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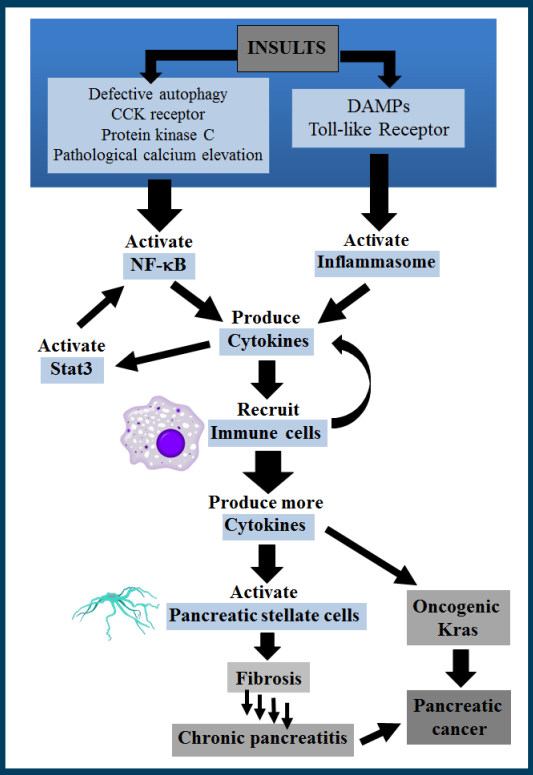

RISK FACTORS FOR PANCREATIC CANCER: UNDERLYING MECHANISMS AND POTENTIAL TARGETS

Topic Editors

Mouad Edderkaoui and Guido Eibl

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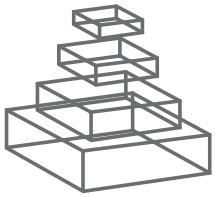

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RISK FACTORS FOR PANCREATIC CANCER: UNDERLYING MECHANISMS AND POTENTIAL TARGETS

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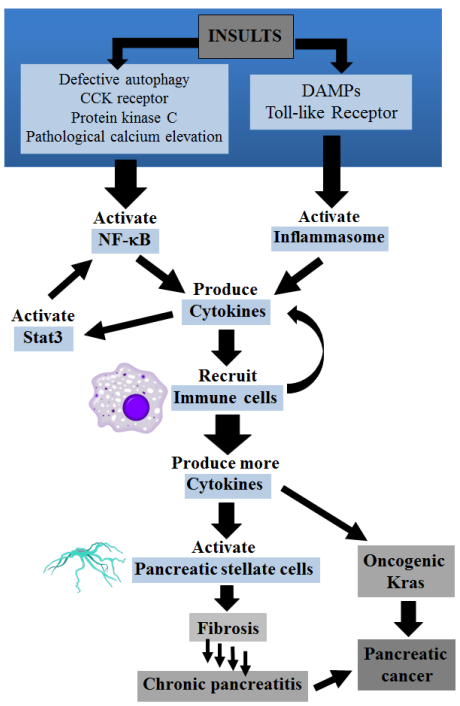

Insults lead to the recruitment of immune cells and the activation of stellate cells leading to chronic pancreatitis and pancreatic cancer. The owner of this image is Dr Edwin Thrower.

Pancreatic Cancer has been and still is one of the deadliest types of human malignancies. The annual mortality rates almost equal incidence rates making this disease virtually universally fatal. The 5-year survival of patients with pancreatic cancer is a dismal 5% or less. Therapeutic strategies are extremely limited with gemcitabine extending the survival by a disappointing few weeks. The failure of several randomized clinical trials in the past decade investigating the therapeutic efficacy of different mono- and combination therapies reflects our limited knowledge of pancreatic cancer biology. In addition, biomarkers for early detection are sorely missing. Several pancreatic cancer risk factors have been identified. Unfortunately, the underlying mechanisms linking these risk factors to cancer development are poorly understood.

Well known possible and probable risk factors for the development of pancreatic cancer are age, smoking, chronic pancreatitis, obesity, and type-2 diabetes mellitus. Age is certainly of the most important risk factors as most cases of pancreatic cancer occur in the elderly population. Smoking ten cigarettes a day increases the risk by 2.6 times and smoking a pack per day increases it by 5 folds. Chronic pancreatitis

increases the risk of pancreatic cancer by up to 13 times. Patients with hereditary forms of chronic pancreatitis have an even higher risk. Obesity, a growing global health problem, increases the risk of pancreatic cancer by about 1.5 fold. Type-2 diabetes mellitus is also associated with an increased risk of pancreatic cancer by at least two-fold. The more recent the onset of diabetes, the stronger the correlation with pancreatic cancer is. In addition, heavy alcohol drinking, a family history of the disease, male gender and African American ethnicity are other risk factors for pancreatic cancer.

Pancreatic cancer is characterized by several genetic alterations including mutations in the Kras proto-oncogene and mutations in the tumor suppressor genes p53 and p16. While Kras mutations are currently thought as early events present in a certain percentage of pancreatic intraepithelial neoplasias (PanINs), known precursor lesions of pancreatic ductal adenocarcinomas, mutations in tumor suppressor genes, e.g. p53, seem to accumulate later during progression. In addition, several intracellular signaling pathways are amplified or enhanced, including the MAPK/ERK and PI3K/AKT signaling modules. Overall, these genetic alterations lead to enhanced and sustained proliferation, resistance to cell death, invasive and metastatic potential, and angiogenesis, all hallmarks of cancers.

The scope of this Research Topic is to collect data and knowledge of how risk factors increase the risk of initiation/progression of pancreatic cancer. Of particular interest are potential underlying molecular mechanisms. Understanding the molecular mechanisms and driving signaling pathways will ultimately allow the development of targeted interventions to disrupt the risk factor-induced cancer development. This Research Topic is interested in a broad range of risk factors, including genetic and environmental, and welcomes original papers, mini and full reviews, and hypothesis papers. Manuscripts that address the effect of combination of risk factors on pancreatic cancer development and progression are of great interest as well.

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Risk factors for pancreatic cancer: underlying mechanisms and potential targets

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Keywords: pancreatic cancer, risk factors, genetic mutations, inflammation, Src kinase

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths among both men and women in the United States and remains essentially without effective therapies. The 5-year survival of patients with pancreatic cancer is a dismal 6% or less. Environmental risk factors such as smoking, diabetes, obesity, and alcoholism play major roles in the promotion of PDAC. However, currently we have limited understanding of how these risk factors promote the disease. The papers presented in this topic illustrate the latest knowledge regarding the mechanisms of pancreatic cancer induction and promotion by major risk factors such as smoking, pancreatitis, alcohol abuse, obesity and diabetes (Kolodecik et al., 2013), and less studied factors such blood group types (Pelzer et al., 2013) and the genetic mutations (Kong et al., 2013; Reznik et al., 2014; Weiss, 2014).

Indeed, an inherited predisposition to PDAC is believed to present in familial cancer syndromes such as the Peutz-Jeghers Syndrome, which is associated with germline mutations in the STK11/LKB1 gene, Familial Atypical Multiple Mole Melanoma syndrome, which results due to germline mutations in the p16/CDKN2A gene, Hereditary Breast-Ovarian Cancer syndrome (BRCA1/2 genes), Hereditary Non-polyposis Colorectal Cancer (mismatch repair genes), and Familial Adenomatous Polyposis syndrome (Reznik et al., 2014). Hereditary causes of pancreatitis, such as the autosomal dominant form caused by germline mutations of the cationic trypsinogen gene, PRSS1, have been indirectly linked to PDAC through early onset chronic pancreatitis with an associated 53-fold increased incidence and approximately 40% of hereditary pancreatitis patients noted to develop pancreatic cancer by age 70 (Weiss, 2014). Finally, more patients with blood group A suffer from PDAC whereas blood group O was less frequent in patients with PDAC (Pelzer et al., 2013). Kras mutations remain the most abundant genetic alteration found in pancreatic cancer patients. Kras mutations may lead to reducing power for ROS detoxification, leading to low ROS levels in pancreatic pre-neoplastic cells and in cancer cells. In adult stem cells and cancer stem cells, low ROS levels have been associated with the formation of a proliferation-permissive intracellular environment and with perseverance of self-renewal capacities. Therefore, it is conceivable that low intracellular ROS levels may contribute significantly to oncogenic Kras-mediated PDAC formation (Kong et al., 2013).

Specific gene-based, gene-product, and marker-based testing for the early detection of pancreatic cancer are currently being developed, with the potential for these to be useful as potential therapeutic targets as well.

Today, the role of stromal cells is highly appreciated for pancreatic cancer development. Immune cell infiltration into the tumor not only fail to contribute to disease eradication but rather due to exhibiting a Th2-type inflammation and immunosuppression is associated with more rapid disease progression, cachexia induction, and reduced survival. Polarization of macrophages toward M2-type correlates with a poor prognosis after surgery in resected patients. High CD163+ and CD204+ cell counts correlate with metastasis and poor prognosis in PDAC patients (Protti and De Monte, 2013; Tan et al., 2014).

Src kinase might serve as a critical mechanistic link between inflammation and cancer, mediating and propagating a cycle between immune and tissue cells that can ultimately lead to the development and progression of cancer (Liu et al., 2013). In addition, there is now compelling evidence that pancreatic stellate cells interact not only with cancer cells themselves, but with several other cell types in the stroma (endothelial cells, immune cells, and possibly neuronal cells) to promote cancer progression. Strategies to target the tumor microenvironment cells are proposed (Wilson et al., 2014). The central role of the mammalian target of rapamycin (mTOR) in mediating a crosstalk between the insulin/IGF-1 and GPCR signaling in pancreatic cancer cells is discussed in depth and strategies, including the use of metformin, to target this signaling pathway in PDAC cells are proposed (Rozengurt, 2014). Finally, a comprehensive review of the latest animal models of pancreatic cancer are discussed, proposing novel tools to study the mechanism of pancreatic cancer initiation and promotion by major risk factors.

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REFERENCES

Kolodecik, T., Shugrue, C., Ashat, M., and Thrower, E. C. (2013). Risk factors for pancreatic cancer: underlying mechanisms and potential targets. *Front. Physiol.* 4:415. doi: 10.3389/fphys.2013.00415

Kong, B., Qia, C., Erkan, M., Kleeff, J., and Michalski, C. W. (2013). Overview on how oncogenic Kras promotes pancreatic carcinogenesis by inducing low intracellular ROS levels. *Front. Physiol.* 4:246. doi: 10.3389/fphys.2013.00246

Liu, S. T., Pham, H., Pandol, S. J., and Ptasznik, A. (2013). Src as the link between inflammation and cancer. *Front. Physiol.* 4:416. doi: 10.3389/fphys.2013.00416

Pelzer, U., Klein, F., Bahra, M., Sinn, M., Dörken, B., Neuhaus, P., et al. (2013). Blood group determinates incidence for pancreatic cancer in Germany. *Front. Physiol.* 4:118. doi: 10.3389/fphys.2013.00118

Protti, M. P., and De Monte, L. (2013). Immune infiltrates as predictive markers of survival in pancreatic cancer patients. *Front. Physiol.* 4:210. doi: 10.3389/fphys.2013.00210

Reznik, R., Hendifar, A. E., and Tuli, R. (2014). Genetic determinants and potential therapeutic targets for pancreatic adenocarcinoma. *Front. Physiol.* 5:87. doi: 10.3389/fphys.2014.00087

Rozengurt, E. (2014). Mechanistic target of rapamycin (mTOR): a point of convergence in the action of insulin/IGF-1 and G protein-coupled receptor agonists in pancreatic cancer cells. *Front. Physiol.* 5:357. doi: 10.3389/fphys.2014.00357

Tan, C. R., Yaffee, P. M., Jamil, L. H., Lo, S. K., Nissen, N., Pandol, S. J., et al. (2014). Pancreatic cancer cachexia: a review of mechanisms and therapeutics. *Front. Physiol.* 5:88. doi: 10.3389/fphys.2014.00088

Weiss, F. U. (2014). Pancreatic cancer risk in hereditary pancreatitis. *Front. Physiol.* 5:70. doi: 10.3389/fphys.2014.00070

Wilson, J. S., Pirola, R. C., and Apte, M. V. (2014). Stars and stripes in pancreatic cancer: role of stellate cells and stroma in cancer progression. *Front. Physiol.* 5:52. doi: 10.3389/fphys.2014.00052

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Risk factors for pancreatic cancer: underlying mechanisms and potential targets

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Purpose of the review: Pancreatic cancer is extremely aggressive, forming highly chemo-resistant tumors, and has one of the worst prognoses. The evolution of this cancer is multi-factorial. Repeated acute pancreatic injury and inflammation are important contributing factors in the development of pancreatic cancer. This article attempts to understand the common pathways linking pancreatitis to pancreatic cancer.

Recent findings: Intracellular activation of both pancreatic enzymes and the transcription factor NF-κB are important mechanisms that induce acute pancreatitis (AP). Recurrent pancreatic injury due to genetic susceptibility, environmental factors such as smoking, alcohol intake, and conditions such as obesity lead to increases in oxidative stress, impaired autophagy and constitutive activation of inflammatory pathways. These processes can stimulate pancreatic stellate cells, thereby increasing fibrosis and encouraging chronic disease development. Activation of oncogenic Kras mutations through inflammation, coupled with altered levels of tumor suppressor proteins (p53 and p16) can ultimately lead to development of pancreatic cancer.

Summary: Although our understanding of pancreatitis and pancreatic cancer has tremendously increased over many years, much remains to be elucidated in terms of common pathways linking these conditions.

Keywords: pancreatitis, pancreatic cancer, inflammation, autophagy, stellate cells, K-ras

INTRODUCTION: PANCREATIC ANATOMY, PHYSIOLOGY, AND PATHOLOGY

The pancreas is a glandular organ of the digestive system consisting of (a) an endocrine component which secretes insulin, glucagon, and stomatostatins, and (b) an exocrine component that produces numerous digestive enzymes and 1500–2000 ml of iso-osmotic alkaline fluid which is released into the small intestine every day. The exocrine pancreas is composed of both acinar and ductal cells; acinar cells (or acini) are responsible for synthesis, storage and secretion of both active (amylase, lipase) and inactive enzymes (zymogens; trypsinogen) (Ogami and Otsuki, 1998). Over 100 years ago it was first documented that the hormone secretin could stimulate pancreatic secretion. Since then it has become clear that pancreatic secretion is maintained and modulated by a complex interaction between neural, hormonal and mucosal factors (Bayliss and Starling, 1902). Gastric acid influx into the small intestine initiates the release of secretin from duodenal S-cells which then stimulates the release of bicarbonate from pancreatic ductal cells to buffer this increase in intestinal acid. Cholecystokinin (CCK) is released from duodenal endocrine I-cells in response to proteins and fats in the small intestine. CCK stimulates acinar cells both directly (Murphy et al., 2008) and indirectly via stimulation of vagal nerve responses which activate muscarinic acetylcholine receptors on the acinar cell. This results in release of pancreatic enzymes into the small intestine. These normal physiological responses can be altered by many factors that can ultimately lead to pathological responses and development of pancreatitis and pancreatic cancer (Bayliss and Starling, 1902; Ogami and Otsuki, 1998; Weiss et al., 2008). This review will focus on common pathways that link the progression from acute to chronic pancreatitis (CP) and finally pancreatic cancer.

EPIDEMIOLOGY

Acute pancreatitis (AP) is a clinical syndrome which begins with acute injury to the pancreas. It is one of the most frequent causes of hospitalization, amounting to nearly 275,000 hospital admissions every year in the United States at a cost of $2.6 billion (Spanier et al., 2008). The most common causes of pancreatitis include alcohol, gallstones, toxins, hyperlipidemia, and trauma, with a small number of cases remaining idiopathic. These factors initiate distinct changes in pancreatic physiology causing pathological activation of digestive enzymes within acinar cells, decreased pancreatic enzyme secretion, increased inflammatory responses and ultimately cell death (Spanier et al., 2008; Peery et al., 2012). Traditionally AP is self-limited with complete resolution of function after the acute event. In some cases there may be tissue scarring and stricture formation leading to pancreatic flow obstruction and recurrent AP. The link between recurrent acute and CP is unclear. Studies have shown that recurrent episodes of pancreatitis set into motion various inflammatory pathways that can lead to immunological and inflammatory responses. This in

turn leads to increased fibrotic tissue formation and stellate cell activation, well known hallmarks of CP.

CP is a fibro-inflammatory disease involving the pancreatic parenchyma which is progressively destroyed and replaced by fibrotic tissues. Histologically, acinar cell damage, mononuclear cell infiltration, and fibrosis are observed (Shrikhande et al., 2003). Traditionally, CP was thought of as a separate disease but years of research have concluded that AP, recurrent AP and CP can be part of the same disease continuum. There are various causes that may lead to CP, but the exact pathophysiology of the disease is still unclear. Three stages of CP development have been described starting with stage one, the pre-pancreatitis phase, which is associated with risk factors for CP such as alcohol, smoking and genetic mutations. This is followed by stage two in the form of AP, with release of inflammatory cytokines. If the attack is severe enough it could activate macrophage dependent stellate cells which ultimately lead to fibrosis, particularly if there is a continuous stimulus causing interplay between pro-inflammatory and anti-inflammatory pathways. Finally there is stage three which is a progression to CP driven by factors that modulate immune responses (Whitcomb, 2011, 2012). Thus CP develops due to complex interactions between an impaired immune response to low grade inflammation and environmental factors that decrease the threshold for recurrent AP like alcohol intake and smoking.

CP has long been thought of as a strong risk factor for pancreatic cancer. Among patients with CP, a meta-analysis has shown a relative risk of 13.3 for developing pancreatic cancer (Raimondi et al., 2010). Chronic inflammation associated with CP facilitates this progression to cancer resulting in the occurrence of three types of precancerous lesions: pancreatic intraepithelial neoplasia (PanINs), intraductal papillary mucinous neoplasms (IPMN), and mucinous cystic neoplasms (MCN). Subsequent evolution of these precursor lesions into pancreatic ductal adenocarcinoma (PDAC) ultimately involves a number of diverse molecular changes (Yonezawa et al., 2008). Despite the strong link between CP and pancreatic cancer, less than 5% of patients with CP actually go on to develop the disease (Raimondi et al., 2010).

Pancreatic cancer is an extremely aggressive, invariably deadly disease without any improvements in patient outcome over the last 2 decades. With over 45,220 new cases of pancreatic cancer diagnosed every year in the USA the estimated number of deaths in 2013 is projected to be around 39,000 making pancreatic cancer the fourth leading cause of cancer deaths in the USA (Yadav and Lowenfels, 2013). The most effective treatment is early resection of the cancer but this is not always possible because of late presentations and aggressive metastasis with chemo-resistance. So only 20% of cases are eligible for surgery and without surgery the median survival is only 6 months with a 5 year survival of 3–5% (Vincent et al., 2011; Siegel et al., 2012; Yadav and Lowenfels, 2013). Pancreatic cancer is not prevalent in patients under 20 years of age; the median age at onset is 71 years (Yadav and Lowenfels, 2013). Hereditary pancreatitis is a severe risk factor for pancreatic cancer with a lifetime risk of developing pancreatic cancer of 40–55% (Yadav and Lowenfels, 2013). Smoking increases the risk of cancer in these patients and lowers the median age of diagnosis from 71 in non-smokers to 56 in smokers (Howes et al., 2004).

Although epidemiology of the disease is well known, the underlying cellular mechanisms of disease initiation and progression are less clear. Chemotherapeutic agents like gemcitabine have been approved for pancreatic cancer not amenable to surgery, but have not shown clear therapeutic effects (Lohr and Jesenofsky, 2009). In order to understand the complexities of molecular mechanisms and drug interactions various mouse models have been developed (Lee et al., 1995; Colby et al., 2008; Jung et al., 2011). In the following sections, common cellular pathways in pancreatitis and pancreatic cancer will be considered, and their role in the transformation of acute to chronic disease, and ultimately cancer, will be discussed.

COMMON CELLULAR PATHWAYS IN TRANSFORMATION OF PANCREATITIS TO PANCREATIC CANCER

Premature activation of digestive zymogens and generation of inflammatory mediators are key initiating events in pancreatitis. Furthermore, these incidents can form the basis for progression from acute to CP and even pancreatic cancer (**Figure 1**). A

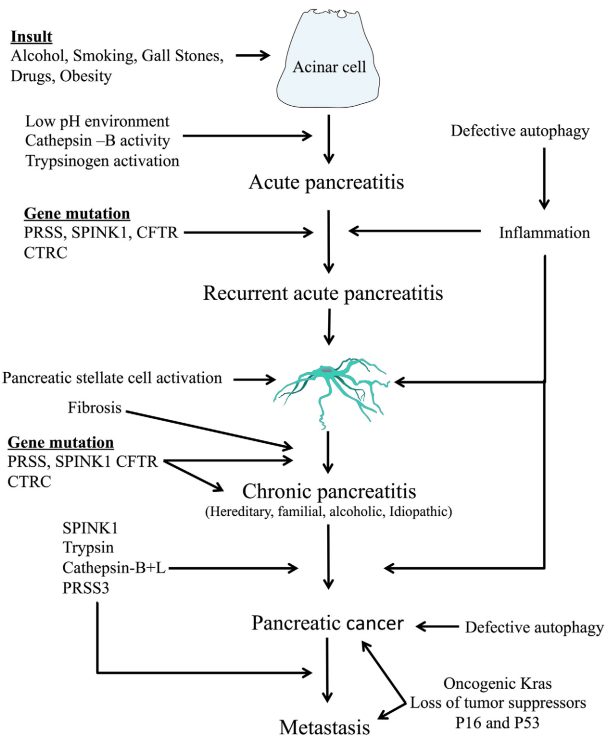

FIGURE 1 | Common pathways associated with disease progression from acute to chronic pancreatitis and pancreatic cancer. Pancreatitis starts with an initiating insult followed by changes in the cellular environment and premature digestive enzyme activation. Mutations of genes associated with trypsinogen activation/inactivation predispose the pancreas to development of disease. As disease progresses defective autophagy, increased inflammation, pancreatic stellate cell activation, and fibrosis occur. Advancement toward pancreatic cancer and metastasis is also associated with defective autophagy, as well as extracellular matrix degradation, cell proliferation, expression of oncogenic Kras and loss of tumor suppressors (e.g., P16 and P53). Autophagy and inflammation are discussed further in **Figures 2**, **3**.

detailed review of these molecular events and their relevance in disease advancement follows.

ROLE OF PREMATURE TRYPSINOGEN ACTIVATION

During pancreatitis lysosomal enzymes are mistargeted to zymogen-containing organelles within the acinar cell. The lysosomal hydrolase cathepsin-B prematurely converts the digestive zymogen, trypsinogen, to its active form, trypsin (Figarella et al., 1988; Gorelick and Matovcik, 1995; Lerch et al., 1995; Wartmann et al., 2010). This conversion requires an acidic pH and cathepsin-B activates trypsinogen in a pH dependent manner (Kukor et al., 2002). In addition, cleavage of trypsinogen to active trypsin requires the folding of its N-terminal upon itself to form a globular molecule, a process which is also pH dependent (Nemoda and Sahin-Toth, 2005). It has been shown that a low pH environment sensitizes acinar cells to secretagogue induced zymogen activation and cell injury. This process is mediated by a vacuolar ATPase (vATPase) and the effects of low pH on zymogen activation can be blocked by the vATPase inhibitor concanamycin (Bhoomagoud et al., 2009). Once trypsinogen has been activated, trypsin can activate more trypsinogen (autoactivation), and additional zymogens, resulting in autodigestion of the pancreas. Inhibition (Van Acker et al., 2002) or genetic deletion (Halangk et al., 2000) of cathepsin B has been shown to attenuate trypsinogen activation and pancreatic inflammation. There are various protective mechanisms to counter trypsinogen activation, mainly through inhibition or degradation of activated trypsin. These mechanisms include inhibition by Serine protease inhibitor, Kazal type 1 also known as pancreatic secretory trypsin inhibitor (SPINK1/PSTI) and degradation by chymotrypsin-C (CTRC). In addition, the lysosomal hydrolase cathepsin-L degrades trypsinogen to an inactive form of trypsin thus providing protection against premature zymogen activation. Paradoxically, when cathepsin-L is genetically deleted there is also a switch from acinar cell necrosis to apoptosis with reduced severity of disease (Wartmann et al., 2010). This indicates that cathepsin L may be involved in additional pathways which contribute to pancreatitis. For the most part though, when these protective mechanisms are overwhelmed there is an increased predisposition to develop pancreatitis.

Activation of trypsinogen is thought to be the initiating event in the cascade of zymogen activation associated with pancreatitis. This is supported by work done in mice lacking trypsinogen-7 ($T^{-/-}$), an ortholog of human cationic trypsinogen (PRSS1). Hyperstimulation with the CCK ortholog cerulein induced zymogen activation and pancreatitis in wild type mice, whereas necrosis and cell death was significantly reduced in $T^{-/-}$ mice (Dawra et al., 2011). However, no effect on inflammation and NFκB activation was observed in $T^{-/-}$ mice (Dawra et al., 2011) suggesting that other mechanisms are also involved in the pathogenesis of AP. Another study found, using a cell free system where acinar cell components can be reconstituted, that activation of other zymogens, such as chymotrypsinogen and procarboxypeptidase, can occur independently of trypsinogen activation (Thrower et al., 2006). Thus development of pancreatitis appears to include both trypsin dependent and independent events.

CP is associated with several genetic mutations related to trypsin activation and inactivation. Cationic trypsinogen (PRSS1) has several mutations which lead to chronic hereditary pancreatitis (Whitcomb et al., 1996). The two most common are replacement of the arginine at position 122 with histidine (R122H) and replacement of the asparagine at position 29 with isoleucine (N29I). These substitutions lead to increased autoactivation of trypsinogen and elevated levels of active trypsin (Chen and Ferec, 2009, 2012). Mutation of SPINK1 which encodes an endogenous trypsin inhibitor has been described as disease-predisposing rather than a disease causing factor (Witt et al., 2000; Chen and Ferec, 2012). Moreover meta-analysis studies conducted in Europe and America has shown idiopathic CP to be strongly associated with SPINK1 mutations (Pfutzer et al., 2000; Threadgold et al., 2002). Chymotrypsin-C (CTRC) protects against intra-cellular trypsin activity by degrading both trypsinogen and trypsin. Mutations in PRSS1 render it resistant to CTRC-dependent degradation (Szabo and Sahin-Toth, 2012) while mutation of CTRC results in an inability to inactivate trypsinogen and trypsin resulting in increased levels of active trypsin (Beer et al., 2013). Cystic fibrosis transmembrane conductance regulator (CFTR), an anion channel, allows the movement of chloride and bicarbonate from ductal cells to the ductal lumen. In mutations of CFTR that lead to decreased bicarbonate conductance, but not chloride, there is a higher risk of idiopathic CP especially when paired with mutation of SPINK1 (Mounzer and Whitcomb, 2013). Ethanol has been shown to reduce CFTR function via depletion of ATP (Judak et al., 2013). Thus, inhibition of CFTR activity whether by genetic mutation or ethanol exposure can lead to both AP and CP (Choudari et al., 1999; Pezzilli et al., 2003).

Pancreatic cancer can also be modulated by pathways associated with trypsinogen activation and inactivation. SPINK1 has been shown to cause cell proliferation in pancreatic cell lines by binding to the epidermal growth factor receptor (EGFR) and stimulating the mitogen-activated protein kinase pathway (MAPK). Both SPINK1 and EGFR were found in PDAC as well as PanINs including early stage PanIN-1A but not in adjacent normal duct cells (Ozaki et al., 2009). A Japanese study of PDAC for 23 patients (20 invasive and 3 non-invasive) found pancreatic trypsinogen in 70% of tumors, but not in any of the non-invasive tumors. The trypsinogen activator, cathepsin-B, was also found in 70% of invasive tumors but not in non-invasive tumors. Metastatic peripancreatic neural plexuses and lymph nodes also stained intensely positive for trypsinogen. In addition, they stained positive for cathepsin B, but only weak to moderate (Ohta et al., 1994). In a more recent paper it has been shown that knockout of cathepsin B is associated with slowed PDAC progression, extended survival and decreased liver metastasis in a mouse model (Gopinathan et al., 2012). This data suggests that pancreatic trypsinogen (expressed in PDAC) and cathepsin-B play a role in PDAC progression and metastasis. Cathepsin-L which can protect against pancreatitis by degrading trypsinogen and trypsin has a very different effect in cancer. In one study, cathepsin-L expression levels in PDAC epithelium was associated with median survival time. The median survival time for tumors expressing high levels of cathepsin-L was 6 months while those expressing

low levels was 22 months (Singh et al., 2013). This difference may be due to the ability of cathepsin-L to degrade extracellular matrix allowing for more tumor growth in those tumors expressing high levels of cathepsin-L. Mesotrypsinogen (PRSS3) has been found to be overexpressed in pancreatic cancer cell lines and promotes cell proliferation and invasion in cell culture, while *in vivo* it causes both tumor growth and metastasis. This data suggests that modulation of the PRSS3 signaling pathway may be a viable approach for treating pancreatic cancer (Jiang et al., 2010).

CALCIUM SIGNALING

Aberrant increases in intracellular calcium levels are critical in acinar cell injury. Localized transient calcium spikes constitute a normal physiologic response whereas a sustained global rise in calcium is a pathological response causing pancreatic injury (Cancela et al., 2002; Petersen et al., 2011). Endoplasmic reticulum ryanodine receptors (RyR) and plasma membrane store operated calcium channels (SOC) are an important means of elevating calcium in pancreatic acinar cells (Glitsch et al., 2002; Parekh, 2003; Husain et al., 2005). For example, mice deficient in the transient receptor potential cation channel, subfamily C, member 3, (TRPC3), a SOC, have reduced calcium elevations in secretagogue, bile acid, and alcohol metabolite-mediated models of pancreatitis (Kim et al., 2009, 2011). Furthermore, ethanol abuse has been shown to impact calcium signaling. Ethanol in the pancreas is converted via non-oxidative pathways into fatty acid ethyl esters (FAEEs) which can cause release of calcium from intracellular stores and premature trypsinogen activation (Wilson et al., 1992; Wilson and Apte, 2003). Ethanol itself does not cause pancreatitis in rats, but it has been reported to worsen cerulein stimulated pancreatitis, suggesting synergistic association. Ethanol causes a dose dependent sensitization of the pancreas to CCK or cerulein mediated pancreatitis. Furthermore, free radicals generated through ethanol metabolism and FAEEs have been shown to damage mitochondrial membranes causing ATP depletion (Wilson and Apte, 2003). This alters the bioenergetics of acinar cells and favors necrosis over apoptosis. ATP is also needed for calcium homeostasis and decreased ATP levels cause further increases in pathological calcium levels in the cytosol (Criddle et al., 2006).

Downstream targets of calcium include Protein-Kinase C (PKC) and the calcium-sensitive phosphatase calcineurin (Gukovskaya et al., 2004; Satoh et al., 2004; Cosen-Binker et al., 2007; Thrower et al., 2008, 2009; Muili et al., 2012). FK506 (Tacrolimus), a macrolide immunosuppressant that inhibits calcineurin has been shown to markedly reduce intra-pancreatic protease activation and pancreatitis severity in cerulein models of pancreatitis (Kim et al., 2011; Muili et al., 2012). Furthermore, pharmacological or genetic blocking of calcineurin also reduces acinar cell injury in a bile-acid induced model of pancreatitis (Muili et al., 2012). Interestingly, recent studies have shown that NFATc1, a calcineurin responsive transcription factor, is associated with aggressive pancreatic cancer and may mediate drug resistance to anticancer agents (Murray et al., 2013). Thus, calcineurin and its downstream effectors may represent attractive therapeutic targets in the treatment of pancreatitis and pancreatic cancer.

AUTOPHAGY

Autophagy is a process of lysosome-mediated degradation and recycling of cellular components, lipids, and proteins. The materials that are marked for degradation are sequestered into double membrane autophagosomes which join with lysosomes to form single membrane autolysosomes, and recycled products are sent back to the cytoplasm. In the basal state this process helps to remove protein aggregates and damaged organelles such as mitochondria and maintain cellular homeostasis (Gukovsky et al., 2013). However, under oxidative stress, hypoxia, pathogen infection, or radiation exposure autophagy increases significantly to protect the cell from further damage. Autophagy can become dysregulated, due to recurrent injury to pancreatic acinar cells, and result in acinar cell vacuolization, trypsinogen activation, and cell death (**Figure 2**) (Gukovsky et al., 2012, 2013).

Impairment of autophagy is a key feature of pancreatitis and chiefly involves defective functional lysosomes. Accumulation of large vacuoles in the acinar cell is one of the hallmark characteristics of pancreatitis and many of these vacuoles are

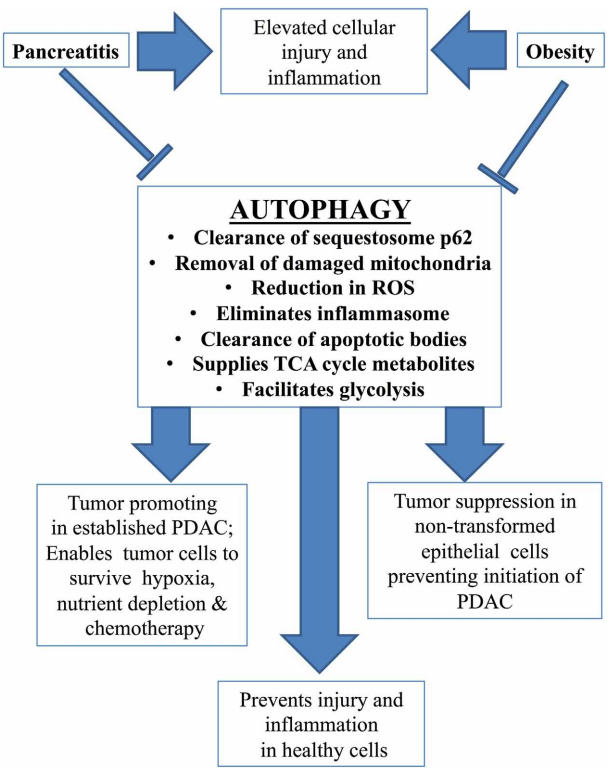

FIGURE 2 | Autophagy and pancreatic disease. Autophagy is responsible for clearance of aggregates of the sequestosome p62, damaged mitochondria, apoptotic bodies, the inflammasome, and reduces levels of reactive oxygen species (ROS). This limits injury and inflammation in healthy cells and prevents neoplastic transformation and initiation of PDAC. Therefore the role of autophagy is normally beneficial. In tumor cells, however, autophagy promotes survival, enabling cancer to resist hypoxia, nutrient depletion, and chemotherapy. Pancreatitis and obesity lead to arrested autophagy resulting in elevated cellular injury and inflammation. This can predispose to chronic disease and even progression to PDAC.

autolysosomes with poorly-degraded contents (Mareninova et al., 2009). Furthermore, increased pancreatic levels of the autophagy marker proteins Atg8/LC3-II accompany this vacuole formation (Fortunato et al., 2009; Mareninova et al., 2009; Grasso et al., 2011; Gukovskaya and Gukovsky, 2012). During pancreatitis, autophagic efficiency and degradation of long-lived proteins are reduced. Lysosomal hydrolytic activity is compromised and alterations in lysosome-associated membrane proteins (LAMPs) are seen (Fortunato et al., 2009; Mareninova et al., 2009; Gukovskaya and Gukovsky, 2012; Gukovsky et al., 2012). In addition, levels of the sequestosome, p62, a multi-purpose protein which mediates autophagic clearance and can itself be degraded by autophagy, are elevated. Collectively, these observations indicate loss of lysosomal function and impairment of autophagic flux in AP. These changes have been observed both in human disease and in experimental models of AP (Fortunato et al., 2009; Mareninova et al., 2009; Grasso et al., 2011; Gukovsky et al., 2011, 2012; Alirezaei et al., 2012).

Deficient autophagy can also mediate pathologic accumulation of active trypsin (Hashimoto et al., 2008; Mareninova et al., 2009; Gukovskaya and Gukovsky, 2012). The respective roles of the lysosomal hydrolases, cathepsins B and L were discussed earlier in this review (section Role of Premature Trypsinogen Activation); cathepsin B activates trypsinogen, forming trypsin, whereas cathepsin L degrades both trypsin and trypsinogen. Malfunctioning lysosomes in pancreatitis allow an imbalance between these two cathepsins, resulting in less cathepsin L and accumulation of active trypsin (Mareninova et al., 2009; Gukovskaya and Gukovsky, 2012). In addition, disruption of endogenous trypsin inhibitors, similar to that seen in cases of CP, can abrogate autophagy (Ohmuraya et al., 2005; Romac et al., 2010). When *Spink-3* (the mouse ortholog of SPINK-1) is compromised, autophagy is impaired and acinar cell vacuolization and pancreatic degeneration occurs. Although impaired autophagy has primarily been investigated in models of AP, the latter evidence indicates a similar role for autophagy in CP. Furthermore, a critical cellular function of efficient autophagy is to limit inflammation; any compromise in autophagy leads to persistent inflammation, which sets the stage for development of CP.

Autophagy and inflammation

Defective autophagy is a key component in promoting persistent inflammatory responses (Levine and Kroemer, 2008; Deretic, 2012). Accumulation of p62 through faulty autophagy can ultimately lead to activation of the transcription factor NF-κB, a critical mediator of inflammation (discussed further in section NF-κB) (Ling et al., 2012; Moscat and Diaz-Meco, 2012). Arrested autophagy also leads to elevations in reactive oxygen species (ROS), due to lack of removal of damaged mitochondria. ROS can activate inflammasomes, large intracellular multiprotein complexes that play a central role in innate immunity (see section Inflammasome) (Nathan and Ding, 2010; Green et al., 2011; Strowig et al., 2012). In addition, inflammasomes are normally eliminated through autophagy; lack of autophagy in pancreatitis therefore maintains their presence in the cell and hence their participation in the inflammatory process (Shi et al., 2012). Finally, impaired autophagy disrupts clearance of apoptotic material from the acinar cell. This leads to secondary necrosis and the release of damage-associated molecular pattern molecules (DAMPs), which induce inflammation. Inflammation is a consistent theme throughout the pancreatic disease continuum; if initial inflammatory events subside, an acute episode results, however persistent inflammation can lead to chronic disease. A more detailed discussion of inflammation and its multi-layered effects follows in section Inflammation and **Figure 3**.

INFLAMMATION

NF-κB

NF-κB is a transcription factor which is involved in many cellular signaling pathways involved in inflammation and stress-induced

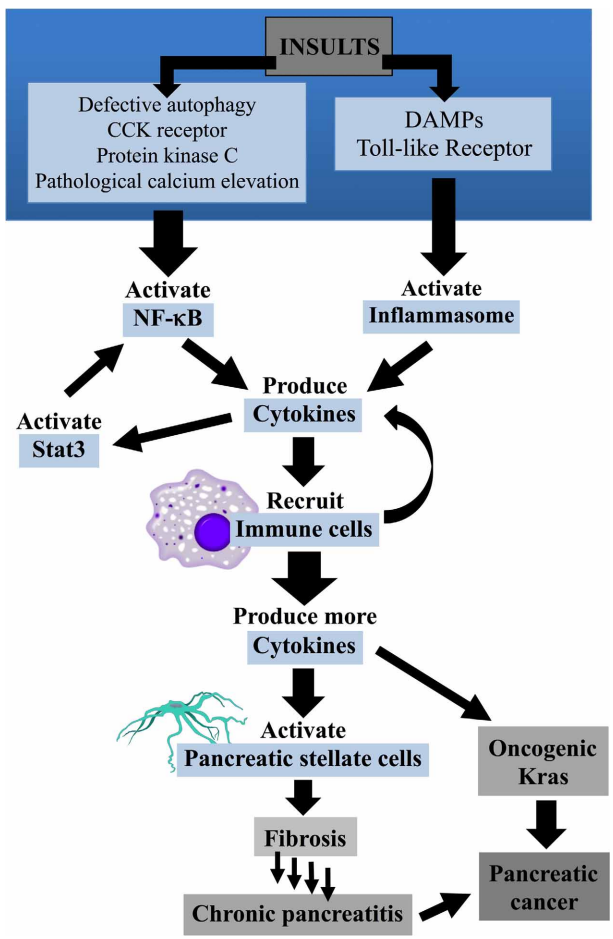

FIGURE 3 | Inflammation and pancreatic disease. Insults lead to the activation of NF-κB and inflammasomes. NF-κB activation leads to the production of cytokines which, in turn, recruit immune cells and activate Stat3. Neutrophils, macrophages and other immune cells infiltrate the pancreas and produce more cytokines amplifying the inflammatory response. Cytokines can lead to the activation of pancreatic stellate cells which can, with repeated bouts of acute pancreatitis lead to fibrosis and the development of chronic pancreatitis. Cytokines can activate oncogenic Kras, a characteristic of nearly 90% of all pancreatic adenocarcinomas. Chronic pancreatitis can also lead to the development of pancreatic cancer.

responses (Senftleben and Karin, 2002). Upon activation NF-κB component RelA/p50 is released from the inhibitor, IκB, and translocates to the nucleus where it increases the expression of pro-inflammatory mediators. Cytokines and adhesion molecules attract additional immune cells and inflammation persists within the pancreas (see section Cytokines and Pancreatitis) (Rakonczay et al., 2008).

Levels of NF-κB rise independently of, but concurrently with, trypsinogen activation (Gukovsky et al., 1998). Pathological rises in calcium levels and activation of PKC isoforms have been implicated in NF-κB activation. Decreased NF-κB activation has been observed following treatment with calcium chelators and experimental data from ethanol and cerulein models of pancreatitis has determined that NF-κB activation is mediated by calcium/calcineurin and PKC pathways (Satoh et al., 2004; Muili et al., 2012).

Ethanol increases the effect of CCK on NF-κB activation via PKC pathways demonstrating the role of alcohol in sensitizing acinar cells to inflammatory responses and pancreatitis (Gukovskaya et al., 2004). The sensitizing effects of alcohol have also been observed in *in vivo* models of the disease; alcohol-fed rats do not experience pancreatitis, but when treated with lipopolysaccharide (LPS; an endotoxin in the cell wall of Gram-negative bacteria) AP develops in the animals. Disease progression occurs leading to acinar cell atrophy and fibrosis, the latter via activation of pancreatic stellate cells (PSCs) [see section Pancreatic Stellate Cells (PSCs)] (Vonlaufen et al., 2011).

The above studies and others point to the detrimental role of NF-κB in pancreatitis. However, some studies have determined it to be beneficial (Gukovsky and Gukovskaya, 2013). For example, transgenic mice with the deletion of IκB, an NF-κB inhibitor, led to constitutive NF-κB activation but a decrease in cerulein-stimulated pancreatitis was observed (Neuhofer et al., 2013). In contrast, transgenic mice overexpressing IκB kinase (IKK2) exhibited high levels of NF-κB activation and spontaneous AP was observed. Over time these mice developed pancreatic damage such as fibrosis, acinar cell atrophy, and inflammatory cell infiltration indicating CP (Huang et al., 2013). One way to reconcile these conflicting results is to point to NF-κB's dual role as promoter of both pro- and anti-inflammatory pathways. Early events, as described above, show NF-κB as the key initiator to the pro-inflammatory cascade of cytokines and other mediators. However, NF-κB can reduce inflammation by limiting apoptosis, necroptosis, and the inflammasome (Algul et al., 2007; Gaiser et al., 2011; Strowig et al., 2012). In addition, NF-κB activation in inflammatory cells may be quite different, if not opposite, than that observed in acinar cells (Treiber et al., 2011).

Persistent NF-κB activation was found in CP as well as 67% of the pancreatic cancer specimens examined in one study (Wang et al., 1999; Sah et al., 2013). Constitutive NF-κB activation promotes low-grade inflammation creating an environment favorable to the development of cancer (Grivennikov et al., 2010). Studies suppressing NF-κB activity have shown a decrease in tumorigenesis or an induction in cytotoxicity in cancer cell lines (Fujioka et al., 2003; Fabre et al., 2012).

NF-κB activation can also occur via a non-canonical (or alternative) pathway which differs from the canonical pathway in its activation and downstream effectors (Sun, 2012). Namely, in the alternative pathway NF-κB activation occurs with the proteasome-mediated processing of the NF-κB component p100 to p52 which then translocates to the nucleus in combination with RelB. Unlike the canonical pathway which depends on the trimeric IKK complex for activation, the alternative pathway relies on NF-κB-inducing kinase (NIK) and IKKα (Sun, 2011). In pancreatic cancer cells NF-κB activation has been shown to occur by both pathways; in the alternative pathway, NIK is upregulated, often due to the suppression of TNF-associated factor 2 (TRAF2) (Nishina et al., 2009; Wharry et al., 2009). In a recent study, NIK upregulation was observed in each of the 55 human PDAC samples examined and 69% of the samples showed decreased expression of the NIK inhibitor, TRAF2 (Doppler et al., 2013).

NF-κB and its effectors have emerged as targets for the development of potential therapies to treat CP and pancreatic cancer. Examples include anti-inflammatory drugs, polyphenols, and proteasomal inhibitors (Carbone and Melisi, 2012; Aravindan et al., 2013; Doppler et al., 2013). Alternative pathway components such as NIK and TRAF2 are key proteins and may prove favorable as targets for therapies. Therapies trying to induce apoptosis in cancer cells are often stymied by high levels of NF-κB limiting apoptosis. To surmount this, therapies are being tested using NF-κB inhibitors, such as proteasomal inhibitors like bortezomib in combination with apoptotic drugs such as gemcitabine (Ahn et al., 2012; Walsby et al., 2012; Salem et al., 2013).

Inflammasome

The inflammasome is a large multi-protein complex concerned with detection of pathogen- and damage-associated molecular patterns (PAMPS and DAMPS) which arise during insult or injury to the pancreas. A typical inflammasome consists of a sensor or scaffolding protein such as a nucleotide oligomerization domain leucine-rich repeat-containing receptor (NLR), an adaptor protein designated ASC, and pro-caspase-1 (Drexler and Yazdi, 2013). During AP, pancreatic acinar cell injury and necrosis causes release of DAMPS, including nuclear DNA, mitochondrial DNA and ATP. Resident macrophages within the pancreas detect these DAMPs via (i) Toll-like receptor-9 (TLR-9) which induces NFkB activation and pro-IL-1β transcription and (ii) plasma membrane purinergic receptor $P2X_7$, which mediates IL-1β maturation through inflammasomal components Nlrp3-ASC. Subsequent generation of IL-1β results in further cytokine production, recruitment of immune cells, and apoptosis (Hoque et al., 2011).

The role of the inflammasome in the pathogenesis of acute alcoholic pancreatitis has also been explored recently (Gu et al., 2013). In alcohol-fed rats, treated with lipopolysaccharide (LPS), pancreatic acinar cells had enhanced expression of cytokines and chemokines, including the inflammasome-associated factors IL-18 and caspase-1. Furthermore, inflammasome mediated responses were found to be initiated through TLR4-signaling. Similar results were observed in acinar cells derived from patients with acute/recurrent pancreatitis.

The inflammasome thus has a central role in promoting chronic inflammation in pancreatitis but its contribution to pancreatic cancer remains largely unexplored. Generation of IL-1β

and IL-18 may be the linking factor between inflammation and tumor initiation/progression although current understanding is limited (Drexler and Yazdi, 2013). In terms of treatment for pancreatitis, targets in the inflammasome pathway merit investigation, although the implication for pancreatic cancer therapy is less clear.

Cytokines and pancreatitis

In the early stages of AP, NF-κB (section NF-κB), and other transcription factors such as activator protein-1 (AP-1) and nuclear factor of activated T-cells (NFAT) are triggered resulting in the production and release of cytokines from the acinar cell. Immune cells such as neutrophils, macrophages, monocytes, and lymphocytes are recruited to the pancreas where they, in turn, produce and secrete additional cytokines resulting in an amplification of the inflammatory response. Key cytokines observed in serum and the pancreas during AP, include the interleukins IL-1β, IL-6, IL-8, as well as tumor necrosis factor (TNF-α) and soluble receptor for tumor necrosis factor (sTNFr); furthermore, serum levels correlate with disease severity (Mayer et al., 2000; Fisic et al., 2013). Anti-inflammatory mediators such as interleukins IL-10, IL-11, IL-22, TNF-α receptors, and IL-1 receptor antagonist (IL-1ra) are produced in an effort to limit the inflammatory response; IL-10 and IL-22 have been shown to reduce AP in experimental animal models (Feng et al., 2012; Koike et al., 2012; Xue et al., 2012; Fisic et al., 2013).

Cytokines released during AP appear to also have roles in CP. In contrast to its beneficial role in AP, IL-10 has been shown to be instrumental in the development of CP in an experimental animal model (Gu et al., 2009). Furthermore, cytokines TGF-β, TNF-α, IL-1, IL-6, and IL-10 have been shown to activate pancreatic stellate cells which could either result in tissue repair or the development of fibrosis [see section Pancreatic Stellate Cells (PSCs)] (Apte et al., 1999; Mews et al., 2002).

Therapies for AP currently under study aim to inhibit pro-inflammatory pathways, such as TNF-α, with neutralizing antibodies, or up-regulate anti-inflammatory cytokines such as IL-10 or IL-22 (Feng et al., 2012; Xue et al., 2012; Sendler et al., 2013). Elevation of anti-inflammatory cytokines as a therapy should be approached with caution though, as up-regulation of cytokines that reduce AP might also predispose to CP. Further study of these pathways is required to resolve these complex issues, prior to development of suitable therapies.

STAT3 and pancreatic cancer

Inflammation has been shown to be a key driver of pancreatic cancer (Guerra et al., 2011; Yadav and Lowenfels, 2013). Immune cells recruited to the pancreas and pancreatic stellate cells together secrete a host of cytokines, growth factors and matrix modifying enzymes that create a microenvironment favorable to PanIN development and progression (Steele et al., 2013). Signal transducer and activator of transcription 3 (Stat3), a transcription factor activated by cytokines such as IL-6 and growth factors such as epidermal growth factor (EGF) is a key mediator of inflammation (Grivennikov et al., 2010). Constitutively active Stat3 has been observed in 30–100% of human pancreatic adenocarcinoma samples examined (Scholz et al., 2003). Stat3 has also been shown to be required for the activation and progression of PDAC (Scholz et al., 2003; Corcoran et al., 2011; Fukuda et al., 2011; Lesina et al., 2011). Interestingly, there is evidence for cross-talk between Stat3 and NF-κB: Stat3 promotes constitutively high levels of NF-κB while NF-κB, in turn, may regulate Stat3 activation by recruiting immune cells that secrete Stat3-activating cytokines (Bollrath and Greten, 2009; Lee et al., 2009; Grivennikov and Karin, 2010).

Like NF-κB, Stat3 is an attractive target for therapies treating pancreatic cancer. Inhibitors of a Stat3 kinase, Jak2, have reduced solid tumor growth in animal models (Hedvat et al., 2009). Two triterpenoids under study in animal models are Stat3 and NF-κB inhibitors (Liby et al., 2010). Such compounds may also lend themselves to be used in combination therapies with other drugs such as gemcitabine.

COX-2 overexpression

The enzymes cyclooxygenase 1 and 2 (COX-1 and 2) are important rate limiting factors in prostaglandin production. Whereas COX-1 is constitutively expressed, there is very little COX-2 immunoreactivity in normal pancreatic acinar cells. However, during inflammation COX-2 is upregulated and in CP it is overexpressed in acinar, islet, and ductal cells. The presence of COX-2 in ductal cells points toward its role in modulating growth factors and cytokines from ductal cells in fibrosis and inflammatory pathways (Eibl et al., 2004). COX-2 has been linked to development of pancreatic dysplasia and PDAC and may form a potential link between CP and subsequent development of pancreatic cancer. Elevated COX-2 has been associated with pancreatic cancer cell proliferation (Sun et al., 2009) and tumor growth (Colby et al., 2008; Mukherjee et al., 2009; Hill et al., 2012). Moreover, a recent study has shown that a combination therapy, involving pharmacologic inhibitors of COX-2 and histone deacetylases (HDAC), a family of enzymes that regulate paramount cellular activities, results in a complete inhibition of tumor growth.

HEAT SHOCK PROTEINS

Heat shock proteins (Hsp) are a family of survival proteins. Their function in AP has often been considered protective although the opposite is true in pancreatic cancer; they largely account for the continued persistence of pancreatic tumors (Bhagat et al., 2002; Banerjee et al., 2013). Triptolide is a naturally derived compound, and its water-soluble pro-drug, Minnelide, have been shown to down-regulate expression of Hsp 70 in pancreatic cancer cells, resulting in cell death (Banerjee et al., 2013). This occurs via decreased glycosylation of the transcription factor Sp1, and subsequent down-regulation of pro-survival pathways like NF-κB. Inhibition of Hsp70 and ultimately cell death follows. Given the efficacy of this drug in preclinical trials, Minnelide studies have now moved to Phase I clinical trials.

PANCREATIC STELLATE CELLS (PSCs)

Pancreatic stellate cells (PSCs) play an essential role in pancreatic fibrosis in CP and pancreatic cancer. These star-shaped cells were first described in 1998 by two independent groups and since then they have been extensively studied (Apte et al., 1998; Bachem et al., 1998). Stellate cells lie in a quiescent state in periacinar, perivascular, and periductal areas and store Vitamin-A lipid

droplets in the cytoplasm (Apte et al., 1998). During pancreatic injury, acinar cells, inflammatory cells, platelets, and endothelial cells produce cytokines and growth factors such as transforming growth factor beta (TGF-β) TNF-α, IL-1, IL-6, and activin A which activate PSCs in a paracrine manner. PSCs also produce a range of growth factors and cytokines themselves and could be activated in an autocrine manner. Upon activation PSCs start expressing α-Smooth muscle actin (α-SMA), with a myofibroblast like phenotype, synthesizing excess extracellular matrix components (ECM) such as collagen-1 and fibronectin (Omary et al., 2007; Vonlaufen et al., 2008; Masamune and Shimosegawa, 2009; Masamune et al., 2009; Erkan et al., 2012a). In addition to their pivotal role in fibrogenesis, PSCs synthesize matrix degradation enzymes like matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases or TIMPS) (Phillips et al., 2003) that remodel the pancreatic parenchyma (Yokota et al., 2002; Omary et al., 2007). Therefore PSCs may play a role in maintenance of pancreatic architecture through regulation of ECM turnover.

PSCs interact with, and may regulate, other pancreatic cell types such as acinar cells and cancer cells. CCK has been shown to initiate acetylcholine release from PSCs which subsequently stimulates exocrine functions in acinar cells (Phillips et al., 2010). These findings suggest a novel role for PSCs in physiological regulation of acinar cells. Whether such an interaction can initiate pathological responses such as those observed in AP, remains to be determined. It has also been reported that PSCs interact with cancer cells and promote cancer progression through multiple mechanisms including elevated proliferation, migration and metastasis (Bachem et al., 2005; Hwang et al., 2008; Vonlaufen et al., 2008; Xu et al., 2010; Mantoni et al., 2011; Erkan et al., 2012a,b). PSCs have been shown to induce epithelial to mesenchymal transition (EMT) in pancreatic cancer cells. EMT is a critical process in cancer progression, which allows a polarized epithelial cell to assume a mesenchymal phenotype, enabling it to acquire invasive and metastatic properties and resistance to apoptosis and therapies. Furthermore, recent studies have shown that PSCs can augment stem cell-like phenotypes in pancreatic cancer cells, enhancing tumorigenicity (Hamada et al., 2012). Interactions between PSCs and other pancreatic cell types therefore appear to be an essential component of pancreatic regulation and disease development. Further research on the role of PSCs in development of pancreatitis and pancreatic cancer is required, given the emerging multi-functional roles these cells play.

Kras

Kras is a guanine nucleotide binding protein and individual Kras proteins act as binary molecular "switches" to activate a range of important cellular signaling pathways. Kras can bind either guanosine triphosphate (GTP) or guanosine diphosphate (GDP). When occupied by GDP, Kras does not activate downstream signaling pathways and is effectively "switched off." Extracellular signals coming from the environment in the form of growth factors, cytokines, damage molecules (DAMPs), hormones, or other molecules activate Kras. These molecules indirectly interact with guanine nucleotide exchange factors (GEFs), replacing GDP for GTP and switching Kras "on." The active Kras subsequently interacts with a wide range of downstream signaling pathways including STAT3, NFκB, COX-2, and Scr. Some of these pathways can generate signals, such as inflammatory mediators that further activate Kras through positive feedback. Normal Kras is rapidly inactivated by GTPase-activating proteins (GAPs) that help hydrolyze GTP to GDP. Although individual Kras molecules may act as a "binary switch," populations of Kras proteins have varying degrees of activity; at the cellular level, Kras is never truly "on or off." It is the *number* of active Kras proteins which define the level of the resulting downstream signals. However, specific point mutations in Kras, particularly those that affect Kras-GAP interactions, limit GTP hydrolysis resulting in sustained activity for Kras. Such pathological responses can ultimately lead to cancer.

Oncogenic Kras was first linked to pancreatic cancer over 20 years ago. The most common mutation in the majority of pancreatic tumors was identified as $Kras^{G12D}$ (Almoguera et al., 1988; Smit et al., 1988). Development of genetic mouse models with this mutation enabled researchers to learn more about pancreatic cancer development, although these models were found to have limitations (Di Magliano and Logsdon, 2013). The mouse models do not exactly match human disease; oncogenic Kras is expressed in all pancreatic cells in mice, unlike pancreatic tumors in humans. A combination of approaches, including the use of human pancreatic cancer cell lines, primary human cultures and human xenograft tumors in mice has yielded a broader view of disease mechanisms.

Mouse models have been used to demonstrate how cellular changes induced during pancreatitis, may actually lead to cancer progression in the presence of a Kras mutation. Induction of AP with the CCK ortholog cerulein in wild-type mice leads to acinar cell damage, infiltration of immune cells, and edema; the level of damage peaking within a 24 h period. Tissue repair rapidly occurs, and normal pancreatic histology is restored within 1 week. In contrast, pancreata from mice with a Kras mutation (the KC and iKras* models) fail to undergo tissue repair after cerulein treatment (Morris et al., 2010; Collins et al., 2012a). In these mice, acinar to ductal metaplasia progresses forming dysplastic ductal structures, surrounded by extensive fibrosis, within 1 week. After 3 weeks, the majority of ductal structures exhibit characteristics of PanINs. With time, higher-grade PanIN lesions populate the pancreas resulting in development of carcinoma.

Merely the presence of a mutant copy of Kras may not be entirely sufficient for development of pancreatic cancer. It is widely thought that a threshold level of mutant Kras activity must be reached for cancer progression to occur (Di Magliano and Logsdon, 2013). In addition, sustained Kras activity may lead to cellular stress which could result in apoptosis or senescence. Factors which allow the cells to overcome the senescence barrier such as inflammation or loss of tumor suppressor genes such as p16 or p53 may allow transformation to cancerous cells. In mouse models of oncogenic Kras, pancreatic lesions rarely progress to carcinoma unless additional mutations are introduced. Tumor suppressors such as p53 and p16 are spontaneously lost at different rates, depending on levels of inflammation and/or Kras activity. KC mice express endogenous levels of oncogenic Kras,

and the tumor suppressor p53 has a tendency to be mutated or lost in the later stages of tumor development (Hingorani et al., 2003). In contrast, mice engineered to express high levels of oncogenic Kras in pancreatic cells (Elastase- CreER;cLGL-KrasG12D, or LGL model), rapidly lose p16 (Ji et al., 2009). These observations are consistent with those seen in patients, whereby pancreatic adenocarcinoma does not occur without the accumulation of multiple genetic alterations, potentially over the course of many years (Yachida et al., 2010). Loss, inactivation, or mutation of a range of tumor suppressors (e.g., Tp53 and p16) is commonly detected in human pancreatic tumors.

Onogenic Kras activation mediates many downstream cellular targets including RAF-mitogen activated protein kinase, Phosphoinositide-3-kinase (PI3K) and RalGDS pathways. The P13-kinase-AKT pathway can play an important role in cell survival and malignant transformation and is Ras dependent (Fernandez-Medarde and Santos, 2011). It has been shown that Kras plays a role in activation of the Hedgehog pathway. Inhibition of the Hedgehog pathway dramatically decreases proliferation of pancreatic cancer cells due to its impact on the cell cycle regulators, Cyclin D1, N-myc, and Wnt proteins (Morton et al., 2007). Since both Notch and Hedgehog pathways are not activated in normal pancreas, it is postulated that there is a link between their activation and molecular and genetic alterations that occur during repetitive cell damage and repair processes.

A more detailed view of the critical role played by Kras in pancreatic disease is beyond the scope of this current review. Kras is an integral player in pancreatic disease progression and may play a role in transition of pancreatitis to pancreatic cancer. Cellular processes involved in pancreatitis, such as inflammation and autophagy, may interact with Kras and its downstream pathways, resulting in pancreatic lesions and PDAC development. The interplay of Kras with autophagy will be discussed further in the next section. Finally, in conjunction with other genetic mutations, Kras can facilitate progression to pancreatic cancer. In terms of therapy for pancreatic cancer, Kras is an attractive target. In mouse models, inactivation of oncogenic Kras results in tumor regression and the animals remain healthy over time with no signs of relapse (Collins et al., 2012a,b; Ying et al., 2012). Thus development of effective inhibitors for Kras, or targeting its downstream effectors such as the kinase Akt or MAP Kinase may be the direction to go in terms of drug development.

AUTOPHAGY AND DEVELOPMENT OF PANCREATIC CANCER

Earlier in this review, the role of autophagy in development of acute and CP was discussed. Autophagy also plays a complex part in the development of pancreatic cancer, with reports indicating both pro-tumorigenic and tumor-suppressive roles (Liang et al., 1999; Yue et al., 2003; Levine and Kroemer, 2008; Guo et al., 2011, 2013; Takamura et al., 2011; Wei et al., 2011; Yang et al., 2011; Aghajan et al., 2012; Mah and Ryan, 2012; White, 2012). PDAC cells have higher basal levels of autophagy than most other types of tumor cells, facilitating their survival under stressful conditions including nutrient deprivation, hypoxia, metabolic stress and chemotherapy (Aghajan et al., 2012). As the tumor environment is hypoxic, autophagy is often induced by hypoxia-inducible factor-α signaling, or adenosine monophosphate activated protein kinase (AMPK), the latter also being associated with pancreatitis (Shugrue et al., 2012). Elevated levels of autophagy in PDAC cells are critical in removal of ROS, preventing DNA damage and maintaining energy homeostasis, thus optimizing PDAC cell survival and proliferation (Yang and Kimmelman, 2011).

In contrast, in non-transformed epithelial cells, PDAC initiation is suppressed by autophagy. ROS production, genomic damage, inflammation, and cellular injury are limited. In addition, oncogenic aggregates of p62 are eliminated. However, as discussed earlier, when impairment of autophagy and lysosomal dysfunction occurs pancreatitis is initiated. This can lead to chronic pancreatic injury and compensatory proliferation of stem cells, resulting in ductal metaplasia and regenerative responses which contribute to tumorigenesis. Pathways such as Notch, Hedgehog, and Wnt-β catenin are activated in pancreatic tissues in CP during the regenerative response and dysregulation of these pathways has been attributed to pancreatic tumorigenesis (Bhanot and Moller, 2009).

Several clinical trials are currently using inhibitors of autophagy, such as hydroxychloroquine (which halts lysosomal acidification and autophagosome degradation), in the treatment of PDAC (Amaravadi et al., 2011). Inhibition of autophagy has been shown to retard growth of pancreatic xenograft tumors in mice, and development of tumors in mice with pancreata containing oncogenic Kras (Yang et al., 2011). However, a recent study demonstrated that treatment of PDAC maybe more complex (Rosenfeldt et al., 2013). In a humanized genetically-modified mouse model of PDAC, the role of autophagy in tumor development was found to be inherently linked to the status of the tumor suppressor p53. Kras mice developed a small number of pre-cancerous lesions that became PDAC over time. However, it was found that mice also lacking the essential autophagy genes *Atg5* or *Atg7* accumulated low grade pre-malignant PanIN lesions, which did not progress to high grade PanINs and PDAC. In contrast, in mice lacking Kras and p53, a loss of autophagy no longer blocked tumor progression, but actually accelerated the onset of tumors and increased uptake of glucose to fuel tumor growth. Furthermore, this study showed that treatment of the mice with hydroxychloroquine actually accelerated tumor formation in mice with onogenic Kras but lacking p53. Thus the role of autophagy in pancreatic cancer is extremely complex and care needs to be taken when designing appropriate therapies.

OBESITY AND PANCREATIC DISEASE

Obesity is a major health problem worldwide and leads to increases in risk for cardiovascular disease, stroke, and a variety of cancers (Hotamisligil and Erbay, 2008; Osborn and Olefsky, 2012). Obesity can result in low grade chronic inflammation which renders patients vulnerable to these diseases, although the underlying cellular mechanisms between obesity and inflammation remain vague (Weisberg et al., 2003; Hotamisligil and Erbay, 2008; Johnson et al., 2012; Osborn and Olefsky, 2012). Obesity is known to increase the number of CD8+ T-cells and decrease T-regulatory cells, promoting recruitment of macrophages (Johnson et al., 2012). Elevated levels of inflammatory mediators such as TNF-α, IL-1β, IL-6,

and IL-18 are seen within adipose tissue and also systemically through inflammasome activation in macrophages (Stienstra et al., 2011). Inflammatory mediators secreted by macrophages further augment general inflammation. In addition, levels of the pro-inflammatory hormone leptin are increased by obesity and decreases in adiponectin, its anti-inflammatory counterpart, are observed. Obesity, or a high fat diet (HFD), can also affect autophagy, increasing ER stress and inflammation (Yang et al., 2010; Hasnain et al., 2012). Obesity inhibits autophagy by activating Akt and mTOR signaling pathways, and down-regulating autophagic genes such as Ulk1/Atg1, Atg5, Atg6/Beclin 1.

Obesity has been linked to increased risk and severity of pancreatitis (Frossard et al., 2009; Navina et al., 2011). Deletion of leptin (*ob/ob*) or the leptin receptor (*db/db*), or administration of an HFD, in mice caused obesity and increased severity of pancreatitis. Following induction of pancreatitis with cerulein, levels of pancreatic IL-1β, IL-6, CCL2/MCP-1, and neutrophil infiltration were much greater in *ob/ob* and *db/db* mice compared to their lean littermates (Zyromski et al., 2008). Furthermore, in a model of AP induced by a combination of IL-12 and IL-18, severe disease occurred in *ob/ob* mice compared to wild type mice (Sennello et al., 2008). Finally, in a model of taurocholate-induced pancreatitis TNF-α levels increased while IL-10 was reduced, resulting in necrosis of adipose tissue (Franco-Pons et al., 2010). Thus obesity-related inflammatory mediators appear to play a pivotal role in severity of pancreatitis.

Obesity and HFD have further been identified as prominent risk factors for pancreatic cancer (Wiseman, 2008). Consumption of an HFD in mice with oncogenic Kras expression increased PanIN formation, fibrosis, inflammation, and PDAC, resulting in reduced survival (Philip et al., 2013). In contrast, control mice lacking Kras expression and fed with HFD, or Kras-expressing mice fed a control diet (CD), showed minimal pancreatic pathology. This model underscores the risk posed by an HFD in humans that express pancreatic oncogenic Kras. Activity of Kras and its downstream effectors such as COX-2 and phospho-ERK are elevated. Infiltration of macrophages into the stroma and activation of quiescent PSCs producing α-SMA and collagen I also occurs. COX-2 forms a positive feed-forward loop thus maintaining Kras activity and further augments inflammation, fibrosis, and recruitment of inflammatory mediators to the pancreas. This ultimately leads to development of PanINs and PDAC. Given that many healthy individuals express oncogenic Kras, consumption of HFD could put them at greater risk of developing PDAC. Consuming a reduced fat diet and ingestion of COX-2 inhibitors could limit pancreatic inflammation and fibrosis and may prevent formation of PanINs and progression to PDAC.

CONCLUSION

Although our knowledge of underlying mechanisms of pancreatitis and pancreatic cancer have advanced in the past few years much remains unknown. Recent studies have strongly implicated smoking, alcohol, and obesity as common etiological factors in pancreatitis-to-cancer pathways. At the cellular level, aberrant zymogen activation, particularly through mutations in trypsinogen, can lead to repeat bouts of AP. This can result in low grade inflammation, autophagy, stellate cell activation, and fibrosis, culminating in chronic disease. Furthermore, oncogenic Kras mutations and modifications of tumor suppressor genes (p16 and p53) may all contribute to progression from CP to PDAC (**Figure 1**). Development of multiple drugs that target various aspects of this complex tapestry of cellular pathways will be paramount in halting disease initiation and progression.

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REFERENCES

Aghajan, M., Li, N., and Karin, M. (2012). Obesity, autophagy and the pathogenesis of liver and pancreatic cancers. *J. Gastroenterol. Hepatol.* 27(Suppl. 2), 10–14. doi: 10.1111/j.1440-1746.2011.07008.x

Ahn, D. W., Seo, J. K., Lee, S. H., Hwang, J. H., Lee, J. K., Ryu, J. K., et al. (2012). Enhanced antitumor effect of combination therapy with gemcitabine and guggulsterone in pancreatic cancer. *Pancreas* 41, 1048–1057. doi: 10.1097/MPA.0b013e318249d62e

Algul, H., Treiber, M., Lesina, M., Nakhai, H., Saur, D., Geisler, F., et al. (2007). Pancreas-specific RelA/p65 truncation increases susceptibility of acini to inflammation-associated cell death following cerulein pancreatitis. *J. Clin. Invest.* 117, 1490–1501. doi: 10.1172/JCI29882

Alirezaei, M., Flynn, C. T., and Whitton, J. L. (2012). Interactions between enteroviruses and autophagy *in vivo*. *Autophagy* 8, 973–975. doi: 10.4161/auto.20160

Almoguera, 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. doi: 10.1016/0092-8674(88)90571-5

Amaravadi, R. K., Lippincott-Schwartz, J., Yin, X. M., Weiss, W. A., Takebe, N., Timmer, W., et al. (2011). Principles and current strategies for targeting autophagy for cancer treatment. *Clin. Cancer Res.* 17, 654–666. doi: 10.1158/1078-0432.CCR-10-2634

Apte, M. V., Haber, P. S., Applegate, T. L., Norton, I. D., McCaughan, G. W., Korsten, M. A., et al. (1998). Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture. *Gut* 43, 128–133. doi: 10.1136/gut.43.1.128

Apte, M. V., Haber, P. S., Darby, S. J., Rodgers, S. C., McCaughan, G. W., Korsten, M. A., et al. (1999). Pancreatic stellate cells are activated by proinflammatory cytokines: implications for pancreatic fibrogenesis. *Gut* 44, 534–541. doi: 10.1136/gut.44.4.534

Aravindan, S., Delma, C. R., Thirugnanasambandan, S. S., Herman, T. S., and Aravindan, N. (2013). Anti-pancreatic cancer deliverables from sea: first-hand evidence on the efficacy, molecular targets and mode of action for multifarious polyphenols from five different brown-algae. *PLoS ONE* 8:e61977. doi: 10.1371/journal.pone.0061977

Bachem, M. G., Schneider, E., Gross, H., Weidenbach, H., Schmid, R. M., Menke, A., et al. (1998). Identification, culture, and characterization of pancreatic stellate cells in rats and humans. *Gastroenterology* 115, 421–432. doi: 10.1016/S0016-5085(98)70209-4

Bachem, M. G., Schunemann, M., Ramadani, M., Siech, M., Beger, H., Buck, A., et al. (2005). Pancreatic carcinoma cells induce fibrosis by stimulating proliferation and matrix synthesis of stellate cells. *Gastroenterology* 128, 907–921. doi: 10.1053/j.gastro.2004.12.036

Banerjee, S., Sangwan, V., McGinn, O., Chugh, R., Dudeja, V., Vickers, S. M., et al. (2013). Triptolide-induced cell death in pancreatic cancer is mediated by O-GlcNAc modification of transcription factor Sp1. *J. Biol. Chem.* 288, 33927–33938. doi: 10.1074/jbc.M113.500983

Bayliss, W. M., and Starling, E. H. (1902). The mechanism of pancreatic secretion. *J. Physiol.* 28, 325–353.

Beer, S., Zhou, J., Szabo, A., Keiles, S., Chandak, G. R., Witt, H., et al. (2013). Comprehensive functional analysis of chymotrypsin C (CTRC) variants reveals distinct loss-of-function mechanisms associated with pancreatitis risk. *Gut* 62, 1616–1624. doi: 10.1136/gutjnl-2012-303090

Bhagat, L., Singh, V. P., Song, A. M., Van Acker, G. J., Agrawal, S., Steer, M. L., et al. (2002). Thermal stress-induced HSP70 mediates protection against intrapancreatic trypsinogen activation and acute pancreatitis in rats. *Gastroenterology* 122, 156–165. doi: 10.1053/gast.2002.30314

Bhanot, U. K., and Moller, P. (2009). Mechanisms of parenchymal injury and signaling pathways in ectatic ducts of chronic pancreatitis: implications for pancreatic carcinogenesis. *Lab. Invest.* 89, 489–497. doi: 10.1038/labinvest.2009.19

Bhoomagoud, M., Jung, T., Atladottir, J., Kolodecik, T. R., Shugrue, C., Chaudhuri, A., et al. (2009). Reducing extracellular pH sensitizes the acinar cell to secretagogue-induced pancreatitis responses in rats. *Gastroenterology* 137, 1083–1092. doi: 10.1053/j.gastro.2009.05.041

Bollrath, J., and Greten, F. R. (2009). IKK/NF-kappaB and STAT3 pathways: central signalling hubs in inflammation-mediated tumour promotion and metastasis. *EMBO Rep.* 10, 1314–1319. doi: 10.1038/embor.2009.243

Cancela, J. M., Van Coppenolle, F., Galione, A., Tepikin, A. V., and Petersen, O. H. (2002). Transformation of local Ca2+ spikes to global Ca2+ transients: the combinatorial roles of multiple Ca2+ releasing messengers. *EMBO J.* 21, 909–919. doi: 10.1093/emboj/21.5.909

Carbone, C., and Melisi, D. (2012). NF-kappaB as a target for pancreatic cancer therapy. *Expert Opin. Ther. Targets* 16(Suppl. 2), S1–S10. doi: 10.1517/14728222.2011.645806

Chen, J. M., and Ferec, C. (2009). Chronic pancreatitis: genetics and pathogenesis. *Annu. Rev. Genomics Hum. Genet.* 10, 63–87. doi: 10.1146/annurev-genom-082908-150009

Chen, J. M., and Ferec, C. (2012). Genetics and pathogenesis of chronic pancreatitis: the 2012 update. *Clin. Res. Hepatol. Gastroenterol.* 36, 334–340. doi: 10.1016/j.clinre.2012.05.003

Choudari, C. P., Lehman, G. A., and Sherman, S. (1999). Pancreatitis and cystic fibrosis gene mutations. *Gastroenterol. Clin. North Am.* 28, 543–549, vii–viii. doi: 10.1016/S0889-8553(05)70072-8

Colby, J. K., Klein, R. D., McArthur, M. J., Conti, C. J., Kiguchi, K., Kawamoto, T., et al. (2008). Progressive metaplastic and dysplastic changes in mouse pancreas induced by cyclooxygenase-2 overexpression. *Neoplasia* 10, 782–796. doi: 10.1593/neo.08330

Collins, M. A., Bednar, F., Zhang, Y., Brisset, J. C., Galban, S., Galban, C. J., et al. (2012a). Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer in mice. *J. Clin. Invest.* 122, 639–653. doi: 10.1172/JCI59227

Collins, M. A., Brisset, J. C., Zhang, Y., Bednar, F., Pierre, J., Heist, K. A., et al. (2012b). Metastatic pancreatic cancer is dependent on oncogenic Kras in mice. *PLoS ONE* 7:e49707. doi: 10.1371/journal.pone.0049707

Corcoran, R. B., Contino, G., Deshpande, V., Tzatsos, A., Conrad, C., Benes, C. H., et al. (2011). STAT3 plays a critical role in KRAS-induced pancreatic tumorigenesis. *Cancer Res.* 71, 5020–5029. doi: 10.1158/0008-5472.CAN-11-0908

Cosen-Binker, L. I., Lam, P. P., Binker, M. G., Reeve, J., Pandol, S., and Gaisano, H. Y. (2007). Alcohol/cholecystokinin-evoked pancreatic acinar basolateral exocytosis is mediated by protein kinase C alpha phosphorylation of Munc18c. *J. Biol. Chem.* 282, 13047–13058. doi: 10.1074/jbc.M611132200

Criddle, D. N., Murphy, J., Fistetto, G., Barrow, S., Tepikin, A. V., Neoptolemos, J. P., et al. (2006). Fatty acid ethyl esters cause pancreatic calcium toxicity via inositol trisphosphate receptors and loss of ATP synthesis. *Gastroenterology* 130, 781–793. doi: 10.1053/j.gastro.2005.12.031

Dawra, R., Sah, R. P., Dudeja, V., Rishi, L., Talukdar, R., Garg, P., et al. (2011). Intra-acinar trypsinogen activation mediates early stages of pancreatic injury but not inflammation in mice with acute pancreatitis. *Gastroenterology* 141, 2210–2217.e2212. doi: 10.1053/j.gastro.2011.08.033

Deretic, V. (2012). Autophagy: an emerging immunological paradigm. *J. Immunol.* 189, 15–20. doi: 10.4049/jimmunol.1102108

Di Magliano, M. P., and Logsdon, C. D. (2013). Roles for KRAS in pancreatic tumor development and progression. *Gastroenterology* 144, 1220–1229. doi: 10.1053/j.gastro.2013.01.071

Doppler, H., Liou, G. Y., and Storz, P. (2013). Downregulation of TRAF2 mediates NIK-induced pancreatic cancer cell proliferation and tumorigenicity. *PLoS ONE* 8:e53676. doi: 10.1371/journal.pone.0053676

Drexler, S. K., and Yazdi, A. S. (2013). Complex roles of inflammasomes in carcinogenesis. *Cancer J.* 19, 468–472. doi: 10.1097/PPO.0000000000000004

Eibl, G., Reber, H. A., Hines, O. J., and Go, V. L. (2004). COX and PPAR: possible interactions in pancreatic cancer. *Pancreas* 29, 247–253. doi: 10.1097/00006676-200411000-00002

Erkan, M., Adler, G., Apte, M. V., Bachem, M. G., Buchholz, M., Detlefsen, S., et al. (2012a). StellaTUM: current consensus and discussion on pancreatic stellate cell research. *Gut* 61, 172–178. doi: 10.1136/gutjnl-2011-301220

Erkan, M., Hausmann, S., Michalski, C. W., Fingerle, A. A., Dobritz, M., Kleeff, J., et al. (2012b). The role of stroma in pancreatic cancer: diagnostic and therapeutic implications. *Nat. Rev. Gastroenterol. Hepatol.* 9, 454–467. doi: 10.1038/nrgastro.2012.115

Fabre, C., Mimura, N., Bobb, K., Kong, S. Y., Gorgun, G., Cirstea, D., et al. (2012). Dual inhibition of canonical and noncanonical NF-kappaB pathways demonstrates significant antitumor activities in multiple myeloma. *Clin. Cancer Res.* 18, 4669–4681. doi: 10.1158/1078-0432.CCR-12-0779

Feng, D., Park, O., Radaeva, S., Wang, H., Yin, S., Kong, X., et al. (2012). Interleukin-22 ameliorates cerulein-induced pancreatitis in mice by inhibiting the autophagic pathway. *Int. J. Biol. Sci.* 8, 249–257. doi: 10.7150/ijbs.3967

Fernandez-Medarde, A., and Santos, E. (2011). Ras in cancer and developmental diseases. *Genes Cancer* 2, 344–358. doi: 10.1177/1947601911411084

Figarella, C., Miszczuk-Jamska, B., and Barrett, A. J. (1988). Possible lysosomal activation of pancreatic zymogens. Activation of both human trypsinogens by cathepsin B and spontaneous acid. Activation of human trypsinogen 1. *Biol. Chem. Hoppe Seyler* 369(Suppl.), 293–298.

Fisic, E., Poropat, G., Bilic-Zulle, L., Licul, V., Milic, S., and Stimac, D. (2013). The role of IL-6, 8, and 10, sTNFr, CRP, and pancreatic elastase in the prediction of systemic complications in patients with acute pancreatitis. *Gastroenterol. Res. Pract.* 2013, 282645. doi: 10.1155/2013/282645

Fortunato, F., Burgers, H., Bergmann, F., Rieger, P., Buchler, M. W., Kroemer, G., et al. (2009). Impaired autolysosome formation correlates with Lamp-2 depletion: role of apoptosis, autophagy, and necrosis in pancreatitis. *Gastroenterology* 137, 350–360, 360.e351–e355. doi: 10.1053/j.gastro.2009.04.003

Franco-Pons, N., Gea-Sorli, S., and Closa, D. (2010). Release of inflammatory mediators by adipose tissue during acute pancreatitis. *J. Pathol.* 221, 175–182. doi: 10.1002/path.2691

Frossard, J. L., Lescuyer, P., and Pastor, C. M. (2009). Experimental evidence of obesity as a risk factor for severe acute pancreatitis. *World J. Gastroenterol.* 15, 5260–5265. doi: 10.3748/wjg.15.5260

Fujioka, S., Sclabas, G. M., Schmidt, C., Niu, J., Frederick, W. A., Dong, Q. G., et al. (2003). Inhibition of constitutive NF-kappa B activity by I kappa B alpha M suppresses tumorigenesis. *Oncogene* 22, 1365–1370. doi: 10.1038/sj.onc.1206323

Fukuda, A., Wang, S. C., Morris, J. P. T., Folias, A. E., Liou, A., Kim, G. E., et al. (2011). Stat3 and MMP7 contribute to pancreatic ductal adenocarcinoma initiation and progression. *Cancer Cell* 19, 441–455. doi: 10.1016/j.ccr.2011.03.002

Gaiser, S., Daniluk, J., Liu, Y., Tsou, L., Chu, J., Lee, W., et al. (2011). Intracellular activation of trypsinogen in transgenic mice induces acute but not chronic pancreatitis. *Gut* 60, 1379–1388. doi: 10.1136/gut.2010.226175

Glitsch, M. D., Bakowski, D., and Parekh, A. B. (2002). Store-operated Ca2+ entry depends on mitochondrial Ca2+ uptake. *EMBO J.* 21, 6744–6754. doi: 10.1093/emboj/cdf675

Gopinathan, A., Denicola, G. M., Frese, K. K., Cook, N., Karreth, F. A., Mayerle, J., et al. (2012). Cathepsin B promotes the progression of pancreatic ductal adenocarcinoma in mice. *Gut* 61, 877–884. doi: 10.1136/gutjnl-2011-300850

Gorelick, F. S., and Matovcik, L. M. (1995). Lysosomal enzymes and pancreatitis. *Gastroenterology* 109, 620–625. doi: 10.1016/0016-5085(95)90355-0

Grasso, D., Ropolo, A., Lo Re, A., Boggio, V., Molejon, M. I., Iovanna, J. L., et al. (2011). Zymophagy, a novel selective autophagy pathway mediated by VMP1-USP9x-p62, prevents pancreatic cell death. *J. Biol. Chem.* 286, 8308–8324. doi: 10.1074/jbc.M110.197301

Green, D. R., Galluzzi, L., and Kroemer, G. (2011). Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science* 333, 1109–1112. doi: 10.1126/science.1201940

Grivennikov, S. I., Greten, F. R., and Karin, M. (2010). Immunity, inflammation, and cancer. *Cell* 140, 883–899. doi: 10.1016/j.cell.2010.01.025

Grivennikov, S. I., and Karin, M. (2010). Dangerous liaisons: STAT3 and NF-kappaB collaboration and crosstalk in cancer. *Cytokine Growth Factor Rev.* 21, 11–19. doi: 10.1016/j.cytogfr.2009.11.005

Gu, H., Werner, J., Bergmann, F., Whitcomb, D. C., Buchler, M. W., and Fortunato, F. (2013). Necro-inflammatory response of pancreatic acinar cells in the pathogenesis of acute alcoholic pancreatitis. *Cell Death Dis.* 4, e816. doi: 10.1038/cddis.2013.354

Gu, R., Shampang, A., Reilly, A., Fisher, D., Glass, W., and Ramsingh, A. I. (2009). IL-10 is pathogenic during the development of coxsackievirus B4-induced chronic pancreatitis. *Virology* 395, 77–86. doi: 10.1016/j.virol.2009.09.005

Guerra, C., Collado, M., Navas, C., Schuhmacher, A. J., Hernandez-Porras, I., Canamero, M., et al. (2011). Pancreatitis-induced inflammation contributes to pancreatic cancer by inhibiting oncogene-induced senescence. *Cancer Cell* 19, 728–739. doi: 10.1016/j.ccr.2011.05.011

Gukovskaya, A. S., and Gukovsky, I. (2012). Autophagy and pancreatitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 303, G993–G1003. doi: 10.1152/ajpgi.00122.2012

Gukovskaya, A. S., Hosseini, S., Satoh, A., Cheng, J. H., Nam, K. J., Gukovsky, I., et al. (2004). Ethanol differentially regulates NF-kappaB activation in pancreatic acinar cells through calcium and protein kinase C pathways. *Am. J. Physiol. Gastrointest. Liver Physiol.* 286, G204–G213. doi: 10.1152/ajpgi.00088.2003

Gukovsky, I., and Gukovskaya, A. (2013). Nuclear factor-kappaB in pancreatitis: jack-of-all-trades, but which one is more important? *Gastroenterology* 144, 26–29. doi: 10.1053/j.gastro.2012.11.016

Gukovsky, I., Gukovskaya, A. S., Blinman, T. A., Zaninovic, V., and Pandol, S. J. (1998). Early NF-κB activation is associated with hormone-induced pancreatitis. *Am. J. Physiol.* 275, G1402–G1414.

Gukovsky, I., Li, N., Todoric, J., Gukovskaya, A., and Karin, M. (2013). Inflammation, autophagy, and obesity: common features in the pathogenesis of pancreatitis and pancreatic cancer. *Gastroenterology* 144, 1199–1209. e1194. doi: 10.1053/j.gastro.2013.02.007

Gukovsky, I., Pandol, S. J., and Gukovskaya, A. S. (2011). Organellar dysfunction in the pathogenesis of pancreatitis. *Antioxid. Redox Signal.* 15, 2699–2710. doi: 10.1089/ars.2011.4068

Gukovsky, I., Pandol, S. J., Mareninova, O. A., Shalbueva, N., Jia, W., and Gukovskaya, A. S. (2012). Impaired autophagy and organellar dysfunction in pancreatitis. *J. Gastroenterol. Hepatol.* 27(Suppl. 2), 27–32. doi: 10.1111/j.1440-1746.2011.07004.x

Guo, J. Y., Chen, H. Y., Mathew, R., Fan, J., Strohecker, A. M., Karsli-Uzunbas, G., et al. (2011). Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev.* 25, 460–470. doi: 10.1101/gad.2016311

Guo, J. Y., Karsli-Uzunbas, G., Mathew, R., Aisner, S. C., Kamphorst, J. J., Strohecker, A. M., et al. (2013). Autophagy suppresses progression of K-ras-induced lung tumors to oncocytomas and maintains lipid homeostasis. *Genes Dev.* 27, 1447–1461. doi: 10.1101/gad.219642.113

Halangk, W., Lerch, M. M., Brandt-Nedelev, B., Roth, W., Ruthenbuerger, M., Reinheckel, T., et al. (2000). Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. *J. Clin. Invest.* 106, 773–781. doi: 10.1172/JCI9411

Hamada, S., Masamune, A., Takikawa, T., Suzuki, N., Kikuta, K., Hirota, M., et al. (2012). Pancreatic stellate cells enhance stem cell-like phenotypes in pancreatic cancer cells. *Biochem. Biophys. Res. Commun.* 421, 349–354. doi: 10.1016/j.bbrc.2012.04.014

Hashimoto, D., Ohmuraya, M., Hirota, M., Yamamoto, A., Suyama, K., Ida, S., et al. (2008). Involvement of autophagy in trypsinogen activation within the pancreatic acinar cells. *J. Cell Biol.* 181, 1065–1072. doi: 10.1083/jcb.200712156

Hasnain, S. Z., Lourie, R., Das, I., Chen, A. C., and McGuckin, M. A. (2012). The interplay between endoplasmic reticulum stress and inflammation. *Immunol. Cell Biol.* 90, 260–270. doi: 10.1038/icb.2011.112

Hedvat, M., Huszar, D., Herrmann, A., Gozgit, J. M., Schroeder, A., Sheehy, A., et al. (2009). The JAK2 inhibitor AZD1480 potently blocks Stat3 signaling and oncogenesis in solid tumors. *Cancer Cell* 16, 487–497. doi: 10.1016/j.ccr.2009.10.015

Hill, R., Li, Y., Tran, L. M., Dry, S., Calvopina, J. H., Garcia, A., et al. (2012). Cell intrinsic role of COX-2 in pancreatic cancer development. *Mol. Cancer Ther.* 11, 2127–2137. doi: 10.1158/1535-7163.MCT-12-0342

Hingorani, S. R., Petricoin, E. F., Maitra, A., Rajapakse, V., King, C., Jacobetz, M. A., et al. (2003). Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* 4, 437–450. doi: 10.1016/S1535-6108(03)00309-X

Hoque, R., Sohail, M., Malik, A., Sarwar, S., Luo, Y., Shah, A., et al. (2011). TLR9 and the NLRP3 inflammasome link acinar cell death with inflammation in acute pancreatitis. *Gastroenterology* 141, 358–369. doi: 10.1053/j.gastro.2011.03.041

Hotamisligil, G. S., and Erbay, E. (2008). Nutrient sensing and inflammation in metabolic diseases. *Nat. Rev. Immunol.* 8, 923–934. doi: 10.1038/nri2449

Howes, N., Lerch, M. M., Greenhalf, W., Stocken, D. D., Ellis, I., Simon, P., et al. (2004). Clinical and genetic characteristics of hereditary pancreatitis in Europe. *Clin. Gastroenterol. Hepatol.* 2, 252–261. doi: 10.1016/S1542-3565(04)00013-8

Huang, H., Liu, Y., Daniluk, J., Gaiser, S., Chu, J., Wang, H., et al. (2013). Activation of nuclear factor-kappaB in acinar cells increases the severity of pancreatitis in mice. *Gastroenterology* 144, 202–210. doi: 10.1053/j.gastro.2012.09.059

Husain, S. Z., Prasad, P., Grant, W. M., Kolodecik, T. R., Nathanson, M. H., and Gorelick, F. S. (2005). The ryanodine receptor mediates early zymogen activation in pancreatitis. *Proc. Natl. Acad. Sci. U.S.A.* 102, 14386–14391. doi: 10.1073/pnas.0503215102

Hwang, R. F., Moore, T., Arumugam, T., Ramachandran, V., Amos, K. D., Rivera, A., et al. (2008). Cancer-associated stromal fibroblasts promote pancreatic tumor progression. *Cancer Res.* 68, 918–926. doi: 10.1158/0008-5472.CAN-07-5714

Ji, B., Tsou, L., Wang, H., Gaiser, S., Chang, D. Z., Daniluk, J., et al. (2009). Ras activity levels control the development of pancreatic diseases. *Gastroenterology* 137, 1072–1082, 1082.e1071–e1076. doi: 10.1053/j.gastro.2009.05.052

Jiang, G. Z., Cao, F. Y., Ren, G. P., Gao, D. L., Bhakta, V., Zhang, Y. H., et al. (2010). PRSS3 promotes tumour growth and metastasis of human pancreatic cancer. *Gut* 59, 1535–1544. doi: 10.1136/gut.2009.200105

Johnson, A. R., Milner, J. J., and Makowski, L. (2012). The inflammation highway: metabolism accelerates inflammatory traffic in obesity. *Immunol. Rev.* 249, 218–238. doi: 10.1111/j.1600-065X.2012.01151.x

Judak, L., Hegyi, P., Rakonczay, Z. Jr., Maleth, J., Gray, M. A., and Venglovecz, V. (2013). Ethanol and its non-oxidative metabolites profoundly inhibit CFTR function in pancreatic epithelial cells which is prevented by ATP supplementation. *Pflugers Arch.* doi: 10.1007/s00424-013-1333-x. [Epub ahead of print].

Jung, I. H., Jung, D. E., Park, Y. N., Song, S. Y., and Park, S. W. (2011). Aberrant Hedgehog ligands induce progressive pancreatic fibrosis by paracrine activation of myofibroblasts and ductular cells in transgenic zebrafish. *PLoS ONE* 6:e27941. doi: 10.1371/journal.pone.0027941

Kim, M. S., Hong, J. H., Li, Q., Shin, D. M., Abramowitz, J., Birnbaumer, L., et al. (2009). Deletion of TRPC3 in mice reduces store-operated Ca2+ influx and the severity of acute pancreatitis. *Gastroenterology* 137, 1509–1517. doi: 10.1053/j.gastro.2009.07.042

Kim, M. S., Lee, K. P., Yang, D., Shin, D. M., Abramowitz, J., Kiyonaka, S., et al. (2011). Genetic and pharmacologic inhibition of the Ca2+ influx channel TRPC3 protects secretory epithelia from Ca2+-dependent toxicity. *Gastroenterology* 140, 2107–2115, 2115.e2101–e2104. doi: 10.1053/j.gastro.2011.02.052

Koike, Y., Kanai, T., Saeki, K., Nakamura, Y., Nakano, M., Mikami, Y., et al. (2012). MyD88-dependent interleukin-10 production from regulatory CD11b(+)Gr-1(high) cells suppresses development of acute cerulein pancreatitis in mice. *Immunol. Lett.* 148, 172–177. doi: 10.1016/j.imlet.2012.08.008

Kukor, Z., Mayerle, J., Kruger, B., Toth, M., Steed, P. M., Halangk, W., et al. (2002). Presence of cathepsin B in the human pancreatic secretory pathway and its role in trypsinogen activation during hereditary pancreatitis. *J. Biol. Chem.* 277, 21389–21396. doi: 10.1074/jbc.M200878200

Lee, H., Herrmann, A., Deng, J. H., Kujawski, M., Niu, G., Li, Z., et al. (2009). Persistently activated Stat3 maintains constitutive NF-kappaB activity in tumors. *Cancer Cell* 15, 283–293. doi: 10.1016/j.ccr.2009.02.015

Lee, M. S., Gu, D., Feng, L., Curriden, S., Arnush, M., Krahl, T., et al. (1995). Accumulation of extracellular matrix and developmental dysregulation in the pancreas by transgenic production of transforming growth factor-beta 1. *Am. J. Pathol.* 147, 42–52.

Lerch, M. M., Saluja, A. K., Runzi, M., Dawra, R., and Steer, M. L. (1995). Luminal endocytosis and intracellular targeting by acinar cells during early biliary pancreatitis in the opossum. *J. Clin. Invest.* 95, 2222–2231. doi: 10.1172/JCI117912

Lesina, M., Kurkowski, M. U., Ludes, K., Rose-John, S., Treiber, M., Kloppel, G., et al. (2011). Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. *Cancer Cell* 19, 456–469. doi: 10.1016/j.ccr.2011.03.009

Levine, B., and Kroemer, G. (2008). Autophagy in the pathogenesis of disease. *Cell* 132, 27–42. doi: 10.1016/j.cell.2007.12.018

Liang, X. H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H., et al. (1999). Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature* 402, 672–676. doi: 10.1038/45257

Liby, K. T., Royce, D. B., Risingsong, R., Williams, C. R., Maitra, A., Hruban, R. H., et al. (2010). Synthetic triterpenoids prolong survival in a transgenic

mouse model of pancreatic cancer. *Cancer Prev. Res. (Phila.)* 3, 1427–1434. doi: 10.1158/1940-6207.CAPR-10-0197

Ling, J., Kang, Y., Zhao, R., Xia, Q., Lee, D. F., Chang, Z., et al. (2012). KrasG12D-induced IKK2/beta/NF-kappaB activation by IL-1alpha and p62 feedforward loops is required for development of pancreatic ductal adenocarcinoma. *Cancer Cell* 21, 105–120. doi: 10.1016/j.ccr.2011.12.006

Lohr, J. M., and Jesenofsky, R. (2009). Pancreatic stellate cells and pancreatic carcinoma: an unholy alliance. *JOP* 10, 472–473.

Mah, L. Y., and Ryan, K. M. (2012). Autophagy and cancer. *Cold Spring Harb. Perspect. Biol.* 4, a008821. doi: 10.1101/cshperspect.a008821

Mantoni, T. S., Lunardi, S., Al-Assar, O., Masamune, A., and Brunner, T. B. (2011). Pancreatic stellate cells radioprotect pancreatic cancer cells through beta1-integrin signaling. *Cancer Res.* 71, 3453–3458. doi: 10.1158/0008-5472.CAN-10-1633

Mareninova, O. A., Hermann, K., French, S. W., O'Konski, M. S., Pandol, S. J., Webster, P., et al. (2009). Impaired autophagic flux mediates acinar cell vacuole formation and trypsinogen activation in rodent models of acute pancreatitis. *J. Clin. Invest.* 119, 3340–3355. doi: 10.1172/JCI38674

Masamune, A., and Shimosegawa, T. (2009). Signal transduction in pancreatic stellate cells. *J. Gastroenterol.* 44, 249–260. doi: 10.1007/s00535-009-0013-2

Masamune, A., Watanabe, T., Kikuta, K., and Shimosegawa, T. (2009). Roles of pancreatic stellate cells in pancreatic inflammation and fibrosis. *Clin. Gastroenterol. Hepatol.* 7, S48–S54. doi: 10.1016/j.cgh.2009.07.038

Mayer, J., Rau, B., Gansauge, F., and Beger, H. G. (2000). Inflammatory mediators in human acute pancreatitis: clinical and pathophysiological implications. *Gut* 47, 546–552. doi: 10.1136/gut.47.4.546

Mews, P., Phillips, P., Fahmy, R., Korsten, M., Pirola, R., Wilson, J., et al. (2002). Pancreatic stellate cells respond to inflammatory cytokines: potential role in chronic pancreatitis. *Gut* 50, 535–541. doi: 10.1136/gut.50.4.535

Morris, J. P. T., Cano, D. A., Sekine, S., Wang, S. C., and Hebrok, M. (2010). Beta-catenin blocks Kras-dependent reprogramming of acini into pancreatic cancer precursor lesions in mice. *J. Clin. Invest.* 120, 508–520. doi: 10.1172/JCI40045

Morton, J. P., Mongeau, M. E., Klimstra, D. S., Morris, J. P., Lee, Y. C., Kawaguchi, Y., et al. (2007). Sonic hedgehog acts at multiple stages during pancreatic tumorigenesis. *Proc. Natl. Acad. Sci. U.S.A.* 104, 5103–5108. doi: 10.1073/pnas.0701158104

Moscat, J., and Diaz-Meco, M. T. (2012). p62: a versatile multitasker takes on cancer. *Trends Biochem. Sci.* 37, 230–236. doi: 10.1016/j.tibs.2012.02.008

Mounzer, R., and Whitcomb, D. C. (2013). Genetics of acute and chronic pancreatitis. *Curr. Opin. Gastroenterol.* 29, 544–551. doi: 10.1097/MOG.0b013e3283639383

Muili, K. A., Ahmad, M., Orabi, A. I., Mahmood, S. M., Shah, A. U., Molkentin, J. D., et al. (2012). Pharmacological and genetic inhibition of calcineurin protects against carbachol-induced pathological zymogen activation and acinar cell injury. *Am. J. Physiol. Gastrointest. Liver Physiol.* 302, G898–G905. doi: 10.1152/ajpgi.00545.2011

Mukherjee, P., Basu, G. D., Tinder, T. L., Subramani, D. B., Bradley, J. M., Arefayene, M., et al. (2009). Progression of pancreatic adenocarcinoma is significantly impeded with a combination of vaccine and COX-2 inhibition. *J. Immunol.* 182, 216–224.

Murphy, J. A., Criddle, D. N., Sherwood, M., Chvanov, M., Mukherjee, R., McLaughlin, E., et al. (2008). Direct activation of cytosolic Ca2+ signaling and enzyme secretion by cholecystokinin in human pancreatic acinar cells. *Gastroenterology* 135, 632–641. doi: 10.1053/j.gastro.2008.05.026

Murray, O. T., Wong, C. C., Vrankova, K., and Rigas, B. (2013). Phospho-sulindac inhibits pancreatic cancer growth: NFATc1 as a drug resistance candidate. *Int. J. Oncol.* 44, 521–529. doi: 10.3892/ijo.2013.2190

Nathan, C., and Ding, A. (2010). Nonresolving inflammation. *Cell* 140, 871–882. doi: 10.1016/j.cell.2010.02.029

Navina, S., Acharya, C., Delany, J. P., Orlichenko, L. S., Baty, C. J., Shiva, S. S., et al. (2011). Lipotoxicity causes multisystem organ failure and exacerbates acute pancreatitis in obesity. *Sci. Transl. Med.* 3, 107ra110. doi: 10.1126/scitranslmed.3002573

Nemoda, Z., and Sahin-Toth, M. (2005). The tetra-aspartate motif in the activation peptide of human cationic trypsinogen is essential for autoactivation control but not for enteropeptidase recognition. *J. Biol. Chem.* 280, 29645–29652. doi: 10.1074/jbc.M505661200

Neuhofer, P., Liang, S., Einwachter, H., Schwerdtfeger, C., Wartmann, T., Treiber, M., et al. (2013). Deletion of IkappaBalpha activates RelA to reduce acute pancreatitis in mice through up-regulation of Spi2A. *Gastroenterology* 144, 192–201. doi: 10.1053/j.gastro.2012.09.058

Nishina, T., Yamaguchi, N., Gohda, J., Semba, K., and Inoue, J. (2009). NIK is involved in constitutive activation of the alternative NF-kappaB pathway and proliferation of pancreatic cancer cells. *Biochem. Biophys. Res. Commun.* 388, 96–101. doi: 10.1016/j.bbrc.2009.07.125

Ogami, Y., and Otsuki, M. (1998). Exocrine pancreatic physiology: overview. *Pancreas* 16, 265–272. doi: 10.1097/00006676-199804000-00010

Ohmuraya, M., Hirota, M., Araki, M., Mizushima, N., Matsui, M., Mizumoto, T., et al. (2005). Autophagic cell death of pancreatic acinar cells in serine protease inhibitor Kazal type 3-deficient mice. *Gastroenterology* 129, 696–705. doi: 10.1016/j.gastro.2005.05.057

Ohta, T., Terada, T., Nagakawa, T., Tajima, H., Itoh, H., Fonseca, L., et al. (1994). Pancreatic trypsinogen and cathepsin B in human pancreatic carcinomas and associated metastatic lesions. *Br. J. Cancer* 69, 152–156. doi: 10.1038/bjc.1994.25

Omary, M. B., Lugea, A., Lowe, A. W., and Pandol, S. J. (2007). The pancreatic stellate cell: a star on the rise in pancreatic diseases. *J. Clin. Invest.* 117, 50–59. doi: 10.1172/JCI30082

Osborn, O., and Olefsky, J. M. (2012). The cellular and signaling networks linking the immune system and metabolism in disease. *Nat. Med.* 18, 363–374. doi: 10.1038/nm.2627

Ozaki, N., Ohmuraya, M., Hirota, M., Ida, S., Wang, J., Takamori, H., et al. (2009). Serine protease inhibitor Kazal type 1 promotes proliferation of pancreatic cancer cells through the epidermal growth factor receptor. *Mol. Cancer Res.* 7, 1572–1581. doi: 10.1158/1541-7786.MCR-08-0567

Parekh, A. B. (2003). Store-operated Ca2+ entry: dynamic interplay between endoplasmic reticulum, mitochondria and plasma membrane. *J. Physiol.* 547, 333–348. doi: 10.1113/jphysiol.2002.034140

Peery, A. F., Dellon, E. S., Lund, J., Crockett, S. D., McGowan, C. E., Bulsiewicz, W. J., et al. (2012). Burden of gastrointestinal disease in the United States: 2012 update. *Gastroenterology* 143, 1179–1187.e1171–1173. doi: 10.1053/j.gastro.2012.08.002

Petersen, O. H., Gerasimenko, O. V., Tepikin, A. V., and Gerasimenko, J. V. (2011). Aberrant Ca(2+) signalling through acidic calcium stores in pancreatic acinar cells. *Cell Calcium* 50, 193–199. doi: 10.1016/j.ceca.2011.02.010

Pezzilli, R., Morselli-Labate, A. M., Mantovani, V., Romboli, E., Selva, P., Migliori, M., et al. (2003). Mutations of the CFTR gene in pancreatic disease. *Pancreas* 27, 332–336. doi: 10.1097/00006676-200311000-00011

Pfutzer, R. H., Barmada, M. M., Brunskill, A. P., Finch, R., Hart, P. S., Neoptolemos, J., et al. (2000). SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. *Gastroenterology* 119, 615–623. doi: 10.1053/gast.2000.18017

Philip, B., Roland, C. L., Daniluk, J., Liu, Y., Chatterjee, D., Gomez, S. B., et al. (2013). A high-fat diet activates oncogenic Kras and COX2 to induce development of pancreatic ductal adenocarcinoma in mice. *Gastroenterology* 145, 1449–1458. doi: 10.1053/j.gastro.2013.08.018

Phillips, P. A., McCarroll, J. A., Park, S., Wu, M. J., Pirola, R., Korsten, M., et al. (2003). Rat pancreatic stellate cells secrete matrix metalloproteinases: implications for extracellular matrix turnover. *Gut* 52, 275–282. doi: 10.1136/gut.52.2.275

Phillips, P. A., Yang, L., Shulkes, A., Vonlaufen, A., Poljak, A., Bustamante, S., et al. (2010). Pancreatic stellate cells produce acetylcholine and may play a role in pancreatic exocrine secretion. *Proc. Natl. Acad. Sci. U.S.A.* 107, 17397–17402. doi: 10.1073/pnas.1000359107

Raimondi, S., Lowenfels, A. B., Morselli-Labate, A. M., Maisonneuve, P., and Pezzilli, R. (2010). Pancreatic cancer in chronic pancreatitis; aetiology, incidence, and early detection. *Best Pract. Res. Clin. Gastroenterol.* 24, 349–358. doi: 10.1016/j.bpg.2010.02.007

Rakonczay, Z. Jr., Hegyi, P., Takacs, T., McCarroll, J., and Saluja, A. K. (2008). The role of NF-kappaB activation in the pathogenesis of acute pancreatitis. *Gut* 57, 259–267. doi: 10.1136/gut.2007.124115

Romac, J. M., Ohmuraya, M., Bittner, C., Majeed, M. F., Vigna, S. R., Que, J., et al. (2010). Transgenic expression of pancreatic secretory trypsin inhibitor-1 rescues SPINK3-deficient mice and restores a normal pancreatic phenotype. *Am. J. Physiol. Gastrointest. Liver Physiol.* 298, G518–G524. doi: 10.1152/ajpgi.00431.2009

Rosenfeldt, M. T., O'Prey, J., Morton, J. P., Nixon, C., MacKay, G., Mrowinska, A., et al. (2013). p53 status determines the role of autophagy in pancreatic tumour development. *Nature* 504, 296–300. doi: 10.1038/nature12865

Sah, R. P., Dudeja, V., Dawra, R. K., and Saluja, A. K. (2013). Cerulein-induced chronic pancreatitis does not require intra-acinar activation of trypsinogen in mice. *Gastroenterology* 144, 1076–1085.e1072. doi: 10.1053/j.gastro.2013.01.041

Salem, K., Brown, C. O., Schibler, J., and Goel, A. (2013). Combination chemotherapy increases cytotoxicity of multiple myeloma cells by modification of nuclear factor (NF)-kappaB activity. *Exp. Hematol.* 41, 209–218. doi: 10.1016/j.exphem.2012.10.002

Satoh, A., Gukovskaya, A. S., Nieto, J. M., Cheng, J. H., Gukovsky, I., Reeve, J. R., et al. (2004). PKC-delta and -epsilon regulate NF-kappaB activation induced by cholecystokinin and TNF-alpha in pancreatic acinar cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 287, G582–G591. doi: 10.1152/ajpgi.00087.2004

Scholz, A., Heinze, S., Detjen, K. M., Peters, M., Welzel, M., Hauff, P., et al. (2003). Activated signal transducer and activator of transcription 3 (STAT3) supports the malignant phenotype of human pancreatic cancer. *Gastroenterology* 125, 891–905. doi: 10.1016/S0016-5085(03)01064-3

Sendler, M., Dummer, A., Weiss, F. U., Kruger, B., Wartmann, T., Scharffetter-Kochanek, K., et al. (2013). Tumour necrosis factor alpha secretion induces protease activation and acinar cell necrosis in acute experimental pancreatitis in mice. *Gut* 62, 430–439. doi: 10.1136/gutjnl-2011-300771

Senftleben, U., and Karin, M. (2002). The IKK/NF-kappa B pathway. *Crit. Care Med.* 30, S18–S26. doi: 10.1097/00003246-200201001-00003

Sennello, J. A., Fayad, R., Pini, M., Gove, M. E., Ponemone, V., Cabay, R. J., et al. (2008). Interleukin-18, together with interleukin-12, induces severe acute pancreatitis in obese but not in nonobese leptin-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 105, 8085–8090. doi: 10.1073/pnas.0804091105

Shi, C. S., Shenderov, K., Huang, N. N., Kabat, J., Abu-Asab, M., Fitzgerald, K. A., et al. (2012). Activation of autophagy by inflammatory signals limits IL-1beta production by targeting ubiquitinated inflammasomes for destruction. *Nat. Immunol.* 13, 255–263. doi: 10.1038/ni.2215

Shrikhande, S. V., Martignoni, M. E., Shrikhande, M., Kappeler, A., Ramesh, H., Zimmermann, A., et al. (2003). Comparison of histological features and inflammatory cell reaction in alcoholic, idiopathic and tropical chronic pancreatitis. *Br. J. Surg.* 90, 1565–1572. doi: 10.1002/bjs.4353

Shugrue, C. A., Alexandre, M., de Villalvilla, A. D., Kolodecik, T. R., Young, L. H., Gorelick, F. S., et al. (2012). Cerulein hyperstimulation decreases AMP-activated protein kinase levels at the site of maximal zymogen activation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 303, G723–G732. doi: 10.1152/ajpgi.00082.2012

Siegel, R., Desantis, C., Virgo, K., Stein, K., Mariotto, A., Smith, T., et al. (2012). Cancer treatment and survivorship statistics, 2012. *CA Cancer J. Clin.* 62, 220–241. doi: 10.3322/caac.21149

Singh, N., Das, P., Datta Gupta, S., Sahni, P., Pandey, R. M., Gupta, S., et al. (2013). Prognostic significance of extracellular matrix degrading enzymes-cathepsin L and matrix metalloproteases-2 [MMP-2] in human pancreatic cancer. *Cancer Invest.* 31, 461–471. doi: 10.3109/07357907.2013.820318

Smit, V. T., Boot, A. J., Smits, A. M., Fleuren, G. J., Cornelisse, C. J., and Bos, J. L. (1988). KRAS codon 12 mutations occur very frequently in pancreatic adenocarcinomas. *Nucleic Acids Res.* 16, 7773–7782. doi: 10.1093/nar/16.16.7773

Spanier, B. W., Dijkgraaf, M. G., and Bruno, M. J. (2008). Trends and forecasts of hospital admissions for acute and chronic pancreatitis in the Netherlands. *Eur. J. Gastroenterol. Hepatol.* 20, 653–658. doi: 10.1097/MEG.0b013e3282f52f83

Steele, C. W., Jamieson, N. B., Evans, T. R., McKay, C. J., Sansom, O. J., Morton, J. P., et al. (2013). Exploiting inflammation for therapeutic gain in pancreatic cancer. *Br. J. Cancer* 108, 997–1003. doi: 10.1038/bjc.2013.24

Stienstra, R., van Diepen, J. A., Tack, C. J., Zaki, M. H., van de Veerdonk, F. L., Perera, D., et al. (2011). Inflammasome is a central player in the induction of obesity and insulin resistance. *Proc. Natl. Acad. Sci. U.S.A.* 108, 15324–15329. doi: 10.1073/pnas.1100255108

Strowig, T., Henao-Mejia, J., Elinav, E., and Flavell, R. (2012). Inflammasomes in health and disease. *Nature* 481, 278–286. doi: 10.1038/nature10759

Sun, S. C. (2011). Non-canonical NF-kappaB signaling pathway. *Cell Res.* 21, 71–85. doi: 10.1038/cr.2010.177

Sun, S. C. (2012). The noncanonical NF-kappaB pathway. *Immunol. Rev.* 246, 125–140. doi: 10.1111/j.1600-065X.2011.01088.x

Sun, W. H., Chen, G. S., Ou, X. L., Yang, Y., Luo, C., Zhang, Y., et al. (2009). Inhibition of COX-2 and activation of peroxisome proliferator-activated receptor gamma synergistically inhibits proliferation and induces apoptosis of human pancreatic carcinoma cells. *Cancer Lett.* 275, 247–255. doi: 10.1016/j.canlet.2008.10.023

Szabo, A., and Sahin-Toth, M. (2012). Increased activation of hereditary pancreatitis-associated human cationic trypsinogen mutants in presence of chymotrypsin C. *J. Biol. Chem.* 287, 20701–20710. doi: 10.1074/jbc.M112.360065

Takamura, A., Komatsu, M., Hara, T., Sakamoto, A., Kishi, C., Waguri, S., et al. (2011). Autophagy-deficient mice develop multiple liver tumors. *Genes Dev.* 25, 795–800. doi: 10.1101/gad.2016211

Threadgold, J., Greenhalf, W., Ellis, I., Howes, N., Lerch, M. M., Simon, P., et al. (2002). The N34S mutation of SPINK1 (PSTI) is associated with a familial pattern of idiopathic chronic pancreatitis but does not cause the disease. *Gut* 50, 675–681. doi: 10.1136/gut.50.5.675

Thrower, E. C., Diaz de Villalvilla, A. P., Kolodecik, T. R., and Gorelick, F. S. (2006). Zymogen activation in a reconstituted pancreatic acinar cell system. *Am. J. Physiol. Gastrointest. Liver Physiol.* 290, G894–G902. doi: 10.1152/ajpgi.00373.2005

Thrower, E. C., Osgood, S., Shugrue, C. A., Kolodecik, T. R., Chaudhuri, A. M., Reeve, J. R. Jr., et al. (2008). The novel protein kinase C isoforms -delta and -epsilon modulate caerulein-induced zymogen activation in pancreatic acinar cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 294, G1344–G1353. doi: 10.1152/ajpgi.00020.2008

Thrower, E. C., Wang, J., Cheriyan, S., Lugea, A., Kolodecik, T. R., Yuan, J., et al. (2009). Protein kinase C delta-mediated processes in cholecystokinin-8-stimulated pancreatic acini. *Pancreas* 38, 930–935. doi: 10.1097/MPA.0b013e3181b8476a

Treiber, M., Neuhofer, P., Anetsberger, E., Einwachter, H., Lesina, M., Rickmann, M., et al. (2011). Myeloid, but not pancreatic, RelA/p65 is required for fibrosis in a mouse model of chronic pancreatitis. *Gastroenterology* 141, 1473–1485, 1485.e1471–e1477. doi: 10.1053/j.gastro.2011.06.087

Van Acker, G. J., Saluja, A. K., Bhagat, L., Singh, V. P., Song, A. M., and Steer, M. L. (2002). Cathepsin B inhibition prevents trypsinogen activation and reduces pancreatitis severity. *Am. J. Physiol. Gastrointest. Liver Physiol.* 283, G794–G800. doi: 10.1152/ajpgi.00363.2001

Vincent, A., Herman, J., Schulick, R., Hruban, R. H., and Goggins, M. (2011). Pancreatic cancer. *Lancet* 378, 607–620. doi: 10.1016/S0140-6736(10)62307-0

Vonlaufen, A., Phillips, P. A., Xu, Z., Goldstein, D., Pirola, R. C., Wilson, J. S., et al. (2008). Pancreatic stellate cells and pancreatic cancer cells: an unholy alliance. *Cancer Res.* 68, 7707–7710. doi: 10.1158/0008-5472.CAN-08-1132

Vonlaufen, A., Phillips, P. A., Xu, Z., Zhang, X., Yang, L., Pirola, R. C., et al. (2011). Withdrawal of alcohol promotes regression while continued alcohol intake promotes persistence of LPS-induced pancreatic injury in alcohol-fed rats. *Gut* 60, 238–246. doi: 10.1136/gut.2010.211250

Walsby, E., Pearce, L., Burnett, A. K., Fegan, C., and Pepper, C. (2012). The Hsp90 inhibitor NVP-AUY922-AG inhibits NF-kappaB signaling, overcomes microenvironmental cytoprotection and is highly synergistic with fludarabine in primary CLL cells. *Oncotarget* 3, 525–534.

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.

Wartmann, T., Mayerle, J., Kahne, T., Sahin-Toth, M., Ruthenburger, M., Matthias, R., et al. (2010). Cathepsin L inactivates human trypsinogen, whereas cathepsin L-deletion reduces the severity of pancreatitis in mice. *Gastroenterology* 138, 726–737. doi: 10.1053/j.gastro.2009.10.048

Wei, H., Wei, S., Gan, B., Peng, X., Zou, W., and Guan, J. L. (2011). Suppression of autophagy by FIP200 deletion inhibits mammary tumorigenesis. *Genes Dev.* 25, 1510–1527. doi: 10.1101/gad.2051011

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., and Ferrante, A. W. Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 112, 1796–1808. doi: 10.1172/JCI19246

Weiss, F. U., Halangk, W., and Lerch, M. M. (2008). New advances in pancreatic cell physiology and pathophysiology. *Best Pract. Res. Clin. Gastroenterol.* 22, 3–15. doi: 10.1016/j.bpg.2007.10.017

Wharry, C. E., Haines, K. M., Carroll, R. G., and May, M. J. (2009). Constitutive non-canonical NFkappaB signaling in pancreatic cancer cells. *Cancer Biol. Ther.* 8, 1567–1576. doi: 10.4161/cbt.8.16.8961

Whitcomb, D. C. (2011). Genetics and alcohol: a lethal combination in pancreatic disease? *Alcohol. Clin. Exp. Res.* 35, 838–842. doi: 10.1111/j.1530-0277.2010.01409.x

Whitcomb, D. C. (2012). Genetics of alcoholic and nonalcoholic pancreatitis. *Curr. Opin. Gastroenterol.* 28, 501–506. doi: 10.1097/MOG.0b013e328356e7f3

Whitcomb, D. C., Gorry, M. C., Preston, R. A., Furey, W., Sossenheimer, M. J., Ulrich, C. D., et al. (1996). Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat. Genet.* 14, 141–145. doi: 10.1038/ng1096-141

White, E. (2012). Deconvoluting the context-dependent role for autophagy in cancer. *Nat. Rev. Cancer* 12, 401–410. doi: 10.1038/nrc3262

Wilson, J. S., and Apte, M. V. (2003). Role of alcohol metabolism in alcoholic pancreatitis. *Pancreas* 27, 311–315. doi: 10.1097/00006676-200311000-00007

Wilson, J. S., Apte, M. V., Thomas, M. C., Haber, P. S., and Pirola, R. C. (1992). Effects of ethanol, acetaldehyde and cholesteryl esters on pancreatic lysosomes. *Gut* 33, 1099–1104. doi: 10.1136/gut.33.8.1099

Wiseman, M. (2008). The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. *Proc. Nutr. Soc.* 67, 253–256. doi: 10.1017/S002966510800712X

Witt, H., Luck, W., Hennies, H. C., Classen, M., Kage, A., Lass, U., et al. (2000). Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. *Nat. Genet.* 25, 213–216. doi: 10.1038/76088

Xu, Z., Vonlaufen, A., Phillips, P. A., Fiala-Beer, E., Zhang, X., Yang, L., et al. (2010). Role of pancreatic stellate cells in pancreatic cancer metastasis. *Am. J. Pathol.* 177, 2585–2596. doi: 10.2353/ajpath.2010.090899

Xue, J., Nguyen, D. T., and Habtezion, A. (2012). Aryl hydrocarbon receptor regulates pancreatic IL-22 production and protects mice from acute pancreatitis. *Gastroenterology* 143, 1670–1680. doi: 10.1053/j.gastro.2012.08.051

Yachida, S., Jones, S., Bozic, I., Antal, T., Leary, R., Fu, B., et al. (2010). Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* 467, 1114–1117. doi: 10.1038/nature09515

Yadav, D., and Lowenfels, A. B. (2013). The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology* 144, 1252–1261. doi: 10.1053/j.gastro.2013.01.068

Yang, L., Li, P., Fu, S., Calay, E. S., and Hotamisligil, G. S. (2010). Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. *Cell Metab.* 11, 467–478. doi: 10.1016/j.cmet.2010.04.005

Yang, S., and Kimmelman, A. C. (2011). A critical role for autophagy in pancreatic cancer. *Autophagy* 7, 912–913. doi: 10.4161/auto.7.8.15762

Yang, S., Wang, X., Contino, G., Liesa, M., Sahin, E., Ying, H., et al. (2011). Pancreatic cancers require autophagy for tumor growth. *Genes Dev.* 25, 717–729. doi: 10.1101/gad.2016111

Ying, H., Kimmelman, A. C., Lyssiotis, C. A., Hua, S., Chu, G. C., Fletcher-Sananikone, E., et al. (2012). Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. *Cell* 149, 656–670. doi: 10.1016/j.cell.2012.01.058

Yokota, T., Denham, W., Murayama, K., Pelham, C., Joehl, R., and Bell, R. H. Jr. (2002). Pancreatic stellate cell activation and MMP production in experimental pancreatic fibrosis. *J. Surg. Res.* 104, 106–111. doi: 10.1006/jsre.2002.6403

Yonezawa, S., Higashi, M., Yamada, N., and Goto, M. (2008). Precursor lesions of pancreatic cancer. *Gut Liver* 2, 137–154. doi: 10.5009/gnl.2008.2.3.137

Yue, Z., Jin, S., Yang, C., Levine, A. J., and Heintz, N. (2003). Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc. Natl. Acad. Sci. U.S.A.* 100, 15077–15082. doi: 10.1073/pnas.2436255100

Zyromski, N. J., Mathur, A., Pitt, H. A., Lu, D., Gripe, J. T., Walker, J. J., et al. (2008). A murine model of obesity implicates the adipokine milieu in the pathogenesis of severe acute pancreatitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 295, G552–G558. doi: 10.1152/ajpgi.90278.2008

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Pancreatic cancer risk in hereditary pancreatitis

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Inflammation is part of the body's immune response in order to remove harmful stimuli—like pathogens, irritants or damaged cells—and start the healing process. Recurrent or chronic inflammation on the other side seems a predisposing factor for carcinogenesis and has been found associated with cancer development. In chronic pancreatitis mutations of the cationic trypsinogen (*PRSS1*) gene have been identified as risk factors of the disease. Hereditary pancreatitis (HP) is a rare cause of chronic pancreatic inflammation with an early onset, mostly during childhood. HP often starts with recurrent episodes of acute pancreatitis and the clinical phenotype is not very much different from other etiologies of the disease. The long-lasting inflammation however generates a tumor promoting environment and represents a major risk factor for tumor development This review will reflect our knowledge concerning the specific risk of HP patients to develop pancreatic cancer.

Keywords: cancer risk, pancreatitis, hereditary pancreatitis, PRSS1, chronic inflammation

INTRODUCTION

Chronic pancreatitis (CP) and carcinoma of the pancreas are common in Western countries. Incidence rates of CP range from 2 to 23 per 100,000 and are around 10 per 100,000 for the incidence and death rate of pancreatic cancer (Dufour and Adamson, 2003; Lévy et al., 2006; Ferlay et al., 2010; Spanier et al., 2013). However, not all CP develops into cancer, even not in the very long-standing cases, and the majority of patients presenting with pancreatic carcinoma have no history of CP.

In a recent pooled analysis by the International Pancreatic Cancer Case-Control Consortium (PanC4) Duell et al. reviewed a total of 5048 cancer cases and 10947 controls. Interestingly, only 6.2% of pancreatic cancer patients reported a history of pancreatitis. Duell et al. calculated a ∼5.6-fold increased pancreatic cancer risk in patients with a history of pancreatitis (Duell et al., 2012). In the first two years following diagnosis of pancreatitis, the risk is even higher (OR: 13.6), probably reflecting increased likelihood of cancer diagnosis in people undergoing medical investigations, and possible misdiagnosis of pancreatic cancer as pancreatitis. The type of pancreatitis was not determined in most of the evaluated studies, preventing a more detailed analysis of the specific risk of acute vs. CP.

Much more than a single inflammatory event, the recurrent or persistent chronic inflammation is regarded as an important risk factor for cancer development, not only in the pancreas, but in many different organs (Mantovani et al., 2008). Observations that tumors often arise at sites of chronic inflammation were first made in the nineteenth century (Balkwill and Mantovani, 2001). Since that time several lines of evidence, based on histologic findings of inflammatory cells in tumor samples and also genetic and molecular analyses have supported the general concept that inflammation and cancer are linked. In addition, epidemiologic studies have shown that chronic inflammation is associated with the development of several types of cancer. Factors that drive the chronic inflammation process are many-fold and include toxins like cigarette smoke, alcohol, microbial infection (helicobacter pylori), autoimmune diseases (M. Chron), inflammatory conditions of unknown origin, a genetic predisposition (hereditary pancreatitis) or a combination of several factors.

Numerous studies which analyzed the pancreatic cancer risk of CP patients reported considerably different results, probably reflecting methodological variation concerning the recruitment, diagnosis and evaluation of patients. This review will mainly focus on the question if pancreatic cancer is especially frequent in those patients that are predisposed to CP by the presence of a *PRSS1* mutation.

HEREDITARY PANCREATITIS

Hereditary pancreatitis is a rare cause of CP with an estimated frequency of 0.3 /100.000 in Western countries. In 1952, Comfort and Steinberg reported a family with hereditary CP over three generations. Affected patients had chronic relapsing pancreatitis with an unusual early onset of the disease (5–23 years) (Comfort and Steinberg, 1952). In 1996 Whitcomb et al. identified from a large HP family with an autosomal dominant inheritance pattern a first genetic defect of the cationic trysinogen gene (*PRSS1*) through sequencing analysis of the 7q35 chromosome region. They identified a G to A transition in exon 3 of the PRSS1 gene that encodes the replacement of Arginine 122 by Histidine (Whitcomb et al., 1996). Trypsins are digestive enzymes that are synthesized and secreted in large amounts by the acinar cells of the exocrine pancreas. Three different trypsinogen isoforms are known and cationic trypsinogen represents 2/3 of the total amount of trypsinogen in the pancreatic juice. Anionic trypsinogen accounts for another 1/3 of the trypsinogen, whereas mesotrypsin is expressed only in small traces. Trypsinogens are synthesized as enzymatically inactive pro-enzymes or zymogens that are stored and released from the secretory compartment of the acinar cell. Under physiological conditions trypsinogens are activated in the duodenum by enterokinase, which is produced by

cells of the duodenal mucosa and which cleaves the N-terminal activation peptide bond and releases the enzymatic activity of trypsins. Trypsin is the main digestive enzyme of the gastrointestinal tract and has autoactivation as well as autolysis properties. Influenced by ambient pH and calcium concentration the protein may therefore either tend to self-activation or self-destruction. Subtle changes in the trypsin protein structure seem sufficient to disrupt the mechanism of normal trypsin activation leading to increased premature intrapancreatic trypsin activation or impaired inactivation. Both ways *PRSS1* mutations may lead to enhanced trypsin activity which eventually increases the risk for recurrent pancreatic injury and inflammation. Since 1996 more than 30 different PRSS1 mutations have been identified (www.uni-leipzig.de/pancreasmutation). The majority of these mutations were reported only in one or a few families and the biochemical analysis of these mutations gave valuable insights in the disease mechanism. Some mutations like K23R, D22G, or D19A are localized in the area where enterokinase activation of trypsinogen occurs. These mutations were found to facilitate trypsin autoactivation (Geisz et al., 2013).

Autoactivation of cationic trypsinogen is also influenced by chymotrypsin C (CTRC), which opposes the trypsin activity by promoting trypsinogen and trypsin degradation (Szmola and Sahin-Tóth, 2007). Chymotrypsin C selectively cleaves the Leu81-Glu82 peptide bond within the Ca2+ binding loop of cationic trypsin. Further degradation and inactivation is then achieved through tryptic cleavage of the Arg122-Val123 peptide bond. Therefore, mutation of either Leu81 or Arg122 blocks chymotrypsin C-mediated trypsin degradation (Szabó and Sahin-Tóth, 2012). The mechanistic basis of increased trypsinogen activation involves either resistance to degradation (N29I, N29T, V39A, R122C, and R122H) and/or increased N-terminal processing by CTRC (A16V and N29I). In hereditary pancreatitis the CTRC-dependent control of cationic trypsinogen autoactivation is disturbed giving rise to intrapancreatic trypsinogen activation. Most frequent *PRSS1* mutations R122H and N29I lead with high penetrance (∼80%) to CP, in most cases with an early onset of symptoms. The A16V and R122C mutants were found to have a more variable disease penetrance ∼40–50% (De Las Heras-Castaño et al., 2009; Grocock et al., 2010). Apart from some variation in disease penetrance the clinical phenotypes of these most relevant HP mutations seem rather comparable and—with the exception of an early onset—resemble the same features of CP of other etiologies.

Lowenfels and colleagues from the International Hereditary Pancreatitis Study Group were one of the first to review the medical records of 246 patients with a diagnosis of HP. Comparison of observed and expected frequency of cancer in this historical group of patients revealed and standardized incidence ratio (SIR) of pancreatic cancer of 53 (95%CI: 23–105). In those individuals that developed pancreatic cancer the mean age at onset of symptoms of pancreatitis was 17.3 ± 6.9 years and mean age at diagnosis of pancreatic cancer was 56.9 ±11.2 years, indicating a high risk of pancreatic cancer several decades (39.6 ± 9.7 years) after the initial onset of pancreatitis(Lowenfels et al., 1997). The risk was not different in males or in females or for different nationalities and the cumulative risk in these patients until the age of 70 was 40%. The diagnosis of HP in the study was mainly based on early onset of pancreatitis, a positive family history and the absence of other known causes of pancreatitis. Today we know that many HP patients have an underlying causative *PRSS1* mutation, but at the time of the study by Lowenfels the genetic testing for *PRSS1* had only just started and therefore could not yet be systematically analyzed.

Such a genotype-phenotype correlation was done in 2004 by Howes et al. on behalf of the European registry of hereditary pancreatitis and pancreatic cancer (EUROPAC) (Howes et al., 2004). Their study cohort comprised 112 families (418 individuals) from 14 countries and included 52% R122H-families, 21% N29I-families, 4% A16V-families and 19% without detectable mutation. The high mutation rate of 81% in HP was much higher than previously reported and presumably due to the strict diagnostic criteria of HP by the EUROPAC group. The authors confirmed that onset of symptoms starts at young age for R122H mutation carriers with a median onset at 10 (95%CI: 8–12) and 14.5 (95%CI: 10–21) for mutation negative patients. Interestingly time to development of exocrine and endocrine failure showed no significant differences, neither by mutation status nor by gender. Still the cumulative risk for exocrine failure or diabetes is much higher in HP (60.2 and 68.6%) than in idiopathic or alcoholic pancreatitis patients. Pancreatic cancer was diagnosed in 26 (6%) patients and arose in individuals carrying any of the common mutations as well as in *PRSS1*-mutation negative families (14x R122H, 7x N29I, 1x A16V and 4x no *PRSS1* mutation). The time to develop cancer was not influenced by mutation status, gender or if the mutation was transmitted from the father or the mother. The study further showed that the cumulative risk of pancreatic cancer is rather negligible until the age of 50 (3.4%) in both sexes. However, after 50 years the risk of pancreatic cancer rises considerably, reaching 18.8% at 70 years and 33.3% at 80 years. In other words: the cumulative risk of pancreatic cancer after onset of symptoms slowly increases from 1.5% at 20 years and 2.5% at 30 years after first symptoms to 25.3% at 60 years and 44% at 70 years after the onset of the disease. The calculated SIR of pancreatic cancer in the EUROPAC cohort after correction for age, smokers, nationality and surgical intervention, was 67 (95%CI: 50–82).

In a national series in 2008 Rebours et al. investigated the SIR of pancreatic adenocarcinoma in an exhaustive cohort of French HP patients (Rebours et al., 2012). In their nation-wide survey genetic laboratories, pediatricians and gastroenterologists contributed 200 individuals from 78 families with know *PRSS1* mutation or the diagnosis of recurrent acute or CP in the absence of known precipitating factors. *PRSS1* mutations were present in 68% (78% R122H, 12% N29I, 10% others) of the study cohort and again the *PRSS1* mutation type did not correlate with the development of pancreatic cancer, which was diagnosed in ten individuals at a median age of 55. The cumulative risk at age 50 was ∼10% and increased to ∼50% at age 75. The SIR of pancreatic cancer in the French cohort was 87 (95%CI: 42–113) for the whole population and seemed higher in females (142; 95%CI: 38–225) compared to males (69; 95%CI: 25–150). Whereas Lowenfels et al. also found a slightly higher SIR in females (73 vs. 46) the results from the EUROPAC study indicated

a higher SIR in men (72 vs. 60). A gender impact therefore seems not generally operative in the development of pancreatic adenocarcinoma.

In comparison to the general population HP patients clearly carry an increased absolute risk of developing pancreatic cancer. Smoking was identified as a main associated risk factor and HP patients therefore should be strongly advised to stay away from tobacco consumption. Diabetes and calcifications are also more frequently seen in patients that develop pancreatic cancer, probably indicating a correlation of the cancer risk not only with the duration but also with the severity of pancreatitis.

PANCREATIC CANCER RISK IN SPORADIC PANCREATITIS OF MUTATION CARRIERS

In the clinical situation HP is diagnosed mainly in patients with idiopathic recurrent acute or CP families. However, sometimes also sporadic cases without a corresponding family history have a positive finding of an HP mutation. Inheritance from unaffected carrier parents, uncertain paternity and spontaneous *de novo* mutations must be considered in such cases (Simon et al., 2002). A recent study by Hamoir et al. identified a total of 17.4% carriers of *CFTR*, *PRSS1*, or *SPINK1* mutations in a cohort of 351 Belgium patients with idiopathic recurrent or CP and no family history (Hamoir et al., 2013). The authors claim that the clinical features were not influenced by the presence of a gene mutation except for an earlier age at onset and a higher incidence of pancreatic cancer, especially in patients with a *CFTR* mutation (four cancer patients had *CFTR* mutations, one a *PRSS1* mutation). The SIR for pancreatic cancer in their cohort was 26.5 (95%CI: 8.6–61.9). However, all cancer patients were also smokers. The authors suggest to "include patients with *CFTR* variants presenting with risk factors in a screening and surveillance program and to strongly advise them not to smoke." Three of the four cancer patients with CFTR mutation carried the p.L997F mutation (2× compound heterozygous, 1× heterozygous) which also had been identified at high frequency in patients with recurrent idiopathic pancreatitis (Gomez Lira et al., 2000). Whereas there is no disagreement concerning the adequacy of a non-smoking advice other reports find a modest increased risk for carriers of disease-relevant *CFTR* mutations (OR:1.4; 95%CI: 1.04–1.89) and are more reluctant concerning the role of *CFTR* mutations as risk factors of pancreatic cancer (Whitcomb, 2004; McWilliams et al., 2010).

CHRONIC INFLAMMATION AND CANCEROGENESIS

The link between CP and pancreatic cancer is unknown to date, but several signaling pathways were identified to become activated in the inflamed pancreas which may trigger cellular transformation and ultimately stimulate the development of pancreatic cancer.

It is generally accepted that inflammation results in repeated DNA damage, error-prone repair-mechanisms and the progressive accumulation of genetic mutations. In the pancreas pre-cancerous histologic changes have been described that are associated with a sequential accumulation of genetic defects. These pancreatic intra-epithelial neoplasms (PanIN) are present in sporadic pancreatic adenocarcinomas and also in patients with a history of CP. Histologically, PanINs progress through stages of increasing architectural and cytological atypia, starting from a low grade dysplasia (PanIN-1A, PanIN-1B) to moderate dysplasia (PanIN-2) and to high grade dysplasia (PanIN-3). First genetic mutations seen in the early stages include *kRas* mutations, followed by *p16/CDKN2A*, *TP53*, and *SMAD4/DPC4* (Hruban et al., 2004). Mutations in all four genes have been recognized as driver mutations that trigger neoplastic transformation and tumor progression (Korc, 2010). In a mouse model kRas mutations were shown to give rise to pancreatic intraepithelial neoplasms and pancreatic cancer and that concomitant mutation of p16, p53 or smad4 greatly enhanced the process of carcinoma formation (Hingorani et al., 2005). These mutations are more frequent in advanced PanIN stages and are well-characterized in invasive pancreatic carcinoma.

Signaling mechanisms involving Hedgehog and Notch, as well as cyclooxygenase 2 (*COX-2*) have also been implicated in the triggering mechanisms that stimulate the generation of pancreatic cancer from pancreatic inflammation (Maitra et al., 2002; Avila and Kissil, 2013; Hamada et al., 2013). COX-2 mediates prostaglandine synthesis which triggers cell proliferation and cytokine synthesis. Cox-2 inhibitors have been demonstrated to have anti-cancer effects and are effective especially in patients with cancers that have a high COX-2 expression. Extensive inflammation exposes the organ tissue to pro-inflammatory cytokines and reactive oxygen species. Local production of both may activate cellular protective mechanisms, including apoptosis and regenerating mechanisms that stimulate cell proliferation in order to rebuild the lost tissue structure. Increased proliferation in the presence of elevated concentrations of potential mutagens such as reactive oxygen species may set an environment where growth promoting mutations accumulate and provide selective growth advantage for individual cell clones.

Another signaling pathway that has been suggested to drive cancerogenesis from inflammation involves NFkB (Karin, 2006). Important cancer-associated genes, such as c-myc, jun B Cyclin D1, TP53, and VEGF are under the control of this transcription factor. In addition it's a major factor controlling the ability of malignant cells to resist apoptosis-based tumor-surveillance mechanisms.

PERSPECTIVE

PANCREATIC CANCER SURVEILLANCE

Today there is no rationale for early diagnostic screening of pancreatic cancer in the general population. It's a rare disease, specific diagnostic markers are missing and a survival benefit of such screening programs has nowhere been demonstrated. However, pancreatic cancer screening is recommended for families and individuals at an elevated risk. Counseling and surveillance guidelines have been established that recommend screening studies as part of peer-reviewed protocols with scientific evaluation and human subjects protection(Brand et al., 2007). Candidates for pancreatic cancer surveillance should carry a >10-fold increased risk for developing pancreatic cancer, which includes individuals with HP.

SURGERY

Generally, the survival rate for patients with CP is poor (Jupp et al., 2010). CP patients tend to die of other causes such as smoking related cancers, cardiovascular disease and alcoholic liver cirrhosis. The potential cancer risk of a persistent inflammation may suggest beneficial effects of anti-inflammatory therapy or surgery for CP. A recent retrospective multicenter study from Japan investigated associated factors with the pancreatic cancer risk in 506 patients with CP (Ueda et al., 2013). Nineteen of 506 enrolled patients developed pancreatic cancer (3.7%) with a SIR of 11.8 (95% CI, 7.1–18.4). Interestingly, among 9 patients with HP, no patient developed pancreatic cancer (follow-up period: 3.4–43.8 years, median, 8.4 years). Among the 352 CP patients who had not received surgical treatment a total of 18 patients (5.1%) developed pancreatic cancer, which otherwise occurred in only 1 (0.7%) of the 147 patients who had undergone surgery for CP. Apparently surgery for CP inhibits the development of pancreatic cancer in those patients.

In addition the study confirmed that patients who continued to drink alcohol after the diagnosis of CP showed a significant higher incidence of pancreatic cancer than those who stopped drinking.

BIOMARKER

The goal for diagnostic screening is the identification of early cancer lesions before the onset of metastasis and tissue invasion. Until today no biomarker in plasma or serum has generally been recommended for screening or diagnosing of pancreatic cancer and there is an urgent need to identify novel markers of pancreatic cancer. The search is on for new strategies that help to improve the sensitivity and specificity of diagnostic procedures.

One example is a study of Yokoi et al. who analyzed proteins from circulating mononuclear cells (MNC) to identify surrogate markers of pancreatic cancer (Yokoi et al., 2011). Continuous interactions between tumor cells and host stroma cells is a fundamental requirement for tumor cell growth, invasion, and metastasis (Fidler et al., 2007). In histologic stainings the stroma typically occupies 70–90% of pancreatic tumors. Among the cellular components of the stroma, MNCs are believed to play a central role in the progression and chemoresistance of tumors (Condeelis and Pollard, 2006; Noonan et al., 2008). Circulating MNCs, such as monocytes and/or macrophages, are recruited into the tumor microenvironment, where they extravasate and differentiate into tumor-associated macrophages (TAMs) (Shojaei et al., 2008). Even small tumors could generate a detectable immune response that may include changes in protein content or phosphorylation of MNCs. Analysing circulating MNCs in a nude mouse model of orthotopic human pancreatic cancer, Yokoi et al. found significant higher Src-expression (c-src tyrosinkinase) and phosphorylation in MNCs from mice bearing tumors. The identified surrogate marker Src may not be a convincing finding so far, but circulating MNCs may represent a good source for the identification of novel biomarkers of early tumor development.

In summary HP markedly increases the risk for pancreatic adenocarcinoma. *PRSS1* and other pancreatitis-associated gene mutations are not directly important in the development of pancreatic cancer, but rather lead to a high-risk inflammatory milieu for the accumulation of oncogenic mutations. The risk is potentiated by known cofactors such as tobacco smoking and, likely, by genetic factors that are yet to be identified. Future genetic and molecular studies will help to a better understanding of the relationship between inflammation and cancerogenesis and may lead to new diagnostic and therapeutic possibilities for those subjects with CP that are at high risk of developing pancreatic carcinoma.

REFERENCES

Avila, J. L., and Kissil, J. L. (2013). Notch signaling in pancreatic cancer: oncogene or tumor suppressor? *Trends Mol. Med.* 19, 320–327. doi: 10.1016/j.molmed.2013.03.003

Balkwill, F., and Mantovani, A. (2001). Inflammation and cancer: back to Virchow? *Lancet* 357, 539–545. doi: 10.1016/S0140-6736(00)04046-0

Brand, R. E., Lerch, M. M., Rubinstein, W. S., Neoptolemos, J. P., Whitcomb, D. C., Hruban, R. H., et al. (2007). Advances in counselling and surveillance of patients at risk for pancreatic cancer. *Gut* 56, 1460–1469. doi: 10.1136/gut.2006.108456

Comfort, M. W., and Steinberg, A. G. (1952). Pedigree of a family with hereditary chronic relapsing pancreatitis. *Gastroenterology* 21, 54–63.

Condeelis, J., and Pollard, J. W. (2006). Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* 124, 263–266. doi: 10.1016/j.cell.2006.01.007

De Las Heras-Castaño, G., Castro-Senosiaín, B., Fontalba, A., López-Hoyos, M., and Sánchez-Juán, P. (2009). Hereditary pancreatitis: clinical features and inheritance characteristics of the R122C mutation in the cationic trypsinogen gene (PRSS1) in six Spanish families. *JOP* 10, 249–255.

Duell, E. J., Lucenteforte, E., Olson, S. H., Bracci, P. M., Li, D., Risch, H. A., et al. (2012). Pancreatitis and pancreatic cancer risk: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). *Ann. Oncol.* 23, 2964–2970. doi: 10.1093/annonc/mds140

Dufour, M. C., and Adamson, M. D. (2003). The epidemiology of alcohol-induced pancreatitis. *Pancreas* 27, 286–290. doi: 10.1097/00006676-200311000-00002

Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C., and Parkin, D. M. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer* 127, 2893–2917. doi: 10.1002/ijc.25516

Fidler, I. J., Kim, S. J., and Langley, R. R. (2007). The role of the organ microenvironment in the biology and therapy of cancer metastasis. *J. Cell. Biochem.* 101, 927–936. doi: 10.1002/jcb.21148

Geisz, A., Hegyi, P., and Sahin-Tóth, M. (2013). Robust autoactivation, chymotrypsin C independence and diminished secretion define a subset of hereditary pancreatitis-associated cationic trypsinogen mutants. *FEBS J.* 280, 2888–2899. doi: 10.1111/febs.12292

Gomez Lira, M., Benetazzo, M. G., Marzari, M. G., Bombieri, C., Belpinati, F., Castellani, C., et al. (2000). High frequency of cystic fibrosis transmembrane regulator mutation L997F in patients with recurrent idiopathic pancreatitis and in newborns with hypertrypsinemia. *Am. J. Hum. Genet.* 66, 2013–2014. doi: 10.1086/302928

Grocock, C. J., Rebours, V., Delhaye, M. N., Andrén-Sandberg, A., Weiss, F. U., Mountford, R., et al. (2010). The variable phenotype of the p.A16V mutation of cationic trypsinogen (PRSS1) in pancreatitis families. *Gut* 59, 357–363. doi: 10.1136/gut.2009.186817

Hamada, S., Masamune, A., and Shimosegawa, T. (2013). Novel therapeutic strategies targeting tumor-stromal interactions in pancreatic cancer. *Front. Physiol.* 4:331. doi: 10.3389/fphys.2013.00331

Hamoir, C., Pepermans, X., Piessevaux, H., Jouret-Mourin, A., Weynand, B., Habyalimana, J. B., et al. (2013). Clinical and morphological characteristics of sporadic genetically determined pancreatitis as compared to idiopathic pancreatitis: higher risk of pancreatic cancer in CFTR variants. *Digestion* 87, 229–239. doi: 10.1159/000348439

Hingorani, S. R., Wang, L., Multani, A. S., Combs, C., Deramaudt, T. B., Hruban, R. H., et al. (2005). Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell* 7, 469–483. doi: 10.1016/j.ccr.2005.04.023

Howes, N., Lerch, M. M., Greenhalf, W., Stocken, D. D., Ellis, I., Simon, P., et al. (2004). Clinical and genetic characteristics of hereditary pancreatitis in Europe. *Clin. Gastroenterol. Hepatol.* 2, 252–261. doi: 10.1016/S1542-3565(04)00013-8

Hruban, R. H., Takaori, K., Klimstra, D. S., Adsay, N. V., Albores-Saavedra, J., Biankin, A. V., et al. (2004). An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. *Am. J. Surg. Pathol.* 28, 977–987. doi: 10.1097/01.pas.0000126675.59108.80

Jupp, J., Fine, D., and Johnson, C. D. (2010). The epidemiology and socioeconomic impact of chronic pancreatitis. *Best Pract. Res. Clin. Gastroenterol.* 24, 219–231. doi: 10.1016/j.bpg.2010.03.005

Karin, M. (2006). Nuclear factor-kappaB in cancer development and progression. *Nature* 441, 431–436. doi: 10.1038/nature04870

Korc, M. (2010). Driver mutations: a roadmap for getting close and personal in pancreatic cancer. *Cancer Biol. Ther.* 10, 588–591. doi: 10.4161/cbt.10.6.13128

Lévy, P., Barthet, M., Mollard, B. R., Amouretti, M., Marion-Audibert, A. M., and Dyard, F. (2006). Estimation of the prevalence and incidence of chronic pancreatitis and its complications. *Gastroenterol. Clin. Biol.* 30, 838–844. doi: 10.1016/S0399-8320(06)73330-9

Lowenfels, A. B., Maisonneuve, P., Dimagno, E. P., Elitsur, Y., Gates, L. K., Perrault, J., et al. (1997). Hereditary pancreatitis and the risk of pancreatic cancer. International Hereditary Pancreatitis Study Group. *J. Natl. Cancer Inst.* 89, 442–446. doi: 10.1093/jnci/89.6.442

Maitra, A., Ashfaq, R., Gunn, C. R., Rahman, A., Yeo, C. J., Sohn, T. A., et al. (2002). Cyclooxygenase 2 expression in pancreatic adenocarcinoma and pancreatic intraepithelial neoplasia: an immunohistochemical analysis with automated cellular imaging. *Am. J. Clin. Pathol.* 118, 194–201. doi: 10.1309/TPG4-CK1C-9V8V-8AWC

Mantovani, A., Allavena, P., Sica, A., and Balkwill, F. (2008). Cancer-related inflammation. *Nature* 454, 436–444. doi: 10.1038/nature07205

McWilliams, R. R., Petersen, G. M., Rabe, K. G., Holtegaard, L. M., Lynch, P. J., Bishop, M. D., et al. (2010). Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations and risk for pancreatic adenocarcinoma. *Cancer* 116, 203–209.

Noonan, D. M., De Lerma Barbaro, A., Vannini, N., Mortara, L., and Albini, A. (2008). Inflammation, inflammatory cells and angiogenesis: decisions and indecisions. *Cancer Metastasis Rev.* 27, 31–40. doi: 10.1007/s10555-007-9108-5

Rebours, V., Lévy, P., and Ruszniewski, P. (2012). An overview of hereditary pancreatitis. *Dig. Liver Dis.* 44, 8–15. doi: 10.1016/j.dld.2011.08.003

Shojaei, F., Zhong, C., Wu, X., Yu, L., and Ferrara, N. (2008). Role of myeloid cells in tumor angiogenesis and growth. *Trends Cell Biol.* 18, 372–378. doi: 10.1016/j.tcb.2008.06.003

Simon, P., Weiss, F. U., Zimmer, K. P., Rand, S., Brinkmann, B., Domschke, W., et al. (2002). Spontaneous and sporadic trypsinogen mutations in idiopathic pancreatitis. *JAMA* 288, 2122. doi: 10.1001/jama.288.17.2122

Spanier, B., Bruno, M. J., and Dijkgraaf, M. G. (2013). Incidence and mortality of acute and chronic pancreatitis in the Netherlands: a nationwide record-linked cohort study for the years 1995-2005. *World J. Gastroenterol.* 19, 3018–3026. doi: 10.3748/wjg.v19.i20.3018

Szabó, A., and Sahin-Tóth, M. (2012). Increased activation of hereditary pancreatitis-associated human cationic trypsinogen mutants in presence of chymotrypsin C. *J. Biol. Chem.* 287, 20701–20710. doi: 10.1074/jbc.M112.360065

Szmola, R., and Sahin-Tóth, M. (2007). Chymotrypsin C (caldecrin) promotes degradation of human cationic trypsin: identity with Rinderknecht's enzyme Y. *Proc. Natl. Acad. Sci. U.S.A.* 104, 11227–11232. doi: 10.1073/pnas.0703714104

Ueda, J., Tanaka, M., Ohtsuka, T., Tokunaga, S., Shimosgawa, T., and Research Committee of Intractable Diseases of the Pancreas. (2013). Surgery for chronic pancreatitis decreases the risk for pancreatic cancer: a multicenter retrospective analysis. *Surgery* 153, 357–364. doi: 10.1016/j.surg.2012.08.005

Whitcomb, D. C. (2004). Inflammation and Cancer V. Chronic pancreatitis and pancreatic cancer. *Am. J. Physiol. Gastrointest Liver Physiol.* 287, G315–G319. doi: 10.1152/ajpgi.00115.2004

Whitcomb, D. C., Gorry, M. C., Preston, R. A., Furey, W., Sossenheimer, M. J., Ulrich, C. D., et al. (1996). Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat. Genet.* 14, 141–145. doi: 10.1038/ng1096-141

Yokoi, K., Hawke, D., Oborn, C. J., Jang, J. Y., Nishioka, Y., Fan, D., et al. (2011). Identification and validation of SRC and phospho-SRC family proteins in circulating mononuclear cells as novel biomarkers for pancreatic cancer. *Transl. Oncol.* 4, 83–91. doi: 10.1593/tlo.10202

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Genetic determinants and potential therapeutic targets for pancreatic adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer deaths in both men and women in the United States, carrying a 5-year survival rate of approximately 5%, which is the poorest prognosis of any solid tumor type. Given the dismal prognosis associated with PDAC, a more thorough understanding of risk factors and genetic predisposition has important implications not only for cancer prevention, but also for screening techniques and the development of personalized therapies. While screening of the general population is not recommended or practicable with current diagnostic methods, studies are ongoing to evaluate its usefulness in people with at least 5- to 10-fold increased risk of PDAC. In order to help identify high-risk populations who would be most likely to benefit from early detection screening tests for pancreatic cancer, discovery of additional pancreatic cancer susceptibility genes is crucial. Thus, specific gene-based, gene-product, and marker-based testing for the early detection of pancreatic cancer are currently being developed, with the potential for these to be useful as potential therapeutic targets as well. The goal of this review is to provide an overview of the genetic basis for PDAC with a focus on germline and familial determinants. A discussion of potential therapeutic targets and future directions in screening and treatment is also provided.

Keywords: pancreatic ductal adenocarcinoma, familiar pancreatic cancer, pancreatic cancer syndromes, pancreatic cancer oncogenes, pancreatic cancer tumor suppressor genes

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer deaths in both men and women in the United States, carrying a 5-year survival rate of approximately 5% (Klein, 2012), which is the poorest prognosis of any solid tumor type. Such outcomes are largely due to the fact that 80% of patients have locally advanced or metastatic disease at diagnosis (Siegel et al., 2013). Furthermore, for the 10–20% of patients who present with resectable disease, the overall 5-year survival rate is only 15–20% and median survival is a dismal 18–24 months (Ducreux et al., 2007). PDAC accounts for approximately 90% of pancreatic neoplasms and is synonomous with the term, "pancreatic cancer;" the remaining 15% of pancreatic tumors are represented by acinar cell carcinoma, pancreatoblastoma, solid pseudopapillary neoplasm, serous cystadenoma and pancreatic neuroendocrine tumors (Li et al., 2004a; Hezel et al., 2006; Maitra et al., 2006; Ducreux et al., 2007). Given the dismal prognosis associated with PDAC, a more thorough understanding of risk factors and genetic predisposition has important implications not only for cancer prevention, but also for development of personalized therapies. The goal of this review is to provide an overview of the genetic basis for PDAC with a focus on germline and familial determinants, with a discussion of potential therapeutic targets also provided.

INHERITED RISK FACTORS

Increasing knowledge of inherited genetic mutations is leading to a better understanding of pancreatic cancer risk, as these genetic variations are known to contribute to both familial and non-familial (sporadic) PDAC. Studies have estimated up to 10% of patients demonstrate an inherited predisposition to PDAC based on familial clustering (Lynch et al., 1990, 1996; Hruban et al., 1998; Schenk et al., 2001; Del Chiaro et al., 2007; Hruban et al., 2010), while two prospective studies from Sweden and Germany have suggested lower rates of 2.7 and 1.9%, respectively (Hemminki and Li, 2003; Bartsch et al., 2004). A systematic review by Permuth-Wey and Egan revealed the proportion of their study population with a positive family history of pancreatic cancer was only 1.3%. In the latter study, the lower rate was attributed to adjusting for shared environmental factors, such as smoking. Additionally, a majority of the weight (82%) of the meta-analysis was contributed by a prospective cohort study, as opposed to case-control studies, which inherently pose potential for increased biases, such as recall and publication (Permuth-Wey and Egan, 2009).

An inherited predisposition to PDAC is believed to occur in three distinct clinical settings. Firstly, familial cancer syndromes have a well-known association. Peutz-Jeghers Syndrome (PJS), which is associated with germline mutations in the STK11/LKB1 gene, leads to a 36% lifetime risk for pancreatic cancer (Hahn et al., 2003); similarly, Familial Atypical Multiple Mole Melanoma (FAMMM) syndrome, which results due to germline mutations in the p16/CDKN2A gene, leads to an approximate 17% lifetime risk for pancreatic cancer (Hahn et al., 2003); other syndromes include Hereditary Breast-Ovarian Cancer (HBOC) syndrome (BRCA1/2 genes), Hereditary Non-polyposis Colorectal Cancer (HNPCC; mismatch repair genes), and Familial Adenomatous Polyposis (FAP) syndrome (APC gene) (**Table 1**). Secondly,

Table 1 | Pancreatic cancer susceptibility genes.

Genes	Associated syndrome	Freq. of mutation (%)	PDAC risk	References
ONCOGENE				
BRAF		30		Maitra et al., 2006; Koorstra et al., 2008
AKT2		10–60		Koorstra et al., 2008
KRAS		30–100		Hezel et al., 2006; Koorstra et al., 2008
TUMOR SUPP				
BRCA1	HBOC		2.0–2.5×	Thompson et al., 2002; Hahn et al., 2003; Lynch et al., 2008; Ferrone et al., 2009
BRCA2	HBOC	3–10	3.5×	Hahn et al., 2003; Koorstra et al., 2008; Lynch et al., 2008; Klein, 2012
PALB2		3–5	10–32×	Jones et al., 2009; Slater et al., 2010; Schneider et al., 2011; Klein, 2012
PTEN				
p16/CDKN2A	FAMMM	80–95	13–22 ×17% LR	Koorstra et al., 2008; Lynch et al., 2008; Bartsch et al., 2012; Klein, 2012
MMR (MLH1,2)	HNPCC	4	3.7% LR	Koorstra et al., 2008; Bartsch et al., 2012; Klein, 2012
APC	FAP		4.5 ×<5% LR	Goggins et al., 2000; Bartsch et al., 2012
TP53	Li-Fraumeni	75–85	7.3×	Koorstra et al., 2008; Bartsch et al., 2012
ATM		<10	2.4×	Maitra et al., 2006; Koorstra et al., 2008
SMAD4/DPC4		50–60		Hezel et al., 2006; Maitra et al., 2006; Koorstra et al., 2008
STK11/KKB1	Peutz-Jeghers		35% lifetime	

HBOC, hereditary breast and ovarian cancer; FAMMM, familial atypical multiple mole melanoma syndrome; FAP, familial adenomatous polyposis; LR, lifetime risk; HNPCC, hereditary non-polyposis colorectal cancer.

hereditary causes of pancreatitis, such as the autosomal dominant form caused by germline mutations of the cationic trypsinogen gene, PRSS1, have been indirectly linked to PDAC through early onset chronic pancreatitis with an associated 53-fold increased incidence and approximately 40% of hereditary pancreatitis patients noted to develop pancreatic cancer by age 70 (Hahn et al., 2003; Hezel et al., 2006; Koorstra et al., 2008). Finally, Familial Pancreatic Cancer (FPC) is defined as two or more first-degree relatives having pancreatic cancer without fulfilling criteria for one of the familial cancer syndromes noted above. Although at significantly increased risk for PDAC, pancreatic cancer patients with a hereditary predisposition have not shown any significant difference in clinical course or median survival when compared to sporadic pancreatic cancer patients (James et al., 2004).

FAMILIAL PANCREATIC CANCER

The presence of an inherited genetic component and possibility of a hereditary pancreatic cancer syndrome was first suggested by several case reports describing familial aggregation of pancreatic cancers (MacDermott and Kramer, 1973; Reimer et al., 1977; Ehrenthal et al., 1987). Lynch et al performed the first systematic study of 18 families with pancreatic cancer in 1990 and subsequent case-control and cohort studies have shown that individuals with a family history of PDAC are at an increased risk of developing pancreatic cancer themselves (Lynch et al., 1990, 1996; Klein et al., 2001). Furthermore, the odds of having a family history of PDAC are 1.9- to 13-fold higher in pancreatic cancer patients compared to healthy controls (Ghadirian et al., 1991; Jacobs et al., 2010; Klein, 2012). Jacobs et al performed a pooled analysis of data from 5 cohort and one case-control study, which estimated the odds of pancreatic cancer to be 1.76-fold higher (95% CI = 1.19–2.61) among individuals with at least one first-degree relative with PDAC compared to those without a family history. This risk was noted to be even higher in those individuals with at least two first-degree relatives with PDAC with an OR = 4.26 (95% CI = 0.48–37.79) (Jacobs et al., 2010). Tersmette et al similarly noted an increased risk among those with a family history of pancreatic cancer, specifically noting that individuals with a pair of affected first-degree relatives had an 18-fold increased risk of developing PDAC and an estimated lifetime risk of 9–18%, while there was an even more significant 57-fold increased risk in FPC kindred with three or more affected family members when compared to the SEER age-adjusted incidence of pancreatic cancer in the US (Tersmette et al., 2001). The National Familial Pancreas Tumor Registry (NFPTR) has similarly concluded that the risk of pancreatic cancer increases with the number of affected first degree relatives (RR of 6.4 with two first-degree relatives; 32% with three first-degree relatives) (Klein et al., 2004). Based on such conclusive findings, the clinical entity of FPC has been defined (Tersmette et al., 2001; Hahn et al., 2003; Rulyak et al., 2003; Brand et al., 2007; Bartsch et al., 2012; Klein, 2012).

The inheritance pattern of FPC is mostly autosomal dominant and demonstrates a heterogenous phenotype (Slater et al., 2010) The genetic mutations responsible for the majority of clustering in families with PDAC have yet to be identified, although germline mutations in high-penetrance genes such as BRCA2 and PALB2 have been established along with mutations in p16/CDKN2A, STK11/LKB1, PRSS1, BRCA1, mismatch repair genes (hMLH1, hMSH2, hMSH6), VHL, and ATM (**Table 1**). Despite sharing many of the same genetic mutations associated with well-established familial cancer syndromes, FPC patients must not fulfill criteria for one of the familial cancer syndromes, and thus likely represent phenotypic variants with the associated influence of environmental risk factors. Brand and Lynch noted that the heterogeneity seen within pancreatic cancer cases in both FPC and familial cancer syndromes may indeed be due to the fact

these are similar entities that fall along a spectrum. Furthermore, they suggested that the heterogeneity of FPC may actually lie in the varying penetrance of the associated deleterious mutations and the interplay of non-genetic factors, such as environmental risk factors noted above (Brand and Lynch, 2006). The exact relationship between affected family members is also an important indicator of risk and serves as the basis of quantitative risk modeling and prediction tools, such as PancPRO, which is a Bayesian prediction model developed at Johns Hopkins as an extension of BRCAPRO and validated using an FPC registry with an observed-to-predicted pancreatic cancer ratio of 0.83 (Wang et al., 2007; Klein, 2012).

Whereas the incidence of sporadic pancreatic cancer dramatically increases with age, with a peak incidence in the seventh to eighth decade, studies examining the impact of age in FPC patients have yielded inconclusive results. Some studies have suggested a younger age of onset (8–10 years younger) in individuals with a family history or known germline mutation (i.e., BRCA2), while other studies have shown no association with age of onset in those with a hereditary predisposition (Lynch et al., 1990; Phelan et al., 1996; Ozcelik et al., 1997; Hruban et al., 1999; Silverman et al., 1999; Lal et al., 2000; Schenk et al., 2001; Hahn et al., 2003; James et al., 2004; Hezel et al., 2006; Brune et al., 2010; Schneider et al., 2011; Klein, 2012). This may be explained by epigenetic factors and the interaction between genetic and environmental factors in the development of pancreatic cancer (McFaul et al., 2006). This differs from the data on early-onset breast cancer defined as less than 50 years of age, where a conclusively stronger association with predisposing germline mutations, such as BRCA1/2, is seen (Langston et al., 1996; Krainer et al., 1997; Couch et al., 2007). Additionally, BRCA1/2 mutations carriers may be more likely to die from aggressive breast or ovarian cancer at an early age, thereby masking an underlying diagnosis of pancreatic cancer. Interestingly, the phenomenon of "anticipation," which is a reduction in the age of onset of hereditary pancreatic cancer with successive generations, has been described in 59–85% of FPC families from studies by the European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer and FaPaCa (German national case collection for FPC) registries (James et al., 2004; McFaul et al., 2006; Schneider et al., 2011). Analysis of 80 affected child-parent pairs by McFaul et al revealed the children died a median of 10 years earlier than the parent, thereby providing strong implications for genetic counseling and secondary screening per the authors (McFaul et al., 2006).

PANCREATIC CANCER SUSCEPTIBILITY GENES

The genetic basis for the majority of PDAC has yet to be discovered. Although several important and high-penetrance genes associated with increased risk of pancreatic cancer have been identified, including BRCA2 and PALB2, it is clear that most cases of pancreatic cancer that demonstrate familial clustering are not explained by known genetic syndromes. This is evident by the fact that only 10–20% of PDAC with familial aggregation results from high-penetrance genes. Novel susceptibility genes in familial aggregating pancreatic cancer still remain to be identified in approximately 80% of affected families, and discovery of such genes is most likely to occur using family-based studies examining linkage or genome-sequencing approaches (Maitra et al., 2006; Bartsch et al., 2012; Klein, 2012).

GERMLINE MUTATIONS

Genes with germline mutations that have been identified in FPC kindred include BRCA2 (and other Fanconi anemia DNA repair pathway genes, including FANCC and FANCG genes), PALB2, PTEN, STK11/LKB1, p16/CDKN2A, TP53, ATM, and PRSS1 (**Table 1**). Those mutations with high-penetrance, including BRCA2, PALB2, and PTEN, are discussed below. Notably, genetically engineered mouse models have been created for many of these mutations (across a wide range of malignancies), thereby allowing for a tractable *in vivo* system to help determine the biologic impact of oncogenic mutations as well as helping establish genotype-phenotype relationships. Furthermore, these mouse models have the potential to identify early markers of disease and associated genetic mutations, as well as providing improved preclinical models for therapeutic targets and initiatives (Hezel et al., 2006).

BRCA2 (FANCONI ANEMIA DNA REPAIR PATHWAY GENE)

The BRCA2 protein is encoded by the BRCA2 gene located on chromosome 13q and functions in the Fanconi anemia pathway, which is partly responsible for genome-maintenance. Genomic integrity is maintained by enabling homologous recombination (HR)-based double-stranded (DS) DNA repair following interstrand crosslinking damage, in addition to acting in intra-S phase DNA damage checkpoint control (van der Heijden et al., 2005; Xia et al., 2006). Therefore, BRCA2 mutant cells exhibit defective HR repair, proliferation arrest, impaired cytokinesis, radioresistant DNA synthesis (due to impairment of intra-S phase DNA damage checkpoint control), genomic instability, and hypersensitivity to DNA damaging agents (e.g., PARP inhibitors, mitomycin, platinum, etc.) (Sharan et al., 1997; Patel et al., 1998; Kraakman-van der Zwet et al., 2002; Xia et al., 2006; Couch et al., 2007). The majority (80%) of BRCA2 germline mutations are nonsense or frameshift mutations, such as the 6174delT mutation and other exon 11 mutations, which lead to the development of premature stop codons and result in truncated and non-functional BRCA2 proteins similar to what is seen in BRCA2-mutated breast cancers (Hahn et al., 2003). Additionally, several rare missense mutations have been detected (Couch et al., 2007). Of note, it is estimated that 1% of the Ashkenazi Jewish population in North America harbors the germline BRCA2 6174delT founder mutation, which has been associated with a 10-fold increased risk of developing pancreatic, breast, prostate, and ovarian cancers (Oddoux et al., 1996; Ozcelik et al., 1997) Interestingly though, the BRCA2 6174delT mutation has been described to have independent origins in both Ashkenazi Jewish and non-Jewish populations (Berman et al., 1996; Hahn et al., 2003). BRCA2-deficient murine models of pancreatic cancer have been established in order to evaluate both diagnostic and therapeutic strategies for FPC. In this setting, biallelic loss of BRCA2 alone and certainly in conjunction with p53 deregulation, has been shown to induce the spectrum of pancreatic ductal neoplasia although after a fairly long latency period (Skoulidis et al., 2010; Feldmann et al., 2011; Rowley et al., 2011). The FANCC (located on chromosome 9q)

and FANCG (located on chromosome 9p) genes are additional Fanconi complementation group genes which have been implicated in the pathogenesis of pancreatic cancer (Goggins et al., 1996; Maitra et al., 2006).

Previous studies analyzing families with known BRCA2 mutations found young-onset breast and/or ovarian cancer BRCA2 mutation carriers to have a 3.5- to 10-fold increased risk and estimated 5% lifetime risk of developing PDAC relative to non-BRCA2 carriers (Breast Cancer Linkage Consortium, 1999; van Asperen et al., 2005). Although the average age of onset of PDAC does not differ between non-BRCA2 and BRCA2 mutated families, it has been noted that the presence of one young-onset case of PDAC in a pancreatic cancer family may be predictive of the presence of a BRCA2 mutation (Couch et al., 2007) Additionally, it has been suggested that BRCA2 germline mutation carriers exhibit at least two different cancer phenotypes, although it is not yet understood which genetic or environmental factors cause each of these phenotypic variations, it may be due to different mutational loci within the BRCA2 gene (Lubinski et al., 2004; Couch et al., 2007). One phenotype demonstrates a preponderance for breast and ovarian cancers, while a second phenotype is associated with familial pancreatic cancer without an increased incidence of, or high penetrance for, breast and/or ovarian cancer (Couch et al., 2007). The overall prevalence of BRCA2 mutations in moderate-risk (two or more affected first-degree relatives) and high-risk (three or more affected first-degree relatives) pancreatic cancer families, was noted by Couch et al to be approximately 6% with a frequency ranging from 3 to 15% for families depending on the number of affected family members (Couch et al., 2007). Other studies have suggested the prevalence of BRCA2 germline mutations to be significantly higher (12–19%) among individuals with a family history of PDAC, albeit those specifically fulfilling criteria for FPC (Murphy et al., 2002; Hahn et al., 2003). Thus, BRCA2 germline mutations are currently the most frequently identified genetic alteration in FPC even in the absence of breast and/or ovarian cancer (Goggins et al., 1996; Ozcelik et al., 1997; Hahn et al., 2003).

Given that approximately 10% of high-risk FPCs are noted to carry BRCA2-truncating mutations, it has been suggested that these individuals undergo genetic screening for the presence of BRCA2 mutations (Couch et al., 2007). The advantages of clinical testing include the possibility for close monitoring for pancreatic, as well as other BRCA2 mutation-associated cancers (breast, ovarian, prostate) in carriers. Although prophylactic surgical intervention to reduce the risk of breast and ovarian cancer onset is acceptable for BRCA2 mutated female carriers from families with numerous individuals affected with breast and/or ovarian cancers, it is unclear whether there would be risk reduction conferred by similar surgeries in women with BRCA2 mutations from families that only display a history of pancreatic cancer (Couch et al., 2007).

BRCA1

HBOC is commonly associated with an inherited germline mutation in one of the BRCA1 or BRCA2 alleles with the remaining functional/wildtype allele lost via somatic mutation (Bryant et al., 2005). As previously noted, BRCA2 mutation carriers far outnumber BRCA1 mutation carriers in both HBOC-associated pancreatic cancer and FPC (Hruban et al., 1999; Hahn et al., 2003; Bartsch et al., 2012). The majority of studies examining the prevalence of pancreatic cancer in BRCA1 mutated patients have shown no increased risk, however, others have estimated a 2- to 2.5-fold increased risk (Thompson et al., 2002; Ferrone et al., 2009). Ferrone et al examined 145 Ashkenazi Jewish pancreatic cancer patients and found no increase in frequency of BRCA1 mutations among this group (Ferrone et al., 2009). In addition, an analysis of 66 familial pancreatic cancer patients from NFPTR kindred with three or more relatives with PDAC did not identify any deleterious BRCA1 germline mutations in these patients (Axilbund et al., 2009). In examining whether BRCA1 mutations confer an increased risk of pancreatic cancer, Moran et al studied 268 British BRCA1 mutation-associated HBOC families to determine whether BRCA-mutations conferred an increased risk of PDAC and found no overall increased risk (Moran et al., 2012). In addition, when specifically analyzing for the BRCA1 185delAG founder mutation in pancreatic cancer patients, it was suggested that BRCA1 germline mutations do not contribute to an increased risk of pancreatic cancer (Schnall and Macdonald, 1996).

PALB2

Germline truncating mutations in the "partner and localizer of BRCA2" (PALB2) gene, which is located on chromosome 16p12 have been identified in approximately 3% of patients with FPC (Jones et al., 2009; Slater et al., 2010). The PALB2 gene encodes for a nuclear protein which co-localizes with BRCA1/2 in nuclear foci, acts as functional bridge between the two proteins, and provides stability to this complex by preventing proteosomal degradation, thereby allowing it to function in HR repair and checkpoint control as part of the Fanconi Anemia DNA repair pathway (Xia et al., 2006). Although it appears that PALB2 allows for stable BRCA2 association with certain nuclear structures, PALB2 is not required for BRCA2 entry into the nucleus. Nearly 50% of nuclear bound BRCA2 is associated with PALB2 and more than 50% of PALB2 is associated with BRCA2, as PALB2 appears to participate in only a subset of cellular responses to DS DNA breaks. Germline BRCA2 missense mutations within the PALB2-binding motif have been shown to disrupt PALB2 binding, thereby disabling BRCA2 function, and PALB2-depleted cells share a phenotype similar to those deficient in BRCA2 function, further highlighting the importance of this complex (Xia et al., 2006). Although mutated PALB2 has been linked with HBOC syndrome and Fanconi Anemia, its role in the pathogenesis of PDAC has only recently been shown. Indeed, according to Slater et al, PALB2 mutation carriers in FPC families demonstrated a 10- to 32-fold increased risk for the development of pancreatic cancer depending on the number of affected family members (Brand et al., 2007; Rahman et al., 2007; Jones et al., 2009; Slater et al., 2010).

Using whole-exome sequencing to examine patients with FPC, Jones et al identified a total of four PALB2 truncating mutations in 3.1% of patients with pancreatic cancer (Jones et al., 2009). While some families with PALB2 stop mutations were noted to have a history of breast and pancreatic cancer, breast cancer was not seen in all families (Jones et al., 2009; Slater et al., 2010).

Further studies have identified additional PALB2 mutations in 1–3% of FPC kindred (Tischkowitz et al., 2009; Slater et al., 2010). Nonsense and frameshift mutations, particularly in exon 11 of the PALB2 gene, result in a variety of premature stop codons and ultimately a truncated PALB2 protein, which is exceedingly rare in the general population and those without cancer (Slater et al., 2010). Additionally, while some studies have suggested an earlier age of onset of pancreatic or breast cancer in those with PALB2 mutations in the setting of FPC, recent studies have not observed similar findings (Slater et al., 2010).

PTEN

Phosphatase and tensin homolog (PTEN) is a major tumor suppressor gene located on chromosome 10q, which encodes a regulator of the NF-kB cytokine network in PDAC. It specifically inhibits activated PI3K (phosphoinositide-3-kinase) and formation of its enzymatic product, phosphorylated phosphatidylinositides (PIP3) (Koorstra et al., 2008). Whereas initiation of PDAC tumorigenesis has been found to be driven by oncogenic KRAS mutations, disease progression has been associated with frequent loss of tumor suppressors within tumor cells, such as the PI3K/PTEN pathway. Possibly due to promoter hypermethylation, aberrant expression and deletion of the PTEN gene has been frequently noted in primary tumor tissue (Asano et al., 2004). Furthermore, the PI3K/PTEN pathway has been reported to be activated in PDAC precursor lesions via activating mutations of PIK3CA, which is the gene that encodes PI3K (Schonleben et al., 2006; Koorstra et al., 2008). Even in the absence of such mutations, it has been observed that the PI3K/AKT pathway is constitutively activated in the majority of pancreatic cancers, through aberrant expression of PTEN as well as amplification or activation of AKT2 kinase (Cheng et al., 1996; Ruggeri et al., 1998; Schlieman et al., 2003; Asano et al., 2004; Reichert et al., 2007; Koorstra et al., 2008). In mouse models, PDAC is driven by combined oncogenic KRAS mutation and haploinsufficient PTEN deficiency, which together promote marked NF-kB activation, its cytokine network (CCL20, CXCL1, IL-6, and IL-23), stromal activation, and immune cell infiltration; these processes shape the pancreatic cancer tumor microenvironment, stimulate the development of peritumoral stroma, and promote local and metastatic progression (Ying et al., 2011). The desmoplastic host response is a hallmark pathologic feature of pancreatic cancer and is characterized by the aforementioned peritumoral stroma consisting of fibroblasts and inflammatory cells. This process is felt to be partly mediated by increased TGF- β levels, and contributes to the decreased tumor vascular density which in turn is felt to compromise delivery of systemic agents and promote radioresistance through hypoxia. As a result, stromal targeting agents are currently under active clinical investigation in patients with locally advanced and metastatic pancreatic cancer (Hezel et al., 2006; Ying et al., 2011). Moreover, constitutively-activated NF-kB and correspondingly upregulated PI3K/AKT signaling have been observed in many primary PDAC cell lines, but not in normal pancreatic tissue specimens suggesting angiogenesis-based pro-survival mechanisms via VEGF, urokinase, and other proinvasive/angiogenic factors (Hezel et al., 2006). Furthermore, activated NF-kB is hypothesized to contribute to pancreatic tumor chemoresistance via upregulation of BCL-2, BCL-XL, and other anti-apoptotic proteins (Hezel et al., 2006).

CARCINOGENESIS AND SOMATIC MUTATIONS

The genetic progression model for PDAC, comparable to that of the adenoma-carcinoma sequence seen in colorectal cancer, results from sequential acquisition of mutations in the proto-oncogene KRAS followed by mutations in tumor suppressor genes such as p16/CDKN2A/INK4A, TP53, and SMAD4 that lead to disturbance in cell cycle regulation, and promote the PanIN-to-PDAC progression (Hruban et al., 2000; Schneider and Schmid, 2003). The noninvasive pancreatic intraepithelial neoplasia (PanIN) lesion may harbor many of the same mutations found in invasive PDAC, although there are likely to be an increasing number of mutations associated with increasing degrees of dysplasia within the PanIN (Hruban et al., 2000). The major genetic alterations leading to sporadic pancreatic cancer are thought to be mutations in the proto-oncogene, KRAS, as well as the p16/CDKN2A/INK4A, TP53, and DPC4/SMAD4 tumor suppressor genes, while mutations in BRCA2, the mismatch repair genes (hMLH1, hMSH2, and hMSH6), and the AKT2 and STK11/LKB1 genes are noted to be rare (Schneider and Schmid, 2003; Hezel et al., 2006). Since p16/CDKN2A and BRCA2 mutations are not detected in the earliest sporadic premalignant pancreatic lesions and are more commonly found in later intermediate and advanced PanIN lesions, supports the hypothesis that these changes likely accumulate and impact the malignant progression of precursor lesions into PDAC rather than participate in cancer initiation (Hezel et al., 2006). It is likely that the relative late event of biallelic loss of BRCA2 in PDAC tumorigenesis is similar and shared between PDAC in those with germline and somatic mutations in the BRCA2 gene (Goggins et al., 2000; Hezel et al., 2006). KRAS gene mutations occur first in the lowest grade of intraductal lesions, known as PanIN-1 and are subsequently followed by p16/CDKN2A gene mutations, which are noted in PanIN-2 (moderately advanced/intermediate grade) lesions; the TP53, SMAD4, and sporadic BRCA2 inactivating mutations are not identified until further progression to a PanIN-3 (high grade) lesion (Hruban et al., 2000; Hezel et al., 2006). Knowledge of the underlying molecular mechanisms involved in pancreatic cancer tumorigenesis will offer new diagnostic and therapeutic options for the treatment and early detection of PDAC and its precursor lesions.

ONCOGENES

Mutated, constitutively activated oncogenes contribute to oncogenesis in PDAC, and include KRAS, BRAF, AKT2, and AIB I (**Table 1**) (Maitra et al., 2006).

KRAS

PDAC harbors the highest incidence of mutations in RAS proteins, which are known to mediate pleiotropic effects, including cell proliferation, differentiation, survival, and migration via GTP-binding cytoplasmic protein activity (Schneider and Schmid, 2003; Hezel et al., 2006). Oncogenic KRAS, located on chromosome 12p, is one of the most frequently mutated genes in

PDAC with over 90% of tumors harboring a KRAS gene mutation (Hruban et al., 1993; Maitra et al., 2006). The vast majority of the KRAS activating point mutations occur at codon 12 and less frequently at codons 13 and 61, thereby resulting in a constitutively activated protein product and downstream stimulatory signals to RAS effector pathways, such as RAF-mitogen-activated protein (MAP) kinase, PI3K, and RalGDS pathways independent of growth factor stimulation (Hruban et al., 1993; Hezel et al., 2006; Maitra et al., 2006; Koorstra et al., 2008). These mutations appear to occur very early in the development of pancreatic neoplasia, as evidenced by the presence of KRAS mutations in noninvasive precursor lesions, including intraductal papillary mucinous neoplasms (IPMN) and PanINs (Hezel et al., 2006; Maitra et al., 2006). KRAS mutations are the first known genetic alterations known to occur sporadically in normal pancreatic tissue, chronic pancreatitis, and smokers; moreover, they are detected in approximately 30% of early pancreatic neoplasms and close to 100% of advanced PDAC lesions. KRAS-mediated oncogenesis has thus been considered a likely necessary event in the development of PDAC (Rozenblum et al., 1997; Hezel et al., 2006). Biomarker studies have suggested KRAS activation alone is unlikely to singlehandedly promote carcinogenesis given the finding of oncogenic KRAS in normal tissues, such as lung, pancreas and colon (Lu et al., 2002). Follow up murine studies have suggested a threshold level of oncogenic KRAS expression is required to initiate transformation through downstream activation of KRAS-effector genes (Ardito et al., 2012; di Magliano and Logsdon, 2013). Although KRAS has been considered an attractive therapeutic target, its specific biochemical properties have made it an elusive target. Oncogenic mutations in KRAS result in a decreased intrinsic rate of GTP hydrolysis and make the molecule insensitive to GTPase activating proteins (GAPs) (Hezel et al., 2006). These oncogenic mutations inhibit the protein's enzymatic activity; thus, an effective KRAS inhibitor would increase the GTPase activity or make the KRAS protein more susceptible to GAPs (Hezel et al., 2006). This differs from the traditional paradigm of attempting to inhibit an oncogene's enzymatic function.

The mammalian Hedgehog family of secreted signaling proteins (Shh, Ihh, and Dhh) regulate the embryonic growth and patterning of many organs, including the pancreas. Activating mutations in Hedgehog proteins have been associated with a variety of cancers. Hedgehog pathway activation, specifically the overexpression of the pathway's principal activating ligand, sonic hedgehog (Shh), has been implicated in both the initiation and maintenance of pancreatic ductal neoplasia as well as more advanced lesions with a relative increase in the expression of Hedgehog ligands observed during pancreatic ductal tumorigenesis. This increased expression of ligands differs from the undetectable expression of Hedgehog ligands in normal human pancreatic ducts. Furthermore, it has been confirmed that the Hedgehog pathway also plays a role in metastases, with inhibition of Hedgehog signaling shown to reduce the incidence of systemic metastasis seen in PDAC xenografts (Berman et al., 2003; Maitra et al., 2006; Koorstra et al., 2008). Studies in pancreatic cancer cell lines have revealed crosstalk between oncogenic KRAS and the Hedgehog signaling pathway, which may suggest oncogenic KRAS plays an important role in activating Hedgehog signaling through the RAF/MEK/MAPK pathway in the absence of Hedgehog ligands during pancreatic tumorigenesis (Koorstra et al., 2008).

BRAF

The BRAF gene found on chromosome 7q encodes a serine/threonine kinase, which is regulated by binding to RAS and also functions in the RAS-RAF-MEK-ERK-MAP kinase pathway (Koorstra et al., 2008). It is mutated in 1/3 of pancreatic cancers with known wild-type KRAS (Calhoun et al., 2003; Maitra et al., 2006; Koorstra et al., 2008). Thus, KRAS and BRAF oncogenes may function in a mutually exclusive manner in the transformation and carcinogenesis of pancreatic cancers; indeed, some studies suggest that a mutation in one of these two genes invariably results in retention of wild-type copies of the other (Maitra et al., 2006; Koorstra et al., 2008). This suggests a potential requirement for either oncogenic KRAS or BRAF-related signal transduction as a critically important step in the malignant transformation of most pancreatic tumors, and also implies that the RAF-MAP signaling pathway plays a critical role in mediating cancer-causing signals in the RAS pathway (Maitra et al., 2006; Koorstra et al., 2008).

AKT2

The AKT2 gene is located on chromosome 19q and encodes a serine-threonine kinase that acts as a downstream effector of the PI3K/AKT pathway. This gene is amplified and overexpressed in approximately 10–60% of PDAC (Ruggeri et al., 1998; Schneider and Schmid, 2003; Hezel et al., 2006; Koorstra et al., 2008). It can be activated by epidermal growth factor, platelet-derived growth factor, and basic fibroblast growth factor, all of which are known to be overexpressed in pancreatic cancer, as well as through the PI3K/AKT pathway (Friess et al., 1996; Schneider and Schmid, 2003; Hezel et al., 2006; Koorstra et al., 2008). AKT signaling has also been linked to enhanced insulin-like growth factor I receptor (IGF-IR) expression in PDAC by promoting invasive potential of cells (Tanno et al., 2001; Schneider and Schmid, 2003; Hezel et al., 2006).

AIB I

Located on chromosome 20q, the AIB I gene is amplified in as many as 60% of PDAC. The nuclear receptor coactivator, Amplified In Breast cancer 1 (AIB I/SRC-3), belongs to the p160/steroid receptor coactivator family (SRC) (Koorstra et al., 2008). AIB I amplification and overexpression are not only detected in hormone-sensitive tumor types, such as breast, ovarian, and prostate cancers, but also in nonsteroid-targeted tumors including colorectal, hepatocellular, and pancreatic cancers (Koorstra et al., 2008).

TUMOR SUPPRESSOR GENES

Tumor suppressor genes are recessive and promote tumor growth when inactivated. Loss of function of several tumor suppressor genes has been observed in PDAC. Biallelic inactivation of these genes can occur via several mechanisms, including intragenic mutation of one allele coupled with loss of the second allele (loss of heterozygosity mutations), deletion of both

alleles (homozygous deletion), or hypermethylation of the gene's promotor resulting in silencing of gene expression. The most common tumor suppressor genes noted to be inactivated in greater than half of all PDAC are p16INK4A/CDKN2A, TP53, and SMAD4/DPC4 (**Table 1**). BRCA2, which is inactivated less frequently (7%), is discussed above (Rozenblum et al., 1997; Hahn and Schmiegel, 1998; Maitra et al., 2006; Koorstra et al., 2008).

P16INK4A/CDKN2A

The p16INK4A/CDKN2A (cyclin-dependent kinase inhibitor 2A) tumor suppressor gene is located on chromosome 9p and encodes the $p16^{INK4A}$ protein, which regulates the cell cycle through the p16/Rb pathway and controls progression through the G_1/S transition. Subsequent inhibition of the cyclin D1/CDK4/6 kinase complex results in inappropriate phosphorylation of Rb and blocks entry into S phase of the cell cycle (Schneider and Schmid, 2003; Hezel et al., 2006). Although germline and sporadic mutations have been identified with carriers of the germline p16-Leiden mutation, having an estimated 17% risk of developing pancreatic cancer by the age of 75, CDKN2A has been identified as one of the most frequently inactivated somatic tumor suppressors in PDAC (Koorstra et al., 2008). Inactivation of the gene during sporadic mutation occurs via homozygous deletion in 40% of cases, loss of heterozygosity in 40%, and gene inactivation through promotor hypermethylation in 15–20% (Caldas et al., 1994; Rozenblum et al., 1997; Maitra et al., 2006; Koorstra et al., 2008). p16INK4A/CDKN2A mutations cooperate with KRAS mutations in the development of PDAC, and are known to accelerate tumor progression in the setting of concurrent p53 mutations (Hezel et al., 2006).

Germline mutations in exon 1α of the p16INK4A/CDKN2A gene are associated with FAMMM syndrome (Gruis et al., 1995; Schneider and Schmid, 2003). In addition to a significantly increased risk of developing melanoma, individuals with FAMMM syndrome have a 20- to 34-fold increased risk of developing PDAC, although the penetrance is much lower potentially suggesting a modulating role by environmental factors (Hahn et al., 2003; Schneider and Schmid, 2003; Hezel et al., 2006; Maitra et al., 2006). Homozygous deletions resulting in inactivation of the p16INK4A/CDKN2A gene also frequently inactivate an adjacent gene on chromosome 9p, MTAP (methylthioadenosine phosphorylase), which is located 100 kilobases telomeric and plays an important role in the synthesis of adenosine. As a result of this coincident inactivation, MTAP function is completely lost in approximately 30% of PDAC and is also under active investigation as a potential therapeutic target using purine biosynthesis inhibitors, such as L-alanosine (Hustinx et al., 2005; Maitra et al., 2006; Koorstra et al., 2008). It has been suggested that use of such a targeted agent may be effective against the 1/3 of PDACs that harbor the deletion of this adjacent gene (Hustinx et al., 2005; Maitra et al., 2006; Koorstra et al., 2008).

TP53

The p53 protein is encoded by the TP53 gene located on chromosome 17p and is responsible for modulating cellular responses to cytotoxic stress by maintaining genomic stability. Specifically, p53 is responsible for regulation of the G_1/S cell cycle checkpoint, maintenance of G_2/M arrest, induction of apoptosis, and protection against genomic rearrangement and accumulation of mutations. It also suppresses cellular transformation caused by oncogenic activation or loss of tumor suppressor pathways; thus, deletion or inactivation of TP53 is associated with aneuploidy, as well as the growth and survival of cells harboring chromosomal aberrations and genetic instability with potential for carcinogenic transformation (Schneider and Schmid, 2003; Hezel et al., 2006; Maitra et al., 2006; Koorstra et al., 2008). The loss of p53 function results in deregulation of two essential controls over cell number, cell proliferation, cell division and cell death (Schneider and Schmid, 2003; Hezel et al., 2006). Notably, TP53 inactivation is the most common somatic alteration in human cancer, has been described in Li-Fraumeni syndrome, and is inactivated in 75–85% of PDAC almost always via intragenic mutation coupled with a somatically acquired loss of the second allele (Redston et al., 1994; Rozenblum et al., 1997; Schneider and Schmid, 2003; Hezel et al., 2006).

Additionally, p53-induced growth arrest is achieved by transactivation of p21. Binding of p53 to DNA stimulates p21 protein production, which negatively regulates the cyclin D/CDK2 complex and prevents the cell from progressing through G_1-S phase. This process also allows time for damaged DNA to be repaired. However, mutated p53 is unable to bind DNA, p21 is not available, and abnormal and deregulated growth occurs as a result. Loss of p21 activity through lack of transactivation has been observed in approximately 30–60% of PDAC specimens (Koorstra et al., 2008).

SMAD4

The SMAD4 gene, also known as DPC4 (deleted in pancreatic carcinoma, locus 4) is located on chromosome 18q21 and is inactivated in approximately 50–60% of PDAC (Hezel et al., 2006; Maitra et al., 2006; Jones et al., 2008). In 30–35% of the tumors, the gene is inactivated by homozygous deletion and by a loss of heterozygosity mutation in another 20–30% of cases. The protein product of the SMAD4 gene functions in transcriptional regulation and localizes to the nucleus following activation of the TGF-β intracellular signaling cascade (Derynck and Zhang, 2003; Hezel et al., 2006; Maitra et al., 2006). Once Smad4 is in the nucleus, it exhibits growth-controlling effects by regulating expression of specific gene targets (Maitra et al., 2006). Loss of SMAD4 interferes with the intracellular signaling cascades downstream from TGF-β, resulting in decreased growth inhibition via loss of pro-apoptotic signaling or via inappropriate G_1/S transition (Koorstra et al., 2008). Although it is assumed that the growth-inhibitory function of TGF-β is important in SMAD4 tumor suppressor activity, data has also suggested a TGF-β independent function of SMAD4, which modulates the interaction of the tumor with the microenvironment. This includes a decrease in pro-angiogenic VEGF expression and an increase in angiogenesis inhibitor TSP-1 (Schneider and Schmid, 2003). Thus, SMAD4 tumor suppressor function may also occur through regulation of an angiogenic mechanism (Schneider and Schmid, 2003; Hezel et al., 2006). Frequent inactivation of the SMAD4 gene appears to be specific to PDAC, as inactivation is rarely noted in other

tumor types or in non-ductal neoplasms of the pancreas (Maitra et al., 2006; Koorstra et al., 2008). Immunohistochemical staining for the Smad4 protein on tissue sections correlates strongly with SMAD4 gene status; thus, immunostaining can be used diagnostically to determine SMAD4 gene status in biopsies and resected tissues, as well as to suggest a pancreatic primary in the setting of occult metastatic adenocarcinoma (Wilentz et al., 2000; Maitra et al., 2006; Koorstra et al., 2008). This is particularly useful since PDAC with loss of Smad4 reportedly demonstrate an increased propensity for distant metastases and thus have a generally poorer prognosis, although SMAD4 gene status is not yet utilized for prognostic stratification (Schneider and Schmid, 2003; Blackford et al., 2009).

STK11/LKB1 AND OTHER TUMOR SUPPRESSOR GENES

Genetic alterations in tumor suppressor genes found in lower frequency (<10%) in pancreatic cancer include STK11/LKB1, MKK4, TGFβR1 (ALK 5, chromosome 9q), TGFβR2 (chromosome 3p), ACVR1β (ALK 4, chromosome 12q), ACVR2 (chromosome 2q), FBXW7 (CDC4), EP300, BRCA2, ATM, and AKT2 (Maitra et al., 2006; Koorstra et al., 2008). These infrequently mutated genes provide further insight into cellular pathways altered in PDAC, as well as potential therapeutic targets for gene-specific therapies (Maitra et al., 2006). The STK11/LKB1 gene on chromosome 19p13 encodes for a serine/threonine kinase that regulates cell polarity and metabolism (Jenne et al., 1998; Schneider and Schmid, 2003; Hezel et al., 2006). Inactivation of the STK11 gene appears to play a role in both hereditary and sporadic PDAC (Schneider and Schmid, 2003; Hezel et al., 2006). Germline mutations in this gene are associated with PJS and are identified in approximately 50% of PJS families who typically present with hamartomatous polyps of the GI tract, pigmented macules of the lips and buccal mucosa, as well as a 36% lifetime risk for the development of pancreatic cancer (>40-fold increased RR) (Giardiello et al., 2000; Sato et al., 2001; Hahn et al., 2003; Hezel et al., 2006). Somatic STK11 mutations have been observed in approximately 5% of sporadic PDAC, particularly those that arise in association within an IPMN, whereas loss of heterozygosity is seen in approximately 25% of patients with IPMN who lack PJS features (Sato et al., 2001; Hezel et al., 2006).

In a smaller percentage of PDAC, intragenic mutations and homozygous deletions of the MKK4 gene are noted. This gene encodes for a component of a stress-activated protein kinase cascade and functions in apoptosis and growth control (Koorstra et al., 2008). MKK4 is preferentially inactivated in specific subsets of pancreatic cancer metastases and less commonly in the primary tumors of the same patients (Xin et al., 2004; Koorstra et al., 2008). Thus, it has been suggested that the MKK4 protein product may function as a suppressor of metastasis in pancreatic cancer, as it is does in breast and prostatic carcinomas (Xin et al., 2004). There is also a noted trend toward worse survival in those patients with loss of MKK4 expression and thus evaluation of MKK4 immunolabeling may have prognostic value. Furthermore, there lies the potential for MKK4 to be a therapeutic target for restoration of the stress-activated protein kinase pathway in advanced PDAC patients (Xin et al., 2004; Koorstra et al., 2008).

BIOMARKERS AND THERAPEUTIC TARGETS

A better understanding of the genetic causes of sporadic and FPC has afforded the opportunity to investigate novel mechanism-based targeted and systemic therapies, as well as predictive and prognostic biomarkers.

TARGETING DNA REPAIR

BRCA1/2, other Fanconi anemia family proteins, and PARP-1/2 among others, function in a coordinated series of early events in DNA damage repair. When this process is impaired, cells become exquisitely sensitive to DNA damaging agents. The potential to exploit this strategy exists in PDAC. Mitomycin C is an alkylating antineoplastic agent that works by inducing interstrand DNA crosslinking with eventual production of DS-breaks. Early xenograft studies by van der Heijden et al showed a more pronounced response of FANCC and BRCA2-deficient pancreatic tumors to mitomycin C relative to Fanconi anemia proficient xenografts (van der Heijden et al., 2005). This enhanced preclinical response to mitomycin C involved cell cycle arrest in late S or G_2/M phase and caspase-dependent apoptosis; similar findings were noted with cyclophosphamide, another DNA interstrand crosslinking agent (van der Heijden et al., 2005). In spite of such promising preclinical data, clinical results with both mitomycin C and cyclophosphamide have been disappointing. In a retrospective analysis by Brunner et al, the combination of 5-fluorouracil and mitomycin C-based chemoradiotherapy demonstrated worse median overall survival (9.7 vs. 12.7 months) and 1 year overall survival (53 vs. 40%) compared to gemcitabine and cisplatin-based chemoradiotherapy in patients with locally advanced PDAC with similar toxicities. (Brunner et al., 2011). A phase II study by Cereda et al looking at salvage therapy with mitomycin C and ifosfamide (analog of cyclophosphamide) in gemcitabine-resistant metastatic pancreatic cancer was closed prematurely based on poor clinical outcomes with 71% of patients experiencing chemotherapy interruption due to progressive disease and 80% of patients demonstrating grade >2 toxicity. This study concluded that the mitomycin C and ifosfamide regimen was considered insufficiently active in gemcitabine-resistant metastatic pancreatic cancer (Cereda et al., 2011).

Poly (ADP-ribose) polymerase-1/2 (PARP-1/2) activity and poly (ADP-ribose) polymerization are essential for the repair of single stranded (SS)-DNA breaks through the base excision repair (BER) pathways (Bryant et al., 2005). Additionally, enhanced PARP-1 expression is seen in many tumor types compared to normal cells, and represents one of the mechanisms by which tumors avoid apoptosis caused by DNA damaging agents (Berger et al., 1978). In the absence of PARP-1, spontaneous SS-breaks can collapse replication forks and trigger HR repair (Bryant et al., 2005). Despite its role in cellular responses to genotoxic stress, in knockout mouse models, PARP-1 has been shown to not be required for survival or fertility in the absence of such insults; thus, PARP-1 can be considered a non-essential DNA repair protein in the setting of functional HR repair mechanisms (Bryant et al., 2005). Inhibition of PARP is therefore known to sensitize tumor cells to cytotoxic agents, such as topoisomerase-I inhibitors and alkylators, which induce DNA damage normally repaired by BER. The resulting increase in HR repair that occurs

in PARP-1 deficient mice is felt to represent an error-free mechanism, which likely explains why the genetic instability in PARP-1 deficient cells is not associated with accumulation of mutations or cancers (Bryant et al., 2005). BRCA2 and other Fanconi Anemia-pathway defective cells are felt to be sensitive to single-agent PARP inhibition and restoring functional BRCA abrogates this activity. (Bryant et al., 2005). Therefore, compromise of both BER and HR repair is felt to result in a lethal persistence and accumulation of recombinogenic lesions, chromosomal instability, cell cycle arrest, and resulting apoptosis partly through use of alternative error-prone repair pathways, such as SS annealing and non-homologous end joining (NHEJ) (Farmer et al., 2005). Bryant et al demonstrated that PARP inhibitors were profoundly cytotoxic to a BRCA2-deficient cell line at low concentrations relative to BRCA2-competent cells and normal cells, thus suggesting potential for a wide therapeutic index (Bryant et al., 2005). Whereas clonogenic survival was significantly reduced following PARP-1 and BRCA2 protein co-depletion in human cells using siRNA, depletion of PARP-2 with BRCA2 had no effect on clonogenic survival following treatment with PARP inhibitors. Depletion of PARP-2 in PARP-1- and BRCA2-depleted cells also did not result in added toxicity. Thus, it has been suggested that PARP-1, rather than PARP-2, is responsible for protection against spontaneously occurring recombinogenic lesions in cells. In turn, these lesions may convert to persistent DS breaks and collapsed replication forks during replication, which may ultimately result in cellular apoptosis in the absence of PARP-1-mediated repair (Bryant et al., 2005). Preclinical studies have demonstrated the potential effectiveness of PARP inhibitors in targeting pancreatic cancers demonstrating biallelic inactivation of the ATM gene and clinical trials are underway investigating the role of PARP-inhibition with DNA damaging agents in patients with or without BRCA-mutations (NCT01908478, NCT01585805) (Williamson et al., 2012).

HEDGEHOG SIGNALING PATHWAY INHIBITION

Aberrant activation of previously quiescent developmental signaling pathways, such as the Hedgehog pathway has been implicated in PDAC tumorigenesis, progression and development of metastases (Koorstra et al., 2008). Targeting of sonic hedgehog, the overexpressed principal activating ligand of the Hedgehog signaling pathway, has been a focus of much investigation (Berman et al., 2003; Maitra et al., 2006). Drugs such as cyclopamine have been developed which specifically inhibit the hedgehog pathway, thereby producing dramatic anti-tumor effects in murine xenograft PDAC models without significant side effects (Berman et al., 2003; Maitra et al., 2006). Given the dramatic results seen with cyclopamine, development of additional inhibitors of the hedgehog pathway, such as IPI-926 and GDC-0449, have and continue to be explored in clinical studies in the treatment of both pancreatic and other solid tumors with varied responses noted thus far (Berman et al., 2003; Maitra et al., 2006; Kelleher, 2011; LoRusso et al., 2011). One such study by Olive et al, examined the addition of IPI-926 to gemcitabine applied in a genetically engineered pancreatic cancermouse model, which demonstrated a significant depletion of tumor-associated stroma and a corresponding increased intratumoral concentration of gemcitabine (Olive et al., 2009). Unfortunately, a follow-up double blind, placebo-controlled phase II study randomizing patients with previously untreated metastatic pancreatic cancer to gemcitabine with or without IPI-926 (saridegib) was discontinued following interim analysis due to inferior survival of the investigational arm. As a result, clinical excitement over hedgehog inhibition has waned. Additionally, given the relationship between the RAS/MAPK and Hedgehog signaling pathways in PDAC, it has been suggested that synergistic targeting of both the RAS and Hedgehog pathways may represent a new therapeutic strategy for the treatment of PDAC (Pasca di Magliano et al., 2006; Koorstra et al., 2008; Mimeault and Batra, 2010; LoRusso et al., 2011).

KRAS AND BEYOND

Benzodiazepine peptidomimetics have been shown to block the post-translational attachment of farnesyl groups to Ras proteins, which are required for attachment to the cellular membrane. In preclinical studies, such farnyltransferase inhibitors restored normal growth patterns to Ras-transformed cells suggesting therapeutic potential in PDAC (James et al., 1993). However, when this was examined in a Phase III clinical study, the addition of tipifarnib (farnyltransferase inhibitor) to gemcitabine in advanced pancreatic cancer did not demonstrate any improvement in overall, 6-month, and 1-year survivals over gemcitabine alone, with acceptable toxicity noted in both arms (Van Cutsem et al., 2004). Based on the significant clinical benefit noted in patients with locally advanced disease, it was suggested that the negative results of this study could be explained by 76% of the patients in the study having metastatic disease and correspondingly large tumor burdens (Van Cutsem et al., 2004). As noted previously, tumors with oncogenic KRAS are often associated with relative drug resistance and poor prognosis (Hezel et al., 2006; Barbie et al., 2009). Thus, the combination of oncogenic KRAS mutation with PTEN-deficiency seen in PDAC promote NF-kB activation and sustained activity of the NF-kB downstream cytokine pathway. This is mediated via an elevated PI3K pathway, which provides yet an additional avenue for targeted therapies for those tumors demonstrating altered PI3K regulation (Ying et al., 2011).

Targeting of KRAS effectors such as mTOR, which act downstream of AKT2 have previously been shown to be activated in PDAC. Targeting of mTOR with an mTOR inhibitor (rapamycin analog) has shown tumor growth inhibition in several PDAC cell lines (Asano et al., 2005; Hezel et al., 2006). Additionally, rapamycin has been shown to inhibit PDAC xenograft growth and metastasis (Bruns et al., 2004). The possible mechanisms by which these agents work include induction of endothelial cell death and tumor vessel thrombosis (Bruns et al., 2004). A phase II study by Wolpin et al examined the use of everolimus in gemcitabine-refractory, metastatic PDAC patients. Single-agent everolimus was well-tolerated, but showed minimal clinical activity with no clear evidence of treatment response, only 21% of patients having stable disease at 2 months, a median PFS of 1.8 months, and an overall survival of 4.5 months (Wolpin et al., 2009).

Dramatic tumor shrinkage was noted in a recent mutated KRAS lung cancer model when treated with a combination of a dual PI3K/mTOR inhibitor and a MEK (MAP/ERK kinase) inhibitor (Engelman et al., 2008). This provides preclinical

feasibility of the concept that targeting KRAS surrogates and downstream targets is potentially a feasible therapeutic strategy. As a result, numerous IV and oral PI3K and MEK inhibitors are in various stages of clinical development and testing (Phase I and II) (Engelman et al., 2008; Ying et al., 2011; Britten, 2013). Given the known cooperation between oncogenic KRAS and PTEN deficiency in PDAC tumorigenesis, further investigation is validated for combined therapies with MEK inhibitors and PI3K or NF-kB inhibitors. This concept of combination therapies with multiple targets is further supported by the poor results seen in Phase I/II studies of single-agent MEK inhibitors (CI-1040, selumetinib), which have shown minimal clinical response and only a marginal improvement in median survival when used alone (Rinehart et al., 2004; Lorusso et al., 2005; Bodoky et al., 2012; Britten, 2013).

GROWTH FACTOR INHIBITION

The epidermal growth factor receptor (EGFR), which consists of 4 separate receptors [HER1 (ErbB-1), HER-2/Neu (ErbB-2), HER-3 (ErbB-3), and HER-4 (ErbB-4)], is overexpressed and plays a distinct role in PDAC (Koorstra et al., 2008). HER-2/neu overexpression is most prominent in well-differentiated PDAC and early-stage precursor lesions, and appears to correlate with degree of dysplasia in the latter (Koorstra et al., 2008). In PDAC, HER-2/neu amplification has been observed in 10–60% of patients. (Stoecklein et al., 2004; Talar-Wojnarowska and Malecka-Panas, 2006; Koorstra et al., 2008). This gene amplication is of interest as it could potentially be a target of trastuzumab, the monoclonal antibody directed against the HER2/neu receptor. (Koorstra et al., 2008). In addition, increased levels of fibroblast growth factor (FGF), FGF-receptor, insulin-like growth factor I (IGF-I), IGF-I receptor, nerve growth factor, and vascular endothelial growth factor (VEGF) have also been reported in PDAC. Targeted therapies directed toward several of these growth factors have been examined with some under active clinical investigation, as noted below (Koorstra et al., 2008).

EGFR

Despite the complexity of the EGFR signaling cascade, which is known to provide a multitude of resistance mechanisms to EGFR-targeted agents in PDAC, the small molecule tyrosine kinase inhibitor (TKI) of EGFR, erlotinib, has been approved in the US. Moore et al conducted a phase III double-blind study randomizing patients with advanced PDAC to gemcitabine with or without erlotinib. A small but statistically significant improvement in PFS, one-year OS, and median OS was seen (Bruns et al., 2000; Li et al., 2004b; Ducreux et al., 2007; Moore et al., 2007). Interestingly, the subset of patients who developed erlotinib-related skin toxicity had a significantly more profound clinical response. It has been hypothesized that these results may be due to a decrease in tumor vasculature mediated through endothelial apoptosis, given that EGFR is expressed not only on tumor cells but also on dividing endothelial cells (Bruns et al., 2000; Li et al., 2004b; Ducreux et al., 2007). Furthermore, the effect of erlotinib may also potentially be due to inhibition of proangiogenic factors (VEGF, IL-8) by EGFR inhibitors, given that activation of the EGF receptor on tumor cells is known to induce the production of VEGF (Bruns et al., 2000; Li et al., 2004b; Ducreux et al., 2007). Attempts to correlate expression of molecular targets, such as EGFR, to outcomes in erlotinib-based therapies have been unsuccessful to date (da Cunha Santos et al., 2010). EGFR expression as quantified by immunohistochemistry techniques is unlikely to identify those tumors predominantly driven by the EGFR signaling pathway and thus would potentially be responsive to EGFR inhibition (Philip et al., 2010). A phase III study investigated the addition of cetuximab to gemcitabine in an unselected patient population (not selected for presence of EGFR mutations) and found no significant improvement in overall or progression-free survival observed relative to gemcitabine alone (Ducreux et al., 2007; Philip et al., 2010). Ongoing and future research focusing on identification of molecular predictors of resistance and sensitivity to EGFR blockade will potentially improve our understanding of such therapies and selected patient response (Philip et al., 2010).

SMAD4/DPC4

Iacobuzio-Donahue et al recently reported on Smad4 as a potential predictor of local vs. distant failure using rapid autopsy specimen of patients with PDAC (Iacobuzio-Donahue et al., 2009). Interestingly, intact Smad4 immunolabeling strongly correlated with a locally destructive phenotype ($p = 0.007$) and cause of death was attributed to local progression in 30% of patients. In a prospective single arm study of locally advanced PDAC patients treated with induction cetuximab, gemcitabine, and oxaliplatin followed by cetuximab, capecitabine, and radiotherapy, Crane et al similarly found Smad4 expression correlated with local rather than distant disease progression and potentially represented a predictive biomarker (Crane et al., 2011). Based on these results, RTOG 1201 is currently randomizing patients to upfront gemcitabine followed by high-intensity capecitabine-based IMRT (63.0 Gy) vs. upfront gemcitabine followed by standard intensity capecitabine-based 3D-CRT (50.4 Gy) vs. upfront FOLFIRINOX followed by standard intensity capecitabine-based 3D-CRT (50.4 Gy) and stratifying patients for intensification of local therapy based on Smad4 status (NCT01921751).

VEGF

It is well-known that VEGF and VEGFR are frequently overexpressed in PDAC. Disruption of VEGF signaling and tumor angiogenesis using soluble VEGFR, VEGF high-affinity binding chimeras, anti-VEGF monoclonal antibodies (e.g., bevacizumab), and ribozymes have shown strong antitumor activity in PDAC mouse xenografts and cultured pancreatic cancer cell lines (Hezel et al., 2006; Koorstra et al., 2008). Unfortunately, clinical results have been disparate. Kindler et al conducted a single arm phase II study investigating the addition of bevacizumab to gemcitabine in patients with advanced PDAC and noted a promising median OS of 8.8 months, PR of 21%, and *SD* of 46% (Kindler et al., 2005). The follow up CALGB phase III placebo-controlled study randomized patients to gemcitabine with or without bevacizumab and found no statistically significant improvement in clinical outcomes (Kindler et al., 2010). Similar disappointing results have been noted with small molecule TKI of VEGFR1-3, axitinib. (Spano et al., 2008). In addition, based on the enhanced radiosensitization seen with the addition of bevacizumab to 5-FU-based

radiotherapy in rectal cancer, similar strategies for radiosensitization could be considered in the treatment of PDAC (Willett et al., 2006; Ducreux et al., 2007). A phase I study evaluating the safety of bevacizumab with concurrent capecitabine-based radiotherapy in locally advanced PDAC initially showed bevacizumab-related GI toxicity of duodenal mucosal ulceration, bleeding, and perforation, with protocol mandated dose reductions in capecitabine required in 43% of patients, thus concluding that further study of bevacizumab with chemoradiotherapy was indicated (Crane et al., 2006). A future therapeutic target may be the VEGF-C, a regulator of lymphangiogenesis, which is noted to be overexpressed in PDAC, and may contribute to the lymphatic spread and metastasis that are commonly seen in pancreatic cancer (Hezel et al., 2006).

IGF-I

Elevated expression of IGF-I has been noted in PDAC tumor cells and their surrounding stroma (Hezel et al., 2006). In addition, there is aberrant activation and constitutive overexpression of the IGF-I receptor (IGF-IR) in approximately 64% of pancreatic tumor cells (Bergmann et al., 1995; Hakam et al., 2003; Ouban et al., 2003; Stoeltzing et al., 2003; Hezel et al., 2006). Furthermore, in a particular PDAC cell line, aberrant expression and activation of IGF-IR via paracrine and autocrine IGF-I signaling was noted to promote cell proliferation and growth-factor-independent survival (Nair et al., 2001). Therefore, inhibition of this pathway using anti-IGF-IR antibodies or expression of truncated IGF-I receptors (via recombinant adenovirus technique) that function as a dominant-negative form of IGF-IR has been examined in the preclinical setting and shown to inhibit the growth of xenograft tumors by up-regulating stressor induced apoptosis, blocking IGF-I and IGF-II induced activation of AKT-1, as well as sensitizing tumor cells to chemotherapy (Maloney et al., 2003; Min et al., 2003; Hezel et al., 2006). Given the encouraging preclinical results, several phase I and II studies of IGF-IR monoclonal antibody and small molecule agents have been pursued (Carboni et al., 2009; Hewish et al., 2009; Kindler et al., 2012). Kindler et al performed a randomized phase II study of ganitumab (AMG 479; monoclonal antibody antagonist of IGF-IR) and gemcitabine vs. gemcitabine and placebo in previously untreated metastatic PDAC. The ganitumab arm demonstrated acceptable toxicity, as well as trends toward improved 6- and 12-month survival, PFS, and overall survival. Given these favorable results, a randomized Phase III study of AMG 479 and gemcitabine in metastatic pancreatic adenocarcinoma was pursued, randomizing patients to AMG 479 (12 or 20 mg/kg) and gemcitabine vs. placebo and gemcitabine. Unfortunately, this study was stopped early for futility based on pre-planned interim analysis (NCT01231347).

FUTURE DIRECTIONS/SCREENING

While screening of the general population is not practicable with current diagnostic methods, studies are ongoing to evaluate its usefulness in people with at least 5- to 10-fold increased risk of PDAC. This would include patients with FPC or carriers of a mutation in an established high-penetrance PDAC susceptibility gene (e.g., BRCA2 or PALB2) with at least one case of pancreatic cancer in a first-degree relative (Brand et al., 2007; Bartsch et al., 2012; Klein, 2012; Canto et al., 2013). Furthermore, it has been suggested that such individuals undergo screening for any extrapancreatic tumors associated with their respective germline mutation prior to the development of any respective clinical symptomatology.

USPSTF Screening Guidelines for PDAC have been given a D recommendation indicating harm outweighing any potential benefit and recommending against routine screening in asymptomatic adults using abdominal palpation, ultrasonography, or serologic markers. International Cancer of the Pancreas Screening (CAPS) Consortium summit recommendations for PDAC concluded that screening is recommended for high-risk individuals, although more evidence is needed regarding optimal management of patients with detected lesions. These high-risk candidates for screening include first degree relatives of patients with PDAC from familial kindred with at least two affected first-degree relatives, patients with PJS, and carriers of p16, BRCA2, or HNPCC mutations with at least one affected first-degree relative. The CAPS Consortium was not able to reach a consensus on the age to initiate screening or stop surveillance, as well as screening intervals, although agreement was made that initial screening should include EUS and/or MRI/MRCP, and not CT or ERCP (Canto et al., 2013). At this time, based on the current knowledge of pancreatic susceptibility genes, affected patients of FPC families should consider being tested for the most frequently inherited genetic defects identified in FPC, BRCA2, PALB2, and ATM germline mutations. The use of PDAC biomarkers, such as CA-19-9 and CEACAM-1, have not yet been validated for clinical use in screening (Bussom and Saif, 2010).

To help identify high-risk populations who would be most likely to benefit from early detection screening tests, discovery of additional pancreatic cancer susceptibility genes is crucial (Brentnall et al., 1999; Canto et al., 2006; Koorstra et al., 2008; Vasen et al., 2011; Klein, 2012). Gene expression patterns in serum and tissue biopsies can be studied using whole-genome assaying, including technologies such serial analysis of gene expression (SAGE), cDNA arrays, and oligonucleotide arrays (i.e., gene chips) (Maitra et al., 2006). Further, specific gene-based, gene-product, and marker-based testing for the early detection of pancreatic cancer are currently being developed, which may include miRNAs, which may also be useful as potential therapeutic targets as well (Koorstra et al., 2008).

REFERENCES

Ardito, C. M., Gruner, B. M., Takeuchi, K. K., Lubeseder-Martellato, C., Teichmann, N., Mazur, P. K., et al. (2012). EGF receptor is required for KRAS-induced pancreatic tumorigenesis. *Cancer Cell* 22, 304–317. doi: 10.1016/j.ccr.2012.07.024

Asano, T., Yao, Y., Zhu, J., Li, D., Abbruzzese, 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-kappaB and c-Myc in pancreatic cancer cells. *Oncogene* 23, 8571–8580. doi: 10.1038/sj.onc.1207902

Asano, T., Yao, Y., Zhu, J., Li, D., Abbruzzese, J. L., and Reddy, S. A. (2005). The rapamycin analog CCI-779 is a potent inhibitor of pancreatic cancer cell proliferation. *Biochem. Biophys. Res. Commun.* 331, 295–302. doi: 10.1016/j.bbrc.2005.03.166

Axilbund, J. E., Argani, P., Kamiyama, M., Palmisano, E., Raben, M., Borges, M., et al. (2009). Absence of germline BRCA1 mutations in familial pancreatic cancer patients. *Cancer Biol. Ther.* 8, 131–135. doi: 10.4161/cbt.8.2.7136

Barbie, D. A., Tamayo, P., Boehm, J. S., Kim, S. Y., Moody, S. E., Dunn, I. F., et al. (2009). Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature* 462, 108–112. doi: 10.1038/nature08460

Bartsch, D. K., Gress, T. M., and Langer, P. (2012). Familial pancreatic cancer–current knowledge. *Nat. Rev. Gastroenterol. Hepatol.* 9, 445–453. doi: 10.1038/nrgastro.2012.111

Bartsch, D. K., Kress, R., Sina-Frey, M., Grutzmann, R., Gerdes, B., Pilarsky, C., et al. (2004). Prevalence of familial pancreatic cancer in Germany. *Int. J. Cancer* 110, 902–906. doi: 10.1002/ijc.20210

Berger, N. A., Adams, J. W., Sikorski, G. W., Petzold, S. J., and Shearer, W. T. (1978). Synthesis of DNA and poly(adenosine diphosphate ribose) in normal and chronic lymphocytic leukemia lymphocytes. *J. Clin. Invest.* 62, 111–118. doi: 10.1172/JCI109094

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. B., Costalas, J., Schultz, D. C., Grana, G., Daly, M., and Godwin, A. K. (1996). A common mutation in BRCA2 that predisposes to a variety of cancers is found in both Jewish Ashkenazi and non-Jewish individuals. *Cancer Res.* 56, 3409–3414.

Berman, D. M., Karhadkar, S. S., Maitra, A., Montes De Oca, R., Gerstenblith, M. R., Briggs, K., et al. (2003). Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. *Nature* 425, 846–851. doi: 10.1038/nature01972

Blackford, A., Serrano, O. K., Wolfgang, C. L., Parmigiani, G., Jones, S., Zhang, X., et al. (2009). SMAD4 gene mutations are associated with poor prognosis in pancreatic cancer. *Clin. Cancer Res.* 15, 4674–4679. doi: 10.1158/1078-0432.CCR-09-0227

Bodoky, G., Timcheva, C., Spigel, D. R., La Stella, P. J., Ciuleanu, T. E., Pover, G., et al. (2012). A phase II open-label randomized study to assess the efficacy and safety of selumetinib (AZD6244 [ARRY-142886]) versus capecitabine in patients with advanced or metastatic pancreatic cancer who have failed first-line gemcitabine therapy. *Invest. New Drugs* 30, 1216–1223. doi: 10.1007/s10637-011-9687-4

Brand, R. E., Lerch, M. M., Rubinstein, W. S., Neoptolemos, J. P., Whitcomb, D. C., Hruban, R. H., et al. (2007). Participants of the fourth international symposium of inherited diseases of the, advances in counselling and surveillance of patients at risk for pancreatic cancer. *Gut* 56, 1460–1469. doi: 10.1136/gut.2006.108456

Brand, R. E., and Lynch, H. T. (2006). Genotype/phenotype of familial pancreatic cancer. *Endocrinol. Metabol. Clin. N. Am.* 35, 405–415. doi: 10.1016/j.ecl.2006.02.015

Breast Cancer Linkage Consortium. (1999). Cancer risks in BRCA2 mutation carriers. *J. Natl. Cancer Inst.* 91, 1310–1316. doi: 10.1093/jnci/91.15.1310

Brentnall, T. A., Bronner, M. P., Byrd, D. R., Haggitt, R. C., and Kimmey, M. B. (1999). Early diagnosis and treatment of pancreatic dysplasia in patients with a family history of pancreatic cancer. *Ann. Intern. Med.* 131, 247–255. doi: 10.7326/0003-4819-131-4-199908170-00003

Britten, C. D. (2013). PI3K and MEK inhibitor combinations: examining the evidence in selected tumor types. *Cancer Chemother. Pharmacol.* 71, 1395–1409. doi: 10.1007/s00280-013-2121-1

Brune, K. A., Lau, B., Palmisano, E., Canto, M., Goggins, M. G., Hruban, R. H., et al. (2010). Importance of age of onset in pancreatic cancer kindreds. *J. Natl. Cancer Inst.* 102, 119–126. doi: 10.1093/jnci/djp466

Brunner, T. B., Sauer, R., and Fietkau, R. (2011). Gemcitabine/cisplatin versus 5-fluorouracil/mitomycin C chemoradiotherapy in locally advanced pancreatic cancer: a retrospective analysis of 93 patients. *Radiat. Oncol.* 6:88. doi: 10.1186/1748-717X-6-88

Bruns, C. J., Koehl, G. E., Guba, M., Yezhelyev, M., Steinbauer, M., Seeliger, H., et al. (2004). Rapamycin-induced endothelial cell death and tumor vessel thrombosis potentiate cytotoxic therapy against pancreatic cancer. *Clin. Cancer Res.* 10, 2109–2119. doi: 10.1158/1078-0432.CCR-03-0502

Bruns, C. J., Solorzano, C. C., Harbison, M. T., Ozawa, S., Tsan, R., Fan, D., 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.

Bryant, H. E., Schultz, N., Thomas, H. D., Parker, K. M., Flower, D., Lopez, E., et al. (2005). Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 434, 913–917. doi: 10.1038/nature03443

Bussom, S., and Saif, M. W. (2010). Methods and rationale for the early detection of pancreatic cancer. Highlights from the "2010 ASCO Gastrointestinal Cancers Symposium." Orlando, FL. January 22–24, 2010. *JOP* 11, 128–130.

Caldas, C., Hahn, S. A., da Costa, L. T., Redston, M. S., Schutte, M., Seymour, A. B., et al. (1994). Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. *Nat. Genet.* 8, 27–32. doi: 10.1038/ng0994-27

Calhoun, E. S., Jones, J. B., Ashfaq, R., Adsay, V., Baker, S. J., Valentine, V., 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. doi: 10.1016/S0002-9440(10)63485-2

Canto, M. I., Goggins, M., Hruban, R. H., Petersen, G. M., Giardiello, F. M., Yeo, C., et al. (2006). Screening for early pancreatic neoplasia in high-risk individuals: a prospective controlled study. *Clin. Gastroenterol. Hepatol.* 4, 766–781, quiz: 665. doi: 10.1016/j.cgh.2006.02.005

Canto, M. I., Harinck, F., Hruban, R. H., Offerhaus, G. J., Poley, J. W., Kamel, I., et al. (2013). International cancer of the pancreas screening (CAPS) Consortium summit on the management of patients with increased risk for familial pancreatic cancer. *Gut* 62, 339–347. doi: 10.1136/gutjnl-2012-303108

Carboni, J. M., Wittman, M., Yang, Z., Lee, F., Greer, A., Hurlburt, W., et al. (2009). BMS-754807, a small molecule inhibitor of insulin-like growth factor-1R/IR. *Mol. Cancer Ther.* 8, 3341–3349. doi: 10.1158/1535-7163.MCT-09-0499

Cereda, S., Reni, M., Rognone, A., Fugazza, C., Ghidini, M., Ceraulo, D., et al. (2011). Salvage therapy with mitomycin and ifosfamide in patients with gemcitabine-resistant metastatic pancreatic cancer: a phase II trial. *Chemotherapy* 57, 156–161. doi: 10.1159/000324865

Cheng, J. Q., Ruggeri, B., Klein, W. M., Sonoda, G., Altomare, D. A., Watson, D. K., et al. (1996). Amplification of AKT2 in human pancreatic cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA. *Proc. Natl. Acad. Sci. U.S.A.* 93, 3636–3641. doi: 10.1073/pnas.93.8.3636

Couch, F. J., Johnson, M. R., Rabe, K. G., Brune, K., de Andrade, M., Goggins, M., et al. (2007). The prevalence of BRCA2 mutations in familial pancreatic cancer. *Cancer Epidemiol. Biomarkers Prev.* 16, 342–346. doi: 10.1158/1055-9965.EPI-06-0783

Crane, C. H., Ellis, L. M., Abbruzzese, J. L., Amos, C., Xiong, H. Q., Ho, L., et al. (2006). Phase I trial evaluating the safety of bevacizumab with concurrent radiotherapy and capecitabine in locally advanced pancreatic cancer. *J. Clin. Oncol.* 24, 1145–1151. doi: 10.1200/JCO.2005.03.6780

Crane, C. H., Varadhachary, G. R., Yordy, J. S., Staerkel, G. A., Javle, M. M., Safran, H., et al. (2011). Phase II trial of cetuximab, gemcitabine, and oxaliplatin followed by chemoradiation with cetuximab for locally advanced (T4) pancreatic adenocarcinoma: correlation of Smad4(Dpc4) immunostaining with pattern of disease progression. *J. Clin. Oncol.* 29, 3037–3043. doi: 10.1200/JCO.2010.33.8038

da Cunha Santos, G., Dhani, N., Tu, D., Chin, K., Ludkovski, O., Kamel-Reid, S., et al. (2010). Molecular predictors of outcome in a phase 3 study of gemcitabine and erlotinib therapy in patients with advanced pancreatic cancer: national cancer institute of Canada clinical trials group study PA.3. *Cancer* 116, 5599–5607. doi: 10.1002/cncr.25393

Del Chiaro, M., Zerbi, A., Falconi, M., Bertacca, L., Polese, M., Sartori, N., et al. (2007). Cancer risk among the relatives of patients with pancreatic ductal adenocarcinoma. *Pancreatology* 7, 459–469. doi: 10.1159/000108963

Derynck, R., and Zhang, Y. E. (2003). Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 425, 577–584. doi: 10.1038/nature02006

di Magliano, M. P., and Logsdon, C. D. (2013). Roles for KRAS in pancreatic tumor development and progression. *Gastroenterology* 144, 1220–1229. doi: 10.1053/j.gastro.2013.01.071

Ducreux, M., Boige, V., Goere, D., Deutsch, E., Ezra, P., Elias, D., et al. (2007). The multidisciplinary management of gastrointestinal cancer. Pancreatic cancer: from pathogenesis to cure. *Best Pract. Res. Clin.Gastroenterol.* 21, 997–1014. doi: 10.1016/j.bpg.2007.10.025

Ehrenthal, D., Haeger, L., Griffin, T., and Compton, C. (1987). Familial pancreatic adenocarcinoma in three generations. A case report and a review of the literature. *Cancer* 59, 1661–1664. doi: 10.1002/1097-0142(19870501)59:9<1661::AID-CNCR2820590923>3.0.CO;2-H

Engelman, J. A., Chen, L., Tan, X., Crosby, K., Guimaraes, A. R., Upadhyay, R., et al. (2008). Effective use of PI3K and MEK inhibitors to treat mutant Kras

G12D and PIK3CA H1047R murine lung cancers. *Nat. Med.* 14, 1351–1356. doi: 10.1038/nm.1890

Farmer, H., McCabe, N., Lord, C. J., Tutt, A. N., Johnson, D. A., Richardson, T. B., et al. (2005). Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 434, 917–921. doi: 10.1038/nature03445

Feldmann, G., Karikari, C., dal Molin, M., Duringer, S., Volkmann, P., Bartsch, D. K., et al. (2011). Inactivation of Brca2 cooperates with Trp53(R172H) to induce invasive pancreatic ductal adenocarcinomas in mice: a mouse model of familial pancreatic cancer. *Cancer Biol. Ther.* 11, 959–968. doi: 10.4161/cbt.11.11.15534

Ferrone, C. R., Levine, D. A., Tang, L. H., Allen, P. J., Jarnagin, W., Brennan, M. F., et al. (2009). BRCA germline mutations in Jewish patients with pancreatic adenocarcinoma. *J. Clin. Oncol.* 27, 433–438. doi: 10.1200/JCO.2008.18.5546

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. doi: 10.1007/BF00202070

Ghadirian, P., Boyle, P., Simard, A., Baillargeon, J., Maisonneuve, P., and Perret, C. (1991). Reported family aggregation of pancreatic cancer within a population-based case-control study in the Francophone community in Montreal, Canada. *Int. J. Pancreatol.* 10, 183–196.

Giardiello, F. M., Brensinger, J. D., Tersmette, A. C., Goodman, S. N., Petersen, G. M., Booker, S. V., et al. (2000). Very high risk of cancer in familial Peutz-Jeghers syndrome. *Gastroenterology* 119, 1447–1453. doi: 10.1053/gast.2000.20228

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. doi: 10.1016/S0002-9440(10)65047-X

Goggins, M., Schutte, M., Lu, J., Moskaluk, C. A., Weinstein, C. L., Petersen, G. M., et al. (1996). Germline BRCA2 gene mutations in patients with apparently sporadic pancreatic carcinomas. *Cancer Res.* 56, 5360–5364.

Gruis, N. A., van der Velden, P. A., Sandkuijl, L. A., Prins, D. E., Weaver-Feldhaus, J., Kamb, A., et al. (1995). Homozygotes for CDKN2 (p16) germline mutation in Dutch familial melanoma kindreds. *Nat. Genet.* 10, 351–353. doi: 10.1038/ng0795-351

Hahn, S. A., Greenhalf, B., Ellis, I., Sina-Frey, M., Rieder, H., Korte, B., et al. (2003). BRCA2 germline mutations in familial pancreatic carcinoma. *J. Natl. Cancer Inst.* 95, 214–221. doi: 10.1093/jnci/95.3.214

Hahn, S. A., and Schmiegel, W. H. (1998). Recent discoveries in cancer genetics of exocrine pancreatic neoplasia. *Digestion* 59, 493–501. doi: 10.1159/000007526

Hakam, A., Fang, Q., Karl, R., and Coppola, D. (2003). Coexpression of IGF-1R and c-Src proteins in human pancreatic ductal adenocarcinoma. *Dig. Dis. Sci.* 48, 1972–1978. doi: 10.1023/A:1026122421369

Hemminki, K., and Li, X. (2003). Familial and second primary pancreatic cancers: a nationwide epidemiologic study from Sweden. *Int. J. Cancer* 103, 525–530. doi: 10.1002/ijc.10863

Hewish, M., Chau, I., and Cunningham, D. (2009). Insulin-like growth factor 1 receptor targeted therapeutics: novel compounds and novel treatment strategies for cancer medicine. *Recent Pat. Anticancer Drug Discov.* 4, 54–72. doi: 10.2174/157489209787002515

Hezel, A. F., Kimmelman, A. C., Stanger, B. Z., Bardeesy, N., and Depinho, R. A. (2006). Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev.* 20, 1218–1249. doi: 10.1101/gad.1415606

Hruban, R. H., Canto, M. I., Goggins, M., Schulick, R., and Klein, A. P. (2010). Update on familial pancreatic cancer. *Adv. Surg.* 44, 293–311. doi: 10.1016/j.yasu.2010.05.011

Hruban, R. H., Goggins, M., Parsons, J., and Kern, S. E. (2000). Progression model for pancreatic cancer. *Clin. Cancer Res.* 6, 2969–2972.

Hruban, R. H., Petersen, G. M., Goggins, M., Tersmette, A. C., Offerhaus, G. J., Falatko, F., et al. (1999). Familial pancreatic cancer. *Ann. Oncol.* 10(Suppl. 4), 69–73. doi: 10.1093/annonc/10.suppl_4.S69

Hruban, R. H., Petersen, G. M., Ha, P. K., and Kern, S. E. (1998). Genetics of pancreatic cancer. From genes to families. *Surg. Oncol. Clin. N. Am.* 7, 1–23.

Hruban, R. H., van Mansfeld, A. D., Offerhaus, G. J., van Weering, D. H., Allison, D. C., Goodman, S. N., et al. (1993). K-ras oncogene activation in adenocarcinoma of the human pancreas. A study of 82 carcinomas using a combination of mutant-enriched polymerase chain reaction analysis and allele-specific oligonucleotide hybridization. *Am. J. Pathol.* 143, 545–554.

Hustinx, S. R., Hruban, R. H., Leoni, L. M., Iacobuzio-Donahue, C., Cameron, J. L., Yeo, C. J., et al. (2005). Homozygous deletion of the MTAP gene in invasive adenocarcinoma of the pancreas and in periampullary cancer: a potential new target for therapy. *Cancer Biol. Ther.* 4, 83–86. doi: 10.4161/cbt.4.1.1380

Iacobuzio-Donahue, C. A., Fu, B., Yachida, S., Luo, M., Abe, H., Henderson, C. M., et al. (2009). DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *J. Clin. Oncol.* 27, 1806–1813. doi: 10.1200/JCO.2008.17.7188

Jacobs, E. J., Chanock, S. J., Fuchs, C. S., Lacroix, A., McWilliams, R. R., Steplowski, E., et al. (2010). Family history of cancer and risk of pancreatic cancer: a pooled analysis from the Pancreatic Cancer Cohort Consortium (PanScan). *Int. J. Cancer* 127, 1421–1428. doi: 10.1002/ijc.25148

James, G. L., Goldstein, J. L., Brown, M. S., Rawson, T. E., Somers, T. C., McDowell, R. S., et al. (1993). Benzodiazepine peptidomimetics: potent inhibitors of Ras farnesylation in animal cells. *Science* 260, 1937–1942. doi: 10.1126/science.8316834

James, T. A., Sheldon, D. G., Rajput, A., Kuvshinoff, B. W., Javle, M. M., Nava, H. R., et al. (2004). Risk factors associated with earlier age of onset in familial pancreatic carcinoma. *Cancer* 101, 2722–2726. doi: 10.1002/cncr.20700

Jenne, D. E., Reimann, H., Nezu, J., Friedel, W., Loff, S., Jeschke, R., et al. (1998). Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. *Nat. Genet.* 18, 38–43. doi: 10.1038/ng0198-38

Jones, S., Hruban, R. H., Kamiyama, M., Borges, M., Zhang, X., Parsons, D. W., et al. (2009). Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. *Science* 324, 217. doi: 10.1126/science.1171202

Jones, S., Zhang, X., Parsons, D. W., Lin, J. C., Leary, R. J., Angenendt, P., et al. (2008). Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 321, 1801–1806. doi: 10.1126/science.1164368

Kelleher, F. C. (2011). Hedgehog signaling and therapeutics in pancreatic cancer. *Carcinogenesis* 32, 445–451. doi: 10.1093/carcin/bgq280

Kindler, H. L., Friberg, G., Singh, D. A., Locker, G., Nattam, S., Kozloff, M., et al. (2005). Phase II trial of bevacizumab plus gemcitabine in patients with advanced pancreatic cancer. *J. Clin. Oncol.* 23, 8033–8040. doi: 10.1200/JCO.2005.01.9661

Kindler, H. L., Niedzwiecki, D., Hollis, D., Sutherland, S., Schrag, D., Hurwitz, H., et al. (2010). Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303). *J. Clin. Oncol.* 28, 3617–3622. doi: 10.1200/JCO.2010.28.1386

Kindler, H. L., Richards, D. A., Garbo, L. E., Garon, E. B., Stephenson, J. J. Jr., Rocha-Lima, C. M., et al. (2012). randomized, A, placebo-controlled phase 2 study of ganitumab (AMG 479) or conatumumab (AMG 655) in combination with gemcitabine in patients with metastatic pancreatic cancer. *Ann. Oncol.* 23, 2834–2842. doi: 10.1093/annonc/mds142

Klein, A. P. (2012). Genetic susceptibility to pancreatic cancer. *Mol. Carcinog.* 51, 14–24. doi: 10.1002/mc.20855

Klein, A. P., Brune, K. A., Petersen, G. M., Goggins, M., Tersmette, A. C., Offerhaus, G. J., et al. (2004). Prospective risk of pancreatic cancer in familial pancreatic cancer kindreds. *Cancer Res.* 64, 2634–2638. doi: 10.1158/0008-5472.CAN-03-3823

Klein, A. P., Hruban, R. H., Brune, K. A., Petersen, G. M., and Goggins, M. (2001). Familial pancreatic cancer. *Cancer J.* 7, 266–273.

Koorstra, J. B., Hustinx, S. R., Offerhaus, G. J., and Maitra, A. (2008). Pancreatic carcinogenesis. *Pancreatology* 8, 110–125. doi: 10.1159/000123838

Kraakman-van der Zwet, M., Overkamp, W. J., van Lange, R. E., Essers, J., van Duijn-Goedhart, A., Wiggers, I., et al. (2002). Brca2 (XRCC11) deficiency results in radioresistant DNA synthesis and a higher frequency of spontaneous deletions. *Mol. Cell. Biol.* 22, 669–679. doi: 10.1128/MCB.22.2.669-679.2002

Krainer, M., Silva-Arrieta, S., FitzGerald, M. G., Shimada, A., Ishioka, C., Kanamaru, R., et al. (1997). Differential contributions of BRCA1 and BRCA2 to early-onset breast cancer. *N. Engl. J. Med.* 336, 1416–1421. doi: 10.1056/NEJM199705153362003

Lal, G., Liu, G., Schmocker, B., Kaurah, P., Ozcelik, H., Narod, S. A., et al. (2000). Inherited predisposition to pancreatic adenocarcinoma: role of family history and germ-line p16, BRCA1, and BRCA2 mutations. *Cancer Res.* 60, 409–416.

Langston, A. A., Malone, K. E., Thompson, J. D., Daling, J. R., and Ostrander, E. A. (1996). BRCA1 mutations in a population-based sample of young women with breast cancer. *N. Engl. J. Med.* 334, 137–142. doi: 10.1056/NEJM199601183340301

Li, D., Xie, K., Wolff, R., and Abbruzzese, J. L. (2004a). Pancreatic cancer. *Lancet* 363, 1049–1057. doi: 10.1016/S0140-6736(04)15841-8

Li, J., Kleeff, J., Giese, N., Buchler, M. W., Korc, M., and Friess, H. (2004b). 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.

Lorusso, P. M., Adjei, A. A., Varterasian, M., Gadgeel, S., Reid, J., Mitchell, D. Y., et al. (2005). Phase I and pharmacodynamic study of the oral MEK inhibitor CI-1040 in patients with advanced malignancies. *J. Clin. Oncol.* 23, 5281–5293. doi: 10.1200/JCO.2005.14.415

LoRusso, P. M., Rudin, C. M., Reddy, J. C., Tibes, R., Weiss, G. J., Borad, M. J., et al. (2011). Phase I trial of hedgehog pathway inhibitor vismodegib (GDC-0449) in patients with refractory, locally advanced or metastatic solid tumors. *Clin. Cancer Res.* 17, 2502–2511. doi: 10.1158/1078-0432.CCR-10-2745

Lu, X., Xu, T., Qian, J., Wen, X., and Wu, D. (2002). Detecting K-ras and p53 gene mutation from stool and pancreatic juice for diagnosis of early pancreatic cancer. *Chin. Med. J.* 115, 1632–1636.

Lubinski, J., Phelan, C. M., Ghadirian, P., Lynch, H. T., Garber, J., Weber, B., et al. (2004). Cancer variation associated with the position of the mutation in the BRCA2 gene. *Fam. Cancer* 3, 1–10. doi: 10.1023/B:FAME.0000026816.32400.45

Lynch, H. T., Fitzsimmons, M. L., Smyrk, T. C., Lanspa, S. J., Watson, P., McClellan, J., et al. (1990). Familial pancreatic cancer: clinicopathologic study of 18 nuclear families. *Am. J. Gastroenterol.* 85, 54–60.

Lynch, H. T., Fusaro, R. M., Lynch, J. F., and Brand, R. (2008). Pancreatic cancer and the FAMMM syndrome. *Fam. Cancer* 7, 103–112. doi: 10.1007/s10689-007-9166-4

Lynch, H. T., Smyrk, T., Kern, S. E., Hruban, R. H., Lightdale, C. J., Lemon, S. J., et al. (1996). Familial pancreatic cancer: a review. *Semin. Oncol.* 23, 251–275.

MacDermott, R. P., and Kramer, P. (1973). Adenocarcinoma of the pancreas in four siblings. *Gastroenterology* 65, 137–139.

Maitra, A., Kern, S. E., and Hruban, R. H. (2006). Molecular pathogenesis of pancreatic cancer. *Best Pract. Res. Clin. Gastroenterol.* 20, 211–226. doi: 10.1016/j.bpg.2005.10.002

Maloney, E. K., McLaughlin, J. L., Dagdigian, N. E., Garrett, L. M., Connors, K. M., Zhou, X. M., et al. (2003). An anti-insulin-like growth factor I receptor antibody that is a potent inhibitor of cancer cell proliferation. *Cancer Res.* 63, 5073–5083.

McFaul, C. D., Greenhalf, W., Earl, J., Howes, N., Neoptolemos, J. P., Kress, R., et al. (2006). Anticipation in familial pancreatic cancer. *Gut* 55, 252–258. doi: 10.1136/gut.2005.065045

Mimeault, M., and Batra, S. K. (2010). Frequent deregulations in the hedgehog signaling network and cross-talks with the epidermal growth factor receptor pathway involved in cancer progression and targeted therapies. *Pharmacol. Rev.* 62, 497–524. doi: 10.1124/pr.109.002329

Min, Y., Adachi, Y., Yamamoto, H., Ito, H., Itoh, F., Lee, C. T., et al. (2003). Genetic blockade of the insulin-like growth factor-I receptor: a promising strategy for human pancreatic cancer. *Cancer Res.* 63, 6432–6441.

Moore, M. J., Goldstein, D., Hamm, J., Figer, A., Hecht, J. R., Gallinger, S., et al. (2007). Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J. Clin. Oncol.* 25, 1960–1966. doi: 10.1200/JCO.2006.07.9525

Moran, A., O'Hara, C., Khan, S., Shack, L., Woodward, E., Maher, E. R., et al. (2012). Risk of cancer other than breast or ovarian in individuals with BRCA1 and BRCA2 mutations. *Fam. Cancer* 11, 235–242. doi: 10.1007/s10689-011-9506-2

Murphy, K. M., Brune, K. A., Griffin, C., Sollenberger, J. E., Petersen, G. M., Bansal, R., et al. (2002). Evaluation of candidate genes MAP2K4, MADH4, ACVR1B, and BRCA2 in familial pancreatic cancer: deleterious BRCA2 mutations in 17%. *Cancer Res.* 62, 3789–3793.

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. doi: 10.1038/sj.onc.1205044

Oddoux, C., Struewing, J. P., Clayton, C. M., Neuhausen, S., Brody, L. C., Kaback, M., et al. (1996). The carrier frequency of the BRCA2 6174delT mutation among Ashkenazi Jewish individuals is approximately 1%. *Nat. Genet.* 14, 188–190. doi: 10.1038/ng1096-188

Olive, K. P., Jacobetz, M. A., Davidson, C. J., Gopinathan, A., McIntyre, D., Honess, D., et al. (2009). Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 324, 1457–1461. doi: 10.1126/science.1171362

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. doi: 10.1016/S0046-8177(03)00291-0

Ozcelik, H., Schmocker, B., Di Nicola, N., Shi, X. H., Langer, B., Moore, M., et al. (1997). Germline BRCA2 6174delT mutations in Ashkenazi Jewish pancreatic cancer patients. *Nat. Genet.* 16, 17–18. doi: 10.1038/ng0597-17

Pasca di Magliano, M., Sekine, S., Ermilov, A., Ferris, J., Dlugosz, A. A., and Hebrok, M. (2006). Hedgehog/Ras interactions regulate early stages of pancreatic cancer. *Genes Dev.* 20, 3161–3173. doi: 10.1101/gad.1470806

Patel, K. J., Yu, V. P., Lee, H., Corcoran, A., Thistlethwaite, F. C., Evans, M. J., et al. (1998). Involvement of Brca2 in DNA repair. *Mol. Cell* 1, 347–357. doi: 10.1016/S1097-2765(00)80035-0

Permuth-Wey, J., and Egan, K. M. (2009). Family history is a significant risk factor for pancreatic cancer: results from a systematic review and meta-analysis. *Fam. Cancer* 8, 109–117. doi: 10.1007/s10689-008-9214-8

Phelan, C. M., Lancaster, J. M., Tonin, P., Gumbs, C., Cochran, C., Carter, R., et al. (1996). Mutation analysis of the BRCA2 gene in 49 site-specific breast cancer families. *Nat. Genet.* 13, 120–122. doi: 10.1038/ng0596-120

Philip, P. A., Benedetti, J., Corless, C. L., Wong, R., O'Reilly, E. M., Flynn, P. J., et al. (2010). Phase III study comparing gemcitabine plus cetuximab versus gemcitabine in patients with advanced pancreatic adenocarcinoma: southwest oncology group-directed intergroup trial S(0205). *J. Clin. Oncol.* 28, 3605–3610. doi: 10.1200/JCO.2009.25.7550

Rahman, N., Seal, S., Thompson, D., Kelly, P., Renwick, A., Elliott, A., et al. (2007). PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nat. Genet.* 39, 165–167. doi: 10.1038/ng1959

Redston, M. S., Caldas, C., Seymour, A. B., Hruban, R. H., da Costa, L., Yeo, C. J., et al. (1994). p53 mutations in pancreatic carcinoma and evidence of common involvement of homocopolymer tracts in DNA microdeletions. *Cancer Res.* 54, 3025–3033.

Reichert, M., Saur, D., Hamacher, R., Schmid, R. M., and Schneider, G. (2007). Phosphoinositide-3-kinase signaling controls S-phase kinase-associated protein 2 transcription via E2F1 in pancreatic ductal adenocarcinoma cells. *Cancer Res.* 67, 4149–4156. doi: 10.1158/0008-5472.CAN-06-4484

Reimer, R. R., Fraumeni, J. F. Jr., Ozols, R. F., and Bender, R. (1977). Pancreatic cancer in father and son. *Lancet* 1, 911. doi: 10.1016/S0140-6736(77)91244-2

Rinehart, J., Adjei, A. A., Lorusso, P. M., Waterhouse, D., Hecht, J. R., Natale, R. B., et al. (2004). Multicenter phase II study of the oral inhibitor, MEK, CI-1040, in patients with advanced non-small-cell lung, breast, colon, and pancreatic cancer. *J. Clin. Oncol.* 22, 4456–4462. doi: 10.1200/JCO.2004.01.185

Rowley, M., Ohashi, A., Mondal, G., Mills, L., Yang, L., Zhang, L., et al. (2011). Inactivation of Brca2 promotes Trp53-associated but inhibits KrasG12D-dependent pancreatic cancer development in mice. *Gastroenterology* 140, 1303–1313.e1–e3. doi: 10.1053/j.gastro.2010.12.039

Rozenblum, E., Schutte, M., Goggins, M., Hahn, S. A., Panzer, S., Zahurak, M., et al. (1997). Tumor-suppressive pathways in pancreatic carcinoma. *Cancer Res.* 57, 1731–1734.

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. doi: 10.1002/(SICI)1098-2744(199802)21:2<81::AID-MC1>3.0.CO;2-R

Rulyak, S. J., Lowenfels, A. B., Maisonneuve, P., and Brentnall, T. A. (2003). Risk factors for the development of pancreatic cancer in familial pancreatic cancer kindreds. *Gastroenterology* 124, 1292–1299. doi: 10.1016/S0016-5085(03)00272-5

Sato, N., Rosty, C., Jansen, M., Fukushima, N., Ueki, T., Yeo, C. J., et al. (2001). STK11/LKB1 Peutz-Jeghers gene inactivation in intraductal papillary-mucinous neoplasms of the pancreas. *Am. J. Pathol.* 159, 2017–2022. doi: 10.1016/S0002-9440(10)63053-2

Schenk, M., Schwartz, A. G., O'Neal, E., Kinnard, M., Greenson, J. K., Fryzek, J. P., et al. (2001). Familial risk of pancreatic cancer. *J. Natl. Cancer Inst.* 93, 640–644. doi: 10.1093/jnci/93.8.640

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. doi: 10.1038/sj.bjc.6601396

Schnall, S. F., and Macdonald, J. S. (1996). Chemotherapy of adenocarcinoma of the pancreas. *Semin. Oncol.* 23, 220–228.

Schneider, G., and Schmid, R. M. (2003). Genetic alterations in pancreatic carcinoma. *Mol. Cancer* 2:15. doi: 10.1186/1476-4598-2-15

Schneider, R., Slater, E. P., Sina, M., Habbe, N., Fendrich, V., Matthai, E., et al. (2011). German national case collection for familial pancreatic cancer (FaPaCa): ten years experience. *Fam. Cancer* 10, 323–330. doi: 10.1007/s10689-010-9414-x

Schonleben, F., Qiu, W., Ciau, N. T., Ho, D. J., Li, X., Allendorf, J. D., et al. (2006). PIK3CA mutations in intraductal papillary mucinous neoplasm/carcinoma of the pancreas. *Clin. Cancer Res.* 12, 3851–3855. doi: 10.1158/1078-0432.CCR-06-0292

Sharan, S. K., Morimatsu, M., Albrecht, U., Lim, D. S., Regel, E., Dinh, C., et al. (1997). Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. *Nature* 386, 804–810. doi: 10.1038/386804a0

Siegel, R., Naishadham, D., and Jemal, A. (2013). Cancer statistics (2013). *CA Cancer J. Clin.* 63, 11–30. doi: 10.3322/caac.21166

Silverman, D. T., Schiffman, M., Everhart, J., Goldstein, A., Lillemoe, K. D., Swanson, G. M., et al. (1999). Diabetes mellitus, other medical conditions and familial history of cancer as risk factors for pancreatic cancer. *Br. J. Cancer* 80, 1830–1837. doi: 10.1038/sj.bjc.6690607

Skoulidis, F., Cassidy, L. D., Pisupati, V., Jonasson, J. G., Bjarnason, H., Eyfjord, J. E., et al. (2010). Germline Brca2 heterozygosity promotes Kras(G12D) -driven carcinogenesis in a murine model of familial pancreatic cancer. *Cancer Cell* 18, 499–509. doi: 10.1016/j.ccr.2010.10.015

Slater, E. P., Langer, P., Niemczyk, E., Strauch, K., Butler, J., Habbe, N., et al. (2010). PALB2 mutations in European familial pancreatic cancer families. *Clin. Genet.* 78, 490–494. doi: 10.1111/j.1399-0004.2010.01425.x

Spano, J. P., Chodkiewicz, C., Maurel, J., Wong, R., Wasan, H., Barone, C., et al. (2008). Efficacy of gemcitabine plus axitinib compared with gemcitabine alone in patients with advanced pancreatic cancer: an open-label randomised phase II study. *Lancet* 371, 2101–2108. doi: 10.1016/S0140-6736(08)60661-3

Stoecklein, N. H., Luebke, A. M., Erbersdobler, A., Knoefel, W. T., Schraut, W., Verde, P. E., et al. (2004). Copy number of chromosome 17 but not HER2 amplification predicts clinical outcome of patients with pancreatic ductal adenocarcinoma. *J. Clin. Oncol.* 22, 4737–4745. doi: 10.1200/JCO.2004.05.142

Stoeltzing, O., Liu, W., Reinmuth, N., Fan, F., Parikh, A. A., Bucana, C. D., et al. (2003). Regulation of hypoxia-inducible factor-1alpha, 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. doi: 10.1016/S0002-9440(10)63460-8

Talar-Wojnarowska, R., and Malecka-Panas, E. (2006). Molecular pathogenesis of pancreatic adenocarcinoma: potential clinical implications. *Med. Sci. Monit.* 12, RA186–RA193.

Tanno, S., Tanno, S., Mitsuuchi, Y., Altomare, D. A., Xiao, G. H., and Testa, J. R. (2001). AKT activation up-regulates insulin-like growth factor I receptor expression and promotes invasiveness of human pancreatic cancer cells. *Cancer Res.* 61, 589–593.

Tersmette, A. C., Petersen, G. M., Offerhaus, G. J., Falatko, F. C., Brune, K. A., Goggins, M., et al. (2001). Increased risk of incident pancreatic cancer among first-degree relatives of patients with familial pancreatic cancer. *Clin. Cancer Res.* 7, 738–744.

Thompson, D., Easton, D. F., and Breast Cancer Linkage Consortium. (2002). Cancer incidence in BRCA1 mutation carriers. *J. Natl. Cancer Inst.* 94, 1358–1365. doi: 10.1093/jnci/94.18.1358

Tischkowitz, M. D., Sabbaghian, N., Hamel, N., Borgida, A., Rosner, C., Taherian, N., et al. (2009). Analysis of the gene coding for the BRCA2-interacting protein PALB2 in familial and sporadic pancreatic cancer. *Gastroenterology* 137, 1183–1186. doi: 10.1053/j.gastro.2009.06.055

van Asperen, C. J., Brohet, R. M., Meijers-Heijboer, E. J., Hoogerbrugge, N., Verhoef, S., Vasen, H. F., et al. (2005). Cancer risks in BRCA2 families: estimates for sites other than breast and ovary. *J. Med. Genet.* 42, 711–719. doi: 10.1136/jmg.2004.028829

Van Cutsem, E., van de Velde, H., Karasek, P., Oettle, H., Vervenne, W. L., Szawlowski, A., 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. doi: 10.1200/JCO.2004.10.112

van der Heijden, M. S., Brody, J. R. Dezentje, D. A., Gallmeier, E., Cunningham, S. C., Swartz, M. J., et al. (2005). *In vivo* therapeutic responses contingent on Fanconi anemia/BRCA2 status of the tumor. *Clin. Cancer Res.* 11, 7508–7515. doi: 10.1158/1078-0432.CCR-05-1048

Vasen, H. F., Wasser, M., van Mil, A., Tollenaar, R. A., Konstantinovski, M., Gruis, N. A., et al. (2011). Magnetic resonance imaging surveillance detects early-stage pancreatic cancer in carriers of a p16-Leiden mutation. *Gastroenterology* 140, 850–856. doi: 10.1053/j.gastro.2010.11.048

Wang, W., Chen, S., Brune, K. A., Hruban, R. H., Parmigiani, G., and Klein, A. P. (2007). PancPRO: risk assessment for individuals with a family history of pancreatic cancer. *J. Clin. Oncol.* 25, 1417–1422. doi: 10.1200/JCO.2006.09.2452

Wilentz, R. E., Su, G. H., Dai, J. L., Sparks, A. B., Argani, P., Sohn, T. A., et al. (2000). Immunohistochemical labeling for dpc4 mirrors genetic status in pancreatic adenocarcinomas : a new marker of DPC4 inactivation. *Am. J. Pathol.* 156, 37–43. doi: 10.1016/S0002-9440(10)64703-7

Willett, C. G., Kozin, S. V., Duda, D. G., di Tomaso, E., Kozak, K. R., Boucher, Y., et al. (2006). Combined vascular endothelial growth factor-targeted therapy and radiotherapy for rectal cancer: theory and clinical practice. *Semin. Oncol.* 33, S35–S40. doi: 10.1053/j.seminoncol.2006.08.007

Williamson, C. T., Kubota, E., Hamill, J. D., Klimowicz, A., Ye, R., Muzik, H., et al. (2012). Enhanced cytotoxicity of PARP inhibition in mantle cell lymphoma harbouring mutations in both ATM and p53. *EMBO Mol. Med.* 4, 515–527. doi: 10.1002/emmm.201200229

Wolpin, B. M., Hezel, A. F., Abrams, T., Blaszkowsky, L. S., Meyerhardt, J. A., Chan, J. A., et al. (2009). Oral mTOR inhibitor everolimus in patients with gemcitabine-refractory metastatic pancreatic cancer. *J. Clin. Oncol.* 27, 193–198. doi: 10.1200/JCO.2008.18.9514

Xia, B., Sheng, Q., Nakanishi, K., Ohashi, A., Wu, J., Christ, N., et al. (2006). Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. *Mol. Cell* 22, 719–729. doi: 10.1016/j.molcel.2006.05.022

Xin, W., Yun, K. J., Ricci, F., Zahurak, M., Qiu, W., Su, G. H., et al. (2004). MAP2K4/MKK4 expression in pancreatic cancer: genetic validation of immunohistochemistry and relationship to disease course. *Clin. Cancer Res.* 10, 8516–8520. doi: 10.1158/1078-0432.CCR-04-0885

Ying, H., Elpek, K. G., Vinjamoori, A., Zimmerman, S. M., Chu, G. C., Yan, H., et al. (2011). PTEN is a major tumor suppressor in pancreatic ductal adenocarcinoma and regulates an NF-kappaB-cytokine network. *Cancer Discov.* 1, 158–169. doi: 10.1158/2159-8290.CD-11-0031

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Overview on how oncogenic Kras promotes pancreatic carcinogenesis by inducing low intracellular ROS levels

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Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease without clearly known disease causes. Recent epidemiological and animal studies suggest that the supplementation of dietary antioxidants (e.g., vitamins C and E) decreases cancer risk, implying that increased reactive oxygen species (ROS) may play a role in pancreatic carcinogenesis. However, oncogenic Kras mutations (e.g., $Kras^{G12D}$), which are present in more than 90% of PDAC, have been proven to foster low intracellular ROS levels. Here, oncogenic Kras activates expression of a series of anti-oxidant genes via Nrf2 (nuclear factor, erythroid derived 2, like 2) and also mediates an unusual metabolic pathway of glutamine to generate NADPH. This can then be used as the reducing power for ROS detoxification, leading collectively to low ROS levels in pancreatic pre-neoplastic cells and in cancer cells. In adult stem cells and cancer stem cells, low ROS levels have been associated with the formation of a proliferation-permissive intracellular environment and with perseverance of self-renewal capacities. Therefore, it is conceivable that low intracellular ROS levels may contribute significantly to oncogenic Kras-mediated PDAC formation.

Keywords: pancreatic cancer, redox equilibrium, reactive oxygen species, oncogenic Kras, pancreatic cancer stem cells, $Kras^{G12D}$

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive tumor entity without clearly known disease causes (Kong et al., 2011; Siegel et al., 2013). Oncogenic *KRAS* (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) mutations (e.g., $KRAS^{G12D}$ or $KRAS^{G12V}$) have been considered as the initiating genetic event for this disease (Kong et al., 2011). Recently, prospective studies have demonstrated that dietary antioxidants (e.g., vitamins C and E) significantly decreased cancer risk, underscoring an important role of the redox equilibrium in the etiology of PDAC (Gong et al., 2010; Banim et al., 2012; Heinen et al., 2012). Furthermore, genetic variations in antioxidant genes seem to modify the risk to develop PDAC in humans (Tang et al., 2010). In line, long-term treatment with δ-tocotrienol (a bioactive vitamin E derivative) which has putative anti-oxidative activity dramatically inhibited $Kras^{G12D}$-driven formation of pancreatic intraepithelial neoplasms (mPanINs) in a genetically engineered mouse model (GEMM) of pancreatic cancer (Husain et al., 2011, 2013; Shin-Kang et al., 2011). These data suggest that a systemic reduction in the production of reactive oxygen species (ROS) may prevent/delay the development of PDAC. Paradoxically, recent studies have also demonstrated that oncogenic $Kras^{G12D}$ mediates activation of metabolic programs, which effectively detoxify ROS and thus reduce ROS levels in pancreatic pre-neoplastic cells and in cancer cells. Furthermore, low intracellular ROS levels seem to be essential for $Kras^{G12D}$-driven carcinogenesis in mice (deNicola et al., 2011; Son et al., 2013). In this case, a "chemo-" preventive effect of dietary antioxidants cannot be explained by reduced intracellular ROS levels in pancreatic epithelial cells. Thus, we reviewed and discussed the potential biological significance of $Kras^{G12D}$-mediated ROS-detoxifying networks.

ONCOGENIC Kras INITIATES PANCREATIC CANCER

Characterization of human cancer genomes confirmed that more than 90% of human PDACs harbor oncogenic KRAS mutations (Almoguera et al., 1988; Smit et al., 1988). The mutated *KRAS* encodes a protein locked in a constitutively active state, leading to persistent downstream signals such as activation of the RAF-MEK-ERK (extracellular signal-regulated kinase) cascade (Barbacid, 1987). The ability of oncogenic KRAS in initiating PDAC has been demonstrated in GEMMs of pancreatic cancer. Here, pancreas-specific expression of $Kras^{G12D}$ recapitulated the whole spectrum of human PDAC pathologies, from its precursor lesions to locally invasive and metastatic entities (Hingorani et al., 2003). Recent studies have demonstrated that the activity of $Kras^{G12D}$ is required for all stages of carcinogenesis including inception, progression and metastasis because inactivation of $Kras^{G12D}$ using genetic approaches invariably reversed the ongoing carcinogenic process (Collins et al., 2012). However, it remains largely elusive how $Kras^{G12D}$ exactly promotes PDAC development.

REACTIVE OXYGEN SPECIES (ROS) METABOLISM

Chemically reactive molecules containing oxygen, which are usually termed as ROS, consist of free radical ROS [e.g., oxygen ions (O_2^-)] and non-radical ROS [e.g., peroxide (H_2O_2)]. The free radical ROS has unpaired electrons in the molecular orbital whereas non-radical ROS contains no unpaired electrons

(Shi et al., 2012). ROS formation, as a natural byproduct of aerobic metabolism, can be derived from exogenous and endogenous sources (Castro and Freeman, 2001). As for the exogenous sources, substances (e.g., metals and chemicals) inducing ROS formation can be directly metabolized to radicals in cells or can trigger intracellular ROS production (Bonney et al., 1991; Halliwell and Aruoma, 1991; Dreher and Junod, 1996; Jaruga and Dizdaroglu, 1996; Wang et al., 1998). Under physiological circumstances, the mitochondrion is an intracellular organelle which is responsible for energy production through cellular respiration. However, the leaking electron from the mitochondrial electron transport chain eventually interacts with oxygen and generates superoxide radicals, producing approximately 98% of the endogenous ROS (Freeman and Crapo, 1982; McCord, 2000; Salvador et al., 2001). Apart from the mitochondrion, biochemical reactions within the endoplasmatic reticulum (ER), the peroxisome or the cytoplasm also generate additional ROS (Butler and Hoey, 1993; Conner and Grisham, 1996; Li and Jackson, 2002; Klaunig and Kamendulis, 2004; Valko et al., 2004). For instance, cytochrome P450 in the ER uses oxygen to oxidize and to detoxify foreign compounds; a process in which ROS are generated (Butler and Hoey, 1993). In addition, membrane-bound NADPH (nicotinamide adenine dinucleotide phosphate) oxidase in immune cells (e.g., neutrophils and macrophages) produces ROS via a biochemical process known as the respiratory burst, which is essential for these cells to eliminate bacteria (Conner and Grisham, 1996).

Since excessive ROS can cause oxidative damage to macromolecules (e.g., DNA and lipids) and can alter intracellular signal transduction (e.g., through NF-κB), intracellular ROS is constantly eliminated via a sophisticated ROS-detoxifying system including non-enzymatic antioxidants (e.g., Vitamins C and E) and enzymatic antioxidants [such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxides (GPX)] (Mates et al., 1999; McCall and Frei, 1999). Notably, the majority of these enzymes require the activity of reduced glutathione (GSH) which further relies on NADPH. In this case, NADPH provides the ultimate reducing power for ROS detoxification. Taken together, both non-enzymatic antioxidants and enzymatic antioxidants act as an "antioxidant network" which maintains a fine-tuned intracellular redox balance (Sies et al., 2005).

$Kras^{G12D}$ MAINTAINS LOW ROS LEVELS IN PDAC CELLS

Until today, it remains elusive how $Kras^{G12D}$ promotes PDAC. Recent studies demonstrated that $Kras^{G12D}$ induces maintenance of low intracellular ROS levels via the transcription factor Nrf2 (nuclear factor, erythroid derived 2, like 2), which is a master switch in the antioxidant network (deNicola et al., 2011). To provide a reducing power for this Nrf2-mediated antioxidant program, $Kras^{G12D}$ promotes a concerted metabolic program (e.g., thorough glutamine and fatty acid) that continually sustains the intracellular NADPH/$NADP^+$ ratio (Khasawneh et al., 2009; Son et al., 2013) (**Figure 1**).

An earlier study demonstrated that ectopic expression of oncogenic Ras increased ROS production through NADPH-oxidase (Nox; Irani et al., 1997). Later on, a follow-up study provided

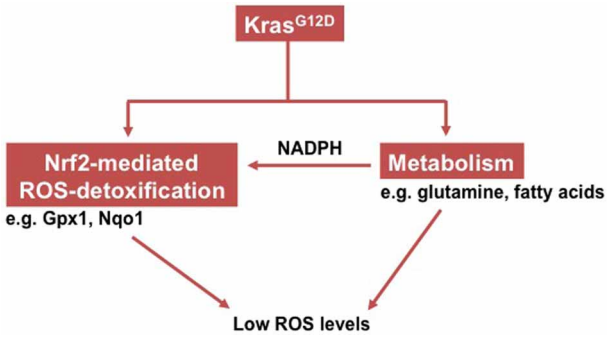

FIGURE 1 | Schema showing how oncogenic Kras induces low intracellular ROS levels.

evidence that such an increased ROS generation is functionally relevant to oncogenic Ras-mediated malignant transformation of NIH3T3 cells (Mitsushita et al., 2004). However, this concept has been challenged by a recent study which substantiated that ROS production was actually repressed by endogenous expression of the $Kras^{G12D}$ allele in mouse cell lines (deNicola et al., 2011). Further investigation uncovered that $Kras^{G12D}$ activated Nrf2 via MAPK pathways (mitogen-activated protein kinase), which then initiated a set of antioxidant programs. Consistently, human PanINs and PDACs exhibit activation of NRF2 and have low ROS levels in comparison to normal pancreatic ducts cells. NRF2, which is negatively regulated by KEAP1 (kelch-like ECH-associated protein 1), controls the expression of a series of proteins involved in different steps of ROS detoxification—such as NADPH generation (Cullinan et al., 2004; McMahon et al., 2006; Hayes and McMahon, 2009). Unlike many other tumor entities such as lung cancers (Shibata et al., 2008; Kim et al., 2010), however, PDACs rarely harbor somatic mutations in either the *KEAP1* or *NRF2* genes that usually result in an active NRF2. Hence, the Nrf2-mediated antioxidant program in PDAC is activated in an oncogenic Kras-dependent manner. In line, silencing of Kras or blockade of the MAPK pathway effectively decreased Nrf2 expression and increased intracellular ROS levels.

As early illustrated, ROS detoxification is a biochemical process that consumes NADPH (NADPH provides the reducing power). Thus, generation and maintenance of constant intracellular NADPH levels is essentially important. In this regard, a previous study demonstrated that $Kras^{G12D}$ enhanced glycolysis of PDAC cells and that it directed glycolytic intermediates into the non-oxidative pentose phosphate pathway (PPP) whereas the NAPDH-producing oxidative arm of the PPP remained unaffected (Ying et al., 2012). These data suggest that PDAC cells might use other NADPH-producing metabolic pathways to maintain intracellular NADPH levels. Indeed, a recent study uncovered a distinct metabolic pathway of glutamine which is used by PDAC cells to generate NADPH (Son et al., 2013). Briefly, glutamine-derived aspartate (Asp) and α-ketoglutarate (α-KG) are converted into oxaloacetate (OAA) via aspartate transaminase (GOT1). The OAA is metabolized into malate by malate dehydrogenase (MDH1) and subsequently into pyruvate by malic

enzyme (ME1). Conversion from malate to pyruvate then creates NADPH, which is important for maintaining the redox balance of PDACs because inactivation of any component of this metabolic pathway increased intracellular ROS levels and affected tumor growth (Cairns et al., 2011). Though the tumor environment of PDAC is usually depleted of glutamine, a recent study substantiated that PDAC cells containing oncogenic Kras showed an increased protein uptake by macropinocytosis. These internalized proteins are then metabolized into glutamine that is fueled into the NDAPH-producing process (Commisso et al., 2013). It has also been demonstrated that the $Kras^{G12D}$-expressing pancreas exhibited increased fatty acid oxidation (Khasawneh et al., 2009). Since fatty acid oxidation is a NADPH-generating process (Jeon et al., 2012), it remains to be defined whether increased fatty acid oxidation also contributes to the maintenance of NADPH levels.

In conclusion, collaboration between Nrf2-mediated ROS detoxification and the NADPH-generating metabolic program collectively contributes to a "reduced" intracellular environment (e.g., low ROS levels). Since both of these depend on the activity of oncogenic Kras, it is conceivable that such an intracellular environment constitutes an important step in pancreatic carcinogenesis.

THE TUMOR-SUPPRESSING FUNCTION OF ANTIOXIDANTS DOES NOT CONTRADICT TUMOR-PROMOTING EFFECTS OF ONCOGENIC Kras-MEDIATED LOW INTRACELLULAR ROS LEVELS

As earlier illustrated, prospective studies have suggested an association between dietary antioxidants and a decreased risk for developing pancreatic cancer (Gong et al., 2010; Banim et al., 2012; Heinen et al., 2012). Besides, certain antioxidants (especially δ-tocotrienol) have chemo-preventive effects in GEMMs of pancreatic cancer (Husain et al., 2011, 2013; Shin-Kang et al., 2011). Interestingly, these data rather point to a tumor-suppressing function of antioxidants in pancreatic cancer. However, the emergence of this evidence does not necessarily argue against the tumor-promoting functions of oncogenic Kras-mediated low intracellular ROS levels. Firstly, the tumor-suppressing function of antioxidants may be attributed to their effects on the immune system and especially T cell immunity. Recently, it has been shown that antitumor T cell immunity plays a crucial role in the early stages of pancreatic carcinogenesis (Bayne et al., 2012; Pylayeva-Gupta et al., 2012). In this regard, the dietary supplementation of antioxidants (e.g., vitamins E or C) has been proven to significantly enhance T cell immunity in humans (Burgess and Johansen, 1976; Meydani et al., 1997; Malmberg et al., 2002). Therefore, antioxidants may execute their tumor-suppressing functions by promoting antitumor immunity. Secondly, it remains largely unknown whether ROS levels in the pancreas (especially in epithelial cells) are actually affected by the intake of dietary antioxidant in humans. Thus, it is difficult to evaluate the contribution of their antioxidative effects to the development of pancreatic cancer. Lastly, some antioxidants display antitumor activities independent of their antioxidative effects. For instance, δ-tocotrienol, which has been used for chemo-prevention of pancreatic cancer in animal studies, contains an unsaturated isoprenoid side chain that has a unique antitumor property (Shin-Kang et al., 2011). Taken together, further studies are required to clarify how/why antioxidants execute their tumor-suppressor functions on oncogenic Kras-mediated low intracellular ROS levels in the pancreas.

LOW ROS LEVELS IN DIFFERENT BIOLOGICAL SYSTEMS

Although the biological significance of such an oncogenic Kras-mediated reductive intracellular environment remains unclear, this phenomenon has been widely described in other biological systems (**Table 1**). For example, when yeast cells are cultured under nutrient-limited conditions, they display a periodic metabolic cycle alternating between glycolysis and respiration. Their cell cycle is tightly restricted to the reductive phase of the metabolic cycle, which guarantees that DNA replication only occurs during glycolysis when the oxidative damage from respiration on the genome is minimal. Such a circadian rhythm that coordinates the metabolic and cell division cycles in situations where resources are limited, simply reflects an evolutionarily conserved means of preserving genome integrity (Chen et al., 2007). Silencing of a DNA checkpoint kinase abolishing such a rhythm allows DNA synthesis outside of the reductive phase but at the cost of increased spontaneous mutation rates. In adult stem cells, a similar nutrient-limited microenvironment (hypoxia) with low intracellular ROS levels also exists (Suda et al., 2011; Zhang and Sadek, 2013). Here, low ROS levels have been shown to be essential for maintaining the stem cell functions of hematopoietic stem cells (HSCs) in that the ROS^{low} cell population expressed high levels of stemness-associated molecules such as Notch1 and telomerase; it also had a higher self-renewal potential than the ROS^{high} population of cells (Jang and Sharkis, 2007). Similarly, mammary epithelial stem cells have low ROS

Table 1 | Cellular systems with low intracellular ROS levels.

References	Species/organ system	Condition	Biological significance
Chen et al., 2007	Yeast	Nutrient-limited	Preserve integrity of the genome
Jang and Sharkis, 2007	Mouse/hematopoietic stem cells	Hypoxic	Reserve stem cell function
Diehn et al., 2009	Mouse/mammary epithelial stem cells	–	Maintain stemness
Diehn et al., 2009	Human/breast CSCs	Cancer microenvironment	Preserve tumor-initiating capacity and radio-resistance
Dong et al., 2013	Cell lines/basal-like breast cancer CSCs	Inhibit ROS production by metabolic reprogramming	Promote CSC-like properties

levels (Diehn et al., 2009). Low ROS levels have been described in another type of "stem" cells—the "cancer stem" cells (CSCs) or "tumor-initiating" cells (TICs) (Shi et al., 2012). Historically, CSCs have been defined as a subset of cancer cells that are responsible for initiation, maintenance and metastasis of cancer (Lapidot et al., 1994). A seminal study demonstrated that human breast CSCs contained lower ROS levels than their non-tumorigenic progeny (Diehn et al., 2009). These low ROS levels rendered the CSCs highly resistant toward irradiation-induced DNA damage and cell death. Consistently, a recent study provided functional evidence that CSC-like properties in basal-like breast cancer are induced, when ROS production is inhibited by metabolic reprogramming of glucose metabolism [e.g., when more NADPH is generated, (Dong et al., 2013)]. Taken together, low ROS levels in other biological systems appear to be associated with stemness properties of cells in mammals or with a proliferation-permissive intracellular environment in low eukaryotic systems, both of which may contribute to oncogenic Kras-mediated carcinogenesis in the pancreas.

LOW ROS LEVELS AND PANCREATIC CARCINOGENESIS

Because early expansion of pancreatic stem/progenitor cells accelerates $Kras^{G12D}$-driven carcinogenesis in mice (Kong et al., 2011), $Kras^{G12D}$-induced low intracellular ROS levels may facilitate expansion of pancreatic stem/progenitor cells by creating a proliferation-permissive intracellular environment. Furthermore, despite a questionable general compliance of PDAC to the CSCs concept, the heterogeneity of pancreatic cancer tissues indicates that a subset of pancreatic cancer cells may have low intracellular ROS levels in comparison to others.

CONCLUSION

The exact contribution of pre-neoplastic and cancer cells with low ROS levels to PDAC initiation, progression and metastasis in humans remains to be defined. Certainly, such a subset of cancer cells may constitute a promising drug target for future therapies. Though the Nrf2-mediated network has been proposed as a potential drug target (Arlt et al., 2012), further studies on the contribution of (low) ROS levels to the aggressiveness of pancreatic cancer are warranted.

REFERENCES

Almoguera, 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. doi: 10.1016/0092-8674(88)90571-5

Arlt, A., Sebens, S., Krebs, S., Geismann, C., Grossmann, M., Kruse, M. L., et al. (2012). Inhibition of the Nrf2 transcription factor by the alkaloid trigonelline renders pancreatic cancer cells more susceptible to apoptosis through decreased proteasomal gene expression and proteasome activity. *Oncogene* doi: 10.1038/onc.2012.493. [Epub ahead of print].

Banim, P. J., Luben, R., McTaggart, A., Welch, A., Wareham, N., Khaw, K. T., et al. (2012). Dietary antioxidants and the aetiology of pancreatic cancer: a cohort study using data from food diaries and biomarkers. *Gut*. doi: 10.1136/gutjnl-2011-301908. [Epub ahead of print].

Barbacid, M. (1987). Ras genes. *Annu. Rev. Biochem.* 56, 779–827.

Bayne, L. J., Beatty, G. L., Jhala, N., Clark, C. E., Rhim, A. D., Stanger, B. Z., et al. (2012). Tumor-derived granulocyte-macrophage colony-stimulating factor regulates myeloid inflammation and T cell immunity in pancreatic cancer. *Cancer Cell* 21, 822–835. doi: 10.1016/j.ccr.2012.04.025

Bonney, R. C., Higham, J. M., Watson, H., Beesley, J. S., Shaw, R. W., and Franks, S. (1991). Phospholipase activity in the endometrium of women with normal menstrual blood loss and women with proven ovulatory menorrhagia. *Br. J. Obstet. Gynaecol.* 98, 363–368. doi: 10.1111/j.1471-0528.1991.tb13426.x

Burgess, A. W., and Johansen, P. M. (1976). Assault: patterns of emergency visits. *J. Psychiatr. Nurs. Ment. Health Serv.* 14, 32–36.

Butler, J., and Hoey, B. M. (1993). The one-electron reduction potential of several substrates can be related to their reduction rates by cytochrome P-450 reductase. *Biochim. Biophys. Acta* 1161, 73–78. doi: 10.1016/0167-4838(93)90198-Z

Cairns, R. A., Harris, I. S., and Mak, T. W. (2011). Regulation of cancer cell metabolism. *Nat. Rev. Cancer* 11, 85–95. doi: 10.1038/nrc2981

Castro, L., and Freeman, B. A. (2001). Reactive oxygen species in human health and disease. *Nutrition* 17 161, 163–165.

Chen, Z., Odstrcil, E. A., Tu, B. P., and McKnight, S. L. (2007). Restriction of DNA replication to the reductive phase of the metabolic cycle protects genome integrity. *Science* 316, 1916–1919. doi: 10.1126/science.1140958

Collins, M. A., Bednar, F., Zhang, Y., Brisset, J. C., Galban, S., Galbán, C. J., et al. (2012). Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer in mice. *J. Clin. Invest.* 122, 639–653. doi: 10.1172/JCI59227

Commisso, C., Davidson, S. M., Soydaner-Azeloglu, R. G., Parker, S. J., Kamphorst, J. J., Hackett, S., et al. (2013). Macropinocytosis of protein is an amino acid supply route in Ras-transformed cells. *Nature* 497, 633–637. doi: 10.1038/nature12138

Conner, E. M., and Grisham, M. B. (1996). Inflammation, free radicals, and antioxidants. *Nutrition* 12, 274–277. doi: 10.1016/S0899-9007(96)00000-8

Cullinan, S. B., Gordan, J. D., Jin, J., Harper, J. W., and Diehl, J. A. (2004). The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase. *Mol. Cell. Biol.* 24, 8477–8486. doi: 10.1128/MCB.24.19.8477-8486.2004

deNicola, G. M., Karreth, F. A., Humpton, T. J., Gopinathan, A., Wei, C., Frese, K., et al. (2011). Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature* 475, 106–109. doi: 10.1038/nature10189

Diehn, M., Cho, R. W., Lobo, N. A., Kalisky, T., Dorie, M. J., Kulp, A. N., et al. (2009). Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature* 458, 780–783. doi: 10.1038/nature07733

Dong, C., Yuan, T., Wu, Y., Wang, Y., Fan, T. W., Miriyala, S., et al. (2013). Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. *Cancer Cell* 23, 316–331. doi: 10.1016/j.ccr.2013.01.022

Dreher, D., and Junod, A. F. (1996). Role of oxygen free radicals in cancer development. *Eur. J. Cancer* 32A, 30–38.

Freeman, B. A., and Crapo, J. D. (1982). Biology of disease: free radicals and tissue injury. *Lab. Invest.* 47, 412–426.

Gong, Z., Holly, E. A., Wang, F., Chan, J. M., and Bracci, P. M. (2010). Intake of fatty acids and antioxidants and pancreatic cancer in a large population-based case-control study in the San Francisco Bay Area. *Int. J. Cancer* 127, 1893–1904. doi: 10.1002/ijc.25208

Halliwell, B., and Aruoma, O. I. (1991). DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. *FEBS Lett.* 281, 9–19. doi: 10.1016/0014-5793(91)80347-6

Hayes, J. D., and McMahon, M. (2009). NRF2 and KEAP1 mutations: permanent activation of an adaptive response in cancer. *Trends Biochem. Sci.* 34, 176–188. doi: 10.1016/j.tibs.2008.12.008

Heinen, M. M., Verhage, B. A., Goldbohm, R. A., and van den Brandt, P. A. (2012). Intake of vegetables, fruits, carotenoids and vitamins C and E and pancreatic cancer risk in The Netherlands Cohort Study. *Int. J. Cancer* 130, 147–158. doi: 10.1002/ijc.25989

Hingorani, S. R., Petricoin, E. F., Maitra, A., Rajapakse, V., King, C., Jacobetz, M. A., et al. (2003). Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* 4, 437–450. doi: 10.1016/S1535-6108(03)00309-X

Husain, K., Centeno, B. A., Chen, D. T., Fulp, W. J., Perez, M., Zhang Lee, G., et al. (2013). Prolonged survival and delayed progression of pancreatic intraepithelial neoplasia in LSL-KrasG12D/+;Pdx-1-Cre mice by vitamin E delta-tocotrienol. *Carcinogenesis* 34, 858–863. doi: 10.1093/carcin/bgt002

Husain, K., Francois, R. A., Yamauchi, T., Perez, M., Sebti, S. M., and Malafa, M. P. (2011). Vitamin E delta-tocotrienol augments the antitumor activity of gemcitabine and suppresses constitutive NF-kappaB activation in pancreatic cancer. *Mol. Cancer Ther.* 10, 2363–2372. doi: 10.1158/1535-7163.MCT-11-0424

Irani, K., Xia, Y., Zweier, J. L., Sollott, S. J., Der, C. J., Fearon, E. R., et al. (1997). Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. *Science* 275, 1649–1652. doi: 10.1126/science.275.5306.1649

Jang, Y. Y., and Sharkis, S. J. (2007). A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. *Blood* 110, 3056–3063. doi: 10.1182/blood-2007-05-087759

Jaruga, P., and Dizdaroglu, M. (1996). Repair of products of oxidative DNA base damage in human cells. *Nucleic Acids Res.* 24, 1389–1394. doi: 10.1093/nar/24.8.1389

Jeon, S. M., Chandel, N. S., and Hay, N. (2012). AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress. *Nature* 485, 661–665. doi: 10.1038/nature11066

Khasawneh, J., Schulz, M. D., Walch, A., Rozman, J., Hrabe de Angelis, M., Klingenspor, M., et al. (2009). Inflammation and mitochondrial fatty acid beta-oxidation link obesity to early tumor promotion. *Proc. Natl. Acad. Sci. U.S.A.* 106, 3354–3359. doi: 10.1073/pnas.0802864106

Kim, Y. R., Oh, J. E., Kim, M. S., Kang, M. R., Park, S. W., Han, J. Y., et al. (2010). Oncogenic NRF2 mutations in squamous cell carcinomas of oesophagus and skin. *J. Pathol.* 220, 446–451. doi: 10.1002/path.2653

Klaunig, J. E., and Kamendulis, L. M. (2004). The role of oxidative stress in carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.* 44, 239–267. doi: 10.1146/annurev.pharmtox.44.101802.121851

Kong, B., Michalski, C. W., Erkan, M., Friess, H., and Kleeff, J. (2011). From tissue turnover to the cell of origin for pancreatic cancer. *Nat. Rev. Gastroenterol.Hepatol.* 8, 467–472. doi: 10.1038/nrgastro.2011.114

Lapidot, T., Sirard, C., Vormoor, J., Murdoch, B., Hoang, T., Caceres-Cortes, J., et al. (1994). A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 367, 645–648. doi: 10.1038/367645a0

Li, C., and Jackson, R. M. (2002). Reactive species mechanisms of cellular hypoxia-reoxygenation injury. *Am. J. Physiol. Cell Physiol.* 282, C227–C241.

Malmberg, K. J., Lenkei, R., Petersson, M., Ohlum, T., Ichihara, F., Glimelius, B., et al. (2002). A short-term dietary supplementation of high doses of vitamin E increases T helper 1 cytokine production in patients with advanced colorectal cancer. *Clin. Cancer Res.* 8, 1772–1778.

Mates, J. M., Perez-Gomez, C., and Nunez de Castro, I. (1999). Antioxidant enzymes and human diseases. *Clin. Biochem.* 32, 595–603. doi: 10.1016/S0009-9120(99)00075-2

McCall, M. R., and Frei, B. (1999). Can antioxidant vitamins materially reduce oxidative damage in humans. *Free Radic. Biol. Med.* 26, 1034–1053. doi: 10.1016/S0891-5849(98)00302-5

McCord, J. M. (2000). The evolution of free radicals and oxidative stress. *Am. J. Med.* 108, 652–659. doi: 10.1016/S0002-9343(00)00412-5

McMahon, M., Thomas, N., Itoh, K., Yamamoto, M., and Hayes, J. D. (2006). Dimerization of substrate adaptors can facilitate cullin-mediated ubiquitylation of proteins by a "tethering" mechanism: a two-site interaction model for the Nrf2-Keap1 complex. *J. Biol. Chem.* 281, 24756–24768. doi: 10.1074/jbc.M601119200

Meydani, S. N., Meydani, M., Blumberg, J. B., Leka, L. S., Siber, G., Loszewski, R., et al. (1997). Vitamin E supplementation and *in vivo* immune response in healthy elderly subjects. A randomized controlled trial. *JAMA* 277, 1380–1386. doi: 10.1001/jama.1997.03540410058031

Mitsushita, J., Lambeth, J. D., and Kamata, T. (2004). The superoxide-generating oxidase Nox1 is functionally required for Ras oncogene transformation. *Cancer Res.* 64, 3580–3585. doi: 10.1158/0008-5472.CAN-03-3909

Pylayeva-Gupta, Y., Lee, K. E., Hajdu, C. H., Miller, G., and Bar-Sagi, D. (2012). Oncogenic Kras-induced GM-CSF production promotes the development of pancreatic neoplasia. *Cancer Cell* 21, 836–847. doi: 10.1016/j.ccr.2012.04.024

Salvador, A., Sousa, J., and Pinto, R. E. (2001). Hydroperoxyl, superoxide and pH gradients in the mitochondrial matrix: a theoretical assessment. *Free Radic. Biol. Med.* 31, 1208–1215. doi: 10.1016/S0891-5849(01)00707-9

Shi, X., Zhang, Y., Zheng, J., and Pan, J. (2012). Reactive oxygen species in cancer stem cells. *Antioxid. Redox Signal.* 16, 1215–1228. doi: 10.1089/ars.2012.4529

Shibata, T., Ohta, T., Tong, K. I., Kokubu, A., Odogawa, R., Tsuta, K., et al. (2008). Cancer related mutations in NRF2 impair its recognition by Keap1-Cul3 E3 ligase and promote malignancy. *Proc. Natl. Acad. Sci. U.S.A.* 105, 13568–13573. doi: 10.1073/pnas.0806268105

Shin-Kang, S., Ramsauer, V. P., Lightner, J., Chakraborty, K., Stone, W., Campbell, S., et al. (2011). Tocotrienols inhibit AKT and ERK activation and suppress pancreatic cancer cell proliferation by suppressing the ErbB2 pathway. *Free Radic. Biol. Med.* 51, 1164–1174. doi: 10.1016/j.freeradbiomed.2011.06.008

Siegel, R., Naishadham, D., and Jemal, A. (2013). Cancer statistics, 2013. *CA Cancer J. Clin.* 63, 11–30. doi: 10.3322/caac.21166

Sies, H., Stahl, W., and Sevanian, A. (2005). Nutritional, dietary and postprandial oxidative stress. *J. Nutr.* 135, 969–972.

Smit, V. T., Boot, A. J., Smits, A. M., Fleuren, G. J., Cornelisse, C. J., and Bos, J. L. (1988). KRAS codon 12 mutations occur very frequently in pancreatic adenocarcinomas. *Nucleic Acids Res.* 16, 7773–7782. doi: 10.1093/nar/16.16.7773

Son, J., Lyssiotis, C. A., Ying, H., Wang, X., Hua, S., Ligorio, M., et al. (2013). Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature* 496, 101–105. doi: 10.1038/nature12040

Suda, T., Takubo, K., and Semenza, G. L. (2011). Metabolic regulation of hematopoietic stem cells in the hypoxic niche. *Cell Stem Cell* 9, 298–310. doi: 10.1016/j.stem.2011.09.010

Tang, H., Dong, X., Day, R. S., Hassan, M. M., and Li, D. (2010). Antioxidant genes, diabetes and dietary antioxidants in association with risk of pancreatic cancer. *Carcinogenesis* 31, 607–613. doi: 10.1093/carcin/bgp310

Valko, M., Izakovic, M., Mazur, M., Rhodes, C. J., and Telser, J. (2004). Role of oxygen radicals in DNA damage and cancer incidence. *Mol. Cell. Biochem.* 266, 37–56. doi: 10.1023/B:MCBI.0000049134.69131.89

Wang, D., Kreutzer, D. A., and Essigmann, J. M. (1998). Mutagenicity and repair of oxidative DNA damage: insights from studies using defined lesions. *Mutat. Res.* 400, 99–115. doi: 10.1016/S0027-5107(98)00066-9

Ying, H., Kimmelman, A. C., Lyssiotis, C. A., Hua, S., Chu, G. C., Fletcher-Sananikone, E., et al. (2012). Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. *Cell* 149, 656–670. doi: 10.1016/j.cell.2012.01.058

Zhang, C. C., and Sadek, H. (2013). Hypoxia and metabolic properties of hematopoietic stem cells. *Antioxid. Redox Signal.* doi: 10.1089/ars.2012.5019. [Epub ahead of print].

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Blood group determinates incidence for pancreatic cancer in Germany

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Background: Genetic risk factors for sporadic pancreatic cancer are largely unknown but actually under high exposure. Findings of correlations between the AB0 blood group system (Chromosome 9q34,1—q34,2) and the risk of pancreatic cancer (PC) in patients from Asia, America and south Europe have already been published. So far it is unclear, whether this correlation between blood group an PC incidence can be found in German patients as well.

Methods: One hundred and sixty-six patients who underwent a resection of PC were evaluated in a period between 2000 and 2010. Blood group reference distribution for the German population is given as: 0: 41%; A: 43%; B: 11%; AB: 5%; Rhesus positive: 85%; Rhesus negative: 15%. Analyses were done using the non-parametric Chi^2-test (p-value two sided; SPSS 19.0).

Results: Median age was 62 (34–82) years. Gender: female 73/44%; male: 93/56%. Observed blood group proportions: 0: 43 (25.9%)/A: 94 (56.6%)/B: 16 (9.6%)/AB: 13 (7.8%)/Rhesus positive: 131 (78.9%)/negative: 35 (21.1%). We detected a significant difference to the German reference distribution of the AB0 system (Chi^2 19.34, df 3, $p < 0.001$). Rhesus factor has no impact on AB0-distribution (Chi^2 4.13, df 3, $p = 0.25$), but differs significantly from reference distribution—probably due to initial AB0-variation (Chi^2 4.82, df 1, $p = 0.028$). The odds ratio for blood group A is 2.01 and for blood group 0 is 0.5.

Conclusions: The incidence of PC in the German cohort is highly associated with the AB0-system as well. More patients with blood group A suffer from PC ($p < 0.001$) whereas blood group 0 was less frequent in patients with PC ($p < 0.001$). Thus, our findings support the results from other non-German surveys. The causal trigger points of this carcinogenesis correlation are still not known.

Keywords: pancreatic cancer, risk factor, ABO blood-group system, determination, genome

INTRODUCTION

Advanced pancreatic cancer holds one of the highest mortality rates of any cancer, with corresponding 5 year survival rate of less than 5% (Adler et al., 2007; Pelzer, 2008). It remains one of the leading causes of cancer-related deaths worldwide, reflected by an incidence of 277,668 new cases and almost the same mortality rate (266,029 cases) per year (Jemal et al., 2010). Due to early disease symptoms being absent, only up to 20% of patients can have their cancer resected with curative intent, however, probably due to early lymphogenic spread or micro metastasis, the 5-year overall survival rate of resected patients is only 15–22% in spite of adjuvant treatment. An effective screening method or test for this devastating cancer is still missing. Established risk factors include a family history of pancreatic cancer, a medical history of hereditary pancreatitis, diabetes type II and cigarette smoking. Established research groups seeking for predefined genome aberrations correlated to pancreatic cancer (Amundadottir et al., 2009; He et al., 2013). In recent time several studies investigating the possible correlation of the AB0 blood group system to pancreatic cancer (Yeo and Lowenfels, 2012) were published. Correlations were found in many populations, exemplary in Turkish patients (Engin et al., 2012), Korean patients (Woo et al., 2013), Japanese patients (Nakao et al., 2011), Italian patients (Iodice et al., 2010), and North American patients (Greer et al., 2010; Wolpin et al., 2010). But there is no overall accordance in all populations. For instance in Chinese patients publications showed inconsistent results without detection of correlations on the one hand (Gong et al., 2012) and proof of coherence on the other hand (Ben et al., 2011). These assured correlations are not consistent over all malignancies (Khalili et al., 2011). Currently no published survey data of German patients or a central Europe cohort exists which could help clarify the possible coherence. For further detection of causality it is important to know whether these findings are

valid for German patients as well, therefore we conducted this investigation.

MATERIALS AND METHODS

Patients who underwent a resection of PC were evaluated in a period between 2000 and 2010. All patients suffered from histologically confirmed pancreatic cancer. Blood type assay from 166 patients (AB0 antigen and Rhesus antigen) were conducted. As reference cohort, healthy blood donors from our department of transfusion medicine were tested, whose blood types showed the same distribution as the reference distribution of the German population. Reference distribution was given as: blood group 0 41%; blood group A 43%; blood group B 11%; blood group AB 5%; Rhesus antigen positive 85% and Rhesus antigen negative 15%. In addition to descriptive analyses non-parametric Chi^2-tests (p-value two sided; SPSS 19.0) were used for comparisons.

RESULTS

The present, non-selected population of patients with pancreatic cancer reflects the general population with PC in Germany as you can find in many trials. The median age was 62 (34–82) years. The gender distribution favors male patients with a percentage of 56% male patients to 44% female patients (**Table 1**). We observed blood group 0 in 43 (25.9%) patients, blood group A in 94 (56.6%) patients, blood group B in 16 (9.6%) patients and blood group AB in 13 (7.8%) patients. These observations differ significantly from the reference distribution of the AB0 system (Chi^2 19.34, df 3, $p < 0.001$). The absolute differences to the expected AB0-distribution were minus 25 patients for blood group 0, plus 23 for blood group A, minus two patients for blood group B and plus five patients for blood group AB. The odds ratio for blood group A is 2.01 and for blood group 0 is 0.5. The Chi^2-tests for the single AB0-characters were as follow: for 0 (Chi^2 15.64, df 1, p < 0.001), for A (Chi^2 12.58, df 1, $p < 0.001$), for B (Chi^2 0.31, df 1, $p = 0.58$), and for AB (Chi^2 2.80, df 1, $p = 0.09$) (**Figure 1**, **Table 2**).

Furthermore we observed the positive rhesus antigen in 131 (78.9%) patients and the negative rhesus antigen in 35 (21.1%) patients. The Rhesus factor has no significant impact on the AB0-distribution (Chi^2 4.13, df 3, $p = 0.25$) within the observed cohort. As compared to the reference cohort, the distribution of the Rhesus factor resulted in a significant difference (Chi^2 4.82, df 1, $p = 0.028$). This observation is possibly boosted due to the initial AB0-variation (**Table 3**).

Table 1 | Patients' characteristics.

Characteristic	Patients
Included	166
Age: median [range]	62 [34–82] years
<60 years	63
60–70 years	69
>70 years	34
Gender	
Female	73
Male	93
Biopsy proven adeno-carcinoma (pancreas)	166

DISCUSSION

In spite of recent advantages in the treatment modalities, likewise the FOLFIRINOX 1st-line regimen (Conroy et al., 2011), the OFF 2nd-line treatment (Pelzer et al., 2011) or the latest data from the gemcitabine/nab-paclitaxel 1st-line treatment, patients suffering from pancreatic adenocarcinoma still have the poorest survival outcome among cancer illnesses at all. Because of the heavy difficulties in the treatment of advanced disease, increased effort was dedicated to detect risk factors and causalities in the carcinogenesis to diagnose patients at earliest point of disease. Descriptions of observed correlations form the basis for further investigations.

The characteristics of our observed patients were in accordance with the appearance of the German clinician in terms of gender and age (Adler et al., 2007). Thus, this cohort is representative for patients with pancreatic carcinoma in Germany. Our findings are not completely identical with the observations of other research groups, but agree with risk lowering in patients with blood group 0. The Korean survey displayed an increased risk for the population with non-blood 0 character (Woo et al., 2013), Turkey findings showed higher risk of patients with blood group A and a lower risk of patients with blood group AB (Engin et al., 2012) whereas Chinese patients were interestingly investigated without blood group risk correlation (Gong et al., 2012). There are some attempts to explain the observed correlations, mainly based on the assumption of collocated signal cascade triggers. The chronic pancreatitis is known to be a risk factor for carcinogenesis. A misfit is that chronic pancreatitis was found to correlate with blood group 0 (Greer et al., 2011) which on the other hand lower the risk of pancreatic cancer. Another way of sourcing is the infection triggered chronic inflammation. Helicobacter pylori infection is also associated with the AB0 genotype mainly due to the AB0 antigen expressions on gastrointestinal epithelium and therefore better adhesion for the Helicobacter colonization. The positive association between the AB0 expression and duodenal and gastric ulcer as well as gastric cancers may base on effects of gastric and pancreatic secretory function disorders. This could have an additional impact on the carcinogenicity of dietary- and smoking-related N-nitrosamine exposures, and thus risk of pancreatic cancer (Risch, 2012).

Furthermore, venous thromboembolic events are also associated with pancreatic cancer known as trousseaus syndrome, first described in 1865. Is there a common base? Von Willebrand factor (vWF) is one mediator for this cause. It has the blood group antigens A and/or B on its surface and carries factor VIII and protects it from degradation. Blood group A1 and B educes a higher level of vWF and factor VIII. Thrombosis may appear as an early observed symptom of the subsequent diagnosis of pancreatic cancer. The activated coagulation was formerly described as an additional trigger associated with poor prognosis and increased angiogenesis. There is supposedly a combined cascade of carcinogenesis activation (Maisonneuve et al., 2009). But, maybe as a result of earlier detection or more effective therapy of thrombosis and thromboembolic

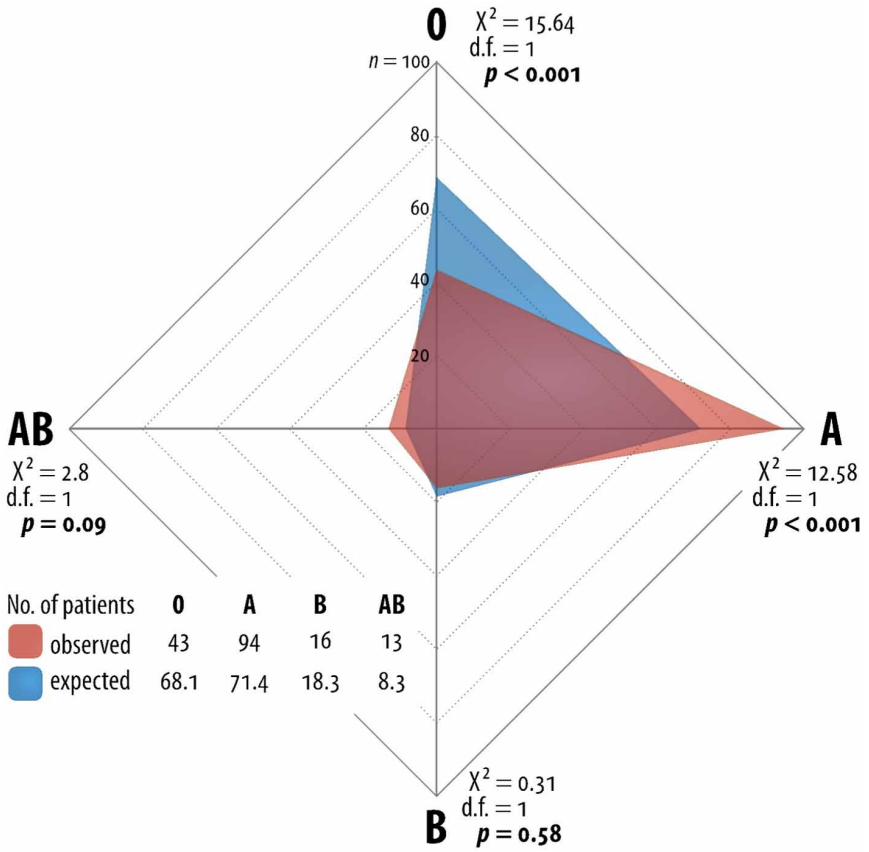

FIGURE 1 | Network grafic—statistical distribution of blood group in Germany and for German patients with pancreatic cancer.

Table 2 | Calculations between single characters of group antigens.

	Observed patients	Expected patients	
0	43	68.1	Chi^2 19.34 df 3 $p < 0.001$
A	94	71.4	
B	16	18.3	
AB	13	8.3	
Rh. neg.	35	24.9	Chi^2 4.82 df 1 $p < 0.028$
Rh. pos.	131	141.1	
Overall	166	166	

Table 3 | Calculations of AB0/Rhesus independence.

Blood group antigen	Rhesus antigen		All
	Negative	Positive	
0			
obs. pts.	5	38	43
exp. pts.	9.1	33.9	
A			
obs. pts.	21	73	94
exp. pts.	19.8	74.2	
B			
obs. pts.	5	11	16
exp. pts.	3.4	12.6	
AB			
obs. pts.	4	9	13
exp. pts.	2.7	10.3	
All			
obs. pts.	35	131	166
exp. pts.			

Rhesus/AB0-proportions are independent (Chi^2 4.13, df 3, $p = 0.248$).

events, recent studies showed no survival disadvantage for cancer patients suffering thrombosis (Riess et al., 2008; Agnelli et al., 2009).

It is noteworthy that other cancer types do not have stringent correlations to the AB0-antigen (Iodice et al., 2010), indicating it to be a special observation in pancreatic cancer disease. But of all above, the feasible research hypothesis is that the single base deletion that generates the 0 blood group underlies the association signal. Additional mapping and laboratory work is mandatory to determine which variants account for the observed correlation (Amundadottir et al., 2009).

The discovery of additional genetic risk factors for this highly lethal cancer type may contribute to novel risk stratifications

and advances in prevention, early detection and therapeutic approaches to pancreatic cancer.

Based on these representative data, we plan a genome mapping project of available data from our adjuvant studies (CONKO 001/005/006), which is under recent approval of the German Society of Cancer.

CONCLUSIONS

The incidence of pancreatic cancer in Germany is significantly associated with the AB0-blood group system. More patients with blood group A suffer from pancreatic cancer ($p < 0.001$) whereas blood group 0 was less frequently observed in patients with pancreatic cancer ($p < 0.001$). Genetic variations in the AB0 locus of 9q34 may influence the pancreatic carcinogenesis and increase the risk for patients with blood group A and tapering the risk for patients with blood group 0.

AUTHOR CONTRIBUTORS

U. Pelzer, M. Bahra, and H. Riess were responsible for the concept and design of the study and the writing of the manuscript. Collecting data and further analysis and interpretation was done by U. Pelzer, M. Bahra, F. Klein, M. Sinn, and H. Riess. O. Meyer, H. Riess, and B. Dörken provided staff and facilities for the investigation. All authors were involved in the provision of patients and the collection and collation of data. All authors reviewed the manuscript and gave their approval.

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REFERENCES

Adler, G., Seufferlein, T., Bischoff, S. C., Brambs, H.-J., Feuerbach, S., Grabenbauer, G., et al. (2007). S3-Guidelines "Exocrine pancreatic cancer" 2007. *Z Gastroenterol.* 45, 487–523.

Agnelli, G., Gussoni, G., Bianchini, C., Verso, M., Mandalà, M., Cavanna, L., et al. (2009). Nadroparin for the prevention of thromboembolic events in ambulatory patients with metastatic or locally advanced solid cancer receiving chemotherapy: a randomised, placebo-controlled, double-blind study. *Lancet Oncol.* 10, 943–949.

Amundadottir, L., Kraft, P., Stolzenberg-Solomon, R. Z., Fuchs, C. S., Petersen, G. M., Arslan, A. A., et al. (2009). Genome-wide association study identifies variants in the ABO locus associated with susceptibility to pancreatic cancer. *Nat. Genet.* 41, 986–990.

Ben, Q., Wang, K., Yuan, Y., and Li, Z. (2011). Pancreatic cancer incidence and outcome in relation to ABO blood groups among Han Chinese patients: a case-control study. *Int. J. Cancer* 128, 1179–1186.

Conroy, T., Desseigne, F., Ychou, M., Bouché, O., Guimbaud, R., Bécouarn, Y., et al. (2011). FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N. Engl. J. Med.* 364, 1817–1825.

Engin, H., Bilir, C., Üstün, H., and Gökmen, A. (2012). ABO blood group and risk of pancreatic cancer in a Turkish population in Western Blacksea region. *Asian Pac. J. Cancer Prev.* 13, 131–133.

Gong, Y., Yang, Y.-S., Zhang, X.-M., Su, M., Wang, J., Han, J.-D., et al. (2012). ABO blood type, diabetes and risk of gastrointestinal cancer in northern China. *World J. Gastroenterol.* 18, 563–569.

Greer, J. B., LaRusch, J., Brand, R. E., O'Connell, M. R., Yadav, D., and Whitcomb, D. C. (2011). ABO blood group and chronic pancreatitis risk in the NAPS2 cohort. *Pancreas* 40, 1188–1194.

Greer, J.-B., Yazer, M.-H., Raval, J.-S., Barmada, M.-M., Brand, R.-E., and Whitcomb, D.-C. (2010). Significant association between ABO blood group and pancreatic cancer. *World J. Gastroenterol.* 16, 5588–5591.

He, M., Wu, C., Xu, J., Guo, H., Yang, H., Zhang, X., et al. (2013). A genome wide association study of genetic loci that influence tumour biomarkers cancer antigen 19-9, carcinoembryonic antigen and α fetoprotein and their associations with cancer risk. *Gut.* doi: 10.1136/gutjnl-2012-303434. [Epub ahead of print].

Iodice, S., Maisonneuve, P., Botteri, E., Sandri, M. T., and Lowenfels, A. B. (2010). ABO blood group and cancer. *Eur. J. Cancer.* 46, 3345–3350.

Jemal, A., Siegel, R., Xu, J., and Ward, E. (2010). Cancer statistics, 2010. *CA Cancer J. Clin.* 60, 277–300.

Khalili, H., Wolpin, B. M., Huang, E. S., Giovannucci, E. L., Kraft, P., Fuchs, C. S., et al. (2011). ABO blood group and risk of colorectal cancer. *Cancer Epidemiol. Biomark. Prev.* 20, 1017–1020.

Maisonneuve, P., Iodice, S., Löhr, J.-M., and Lowenfels, A. B.. (2009). Re: ABO blood group and the risk of pancreatic cancer. *J. Natl. Cancer Inst.* 101, 1156; author reply 1156–1157.

Nakao, M., Matsuo, K., Hosono, S., Ogata, S., Ito, H., Watanabe, M., et al. (2011). ABO blood group alleles and the risk of pancreatic cancer in a Japanese population. *Cancer Sci.* 102, 1076–1080.

Pelzer, U. (2008). First-line chemotherapy in advanced pancreatic cancer. *Recent Res. Cancer Res.* 177, 57–60.

Pelzer, U., Schwaner, I., Stieler, J., Adler, M., Seraphin, J., Dörken, B., et al. (2011). Best supportive care (BSC) versus oxaliplatin, folinic acid and 5-fluorouracil (OFF) plus BSC in patients for second-line advanced pancreatic cancer: a phase III-study from the German CONKO-study group. *Eur. J. Cancer.* 47, 1676–1681.

Pelzer, U., Bahra, M., Sinn, M., Dörken, B., Neuhaus, P., Meyer, O., et al. (2012). "AB0 Blood group and risk of pancreatic cancer in a German single center cohort," in *Annual Meeting of the German, Austrian and Swiss Societies for Hematology and Oncology* (Stuttgart), A: 684.

Riess, H., Pelzer, U., Hilbig, A., Stieler, J., Opitz, B., Scholten, T., et al. (2008). Rationale and design of PROSPECT-CONKO 004: a prospective, randomized trial of simultaneous pancreatic cancer treatment with enoxaparin and chemotherapy. *BMC Cancer.* 8:361. doi: 10.1186/1471-2407-8-361

Risch, H. A. (2012). Pancreatic cancer: helicobacter pylori colonization, N-nitrosamine exposures, and ABO blood group. *Mol. Carcinog.* 51, 109–118.

Wolpin, B. M., Kraft, P., Xu, M., Steplowski, E., Olsson, M. L., Arslan, A. A., et al. (2010). Variant ABO blood group alleles, secretor status, and risk of pancreatic cancer: results from the pancreatic cancer cohort consortium. *Cancer Epidemiol. Biomark. Prev.* 19, 3140–3149.

Woo, S. M., Joo, J., Lee, W. J., Park, S.-J., Han, S.-S., Kim, T. H., et al. (2013). Risk of pancreatic cancer in relation to ABO blood group and hepatitis C virus infection in Korea: a case-control study. *J. Korean Med. Sci.* 28, 247–251.

Yeo, T. P., and Lowenfels, A. B. (2012). Demographics and epidemiology of pancreatic cancer. *Cancer. J.* 18, 477–484.

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Immune infiltrates as predictive markers of survival in pancreatic cancer patients

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Pancreatic cancer is a devastating disease with dismal prognosis. The tumor microenvironment is composed by multiple cell types, molecular factors, and extracellular matrix forming a strong desmoplastic reaction, which is a hallmark of the disease. A complex cross-talk between tumor cells and the stroma exists with reciprocal influence that dictates tumor progression and ultimately the clinical outcome. In this context, tumor infiltrating immune cells through secretion of chemokine and cytokines exert an important regulatory role. Here we review the correlation between the immune infiltrates, evaluated on tumor samples of pancreatic cancer patients underwent surgical resection, and disease free and/or overall survival after surgery. Specifically, we focus on tumor infiltrating lymphocytes (TILs), mast cells (MCs) and macrophages that all contribute to a Th2-type inflammatory and immunosuppressive microenvironment. In these patients tumor immune infiltrates not only do not contribute to disease eradication but rather the features of Th2-type inflammation and immunosuppression is significantly associated with more rapid disease progression and reduced survival.

Keywords: pancreatic cancer, tumor infiltrating lymphocytes, macrophages, mast cells, survival predictive factor, univariate and multivariate analyses

INTRODUCTION

A relationship between tumors and immune system exists (Schreiber et al., 2011). Indeed, multistep carcinogenesis results from a cross-talk between cancer-cell-intrinsic factors and host immune system (cell-extrinsic) effects (Zitvogel et al., 2006). This cross-talk leads to different outcomes that are well explained by the concept of the three Es of cancer immunoediting (Dunn et al., 2004). At early stages immunosurveillance is responsible for tumor rejection (Elimination phase), in advanced stages the immune system prevents tumor outgrowth and edits tumor immunogenicity (Equilibrium phase) with the appearance in late stages of tumor cell variants that are no longer recognized by the immune system but rather tumors develop strategies to redirect infiltrating immune cells toward a pro-tumorigenic phenotype (Escape phase) (Dunn et al., 2004; Schreiber et al., 2011). The mechanisms of immune escape have been recently recognized (Hanahan and Weinberg, 2011; Hanahan and Coussens, 2012) as an emerging hallmark of cancer.

Tumors are complex organs composed by tumor cells as well as a variety of cells and factors forming the tumor microenvironment. Cells present in the tumor microenvironment are cancer associated fibroblasts (CAFs), endothelial cells, pericytes and immune cells, among which macrophages, dendritic cells (DCs), natural killer cells, mast cells (MCs), granulocytes, B cells and naïve and memory T cells [including cytotoxic $CD8^+$ T cells and different subsets of $CD4^+$ T and regulatory T cells (Tregs)]. All these cell types and their released factors interact with each other and determine the cytokine/chemokine milieu, which ultimately have an impact on tumor regression or progression.

Studies in several tumors have evaluated the association between anti-tumor immunity and cancer prognosis but only in recent years the development of more accurate methods for analysis of immune infiltrates has allowed the identification of the features of productive anti-tumor immunity (Fridman et al., 2011). Large-scale studies have then revealed the prognostic and predictive impact of immune infiltrates (Pages et al., 2005; Galon et al., 2006; Denkert et al., 2010) and international efforts have been put together to standardize predictive immune scores for prognosis in several tumor histotypes (Galon et al., 2012).

Pancreatic ductal adenocarcinoma (PDAC) is a very aggressive disease with dismal prognosis (Hidalgo, 2010). The tumor microenvironment is characterized by a strong desmoplastic reaction, which is a hallmark of the disease and it is believed to play a role in carcinogenesis and in tumor progression through its effects on angiogenesis, resistance to therapy and metastatic spread of tumor cells (Kleeff et al., 2007; Erkan et al., 2012). Fibrosis is due to activation by tumor and immune cells of pancreatic stellate cells, which are responsible for extracellular matrix deposition. Importantly, fibrogenesis is differentially regulated by Th1 (i.e., IFN-γ) and Th2 (i.e., IL-4, IL-5, and IL-13) cytokines, which exert opposing roles by promoting collagen degradation and synthesis, respectively (Wynn, 2004). Th1 or Th2 polarized immune

Abbreviations: CAFs, cancer associated fibroblasts; DCs, dendritic cells; FRβ, folate receptor β; LNs, lymph nodes; MCs, mast cells; PanIN, precursor lesions; PDAC, pancreatic ductal adenocarcinoma; TAMs, tumor associated macrophages; TILs, tumor infiltrating lymphocytes; Tregs, T regulatory cells; TSLP, thymic stromal lymphopoietin.

cells may thus differentially contribute to fibrosis in PDAC and possibly influence tumor progression.

The role of immune cells in pancreatic cancer development and progression has been discussed elsewhere (Clark et al., 2009; Evans and Costello, 2012; Wachsmann et al., 2012; Vonderheide and Bayne, 2013). We refer readers interested in exhaustive summaries of the field to those reviews. We focus here on studies in human samples in which a correlation between immune infiltrates and clinicopathologic features of PDAC that have prognostic significance and an impact on patients' survival has been documented. These studies are summarized in **Table 1**.

TUMOR INFILTRATING LYMPHOCYTES

Tumor infiltrating lymphocytes (TILs) are present in several solid tumors and key features such as their distribution, density, and function dictate their anti-*versus* pro-tumor activity (Galon et al., 2006; Fridman et al., 2011, 2012). The major anti-tumor effectors are memory ($CD45RO^+$) cytotoxic $CD8^+$ T cells while more complex is the role of $CD4^+$ T cells that depends on the pattern of cytokines produced. Several $CD4^+$ T cell subsets have been described (Ruffell et al., 2010; Zhu et al., 2010): Th1 producing IFN-γ, Th2 producing IL-4, IL-5 and IL-13, Th17 producing IL-17, Th22 producing IL-22 and immunosuppressive Tregs. The role of the different subsets in tumor immunity is still under debate (Kennedy and Celis, 2008; Ruffell et al., 2010; Fridman et al., 2012).

Table 1 | Tumor infiltrating immune cells as predictors of the clinical outcome after surgery in pancreatic cancer patients.

Immune cells in the tumor	Predictive marker of favorable clinical outcome	References
T cells	High CD4/8($^{+/+}$) counts[a]	Fukunaga et al., 2004
	$CD4^{+high}/CD8^{+high}$ counts[b]	Ino et al., 2013
	GATA-3^+/T-bet^+ TILs ratio below the median value[c]	De Monte et al., 2011
	$CD4^{+high}/CD8^{+high}/\%Treg^{low}$ counts[d]	Ino et al., 2013
Mast cells	Low counts[e]	Strouch et al., 2010
	Low counts in the intratumor border zone[f]	Cai et al., 2011
	Counts below the MCs score[g]	Chang et al., 2011
Macrophages	Low $CD163^+/CD204^+$ cells infiltration[h]	Kurahara et al., 2011
	Low $FR\beta^+$ macrophages infiltration[i]	Kurahara et al., 2012
	Low M2 macrophages ($CD163^+/CD204^+$ cells) infiltration[j]	Ino et al., 2013

[a] *T cell counts were considered high for $CD4^+ \geq 20$ and $CD8^+ \geq 100$, corresponding to average numbers of 5 fields.*

[b] *High and low are based on the median values of $CD4^+$ and $CD8^+$ T cell counts.*

[c] *Patients were categorized in two groups based on the median value of the ratio of the percentage of GATA-3^+/T-bet^+ TILs.*

[d] *Patients were categorized based on the average values of $CD4^+$ T cells and $CD8^+$ T cell counts and of the percentage of Tregs.*

[e] *MCs counts were defined low if <8 and high if >13.*

[f] *Patients were categorized in two groups based on the median values of MCs counts.*

[g] *MCs score was set at 3.68 and it was defined as the ratio of the number of MCs to the percentage of $CD45^+$ cells.*

[h] *Four grade infiltrations were considered: weak ($<20/mm^2$), moderate ($>20<40/mm^2$), strong ($>40<60/mm^2$), and massive ($>60/mm^2$). Low correspond to weak plus moderate; high correspond to strong plus massive.*

[i] *Patients were categorized in two groups based on the median values of $FR\beta^+$ macrophages counts.*

[j] *Patients were categorized in two groups based on the median values M2 macrophages counts.*

In PDAC few studies have addressed the role of TILs in anti-tumor or pro-tumor activity and the correlation between those infiltrates and the clinical outcome.

The presence of TILs in PDAC was first reported in a study (Ademmer et al., 1998), which found that lymphocytes were typically localized as aggregates in the fibrotic interstitial tissue while very few cells reached the epithelial tumor cells. The amount of $CD4^+$ and $CD8^+$ T cells was variable among samples and showed a predominant $CD45RO^+$ memory phenotype. Only few T cells were found in normal pancreatic tissue. Kalthoff and collaborators (Von Bernstorff et al., 2001) confirmed that TILs do not reach tumor cells in significant numbers, being "trapped" in the peritumoral tissue. Heterogeneous TILs distribution with both focal areas of high accumulation, mainly at the periphery of the tumor, and areas with diffusely scattered cells was reported in (Ryschich et al., 2005). In this study a survival analysis performed on 24 patients showed that median survival of patients with high density of $CD8^+$ T cells was, although not statistically significant, considerably higher than that of the group with low $CD8^+$ T cells density. Statistical significance was reached in Fukunaga et al. (2004), in which 80 patients were analyzed. The overall survival rate was significantly longer in patients with $CD8^+$ but not $CD4^+$ T cell infiltration and highest for patients positive for both T cells populations CD8/CD4($^{+/+}$) (positivity was defined as average counts from 5 fields $\geq$100 for CD8 and $\geq$20 for CD4). Interestingly, the Authors found a negative correlation between CD8/CD4($^{+/+}$) infiltration and both tumor depth and TNM stage and in multivariate analysis the CD8/CD4($^{+/+}$) infiltration was confirmed as an independent prognostic factor of survival.

The presence of $FoxP3^+CD4^+CD25^+$ T regulatory cells (Tregs) possibly with immunosuppressive activity was evaluated in tumor tissues, inflammatory tissue and draining lymph nodes (LNs) in 198 PDAC patients and 15 patients with non-neoplastic lesions (Hiraoka et al., 2006). Tregs infiltration was localized in cancer stroma in areas of invasion. The prevalence of Tregs was significantly higher in PDAC than in inflammatory areas and in non-neoplastic lesions while no differences were observed in the LNs. When patients were divided into two groups based on values higher and lower than the average, the low Tregs group showed significantly better survival than the high Tregs group did. The Authors analyzed Tregs also in precursors lesions (PanIN) and interestingly found a significant increase of Tregs prevalence during progression from low-grade PanIN to invasive carcinoma (Hiraoka et al., 2006). In the same study intraepithelial $CD8^+$ T cells inversely correlated with Tregs infiltration in the stroma: indeed they were

present in PanIN-1, significantly decreased in PanIN-2 and drastically diminished to few in PanIN-3. More recently, in a large retrospective study of 212 tumor samples, the same Authors (Ino et al., 2013) reported that in multivariate analysis the prevalence of tumor infiltrating $CD4^+$ $T^{high}/CD8^+$ $T^{high}/\%Treg^{low}$ significantly correlated with longer survival and had a higher hazard ratio.

The characterization of TILs polarization in PDAC was initially reported in (Tassi et al., 2008), in which the presence of Th2, Th1 and Tregs cells in tumor samples was evaluated by immunohistochemistry using specific antibodies for GATA-3 (i.e., to detect Th2 cells), T-bet (i.e., to detect Th1 cells) and FoxP3. In this study PDAC patients undergoing surgery had circulating carcinoembryonic antigen-specific $CD4^+$ Th2 cells in the presence of conserved anti-viral Th1 immunity. Furthermore, analysis of TILs in tumor samples from the same patients showed that, in agreement with the data in the blood, the number of lymphoid cells expressing GATA-3 was significantly superior to that of lymphoid cells expressing T-bet. FoxP3 was also expressed in lymphoid cells, as previously reported (Hiraoka et al., 2006), and cells expressing FoxP3 were in greater proportion relative to T-bet but in lower proportion relative to GATA-3. More recently, we analyzed 69 tumor samples and found that the amount of TILs differed among the samples and that in all but one case the percentage of GATA-3^+ was significantly higher than that of T-bet^+ TILs (De Monte et al., 2011). To compare samples with different amounts of TILs we used the ratio of the percentage of GATA-3^+/T-bet^+ TILs and performed survival analysis. We found that patients with a ratio inferior to the median value had a statistically prolonged survival. Multivariate analysis stratifying for tumor stage, grading, size, site, patient performance status, gender, age, surgical resection margins, postoperative CA19.9 value, and postoperative treatment confirmed that the ratio was independently predictive of both disease-free and overall survival. We also identified a complex cross-talk among tumor cells, CAFs and DCs that implicates (i) the secretion of pro-inflammatory cytokines (i.e., TNF-α and IL-1β) by tumor cells with (ii) activation of CAFs to secrete the thymic stromal lymphopoietin (TSLP), (iii) activation by CAFs-derived TSLP of resident DCs with Th2 polarizing capability and which secrete Th2 attracting chemokines, and (iv) migration of TSLP activated DCs, possibly tumor antigen-loaded, to draining LNs where Th2 cell priming occurs. Interestingly, epithelial cells derived TSLP was also correlated with the presence of Th2 inflammation in breast carcinoma (Pedroza-Gonzalez et al., 2011).

Collectively, intraepithelial $CD8^+$ T cells infiltration is very rare in PDAC. $CD4^+$ and $CD8^+$ T cells are predominantly present in the stroma either dispersed or in aggregates, mainly at the periphery of the tumor. The prevalence of $CD4^+$ $T^{high}/CD8^+$ $T^{high}/\%Treg^{low}$ and the ratio of the percentage of GATA-3^+/T-bet^+ TILs in the tumor stroma were found to be independent predictive factors of overall survival after surgery in PDAC patients. An open issue remains as to the antigen-specificity of tumor infiltrating T cells.

MAST CELLS

MCs have been extensively studied for their role in allergic and anaphylactic reactions during which FcεRI aggregation leads to degranulation and release of multiple mediators (Galli et al., 2005, 2008). They are also known to be critical players in inflammatory diseases where they act through "selective" release of mediators without degranulation (Theoharides et al., 2007).

MCs infiltration is a relevant component of the tumor microenvironment in a number of human malignancies (Theoharides et al., 2007). MCs accumulate in the tumor stroma in response to tumor-derived chemoattractants such as MCP-1 and RANTES. However, there is no general agreement on their role in cancer. Indeed, MCs counts were shown to correlate with either favorable or poor prognosis depending on the tumor (Theoharides and Conti, 2004; Khazaie et al., 2011; Ribatti and Crivellato, 2012). MCs can exert pro-tumorigenic effects by secreting factors like VEGF and IL-8 that promote tumor angiogenesis, tumor growth factors (i.e., PDGF, NGF, SCF) and proteases that facilitate metastases. On the other hand, high MCs counts in draining LNs were found to correlate with better prognosis in human breast cancer where a mechanism involving allergy-like degranulation with inhibitory effects on tumor cell growth was hypothesized (Theoharides and Conti, 2004). This dual role is tentatively explained with their different mechanisms of secretion: inflammation-driven selective secretion is pro-tumorigenic while allergy-like degranulation is anti-tumor.

In PDAC MCs were found in significant higher numbers in tumor tissue compared to normal pancreas (Esposito et al., 2002). MCs were located around ducts, blood vessels and nerves in the connective tissue without particular clustering around neoplastic cells. In a more extensive analysis of 137 patients, the same Authors (Esposito et al., 2004) also showed that MCs number correlated with the presence of LNs metastases and intratumor microvessel density. Moreover, patients with low numbers of infiltrating MCs compared with those with high numbers had a tendency toward a longer survival.

More recently, a study (Strouch et al., 2010) on 53 tumor specimens found that increased MCs infiltration correlated with higher-grade tumors. Recurrence-free and disease-specific survival was found significantly worse in patients with high MCs counts compared to those with low counts. Interestingly, patients with PDAC had higher serum tryptase activity than patients with benign disease. Furthermore, *in vitro* experiments using cell lines demonstrated a cross-talk between tumor cells that secrete MCs attracting factor(s) and MCs, which in turn release cancer cell growth and pro-invasive factor(s).

Particular attention to MCs distribution in different tumor areas was the focus of a study (Cai et al., 2011), which evaluated in 103 patients MCs infiltration in intratumoral and peritumoral areas and further in their border and center zones. In this correlative study the Authors found that in the intratumoral border zone, but not in the peritumoral or in the intratumoral center zone, high MCs counts were associated with LNs metastasis, tumor stage, lymphatic, and microvascular invasion. Significantly, high

intratumoral border zone infiltration was identified as an independent prognostic factor of overall survival in resected patients, underlying the relevance of zone-specific distribution of MCs in PDAC.

High MCs infiltration was further confirmed as a negative predictive marker of survival in another study (Chang et al., 2011), which comprised 67 tumor samples. However, in multivariate analysis the MCs score used to stratify the patients did not reach statistical significance.

Collectively, the number of infiltrating MCs was found increased in PDAC compared to normal pancreas. A correlation between increased MCs numbers and the presence of LNs metastasis, tumor grade, intratumor microvessel density, and lymphatic and microvascular invasion was observed. The degree of MCs infiltration was identified as a predictive marker of patients' survival in the majority of studies.

MACROPHAGES

Solid tumors are frequently infiltrated by tumor-associated macrophages (TAMs), which are driven by tumor and T cell derived cytokines (especially IL-4, IL-10, and IL-13) to acquire an "alternatively activated" M2 phenotype with pro-tumor properties (Mantovani et al., 2002; Gordon, 2003). This M2-type macrophages are opposed to the "classic" M1-type that are activated by Th1 cytokines (IFN-γ, IL-1β) and are endowed with anti-tumor properties (Lewis and Pollard, 2006). TAMs receive signals from diverse cells within the tumor microenvironment and promote tumor growth and progression through regulation of angiogenesis, production of soluble mediators, which support proliferation, survival and invasive properties of tumor cells, and direct and indirect immunosuppression/modulation of lymphoid cells function (Mantovani et al., 2002; Qian and Pollard, 2010; Balkwill and Mantovani, 2012; Ruffell et al., 2012).

$CD68^+$ cells were found increased in PDAC compared to normal pancreatic tissue: however, in 137 tumor samples no significant correlation with cumulative survival was demonstrated (Esposito et al., 2004). A more accurate analysis of TAM polarization was performed on 76 patients samples by immunohistochemistry using both anti-CD68 (i.e., pan macrophage) and anti-CD163 and anti-CD204 antibodies (i.e., which should preferentially stain M2-type macrophages) (Kurahara et al., 2011). The number of $CD68^+$ cells varied among the samples examined: some tumors were extensively infiltrated while others had only sparse $CD68^+$ cells infiltration. $CD163^+$ and $CD204^+$ cells were present within the same areas and the counts were lower than the number of total $CD68^+$ cells. Interestingly, the number of $CD163^+$ and $CD204^+$ better then $CD68^+$ cells correlated with LNs metastasis (Kurahara et al., 2011). Patients were stratified into two groups based on mean values of $CD68^+$ or $CD163^+/CD204^+$ cell counts. The Authors found that lymphatic vessel density in the invasive front was significantly higher for high $CD163^+/CD204^+$ tumor samples compared to low samples but not statistically significant difference was found between the high and low $CD68^+$ infiltration. The data suggests that increased M2-type infiltration in the invasive front might have a role in lymph-angiogenesis and lymphatic metastatic spread in PDAC. When the prognostic impact of TAMs infiltration was assessed, the prognosis was significantly poorer in the high $CD163^+/CD204^+$ group compared with the low. Whereas, although the high $CD68^+$ tended to have a poor prognosis compared with the low $CD68^+$, no significant difference in the survival rate between the high and low $CD68^+$ cell counts was found.

In a following study (Kurahara et al., 2013), the same Authors showed a strong association among the density of VEGF-C expressing M2-type TAMs in regional LNs, nodal lymphatic vessel density and the incidence of isolated tumor cells in pN0 pancreatic cancer, further suggesting that M2-polarized TAMs may indeed facilitate nodal lymph-angiogenesis and promote lymph nodes micro-metastases.

Infiltration of macrophages that express the folate receptor β (FRβ) [i.e., a marker expressed in M2-type macrophages (Puig-Kroger et al., 2009)] was also investigated in PDAC (Kurahara et al., 2012). $FR\beta^+$ macrophages were prominent in perivascular areas of the tumor invasive front and when in high numbers they showed (i) a positive association with high tumor microvessel density, (ii) a high incidence of hematogenous metastasis, and (iii) poor prognosis in PDAC patients (Kurahara et al., 2012).

In agreement with an inverse correlation between M2-type TAMs infiltration and disease survival, a recent study (Ino et al., 2013), which included 212 patients, reported that high $CD163^+$ and $CD204^+$ cells infiltration was significantly associated with both shorter disease-free and overall survival. In the same study the presence of M2-type macrophages and the percentage of Tregs correlated with the presence of venous invasion.

Another study (Tjomsland et al., 2011) evaluated macrophage infiltration in PDAC samples by CD68 and CD163 gene expression analysis and found that, in contrast with the studies reported above, high CD163 expression correlated with longer survival. However, clinical correlation was done in a limited number of samples (30 patients) compared to the ones reported above (Kurahara et al., 2011, 2012, 2013; Ino et al., 2013) and based on CD163 gene expression rather then actual macrophage counts.

Collectively, higher numbers of $CD68^+$ cells were found in PDAC samples compared to normal pancreas. Functional polarization toward M2-type correlates with a poor prognosis after surgery in resected patients. High $CD163^+$ and $CD204^+$ cell counts in perivascular areas of the tumor invasive front correlate with lymphangiogensis, high tumor micro-vessel density, LNs occult metastasis and poor prognosis in PDAC patients.

CONCLUDING REMARKS

Several studies have demonstrated that tumor antigens specific T cells are present in the circulation of PDAC patients (Laheru and Jaffee, 2005). However, the presence of a Th2-type inflammatory and immunosuppressive microenvironment questions the possibility that anti-tumor Th1 effectors reach the tumor and eventually maintain their effector functions. Future therapeutic approaches in PDAC should implement the efficacy of Th1 effectors by a combination of active and adoptive immunotherapy (Mellman et al., 2011) and strategies, such as the use of immunomodulators and/or therapy with agonistic CD40 that has proved to be efficacious in PDAC patients (Beatty et al., 2011),

aimed at redirecting Th2 toward Th1-type inflammation in the tumor microenvironment. Moreover, since extensive immunohistochemical evaluations in bioptic material are not feasible, it will be interesting to compare the results of the studies reported here in surgical specimens from patients undergoing neoadjuvant therapies such as chemo or immunotherapy.

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REFERENCES

Ademmer, K., Ebert, M., Muller-Ostermeyer, F., Friess, H., Buchler, M. W., Schubert, W., et al. (1998). Effector T lymphocyte subsets in human pancreatic cancer: detection of CD8+CD18+ cells and CD8+CD103+ cells by multi-epitope imaging. *Clin. Exp. Immunol.* 112, 21–26. doi: 10.1046/j.1365-2249.1998.00546.x

Balkwill, F. R., and Mantovani, A. (2012). Cancer-related inflammation: common themes and therapeutic opportunities. *Semin. Cancer Biol.* 22, 33–40. doi: 10.1016/j.semcancer.2011.12.005

Beatty, G. L., Chiorean, E. G., Fishman, M. P., Saboury, B., Teitelbaum, U. R., Sun, W., et al. (2011). CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science* 331, 1612–1616. doi: 10.1126/science.1198443

Cai, S. W., Yang, S. Z., Gao, J., Pan, K., Chen, J. Y., Wang, Y. L., et al. (2011). Prognostic significance of mast cell count following curative resection for pancreatic ductal adenocarcinoma. *Surgery* 149, 576–584. doi: 10.1016/j.surg.2010.10.009

Chang, D. Z., Ma, Y., Ji, B., Wang, H., Deng, D., Liu, Y., et al. (2011). Mast cells in tumor microenvironment promotes the *in vivo* growth of pancreatic ductal adenocarcinoma. *Clin. Cancer Res.* 17, 7015–7023. doi: 10.1158/1078-0432.CCR-11-0607

Clark, C. E., Beatty, G. L., and Vonderheide, R. H. (2009). Immunosurveillance of pancreatic adenocarcinoma: insights from genetically engineered mouse models of cancer. *Cancer Lett.* 279, 1–7. doi: 10.1016/j.canlet.2008.09.037

De Monte, L., Reni, M., Tassi, E., Clavenna, D., Papa, I., Recalde, H., et al. (2011). Intratumor T helper type 2 cell infiltrate correlates with cancer-associated fibroblast thymic stromal lymphopoietin production and reduced survival in pancreatic cancer. *J. Exp. Med.* 208, 469–478. doi: 10.1084/jem.20101876

Denkert, C., Loibl, S., Noske, A., Roller, M., Muller, B. M., Komor, M., et al. (2010). Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. *J. Clin. Oncol.* 28, 105–113. doi: 10.1200/JCO.2009.23.7370

Dunn, G. P., Old, L. J., and Schreiber, R. D. (2004). The three Es of cancer immunoediting. *Annu. Rev. Immunol.* 22, 329–360. doi: 10.1146/annurev.immunol.22.012703.104803

Erkan, M., Hausmann, S., Michalski, C. W., Fingerle, A. A., Dobritz, M., Kleeff, J., et al. (2012). The role of stroma in pancreatic cancer: diagnostic and therapeutic implications. *Nat. Rev. Gastroenterol. Hepatol.* 9, 454–467.

Esposito, I., Kleeff, J., Bischoff, S. C., Fischer, L., Collecchi, P., Iorio, M., et al. (2002). The stem cell factor-c-kit system and mast cells in human pancreatic cancer. *Lab. Invest.* 82, 1481–1492.

Esposito, I., Menicagli, M., Funel, N., Bergmann, F., Boggi, U., Mosca, F., et al. (2004). Inflammatory cells contribute to the generation of an angiogenic phenotype in pancreatic ductal adenocarcinoma. *J. Clin. Pathol.* 57, 630–636. doi: 10.1136/jcp.2003.014498

Evans, A., and Costello, E. (2012). The role of inflammatory cells in fostering pancreatic cancer cell growth and invasion. *Front. Physiol.* 3:270. doi: 10.3389/fphys.2012.00270

Fridman, W. H., Galon, J., Pages, F., Tartour, E., Sautes-Fridman, C., and Kroemer, G. (2011). Prognostic and predictive impact of intra- and peritumoral immune infiltrates. *Cancer Res.* 71, 5601–5605. doi: 10.1158/0008-5472.CAN-11-1316

Fridman, W. H., Pages, F., Sautes-Fridman, C., and Galon, J. (2012). The immune contexture in human tumours: impact on clinical outcome. *Nat. Rev. Cancer* 12, 298–306. doi: 10.1038/nrc3245

Fukunaga, A., Miyamoto, M., Cho, Y., Murakami, S., Kawarada, Y., Oshikiri, T., et al. (2004). CD8+ tumor-infiltrating lymphocytes together with CD4+ tumor-infiltrating lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma. *Pancreas* 28, e26–e31. doi: 10.1097/00006676-200401000-00023

Galli, S. J., Grimbaldeston, M., and Tsai, M. (2008). Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. *Nat. Rev. Immunol.* 8, 478–486. doi: 10.1038/nri2327

Galli, S. J., Nakae, S., and Tsai, M. (2005). Mast cells in the development of adaptive immune responses. *Nat. Immunol.* 6, 135–142. doi: 10.1038/ni1158

Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pages, C., et al. (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 313, 1960–1964. doi: 10.1126/science.1129139

Galon, J., Pages, F., Marincola, F. M., Angell, H. K., Thurin, M., Lugli, A., et al. (2012). Cancer classification using the Immunoscore: a worldwide task force. *J. Transl. Med.* 10, 205. doi: 10.1186/1479-5876-10-205

Gordon, S. (2003). Alternative activation of macrophages. *Nat. Rev. Immunol.* 3, 23–35. doi: 10.1038/nri978

Hanahan, D., and Coussens, L. M. (2012). Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell* 21, 309–322. doi: 10.1016/j.ccr.2012.02.022

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi: 10.1016/j.cell.2011.02.013

Hidalgo, M. (2010). Pancreatic cancer. *N. Engl. J. Med.* 362, 1605–1617. doi: 10.1056/NEJMra0901557

Hiraoka, N., Onozato, K., Kosuge, T., and Hirohashi, S. (2006). Prevalence of FOXP3+ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. *Clin. Cancer Res.* 12, 5423–5434. doi: 10.1158/1078-0432.CCR-06-0369

Ino, Y., Yamazaki-Itoh, R., Shimada, K., Iwasaki, M., Kosuge, T., Kanai, Y., et al. (2013). Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. *Br. J. Cancer* 108, 914–923. doi: 10.1038/bjc.2013.32

Kennedy, R., and Celis, E. (2008). Multiple roles for CD4+ T cells in anti-tumor immune responses. *Immunol. Rev.* 222, 129–144. doi: 10.1111/j.1600-065X.2008.00616.x

Khazaie, K., Blatner, N. R., Khan, M. W., Gounari, F., Gounaris, E., Dennis, K., et al. (2011). The significant role of mast cells in cancer. *Cancer Metastasis Rev.* 30, 45–60. doi: 10.1007/s10555-011-9286-z

Kleeff, J., Beckhove, P., Esposito, I., Herzig, S., Huber, P. E., Lohr, J. M., et al. (2007). Pancreatic cancer microenvironment. *Int. J. Cancer* 121, 699–705. doi: 10.1002/ijc.22871

Kurahara, H., Shinchi, H., Mataki, Y., Maemura, K., Noma, H., Kubo, F., et al. (2011). Significance of M2-polarized tumor-associated macrophage in pancreatic cancer. *J. Surg. Res.* 167, e211–e219. doi: 10.1016/j.jss.2009.05.026.

Kurahara, H., Takao, S., Kuwahata, T., Nagai, T., Ding, Q., Maeda, K., et al. (2012). Clinical significance of folate receptor beta-expressing tumor-associated macrophages in pancreatic cancer. *Ann. Surg. Oncol.* 19, 2264–2271. doi: 10.1245/s10434-012-2263-0

Kurahara, H., Takao, S., Maemura, K., Mataki, Y., Kuwahata, T., Maeda, K., et al. (2013). M2-polarized tumor-associated macrophage infiltration of regional lymph nodes is associated with nodal lymphangiogenesis and occult nodal involvement in pN0 pancreatic cancer. *Pancreas* 42, 155–159. doi: 10.1097/MPA.0b013e318254f2d1

Laheru, D., and Jaffee, E. M. (2005). Immunotherapy for pancreatic cancer – science driving clinical progress. *Nat. Rev. Cancer* 5, 459–467. doi: 10.1038/nrc1630

Lewis, C. E., and Pollard, J. W. (2006). Distinct role of macrophages in different tumor microenvironments. *Cancer Res.* 66, 605–612. doi: 10.1158/0008-5472.CAN-05-4005

Mantovani, A., Sozzani, S., Locati, M., Allavena, P., and Sica, A. (2002). Macrophage polarization: tumor-associated macrophages

as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol.* 23, 549–555. doi: 10.1016/S1471-4906(02)02302-5

Mellman, I., Coukos, G., and Dranoff, G. (2011). Cancer immunotherapy comes of age. *Nature* 480, 480–489. doi: 10.1038/nature10673

Pages, F., Berger, A., Camus, M., Sanchez-Cabo, F., Costes, A., Molidor, R., et al. (2005). Effector memory T cells, early metastasis, and survival in colorectal cancer. *N. Engl. J. Med.* 353, 2654–2666. doi: 10.1056/NEJMoa051424

Pedroza-Gonzalez, A., Xu, K., Wu, T. C., Aspord, C., Tindle, S., Marches, F., et al. (2011). Thymic stromal lymphopoietin fosters human breast tumor growth by promoting type 2 inflammation. *J. Exp. Med.* 208, 479–490. doi: 10.1084/jem.20102131

Puig-Kroger, A., Sierra-Filardi, E., Dominguez-Soto, A., Samaniego, R., Corcuera, M. T., Gomez-Aguado, F., et al. (2009). Folate receptor beta is expressed by tumor-associated macrophages and constitutes a marker for M2 anti-inflammatory/regulatory macrophages. *Cancer Res.* 69, 9395–9403. doi: 10.1158/0008-5472.CAN-09-2050

Qian, B. Z., and Pollard, J. W. (2010). Macrophage diversity enhances tumor progression and metastasis. *Cell* 141, 39–51. doi: 10.1016/j.cell.2010.03.014

Ribatti, D., and Crivellato, E. (2012). Mast cells, angiogenesis, and tumour growth. *Biochim. Biophys. Acta* 1822, 2–8. doi: 10.1016/j.bbadis.2010.11.010

Ruffell, B., Affara, N. I., and Coussens, L. M. (2012). Differential macrophage programming in the tumor microenvironment. *Trends Immunol.* 33, 119–126. doi: 10.1016/j.it.2011.12.001

Ruffell, B., Denardo, D. G., Affara, N. I., and Coussens, L. M. (2010). Lymphocytes in cancer development: polarization towards pro-tumor immunity. *Cytokine Growth Factor Rev.* 21, 3–10. doi: 10.1016/j.cytogfr.2009.11.002

Ryschich, E., Notzel, T., Hinz, U., Autschbach, F., Ferguson, J., Simon, I., et al. (2005). Control of T-cell-mediated immune response by HLA class I in human pancreatic carcinoma. *Clin. Cancer Res.* 11, 498–504.

Schreiber, R. D., Old, L. J., and Smyth, M. J. (2011). Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* 331, 1565–1570. doi: 10.1126/science.1203486

Strouch, M. J., Cheon, E. C., Salabat, M. R., Krantz, S. B., Gounaris, E., Melstrom, L. G., et al. (2010). Crosstalk between mast cells and pancreatic cancer cells contributes to pancreatic tumor progression. *Clin. Cancer Res.* 16, 2257–2265. doi: 10.1158/1078-0432.CCR-09-1230

Tassi, E., Gavazzi, F., Albarello, L., Senyukov, V., Longhi, R., Dellabona, P., et al. (2008). Carcinoembryonic antigen-specific but not antiviral CD4+ T cell immunity is impaired in pancreatic carcinoma patients. *J. Immunol.* 181, 6595–6603.

Theoharides, T. C., and Conti, P. (2004). Mast cells: the Jekyll and Hyde of tumor growth. *Trends Immunol.* 25, 235–241. doi: 10.1016/j.it.2004.02.013

Theoharides, T. C., Kempuraj, D., Tagen, M., Conti, P., and Kalogeromitros, D. (2007). Differential release of mast cell mediators and the pathogenesis of inflammation. *Immunol. Rev.* 217, 65–78. doi: 10.1111/j.1600-065X.2007.00519.x

Tjomsland, V., Niklasson, L., Sandstrom, P., Borch, K., Druid, H., Bratthall, C., et al. (2011). The desmoplastic stroma plays an essential role in the accumulation and modulation of infiltrated immune cells in pancreatic adenocarcinoma. *Clin. Dev. Immunol.* 2011, 212810. doi: 10.1155/2011/212810

Von Bernstorff, W., Voss, M., Freichel, S., Schmid, A., Vogel, I., Johnk, C., et al. (2001). Systemic and local immunosuppression in pancreatic cancer patients. *Clin. Cancer Res.* 7, 925S–932S.

Vonderheide, R. H., and Bayne, L. J. (2013). Inflammatory networks and immune surveillance of pancreatic carcinoma. *Curr. Opin. Immunol.* 25, 200–205. doi: 10.1016/j.coi.2013.01.006

Wachsmann, M. B., Pop, L. M., and Vitetta, E. S. (2012). Pancreatic ductal adenocarcinoma: a review of immunologic aspects. *J. Investig. Med.* 60, 643–663.

Wynn, T. A. (2004). Fibrotic disease and the T(H)1/T(H)2 paradigm. *Nat. Rev. Immunol.* 4, 583–594. doi: 10.1038/nri1412

Zhu, J., Yamane, H., and Paul, W. E. (2010). Differentiation of effector CD4 T cell populations (*). *Annu. Rev. Immunol.* 28, 445–489. doi: 10.1146/annurev-immunol-030409-101212

Zitvogel, L., Tesniere, A., and Kroemer, G. (2006). Cancer despite immunosurveillance: immunoselection and immunosubversion. *Nat. Rev. Immunol.* 6, 715–727. doi: 10.1038/nri1936

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Stars and stripes in pancreatic cancer: role of stellate cells and stroma in cancer progression

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Pancreatic cancer is a devastating disease with an unacceptably high mortality to incidence ratio. Traditional therapeutic approaches such as surgery in combination with chemo- or radiotherapy have had limited efficacy in improving the outcome of this disease. Up until just under a decade ago, the prominent desmoplastic reaction which is a characteristic of the majority of pancreatic ductal adenocarcinomas (PDAC) had been largely ignored. However, since the identification of the pancreatic stellate cell (PSC) as the key cell responsible for the production of the collagenous stroma in PDAC, increasing attention has been paid to the role of the stromal reaction in pancreatic cancer pathobiology. There is now compelling evidence that PSCs interact not only with cancer cells themselves, but with several other cell types in the stroma (endothelial cells, immune cells, and possibly neuronal cells) to promote cancer progression. This review summarizes current knowledge in the field about the influence of PSCs and the stromal microenvironment on cancer behavior and discusses novel therapeutic approaches which reflect an increasing awareness amongst clinicians and researchers that targeting cancer cells alone is no longer sufficient to improve patient outcome and that combinatorial treatments targeting the stroma as well as the cancer cells will be required to change the clinical course of this disease.

Keywords: pancreatic cancer, pancreatic stellate cells, desmoplastic/stromal reaction, stromal-tumor interactions, stromal therapeutic targets

INTRODUCTION

Pancreatic cancer (pancreatic ductal adenocarcinoma; PDAC) is a lethal disease. It is the fourth leading cause of cancer related death in developed countries (Jemal et al., 2011; Siegel et al., 2013). Five year survival is at best 6% and survival beyond 12 months is unusual. Only 20% of patients are deemed suitable for attempted curative resection. Chemotherapy confers marginal benefit while the benefit of radiotherapy is debated. There are several reasons for this grim outlook. As the pancreas is a retroperitoneal organ, cancers in its body and tail present late, often with considerable local and distant spread. Early symptoms are often non-specific. There are no biomarkers for the disease.

Risk factors for pancreatic cancer include age, smoking, race, diabetes, and chronic pancreatitis. The strongest known risk factor for pancreatic cancer is chronic pancreatitis. Patients with a history of more than 5 years chronic pancreatitis have a greater than 14-fold risk of developing pancreatic cancer compared to the general population (Chu et al., 2007; Pandol et al., 2012). A significant proportion (40%) of patients with hereditary pancreatitis is at increased risk of developing pancreatic cancer (Whitcomb and Greer, 2009). For patients with tropical pancreatitis, a 100-fold increased risk and an earlier onset of pancreatic cancer has been reported (Chari et al., 1994; Whitcomb, 2004). The mechanisms underlying this increased propensity for patients with chronic pancreatitis to develop pancreatic cancer are not fully elucidated although recent studies suggest that several signaling pathways known to be active in inflammatory disease may be involved in driving this process (Thomasova et al., 2012).

Histologically, PDAC is characterized by an extensive and dense desmoplastic/fibrotic stroma in which cancer cells are embedded (**Figure 1**). It has now been unequivocally shown that the principal effector cells responsible for the production of this stroma are pancreatic stellate cells (PSCs) (Apte et al., 2004). Considerable evidence has also accumulated in recent years to indicate that this abundant stroma can no longer be considered a mere bystander in pancreatic cancer pathobiology, but should be recognized as a critical player in cancer progression.

This review will concentrate on the interactions between PSCs and pancreatic cancer cells and will also touch upon recent reports about the interactions between PSCs and other stromal cells (endothelial, immune, and nerve cells), all of which have the potential to influence local growth and distant spread of pancreatic tumors.

PANCREATIC STELLATE CELLS (PSCs)

PSCs were first described by Watari et al. (1982). These resident cells of the pancreas are predominantly periacinar in location and comprise 4–7% of total pancreatic parenchymal cells. In the healthy pancreas, PSCs are in a quiescent state and exhibit abundant vitamin A containing lipid droplets in their cytoplasm (Apte et al., 1998). Similar cells exist in the liver—hepatic stellate cells (HSCs). HSCs were first described by Kupffer in 1876 but were

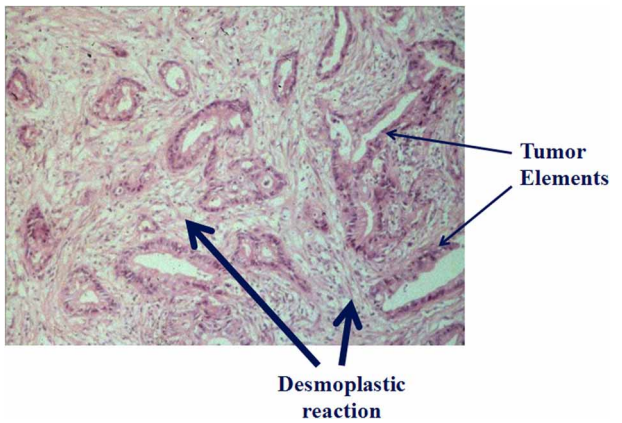

FIGURE 1 | Histology of pancreatic cancer: *Hematoxylin* and eosin stained section of a human pancreatic cancer section showing tumor elements (thin arrows) embedded in an abundant collagenous stroma (thick arrows) (*previously unpublished figure*).

brought into modern prominence by the work of Ito (1951) and Wake et al. (1987). Since that time, HSCs have been acknowledged as the principal site of storage of vitamin A in the body as well as being (when activated) the principal effector cells of liver fibrosis. It is now well-established that HSCs have a range of functions encompassing extracellular matrix (ECM) homeostasis, fibrosis, retinoid metabolism, liver development and regeneration, and immunomodulation (Lee and Friedman, 2011).

PSCs were first isolated by Apte et al. (1998) and this achievement opened up the field of pancreatic fibrogenesis as the cells could now be studied *in vitro* and *in vivo*. Since 1998, PSCs have been extensively characterized and their roles in fibrogenesis and tumor stromal interactions have been delineated in some detail (Apte et al., 2012).

PSCs express desmin, glial fibrillary acid protein (GFAP), vimentin, and nestin (intermediate filament proteins) as well as the neuroectodermal markers such as nerve growth factor (NGF) and neural cell adhesion molecule; the expression of these selective markers differentiates PSCs from fibroblasts (**Figure 2**). At the ultrastructural level they feature a prominent rough endoplasmic reticulum, collagen fibrils, and lipid droplets surrounding a central nucleus. With their ability to produce ECM proteins as well as the enzymes that degrade ECM proteins [matrix metalloproteinases (MMPS)], and inhibitors of MMPs [tissue inhibitors of metalloproteinases (TIMPS)], PSCs are thought to play a primary role in maintenance of normal pancreatic architecture. However, when activated, during pancreatic injury, the cells lose their lipid droplets, express α-smooth muscle *actin* (α-SMA), proliferate, migrate, and produce excessive amounts of ECM proteins, resulting in a loss of the balance between ECM production and degradation and leading eventually to fibrosis. During an acute episode of pancreatic injury, PSCs are activated early, and secrete excess ECM proteins that lay down a lattice for regenerating epithelial cells. As the injury resolves, activated PSCs are lost most likely through apoptosis (Tahara et al., 2008; Vonlaufen et al., 2011). MMPs secreted by the remaining PSCs degrade the excess fibrosis resulting in restitution of normal pancreatic histology. However, with repeated or sustained injury, PSCs can attain a perpetually activated state, since the cells can secrete their own cytokines and growth factors, which in turn can activate PSCs via selected receptors on the cell surface (Mews et al., 2002; Masamune et al., 2009). Thus, even in the absence of the original triggers, PSCs can remain in their activated state eventually being responsible for the development of pathological, often irreversible fibrosis.

While most of the initial research attention was directed toward elucidating the mechanisms responsible for PSC-mediated pancreatic fibrosis, it is becoming increasingly clear that PSCs may have several additional functions in health and disease. These include:

i. Role in pancreatic exocrine secretion: The secretagogue cholecystokinin (CCK) has been shown to directly stimulate exocrine secretion from rodent pancreatic acinar cells by binding to CCK receptors on the cell surface. However, there has been some controversy in the published literature regarding the direct effects of CCK on human pancreatic acinar cells, with Ji et al. reporting in 2001 and 2002 (Ji et al., 2001, 2002) that human acinar cells did not exhibit functional CCK receptors, a finding that was later countered by Murphy et al. (2008) who reported that isolated human pancreatic acini responded to physiological CCK concentrations by exhibiting the expected oscillatory rise in cytosolic calcium and by secreting amylase. In view of the close association of PSCs with the basolateral aspects of acinar cells, it has been postulated that in the human pancreas, PSCs may provide an alternative/additional pathway for CCK-mediated enzyme secretion by acting as intermediary cells in the CCK-stimulated secretory pathway. This concept is supported by the observations that (a) PSCs express both types of (CCK) receptors; (b) upon exposure to CCK, PSCs synthesize and secrete acetylcholine which can then act on muscarinic receptors on acinar cells leading to digestive enzyme release; and (c) PSC-mediated amylase secretion by acinar cells can be inhibited by the muscarinic receptor antagonist, atropine (Phillips et al., 2010).
ii. Role in innate immunity (Masamune et al., 2008a; Shimizu et al., 2012): PSCs express Toll-like receptors (TLR 2, 3, 4, 5, and 9) which recognize foreign pathogen-associated molecular patterns (PAMPs) and have been shown to be able to phagocytose necrotic and apoptotic cells. These functions suggest that the cells may have an "innate" immune function which protects local parenchyma, thereby limiting tissue damage during early pancreatic injury. However, the role of PSCs in acquired immunity is not as clear. Unlike their hepatic counterparts, PSCs do not express any antigen-presenting cell markers such as MHC class II or HLA-DR molecules. The reason for this difference between HSCs and PSCs is not known, but may reflect the fact that HSCs are routinely exposed to numerous antigens via the portal circulation resulting in the cells acquiring functions of antigen presenting cells, while PSCs are relatively protected within the pancreas.
iii. Role as progenitor cells (Mato et al., 2009; Kordes et al., 2013): Mato et al. (2009) used mitoxantrone (a compound that acts

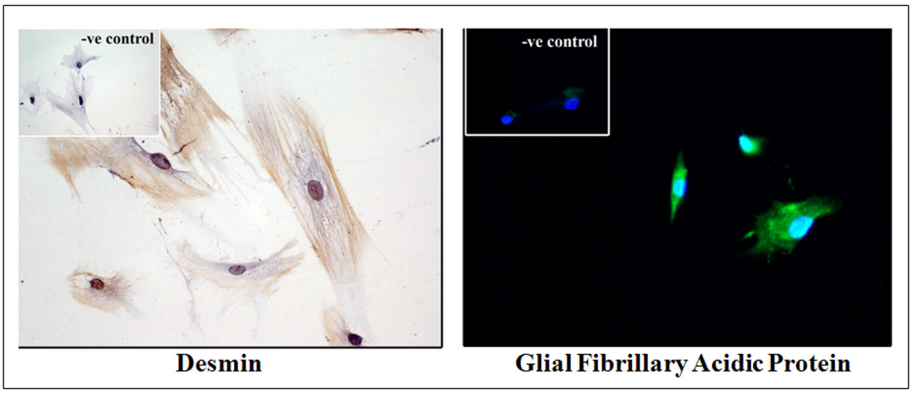

FIGURE 2 | Human pancreatic stellate cells (PSCs) in culture. Immunocytochemical analysis of primary cultures of human PSCs exhibiting desmin and glial fibrillary acidic protein (GFAP) staining. Insets: Negative control (*previously unpublished figure*).

through multidrug transport systems) to isolate and expand a population of mitoxantrone-resistant pancreatic cells from lactating rats. They reported that these selected cells exhibited a morphology identical to PSCs, with vitamin-A containing lipid droplets in the cytoplasm. The cells also expressed ABCG2 transporter (ATP-binding cassette G2 transporter—a stem cell marker) and when incubated with an appropriate differentiating medium, were able to secrete insulin. More intriguingly, a recent study by Kordes et al. (2013) has reported that clonally expanded rat PSCs, when injected into hepatectomized recipient rats, were able to migrate to the liver and to reconstitute large parts of the liver by differentiating into hepatocytes and cholangiocytes, whereas muscle fibroblast did not show any such transformations.

iv. Role in cancer progression (Apte et al., 2013): There is now incontrovertible evidence from both *in vivo* and *in vitro* studies for a central role for PSCs in promoting local growth of pancreatic tumors as well as facilitating regional and distant spread of pancreatic cancer cells.

INTERACTIONS BETWEEN PSC AND PANCREATIC CANCER CELLS, ENDOTHELIAL CELLS, IMMUNE CELLS, AND NEURAL CELLS

EVIDENCE FROM *IN VIVO* STUDIES

The role of PSCs in pancreatic cancer biology was initially studied in xenograft models and more recently has been examined using transgenic animal models of the disease. The earliest study in this area was published by Bachem et al. (2005), who used a subcutaneous xenograft model in immunocompromised mice to demonstrate increased growth of pancreatic cancer cells when co-injected with PSCs into the flanks of mice. The tumors produced in mice injected with both cell types were significantly larger than those in mice injected with cancer cells alone, exhibiting increased fibrosis as well as enhanced cancer cell proliferation. These observations suggested that, in addition to producing the collagenous stroma, PSCs also directly stimulated cancer cell growth.

Although the above findings were of interest, it is well-known that subcutaneous xenograft models of pancreatic cancer have an important limitation—the natural tumor microenvironment is absent in these models. Therefore, orthotopic tumors produced by implantation/injection of cancer cells directly into the pancreas are a preferred option. Such cells would be exposed to the same microenvironment as may be expected in human pancreatic cancer and would also have the capacity to metastasize, further simulating the human condition.

In recent years, several studies have reported orthotopic models of pancreatic cancer involving direct implantation/injection into the mouse pancreas of human pancreatic cancer cells (MiaPaCa-2, BxPC-1, AsPC-1) with or without human PSCs (hPSCs) (Hwang et al., 2008; Vonlaufen et al., 2008a; Xu et al., 2010). The presence of hPSCs enhanced local tumor growth as well as regional and distant metastasis. Tumors composed of both cancer cells and hPSCs exhibited (i) bands of fibrosis (resembling desmoplasia) containing α-SMA positive (activated) PSCs (**Figure 3**); and (ii) increased proliferation and decreased apoptosis of cancer cells, suggesting that the presence of PSCs increased the survival of cancer cells. These observations concur with those seen with hPSCs and tumor cells *in vitro* (vide infra) and support a role for PSCs in pancreatic cancer progression.

Orthotopic tumors produced by cancer cells + PSCs also exhibited enhanced angiogenesis (as indicated by the upregulation of the endothelial cell marker CD31) compared to tumors produced by the injection of cancer cells only, suggesting that PSCs stimulate angiogenesis in pancreatic cancer (Xu et al., 2010). It must be noted here however, that angiogenesis in human pancreatic cancers may be more complex than that observed in orthotopic models. Indeed, the central areas of advanced pancreatic tumors in humans are known to be very poorly perfused and hypoxic, with only a few blood vessels evident on histological examination; it is only the invading front of the cancers that manifests neoangiogenesis (Erkan et al., 2009). These findings are supported by *in vitro* work indicating that while the inductive effect of PSCs on angiogenesis is well-demonstrated under normoxic conditions, the same cannot be demonstrated under hypoxic conditions (Erkan et al., 2009). Thus, the overall influence of PSCs on angiogenesis in pancreatic cancer (taking into account the differences in oxygenation within the tumor) remains to be fully clarified.

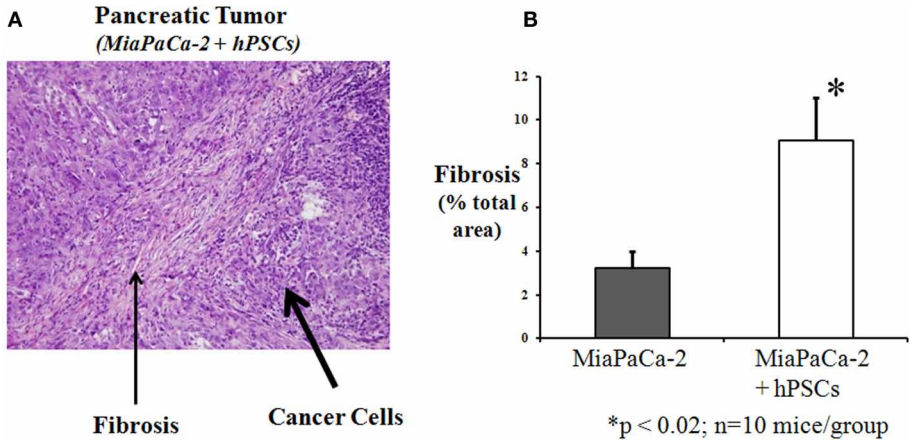

FIGURE 3 | Orthotopic pancreatic tumor produced by injecting a mixture of human pancreatic cancer cells (MiaPaCa-2) and human pancreatic stellate cells into the pancreas of nude mice. (A) H and E staining of tumor section showing prominent areas of fibrosis (desmoplasia) within the tumor. Reprinted with permission Vonlaufen et al. (2008a). **(B)** Fibrosis was quantitated by morphometry of Masson's stained sections (not shown). The graph depicts the significant increase in fibrosis in tumors produced by injection of a mixture of PSCs and cancer cells (MiaPaCa-2), compared to cancer cells alone. $*p < 0.02$; $n = 10$ mice/group (*previously unpublished data*).

One of the well-documented features of human pancreatic cancer is its resistance to chemotherapeutic agents and to radiotherapy. It is possible that this resistance may be mediated, at least in part, by the dense stroma produced by PSCs (Hanahan and Weinberg, 2011). In support of this notion, it has been shown that sequestration of chemotherapeutic agents such as gemcitabine can occur in the tumor stroma, effectively reducing the amount of the drug that can reach cancer cells (Olive et al., 2009). Furthermore, Mantoni et al. (2011) have reported that PSCs protect cancer cells from radiation via a β1-integrin dependent pathway.

As indicated above, in orthotopic models, PSCs have been shown to promote tumor metastasis. Traditionally, only cancer cells have been thought to possess metastatic capabilities, which allow the cells to intravasate into blood vessels or lymphatics, travel through the circulation, and extravasate at distant sites. This concept has been challenged by the findings of Xu et al. (2010) who, using a gender mismatch approach have demonstrated that PSCs from the primary tumor can also be detected at distant metastatic sites. The authors injected a mixture of male human PSCs and female cancer cells (AsPC-1 cell line from a female patient), into the pancreas of female mice. Using fluorescent *in situ* hybridization, y chromosome positive cells were detected not only in the primary tumors (as expected) but also within metastatic nodules in the mediastinum, liver, and diaphragm. These observations indicate that PSCs can travel to distant metastatic sites (possibly with cancer cells), where they may be reasonably postulated to play a role in the seeding, survival, and proliferation of cancer cells. A subsequent study reported similar findings in a model of lung cancer (Duda et al., 2010) suggesting that metastasis can no longer be considered the sole preserve of cancer cells.

In contrast to subcutaneous and orthotopic models where tumors are produced in immunocompromised mice by xenografts of human pancreatic cancer cells and PSCs, some genetically engineered mouse (GEM) models exhibit the development of spontaneous pancreatic cancer with a prominent endogenously produced stromal reaction (Guerra and Barbacid, 2013). These models include KPC mice ($Kras^{LSL-G12D/+}$; $Trp53^{LSL-R172H/+}$; $Pdx^{cre/+}$), KPGC mice ($Kras^{LSL-G12D/+}$; $Trp53^{LSL-R172H/+}$; $R26^{LSL-GFP/+}$; $Pdx^{cre/+}$), and TGFβ type II receptor organ specific knockout in the mouse pancreas ($Kras^{LSL-G12D/+}$; $TGF\beta r2^{floxflox}$; $Ptf1a^{cre/+}$). The lesions in these models progress from preinvasive ductal changes (PanIN lesions) to overt carcinoma and metastases, with an associated progressive increase in the surrounding stromal reaction. Importantly, activated PSCs have been observed in the earliest PanIN lesions (Ijichi et al., 2011; Apte et al., 2013). These GEM models provide an additional *in vivo* tool to assess the interactions between cancer cells and an endogenous stromal reaction and also to trial new therapeutic strategies in pancreatic cancer.

Evasion of the immune system is a well-recognized feature of pancreatic cancer (Bayne et al., 2012). Pancreatic cancer tissue is infiltrated with immune cells, such as T cells, B cells, NK cells, neutrophils, and macrophages as well as myeloid-derived suppressor cells (as the name suggests, MDSCs have a largely immunosuppressive function) (Apte et al., 2013; Ene-Obong et al., 2013; Hamada et al., 2013; Ino et al., 2013). Higher levels of CD8+ T cell infiltration have been shown to correlate with a better survival (Ene-Obong et al., 2013; Ino et al., 2013), while macrophage and neutrophil infiltration as well as high levels of MDSCs have been reported to be associated with poor survival (Gabitass et al., 2011; Ino et al., 2013). It has been demonstrated that cancer cells can evade the host immune system by producing granulocyte-macrophage colony-stimulating factor to suppress anti-tumor T cell immunity (Bayne et al., 2012).

Recent studies suggest that PSCs may also aid immune evasion. PSCs in the stroma of PanIN lesions and around cancer cells produce galectin-1, a β-galactoside-binding protein (Chen et al., 2012), that binds to N-acetyllactosamine on membrane

glycoproteins and induces apoptosis in T cells thus suppressing the immune response (Tang et al., 2012). Ene-Obong et al. (2013) have reported that activated PSCs reduce the migration of CD8 positive T cells toward cancer cells in both human PDAC and the KPC mouse model of pancreatic cancer. Fibroblast activation protein-α (FAP-α), known to be expressed by stromal cells, is another protein that has been reported to disrupt anti-tumor immunity. Depletion of the cells expressing FAP-α enabled immune response-associated tumor regression, supporting the notion that FAP-α might act as an immune suppressor in pancreatic cancer (Kraman et al., 2010). Most recently, another type of immune cell, the mast cell, has been reported to play a role in pancreatic cancer progression. Using an orthotopic model of pancreatic cancer, Chang et al. (2013) have reported that cancer growth is significantly hampered in mast cell deficient Kit mice, while the reconstitution of mast cells in these mice from the bone marrow of wild type mice significantly enhanced tumor growth. Interestingly, as detailed later in this review, PSCs have been shown to activate mast cells *in vitro* (Ma et al., 2013), suggesting cross-talk between these two cell types in the stroma.

Taken together, the above studies suggest that PSCs may negatively modulate immune responses.

EVIDENCE FROM VITRO STUDIES

Findings derived from mouse models and observations on resected human tissue are supported by a number of *in vitro* studies which have confirmed a close bi-directional interaction between pancreatic cancer cells and PSCs.

When PSCs are exposed to cancer cells (either by co-culture or by using conditioned media), they are activated and manifest increased proliferation, migration, and ECM production (Apte and Wilson, 2012). In turn, PSCs stimulate cancer cell proliferation and inhibit cancer cell apoptosis thereby facilitating cancer cell survival (Vonlaufen et al., 2008b). PSCs have also been shown to promote cancer cell migration, during which cancer cells exhibit features of epithelial-mesenchymal transition (EMT) namely, decreased levels of epithelial markers such as E-cadherin concurrent with increased expression of mesenchymal markers (vimentin and Snail) (Fujiwara et al., 2013). It is possible that EMT is responsible (at least in part) for the PSC-induced increased migration of cancer cells. Most recently, a study by Bachem et al. (Lu et al., 2014) has demonstrated that PSC-induced cancer cell migration is dependent on collagen I secreted by PSCs; interaction of cancer cells with collagen I enhances the α2/β1 integrin-focal adhesion kinase (FAK) signaling pathway that regulates migration of cancer cells.

While the above effects of PSCs on cancer cells are of significant interest, researchers have also been mindful of the known heterogeneity of pancreatic cancer with respect to rate of progression. This has led to studies examining whether all PSCs uniformly exert the same effects on cancer cells. Interestingly, a subpopulation of PSCs that express CD10 (a cell membrane associated matrix metalloproteinase), has been reported to induce significantly greater effects on cancer cell proliferation and invasion than $CD10^-$ PSCs (Ikenaga et al., 2010). These findings indicate that functional heterogeneity between PSC populations may dictate the ultimate effects of these cells on cancer cell behavior.

One of the major factors responsible for the poor prognosis of pancreatic cancer is its propensity for recurrence, with recurrent tumors postulated to arise from a niche of drug resistant cancer stem cells. Recent evidence suggests that PSCs may play a role in facilitating such a stem cell niche in pancreatic cancer. Hamada et al. (2012) have reported that pancreatic cancer cells in co-culture with PSCs show increased expression of stem cell related genes such as nestin, ABCGZ, and LIN28, supporting the possibility that a PSC-facilitated cancer stem cell niche may be one of the factors responsible for recurrence of pancreatic cancer.

As the interactions between cancer cells and PSCs have become increasingly recognized, factors mediating these interactions have also attracted much interest. The increased proliferation of PSCs induced by cancer cells is likely mediated by platelet-derived growth factor (PDGF, a known mitogen for many cell types), which stimulates mitogen-activated protein kinase signaling (MAPK) in PSCs (Vonlaufen et al., 2008a). Recent studies have also implied that cancer cell-stimulated PSC proliferation is mediated by cyclooxygenase 2 (the inducible form of cyclooxygenases, which are enzymes involved in the conversion of arachidonic acid to prostaglandin Yoshida et al., 2005) and by trefoil factor 1 (a stable secretory protein that is upregulated in pancreatic cancer but is not expressed in normal pancreas) (Arumugam et al., 2011). The increase in ECM synthesis by PSCs upon exposure to cancer cells is thought to be mediated by transforming growth factor beta 1 (TGFβ1) and fibroblast growth factor 2 (FGF2) (Bachem et al., 2005).

Factors mediating the effects of PSCs on cancer cells remain to be fully elucidated. Since cancer cells express receptors for PDGF and PSCs have the capacity to secrete PDGF, it has been postulated that this growth factor mediates the PSC-induced proliferation of cancer cells (Vonlaufen et al., 2008a). PSCs also secrete a cell adhesion protein named periostin, which has been found to increase the growth of cancer cells and their resistance to serum starvation and hypoxia (Erkan et al., 2007). Other candidate mediators that require further study include growth factors such as EGF, insulin-like growth factor (IGF), hepatocyte growth factor (HGF), and TGFβ as well as a variety of proinflammatory cytokines. Notably, ERK1/2 and Akt have been identified as the intracellular signaling pathways that regulate the response of cancer cells (increased migration, invasion, and colony formation) to PSC secretions (Hwang et al., 2008; Vonlaufen et al., 2008a).

The observed effects of PSCs on angiogenesis and metastatic spread *in vivo* (described earlier) are strongly supported by *in vitro* studies. PSCs have been shown to stimulate tube formation (a measure of angiogenesis) of human microvascular endothelial cells, an effect mediated by vascular endothelial growth factor (VEGF) secreted by PSCs (Xu et al., 2010). Under normoxic conditions, PSCs also induce endothelial cell proliferation, an effect again mediated by VEGF (Erkan et al., 2009). However, this proliferative effect of PSCs on endothelial cells was inhibited under hypoxic conditions (simulating the hypoxia in the center of a dense desmoplastic stroma), particularly in the presence of cancer cells (Erkan et al., 2009). On

the other hand, hypoxia itself was shown to significantly increase PSC activation and ECM synthesis (Masamune et al., 2008b). Thus, the interplay between vessel density/oxygenation at different sites within the tumor (central vs. peripheral) and PSC activation, as well as the influence of PSCs on endothelial cell function under varying oxygen concentrations requires further study.

The ability of PSCs to travel from the primary tumor to metastatic sites (noted earlier) implies that PSCs can migrate through an endothelial layer. Using a Boyden chamber method with a porous membrane coated by a monolayer of endothelial cells, Xu et al. (2010) have shown that PSCs can invade and migrate through the endothelial cell layer, an effect that is enhanced in the presence of cancer cell secretions. This cancer cell-induced transendothelial migration of PSCs is mediated by PDGF in cancer cell secretions.

As noted earlier, pancreatic cancer cells have the ability to escape immune surveillance despite the presence of significant leukocyte infiltration in the stroma. There is *in vivo* evidence to suggest that PSCs may play a role in this immune evasion by sequestering CD8+ T cells and reducing their infiltration around tumor cells, thus preventing the T cells from exerting their antitumor effects. *In vitro* support for this concept comes from studies showing that PSCs exert a chemotactic effect on CD8+ T cells, and that this effect is mediated by the PSC-derived chemokine CXCL12 (Ene-Obong et al., 2013). Interactions between PSCs and mast cells have also been recently characterized (Ma et al., 2013). PSCs have been shown to activate mast cells *in vitro* promoting tryptase and IL13 release from the latter; these mast cell-derived factors have been shown to stimulate cancer cell proliferation. Mast cells also induce PSC proliferation, an effect mediated by IL13. Most recently, IL6 secreted by PSCs has been implicated in PSC-induced migration of the immunosuppressive cells MDSCs (Mace et al., 2013); as noted previously, high levels of MDSCs in pancreatic cancer tissue have been associated with reduced overall survival (Gabitass et al., 2011).

Compared to the interactions of PSCs with cancer cells, endothelial cells, and immune cells described above, little is known about the interaction of PSCs with neural elements in the desmoplastic reaction. However, extensive neural remodeling is known to occur in pancreatic cancer with the cancer stroma revealing neural hypertrophy and increased neural density (Ceyhan et al., 2010). It noteworthy that PSCs themselves express the neural markers GFAP and nestin, and also produce the neurotrophic factors NGF, brain-derived neurotrophic factor, and neurotrophin 45 (Haas et al., 2009; Demir et al., 2012). Thus, it would be reasonable to postulate that PSCs may act as neural elements in the tumor stroma, affecting the growth of nerves (via secretion of ECM components collagen and fibronectin and the neurotrophic factors noted above) and survival of cancer cells that express receptors for neurotrophic factors. This hypothesis is supported by a report by Ceyhan et al. (2009) demonstrating a positive correlation between the extent of desmoplasia and the degree of neural invasion in human PDAC.

Figure 4 summarizes the interactions between PSCs and pancreatic cancer cells as well as those between PSCs and other stromal cells that may promote cancer growth and spread.

DO PANCREATIC STELLATE CELLS PLAY A ROLE IN THE EARLIEST STAGES OF PANCREATIC CANCER?

While the role of PSCs in advanced pancreatic cancer is now well-accepted, evidence is also accumulating to suggest that PSCs may be activated at the earliest stages of pancreatic carcinogenesis, i.e., around PanIN lesions. Pandol et al. (2012) have described a distinct stromal reaction comprising extensive collagen deposition and α-SMA positive activated PSCs around PanIN lesions (**Figure 5**) which eventually lead to overt pancreatic cancer in a mouse model overexpressing $Kras^{G12D}$. Similarly periostin (solely expressed by PSCs) has been observed in intraductal papillary mucinous neoplasms of the human pancreas (Fukushima et al., 2008), further supporting the idea that PSCs are activated early in the neoplastic process. Recent *in vitro* studies have confirmed an interaction between PanIN cells and PSCs. Exposure of PSCs to PanIN cells isolated from $Kras^{G12D}$ mice significantly increased PSC proliferation, activation (α-SMA), fibronectin synthesis, and MMP expression (Pandol et al., 2012), indicating that preneoplastic cells have the capacity to activate PSCs in the early stages of carcinogenesis.

Based on findings reported by Funahashi et al. (2007), reciprocal effects of PSCs on PanIN cells which could facilitate progression to overt PDAC may also be postulated. The authors have shown that nimesulide, a selective inhibitor of COX-2 (which as noted earlier, is expressed by PSCs and implicated in PSC-cancer interactions), retards the progression of pancreatic cancer precursor lesions in a GEM model.

THERAPEUTIC TARGETING OF STROMA IN PANCREATIC CANCER

Clinical outcome in pancreatic cancer has not improved significantly over many decades. The usual regimens of surgery, radiotherapy, and chemotherapy benefit only a small minority of patients, and even in these patients, the chances of recurrence and emergence of chemoresistant cancers are high. The majority of patients are either not suitable for surgery at diagnosis or develop resistance to single chemotherapeutic agents. In a bid to address drug resistance, combination therapies have been trialed where the standard chemotherapeutic agent gemcitabine is combined with other agents such as Folfirinox (comprising 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin) or with targeted drugs such as growth factor inhibitors or with soluble taxanes such as Abraxane. The most recent studies combining gemcitabine with Abraxane (nab-Paclitaxel) (Von Hoff et al., 2013) or Folfirinox (Conroy et al., 2011) have reported an increase in overall survival, but the benefit is marginal (a few months increased survival). Thus, it is clear that a new approach is required to improve the prognosis of this disease.

For reasons already discussed, strategies are now being developed to target not only cancer cells but also the desmoplastic reaction, and initial studies have been focused on ways to inhibit PSC activation.

One of the signaling factors known to mediate PSC activation is the Hedgehog pathway (which is essential for embryonic development, but usually not detectable in adult healthy pancreas) (Bailey et al., 2008). This pathway has been also been implicated in stem cell regulation and neoplasia (Thayer et al., 2003). Binding

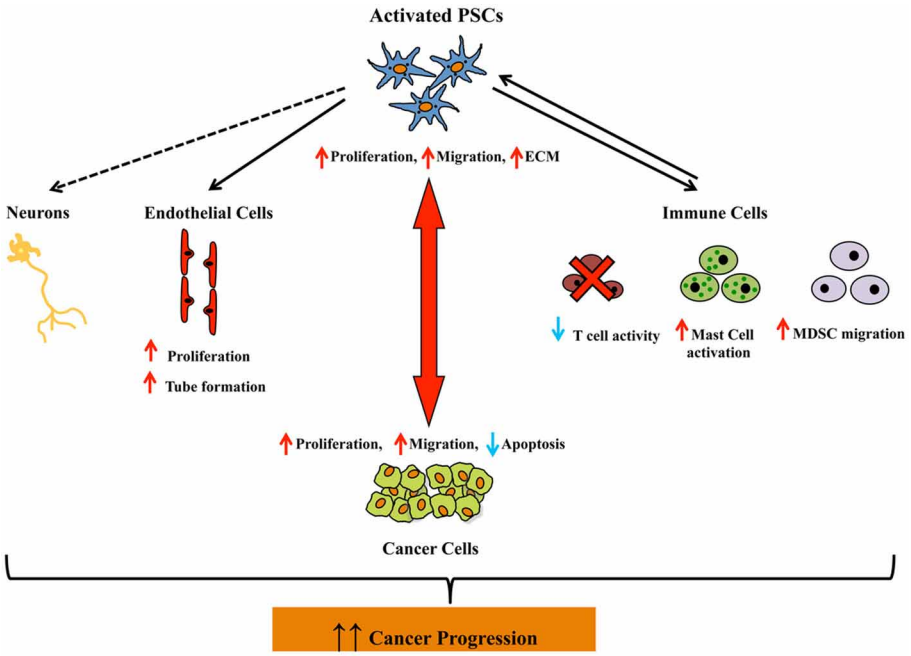

FIGURE 4 | Schematic diagram depicting the interactions between activated pancreatic stellate cells and pancreatic cancer cells, as well as pancreatic stellate cells and other stromal cells (endothelial cells, immune cells, and neuronal cells), all of which may promote pancreatic cancer progression. The dashed arrow between PSCs and neuronal cells indicates that although an interaction between these two cell types may be reasonably postulated, direct experimental evidence in support of this concept has not yet been reported. Abbreviations: PSCs, pancreatic stellate cells; MC, mast cells; MDSC, myeloid derived suppressor cells; ECM, extracellular matrix.

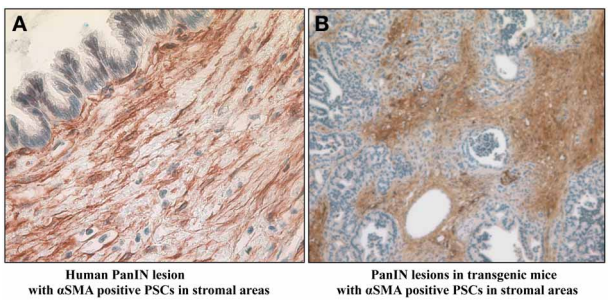

FIGURE 5 | Presence of α-smooth muscle actin positive activated pancreatic stellate cells in stroma surrounding early PanIN lesions in (A) humans and (B) transgenic mice. Reprinted with permission Apte et al. (2013).

of the Hedgehog ligand (Sonic, Indian, and Desert Hedgehog) to its receptor Patched releases the co-receptor Smoothened from repression and results in translocation of the transcription factor Gli-1 from the cytoplasm to the nucleus where it regulates genes involved in cell differentiation, proliferation, apoptosis, adhesion, and migration. Abnormal activation of Hedgehog pathway has been reported in several cancers including basal cell carcinoma, lung, prostate, and pancreatic cancer. Inhibition of Smoothened by cyclopamine or its derivative IPI-926 in transgenic models of pancreatic cancer has been recently studied (Feldmann et al., 2007; Olive et al., 2009). Cyclopamine marginally increased median survival by 6 days, while treatment of mice with IPI-926 in combination with gemcitabine, resulted in increased delivery of the chemotherapeutic agent to cancer cells, but had only a transient effect on improved blood vessel density and extension of median survival. Subsequently, Hwang et al. (2012) used another Smoothened inhibitor AZD8542 in an orthotopic model of pancreatic cancer produced by implantation of a mixture of PSCs and cancer cells in the pancreas. AZD8542 was reported to reduce tumor volume, metastasis, and Hedgehog downstream signaling activity. Based on these encouraging pre-clinical reports, clinical trials using Hedgehog inhibitors were commenced. Unfortunately the phase II trial with IPI-926 had to be abandoned prematurely due to decreased survival of patients in the treatment arm. The lack of translation of the preclinical findings to the clinical setting may reflect the fact that preclinical models do not fully capture the heterogeneity of human pancreatic cancer, or that the preclinical findings need to be better confirmed using a range of experimental settings.

Taxanes such as Paclitaxel and Docetaxel have been used as chemotherapeutic agents in a variety of cancers. The compounds act by preventing microtubule depolymerization and interfering with the cell cycle, but their use is hampered by their toxicity and insolubility in water. Nanoparticle albumin complexed paclitaxel (nab-paclitaxel) was developed to overcome the issues of solubility and to enhance drug delivery through albumin facilitated receptor-mediated transcytosis (Yardley, 2013). Administration of nab-paclitaxel alone or in combination with gemcitabine in a patient-tumor-derived subcutaneous xenograft model depleted

the stroma in the tumors and increased perfusion via an increase in blood vessel diameter with consequent improved delivery of gemcitabine to tumor cells (Von Hoff et al., 2011). The mechanisms mediating the effects of nab-paclitaxel on the stroma are unknown. However, with regard to the anti-cancer effects, it is postulated that the albumin in nab-paclitaxel is bound by secreted protein acidic and rich in cysteine (SPARC), an albumin binding glycoprotein that is overexpressed in pancreatic cancer stroma (Neuzillet et al., 2013), leading to accumulation of nab-paclitaxel near tumor cells (Yardley, 2013). Furthermore, nab-paclitaxel may increase the availability of gemcitabine within tumor tissue by inducing the generation of reactive oxygen species within cancer cells, leading to inhibition of cytidine deaminase and consequently decreased metabolic inactivation of gemcitabine (Yardley, 2013). As noted earlier, a recent Phase 3 trial has compared the effects of nab-paclitaxel plus gemcitabine to gemcitabine alone in patients with metastatic pancreatic cancer (Von Hoff et al., 2013). The combination was found to significantly improve overall survival as well as progression-free survival compared to gemcitabine alone (8.5 vs. 6.7 months and 5.5 vs. 3.7 months, respectively). Although the improvements may be regarded as modest, the results support the concept that targeting the stroma in addition to cancer cells may be a potentially beneficial approach.

With regard to targeting the immune cells in PDAC stroma, Beatty et al. (2011) have demonstrated in the KPC mouse model of pancreatic cancer that activation of CD40, a member of the TNF receptor superfamily, activates macrophages in the stroma and results in apoptosis of cancer cells as well as a reduction in stromal collagen. Activation of CD40 was achieved by systemic administration of a CD40 agonist monoclonal antibody to KPC mice. Using a similar approach in a Phase I study in a small number of chemotherapy-naïve advanced pancreatic cancer patients, the authors have reported that the antibody in combination with gemcitabine was well-tolerated with some evidence of anti-tumor activity, but with heterogeneous responses particularly with regard to metastatic lesions (Beatty et al., 2013). Thus, larger randomized controlled trials will be needed before the role of a CD40 agonist monoclonal antibody in pancreatic cancer treatment can be clearly determined.

Other compounds that have been used to target the stroma, but so far only in preclinical models, include:

i. Angiotensin II receptor antagonists: Angiotensin II, a component of the renin-angiotensin system, has been shown to induce PSC proliferation, ECM synthesis and migration, and to increase the production of growth factors by PSCs. Thus, angiotensin II receptor blockade, already in clinical use in hypertension, has been recently assessed in a subcutaneous xenograft model of pancreatic cancer. Using the inhibitor olmasartan, Masamune et al. (2013) report a significant decrease in primary tumor growth accompanied by decreased α-SMA staining and ECM production in mice injected with a mixture of PSCs and cancer cells, but not in mice injected with cancer cells alone. Similarly, using losartan (another Angiotensin II receptor inhibitor), Chauhan et al. (2013) have reported decreased αSMA positive cells, and reduced collagen and hyaluronan production in the stroma of pancreatic cancer in an orthotopic mouse model.
ii. Pirfenidone (a pyridone compound known to be an effective antifibrotic agent in idiopathic pulmonary fibrosis): Treatment with this compound using subcutaneous and orthotopic models of pancreatic cancer has been reported to decrease the growth of tumors produced by the injection of a mixture of pancreatic cancer cells and PSCs, but not that of tumors produced by cancer cells alone (Kozono et al., 2013). *In vitro* studies showed that pirfenidone inhibited PSC proliferation, invasion, and migration, and interrupted the interaction between pancreatic cancer cells and PSCs; these effects were associated with decreased expression of PDGF-A, HGF, periostin, collagen type I, and fibronectin in PSCs, as well as reduced PSC activation (decreased α-SMA expression) (Kozono et al., 2013). The findings suggest that pirfenidone regulates PSC function and inhibits cancer growth.
iii. PEGylated human recombinant PH20 hyaluronidase (PEGPH20): This compound enzymatically degrades one of the predominant components of the ECM, hyaluronan. PEGPH20 treatment of KPC mice resulted in stromal depletion and decompression of tumor vessels leading to an increase in tumor vascular patency without increasing vessel density. PEGPH20 also increased fenestrations in endothelia and interendothelial junction gaps that increased the permeability of the endothelium to macromolecules. When combined with gemcitabine, PEGPH20 treatment improved the delivery of gemcitabine to tumor cells inhibiting tumor growth and extending the median survival of the mice (Provenzano et al., 2012; Jacobetz et al., 2013).
iv. Phytonutrients ellagic acid and embelin: Ellagic acid is a polyphenol found in a variety of nuts and fruit, while embelin is a phytochemical from a Japanese herb Arsidae Japonicae. These compounds have been reported to decrease proliferation and increase apoptosis of cancer cell as well as stellate cells resulting in significantly reduced tumor volumes in a xenograft model of pancreatic cancer (Edderkaoui et al., 2013).

In conclusion, it is now abundantly clear that the prominent stromal/desmoplastic reaction of pancreatic cancer can no longer be dismissed as a mere epiphenomenon of carcinogenesis. Indeed, available evidence strongly indicates that this stromal reaction, and in particular the cells responsible for its production, PSCs, likely play a key role at the earliest stages of pancreatic cancer development. Therefore, all components of this reaction (stromal cells and collagenous matrix) warrant attention as potentially useful, additional therapeutic targets in this disease. The challenge in this field of research will be to ensure that preclinical testing is carried out with experimental models (or a range of models) that not only closely simulate the pathology, but also account for the heterogeneity of human pancreatic cancer, so as to successfully translate research findings into clinically effective therapies.

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REFERENCES

Apte, M. V., Haber, P. S., Applegate, T. L., Norton, I. D., McCaughan, G. W., Korsten, M. A., et al. (1998). Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture. *Gut* 43, 128–133. doi: 10.1136/gut.43.1.128

Apte, M. V., Park, S., Phillips, P. A., Santucci, N., Goldstein, D., Kumar, R. K., et al. (2004). Desmoplastic reaction in pancreatic cancer: role of pancreatic stellate cells. *Pancreas* 29, 179–187. doi: 10.1097/00006676-200410000-00002

Apte, M. V., Pirola, R. C., and Wilson, J. S. (2012). Pancreatic stellate cells: a starring role in normal and diseased pancreas. *Front. Physiol.* 3:344. doi: 10.3389/fphys.2012.00344

Apte, M. V., and Wilson, J. S. (2012). Dangerous liaisons: pancreatic stellate cells and pancreatic cancer cells. *J. Gastroenterol. Hepatol.* 27(Suppl. 2), 69–74. doi: 10.1111/j.1440-1746.2011.07000.x

Apte, M. V., Wilson, J. S., Lugea, A., and Pandol, S. J. (2013). A starring role for stellate cells in the pancreatic cancer microenvironment. *Gastroenterology* 144, 1210–1219. doi: 10.1053/j.gastro.2012.11.037

Arumugam, T., Brandt, W., Ramachandran, V., Moore, T. T., Wang, H., May, F. E., et al. (2011). Trefoil factor 1 stimulates both pancreatic cancer and stellate cells and increases metastasis. *Pancreas* 40, 815–822. doi: 10.1097/MPA.0b013e31821f6927

Bachem, M. G., Schunemann, M., Ramadani, M., Siech, M., Beger, H., Buck, A., et al. (2005). Pancreatic carcinoma cells induce fibrosis by stimulating proliferation and matrix synthesis of stellate cells. *Gastroenterology* 128, 907–921. doi: 10.1053/j.gastro.2004.12.036

Bailey, J. M., Swanson, B. J., Hamada, T., Eggers, J. P., Singh, P. K., Caffery, T., et al. (2008). Sonic hedgehog promotes desmoplasia in pancreatic cancer. *Clin. Cancer Res.* 14, 5995–6004. doi: 10.1158/1078-0432.CCR-08-0291

Bayne, L. J., Beatty, G. L., Jhala, N., Clark, C. E., Rhim, A. D., Stanger, B. Z., et al. (2012). Tumor-derived granulocyte-macrophage colony-stimulating factor regulates myeloid inflammation and T cell immunity in pancreatic cancer. *Cancer Cell* 21, 822–835. doi: 10.1016/j.ccr.2012.04.025

Beatty, G. L., Chiorean, E. G., Fishman, M. P., Saboury, B., Teitelbaum, U. R., Sun, W., et al. (2011). CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science* 331, 1612–1616. doi: 10.1126/science.1198443

Beatty, G. L., Torigian, D. A., Chiorean, E. G., Saboury, B., Brothers, A., Alavi, A., et al. (2013). A phase I study of an agonist CD40 monoclonal antibody (CP-870, 893) in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma. *Clin. Cancer Res.* 19, 6286–6295. doi: 10.1158/1078-0432.CCR-13-1320

Ceyhan, G. O., Bergmann, F., Kadihasanoglu, M., Altintas, B., Demir, I. E., Hinz, U., et al. (2009). Pancreatic neuropathy and neuropathic pain–a comprehensive pathomorphological study of 546 cases. *Gastroenterology* 136, 177.e1–186.e1. doi: 10.1053/j.gastro.2008.09.029

Ceyhan, G. O., Schafer, K. H., Kerscher, A. G., Rauch, U., Demir, I. E., Kadihasanoglu, M., et al. (2010). Nerve growth factor and artemin are paracrine mediators of pancreatic neuropathy in pancreatic adenocarcinoma. *Ann. Surg.* 251, 923–931. doi: 10.1097/SLA.0b013e3181d974d4

Chang, D. Z., Ma, Y., Ji, B., Wang, H., Deng, D., Liu, Y., et al. (2013). Mast cells in tumor microenvironment promotes the *in vivo* growth of pancreatic ductal adenocarcinoma. *Clin. Cancer Res.* 17, 7015–7023. doi: 10.1158/1078-0432.CCR-11-0607

Chari, S. T., Mohan, V., Pitchumoni, C. S., Viswanathan, M., Madanagopalan, N., and Lowenfels, A. B. (1994). Risk of pancreatic carcinoma in tropical calcifying pancreatitis: an epidemiologic study. *Pancreas* 9, 62–66. doi: 10.1097/00006676-199401000-00009

Chauhan, V. P., Martin, J. D., Liu, H., Lacorre, D. A., Jain, S. R., Kozin, S. V., et al. (2013). Angiotensin inhibition enhances drug delivery and potentiates chemotherapy by decompressing tumour blood vessels. *Nat. Commun.* 4, 2516. doi: 10.1038/ncomms3516

Chen, R., Pan, S., Ottenhof, N. A., de Wilde, R. F., Wolfgang, C. L., Lane, Z., et al. (2012). Stromal galectin-1 expression is associated with long-term survival in resectable pancreatic ductal adenocarcinoma. *Cancer Biol. Ther.* 13, 899–907. doi: 10.4161/cbt.20842

Chu, G. C., Kimmelman, A. C., Hezel, A. F., and DePinho, R. A. (2007). Stromal biology of pancreatic cancer. *J. Cell. Biochem.* 101, 887–907. doi: 10.1002/jcb.21209

Conroy, T., Desseigne, F., Ychou, M., Bouche, O., Guimbaud, R., Becouarn, Y., et al. (2011). FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N. Engl. J. Med.* 364, 1817–1825. doi: 10.1056/NEJMoa1011923

Demir, I. E., Friess, H., and Ceyhan, G. O. (2012). Nerve-cancer interactions in the stromal biology of pancreatic cancer. *Front. Physiol.* 3:97. doi: 10.3389/fphys.2012.00097

Duda, D. G., Duyverman, A. M., Kohno, M., Snuderl, M., Steller, E. J., Fukumura, D., et al. (2010). Malignant cells facilitate lung metastasis by bringing their own soil. *Proc. Natl. Acad. Sci. U.S.A.* 107, 21677–21682. doi: 10.1073/pnas.1016234107

Edderkaoui, M., Lugea, A., Hui, H., Eibl, G., Lu, Q. Y., Moro, A., et al. (2013). Ellagic acid and embelin affect key cellular components of pancreatic adenocarcinoma, cancer, and stellate cells. *Nutr. Cancer* 65, 1232–1244. doi: 10.1080/01635581.2013.832779

Ene-Obong, A., Clear, A. J., Watt, J., Wang, J., Fatah, R., Riches, J. C., et al. (2013). Activated pancreatic stellate cells sequester CD8+ T-cells to reduce their infiltration of the juxtatumoral compartment of pancreatic ductal adenocarcinoma. *Gastroenterology.* 145, 1121–1132. doi: 10.1053/j.gastro.2013.07.025

Erkan, M., Kleeff, J., Gorbachevski, A., Reiser, C., Mitkus, T., Esposito, I., et al. (2007). Periostin creates a tumor-supportive microenvironment in the pancreas by sustaining fibrogenic stellate cell activity. *Gastroenterology* 132, 1447–1464. doi: 10.1053/j.gastro.2007.01.031

Erkan, M., Reiser-Erkan, C., Michalski, C. W., Deucker, S., Sauliunaite, D., Streit, S., et al. (2009). Cancer-stellate cell interactions perpetuate the hypoxia-fibrosis cycle in pancreatic ductal adenocarcinoma. *Neoplasia* 11, 497–508. doi: 10.1593/neo.81618

Feldmann, G., Dhara, S., Fendrich, V., Bedja, D., Beaty, R., Mullendore, M., et al. (2007). Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers. *Cancer Res.* 67, 2187–2196. doi: 10.1158/0008-5472.CAN-06-3281

Fujiwara, K., Ohuchida, K., Ohtsuka, T., Mizumoto, K., Shindo, K., Ikenaga, N., et al. (2013). Migratory activity of CD105+ pancreatic cancer cells is strongly enhanced by pancreatic stellate cells. *Pancreas* 2042, 1283–1290. doi: 10.1097/MPA.0b013e318293e7bd

Fukushima, N., Kikuchi, Y., Nishiyama, T., Kudo, A., and Fukayama, M. (2008). Periostin deposition in the stroma of invasive and intraductal neoplasms of the pancreas. *Mod. Pathol.* 21, 1044–1053. doi: 10.1038/modpathol.2008.77

Funahashi, H., Satake, M., Dawson, D., Huynh, N. A., Reber, H. A., Hines, O. J., et al. (2007). Delayed progression of pancreatic intraepithelial neoplasia in a conditional Kras(G12D) mouse model by a selective cyclooxygenase-2 inhibitor. *Cancer Res.* 67, 7068–7071. doi: 10.1158/0008-5472.CAN-07-0970

Gabitass, R. F., Annels, N. E., Stocken, D. D., Pandha, H. A., and Middleton, G. W. (2011). Elevated myeloid-derived suppressor cells in pancreatic, esophageal and gastric cancer are an independent prognostic factor and are associated with significant elevation of the Th2 cytokine interleukin-13. *Cancer Immunol. Immunother.* 60, 1419–1430. doi: 10.1007/s00262-011-1028-0

Guerra, C., and Barbacid, M. (2013). Genetically engineered mouse models of pancreatic adenocarcinoma. *Mol. Oncol.* 7, 232–247. doi: 10.1016/j.molonc.2013.02.002

Haas, S. L., Fitzner, B., Jaster, R., Wiercinska, E., Gaitantzi, H., Jesnowski, R., et al. (2009). Transforming growth factor-beta induces nerve growth factor expression in pancreatic stellate cells by activation of the ALK-5 pathway. *Growth Factors* 27, 289–299. doi: 10.1080/08977190903132273

Hamada, S., Masamune, A., and Shimosegawa, T. (2013). Inflammation and pancreatic cancer: disease promoter and new therapeutic target. *J. Gastroenterol.* doi: 10.1007/s00535-013-0915-x. [Epub ahead of print].

Hamada, S., Masamune, A., Takikawa, T., Suzuki, N., Kikuta, K., Hirota, M., et al. (2012). Pancreatic stellate cells enhance stem cell-like phenotypes in pancreatic cancer cells. *Biochem. Biophys. Res. Commun.* 421, 349–354. doi: 10.1016/j.bbrc.2012.04.014

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi: 10.1016/j.cell.2011.02.013

Hwang, R. F., Moore, T., Arumugam, T., Ramachandran, V., Amos, K. D., Rivera, A., et al. (2008). Cancer-associated stromal fibroblasts promote pancreatic tumor progression. *Cancer Res.* 68, 918–926. doi: 10.1158/0008-5472.CAN-07-5714

Hwang, R. F., Moore, T. T., Hattersley, M. M., Scarpitti, M., Yang, B., Devereaux, E., et al. (2012). Inhibition of the hedgehog pathway targets the tumor-associated

stroma in pancreatic cancer. *Mol. Cancer Res.* 10, 1147–1157. doi: 10.1158/1541-7786.MCR-12-0022

Ijichi, H., Chytil, A., Gorska, A. E., Aakre, M. E., Bierie, B., Tada, M., et al. (2011). Inhibiting Cxcr2 disrupts tumor-stromal interactions and improves survival in a mouse model of pancreatic ductal adenocarcinoma. *J. Clin. Invest.* 121, 4106–4117. doi: 10.1172/JCI42754

Ikenaga, N., Ohuchida, K., Mizumoto, K., Cui, L., Kayashima, T., Morimatsu, K., et al. (2010). CD10+ pancreatic stellate cells enhance the progression of pancreatic cancer. *Gastroenterology* 139, 1041–1051, 1051.e1–1051.e8. doi: 10.1053/j.gastro.2010.05.084

Ino, Y., Yamazaki-Itoh, R., Shimada, K., Iwasaki, M., Kosuge, T., Kanai, Y., et al. (2013). Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. *Br. J. Cancer* 108, 914–923. doi: 10.1038/bjc.2013.32

Ito, T. (1951). Cytological studies on stellate cells of Kupffer and fat storing cells in the capillary wall of the human liver (Japanese abstract). *Acta Anat. Nippon* 26:42.

Jacobetz, M. A., Chan, D. S., Neesse, A., Bapiro, T. E., Cook, N., Frese, K. K., et al. (2013). Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. *Gut* 62, 112–120. doi: 10.1136/gutjnl-2012-302529

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., and Forman, D. (2011). Global cancer statistics. *CA Cancer J. Clin.* 61, 69–90. doi: 10.3322/caac.20107

Ji, B., Bi, Y., Simeone, D., Mortensen, R. M., and Logsdon, C. D. (2001). Human pancreatic acinar cells lack functional responses to cholecystokinin and gastrin. *Gastroenterology* 121, 1380–1390. doi: 10.1053/gast.2001.29557

Ji, B., Bi, Y., Simeone, D., Mortensen, R. M., and Logsdon, C. D. (2002). Human pancreatic acinar cells do not respond to cholecystokinin. *Pharmacol. Toxicol.* 91, 327–332. doi: 10.1034/j.1600-0773.2002.910610.x

Kordes, C., Sawitza, I., Gotze, S., and Haussinger, D. (2013). Stellate cells from rat pancreas are stem cells and can contribute to liver regeneration. *PLoS ONE* 7:e51878. doi: 10.1371/journal.pone.0051878

Kozono, S., Ohuchida, K., Eguchi, D., Ikenaga, N., Fujiwara, K., Cui, L., et al. (2013). Pirfenidone inhibits pancreatic cancer desmoplasia by regulating stellate cells. *Cancer Res.* 73, 2345–2356. doi: 10.1158/0008-5472.CAN-12-3180

Kraman, M., Bambrough, P. J., Arnold, J. N., Roberts, E. W., Magiera, L., Jones, J. O., et al. (2010). Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. *Science* 330, 827–830. doi: 10.1126/science.1195300

Lee, U. E., and Friedman, S. L. (2011). Mechanisms of hepatic fibrogenesis. *Best Pract. Res. Clin. Gastroenterol.* 25, 195–206. doi: 10.1016/j.bpg.2011.02.005

Lu, J., Zhou, S., Siech, M., Habisch, H., Seufferlein, T., and Bachem, M. G. (2014). Pancreatic stellate cells promote hapto-migration of cancer cells through collagen I-mediated signalling pathway. *Br. J. Cancer*. 110, 409–420. doi: 10.1038/bjc.2013.706

Ma, Y., Hwang, R. F., Logsdon, C. D., and Ullrich, S. E. (2013). Dynamic mast cell-stromal cell interactions promote growth of pancreatic cancer. *Cancer Res.* 73, 3927–3937. doi: 10.1158/0008-5472.CAN-12-4479

Mace, T. A., Bloomston, M., and Lesinski, G. B. (2013). Pancreatic cancer-associated stellate cells: a viable target for reducing immunosuppression in the tumor microenvironment. *Oncoimmunology* 2:e24891. doi: 10.4161/onci.24891

Mantoni, T. S., Lunardi, S., Al-Assar, O., Masamune, A., and Brunner, T. B. (2011). Pancreatic stellate cells radioprotect pancreatic cancer cells through beta1-integrin signaling. *Cancer Res.* 71, 3453–3458. doi: 10.1158/0008-5472.CAN-10-1633

Masamune, A., Hamada, S., Kikuta, K., Takikawa, T., Miura, S., Nakano, E., et al. (2013). The angiotensin II type I receptor blocker olmesartan inhibits the growth of pancreatic cancer by targeting stellate cell activities in mice. *Scand. J. Gastroenterol.* 48, 602–609. doi: 10.3109/00365521.2013.777776

Masamune, A., Kikuta, K., Watanabe, T., Satoh, K., Hirota, M., and Shimosegawa, T. (2008b). Hypoxia stimulates pancreatic stellate cells to induce fibrosis and angiogenesis in pancreatic cancer. *Am. J. Physiol. Gastrointest. Liver Physiol.* 295, G709–G717. doi: 10.1152/ajpgi.90356.2008

Masamune, A., Kikuta, K., Watanabe, T., Satoh, K., Satoh, A., and Shimosegawa, T. (2008a). Pancreatic stellate cells express Toll-like receptors. *J. Gastroenterol.* 43, 352–362. doi: 10.1007/s00535-008-2162-0

Masamune, A., Watanabe, T., Kikuta, K., and Shimosegawa, T. (2009). Roles of pancreatic stellate cells in pancreatic inflammation and fibrosis. *Clin. Gastroenterol. Hepatol.* 7, S48–S54. doi: 10.1016/j.cgh.2009.07.038

Mato, E., Lucas, M., Petriz, J., Gomis, R., and Novials, A. (2009). Identification of a pancreatic stellate cell population with properties of progenitor cells: new role for stellate cells in the pancreas. *Biochem. J.* 421, 181–191. doi: 10.1042/BJ20081466

Mews, P., Phillips, P., Fahmy, R., Korsten, M., Pirola, R., Wilson, J., et al. (2002). Pancreatic stellate cells respond to inflammatory cytokines: potential role in chronic pancreatitis. *Gut* 50, 535–541. doi: 10.1136/gut.50.4.535

Murphy, J. A., Criddle, D. N., Sherwood, M., Chvanov, M., Mukherjee, R., McLaughlin, E., et al. (2008). Direct activation of cytosolic Ca2+ signaling and enzyme secretion by cholecystokinin in human pancreatic acinar cells. *Gastroenterology* 135, 632–641. doi: 10.1053/j.gastro.2008.05.026

Neuzillet, C., Tijeras-Raballand, A., Cros, J., Faivre, S., Hammel, P., and Raymond, E. (2013). Stromal expression of SPARC in pancreatic adenocarcinoma. *Cancer Metastasis Rev.* 32, 585–602. doi: 10.1007/s10555-013-9439-3

Olive, K. P., Jacobetz, M. A., Davidson, C. J., Gopinathan, A., McIntyre, D., Honess, D., et al. (2009). Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 324, 1457–1461. doi: 10.1126/science.1171362

Pandol, S., Gukovskaya, A., Edderkoui, M., Dawson, D., Eibl, G., and Lugea, A. (2012). Epidemiology, risk factors, and the promotion of pancreatic cancer: role of the stellate cell. *J. Gastroenterol. Hepatol.* 27(Suppl. 2), 127–134. doi: 10.1111/j.1440-1746.2011.07013.x

Phillips, P. A., Yang, L., Shulkes, A., Vonlaufen, A., Poljak, A., Bustamante, S., et al. (2010). Pancreatic stellate cells produce acetylcholine and may play a role in pancreatic exocrine secretion. *Proc. Natl. Acad. Sci. U.S.A.* 107, 17397–17402. doi: 10.1073/pnas.1000359107

Provenzano, P. P., Cuevas, C., Chang, A. E., Goel, V. K., Von Hoff, D. D., and Hingorani, S. R. (2012). Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell* 21, 418–429. doi: 10.1016/j.ccr.2012.01.007

Shimizu, K., Hashimoto, K., Tahara, J., Imaeda, H., Andoh, A., and Shiratori, K. (2012). Pancreatic stellate cells do not exhibit features of antigen-presenting cells. *Pancreas* 41, 422–427. doi: 10.1097/MPA.0b013e31822e673b

Siegel, R., Naishadham, D., and Jemal, A. (2013). Cancer statistics, (2013). *CA Cancer J. Clin.* 63, 11–30. doi: 10.3322/caac.21166

Tahara, J., Shimizu, K., and Shiratori, K. (2008). Engulfment of necrotic acinar cells by pancreatic stellate cells inhibits pancreatic fibrogenesis. *Pancreas* 37, 69–74. doi: 10.1097/MPA.0b013e318160a5cb

Tang, D., Yuan, Z., Xue, X., Lu, Z., Zhang, Y., Wang, H., et al. (2012). High expression of Galectin-1 in pancreatic stellate cells plays a role in the development and maintenance of an immunosuppressive microenvironment in pancreatic cancer. *Int. J. Cancer* 130, 2337–2348. doi: 10.1002/ijc.26290

Thayer, S. P., di Magliano, M. P., Heiser, P. W., Nielsen, C. M., Roberts, D. J., Lauwers, G. Y., et al. (2003). Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature* 425, 851–856. doi: 10.1038/nature02009

Thomasova, D., Mulay, S. R., Bruns, H., and Anders, H. J. (2012). p53-independent roles of MDM2 in NF-kappaB signaling: implications for cancer therapy, wound healing, and autoimmune diseases. *Neoplasia* 14, 1097–1101. doi: 10.1593/neo.121534

Von Hoff, D. D., Ervin, T., Arena, F. P., Chiorean, E. G., Infante, J., Moore, M., et al. (2013). Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N. Engl. J. Med.* 369, 1691–1703. doi: 10.1056/NEJMoa1304369

Von Hoff, D. D., Ramanathan, R. K., Borad, M. J., Laheru, D. A., Smith, L. S., Wood, T. E., et al. (2011). Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial. *J. Clin. Oncol.* 29, 4548–4554. doi: 10.1200/JCO.2011.36.5742

Vonlaufen, A., Joshi, S., Qu, C., Phillips, P. A., Xu, Z., Parker, N. R., et al. (2008a). Pancreatic stellate cells: partners in crime with pancreatic cancer cells. *Cancer Res.* 68, 2085–2093. doi: 10.1158/0008-5472.CAN-07-2477

Vonlaufen, A., Phillips, P. A., Xu, Z., Goldstein, D., Pirola, R. C., Wilson, J. S., et al. (2008b). Pancreatic stellate cells and pancreatic cancer cells: an unholy alliance. *Cancer Res.* 68, 7707–7710. doi: 10.1158/0008-5472.CAN-08-1132

Vonlaufen, A., Phillips, P. A., Xu, Z., Zhang, X., Yang, L., Pirola, R. C., et al. (2011). Withdrawal of alcohol promotes regression while continued alcohol intake promotes persistence of LPS-induced pancreatic injury in alcohol-fed rats. *Gut* 60, 238–246. doi: 10.1136/gut.2010.211250

Wake, K., Motomatsu, K., and Senoo, H. (1987). Stellate cells storing retinol in the liver of adult lamprey, *Lampetra japonica*. *Cell Tissue Res.* 249, 289–299. doi: 10.1007/BF00215511

Watari, N., Hotta, Y., and Mabuchi, Y. (1982). Morphological studies on a vitamin A-storing cell and its complex with macrophage observed in mouse pancreatic

tissues following excess vitamin A administration. *Okajimas Folia Anat. Jpn.* 58, 837–858.

Whitcomb, D., and Greer, J. (2009). Germ-line mutations, pancreatic inflammation, and pancreatic cancer. *Clin. Gastroenterol. Hepatol.* 7, S29–S34. doi: 10.1016/j.cgh.2009.07.032

Whitcomb, D. C. (2004). Inflammation and Cancer V. Chronic pancreatitis and pancreatic cancer. *Am. J. Physiol. Gastrointest. Liver Physiol.* 287, G315–G319. doi: 10.1152/ajpgi.00115.2004

Xu, Z., Vonlaufen, A., Phillips, P. A., Fiala-Beer, E., Zhang, X., Yang, L., et al. (2010). Role of pancreatic stellate cells in pancreatic cancer metastasis. *Am. J. Pathol.* 177, 2585–2596. doi: 10.2353/ajpath.2010.090899

Yardley, D. A. (2013). nab-Paclitaxel mechanisms of action and delivery. *J. Control. Release* 170, 365–372. doi: 10.1016/j.jconrel.2013.05.041

Yoshida, S., Ujiki, M., Ding, X. Z., Pelham, C., Talamonti, M. S., Bell, R. H., et al. (2005). Pancreatic stellate cells (PSCs) express cyclooxygenase-2 (COX-2) and pancreatic cancer stimulates COX-2 in PSCs. *Mol. Cancer* 4, 27. doi: 10.1186/1476-4598-4-27

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Src as the link between inflammation and cancer

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Although a causal link between chronic inflammation and cancer has been established, the exact molecular mechanism linking inflammation to cancer remains largely unknown. It was previously postulated that molecular switches responsible for cancer cell development, and for infiltration of inflammatory cells into cancer, were divided into a distinct set of intracellular proteins and signaling pathways. However, recent evidence suggests that both tumor cells and tumor-infiltrating immune cells utilize the same kinases, mostly that of Src family, to facilitate cancer development and progression. In the past few years several groups have found that Src activation both in cancer and inflammatory cells is mainly driven by pro-inflammatory cytokines within the tumor microenvironment. Here we evaluate the cross talks between Src kinase pathways in immune cells and cancer cells. We conclude that Src might serve as a critical mechanistic link between inflammation and cancer, mediating and propagating a cycle between immune and tissue cells that can ultimately lead to the development and progression of cancer.

Keywords: inflammation, cancer, Src, cytokines, chronic pancreatitis, pancreatic cancer

INTRODUCTION

Inflammation is a vital defensive response that serve critical roles in a variety of physiological situations, and when dysregulated, can contribute to the pathogenesis of many diseases. Chronic inflammation is a well-documented risk for promoting cancer (Coussens and Werb, 2002; Balkwill et al., 2005; Mantovani et al., 2008), particularly in the pancreas and GI tract (Guerra et al., 2007; Terzić et al., 2010). Chronic pancreatitis is long-standing inflammation of the pancreas associated with an increased risk (∼20-fold) for pancreatic cancer. This projects a serious clinical problem as pancreatic cancer is a highly lethal disease with the worst prognosis of all the major malignancies; for all stages combined, and a 5-year survival rate of 5% (Yadav et al., 2011). Similarly, uncontrolled inflammatory bowel disease poses a significant risk factor for colorectal cancer. When compared to the general population matched for age, sex, and years at risk, there is a 18-fold increase in Crohn's disease and a 19-fold increase in ulcerative colitis, (Bernstein et al., 2001; Eaden et al., 2001; Itzkowitz and Yio, 2004; Ullman and Itzkowitz, 2011). Interestingly, many environmental cancer risk factors, including alcohol overuse, smoking, chronic infections and obesity, can trigger some form of chronic inflammation, largely in the pancreas and colon (Trinchieri, 2012). These environmental risk factors seemingly facilitate the development and progression of cancer mostly through the induction of chronic persistent inflammation in these tissues.

Although many studies point to an association between inflammation and cancer, the mechanistic signaling basis of this linkage is not well understood. The importance of Src family kinases in colon and pancreatic cancer development is known for many years and is well established (Staley et al., 1997; Lutz et al., 1998; Aligayer et al., 2002). Recent evidence has shown that Src signaling network is also very important in movement and infiltration of immune cells into tumor (Balkwill, 2004; Kulbe et al., 2004). Several groups have found that Src activation in cancer and immune inflammatory cells are mediated by inflammatory cytokines within the tumor microenvironment. Given that Src is overactive in both tumor cells and in tumor-infiltrating immune cells, and is also involved in cytokine-mediated cross talk between cancer and inflammatory cells—Src may be a critical link between inflammation and cancer. We illustrate and expound on this concept using the model of chronic pancreatitis and pancreatic cancer.

PERSISTENT INFLAMMATION INCREASES CANCER RISK IN PANCREAS

Chronic pancreatitis highlights an important role for chronic inflammation in the development of cancer. Chronic pancreatitis is the most consistent risk factor for pancreatic cancer and alone increases the risk of developing pancreatic cancer by 10–20-fold (Dítě et al., 2012). Many of the environmental cancer risk factors can initially induce chronic inflammation that subsequently leads to pancreatic cancer. Recurrent pancreatic injury from alcohol abuse, smoking, high-fat diet, diabetes, and genetic predisposition, induces a pro-inflammatory environment consisting of various types of immune cells, cytokines, chemokines, and growth factors that, when dysregulated and persistent, can ultimately lead to the development and progression of cancer (Lowenfels et al., 2001; Shoelson et al., 2007; Pannala et al., 2009; Momi et al., 2012).

Abbreviations: LPS, lipopolysaccharides; TNF-α, tumor necrosis factor; IL-1, interleukine 1; IL-6, interleukine 6; MCP-1, monocyte chemoattractant protein-1; MIP-1, macrophage inflammatory protein 1; MIP-2, macrophage inflammatory protein 2; SDF-1, stromal cell-derived factor 1; PI3K, phosphatidylinositol-3 kinase.

Alcohol abuse is a major cause of acute and chronic pancreatitis. The disease usually presents as an acute episode of pancreatitis and progress with additional exacerbations that can lead to chronic pancreatitis, characterized by a sequence of necrotic and fibrotic events. The initial tissue injures are associated with cytokine release during necro-inflammation and appears to include premature intracellular activation of digestive enzymes, leading to autodigestion. Alcohol metabolism causes release of endogenous hydrolases from pancreatic lysozymes, which are responsible for premature activation of trypsinogen leading to intrapancreatic autodigestion and inflammation (Talamini et al., 1999). Reactive oxygen species generated results in further pancreatic tissue injury, and further release of pro-inflammatory cytokines and chemokines (Shi et al., 2005).

In addition, alcohol when combined with cigarette smoking exacerbates the chronic inflammatory process (Go et al., 2005; Maisonneuve et al., 2005; Wiśniewska et al., 2013). Cigarette smoking contributes to the development of chronic pancreatitis by inducing cytokine release and inflammation. Smoking is the major risk factor for the development of pancreatic cancer accounting for 20–30% of cases (Lowenfels et al., 2001). In experimental models, nicotine stimulated an acute inflammatory reaction in the pancreas, which progressed to chronic pancreatitis after repeated sessions of smoking-induced acute pancreatic inflammation. These nicotine-induced inflammatory events are clearly associated with the release of pro-inflammatory cytokines (Nordskog et al., 2003).

Both central and overall obesity are associated with increased risk for pancreatic cancer (Pannala et al., 2009). Although the exact mechanism of obesity to pancreatic cancer is unclear, the major issues revolve around chronic inflammation, glucose intolerance, hyperinsulinemia, insulin resistance, and oxidative stress. Inflammation, along with the immune system plays a vital role in the development of insulin resistance, diabetes, and ultimately pancreatic cancer. Adipose tissue is involved in the release of cytokines and chemokines including TNF-α, IL-6, MCP-1, CXCL12, CCL5, CCL20, that lead to the recruitment of pro-inflammatory cells into adipose tissue (Shoelson et al., 2007; Sell et al., 2012). Obese individuals also exhibit lower circulating levels of anti-inflammatory adipokines that sustains a low-grade systemic inflammation.

Hereditary pancreatitis is a rare autosomal dominant condition caused by gain-of-function mutations in the cationic trypsinogen gene (PRSS1) and is responsible for <1% of all forms of pancreatitis. Mutant PRSS1 gene causes premature activation or impairs the deactivation of trypsin leading to recurrent injury, cytokine release, and inflammation. The risk of developing pancreatic cancer is 53 times higher when compared to the risk in unaffected individuals. Of the patients who progress to chronic pancreatitis, the risk of developing pancreatic cancer by age 70 years is approximately 40%. Pancreatic inflammation also occurs at a much younger age in this group of patients. In addition, Lowenfels et al. reported a 2-fold increased risk of developing pancreatic cancer in smokers with hereditary pancreatitis as compared to non-smokers. Pancreatic cancer also developed 20 years earlier in smokers than in non-smokers (Howes et al., 2004; Rebours et al., 2009), suggesting that nicotine-induced release of cytokines and inflammation can rapidly accelerate the promotion and development of cancer in these patients.

INFLAMMATORY CELLS INFILTRATE TUMOR IN PANCREAS

Since the role of various immune cells (including lymphocytes, granulocytes, and macrophages) in pancreatic inflammation and cancer has been discussed elsewhere (Mantovani et al., 2008), this review will focus on studies of macrophages as Src kinase-dependent and cytokine-mediated linkage between inflammation and cancer seems most apparent in these cells. Tumor-associated macrophages are key players in pancreatic inflammation and cancer and an important source of cytokines (Feig et al., 2012; Liou et al., 2013). As described above, chronic pancreatitis is often initiated by environmental risk factors, leads to permanent damage of pancreas, and is a consistent risk factor for pancreatic cancer. Chronic pancreatitis is characterized by marked stroma formation with a high number of infiltrating macrophages and myofiroblastic-like stellate cells, which are believed to play a central role in initiating inflammation and disease progression (Erkan et al., 2012). In response to pancreatic injury (alcohol abuse, cigarette smoking, obesity, mutations in genetically predisposed persons, etc.), inflammatory signals and chemokines production are upregulated leading to infiltration of leukocytes and stellate cells to the damaged acinar cells. Inflammatory cells that are recruited in turn secrete several cytokines, including chemokines, interleukins, and interferons, that contribute to cancer growth, invasion, and metastasis (**Figure 1**, **Table 1**).

Numerous experimental studies have suggested an important role of macrophages in generating the microenvironment for both chronic pancreatitis and tumor cells, thus highlighting a similarity between stroma composition in chronic pancreatitis and pancreatic cancer. Macrophages are derived from circulating peripheral monocytes mostly in response to chemokine monocyte chemoattractant protein 1 (MCP-1). Several other chemokines, including MIP-1, MIP-2, and SDF-1, are also increased at the site of inflammation attracting leukocytes and tissue precursors to the injured pancreas (Spaeth et al., 2008). In turn, macrophages, other leukocytes, and stellate cells, which all infiltrate the tumor, release cytokines, including IL-1, IL-6 and TNF that directly effect cancer cell proliferation, and movement/attachment. This process promotes cancer development and progression (**Figure 1**, **Table 1**). It is also possible that the cytokine-mediated persistent activation of certain key intracellular signaling pathways, which occurs during chronic inflammation, might inhibit apoptosis and prevent the elimination of genetically altered, precancerous and cancerous cells.

Src ACTIVATION CONTRIBUTES TO BOTH INFLAMMATION AND CANCER IN PANCREAS

Src was the first transforming protein discovered and isolated (Rous, 1911; Stehelin et al., 1976; Brugge and Erikson, 1997) and was also the first gene product with protein tyrosine kinase activity (Hunter and Sefton, 1980). The Src family kinases comprise of nine non-receptor protein tyrosine kinases that share similar structure and function. Src family kinases have a critical role in cell adhesion, proliferation, survival, and invasion, including cell movement, and activation of cytokine receptors.

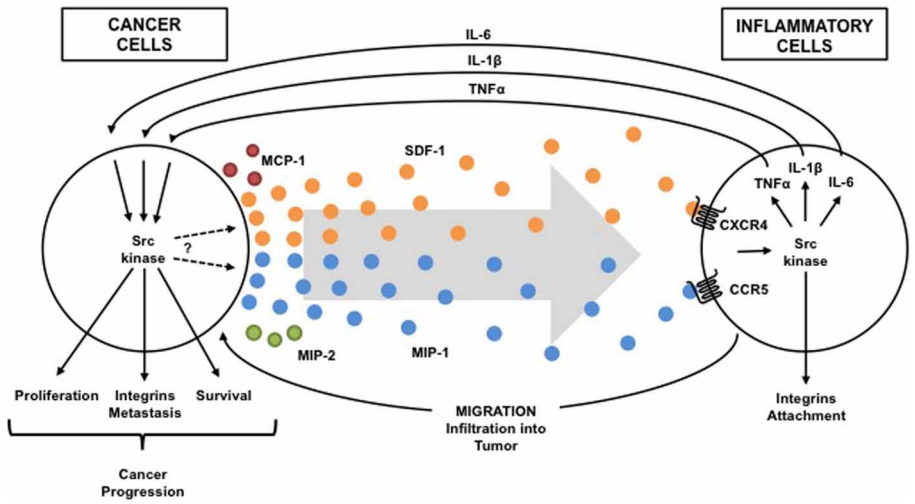

FIGURE 1 | Src-dependent cross talk between inflammatory and cancer cells. Src activation is driven in both inflammatory cells and cancer cells by pro-inflammatory cytokines within the tumor microenvironment. Activation of Src kinases in immune cells by tumor-secreted cytokines (chemokines: SDF-1, MIP-1, MCP-1, MIP-2, etc.) induces production of cytokines (TNF-a, IL-1b, IL-6, etc.) that reciprocally activate Src in cancer cells to promote cancer progression and to induce more cytokine (chemokine) that attract more inflammatory cells into the tumor. Thus, a Src-dependent condition exists in which one problem causes another problem that makes the first problem worse (vicious cycle).

Table 1 | Src tyrosine kinase family and cytokine/chemokine interaction in immune and cancer cells.

Src TYROSINE KINASE FAMILY MEDIATE CYTOKINE/CHEMOKINE PRODUCTION			
Src kinase family	**Cytokines/chemokines**	**Source**	**References**
Src	MIP-1α, MCP-1, MIP-2	Acinar cells	Ramnath et al., 2009
Lyn	TNF-α	Macrophages	Tomkowicz et al., 2006
Hck	TNF-α, IL-6	Colorectal cancer	Smolinska et al., 2011
Src	TNF-α, IL-6	Macrophages	Sarang et al., 2011
Lyn	IL-1β	Macrophages	Cheung et al., 2008
CYTOKINES ACTIVATE Src TYROSINE KINASE FAMILY			
Cytokines	**Src kinase family**	**Source**	
TNF-α	Src	Acinar cells	Satoh et al., 2005
IL-6	Src	Gastric cancer cells	Lin et al., 2007
IL-6	Hck	Myeloma cells	Podar et al., 2004
IL-6	Fyn, Lyn Hck	Myeloma cells	Hallek et al., 1997
CHEMOKINES ACTIVATE Src TYROSINE KINASE FAMILY			
Chemokines	**Src kinase family**	**Source**	
SDF-1	Src	Ductal cells	Kayali et al., 2003
SDF-1	Lyn	Macrophages	Malik et al., 2008
SDF-1	Lyn	B lymphocytes	Nakata et al., 2006
SDF-1	Lck	T lymphocytes	Inngjerdingen et al., 2002
MIP-1-β	Lyn	Macrophages	Tomkowicz et al., 2006
RANTES	Lyn	Macrophages	Cheung et al., 2009

Numerous groups have found that hyper-activation and/or over-expression of Src family kinases are critical to various types of cancers.

Expression of several members of the Src-family tyrosine kinases, including Src, Fyn, Yes, Fgr and Lyn has been demonstrated in pancreatic cancer cell lines and primary cells. The expression of Lyn kinase is the most abundant in these cells (Fu et al., 2006). Numerous studies have shown that elevated Src-family kinase activity in human pancreatic carcinomas (when compared to normal pancreatic cells) not only contributes to pancreatic cancer growth, but also to invasion and metastasis (Lutz et al., 1998; Trevino et al., 2006; Yokoi et al., 2011). Src kinases and oncogenic Ras, PI3K, p38MAPK and Dynamin-2 have been shown to co-operatively stimulate the growth, metastatic migration and invasion of pancreatic carcinoma (Summy et al., 2005; Shields et al., 2011).

Src activation has been observed in circulating blood monocytes and tissue macrophages in chronic pancreatitis, as well as in tumor-associated macrophages and acinar cells in pancreatic cancer (Yokoi et al., 2011). Elevated level of activity of Src in inflammatory monocytes/macrophages was proposed as a biomarker for pancreatic cancer (Coppola, 2000; Yokoi et al., 2011). However, no oncogenic mutations responsible for Src activation in inflammatory and cancer cells in the pancreas have yet been identified. Thus, Src activation is likely a result of underlying inflammation and the consequence of a cytokine-mediated inflammatory microenvironment during malignant transformation and progression. It seems that the signal activating Src kinases is within the inflammatory microenvironment without the necessity of the Src mutation. Consequently, several groups have found that Src activation is driven by pro-inflammatory cytokines, and inversely, the cytokine production is driven by Src kinases, in various types of cancer and inflammatory cells, as summarized in **Table 1**.

As previously discussed, in response to pancreatic injury, chemokine production is upregulated leading to infiltration of leukocytes and stellate cells to the injured acinar cells. Rather limited information is available on the exact role of Src kinases in chemokine production in pancreatic inflammation and cancer (note the question mark in the **Figure 1**). However, Src kinases involvement in the secretion of several chemokines was demonstrated in pancreatic acinar cells (Ramnath et al., 2009) and ductal cells (Ungefroren et al., 2011). The pretreatment of pancreatic acini with Src kinase inhibitors markedly decreased MCP-1, MIP-1, and MIP-2 production after stimulation with the substance-P (Ramnath et al., 2009). Substance-P is known to play a role in pathogenesis of cerulein-induced pancreatitis and pancreatic cancer invasion (Ramnath and Bhatia, 2006; Ito et al., 2007).

Accordingly, it also has been shown that the expression of CCR5 receptor for MIP-1, MCP-2 and RANTES, is upregulated in chronic pancreatitis in human tissue, as compared with the healthy pancreas, and the majority of CCR5-positive cells were infiltrating macrophages (Goecke et al., 2000). Similarly, the expression of the CCR5 chemokine receptor and its ligands (MIP-1, MCP-2, RANTES) was significantly increased in the mouse pancreas during cerulein-induced pancreatitis (Goecke et al., 2000; Duell et al., 2006). On the other hand, the SDF-1 chemokine signaling in pancreas and in the other tissues is also dependent on Src family kinases (Takatomo et al., 2000; Nakata et al., 2006; Malik et al., 2008). Src family kinases are downstream intracellular targets of CXCR4 receptor, and are required for the SDF-1—mediated cell movement and attachment (Nakata et al., 2006; Malik et al., 2008). The SDF-1-CXCR4 ligand receptor axis induces pancreatic cancer cell invasion, and the Src-mediated SDF-1 signaling is also an obligatory component of pancreatic regeneration (Takatomo et al., 2000; Kayali et al., 2003; Gao et al., 2010).

In addition, we have previously shown the SDF-1-CXCR4-Src signaling axis is crucial for the movement and invasiveness of inflammatory leukocytes, in a variety of pathological contexts ranging from inflammation to cancer (Nakata et al., 2006; Chen et al., 2008; Malik et al., 2008). Several studies have shown that in human primary leukocytes, Src family members, particularly Lyn and Lck, are required for CXCR4-dependent cell movement and infiltration into various inflamed tissues (Inngjerdingen et al., 2002; Malik et al., 2008). The SDF-1-mediated activation of Lyn kinase in monocytes, modifies integrin activity through inside-out signaling, and transiently destabilizes monocyte/endothelial cell interactions, facilitating full monocyte detachment from endothelium and penetration into inflamed tissue (Nakata et al., 2006; Chen et al., 2008; Malik et al., 2008). Importantly, Lyn is also required for TNF-α and IL-1β production in inflammatory macrophages during stimulation with the CCR5 receptor ligands (Tomkowicz et al., 2006; Cheung et al., 2008, 2009). The other Src family members, Src and Hck, have been shown to play a critical role in IL-6 production in osteoblasts and inflammatory macrophages, respectively (Smolinska et al., 2011; Peruzzi et al., 2012). IL-6 is required for the maintenance and progression of pancreatic cancer precursor lesions, and thus is required for pancreatic cancer growth (Zhang et al., 2013).

In summary, Src family kinases have been demonstrated to be important in the activation of macrophage, dendritic cells, neutrophils and natural killer cells in normal tissues (Ptasznik et al., 1995, 1996; Abram and Lowell, 2008; Malik et al., 2008). It has also been shown to control production of cytokine TNF-alpha stimulated by LPS in normal cells (Orlicek et al., 1999; Sarang et al., 2011; Okenwa et al., 2013). Thus, Src affects both innate and adaptive immune responses in normal cells. Consequently, the elevated and dysregulated Src activity may play a key role in initiation of the invasive cell phenotype both in infiltrating immune cells and precancerous cells. However, its most robust effects are from the production of cytokines and alterations of cell movement/attachment. In fact, the Src family kinase signaling network is the go between that relay crucial cytokine signals from inflammatory cells to cancer cells, and conversely, within the tumor microenvironment (**Figure 1**). The Src-mediated stimulatory effects on malignant cell proliferation and inhibitory effect on cell death, leads to the accumulation of malignant cells and thus increases the total mass of the tumor. Consequently, this elevates the production of pro-inflammatory cytokines, including chemokines, by the tumor which further leads to the recruitment and activation of additional leukocytes that results in a cycle (as depicted in the **Figure 1**) leading to cancer development and progression.

CONCLUDING REMARKS

The Src kinases-dependent signaling that link immune system with normal tissue plays a vital role in regulating and coordinating immune defense responses. The cross talk between Src kinase pathways in immune cells and Src kinase-mediated pathways in target tissue cells is mediated via cytokine signals elicited by these cells. These Src-dependent signaling pathways, when hyper-activated and dysregulated, can lead to the development of chronic inflammation that predispose to cancer. Src activation both in infiltrating immune cells and cancer precursor lesions is driven by pro-inflammatory cytokines within tumor-promoting microenvironment. This leads to a vicious cycle in which Src activation increases cytokine production that again induces Src activation, leading to invasive inflammatory cell and cancer cell phenotypes. Thus, elucidating the Src-dependent cross talk signaling mechanisms that link inflammatory cells with cancer cells, may facilitate the design of new pharmacological agents for

the concurrent treatment of tumor-promoting inflammation and cancer. Pancreatic cancer, because of its robust cytokine mediated interactions between the tumor cells and tumor microenvironment, can be used in designing new agents for the inhibition of the linkage between inflammation and cancer.

REFERENCES

Abram, C. L., and Lowell, C. A. (2008). The diverse functions of Src family kinases in macrophages. *Front. Biosci.* 13, 4426–4450. doi: 10.2741/3015

Aligayer, H., Boyd, D. D., Heiss, M. M., Abdalla, E. K., Curley, S. A., and Gallick, G. E. (2002). Activation of Src kinase in primary colorectal carcinoma: an indicator of poor clinical prognosis. *Cancer* 94, 344–351. doi: 10.1002/cncr.10221

Balkwill, F. (2004). The significance of cancer cell expression of the chemokine receptor CXCR4. *Semin. Cancer Biol.* 14, 171–179. doi: 10.1016/j.semcancer.2003.10.003

Balkwill, F., Charles, K. A., and Mantovani, A. (2005). Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell* 7, 211–217. doi: 10.1016/j.ccr.2005.02.013

Bernstein, C. N., Blanchard, J. F., Kliewer, E., and Wajda, A. (2001). Cancer risk in patients with inflammatory bowel disease: a population-based study. *Cancer* 91, 854–862. doi: 10.1002/1097-0142(20010215)91:4<854::AID-CNCR1073>3.0.CO;2-Z

Brugge, J. S., and Erikson, R. L. (1997). Identification of a transformation-specific antigen induced by an avian sarcome virus. *Nature* 269, 346–348. doi: 10.1038/269346a0

Chen, Y. Y., Malik, M., Tomkowicz, B. E., Collman, R. G., and Ptasznik, A. (2008). BCR-ABL1 alters SDF-1alpha-mediated adhesive responses through the beta2 integrin LFA-1 in leukemia cells. *Blood* 111, 5182–5186. doi: 10.1182/blood-2007-10-117705

Cheung, R., Malik, M., Ravyn, V., Tomkowicz, B., Ptasznik, A., and Collman, R. G. (2009). An arrestin-dependent multi-kinase signaling complex mediates MIP-1beta/CCL4 signaling and chemotaxis of primary human macrophages. *J. Leukoc. Biol.* 86, 833–845. doi: 10.1189/jlb.0908551

Cheung, R., Ravyn, V., Wang, L., Ptasznik, A., and Collman, R. G. (2008). Signaling mechanism of HIV-1 gp120 and virion-induced IL-1beta release in primary human macrophages. *J. Immunol.* 180, 6675–6684.

Coppola, D. (2000). Molecular prognostic markers in pancreatic cancer. *Cancer Control* 7, 421–427.

Coussens, L. M., and Werb, Z. (2002). Inflammation and cancer. *Nature* 420, 860–867. doi: 10.1038/nature01322

Dítě, P., Hermanová, M., Trna, J., Novotný, I., Růžièka, M., Liberda, M., et al. (2012). The role of chronic inflammation: chronic pancreatitis as a risk factor of pancreatic cancer. *Dig. Dis.* 30, 277–283. doi: 10.1159/000336991

Duell, E. J., Casella, D. P., Burk, R. D., Kelsey, K. T., and Holly, E. A. (2006). Inflammation, genetic polymorphisms in proinflammatory genes TNF-A, RANTES, and CCR5, and risk of pancreatic adenocarcinoma. *Cancer Epidemiol. Biomarkers Prev.* 15, 726–731. doi: 10.1158/1055-9965.EPI-05-0797

Eaden, J. A., Abrams, K. R., and Mayberry, J. F. (2001). The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* 48, 526–535. doi: 10.1136/gut.48.4.526

Erkan, M., Hausmann, S., Michalski, C. W., Fingerle, A. A., Dobritz, M., Kleeff, J., et al. (2012). The role of stroma in pancreatic cancer: diagnostic and therapeutic implications. *Nat. Rev. Gastroenterol. Hepatol.* 9, 454–467. doi: 10.1038/nrgastro.2012.115

Feig, C., Gopinathan, A., Neesse, A., Chan, D. S., Cook, N., and Tuveson, D. A. (2012). The pancreas cancer microenvironment. *Clin. Cancer Res.* 18, 4266–4276. doi: 10.1158/1078-0432.CCR-11-3114

Fu, Y., Zagozdzon, R., Avraham, R., and Avraham, H. K. (2006). CHK negatively regulates Lyn kinase and suppresses pancreatic cancer invasion. *Int. J. Oncol.* 29, 1453–1458.

Gao, Z., Wang, X., Wu, K., Zhao, Y., and Hu, G. (2010). Pancreatic stellate cells increase the invasion of human pancreatic cancer cells through the stromal cell-derived factor-1/CXCR4 axis. *Pancreatology* 10, 186–193. doi: 10.1159/000236012

Go, V. L., Gukovskaya, A., and Pandol, S. J. (2005). Alcohol and pancreatic cancer. *Alcohol* 35, 205–211. doi: 10.1016/j.alcohol.2005.03.010

Goecke, H., Forssmann, U., Uguccioni, M., Friess, H., Conejo-Garcia, J. R., Zimmermann, A., et al. (2000). Macrophages infiltrating the tissue in chronic pancreatitis express the chemokine receptor CCR5. *Surgery* 128, 806–814. doi: 10.1067/msy.2000.108613

Guerra, C., Schuhmacher, A. J., Cañamero, M., Grippo, P. J., Verdaguer, L., Pérez-Gallego, L., et al. (2007). Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. *Cancer Cell* 11, 291–302. doi: 10.1016/j.ccr.2007.01.012

Hallek, M., Neumann, C., Schäffer, M., Danhauser-Riedl, S., von Bubnoff, N., de Vos, G., et al. (1997). Signal transduction of interleukin-6 involves tyrosine phosphorylation of multiple cytosolic proteins and activation of Src-family kinases Fyn, Hck, and Lyn in multiplemyeloma cell lines. *Exp. Hematol.* 25, 1367–1377.

Howes, N., Lerch, M. M., Greenhalf, W., Stocken, D. D., Ellis, I., Simon, P., et al. (2004). Clinical and genetic characteristics of hereditary pancreatitis in Europe. *Clin. Gastroenterol. Hepatol.* 2, 252–261. doi: 10.1016/S1542-3565(04)00013-8

Hunter, T., and Sefton, B. M. (1980). Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. *Proc. Natl. Acad. Sci. U.S.A.* 77, 1311–1315. doi: 10.1073/pnas.77.3.1311

Inngjerdingen, M., Torgersen, K. M., and Maghazachi, A. A. (2002). Lck is required for stromal cell-derived factor 1 alpha (CXCL12)-induced lymphoid cell chemotaxis. *Blood.* 15, 99, 4318–4325. doi: 10.1182/blood.V99.12.4318

Ito, Y., Lugea, A., Pandol, S. J., and McCuskey, R. S. (2007). Substance P mediates cerulein-induced pancreatic microcirculatory dysfunction in mice. *Pancreas* 34, 138–143. doi: 10.1097/01.mpa.0000246663.30751.24

Itzkowitz, S. H., and Yio, X. (2004). Inflammation and cancer IV. colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 287, G7–G17. doi: 10.1152/ajpgi.00079.2004

Kayali, A. G., Van Gunst, K., Campbell, I. L., Stotland, A., Kritzik, M., Liu, G., et al. (2003). The stromal cell-derived factor-1alpha/CXCR4 ligand-receptor axis is critical for progenitor survival and migration in the pancreas. *J. Cell Biol.* 163, 859–869. doi: 10.1083/jcb.200304153

Kulbe, H., Levinson, N. R., Balkwill, F., and Wilson, J. L. (2004). The chemokine network in cancer–much more than directing cell movement. *Int. J. Dev. Biol.* 48, 489–496. doi: 10.1387/ijdb.041814hk

Lin, M. T., Lin, B. R., Chang, C. C., Chu, C. Y., Su, H. J., Chen, S. T., et al. (2007). IL-6 induces AGS gastric cancer cell invasion via activation of the c-Src/RhoA/ROCK signaling pathway. *Int. J. Cancer.* 120, 2600–2608. doi: 10.1002/ijc.22599

Liou, G. Y., Döppler, H., Necela, B., Krishna, M., Crawford, H. C., Raimondo, M., et al. (2013). Macrophage-secreted cytokines drive pancreatic acinar-to-ductal metaplasia through NF-κB and MMPs. *J. Cell Biol.* 202, 563–577. doi: 10.1083/jcb.201301001

Lowenfels, A. B., Maisonneuve, P., Whitcomb, D. C., Lerch, M. M., and DiMagno, E. P. (2001). Cigarette smoking as a risk factor for pancreatic cancer in patients with hereditary pancreatitis. *JAMA* 286, 169–170. doi: 10.1001/jama.286.2.169

Lutz, M. P., Esser, I. B., Flossmann-Kast, B. B., Vogelmann, R., Lührs, H., Friess, H., et al. (1998). Overexpression and activation of the tyrosine kinase Src in human pancreatic carcinoma. *Biochem. Biophys. Res. Commun.* 243, 503–508. doi: 10.1006/bbrc.1997.8043

Maisonneuve, P., Lowenfels, A. B., Müllhaupt, B., Cavallini, G., Lankisch, P. G., Andersen, J. R., et al. (2005). Cigarette smoking accelerates progression of alcoholic chronic pancreatitis. *Gut* 54, 510–514. doi: 10.1136/gut.2004.039263

Malik, M., Chen, Y. Y., Kienzle, M. F., Tomkowicz, B. E., Collman, R. G., and Ptasznik, A. (2008). Monocyte migration and LFA-1-mediated attachment to brain microvascular endothelia is regulated by SDF-1 alpha through Lyn kinase. *J. Immunol.* 181, 4632–4637.

Mantovani, A., Allavena, P., Sica, A., and Balkwill, F. (2008). Cancer-related inflammation. *Nature* 454, 436–444. doi: 10.1038/nature07205

Momi, N., Kaur, S., Ponnusamy, M. P., Kumar, S., Wittel, U. A., and Batra, S. K. (2012). Interplay between smoking-induced genotoxicity and altered signaling in pancreatic carcinogenesis. *Carcinogenesis* 33, 1617–1628. doi: 10.1093/carcin/bgs186

Nakata, Y., Tomkowicz, B., Gewirtz, A. M., and Ptasznik, A. (2006). Integrin inhibition through Lyn-dependent cross talk from CXCR4 chemokine receptors in normal human CD34+ marrow cells. *Blood* 107, 4234–4239. doi: 10.1182/blood-2005-08-3343

Nordskog, B. K., Blixt, A. D., Morgan, W. T., Fields, W. R., and Hellmann, G. M. (2003). Matrix-degrading and pro-inflammatory changes in human vascular endothelial cells exposed to cigarette smoke condensate. *Cardiovasc. Toxicol.* 3, 101–117. doi: 10.1385/CT:3:2:101

Okenwa, C., Kumar, A., Rego, D., Konarski, Y., Nilchi, L., Wright, K., et al. (2013). SHP-1-Pyk2-Src protein complex and p38 MAPK pathways independently

regulate IL-10 production in lipopolysaccharide-stimulated macrophages. *J. Immunol.* 191, 2589–2603. doi: 10.4049/jimmunol.1300466

Orlicek, S. L., Hanke, J. H., and English, B. K. (1999). The src family-selective tyrosine kinase inhibitor PP1 blocks LPS and IFN-gamma-mediated TNF and iNOS production in murine macrophages. *Shock* 12, 350–354. doi: 10.1097/00024382-199911000-00004

Pannala, R., Basu, A., Petersen, G. M., and Chari, S. T. (2009). New-onset diabetes: a potential clue to the early diagnosis of pancreatic cancer. *Lancet Oncol.* 10, 88–95. doi: 10.1016/S1470-2045(08)70337-1

Peruzzi, B., Cappariello, A., Del Fattore, A., Rucci, N., De Benedetti, F., and Teti, A. (2012). c-Src and IL-6 inhibit osteoblast differentiation and integrate IGFBP5 signalling. *Nat. Commun.* 3, 630. doi: 10.1038/ncomms1651

Podar, K., Mostoslavsky, G., Sattler, M., Tai, Y. T., Hayashi, T., Catley, L. P., et al. (2004). Critical role for hematopoietic cell kinase (Hck)-mediated phosphorylation of Gab1 and Gab2 docking proteins in interleukin 6-induced proliferation and survival of multiple myeloma cells. *J. Biol. Chem.* 279, 21658–21665. doi: 10.1074/jbc.M305783200

Ptasznik, A., Prossnitz, E. R., Yoshikawa, D., Smrcka, A., Traynor-Kaplan, A. E., and Bokoch, G. M. (1996). A tyrosine kinase signaling pathway accounts for the majority of phosphatidylinositol 3,4,5-trisphosphate formation in chemoattractant-stimulated human neutrophils. *J. Biol. Chem.* 271, 25204–25207. doi: 10.1074/jbc.271.41.25204

Ptasznik, A., Traynor-Kaplan, A., and Bokoch, G. M. (1995). G protein-coupled chemoattractant receptors regulate Lyn tyrosine kinase.Shc adapter protein signaling complexes. *J. Biol. Chem.* 270, 19969–19973. doi: 10.1074/jbc.270.34.19969

Ramnath, R. D., and Bhatia, M. (2006). Substance P treatment stimulates chemokine synthesis in pancreatic acinar cells via the activation of NF-kappaB. *Am. J. Physiol. Gastrointest. Liver Physiol.* 291, G1113–G1119. doi: 10.1152/ajpgi.00177.2006

Ramnath, R. D., Sun, J., and Bhatia, M. (2009). Involvement of SRC family kinases in substance P-induced chemokine production in mouse pancreatic acinar cells and its significance in acute pancreatitis. *J. Pharmacol. Exp. Ther.* 329, 418–428. doi: 10.1124/jpet.108.148684

Rebours, V., Boutron-Ruault, M. C., Schnee, M., Férec, C., Le Maréchal, C., Hentic, O., et al. (2009). The natural history of hereditary pancreatitis: a national series. *Gut* 58, 97–103. doi: 10.1136/gut.2008.149179

Rous, P. (1911). A sarcoma of the fowl transmissible by an aget seperable from the tumor cells. *J Exp. Med.* 13, 397–411. doi: 10.1084/jem.13.4.397

Sarang, Z., Koroskenyi, K., Pallai, A., Duro, E., Melino, G., Griffin, M., et al. (2011). Translutaminase 2 null macrophages respond to lipopolysaccharide stimulation by elevated proinflammatory ctyokine production due to an enhanced αvβ3 integrin-induced Src tyrosine kinase sinaling. *Immunol. Lett.* 138, 71–78. doi: 10.1016/j.imlet.2011.03.004

Satoh, A., Gukovskaya, A. S., Edderkaoui, M., Daghighian, M. S., Reeve, J. R. Jr, Shimosegawa, T., et al. (2005). Tumor necrosis factor-alpha mediates pancreatitis responses in acinar cells via protein kinase C and proline-rich tyrosine kinase 2. *Gastroenterology* 129, 639–651. doi: 10.1016/j.gastro.2005.05.005

Sell, H., Habich, C., and Eckel, J. (2012). Adaptive immunity in obesity and insulin resistance. *Nat. Rev. Endocrinol.* 8, 709–716. doi: 10.1038/nrendo.2012.114

Shi, C., Andersson, R., Zhao, X., and Wang, X. (2005). Potential role of reactive oxygen species in pancreatitis-associated multiple organ dysfunction. *Pancreatology* 5, 492–500. doi: 10.1159/000087063

Shields, D. J., Murphy, E. A., Desgrosellier, J. S., Mielgo, A., Lau, S. K., Barnes, L. A., et al. (2011). Oncogenic Ras/Src cooperativity in pancreatic neoplasia. *Oncogene* 30, 2123–2134. doi: 10.1038/onc.2010.589

Shoelson, S. E., Herrero, L., and Naaz, A. (2007). Obesity, inflammation, and insulin resistance. *Gastroenterology* 132, 2169–2180. doi: 10.1053/j.gastro.2007.03.059

Smolinska, M. J., Page, T. H., Urbaniak, A. M., Mutch, B. E., and Horwood, N. J. (2011). Hck tyrosine kinase regulates TLR4-induced TNF and IL-6 production via AP-1. *J. Immunol.* 187, 6043–6051. doi: 10.4049/jimmunol.1100967

Spaeth, E., Klopp, A., Dembinski, J., Andreeff, M., and Marini, F. (2008). Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells. *Gene Ther.* 15, 730–738. doi: 10.1038/gt.2008.39

Staley, C. A., Parikh, N. U., and Gallick, G. E. (1997). Decreased tumorigenicity of a human colon adenocarcinoma cell line by an antisense expression vector specific for c-Src. *Cell Growth Differ.* 8, 269–274.

Stehelin, D., Varmus, H. E., Bishop, J. M., and Vogt, P. K. (1976). DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* 260, 170–173. doi: 10.1038/260170a0

Summy, J. M., Trevino, J. G., Baker, C. H., and Gallick, G. E. (2005). c-Src regulates constitutive and EGF-mediated VEGF expression in pancreatic tumor cells through activation of phosphatidyl inositol-3 kinase and p38 MAPK. *Pancreas* 31, 263–274. doi: 10.1097/01.mpa.0000178280.50534.0c

Takatomo, K., Ryo, H., Yoshiharu, M., Jun, I., Shoichiro, T., Sanae, N., et al. (2000). Expression of stromal cell-derived factor 1 and CXCR4 ligand receptor system in pancreatic cancer: a possible role for tumor progression. *Clin. Cancer Res.* 6, 3530–3535.

Talamini, G., Bassi, C., Falconi, M., Sartori, N., Salvia, R., Rigo, L., et al. (1999). Alcohol and smoking as risk factors in chronic pancreatitis and pancreatic cancer. *Dig. Dis. Sci.* 44, 1303–1311. doi: 10.1023/A:1026670911955

Terzić, J., Grivennikov, S., Karin, E., and Karin, M. (2010). Inflammation and colon cancer. *Gastroenterology* 138, 2101–2114. doi: 10.1053/j.gastro.2010.01.058

Tomkowicz, B., Lee, C., Ravyn, V., Cheung, R., Ptasznik, A., and Collman, R. G. (2006). The Src kinase Lyn is required for CCR5 signaling in response to MIP-1beta and R5 HIV-1 gp120 in human macrophages. *Blood* 108, 1145–1150. doi: 10.1182/blood-2005-12-012815

Trevino, J. G., Summy, J. M., Lesslie, D. P., Parikh, N. U., Hong, D. S., Lee, F. Y., et al. (2006). Inhibition of SRC expression and activity inhibits tumor progression and metastasis of human pancreatic adenocarcinoma cells in an orthotopic nude mouse model. *Am. J. Pathol.* 168, 962–972. doi: 10.2353/ajpath.2006.050570

Trinchieri, G. (2012). Cancer and inflammation: an old intuition with rapidly evolving new concepts. *Annu. Rev. Immunol.* 30, 677–706. doi: 10.1146/annurev-immunol-020711-075008

Ullman, T. A., and Itzkowitz, S. H. (2011). Intestinal inflammation and cancer. *Gastroenterology* 140, 1807–1816. doi: 10.1053/j.gastro.2011.01.057

Ungefroren, H., Sebens, S., Groth, S., Gieseler, F., and Fändrich, F. (2011). Differential roles of Src in transforming growth factor-ß regulation of growth arrest, epithelial-to-mesenchymal transition and cell migration in pancreatic ductal adenocarcinoma cells. *Int. J. Oncol.* 38, 797–805. doi: 10.3892/ijo.2011.897

Wiśniewska, E., Dylik, A., Kulza, M., Florek, E., Piekoszewski, W., Señczuk-Przybylowska, M., et al. (2013). Exposure to ethanol and tobacco smoke in relation to level of PCNA antigen expression in pancreatic and hepatic rat cells. *Pharmacol. Rep.* 65, 914–926.

Yadav, D., Timmons, L., Benson, J. T., Dierkhising, R. A., and Chari, S. T. (2011). Incidence, prevalence, and survival of chronic pancreatitis: a population-based study. *Am. J. Gastroenterol.* 106, 2192–2199. doi: 10.1038/ajg.2011.328

Yokoi, K., Hawke, D., Oborn, C. J., Jang, J. Y., Nishioka, Y., Fan, D., et al. (2011). Identification and validation of SRC and phospho-SRC family proteins in circulating mononuclear cells as novel biomarkers for pancreatic cancer. *Transl. Oncol.* 4, 83–91.

Zhang, Y., Yan, W., Collin, M., Bednar, F., Rakshit, S., Zetter, B., et al. (2013). Interleukin-6 is required for pancreatic cancer progression by promoting MAPK signaling activation and oxidative stress resistance. *Cancer Res.* 73, 6359–6374. doi: 10.1158/0008-5472.CAN-13-1558-T

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Diabetes, pancreatic cancer, and metformin therapy

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Pancreatic cancer carries a poor prognosis as most patients present with advanced disease and preferred chemotherapy regimens offer only modest effects on survival. Risk factors include smoking, obesity, heavy alcohol, and chronic pancreatitis. Pancreatic cancer has a complex relationship with diabetes, as diabetes can be both a risk factor for pancreatic cancer and a result of pancreatic cancer. Insulin, insulin-like growth factor-1 (IGF-1), and certain hormones play an important role in promoting neoplasia in diabetics. Metformin appears to reduce risk for pancreatic cancer and improve survival in diabetics with pancreatic cancer primarily by decreasing insulin/IGF signaling, disrupting mitochondrial respiration, and inhibiting the mammalian target of rapamycin (mTOR) pathway. Other potential anti-tumorigenic effects of metformin include the ability to downregulate specificity protein transcription factors and associated genes, alter microRNAs, decrease cancer stem cell proliferation, and reduce DNA damage and inflammation. Here, we review the most recent knowledge on risk factors and treatment of pancreatic cancer and the relationship between diabetes, pancreatic cancer, and metformin as a potential therapy.

Keywords: metformin, pancreatic cancer, diabetes, mTOR, AMPK, insulin, IGF-1

INTRODUCTION

EPIDEMIOLOGY

Pancreatic cancer is the twelfth most common cancer in the US but represents the fourth leading cause of cancer death in both men and women (Howlader et al., 2014). The prognosis is extremely poor with a 5-year survival rate of only 6.7% as pancreatic cancer is usually asymptomatic in the early stages of disease and most cases are diagnosed relatively late (Howlader et al., 2014). Treatment and advances in early detection are of crucial importance.

RISK FACTORS

Smoking is a well-known risk factor for pancreatic cancer and is estimated to contribute to 20–30% of all cases of pancreatic cancer (Iodice et al., 2008). A meta-analysis including 82 studies showed that smokers have a 75% increased risk of pancreatic cancer compared to non-smokers and that the increased risk persists at least 10 years after smoking cessation (Iodice et al., 2008). A meta-analysis from 2012 suggested that risk of pancreatic cancer initially increases with cigarette amount but levels off at higher intensities of cigarette smoking, indicating that quantity has some role in determining risk (Zou et al., 2014).

A meta-analysis from 2010 found that individuals with chronic pancreatitis had a 13.3-fold higher risk of developing pancreatic cancer and a 5.8-fold increased risk after excluding cases diagnosed within 2 years of cancer diagnosis (Raimondi et al., 2010). However, despite this strong relationship, only about 5% of patients with chronic pancreatitis will actually develop pancreatic cancer in a 20 year period (Raimondi et al., 2010). Hereditary pancreatitis is a rare autosomal dominant disease due to a mutation in the gene encoding trypsinogen in which patients develop chronic pancreatitis at a young age (under 30). The cumulative risk of developing pancreatic cancer is 40% by age 70 (Lowenfels et al., 1997).

A recent meta-analysis which evaluated risk based on different intensities of alcohol consumption provided evidence that heavy alcohol consumption (defined as >3 drinks per day) increases risk for pancreatic cancer by 22%, independent of tobacco use, whereas moderate alcohol consumption did not carry an increased risk (Tramacere et al., 2010).

Another important risk factor is body mass index, which has been associated with an elevated risk of pancreatic cancer in several studies (Larsson et al., 2007; Arslan et al., 2010; Jiao et al., 2010; Genkinger et al., 2011). In a pooled analysis of 14 cohort studies, risk for pancreatic cancer was 47% greater for individuals with BMI>30. Higher waist to hip ratio was also found to be positively associated with pancreatic cancer, suggesting that central obesity in particular may confer risk (Genkinger et al., 2011). Lastly, a recent meta-analysis involving more than 3-million individuals identified tobacco use, obesity, and heavy alcohol, among a host of other factors, as the 3 most important risk factors for pancreatic cancer while vegetable and fruit consumption offered the greatest protection against pancreatic diseases (Alsamarrai et al., 2014).

DIABETES AND PANCREATIC CANCER

DIABETES MELLITUS AS A RISK FACTOR FOR PANCREATIC CANCER

Diabetes mellitus (DM) or glucose intolerance may be present in up to 75% of patients with pancreatic cancer, a figure much higher than in other cancer types in whom the prevalence is no more than 30% (Permert et al., 1993b; Aggarwal et al., 2013). The relationship between DM and pancreatic cancer is bi-directional, as studies point to both increased risk of pancreatic cancer in those with long-term diabetes, as well as greater incidence of diabetes in sync with the development of pancreatic cancer (Li, 2012). Many studies evaluating DM as a risk factor have focused on patients with DM diagnosed several years prior to the time of pancreatic cancer diagnosis in order to exclude cases of DM that are a result of pancreatic cancer. This follows from the assumption that pancreatic cancer is rapidly fatal and therefore DM diagnosed several years prior to cancer diagnosis would unlikely be from the cancer (Li, 2012).

In a recent pooled analysis, after adjusting for age, gender, prior involved study, alcohol use, smoking, BMI, and family history of pancreatic cancer, patients with DM had a 40% increased risk of pancreatic cancer (Elena et al., 2013). This analysis excluded cases developing within 2 years, providing evidence for DM as a risk factor rather than just a result of pancreatic cancer. A meta-analysis of 20 studies conducted in 1995 showed that patients with DM for 5 or more years had a two-fold increased risk (Everhart and Wright, 1995). In another 2005 meta-analysis which included 36 studies, individuals with DM for >5 years had a 50% increased risk of pancreatic cancer (Huxley et al., 2005). Diabetes is characterized by hyperglycemia and insulin resistance, which can both contribute to tumor formation. In a prospective nested case-control study, higher levels of proinsulin, a marker of peripheral insulin resistance, was found to be associated with pancreatic cancer, independent of hemoglobin A1c, suggesting that insulin resistance may be a stronger carcinogenic contributor than hyperglycemia (Wolpin et al., 2013). This finding was supported by the fact that lower levels of adiponectin, which functions to enhance insulin sensitivity, was associated with increased pancreatic cancer risk (Bao et al., 2013).

MECHANISMS OF RISK

DM and associated obesity may lead to increased risk for cancer through several mechanisms (**Figure 1**). Individuals with DM2 often have peripheral insulin resistance and develop compensatory hyperinsulinemia (Godsland, 2009). Insulin is a growth promoting hormone and acts by increasing cell proliferation, decreasing apoptosis, increasing glucose utilization, and enhancing responsiveness to other growth factors; all of these actions are important for cancer progression (Ding et al., 2000; Draznin, 2011). Insulin also decreases insulin-like growth factor (IGF) binding protein production thereby increasing the amount of bioavailable IGF-1 (Powell et al., 1991). IGF-1 is a more potent mitogen than insulin and promotes pancreatic cancer cell proliferation and invasion while inhibiting the tumor suppressor phosphatase and tensin homolog (PTEN, Ma et al., 2010). IGF-1 receptor binding leads to activation of the PI3K/Akt and the Raf/MAPK pathways, which promote cell proliferation and inhibit apoptosis (Pollak et al., 2004).

DM and obesity are associated with other hormonal alterations that may promote neoplasia as well. Adiponectin is a hormone that has been shown to limit angiogenesis, promote apoptosis, and decrease inflammation. DM is associated with low circulating levels of adiponectin, an effect that may promote carcinogenesis (Bao et al., 2011). Leptin is a mitogenic hormone which is increased in obesity. It promotes angiogenesis, inhibits apoptosis, and activates the PI3K/Akt and STAT pathways, which promote cell growth and survival (Bao et al., 2011).

PANCREATIC CANCER-ASSOCIATED DIABETES MELLITUS

Several studies have suggested that DM is not just a risk factor, but also a consequence of pancreatic cancer. One study looked at the temporal association between DM and pancreatic cancer and found a marked increase in cases of DM starting at 36 months prior to diagnosis, with cases continuing to increase up to cancer diagnosis, suggesting that the cancer itself was likely the etiologic factor (Chari et al., 2008). A meta-analysis of 35 cohort studies showed DM was associated with a 94% increased risk of pancreatic cancer. Interestingly, risk decreased with duration of diabetes (5.38 for <1 year, 1.95 for 1–4 years, 1.49 for 5–9 years, 1.47 for ≥10 years) providing support that much of diabetes in pancreatic cancer patients is caused by the cancer itself (Ben et al., 2011).

Pancreatic cancer-induced DM is thought to be a paraneoplastic phenomenon involving release of products from the tumor rather than a result of destruction of the pancreas due to malignant infiltration (Pannala et al., 2008; Aggarwal et al., 2012). This hypothesis is supported by a study which showed that the prevalence of DM in patients with pancreatic cancer was not related to tumor stage or location, as would be expected if the DM were a result of tumor infiltration (Pannala et al., 2008). Furthermore, several studies have shown resolution of DM after tumor resection in individuals with pancreatic cancer (Permert et al., 1993a; Fogar et al., 1994; Pannala et al., 2008; White et al., 2011). A study by Aggarwal et al. (2012) showed that the hormone adrenomedullin is upregulated at the mRNA and protein level in pancreata from patients with pancreatic cancer. *In vitro* studies indicate that adrenomedullin impairs glucose-stimulated insulin secretion in β-cells, and adrenomedullin overexpression in mouse pancreatic cancer tissues is associated with increased glucose intolerance suggesting that adrenomedullin is an important mediator of cancer-induced DM (Aggarwal et al., 2012). Other potential mediators found upregulated in pancreatic cancer include S-100A8 N-terminal peptide, which was shown to alter glucose catabolism *in vitro* (Basso et al., 2006), and islet amyloid polypeptide which causes insulin resistance (Permert et al., 1994). One review suggested that pancreatic cancer-associated insulin resistance may be mediated by release of cytokines, adipokines, and non-esterified fatty acids from adipose tissue inflammation (Sah et al., 2013).

STANDARDS OF THERAPY

Surgical resection is the only cure for pancreatic adenocarcinoma although 80% of patients have unresectable disease at the time of presentation (Campen et al., 2011). Pancreaticoduodenectomy and distal pancreatectomy with or without splenectomy are

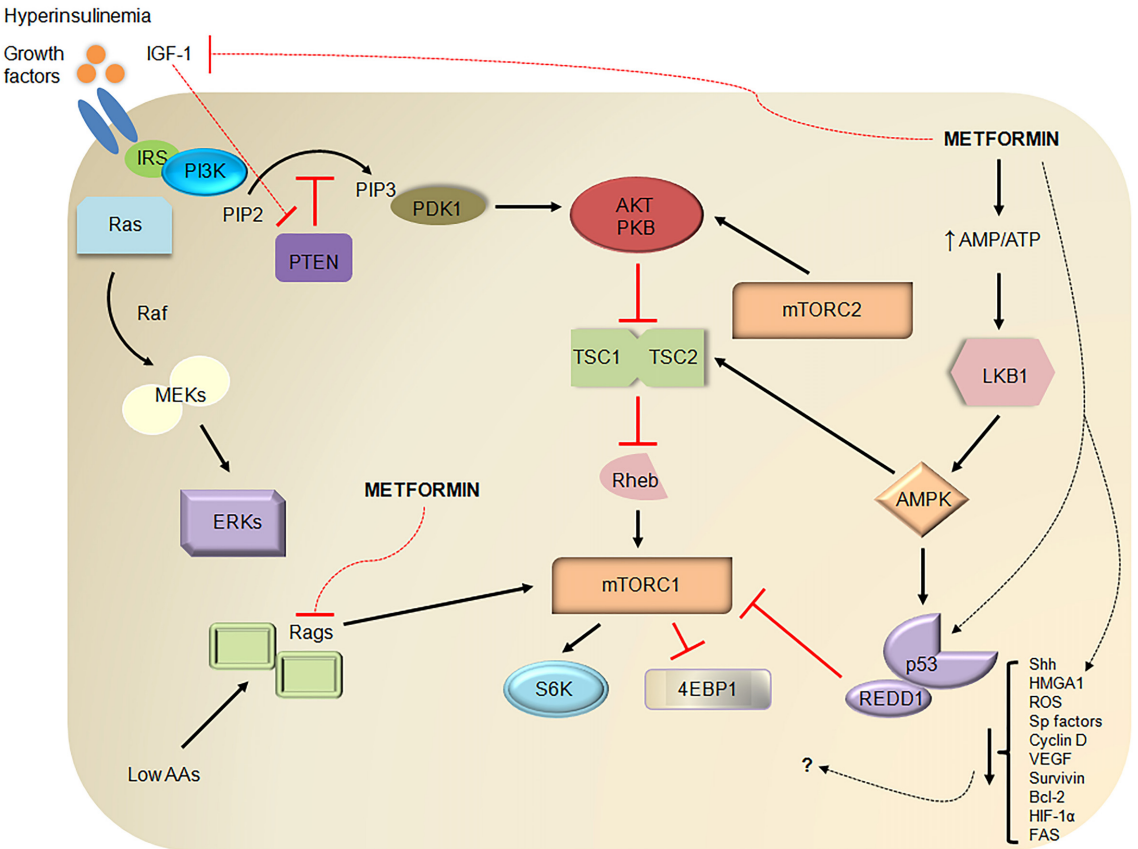

FIGURE 1 | Metformin demonstrates antitumor properties through several pathways. Diabetes mellitus type 2 (DM2) is often characterized by insulin resistance, hyperglycemia, and compensatory hyperinsulinemia. Insulin increases IGF-1 levels by displacing IGF-1 from common binding proteins, stimulating hepatic growth hormone signaling, and decreasing IGF-binding protein production. Like other growth factors, insulin and IGF-1, upon binding to their respective growth factor receptors, can promote pancreatic cancer development through MAPK/ERK or Ras/Raf/MEK/ERK signaling and PI3K/Akt/mTOR signaling. For example, IGF-1 binding to the IGF-1 receptor recruits and activates PI3K via adaptor proteins such as IRS, converts PIP2 to PIP3 (a process that is inhibited by PTEN), activates Akt/PKB through PDK1- and mTORC2-mediated phosphorylation, and inhibits formation of TSC1-TSC2 thereby releasing its inhibition on Rheb (an mTORC1 activator). Activated mTORC1 is a key regulator of cell growth, metabolism, survival, and proliferation through downstream mediators such as 4EBP1 and S6K. Metformin has been known to uncouple the electron transport chain at complex I leading to decreased ATP production and activation of LKB1 and AMPK. AMPK is a stabilizer of TSC1-TSC2 and activator of p53, a tumor suppressor. Independent of AMPK, metformin increases p53-dependent expression of REDD1, an inhibitor of mTORC1, and inhibits mTORC1 by inhibiting Rags. Metformin also reduces hyperinsulinemia and IGF-1 levels and offers further antitumor effects by reducing levels of Shh, HMGA1, ROS, Sp transcription factors, Sp-related oncogenic proteins (cyclin D1, VEGF, survivin, Bcl-2, FAS), and HIF-1α through relatively unknown mechanisms. Dashed lines represent putative or suggested pathways while red lines represent inhibitory pathways. IGF-1, insulin-like growth factor-1; IRS, insulin receptor substrate; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PTEN, phosphatase and tensin homolog; MAPK, mitogen-activated protein kinase; MEKs, MAPK kinases; ERKs, extracellular signal regulated kinases; PDK1, phosphoinositide-dependent kinase 1; PKB, protein kinase B or Akt; TSC1, tuberous sclerosis complex 1; Rheb, Ras homolog enriched in brain; mTOR, mammalian target of rapamycin; 4EBP1, eukaryotic initiation factor 4E binding protein 1; S6K, S6 kinase; AMP, adenosine monophosphate; LKB1, liver kinase B1; AMPK, AMP-activated protein kinase; AAs, amino acids; Rags, Rag GTPases; REDD1, regulated in development and DNA damage responses 1; Shh, Sonic hedgehog; HMGA1, high mobility group AT-hook 1; ROS, reactive oxygen species; Sp, specificity protein; VEGF, vascular endothelial growth factor; Bcl-2, B-cell lymphoma-2; HIF-1α, hypoxia-inducible factor-1 alpha; FAS, fatty acid synthase.

performed in patients with tumors in the head and body/tail of the pancreas, respectively (De La Cruz et al., 2014). Given that median survival is poor even in patients that undergo surgical resection, most patients are offered adjuvant chemotherapy with gemcitabine or fluorouracil ± chemoradiation (De La Cruz et al., 2014).

Patients with locally advanced, unresectable, or borderline resectable disease may receive neoadjuvant chemotherapy ± radiotherapy in an attempt to downstage the disease (Seufferlein et al., 2012). For metastatic disease, treatment options address palliation and improved survival. Gemcitabine has been used to treat metastatic pancreatic adenocarcinoma since 1997 when it produced a one year survival benefit compared to 5-FU and remains the chemotherapy of choice in patients with poor functional status and advanced disease (Burris et al., 1997; Ghosn et al., 2014). In 2011, FOLFIRINOX (fluorouracil, leucovorin, irinotecan, and oxaliplatin) produced a significant survival benefit compared to patients treated with gemcitabine monotherapy

(Conroy et al., 2011). Gemcitabine plus nab-paclitaxel produces less adverse effects than FOLFIRINOX and offers the second best overall survival in those with good functional status (Ghosn et al., 2014). Gemcitabine plus erlotinib shows improved survival vs. monotherapy, but survival benefit remains minimal (Ghosn et al., 2014). Despite the fact that chemotherapy confers some survival benefit, this benefit is modest and exploration of new therapies is essential.

METFORMIN AND PANCREATIC CANCER

METFORMIN REDUCES RISK FOR PANCREATIC CANCER

Metformin is one of the most widely prescribed oral agents for the treatment of DM2. Evans et al. (2005) were among the first to suggest metformin as an anti-cancer therapy. However, as early as 2001, one study demonstrated that metformin prevented pancreatic cancer development in hamsters treated with a pancreatic carcinogen (Schneider et al., 2001). A study in 2009 with 62,809 patients compared cancer risk in patients on different kinds of diabetic therapies and found that insulin therapy increased the risk of pancreatic cancer compared to metformin therapy (Currie et al., 2009). Furthermore, compared to patients that were on insulin alone, patients with metformin added to insulin had a significantly reduced risk of developing solid tumors (Currie et al., 2009). A recent meta-analysis showed that patients with diabetes who were taking metformin had a significantly reduced risk of pancreatic cancer (Wang et al., 2014). Consistent with this finding, an observational study of 302 diabetic patients with pancreatic cancer found that metformin users showed significantly increased survival times as compared to non-users (15.2 months vs. 11.1 months, Sadeghi et al., 2012).

MECHANISMS OF ACTION

Although there is much to be learned about metformin's mechanism of action in cancer (**Figure 1**), most studies suggest that metformin acts primarily through its effect on AMP-activated protein kinase (AMPK, Kalender et al., 2010; Ben Sahra et al., 2011). Metformin inhibits complex I of the electron transport chain (El-Mir et al., 2000), which decreases adenosine triphosphate (ATP) production and leads to AMPK activation. AMPK activation leads to disruption of insulin/IGF-1 signaling through inhibition of mammalian target of rapamycin (mTOR, Rozengurt et al., 2010). Inhibition of mTOR signaling, in turn, results in decreased protein synthesis and cell growth, which are important for cancer survival (**Figure 1**). Metformin can also inhibit mTOR signaling through activation of AMPK independent pathways including Rag GTPase (Kalender et al., 2010) and REDD1 (Ben Sahra et al., 2011). AMPK-induced activation of tumor suppressor 53 (p53) and subsequent cell cycle arrest represents another potential mechanism of action of metformin in pancreatic cancer models (Jalving et al., 2010). Activation of AMPK in the liver, muscle, adipose tissue, and pancreas also results in reduced levels of insulin and IGF-1 (Jalving et al., 2010).

Indeed, mTOR and mitochondrial energy signaling pathways have increasingly been the focus of recent investigations on the antitumor properties of metformin in pancreatic cancer. Early studies demonstrated that metformin induced inhibition of mTORC1 activity and growth of human pancreatic cancer xenografts (Kisfalvi et al., 2009). Concentrations of metformin approximate to plasma levels found in patients with DM2 taking the drug (0.05 mM or 0.1 mM) similarly inhibited mTORC1 activity in a dose-dependent manner *in vitro* (Sinnett-Smith et al., 2012). Interestingly, metformin-induced mTORC1 inhibition was significantly enhanced in pancreatic cancer cells cultured in physiologic glucose concentrations (5 mM) compared to supraphysiologic levels (25 mM) highlighting the concept that cancer cells, at lower ambient glucose concentrations, rely more on mitochondrial oxidative phosphorylation for ATP generation and are therefore more sensitized to mitochondrial respiration inhibitors (Sinnett-Smith et al., 2012; Rozengurt, 2014). Furthermore, synergistic enhancements in ATP depletion and pancreatic cancer cell growth suppression were demonstrated when metformin was added to an inhibitor of glycolysis, 2-deoxyglucose (2-DG), *in vitro* (Cheng et al., 2014).

Recent studies have shown that pancreatic cancer cell growth (*in vitro* and *in vivo*) is also dependent on glutamine metabolism reprogrammed by oncogenic *Kras* via a unique pathway involving aspartate transaminase (GOT1) that leads to a maintenance of essential cellular redox states in the mitochondria (Son et al., 2013). Moreover, pancreatic cancer cells responsible for relapse that survive oncogene ablation have increased expression of genes involving mitochondrial function and reliance on glycolysis and mitochondrial respiration for energy metabolism in *Kras* mouse models (Viale et al., 2014). These latest insights offer exciting future therapeutic strategies in pancreatic cancer by targeting *Kras* signaling in combination with using mitochondrial respiration inhibitors such as metformin. Further identification of novel therapeutic targets will rely, as they have before, on the development of tools critical to our understanding of pancreatic cancer such as genetically engineered mouse models (GEMMs) like the $Kras^{G12D}$ and embryonic stem cell (ESC)-based mouse models (Kirk, 2012; Saborowski et al., 2014).

Rapamycin and active-site mTOR inhibitors have been shown to increase Akt phosphorylation and ERK activation in pancreatic cancer cells *in vitro*, respectively, and highlight the feedback mechanisms that potentially counterbalance the antitumor effects of mTOR inhibitors (Soares et al., 2013). Metformin's antitumor effects, however, occur without stimulating Akt and ERK activation, and therefore, metformin in combination with mTOR inhibitors represents a future direction to improve clinical efficacy in pancreatic cancer (Soares et al., 2013). Indeed, metformin with rapamycin is now under ongoing clinical investigation (phase I and II) in the treatment of advanced pancreatic cancer (NCT02048384). Of note, PTEN deficiencies in $Kras^{G12D}$ mice models have been shown to promote NF-κB and associated cytokine activation and development of metastatic pancreatic cancer (Ying et al., 2011). Treatment with rapamycin conferred a significant survival advantage in *Kras* mice models deficient of PTEN compared to those lacking PTEN deficiencies *in vivo* (Morran et al., 2014). These studies intriguingly identify particular subsets of pancreatic cancer, those with *Kras* mutations and PTEN deficiencies, that may be more responsive to treatment with mTOR inhibitors and/or inhibitors of MAPK/ERK, PI3K, and NF-κB (mediators of converging signaling pathways).

Table 1 | The preclinical development of metformin in pancreatic cancer.

Study	Source in which metformin demonstrated antitumor activity
Cheng et al., 2014	MiaPaCa-2 and Capan-2 cells (*in vitro*, ± glycolytic inhibitor 2-DG)
Das et al., 2014	Panc-1 and AsPC-1 cells (*in vitro*)
Fasih et al., 2014	Panc-1 and MiaPaCa-2 cells (*in vitro*, ± ionizing radiation ± gemcitabine)
Nair et al., 2014	Panc-28, L3.6pL, and Panc-1 cells (*in vitro*)
Snima et al., 2014	MiaPaCa-2 cells (*in vitro*, in metformin-containing *O*-carboxymethyl chitosan nanoparticles)
Yue et al., 2014	BxPC-3 and Panc-1 cells (*in vitro*, + aspirin)
Gou et al., 2013	AsPC-1 and SW1990 cells (*in vitro*) and AsPC-1 and SW1990 tumor xenografts in nude mice (*in vivo*)
Karnevi et al., 2013	BxPC-3, Panc-1, and MiaPaCa-2 cells (*in vitro*)
Kisfalvi et al., 2013	Panc-1 and MiaPaCa-2 tumor xenografts in nude mice (*in vivo*)
Lonardo et al., 2013	Primary human pancreatic ductal adenocarcinoma (PDAC) cells and spheres (*in vitro*, ± gemcitabine) and tumor xenografts in nude mice (*in vivo*, ± gemcitabine and smoothened inhibitor SIBI-C1)
Nair et al., 2013	Panc-28, Panc-1, and L3.6pL cells (*in vitro*) and L3.6pL tumor xenografts in nude mice (*in vivo*)
Soares et al., 2013	Panc-1 cells (*in vitro*)
Yan et al., 2013	MiaPaCa and KP cells (*in vitro*)
Bao et al., 2012	Gemcitabine-sensitive and gemcitabine-resistant AsPC-1 and MiaPaCa-2 cells (*in vitro*) and MiaPaCa-2 tumor xenografts in mice (*in vivo*)
Li et al., 2012	Sw1990 tumor xenografts in nude mice (*in vivo*)
Sinnett-Smith et al., 2012	Panc-1 and MiaPaCa-2 cells (*in vitro*)
Snima et al., 2012	MiaPaCa-2 cells (*in vitro*, in metformin-containing *O*-carboxymethyl chitosan nanoparticles)
Kisfalvi et al., 2009	Panc-1 and MiaPaCa-2 tumor xenografts in nude mice (*in vivo*)
Wang et al., 2008	SW1990, AsPC-1, BxPc-3, and Panc-1 cells (*in vitro*)

Other studies have shown that metformin treatment leads to downregulation of members of the specificity protein (Sp) transcription factor family and target genes involved in tumorigenesis including Bcl-2, survivin, cyclin D1, vascular endothelial growth factor, and fatty acid synthase (FAS, Nair et al., 2013, 2014). In particular, decrease in cyclin D1 induced cell cycle arrest in prostate cancer cells (Ben Sahra et al., 2008). Metformin may also inhibit FAS in the context of available cholesterol and glucose-derived acetyl-CoA in pancreatic cancer cells (Cantoria et al., 2014). In addition, *in vitro* studies showed that metformin alters profiles of microRNAs that regulate apoptosis, inhibit proliferation and invasion, and are linked to reduced expression of the oncogene HMGA1 (Li et al., 2012). Metformin can also affect proliferation of cancer stem cells, and this effect may contribute to its ability to limit tumor growth (Gou et al., 2013). Other potential anti-tumorigenic effects of metformin include the ability to reduce endogenous reactive oxygen species (ROS) and associated DNA damage (Algire et al., 2012), reduce Sonic hedgehog (Shh) expression (Nakamura et al., 2014), and induce anti-inflammatory responses (Cufí et al., 2010; Hirsch et al., 2013; Zhao et al., 2014).

Metformin has demonstrated antitumor activity against pancreatic cancer in numerous preclinical studies (**Table 1**). A recent study highlighted the ability of metformin to prevent progression of pancreatic intraepithelial neoplasia (PanIN) lesions to pancreatic cancer in transgenic mice as well (Mohammed et al., 2013). There are several clinical trials (phase I and II, https://clinicaltrials.gov) involving metformin in the treatment of pancreatic cancer. The majority involve metformin in combination therapies, given that metformin is unlikely to produce a desired efficacy to serve as monotherapy in pancreatic cancer. However, its attractiveness as part of combinatorial therapy lies, in part, in its inexpensiveness (as low as $4 per month) and well-tolerated toxicity profile (common toxicities being gastrointestinal). As discussed above, metformin offers synergistic activity across several but converging signaling pathways important to tumorigenesis. Addition of metformin may also reduce effective doses of other chemotherapeutic agents needed to treat a variety of cancers (Iliopoulos et al., 2011).

Undoubtedly, there remains a need for further understanding of: (1) anticancer mechanisms of metformin, particularly those involving, but not limited to, mTOR signaling (upstream and downstream) and mitochondrial energy metabolism, (2) pharmacokinetic and pharmacodynamic properties of metformin, and (3) relationships between risk factors such as DM and development and progression of pancreatic cancer to identify further molecular targets and advance potential therapies. For now, the future remains bright for metformin as the scientific community eagerly awaits the results of its continued development as a treatment for pancreatic cancer.

REFERENCES

Aggarwal, G., Kamada, P., and Chari, S. T. (2013). Prevalence of diabetes mellitus in pancreatic cancer compared to common cancers. *Pancreas* 42, 198–201. doi: 10.1097/MPA.0b013e3182592c96

Aggarwal, G., Ramachandran, V., Javeed, N., Arumugam, T., Dutta, S., Klee, G. G., et al. (2012). Adrenomedullin is up-regulated in patients with pancreatic cancer and causes insulin resistance in β cells and mice. *Gastroenterology* 143, 1510–1517. doi: 10.1053/j.gastro.2012.08.044

Algire, C., Moiseeva, O., Deschênes-Simard, X., Amrein, L., Petruccelli, L., Birman, E., et al. (2012). Metformin reduces endogenous reactive oxygen species and associated DNA damage. *Cancer Prev. Res. (Phila.)* 5, 536–543. doi: 10.1158/1940-6207.CAPR-11-0536

Alsamarrai, A., Das, S. L. M., Windsor, J. A., and Petrov, M. S. (2014). Factors that affect risk for pancreatic disease in the general population: a systematic review and meta-analysis of prospective cohort studies. *Clin. Gastroenterol. Hepatol.* 12, 1635.e5–1644.e5. doi: 10.1016/j.cgh.2014.01.038

Arslan, A. A., Helzlsouer, K. J., Kooperberg, C., Shu, X. O., Steplowski, E., Bueno-de-Mesquita, H. B., et al. (2010). Anthropometric measures, body mass index, and pancreatic cancer: a pooled analysis from the Pancreatic Cancer Cohort Consortium (PanScan). *Arch. Intern. Med.* 170, 791–802. doi: 10.1001/archinternmed.2010.63

Bao, B., Wang, Z., Ali, S., Ahmad, A., Azmi, A. S., Sarkar, S. H., et al. (2012). Metformin inhibits cell proliferation, migration and invasion by attenuating CSC function mediated by deregulating miRNAs in pancreatic cancer cells. *Cancer Prev. Res.* 5, 355–364. doi: 10.1158/1940-6207.CAPR-11-0299

Bao, B., Wang, Z., Li, Y., Kong, D., Ali, S., Banerjee, S., et al. (2011). The complexities of obesity and diabetes with the development and progression of pancreatic cancer. *Biochim. Biophys. Acta* 1815, 135–146. doi: 10.1016/j.bbcan.2010.11.003

Bao, Y., Giovannucci, E. L., Kraft, P., Stampfer, M. J., Ogino, S., Ma, J., et al. (2013). A prospective study of plasma adiponectin and pancreatic cancer risk in five US cohorts. *J. Natl. Cancer Inst.* 105, 95–103. doi: 10.1093/jnci/djs474

Basso, D., Greco, E., Fogar, P., Pucci, P., Flagiello, A., Baldo, G., et al. (2006). Pancreatic cancer-derived S-100A8 N-terminal peptide: a diabetes cause? *Clin. Chim. Acta* 372, 120–128. doi: 10.1016/j.cca.2006.03.027

Ben, Q., Xu, M., Ning, X., Liu, J., Hong, S., Huang, W., et al. (2011). Diabetes mellitus and risk of pancreatic cancer: a meta-analysis of cohort studies. *Eur. J. Cancer* 47, 1928–1937. doi: 10.1016/j.ejca.2011.03.003

Ben Sahra, I., Laurent, K., Loubat, A., Giorgetti-Peraldi, S., Colosetti, P., Auberger, P., et al. (2008). The antidiabetic drug metformin exerts an antitumoral effect *in vitro* and *in vivo* through a decrease of cyclin D1 level. *Oncogene* 27, 3576–3586. doi: 10.1038/sj.onc.1211024

Ben Sahra, I., Regazzetti, C., Robert, G., Laurent, K., Le Marchand-Brustel, Y., Auberger, P., et al. (2011). Metformin, independent of AMPK, induces mTOR inhibition and cell-cycle arrest through REDD1. *Cancer Res.* 71, 4366–4372. doi: 10.1158/0008-5472.CAN-10-1769

Burris, H. A., Moore, M. J., Andersen, J., Green, M. R., Rothenberg, M. L., Modiano, M. R., et al. (1997). Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J. Clin. Oncol.* 15, 2403–2413.

Campen, C. J., Dragovich, T., and Baker, A. F. (2011). Management strategies in pancreatic cancer. *Am. J. Health Syst. Pharm.* 68, 573–584. doi: 10.2146/ajhp100254

Cantoria, M. J., Boros, L. G., and Meuillet, E. J. (2014). Contextual inhibition of fatty acid synthesis by metformin involves glucose-derived acetyl-CoA and cholesterol in pancreatic tumor cells. *Metabolomics* 10, 91–104. doi: 10.1007/s11306-013-0555-4

Chari, S. T., Leibson, C. L., Rabe, K. G., Timmons, L. J., Ransom, J., de Andrade, M., et al. (2008). Pancreatic cancer-associated diabetes mellitus: prevalence and temporal association with diagnosis of cancer. *Gastroenterology* 134, 95–101. doi: 10.1053/j.gastro.2007.10.040

Cheng, G., Zielonka, J., McAllister, D., Tsai, S., Dwinell, M. B., and Kalyanaraman, B. (2014). Profiling and targeting of cellular bioenergetics: inhibition of pancreatic cancer cell proliferation. *Br. J. Cancer* 111, 85–93. doi: 10.1038/bjc.2014.272

Conroy, T., Desseigne, F., Ychou, M., Bouché, O., Guimbaud, R., Bécouarn, Y., et al. (2011). FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N. Engl. J. Med.* 364, 1817–1825. doi: 10.1056/NEJMoa1011923

Cufí, S., Vazquez-Martin, A., Oliveras-Ferraros, C., Martin-Castillo, B., Joven, J., and Menendez, J. A. (2010). Metformin against TGFβ-induced epithelial-to-mesenchymal transition (EMT): from cancer stem cells to aging-associated fibrosis. *Cell Cycle* 9, 4461–4468. doi: 10.4161/cc.9.22.14048

Currie, C. J., Poole, C. D., and Gale, E. A. (2009). The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. *Diabetologia* 52, 1766–1777. doi: 10.1007/s00125-009-1440-6

Das, A., De, A., Maity, G., Banerjee, S., and Banerjee, S. (2014). The anti-diabetic drug metformin perturbs proliferation and cellular differentiation of pancreatic cancer cells: a key mechanism of its anticancer activity (784.6). *FASEB J.* 28, 784–786. Available online at: http://www.fasebj.org.mlprox.csmc.edu/content/28/1_Supplement/784.6.abstract

De La Cruz, M. S., Young, A. P., and Ruffin, M. T. (2014). Diagnosis and management of pancreatic cancer. *Am. Fam. Physician* 89, 626–632.

Ding, X. Z., Fehsenfeld, D. M., Murphy, L. O., Permert, J., and Adrian, T. E. (2000). Physiological concentrations of insulin augment pancreatic cancer cell proliferation and glucose utilization by activating MAP kinase, PI3 kinase and enhancing GLUT-1 expression. *Pancreas* 21, 310–320. doi: 10.1097/00006676-200010000-00014

Draznin, B. (2011). Mechanism of the mitogenic influence of hyperinsulinemia. *Diabetol. Metab. Syndr.* 3:10. doi: 10.1186/1758-5996-3-10

Elena, J. W., Steplowski, E., Yu, K., Hartge, P., Tobias, G. S., Brotzman, M. J., et al. (2013). Diabetes and risk of pancreatic cancer: a pooled analysis from the pancreatic cancer cohort consortium. *Cancer Causes Control* 24, 13–25. doi: 10.1007/s10552-012-0078-8

El-Mir, M. Y., Nogueira, V., Fontaine, E., Avéret, N., Riqoulet, M., and Leverve, X. (2000). Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. *J. Biol. Chem.* 275, 223–228. doi: 10.1074/jbc.275.1.223

Evans, J. M., Donnelly, L. A., Emslie-Smith, A. M., Alessi, D. R., and Morris, A. D. (2005). Metformin and reduced risk of cancer in diabetic patients. *BMJ* 330, 1304–1305. doi: 10.1136/bmj.38415.708634.F7

Everhart, J., and Wright, D. (1995). Diabetes mellitus as a risk factor for pancreatic cancer. A meta-analysis. *JAMA* 273, 1605–1609.

Fasih, A., Elbaz, H. A., Hüttemann, M., Konski, A. A., and Zielske, S. P. (2014). Radiosensitization of pancreatic cancer cells by metformin through the AMPK pathway. *Radiat. Res.* 182, 50–59. doi: 10.1667/RR13568.1

Fogar, P., Pasquali, C., Basso, D., Sperti, C., Panozzo, M. P., Tessari, G., et al. (1994). Diabetes mellitus in pancreatic cancer follow-up. *Anticancer Res.* 14, 2827–2830.

Genkinger, J. M., Spiegelman, D., Anderson, K. E., Bernstein, L., van den Brandt, P. A., Calle, E. E., et al. (2011). A pooled analysis of 14 cohort studies of anthropometric factors and pancreatic cancer risk. *Int. J. Cancer* 129, 1708–1717. doi: 10.1002/ijc.25794

Ghosn, M., Kourie, H. R., El Karak, F., Hanna, C., Antoun, J., and Nasr, D. (2014). Optimum chemotherapy in the management of metastatic pancreatic cancer. *World J. Gastroenterol.* 20, 2352–2357. doi: 10.3748/wjg.v20.i9.2352

Godsland, I. F. (2009). Insulin resistance and hyperinsulinaemia in the development and progression of cancer. *Clin. Sci.* 118, 315–332. doi: 10.1042/CS20090399

Gou, S., Cui, P., Li, X., Shi, P., Liu, T., and Wang, C. (2013). Low concentrations of metformin selectively inhibit $CD133^+$ cell proliferation in pancreatic cancer and have anticancer action. *PLoS ONE* 8:e63969. doi: 10.1371/journal.pone.0063969

Hirsch, H. A., Iliopoulos, D., and Struhl, K. (2013). Metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth. *Proc. Natl. Acad. Sci. U.S.A.* 110, 972–977. doi: 10.1073/pnas.1221055110

Howlader, N., Noone, A. M., Krapcho, M., Garshell, J., Miller, D., Altekruse, S. F., et al. (ed.). (2014). *SEER Cancer Statistics Review, 1975-2011*, Bethesda, MD; National Cancer Institute. Available online at: http://seer.cancer.gov/csr/1975_2011/

Huxley, R., Ansary-Moghaddam, A., Berrington de González, A., Barzi, F., and Woodward, M. (2005). Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies. *Br. J. Cancer* 92, 2076–2083. doi: 10.1038/sj.bjc.6602619

Iliopoulos, D., Hirsch, H. A., and Struhl, K. (2011). Metformin decreases the dose of chemotherapy for prolonging tumor remission in mouse xenografts involving multiple cancer cell types. *Cancer Res.* 71, 3196–3201. doi: 10.1158/0008-5472.CAN-10-3471

Iodice, S., Gandini, S., Maisonneuve, P., and Lowenfels, A. B. (2008). Tobacco and the risk of pancreatic cancer: a review and meta-analysis. *Langenbecks Arch. Surg.* 393, 535–545. doi: 10.1007/s00423-007-0266-2

Jalving, M., Gietema, J. A., Lefrandt, J. D., de Jong, S., Reyners, A. K., Gans, R. O., et al. (2010). Metformin: taking away the candy for cancer? *Eur. J. Cancer* 46, 2369–2380. doi: 10.1016/j.ejca.2010.06.012

Jiao, L., Berrington de Gonzalez, A., Hartge, P., Pfeiffer, R. M., Park, Y., Freedman, D. M., et al. (2010). Body mass index, effect modifiers, and risk of pancreatic cancer: a pooled study of seven prospective cohorts. *Cancer Causes Control* 21, 1305–1314. doi: 10.1007/s10552-010-9558-x

Kalender, A., Selvaraj, A., Kim, S. Y., Gulati, P., Brûlé, S., Viollet, B., et al. (2010). Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner. *Cell Metab.* 11, 390–401. doi: 10.1016/j.cmet.2010.03.014

Karnevi, E., Said, K., Andersson, R., and Rosendahl, A. H. (2013). Metformin-mediated growth inhibition involves suppression of the IGF-I receptor signalling pathway in human pancreatic cancer cells. *BMC Cancer* 13:325. doi: 10.1186/1471-2407-13-235

Kirk, R. (2012). Pancreatic cancer: mouse models link metabolism and deubiquitination with Kras. *Nat. Rev. Clin. Oncol.* 9, 368. doi: 10.1038/nrclinonc.2012.85

Kisfalvi, K., Eibl, G., Sinnett-Smith, J., and Rozengurt, E. (2009). Metformin disrupts crosstalk between G protein-coupled receptor and insulin receptor signaling systems and inhibits pancreatic cancer growth. *Cancer Res.* 69, 6539–6545. doi: 10.1158/0008-5472.CAN-09-0418

Kisfalvi, K., Moro, A., Sinnett-Smith, J., Eibl, G., and Rozengurt, E. (2013). Metformin inhibits the growth of human pancreatic cancer xenografts. *Pancreas* 42, 781–785. doi: 10.1097/MPA.0b013e31827aec40

Larsson, S. C., Orsini, N., and Wolk, A. (2007). Body mass index and pancreatic cancer risk: a meta-analysis of prospective studies. *Int. J. Cancer* 120, 1993–1998. doi: 10.1002/ijc.22535

Li, D. (2012). Diabetes and pancreatic cancer. *Mol. Carcinog.* 51, 64–74. doi: 10.1002/mc.20771

Li, W., Yuan, Y., Huang, L., Qiao, M., and Zhang, Y. (2012). Metformin alters the expression profiles of microRNAs in human pancreatic cancer cells. *Diabetes Res. Clin. Pract.* 96, 187–195. doi: 10.1016/j.diabres.2011.12.028

Lonardo, E., Cioffi, M., Sancho, P., Sanchez-Ripoll, Y., Trabulo, S. M., Dorado, J., et al. (2013). Metformin targets the metabolic Achilles heel of human pancreatic cancer stem cells. *PLoS ONE* 8:e76518. doi: 10.1371/journal.pone.0076518

Lowenfels, A. B., Maisonneuve, P., DiMagno, E. P., Elitsur, Y., Gates, L. K. Jr., Perrault, J., et al. (1997). Hereditary pancreatitis and the risk of pancreatic cancer. *J. Natl. Cancer Inst.* 89, 442–446. doi: 10.1093/jnci/89.6.442

Ma, J., Sawai, H., Matsuo, Y., Ochi, N., Yasuda, A., Takahashi, H., et al. (2010). IGF-1 mediates PTEN suppression and enhances cell invasion and proliferation via activation of the IGF 1/PI3K/Akt signaling pathway in pancreatic cancer cells. *J. Surg. Res.* 160, 90–101. doi: 10.1016/j.jss.2008.08.016

Mohammed, A., Janakiram, N. B., Brewer, M., Ritchie, R. L., Marya, A., Lightfoot, S., et al. (2013). Antidiabetic drug metformin prevents progression of pancreatic cancer by targeting in part cancer stem cells and mTOR signaling. *Transl. Oncol.* 6, 649–659. doi: 10.1593/tlo.13556

Morran, D. C., Wu, J., Jamieson, N. B., Mrowinska, A., Kalna, G., Karim, S. A., et al. (2014). Targeting mTOR dependency in pancreatic cancer. *Gut* 0, 1481–1489. doi: 10.1136/gutjnl-2013-306202

Nair, V., Pathi, S., Jutooru, I., Sreevalsan, S., Basha, R., Abdelrahim, M., et al. (2013). Metformin inhibits pancreatic cancer cell and tumor growth and downregulates Sp transcription factors. *Carcinogenesis* 34, 2870–2879. doi: 10.1093/carcin/bgt231

Nair, V., Sreevalsan, S., Basha, R., Abdelrahim, M., Abudayyeh, A., Hoffman, A. R., et al. (2014). Mechanism of metformin-dependent inhibition of mammalian target of rapamycin (mTOR) and Ras activity in pancreatic cancer: role of specificity protein (Sp) transcription factors. *J. Biol. Chem.* 289, 27692–27701. doi: 10.1074/jbc.M114.592576

Nakamura, M., Ogo, A., Yamura, M., Yamaguchi, Y., and Nakashima, H. (2014). Metformin suppresses Sonic hedgehog expression in pancreatic cancer cells. *Anticancer Res.* 34, 1765–1769.

Pannala, R., Leirness, J. B., Bamlet, W. R., Basu, A., Petersen, G. M., and Chari, S. T. (2008). Prevalence and clinical profile of pancreatic cancer-associated diabetes mellitus. *Gastroenterology* 134, 981–987. doi: 10.1053/j.gastro.2008.01.039

Permert, J., Ihse, I., Jorfeldt, L., von Schenck, H., Arnquist, H. J., and Larsson, J. (1993a). Improved glucose metabolism after subtotal pancreatectomy for pancreatic cancer. *Br. J. Surg.* 80, 1047–1050. doi: 10.1002/bjs.1800800841

Permert, J., Ihse, I., Jorfeldt, L., von Schenck, H., Arnqvist, H. J., and Larsson, J. (1993b). Pancreatic cancer is associated with impaired glucose metabolism. *Eur. J. Surg.* 159, 101–107.

Permert, J., Larsson, J., Westermark, G. T., Herrington, M. K., Christmanson, L., Pour, P. M., et al. (1994). Islet amyloid polypeptide in patients with pancreatic cancer and diabetes. *N. Engl. J. Med.* 330, 313–318. doi: 10.1056/NEJM199402033300503

Pollak, M. N., Schernhammer, E. S., and Hankinson, S. E. (2004). Insulin-like growth factors and neoplasia. *Nat. Rev. Cancer* 4, 505–518. doi: 10.1038/nrc1387

Powell, D. R., Suwanichkul, A., Cubbage, M. L., DePaolis, L. A., Snuggs, M. B., and Lee, P. D. (1991). Insulin inhibits transcription of the human gene for insulin-like growth factor-binding protein-1. *J. Biol. Chem.* 266, 18868–18876.

Raimondi, S., Lowenfels, A. B., Morselli-Labate, A. M., Maisonneuve, P., and Pezzilli, R. (2010). Pancreatic cancer in chronic pancreatitis; aetiology, incidence, and early detection. *Best Pract. Res. Clin. Gastroenterol.* 24, 349–358. doi: 10.1016/j.bpg.2010.02.007

Rozengurt, E. (2014). Mechanistic target of rapamycin (mTOR): a point of convergence in the action of insulin/IGF-1 and G protein-coupled receptor agonists in pancreatic cancer cells. *Front. Physiol.* 5:357. doi: 10.3389/fphys.2014.00357

Rozengurt, E., Sinnett-Smith, J., and Kisfalvi, K. (2010). Crosstalk between insulin/insulin-like growth factor-1 receptors and G protein-coupled receptor signaling systems: a novel target for the antidiabetic drug metformin in pancreatic cancer. *Clin. Cancer Res.* 16, 2505–2511. doi: 10.1158/1078-0432.CCR-09-2229

Saborowski, M., Saborowski, A., Morris, J. P., Bosbach, B., Dow, L. E., Pelletier, J., et al. (2014). A modular and flexible ESC-based mouse model of pancreatic cancer. *Genes Dev.* 28, 85–97. doi: 10.1101/gad.232082.113

Sadeghi, N., Abbruzzese, J. L., Yeung, S. J., Hassan, M., and Li, D. (2012). Metformin use is associated with better survival of diabetic patients with pancreatic cancer. *Clin. Cancer Res.* 18, 2905–2912. doi: 10.1158/1078-0432.CCR-11-2994

Sah, R. P., Nagpal, S. J., Mukhopadhyay, D., and Chari, S. T. (2013). New insights into pancreatic cancer-induced paraneoplastic diabetes. *Nat. Rev. Gastroenterol. Hepatol.* 10, 423–433. doi: 10.1038/nrgastro.2013.49

Schneider, M. B., Matsuzaki, H., Haorah, J., Ulrich, A., Standop, J., Ding, X. Z., et al. (2001). Prevention of pancreatic cancer induction in hamsters by metformin. *Gastroenterology* 120, 1263–1270. doi: 10.1053/gast.2001.23258

Seufferlein, T., Bachet, J. B., Van Cutsem, E., and Rougier, P. (2012). Pancreatic adenocarcinoma: ESMO-ESDO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 23(Suppl. 7), vii33–vii40. doi: 10.1093/annonc/mds224

Sinnett-Smith, J., Kisfalvi, K., Kui, R., and Rozengurt, E. (2012). Metformin inhibition of mTORC1 activation, DNA synthesis and proliferation in pancreatic cancer cells: dependence on glucose concentration and role of AMPK. *Biochem. Biophys. Res. Commun.* 430, 352–357. doi: 10.1016/j.bbrc.2012.11.010

Snima, K. S., Jayakumar, R., and Lakshmanan, V. K. (2014). *In vitro* and *in vivo* biological evaluation of O-carboxymethyl chitosan encapsulated metformin nanoparticles for pancreatic cancer therapy. *Pharm. Res.* doi: 10.1007/s11095-014-1425-0. [Epub ahead of print].

Snima, K. S., Jayakumar, R., Unnikrishnan, A. G., Nair, S. V., and Lakshmanan, V. K. (2012). O-Carboxymethyl chitosan nanoparticles for metformin delivery to pancreatic cancer cells. *Carbohydr. Polym.* 89, 1003–1007. doi: 10.1016/j.carbpol.2012.04.050

Soares, H. P., Ni, Y., Kisfalvi, K., Sinnett-Smith, J., and Rozengurt, E. (2013). Different patterns of Akt and ERK feedback activation in response to rapamycin, active-site mTOR inhibitors and metformin in pancreatic cancer cells. *PLoS ONE* 8:e57289. doi: 10.1371/journal.pone.0057289

Son, J., Lyssiotis, C. A., Ying, H., Wang, X., Hua, S., Ligorio, M., et al. (2013). Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature* 496, 101–105. doi: 10.1038/nature12040

Tramacere, I., Scotti, L., Jenab, M., Bagnardi, V., Bellocco, R., Rota, M., et al. (2010). Alcohol drinking and pancreatic cancer risk: a meta-analysis of the dose-risk relation. *Int. J. Cancer* 126, 1474–1486. doi: 10.1002/ijc.24936

Viale, A., Pettazzoni, P., Lyssiotis, C. A., Ying, H., Sánchez, N., Marchesini, M., et al. (2014). Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. *Nature* 514, 628–632. doi: 10.1038/nature13611

Wang, L. W., Li, Z. S., Zou, D. W., Jin, Z. D., Gao, J., and Xu, G. M. (2008). Metformin induces apoptosis of pancreatic cancer cells. *World J. Gastroenterol.* 14, 7192–7198. doi: 10.3748/wjg.14.7192

Wang, Z., Lai, S. T., Xie, L., Zhao, J. D., Ma, N. Y., Zhu, J., et al. (2014). Metformin is associated with reduced risk of pancreatic cancer in patients with type 2 diabetes mellitus: a systematic review and meta-analysis. *Diabetes Res. Clin. Pract.* S0168–S8227, 00190–00199. doi: 10.1016/j.diabres.2014.04.007

White, M. A., Agle, S. C., Fuhr, H. M., Mehaffey, J. H., Waibel, B. H., and Zervos, E. E. (2011). Impact of pancreatic cancer and subsequent resection on glycemic control in diabetic and nondiabetic patients. *Am. Surg.* 77, 1032–1037.

Wolpin, B. M., Bao, Y., Qian, Z. R., Wu, C., Kraft, P., Ogino, S., et al. (2013). Hyperglycemia, insulin resistance, impaired pancreatic β-cell function, and risk of pancreatic cancer. *J. Natl. Cancer Inst.* 105, 1027–1035. doi: 10.1093/jnci/djt123

Yan, G., Freeman, T., and Lanza-Jacoby, S. (2013). Limiting glucose availability with metformin increased reactive oxygen species, decreased cell survival, and promoted apoptosis in human and mouse pancreatic cancer cells. *Cancer Res.* 73(8 Suppl. 1), Abstr:5405. doi: 10.1158/1538-7445.AM2013-5405

Ying, H., Elpek, K. G., Vinjamoori, A., Zimmerman, S. M., Chu, G. C., Yan, H., et al. (2011). PTEN is a major tumor suppressor in pancreatic ductal adenocarcinoma and regulates an NF-κB-cytokine network. *Cancer Disc.* 1, 158–169. doi: 10.1158/2159-8290.CD-11-0031

Yue, W., Yang, C. S., DiPaola, R. S., and Tan, X. L. (2014). Repurposing of metformin and aspirin by targeting AMPK-mTOR and inflammation for pancreatic cancer prevention and treatment. *Cancer Prev. Res.* 7, 388–397. doi: 10.1158/1940-6207.CAPR-13-0337

Zhao, Z., Cheng, X., Wang, Y., Han, R., Li, L., Xiang, T., et al. (2014). Metformin inhibits the IL-6-induced epithelial-mesenchymal transition and

lung adenocarcinoma growth and metastasis. *PLoS ONE* 9:e95884. doi: 10.1371/journal.pone.0095884

Zou, L., Zhong, R., Shen, N., Chen, W., Zhu, B., Ke, J., et al. (2014). Non-linear dose-response relationship between cigarette smoking and pancreatic cancer risk: evidence from a meta-analysis of 42 observational studies. *Eur. J. Cancer* 50, 193–203. doi: 10.1016/j.ejca.2013.08.014

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Mechanistic target of rapamycin (mTOR): a point of convergence in the action of insulin/IGF-1 and G protein-coupled receptor agonists in pancreatic cancer cells

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Pancreatic ductal adenocarcinoma (PDAC), the most common form of pancreatic cancer, is one of the most lethal human diseases. PDAC is now the fourth leading cause of cancer mortality in both men and women and deaths due to PDAC are projected to increase dramatically. Novel targets and agents for chemoprevention are urgently needed and will most likely arise from a more detailed understanding of the signaling mechanisms that stimulate the promotion and progression of sub-malignant cells into pancreatic cancer cells and from the identification of modifiable risk factors for PDAC. Many epidemiological studies have linked obesity and long-standing type 2 diabetes mellitus (T2DM) with increased risk and worse clinical outcomes for developing PDAC. These diet-related metabolic disorders are multifaceted but characterized by peripheral insulin resistance, compensatory overproduction of insulin and increased bioavailability of insulin-like growth factor-1 (IGF-1). Mounting evidence indicates that the insulin/IGF-1 receptor system plays a critical role in PDAC development and multiple studies support the notion that crosstalk between the insulin receptor and heptahelical G protein-coupled receptor (GPCR) signaling systems is an important element in the biological responses elicited by these signaling systems, including cell proliferation. This article highlights the central role of the mechanistic target of rapamycin (mTOR) in mediating crosstalk between insulin/IGF-1 and GPCR signaling in pancreatic cancer cells and proposes strategies, including the use of metformin, to target this signaling system in PDAC cells.

Keywords: Akt, PI3K, PKC, S6 kinase, neurotensin

Pancreatic ductal adenocarcinoma (PDAC), the most common form of pancreatic cancer, is one of the most lethal human diseases. Indeed, the overall 5-year survival rate is a dismal 6% and the median survival period of 4–6 months. The incidence of this disease in the US is estimated to increase to more than 44,000 new cases in 2014 and is now the fourth leading cause of cancer mortality in both men and women (Siegel et al., 2014). Total deaths due to PDAC are projected to increase dramatically (Rahib et al., 2014). Novel targets and agents for chemoprevention are urgently needed and will most likely arise from a more detailed understanding of the signaling mechanisms that stimulate the promotion and progression of sub-malignant cells into pancreatic cancer cells and from the identification of modifiable risk factors for PDAC. In this context, it is recognized that PDAC arises from the progression of precursor lesions, the most common of which are pancreatic intraepithelial neoplasias (PanINs). Progression from these non-invasive lesions to invasive cancer is associated with the accumulation of genetic alterations (Murphy et al., 2013), including activating mutations in the *KRAS* oncogene which appears in ∼90% of PDACs as well as inactivating mutations in tumor suppressors genes, including p53, p16, and SMAD4 (Murphy et al., 2013). It is generally accepted that progression of pancreatic carcinogenesis requires dysregulation of a set of signaling pathways leading to sustained cell proliferation (Jones et al., 2008). The focus of this brief article is on the central role of the mechanistic/mammalian target of rapamycin (mTOR) in mediating insulin/IGF-1 and G protein-coupled receptor (GPCR) signaling leading to proliferation of pancreatic cancer cells. Subsequently, strategies to target this pathway in PDAC cells are proposed.

OBESITY, TYPE 2 DIABETES, AND PDAC

In addition to smoking, chronic pancreatitis and a family history of PDAC (Kolodecik et al., 2014), many epidemiological studies have linked obesity and long-standing type 2 diabetes mellitus (T2DM) with increased risk and worse clinical outcomes for developing PDAC (Arslan et al., 2010; Giovannucci et al., 2010). These diet-related metabolic disorders are multifaceted but characterized by peripheral insulin resistance, compensatory overproduction of insulin and increased bioavailability of IGF-1 (Alemán et al., 2014). Given the complex organization of the pancreatic microcirculation, locally overproduced insulin by β cells is thought to act directly on insulin receptors expressed by exocrine pancreatic cells. The highly related insulin-like growth factor-1 (IGF-1) receptor (IGF-1R) and hybrids of IGF-1R and

insulin receptors can also be activated by insulin (Taniguchi et al., 2006), in particular at the high concentrations of intra-pancreatic insulin. Accordingly, PDAC cells express insulin and IGF-1 receptors and over-express insulin receptor substrate (IRS)-1 and IRS-2 and PDAC (but not normal) tissue expresses activated IGF-1R and IGF-1 (Rozengurt et al., 2010). Silencing the expression of IGF-1R in pancreatic cancer cells inhibits their growth and metastasis (Subramani et al., 2014) and the beneficial effects of calorie restriction in pancreatic cancer models appear mediated through the IGF-1/IGF-1R axis (Harvey et al., 2014). Reciprocally, the promoting effects of high calorie diet have been associated with an increase in the circulating levels of insulin and IGF-1 (Dawson et al., 2013). Interestingly, IGF-1 and orthotopically transplanted PDAC growth were decreased in liver-specific IGF-1-deficient mice and restored by IGF-1 administration (Lashinger et al., 2013). Inactivation of p53, as seen during the progression of 50–75% of PDAC, has been recognized to potently up-regulate the insulin/IGF-1 pathway (Feng and Levine, 2010) and gene variations in the IGF-1 signaling system have been associated with worse survival in PDAC (Dong et al., 2010). Collectively, these studies underscore the significance of the insulin/IGF-1 signaling pathway in PDAC development. Accordingly, elucidation of the signaling pathways triggered by insulin/IGF-1 and the crosstalk mechanisms between the insulin/IGF-1R and other signaling pathways in PDAC cells is likely to facilitate the identification of new targets for therapeutic and chemo-preventive interventions.

INSULIN/IGF-1 SIGNALING, PI3K/Akt/mTOR AND PDAC

In most cells, binding of insulin to its tetrameric receptor induces activation of the receptor tyrosine kinase and autophosphorylation, followed by docking and tyrosine phosphorylation of adaptor proteins, including insulin receptor substrates (IRS 1–4) and Shc which propagate downstream signals (Metz and McGarry Houghton, 2011). The insulin receptor exhibits a high degree of homology with the IGF-1R, especially in their tyrosine kinase domains. Furthermore, the insulin and IGF-1 receptors form heterodimers that bind IGF-2, another ligand of the IGF family produced by cancer cells. As illustrated in **Figure 1**, a key insulin/IGF1R-induced pathway via IRS is class I phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR (Taniguchi et al., 2006; Zoncu et al., 2011). PI3K catalyzes the synthesis of phosphatidylinositol (3,4,5)-trisphosphate (PIP_3), a membrane lipid second messenger that coordinates the localization and activation of downstream effectors, including the isoforms of the Akt family (Franke, 2008). The Akts possess a PH domain and conserved residues (Thr^{308} and Ser^{473} in Akt1, the most commonly expressed isoform in normal cells) which are critical for Akt activation. Specifically, Akt translocated to the plasma membrane in response to products of PI3K, is activated by phosphorylation at Thr^{308} in the kinase activation loop and at Ser^{473} in the hydrophobic motif. The PI3K/Akt/mTOR pathway plays a pivotal role in promoting the proliferation and survival of PDAC cells (Asano et al., 2005), is activated in pancreatic cancer tissues, and limits catabolic processes, including autophagy (Lee et al., 2010). Interestingly, the Akt2 gene is amplified or activated in a subset of pancreatic carcinomas (Ruggeri et al., 1998). Collectively, these findings imply that mTOR signaling plays an important role in obesity-induced pancreatic cancer and is a potential target for chemoprevention.

mTOR, a master regulator of cell metabolism, growth and proliferation, functions as a catalytic subunit in two distinct multi-protein complexes, mTORC1 and mTORC2 (Beauchamp and Platanias, 2013). mTORC1, characterized by the substrate binding subunit Raptor senses both nutrients and growth factors (Dibble and Manning, 2013). As indicated in **Figure 1**, mTORC1 phosphorylates and controls at least two regulators of protein synthesis, the 40S ribosomal protein subunit S6 kinase (S6K) and the inhibitor of protein synthesis 4E-binding protein 1 (4EBP1) which promote protein synthesis and plays a critical role in the regulation of cellular metabolism (Dibble and Manning, 2013). mTORC1 is acutely inhibited by rapamycin whereas mTORC2, which is characterized by Rictor and mSin1, is not inhibited by short-term treatment with this agent.

The heterodimer of the tumor suppressor tuberous sclerosis complex 2 (TSC2; tuberin) and TSC1 (hamartin) represses mTORC1 signaling by acting as the GTPase-activator protein for the small G protein Rheb (Ras homolog enriched in brain), a potent activator of mTORC1 in its GTP-bound state. Phosphorylation of TSC2 by Akt and/or ERK/p90RSK (at different sites) uncouples TSC1/TSC2 from Rheb, leading to Rheb-GTP accumulation and mTORC1 activation (**Figure 1**). The Rag GTPases (RAGA/B and RAGC/D), in conjunction with the adaptor Ragulator, activate mTORC1 in response to amino acids, by promoting mTORC1 translocation to lysosomal membranes that contain Rheb-GTP (Bar-Peled and Sabatini, 2014). Phosphatase and tensin homolog (PTEN) opposes PI3K by degrading PIP_3 to PIP_2 thereby inactivating Akt and mTOR signaling (Song et al., 2012). The adaptor protein Shc binds to autophosphorylated IGF-1R to stimulate Grb2/SOS-mediated Ras activation (GTP loading) leading to Raf/MEK/ERK activation (**Figure 1**). As will be discussed below, insulin/IGF-1-induced signaling cross-talks with pathways triggered through other receptors systems expressed by PDAC cells thereby forming complex networks.

In addition to be phosphorylated at multiple Tyr residues that promote downstream signaling, the IRS family is also phosphorylated at multiple serine and threonine residues that attenuate signaling and promote degradation. In this context, it is important that activation of the mTORC1/S6K axis inhibits IRS-1 function following its phosphorylation at multiple residues, including $Ser^{636/639}$ by mTORC1 and $Ser^{307/636/1001}$ by S6K (Tanti and Jager, 2009). Accordingly, treatment of PDAC cells with rapamycin caused a striking increase in Akt phosphorylation at Ser^{473} while exposure to active-site inhibitors of mTOR (e.g., KU63794 and PP242) abrogated Akt phosphorylation at this site in PDAC cells (Soares et al., 2013). Conversely, active-site inhibitors of mTOR caused a marked increase in ERK activation whereas rapamycin did not have any stimulatory effect on ERK activation in PDAC cells (Soares et al., 2013). These results imply that first and second generation of mTOR inhibitors promote over-activation of different pro-oncogenic pathways in PDAC cells, suggesting that suppression of feed-back loops should be a major consideration in the use of these inhibitors for PDAC therapy.

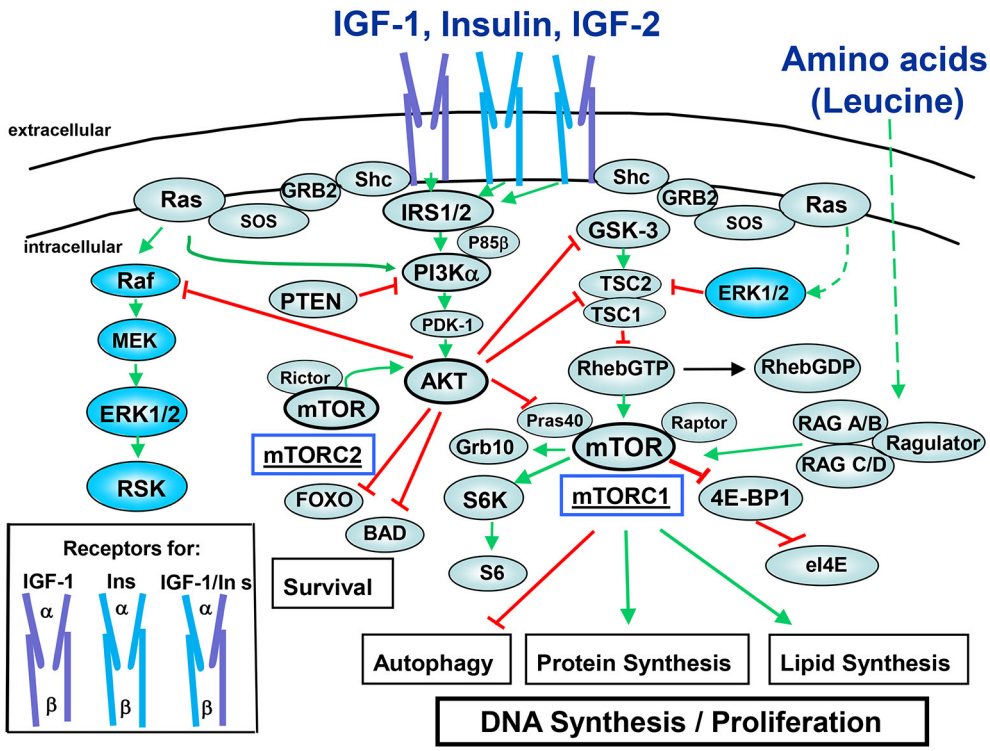

FIGURE 1 | Insulin/IGF-1 signaling pathways. The receptors for the peptides of the insulin family peptides consist of ligand-binding α chain and tyrosine kinase-containing β chain (cartoons in the box). Insulin binds to the insulin receptors (InsR) with high affinity while it binds to IGF-1R at higher concentrations. Insulin also binds to hybrid receptors (IGF-1R/InsR). IGF-1 binds to the IGF-1R and to hybrid receptors with high affinity. IGF-2 binds to the InsR-A, IGF-1R, and IGF-1R/InsR-A hybrid receptor. For the sake clarity, negative feedback loops mediated by downstream components of the pathway (e.g., mTORC1, S6K) that restrain the activity of upstream components (e.g., IGF-1R, IRS) have not been included. The signaling network depicted in this figure is discussed in the text. Note that the IGF-1R and hybrid IGF-1R/InsRs couple more efficiently to Shc/Grb2/SOS providing an explanation for the increased ability of IGF-1 to induce ERK activation as compared with insulin. Green lines indicate stimulatory interactions while red lines indicate inhibitory interactions.

CROSSTALK BETWEEN INSULIN/IGF-1 RECEPTOR AND G PROTEIN-COUPLED RECEPTOR SIGNALING SYSTEMS IN PDAC

Many studies support the notion that crosstalk between the insulin receptor and heptahelical GPCR signaling systems is implicated in a variety of normal and abnormal processes, including cardiovascular and renal pathologies in obesity, metabolic syndrome and T2DM. Many GPCRs and their cognate agonists also mediate autocrine/paracrine growth stimulation in a variety of cancer cells and dramatically synergize with insulin/IGF-1 in inducing mitogenic signaling (Rozengurt, 1986). A recent characterization of cancer genomes demonstrated frequent mutations in GPCRs and G proteins (Kan et al., 2010). Consequently, we hypothesized that crosstalk between insulin/IGF-1 receptor and GPCR signaling systems is also a mechanism for enhancing the development of pancreatic cancer (Rozengurt et al., 2010). Accordingly, PDAC cells and tissues express multiple mitogenic GPCRs, including receptors that recognize neurotensin, angiotensin II and substance P (Rozengurt et al., 2010) and a broad-spectrum GPCR antagonist inhibited the growth of PDAC cells *in vivo* (Guha et al., 2005). Using PDAC cells in culture, we demonstrated positive crosstalk between insulin receptor and GPCR signaling systems (Kisfalvi et al., 2009).

Many GPCRs activate G proteins of the Gq family, promoting its dissociation into Gαq and Gβγ and the exchange of GDP bound to Gαq for GTP (Rozengurt, 2007). The resulting GTP-Gαq complex activates the β isoforms of phospholipase C (PLC), identified as one of the "core" signaling pathways that undergo somatic alterations in nearly all pancreatic cancers (Jones et al., 2008). As shown in **Figure 2**, PLCβ produces second messengers that activate members of the protein kinase C (PKC) family which, in turn, phosphorylate and activate the protein kinases of the protein kinase D (PKD) family, including PKD1, PKD2, and PKD3 (Rozengurt et al., 2005). The PKC/PKD axis induces MEK/ERK/p90RSK activation, at least in part by direct phosphorylation of RIN1 and thereby potentiates K-Ras signaling (Rozengurt et al., 2005). In addition, PKDs can promote COX-2-mediated production of PGE2 which can bind to their own receptors after exiting the cells (**Figure 2**). PKDs are rapidly activated by GPCR agonists in PDAC cells (Guha et al., 2002; Rey et al., 2003a,b; Yuan and Rozengurt, 2008), are over-expressed in PDAC tissues (Harikumar et al., 2010) and PKD over-expression in PDAC cell lines promotes their proliferation (Kisfalvi et al.,

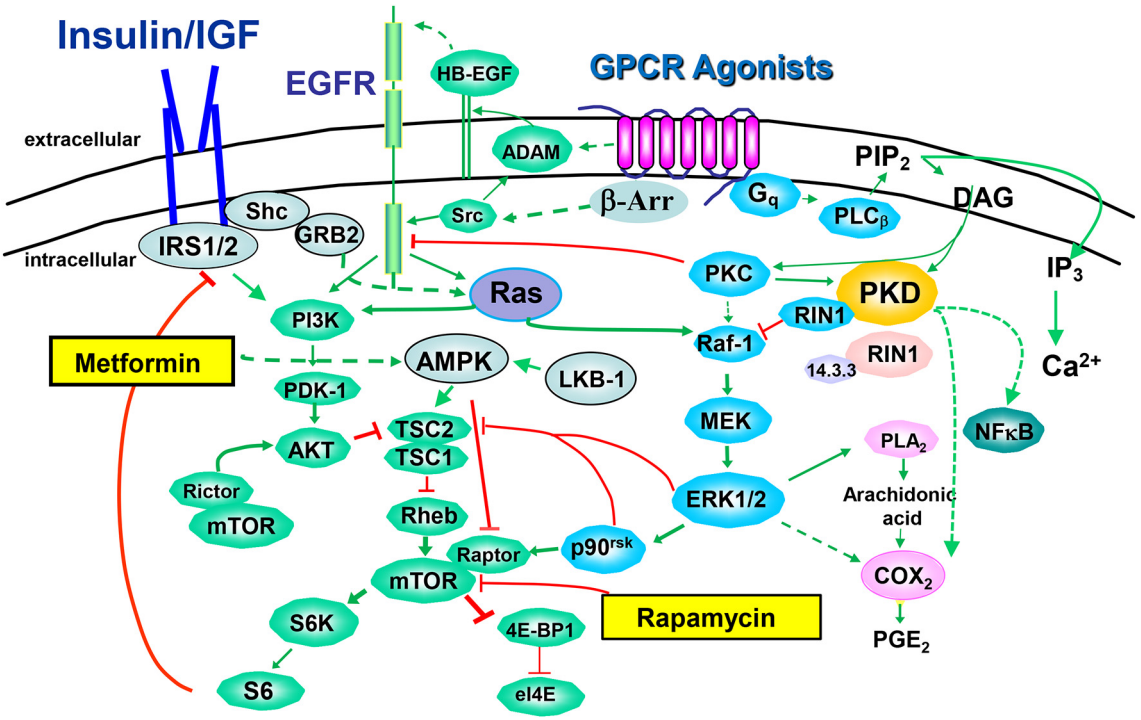

FIGURE 2 | Crosstalk between insulin/IGF-1 receptors and GPCR signaling systems. The binding of an agonistic ligand to its cognate GPCR triggers the activation of multiple signal transduction pathways via heterotrimeric G proteins, including Gq/11. GPCRS also signal via arrestin (β-Arr) in a G protein-independent manner. A rapid increase in the activity of phospholipases C leads to the synthesis of lipid-derived second messengers, Ca^{2+} fluxes and subsequent activation of protein phosphorylation cascades, including PKC/PKD, Raf/MEK/ERK and Akt/mTOR/p70S6K. The EGFR has emerged as a transducer in the signaling by GPCRs, a process termed EGFR transactivation, and promoted by the release of heparin-binding epidermal growth factor (HB-EGF) through the activation of a disintegrin and metalloprotease (ADAM). The pathways stimulated by GPCRs are extensively interconnected by synergistic and antagonistic cross-talks that play a critical role in signal transmission, integration and dissemination. In this context, mTOR emerges as a critical point of convergence in the action of insulin/IGR-1R, EGFR, and GPCRs. Rapamycin, an allosteric inhibitor of mTORC1 and metformin, an inhibitor of mitochondrial function that indirectly (broken lines) stimulates AMPK, are also included.

2010) and invasion (Ochi et al., 2011). Furthermore, a novel PKD inhibitor blocks pancreatic cancer cell growth *in vitro* and *in vivo* (Harikumar et al., 2010).

GPCR agonists also stimulate mTORC1 through at least two converging mechanisms: EGFR transactivation and ERK-mediated phosphorylation of TSC2 (Rozengurt, 2007; Foster and Fingar, 2010; Rozengurt et al., 2010). Transactivation of the EGFR is mediated by the rapid generation of EGFR ligands through proteolysis of membrane-bound precursors proteins and via intracellular phosphorylation of EGFR mediated by Src (Santiskulvong and Rozengurt, 2007). The importance of EGFR has been demonstrated in transgenic mice models in which pancreas-specific deletion of EGFR prevented *Kras*-induced development of PDAC (Ardito et al., 2012).

We hypothesize that the concomitant activation of PI3K/Akt (through insulin/IGF-1 and EGF receptors), PKD/ERK (via agonist-induced Gq signaling) and mTORC1 (synergistically through PI3K/Akt induced by insulin/IGF-1R and EGFR and GPCR-stimulated ERK/p90RSK) in PDAC cells potently stimulates DNA synthesis and proliferation of these cancer cells, and thus provide potential targets for chemotherapeutic intervention (**Figure 2**). Since both the ERK and PI3K pathways are effectors of KRAS, activating mutations of *KRAS* reinforce the crosstalk between insulin/IGF-1 receptor and GPCR signaling systems, thereby increasing the robustness of the network induced by insulin/IGF-1 and GPCR agonists in pancreatic cancer cells.

METFORMIN, AMPK, AND PDAC

Metformin (1,1-dimethylbiguanide hydrochloride) is the most widely prescribed drug for treatment of T2DM worldwide. Although it has been in clinical use for decades, its precise molecular mechanism of action remains incompletely understood. The primary systemic effect of metformin is the lowering of blood glucose levels through reduced hepatic gluconeogenesis and improved insulin sensitivity by increasing glucose uptake in peripheral tissues, including skeletal muscles and adipose tissue (Shaw et al., 2005). Metformin also reduces the circulating levels of insulin and IGF-1 in both diabetic and non-diabetic patients (Berker et al., 2004; Goodwin et al., 2008).

At the cellular level, metformin indirectly stimulates AMP–activated protein kinase (AMPK) activation (Hawley et al., 2010), though other cellular mechanisms of action have been proposed, especially at high concentrations (Sahra et al., 2008; Kalender et al., 2010). Metformin does not act directly on AMPK but inhibits complex I activity of the mitochondrial respiratory chain (El-Mir et al., 2000; Owen et al., 2000), resulting in reduced ATP

synthesis and increase in cellular AMP and ADP. AMPK is a conserved sensor of cellular energy being activated when ATP concentrations decrease and 5′-AMP concentrations increase (Kahn et al., 2005; Oakhill et al., 2011). Interestingly, AMPK is also implicated in the regulation of epithelial cell polarity (Mirouse et al., 2007), which is lost in advanced PanINs (Hingorani et al., 2003).

AMPK exists as a heterotrimer, composed of the catalytic kinase α subunit and two regulatory subunits, β and γ (Kahn et al., 2005). AMP directly binds to the AMPK γ subunit, causing allosteric activation and preventing dephosphorylation of Thr^{172} in the activation loop of the α subunit (Gowans et al., 2013). The tumor suppressor LKB-1/STK11 (Liver kinase B1/serine–threonine kinase 11) is the major kinase phosphorylating the AMPK activation loop. LKB-1/STK11 is mutated in the Peutz-Jegher syndrome (Kahn et al., 2005), characterized by predisposition to GI neoplasms, including PDAC.

AMPK is thought to inhibit mTORC1 function at three levels: (1) AMPK stimulates TSC2 function via phosphorylation on Ser^{1345} (Inoki et al., 2003, 2006; Shaw et al., 2004), leading to accumulation of Rheb-GDP (the inactive form) and thereby to inhibition of mTORC1 activation; (2) AMPK inhibits mTORC1 by direct phosphorylation of Raptor (on Ser^{722} and Ser^{792}), which disrupts its association with mTOR (Gwinn et al., 2008); (3) Insulin/IGF-1-induced mTORC1 activation is also attenuated by AMPK by direct phosphorylation of IRS-1 on Ser^{794}, a site that interferes with PI3K activation (Tzatsos and Tsichlis, 2007; Ning and Clemmons, 2010). Metformin, at high concentrations, also inhibits mTORC1 via AMPK-independent pathways, targeting Rag GTPases and/or REDD1 (Kalender et al., 2010; Ben Sahra et al., 2011). Since mTORC1 is a key site of signaling crosstalk in PDAC cells, we examined whether metformin opposes positive crosstalk between insulin/IGF-1 receptors and GPCR signaling systems in these cells.

In designing mechanistic experiments with metformin or other inhibitors of mitochondrial respiration such as the natural alkaloid berberine, it is important to use physiological concentrations of glucose in the culture medium. Cancer cells use aerobic glycolysis when the glucose concentration in the medium is very high but retain significant capacity of oxidative phosphorylation (Rossignol et al., 2004; Imamura et al., 2009; Vander Heiden et al., 2009). Thus, when cultured in regular DMEM (which contains 25 mM glucose), cells derive most of the ATP from glycolysis. In contrast, when the concentration of ambient glucose is physiological (∼5 mM) and glucose uptake rates are lower, cells derive part of their ATP from mitochondrial oxidative phosphorylation (Vazquez et al., 2010) and hence, are more sensitive to mild inhibitors of mitochondrial function, like metformin. Our results demonstrated that metformin prevented mTORC1 signaling in PDAC cells (Kisfalvi et al., 2009) and that the inhibitory effect of low doses of metformin on mTORC1 was markedly enhanced when PDAC cells were cultured in medium containing physiological concentrations of glucose (Sinnett-Smith et al., 2013; Soares et al., 2013). In this context, most previous studies *in vitro* with multiple cell types have used high concentrations of this agent to elicit effects [e.g., 5–30 mM], a condition that can lead to off-target effects. In addition to inhibit mTORC1, our results demonstrated that metformin prevented ERK activation in PDAC cells (Soares et al., 2013). Interestingly, the effects of metformin on Akt and ERK activation are strikingly different from allosteric or active-site mTOR inhibitors in PDAC cells, though all these agents potently inhibited the mTORC1/S6K axis (Soares et al., 2013). Furthermore, administration of metformin inhibited the growth of aggressive PDAC cells in xenograft models (Kisfalvi et al., 2013). Collectively, these studies imply that metformin inhibits mitogenic signaling, including mTORC1, ERK, and proliferation in PDAC cells and raise the attractive possibility that this anti-diabetic agent could offer a novel approach for the chemoprevention of PDAC (Rozengurt et al., 2010; Yue et al., 2014).

In line with this possibility, a number of epidemiological studies suggested a link between administration of metformin and reduced incidence of a variety of cancers in T2DM patients, including PDAC (Li et al., 2009; DeCensi et al., 2010; Lee et al., 2011; Bodmer et al., 2012; Franciosi et al., 2013; Zhang et al., 2013). Interestingly, metformin use in T2DM patients with PDAC was associated to better survival (Sadeghi et al., 2012). However, a meta-analysis of nine observational studies showed a trend but failed to show a significant association between metformin and PDAC risk (Singh et al., 2013). Methodological limitations and biases that potentially exaggerate the beneficial effects of metformin in observational studies have been identified (Gandini et al., 2014). In any case, epidemiological associations do not establish causation, but support the need for understanding mechanism(s) of action and for prospective clinical studies. For example, it will be of great interest to test anti-cancer effects of metformin on PDAC cells with complex I mutations that render them hypersensitive to inhibitors (Birsoy et al., 2014).

The elucidation of the mechanism(s) by which metformin targets cancer cells is key for advancing the field as can lead to novel therapeutic strategies, including the identification of specific patient populations that ultimately will benefit from metformin administration, the generation of preliminary biomarker evidence of target inhibition, will stimulate the development of second generation drugs and the design of combinatorial interventions.

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REFERENCES

Alemán, J. O., Eusebi, L. H., Ricciardiello, L., Patidar, K., Sanyal, A. J., and Holt, P. R. (2014). Mechanisms of obesity-induced gastrointestinal neoplasia. *Gastroenterology* 146, 357–373. doi: 10.1053/j.gastro.2013.11.051

Ardito, C. M., Grüner, B. M., Takeuchi, K. K., Lubeseder-Martellato, C., Teichmann, N., Mazur, P. K., et al. (2012). EGF Receptor is required for KRAS-induced pancreatic tumorigenesis. *Cancer Cell* 22, 304–317. doi: 10.1016/j.ccr.2012.07.024

Arslan, A. A., Helzlsouer, K. J., Kooperberg, C., Shu, X.-O., Steplowski, E., Bueno-de-Mesquita, H. B., et al. (2010). Anthropometric measures, body mass index,

and pancreatic cancer: a pooled analysis from the Pancreatic Cancer Cohort Consortium (PanScan). *Arch. Intern. Med.* 170, 791–802. doi: 10.1001/archinternmed.2010.63

Asano, T., Yao, Y., Shin, S., McCubrey, J., Abbruzzese, J. L., and Reddy, S. A. (2005). Insulin receptor substrate is a mediator of phosphoinositide 3-kinase activation in quiescent pancreatic cancer cells. *Cancer Res.* 65, 9164–9168. doi: 10.1158/0008-5472.CAN-05-0779

Bar-Peled, L., and Sabatini, D. M. (2014). Regulation of mTORC1 by amino acids. *Trends Cell Biol.* 24, 400–406. doi: 10.1016/j.tcb.2014.03.003

Beauchamp, E. M., and Platanias, L. C. (2013). The evolution of the TOR pathway and its role in cancer. *Oncogene* 32, 3923–3932. doi: 10.1038/onc.2012.567

Ben Sahra, I., Regazzetti, C., Robert, G., Laurent, K., Le Marchand-Brustel, Y., Auberger, P., et al. (2011). Metformin, independent of AMPK, induces mTOR inhibition and cell-cycle arrest through REDD1. *Cancer Res.* 71, 4366–4372. doi: 10.1158/0008-5472.CAN-10-1769

Berker, B., Emral, R., Demirel, C., Corapcioglu, D., Unlu, C., and Kose, K. (2004). Increased insulin-like growth factor-I levels in women with polycystic ovary syndrome, and beneficial effects of metformin therapy. *Gynecol. Endocrinol.* 19, 125–133. doi: 10.1080/09513590400007309

Birsoy, K., Possemato, R., Lorbeer, F. K., Bayraktar, E. C., Thiru, P., Yucel, B., et al. (2014). Metabolic determinants of cancer cell sensitivity to glucose limitation and biguanides. *Nature* 508, 108–112. doi: 10.1038/nature13110

Bodmer, M., Becker, C., Meier, C., Jick, S. S., and Meier, C. R. (2012). Use of antidiabetic agents and the risk of pancreatic cancer: a case-control analysis. *Am. J. Gastroenterol.* 107, 620–626. doi: 10.1038/ajg.2011.483

Dawson, D. W., Hertzer, K., Moro, A., Donald, G., Chang, H. H., Go, V. L., et al. (2013). High fat, high calorie diet promotes early pancreatic neoplasia in the conditional KrasG12D mouse model. *Cancer Prev. Res. (Phila).* 6, 1064–1073. doi: 10.1158/1940-6207.CAPR-13-0065

DeCensi, A., Puntoni, M., Goodwin, P., Cazzaniga, M., Gennari, A., Bonanni, B., et al. (2010). Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. *Cancer Prev. Res.* 3, 1451–1461. doi: 10.1158/1940-6207.CAPR-10-0157

Dibble, C. C., and Manning, B. D. (2013). Signal integration by mTORC1 coordinates nutrient input with biosynthetic output. *Nat. Cell Biol.* 15, 555–564. doi: 10.1038/ncb2763

Dong, X., Javle, M., Hess, K. R., Shroff, R., Abbruzzese, J. L., and Li, D. (2010). Insulin-like growth factor axis gene polymorphisms and clinical outcomes in pancreatic cancer. *Gastroenterology* 139, 464–473. doi: 10.1053/j.gastro.2010.04.042

El-Mir, M. Y., Nogueira, V., Fontaine, E., Averet, N., Rigoulet, M., and Leverve, X. (2000). Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. *J. Biol. Chem.* 275, 223–228. doi: 10.1074/jbc.275.1.223

Feng, Z., and Levine, A. J. (2010). The regulation of energy metabolism and the IGF-1/mTOR pathways by the p53 protein. *Trends Cell Biol.* 20, 427–434. doi: 10.1016/j.tcb.2010.03.004

Foster, K. G., and Fingar, D. C. (2010). Mammalian target of rapamycin (mTOR): conducting the cellular signaling symphony. *J. Biol. Chem.* 285, 14071–14077. doi: 10.1074/jbc.R109.094003

Franciosi, M., Lucisano, G., Lapice, E., Strippoli, G. F., Pellegrini, F., and Nicolucci, A. (2013). Metformin therapy and risk of cancer in patients with type 2 diabetes: systematic review. *PLoS ONE* 8:e71583. doi: 10.1371/journal.pone.0071583

Franke, T. F. (2008). PI3K/Akt: getting it right matters. *Oncogene* 27, 6473–6488. doi: 10.1038/onc.2008.313

Gandini, S., Puntoni, M., Heckman-Stoddard, B. M., Dunn, B. K., Ford, L., DeCensi, A., et al. (2014). Metformin and cancer risk and mortality: a systematic review and meta-analysis taking into account biases and confounders. *Cancer Prev. Res.* 7, 867–885. doi: 10.1158/1940-6207.CAPR-13-0424

Giovannucci, E., Harlan, D. M., Archer, M. C., Bergenstal, R. M., Gapstur, S. M., Habel, L. A., et al. (2010). Diabetes and cancer: a consensus report. *CA Cancer J. Clin.* 60, 207–221. doi: 10.3322/caac.20078

Goodwin, P., Pritchard, K., Ennis, M., Clemons, M., Graham, M., and Fantus, I. G. (2008). Insulin-lowering effects of metformin in women with early breast cancer. *Clin. Breast Cancer* 8:501. doi: 10.3816/CBC.2008.n.060

Gowans, G. J., Hawley, S. A., Ross, F. A., and Hardie, D. G. (2013). AMP is a true physiological regulator of AMP-activated protein kinase by both allosteric activation and enhancing net phosphorylation. *Cell Metab.* 18, 556–566. doi: 10.1016/j.cmet.2013.08.019

Guha, S., Eibl, G., Kisfalvi, K., Fan, R. S., Burdick, M., Reber, H., et al. (2005). Broad-spectrum G protein-coupled receptor antagonist, [D-Arg1,D-Trp5,7,9,Leu11]SP: a dual inhibitor of growth and angiogenesis in pancreatic cancer. *Cancer Res.* 65, 2738–2745. doi: 10.1158/0008-5472.CAN-04-3197

Guha, S., Rey, O., and Rozengurt, E. (2002). Neurotensin induces protein kinase C-dependent protein kinase D activation and DNA synthesis in human pancreatic carcinoma cell line PANC-1. *Cancer Res.* 62, 1632–1640.

Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Mery, A., Vasquez, D. S., et al. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol. Cell* 30, 214–226. doi: 10.1016/j.molcel.2008.03.003

Harikumar, K. B., Kunnumakkara, A. B., Ochi, N., Tong, Z., Deorukhkar, A., Sung, B., et al. (2010). A novel small-molecule inhibitor of protein kinase D blocks pancreatic cancer growth *in vitro* and *in vivo*. *Mol. Cancer Ther.* 9, 1136–1146. doi: 10.1158/1535-7163.MCT-09-1145

Harvey, A. E., Lashinger, L. M., Hays, D., Harrison, L. M., Lewis, K., Fischer, S. M., et al. (2014). Calorie restriction decreases murine and human pancreatic tumor cell growth, nuclear factor-κb activation, and inflammation-related gene expression in an insulin-like growth factor-1-dependent manner. *PLoS ONE* 9:e94151. doi: 10.1371/journal.pone.0094151

Hawley, S. A., Ross, F. A., Chevtzoff, C., Green, K. A., Evans, A., Fogarty, S., et al. (2010). Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. *Cell Metab.* 11, 554–565. doi: 10.1016/j.cmet.2010.04.001

Hingorani, S. R., Petricoin, E. F., Maitra, A., Rajapakse, V., King, C., Jacobetz, M. A., et al. (2003). Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* 4, 437–450. doi: 10.1016/S1535-6108(03)00309-X

Imamura, H., Huynh Nhat, K. P., Togawa, H., Saito, K., Iino, R., Kato-Yamada, Y., et al. (2009). Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators. *Proc. Natl. Acad. Sci. U.S.A.* 106, 15651–15656. doi: 10.1073/pnas.0904764106

Inoki, K., Ouyang, H., Zhu, T., Lindvall, C., Wang, Y., Zhang, X., et al. (2006). TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. *Cell* 126, 955–968. doi: 10.1016/j.cell.2006.06.055

Inoki, K., Zhu, T., and Guan, K. L. (2003). TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 115, 577–590. doi: 10.1016/S0092-8674(03)00929-2

Jones, S., Zhang, X., Parsons, D. W., Lin, J. C., Leary, R. J., Angenendt, P., et al. (2008). Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 321, 1801–1806. doi: 10.1126/science.1164368

Kahn, B. B., Alquier, T., Carling, D., and Hardie, D. G. (2005). AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab.* 1, 15–25. doi: 10.1016/j.cmet.2004.12.003

Kalender, A., Selvaraj, A., Kim, S. Y., Gulati, P., Brulé, S., Viollet, B., et al. (2010). Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner. *Cell Metabol.* 11, 390–401. doi: 10.1016/j.cmet.2010.03.014

Kan, Z., Jaiswal, B. S., Stinson, J., Janakiraman, V., Bhatt, D., Stern, H. M., et al. (2010). Diverse somatic mutation patterns and pathway alterations in human cancers. *Nature* 466, 869–873. doi: 10.1038/nature09208

Kisfalvi, K., Eibl, G., Sinnett-Smith, J., and Rozengurt, E. (2009). Metformin disrupts crosstalk between G protein-coupled receptor and insulin receptor signaling systems and inhibits pancreatic cancer growth. *Cancer Res.* 69, 6539–6545. doi: 10.1158/0008-5472.CAN-09-0418

Kisfalvi, K., Hurd, C., Guha, S., and Rozengurt, E. (2010). Induced overexpression of protein kinase D1 stimulates mitogenic signaling in human pancreatic carcinoma PANC-1 cells. *J. Cell Physiol.* 223, 309–316. doi: 10.1002/jcp.22036

Kisfalvi, K., Moro, A., Sinnett-Smith, J., Eibl, G., and Rozengurt, E. (2013). Metformin inhibits the growth of human pancreatic cancer xenografts. *Pancreas* 42, 781–785. doi: 10.1097/MPA.0b013e31827aec40

Kolodecik, T., Shugrue, C., Ashat, M., and Thrower, E. C. (2014). Risk factors for pancreatic cancer: underlying mechanisms and potential targets. *Front. Physiol.* 4:415. doi: 10.3389/fphys.2013.00415

Lashinger, L. M., Harrison, L. M., Rasmussen, A. J., Logsdon, C. D., Fischer, S. M., McArthur, M. J., et al. (2013). Dietary energy balance modulation of Kras- and Ink4a/Arf+/–driven pancreatic cancer: the role of insulin-like growth factor-I. *Cancer Prev. Res.* 6, 1046–1055. doi: 10.1158/1940-6207.CAPR-13-0185

Lee, J. W., Park, S., Takahashi, Y., and Wang, H.-G. (2010). The association of AMPK with ULK1 regulates autophagy. *PLoS ONE* 5:e15394. doi: 10.1371/journal.pone.0015394

Lee, M. S., Hsu, C. C., Wahlqvist, M. L., Tsai, H. N., Chang, Y. H., and Huang, Y. C. (2011). Type 2 diabetes increases and metformin reduces total, colorectal, liver and pancreatic cancer incidences in Taiwanese: a representative population prospective cohort study of 800,000 individuals. *BMC Cancer* 11:20. doi: 10.1186/1471-2407-11-20

Li, D., Yeung, S.-C. J., Hassan, M. M., Konopleva, M., and Abbruzzese, J. L. (2009). Anti-diabetic therapies affect risk of pancreatic cancer. *Gastroenterology* 137, 482–488. doi: 10.1053/j.gastro.2009.04.013

Metz, H. E., and McGarry Houghton, A. (2011). Insulin receptor substrate regulation of phosphoinositide 3-kinase. *Clin. Cancer Res.* 17, 206–211. doi: 10.1158/1078-0432.CCR-10-0434

Mirouse, V., Swick, L. L., Kazgan, N., St Johnston, D., and Brenman, J. E. (2007). LKB1 and AMPK maintain epithelial cell polarity under energetic stress. *J. Cell Biol.* 177, 387–392. doi: 10.1083/jcb.200702053

Murphy, S. J., Hart, S. N., Lima, J. F., Kipp, B. R., Klebig, M., Winters, J. L., et al. (2013). Genetic alterations associated with progression from pancreatic intraepithelial neoplasia to invasive pancreatic tumor. *Gastroenterology* 145, 1098–1109.e1091. doi: 10.1053/j.gastro.2013.07.049

Ning, J., and Clemmons, D. R. (2010). AMP-activated protein kinase inhibits IGF-I signaling and protein synthesis in vascular smooth muscle cells via stimulation of insulin receptor substrate 1 S794 and tuberous sclerosis 2 S1345 phosphorylation. *Mol. Endocrinol.* 24, 1218–1229. doi: 10.1210/me.2009-0474

Oakhill, J. S., Steel, R., Chen, Z.-P., Scott, J. W., Ling, N., Tam, S., et al. (2011). AMPK Is a direct adenylate charge-regulated protein kinase. *Science* 332, 1433–1435. doi: 10.1126/science.1200094

Ochi, N., Tanasanvimon, S., Matsuo, Y., Tong, Z., Sung, B., Aggarwal, B. B., et al. (2011). Protein kinase D1 promotes anchorage-independent growth, invasion, and angiogenesis by human pancreatic cancer cells. *J. Cell Physiol.* 226, 1074–1081. doi: 10.1002/jcp.22421

Owen, M. R., Doran, E., and Halestrap, A. P. (2000). Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem. J.* 348, 607–614. doi: 10.1042/0264-6021:3480607

Rahib, L., Smith, B. D., Aizenberg, R., Rosenzweig, A. B., Fleshman, J. M., and Matrisian, L. M. (2014). Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res.* 74, 2913–2921. doi: 10.1158/0008-5472.CAN-14-0155

Rey, O., Yuan, J., and Rozengurt, E. (2003a). Intracellular redistribution of protein kinase D2 in response to G- protein-coupled receptor agonists. *Biochem. Biophys. Res. Commun.* 302, 817–824. doi: 10.1016/S0006-291X(03)00269-9

Rey, O., Yuan, J., Young, S. H., and Rozengurt, E. (2003b). Protein kinase C nu/protein kinase D3 nuclear localization, catalytic activation, and intracellular redistribution in response to G protein-coupled receptor agonists. *J. Biol. Chem.* 278, 23773–23785. doi: 10.1074/jbc.M300226200

Rossignol, R., Gilkerson, R., Aggeler, R., Yamagata, K., Remington, S. J., and Capaldi, R. A. (2004). Energy substrate modulates mitochondrial structure and oxidative capacity in cancer cells. *Cancer. Res.* 64, 985–993. doi: 10.1158/0008-5472.CAN-03-1101

Rozengurt, E. (1986). Early signals in the mitogenic response. *Science* 234, 161–166. doi: 10.1126/science.3018928

Rozengurt, E. (2007). Mitogenic signaling pathways induced by G protein-coupled receptors. *J. Cell. Physiol.* 213, 589–602. doi: 10.1002/jcp.21246

Rozengurt, E., Rey, O., and Waldron, R. T. (2005). Protein kinase D signaling. *J. Biol. Chem.* 280, 13205–13208. doi: 10.1074/jbc.R500002200

Rozengurt, E., Sinnett-Smith, J., and Kisfalvi, K. (2010). Crosstalk between insulin/insulin-like growth factor-1 receptors and G protein-coupled receptor signaling systems: a novel target for the antidiabetic drug metformin in pancreatic cancer. *Clin. Cancer Res.* 16, 2505–2511. doi: 10.1158/1078-0432.CCR-09-2229

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.

Sadeghi, N., Abbruzzese, J. L., Yeung, S.-C. J., Hassan, M., and Li, D. (2012). Metformin use is associated with better survival of diabetic patients with pancreatic cancer. *Clin. Cancer. Res.* 18, 2905–2912. doi: 10.1158/1078-0432.CCR-11-2994

Sahra, I. B., Laurent, K., Loubat, A., Giorgetti-Peraldi, S., Colosetti, P., Auberger, P., et al. (2008). The antidiabetic drug metformin exerts an antitumoral effect *in vitro* and *in vivo* through a decrease of cyclin D1 level. *Oncogene* 27:3576. doi: 10.1038/sj.onc.1211024

Santiskulvong, C., and Rozengurt, E. (2007). Protein kinase Calpha mediates feedback inhibition of EGF receptor transactivation induced by G(q)-coupled receptor agonists. *Cell Signal.* 19, 1348–1357. doi: 10.1016/j.cellsig.2007.01.006

Shaw, R. J., Bardeesy, N., Manning, B. D., Lopez, L., Kosmatka, M., DePinho, R. A., et al. (2004). The LKB1 tumor suppressor negatively regulates mTOR signaling. *Cancer Cell* 6, 91–99. doi: 10.1016/j.ccr.2004.06.007

Shaw, R. J., Lamia, K. A., Vasquez, D., Koo, S. H., Bardeesy, N., Depinho, R. A., et al. (2005). The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science* 310, 1642–1646. doi: 10.1126/science.1120781

Siegel, R., Ma, J., Zou, Z., and Jemal, A. (2014). Cancer statistics, 2014. *CA Cancer J. Clin.* 64, 9–29. doi: 10.3322/caac.21208

Singh, S., Singh, P. P., Singh, A. G., Murad, M. H., McWilliams, R. R., and Chari, S. T. (2013). Anti-diabetic medications and risk of pancreatic cancer in patients with diabetes mellitus: a systematic review and meta-analysis. *Am. J. Gastroenterol.* 108, 510–519. doi: 10.1038/ajg.2013.7

Sinnett-Smith, J., Kisfalvi, K., Kui, R., and Rozengurt, E. (2013). Metformin inhibition of mTORC1 activation, DNA synthesis and proliferation in pancreatic cancer cells: dependence on glucose concentration and role of AMPK. *Biochem. Biophys. Res. Commun.* 430, 352–3527. doi: 10.1016/j.bbrc.2012.11.010

Soares, H. P., Ni, Y., Kisfalvi, K., Sinnett-Smith, J., and Rozengurt, E. (2013). Different patterns of Akt and ERK feedback activation in response to rapamycin, active-site mTOR inhibitors and metformin in pancreatic cancer cells. *PLoS ONE* 8:e57289. doi: 10.1371/journal.pone.0057289

Song, M. S., Salmena, L., and Pandolfi, P. P. (2012). The functions and regulation of the PTEN tumour suppressor. *Nat. Rev. Mol. Cell Biol.* 13, 283–296. doi: 10.1038/nrm3330

Subramani, R., Lopez-Valdez, R., Arumugam, A., Nandy, S., Boopalan, T., and Lakshmanaswamy, R. (2014). Targeting insulin-like growth factor 1 receptor inhibits pancreatic cancer growth and metastasis. *PLoS ONE* 9:e97016. doi: 10.1371/journal.pone.0097016

Taniguchi, C. M., Emanuelli, B., and Kahn, C. R. (2006). Critical nodes in signalling pathways: insights into insulin action. *Nat. Rev. Mol. Cell. Biol.* 7, 85–96. doi: 10.1038/nrm1837

Tanti, J.-F., and Jager, J. (2009). Cellular mechanisms of insulin resistance: role of stress-regulated serine kinases and insulin receptor substrates (IRS) serine phosphorylation. *Curr. Opin. Pharmacol.* 9, 753–762. doi: 10.1016/j.coph.2009.07.004

Tzatsos, A., and Tsichlis, P. N. (2007). Energy depletion inhibits phosphatidylinositol 3-kinase/Akt signaling and induces apoptosis via AMP-activated protein kinase-dependent phosphorylation of IRS-1 at Ser-794. *J. Biol. Chem.* 282, 18069–18082. doi: 10.1074/jbc.M610101200

Vander Heiden, M. G., Cantley, L. C., and Thompson, C. B. (2009). Understanding the warburg effect: the metabolic requirements of cell proliferation. *Science* 324, 1029–1033. doi: 10.1126/science.1160809

Vazquez, A., Liu, J., Zhou, Y., and Oltvai, Z. N. (2010). Catabolic efficiency of aerobic glycolysis: the Warburg effect revisited. *BMC Syst. Biol.* 4:58. doi: 10.1186/1752-0509-4-58

Yuan, J., and Rozengurt, E. (2008). PKD, PKD2, and p38 MAPK mediate Hsp27 serine-82 phosphorylation induced by neurotensin in pancreatic cancer PANC-1 cells. *J. Cell Biochem.* 103, 648–662. doi: 10.1002/jcb.21439

Yue, W., Yang, C. S., DiPaola, R. S., and Tan, X.-L. (2014). Repurposing of metformin and aspirin by targeting AMPK-mTOR and inflammation for pancreatic cancer prevention and treatment. *Cancer Prev. Res.* 7, 388–397. doi: 10.1158/1940-6207.CAPR-13-0337

Zhang, P., Li, H., Tan, X., Chen, L., and Wang, S. (2013). Association of metformin use with cancer incidence and mortality: a meta-analysis. *Cancer Epidemiol.* 37, 207–218. doi: 10.1016/j.canep.2012.12.009

Zoncu, R., Efeyan, A., and Sabatini, D. M. (2011). mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat. Rev. Mol. Cell. Biol.* 12, 21–35. doi: 10.1038/nrm3025

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Pancreatic cancer cachexia: a review of mechanisms and therapeutics

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Over the last decade, we have gained new insight into the pathophysiology of cachexia associated with pancreatic cancer. Unfortunately, its treatment is complex and remains a challenge. Pancreatic cancer cachexia is a multifactorial syndrome characterized by uncompensated adipose tissue and skeletal muscle loss in the setting of anorexia that leads to progressive functional impairment. This paper will review the current concepts of pancreatic cancer cachexia, its assessment and pathophysiology as well as current and future treatments. The successful management of pancreatic cancer cachexia will likely require a multimodal approach that includes nutritional support and combination pharmaceutical interventions.

Keywords: pancreatic cancer, cachexia, anorexia, catabolism, multimodal therapy

INTRODUCTION

Cachexia is a ubiquitous characteristic of pancreatic cancer and develops in approximately 80% of pancreatic cancer patients during their disease course (Fearon et al., 2006b). Up to one-third die from complications associated with cachexia through immobility, severe respiratory muscle impairment resulting in cardiopulmonary failure, and impaired immunity (Bachmann et al., 2009). Cachexia is a complex metabolic disorder that involves features of anorexia, anemia, and loss of adipose and skeletal muscle mass. In pancreatic cancer patients, it has been associated with reduced physical function, lower response rates to chemotherapy and radiation treatment, and decreased survival (Dewys et al., 1980; Moses et al., 2004; Bachmann et al., 2008, 2009). Pre-operative evidence of cachexia in pancreatic cancer patients has been also associated with worse postoperative outcome after pancreaticoduodenectomy (Pausch et al., 2012).

Although new insights to the pathogenesis of pancreatic cancer cachexia have been gained over the past decade, the underlying mechanisms leading to this syndrome are not fully understood. There continues to be an active search for potential targets and effective treatment. This article reviews the current concepts and management of this clinical dilemma.

DEFINITION AND CLASSIFICATION OF CANCER CACHEXIA

Cachexia has been recognized as a common complication of cancer. In 2011, an international consensus defined cancer cachexia as a multifactorial syndrome characterized by ongoing loss of skeletal muscle mass that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment (Fearon et al., 2011). Diagnostic criteria include weight loss greater than 5% over the past 6 months, weight loss greater than 2% in individuals with body mass index (BMI) less than 20 kg/m^2, or evidence of sarcopenia with any degree of weight loss greater than 2% (**Table 1**). Evidence of sarcopenia is defined as appendicular skeletal muscle index less than 7.26 kg/m^2 in males and less than 5.45 kg/m^2 in females determined by dual energy X-ray absorptiometry (DEXA). Based on these criteria, the majority of pancreatic cancer patients have cachexia at the time of diagnosis (Fearon et al., 2006b).

Cancer cachexia develops progressively through a spectrum. The international consensus identified three stages of cachexia: precachexia, cachexia, and refractory cachexia (Fearon et al., 2011). Severity is classified based on the degree of depletion of energy stores and body protein mass (using BMI) and the rate of ongoing weight loss. In precachexia, patients demonstrate early clinical and metabolic signs including anorexia and impaired glucose tolerance preceding substantial involuntary weight loss. Patients then develop progressive weight loss and meet the criteria for cachexia as previously defined. Cachexia becomes clinically refractory as a result of progressive cancer unresponsive to therapy. In this stage, there is active catabolism, and patients have worsening physical function with a life expectancy of less than 3 months.

ASSESSMENT OF CANCER CACHEXIA

Since cachexia is a multifactorial syndrome, its evaluation should involve assessment for various features as summarized in **Table 2**: anorexia or reduced food intake, catabolic drivers, muscle mass and strength, and functional and psychosocial effects (Fearon et al., 2011). Some of these different characteristics of cancer cachexia have been found to be adverse prognostic indicators. A recent study showed that weight loss (>10% weight loss), reduced food intake (<1500 kcal/day), and evidence of systemic inflammation [C-reactive protein (CRP) > 10 mg/L] identified

Table 1 | Diagnosis of cancer cachexia (Fearon et al., 2011).

Weight loss greater than 5% over the past 6 months; or Weight loss greater than 2% in individuals with BMI less than 20 kg/m^2; or Evidence of sarcopenia* with weight loss greater than 2%

**Sarcopenia defined as appendicular skeletal muscle index in males < 7.26 kg/m^2 and in females < 5.45 kg/m^2 determined by DEXA.*

Table 2 | Assessment of cancer cachexia.

Areas of assessment	Methods
Reduced food intake/ anorexia	Patients estimate overall food intake Third-party assessment of food intake (family member) Assess for mechanical factors contributing to reduced intake
Hypercatabolism	Serum CRP levels Responsiveness to treatment and rate of disease progression
Muscle mass and strength	Cross-sectional imaging with CT or MRI DEXA: appendicular skeletal muscle index Anthropometry: mid-upper-arm muscle area Bioimpedance analysis: whole body fat-free mass index
Physical and psychosocial functioning	EORTC QLQ-C30 ECOG questionnaire Karnofsky performance score Electric activity meters Checklists of specific activities

CRP, C-reactive protein; CT, computed tomography; MRI, magnetic resonance imaging; DEXA, dual-energy X-ray absorptiometry; EORTC QLQ-C30, European Organization for Research and Treatment of Cancer quality of life questionnaire C-30; ECOG, Eastern Cooperative Oncology Group.

pancreatic cancer patients with reduced subjective and objective functional ability. Patients with at least two of these components had a significantly worse prognosis (Fearon et al., 2006b).

Evaluation of food intake should be routinely performed. At the minimum, patients can be asked to estimate their overall food intake in relation to normal intake with dietary or recall records. Another simple method for prospective third-party assessment of food intake is the percentile calculation of food consumed at each meal by a family member (Bruera and Sweeney, 2000). Patients should also be evaluated for underlying factors that contribute to reduced food intake, such as lack of appetite, chemosensory disturbances, dysphagia, decreased gastrointestinal (GI) motility, pain, and fatigue.

A key component of pancreatic cancer cachexia is hypercatabolism due to direct tumor metabolism, systemic inflammation, or other tumor-mediated effects. Hypercatabolism due to systemic inflammation can be assessed using serum CRP levels (Moses et al., 2009). Indirect indices of catabolism include responsiveness to chemotherapy and rate of disease progression.

Cancer cachexia is characterized by ongoing skeletal muscle loss. There are various methods for muscle mass assessment: cross-sectional imaging with computed tomography (CT) or magnetic resonance imaging (MRI); appendicular skeletal muscle index obtained from DEXA; mid-upper-arm muscle area by anthropometry; and whole body fat-free mass index determined by bioimpedance analysis (Simons et al., 1995; Prado et al., 2009; Fearon et al., 2011; Di Sebastiano and Mourtzakis, 2012). Imaging-based methods of muscle mass assessment can quantify changes in body composition, including skeletal muscle wasting, altered distribution of body fat, and pathological accumulation of lipids in various tissues. MRI can measure the volume of the quadriceps muscle with a coefficient of variation < 1%. Diagnostic CT scans can be used to estimate abdominal muscle cross-sectional area at the L3 level, which can be extrapolated to whole body lean body mass. These modalities are usually reserved for research purposes and not routinely used in the clinic.

A comprehensive approach, including history, physical examination, and various imaging studies can aid in recognizing the phenomenon termed sarcopenic obesity or cachexia hidden in the context of obesity. Even at the time of diagnosis, approximately 40% of overweight or obese pancreatic cancer patients have substantial ongoing skeletal muscle wasting (Tan et al., 2009). Early detection of sarcopenic obesity is important because it has been shown to be an independent prognostic factor for decreased survival in pancreatic cancer patients (Tan et al., 2009).

Cancer cachexia can have a profound adverse effect on physical and psychosocial functioning. Patients report altered body images, which can significantly impact emotions and relationships. The method of choice for evaluating functional effects of cancer cachexia is the routine use of patient-reported physical functioning. This assessment can be obtained using the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire (QLQ)-C30 or the Eastern Cooperative Oncology Group (ECOG) questionnaire. Physician-reported physical activity (Karnofsky performance score) and objective methodologies such as electric activity meters or checklists of specific activities can also be used to assess physical functioning. A recent study with subjects wearing an electric activity monitor showed that the level of physical activity in cachectic cancer patients is reduced by about 40% (Dahele et al., 2007).

MECHANISMS OF CANCER CACHEXIA

The pathophysiology of cancer cachexia is characterized by negative protein and energy balance driven by a combination of reduced food intake and increased metabolism (Fearon, 2008, 2012; Fearon et al., 2011). This process involves complex interactions between the host and the tumor (**Figure 1**). There are mechanical factors that contribute to reduced food intake. There is evidence that anorexia and hypercatabolism are driven by cytokines, circulating hormones, neuropeptides, neurotransmitters, and tumor-derived factors. In addition, recent studies have discovered other potentially significant processes involved in pancreatic cancer cachexia, including neural invasion and abnormalities in the muscle microenvironment. This section will review

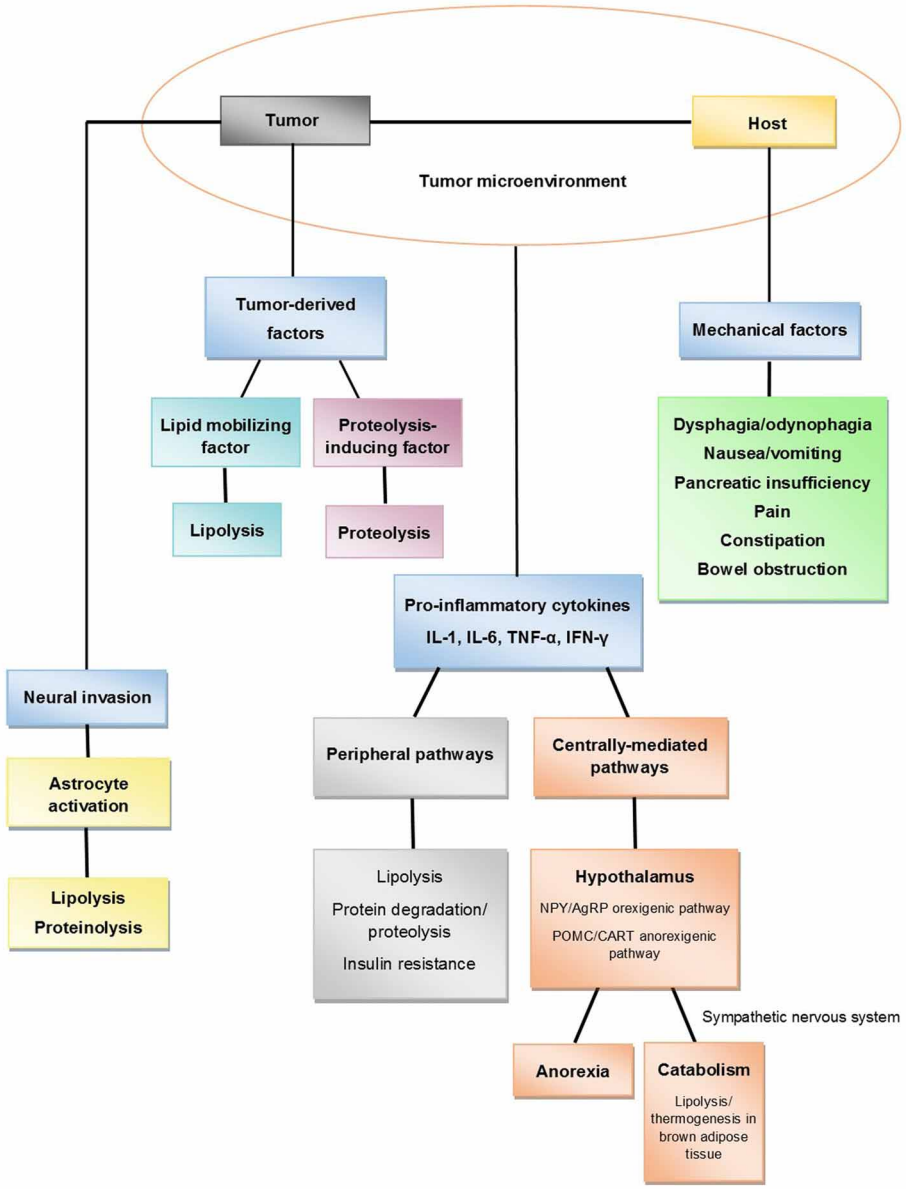

FIGURE 1 | Proposed mechanisms of pancreatic cancer cachexia.

current proposed mechanisms that lead to the development of this disease process.

MECHANICAL FACTORS

Reduced food intake can promote and maintain cancer-associated weight loss (Wigmore et al., 1997b). Mechanical digestive abnormalities can result in a lack of appetite and reduced food intake. Patients with pancreatic cancer suffer from pain, fatigue, nausea, dysphagia, gastroparesis, duodenal stenosis, pancreatic insufficiency and malabsorption, and constipation (Deutsch and Kolhouse, 2004). These symptoms are the direct consequence of tumor invasion, which can result in the obstruction of the pancreatic duct and/or GI tract, particularly the second portion of the duodenum. Many patients will undergo pancreaticoduodenectomy for the resection of a pancreatic head mass. This procedure can worsen pancreatic insufficiency and decrease oral intake.

CYTOKINES AND SYSTEMIC INFLAMMATION

Systemic inflammation plays an important role in the pathophysiology of pancreatic cancer cachexia. Elevated CRP levels (CRP $>$ 10 mg/L), an indirect measure of systemic inflammation, has been associated with cachexia and poor prognosis in pancreatic cancer patients (Fearon et al., 2006b). Elevated cytokine levels, including IL-6 and IL-10, have been associated with poor performance, weight loss, and decreased survival in pancreatic

cancer patients (Ebrahimi et al., 2004; Moses et al., 2009). Recent evidence strongly suggest that cytokines produced by tumor cells or released by the host as a response to the cancer affect various pathways that lead to anorexia and a hypercatabolic state (**Figure 1**). These pathways can be divided into central pathways, which are hypothalamus-mediated, and peripheral pathways, which involve direct lipolysis and proteolysis.

Centrally-mediated pathways

Under normal conditions, energy homeostasis is a highly regulated system of controls. The hypothalamus controls energy intake by integrating peripheral signals that convey information on energy and adiposity status. These inputs are transduced into neuronal responses and, via signaling pathways, behavioral responses. Current evidence suggests that systemic inflammation plays a critical role in inducing cancer anorexia by triggering a complex neurochemical cascade (Laviano et al., 2003; Fearon et al., 2013; Suzuki et al., 2013; Tuca et al., 2013). Increased cytokine expression from tumor growth prevents the hypothalamus from responding appropriately to peripheral signals by persistent stimulation of anorexigenic pathways and inhibition of orexigenic pathways (Suzuki et al., 2013; Tuca et al., 2013).

Cancer anorexia may be partially due to derangement of peripheral signaling transduction into neuronal responses by the hypothalamus. There are two pathways that control energy expenditure and food intake within the hypothalamus: neuropeptide Y (NPY)/Agouti-related peptide (AgRP) neurons that stimulate energy intake and pro-opiomelanocortin (POMC)/cocaine and amphetamine-regulated transcript (CART) neurons that inhibit intake. Some studies suggest that cancer cachexia is associated with hyperactivation of the POMC/CART pathway which may be triggered by IL-1 and other pro-inflammatory cytokines (Wisse et al., 2001; Marks and Cone, 2003; Marks et al., 2003; Scarlett et al., 2007).

Leptin is a protein involved in regulating energy intake and expenditure. Leptin reduces appetite and increases energy expenditure via the central nervous system (CNS). Through feedback signaling, leptin controls the production, and activation of hypothalamic neuropeptides that regulate food intake and energy expenditure, including NPY and corticotropin-releasing factor (CRF). Since leptin is primarily released by adipose tissue, decreased body fat mass or starvation leads to a decrease in leptin levels. Low leptin levels allow for increased production, release, and action of NPY, a potent orexigenic peptide, which subsequently results in the activation of the NPY/AgRP pathway. Additionally, low leptin levels result in decreased activity of anorexigenic neuropepetides, such as CRF and melanocortin. Studies have demonstrated that cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin1 (IL-1) increase the expression of leptin mRNA in adipocytes and plasma levels of leptin despite starvation (Grunfeld et al., 1996; Janik et al., 1997; Sarraf et al., 1997; Finck et al., 1998). Therefore, an increased leptin level may contribute to cancer anorexia by preventing the normal compensatory mechanisms that should occur with decreased food intake. However, other studies have shown that these cytokines can induce anorexia even in the absence of leptin (Faggioni et al., 1997). Animal and clinical studies have also demonstrated that leptin levels are not elevated in tumor-bearing rats and patients with cancer cachexia (Simons et al., 1997; Wallace et al., 1998; Mantovani et al., 2000; Bing et al., 2001). Recent evidence suggests that in cancer cachexia IL-1 and TNF-α mimic leptin signaling and interfere with the orexigenic response to reduced leptin levels (Inui, 1999; Suzuki et al., 2013). Therefore, even with decreased adiposity, there continues to be suppression of the orexigenic response and stimulation of the anorexigenic pathway, resulting in unopposed anorexia and increased energy expenditure.

Serotonin may also play an important role in the development of cancer anorexia through the melanocortin system. Studies have established that IL-1 stimulates the release of hypothalamic serotonin (Shintani et al., 1993). Elevated serotonin levels, in turn, contribute to the persistent activation of POMC/CART neurons, resulting in decreased appetite and anorexia (Heisler et al., 2002). Studies have demonstrated elevated plasma and cerebrospinal fluid concentrations of tryptophan, a precursor of serotonin, in patients with evidence of cancer cachexia compared to healthy controls or cancer patients without cachexia (Cangiano et al., 1990). Plasma tryptophan concentration normalized, and food intake improved after tumor removal (Cangiano et al., 1994). These findings suggest that hypothalamic serotonin may be an important factor in the pathogenesis of cancer cachexia and a potential therapeutic target.

These hypothalamic pathways and neuropeptides also have catabolic effects. The POMC/CART anorexigenic pathway increases the sympathetic nervous system activity, which causes induction of mitochondrial uncoupling proteins, such as UCP-1 and UCP-2 (Li et al., 2002; Arruda et al., 2010). UCP-1 channels protons across the inner mitochondrial membrane without ATP production, resulting in thermogenesis and energy expenditure in brown adipose tissue (Li et al., 2002; Arruda et al., 2010).

Peripheral pathways

Cytokines not only corroborate and sustain the neurochemical changes responsible for anorexia, they have also been shown to induce lipolysis, muscle catabolism, and the hepatic acute phase protein response (APPR) through various pathways. These processes lead to the development of uncompensated loss of muscle and adipose tissue mass.

TNF-α. TNF-α was first identified as a cachexia-inducing factor in chronic diseases. It may have properties that promote lipolysis, impair lipogenesis, and induce muscle degradation. It has been shown to induce lipolysis *in vitro* with increases in glycerol release in mouse and human adipocytes, likely through down-regulation of perilipin expression (Rydén et al., 2004). Perilipin coats intracellular lipid droplets and acts as a barrier to lipolysis. Decreased perilipin expression subsequently enables hormone-sensitive lipase (HSL), a key regulator of lipolysis, to access the surface of lipid droplets for breakdown (Zhang et al., 2002; Rydén et al., 2004). TNF-α also has an inhibitory effect on adipocyte differentiation, resulting in impaired lipogenesis (Cawthorn et al., 2007; Hammarstedt et al., 2007).

Animal studies also suggest that TNF-α is involved in muscle loss in cancer cachexia. Mouse models have shown that TNF-α may induce muscle protein degradation through formation of

reactive oxygen species (ROS). Oxidative stress results in the activation of nuclear factor κB (NFκB) which, in turn, activates the ubiquitin-proteasome pathway (Llovera et al., 1998; Li and Reid, 2000). Moreover, TNF-α has been shown to increase expression of the 1.2- and 2.4-kb transcripts of ubiquitin and the ubiquitin ligase atrogin 1/MAFbx in skeletal muscle (Llovera et al., 1998; Li and Reid, 2000). In addition to protein degradation, TNF-α has been shown to inhibit myogenesis *in vitro* through NFκB-mediated downregulation of MyoD transcripts (Guttridge et al., 2000).

Although these findings suggest a role for TNF-α in lipolysis and proteolysis, its importance in cancer cachexia is an active area of debate. Results from studies measuring levels of TNF-α in patients with cancer cachexia have been conflicting. Some studies have shown detectable levels of TNF-α in the serum of pancreatic cancer patients with TNF-α levels inversely correlating with body weight and BMI; other studies involving patients with advanced cancers have shown no correlation between circulating TNF-α levels, weight loss, and anorexia (Maltoni et al., 1997; Karayiannakis et al., 2001; Rydén et al., 2008). Therefore, the origin and relevance of TNF-α to cancer cachexia remains unclear.

IL-6. IL-6 is another important cytokine in pancreatic cancer cachexia. IL-6 secretion is induced by TNF-α; it acts synergistically with TNF-α in many of its actions including stimulation of other cytokines. Circulating levels of IL-6 correlate with weight loss and reduced survival in pancreatic cancer patients (Ebrahimi et al., 2004; Martignoni et al., 2005; Moses et al., 2009). Although the role of IL-6 in lipolysis is not well established, a recent study has shown enhanced IL-6 signaling in brown adipose tissue in cachectic tumor-bearing mice suggesting that it may play a direct role in the activation of thermogenesis (Tsoli et al., 2012). More importantly, IL-6 is known to activate the hepatic APPR and trigger tissue catabolism. The murine C-26 cachexia model has shown that increasing levels of IL-6 correlated with the development of cachexia; treatment with an IL-6 neutralizing antibody attenuated the development of weight loss (Strassmann et al., 1992). Moses et al. found that pancreatic cancer patients with cachexia had elevated CRP levels and stimulated IL-6 production (Moses et al., 2009). Although various cytokines and hormones affect hepatocyte protein metabolism, IL-6 is known as the principal regulator of APPR in human hepatocytes (Castell et al., 1990). There is a strong correlation between increased peripheral blood mononuclear cells (PBMC) production of IL-6 and the presence of elevated APPR (Martignoni et al., 2005, 2009; Moses et al., 2009). The activation of hepatic APPR subsequently results in hypercatabolism through reprioritization of body protein metabolism from skeletal muscle to production of acute phase proteins (Fearon et al., 1999). There appears to be a two- to three-fold increase in fibrinogen production and increase in serum CRP levels (Preston et al., 1998). Production of these acute phase proteins by the liver is associated with mobilization of peripheral amino acid stores primarily from skeletal muscle contributing to the loss of lean tissue and catabolism. Overproduction of IL-6 and elevated APPR have been associated with decreased survival in patients with pancreatic cancer cachexia (Moses et al., 2009).

TUMOR-DERIVED FACTORS

In addition to cytokines and systemic inflammation, tumor-derived factors contribute to metabolic abnormalities that give rise to pancreatic cancer cachexia. Two of the most well studied factors are lipid mobilizing factor (LMF) and proteolysis-inducing factor (PIF). The presence and role of other factors that contribute to pancreatic cancer cachexia are currently being investigated.

Lipid mobilizing factor

Todorov et al. isolated a LMF from a cachexia-inducing murine tumor (MAC16 adenocarcinoma) model and the urine of patients with unresectable pancreatic cancer and weight loss (Todorov et al., 1998). The material was 43 kDA and was found to be homologous with the plasma protein zinc-α2-glycoprotein (ZAG) (Todorov et al., 1998). Pancreatic cancer patients with weight loss generally had LMF/ZAG in the urine, but it was absent from patients without weight loss or normal subjects (Todorov et al., 1998). A recent study identifying serum proteins involved in pancreatic cancer cachexia identified LMF/ZAG as a possible marker (Felix et al., 2011). Moreover, immunohistochemical analysis demonstrated LMF/ZAG expression in pancreatic cancer cells and in the peritumoral stroma (Felix et al., 2011). Patients with cachexia had stronger immunostaining compared to pancreatic cancer patients without cachexia or normal subjects (Felix et al., 2011).

In vivo studies have shown that LMF/ZAG causes selective loss of carcass fat without change in body water or nonfat mass (Hirai et al., 1998). LMF/ZAG directly induces lipolysis by stimulating adenylate cyclase in a GTP-dependent process; this process is postulated to be mediated by β3 adrenergic receptors (Hirai et al., 1998; Khan and Tisdale, 1999; Russell et al., 2002). Hirai et al. showed an increase in serum levels of glycerol and 3-hydroxybutyrate after treating mice with LMF/ZAG. They also showed a significant increase in oxygen uptake by brown adipose tissue suggesting that LMF/ZAG promotes lipid utilization (Hirai et al., 1998). In addition, LMF/ZAG has been shown to increase lipid oxidation using the production of $^{14}CO_2$ from [^{14}C-carboxy]triolein (Russell and Tisdale, 2002). This function is achieved by directly activating the expression of mitochondrial UCPs. LMF/ZAG induces increased expression of UCP-1, UCP-2, and UCP-3 in brown adipose tissue, and UCP-2 in skeletal muscle and liver (Bing et al., 2002). The effect of LMF/ZAG on lipid oxidation and utilization is also likely mediated by β3 adrenergic receptors. LMF/ZAG also increases the sensitivity of white adipose tissues to the lipolytic effects of other stimuli, including catecholamines (Islam-Ali et al., 2001). Adipocyte plasma membranes have Gs α-subunits and Gi α-subunits, which stimulate and inhibit adenylate cyclase, respectively. LMF/ZAG increases Gαs expression and decreases Gαi expression, which favor mobilization of lipid stores from adipocytes and facilitate a catabolic state (Islam-Ali et al., 2001). LMF/ZAG not only increases lipid mobilization through various pathways but it also increases substrate utilization and activates mitochondrial oxidative pathways in brown adipose tissue resulting in lipolysis, increased energy expenditure, and hypercatabolism.

PROTEOLYSIS-INDUCING FACTOR

PIF was discovered in 1996 using a MAC16 adenocarcinoma mouse model of cachexia. Todorov et al. reported the discovery of a glycoprotein of molecular mass 24 kDa that produced cachexia *in vivo* by inducing skeletal muscle catabolism (Todorov et al., 1996). The same material was isolated from urine of cachectic cancer patients, but not from patients with weight loss due to trauma, cancer patients with little or no weight loss, and normal subjects. PIF was detected in the urine of 80% of pancreatic cancer patients with significantly greater total weight loss and rate of weight loss than patients who did not have PIF in their urine (Wigmore et al., 2000b). Immunochemistry demonstrated that the 24 kDa material is present in the cytoplasm of GI tumors, including pancreatic adenocarcinoma (Cabal-Manzano et al., 2001). Enzymatic degradation of PIF suggests that it consists of a peptide core with molecular weight 4000 Da that is extensively *N*- and *O*-glycosylated to give a total molecular mass of 24 kDa (Todorov et al., 1997). Examination of the sequence of the human genome revealed the gene for the polypeptide core of PIF is located in chromosome 12; two proteins, dermicidin and Y-P30, have been reported to have 100% homology (Schittek et al., 2001; Cunningham et al., 2002). However, enzymatic degradation has shown that the oligosaccharide chains are essential for the biologic activity of PIF (Todorov et al., 1997).

When administered intravenously to normal mice, PIF isolated from urine of cancer cachexia patients induced cachexia without reduction in food and water intake (Cariuk et al., 1997). Analysis of body composition demonstrated that the majority of weight loss involved loss of lean body mass (Cariuk et al., 1997). This decrease in lean body mass had two components: an increase in protein degradation by 50% and a decrease in protein synthesis by 50% observed in gastrocnemius muscle (Lorite et al., 1997). Some studies suggest that PIF-mediated protein degradation may involve the ubiquitin-proteasome proteolytic pathway. Administration of PIF to normal mice caused an increase in mRNA levels for ubiquitin, $E2_{14k}$ and the C9 proteasome subunit. Therefore, protein degradation likely occurs through increased expression of the ubiquitin-proteosome pathway in skeletal muscle; this process is thought to be mediated by the activation of NFκB (Lorite et al., 2001; Whitehouse and Tisdale, 2003; Wyke and Tisdale, 2005). PIF has also been shown to induce total protein degradation and myosin depletion while actin levels remain unchanged (Wyke and Tisdale, 2005).

The mechanism for NFκB activation by PIF is not fully understood. It does involve release of arachidonic acid from membrane phospholipids with rapid metabolism to eicosanoids by phospholipase A_2 (PLA_2) (Smith and Tisdale, 2003). PIF has been shown to increase expression of PLA_2 (Smith and Tisdale, 2003). One of the eicosanoids formed in response to PIF, 15-hydroxyeicosatetraenoic acid (15-HETE), can induce muscle degradation in murine muscle cells (Wyke et al., 2005). 15-HETE may be involved in the activation of protein kinase C (PKC), which is important in the activation of NADPH oxidase (Whitehouse et al., 2003; Smith et al., 2004; Wyke et al., 2005). Activation of NADPH oxidase and generation of ROS play a key role in PIF-induced expression of the ubiquitin-proteasome pathway leading to muscle degradation (Smith et al., 2004; Russell et al., 2007). Increased ROS activates IκB kinase (IKK) which leads to phosphorylation and degradation of IκB; this process, in turn, releases NFκB from its inactive cytosolic complex (Smith et al., 2004).

PIF not only results in protein degradation, it also causes inhibition of protein synthesis. PIF induces the activation/phosphorylation of double-stranded RNA-dependent protein kinase (PKR) (Eley and Tisdale, 2007). The activation of PKR leads to the phosphorylation of eIF2, which inhibits translation initiation and protein synthesis (Eley and Tisdale, 2007). In addition, PKR is known to activate IKK resulting in the nuclear accumulation of NFκB and increased expression and activity of the ubiquitin-proteasome pathway (Zamanian-Daryoush et al., 2000).

In addition to its direct effects on skeletal muscles, PIF may play a role in increasing hepatic cytokine production. Treatment of cultures of human hepatocytes with PIF resulted in activation of NFκB and signal transducers and activators of transcription (STAT3), which caused an increased production of IL-6, IL-8, and CRP, as well as decreased production of transferrin (Watchorn et al., 2001). A similar effect was observed in human Kupffer cells and monocytes and resulted in increased production of TNF-α, IL-6, and IL-8 (Watchorn et al., 2005). PIF may likely contribute to APPR seen in pancreatic cancer cachexia.

OTHER PROPOSED MECHANISMS

Pax7 dysregulation

A recent study provides evidence for a different pathway involved in pancreatic cancer related muscle wasting. Pax7 is a self-renewing transcription factor expressed in various muscle cells, including satellite cells and other myogenic progenitor cells. He et al. demonstrated that NFκB activation in satellite cells resulted in the dysregulation of Pax7, which suppressed expression of MyoD and myogenin (Olguin and Olwin, 2004; He et al., 2013). This process subsequently blocked myogenic differentiation and inhibited myoblast fusion leading to impaired regeneration and muscle wasting (He et al., 2013). They also demonstrated that Pax7 was induced by serum factors from cachectic mice and pancreatic cancer patients in an NFκB-dependent manner both *in vitro* and *in vivo* (He et al., 2013). However, it remains unclear what circulating factors lead to NFκB activation and Pax7 dysregulation.

Neural invasion

Recent studies have shown that neural invasion, which commonly occurs in pancreatic cancer, is related to cachexia and astrocyte activation in pancreatic cancer patients (Mitsunaga et al., 2008; Imoto et al., 2012). Nerve damage from intraneural tumors of pancreatic cancer can activate astrocytes and microglia in the spinal cord. These activated astrocytes subsequently induce lipolysis and muscle atrophy, although the mechanisms leading to cachexia require further investigation (Imoto et al., 2012). These activated astrocytes may increase sympathetic nervous system activity, which is known to cause lipolysis in adipose tissue and muscle atrophy (Li et al., 2002).

MANAGEMENT OF CANCER CACHEXIA

Clinical management of cachexia is currently limited and complex. Best supportive care practices are important in managing secondary causes of anorexia including pain, nausea, pancreatic insufficiency, and constipation. In addition, current treatment strategies are based on the following factors: oncological therapy with optimal control of the tumor; nutritional support; and pharmacological treatment. Since cancer cachexia is a multifactorial syndrome, successful treatment will likely involve a multimodal approach.

NUTRITIONAL SUPPORT

Nutritional risk is highest among pancreatic cancer patients (Bozzetti and Group, 2009). Early involvement of dieticians and nutrition assessment programs are essential to guide management. Nutritional support is an integral part of pancreatic cancer cachexia management and involves providing dietary advice, oral nutritional supplementation, enteral nutrition, and parenteral nutrition (Ottery, 1996; Nitenberg and Raynard, 2000; Jatoi and Loprinzi, 2001; el-Kamar et al., 2003).

Dietary recommendations can significantly increase oral caloric and protein intake (Ovesen et al., 1993). Several studies evaluating the role of oral nutritional supplementation among patients with pancreatic cancer demonstrated improvement in weight and appetite (Fearon et al., 2003; Bauer and Capra, 2005). Oral supplementation with compounds such as L-Carnitine and omega-3 fatty acids may have benefits as well (Barber et al., 1999; Kraft et al., 2012). A small multicenter randomized double-blind trial demonstrated a significant improvement in weight and body mass composition as well as quality of life with L-Carnitine supplementation in patients with advanced pancreatic cancer (Kraft et al., 2012).

In patients with swallowing difficulties or severe dysphagia, a complete enteral diet can be administered using a nasogastric tube or gastrostomy tube. Enteral feeding can be associated with significant morbidity due to aspiration, pneumonia, and diarrhea. In a select group of patients with bowel dysfunction and progressive weight loss despite enteral support, parenteral nutrition may provide a temporary benefit or stabilization in nutritional status (Pelzer et al., 2010).

Artificial nutrition can limit nutritional deterioration in cachectic cancer patients and improve certain metabolic and nutritional indices. However, the nutritional response is typically limited. It is also lower than responses observed in malnourished non-cancer patients receiving equivalent artificial nutrition (Nixon et al., 1981). Patients with pancreatic cancer cachexia require a multimodal approach to disease management (DeWys, 1979).

PHARMACOLOGICAL APPROACH

Various drugs have been studied in the treatment of cancer cachexia. Their mechanisms of action are based on modulation of cytokines, hormones, or other pathways involved in the pathophysiology of cancer cachexia. **Table 3** summarizes drugs and their pharmacologic activity with proven or potential effects on pancreatic cancer cachexia.

Progestogens

Megestrol acetate is a semi-synthetic progesterone currently used as an appetite stimulant. When megestrol acetate was first introduced in the treatment of disseminated breast and endometrial cancer, patients developed weight gain and increased appetite as a side effect. Multiple trials demonstrated that megestrol acetate (480–800 mg/day) resulted in significant improvement in appetite, food intake, nausea, and weight gain among patients with cancer cachexia, including those with pancreatic cancer (Bruera et al., 1990; Loprinzi et al., 1990, 1993a; Westman et al., 1999; Deutsch and Kolhouse, 2004). In 1993, the Food and Drug Administration (FDA) approved megestrol acetate for the treatment of cancer anorexia-cachexia syndrome as well as cachexia due to chronic conditions, including human acquired immunodeficiency syndrome (AIDS). Megestrol acetate is typically well-tolerated with low incidence of adverse effects, such as rash, adrenal insufficiency, hyperglycemia, and thromboembolic events. The increase in thromboembolism has an incidence of less than 5% (Loprinzi et al., 1990). Since its approval, various meta-analyses have confirmed that megestrol acetate increases appetite, weight, and quality of life compared to placebo or other drugs potentially active in the management of cancer cachexia (cisapride, dronabinol, corticosteroids, nandrolone) (Pascual López et al., 2004; Leśniak et al., 2008). The efficacy of megestrol acetate appears to be dose-dependent (Loprinzi et al., 1993a). Based on body composition analysis, megestrol acetate causes weight gain predominantly from an increase in adipose tissue and less from an increase in body fluid (Loprinzi et al., 1993b). There was no improvement in survival demonstrated in patients treated with megestrol acetate (Westman et al., 1999; Leśniak et al., 2008).

The pharmacologic activity of megestrol acetate in appetite stimulation and weight gain may be related to decreased production and release of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) and stimulation of NPY in the hypothalamus (McCarthy et al., 1994; Mantovani et al., 1998a,b). Another progestogen, medroxyprogesterone acetate, was shown to decrease *in vitro* production of cytokines and serotonin by PBMC of cancer patients (Mantovani et al., 1998a,b).

Corticosteroids

Corticosteroids are effective in inducing an increase in appetite, food intake, weight gain, and sense of well-being (Willox et al., 1984; Bruera et al., 1985; Loprinzi et al., 1999). However, the effects are short lived (less than 4 weeks) and associated with long-term side effects, such as insulin resistance, fluid retention, steroid-induced myopathy, skin fragility, adrenal insufficiency, and sleep and cognitive disorders (Loprinzi et al., 1999). The mechanism of action in cancer cachexia is not well understood but is likely related to the inhibition of IL-1, TNF-α, and leptin as well as the stimulation of NPY (Plata-Salamán, 1991). Because of their short term symptomatic benefits but long term adverse effects, corticosteroids may be useful in patients with short expected survival.

Cannabinoids

Dronabinol is effective in reducing nausea and increasing appetite with associated weight stabilization. A phase II trial showed that

Table 3 | Pharmacological approach to pancreatic cancer cachexia.

Drugs	Mechanism of action	References	Level of evidence
Progestogens (megestrol acetate and medroxyprogesterone acetate)	Appetite stimulation Decrease production and release of cytokines (IL-1, IL-6, TNF-α) Stimulation of NPY Decrease production of serotonin in PBMC	Bruera et al., 1990; Loprinzi et al., 1990, 1993a,b; McCarthy et al., 1994; Mantovani et al., 1998a,b; Westman et al., 1999; Deutsch and Kolhouse, 2004; Pascual López et al., 2004; Leśniak et al., 2008	I
Corticosteroids (prednisone, dexamethasone, methylprednisolone)	Appetite stimulation Not well understood Likely from inhibition of IL-1, TNF-α, and leptin Stimulation of NPY	Willox et al., 1984; Bruera et al., 1985; Plata-Salamán, 1991; Loprinzi et al., 1999;	I
Cannabinoids (dronabinol)	Appetite stimulation, anti-emetic Interaction with endorphin receptors Interference with IL-1 synthesis Activation of cannabinoid receptor involved in the neurochemical circuit of leptin Inhibition of prostaglandin synthesis	Nelson et al., 1994; Jatoi et al., 2002	II
NSAIDs (COX-2 inhibitors, indomethacin, ibuprofen)	Anti-inflammatory Decrease production and release of acute phase proteins and pro-inflammatory cytokines Inhibit prostaglandin synthesis	Gelin et al., 1991a,b; Lundholm et al., 1994; McMillan et al., 1995, 1997, 1999; Preston et al., 1995; Wigmore et al., 1995; Lai et al., 2008	II
Thalidomide	Anti-inflammatory and immunomodulatory properties Downregulate production of TNF-α and other cytokines Inhibit NFκB Downregulate COX-2 Inhibit angiogenesis	Sampaio et al., 1991; Bruera et al., 1999; Gordon et al., 2005	II
Omega-3 fatty acids (eicosapentaenoic acid)	Anti-inflammatory and immunomodulatory properties Decrease production of cytokines (IL-1, IL-6, TNF-α) Inhibit downstream effects of LMF and PIF	Tisdale and Beck, 1991; Meydani et al., 1993; Tisdale, 1996; Wigmore et al., 1996, 1997a, 2000b; Barber et al., 1999; Hussey and Tisdale, 1999; Jatoi et al., 2004; Fearon et al., 2006a	Conflicting

NSAIDs, non-steroidal anti-inflammatory drugs; NPY, neuropeptide Y; IL-1, interleukin-1; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; PBMC, peripheral blood mononuclear cells; NFκB, nuclear factor κB; COX-2, cyclooxygenase-2; LMF, lipid mobilizing factor; PIF, proteolysis-inducing factor.

dronabinol reduced anorexia in 68% of patients, but 16% of patients had to suspend treatment due to toxicity (Nelson et al., 1994). Dronabinol has many adverse effects on the CNS. The main side effects include euphoria, hallucinations, psychosis, vertigo, and cardiovascular disorders. Appetite stimulation appears to be mediated by interaction with endorphin receptors, interference with IL-1 synthesis, activation of cannabinoid receptors involved in the neurochemical circuit of leptin, and prostaglandin synthesis inhibition.

A controlled clinical trial by Jatoi et al. compared megestrol acetate and dronabinol in patients with cancer cachexia (Jatoi et al., 2002). A total of 469 patients were treated with megestrol acetate 800 mg/day or dronabinol 2.5 mg/12 h or both. There was a greater increase in appetite and weight in the megestrol acetate group compared to the dronabinol group: 75 vs. 49% ($P = 0.0001$) for appetite, respectively and 11 vs. 3% ($P = 0.02$) for weight gain of at least 10% from baseline, respectively (Jatoi et al., 2002). The combination treatment group resulted in no significant differences in appetite and weight when compared to the megestrol acetate only group (66 vs. 75%, $P = 0.17$, for appetite and 8 vs. 11%, $P = 0.43$, for =10% weight gain, respectively). Megestrol acetate appears to be superior to dronabinol although the cannabinoid is still able to trigger an increase in appetite and reduction in nausea. It serves as an alternative option as an appetite stimulant and anti-emetic.

Anti-inflammatory agents

Systemic inflammation is an important contributor to the pathophysiology of pancreatic cancer cachexia. Pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6, have been implicated in the development of cancer cachexia and have been shown to exhibit synergistic effects. Therefore, multiple therapeutic

strategies have been developed to curtail the inflammatory response by blocking the synthesis or action of cytokines.

Non-steroidal anti-inflammatory drugs (NSAIDs), including cyclooxygenase-2 (COX-2) inhibitors, indomethacin, and ibuprofen, reduce release of acute phase proteins and cytokines (McMillan et al., 1995; Preston et al., 1995; Wigmore et al., 1995). In animal studies, inhibition of prostaglandin synthesis attenuated tumor progression and decreased incidence of cancer cachexia (Gelin et al., 1991a,b). One possible explanation is that cytokines depend on signal transduction mediated by eicosanoids; NSAIDs inhibit prostaglandin synthesis and thereby block downstream effects of systemic inflammation. Lundholm et al. demonstrated that indomethacin use may prolong survival in cachectic patients with metastatic solid tumors, including pancreatic cancer (Lundholm et al., 1994). Other controlled clinical trials have shown that ibuprofen can decrease CRP levels, increase body weight and muscle mass, and improve quality of life, especially when combined with progestogens (Wigmore et al., 1995; McMillan et al., 1997; Lai et al., 2008). McMillan et al. recruited 73 patients with advanced GI cancers, predominantly pancreatic cancer (67% of patients), and cancer cachexia (McMillan et al., 1999). This multicenter randomized controlled trial demonstrated that taking ibuprofen (1200 mg/day) combined with megestrol acetate (480 mg/day) resulted in a significant increase in weight and improved quality of life compared to patients taking megestrol acetate alone (McMillan et al., 1999). Observed side effects were similar in both groups including venous thrombosis, upper GI bleeding, and ascites. However, due to disease progression, the attrition rate was quite high with 63% of patients failing to complete the 12-week assessment. These preliminary results are promising, but further larger studies are needed to evaluate the clinical role of NSAIDs in the management of pancreatic cancer cachexia.

Thalidomide is known to have anti-inflammatory and immunomodulatory properties. It has been shown to downregulate the production of TNF-α and other cytokines, inhibit NFκB, downregulate COX-2, and inhibit angiogenesis (Sampaio et al., 1991). Multiple small studies have demonstrated the efficacy of thalidomide in improving appetite, weight gain, and sensation of well-being (Bruera et al., 1999; Gordon et al., 2005). Gordon et al. reports a single-center double-blind placebo-controlled randomized clinical trial of thalidomide in 50 pancreatic cancer patients with cachexia. Patients were randomized to take thalidomide 200 mg/day or placebo. Patients in the thalidomide group compared to the placebo group had a significant improvement in weight (0.37 vs. −2.21 kg, $P = 0.005$) and lean body mass (1.0 cm^3 in arm muscle mass vs. −4.46 cm^3, $P = 0.002$) after 4 weeks (Gordon et al., 2005). Thalidomide was overall well-tolerated. Adverse reactions included peripheral neuropathy, dizziness, somnolence, constipation, rash, and possible increased risk of venous thromboembolism in the setting of malignancy. These initial results are positive but further clinical trials are needed to confirm the efficacy of thalidomide in treating pancreatic cancer cachexia.

The omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are known to have immunomodulatory properties and have been shown to suppress production of pro-inflammatory cytokines, including IL-1, TNF-α, and IL-6 by PBMC (Meydani et al., 1993; Wigmore et al., 1997a). EPA may also inhibit the downstream effects of LMF and PIF (Tisdale and Beck, 1991; Tisdale, 1996; Hussey and Tisdale, 1999). Early studies associated fish oil supplementation containing both EPA and DHA as well as high-purity EPA administration with weight stabilization in patients with unresectable pancreatic cancer (Wigmore et al., 1996, 2000b). A small pilot study also showed that the use of EPA with oral nutritional supplements resulted in significant increase in weight, dietary intake, and performance status in cachectic patients with advanced pancreatic cancer (Barber et al., 1999). However, recent data from a multicenter double-blind placebo-controlled randomized clinical trial suggest that single agent EPA administration is not effective in treating cancer cachexia (Fearon et al., 2006a). Another multicenter clinical trial comparing the effects of EPA supplement, megestrol acetate, and combination treatment found that megestrol acetate alone is more effective than EPA in increasing weight (Jatoi et al., 2004). EPA was comparable to megestrol acetate with respect to appetite gain, survival, and quality of life (Jatoi et al., 2004). Combination therapy did not have additional benefits to megestrol acetate alone (Jatoi et al., 2004). The role of EPA in cancer cachexia management remains uncertain although recent data suggest that EPA supplementation may not be effective as a single agent or even in combination regimens in the management of pancreatic cancer cachexia.

Pancreatic cancer cachexia is a complex multifactorial syndrome. Successful management may require a multimodal approach with nutritional supplementation and pharmacological treatment. Recent data from a large multicenter trial suggest that combination therapy with megestrol acetate (320 mg/day), EPA supplementation, L-carnitine (4 g/day), and thalidomide (200 mg/day) is significantly more effective in improving lean body mass and appetite than single agents (Mantovani et al., 2010). Combination pharmacological therapy with nutritional supplementation in the context of best supportive care may be the appropriate approach to pancreatic cancer cachexia management.

FUTURE DIRECTIONS

Current clinical management of pancreatic cancer cachexia is limited. None of the available therapies have shown lasting effects on weight stabilization and improvement in survival. Development of effective treatment for this disease remains a challenge.

Recent studies have focused on targeted therapies with anti-inflammatory properties (**Table 4**). IL-6 is a promising target, but many of the studies involving IL-6 antibodies have been in patients with advanced non-small cell lung cancer (NSCLC) and cachexia (Rigas et al., 2010; Schuster et al., 2010; Bayliss et al., 2011; Ando et al., 2013). Rigas and Schuster et al. reported a phase II randomized, double-blind, placebo-controlled trial with NSCLC patients evaluating the safety and efficacy of ALD518 (also known as BMS-945429), a humanized monoclonal IL-6 antibody, in treating cancer cachexia. ALD518 showed promising beneficial results. It increased hemoglobin levels and prevented loss of lean body mass (Rigas et al., 2010; Schuster et al., 2010). There was also a statistically significant improvement in fatigue score in the ALD518 group vs. placebo group that persisted over

Table 4 | Investigational drugs for the treatment of cancer cachexia.

ClinicalTrials.gov identifier	Title	Phase	Mechanism of action	Sponsor	References
NCT01206335	Open label study with OHR/AVR118 in advanced cancer patients with anorexia-cachexia	II	Broad spectrum peptide-nucleic acid immunomodulator targeting cytokine production (including TNF-α and IL-6)	Ohr Pharmaceutical Inc.	ClinicalTrials.gov. Open label study with OHR/AVR118 in advanced cancer patients with anorexia-cachexia
NCT01433263	Clinical study BYM338 for the treatment of unintentional weight loss in patients with cancer of the lungs or the pancreas	II	Human monoclonal antibody against activin receptor type 2B (ACVR2B)	Novartis Pharmaceuticals	ClinicalTrials.gov. Clinical study of BYM338 for the treatment of unintentional weight loss in patients with cancer of the lung or the pancreas
NCT01505530	A phase 2 study of LY2495655 in participants with pancreatic cancer	II	Humanized monoclonal antibody against myostatin	Eli Lilly and Company	ClinicalTrials.gov. A Phase 2 study of LY2495655 in participants with pancreatic cancer

a 12 week period (Rigas et al., 2010). ALD518 was safe and well-tolerated (Rigas et al., 2010; Schuster et al., 2010).

Another agent with anti-inflammatory activity is OHR/AVR118, a broad-spectrum peptide-nucleic acid immune modulator that targets both TNF-α and IL-6. A phase II study involving patients with advanced cancer and cachexia showed an improvement in anorexia, dyspepsia, strength, and depression (Chasen et al., 2011). A phase IIb study is currently ongoing to assess the efficacy of OHR/AVR118 in improving appetite and enhancing weight, lean body mass, strength, and quality of life (ClinicalTrials.gov. Open label study with OHR/AVR118 in advanced cancer patients with anorexia-cachexia). Further studies are needed to evaluate the safety and efficacy of these agents in patients with pancreatic cancer cachexia.

Myostatin and activin are involved in regulating skeletal muscle mass and function via the activin type IIB (ActRIIB) receptor. They inhibit myogenesis and the Akt/mTOR pathway involved in muscle protein synthesis and increase the expression of ubiquitin ligases to increase muscle degradation. Studies have investigated the therapeutic potential of inhibiting myostatin and ActRIIB in treating cancer cachexia. In pre-clinical studies, inhibition of ActRIIB prevented muscle wasting and prolonged survival in C-26 tumor-bearing mice (Zhou et al., 2010). BYM338 is a myostatin inhibitor developed by Novartis (Hanover, NJ, USA) to treat cancer cachexia. A multicenter, randomized, double-blind, placebo-controlled phase II trial is currently underway to investigate the efficacy of BYM338 in attenuating loss of body mass in cachectic patients with stage IV NSCLC or stage III/IV pancreatic cancer (ClinicalTrials.gov. Clinical study of BYM338 for the treatment of unintentional weight loss in patients with cancer of the lung or the pancreas). LY2495655 is another humanized antimyostatin antibody currently under investigation. A multicenter, randomized, double-blind, placebo-controlled phase II trial in patients with locally advanced or metastatic pancreatic cancer is ongoing to evaluate the efficacy of two different doses of LY2495655 in combination with gemcitabine in improving survival as well as lean body mass and physical performance (ClinicalTrials.gov. A Phase 2 study of LY2495655 in participants with pancreatic cancer).

CONCLUSION

Approximately 80% of pancreatic cancer patients develop cachexia during the disease course and up to 30% die from cachexia-related complications (Fearon et al., 2006b; Bachmann et al., 2009). Pancreatic cancer cachexia is a multifactorial syndrome characterized by anorexia and hypercatabolism that are mediated by mechanical factors, pro-inflammatory cytokines, neuropeptides, hormones, and tumor-derived factors. In pancreatic cancer, energy homeostasis is compromised and oriented toward a continuous suppression of appetite and increased energy expenditure. This state leads to uncompensated loss of skeletal muscle and adipose tissue mass.

Further research is needed to elucidate the intricate mechanisms involved in the induction and maintenance of pancreatic cancer cachexia to aid in the development of future therapeutic targets. The management of cachexia remains limited but is currently an active area of research. The use of targeted immunotherapies have shown promising preliminary results. The future management of pancreatic cancer cachexia will likely involve a multimodal approach with nutritional support, combination agents and possible targeted therapies to improve quality of life, lean body mass, and even survival of pancreatic cancer patients.

REFERENCES

Ando, K., Takahashi, F., Motojima, S., Nakashima, K., Kaneko, N., Hoshi, K., et al. (2013). Possible role for tocilizumab, an anti-interleukin-6 receptor antibody, in treating cancer cachexia. *J. Clin. Oncol.* 31, e69–e72. doi: 10.1200/JCO.2012.44.2020

Arruda, A. P., Milanski, M., Romanatto, T., Solon, C., Coope, A., Alberici, L. C., et al. (2010). Hypothalamic actions of tumor necrosis factor alpha provide the thermogenic core for the wastage syndrome in cachexia. *Endocrinology* 151, 683–694. doi: 10.1210/en.2009-0865

Bachmann, J., Heiligensetzer, M., Krakowski-Roosen, H., Büchler, M. W., Friess, H., and Martignoni, M. E. (2008). Cachexia worsens prognosis in patients with resectable pancreatic cancer. *J. Gastrointest. Surg.* 12, 1193–1201. doi: 10.1007/s11605-008-0505-z

Bachmann, J., Ketterer, K., Marsch, C., Fechtner, K., Krakowski-Roosen, H., Buchler, M. W., et al. (2009). Pancreatic cancer related cachexia: influence on metabolism and correlation to weight loss and pulmonary function. *BMC Cancer* 9:255. doi: 10.1186/1471-2407-9-255

Barber, M. D., Ross, J. A., Voss, A. C., Tisdale, M. J., and Fearon, K. C. (1999). The effect of an oral nutritional supplement enriched with fish oil on weight-loss in patients with pancreatic cancer. *Br. J. Cancer* 81, 80–86. doi: 10.1038/sj.bjc.6690654

Bauer, J. D., and Capra, S. (2005). Nutrition intervention improves outcomes in patients with cancer cachexia receiving chemotherapy–a pilot study. *Support. Care Cancer* 13, 270–274. doi: 10.1007/s00520-004-0746-7

Bayliss, T. J., Smith, J. T., Schuster, M., Dragnev, K. H., and Rigas, J. R. (2011). A humanized anti-IL-6 antibody (ALD518) in non-small cell lung cancer. *Expert Opin. Biol. Ther.* 11, 1663–1668. doi: 10.1517/14712598.2011.627850

Bing, C., Russell, S. T., Beckett, E. E., Collins, P., Taylor, S., Barraclough, R., et al. (2002). Expression of uncoupling proteins-1, -2 and -3 mRNA is induced by an adenocarcinoma-derived lipid-mobilizing factor. *Br. J. Cancer* 86, 612–618. doi: 10.1038/sj.bjc.6600101

Bing, C., Taylor, S., Tisdale, M. J., and Williams, G. (2001). Cachexia in MAC16 adenocarcinoma: suppression of hunger despite normal regulation of leptin, insulin and hypothalamic neuropeptide Y. *J. Neurochem.* 79, 1004–1012. doi: 10.1046/j.1471-4159.2001.00639.x

Bozzetti, F., and Group, S. W. (2009). Screening the nutritional status in oncology: a preliminary report on 1,000 outpatients. *Support. Care Cancer* 17, 279–284. doi: 10.1007/s00520-008-0476-3

Bruera, E., MacMillan, K., Kuehn, N., Hanson, J., and MacDonald, R. N. (1990). A controlled trial of megestrol acetate on appetite, caloric intake, nutritional status, and other symptoms in patients with advanced cancer. *Cancer* 66, 1279–1282. doi: 10.1002/1097-0142(19900915)66:6%3C1279::AID-CNCR2820660630%3E3.0.CO;2-R

Bruera, E., Neumann, C. M., Pituskin, E., Calder, K., Ball, G., and Hanson, J. (1999). Thalidomide in patients with cachexia due to terminal cancer: preliminary report. *Ann. Oncol.* 10, 857–859. doi: 10.1023/A:1008329821941

Bruera, E., Roca, E., Cedaro, L., Carraro, S., and Chacon, R. (1985). Action of oral methylprednisolone in terminal cancer patients: a prospective randomized double-blind study. *Cancer Treat. Rep.* 69, 751–754.

Bruera, E., and Sweeney, C. (2000). Cachexia and asthenia in cancer patients. *Lancet Oncol.* 1, 138–147. doi: 10.1016/S1470-2045(00)00033-4

Cabal-Manzano, R., Bhargava, P., Torres-Duarte, A., Marshall, J., and Wainer, I. W. (2001). Proteolysis-inducing factor is expressed in tumours of patients with gastrointestinal cancers and correlates with weight loss. *Br. J. Cancer* 84, 1599–1601. doi: 10.1054/bjoc.2001.1830

Cangiano, C., Cascino, A., Ceci, F., Laviano, A., Mulieri, M., Muscaritoli, M., et al. (1990). Plasma and CSF tryptophan in cancer anorexia. *J. Neural Transm. Gen. Sect.* 81, 225–233. doi: 10.1007/BF01245044

Cangiano, C., Testa, U., Muscaritoli, M., Meguid, M. M., Mulieri, M., Laviano, A., et al. (1994). Cytokines, tryptophan and anorexia in cancer patients before and after surgical tumor ablation. *Anticancer Res.* 14, 1451–1455.

Cariuk, P., Lorite, M. J., Todorov, P. T., Field, W. N., Wigmore, S. J., and Tisdale, M. J. (1997). Induction of cachexia in mice by a product isolated from the urine of cachectic cancer patients. *Br. J. Cancer* 76, 606–613. doi: 10.1038/bjc.1997.433

Castell, J. V., Gómez-Lechón, M. J., David, M., Fabra, R., Trullenque, R., and Heinrich, P. C. (1990). Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6. *Hepatology* 12, 1179–1186. doi: 10.1002/hep.1840120517

Cawthorn, W. P., Heyd, F., Hegyi, K., and Sethi, J. K. (2007). Tumour necrosis factor-alpha inhibits adipogenesis via a beta-catenin/TCF4(TCF7L2)-dependent pathway. *Cell Death Differ.* 14, 1361–1373. doi: 10.1038/sj.cdd.4402127

Chasen, M., Hirschman, S. Z., and Bhargava, R. (2011). Phase II study of the novel peptide-nucleic acid OHR118 in the management of cancer-related anorexia/cachexia. *J. Am. Med. Dir. Assoc.* 12, 62–67. doi: 10.1016/j.jamda.2010.02.012

ClinicalTrials.gov. A Phase 2 study of LY2495655 in participants with pancreatic cancer. Available online at: http://clinicaltrials.gov/ct2/show/NCT01505530. (Accessed December 2013).

ClinicalTrials.gov. Clinical study of BYM338 for the treatment of unintentional weight loss in patients with cancer of the lung or the pancreas. Available online at: http://clinicaltrials.gov/ct2/show/NCT01433263. (Accessed December 2013).

ClinicalTrials.gov. Open label study with OHR/AVR118 in advanced cancer patients with anorexia-cachexia. Available online at: http://clinicaltrials.gov/ct2/show/NCT01206335. (Accessed December 2013).

Cunningham, T. J., Jing, H., Akerblom, I., Morgan, R., Fisher, T. S., and Neveu, M. (2002). Identification of the human cDNA for new survival/evasion peptide (DSEP): studies *in vitro* and *in vivo* of overexpression by neural cells. *Exp. Neurol.* 177, 32–39. doi: 10.1006/exnr.2002.7979

Dahele, M., Skipworth, R. J., Wall, L., Voss, A., Preston, T., and Fearon, K. C. (2007). Objective physical activity and self-reported quality of life in patients receiving palliative chemotherapy. *J. Pain Symptom Manage.* 33, 676–685. doi: 10.1016/j.jpainsymman.2006.09.024

Deutsch, J., and Kolhouse, J. F. (2004). Assessment of gastrointestinal function and response to megesterol acetate in subjects with gastrointestinal cancers and weight loss. *Support. Care Cancer* 12, 503–510. doi: 10.1007/s00520-004-0615-4

DeWys, W. D. (1979). Anorexia as a general effect of cancer. *Cancer* 43, 2013–2019. doi: 10.1002/1097-0142(197905)43:5+%3C2013::AID-CNCR2820430709%3E3.0.CO;2-Z

Dewys, W. D., Begg, C., Lavin, P. T., Band, P. R., Bennett, J. M., Bertino, J. R., et al. (1980). Prognostic effect of weight loss prior to chemotherapy in cancer patients. *Am. J. Med.* 69, 491–497. doi: 10.1016/S0149-2918(05)80001-3

Di Sebastiano, K. M., and Mourtzakis, M. (2012). A critical evaluation of body composition modalities used to assess adipose and skeletal muscle tissue in cancer. *Appl. Physiol. Nutr. Metab.* 37, 811–821. doi: 10.1139/h2012-079

Ebrahimi, B., Tucker, S. L., Li, D., Abbruzzese, J. L., and Kurzrock, R. (2004). Cytokines in pancreatic carcinoma: correlation with phenotypic characteristics and prognosis. *Cancer* 101, 2727–2736. doi: 10.1002/cncr.20672

Eley, H. L., and Tisdale, M. J. (2007). Skeletal muscle atrophy, a link between depression of protein synthesis and increase in degradation. *J. Biol. Chem.* 282, 7087–7097. doi: 10.1074/jbc.M610378200

el-Kamar, F. G., Grossbard, M. L., and Kozuch, P. S. (2003). Metastatic pancreatic cancer: emerging strategies in chemotherapy and palliative care. *Oncologist* 8, 18–34. doi: 10.1634/theoncologist.8-1-18

Faggioni, R., Fuller, J., Moser, A., Feingold, K. R., and Grunfeld, C. (1997). LPS-induced anorexia in leptin-deficient (ob/ob) and leptin receptor-deficient (db/db) mice. *Am. J. Physiol.* 273, R181–R186.

Fearon, K., Arends, J., and Baracos, V. (2013). Understanding the mechanisms and treatment options in cancer cachexia. *Nat. Rev. Clin. Oncol.* 10, 90–99. doi: 10.1038/nrclinonc.2012.209

Fearon, K. C. (2008). Cancer cachexia: developing multimodal therapy for a multidimensional problem. *Eur. J. Cancer* 44, 1124–1132. doi: 10.1016/j.ejca.2008.02.033

Fearon, K. C. (2012). The 2011 ESPEN Arvid Wretlind lecture: cancer cachexia: the potential impact of translational research on patient-focused outcomes. *Clin. Nutr.* 31, 577–582. doi: 10.1016/j.clnu.2012.06.012

Fearon, K. C., Barber, M. D., Falconer, J. S., McMillan, D. C., Ross, J. A., and Preston, T. (1999). Pancreatic cancer as a model: inflammatory mediators, acute-phase response, and cancer cachexia. *World J. Surg.* 23, 584–588. doi: 10.1007/PL00012351

Fearon, K. C., Barber, M. D., Moses, A. G., Ahmedzai, S. H., Taylor, G. S., Tisdale, M. J., et al. (2006a). Double-blind, placebo-controlled, randomized study of eicosapentaenoic acid diester in patients with cancer cachexia. *J. Clin. Oncol.* 24, 3401–3407. doi: 10.1200/JCO.2005.04.5724

Fearon, K., Strasser, F., Anker, S. D., Bosaeus, I., Bruera, E., Fainsinger, R. L., et al. (2011). Definition and classification of cancer cachexia: an

international consensus. *Lancet Oncol.* 12, 489–495. doi: 10.1016/S1470-2045(10)70218-7

Fearon, K. C., Von Meyenfeldt, M. F., Moses, A. G., Van Geenen, R., Roy, A., Gouma, D. J., et al. (2003). Effect of a protein and energy dense N-3 fatty acid enriched oral supplement on loss of weight and lean tissue in cancer cachexia: a randomised double blind trial. *Gut* 52, 1479–1486. doi: 10.1136/gut.52.10.1479

Fearon, K. C., Voss, A. C., Hustead, D. S., and Group, C. C. (2006b). Definition of cancer cachexia: effect of weight loss, reduced food intake, and systemic inflammation on functional status and prognosis. *Am. J. Clin. Nutr*. 83, 1345–1350.

Felix, K., Fakelman, F., Hartmann, D., Giese, N. A., Gaida, M. M., Schnölzer, M., et al. (2011). Identification of serum proteins involved in pancreatic cancer cachexia. *Life Sci*. 88, 218–225. doi: 10.1016/j.lfs.2010.11.011

Finck, B. N., Kelley, K. W., Dantzer, R., and Johnson, R. W. (1998). *In vivo* and *in vitro* evidence for the involvement of tumor necrosis factor-alpha in the induction of leptin by lipopolysaccharide. *Endocrinology* 139, 2278–2283.

Gelin, J., Andersson, C., and Lundholm, K. (1991a). Effects of indomethacin, cytokines, and cyclosporin A on tumor growth and the subsequent development of cancer cachexia. *Cancer Res*. 51, 880–885.

Gelin, J., Moldawer, L. L., Lönnroth, C., Sherry, B., Chizzonite, R., and Lundholm, K. (1991b). Role of endogenous tumor necrosis factor alpha and interleukin 1 for experimental tumor growth and the development of cancer cachexia. *Cancer Res*. 51, 415–421.

Gordon, J. N., Trebble, T. M., Ellis, R. D., Duncan, H. D., Johns, T., and Goggin, P. M. (2005). Thalidomide in the treatment of cancer cachexia: a randomised placebo controlled trial. *Gut* 54, 540–545. doi: 10.1136/gut.2004.047563

Grunfeld, C., Zhao, C., Fuller, J., Pollack, A., Moser, A., Friedman, J., et al. (1996). Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters. *J. Clin. Invest*. 97, 2152–2157. doi: 10.1172/JCI118653

Guttridge, D. C., Mayo, M. W., Madrid, L. V., Wang, C. Y., and Baldwin, A. S. (2000). NF-kappaB-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia. *Science* 289, 2363–2366. doi: 10.1126/science.289.5488.2363

Hammarstedt, A., Isakson, P., Gustafson, B., and Smith, U. (2007). Wnt-signaling is maintained and adipogenesis inhibited by TNFalpha but not MCP-1 and resistin. *Biochem. Biophys. Res. Commun*. 357, 700–706. doi: 10.1016/j.bbrc.2007.03.202

He, W. A., Berardi, E., Cardillo, V. M., Acharyya, S., Aulino, P., Thomas-Ahner, J., et al. (2013). NF-κB-mediated Pax7 dysregulation in the muscle microenvironment promotes cancer cachexia. *J. Clin. Invest*. 123, 4821–4835. doi: 10.1172/JCI68523

Heisler, L. K., Cowley, M. A., Tecott, L. H., Fan, W., Low, M. J., Smart, J. L., et al. (2002). Activation of central melanocortin pathways by fenfluramine. *Science* 297, 609–611. doi: 10.1126/science.1072327

Hirai, K., Hussey, H. J., Barber, M. D., Price, S. A., and Tisdale, M. J. (1998). Biological evaluation of a lipid-mobilizing factor isolated from the urine of cancer patients. *Cancer Res*. 58, 2359–2365.

Hussey, H. J., and Tisdale, M. J. (1999). Effect of a cachectic factor on carbohydrate metabolism and attenuation by eicosapentaenoic acid. *Br. J. Cancer* 80, 1231–1235. doi: 10.1038/sj.bjc.6690490

Imoto, A., Mitsunaga, S., Inagaki, M., Aoyagi, K., Sasaki, H., Ikeda, M., et al. (2012). Neural invasion induces cachexia via astrocytic activation of neural route in pancreatic cancer. *Int. J. Cancer* 131, 2795–2807. doi: 10.1002/ijc.27594

Inui, A. (1999). Cancer anorexia-cachexia syndrome: are neuropeptides the key? *Cancer Res*. 59, 4493–4501.

Islam-Ali, B., Khan, S., Price, S. A., and Tisdale, M. J. (2001). Modulation of adipocyte G-protein expression in cancer cachexia by a lipid-mobilizing factor (LMF). *Br. J. Cancer* 85, 758–763. doi: 10.1054/bjoc.2001.1992

Janik, J. E., Curti, B. D., Considine, R. V., Rager, H. C., Powers, G. C., Alvord, W. G., et al. (1997). Interleukin 1 alpha increases serum leptin concentrations in humans. *J. Clin. Endocrinol. Metab*. 82, 3084–3086.

Jatoi, A., and Loprinzi, C. L. (2001). Current management of cancer-associated anorexia and weight loss. *Oncology (Williston Park)* 15, 497–502, 508. discussion: 508–510.

Jatoi, A., Rowland, K., Loprinzi, C. L., Sloan, J. A., Dakhil, S. R., MacDonald, N., et al. (2004). An eicosapentaenoic acid supplement versus megestrol acetate versus both for patients with cancer-associated wasting: a North Central Cancer Treatment Group and National Cancer Institute of Canada collaborative effort. *J. Clin. Oncol*. 22, 2469–2476. doi: 10.1200/JCO.2004.06.024

Jatoi, A., Windschitl, H. E., Loprinzi, C. L., Sloan, J. A., Dakhil, S. R., Mailliard, J. A., et al. (2002). Dronabinol versus megestrol acetate versus combination therapy for cancer-associated anorexia: a North Central Cancer Treatment Group study. *J. Clin. Oncol*. 20, 567–573. doi: 10.1200/JCO.20.2.567

Karayiannakis, A. J., Syrigos, K. N., Polychronidis, A., Pitiakoudis, M., Bounovas, A., and Simopoulos, K. (2001). Serum levels of tumor necrosis factor-alpha and nutritional status in pancreatic cancer patients. *Anticancer Res*. 21, 1355–1358.

Khan, S., and Tisdale, M. J. (1999). Catabolism of adipose tissue by a tumour-produced lipid-mobilising factor. *Int. J. Cancer* 80, 444–447. doi: 10.1002/(SICI)1097-0215(19990129)80:3%3C444::AID-IJC18%3E3.3.CO;2-L

Kraft, M., Kraft, K., Gartner, S., Mayerle, J., Simon, P., Weber, E., et al. (2012). L-Carnitine-supplementation in advanced pancreatic cancer (CARPAN)–a randomized multicentre trial. *Nutr. J.* 11:52. doi: 10.1186/1475-2891-11-52

Lai, V., George, J., Richey, L., Kim, H. J., Cannon, T., Shores, C., et al. (2008). Results of a pilot study of the effects of celecoxib on cancer cachexia in patients with cancer of the head, neck, and gastrointestinal tract. *Head Neck* 30, 67–74. doi: 10.1002/hed.20662

Laviano, A., Meguid, M. M., and Rossi-Fanelli, F. (2003). Cancer anorexia: clinical implications, pathogenesis, and therapeutic strategies. *Lancet Oncol*. 4, 686–694. doi: 10.1016/S1470-2045(03)01247-6

Leśniak, W., Bała, M., Jaeschke, R., and Krzakowski, M. (2008). Effects of megestrol acetate in patients with cancer anorexia-cachexia syndrome–a systematic review and meta-analysis. *Pol. Arch. Med. Wewn*. 118, 636–644.

Li, G., Klein, R. L., Matheny, M., King, M. A., Meyer, E. M., and Scarpace, P. J. (2002). Induction of uncoupling protein 1 by central interleukin-6 gene delivery is dependent on sympathetic innervation of brown adipose tissue and underlies one mechanism of body weight reduction in rats. *Neuroscience* 115, 879–889. doi: 10.1016/S0306-4522(02)00447-5

Li, Y. P., and Reid, M. B. (2000). NF-kappaB mediates the protein loss induced by TNF-alpha in differentiated skeletal muscle myotubes. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 279, R1165–R1170.

Llovera, M., Carbó, N., López-Soriano, J., García-Martínez, C., Busquets, S., Alvarez, B., et al. (1998). Different cytokines modulate ubiquitin gene expression in rat skeletal muscle. *Cancer Lett*. 133, 83–87. doi: 10.1016/S0304-3835(98)00216-X

Loprinzi, C. L., Ellison, N. M., Schaid, D. J., Krook, J. E., Athmann, L. M., Dose, A. M., et al. (1990). Controlled trial of megestrol acetate for the treatment of cancer anorexia and cachexia. *J. Natl. Cancer Inst*. 82, 1127–1132. doi: 10.1093/jnci/82.13.1127

Loprinzi, C. L., Kugler, J. W., Sloan, J. A., Mailliard, J. A., Krook, J. E., Wilwerding, M. B., et al. (1999). Randomized comparison of megestrol acetate versus dexamethasone versus fluoxymesterone for the treatment of cancer anorexia/cachexia. *J. Clin. Oncol*. 17, 3299–3306.

Loprinzi, C. L., Michalak, J. C., Schaid, D. J., Mailliard, J. A., Athmann, L. M., Goldberg, R. M., et al. (1993a). Phase III evaluation of four doses of megestrol acetate as therapy for patients with cancer anorexia and/or cachexia. *J. Clin. Oncol.* 11, 762–767.

Loprinzi, C. L., Schaid, D. J., Dose, A. M., Burnham, N. L., and Jensen, M. D. (1993b). Body-composition changes in patients who gain weight while receiving megestrol acetate. *J. Clin. Oncol*. 11, 152–154.

Lorite, M. J., Cariuk, P., and Tisdale, M. J. (1997). Induction of muscle protein degradation by a tumour factor. *Br. J. Cancer* 76, 1035–1040. doi: 10.1038/bjc.1997.504

Lorite, M. J., Smith, H. J., Arnold, J. A., Morris, A., Thompson, M. G., and Tisdale, M. J. (2001). Activation of ATP-ubiquitin-dependent proteolysis in skeletal muscle *in vivo* and murine myoblasts *in vitro* by a proteolysis-inducing factor (PIF). *Br. J. Cancer* 85, 297–302. doi: 10.1054/bjoc.2001.1879

Lundholm, K., Gelin, J., Hyltander, A., Lönnroth, C., Sandström, R., Svaninger, G., et al. (1994). Anti-inflammatory treatment may prolong survival in undernourished patients with metastatic solid tumors. *Cancer Res*. 54, 5602–5606.

Maltoni, M., Fabbri, L., Nanni, O., Scarpi, E., Pezzi, L., Flamini, E., et al. (1997). Serum levels of tumour necrosis factor alpha and other cytokines do not correlate with weight loss and anorexia in cancer patients. *Support Care Cancer* 5, 130–135. doi: 10.1007/BF01262570

Mantovani, G., Macciò, A., Lai, P., Massa, E., Ghiani, M., and Santona, M. C. (1998a). Cytokine activity in cancer-related anorexia/cachexia: role of megestrol acetate and medroxyprogesterone acetate. *Semin Oncol.* 25(2 Suppl. 6), 45–52.

Mantovani, G., Macciò, A., Lai, P., Massa, E., Ghiani, M., and Santona, M. C. (1998b). Cytokine involvement in cancer anorexia/cachexia: role of megestrol acetate and medroxyprogesterone acetate on cytokine downregulation

and improvement of clinical symptoms. *Crit. Rev. Oncog.* 9, 99–106. doi: 10.1615/CritRevOncog.v9.i2.10

Mantovani, G., Macciò, A., Madeddu, C., Serpe, R., Massa, E., Dessì, M., et al. (2010). Randomized phase III clinical trial of five different arms of treatment in 332 patients with cancer cachexia. *Oncologist* 15, 200–211. doi: 10.1634/theoncologist.2009-0153

Mantovani, G., Macciò, A., Mura, L., Massa, E., Mudu, M. C., Mulas, C., et al. (2000). Serum levels of leptin and proinflammatory cytokines in patients with advanced-stage cancer at different sites. *J. Mol. Med. (Berl.)* 78, 554–561. doi: 10.1007/s001090000137

Marks, D., and Cone, R. D. (2003). The role of the melanocortin-3 receptor in cachexia. *Ann. N.Y. Acad. Sci.* 994, 258–266. doi: 10.1111/j.1749-6632.2003.tb03188.x

Marks, D. L., Butler, A. A., Turner, R., Brookhart, G., and Cone, R. D. (2003). Differential role of melanocortin receptor subtypes in cachexia. *Endocrinology* 144, 1513–1523. doi: 10.1210/en.2002-221099

Martignoni, M. E., Dimitriu, C., Bachmann, J., Krakowski-Rosen, H., Ketterer, K., Kinscherf, R., et al. (2009). Liver macrophages contribute to pancreatic cancer-related cachexia. *Oncol. Rep.* 21, 363–369. doi: 10.3892/or_00000231

Martignoni, M. E., Kunze, P., Hildebrandt, W., Künzli, B., Berberat, P., Giese, T., et al. (2005). Role of mononuclear cells and inflammatory cytokines in pancreatic cancer-related cachexia. *Clin. Cancer Res.* 11, 5802–5808. doi: 10.1158/1078-0432.CCR-05-0185

McCarthy, H. D., Crowder, R. E., Dryden, S., and Williams, G. (1994). Megestrol acetate stimulates food and water intake in the rat: effects on regional hypothalamic neuropeptide Y concentrations. *Eur. J. Pharmacol.* 265, 99–102. doi: 10.1016/0014-2999(94)90229-1

McMillan, D. C., Leen, E., Smith, J., Sturgeon, C., Preston, T., Cooke, T. G., et al. (1995). Effect of extended ibuprofen administration on the acute phase protein response in colorectal cancer patients. *Eur. J. Surg. Oncol.* 21, 531–534. doi: 10.1016/S0748-7983(95)97157-2

McMillan, D. C., O'Gorman, P., Fearon, K. C., and McArdle, C. S. (1997). A pilot study of megestrol acetate and ibuprofen in the treatment of cachexia in gastrointestinal cancer patients. *Br. J. Cancer* 76, 788–790. doi: 10.1038/bjc.1997.463

McMillan, D. C., Wigmore, S. J., Fearon, K. C., O'Gorman, P., Wright, C. E., and McArdle, C. S. (1999). A prospective randomized study of megestrol acetate and ibuprofen in gastrointestinal cancer patients with weight loss. *Br. J. Cancer* 79, 495–500. doi: 10.1038/sj.bjc.6690077

Meydani, S. N., Lichtenstein, A. H., Cornwall, S., Meydani, M., Goldin, B. R., Rasmussen, H., et al. (1993). Immunologic effects of national cholesterol education panel step-2 diets with and without fish-derived N-3 fatty acid enrichment. *J. Clin. Invest.* 92, 105–113. doi: 10.1172/JCI116537

Mitsunaga, S., Kinoshita, T., Hasebe, T., Nakagohri, T., Konishi, M., Takahashi, S., et al. (2008). Low serum level of cholinesterase at recurrence of pancreatic cancer is a poor prognostic factor and relates to systemic disorder and nerve plexus invasion. *Pancreas* 36, 241–248. doi: 10.1097/MPA.0b013e3181 5b6b2b

Moses, A. G., Maingay, J., Sangster, K., Fearon, K. C., and Ross, J. A. (2009). Pro-inflammatory cytokine release by peripheral blood mononuclear cells from patients with advanced pancreatic cancer: relationship to acute phase response and survival. *Oncol. Rep.* 21, 1091–1095. doi: 10.3892/or_00000328

Moses, A. W., Slater, C., Preston, T., Barber, M. D., and Fearon, K. C. (2004). Reduced total energy expenditure and physical activity in cachectic patients with pancreatic cancer can be modulated by an energy and protein dense oral supplement enriched with n-3 fatty acids. *Br. J. Cancer* 90, 996–1002. doi: 10.1038/sj.bjc.6601620

Nelson, K., Walsh, D., Deeter, P., and Sheehan, F. (1994). A phase II study of delta-9-tetrahydrocannabinol for appetite stimulation in cancer-associated anorexia. *J. Palliat. Care* 10, 14–18.

Nitenberg, G., and Raynard, B. (2000). Nutritional support of the cancer patient: issues and dilemmas. *Crit. Rev. Oncol. Hematol.* 34, 137–168. doi: 10.1016/S1040-8428(00)00048-2

Nixon, D. W., Lawson, D. H., Kutner, M., Ansley, J., Schwarz, M., Heymsfield, S., et al. (1981). Hyperalimentation of the cancer patient with protein-calorie undernutrition. *Cancer Res.* 41, 2038–2045.

Olguin, H. C., and Olwin, B. B. (2004). Pax-7 up-regulation inhibits myogenesis and cell cycle progression in satellite cells: a potential mechanism for self-renewal. *Dev. Biol.* 275, 375–388. doi: 10.1016/j.ydbio.2004.08.015

Ottery, F. (1996). Supportive nutritional management of the patient with pancreatic cancer. *Oncology (Williston Park)* 10, 26–32.

Ovesen, L., Allingstrup, L., Hannibal, J., Mortensen, E. L., and Hansen, O. P. (1993). Effect of dietary counseling on food intake, body weight, response rate, survival, and quality of life in cancer patients undergoing chemotherapy: a prospective, randomized study. *J. Clin. Oncol.* 11, 2043–2049.

Pascual López, A., Roqué i Figuls, M., Urrútia Cuchi, G., Berenstein, E. G., Almenar Pasies, B., Balcells Alegre, M., et al. (2004). Systematic review of megestrol acetate in the treatment of anorexia-cachexia syndrome. *J. Pain Symptom Manage.* 27, 360–369. doi: 10.1016/j.jpainsymman.2003.09.007

Pausch, T., Hartwig, W., Hinz, U., Swolana, T., Bundy, B. D., Hackert, T., et al. (2012). Cachexia but not obesity worsens the postoperative outcome after pancreatoduodenectomy in pancreatic cancer. *Surgery* 152, S81–S88. doi: 10.1016/j.surg.2012.05.028

Pelzer, U., Arnold, D., Gövercin, M., Stieler, J., Doerken, B., Riess, H., et al. (2010). Parenteral nutrition support for patients with pancreatic cancer. Results of a phase II study. *BMC Cancer* 10:86. doi: 10.1186/1471-2407-10-86

Plata-Salamán, C. R. (1991). Dexamethasone inhibits food intake suppression induced by low doses of interleukin-1 beta administered intracerebroventricularly. *Brain Res. Bull.* 27, 737–738. doi: 10.1016/0361-9230(91)90055-O

Prado, C. M., Birdsell, L. A., and Baracos, V. E. (2009). The emerging role of computerized tomography in assessing cancer cachexia. *Curr. Opin. Support. Palliat. Care* 3, 269–275. doi: 10.1097/SPC.0b013e328331124a

Preston, T., Fearon, K. C., McMillan, D. C., Winstanley, F. P., Slater, C., Shenkin, A., et al. (1995). Effect of ibuprofen on the acute-phase response and protein metabolism in patients with cancer and weight loss. *Br. J. Surg.* 82, 229–234. doi: 10.1002/bjs.1800820233

Preston, T., Slater, C., McMillan, D. C., Falconer, J. S., Shenkin, A., and Fearon, K. C. (1998). Fibrinogen synthesis is elevated in fasting cancer patients with an acute phase response. *J. Nutr.* 128, 1355–1360.

Rigas, J. R., Schuster, M., Orlov, S. V., Milovanovic, B., Prabhash, K., Smith, J. T., et al. (2010). Effect of ALD518, a humanized anti-IL-6 antibody, on lean body mass loss and symptoms in patients with advanced non-small cell lung cancer (NSCLC): results of a phase II randomized, double-blind safety and efficacy trial. *J. Clin. Oncol.* 28:abstract 7622.

Russell, S. T., Eley, H., and Tisdale, M. J. (2007). Role of reactive oxygen species in protein degradation in murine myotubes induced by proteolysis-inducing factor and angiotensin II. *Cell Signal.* 19, 1797–1806. doi: 10.1016/j.cellsig.2007.04.003

Russell, S. T., Hirai, K., and Tisdale, M. J. (2002). Role of beta3-adrenergic receptors in the action of a tumour lipid mobilizing factor. *Br. J. Cancer* 86, 424–428. doi: 10.1038/sj.bjc.6600086

Russell, S. T., and Tisdale, M. J. (2002). Effect of a tumour-derived lipid-mobilising factor on glucose and lipid metabolism *in vivo*. *Br. J. Cancer* 87, 580–584. doi: 10.1038/sj.bjc.6600493

Rydén, M., Agustsson, T., Laurencikiene, J., Britton, T., Sjölin, E., Isaksson, B., et al. (2008). Lipolysis–not inflammation, cell death, or lipogenesis–is involved in adipose tissue loss in cancer cachexia. *Cancer* 113, 1695–1704. doi: 10.1002/cncr.23802

Rydén, M., Arvidsson, E., Blomqvist, L., Perbeck, L., Dicker, A., and Arner, P. (2004). Targets for TNF-alpha-induced lipolysis in human adipocytes. *Biochem. Biophys. Res. Commun.* 318, 168–175. doi: 10.1016/j.bbrc.2004.04.010

Sampaio, E. P., Sarno, E. N., Galilly, R., Cohn, Z. A., and Kaplan, G. (1991). Thalidomide selectively inhibits tumor necrosis factor alpha production by stimulated human monocytes. *J. Exp. Med.* 173, 699–703. doi: 10.1084/jem.173.3.699

Sarraf, P., Frederich, R. C., Turner, E. M., Ma, G., Jaskowiak, N. T., Rivet, D. J., et al. (1997). Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. *J. Exp. Med.* 185, 171–175. doi: 10.1084/jem.185.1.171

Scarlett, J. M., Jobst, E. E., Enriori, P. J., Bowe, D. D., Batra, A. K., Grant, W. F., et al. (2007). Regulation of central melanocortin signaling by interleukin-1 beta. *Endocrinology* 148, 4217–4225. doi: 10.1210/en.2007-0017

Schittek, B., Hipfel, R., Sauer, B., Bauer, J., Kalbacher, H., Stevanovic, S., et al. (2001). Dermcidin: a novel human antibiotic peptide secreted by sweat glands. *Nat. Immunol.* 2, 1133–1137. doi: 10.1038/ni732

Schuster, M., Rigas, J. R., Orlov, S. V., Milovanovic, B., Prabhash, K., Smith, J. T., et al. (2010). ALD518, a humanized anti-IL-6 antibody, treats anemia in patients with advanced non-small cell lung cancer (NSCLC): results of a phase II,

randomized, double-blind, placebo-controlled trial. *J. Clin. Oncol*. 28:abstract 7631.

Shintani, F., Kanba, S., Nakaki, T., Nibuya, M., Kinoshita, N., Suzuki, E., et al. (1993). Interleukin-1 beta augments release of norepinephrine, dopamine, and serotonin in the rat anterior hypothalamus. *J. Neurosci*. 13, 3574–3581.

Simons, J. P., Schols, A. M., Campfield, L. A., Wouters, E. F., and Saris, W. H. (1997). Plasma concentration of total leptin and human lung-cancer-associated cachexia. *Clin. Sci. (Lond.)* 93, 273–277.

Simons, J. P., Schols, A. M., Westerterp, K. R., ten Velde, G. P., and Wouters, E. F. (1995). The use of bioelectrical impedance analysis to predict total body water in patients with cancer cachexia. *Am. J. Clin. Nutr*. 61, 741–745.

Smith, H. J., and Tisdale, M. J. (2003). Signal transduction pathways involved in proteolysis-inducing factor induced proteasome expression in murine myotubes. *Br. J. Cancer* 89, 1783–1788. doi: 10.1038/sj.bjc.6601328

Smith, H. J., Wyke, S. M., and Tisdale, M. J. (2004). Role of protein kinase C and NF-kappaB in proteolysis-inducing factor-induced proteasome expression in C(2)C(12) myotubes. *Br. J. Cancer* 90, 1850–1857. doi: 10.1038/sj.bjc.6601767

Strassmann, G., Fong, M., Kenney, J. S., and Jacob, C. O. (1992). Evidence for the involvement of interleukin 6 in experimental cancer cachexia. *J. Clin. Invest*. 89, 1681–1684. doi: 10.1172/JCI115767

Suzuki, H., Asakawa, A., Amitani, H., Fujitsuka, N., Nakamura, N., and Inui, A. (2013). Cancer cachexia pathophysiology and translational aspect of herbal medicine. *Jpn. J. Clin. Oncol*. 43, 695–705. doi: 10.1093/jjco/hyt075

Tan, B. H., Birdsell, L. A., Martin, L., Baracos, V. E., and Fearon, K. C. (2009). Sarcopenia in an overweight or obese patient is an adverse prognostic factor in pancreatic cancer. *Clin. Cancer Res*. 15, 6973–6979. doi: 10.1158/1078-0432.CCR-09-1525

Tisdale, M. J. (1996). Inhibition of lipolysis and muscle protein degradation by EPA in cancer cachexia. *Nutrition* 12, S31–S33. doi: 10.1016/0899-9007(95)00066-6

Tisdale, M. J., and Beck, S. A. (1991). Inhibition of tumour-induced lipolysis *in vitro* and cachexia and tumour growth *in vivo* by eicosapentaenoic acid. *Biochem. Pharmacol*. 41, 103–107. doi: 10.1016/0006-2952(91)90016-X

Todorov, P., Cariuk, P., McDevitt, T., Coles, B., Fearon, K., and Tisdale, M. (1996). Characterization of a cancer cachectic factor. *Nature* 379, 739–742. doi: 10.1038/379739a0

Todorov, P. T., Deacon, M., and Tisdale, M. J. (1997). Structural analysis of a tumor-produced sulfated glycoprotein capable of initiating muscle protein degradation. *J. Biol. Chem*. 272, 12279–12288. doi: 10.1074/jbc.272.19.12279

Todorov, P. T., McDevitt, T. M., Meyer, D. J., Ueyama, H., Ohkubo, I., and Tisdale, M. J. (1998). Purification and characterization of a tumor lipid-mobilizing factor. *Cancer Res*. 58, 2353–2358.

Tsoli, M., Moore, M., Burg, D., Painter, A., Taylor, R., Lockie, S. H., et al. (2012). Activation of thermogenesis in brown adipose tissue and dysregulated lipid metabolism associated with cancer cachexia in mice. *Cancer Res*. 72, 4372–4382. doi: 10.1158/0008-5472.CAN-11-3536

Tuca, A., Jimenez-Fonseca, P., and Gascón, P. (2013). Clinical evaluation and optimal management of cancer cachexia. *Crit. Rev. Oncol. Hematol*. 88, 625–636. doi: 10.1016/j.critrevonc.2013.07.015

Wallace, A. M., Sattar, N., and McMillan, D. C. (1998). Effect of weight loss and the inflammatory response on leptin concentrations in gastrointestinal cancer patients. *Clin. Cancer Res*. 4, 2977–2979.

Watchorn, T. M., Dowidar, N., Dejong, C. H., Waddell, I. D., Garden, O. J., and Ross, J. A. (2005). The cachectic mediator proteolysis inducing factor activates NF-kappaB and STAT3 in human Kupffer cells and monocytes. *Int. J. Oncol*. 27, 1105–1111.

Watchorn, T. M., Waddell, I., Dowidar, N., and Ross, J. A. (2001). Proteolysis-inducing factor regulates hepatic gene expression via the transcription factors NF-(kappa)B and STAT3. *FASEB J*. 15, 562–564.

Westman, G., Bergman, B., Albertsson, M., Kadar, L., Gustavsson, G., Thaning, L., et al. (1999). Megestrol acetate in advanced, progressive, hormone-insensitive cancer. Effects on the quality of life: a placebo-controlled, randomised, multicentre trial. *Eur. J. Cancer* 35, 586–595. doi: 10.1016/S0959-8049(98)00398-0

Whitehouse, A. S., Khal, J., and Tisdale, M. J. (2003). Induction of protein catabolism in myotubes by 15(S)-hydroxyeicosatetraenoic acid through increased expression of the ubiquitin-proteasome pathway. *Br. J. Cancer* 89, 737–745. doi: 10.1038/sj.bjc.6601184

Whitehouse, A. S., and Tisdale, M. J. (2003). Increased expression of the ubiquitin-proteasome pathway in murine myotubes by proteolysis-inducing factor (PIF) is associated with activation of the transcription factor NF-kappaB. *Br. J. Cancer* 89, 1116–1122. doi: 10.1038/sj.bjc.6601132

Wigmore, S. J., Barber, M. D., Ross, J. A., Tisdale, M. J., and Fearon, K. C. (2000b). Effect of oral eicosapentaenoic acid on weight loss in patients with pancreatic cancer. *Nutr. Cancer* 36, 177–184. doi: 10.1207/S15327914NC3602_6

Wigmore, S. J., Falconer, J. S., Plester, C. E., Ross, J. A., Maingay, J. P., Carter, D. C., et al. (1995). Ibuprofen reduces energy expenditure and acute-phase protein production compared with placebo in pancreatic cancer patients. *Br. J. Cancer* 72, 185–188. doi: 10.1038/bjc.1995.300

Wigmore, S. J., Fearon, K. C., Maingay, J. P., and Ross, J. A. (1997a). Down-regulation of the acute-phase response in patients with pancreatic cancer cachexia receiving oral eicosapentaenoic acid is mediated via suppression of interleukin-6. *Clin. Sci. (Lond.)* 92, 215–221.

Wigmore, S. J., Plester, C. E., Ross, J. A., and Fearon, K. C. (1997b). Contribution of anorexia and hypermetabolism to weight loss in anicteric patients with pancreatic cancer. *Br. J. Surg*. 84, 196–197. doi: 10.1002/bjs.1800840214

Wigmore, S. J., Ross, J. A., Falconer, J. S., Plester, C. E., Tisdale, M. J., Carter, D. C., et al. (1996). The effect of polyunsaturated fatty acids on the progress of cachexia in patients with pancreatic cancer. *Nutrition* 12, S27–S30. doi: 10.1016/0899-9007(95)00078-X

Wigmore, S. J., Todorov, P. T., Barber, M. D., Ross, J. A., Tisdale, M. J., and Fearon, K. C. (2000b). Characteristics of patients with pancreatic cancer expressing a novel cancer cachectic factor. *Br. J. Surg*. 87, 53–58. doi: 10.1046/j.1365-2168.2000.01317.x

Willox, J. C., Corr, J., Shaw, J., Richardson, M., Calman, K. C., and Drennan, M. (1984). Prednisolone as an appetite stimulant in patients with cancer. *Br. Med. J. (Clin. Res. Ed.)*. 288, 27. doi: 10.1136/bmj.288.6410.27

Wisse, B. E., Frayo, R. S., Schwartz, M. W., and Cummings, D. E. (2001). Reversal of cancer anorexia by blockade of central melanocortin receptors in rats. *Endocrinology* 142, 3292–3301. doi: 10.1210/endo.142.8.8324

Wyke, S. M., Khal, J., and Tisdale, M. J. (2005). Signalling pathways in the induction of proteasome expression by proteolysis-inducing factor in murine myotubes. *Cell Signal*. 17, 67–75. doi: 10.1016/j.cellsig.2004.05.015

Wyke, S. M., and Tisdale, M. J. (2005). NF-kappaB mediates proteolysis-inducing factor induced protein degradation and expression of the ubiquitin-proteasome system in skeletal muscle. *Br. J. Cancer* 92, 711–721. doi: 10.1038/sj.bjc.6602402

Zamanian-Daryoush, M., Mogensen, T. H., DiDonato, J. A., and Williams, B. R. (2000). NF-kappaB activation by double-stranded-RNA-activated protein kinase (PKR) is mediated through NF-kappaB-inducing kinase and IkappaB kinase. *Mol. Cell Biol*. 20, 1278–1290. doi: 10.1128/MCB.20.4.1278-1290.2000

Zhang, H. H., Halbleib, M., Ahmad, F., Manganiello, V. C., and Greenberg, A. S. (2002). Tumor necrosis factor-alpha stimulates lipolysis in differentiated human adipocytes through activation of extracellular signal-related kinase and elevation of intracellular cAMP. *Diabetes* 51, 2929–2935. doi: 10.2337/diabetes.51.10.2929

Zhou, X., Wang, J. L., Lu, J., Song, Y., Kwak, K. S., Jiao, Q., et al. (2010). Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival. *Cell* 142, 531–543. doi: 10.1016/j.cell.2010.07.011

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Utilizing past and present mouse systems to engineer more relevant pancreatic cancer models

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The study of pancreatic cancer has prompted the development of numerous mouse models that aim to recapitulate the phenotypic and mechanistic features of this deadly malignancy. This review accomplishes two tasks. First, it provides an overview of the models that have been used as representations of both the neoplastic and carcinoma phenotypes. Second, it presents new modeling schemes that ultimately will serve to more faithfully capture the temporal and spatial progression of the human disease, providing platforms for improved understanding of the role of non-epithelial compartments in disease etiology as well as evaluating therapeutic approaches.

Keywords: mouse model, pancreatic cancer, inducible, conditional, FLP/FRT, Kras

INTRODUCTION

There has been a noticeable increase (near doubling) in the 5-year survival of pancreatic cancer patients, though the number remains quite low at about 6–7% (SEER Stat Fact Sheets: Pancreas Cancer—NCI). Much of this stems from a few more potent clinical therapies [folfirinox (Papadatos-Pastos et al., 2014), nab-paclitaxol (Borazanci and Von Hoff, 2014), and various combinations with gemcitabine (Tian et al., 2013)] that improve on previous survival rates. Thus, begins a new drive to employ relevant preclinical models with which to test novel drugs that can further improve patient survival. Indeed, there are already a few mouse models that can be used, with KPC mice (as described below) being one model that currently boasts a strong recapitulation of the paradigm observed in human pancreatic adenocarcinoma. Yet, further advances on mouse models will not only generate additional preclinical models but, perhaps more importantly, demonstrate the utility of newer diagnostic and/or therapeutic targets. The main objective of this review is to highlight past and present mouse models of pancreatic cancer [see (Guerra and Barbacid, 2013) for a more thorough review of current models] in order to propose continued engineering of more relevant mouse systems. These future models could then be employed to better understand the role of non-parenchymal compartments during the development of disease as well as build inducible systems that allow multiple allelic changes at various intervals.

TRANSGENIC MODELS

Initially, development of cancer in mouse pancreas was demonstrated by targeting Myc and TGFα to mouse pancreatic acinar cells (EL-Myc and EL-TGFα), which demonstrated acinar-to-ductal metaplasia leading to exocrine carcinoma with focally distinct ductal-like lesions (Sandgren et al., 1990, 1991, 1993; Grippo and Sandgren, 2012). Previous targeting of oncogene expression via the elastase (EL) promoter proved effective at inducing exocrine pancreatic neoplasms in transgenic mice, including EL-SV40 TAg and EL-Hras (Ornitz et al., 1985, 1987; Quaife et al., 1987). These two models developed acinar hyperplasia (Ornitz et al., 1987) and carcinoma (Quaife et al., 1987) while EL-TGFα mice produced severe fibrosis, tubular complexes, and aberrant cell morphology (Sandgren et al., 1993). Older EL-TGFα mice eventually develop carcinoma, and tumor development was enhanced in a p53 null background and concomitant with partial or whole loss of INK4a or SMAD4 (Wagner et al., 2001). The metaplasia in EL-TGFα/p53$^{+/-}$ mice was characterized along with its genomic signature (Schreiner et al., 2003) and increased expression of Pdx1, a gene necessary for pancreas development and often expressed in pancreatic cancer, was observed in mice with overexpression of TGFα (Song et al., 1999). Additionally, the EL-KRAS model, which directs human mutant KRAS transgene expression to pancreatic acinar cells via a rat elastase driver, demonstrates a common pancreatic cancer histotype by inducing neoplastic, ductal lesions (Grippo et al., 2003), often referred to as cystic papillary neoplasms (CPNs) similar to human cystic neoplasms including IPMN and MCN (Hruban et al., 2006).

CONDITIONAL MODELS

Conditional systems have become an asset to the mouse-modeling field as they provide tissue specific targeting of genes. One prominent targeting strategy included *Pdx1* and *Ptf1a* or *p48*-driven expression of Cre recombinase in mice with

flanking Lox elements (floxed) that, upon Cre-mediated recombination, generated a mutant Kras in the endogenous mouse allele. These mice developed ductal lesions and mPanINs that occasionally progressed to invasive cancer (Hingorani et al., 2003). This model laid the foundation for the generation of the LSL-*Kras*$^{G12D/+}$;LSL-*Trp53*$^{R172H/+}$;*Pdx*1-*Cre* (KPC) model which demonstrates a highly metastatic carcinoma that resembles human disease (Hingorani et al., 2005). Models such as this one have allowed for the characterization of biomarkers in pancreatic cancer from disease initiation to metastasis (Mirus et al., 2014). It is important to note that these floxed alleles can be targeted to other cell types in the pancreas as demonstrated by expression of the LSL−*Kras*$^{G12D/+}$ allele in Nestin positive cells leading to mPanINs (Carriere et al., 2007) and caerulein-induced PDAC (Carriere et al., 2011b).

Following the use of these models, other conditional targets were generated utilizing similar technology. Since Transforming Growth Factor β (TGFβ) signaling is commonly disrupted in cancer (Principe et al., 2014) and highly so in pancreatic cancer (Jones et al., 2008), LSL-*Kras*$^{G12D/+}$;*Tgfbr2*$^{flox/flox}$;*Ptf*1*a*$^{Cre/+}$ mice were generated to simultaneously express mutant *Kras*^{G12D} and loss of the type 2 TGFβ receptor (*Tgfbr2*) in pancreatic epithelium. This model demonstrated an aggressive form of pancreatic ductal adenocarcinoma (PDAC) and explored the role of TGFβ signaling in the development of the disease (Ijichi et al., 2006). As loss of downstream TGFβ target SMAD4 is common in pancreatic cancer (Hahn et al., 1996), LSL-*Kras*$^{G12D/+}$;*Dpc4*$^{flox/+}$;*Pdx*1-*Cre* and LSL-*Kras*$^{G12D/+}$;*Dpc4*$^{flox/+}$;Ptf1a$^{Cre/+}$ were generated to conditionally express *Kras*^{G12D} in concert with *Smad4/Dpc4* haploinsufficiency in the pancreas, thereby inducing MCNs and subsequent PDAC (Izeradjene et al., 2007). Additionally, IPMN-like lesions accompanied by PDAC and metastatic disease were shown with the LSL-*Kras*$^{G12D/+}$;*Smad4*$^{flox/flox}$;*Pdx1-Cre* model (Bardeesy et al., 2006; Kojima et al., 2007).

Considering the implications for loss/inactivation of *p16*^{Ink4a} and *p19*^{Arf} in cellular transformation, a variety of models have pursued this target in concert with pancreas-specific mutations. An *MT-TGFα;Ink4a/Arf*$^{-/-}$ model was generated, ultimately demonstrating a serous cystadenoma (SCA) phenotype that resembled human disease (Bardeesy et al., 2002). Following the creation of this model, pancreas-specific *Kras* targeting was coupled with a floxed *Ink4a/Arf* locus. These LSL-*Kras*^{G12D};Ink4a/Arf$^{flox/flox}$;Pdx1-Cre mice presented with invasive, metastatic disease consistent with human disease (Aguirre et al., 2003). In addition, the LSL-*Kras*$^{G12D/+}$;*p16*$^{flox/flox}$;*Pdx1-Cre* model directed the knockout of the *p16*^{Ink4a} tumor suppressor gene in pancreatic epithelium. These mice developed mPanINs, PDAC, and metastases (Qiu et al., 2011). Characterization of this tumor suppressive axis also prompted the generation of LSL-*Kras*$^{G12D/+}$;*Rb*$^{flox/flox}$;*Pdx1-Cre* mice to assess the role of *Rb* inactivation and PDAC progression. These mice exhibited accelerated mPanIN progression and rapid PDAC development (Carriere et al., 2011a).

The activation of mutant *Kras* and heparin-binding epidermal growth factor-like growth factor (*HB-EGF*) by the Means group also demonstrated conditional targeting of two oncogenic events. These mice featured rapid progression into the early stages of pancreatic cancer (Ray et al., 2014).

The tumor stroma's control of tumor growth was explored by utilizing two conditional models of pancreatic cancer. *Shh*$^{flox/flox}$;*Pdx1-Cre*;LSL-*Kras*$^{G12D/+}$;*p53*$^{flox/+}$;*Rosa26*$^{LSL-YFP}$ (ShhPKCY) mice were generated to delete Sonic Hedgehog (SHH) in the context on PDAC. Due to lack of SHH, these mice presented with less tumor stroma yet more aggressive, proliferative tumors. This phenotype was also shown utilizing a Smoothened inhibitor in KPC mice. Additionally, VEGFR inhibition promoted SHH-deficient tumor survival, demonstrating that SHH-formed stroma limits tumor growth by restricting tumor angiogenesis. (Rhim et al., 2014).

Additional study of the tumor stroma's contribution to cancer growth was explored via the generation of a mouse model that crosses LSL-*Kras*$^{G12D/+}$;*Tgfbr2*$^{flox/flox}$;*Ptf1a*$^{Cre/+}$ mice to αSMA-tk transgenic mice. Depletion of αSMA^{+} myofibroblasts in the context of mPanINs or PDAC resulted in reduced survival characterized by hypoxia, EMT, and cancer stem cells. In addition, this model was characterized by the increase in regulatory T cells infiltrating myofibroblast-depleted tumors. Similar results were shown when the KPC model was used in cross with the αSMA-tk transgenic (Ozdemir et al., 2014).

Both of these studies hold implications for the future of stromal-directed therapies for the treatment of PDAC. Although mouse models have been successful for such therapies (Olive et al., 2009), the recapitulation of these results in clinical trials has largely failed. Rhim and Ozdemir demonstrated that tumor stroma provided a protective effect for the host. Therefore, targeting the stroma may create a more aggressive form of PDAC. As noted by Gore and Korc, the stroma's capacity for both benefit and damage must be further explored in mouse models before potential therapies are reapplied in human trials (Neesse et al., 2011; Gore and Korc, 2014). However, ablation of a subpopulation of stromal cells (FAP+ cells) permitted immune control of tumor growth and uncovered the efficacy of immunotherapeutic antibodies (anti-CTLA-4 or anti-PD-L1), which resulted in acute tumor regression (Kraman et al., 2010; Feig et al., 2013). More recently it has been shown that VDR acts as a master transcriptional regulator of PSCs to reprise the quiescent state, resulting in induced stromal remodeling, increased intratumoral gemcitabine, reduced tumor volume, and a 57% increase in survival compared to chemotherapy alone (Sherman et al., 2014). The distinct outcome of these studies underscores the need to better understand the role of desmoplastic stroma in pancreatic cancer.

INDUCIBLE/CONDITIONAL MOUSE MODELING SYSTEMS OF PANCREATIC CANCER

While the described conditional modeling systems have provided invaluable insight into disease incidence and progression, they do not fully capture the temporal component of human mutations observed in the clinic. For instance, in systems relying on *Pdx* or *Ptf1* driven Cre, recombination occurs at E8.5 (Ohlsson et al., 1993) or E9.5 (Obata et al., 2001), respectively. While embryonic recombination often shortens the time to a cancer or neoplastic phenotype, the effects of these mutations on pancreatic

development are not fully understood, and do not faithfully mimic the spontaneous mutations that occur in the fully formed gland of an adult human patient.

In recent years, conditional and inducible systems have prompted the unique ability to control when and where genes are expressed. In particular, the development of CreERT technology (Feil et al., 1996, 1997) has prompted an array of tissue specific, temporally-controlled targeting models. Both the *ElastaseCreERT2* (Desai et al., 2007) and the *Ptf1a*$^{Cre\text{-}ERTM}$ (Kopinke et al., 2012) systems have advanced the field of pancreatic cancer modeling by providing a means for inducibly targeting pancreatic epithelium. Both of these systems feature a Cre recombinase cassette fused to a Tamoxifen-responsive mutant estrogen-receptor element that is driven by an acinar cell specific promoter region. The Cre recombinase in each of these systems is then able to activate gene expression in a loxP-mediated system. The utility of the CreERT system was further demonstrated in the *Kras*^{G12D};*Rosa26*^{NIC};*Pdx1-CreERT* model, which temporally controlled the expression of *Notch* and *Kras* and showed synergistic effects between the two proteins with respect to mPanIN progression (De La et al., 2008).

*iKras** MODELS

The Pasca di Magliano group has also generated several models that represent the full utilization of both spatial and temporal control of gene expression. The *iKras** model functions through the transgenesis of three different types of mice. In these mice, the *Ptf1a* allele drives Cre expression (Kawaguchi et al., 2002), which, in turn, excises a stop cassette bound by two loxP sites. This stop cassette functions to inhibit the reverse tetracycline transactivator (rtTa) for an IRES-EGFP cassette at the R26 locus (Belteki et al., 2005). Since *Ptf1a*$^{Cre/+}$ is mostly pancreas specific, the excision of the stop cassette allows for the expression of both rtTa and EGFP in the pancreatic epithelium beginning during embryogenesis (Collins et al., 2012a).

Administering doxycycline to these animals leads to activation of rtTa and subsequent *Kras** expression through a TetO-*Kras*^{G12D} transgene using rat mutant *Kras* (Fisher et al., 2001). This inducible system provides a strong platform to explore several relevant issues. First, the mutation of *Kras* can be expressed in adult tissues, which is far more relevant to PanIN progression to cancer observed in humans. In addition, it allows for the abrogation of oncogenic *Kras* expression at various stages of cancer development and thus the study of the dependence of developing lesions and cancer on mutant *Kras*. Also, this system can be employed to investigate carcinogenesis in the context of tumor suppressor inactivation or additional oncogene activation. iKras*-p53$^{+/-}$ mice were also generated to illustrate the development of PDAC when mutant *Kras* is paired with the concurrent inactivation of this tumor suppressor gene (Collins et al., 2012a). This model provides a framework examining various features of oncogenic *Kras* in PDAC development. Inhibition of mutant *Kras* expression through doxycycline removal and subsequent reversion to a more normal phenotype supports continued efforts to target mutant *Kras* as a therapeutic option and eventual translation to the clinic.

Furthermore, the Pasca di Magliano group generated a model that inducibly and conditionally activated *Kras* and a mutant p53 allele (Collins et al., 2012b). These mice featured the same iKras* system described above with an additional mutant p53 allele preceded by a loxP-bound STOP cassette. Therefore, the same *Ptf1a*-driven Cre-recombinase that activates the rtTa for iKras* expression will also activate the mutant p53^{R172H} (p53*) allele (Olive et al., 2004) by excising the preceding STOP cassette. However, in these iKras*p53* mice, oncogenic *Kras* is not activated until doxycycline administration. This model demonstrated a dual functionality by allowing the simultaneous, pancreas-specific targeting of two alleles (iKras* and p53*) and the inducible/reversible expression of oncogenic *Kras* (Collins et al., 2012b). Although the conditional LSL-*Kras*$^{G12D/+}$;LSL-*Trp53*$^{R172H/+}$;*Pdx1-Cre* (KPC) model (Hingorani et al., 2005) of PDAC demonstrated a close mimicking of the human disease, it lacked inducible control of *Kras*. This type of control over mutant *Kras* expression allowed for the study of its role in primary and metastatic tumor maintenance when expressed concurrently with mutant p53 (Collins et al., 2012b) and the demonstration of mutant *Kras*-dependence on more aggressive and metastatic pancreatic cancer.

LSL-*Kras*$^{+/G12Vgeo}$;*EL-tTA/tetO-Cre* MODELS

Additionally, the Barbacid group generated a model that accomplishes both temporal and spatial targeting of oncogenic Kras using a different mutant variant (G12V vs. G12D). By crossing a LSL-*Kras*$^{+/G12Vgeo}$ knockin strain (Guerra et al., 2003) to EL-tTA/tetO-Cre mice, their group was able to obtain an inducible system of endogenous *Kras*^{G12V} mediated by doxycycline control of Cre recombinase activity (Guerra et al., 2007). Essentially, removing doxycycline in this tet-off system permits an elastase-driven Cre specific to acinar and centroacinar cells of the pancreas. The Cre changes LSL-*Kras*^{G12Vgeo} into the active, oncogenic *Kras*^{G12Vgeo} by excising the loxP sites that contain a stop cassette. The utility of this system is further advanced by the detection of cells that ultimately end up with *Kras*^{G12V} expression. A knockin of IRES-*geo* into the 3′ untranslated sequences of the *Kras* allele allows for LacZ expression when the LSL cassette is removed (Guerra et al., 2003). LacZ encodes β-galactosidase, which is then detectable via histochemical staining. Initially, this system was used to induce expression of oncogenic *Kras* at E16.5, leading to the production of mPanIN lesions that could advance in severity following caerulein administration (Guerra et al., 2007). Surprisingly, doxycycline removal in adult stages resulted in widespread expression of *Kras*^{G12V} in adult acinar cells with no phenotypic consequences. Interestingly, adult mice that express *Kras*^{G12V} in the acinar cell compartment develop mPanINs and PDAC in the context of pancreatitis.

To explore the resistance of postnatal acinar cells to transformation via the expression of *Kras*, the Barbacid group also characterized the role of several tumor suppressors. These acinar cells were resistant to transformation even in the absence of tumor suppressors. *Kras*$^{+/G12V}$;p16*Ink4a*/p19*Arf*$^{flox/flox}$;*EL*-tTA/*tetO*-Cre and *Kras*$^{+/G12V}$;*Trp53*$^{flox/flox}$;*EL*-tTA/*tetO*-Cre mice were generated and given doxycycline from birth until P60 (Guerra et al., 2011). Acting under the same tet-off system as

described above, these mice, when taken off doxycycline, were subject to expression of Cre recombinase in acinar and centroacinar cells of the pancreas. However, instead of just activating *Kras*, the Cre simultaneously excised the floxed p16*Ink4a*/p19*Arf* or *Trp*53 alleles. These models, when combined with caerulein-induced pancreatitis, presented an invasive, metastatic PDAC phenotype (Guerra et al., 2011).

TVA-RCAS MODELS

Another mouse model system that features viral delivery for eventual induction of gene expression or loss of cell targets demonstrates the versatility of this field and another avenue for creating complex inducible/conditional schemes. Varmus and colleagues generated a model that introduced a replication-competent avian leukosis sarcoma virus long-terminal repeat with splice acceptor (ALSV-A-based RCAS) vector to mice that expressed the ALSV-A receptor, TVA, (Orsulic, 2002) under the control of the elastase promoter (Lewis et al., 2003). This elastase-*tva* model allowed somatic acinar cells of the pancreas to incorporate RCAS-delivered genes, such as polyoma virus middle T antigen (PyMT) (Gottlieb and Villarreal, 2001) or c-Myc, into the host cell genome. These elastase-*tva* mice were crossed to *Ink4a/Arf* null mice to create models characterizing the phenotype resulting from these initiating oncogenic events (Lewis et al., 2003). They found that PyMT and c-Myc induced different types of pancreatic tumors, illustrating the impact of the initiating lesion on resulting tumor pathology.

The development of this TVA-RCAS model was further expanded with the coupling of the elastase-*tva* mice with *Trp53* flox;*Ptf1a-Cre* (Jonkers et al., 2001) (Kawaguchi et al., 2002) mice (Morton et al., 2008). In this model, delivery of the PyMT oncogene is accompanied by the pancreas-specific deletion of the tumor suppressor, *Trp53*. Results of this model showed metastatic disease to the liver. In addition, the elastase-*tva*;$Trp53^{flox/flox}$;$Ptf1a^{Cre/+}$ mice were crossed to $Ink4a/Arf^{flox/+}$ (Krimpenfort et al., 2001) mice to assess tumor development in the context of a simultaneous p53 deficiency and *Ink4a/Arf* single allele deletion. Results of this model elucidated a much more aggressive tumor model after PyMT activation via virus administration (Morton et al., 2008). This model succeeds as an example of both conditional and temporal control of gene expression by combining both pancreas–specific deletion of *Trp53* via Cre-recombinase activity and acinar cell-directed, inducible PyMT expression via elastase-*tva* targeting.

Lewis and his group expounded upon these findings by crossing the *elastase-tva* model with LSL-$Kras^{G12D}$;$Ptf1a^{Cre/+}$ mice (Hingorani et al., 2003) to assess the impact of activated Wnt signaling in the context of KRAS-induced pancreatic tumorigenesis (Sano et al., 2014). These mice were injected with chick fibroblasts that produced ALSV-A-based RCAS vectors encoding Wnt1 or a GFP control, ultimately resulting in host genome uptake of these genes in pancreatic acinar cells and their progenitors. Thus, this model allowed for the targeting of Wnt1 to the pancreatic epithelium and subsequent characterization of its signaling activity when introduced in concert with *Kras* activation. They found that in this context, activated Wnt signaling induced the formation of mucinous cystic neoplasms (MCN). Interestingly, these mice displayed higher Wnt signaling in the stroma of the MCNs, rather than in the cyst epithelium, which is consistent with MCN patient data (Sano et al., 2014). These results suggest that Wnt ligands may act in a paracrine fashion to stimulate MCN development.

EXPLORING INDUCIBLE/CONDITIONAL SYSTEMS COUPLED WITH EPIGENETIC EVENTS

The significance of factors external to genomic changes in these models must not be overlooked. Multiple mutant *Kras*-expressing models have demonstrated the contribution of inflammation and dietary aspects to pancreatic cancer pathogenesis, improving our understanding of pancreatic cancer and pancreatitis as well as the interplay between the two. It was shown that high levels of Ras activity in cLGL-$Kras^{G12V}$;EL-*CreERT* generated high levels of fibrosis and inflammation that mimicked chronic pancreatitis. Since elevated Ras activity is also found in PDAC, this finding provided a mechanistic link between pancreatic cancer and chronic pancreatitis (Ji et al., 2009; Logsdon and Ji, 2009).

Other mechanisms have been explored with respect to inflammatory insult and subsequent neoplastic and cancerous phenotypes. Utilizing a breadth of models, Jack's group established that chronic pancreatitis may provide enough insult for insulin-expressing endocrine cells to become susceptible to KRAS-induced transformation (Gidekel Friedlander et al., 2009). Logsdon and colleagues also demonstrated that with caerulein induction of acute pancreatitis in the presence of inducible mutant *Kras* (LSL-$Kras^{G12V}$;EL-*CreERT*) there was NF-κB mediated amplification of Ras activity. These mice presented with chronic inflammation and mPanIN lesions that subsided with the inhibition of Cox-2 or deletion of *IKK2* (Daniluk et al., 2012). This effect was also demonstrated in KC mice with loss of Cox-2 despite the additional loss of pTEN, highlighting the potential role of AKT activation in chemoresistance (Hill et al., 2012). Likewise, the LSL-$Kras^{G12V}$;EL-*CreERT* model was used in a cross with Cox-2 conditional knockout mice to study the effects of high fat diets on PDAC. LSL-$Kras^{G12V}$;EL-*CreERT* mice fed high fat diet presented with increased fibrosis, mPanINs, and PDAC compared to no increased mPanIN lesions or PDAC in $COX^{flox/flox}$;LSL-$Kras^{G12V}$;EL-*CreERT* mice fed the same diet (Philip et al., 2013). Similarly, KC mice were shown to generate mPanIN lesions at an earlier onset following a high fat, high calorie diet with a subsequent increase in infiltration of macrophages and T cells in an expanded stromal bed (Dawson et al., 2013).

Progression of mPanINs and PDAC has also been explored in the context of inhibitors to the Ras signaling pathway. Gefitinib, an EGFR inhibitor, was given to LSL-$Kras^{G12D/+}$;$Ptf1a^{Cre/+}$ mice, demonstrating a prevention of mPanIN and PDAC development (Mohammed et al., 2010). Similarly, it was shown that inhibition of EGFR does not allow for RAS levels sufficient for the transformation seen in PDAC (Ardito et al., 2012; Navas et al., 2012).

FUTURE APPLICATIONS OF INDUCIBLE/CONDITIONAL MODELING SYSTEMS

The mouse-modeling field has capitalized on conditional and/or inducible Cre-lox technology to target gene expression in numerous cell types. However, the overwhelming majority of pancreatic

cancer models rely on Cre-lox to drive oncogenic *Kras* in the pancreatic epithelium, excluding the use of non-epithelial Cre systems and limiting the ability to target other cells types involved in carcinogenesis. Therefore, utilizing non-Cre-lox driven systems to target mutant *Kras* to pancreatic epithelium will allow compatibility with a vast array of preexisting Cre-lox systems that target genetic changes to additional cell types including the stroma and hematopoietic cell compartments.

The use of single transgenic or knockin systems in combination with Cre-lox models that target non-parenchymal cells in the pancreas can circumvent some of the limitations that arise when using Cre-lox to drive an initiating event like mutant *Kras*.

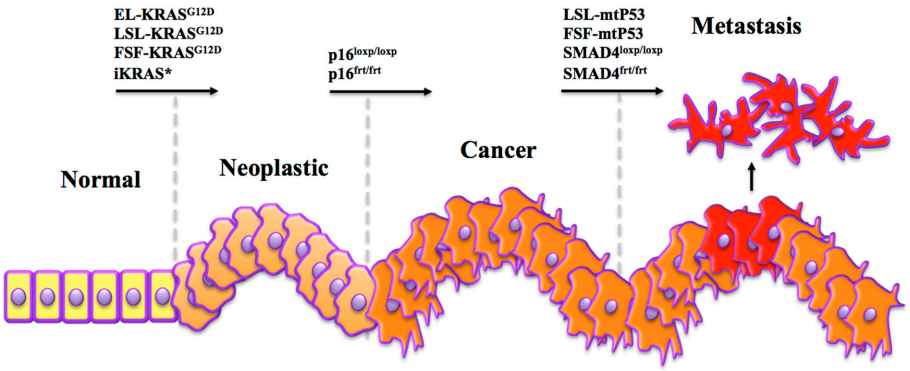

FIGURE 1 | Mimicking human tumorigenesis through temporal modeling of pancreatic cancer. A key difference between human pancreatic cancer and commonly used mouse models is in the timing of mutations. In human patients, *Kras* mutations are often considered an initiating event, occurring in adult cells, soon followed by mutations to *p16*, and later *p53* and/or *SMAD4*. Yet in most models, *Kras* and altered tumor suppressor genes are induced simultaneously in the developing embryo. Despite a human-like histotype, these models have yet to be accurate predictors of outcomes observed in clinical trials. Therefore, we propose that using combinations of several systems to drive sequential *Kras*, *p16*, and *SMAD4/p53* mutations may lead to more human-like disease that responds to therapy more like that observed in the clinic.

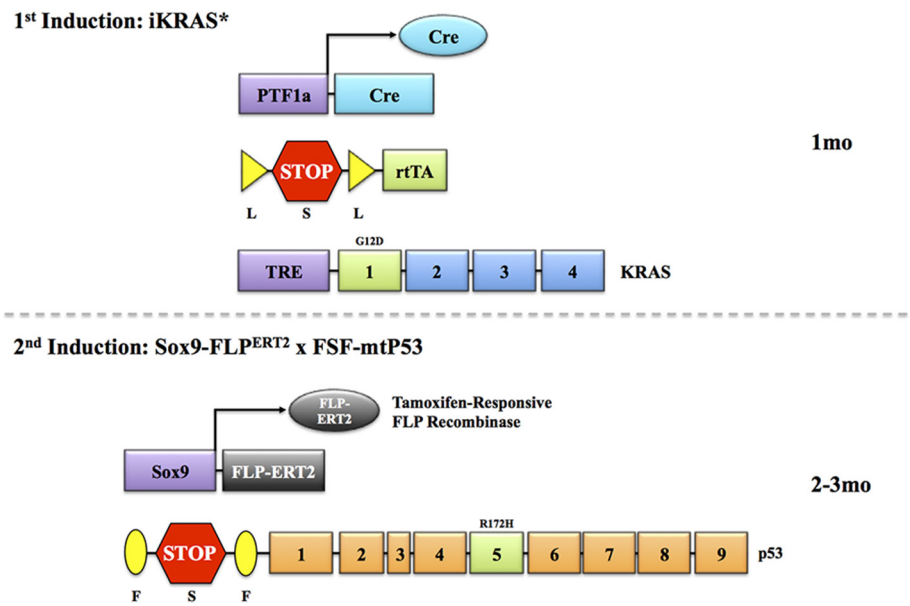

FIGURE 2 | Temporal modeling via two inducible systems. In order to address the issue of successive induction of mutations as they occur in human, several modeling systems can be employed. In this example, as designed by the Pasca di Magliano group, expression of Cre-recombinase is driven by the *Ptf1a* promoter. This is combined with a LSL cassette followed by an rtTA sequence. In the presence of Cre, the stop codon is excised, and rtTA is transcribed. This allows for interaction with a third transgene, a TRE-*Kras*. When doxycycline is administered, oncogenic *Kras* expression is induced. By activating this system at 1 month, it would allow a simulated *Kras* mutation in near-adult tissues. Once lesions manifest, this can be followed by the induction of a second transgene, a mutant *p53* driven by a *Sox9*-FLP^{ERT2} recombinase. This will excise a stop codon in front of a mutant *p53* sequence in the presence of tamoxifen, and drive mutant *p53* expression. The p16 allele could also be engineered in the same manner. Timing of these events will likely have to be determined empirically, as mutant *Kras* expression in adult pancreas may not lead to the development of neoplastic lesions without an external stimulus (like caerulein). Indeed, a third allelic alteration may be necessary to drive a more aggressive metastatic phenotype (see **Figure 3**).

The EL-KRAS model may be a prime candidate for combined Cre-lox targeting of other cell types, as these mice develop acinar-to-ductal metaplasia and cystic papillary neoplasms (CPN) that resemble human cystic disease in the pancreas. These lesions did progress to PDAC in a p16 null background or acinar carcinoma when in a p53 null background (personal communication with Dr. Eric Sandgren).

EL-TGFα (Sandgren et al., 1990) and *Mist1*$^{KrasG12D/+}$ (Tuveson et al., 2006) models can serve as potential neoplastic drivers used in concert with Cre-lox targeting. EL-TGFα mice have been employed in combination with p53 loss (Greten et al., 2001; Schreiner et al., 2003) to generate a model of advanced pancreatic cancer with hallmark genetic features (loss of p16, inactivation of Cdkn2a) reminiscent in human disease and, in combination with mutant *Kras*, development of CPN that resembles human IPMN (Siveke et al., 2007). EL-TGFα does lead to proliferation of acinar cells and fibroblasts and focally generated metaplastic lesions derived from acini (Sandgren et al., 1990). Yet, there was no reported observation of neoplasia or more advanced lesions in this model. *Mist1*$^{KrasG12D/+}$ mice developed a predictable lethal pancreatic cancer phenotype characterized by acinar metaplasia and dysplasia in its early stages (Tuveson et al., 2006). Despite being a strong model of the pancreatic neoplasia to cancer paradigm as an ectopic model of mutant *Kras* expression, *Mist1*$^{KrasG12D/+}$ mice did, rather unexpectedly, develop hepatocellular carcinoma (Tuveson et al., 2006). This feature of the model may be of potential concern when attempting to evaluate the phenotypes of genetically engineered mice that employ this particular initiating event. However, an inducible targeting of LSL-*Kras*$^{G12D/+}$ with *Mist1*$^{CreERT2/+}$ produced mPanIN lesions, indicating the relevance of the *Mist1*-expressing compartment in the origins of PDAC (Habbe et al., 2008). Although EL-KRAS

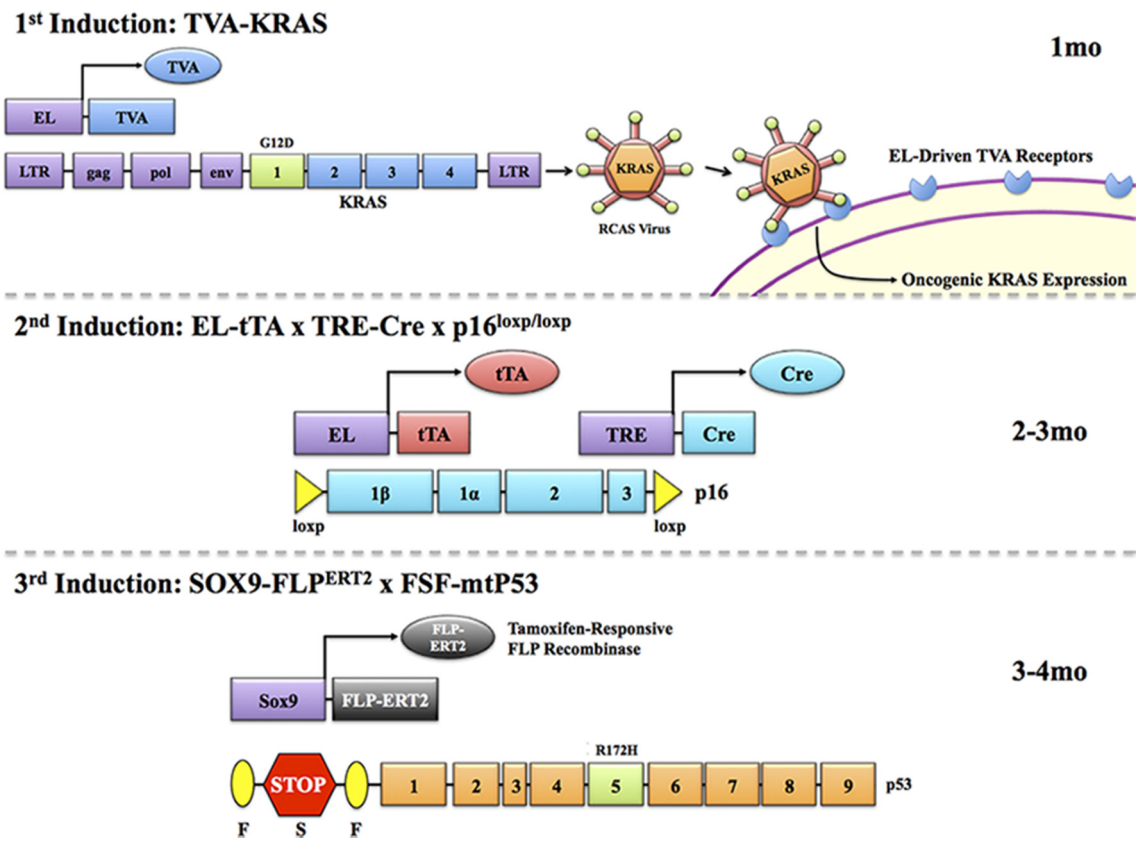

FIGURE 3 | Temporal modeling via three inducible systems. As human malignancies often involve several mutations, a compound inducible system may be employed to target three successive transgenes to the same cell type. For example, mt*Kras* may be first induced through a TVA/RCAS virus system. In this system, expression of a TVA receptor is targeted to the pancreas via the elastase promoter. Upon reaching adulthood, animals can be administered a RCAS virus coding for the mt*Kras* gene. This will interact only with cells expressing the TVA receptor, allowing for targeted and inducible expression of KRAS in the pancreas. A second mutation, such as loss of p16, can then be induced in the same cells via an elastase driven tTA that, in the presence of doxycycline, will induce expression of Cre through TRE-Cre. Combining this with a p16$^{flox/flox}$ gene will allow for doxycycline-induced loss of the p16 gene, and the second genetic hit. Finally, a tamoxifen-responsive *Sox9*-FLP^{ERT2} can target cells expressing ductal markers (including those having undergone acinar-ductal metaplasia), allowing for inducible expression of mtP53 via an FSF cassette, providing the third genetic hit as it often occurs in humans. It is important at each induction point that promoter/gene regulatory elements employed to run the next step be evaluated in the previous model. Hence, acinar-specific markers (eg., Amylase) should be assessed in pancreas following mutant *Kras* expression (TVA/RCAS delivery) and Sox9 antibodies should be used to demonstrate Sox9 expression in mtKras expressing pancreas with loss of p16. This would need to be done at the empirically derived time points (times provided in this figure are merely considerations) when the next induction is scheduled to begin.

mice do, on occasion, develop PanIN-like lesions, these are not the predominant histotype in the pancreas, as PanIN lesions are more frequently observed in human disease. Nonetheless, these transgenic approaches are compatible with non-mutant Kras driving Cre-lox systems and may prove useful in understanding disease etiology in combination with genetic manipulations in other cell compartments. These models do have utility with future approaches, though they lack recapitulation of the predominant clinical histotype (PanIN to PDAC).

Therefore, a FLP/FRT KRAS model poses the most promise for inducing *Kras* mutations that result in a PanIN-like phenotype while allowing the use of Cre-lox to target different genetic events in other cell types. In a manner similar to the Cre-lox system, FLP/FRT utilizes a recombinase called flippase to target FLP recombinase targets that flank an endogenous gene (Dymecki, 1996). Unlike Cre, which is derived from P1 bacteriophage, the FLP recombinase is derived from *Saccharomyces cerevisiae* (Sadowski, 1995). Ideally, a desirable model would involve the generation of a pancreas-specific FLP directed toward a FRT target sequence that flanks a stop codon upstream of oncogenic *Kras*. At this point, a pancreas-specific FLP may be possible with the intraductal injection of an adenovirus FLP or the generation of an EL-tTA;TetO-FLP;FSF-$Kras^{G12D/+}$ mouse. Ideally, this mechanism would drive mutant *Kras* in a near identical fashion as *EL-Cre*;LSL-$Kras^{G12D/+}$ while still allowing for the targeting of non-epithelial cell types with Cre-lox.

While this type of model would increase our understanding of the contributions of stromal, hematopoietic, and other cell types to pancreatic carcinogenesis, the ultimate goal of such a system would be the design of a layered model that is simultaneously and/or sequentially inducible. Mimicking a temporal progression of gene mutations in specific cellular compartments requires the use of multiple systems employing different modes of induction. As described, the CreERT system has been well established for many gene targets but alone can only deliver multiple mutations simultaneously (Frese and Tuveson, 2007). Young and colleagues demonstrated the potential of the FLP/FRT system when coupled with Cre-lox in lung tissue. They generated mice with an Flp inducible allele of $Kras^{G12D}$ and Cre driven mutation of the tumor suppressor, *p53* (Young et al., 2011). The FLP-FRT system, FSF-$Kras^{G12D}$, was induced through an adenovirus or lentivirus expressing Flpo, a version of Flp optimized

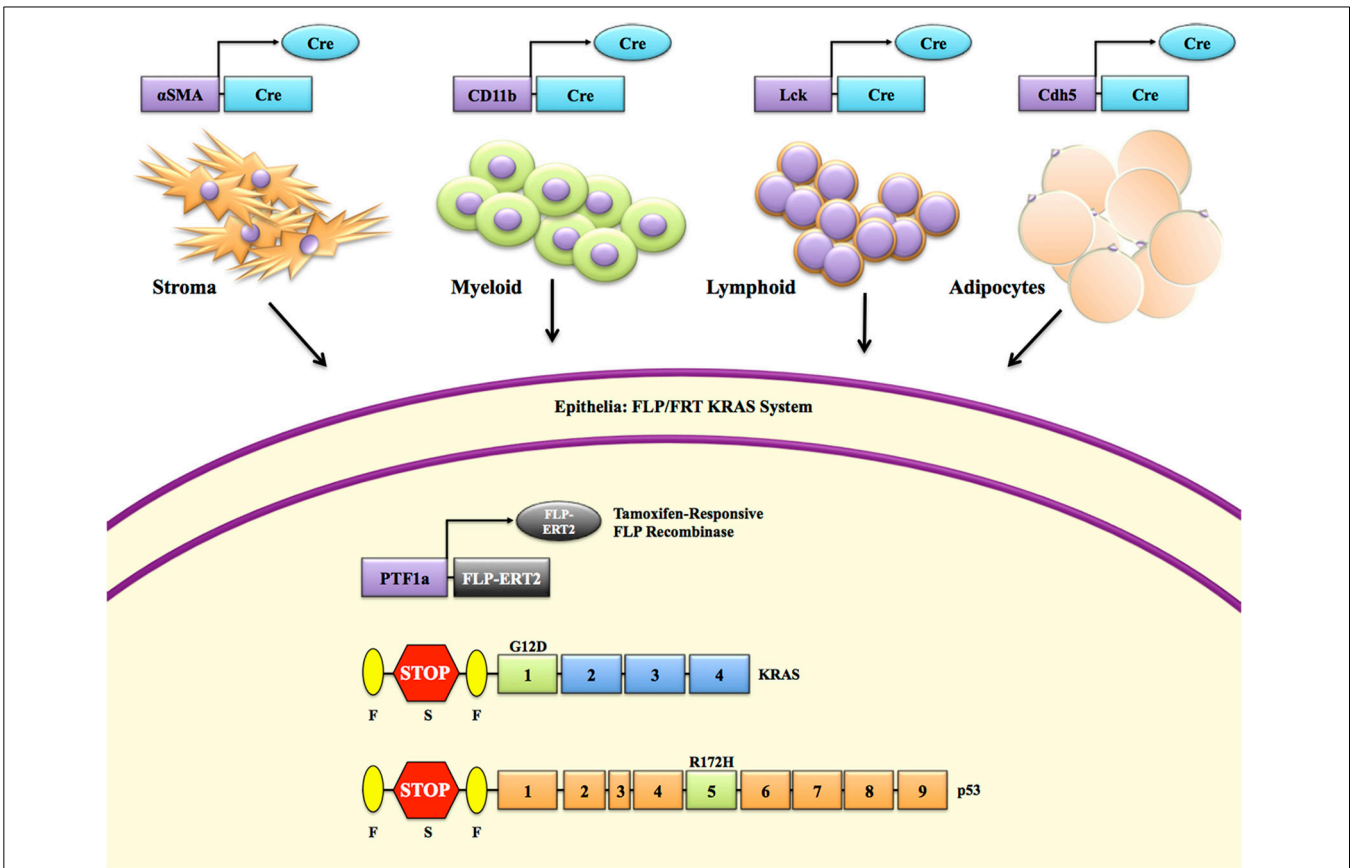

FIGURE 4 | Spatial modeling of pancreatic cancer to explore cross compartmental interactions. Cre-loxP is the most widely used conditional targeting system. This is also true in models of pancreatic cancer, where it is primarily used to drive mtKRAS via a loxP-stop-loxP (LSL) cassette. However, reliance on Cre-loxP to induce a *Kras* mutation limits our ability to target other pertinent cell types in the tumor microenvironment. Should mt*Kras* be induced by another system, for example a *Ptf1a*-FLP-driven Frt-stop-Frt (FSF) cassette, which would allow compatibility with one of the several hundred possible Cre-loxP combinations. For instance, an α*SMA-Cre* to explore the contributions of pancreas stellate cells to tumorigenesis, *CD11b-Cre* to target myeloid cells, *Lck-Cre* to target lymphoid cells, or *Cdh5-Cre* to target mature adipocytes (See **Table 1**).

Table 1 | Tissue Specific Cre-lox Targeting Systems.

Compartment	Cell/Tissue Type	Targeting Model	Reference
Epithelium	Pancreatic epithelium, antral stomach, and duodenum in neonates. Pancreatic beta islet cells in adults.	*Pdx1-Cre*	Hingorani et al., 2003
	Pancreatic acinar cells	*ElastaseCreERT2*	Desai et al., 2007
	Pancreatic acinar cells	*p48-Cre* *Ptf1a*$^{Cre/+}$ *Ptf1a*$^{Cre-ERTM}$	Hingorani et al., 2003; Kopinke et al., 2012
	Pancreatic acinar cells	*Mist1*$^{Cre-ERT2/+}$	Tuveson et al., 2006
Mesenchyme	Myofibroblast	*αSMA-Cre*	Wu et al., 2007
	Myofibroblast	*Vim-Cre*	Troeger et al., 2012
	Smooth muscle	*SMA-CreERT2*	Wendling et al., 2009
	Interstitial stroma of mature tissues—prostate, forestomach, skin	*Fsp1-Cre*	Bhowmick et al., 2004; Teng et al., 2011
	Bone, cartilage	*Dermo1-Cre* *Twist2-Cre*	Yu et al., 2003; Chen et al., 2008; Liu et al., 2010
	Pancreatic exocrine lineages	*Nestin-Cre*	Delacour et al., 2004
	Dermis, lung, pericardial connective tissue, blood vessel wall, splenic capsule, mesangial cells of glomerulus	*Col1a2-CreERT*	Zheng et al., 2002; Riopel et al., 2013
	Nestin-negative mesenchymal progenitors	*Prx1-Cre*	Greenbaum et al., 2013
Hematopoietic	CD4+ T Cells	*CD4-Cre*	Tanigaki et al., 2004
	Peripheral CD8+ T Cells	*CD8a-Cre*	Maekawa et al., 2008
	Liver and T lymphocytes after IFN or pI-pC induction	*Mx1-Cre*	Alonzi et al., 2001
	Myeloid lineage	*Cd11b-Cre*	Boillee et al., 2006
	Macrophages, granulocytes, possibly other myeloid derived cells	*LysM-Cre*	Clausen et al., 1999
	T lymphocytes and thymocytes	*Lck-Cre*	Tomita et al., 2003; Choi et al., 2013
	Hematopoietic cell lineages to peripheral blood, bone marrow, and spleen [Ectopic expression in PDAC (Fernandez-Zapico et al., 2005)]	*Vav1-Cre*	Daria et al., 2008
	Neutrophils, monocytes/macrophages, some dendritic cells	*Lactotransferrin-Cre*	Kovacic et al., 2014
	Hematopoietic stem cells/progeny	*Pf4-Cre*	Calaminus et al., 2012
	Immature B lymphocytes	*CD19-Cre*	Zhang et al., 2012
	Lymphoid and granulocyte-monocyte progenitors	*Flt3-Cre*	Buza-Vidas et al., 2011
Adipose	Brown and white adipose tissue	*aP2-Cre* *FABP4-Cre*	Cole et al., 2012
	Brown and white adipose tissue	*aP2-CreERT2*	Dali-Youcef et al., 2007
	Muscle, white adipose tissue, brain	*GLUT4-Cre*	Lin and Accili, 2011
	Brown and white adipocytes, skeletal muscle, dermis	*Myf5-Cre*	Sanchez-Gurmaches and Guertin, 2014
	Brown and white adipose tissue	*Adipoq-Cre*	Berry and Rodeheffer, 2013
	Mature adipocytes	*Cdh5-Cre*	Berry and Rodeheffer, 2013
	White adipocytes	*PdgfRα-Cre*	Berry and Rodeheffer, 2013
	White, inguinal white, and brown adipose tissue	*Retn-Cre*	Mullican et al., 2013

for mammalian use. Utilization of this mammalian version of Flp, as opposed to Flpe, was utilized due to its higher recombination efficiency (Farley et al., 2000). Injection of the adenovirus/lentivirus activates mutant *Kras* and results in numerous lung tumors, ultimately confirming that FSF-*Kras*^{G12D} results in a phenotype similar to LSL-*Kras*$^{G12D/+}$allele. This virus-driven FLP-FRT was coupled with a tamoxifen-driven p53 mutation via Cre recombinase activity (Young et al., 2011).

The TVA-RCAS targeting of epithelial tissue and subsequent stromal phenotype indicates further opportunity for the utilization of this system to target other cell types simultaneously. For example, the conditional nature of this model would allow for the targeting of genes to the stroma via a TVA-RCAS system utilizing a driver such as αSMA or Vimentin. Taking this further, the possibility arises for generation of a trigenic model. Utilizing Cre-lox, FLP/FRT, and TVA-RCAS targeting methods in the same mouse would provide a novel way to target several different cell types in both a conditional and inducible manner.

ADVANCING THE UTILITY OF INDUCIBLE/CONDITIONAL MODELING

While the aforementioned models are undoubtedly technological achievements, their ability to faithfully recapitulate human disease is still limited. Clinically, at least two gene mutations occur

to produce PDAC. *Kras* is believed to be the first mutation in a series of transformation events that lead to PDAC in adults. Subsequent major mutations include those to p53, SMAD4, or $p16^{INK4a}$, among several others (Hezel et al., 2006). With current mouse models, recombination events affecting *Kras* and these other genes occur either during embryonic development or concomitantly sometime after pancreas formation, in the case of inducible systems. However, they fail to capture the step-wise mutation process that occurs in the adult pancreata of human patients.

Layering multiple inducible systems to target the same cell type and cause multiple mutations in a step-wise manner would assist in capturing a more faithful representation of human disease progression (**Figure 1**). For example, targeting *Kras* with an EL-tTA or EL-TVA system would provide a mechanism for issuing the first hit of genetic instability in both a temporal and tissue-specific manner. However, it should be noted that elastase targeting in these systems may be dramatically inefficient after pancreas cells advance to a ductal and/or abnormal phenotype. Ablation of a second gene such as *p53*, *SMAD4*, or $p16^{INK4a}$ could then be controlled by a Cre-ERT2 system directed toward the same cells expressing mutant KRAS (**Figure 2**). Finally, a third system, the FLP/FRT, could be utilized to mutate a third gene in an effort to drive metastatic phenotypes. This trigenic model, which is just one example of many possible inducible/conditional mutation schemes, would better serve to mimic the progressive nature of PDAC (**Figure 3**). However, generation of such models inherently results in very complex breeding patterns. Additionally, once these trigenic mice are established the induction of different mutations requires a labor-intensive injection scheme and administration of doxycycline over extended periods of time.

From a functional standpoint, the utilization of inducible/conditional drivers other than Cre recombinase for the activation of mutant KRAS allows for subsequent Cre-lox targeting of cell types outside the epithelial compartment (**Figure 4**). Strategically, withholding Cre-lox targeting of *Kras* encourages the use of abundant, pre-existing Cre-lox systems (**Table 1**) that can target stromal, hematopoietic, and adipose compartments. However, this type of modeling is not necessarily relevant from a clinical standpoint, due lack of evidence that these non-epithelial mutations are common in human PDAC. Nevertheless, this approach allows for more rigorous evaluation of the contributions that different components of the tumor microenvironment (TME) have on carcinogenesis. Insight into the mechanism behind TME involvement in tumor progression and metastatic phenotypes may provide strategies and the rationale for targeting these compartments with certain therapeutic agents. These inducible/conditional systems will be highly relevant in studying the therapeutic value of a genetic target in mature tumors and not at the initiation stages. For instance, a model with expression of oncogenic $Kras^{G12V}$ and deletion of p53 with an EL-tTA FLP system used in conjunction with ablation of a target gene, such as EGFR, by an ubiquitous Cre-ERT2 system is under development in the Barbacid laboratory.

The goal of such systems is to recapitulate the human condition, which can only be done in part. Indeed, mouse models are simply that—models that will never completely recapitulate human PDAC. It is critical to generate these models in a clean background strain to eliminate the potential causative role that genetic variability among chimerics may play when comparing test and control animals, particularly as the complexity of these models increases. The layering of multiple schemes lends itself to amplifying the anomalies produced by one model and potentially augmenting those in another system as they are combined. Despite these caveats, current and future inducible and/or conditional models will lead to a more faithful representation of human disease, which is essential to teasing out the phenotypic and mechanistic aspects of pancreatic cancer that will ultimately improve outcomes in the clinic.

REFERENCES

Aguirre, A. J., Bardeesy, N., Sinha, M., Lopez, L., Tuveson, D. A., Horner, J., et al. (2003). Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. *Genes Dev.* 17, 3112–3126. doi: 10.1101/gad.1158703

Alonzi, T., Maritano, D., Gorgoni, B., Rizzuto, G., Libert, C., and Poli, V. (2001). Essential role of STAT3 in the control of the acute-phase response as revealed by inducible gene inactivation [correction of activation] in the liver. *Mol. Cell. Biol.* 21, 1621–1632. doi: 10.1128/MCB.21.5.1621-1632.2001

Ardito, C. M., Gruner, B. M., Takeuchi, K. K., Lubeseder-Martellato, C., Teichmann, N., Mazur, P. K., et al. (2012). EGF receptor is required for KRAS-induced pancreatic tumorigenesis. *Cancer Cell* 22, 304–317. doi: 10.1016/j.ccr.2012.07.024

Bardeesy, N., Cheng, K. H., Berger, J. H., Chu, G. C., Pahler, J., Olson, P., et al. (2006). Smad4 is dispensable for normal pancreas development yet critical in progression and tumor biology of pancreas cancer. *Genes Dev.* 20, 3130–3146. doi: 10.1101/gad.1478706

Bardeesy, N., Morgan, J., Sinha, M., Signoretti, S., Srivastava, S., Loda, M., et al. (2002). Obligate roles for p16(Ink4a) and p19(Arf)-p53 in the suppression of murine pancreatic neoplasia. *Mol. Cell. Biol.* 22, 635–643. doi: 10.1128/MCB.22.2.635-643.2002

Belteki, G., Haigh, J., Kabacs, N., Haigh, K., Sison, K., Costantini, F., et al. (2005). Conditional and inducible transgene expression in mice through the combinatorial use of Cre-mediated recombination and tetracycline induction. *Nucleic Acids Res.* 33:e51. doi: 10.1093/nar/gni051

Berry, R., and Rodeheffer, M. S. (2013). Characterization of the adipocyte cellular lineage *in vivo*. *Nat. Cell Biol.* 15, 302–308. doi: 10.1038/ncb2696

Bhowmick, N. A., Chytil, A., Plieth, D., Gorska, A. E., Dumont, N., Shappell, S., et al. (2004). TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. *Science* 303, 848–851. doi: 10.1126/science.1090922

Boillee, S., Yamanaka, K., Lobsiger, C. S., Copeland, N. G., Jenkins, N. A., Kassiotis, G., et al. (2006). Onset and progression in inherited ALS determined by motor neurons and microglia. *Science* 312, 1389–1392. doi: 10.1126/science.1123511

Borazanci, E., and Von Hoff, D. D. (2014). Nab-paclitaxel and gemcitabine for the treatment of patients with metastatic pancreatic cancer. *Expert Rev. Gastroenterol. Hepatol.* 8, 739–747. doi: 10.1586/17474124.2014.925799

Buza-Vidas, N., Woll, P., Hultquist, A., Duarte, S., Lutteropp, M., Bouriez-Jones, T., et al. (2011). FLT3 expression initiates in fully multipotent mouse hematopoietic progenitor cells. *Blood* 118, 1544–1548. doi: 10.1182/blood-2010-10-316232

Calaminus, S. D., Guitart, A. V., Sinclair, A., Schachtner, H., Watson, S. P., Holyoake, T. L., et al. (2012). Lineage tracing of Pf4-Cre marks hematopoietic stem cells and their progeny. *PLoS ONE* 7:e51361. doi: 10.1371/journal.pone.0051361

Carriere, C., Gore, A. J., Norris, A. M., Gunn, J. R., Young, A. L., Longnecker, D. S., et al. (2011a). Deletion of Rb accelerates pancreatic carcinogenesis by oncogenic Kras and impairs senescence in premalignant lesions. *Gastroenterology* 141, 1091–1101. doi: 10.1053/j.gastro.2011.05.041

Carriere, C., Seeley, E. S., Goetze, T., Longnecker, D. S., and Korc, M. (2007). The Nestin progenitor lineage is the compartment of origin for pancreatic intraepithelial neoplasia. *Proc. Natl. Acad. Sci. U.S.A.* 104, 4437–4442. doi: 10.1073/pnas.0701117104

Carriere, C., Young, A. L., Gunn, J. R., Longnecker, D. S., and Korc, M. (2011b). Acute pancreatitis accelerates initiation and progression to pancreatic cancer in

mice expressing oncogenic Kras in the nestin cell lineage. *PLoS ONE* 6:e27725. doi: 10.1371/journal.pone.0027725

Chen, H., Zhuang, F., Liu, Y. H., Xu, B., Del Moral, P., Deng, W., et al. (2008). TGF-beta receptor II in epithelia versus mesenchyme plays distinct roles in the developing lung. *Eur. Respir. J.* 32, 285–295. doi: 10.1183/09031936.00165407

Choi, Y. I., Duke-Cohan, J. S., Tan, J., Gui, J., Singh, M. K., Epstein, J. A., et al. (2013). Plxnd1 expression in thymocytes regulates their intrathymic migration while that in thymic endothelium impacts medullary topology. *Front. Immunol.* 4:392. doi: 10.3389/fimmu.2013.00392

Clausen, B. E., Burkhardt, C., Reith, W., Renkawitz, R., and Forster, I. (1999). Conditional gene targeting in macrophages and granulocytes using LysMcre mice. *Transgenic Res.* 8, 265–277.

Cole, B. K., Morris, M. A., Grzesik, W. J., Leone, K. A., and Nadler, J. L. (2012). Adipose tissue-specific deletion of 12/15-lipoxygenase protects mice from the consequences of a high-fat diet. *Mediators Inflamm.* 2012:851798. doi: 10.1155/2012/851798

Collins, M. A., Bednar, F., Zhang, Y., Brisset, J. C., Galban, S., Galban, C. J., et al. (2012a). Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer in mice. *J. Clin. Invest.* 122, 639–653. doi: 10.1172/JCI59227

Collins, M. A., Brisset, J. C., Zhang, Y., Bednar, F., Pierre, J., Heist, K. A., et al. (2012b). Metastatic pancreatic cancer is dependent on oncogenic Kras in mice. *PLoS ONE* 7:e49707. doi: 10.1371/journal.pone.0049707

Dali-Youcef, N., Mataki, C., Coste, A., Messaddeq, N., Giroud, S., Blanc, S., et al. (2007). Adipose tissue-specific inactivation of the retinoblastoma protein protects against diabesity because of increased energy expenditure. *Proc. Natl. Acad. Sci. U.S.A.* 104, 10703–10708. doi: 10.1073/pnas.0611568104

Daniluk, J., Liu, Y., Deng, D., Chu, J., Huang, H., Gaiser, S., et al. (2012). An NF-kappaB pathway-mediated positive feedback loop amplifies Ras activity to pathological levels in mice. *J. Clin. Invest.* 122, 1519–1528. doi: 10.1172/JCI59743

Daria, D., Filippi, M. D., Knudsen, E. S., Faccio, R., Li, Z., Kalfa, T., et al. (2008). The retinoblastoma tumor suppressor is a critical intrinsic regulator for hematopoietic stem and progenitor cells under stress. *Blood* 111, 1894–1902. doi: 10.1182/blood-2007-02-071746

Dawson, D. W., Hertzer, K., Moro, A., Donald, G., Chang, H. H., Go, V. L., et al. (2013). High-fat, high-calorie diet promotes early pancreatic neoplasia in the conditional KrasG12D mouse model. *Cancer Prev. Res. (Phila.)* 6, 1064–1073. doi: 10.1158/1940-6207.CAPR-13-0065

Delacour, A., Nepote, V., Trumpp, A., and Herrera, P. L. (2004). Nestin expression in pancreatic exocrine cell lineages. *Mech. Dev.* 121, 3–14. doi: 10.1016/j.mod.2003.11.004

De La, O. J., Emerson, L. L., Goodman, J. L., Froebe, S. C., Illum, B. E., Curtis, A. B., et al. (2008). Notch and Kras reprogram pancreatic acinar cells to ductal intraepithelial neoplasia. *Proc. Natl. Acad. Sci. U.S.A.* 105, 18907–18912. doi: 10.1073/pnas.0810111105

Desai, B. M., Oliver-Krasinski, J., De Leon, D. D., Farzad, C., Hong, N., Leach, S. D., et al. (2007). Preexisting pancreatic acinar cells contribute to acinar cell, but not islet beta cell, regeneration. *J. Clin. Invest.* 117, 971–977. doi: 10.1172/JCI29988

Dymecki, S. M. (1996). Flp recombinase promotes site-specific DNA recombination in embryonic stem cells and transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.* 93, 6191–6196.

Farley, F. W., Soriano, P., Steffen, L. S., and Dymecki, S. M. (2000). Widespread recombinase expression using FLPeR (flipper) mice. *Genesis* 28, 106–110. doi: 10.1002/1526-968X(200011/12)28:3/43.0.CO;2-T

Feig, C., Jones, J. O., Kraman, M., Wells, R. J., Deonarine, A., Chan, D. S., et al. (2013). Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc. Natl. Acad. Sci. U.S.A.* 110, 20212–20217. doi: 10.1073/pnas.1320318110

Feil, R., Brocard, J., Mascrez, B., Lemeur, M., Metzger, D., and Chambon, P. (1996). Ligand-activated site-specific recombination in mice. *Proc. Natl. Acad. Sci. U.S.A.* 93, 10887–10890.

Feil, R., Wagner, J., Metzger, D., and Chambon, P. (1997). Regulation of Cre recombinase activity by mutated estrogen receptor ligand-binding domains. *Biochem. Biophys. Res. Commun.* 237, 752–757. doi: 10.1006/bbrc.1997.7124

Fernandez-Zapico, M. E., Gonzalez-Paz, N. C., Weiss, E., Savoy, D. N., Molina, J. R., Fonseca, R., et al. (2005). Ectopic expression of VAV1 reveals an unexpected role in pancreatic cancer tumorigenesis. *Cancer Cell* 7, 39–49. doi: 10.1016/j.ccr.2004.11.024

Fisher, G. H., Wellen, S. L., Klimstra, D., Lenczowski, J. M., Tichelaar, J. W., Lizak, M. J., et al. (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. doi: 10.1101/gad.947701

Frese, K. K., and Tuveson, D. A. (2007). Maximizing mouse cancer models. *Nat. Rev. Cancer* 7, 645–658. doi: 10.1038/nrc2192

Gidekel Friedlander, S. Y., Chu, G. C., Snyder, E. L., Girnius, N., Dibelius, G., Crowley, D., et al. (2009). Context-dependent transformation of adult pancreatic cells by oncogenic K-Ras. *Cancer Cell* 16, 379–389. doi: 10.1016/j.ccr.2009.09.027

Gore, J., and Korc, M. (2014). Pancreatic cancer stroma: friend or foe? *Cancer Cell* 25, 711–712. doi: 10.1016/j.ccr.2014.05.026

Gottlieb, K. A., and Villarreal, L. P. (2001). Natural biology of polyomavirus middle T antigen. *Microbiol. Mol. Biol. Rev.* 65, 288–318. doi: 10.1128/MMBR.65.2.288-318.2001

Greenbaum, A., Hsu, Y. M., Day, R. B., Schuettpelz, L. G., Christopher, M. J., Borgerding, J. N., et al. (2013). CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. *Nature* 495, 227–230. doi: 10.1038/nature11926

Greten, F. R., Wagner, M., Weber, C. K., Zechner, U., Adler, G., and Schmid, R. M. (2001). TGF alpha transgenic mice. A model of pancreatic cancer development. *Pancreatology* 1, 363–368. doi: 10.1159/000055835

Grippo, 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.

Grippo, P. J., and Sandgren, E. P. (2012). Acinar-to-ductal metaplasia accompanies c-myc-induced exocrine pancreatic cancer progression in transgenic rodents. *Int. J. Cancer* 131, 1243–1248. doi: 10.1002/ijc.27322

Guerra, C., and Barbacid, M. (2013). Genetically engineered mouse models of pancreatic adenocarcinoma. *Mol. Oncol.* 7, 232–247. doi: 10.1016/j.molonc.2013.02.002

Guerra, C., Collado, M., Navas, C., Schuhmacher, A. J., Hernandez-Porras, I., Canamero, M., et al. (2011). Pancreatitis-induced inflammation contributes to pancreatic cancer by inhibiting oncogene-induced senescence. *Cancer Cell* 19, 728–739. doi: 10.1016/j.ccr.2011.05.011

Guerra, C., Mijimolle, N., Dhawahir, A., Dubus, P., Barradas, M., Serrano, M., et al. (2003). Tumor induction by an endogenous K-ras oncogene is highly dependent on cellular context. *Cancer Cell* 4, 111–120. doi: 10.1016/S1535-6108(03)00191-0

Guerra, C., Schuhmacher, A. J., Canamero, M., Grippo, P. J., Verdaguer, L., Perez-Gallego, L., et al. (2007). Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. *Cancer Cell* 11, 291–302. doi: 10.1016/j.ccr.2007.01.012

Habbe, N., Shi, G., Meguid, R. A., Fendrich, V., Esni, F., Chen, H., et al. (2008). Spontaneous induction of murine pancreatic intraepithelial neoplasia (mPanIN) by acinar cell targeting of oncogenic Kras in adult mice. *Proc. Natl. Acad. Sci. U.S.A.* 105, 18913–18918. doi: 10.1073/pnas.0810097105

Hahn, S. A., Schutte, M., Hoque, A. T., Moskaluk, C. A., Da Costa, L. T., Rozenblum, E., et al. (1996). DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science* 271, 350–353.

Hezel, A. F., Kimmelman, A. C., Stanger, B. Z., Bardeesy, N., and Depinho, R. A. (2006). Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev.* 20, 1218–1249. doi: 10.1101/gad.1415606

Hill, R., Li, Y., Tran, L. M., Dry, S., Calvopina, J. H., Garcia, A., et al. (2012). Cell intrinsic role of COX-2 in pancreatic cancer development. *Mol. Cancer Ther.* 11, 2127–2137. doi: 10.1158/1535-7163.MCT-12-0342

Hingorani, S. R., Petricoin, E. F., Maitra, A., Rajapakse, V., King, C., Jacobetz, M. A., et al. (2003). Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* 4, 437–450. doi: 10.1016/S1535-6108(03)00309-X

Hingorani, S. R., Wang, L., Multani, A. S., Combs, C., Deramaudt, T. B., Hruban, R. H., et al. (2005). Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell* 7, 469–483. doi: 10.1016/j.ccr.2005.04.023

Hruban, R. H., Adsay, N. V., Albores-Saavedra, J., Anver, M. R., Biankin, A. V., Boivin, G. P., et al. (2006). Pathology of genetically engineered mouse models of pancreatic exocrine cancer: consensus report and recommendations. *Cancer Res.* 66, 95–106. doi: 10.1158/0008-5472.CAN-05-2168

Ijichi, H., Chytil, A., Gorska, A. E., Aakre, M. E., Fujitani, Y., Fujitani, S., et al. (2006). Aggressive pancreatic ductal adenocarcinoma in mice caused by pancreas-specific blockade of transforming growth factor-beta signaling in cooperation with active Kras expression. *Genes Dev.* 20, 3147–3160. doi: 10.1101/gad.1475506

Izeradjene, K., Combs, C., Best, M., Gopinathan, A., Wagner, A., Grady, W. M., et al. (2007). Kras(G12D) and Smad4/Dpc4 haploinsufficiency cooperate to induce mucinous cystic neoplasms and invasive adenocarcinoma of the pancreas. *Cancer Cell* 11, 229–243. doi: 10.1016/j.ccr.2007.01.017

Ji, B., Tsou, L., Wang, H., Gaiser, S., Chang, D. Z., Daniluk, J., et al. (2009). Ras activity levels control the development of pancreatic diseases. *Gastroenterology* 137, 1072–1082; 1082, e1071–e1076. doi: 10.1053/j.gastro.2009.05.052

Jones, S., Zhang, X., Parsons, D. W., Lin, J. C., Leary, R. J., Angenendt, P., et al. (2008). Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 321, 1801–1806. doi: 10.1126/science.1164368

Jonkers, J., Meuwissen, R., Van Der Gulden, H., Peterse, H., Van Der Valk, M., and Berns, A. (2001). Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. *Nat. Genet.* 29, 418–425. doi: 10.1038/ng747

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. doi: 10.1038/ng959

Kojima, K., Vickers, S. M., Adsay, N. V., Jhala, N. C., Kim, H. G., Schoeb, T. R., et al. (2007). Inactivation of Smad4 accelerates Kras(G12D)-mediated pancreatic neoplasia. *Cancer Res.* 67, 8121–8130. doi: 10.1158/0008-5472.CAN-06-4167

Kopinke, D., Brailsford, M., Pan, F. C., Magnuson, M. A., Wright, C. V., and Murtaugh, L. C. (2012). Ongoing Notch signaling maintains phenotypic fidelity in the adult exocrine pancreas. *Dev. Biol.* 362, 57–64. doi: 10.1016/j.ydbio.2011.11.010

Kovacic, B., Hoelbl-Kovacic, A., Fischhuber, K. M., Leitner, N. R., Gotthardt, D., Casanova, E., et al. (2014). Lactotransferrin-Cre reporter mice trace neutrophils, monocytes/macrophages and distinct subtypes of dendritic cells. *Haematologica* 99, 1006–1015. doi: 10.3324/haematol.2013.097154

Kraman, M., Bambrough, P. J., Arnold, J. N., Roberts, E. W., Magiera, L., Jones, J. O., et al. (2010). Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. *Science* 330, 827–830. doi: 10.1126/science.1195300

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. doi: 10.1038/35092584

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. doi: 10.1101/gad.1140403

Lin, H. V., and Accili, D. (2011). Reconstitution of insulin action in muscle, white adipose tissue, and brain of insulin receptor knock-out mice fails to rescue diabetes. *J. Biol. Chem.* 286, 9797–9804. doi: 10.1074/jbc.M110.210807

Liu, Y., Wang, L., Fatahi, R., Kronenberg, M., Kalajzic, I., Rowe, D., et al. (2010). Isolation of murine bone marrow derived mesenchymal stem cells using Twist2 Cre transgenic mice. *Bone* 47, 916–925. doi: 10.1016/j.bone.2010.07.022

Logsdon, C. D., and Ji, B. (2009). Ras activity in acinar cells links chronic pancreatitis and pancreatic cancer. *Clin. Gastroenterol. Hepatol.* 7, S40–S43. doi: 10.1016/j.cgh.2009.07.040

Maekawa, Y., Minato, Y., Ishifune, C., Kurihara, T., Kitamura, A., Kojima, H., et al. (2008). Notch2 integrates signaling by the transcription factors RBP-J and CREB1 to promote T cell cytotoxicity. *Nat. Immunol.* 9, 1140–1147. doi: 10.1038/ni.1649

Mirus, J. E., Zhang, Y., Hollingsworth, M. A., Solan, J. L., Lampe, P. D., and Hingorani, S. R. (2014). Spatiotemporal proteomic analyses during pancreas cancer progression identifies STK4 as a novel candidate biomarker for early stage disease. *Mol. Cell. Proteomics*. doi: 10.1074/mcp.M113.036517. [Epub ahead of print].

Mohammed, A., Janakiram, N. B., Li, Q., Madka, V., Ely, M., Lightfoot, S., et al. (2010). The epidermal growth factor receptor inhibitor gefitinib prevents the progression of pancreatic lesions to carcinoma in a conditional LSL-KrasG12D/+ transgenic mouse model. *Cancer Prev. Res. (Phila.)* 3, 1417–1426. doi: 10.1158/1940-6207.CAPR-10-0038

Morton, J. P., Klimstra, D. S., Mongeau, M. E., and Lewis, B. C. (2008). Trp53 deletion stimulates the formation of metastatic pancreatic tumors. *Am. J. Pathol.* 172, 1081–1087. doi: 10.2353/ajpath.2008.070778

Mullican, S. E., Tomaru, T., Gaddis, C. A., Peed, L. C., Sundaram, A., and Lazar, M. A. (2013). A novel adipose-specific gene deletion model demonstrates potential pitfalls of existing methods. *Mol. Endocrinol.* 27, 127–134. doi: 10.1210/me.2012-1267

Navas, C., Hernandez-Porras, I., Schuhmacher, A. J., Sibilia, M., Guerra, C., and Barbacid, M. (2012). EGF receptor signaling is essential for k-ras oncogene-driven pancreatic ductal adenocarcinoma. *Cancer Cell* 22, 318–330. doi: 10.1016/j.ccr.2012.08.001

Neesse, A., Michl, P., Frese, K. K., Feig, C., Cook, N., Jacobetz, M. A., et al. (2011). Stromal biology and therapy in pancreatic cancer. *Gut* 60, 861–868. doi: 10.1136/gut.2010.226092

Obata, J., Yano, M., Mimura, H., Goto, T., Nakayama, R., Mibu, Y., et al. (2001). p48 subunit of mouse PTF1 binds to RBP-Jkappa/CBF-1, the intracellular mediator of Notch signalling, and is expressed in the neural tube of early stage embryos. *Genes Cells* 6, 345–360. doi: 10.1046/j.1365-2443.2001.00422.x

Ohlsson, H., Karlsson, K., and Edlund, T. (1993). IPF1, a homeodomain-containing transactivator of the insulin gene. *EMBO J.* 12, 4251–4259.

Olive, K. P., Jacobetz, M. A., Davidson, C. J., Gopinathan, A., Mcintyre, D., Honess, D., et al. (2009). Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 324, 1457–1461. doi: 10.1126/science.1171362

Olive, K. P., Tuveson, D. A., Ruhe, Z. C., Yin, B., Willis, N. A., Bronson, R. T., et al. (2004). Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. *Cell* 119, 847–860. doi: 10.1016/j.cell.2004.11.004

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.

Ornitz, D. M., Palmiter, R. D., Messing, A., Hammer, R. E., Pinkert, C. A., and Brinster, R. L. (1985). Elastase I promoter directs expression of human growth hormone and SV40 T antigen genes to pancreatic acinar cells in transgenic mice. *Cold Spring Harb. Symp. Quant. Biol.* 50, 399–409.

Orsulic, S. (2002). An RCAS-TVA-based approach to designer mouse models. *Mamm. Genome* 13, 543–547. doi: 10.1007/s00335-002-4003-4

Ozdemir, B. C., Pentcheva-Hoang, T., Carstens, J. L., Zheng, X., Wu, C. C., Simpson, T. R., et al. (2014). Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. *Cancer Cell* 25, 719–734. doi: 10.1016/j.ccr.2014.04.005

Papadatos-Pastos, D., Thillai, K., Rabbie, R., Ross, P., and Sarker, D. (2014). FOLFIRINOX - a new paradigm in the treatment of pancreatic cancer. *Expert Rev. Anticancer Ther.* 14, 1115–1125. doi: 10.1586/14737140.2014.957188

Philip, B., Roland, C. L., Daniluk, J., Liu, Y., Chatterjee, D., Gomez, S. B., et al. (2013). A high-fat diet activates oncogenic Kras and COX2 to induce development of pancreatic ductal adenocarcinoma in mice. *Gastroenterology* 145, 1449–1458. doi: 10.1053/j.gastro.2013.08.018

Principe, D. R., Doll, J. A., Bauer, J., Jung, B., Munshi, H. G., Bartholin, L., et al. (2014). TGF-beta: duality of function between tumor prevention and carcinogenesis. *J. Natl. Cancer Inst.* 106:djt369. doi: 10.1093/jnci/djt369

Qiu, W., Sahin, F., Iacobuzio-Donahue, C. A., Garcia-Carracedo, D., Wang, W. M., Kuo, C. Y., et al. (2011). Disruption of p16 and activation of Kras in pancreas increase ductal adenocarcinoma formation and metastasis *in vivo*. *Oncotarget* 2, 862–873.

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.

Ray, K. C., Moss, M. E., Franklin, J. L., Weaver, C. J., Higginbotham, J., Song, Y., et al. (2014). Heparin-binding epidermal growth factor-like growth factor eliminates constraints on activated Kras to promote rapid onset of pancreatic neoplasia. *Oncogene* 33, 823–831. doi: 10.1038/onc.2013.3

Rhim, A. D., Oberstein, P. E., Thomas, D. H., Mirek, E. T., Palermo, C. F., Sastra, S. A., et al. (2014). Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell* 25, 735–747. doi: 10.1016/j.ccr.2014.04.021

Riopel, M. M., Li, J., Liu, S., Leask, A., and Wang, R. (2013). Beta1 integrin-extracellular matrix interactions are essential for maintaining exocrine pancreas architecture and function. *Lab. Invest.* 93, 31–40. doi: 10.1038/labinvest.2012.147

Sadowski, P. D. (1995). The Flp recombinase of the 2-microns plasmid of Saccharomyces cerevisiae. *Prog. Nucleic Acid Res. Mol. Biol.* 51, 53–91.

Sanchez-Gurmaches, J., and Guertin, D. A. (2014). Adipocyte lineages: tracing back the origins of fat. *Biochim. Biophys. Acta* 1842, 340–351. doi: 10.1016/j.bbadis.2013.05.027

Sandgren, E. P., Luetteke, 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., Luetteke, 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.

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. U.S.A.* 88, 93–97.

Sano, M., Driscoll, D. R., De Jesus-Monge, W. E., Klimstra, D. S., and Lewis, B. C. (2014). Activated wnt signaling in stroma contributes to development of pancreatic mucinous cystic neoplasms. *Gastroenterology* 146, 257–267. doi: 10.1053/j.gastro.2013.09.044

Schreiner, B., Baur, D. M., Fingerle, A. A., Zechner, U., Greten, F. R., Adler, G., et al. (2003). Pattern of secondary genomic changes in pancreatic tumors of Tgf alpha/Trp53$^{+/-}$ transgenic mice. *Genes Chromosomes Cancer* 38, 240–248. doi: 10.1002/gcc.10285

Sherman, M. H., Yu, R. T., Engle, D. D., Ding, N., Atkins, A. R., Tiriac, H., et al. (2014). Vitamin d receptor-mediated stromal reprogramming suppresses pancreatitis and enhances pancreatic cancer therapy. *Cell* 159, 80–93. doi: 10.1016/j.cell.2014.08.007

Siveke, J. T., Einwachter, H., Sipos, B., Lubeseder-Martellato, C., Kloppel, G., and Schmid, R. M. (2007). Concomitant pancreatic activation of Kras(G12D) and Tgfa results in cystic papillary neoplasms reminiscent of human IPMN. *Cancer Cell* 12, 266–279. doi: 10.1016/j.ccr.2007.08.002

Song, S. Y., Gannon, M., Washington, M. K., Scoggins, C. R., Meszoely, I. M., Goldenring, J. R., et al. (1999). Expansion of Pdx1-expressing pancreatic epithelium and islet neogenesis in transgenic mice overexpressing transforming growth factor alpha. *Gastroenterology* 117, 1416–1426.

Tanigaki, K., Tsuji, M., Yamamoto, N., Han, H., Tsukada, J., Inoue, H., et al. (2004). Regulation of alphabeta/gammadelta T cell lineage commitment and peripheral T cell responses by Notch/RBP-J signaling. *Immunity* 20, 611–622. doi: 10.1016/S1074-7613(04)00109-8

Teng, Y., Kanasaki, K., Bardeesy, N., Sugimoto, H., and Kalluri, R. (2011). Deletion of Smad4 in fibroblasts leads to defective chondrocyte maturation and cartilage production in a TGFbeta type II receptor independent manner. *Biochem. Biophys. Res. Commun.* 407, 633–639. doi: 10.1016/j.bbrc.2011.02.142

Tian, W., Ding, W., Kim, S., Xu, X., Pan, M., and Chen, S. (2013). Efficacy and safety profile of combining agents against epidermal growth factor receptor or vascular endothelium growth factor receptor with gemcitabine-based chemotherapy in patients with advanced pancreatic cancer: a meta-analysis. *Pancreatology* 13, 415–422. doi: 10.1016/j.pan.2013.04.195

Tomita, S., Jiang, H. B., Ueno, T., Takagi, S., Tohi, K., Maekawa, S., et al. (2003). T cell-specific disruption of arylhydrocarbon receptor nuclear translocator (Arnt) gene causes resistance to 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced thymic involution. *J. Immunol.* 171, 4113–4120. doi: 10.4049/jimmunol.171.8.4113

Troeger, J. S., Mederacke, I., Gwak, G. Y., Dapito, D. H., Mu, X., Hsu, C. C., et al. (2012). Deactivation of hepatic stellate cells during liver fibrosis resolution in mice. *Gastroenterology* 143, 1073–1083, e1022. doi: 10.1053/j.gastro.2012.06.036

Tuveson, D. A., Zhu, L., Gopinathan, A., Willis, N. A., Kachatrian, L., Grochow, R., et al. (2006). Mist1-KrasG12D knock-in mice develop mixed differentiation metastatic exocrine pancreatic carcinoma and hepatocellular carcinoma. *Cancer Res.* 66, 242–247. doi: 10.1158/0008-5472.CAN-05-2305

Wagner, M., Greten, F. R., Weber, C. K., Koschnick, S., Mattfeldt, T., Deppert, W., et al. (2001). A murine tumor progression model for pancreatic cancer recapitulating the genetic alterations of the human disease. *Genes Dev.* 15, 286–293. doi: 10.1101/gad.184701

Wendling, O., Bornert, J. M., Chambon, P., and Metzger, D. (2009). Efficient temporally-controlled targeted mutagenesis in smooth muscle cells of the adult mouse. *Genesis* 47, 14–18. doi: 10.1002/dvg.20448

Wu, Z., Yang, L., Cai, L., Zhang, M., Cheng, X., Yang, X., et al. (2007). Detection of epithelial to mesenchymal transition in airways of a bleomycin induced pulmonary fibrosis model derived from an alpha-smooth muscle actin-Cre transgenic mouse. *Respir. Res.* 8:1. doi: 10.1186/1465-9921-8-1

Young, N. P., Crowley, D., and Jacks, T. (2011). Uncoupling cancer mutations reveals critical timing of p53 loss in sarcomagenesis. *Cancer Res.* 71, 4040–4047. doi: 10.1158/0008-5472.CAN-10-4563

Yu, K., Xu, J., Liu, Z., Sosic, D., Shao, J., Olson, E. N., et al. (2003). Conditional inactivation of FGF receptor 2 reveals an essential role for FGF signaling in the regulation of osteoblast function and bone growth. *Development* 130, 3063–3074. doi: 10.1242/dev.00491

Zhang, S., Zhou, X., Lang, R. A., and Guo, F. (2012). RhoA of the Rho family small GTPases is essential for B lymphocyte development. *PLoS ONE* 7:e33773. doi: 10.1371/journal.pone.0033773

Zheng, B., Zhang, Z., Black, C. M., De Crombrugghe, B., and Denton, C. P. (2002). Ligand-dependent genetic recombination in fibroblasts: a potentially powerful technique for investigating gene function in fibrosis. *Am. J. Pathol.* 160, 1609–1617. doi: 10.1016/S0002-9440(10)61108-X

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