentiated state by Notch signaling (Apelqvist et

**Figure 5.** Cell differentiation programs in pancreatic development and PDAC. On the left, the various stages of normal pancreatic development are portrayed as well as the key genes involved at various steps. Sonic hedgehog (*Shh*) is expressed in foregut endoderm, and expression is repressed in cells that are destined to form the pancreas. Multipotent cells express *Pdx1*, *Ptf1a*, and *Hlxb9*. *Fgf10* serves to promote expansion of these cells. Repression of Notch signaling allows this population of cells to differentiate into the different pancreatic lineages depicted below. Genes that are reactivated in inflammation, regeneration, or PDAC progression are shown on the right. *Shh* is expressed in PanINs and PDAC, as is *Pdx1* and Notch.



al. 1999; Jensen et al. 2000; Moshous et al. 2001; Hald et al. 2003; Murtaugh et al. 2003). Mesenchymal FGF10 promotes the expansion of these progenitor cells (Hart et al. 2003) in a manner reminiscent of lung development (Hogan 1999); FGF10 may also serve to integrate morphogenesis and differentiation by simultaneously regulating Notch signaling and cell division (Norgaard et al. 2003). Following Notch repression, *Pdx1*<sup>+</sup> progenitors are capable of differentiating into distinct pancreatic lineages (Esni et al. 2004) through the regulated activity of numerous transcription factors (for review, see Murtaugh and Melton 2003). The balance between endocrine and acinar cells seems to be regulated by the activity of TGF- $\beta$  family members (for review, see Kim and Hebrok 2001), and more recent studies have demonstrated a role for Wnt signaling in pancreatic development (Dessimoz et al. 2005; H.J. Kim et al. 2005; Murtaugh et al. 2005). The determinants of ductal lineage specification are largely unknown.

The evidence that embryonic programs re-emerge during the development of pancreatic tumors comes from two lines of investigation: characterization of gene expression and functional analyses. Expression studies have demonstrated the “reactivation” of several embryonic genes during pancreatic carcinogenesis (Fig. 5). PDX1, whose expression in the adult is mostly limited to pancreatic  $\beta$  cells, is expressed in nearly half of PDACs, where it carries a poor prognosis (Koizumi et al. 2003). SHH is normally absent from the pancreas throughout development and adult life but is expressed in PanINs and PDAC, with expression levels that correlate with the grade of the lesion (Berman et al. 2003; Thayer et al. 2003). Similarly, Notch signaling is repressed during development to allow pancreatic differentiation, but Notch signaling components are abundantly expressed in PanIN lesions and PDACs (Miyamoto et al. 2003). Although the role of Wnt signaling in PDAC pathogenesis remains to be defined, stabilization of  $\beta$ -catenin is frequently observed in pancreatoblastoma, a rare pediatric tumor of the pancreas (Abraham et al. 2001).

Functional data also support the importance of “reactivation” of embryonic programs—in particular SHH—in PDAC pathogenesis. Blocking SHH signaling with the inhibitor cyclopamine causes human PDAC cells to undergo apoptosis in vitro and lose tumorigenicity in xenograft assays (Thayer et al. 2003). Furthermore, activation of hedgehog signaling in immortalized human pancreatic ductal cells induces a PanIN-like transcriptional “signature” (Prasad et al. 2005). Importantly, this signature includes several extrapancreatic markers of the foregut. This is consistent with the finding that ectopic hedgehog expression within the pancreatic domain leads to “intestinalization” of the pancreatic epithelium (Apelqvist et al. 1997; Thayer et al. 2003) and suggests that adoption of an intestinal phenotype is an important step in the formation of incipient PDAC. Although not directly studied in PDAC, cooperation of the RAS and Notch signaling pathways in transformation and other biological processes has been demonstrated in several in vitro and in vivo systems (Weijzen et al. 2002; Kiaris et

al. 2004; Sundaram 2005). The applicability of these studies to PDAC has added significance in light of the identification of Notch pathway activity in a candidate precursor, the centroacinar cells (see below in next section).

*Origins of pancreatic cancer.* An emerging hypothesis that is being explored in PDAC and many other solid tumors is that cancer precursors arise from stem cells—cells with the unique potential to self-renew and to differentiate into multiple lineages—that exist within adult tissues. While there is good evidence for such a model in the hematopoietic system, where at least a subset of leukemias is derived from stem cells (Passegue et al. 2004), the “cell of origin” for most solid malignancies, including the pancreas, is unknown. The stem-cell origin hypothesis is supported by evidence that brain tumors arise from CD133<sup>+</sup> neural stem cells (for review, see Singh et al. 2004a), and recent studies are suggestive of a stem cell origin for cancer of the lung (C.F. Kim et al. 2005) and prostate (Maitland and Collins 2005). For other cancers, it remains to be determined whether tumors originate from a resident tissue stem cell, and whether highly tumorigenic cells within a cancer reflect the persistence of such a cell (see discussion of Cancer Stem Cells below).

Recent observations have fostered a more dynamic view of stem cells in which “stem-ness” represents a differentiation state rather than a discrete entity (Blau et al. 2001). Thus, it is possible that cells with stem cell activity may arise by “transdifferentiation” or “dedifferentiation” of other cell types. A byproduct of this model is the notion of “facultative” stem cells—differentiated cells that have the potential to be stimulated to assume a stem cell role. Based on studies of cell renewal and differentiation, Bonner-Weir (Bonner-Weir and Sharma 2002) has argued that all or nearly all of the pancreatic ductal cells are potential facultative stem cells, with the capacity to differentiate into both endocrine and exocrine lineages. In rats subjected to partial pancreatectomy, the exocrine and endocrine compartments exhibit increased cell division, and cells expressing the progenitor cell marker *Pdx1* have been described as “dedifferentiating” and expanding from the pancreatic ducts (Bonner-Weir et al. 1997; Sharma et al. 1999). The appearance of cells with a progenitor phenotype is also observed in a variety of rodent models of pancreatic damage (Vinik et al. 1997; Kritzik et al. 1999; Scoggins et al. 2000). While these observations are consistent with a possible facultative activity of rodent duct cells, it should be noted that the extent of pancreatic “regeneration” differs significantly depending on the method, and thus extent, of injury (e.g., recovery from pancreatitis is more robust than regeneration following pancreatectomy), and the contribution by such ductal cells to other pancreatic lineages has not been analyzed directly. It is noteworthy that certain paracrine signals implicated in the regulation of ductal proliferation in these injury models—TGF- $\alpha$ /EGF and HGF as initiating factors and TGF- $\beta$  as an inhibitor—are also engaged during PDAC tumorigenesis



(Arnush et al. 1996; Friess et al. 1996; Bonner-Weir et al. 1997).

PDACs resemble pancreatic duct cells at the histologic level, displaying cuboidal shape, ductal antigen expression, and growth into tubular structures (Solcia et al. 1995), thereby prompting the widely held view that this malignancy arises from ductal cells. Consistent with this idea, the targeting of key PDAC mutations, *K-RAS* and *INK4A*, to the entire pancreas in the mouse yields lesions exclusively in ducts, suggesting a unique propensity for transformation of this cell type (see mouse modeling section below). Nevertheless, several observations hint at alternative possibilities. For example, acinar-to-ductal metaplasia is frequently seen in association with carcinoma, suggesting a potential acinar origin (Parsa et al. 1985). Indeed, both rat and hamster carcinogen models of pancreatic cancers (Pour 1997; Jimenez et al. 1999) and early models involving genetically altered mice (Jhappan et al. 1990; Sandgren et al. 1990; Wagner et al. 1998b, 2001) all exhibit metaplastic histologies. In these models, acinar cells are lost, either due to direct damage or apparent transdifferentiation, and duct-like tubular complexes emerge and proliferate, although the relationship of these complexes to PDAC remains unclear (Hruban et al. 2006a). Recent studies using genetic lineage markers provide additional evidence that acinar-to-ductal transdifferentiation can account for many new ducts appearing in damaged pancreatic tissues (Means et al. 2005). There is also evidence suggesting an endocrine origin for PDAC including the observation that mouse islet cell cultures expressing the *polyoma virus middle T* (*PyMT*) oncogene proceed to form pancreatic cancers when transplanted into histocompatible mice (Yoshida and Hanahan 1994). Moreover, the focal expression of nonductal lineage markers, including endocrine factors and pancreatic enzymes, indicates that there may be developmental plasticity of the tumorigenic process (for review, see Klimstra 1998). The complexities in tracing the cell of origin of PDAC should not be surprising given the close developmental relationships of the pancreatic cell types and the known propensity of endodermal lineages to transdifferentiate in vitro and in vivo (Tosh and Slack 2002). Finally, in the context of a stem cell model for tumor initiation, PDAC could arise from a rare precursor population in the pancreas.

It is also possible that there is no unique “cell of origin” for PDAC. In brain tumors, for example, mutations of *INK4A/ARF* and *EGFR* in either neural stem cells or differentiated astrocytes of mice give rise to malignant gliomas with indistinguishable tumor phenotypes (Bachoo et al. 2002). Thus, it may be that specific genetic alterations, rather than the identity of the target cell, define the ensuing malignant phenotype. The highly specific mutational profiles of the different types of pancreatic cancers suggest that this concept may be relevant to pancreatic neoplasia (Table 1).

One strategy to identify the cellular origin of pancreatic cancer is to focus on stem cells in the adult pancreas. Pancreatic stem cells with the capacity to give rise to  $\beta$

cells have long been sought for their therapeutic potential in type I diabetes. Despite extensive investigation, such cells have not been isolated, and it appears that the majority of  $\beta$  cells in vivo are generated by replication of existing  $\beta$  cells rather than formation of new  $\beta$  cells from stem cells (Dor et al. 2004). However, these results do not preclude the possibility that a stem cell exists in the pancreas. As mentioned above, a facultative stem cell might be called into action only under particular conditions of stress. Furthermore, a pancreatic facultative stem cell might have a differentiation potential that is limited to acinar and duct cells. Recent observations have identified a candidate for such a cell, the pancreatic centroacinar cell (CAC). CACs are strategically located at the junction of the acinar and ductal compartments and exhibit ultrastructural features of duct cells. Notably, Notch signaling remains selectively active in these adult cells, reflecting the persistence of an embryonic program that functions to repress differentiation.

Further evidence of the importance of CACs in PDAC initiation comes from mice with a pancreas-specific deletion of the *PTEN* gene. Such mice exhibit a metaplasia-to-carcinoma sequence that is preceded by the proliferative expansion of CACs that continue to exhibit active Notch signaling (Stanger et al. 2005). If CACs are determined, through more rigorous investigation, to represent a true cell of origin for PDAC, several important questions will need to be addressed: Does this cell represent a stem cell that functions during normal pancreatic homeostasis? What features of this cell make it susceptible to the transforming activity of particular oncogenes? Finally, do different PDAC-associated lesions—PanIN, MCNs, and IPMN—arise from a single cell that has been subjected to different genetic “hits”? Or are there multiple cell types that are susceptible to transformation, each of which is capable of giving rise to tumors with a distinct or overlapping biological behavior?

**Cancer stem cells.** Another feature that links development and cancer is that both embryos and tumors are composed of heterogeneous cell types. Some time ago, it was recognized that only a small fraction of tumor cells has the capacity to reconstitute clonogenic growth in vitro and in vivo (Fidler and Hart 1982; Heppner 1984). More recent studies have provided strong evidence to explain this observation through the existence, within several types of human tumors, of a small number of cells with stem/progenitor characteristics. Such cells can be identified on the basis of their cell surface profile and have the capacity to efficiently reconstitute tumors. Seminal studies with acute myelogenous leukemia (AML) demonstrated that a small fraction of tumor cells, comprising 0.1%–1% of the total, were the only cells capable of transferring leukemia following transplantation into an immunodeficient mouse (Lapidot et al. 1994; Bonnet and Dick 1997). Tumor-reconstituting cells have also been described in solid organs, including cancer of the breast, brain, and prostate (Al-Hajj et al. 2003; Singh et al. 2004b; Collins et al. 2005), and candi-

dates have been found in lung neoplasia and melanoma (Fang et al. 2005; C.F. Kim et al. 2005).

It is currently unknown whether pancreatic cancers also harbor so-called “cancer stem cells.” But since most cancer therapies target the bulk of tumor cells, and since tumor stem cells (like their normal tissue counterparts) may be more resistant to chemotherapy, the question is not merely an academic one. Indeed, it is possible that a mechanism to ensure cancer stem cell renewal is a required aspect of PDAC pathogenesis and maintenance. Specifically, expression of the *Bmi* proto-oncogene is required for self-renewal of both hematopoietic stem cells and leukemia stem cells (for review, see Pardal et al. 2003). As BMI acts by repressing *INK4A/ARF*, the invariable loss of the *INK4A/ARF* locus in PDAC may reflect a mechanism by which pancreatic cancers ensure self-renewal of a “stem cell” population. Ultimately, if such tumor-maintaining cells are found to exist in PDAC, comparison with the cell(s) from which PDAC arises (cell of origin) may provide great insight into the molecular and cellular pathogenesis of the disease.

### Genetically engineered mouse models of PDAC

The recurrent spectrum and the sequential appearance of specific mutational events point toward a defined molecular program for PDAC progression. Genetically en-

gineered mice (GEM) have provided tractable in vivo systems to dissect the biological impact of oncogenic mutations in a wide number of malignancies (for review of other animal models of cancer, see Van Dyke and Jacks 2002). Beyond establishing such genotype–phenotype relationships, these GEM have the potential to identify early markers of disease, pinpoint cooperating genetic alterations, and provide better preclinical models to inform therapeutic initiatives. A wide range of mouse models of pancreatic cancer has been built using varied transgenic and gene targeting approaches. A key consideration has been deciding how to target mutant alleles to the pancreas, or to specific pancreatic cell lineages. Advances in pancreatic developmental biology have enabled a generation of refined genetically engineered mouse models that closely mirror many of the genetic and histologic characteristics of the human disease (Table 2). Furthermore, recent histopathologic review of these models has led to a consensus view among pancreatic pathologists, creating a foundation for more accurate comparisons across the different genetically engineered mouse models and their relationship to the human disease (Hruban et al. 2006a).

*Targeting oncogenes to the acinar cell compartment.* Early attempts to model exocrine pancreatic cancer used acinar-specific transgene expression, taking advantage of the wide availability of promoters capable

**Table 2.** Mouse models of pancreatic cancer

Gene/promoter	Phenotype of mouse
Transgenics with predominantly acinar phenotypes	
T-Ag/ elastase	Acinar cell carcinoma
Hras/ elastase	Acinar cell carcinoma
TGF- $\alpha$ / elastase	Acinar cell carcinoma
	Develop mixed acinar-ductal tumors on a p53 <sup>+/-</sup> background
TGF- $\alpha$ / metallothionein	Tubular metaplasia. Develop lesions resembling serous cystadenomas on Ink4a/Arf- or p53-null background.
c-myc/ elastase	Mixed acinar-ductal tumors
KrasG12D/ Mist1	Acinar cell carcinoma
Transgenics using the RCAS TVA system	
c-myc/ elastase	Islet cell tumors in Ink4a/Arf-null mice
PyMT/ elastase	Mixed acinar-ductal tumors in Ink4a/Arf-null mice.
Activated Kras knock-in GEM	
<i>Kras</i> <sup>G12D</sup> <i>Pdx1-Cre</i>	Spectrum of PanINs and some mice develop PDAC with long latency
<i>Kras</i> <sup>G12D</sup> <i>Pdx1-Cre</i>	Develop PDAC with shorter latency than <i>Kras</i> <sup>G12D</sup> alone.
<i>Ink4a</i> <sup>-/-</sup>	
<i>Kras</i> <sup>G12D</sup> <i>Pdx1-Cre</i>	Develop PDAC with high penetrance and short latency. Micrometastatic disease.
<i>Ink4a/Arf</i> <sup>-/-</sup>	
<i>Kras</i> <sup>G12D</sup> <i>Pdx1-Cre</i>	Develop PDAC with high penetrance. Gross metastatic disease. LOH of wild-type p53 allele.
<i>p53</i> <sup>R273H</sup> or <i>p53</i> <sup>+/-</sup>	
<i>Kras</i> <sup>G12D</sup> <i>Pdx1-Cre</i>	Develop PDAC with longer latency than Ink/Arf-null mice. Gross metastatic disease. LOH of wild-type <i>Ink4a/Arf</i> allele.
<i>Ink4a/Arf</i> <sup>+/-</sup>	
<i>Kras</i> <sup>G12D</sup> <i>Pdx1-Cre</i>	Develop PDAC with high penetrance and shorter latency than p53 <sup>+/-</sup> . LOH of wild-type p53 allele and loss of Ink4a expression.
<i>p53</i> <sup>+/-</sup> <i>Ink4a</i> <sup>+/-</sup>	
Other related GEM with PDAC or precursor phenotypes	
<i>Pten</i> <sup>-/-</sup> <i>Pdx1-Cre</i>	Ductal metaplasia with a fraction of the mice developing PDAC.
<i>Pdx1-Shh</i>	Ductal-intestinal metaplasia

Hezel et al.

of targeting this compartment. Transgenic mice that express SV40 large T antigen (T-Ag) (Ornitz et al. 1987; Glasner et al. 1992), activated *H-RAS* (Quaife et al. 1987), or c-Myc (Sandgren et al. 1991, 1993) in the acini using the Elastase (Ela) promoter develop acinar cell carcinomas, although Ela-Myc tumors progress to mixed acinar-ductal histology. Transgenic mice expressing TGF- $\alpha$  in the acinar cells (Ela-TGF- $\alpha$ ) on a *p53*-deficient background develop mixed acinar-ductal tumors or cystic acinar tumors (Wagner et al. 2001). Metallothionein-TGF- $\alpha$  (MT-TGF- $\alpha$ ) transgenic mice also have acinar TGF- $\alpha$  expression but do not develop PDAC even in the context of *Trp53* and *Ink4a/Arf* deficiency. Instead, these tumor suppressor mutations cooperate to promote benign pancreatic ductal lesions resembling serous cystadenomas in humans (Bardeesy et al. 2002a).

Acinar cells have also been targeted in a more recent report using the RCAS-TVA system. This refined transgenesis approach involves the somatic delivery of retroviruses encoding genes of interest to specific cellular compartments (Orsulic 2002; Pao et al. 2003). In studies exploring the differential impact of specific oncogenes, TVA (the receptor for the avian leukosis sarcoma virus subgroup A [ALSV-A]) was placed under the control of the elastase promoter that is active in the acinar cells, creating *elastase-tva* transgenic mice (Lewis et al. 2003). The delivery of ALSV-A-based RCAS vectors encoding either c-Myc or PyMT antigen to *elastase-tva Ink4a/Arf*-null mice yielded pancreatic tumors with distinct histological phenotypes. The c-Myc-transduced animals developed only islet cell tumors, whereas PyMT-transduced mice developed pancreatic tumors of mixed acinar and ductal features. In the setting of intact *Ink4a/Arf*, PyMT-transduced mice developed PanIN-like lesions in a subset of cases, suggesting a role for *Ink4a/Arf* in restraining PanIN progression. The complex pattern of tumors with distinct lineages despite the targeting of the elastase-positive compartment (predominantly acinar) may relate to the introduction of RCAS viruses on postnatal day 2. At this stage, *elastase-tva* displayed a more extensive distribution throughout the pancreas compared with the acinar-specific expression in the adult pancreas. These studies illustrate how both the specific identity of an oncogene and the “developmental context” may influence the neoplastic phenotype.

**Activated *Kras* GEM.** *Kras* activation is the defining lesion in PDAC, prompting the generation of GEM in which activated *Kras* transgenes have been targeted to specific lineages or a *Kras* knock-in allele has been activated throughout the pancreatic epithelium via Cre recombinase expression. The knock-in studies used a *Kras*<sup>G12D</sup> allele (referred to as *LSL-Kras*<sup>G12D</sup>) that is expressed at the same level and in the same cell types as the endogenous gene after Cre-mediated excision of a LoxP-flanked Stopper element. This system mimics the acquisition of such activating point mutations in human cancers.

Pdx1-Cre and Ptf1-p48-Cre deleter strains have been used to activate *Kras* and induce mutations in the *Ink4a/*

*Arf* and/or *p53* tumor suppressor loci in the pancreas. The Pdx1 and Ptf1-p48 promoters are active in the common progenitors of all pancreatic cell types with relatively restricted expression outside of the pancreas (Pdx1 is also expressed in the developing duodenum and stomach, and Ptf1-p48 is expressed in the cerebellum) (Kawaguchi et al. 2002; Gu et al. 2003). Cre-induced activation of *LSL-Kras*<sup>G12D</sup> leads to the rapid development of PanIN lesions in the first few weeks of life (Aguirre et al. 2003; Hingorani et al. 2003). A related knock-in stopper *Kras*<sup>G12V</sup>-IRES-lacZ allele produced PanINs when combined with a *CDK4*<sup>R24C</sup> point mutant allele, which is refractory to INK4A inhibition (Guerra et al. 2003). In the *Kras*<sup>G12D</sup>-mutant GEM, the resulting neoplasms show a gradual, age-dependent progression of lesions resembling human PanINs-I-III and the development of frank PDAC after a long latency. Additionally, the presence of normal ducts within these pancreases, all of which harbor the activated *Kras*<sup>G12D</sup> allele, conveys that other events, beyond *Kras* activation, are required to initiate neoplastic changes. In summary, these studies clearly illustrate the potent role of *Kras*<sup>G12D</sup> in initiating PanIN development, while also indicating that other rate-limiting events are likely to constrain progression of these *Kras*<sup>G12D</sup>-driven neoplasms toward high-grade PanINs and PDAC.

#### Exploring tumor suppressor function in PDAC progres-

sion. The tumor suppressor roles of *p53* and *Ink4a/Arf* have been investigated against the backdrop of the Pdx1-Cre LSL-*Kras* system. Mice with a pancreas-specific deletion of *Ink4a/Arf* or *p53* do not develop pancreatic neoplasia. However, when *Kras* activation is combined with mutations of either tumor suppressor, a rapidly progressive and lethal PDAC phenotype emerges. Pdx1-Cre LSL-*Kras* mice, homozygous for a conditional *Ink4a/Arf* allele, uniformly develop invasive PDAC by 7–11 wk (Aguirre et al. 2003); animals heterozygous for *Ink4a/Arf* also developed PDAC but with longer latency (Bardeesy et al. 2006). These PDAC lesions showed histologic and molecular resemblance to the human disease including the association with high-grade PanINs and a proliferating stroma. Similarly, rapidly progressive PDAC occurs with the combination of the *Kras*<sup>G12D</sup> allele with a conditional null allele of *p53* (Bardeesy et al. 2006) or a *p53* knock-in allele (*p53*<sup>R273H</sup>, a point mutant observed in Li-Fraumeni syndrome) (Hingorani et al. 2005). Overall, these results demonstrate that *Ink4a/Arf* and *p53* do not play a primary role in the onset of PanIN but, rather, form a critical barrier in blocking progression of PanIN initiated by *Kras*<sup>G12D</sup>. The observed contributions of *Kras* to PanIN initiation and of *Ink4a/Arf* and *p53* to PDAC progression in the mouse fit well with the sequential appearance of *Kras* mutations in the earliest-stage PanIN and subsequent *Ink4a/Arf* and *p53* mutations in more advanced lesions. It is notable that despite the activation of *Kras* and deletion of tumor suppressors in all pancreatic lineages, no neoplasia of the acini or islets was apparent.



As noted above, a significant proportion of human PDACs show mutational inactivation of each of the *Ink4a*, *Arf*, and *p53* tumor suppressors, and hence a key question is in determining the relative roles of these genes. In the *Kras* *Ink4a*/*Arf* model, all PDACs retain *p53* function, indicating that loss of *p53* is not obligate for tumor progression in the mouse (Aguirre et al. 2003; Bardeesy et al. 2006). In contrast, PDACs in the *Kras* *p53*<sup>R273H</sup> mice—and in mice with a heterozygous *p53* deletion—retain both *Ink4a* and *Arf* while losing expression of the wild-type *p53* allele (Hingorani et al. 2005; Bardeesy et al. 2006). One interpretation of these results is that *Arf* and *p53* function in a common pathway to suppress PDAC and that *Ink4a* loss may not be a critical event in murine PDAC progression. On the other hand, studies of *Kras* mice with combined *p53* and *Ink4a* (but intact *Arf*) heterozygous deletions reveal loss of *Ink4a* in most tumors—via deletion or promoter hypermethylation of the wild-type allele—in addition to loss of wild-type *p53* (Bardeesy et al. 2006). Moreover, these animals develop PDAC with shorter latency than mice with heterozygous *p53* deletion and wild-type *Ink4a*. Finally, germline homozygous deletion of *Ink4a* accelerates the development of PDAC in *Kras* mice, although the latency is longer than in mice with deletion of both components of the *Ink4a*/*Arf* locus (Bardeesy et al. 2006). Together, these results suggest that each of these tumor suppressors contributes to the control of PDAC progression in the mouse; specifically, loss of *Arf* and *p53* appears to have redundant effects on tumorigenesis, and either of these lesions may cooperate with *Ink4a* loss. While the retention of *Ink4a* in the mice engineered to sustain *p53* mutations may point to a reduced function of this tumor suppressor in murine PDAC, it is also possible that engineered pre-existing *p53* mutations early during PanIN progression may create a context in which there is reduced selective pressure to mutate *Ink4a*, possibly by facilitating other genetic events that deregulate the *Ink4a*–Rb pathway. A notable feature of each of these models is the maintenance of wild-type *Smad4* expression in all tumors. Engineering mutant *Smad4* alleles within the context above described models will be important in uncovering the specific role of this tumor suppressor in PDAC progression.

Considering these data from the mouse in relation to the known genetics of human PanIN progression, it should be pointed out that *p53* mutations are not observed in PanIN-I or PanIN-II, whereas these lesions show frequent *Ink4a* loss; therefore, the presence of early *p53* mutations may create biological conditions not normally encountered in the pathogenesis of PDAC (Hruban et al. 2000a,b). On the other hand, there is some evidence that the circuitry regulating induction of *Arf*, *p53*, and *Ink4a* in mouse cells may differ from that in human cells; hence there may not be complete overlap of the biological function of these tumor suppressors across species (Rangarajan et al. 2004). Specifically, in human somatic cells experiencing oxidative stress and chronic proliferation, the ensuing shortening of telomeres is likely to activate a DNA damage response pathway lead-

ing to *p53* induction (Sharpless and DePinho 2002) (see above). In contrast, the long telomeres and constitutive telomerase activity in the mouse ensure that advancing murine PanINs do not exhibit telomere erosion and consequent activation of *p53* (Prowse and Greider 1995). With respect to *Arf* and *Ink4a*, murine cells grown in vitro show strong activation of *Arf* by stress stimuli such as high oxygen tensions, high serum, and oncogene expression, whereas stress stimuli in human cells preferentially activate *Ink4a* (Collins and Sedivy 2003; Brookes et al. 2004). It remains to be determined whether there are, indeed, cross-species differences in the oncogenic circuitry of PDAC and how additional perturbations to the model, such as telomere dysfunction, may affect genetic and biological pressures in this disease. Notwithstanding these potential differences, it is clear that the signature mutations associated with human PDAC also contribute to the pathogenesis of the murine tumors.

The different combinations of tumor suppressor gene mutations, in conjunction with *Kras*<sup>G12D</sup> expression, produce tumors with varying spectra of clinical and histological features. While tumors of all genotypes are locally invasive and show micro-metastases, gross metastases appear to be restricted to mice engineered to sustain heterozygous tumor suppressor deletions (*Ink4a*/*Arf*<sup>lox/+</sup> or *p53*<sup>lox/+</sup> *Ink4a*<sup>lox/+</sup> mice) and do not appear in mice with engineered homozygous deletions (Aguirre et al. 2003; Hingorani et al. 2005; Bardeesy et al. 2006). This may reflect the fact that the homozygous models develop multifocal tumors resulting in a rapidly lethal tumor burden, whereas the longer latency of heterozygous models affords the time for clonal maturation, progression, and metastasis. With respect to the impact of genotype on tumor histology, *p53* deficiency is associated with a higher prevalence of well-differentiated ductal adenocarcinoma compared with the *Ink4a*/*Arf*-deficient animals (Hingorani et al. 2005; Bardeesy et al. 2006). Conversely, undifferentiated sarcomatoid histology, a feature of the *Ink/Arf* model, is significantly reduced in *p53*-deficient models. In humans, ductal adenocarcinoma histology predominates, and the sarcomatoid subtype is an uncommon variant of PDAC with more aggressive clinical behavior, although having a comparable spectrum of genetic lesions. These mouse models collectively recapitulate these histologic variants, albeit at different frequencies from those seen in spontaneous human tumors. Overall, these observations suggest that tumor suppressor lesions influence the cell differentiation phenotypes of the resulting tumors.

*Mouse models and insights into the PDAC cell of origin.* The use of different approaches to express activated *Kras* in the pancreas or in specific pancreatic lineages has begun to provide insights into the PDAC cell of origin. As indicated above, in the case of the Pdx1-Cre LSL-*Kras* models, all pancreatic cells including islets and acinar cells harbor an activated *Kras*<sup>G12D</sup> allele and inactivating tumor suppressor mutations, yet the only neoplastic phenotype elicited is prominent PanINs and PDAC, while islet and acinar cancers are not observed.

Hezel et al.

Such observations favor the view that only certain cell types within the pancreas are susceptible to transforming effects of physiological *Kras*<sup>G12D</sup> expression. On the surface, such observations would favor the view of a ductal origin or possibly a centroacinar cell origin. At the same time, while PanINs may appear to arise from differentiated ducts, it is worth noting that the targeting of activated *Kras* to mature ductal cells using the cytokeratin-19 promoter failed to produce PanINs or PDAC, resulting, instead, in periductal inflammation (Brembeck et al. 2003). In a separate set of experiments, elastase-directed activated *Kras* transgene expression in the acinar cells yielded a spectrum of neoplasms including acinar carcinomas and ductal lesions with resemblance to PanIN (Brembeck et al. 2003; Grippo et al. 2003). A similar phenotype was observed with the targeting of *Kras* to the *Mist1* locus, which is expressed at low levels early in pancreatic development and at higher levels in the adult acinar compartment (Tuveson et al. 2006). Taken together, these studies suggest that the ensuing *Kras*-driven neoplastic phenotypes are determined by the cell type, cellular differentiation state, and/or the level of *Kras* expression. The close recapitulation in the *Pdx1*-Cre LSL-*Kras* models of human PanIN to PDAC progression suggests that these mouse neoplasms may share a common cellular origin with the human counterpart, and, thus, a detailed analysis of incipient neoplasms in this model might provide insights into the cellular compartment(s) susceptible to transformation. It is tempting to speculate that the activation of *Kras* in all cell types within the pancreas using the *Pdx1*-driven Cre may target a uniquely susceptible cell compartment such as a pancreatic duct precursor cell, a centroacinar cell, or an as-yet-uncharacterized cell type.

*Genomic instability profiles in GEM.* Widespread chromosomal instability is a defining characteristic of human PDAC. While the presence of such complex karyotypes in the vast majority of human carcinomas has long been documented, the pathogenetic significance in driving tumorigenesis and the underlying genome instability mechanisms are areas of ongoing study and discussion (Duesberg and Rasnick 2000; Rajagopalan and Lengauer 2004). Origins of chromosomal instability in cancer are likely to include diverse defects in the mitotic spindle apparatus, various checkpoint pathways such as p53, telomere dysfunction, increased ROS, and DNA repair pathways such as nucleotide excision repair (NER) and nonhomologous end-joining (Sharpless et al. 2001b; Maser and DePinho 2002; Woo and Poon 2004; Cimini and Degraffi 2005; Kops et al. 2005). The observation of polyploidy in association with aging and senescence and its observation in premalignant lesions have suggested that such an intermediate karyotype may play a role in the acquisition of aneuploidy (Storchova and Pellman 2004). It is likely that a collusion of defects in many of the above pathways contribute to the rampant instability profile of human PDAC.

The recent genomic analyses, using both array-CGH studies and spectral karyotyping (SKY), have provided

both quantitative and qualitative measures of genomic instability among several of the above-described mouse PDAC models. Importantly, these studies have suggested that some of the mechanisms driving genomic instability are present in these models (Schreiner et al. 2003; Hingorani et al. 2005; Bardeesy et al. 2006). In general, all of the evaluated PDAC models have shown evidence of global genomic alteration, and the comparison of specific models has pointed toward possible influences of genotype on genomic stability. p53 mutant tumors demonstrate a modest increase in copy number alterations in comparison with PDACs arising on an *Ink4a/Arf* mutant background (Bardeesy et al. 2006). In addition to these differences in overall genomic complexity among cohorts, the patterns of regional genomic changes fell into groups when analyzed by nonhierarchical clustering algorithms. Moreover, specific regions of alteration were associated with particular genotypes; tumors harboring p53 mutations showed frequent *Myc* amplifications, while those associated with *Ink4a/Arf* mutations showed highly recurrent *Kras* amplifications (Bardeesy et al. 2006).

Defining the anatomy of genomic rearrangements by SKY analysis of mouse PDAC has documented chromosomal aberrations (e.g., NRTs, tetraploidy, and whole chromosomal gains and losses) reminiscent of the human cytogenetic profiles (Hingorani et al. 2005). At the same time, it is worth noting that the absolute degree of chromosomal structural aberrations in the current collection of GEM appears less than that of human PDAC. Modeling genomic instability in other mouse cancer models has been achieved using engineered mutants with defects in several of these processes including DNA repair genes, checkpoint controls, and telomere maintenance. As we have speculated above, engineering telomere-based crisis into the various genetically engineered mouse models may prove useful in driving comparable levels of NRTs, amplifications, and deletions. Other key areas that merit further study for potential effects on genomic stability are the mitotic spindle dynamics, DNA repair mechanisms including mismatch repair and nonhomologous recombination, and the initiation of PDAC in older adult mice rather than in embryonic stages. The identification of syntenic regions of genomic gain and loss across mouse and human PDAC data sets may enhance cancer gene discovery efforts. Indeed, the use of cross-species comparison to discern a *Kras* oncogenic expression signature has offered support to this idea, demonstrating the utility of using murine tumors to uncover patterns of gene expression that are present in human cancers (Sweet-Cordero et al. 2005).

### Major challenges and opportunities in pancreas cancer

Important insights into PDAC biology have been made. A focus on familial and epidemiologic risk factors, pathologic progression, and molecular characterization has pointed toward key processes and pathways governing PDAC genesis and evolution. An increasing number of the genetic changes have been experimentally verified

across a range of systems, and the recent development of mouse model systems has allowed for functional analysis of the various PDAC mutations *in vivo*. Important roles of developmental pathways, including Notch and SHH, have been identified and have pointed toward a possible cell of origin for the disease. Overall, the generation of robust model systems and the availability of comprehensive molecular profiles have created a strong foundation for ongoing discovery.

Key outstanding questions remain. In terms of basic biology, these include definitively establishing the cell(s) of origin, understanding the role of transdifferentiation, and the identification and definition of a cancer stem cell population in PDAC. In addition, the role of the mesenchyme and stroma, which influence pancreatic development and are prominent components of PDAC, must be defined. Progress in reducing the tremendous morbidity and mortality could come with ongoing efforts focused on understanding the biology of early disease states and identifying markers of disease. Along these lines, the research community should pay particular attention to the evolution of PanIN and other premalignant lesions into PDAC, focusing on critical shifts in the behavior of these lesions and how they relate to underlying molecular alterations, checkpoint responses, genomic complexity, and the activation of key signaling cascades. Linking such molecular processes that are indicative of a histopathologic stage of disease with a screening test (be it a molecular imaging reagent or proteomic marker present in serum) could have a profound impact on clinical practice and patient outcome.

The capacity to accurately model PDAC in the mouse has created the opportunity to study the biologic effect of cancer genes; to characterize genomic instability, angiogenesis, and the tumor microenvironment; and to provide insight into pathways and molecules that could serve as targets for therapy. Moreover, these models serve as a valuable resource to test candidate compounds for their therapeutic potential. The development of inducible PDAC models is an important priority. Studies of transgenic melanoma and lung adenocarcinoma models directed by inducible *H-* and *K-ras* alleles, respectively, have shown that sustained mutant *RAS* activity is necessary for both the initiation of tumorigenesis and for maintenance of the transformed state (Chin et al. 1999b; Fisher et al. 2001). Existing evidence suggests that *K-RAS* is critical for PDAC maintenance. An inducible model could serve to validate this *in vivo* and uncover precisely how *K-RAS* may function to support advanced PDAC. Expression profiling analysis using such an inducible system could point toward downstream *RAS* effectors and potential therapeutic targets (Bardeesy et al. 2005). Additionally, inducible model systems could provide serum proteomic profiles specifically regulated by *K-RAS* activity across the spectrum of disease states, from incipient PanIN, to PDAC, and ultimately metastatic disease. These could lead to the better selection of disease markers for future clinical screening tests.

Therapeutic options can be advanced through the development of drugs targeting key pathways and mol-

ecules. As discussed in detail, the inability to pharmacologically target *K-RAS* leaves the research community with known downstream pathways, supporting signaling networks, and large numbers of oncogene candidates. Presently known *RAS* effectors (MEK, PI3K components, etc.) (see Fig. 3 for further detail) and other relevant pathways including SHH and Notch have inhibitors in various stages of development. Multi-agent targeted combinations are likely to be required. The rational construction of such regimens will require consideration of disease biology as well as the understanding of how targets function in normal organisms. Mouse model systems may allow us to best address this preclinically, saving valuable resources for the clinical testing of those combinations most likely to benefit patients. Given the collusion of technologies across the spectrum of proteomic, molecular-genetic, imaging, and biologic fields, and the recent advances in PDAC research, the promise of scientific and medical progress appears to be within reach.

## Acknowledgments

We thank Anirban Maitra and Anna Means for their critical reading of the manuscript and insightful comments, and Gregory Lauwers (Department of Pathology, Massachusetts General Hospital) for generously contributing his expertise and providing the images used in Figure 2. The original illustrations used in Figures 1 and 4 were created by Josh Berta. A.F.H. was supported by the PanCAN/ASCO Samuel Stroum young investigator award. B.Z.S. was supported by NIH K08 DK064136. A.C.K. is a recipient of the Leonard B. Holman Research Pathway fellowship. N.B. was supported by NIH K01CA104647A-01A1 and grants from NCI/SAIC Frederick and the Harvard Stem Cell Institute. R.A.D. is an American Cancer Society Research Professor and an Ellison Medical Foundation Senior Scholar, and is supported by the Robert A. and Renee E. Belfer Foundation, Institute for Innovative Cancer Science, and NIH U01CA84313-07 and P01CA117969-01 grants.

## References

- Abraham, S.C., Wu, T.T., Klimstra, D.S., Finn, L.S., Lee, J.H., Yeo, C.J., Cameron, J.L., and Hruban, R.H. 2001. Distinctive molecular genetic alterations in sporadic and familial adenomatous polyposis-associated pancreatoblastomas: Frequent alterations in the APC/ $\beta$ -catenin pathway and chromosome 11p. *Am. J. Pathol.* **159**: 1619–1627.
- Adsay, N.V., Merati, K., Basturk, O., Iacobuzio-Donahue, C., Levi, E., Cheng, J.D., Sarkar, F.H., Hruban, R.H., and Klimstra, D.S. 2004. Pathologically and biologically distinct types of epithelium in intraductal papillary mucinous neoplasms: Delineation of an 'intestinal' pathway of carcinogenesis in the pancreas. *Am. J. Surg. Pathol.* **28**: 839–848.
- Agbunag, C. and Bar-Sagi, D. 2004. Oncogenic *K-ras* drives cell cycle progression and phenotypic conversion of primary pancreatic duct epithelial cells. *Cancer Res.* **64**: 5659–5663.
- Aguirre, A.J., Bardeesy, N., Sinha, M., Lopez, L., Tuveson, D.A., Horner, J., Redston, M.S., and DePinho, R.A. 2003. Activated *Kras* and *Ink4a/Arf* deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. *Genes & Dev.* **17**: 3112–3126.
- Aguirre, A.J., Brennan, C., Bailey, G., Sinha, R., Feng, B., Leo, C.,



- Zhang, Y., Zhang, J., Gans, J.D., Bardeesy, N., et al. 2004. High-resolution characterization of the pancreatic adenocarcinoma genome. *Proc. Natl. Acad. Sci.* **101**: 9067–9072.
- Akhurst, R.J. and Derynck, R. 2001. TGF- $\beta$  signaling in cancer—A double-edged sword. *Trends Cell Biol.* **11**: S44–S51.
- Al-Hajj, M., Wicha, M.S., Benito-Hernandez, A., Morrison, S.J., and Clarke, M.F. 2003. Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci.* **100**: 3983–3988.
- Almoguer, C., Shibata, D., Forrester, K., Martin, J., Arnheim, N., and Perucho, M. 1988. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. *Cell* **53**: 549–554.
- Altomare, D.A., Tanno, S., De Rienzo, A., Klein-Szanto, A.J., Tanno, S., Skele, K.L., Hoffman, J.P., and Testa, J.R. 2003. Frequent activation of AKT2 kinase in human pancreatic carcinomas. *J. Cell. Biochem.* **88**: 470–476.
- Apelqvist, A., Ahlgren, U., and Edlund, H. 1997. Sonic hedgehog directs specialised mesoderm differentiation in the intestine and pancreas. *Curr. Biol.* **7**: 801–804.
- Apelqvist, A., Li, H., Sommer, L., Beatus, P., Anderson, D.J., Honjo, T., Hrabe de Angelis, M., Lendahl, U., and Edlund, H. 1999. Notch signalling controls pancreatic cell differentiation. *Nature* **400**: 877–881.
- Argani, P., Rosty, C., Reiter, R.E., Wilentz, R.E., Murugesan, S.R., Leach, S.D., Ryu, B., Skinner, H.G., Goggins, M., Jaffee, E.M., et al. 2001. Discovery of new markers of cancer through serial analysis of gene expression: Prostate stem cell antigen is overexpressed in pancreatic adenocarcinoma. *Cancer Res.* **61**: 4320–4324.
- Arlt, A., Gehr, A., Muerkoster, S., Vorndamm, J., Kruse, M.L., Folsch, U.R., and Schafer, H. 2003. Role of NF- $\kappa$ B and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine-induced cell death. *Oncogene* **22**: 3243–3251.
- Armengol, G., Knuutila, S., Lluís, F., Capella, G., Miro, R., and Caballin, M.R. 2000. DNA copy number changes and evaluation of MYC, IGF1R, and FES amplification in xenografts of pancreatic adenocarcinoma. *Cancer Genet. Cytogenet.* **116**: 133–141.
- Arnush, M., Gu, D., Baugh, C., Sawyer, S.P., Mroczkowski, B., Krahl, T., and Sarvetnick, N. 1996. Growth factors in the regenerating pancreas of  $\gamma$ -interferon transgenic mice. *Lab. Invest.* **74**: 985–990.
- Arsura, M., Mercurio, F., Oliver, A.L., Thorgeirsson, S.S., and Sonenshein, G.E. 2000. Role of the I $\kappa$ B kinase complex in oncogenic Ras- and Raf-mediated transformation of rat liver epithelial cells. *Mol. Cell. Biol.* **20**: 5381–5391.
- Artandi, S.E., Chang, S., Lee, S.L., Alson, S., Gottlieb, G.J., Chin, L., and DePinho, R.A. 2000. Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. *Nature* **406**: 641–645.
- Asano, T., Yao, Y., Zhu, J., Li, D., Abbuzzese, J.L., and Reddy, S.A. 2004. The PI 3-kinase/Akt signaling pathway is activated due to aberrant Pten expression and targets transcription factors NF- $\kappa$ B and c-Myc in pancreatic cancer cells. *Oncogene* **23**: 8571–8580.
- . 2005. The rapamycin analog CCI-779 is a potent inhibitor of pancreatic cancer cell proliferation. *Biochem. Biophys. Res. Commun.* **331**: 295–302.
- Baas, A.F., Kuipers, J., van der Wel, N.N., Batlle, E., Koerten, H.K., Peters, P.J., and Clevers, H.C. 2004. Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD. *Cell* **116**: 457–466.
- Baccarini, M. 2005. Second nature: Biological functions of the Raf-1 'kinase.' *FEBS Lett.* **579**: 3271–3277.
- Bachoo, R.M., Maher, E.A., Ligon, K.L., Sharpless, N.E., Chan, S.S., You, M.J., Tang, Y., DeFrances, J., Stover, E., Weissleder, R., et al. 2002. Epidermal growth factor receptor and Ink4a/Arf. Convergent mechanisms governing terminal differentiation and transformation along the neural stem cell to astrocyte axis. *Cancer Cell* **1**: 269–277.
- Bardeesy, N., Morgan, J., Sinha, M., Signoretti, S., Srivastava, S., Loda, M., Merlino, G., and DePinho, R.A. 2002a. Obligate roles for p16(Ink4a) and p19(Arf)-p53 in the suppression of murine pancreatic neoplasia. *Mol. Cell. Biol.* **22**: 635–643.
- Bardeesy, N., Sinha, M., Hezel, A.F., Signoretti, S., Hathaway, N.A., Sharpless, N.E., Loda, M., Carrasco, D.R., and DePinho, R.A. 2002b. Loss of the Lkb1 tumour suppressor provokes intestinal polyposis but resistance to transformation. *Nature* **419**: 162–167.
- Bardeesy, N., Kim, M., Xu, J., Kim, R.S., Shen, Q., Bosenberg, M.W., Wong, W.H., and Chin, L. 2005. Role of epidermal growth factor receptor signaling in RAS-driven melanoma. *Mol. Cell. Biol.* **25**: 4176–4188.
- Bardeesy, N., Aguirre, A., Chu, G., Cheng, K., Lopez, L., Hezel, A.F., Feng, B., Brennan, C., Weissleder, R., Mahmood, U., et al. 2006. Both p16Ink4a and the p19Arf-p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse. *Proc. Natl. Acad. Sci.* **103**: 5947–5952.
- Barton, C.M., Hall, P.A., Hughes, C.M., Gullick, W.J., and Lemoine, N.R. 1991. Transforming growth factor  $\alpha$  and epidermal growth factor in human pancreatic cancer. *J. Pathol.* **163**: 111–116.
- Bashyam, M.D., Bair, R., Kim, Y.H., Wang, P., Hernandez-Bousard, T., Karikari, C.A., Tibshirani, R., Maitra, A., and Pollack, J.R. 2005. Array-based comparative genomic hybridization identifies localized DNA amplifications and homozygous deletions in pancreatic cancer. *Neoplasia* **7**: 556–562.
- Beachy, P.A., Karhadkar, S.S., and Berman, D.M. 2004. Tissue repair and stem cell renewal in carcinogenesis. *Nature* **432**: 324–331.
- Beausejour, C.M., Krtolica, A., Galimi, F., Narita, M., Lowe, S.W., Yaswen, P., and Campisi, J. 2003. Reversal of human cellular senescence: Roles of the p53 and p16 pathways. *EMBO J.* **22**: 4212–4222.
- Benanti, J.A. and Galloway, D.A. 2004. Normal human fibroblasts are resistant to RAS-induced senescence. *Mol. Cell. Biol.* **24**: 2842–2852.
- Bergmann, U., Funatomi, H., Yokoyama, M., Beger, H.G., and Korc, M. 1995. Insulin-like growth factor I overexpression in human pancreatic cancer: Evidence for autocrine and paracrine roles. *Cancer Res.* **55**: 2007–2011.
- Berman, D.M., Karhadkar, S.S., Maitra, A., Montes De Oca, R., Gerstenblith, M.R., Briggs, K., Parker, A.R., Shimada, Y., Eshleman, J.R., Watkins, D.N., et al. 2003. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. *Nature* **425**: 846–851.
- Berrington de Gonzalez, A., Sweetland, S., and Spencer, E. 2003. A meta-analysis of obesity and the risk of pancreatic cancer. *Br. J. Cancer* **89**: 519–523.
- Bharti, A.C. and Aggarwal, B.B. 2002. Nuclear factor- $\kappa$  B and cancer: Its role in prevention and therapy. *Biochem. Pharmacol.* **64**: 883–888.
- Biankin, A.V., Morey, A.L., Lee, C.S., Kench, J.G., Biankin, S.A., Hook, H.C., Head, D.R., Hugh, T.B., Sutherland, R.L., and Henshall, S.M. 2002. DPC4/Smad4 expression and outcome in pancreatic ductal adenocarcinoma. *J. Clin. Oncol.* **20**: 4531–4542.
- Blau, H.M., Brazelton, T.R., and Weimann, J.M. 2001. The evolving concept of a stem cell: Entity or function? *Cell* **105**: 829–841.

- Bonner-Weir, S. and Sharma, A. 2002. Pancreatic stem cells. *J. Pathol.* **197**: 519–526.
- Bonner-Weir, S., Stubbs, M., Reitz, P., Taneja, M., and Smith, F.E. 1997. Partial pancreatectomy as a model of pancreatic regeneration. In *Pancreatic growth and regeneration* (ed. N. Sarvetnick), pp. 138–153. Karger Landes Systems, Basel, Switzerland.
- Bonnet, D. and Dick, J.E. 1997. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat. Med.* **3**: 730–737.
- Borg, A., Sandberg, T., Nilsson, K., Johannsson, O., Klinker, M., Masback, A., Westerdaal, J., Olsson, H., and Ingvar, C. 2000. High frequency of multiple melanomas and breast and pancreas carcinomas in CDKN2A mutation-positive melanoma families. *J. Natl. Cancer Inst.* **92**: 1260–1266.
- Boschman, C.R., Stryker, S., Reddy, J.K., and Rao, M.S. 1994. Expression of p53 protein in precursor lesions and adenocarcinoma of human pancreas. *Am. J. Pathol.* **145**: 1291–1295.
- Brembeck, F.H., Schreiber, F.S., Deramandt, T.B., Craig, L., Rhoades, B., Swain, G., Grippo, P., Stoffers, D.A., Silberg, D.G., and Rustgi, A.K. 2003. The mutant K-ras oncogene causes pancreatic periductal lymphocytic infiltration and gastric mucous neck cell hyperplasia in transgenic mice. *Cancer Res.* **63**: 2005–2009.
- Brookes, S., Rowe, J., Ruas, M., Llanos, S., Clark, P.A., Lomax, M., James, M.C., Vatcheva, R., Bates, S., Vousden, K.H., et al. 2002. INK4a-deficient human diploid fibroblasts are resistant to RAS-induced senescence. *EMBO J.* **21**: 2936–2945.
- Brookes, S., Rowe, J., Gutierrez Del Arroyo, A., Bond, J., and Peters, G. 2004. Contribution of p16(INK4a) to replicative senescence of human fibroblasts. *Exp. Cell Res.* **298**: 549–559.
- Brugge, W.R., Lauwers, G.Y., Sahani, D., Fernandez-del Castillo, C., and Warshaw, A.L. 2004. Cystic neoplasms of the pancreas. *N. Engl. J. Med.* **351**: 1218–1226.
- Brummelkamp, T.R., Bernards, R., and Agami, R. 2002. A system for stable expression of short interfering RNAs in mammalian cells. *Science* **296**: 550–553.
- Bruns, C.J., Solorzano, C.C., Harbison, M.T., Ozawa, S., Tsan, R., Fan, D., Abbruzzese, J., Traxler, P., Buchdunger, E., Radinsky, R., et al. 2000. Blockade of the epidermal growth factor receptor signaling by a novel tyrosine kinase inhibitor leads to apoptosis of endothelial cells and therapy of human pancreatic carcinoma. *Cancer Res.* **60**: 2926–2935.
- Bruns, C.J., Koehl, G.E., Guba, M., Yezhelyev, M., Steinbauer, M., Seeliger, H., Schwend, A., Hoehn, A., Jauch, K.W., and Geissler, E.K. 2004. Rapamycin-induced endothelial cell death and tumor vessel thrombosis potentiate cytotoxic therapy against pancreatic cancer. *Clin. Cancer Res.* **10**: 2109–2119.
- Calhoun, E.S., Jones, J.B., Ashfaq, R., Adsay, V., Baker, S.J., Valentine, V., Hempen, P.M., Hilgers, W., Yeo, C.J., Hruban, R.H., et al. 2003. BRAF and FBXW7 (CDC4, FBW7, AGO, SEL10) mutations in distinct subsets of pancreatic cancer: Potential therapeutic targets. *Am. J. Pathol.* **163**: 1255–1260.
- Camonis, J.H. and White, M.A. 2005. Ral GTPases: Corrupting the exocyst in cancer cells. *Trends Cell Biol.* **15**: 327–332.
- Campbell, S.L., Khosravi-Far, R., Rossman, K.L., Clark, G.J., and Der, C.J. 1998. Increasing complexity of Ras signaling. *Oncogene* **17**: 1395–1413.
- Cantley, L.C. 2002. The phosphoinositide 3-kinase pathway. *Science* **296**: 1655–1657.
- Chandler, N.M., Canete, J.J., and Callery, M.P. 2004. Increased expression of NF- $\kappa$ B subunits in human pancreatic cancer cells. *J. Surg. Res.* **118**: 9–14.
- Cheng, J.Q., Ruggeri, B., Klein, W.M., Sonoda, G., Altomare, D.A., Watson, D.K., and Testa, J.R. 1996. Amplification of AKT2 in human pancreatic cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA. *Proc. Natl. Acad. Sci.* **93**: 3636–3641.
- Chien, Y. and White, M.A. 2003. RAL GTPases are linchpin modulators of human tumour-cell proliferation and survival. *EMBO Rep.* **4**: 800–806.
- Chin, L., Pomerantz, J., Polsky, D., Jacobson, M., Cohen, C., Cordon-Cardo, C., Horner II, J.W., and DePinho, R.A. 1997. Cooperative effects of INK4a and ras in melanoma susceptibility in vivo. *Genes & Dev.* **11**: 2822–2834.
- Chin, L., Artandi, S.E., Shen, Q., Tam, A., Lee, S.L., Gottlieb, G.J., Greider, C.W., and DePinho, R.A. 1999a. p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. *Cell* **97**: 527–538.
- Chin, L., Tam, A., Pomerantz, J., Wong, M., Holash, J., Bardeesy, N., Shen, Q., O'Hagan, R., Pantginis, J., Zhou, H., et al. 1999b. Essential role for oncogenic Ras in tumour maintenance. *Nature* **400**: 468–472.
- Chin, K., de Solorzano, C.O., Knowles, D., Jones, A., Chou, W., Rodriguez, E.G., Kuo, W.L., Lung, B.M., Chew, K., Myambo, K., et al. 2004. In situ analyses of genome instability in breast cancer. *Nat. Genet.* **36**: 984–988.
- Cimini, D. and Degraffi, F. 2005. Aneuploidy: A matter of bad connections. *Trends Cell Biol.* **15**: 442–451.
- Collado, M., Gil, J., Efeyan, A., Guerra, C., Schuhmacher, A.J., Barradas, M., Benguria, A., Zaballos, A., Flores, J.M., Barbacid, M., et al. 2005. Tumour biology: Senescence in pre-malignant tumours. *Nature* **436**: 642.
- Collins, C.J. and Sedivy, J.M. 2003. Involvement of the INK4a/Arf gene locus in senescence. *Aging Cell* **2**: 145–150.
- Collins, A.T., Berry, P.A., Hyde, C., Stower, M.J., and Maitland, N.J. 2005. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res.* **65**: 10946–10951.
- Cooper, H.S. 1998. Benign epithelial polyps of the intestines. In *Pathology of the gastrointestinal tract* (eds. S.-C. Ming and H. Goldman), pp. 819–853. Williams & Wilkins, Baltimore.
- Corradetti, M.N., Inoki, K., Bardeesy, N., DePinho, R.A., and Guan, K.L. 2004. Regulation of the TSC pathway by LKB1: Evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome. *Genes & Dev.* **18**: 1533–1538.
- Corso, S., Comoglio, P.M., and Giordano, S. 2005. Cancer therapy: Can the challenge be MET? *Trends Mol. Med.* **11**: 284–292.
- Crnogorac-Jurcovic, T., Efthimiou, E., Nielsen, T., Loader, J., Terris, B., Stamp, G., Baron, A., Scarpa, A., and Lemoine, N.R. 2002. Expression profiling of microdissected pancreatic adenocarcinomas. *Oncogene* **21**: 4587–4594.
- Cross, M.J. and Claesson-Welsh, L. 2001. FGF and VEGF function in angiogenesis: Signalling pathways, biological responses and therapeutic inhibition. *Trends Pharmacol. Sci.* **22**: 201–207.
- Davies, H., Bignell, G.R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M.J., Bottomley, W., et al. 2002. Mutations of the BRAF gene in human cancer. *Nature* **417**: 949–954.
- Dessimoz, J., Bonnard, C., Huelsken, J., and Grapin-Botton, A. 2005. Pancreas-specific deletion of  $\beta$ -catenin reveals Wnt-dependent and Wnt-independent functions during development. *Curr. Biol.* **15**: 1677–1683.
- Di Renzo, M.F., Poulson, R., Olivero, M., Comoglio, P.M., and Lemoine, N.R. 1995. Expression of the Met/hepatocyte growth factor receptor in human pancreatic cancer. *Cancer Res.* **55**: 1129–1138.

- Dong, Q.G., Sclabas, G.M., Fujioka, S., Schmidt, C., Peng, B., Wu, T., Tsao, M.S., Evans, D.B., Abbruzzese, J.L., McDonnell, T.J., et al. 2002. The function of multiple I $\kappa$ B: NF- $\kappa$ B complexes in the resistance of cancer cells to Taxol-induced apoptosis. *Oncogene* **21**: 6510–6519.
- Dor, Y., Brown, J., Martinez, O.I., and Melton, D.A. 2004. Adult pancreatic  $\beta$ -cells are formed by self-duplication rather than stem-cell differentiation. *Nature* **429**: 41–46.
- Drayton, S., Rowe, J., Jones, R., Vatcheva, R., Cuthbert-Heavens, D., Marshall, J., Fried, M., and Peters, G. 2003. Tumor suppressor p16INK4a determines sensitivity of human cells to transformation by cooperating cellular oncogenes. *Cancer Cell* **4**: 301–310.
- Duda, D.G., Sunamura, M., Lefter, L.P., Furukawa, T., Yokoyama, T., Yatsuoka, T., Abe, T., Inoue, H., Motoi, F., Egawa, S., et al. 2003. Restoration of SMAD4 by gene therapy reverses the invasive phenotype in pancreatic adenocarcinoma cells. *Oncogene* **22**: 6857–6864.
- Duesberg, P. and Rasnick, D. 2000. Aneuploidy, the somatic mutation that makes cancer a species of its own. *Cell Motil. Cytoskeleton* **47**: 81–107.
- Eberle, M.A., Pfutzer, R., Pogue-Geile, K.L., Bronner, M.P., Crispin, D., Kimmey, M.B., Duerr, R.H., Kruglyak, L., Whitcomb, D.C., and Brentnall, T.A. 2002. A new susceptibility locus for autosomal dominant pancreatic cancer maps to chromosome 4q32–34. *Am. J. Hum. Genet.* **70**: 1044–1048.
- Ebert, M., Yokoyama, M., Kobrin, M.S., Friess, H., Lopez, M.E., Buchler, M.W., Johnson, G.R., and Korc, M. 1994. Induction and expression of amphiregulin in human pancreatic cancer. *Cancer Res.* **54**: 3959–3962.
- Ellenrieder, V., Hendler, S.F., Boeck, W., Seufferlein, T., Menke, A., Ruhland, C., Adler, G., and Gress, T.M. 2001a. Transforming growth factor  $\beta$ 1 treatment leads to an epithelial-mesenchymal transdifferentiation of pancreatic cancer cells requiring extracellular signal-regulated kinase 2 activation. *Cancer Res.* **61**: 4222–4228.
- Ellenrieder, V., Hendler, S.F., Ruhland, C., Boeck, W., Adler, G., and Gress, T.M. 2001b. TGF- $\beta$ -induced invasiveness of pancreatic cancer cells is mediated by matrix metalloproteinase-2 and the urokinase plasminogen activator system. *Int. J. Cancer* **93**: 204–211.
- Elliott, R.L. and Blobel, G.C. 2005. Role of transforming growth factor  $\beta$  in human cancer. *J. Clin. Oncol.* **23**: 2078–2093.
- Esni, F., Ghosh, B., Biankin, A.V., Lin, J.W., Albert, M.A., Yu, X., MacDonald, R.J., Civin, C.I., Real, F.X., Pack, M.A., et al. 2004. Notch inhibits Ptf1 function and acinar cell differentiation in developing mouse and zebrafish pancreas. *Development* **131**: 4213–4224.
- Everhart, J. and Wright, D. 1995. Diabetes mellitus as a risk factor for pancreatic cancer. A meta-analysis. *JAMA* **273**: 1605–1609.
- Fang, D., Nguyen, T.K., Leishear, K., Finko, R., Kulp, A.N., Hotz, S., Van Belle, P.A., Xu, X., Elder, D.E., and Herlyn, M. 2005. A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res.* **65**: 9328–9337.
- Feig, L.A. 2003. Ral-GTPases: Approaching their 15 minutes of fame. *Trends Cell Biol.* **13**: 419–425.
- Fernandez-Zapico, M.E., Gonzalez-Paz, N.C., Weiss, E., Savoy, D.N., Molina, J.R., Fonseca, R., Smyrk, T.C., Chari, S.T., Urrutia, R., and Billadeau, D.D. 2005. Ectopic expression of VAV1 reveals an unexpected role in pancreatic cancer tumorigenesis. *Cancer Cell* **7**: 39–49.
- Ferrara, N., Gerber, H.P., and LeCouter, J. 2003. The biology of VEGF and its receptors. *Nat. Med.* **9**: 669–676.
- Fidler, I.J. and Hart, I.R. 1982. Biological diversity in metastatic neoplasms: Origins and implications. *Science* **217**: 998–1003.
- Fisher, G.H., Wellen, S.L., Klimstra, D., Lenczowski, J.M., Tichelaar, J.W., Lizak, M.J., Whitsett, J.A., Koretsky, A., and Varmus, H.E. 2001. Induction and apoptotic regression of lung adenocarcinomas by regulation of a K-Ras transgene in the presence and absence of tumor suppressor genes. *Genes & Dev.* **15**: 3249–3262.
- Fleming, J.B., Shen, G.L., Holloway, S.E., Davis, M., and Brecken, R.A. 2005. Molecular consequences of silencing mutant K-ras in pancreatic cancer cells: Justification for K-ras-directed therapy. *Mol. Cancer Res.* **3**: 413–423.
- Friess, H., Yamanaka, Y., Buchler, M., Berger, H.G., Kobrin, M.S., Baldwin, R.L., and Korc, M. 1993. Enhanced expression of the type II transforming growth factor  $\beta$  receptor in human pancreatic cancer cells without alteration of type III receptor expression. *Cancer Res.* **53**: 2704–2707.
- Friess, H., Yamanaka, Y., Kobrin, M.S., Do, D.A., Buchler, M.W., and Korc, M. 1995. Enhanced erbB-3 expression in human pancreatic cancer correlates with tumor progression. *Clin. Cancer Res.* **1**: 1413–1420.
- Friess, H., Berberat, P., Schilling, M., Kunz, J., Korc, M., and Buchler, M.W. 1996. Pancreatic cancer: The potential clinical relevance of alterations in growth factors and their receptors. *J. Mol. Med.* **74**: 35–42.
- Friess, H., Wang, L., Zhu, Z., Gerber, R., Schroder, M., Fukuda, A., Zimmermann, A., Korc, M., and Buchler, M.W. 1999. Growth factor receptors are differentially expressed in cancers of the papilla of Vater and pancreas. *Ann. Surg.* **230**: 767–774; discussion 774–765.
- Fuchs, C.S., Colditz, G.A., Stampfer, M.J., Giovannucci, E.L., Hunter, D.J., Rimm, E.B., Willett, W.C., and Speizer, F.E. 1996. A prospective study of cigarette smoking and the risk of pancreatic cancer. *Arch. Intern. Med.* **156**: 2255–2260.
- Fujioka, S., Sclabas, G.M., Schmidt, C., Frederick, W.A., Dong, Q.G., Abbruzzese, J.L., Evans, D.B., Baker, C., and Chiao, P.J. 2003. Function of nuclear factor  $\kappa$ B in pancreatic cancer metastasis. *Clin. Cancer Res.* **9**: 346–354.
- Fukasawa, M. and Korc, M. 2004. Vascular endothelial growth factor-trap suppresses tumorigenicity of multiple pancreatic cancer cell lines. *Clin. Cancer Res.* **10**: 3327–3332.
- Furukawa, T., Duguid, W.P., Kobari, M., Matsuno, S., and Tsao, M.S. 1995. Hepatocyte growth factor and Met receptor expression in human pancreatic carcinogenesis. *Am. J. Pathol.* **147**: 889–895.
- Gapstur, S.M., Gann, P.H., Lowe, W., Liu, K., Colangelo, L., and Dyer, A. 2000. Abnormal glucose metabolism and pancreatic cancer mortality. *JAMA* **283**: 2552–2558.
- Garnett, M.J. and Marais, R. 2004. Guilty as charged: B-Raf is a human oncogene. *Cancer Cell* **6**: 313–319.
- Garraway, L.A., Widlund, H.R., Rubin, M.A., Getz, G., Berger, A.J., Ramaswamy, S., Beroukhi, R., Milner, D.A., Granter, S.R., Du, J., et al. 2005. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature* **436**: 117–122.
- Ghosh, S., May, M.J., and Kopp, E.B. 1998. NF- $\kappa$ B and Rel proteins: Evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* **16**: 225–260.
- Giardiello, F.M., Brensinger, J.D., Tersmette, A.C., Goodman, S.N., Petersen, G.M., Booker, S.V., Cruz-Correa, M., and Offerhaus, J.A. 2000. Very high risk of cancer in familial Peutz-Jeghers syndrome. *Gastroenterology* **119**: 1447–1453.
- Gisselsson, D., Jonson, T., Petersen, A., Strombeck, B., Dal Cin, P., Hoglund, M., Mitelman, F., Mertens, F., and Mandahl, N. 2001. Telomere dysfunction triggers extensive DNA fragmentation and evolution of complex chromosome abnormalities in human malignant tumors. *Proc. Natl. Acad. Sci.*



- 98: 12683–12688.
- Glasner, S., Memoli, V., and Longnecker, D.S. 1992. Characterization of the ELSV transgenic mouse model of pancreatic carcinoma. Histologic type of large and small tumors. *Am. J. Pathol.* **140**: 1237–1245.
- Goggins, M., Hruban, R.H., and Kern, S.E. 2000. BRCA2 is inactivated late in the development of pancreatic intraepithelial neoplasia: Evidence and implications. *Am. J. Pathol.* **156**: 1767–1771.
- Goldstein, A.M., Fraser, M.C., Struewing, J.P., Hussussian, C.J., Ranade, K., Zametkin, D.P., Fontaine, L.S., Organic, S.M., Dracopoli, N.C., Clark Jr., W.H., et al. 1995. Increased risk of pancreatic cancer in melanoma-prone kindreds with p16INK4 mutations [see comments]. *N. Engl. J. Med.* **333**: 970–974.
- Goldstein, A.M., Struewing, J.P., Chidambaram, A., Fraser, M.C., and Tucker, M.A. 2000. Genotype–phenotype relationships in U.S. melanoma-prone families with CDKN2A and CDK4 mutations. *J. Natl. Cancer Inst.* **92**: 1006–1010.
- Gomez del Pulgar, T., Benitah, S.A., Valeron, P.F., Espina, C., and Lacal, J.C. 2005. Rho GTPase expression in tumorigenesis: Evidence for a significant link. *Bioessays* **27**: 602–613.
- Gorunova, L., Hoglund, M., Andren-Sandberg, A., Dawiskiba, S., Jin, Y., Mitelman, F., and Johansson, B. 1998. Cytogenetic analysis of pancreatic carcinomas: Intratumor heterogeneity and nonrandom pattern of chromosome aberrations. *Genes Chromosomes Cancer* **23**: 81–99.
- Greenberg, R.A., Chin, L., Femino, A., Lee, K.H., Gottlieb, G.J., Singer, R.H., Greider, C.W., and DePinho, R.A. 1999. Short dysfunctional telomeres impair tumorigenesis in the INK4a(Δ2/3) cancer-prone mouse. *Cell* **97**: 515–525.
- Grippio, P.J., Nowlin, P.S., Demeure, M.J., Longnecker, D.S., and Sandgren, E.P. 2003. Preinvasive pancreatic neoplasia of ductal phenotype induced by acinar cell targeting of mutant Kras in transgenic mice. *Cancer Res.* **63**: 2016–2019.
- Grutzmann, R., Foerder, M., Alldinger, I., Staub, E., Brummen-dorf, T., Ropcke, S., Li, X., Kristiansen, G., Jesnowski, R., Sipos, B., et al. 2003. Gene expression profiles of microdissected pancreatic ductal adenocarcinoma. *Virchows Arch.* **443**: 508–517.
- Gu, G., Dubauskaite, J., and Melton, D.A. 2002. Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development* **129**: 2447–2457.
- Gu, G., Brown, J.R., and Melton, D.A. 2003. Direct lineage tracing reveals the ontogeny of pancreatic cell fates during mouse embryogenesis. *Mech. Dev.* **120**: 35–43.
- Guerra, C., Mijimolle, N., Dhawahir, A., Dubus, P., Barradas, M., Serrano, M., Campuzano, V., and Barbacid, M. 2003. Tumor induction by an endogenous K-ras oncogene is highly dependent on cellular context. *Cancer Cell* **4**: 111–120.
- Gysin, S., Lee, S.H., Dean, N.M., and McMahon, M. 2005. Pharmacologic inhibition of RAF → MEK → ERK signaling elicits pancreatic cancer cell cycle arrest through induced expression of p27Kip1. *Cancer Res.* **65**: 4870–4880.
- Hahn, S.A., Schutte, M., Hoque, A.T., Moskaluk, C.A., da Costa, L.T., Rozenblum, E., Weinstein, C.L., Fischer, A., Yeo, C.J., Hruban, R.H., et al. 1996. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1 [see comments]. *Science* **271**: 350–353.
- Hahn, W.C., Counter, C.M., Lundberg, A.S., Beijersbergen, R.L., Brooks, M.W., and Weinberg, R.A. 1999. Creation of human tumour cells with defined genetic elements. *Nature* **400**: 464–468.
- Hald, J., Hjorth, J.P., German, M.S., Madsen, O.D., Serup, P., and Jensen, J. 2003. Activated Notch1 prevents differentiation of pancreatic acinar cells and attenuate endocrine development. *Dev. Biol.* **260**: 426–437.
- Han, H., Bearss, D.J., Browne, L.W., Calaluze, R., Nagle, R.B., and Von Hoff, D.D. 2002. Identification of differentially expressed genes in pancreatic cancer cells using cDNA microarray. *Cancer Res.* **62**: 2890–2896.
- Hanson, J.L., Hawke, N.A., Kashatus, D., and Baldwin, A.S. 2004. The nuclear factor κB subunits RelA/p65 and c-Rel potentiate but are not required for Ras-induced cellular transformation. *Cancer Res.* **64**: 7248–7255.
- Harada, T., Okita, K., Shiraishi, K., Kusano, N., Kondoh, S., and Sasaki, K. 2002. Interglandular cytogenetic heterogeneity detected by comparative genomic hybridization in pancreatic cancer. *Cancer Res.* **62**: 835–839.
- Hardie, D.G. 2005. New roles for the LKB1 → AMPK pathway. *Curr. Opin. Cell Biol.* **17**: 167–173.
- Hart, A., Papadopoulos, S., and Edlund, H. 2003. Fgf10 maintains notch activation, stimulates proliferation, and blocks differentiation of pancreatic epithelial cells. *Dev. Dyn.* **228**: 185–193.
- Hayden, M.S. and Ghosh, S. 2004. Signaling to NF-κB. *Genes & Dev.* **18**: 2195–2224.
- Hebrok, M., Kim, S.K., and Melton, D.A. 1998. Notochord repression of endodermal Sonic hedgehog permits pancreas development. *Genes & Dev.* **12**: 1705–1713.
- Heidenblad, M., Schoenmakers, E.F., Jonson, T., Gorunova, L., Veltman, J.A., van Kessel, A.G., and Hoglund, M. 2004. Genome-wide array-based comparative genomic hybridization reveals multiple amplification targets and novel homozygous deletions in pancreatic carcinoma cell lines. *Cancer Res.* **64**: 3052–3059.
- Heinmoller, E., Dietmaier, W., Zirngibl, H., Heinmoller, P., Scaringe, W., Jauch, K.W., Hofstadter, F., and Ruschoff, J. 2000. Molecular analysis of microdissected tumors and preneoplastic intraductal lesions in pancreatic carcinoma. *Am. J. Pathol.* **157**: 83–92.
- Hemminki, A., Markie, D., Tomlinson, I., Avizienyte, E., Roth, S., Loukola, A., Bignell, G., Warren, W., Aminoff, M., Hoglund, P., et al. 1998. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature* **391**: 184–187.
- Heppner, G.H. 1984. Tumor heterogeneity. *Cancer Res.* **44**: 2259–2265.
- Herbig, U., Jobling, W.A., Chen, B.P., Chen, D.J., and Sedivy, J.M. 2004. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). *Mol. Cell* **14**: 501–513.
- Hingorani, S.R., Petricoin, E.F., Maitra, A., Rajapakse, V., King, C., Jacobetz, M.A., Ross, S., Conrads, T.P., Veenstra, T.D., Hitt, B.A., et al. 2003. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* **4**: 437–450.
- Hingorani, S.R., Wang, L., Multani, A.S., Combs, C., Deramaut, T.B., Hruban, R.H., Rustgi, A.K., Chang, S., and Tuveson, D.A. 2005. Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell* **7**: 469–483.
- Hirano, T., Shino, Y., Saito, T., Komoda, F., Okutomi, Y., Takeda, A., Ishihara, T., Yamaguchi, T., Saisho, H., and Shirasawa, H. 2002. Dominant negative MEKK1 inhibits survival of pancreatic cancer cells. *Oncogene* **21**: 5923–5928.
- Hogan, B.L. 1999. Morphogenesis. *Cell* **96**: 225–233.
- Holzmann, K., Kohlhammer, H., Schwaenen, C., Wessendorf, S., Kestler, H.A., Schwoerer, A., Rau, B., Radlwimmer, B., Dohner, H., Lichter, P., et al. 2004. Genomic DNA-chip hybridization reveals a higher incidence of genomic amplifica-

- tions in pancreatic cancer than conventional comparative genomic hybridization and leads to the identification of novel candidate genes. *Cancer Res.* **64**: 4428–4433.
- Hoshida, T., Sunamura, M., Duda, D.G., Egawa, S., Miyazaki, S., Shineha, R., Hamada, H., Ohtani, E., Satomi, S., and Matsuno, S. 2002. Gene therapy for pancreatic cancer using an adenovirus vector encoding soluble flt-1 vascular endothelial growth factor receptor. *Pancreas* **25**: 111–121.
- Hotz, H.G., Hines, O.J., Hotz, B., Foitzik, T., Buhr, H.J., and Reber, H.A. 2003. Evaluation of vascular endothelial growth factor blockade and matrix metalloproteinase inhibition as a combination therapy for experimental human pancreatic cancer. *J. Gastrointest. Surg.* **7**: 220–227; discussion 227–228.
- Hruban, R.H., Goggins, M., Parsons, J., and Kern, S.E. 2000a. Progression model for pancreatic cancer. *Clin. Cancer Res.* **6**: 2969–2972.
- Hruban, R.H., Wilentz, R.E., and Kern, S.E. 2000b. Genetic progression in the pancreatic ducts. *Am. J. Pathol.* **156**: 1821–1825.
- Hruban, R.H., Adsay, N.V., Albores-Saavedra, J., Compton, C., Garrett, E.S., Goodman, S.N., Kern, S.E., Klimstra, D.S., Kloppel, G., Longnecker, D.S., et al. 2001. Pancreatic intraepithelial neoplasia: A new nomenclature and classification system for pancreatic duct lesions. *Am. J. Surg. Pathol.* **25**: 579–586.
- Hruban, R.H., Adsay, N.V., Albores-Saavedra, J., Anver, M.R., Biankin, A.V., Boivin, G.P., Furth, E.E., Furukawa, T., Klein, A., Klimstra, D.S., et al. 2006a. Pathology of genetically engineered mouse models of pancreatic exocrine cancer: Consensus report and recommendations. *Cancer Res.* **66**: 95–106.
- Hruban, R.H., Bishop Pitman, M., and Klimstra, D.S. 2006b *Atlas of tumor pathology: Tumors of the pancreas. Fourth Series* (series eds. L.H. Sobin and J. Albores-Saavedra) ARP Press, Washington, DC.
- Hustinx, S.R., Leoni, L.M., Yeo, C.J., Brown, P.N., Goggins, M., Kern, S.E., Hruban, R.H., and Maitra, A. 2005. Concordant loss of MTAP and p16/CDKN2A expression in pancreatic intraepithelial neoplasia: Evidence of homozygous deletion in a noninvasive precursor lesion. *Mod. Pathol.* **18**: 959–963.
- Iacobuzio-Donahue, C.A., Maitra, A., Shen-Ong, G.L., van Heek, T., Ashfaq, R., Meyer, R., Walter, K., Berg, K., Hollingsworth, M.A., Cameron, J.L., et al. 2002. Discovery of novel tumor markers of pancreatic cancer using global gene expression technology. *Am. J. Pathol.* **160**: 1239–1249.
- Iacobuzio-Donahue, C.A., Maitra, A., Olsen, M., Lowe, A.W., van Heek, N.T., Rosty, C., Walter, K., Sato, N., Parker, A., Ashfaq, R., et al. 2003. Exploration of global gene expression patterns in pancreatic adenocarcinoma using cDNA microarrays. *Am. J. Pathol.* **162**: 1151–1162.
- Ingham, P.W. and McMahon, A.P. 2001. Hedgehog signaling in animal development: Paradigms and principles. *Genes & Dev.* **15**: 3059–3087.
- Ishimura, N., Yamasawa, K., Karim Rumi, M.A., Kadowaki, Y., Ishihara, S., Amano, Y., Nio, Y., Higami, T., and Kinoshita, Y. 2003. BRAF and K-ras gene mutations in human pancreatic cancers. *Cancer Lett.* **199**: 169–173.
- Ishiwata, T., Kornmann, M., Beger, H.G., and Korc, M. 1998. Enhanced fibroblast growth factor 5 expression in stromal and exocrine elements of the pancreas in chronic pancreatitis. *Gut* **43**: 134–139.
- Itakura, J., Ishiwata, T., Friess, H., Fujii, H., Matsumoto, Y., Buchler, M.W., and Korc, M. 1997. Enhanced expression of vascular endothelial growth factor in human pancreatic cancer correlates with local disease progression. *Clin. Cancer Res.* **3**: 1309–1316.
- Jackson, E.L., Willis, N., Mercer, K., Bronson, R.T., Crowley, D., Montoya, R., Jacks, T., and Tuveson, D.A. 2001. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. *Genes & Dev.* **15**: 3243–3248.
- Jacobs, J.J. and de Lange, T. 2004. Significant role for p16 in p53-independent telomere-directed senescence. *Curr. Biol.* **14**: 2302–2308.
- Jaffee, E.M., Hruban, R.H., Canto, M., and Kern, S.E. 2002. Focus on pancreas cancer. *Cancer Cell* **2**: 25–28.
- Janda, E., Lehmann, K., Killisch, I., Jechlinger, M., Herzig, M., Downward, J., Beug, H., and Grunert, S. 2002. Ras and TGF $\beta$  cooperatively regulate epithelial cell plasticity and metastasis: Dissection of Ras signaling pathways. *J. Cell Biol.* **156**: 299–313.
- Jaster, R. 2004. Molecular regulation of pancreatic stellate cell function. *Mol. Cancer* **3**: 26.
- Jenne, D.E., Reimann, H., Nezu, J., Friedel, W., Loff, S., Jeschke, R., Muller, O., Back, W., and Zimmer, M. 1998. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. *Nat. Genet.* **18**: 38–43.
- Jensen, J., Pedersen, E.E., Galante, P., Hald, J., Heller, R.S., Ishibashi, M., Kageyama, R., Guillemot, F., Serup, P., and Madsen, O.D. 2000. Control of endodermal endocrine development by Hes-1. *Nat. Genet.* **24**: 36–44.
- Jhappan, C., Stahle, C., Harkins, R.N., Fausto, N., Smith, G.H., and Merlino, G.T. 1990. TGF $\alpha$  overexpression in transgenic mice induces liver neoplasia and abnormal development of the mammary gland and pancreas. *Cell* **61**: 1137–1146.
- Jimenez, R.E., Z'Graggen, K., Hartwig, W., Graeme-Cook, F., Warshaw, A.L., and Fernandez-del Castillo, C. 1999. Immunohistochemical characterization of pancreatic tumors induced by dimethylbenzanthracene in rats. *Am. J. Pathol.* **154**: 1223–1229.
- Kadesch, T. 2004. Notch signaling: The demise of elegant simplicity. *Curr. Opin. Genet. Dev.* **14**: 506–512.
- Karin, M. and Ben-Neriah, Y. 2000. Phosphorylation meets ubiquitination: The control of NF- $\kappa$ B activity. *Annu. Rev. Immunol.* **18**: 621–663.
- Kawaguchi, Y., Cooper, B., Gannon, M., Ray, M., MacDonald, R.J., and Wright, C.V. 2002. The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nat. Genet.* **32**: 128–134.
- Kiaris, H., Politi, K., Grimm, L.M., Szabolcs, M., Fisher, P., Efstratiadis, A., and Artavanis-Tsakonas, S. 2004. Modulation of Notch signaling elicits signature tumors and inhibits hras1-induced oncogenesis in the mouse mammary epithelium. *Am. J. Pathol.* **165**: 695–705.
- Kim, S.K. and Hebrok, M. 2001. Intercellular signals regulating pancreas development and function. *Genes & Dev.* **15**: 111–127.
- Kim, C.F., Jackson, E.L., Woolfenden, A.E., Lawrence, S., Babar, I., Vogel, S., Crowley, D., Bronson, R.T., and Jacks, T. 2005. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* **121**: 823–835.
- Kim, H.J., Schleiffarth, J.R., Jessurun, J., Sumanas, S., Petryk, A., Lin, S., and Ekker, S.C. 2005. Wnt5 signaling in vertebrate pancreas development. *BMC Biol.* **3**: 23.
- Kinzler, K.W. and Vogelstein, B. 1996. Lessons from hereditary colorectal cancer. *Cell* **87**: 159–170.
- Kleeff, J., Ishiwata, T., Kumbasar, A., Friess, H., Buchler, M.W., Lander, A.D., and Korc, M. 1998. The cell-surface heparan sulfate proteoglycan glypican-1 regulates growth factor action in pancreatic carcinoma cells and is overexpressed in human pancreatic cancer. *J. Clin. Invest.* **102**: 1662–1673.
- Kleeff, J., Kothari, N.H., Friess, H., Fan, H., and Korc, M. 2004.

- Adenovirus-mediated transfer of a truncated fibroblast growth factor (FGF) type I receptor blocks FGF-2 signaling in multiple pancreatic cancer cell lines. *Pancreas* **28**: 25–30.
- Klimstra, D.S. 1998. Cell lineage in pancreatic neoplasms. In *Pancreatic cancer: Advances in molecular pathology, diagnosis & clinical management* (eds. F.S. Sarkar and M.C. Duggan), pp. 21–48. Eaton Publishing, Natick, MA.
- Klimstra, D.S. and Longnecker, D.S. 1994. K-ras mutations in pancreatic ductal proliferative lesions. *Am. J. Pathol.* **145**: 1547–1550.
- Kobrin, M.S., Yamanaka, Y., Friess, H., Lopez, M.E., and Korc, M. 1993. Aberrant expression of type I fibroblast growth factor receptor in human pancreatic adenocarcinomas. *Cancer Res.* **53**: 4741–4744.
- Koizumi, M., Doi, R., Toyoda, E., Masui, T., Tulachan, S.S., Kawaguchi, Y., Fujimoto, K., Gittes, G.K., and Imamura, M. 2003. Increased PDX-1 expression is associated with outcome in patients with pancreatic cancer. *Surgery* **134**: 260–266.
- Kops, G.J., Weaver, B.A., and Cleveland, D.W. 2005. On the road to cancer: Aneuploidy and the mitotic checkpoint. *Nat. Rev. Cancer* **5**: 773–785.
- Korc, M., Chandrasekar, B., Yamanaka, Y., Friess, H., Buchier, M., and Beger, H.G. 1992. 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.* **90**: 1352–1360.
- Kornmann, M., Ishiwata, T., Beger, H.G., and Korc, M. 1997. Fibroblast growth factor-5 stimulates mitogenic signaling and is overexpressed in human pancreatic cancer: Evidence for autocrine and paracrine actions. *Oncogene* **15**: 1417–1424.
- Kornmann, M., Ishiwata, T., Matsuda, K., Lopez, M.E., Fukahi, K., Asano, G., Beger, H.G., and Korc, M. 2002. IIIc isoform of fibroblast growth factor receptor 1 is overexpressed in human pancreatic cancer and enhances tumorigenicity of hamster ductal cells. *Gastroenterology* **123**: 301–313.
- Krimpenfort, P., Quon, K.C., Mooi, W.J., Loonstra, A., and Berns, A. 2001. Loss of p16Ink4a confers susceptibility to metastatic melanoma in mice. *Nature* **413**: 83–86.
- Krishnamurthy, J., Torrice, C., Ramsey, M.R., Kovalev, G.I., Al-Regaiey, K., Su, L., and Sharpless, N.E. 2004. Ink4a/Arf expression is a biomarker of aging. *J. Clin. Invest.* **114**: 1299–1307.
- Kritzik, M.R., Jones, E., Chen, Z., Krakowski, M., Krah, T., Good, A., Wright, C., Fox, H., and Sarvetnick, N. 1999. PDX-1 and Msx-2 expression in the regenerating and developing pancreas. *J. Endocrinol.* **163**: 523–530.
- Kuniyasu, H., Abbruzzese, J.L., Cleary, K.R., and Fidler, I.J. 2001. Induction of ductal and stromal hyperplasia by basic fibroblast growth factor produced by human pancreatic carcinoma. *Int. J. Oncol.* **19**: 681–685.
- Kurahara, H., Takao, S., Maemura, K., Shinchi, H., Natsugoe, S., and Aikou, T. 2004. Impact of vascular endothelial growth factor-C and -D expression in human pancreatic cancer: Its relationship to lymph node metastasis. *Clin. Cancer Res.* **10**: 8413–8420.
- Laghi, L., Orbetegui, O., Bianchi, P., Zerbi, A., Di Carlo, V., Boland, C.R., and Malesci, A. 2002. Common occurrence of multiple K-RAS mutations in pancreatic cancers with associated precursor lesions and in biliary cancers. *Oncogene* **21**: 4301–4306.
- Lai, E.C. 2004. Notch signaling: Control of cell communication and cell fate. *Development* **131**: 965–973.
- Lal, G., Liu, L., Hogg, D., Lassam, N.J., Redston, M.S., and Gallinger, S. 2000. Patients with both pancreatic adenocarcinoma and melanoma may harbor germline CDKN2A mutations. *Genes Chromosomes Cancer* **27**: 358–361.
- Lapidot, T., Sirard, C., Vormoor, J., Murdoch, B., Hoang, T., Caceres-Cortes, J., Minden, M., Paterson, B., Caligiuri, M.A., and Dick, J.E. 1994. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* **367**: 645–648.
- Lebowitz, P.F., Davide, J.P., and Prendergast, G.C. 1995. Evidence that farnesyltransferase inhibitors suppress Ras transformation by interfering with Rho activity. *Mol. Cell. Biol.* **15**: 6613–6622.
- Lemoine, N.R., Jain, S., Hughes, C.M., Staddon, S.L., Maillet, B., Hall, P.A., and Kloppel, G. 1992. Ki-ras oncogene activation in preinvasive pancreatic cancer. *Gastroenterology* **102**: 230–236.
- Lerner, E.C., Zhang, T.T., Knowles, D.B., Qian, Y., Hamilton, A.D., and Sefti, S.M. 1997. Inhibition of the prenylation of K-Ras, but not H- or N-Ras, is highly resistant to CAAX peptidomimetics and requires both a farnesyltransferase and a geranylgeranyltransferase I inhibitor in human tumor cell lines. *Oncogene* **15**: 1283–1288.
- Levy, L. and Hill, C.S. 2005. Smad4 dependency defines two classes of transforming growth factor  $\beta$  (TGF- $\beta$ ) target genes and distinguishes TGF- $\beta$ -induced epithelial–mesenchymal transition from its antiproliferative and migratory responses. *Mol. Cell. Biol.* **25**: 8108–8125.
- Lewis, B.C., Klimstra, D.S., and Varmus, H.E. 2003. The c-myc and PyMT oncogenes induce different tumor types in a somatic mouse model for pancreatic cancer. *Genes & Dev.* **17**: 3127–3138.
- Li, D., Xie, K., Wolff, R., and Abbruzzese, J.L. 2004. Pancreatic cancer. *Lancet* **363**: 1049–1057.
- Li, J., Kleeff, J., Giese, N., Buchler, M.W., Korc, M., and Friess, H. 2004. Gefitinib ('Iressa,' ZD1839), a selective epidermal growth factor receptor tyrosine kinase inhibitor, inhibits pancreatic cancer cell growth, invasion, and colony formation. *Int. J. Oncol.* **25**: 203–210.
- Lim, K.H. and Counter, C.M. 2005. Reduction in the requirement of oncogenic Ras signaling to activation of PI3K/AKT pathway during tumor maintenance. *Cancer Cell* **8**: 381–392.
- Lim, K.H., Baines, A.T., Fiordalisi, J.J., Shipitsin, M., Feig, L.A., Cox, A.D., Der, C.J., and Counter, C.M. 2005. Activation of RalA is critical for Ras-induced tumorigenesis of human cells. *Cancer Cell* **7**: 533–545.
- Liptay, S., Weber, C.K., Ludwig, L., Wagner, M., Adler, G., and Schmid, R.M. 2003. Mitogenic and antiapoptotic role of constitutive NF- $\kappa$ B/Rel activity in pancreatic cancer. *Int. J. Cancer* **105**: 735–746.
- Liu, L., Dilworth, D., Gao, L., Monzon, J., Summers, A., Lassam, N., and Hogg, D. 1999. Mutation of the CDKN2A 5' UTR creates an aberrant initiation codon and predisposes to melanoma. *Nat. Genet.* **21**: 128–132.
- Lizcano, J.M., Goransson, O., Toth, R., Deak, M., Morrice, N.A., Boudeau, J., Hawley, S.A., Udd, L., Makela, T.P., Hardie, D.G., et al. 2004. LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *EMBO J.* **23**: 833–843.
- Lohr, M., Maisonneuve, P., and Lowenfels, A.B. 2000. K-Ras mutations and benign pancreatic disease. *Int. J. Pancreatol.* **27**: 93–103.
- Lohr, M., Schmidt, C., Ringel, J., Kluth, M., Muller, P., Nizze, H., and Jesnowski, R. 2001. Transforming growth factor- $\beta$ 1 induces desmoplasia in an experimental model of human pancreatic carcinoma. *Cancer Res.* **61**: 550–555.



Hezel et al.

- Lowe, S.W. and Sherr, C.J. 2003. Tumor suppression by Ink4a-Arf: Progress and puzzles. *Curr. Opin. Genet. Dev.* **13**: 77–83.
- Lowenfels, A.B., Maisonneuve, P., DiMaggio, E.P., Elitsur, Y., Gates Jr., L.K., Perrault, J., and Whitcomb, D.C. 1997. Hereditary pancreatitis and the risk of pancreatic cancer. International Hereditary Pancreatitis Study Group. [see comments]. *J. Natl. Cancer Inst.* **89**: 442–446.
- Luttges, J., Schlehe, B., Menke, M.A., Vogel, I., Henne-Bruns, D., and Kloppel, G. 1999. The K-ras mutation pattern in pancreatic ductal adenocarcinoma usually is identical to that in associated normal, hyperplastic, and metaplastic ductal epithelium. *Cancer* **85**: 1703–1710.
- Luttges, J., Gahldari, H., Brocker, V., Schwarte-Waldhoff, I., Henne-Bruns, D., Kloppel, G., Schmiegel, W., and Hahn, S.A. 2001. Allelic loss is often the first hit in the biallelic inactivation of the p53 and DPC4 genes during pancreatic carcinogenesis. *Am. J. Pathol.* **158**: 1677–1683.
- Lynch, H.T., Smyrk, T., Kern, S.E., Hruban, R.H., Lightdale, C.J., Lemon, S.J., Lynch, J.F., Fusaro, L.R., Fusaro, R.M., and Ghadirani, P. 1996. Familial pancreatic cancer: A review. *Semin. Oncol.* **23**: 251–275.
- Lynch, H.T., Brand, R.E., Hogg, D., Deters, C.A., Fusaro, R.M., Lynch, J.F., Liu, L., Knezetic, J., Lassam, N.J., Goggins, M., et al. 2002. Phenotypic variation in eight extended CDKN2A germline mutation familial atypical multiple mole melanoma-pancreatic carcinoma-prone families: The familial atypical mole melanoma-pancreatic carcinoma syndrome. *Cancer* **94**: 84–96.
- Mahlamaki, E.H., Hoglund, M., Gorunova, L., Karhu, R., Dawiskiba, S., Andren-Sandberg, A., Kallioniemi, O.P., and Johansson, B. 1997. Comparative genomic hybridization reveals frequent gains of 20q, 8q, 11q, 12p, and 17q, and losses of 18q, 9p, and 15q in pancreatic cancer. *Genes Chromosomes Cancer* **20**: 383–391.
- Mahlamaki, E.H., Barlund, M., Tanner, M., Gorunova, L., Hoglund, M., Karhu, R., and Kallioniemi, A. 2002. Frequent amplification of 8q24, 11q, 17q, and 20q-specific genes in pancreatic cancer. *Genes Chromosomes Cancer* **35**: 353–358.
- Maitland, N.J. and Collins, A. 2005. A tumour stem cell hypothesis for the origins of prostate cancer. *BJU Int.* **96**: 1219–1223.
- Maitra, A., Adsay, N.V., Argani, P., Iacobuzio-Donahue, C., De Marzo, A., Cameron, J.L., Yeo, C.J., and Hruban, R.H. 2003. Multicomponent analysis of the pancreatic adenocarcinoma progression model using a pancreatic intraepithelial neoplasia tissue microarray. *Mod. Pathol.* **16**: 902–912.
- Maitra, A., Fukushima, N., Takaori, K., and Hruban, R.H. 2005. Precursors to invasive pancreatic cancer. *Adv. Anat. Pathol.* **12**: 81–91.
- Malats, N., Casals, T., Porta, M., Guarner, L., Estivill, X., and Real, F.X. 2001. Cystic fibrosis transmembrane regulator (CFTR) DeltaF508 mutation and 5T allele in patients with chronic pancreatitis and exocrine pancreatic cancer. PANKRAS II Study Group. *Gut* **48**: 70–74.
- Maloney, E.K., McLaughlin, J.L., Dagdigian, N.E., Garrett, L.M., Connors, K.M., Zhou, X.M., Blattler, W.A., Chittenden, T., and Singh, R. 2003. An anti-insulin-like growth factor I receptor antibody that is a potent inhibitor of cancer cell proliferation. *Cancer Res.* **63**: 5073–5083.
- Malumbres, M. and Barbacid, M. 2003. RAS oncogenes: The first 30 years. *Nat. Rev. Cancer* **3**: 459–465.
- Maser, R.S. and DePinho, R.A. 2002. Connecting chromosomes, crisis, and cancer. *Science* **297**: 565–569.
- Massague, J., Blain, S.W., and Lo, R.S. 2000. TGF $\beta$  signaling in growth control, cancer, and heritable disorders. *Cell* **103**: 295–309.
- Matsubayashi, H., Fukushima, N., Sato, N., Brune, K., Canto, M., Yeo, C.J., Hruban, R.H., Kern, S.E., and Goggins, M. 2003. Polymorphisms of SPINK1 N34S and CFTR in patients with sporadic and familial pancreatic cancer. *Cancer Biol. Ther.* **2**: 652–655.
- Mayo, M.W., Wang, C.Y., Cogswell, P.C., Rogers-Graham, K.S., Lowe, S.W., Der, C.J., and Baldwin Jr., A.S. 1997. Requirement of NF- $\kappa$ B activation to suppress p53-independent apoptosis induced by oncogenic Ras. *Science* **278**: 1812–1815.
- McCormick, F. 1998. Going for the GAP. *Curr. Biol.* **8**: R673–R674.
- McWilliams, R., Highsmith, W.E., Rabe, K.G., de Andrade, M., Tordsen, L.A., Holtegaard, L.M., and Petersen, G.M. 2005. Cystic fibrosis transmembrane regulator gene carrier status is a risk factor for young onset pancreatic adenocarcinoma. *Gut* **54**: 1661–1662.
- Means, A.L., Meszoely, I.M., Suzuki, K., Miyamoto, Y., Rustgi, A.K., Coffey Jr., R.J., Wright, C.V., Stoffers, D.A., and Leach, S.D. 2005. Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates. *Development* **132**: 3767–3776.
- Michaud, D.S., Giovannucci, E., Willett, W.C., Colditz, G.A., Stampfer, M.J., and Fuchs, C.S. 2001. Physical activity, obesity, height, and the risk of pancreatic cancer. *JAMA* **286**: 921–929.
- Min, Y., Adachi, Y., Yamamoto, H., Ito, H., Itoh, F., Lee, C.T., Nadaf, S., Carbone, D.P., and Imai, K. 2003. Genetic blockade of the insulin-like growth factor-I receptor: A promising strategy for human pancreatic cancer. *Cancer Res.* **63**: 6432–6441.
- Missiaglia, E., Blaveri, E., Terris, B., Wang, Y.H., Costello, E., Neoptolemos, J.P., Crnogorac-Jurcevic, T., and Lemoine, N.R. 2004. Analysis of gene expression in cancer cell lines identifies candidate markers for pancreatic tumorigenesis and metastasis. *Int. J. Cancer* **112**: 100–112.
- Mitin, N., Rossman, K.L., and Der, C.J. 2005. Signaling interplay in Ras superfamily function. *Curr. Biol.* **15**: R563–R574.
- Miyamoto, Y., Maitra, A., Ghosh, B., Zechner, U., Argani, P., Iacobuzio-Donahue, C.A., Sriuranpong, V., Iso, T., Meszoely, I.M., Wolfe, M.S., et al. 2003. Notch mediates TGF  $\alpha$ -induced changes in epithelial differentiation during pancreatic tumorigenesis. *Cancer Cell* **3**: 565–576.
- Moshous, D., Callebaut, I., de Chasseval, R., Corneo, B., Cavazzana-Calvo, M., Le Deist, F., Tezcan, I., Sanal, O., Bertrand, Y., Philippe, N., et al. 2001. Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell* **105**: 177–186.
- Moskaluk, C.A., Hruban, R.H., and Kern, S.E. 1997. p16 and K-ras gene mutations in the intraductal precursors of human pancreatic adenocarcinoma. *Cancer Res.* **57**: 2140–2143.
- Muerkoster, S., Arlt, A., Sipos, B., Witt, M., Grossmann, M., Kloppel, G., Kalthoff, H., Folsch, U.R., and Schafer, H. 2005. Increased expression of the E3-ubiquitin ligase receptor subunit  $\beta$ TRCP1 relates to constitutive nuclear factor- $\kappa$ B activation and chemoresistance in pancreatic carcinoma cells. *Cancer Res.* **65**: 1316–1324.
- Murphy, K.M., Brune, K.A., Griffin, C., Sollenberger, J.E., Petersen, G.M., Bansal, R., Hruban, R.H., and Kern, S.E. 2002. Evaluation of candidate genes MAP2K4, MADH4, ACVR1B, and BRCA2 in familial pancreatic cancer: Deleterious BRCA2 mutations in 17%. *Cancer Res.* **62**: 3789–3793.
- Murtaugh, L.C. and Melton, D.A. 2003. Genes, signals, and lineages in pancreas development. *Annu. Rev. Cell Dev. Biol.*

- 19: 71–89.
- Murtaugh, L.C., Stanger, B.Z., Kwan, K.M., and Melton, D.A. 2003. Notch signaling controls multiple steps of pancreatic differentiation. *Proc. Natl. Acad. Sci.* **100**: 14920–14925.
- Murtaugh, L.C., Law, A.C., Dor, Y., and Melton, D.A. 2005.  $\beta$ -Catenin is essential for pancreatic acinar but not islet development. *Development* **132**: 4663–4674.
- Nair, P.N., De Armond, D.T., Adamo, M.L., Strodel, W.E., and Freeman, J.W. 2001. Aberrant expression and activation of insulin-like growth factor-1 receptor (IGF-1R) are mediated by an induction of IGF-1R promoter activity and stabilization of IGF-1R mRNA and contributes to growth factor independence and increased survival of the pancreatic cancer cell line MIA PaCa-2. *Oncogene* **20**: 8203–8214.
- Nakamura, T., Furukawa, Y., Nakagawa, H., Tsunoda, T., Ohigashi, H., Murata, K., Ishikawa, O., Ohgaki, K., Kashimura, N., Miyamoto, M., et al. 2004. Genome-wide cDNA microarray analysis of gene expression profiles in pancreatic cancers using populations of tumor cells and normal ductal epithelial cells selected for purity by laser microdissection. *Oncogene* **23**: 2385–2400.
- Neglia, J.P., FitzSimmons, S.C., Maisonneuve, P., Schoni, M.H., Schoni-Affolter, F., Corey, M., and Lowenfels, A.B. 1995. The risk of cancer among patients with cystic fibrosis. Cystic Fibrosis and Cancer Study Group. *N. Engl. J. Med.* **332**: 494–499.
- Ng, S.S.W., Tsao, M.S., Chow, S., and Hedley, D.W. 2000. Inhibition of phosphatidylinositol 3-kinase enhances gemcitabine-induced apoptosis in human pancreatic cancer cells. *Cancer Res.* **60**: 5451–5455.
- Nielsen, G.P., Stemmer-Rachamimov, A.O., Shaw, J., Roy, J.E., Koh, J., and Louis, D.N. 1999. Immunohistochemical survey of p16INK4A expression in normal human adult and infant tissues. *Lab. Invest.* **79**: 1137–1143.
- Norgaard, G.A., Jensen, J.N., and Jensen, J. 2003. FGF10 signaling maintains the pancreatic progenitor cell state revealing a novel role of Notch in organ development. *Dev. Biol.* **264**: 323–338.
- Nowak, N.J., Gaile, D., Conroy, J.M., McQuaid, D., Cowell, J., Carter, R., Goggins, M.G., Hruban, R.H., and Maitra, A. 2005. Genome-wide aberrations in pancreatic adenocarcinoma. *Cancer Genet. Cytogenet.* **161**: 36–50.
- Offield, M.F., Jetton, T.L., Labosky, P.A., Ray, M., Stein, R.W., Magnuson, M.A., Hogan, B.L., and Wright, C.V. 1996. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* **122**: 983–995.
- Oft, M., Akhurst, R.J., and Balmain, A. 2002. Metastasis is driven by sequential elevation of H-ras and Smad2 levels. *Nat. Cell Biol.* **4**: 487–494.
- Ogawa, T., Takayama, K., Takakura, N., Kitano, S., and Ueno, H. 2002. Anti-tumor angiogenesis therapy using soluble receptors: Enhanced inhibition of tumor growth when soluble fibroblast growth factor receptor-1 is used with soluble vascular endothelial growth factor receptor. *Cancer Gene Ther.* **9**: 633–640.
- O'Hagan, R.C., Chang, S., Maser, R.S., Mohan, R., Artandi, S.E., Chin, L., and DePinho, R.A. 2002. Telomere dysfunction provokes regional amplification and deletion in cancer genomes. *Cancer Cell* **2**: 149–155.
- Ohlsson, H., Karlsson, K., and Edlund, T. 1993. IPF1, a homeodomain-containing transactivator of the insulin gene. *EMBO J.* **12**: 4251–4259.
- Ohta, T., Yamamoto, M., Numata, M., Iseki, S., Tsukioka, Y., Miyashita, T., Kayahara, M., Nagakawa, T., Miyazaki, I., Nishikawa, K., et al. 1995. Expression of basic fibroblast growth factor and its receptor in human pancreatic carcinomas. *Br. J. Cancer* **72**: 824–831.
- Okami, K., Wu, L., Riggins, G., Cairns, P., Goggins, M., Evron, E., Halachmi, N., Ahrendt, S.A., Reed, A.L., Hilgers, W., et al. 1998. Analysis of PTEN/MMAC1 alterations in aerodigestive tract tumors. *Cancer Res.* **58**: 509–511.
- Omer, C.A. and Kohl, N.E. 1997. CA1A2X-competitive inhibitors of farnesyltransferase as anti-cancer agents. *Trends Pharmacol. Sci.* **18**: 437–444.
- Orlowski, R.Z. and Baldwin Jr., A.S. 2002. NF- $\kappa$ B as a therapeutic target in cancer. *Trends Mol. Med.* **8**: 385–389.
- Ornitz, D.M., Hammer, R.E., Messing, A., Palmiter, R.D., and Brinster, R.L. 1987. Pancreatic neoplasia induced by SV40 T-antigen expression in acinar cells of transgenic mice. *Science* **238**: 188–193.
- Orsulic, S. 2002. An RCAS-TVA-based approach to designer mouse models. *Mamm. Genome* **13**: 543–547.
- Ory, S. and Morrison, D.K. 2004. Signal transduction: Implications for Ras-dependent ERK signaling. *Curr. Biol.* **14**: R277–R278.
- Ossipova, O., Bardeesy, N., DePinho, R.A., and Green, J.B. 2003. LKB1 (XEEK1) regulates Wnt signalling in vertebrate development. *Nat. Cell Biol.* **5**: 889–894.
- Ouban, A., Muraca, P., Yeatman, T., and Coppola, D. 2003. Expression and distribution of insulin-like growth factor-1 receptor in human carcinomas. *Hum. Pathol.* **34**: 803–808.
- Paciucci, R., Vila, M.R., Adell, T., Diaz, V.M., Tora, M., Nakamura, T., and Real, F.X. 1998. Activation of the urokinase plasminogen activator/urokinase plasminogen activator receptor system and redistribution of E-cadherin are associated with hepatocyte growth factor-induced motility of pancreas tumor cells overexpressing Met. *Am. J. Pathol.* **153**: 201–212.
- Paliwal, S., Pande, S., Kovi, R.C., Sharpless, N.E., Bardeesy, N., and Grossman, S.R. 2006. Targeting of C-terminal binding protein (CtBP) by ARF results in p53-independent apoptosis. *Mol. Cell Biol.* **26**: 2360–2372.
- Pao, W., Klimstra, D.S., Fisher, G.H., and Varmus, H.E. 2003. Use of avian retroviral vectors to introduce transcriptional regulators into mammalian cells for analyses of tumor maintenance. *Proc. Natl. Acad. Sci.* **100**: 8764–8769.
- Pardal, R., Clarke, M.F., and Morrison, S.J. 2003. Applying the principles of stem-cell biology to cancer. *Nat. Rev. Cancer* **3**: 895–902.
- Parsa, I., Longnecker, D.S., Scarpelli, D.G., Pour, P., Reddy, J.K., and Lefkowitz, M. 1985. Ductal metaplasia of human exocrine pancreas and its association with carcinoma. *Cancer Res.* **45**: 1285–1290.
- Pasca di Magliano, M., and Hebrok, M. 2003. Hedgehog signaling in cancer formation and maintenance. *Nat. Rev. Cancer* **3**: 903–911.
- Passequé, E., Wagner, E.F., and Weissman, I.L. 2004. JunB deficiency leads to a myeloproliferative disorder arising from hematopoietic stem cells. *Cell* **119**: 431–443.
- Peng, B., Fleming, J.B., Breslin, T., Grau, A.M., Fojioka, S., Abbruzzese, J.L., Evans, D.B., Ayers, D., Wathen, K., Wu, T., et al. 2002. Suppression of tumorigenesis and induction of p15(ink4b) by Smad4/DPC4 in human pancreatic cancer cells. *Clin. Cancer Res.* **8**: 3628–3638.
- Perugini, R.A., McDade, T.P., Vittimberga Jr., F.J., and Callery, M.P. 2000. Pancreatic cancer cell proliferation is phosphatidylinositol 3-kinase dependent. *J. Surg. Res.* **90**: 39–44.
- Petersen, G.M. and Hruban, R.H. 2003. Familial pancreatic cancer: Where are we in 2003? *J. Natl. Cancer Inst.* **95**: 180–181.
- Pomerantz, J., Schreiber-Agus, N., Liegeois, N.J., Silverman, A., Alland, L., Chin, L., Potes, J., Chen, K., Orlow, I., Lee, H.W., et al. 1998. The Ink4a tumor suppressor gene product,

- p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53. *Cell* **92**: 713–723.
- Pour, P.M. 1997. The role of Langerhans islets in pancreatic ductal adenocarcinoma. *Front. Biosci.* **2**: D271–D282.
- Prasad, N.B., Biankin, A.V., Fukushima, N., Maitra, A., Dhara, S., Elkahoul, A.G., Hruban, R.H., Goggins, M., and Leach, S.D. 2005. Gene expression profiles in pancreatic intraepithelial neoplasia reflect the effects of Hedgehog signaling on pancreatic ductal epithelial cells. *Cancer Res.* **65**: 1619–1626.
- Prowse, K.R. and Greider, C.W. 1995. Developmental and tissue-specific regulation of mouse telomerase and telomere length. *Proc. Natl. Acad. Sci.* **92**: 4818–4822.
- Qi, Y., Gregory, M.A., Li, Z., Brousal, J.P., West, K., and Hann, S.R. 2004. p19ARF directly and differentially controls the functions of c-Myc independently of p53. *Nature* **431**: 712–717.
- Quaife, C.J., Pinkert, C.A., Ornitz, D.M., Palmiter, R.D., and Brinster, R.L. 1987. Pancreatic neoplasia induced by ras expression in acinar cells of transgenic mice. *Cell* **48**: 1023–1034.
- Radtke, F. and Raj, K. 2003. The role of Notch in tumorigenesis: Oncogene or tumour suppressor? *Nat. Rev. Cancer* **3**: 756–767.
- Rajagopalan, H. and Lengauer, C. 2004. Aneuploidy and cancer. *Nature* **432**: 338–341.
- Ramirez, R.D., Morales, C.P., Herbert, B.S., Rohde, J.M., Passons, C., Shay, J.W., and Wright, W.E. 2001. Putative telomere-independent mechanisms of replicative aging reflect inadequate growth conditions. *Genes & Dev.* **15**: 398–403.
- Rane, S.G., Lee, J.H., and Lin, H.M. 2006. Transforming growth factor- $\beta$  pathway: Role in pancreas development and pancreatic disease. *Cytokine Growth Factor Rev.* **17**: 107–119.
- Rangarajan, A., Hong, S.J., Gifford, A., and Weinberg, R.A. 2004. Species- and cell type-specific requirements for cellular transformation. *Cancer Cell* **6**: 171–183.
- Rocha, S., Campbell, K.J., and Perkins, N.D. 2003. p53- and Mdm2-independent repression of NF- $\kappa$ B transactivation by the ARF tumor suppressor. *Mol. Cell* **12**: 15–25.
- Rodriguez-Viciana, P., Warne, P.H., Vanhaesebroeck, B., Waterfield, M.D., and Downward, J. 1996. Activation of phosphoinositide 3-kinase by interaction with Ras and by point mutation. *EMBO J.* **15**: 2442–2451.
- Rosty, C., Christa, L., Kuzdzal, S., Baldwin, W.M., Zahurak, M.L., Carnot, F., Chan, D.W., Canto, M., Lillemoe, K.D., Cameron, J.L., et al. 2002. Identification of hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein 1 as a biomarker for pancreatic ductal adenocarcinoma by protein biochip technology. *Cancer Res.* **62**: 1868–1875.
- Rowland-Goldsmith, M.A., Maruyama, H., Kusama, T., Ralli, S., and Korc, M. 2001. Soluble type II transforming growth factor- $\beta$  (TGF- $\beta$ ) receptor inhibits TGF- $\beta$  signaling in COLO-357 pancreatic cancer cells in vitro and attenuates tumor formation. *Clin. Cancer Res.* **7**: 2931–2940.
- Rowland-Goldsmith, M.A., Maruyama, H., Matsuda, K., Idezawa, T., Ralli, M., Ralli, S., and Korc, M. 2002. Soluble type II transforming growth factor- $\beta$  receptor attenuates expression of metastasis-associated genes and suppresses pancreatic cancer cell metastasis. *Mol. Cancer Ther.* **1**: 161–167.
- Rozenblum, E., Schutte, M., Goggins, M., Hahn, S.A., Panzer, S., Zahurak, M., Goodman, S.N., Sohn, T.A., Hruban, R.H., Yeo, C.J., et al. 1997. Tumor-suppressive pathways in pancreatic carcinoma. *Cancer Res.* **57**: 1731–1734.
- Ruas, M. and Peters, G. 1998. The p16INK4a/CDKN2A tumor suppressor and its relatives. *Biochim. Biophys. Acta* **1378**: F115–F177.
- Ruggeri, B.A., Huang, L., Wood, M., Cheng, J.Q., and Testa, J.R. 1998. Amplification and overexpression of the AKT2 oncogene in a subset of human pancreatic ductal adenocarcinomas. *Mol. Carcinog.* **21**: 81–86.
- Sahin, F., Maitra, A., Argani, P., Sato, N., Maehara, N., Montgomery, E., Goggins, M., Hruban, R.H., and Su, G.H. 2003. Loss of Stk11/Lkb1 expression in pancreatic and biliary neoplasms. *Mod. Pathol.* **16**: 686–691.
- Saimura, M., Nagai, E., Mizumoto, K., Maehara, N., Minamishima, Y.A., Katano, M., Matsumoto, K., Nakamura, T., and Tanaka, M. 2002. Tumor suppression through angiogenesis inhibition by SUIT-2 pancreatic cancer cells genetically engineered to secrete NK4. *Clin. Cancer Res.* **8**: 3243–3249.
- Samuels, Y. and Velculescu, V.E. 2004. Oncogenic mutations of PIK3CA in human cancers. *Cell Cycle* **3**: 1221–1224.
- Sandgren, E.P., Luetkeke, N.C., Palmiter, R.D., Brinster, R.L., and Lee, D.C. 1990. Overexpression of TGF $\alpha$  in transgenic mice: Induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. *Cell* **61**: 1121–1135.
- Sandgren, E.P., Quaife, C.J., Paulovich, A.G., Palmiter, R.D., and Brinster, R.L. 1991. Pancreatic tumor pathogenesis reflects the causative genetic lesion. *Proc. Natl. Acad. Sci.* **88**: 93–97.
- Sandgren, E.P., Luetkeke, N.C., Qiu, T.H., Palmiter, R.D., Brinster, R.L., and Lee, D.C. 1993. Transforming growth factor  $\alpha$  dramatically enhances oncogene-induced carcinogenesis in transgenic mouse pancreas and liver. *Mol. Cell. Biol.* **13**: 320–330.
- Sato, N., Mizumoto, K., Nakamura, M., Nakamura, K., Kusumoto, M., Niiyama, H., Ogawa, T., and Tanaka, M. 1999. Centrosome abnormalities in pancreatic ductal carcinoma. *Clin. Cancer Res.* **5**: 963–970.
- Sato, N., Mizumoto, K., Nakamura, M., Maehara, N., Minamishima, Y.A., Nishio, S., Nagai, E., and Tanaka, M. 2001a. Correlation between centrosome abnormalities and chromosomal instability in human pancreatic cancer cells. *Cancer Genet. Cytogenet.* **126**: 13–19.
- Sato, N., Rosty, C., Jansen, M., Fukushima, N., Ueki, T., Yeo, C.J., Cameron, J.L., Iacobuzio-Donahue, C.A., Hruban, R.H., and Goggins, M. 2001b. STK11/LKB1 Peutz-Jeghers gene inactivation in intraductal papillary-mucinous neoplasms of the pancreas. *Am. J. Pathol.* **159**: 2017–2022.
- Sato, N., Fukushima, N., Maitra, A., Iacobuzio-Donahue, C.A., van Heek, N.T., Cameron, J.L., Yeo, C.J., Hruban, R.H., and Goggins, M. 2004a. Gene expression profiling identifies genes associated with invasive intraductal papillary mucinous neoplasms of the pancreas. *Am. J. Pathol.* **164**: 903–914.
- Sato, N., Fukushima, N., Matsubayashi, H., and Goggins, M. 2004b. Identification of maspin and S100P as novel hypomethylation targets in pancreatic cancer using global gene expression profiling. *Oncogene* **23**: 1531–1538.
- Schenk, M., Schwartz, A.G., O'Neal, E., Kinnard, M., Greenson, J.K., Fryzek, J.P., Ying, G.S., and Garabrant, D.H. 2001. Familial risk of pancreatic cancer. *J. Natl. Cancer Inst.* **93**: 640–644.
- Schleger, C., Arens, N., Zentgraf, H., Bleyl, U., and Verbeke, C. 2000. Identification of frequent chromosomal aberrations in ductal adenocarcinoma of the pancreas by comparative genomic hybridization (CGH). *J. Pathol.* **191**: 27–32.
- Schlieman, M.G., Fahy, B.N., Ramsamooj, R., Beckett, L., and Bold, R.J. 2003. Incidence, mechanism and prognostic value of activated AKT in pancreas cancer. *Br. J. Cancer* **89**: 2110–2115.
- Schreiner, B., Baur, D.M., Fingerle, A.A., Zechner, U., Greten, F.R., Adler, G., Sipos, B., Kloppel, G., Hameister, H., and Schmid, R.M. 2003. Pattern of secondary genomic changes in pancreatic tumors of Tgfa/Trp53+/- transgenic mice.



- Genes Chromosomes Cancer* **38**: 240–248.
- Schwarte-Waldhoff, I., Volpert, O.V., Bouck, N.P., Sipos, B., Hahn, S.A., Klein-Scory, S., Luttes, J., Kloppel, G., Graeven, U., Eilert-Micus, C., et al. 2000. Smad4/DPC4-mediated tumor suppression through suppression of angiogenesis. *Proc. Natl. Acad. Sci.* **97**: 9624–9629.
- Sclabas, G.M., Fujioka, S., Schmidt, C., Evans, D.B., and Chiao, P.J. 2003. NF- $\kappa$ B in pancreatic cancer. *Int. J. Gastrointest. Cancer* **33**: 15–26.
- Scoggins, C.R., Meszoely, I.M., Wada, M., Means, A.L., Yang, L., and Leach, S.D. 2000. p53-dependent acinar cell apoptosis triggers epithelial proliferation in duct-ligated murine pancreas. *Am. J. Physiol. Gastrointest. Liver Physiol.* **279**: G827–G836.
- Seo, Y., Baba, H., Fukuda, T., Takashima, M., and Sugimachi, K. 2000. High expression of vascular endothelial growth factor is associated with liver metastasis and a poor prognosis for patients with ductal pancreatic adenocarcinoma. *Cancer* **88**: 2239–2245.
- Serrano, M., Lin, A.W., McCurrach, M.E., Beach, D., and Lowe, S.W. 1997. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* **88**: 593–602.
- Shah, S.A., Potter, M.W., Hedeshian, M.H., Kim, R.D., Chari, R.S., and Callery, M.P. 2001. PI-3' kinase and NF- $\kappa$ B cross-signaling in human pancreatic cancer cells. *J. Gastrointest. Surg.* **5**: 603–612; discussion 612–613.
- Sharer, N., Schwarz, M., Malone, G., Howarth, A., Painter, J., Super, M., and Braganza, J. 1998. Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. *N. Engl. J. Med.* **339**: 645–652.
- Sharma, A., Zangen, D.H., Reitz, P., Taneja, M., Lissauer, M.E., Miller, C.P., Weir, G.C., Habener, J.F., and Bonner-Weir, S. 1999. The homeodomain protein IDX-1 increases after an early burst of proliferation during pancreatic regeneration. *Diabetes* **48**: 507–513.
- Sharpless, N.E. and DePinho, R.A. 1999. The INK4A/ARF locus and its two gene products. *Curr. Opin. Genet. Dev.* **9**: 22–30.
- . 2002. p53: Good cop/bad cop. *Cell* **110**: 9–12.
- . 2005. Cancer: Crime and punishment. *Nature* **436**: 636–637.
- Sharpless, N.E., Bardeesy, N., Lee, K.H., Carrasco, D., Castrillon, D.H., Aguirre, A.J., Wu, E.A., Horner, J.W., and DePinho, R.A. 2001a. Loss of p16Ink4a with retention of p19Arf predisposes mice to tumorigenesis. *Nature* **413**: 86–91.
- Sharpless, N.E., Ferguson, D.O., O'Hagan, R.C., Castrillon, D.H., Lee, C., Farazi, P.A., Alson, S., Fleming, J., Morton, C.C., Frank, K., et al. 2001b. Impaired nonhomologous end-joining provokes soft tissue sarcomas harboring chromosomal translocations, amplifications, and deletions. *Mol. Cell* **8**: 1187–1196.
- Shaw, R.J., Bardeesy, N., Manning, B.D., Lopez, L., Kosmatka, M., DePinho, R.A., and Cantley, L.C. 2004a. The LKB1 tumor suppressor negatively regulates mTOR signaling. *Cancer Cell* **6**: 91–99.
- Shaw, R.J., Kosmatka, M., Bardeesy, N., Hurley, R.L., Witters, L.A., DePinho, R.A., and Cantley, L.C. 2004b. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc. Natl. Acad. Sci.* **101**: 3329–3335.
- Shaw, R.J., Lamia, K.A., Vasquez, D., Koo, S.H., Bardeesy, N., Depinho, R.A., Montminy, M., and Cantley, L.C. 2005. The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science* **310**: 1642–1646.
- Sherr, C.J. 2001. The INK4a/ARF network in tumour suppression. *Nat. Rev. Mol. Cell Biol.* **2**: 731–737.
- Sherr, C.J. and DePinho, R.A. 2000. Cellular senescence: Mitotic clock or culture shock? *Cell* **102**: 407–410.
- Siegel, P.M. and Massague, J. 2003. Cytostatic and apoptotic actions of TGF- $\beta$  in homeostasis and cancer. *Nat. Rev. Cancer* **3**: 807–821.
- Signoretti, S., Waltregny, D., Dilks, J., Isaac, B., Lin, D., Garraway, L., Yang, A., Montironi, R., McKeon, F., and Loda, M. 2000. p63 is a prostate basal cell marker and is required for prostate development. *Am. J. Pathol.* **157**: 1769–1775.
- Singh, S.K., Clarke, I.D., Hide, T., and Dirks, P.B. 2004a. Cancer stem cells in nervous system tumors. *Oncogene* **23**: 7267–7273.
- Singh, S.K., Hawkins, C., Clarke, I.D., Squire, J.A., Bayani, J., Hide, T., Henkelman, R.M., Cusimano, M.D., and Dirks, P.B. 2004b. Identification of human brain tumour initiating cells. *Nature* **432**: 396–401.
- Sirivatanauksorn, V., Sirivatanauksorn, Y., Gorman, P.A., Davidson, J.M., Sheer, D., Moore, P.S., Scarpa, A., Edwards, P.A., and Lemoine, N.R. 2001. Non-random chromosomal rearrangements in pancreatic cancer cell lines identified by spectral karyotyping. *Int. J. Cancer* **91**: 350–358.
- Sjölund, J., Manetopoulos, C., Stockhausen, M.T., and Axelson, H. 2005. The Notch pathway in cancer: Differentiation gone awry. *Eur. J. Cancer* **41**: 2620–2629.
- Solcia, E., Capella, C., and Kloppel, G. 1995. *Tumors of the pancreas*. Armed Forces Institute for Pathology, Washington, DC.
- Stanger, B.Z., Stiles, B., Lauwers, G.Y., Bardeesy, N., Mendoza, M., Wang, Y., Greenwood, A., Cheng, K.H., McLaughlin, M., Brown, D., et al. 2005. Pten constrains centroacinar cell expansion and malignant transformation in the pancreas. *Cancer Cell* **8**: 185–195.
- Stoeltzing, O., Liu, W., Reinmuth, N., Fan, F., Parikh, A.A., Bucana, C.D., Evans, D.B., Semenza, G.L., and Ellis, L.M. 2003. Regulation of hypoxia-inducible factor-1 $\alpha$ , vascular endothelial growth factor, and angiogenesis by an insulin-like growth factor-I receptor autocrine loop in human pancreatic cancer. *Am. J. Pathol.* **163**: 1001–1011.
- Stolzenberg-Solomon, R.Z., Graubard, B.I., Chari, S., Limburg, P., Taylor, P.R., Virtamo, J., and Albanes, D. 2005. Insulin, glucose, insulin resistance, and pancreatic cancer in male smokers. *JAMA* **294**: 2872–2878.
- Storchova, Z. and Pellman, D. 2004. From polyploidy to aneuploidy, genome instability and cancer. *Nat. Rev. Mol. Cell Biol.* **5**: 45–54.
- Su, G.H., Hruban, R.H., Bansal, R.K., Bova, G.S., Tang, D.J., Shekher, M.C., Westerman, A.M., Entius, M.M., Goggins, M., Yeo, C.J., et al. 1999. Germline and somatic mutations of the STK11/LKB1 Peutz-Jeghers gene in pancreatic and biliary cancers. *Am. J. Pathol.* **154**: 1835–1840.
- Sugimoto, M., Kuo, M.L., Roussel, M.F., and Sherr, C.J. 2003. Nucleolar Arf tumor suppressor inhibits ribosomal RNA processing. *Mol. Cell* **11**: 415–424.
- Sundaram, M.V. 2005. The love-hate relationship between Ras and Notch. *Genes & Dev.* **19**: 1825–1839.
- Suwa, H., Ohshio, G., Imamura, T., Watanabe, G., Arai, S., Imamura, M., Narumiya, S., Hiai, H., and Fukumoto, M. 1998. Overexpression of the rhoC gene correlates with progression of ductal adenocarcinoma of the pancreas. *Br. J. Cancer* **77**: 147–152.
- Sweet-Cordero, A., Mukherjee, S., Subramanian, A., You, H., Roix, J.J., Ladd-Acosta, C., Mesirov, J., Golub, T.R., and Jacks, T. 2005. An oncogenic KRAS2 expression signature identified by cross-species gene-expression analysis. *Nat. Genet.* **37**: 48–55.
- Taipale, J. and Beachy, P.A. 2001. The Hedgehog and Wnt sig-

- nalling pathways in cancer. *Nature* **411**: 349–354.
- Tang, R.F., Itakura, J., Aikawa, T., Matsuda, K., Fujii, H., Korc, M., and Matsumoto, Y. 2001. Overexpression of lymphangiogenic growth factor VEGF-C in human pancreatic cancer. *Pancreas* **22**: 285–292.
- Tang, B., Vu, M., Booker, T., Santner, S.J., Miller, F.R., Anver, M.R., and Wakefield, L.M. 2003. TGF- $\beta$  switches from tumor suppressor to prometastatic factor in a model of breast cancer progression. *J. Clin. Invest.* **112**: 1116–1124.
- Taniguchi, T., Tischkowitz, M., Ameziane, N., Hodgson, S.V., Mathew, C.G., Joenje, H., Mok, S.C., and D'Andrea, A.D. 2003. Disruption of the Fanconi anemia–BRCA pathway in cisplatin-sensitive ovarian tumors. *Nat. Med.* **9**: 568–574.
- Tascilar, M., Skinner, H.G., Rosty, C., Sohn, T., Wilentz, R.E., Offerhaus, G.J., Adsay, V., Abrams, R.A., Cameron, J.L., Kern, S.E., et al. 2001. The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. *Clin. Cancer Res.* **7**: 4115–4121.
- ten Dijke, P. and Hill, C.S. 2004. New insights into TGF- $\beta$ –Smad signalling. *Trends Biochem. Sci.* **29**: 265–273.
- Thayer, S.P., di Magliano, M.P., Heiser, P.W., Nielsen, C.M., Roberts, D.J., Lauwers, G.Y., Qi, Y.P., Gysin, S., Fernandez-del Castillo, C., Yajnik, V., et al. 2003. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature* **425**: 851–856.
- Thomas, A.M., Santarsiero, L.M., Lutz, E.R., Armstrong, T.D., Chen, Y.C., Huang, L.Q., Laheru, D.A., Goggins, M., Hruban, R.H., and Jaffee, E.M. 2004. Mesothelin-specific CD8<sup>+</sup> T cell responses provide evidence of in vivo cross-priming by antigen-presenting cells in vaccinated pancreatic cancer patients. *J. Exp. Med.* **200**: 297–306.
- Thompson, D. and Easton, D.F. 2002. Cancer incidence in BRCA1 mutation carriers. *J. Natl. Cancer Inst.* **94**: 1358–1365.
- Tokunaga, T., Abe, Y., Tsuchida, T., Hatanaka, H., Oshika, Y., Tomisawa, M., Yoshimura, M., Ohnishi, Y., Kijima, H., Yamazaki, H., et al. 2002. Ribozyme mediated cleavage of cell-associated isoform of vascular endothelial growth factor inhibits liver metastasis of a pancreatic cancer cell line. *Int. J. Oncol.* **21**: 1027–1032.
- Tomioka, D., Maehara, N., Kuba, K., Mizumoto, K., Tanaka, M., Matsumoto, K., and Nakamura, T. 2001. Inhibition of growth, invasion, and metastasis of human pancreatic carcinoma cells by NK4 in an orthotopic mouse model. *Cancer Res.* **61**: 7518–7524.
- Tonon, G., Wong, K.K., Maulik, G., Brennan, C., Feng, B., Zhang, Y., Khatri, D.B., Protopopov, A., You, M.J., Aguirre, A.J., et al. 2005. High-resolution genomic profiles of human lung cancer. *Proc. Natl. Acad. Sci.* **102**: 9625–9630.
- Tosh, D. and Slack, J.M. 2002. How cells change their phenotype. *Nat. Rev. Mol. Cell Biol.* **3**: 187–194.
- Tuveson, D.A., Shaw, A.T., Willis, N.A., Silver, D.P., Jackson, E.L., Chang, S., Mercer, K.L., Grochow, R., Hock, H., Crowley, D., et al. 2004. Endogenous oncogenic K-ras(G12D) stimulates proliferation and widespread neoplastic and developmental defects. *Cancer Cell* **5**: 375–387.
- Tuveson, D.A., Zhu, L., Gopinathan, A., Willis, N.A., Kachatrian, L., Grochow, R., Pin, C.L., Mitin, N.Y., Taparowsky, E.J., Gimotty, P.A., et al. 2006. Mist1-KrasG12D knock-in mice develop mixed differentiation metastatic exocrine pancreatic carcinoma and hepatocellular carcinoma. *Cancer Res.* **66**: 242–247.
- Van Cutsem, E., van de Velde, H., Karasek, P., Oettle, H., Verenne, W.L., Szawlowski, A., Schoffski, P., Post, S., Verslype, C., Neumann, H., et al. 2004. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. *J. Clin. Oncol.* **22**: 1430–1438.
- van der Heijden, M.S., Brody, J.R., Gallmeier, E., Cunningham, S.C., Dezentje, D.A., Shen, D., Hruban, R.H., and Kern, S.E. 2004. Functional defects in the Fanconi anemia pathway in pancreatic cancer cells. *Am. J. Pathol.* **165**: 651–657.
- Van Dyke, T. and Jacks, T. 2002. Cancer modeling in the modern era: Progress and challenges. *Cell* **108**: 135–144.
- van Heek, N.T., Meeker, A.K., Kern, S.E., Yeo, C.J., Lillemoe, K.D., Cameron, J.L., Offerhaus, G.J., Hicks, J.L., Wilentz, R.E., Goggins, M.G., et al. 2002. Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia. *Am. J. Pathol.* **161**: 1541–1547.
- Venkitaraman, A.R. 2002. Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell* **108**: 171–182.
- Vinik, A.I., Pittenger, G.L., Rafaeloff, R., Rosenberg, L., and Duguid, W. 1997. Determinants of pancreatic islet cell neogenesis—The cellophane wrap model. In *Pancreatic growth and regeneration* (ed. N. Sarvetnick), pp. 183–217. Karger Landes Systems, Basel, Switzerland.
- Vivanco, I. and Sawyers, C.L. 2002. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat. Rev. Cancer* **2**: 489–501.
- von Marschall, Z., Cramer, T., Hocker, M., Burde, R., Plath, T., Schirmer, M., Heidenreich, R., Breier, G., Riecken, E.O., Wiedenmann, B., et al. 2000. De novo expression of vascular endothelial growth factor in human pancreatic cancer: Evidence for an autocrine mitogenic loop. *Gastroenterology* **119**: 1358–1372.
- Wagner, M., Lopez, M.E., Cahn, M., and Korc, M. 1998a. Suppression of fibroblast growth factor receptor signaling inhibits pancreatic cancer growth in vitro and in vivo. *Gastroenterology* **114**: 798–807.
- Wagner, M., Luhrs, H., Kloppel, G., Adler, G., and Schmid, R.M. 1998b. Malignant transformation of duct-like cells originating from acini in transforming growth factor transgenic mice. *Gastroenterology* **115**: 1254–1262.
- Wagner, M., Kleeff, J., Friess, H., Buchler, M.W., and Korc, M. 1999. Enhanced expression of the type II transforming growth factor- $\beta$  receptor is associated with decreased survival in human pancreatic cancer. *Pancreas* **19**: 370–376.
- Wagner, M., Greten, F.R., Weber, C.K., Koschnick, S., Mattfeldt, T., Deppert, W., Kern, H., Adler, G., and Schmid, R.M. 2001. A murine tumor progression model for pancreatic cancer recapitulating the genetic alterations of the human disease. *Genes & Dev.* **15**: 286–293.
- Wang, R.N., Rehfeld, J.F., Nielsen, F.C., and Kloppel, G. 1997. Expression of gastrin and transforming growth factor- $\alpha$  during duct to islet cell differentiation in the pancreas of duct-ligated adult rats. *Diabetologia* **40**: 887–893.
- Wang, W., Abbruzzese, J.L., Evans, D.B., Larry, L., Cleary, K.R., and Chiao, P.J. 1999. The nuclear factor- $\kappa$  B RelA transcription factor is constitutively activated in human pancreatic adenocarcinoma cells. *Clin. Cancer Res.* **5**: 119–127.
- Warshaw, A.L. and Fernandez-del Castillo, C. 1992. Pancreatic carcinoma. *N. Engl. J. Med.* **326**: 455–465.
- Weijnen, S., Rizzo, P., Braid, M., Vaishnav, R., Jonkheer, S.M., Zlobin, A., Osborne, B.A., Gottipati, S., Aster, J.C., Hahn, W.C., et al. 2002. Activation of Notch-1 signaling maintains the neoplastic phenotype in human Ras-transformed cells. *Nat. Med.* **8**: 979–986.
- Wennerberg, K., Rossman, K.L., and Der, C.J. 2005. The Ras superfamily at a glance. *J. Cell Sci.* **118**: 843–846.
- Whelan, A.J., Bartsch, D., and Goodfellow, P.J. 1995. Brief report: A familial syndrome of pancreatic cancer and melanoma with a mutation in the CDKN2 tumor-suppressor

- gene [see comments]. *N. Engl. J. Med.* **333**: 975–977.
- Whitcomb, D.C., Gorry, M.C., Preston, R.A., Furey, W., Sossenheimer, M.J., Ulrich, C.D., Martin, S.P., Gates Jr., L.K., Amann, S.T., Toskes, P.P., et al. 1996. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene [see comments]. *Nat. Genet.* **14**: 141–145.
- Wilentz, R.E., Geradts, J., Maynard, R., Offerhaus, G.J., Kang, M., Goggins, M., Yeo, C.J., Kern, S.E., and Hruban, R.H. 1998. Inactivation of the p16<sup>INK4A</sup> tumor-suppressor gene in pancreatic duct lesions: Loss of intranuclear expression. *Cancer Res.* **58**: 4740–4744.
- Wilentz, R.E., Iacobuzio-Donahue, C.A., Argani, P., McCarthy, D.M., Parsons, J.L., Yeo, C.J., Kern, S.E., and Hruban, R.H. 2000. Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: Evidence that DPC4 inactivation occurs late in neoplastic progression. *Cancer Res.* **60**: 2002–2006.
- Wittinghofer, A., Scheffzek, K., and Ahmadian, M.R. 1997. The interaction of Ras with GTPase-activating proteins. *FEBS Lett.* **410**: 63–67.
- Woo, R.A. and Poon, R.Y. 2004. Activated oncogenes promote and cooperate with chromosomal instability for neoplastic transformation. *Genes & Dev.* **18**: 1317–1330.
- Xing, H.R., Cordon-Cardo, C., Deng, X., Tong, W., Campodonico, L., Fuks, Z., and Kolesnick, R. 2003. Pharmacologic inactivation of kinase suppressor of ras-1 abrogates Ras-mediated pancreatic cancer. *Nat. Med.* **9**: 1266–1268.
- Xiong, H.Q., Abbruzzese, J.L., Lin, E., Wang, L., Zheng, L., and Xie, K. 2004. NF- $\kappa$ B activity blockade impairs the angiogenic potential of human pancreatic cancer cells. *Int. J. Cancer* **108**: 181–188.
- Yamanaka, Y., Friess, H., Buchler, M., Beger, H.G., Uchida, E., Onda, M., Kobrin, M.S., and Korc, M. 1993a. Overexpression of acidic and basic fibroblast growth factors in human pancreatic cancer correlates with advanced tumor stage. *Cancer Res.* **53**: 5289–5296.
- Yamanaka, Y., Friess, H., Kobrin, M.S., Buchler, M., Beger, H.G., and Korc, M. 1993b. Coexpression of epidermal growth factor receptor and ligands in human pancreatic cancer is associated with enhanced tumor aggressiveness. *Anticancer Res.* **13**: 565–569.
- Yamano, M., Fujii, H., Takagaki, T., Kadowaki, N., Watanabe, H., and Shirai, T. 2000. Genetic progression and divergence in pancreatic carcinoma. *Am. J. Pathol.* **156**: 2123–2133.
- Yoshida, T. and Hanahan, D. 1994. Murine pancreatic ductal adenocarcinoma produced by in vitro transduction of polyoma middle T oncogene into the islets of Langerhans. *Am. J. Pathol.* **145**: 671–684.
- Zavadil, J. and Bottinger, E.P. 2005. TGF- $\beta$  and epithelial-to-mesenchymal transitions. *Oncogene* **24**: 5764–5774.
- Zhang, Y., Xiong, Y., and Yarbrough, W.G. 1998. ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. *Cell* **92**: 725–734.
- Zhu, J., Woods, D., McMahon, M., and Bishop, J.M. 1998. Senescence of human fibroblasts induced by oncogenic Raf. *Genes & Dev.* **12**: 2997–3007.
- Zindy, F., Quelle, D.E., Roussel, M.F., and Sherr, C.J. 1997. Expression of the p16<sup>INK4a</sup> tumor suppressor versus other INK4 family members during mouse development and aging. *Oncogene* **15**: 203–211.





## Genetics and biology of pancreatic ductal adenocarcinoma

Aram F. Hezel, Alec C. Kimmelman, Ben Z. Stanger, et al.

*Genes Dev.* 2006, **20**:

Access the most recent version at doi:[10.1101/gad.1415606](https://doi.org/10.1101/gad.1415606)

---

### References

This article cites 376 articles, 116 of which can be accessed free at:  
<http://genesdev.cshlp.org/content/20/10/1218.full.html#ref-list-1>

### License

### Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

