

The Role of the Tumor Microenvironment in Invasion and Metastasis of Pancreatic Cancer

Kelly Foley, Lei Zheng

Kelly Foley, The Sidney Kimmel Comprehensive Cancer Center, Department of Oncology, The Skip Viragh Center for Pancreatic Cancer, The Sol Goldman Pancreatic Cancer Center, Graduate Program in Cellular and Molecular Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland, the United States

Lei Zheng, The Sidney Kimmel Comprehensive Cancer Center, Department of Oncology, The Skip Viragh Center for Pancreatic Cancer, The Sol Goldman Pancreatic Cancer Center, Department of Surgery, The Johns Hopkins University School of Medicine, Baltimore, Maryland, the United States

Correspondence to: Lei Zheng, MD, PhD, 488 Bunting Blaustein CRB I, 1650 Orleans Street, Baltimore, MD 21231, Maryland, the United States. lzheng6@jhmi.edu

Telephone: +1-410-502-6241 Fax: +1-410-614-8216

Received: August 15, 2013 Revised: September 27, 2013

Accepted: September 29, 2013

Published online: November 18, 2013

ABSTRACT

Pancreatic cancer, a devastating disease with a median overall survival of six months, is unique because the vast majority of the tumor is composed of stromal cells. The stroma, primarily composed of fibroblast cells, immune cell, and extracellular matrix proteins, has proven to be a major obstacle in the treatment of pancreatic cancer because the signaling pathways that promote metastasis are poorly understood. Recently, the hedgehog (Hh) and semaphorin signaling pathways have been identified as playing important roles in pancreatic cancer metastasis. The hedgehog pathway has been shown to regulate both cancer stem cell (CSCs) maintenance as well as growth factor secretion by stromal cells. Inhibition of the Hh pathway has been proven to be an effective therapeutic strategy that results in not only a decrease in the therapeutically problematic CSCs but also a decrease in the stromal compartment, which results in increased tumor vascularization and pharmacodelivery. Additionally, the semaphorin genes undergo frequent copy number alterations in pancreatic cancer. In other epithelial-derived cancers, semaphorin signaling has been shown to mediate tumor cell invasion into the nerves and blood vessels, as well as increase the density of nerves within the tumor. Because of the frequency of perineural invasion and associated pain in pancreatic cancer, the role of semaphorin signaling in pancreatic cancer metastasis should be further explored. Further

understanding of the invasion-promoting pathways in the tumor microenvironment will aid in our understanding of how the tumor microenvironment regulates pancreatic cancer progression and will help to identify novel targets for improving therapies.

© 2013 ACT. All rights reserved.

Key words: Pancreatic cancer; Invasion; Metastasis; Tumor microenvironment; Epithelial-to-mesenchymal transition

Foley K, Zheng L. The Role of the Tumor Microenvironment in Invasion and Metastasis of Pancreatic Cancer. *Journal of Tumor* 2013; 1(6): 32-42 Available from: URL: <http://www.ghrnet.org/index.php/jt>

INTRODUCTION

Pancreatic cancer remains the fourth leading cause of cancer-related mortality in the United States. Thus far, surgical resection is the only potential cure for pancreatic cancer. However, because of the aggressive nature of this disease, the lack of early symptoms, and limitations in diagnostic tools, few patients present with locally resectable disease. Approximately 80% of patients present with locally advanced or metastatic disease at the time of diagnosis, which results in a median survival of approximately ten and six months, respectively, following diagnosis. Despite improvements in surgical techniques and chemotherapy, the number of cases and deaths from pancreatic cancer has steadily increased over the last five to ten years with the five-year survival rate for patients with pancreatic cancer remaining steady at less than 5%^[1].

Even if pancreatic cancer is diagnosed early and is resectable, many patients have already developed distant micrometastases, and metastatic pancreatic cancer responds poorly to conventional chemotherapeutics^[1]. Therefore, because of the wide prevalence of metastatic pancreatic cancer, it is imperative that treatment strategies aimed at preventing and treating pancreatic cancer metastasis be developed.

Pancreatic cancer is one of the most stromal-rich cancers. In many cases, only 10-40% of the pancreas tumor is composed of tumor epithelial cells, while the remainder is composed of stroma. The stroma is very heterogeneous and consists of pancreatic stellate cells (PSC), fibroblasts, myofibroblasts, immune cells, blood

vessels, extracellular matrix (ECM), cytokines and growth factors^[2]. Because of the excessive desmoplasia, pancreatic cancers also tend to be hypovascular, which impedes pharmacodelivery. In addition to drug delivery, the stroma has proven to be a major obstacle in the treatment of pancreatic cancer because of limited knowledge on the cross-talk between the tumor and stroma in the tumor microenvironment.

MOLECULAR PATHOGENESIS OF PANCREATIC CANCER

The first step in understanding pancreatic tumor progression and metastasis is to elucidate the genetic alterations that occur during malignant transformation. Advancements in modern molecular techniques have made it possible to determine the genetic alterations that occur at each stage of not only pancreatic cancer development, but also colon, cervical and breast cancer. It is now widely accepted that pancreatic ductal adenocarcinoma (PDA) undergoes a distinct set of genetic alterations, which correlate with histologically well-defined precursor stages, during the progression from normal pancreatic ducts to invasive PDA. The first stage in the transformation of pancreatic ducts is the development of a pancreatic intraepithelial neoplasm (PanIN1A/1B), which is characterized by a G12D mutation in *Kras* that occurs in 80-95% of PDA cases and an overexpression of *Her-2/neu* in approximately 70% of PDA cases. The second stage, PanIN2, is characterized by a mutation or loss of heterozygosity at the *p16* locus, which encodes for a tumor suppressor involved in regulating the cell cycle. The prevalence of *p16* loss increases with increasing levels of atypia. The final pre-invasive stage of PDA, PanIN3, is characterized by the inactivation of *p53* in 50-75% of cases. Other mutations that occur during this stage in a large percentage of PDA patients include *BRCA2*, *SMAD4* and *DPC4*^[3].

Recently, it was discovered that the clonal populations of pancreatic cancer cells that give rise to the distant metastases are represented in the primary tumor, and metastasis from the primary tumor occurs late during pancreatic cancer progression. Whole exome sequencing and copy number analysis was previously performed on 24 invasive PDAs, and a total of 1562 somatic mutations were detected with 25.5% of the mutations being synonymous, 62.4% being missense, 3.8% being nonsense, 5.0% being small insertions and deletions and 3.3% of the mutations occurring in splice sites or within the untranslated region (UTR). Overall, Jones *et al.*^[4] found approximately 63 genetic alterations per cancer. The majority of the alterations were point mutations, but amplifications and deletions were also detected. Not surprisingly, many of the genetic alterations that occur in pancreatic cancer are in pathways involved in DNA damage control, cell cycle regulation, Hedgehog (Hh) signaling, homophilic cell adhesion, small GTPase signaling, transforming growth factor- β (TGF- β) signaling, regulation of invasion and integrin signaling^[5].

Further genetic analysis of the metastatic lesions from these patients revealed two categories of mutations. The first category of mutations is termed 'founder' mutations, and they account for approximately 64% of all mutations found in each tumor. The second category of mutations termed 'progressor' mutations included approximately 36% of all mutations found in each patient and was present in one or more distant metastases but not the primary tumor^[6]. This suggests that the majority of genetic alterations in pancreatic cancer occur before metastatic spread of the disease. The genetic alterations that occur in the parental clone tend to be more deleterious than the alterations found in distant sites, with many of these

mutations contributing to chromosome instability. Additionally, it was found that all mutations found in metastatic lesions were clonal. In other words, these mutations were found in almost all cells located at the distant site, and thus, were present in the cell that invaded from the primary tumor and established the distant metastatic lesions.

A mathematical model was used by the authors to estimate the timing of the genetic alterations. Yachida *et al.*^[6] concluded that the time from tumor initiation to the birth of the cell that gives rise to the parental clone containing all of the mutations at the primary site is approximately 11.7 years. It takes approximately another 6.8 years for the parental clone to invade and colonize a distant site and 2.7 years until the patients' death. Therefore, metastasis occurs late during pancreatic tumor development. These findings are of particular importance because it confirms that there is a large window of opportunity for diagnosis and treatment before distant metastases occur and treatment options become limited. The challenges now are to develop early detection methods, identify metastasis-promoting pathways and develop treatments to target the metastasis-promoting pathways.

MOLECULAR MECHANISMS OF PANCREATIC CANCER METASTASIS

Migration and invasion

The processes tumor cells use to migrate to distant tissues are similar to the normal physiological processes that occur during embryonic development, wound healing and immune cell trafficking^[7]. The process of cell trafficking in both neoplastic cells as well as non-neoplastic cells involves a continuous cycle of polarization of the leading edge of the cell, elongation, attachment of the leading edge, detachment of the trailing edge and contraction of the cell. Because the ECM acts as the substrate for cell migration, many ECM proteins are important in this process. In addition, the cancer cell must invade the ECM of its original tissue in order to enter the blood stream, lymphatics or nearby nerves and travel to distant organ sites.

The first step in cell migration is initiated by growth factors and chemokines, such as epidermal growth factor (EGF), LPA, insulin-like growth factor-1 (IGF-1) and CXCR4/SDF1, and involves the development of cell protrusions, called pseudopods, containing filamentous actin and other structural and signaling proteins. Actin polymerizes into filaments^[8,9] at the leading edge of the cell to stabilize the cell protrusion and allow the cell to contact and bind the adjacent ECM via adhesion molecules, primarily integrins^[10]. Transmembrane integrins couple to the actin cytoskeleton via adaptor proteins and activate kinases to phosphorylate and dephosphorylate key proteins^[10].

The clustering of integrins and other adhesion molecules leads to the recruitment of cell proteases that degrade the ECM components enabling the cell to invade into the underlying blood vessels. Surface proteases cluster at the substrate binding sites^[11] and cleave collagen, fibronectin and laminins, as well as pro-MMPs to create active, soluble MMPs such as MMP2^[12,13,14]. MMPs degrade fibronectin, collagen, laminin and proteoglycans. MMPs also break cell-cell and cell-ECM connections by cleaving adhesion molecules like *E-cadherin* and *CD44*^[15,16].

Elongation of the cell occurs both before and during the formation of focal contacts. Myosin light chain kinase (MLCK) phosphorylates and activates myosin light chain (MLC) to activate myosin II. Active myosin II binds actin filaments to generate contraction of the cell^[17,18]. Dephosphorylation of MLC is performed by MLC phosphatase (MLCtase) to stop the contraction cycle. Assembly and contraction

are regulated by Rho and ROCK^[17,19].

Finally, the trailing end of the cell must detach. Actin severing proteins such as gelsolin and cofilin break up the formed actin filaments so that they can be recycled and reform at the leading edge of the cell. Calpain cleaves the focal contacts between the integrins and ECM proteins by cleaving the cytoplasmic tail of many integrins ($\beta 1$ and $\beta 2$)^[20,21]. In addition, proteolytic cleavage also helps to weaken the focal contacts^[22].

The major producer of pro-migration and anti-migration signals in pancreatic cancer is the stroma. The stroma, particularly fibroblast cells, produces fibroblast growth factor (FGF), IGF-1 and hepatocyte growth factor (HGF), which all bind receptors on the tumor cells to enable the cells to proliferate and gain motility^[23,24,25]. In particular, HGF stimulation of PDA cells acting through c-Met and neurophilin-1 results in increased invasion of these cells^[26]. Thus, treatment with an anti-uPAR (urokinase-type plasminogen activator receptor) antibody to inhibit c-Met and IGF-1 receptor-mediated invasion decreases PDA metastases in mice^[27].

In addition, it has been shown that activated PSCs produce periostin, which accumulates in the ECM and promotes PDA cell invasion via $\alpha 6\beta 4$ -integrin^[28,29]. Additionally, Rachagani *et al.*^[30] found that silencing of MUC4, a type-1 transmembrane mucin, resulted in decreased invasion of PDA cells through FGFR1 stabilization and pFAK, pMKK7, pJNK, pc-Jun and the PI-3K/Akt pathway. The resulting decrease in MUC4 expression led to a downregulation in mesenchymal-associated proteins, such as vimentin, N-cadherin, Twist, Slug and Zeb1, and an upregulation in epithelial-associated proteins, such as *E-cadherin*, Occludin, Cytokeratin-18 and Caspase-9^[31].

Additionally, it has been shown that many surface proteins increase invasion of PDA cells. Specifically, Marchesi *et al.*^[32] found that CXCR4, which is frequently expressed on the surface of pancreatic tumor cells, promotes motility, invasion, survival and proliferation of PDA cells. Alternatively, Wey *et al.*^[33] have shown that overexpression of VEGFR-1 induces migration and invasion of PDA cells in an autocrine fashion. Therefore, because of the known role of VEGF in angiogenesis, the authors suggest that therapeutic targeting of VEGFR-1 would not only inhibit tumor migration but also suppress angiogenesis and thus primary tumor growth^[33].

Because of the extensive stromal compartment present in PDA and the recent discovery that migration signals originate from lymphocytes and stromal cells in the tumor microenvironment, it has become increasingly important to understand the molecular cross-talk between these cells and tumor cells. This knowledge will not only help to reduce the stromal compartment and enable enhanced delivery of chemotherapeutics to the tumor site but will also help identify important targets for reducing further spread of this highly metastatic disease.

Epithelial-mesenchymal transition

One of the more well-characterized mechanisms of cell movement and invasion is called the epithelial-mesenchymal transition (EMT)^[34]. EMT is a normal morphogenic process that occurs during embryonic development and is therefore silenced during adulthood. During tumor progression, however, the polar and basal membrane anchored epithelial tumor cells can dedifferentiate into cells with more mesenchymal-like characteristics. The first step in this transition is the loss of cell-cell junctions, such as desmosomes, tight junctions and adherence junctions, which can occur through loss-of-function mutations in cadherins or catenins, upregulation in the expression of proteases that cleave cadherins^[34,35,36,37], gene

silencing by cadherin promoter methylation or downregulation of growth factor receptor expression^[34]. In particular, cytokines produced by the tumor microenvironment, such as HGF, can downregulate cadherins. The changes in cell adhesive properties are also accompanied by changes in gene expression. In particular, cells undergoing EMT lose cyokeratin expression as well as claudin, occuldin, desmoplakin, Laminin I, Type IV collagen and *E-cadherin* expression. While these cells lose epithelial marker expression, they begin to express proteins more characteristic of mesenchymal cells such as N-cadherin, vimentin, fibronectin, laminin 5, Type I collagen, Tenascin C, $\alpha 5\beta 1$ and $\alpha v\beta 6$. All of these changes are induced by growth factors and cytokines from the tumor microenvironment acting on the transcription factors slug, snail, Zeb1, Zeb2 and twist that regulate the expression of these proteins^[38]. The signaling pathways that regulate intracellular transcription factor activity have the ability to orchestrate all of the steps of the invasion-metastasis cascade, except for colonization^[39]. Additionally, during EMT, tumor cells will lose polarity and undergo cytoskeletal and organelle rearrangement and redistribution^[40].

EMT can also be induced by activating Kras-mutations or Her2 overexpression^[41,42]. In particular, Botta *et al.*^[42] found that constitutive K-Ras^{G12D} activation of ERK2 regulates invasion of PDA cells through MMP-1. In addition, EMT is induced by ECM factors (e.g. type 1 collagen, hyaluronic acid), cancer-associated fibroblasts, immune cells and many signaling pathways including Hh, TGF- β , EGF, FGF, HGF or IGF in PDA^[43,44]. Specifically, Zheng *et al.*^[45] have shown that tyrosine 23 phosphorylated, cell-surface AnxA2 is required for TGF- β induced, Rho-mediated EMT, which links AnxA2, a protein involved in GTPase-mediated cytoskeletal rearrangement, with the process of EMT in pancreatic cancer.

MicroRNAs have also been implicated in regulating EMT. In particular, the miR-200 family has been shown to be a potent negative inhibitor of EMT that works through regulating Zeb1 and Zeb2 to promote *E-cadherin* expression^[46,47] in lung adenocarcinoma. In pancreatic cancer, miR-197 induces EMT by targeting p120 catenin^[48]. Conversely *E-cadherin* expression has also been shown to be regulated by Src kinase, and this has been shown to be correlated with EMT^[49].

TGF- β is believed to be the major inducer of EMT in PDA cells. TGF- $\beta 1$ is overexpressed in many patients with PDA and is associated with poor prognosis^[50]. Interestingly, Kras^{G12D} expressing pancreatic tumors with deletions in the *DPC4/Smad4* and *TIF1 γ* genes develop few metastases. This suggests that Kras-dependent activation of the MEK-ERK signaling pathway is essential for TGF- β -induced EMT^[51]. Additionally, Gordon *et al.*^[52] found that loss of the TGF- β RIII was necessary for EMT and invasion of PDA cells. Furthermore, Smad4 was found to be a negative regulator of STAT3. Therefore, loss of Smad4, which occurs frequently during PDA development, results in unregulated STAT3 activation, which increases that results in increased invasion of PDA cells^[53].

Finally, bone morphogenetic proteins (BMPs) play an important role in the EMT of PDA cells. In particular, BMP-2, BMP-4 and BMP-7 were shown to induce EMT in the Panc-1 cell line. The BMP-mediated invasiveness of Panc-1 cells is partly due to increased expression of MMP-2^[54]. Additionally, BMP reduces the expression of the TGF- β RIII, which is an inhibitory receptor. Smad1, which is required for BMP-induced loss of TGF- β RIII expression and invasiveness, is partially responsible for BMP-mediated MMP-2 upregulation.

Until recently, the EMT process has been difficult to study *in vitro* and has been limited to evaluating gene expression changes

under different condition. However, three-dimensional cultures were recently developed and are currently being used for studying other solid tumors such as breast cancer to evaluate the interaction between tumor cells and cells in the surrounding tumor microenvironment *in vitro*^[55]. This assay is superior to previously utilized two-dimensional systems such as monolayer cultures or transwell assays because it allows the tumor cells and supporting stromal cells to directly interact mimicking the actual interaction that occurs in solid tumors between the tumor and ECM^[56]. Thus, because of the importance of the tumor microenvironment in pancreatic cancer, three-dimensional cultures are currently being utilized and optimized for studying EMT in PDA and should help to shed light on the signals required for these processes.

Intravasation and extravasation

Following EMT, the tumor cells must leave their site of origin and enter the lumen of blood, lymphatic and nerve vessels. This begins with tumor-associated macrophages (TAMs) secreting chemokines and cytokines, such as EGF, to attract the tumor cell to the vessel^[57]. Chemotaxis of the tumor cell towards lymphatic vessels is primarily facilitated by CCL21 or CXCL12, which are produced by lymphatic endothelial cells and bind CCR7 or CXCR4 receptors on the PDA cells^[32,58]. Intravasation is achieved either by the production of membrane extensions through gaps in the endothelial wall or by passively entering vessels through preexisting entry sites^[59]. Once in the vessel, the tumor cells are subjected to a lot of force and mechanical stress from the fluids in the vessel as well as immune surveillance. For this reason, very few tumor cells survive in the vessels^[60].

The mechanisms of extravasation are similar to those of intravasation and diapedesis of leukocytes at sites of inflammation. Extravasation depends not only on tumor-endothelial cell interactions but also vascular permeability. The receptors over expressed on cancer cells, such as CXCR4/CXCR7, CXCR6, CD44, VLA and AnxA2 must respond to the ligands in the target organ such as SDF-1, CXCL16, hyaluronic acid, FN, GAS-6 and AnxA2^[61]. Similar to invasion and intravasation, extravasation occurs when the cancer cells extend pseudopodia, penetrate endothelial cell-cell junctions and degrade the ECM.

Little is known about the signals responsible for intravasation and extravasation of pancreatic cancer, because the lack of a good *in vitro* or *in vivo* model has limited research in this area. Recently, our group developed a mouse model of liver metastasis in which we inject tumor cells into the splenic vasculature of mice, enabling the cells to directly enter the circulation and home to the liver, which is the primary site of metastasis in PDA^[45]. Using this model, we are able to manipulate the tumor cells to identify important molecules on the PDA cells necessary for extravasation and colonization of distant organs. Additionally, through the use of different knockout mouse strains, we are able to identify important molecules at the distant sites that are necessary for extravasation and colonization. Data from these studies is forthcoming and has identified a pathway unique to PDA that is important for intravasation, extravasation and colonization of distant organ sites. Finally, several transgenic mouse models including a *Kras*, *p53*, *Pdx-1 Cre*, *Rosa^{YFP}* mouse have been developed to track pancreatic epithelial cells during tumor progression. Using this model, the authors demonstrated that pancreatic epithelial cells undergo the process of EMT extremely early and are able to invade the basement membrane, enter the blood stream and metastasize to the liver as early as the PanIN2 stage^[62]. The development of mice with labeled tumor and vascular cells will

make it possible to evaluate the signals required for intravasation and extravasation using *in vivo* cellular imaging techniques.

Colonization

The site of tumor cell colonization depends largely on the molecules expressed on the tumor cells, the endothelial cells of the distant organ as well as the microenvironment at the distant site. For example, the CXCL12 ligand expressed in the lung, liver, bone marrow and lymph nodes binds the CXCR4 receptor expressed on breast cancer cells. Therefore, the lung, liver, bone marrow and lymph nodes are all common sites of metastasis for breast cancer^[63].

While the expression of various surface proteins determines the distant site the tumor cell will travel to, the tumor microenvironment determines if the tumor cell will survive once there. Once cancer cells extravasate they can undergo apoptosis, proliferate or remain dormant. Most cells die quickly after extravasation^[64]. Of the few that remain, only 0.01% are able to proliferate and form macrometastases. The major factor preventing proliferation of these cells is a sufficient blood supply. Many tumor cells will remain dormant for years until they acquire mutations that allow them to proliferate in a hypoxic environment, explaining why many patients experience remission after many successful rounds of chemotherapy^[65]. Thus, organs that promote angiogenesis are common sites of metastasis.

STEM CELLS IN PANCREATIC CANCER METASTASIS

Cancer stem cells (CSCs) have recently emerged as a new potential therapeutic target for the treatment of cancer, including pancreatic cancer. CSCs are a phenotypically distinct subtype of cells found in all tumors and are characterized by enhanced tumor-initiating potential, self-renewal and the ability to form any of the cells found in the primary tumor^[66]. Because of these properties, CSCs are readily able to colonize distant organs. It is therefore suggested that circulating tumor cells may in fact be CSCs. Additionally, CSCs are of particular interest because they tend to be highly resistant to chemotherapeutics (including gemcitabine), they have inherent metastatic potential^[67] and their presence in the tumor is associated with poor survival^[68].

One of the major controversies and challenges surrounding CSCs, also referred to as tumor-initiating cells, is establishing an accurate method to identify and characterize these cells. Mouse models and colony forming assays have previously been used to identify CSCs^[69]. However, the preferred method for identifying these cells would be through the use of cell surface markers because this would allow for the cells to be isolated from the whole tumor and characterized functionally.

The markers used to identify pancreatic CSCs *in vivo* include CD44+, CD24+, ESA+, CD133+, ALDH+ and c-Met^[67,68,70,71]. For many of these markers, it is unclear if they are important functionally to the CSC or are simply a phenotypic marker. Interestingly, because these markers are not necessarily expressed together on CSCs, depending on the markers used a different subset of CSCs will be isolated^[68,72]. Therefore, it may be more important to determine the functional role of these cells in order to determine the best therapeutic strategy for eliminating these cells from the tumor. Perhaps subsets of CSCs are important in tumor-initiation, while different subsets are important for metastasis to distant sites. Hermann PC *et al.*^[66] found that a subpopulation of CD133+ CXCR4+ CSCs in pancreatic tumors were essential for tumor metastasis, such that, when these cells were eliminated from the tumor, all metastatic potential was lost.

Not only is the function of these CSC subsets important, but their interaction with distant niches may be equally if not more important. Therefore, it is important that these subsets be further examined and characterized.

Recent studies have found a correlation between CSCs and the EMT associated with invasion and metastasis. In particular, ALDH+ cells in pancreatic cancer tend to be more invasive than the remaining ALDH- subpopulation, and these cells also have characteristics of EMT^[67,72].

Several signaling pathways, including the Hh pathway, have been implicated in regulating the self-renewal properties of normal stem cells and CSCs^[73]. Therefore, these pathways may serve as excellent targets for eliminating CSCs in tumors. A recent Phase II clinical trial using gemcitabine in combination with IPI-926, a novel inhibitor of the Hh pathway, in patients with metastatic pancreatic cancer demonstrated enhanced responses to cytotoxic chemotherapy^[74]. Additional pre-clinical studies have shown a decrease in the frequency of CSCs and decreased tumor formation and metastasis following inhibition of the Hh pathway^[75,76]. However, the direct mechanism of action is unclear from these studies.

CSCs have emerged as a new potential therapeutic target to prevent and treat metastatic pancreatic cancer. However, further characterization of these cells and the pathways that promote invasion and metastasis is warranted. In addition, it is important to understand the interaction of CSCs and tumor cells in the tumor microenvironment in order to target this niche within the primary tumor as well as in distant organ sites to prevent further metastatic spread.

THE ROLE OF THE TUMOR MICROENVIRONMENT IN PANCREATIC CANCER METASTASIS

In addition to the role of the CSC compartment in the progression of pancreatic cancer, the tumor microenvironment has gained considerable attention in recent years. A hallmark of pancreatic cancer is the extensive desmoplastic reaction that occurs during tumor progression. This dense desmoplastic reaction consists of primarily fibroblasts, PSCs and an extensive deposition of ECM proteins (collagen I, collagen III, fibronectin), as well as endothelial cells, immune cells, nerves and lymphatic vessels. Clinically, the desmoplastic reaction is associated with a less favorable outcome. It is believed that signaling from the tumor microenvironment helps promote tumor growth, metastatic spread and drug resistance^[77]. Thus, it is important to understand tumor-stroma interactions in order to develop therapies and improve patient outcomes.

Fibroblasts and pancreatic stellate cells

PSCs, believed to arise from mesenchymal, endodermal and neuroectodermal cell origins, express glial fibrillary acidic protein and desmin in normal tissues. However, in the case of cancer, these cells, derived from quiescent PSCs, fibroblasts, bone marrow-derived cells or EMT, become activated, acquire characteristics of myofibroblasts and begin to express α -smooth muscle actin (α -SMA). The primary mechanism by which PSCs promote tumorigenesis and metastasis is through the secretion of various cytokines, such as TGF- β , HGF, FGF, EGF, IGF-1 and Tenascin C (TnC). In particular, co-culture of tumor cells and their associated fibroblasts or with conditioned media from fibroblast cultures has been shown to promote invasiveness of the tumor cells^[78], which is at least in part mediated by AnxA2^[45]. Co-

culture of tumor cells and stromal cells results in an upregulation of the expression of several different gene families including members of the CXC/CC chemokine family, hyaluronan synthase 2 (HAS2) and matrix metalloproteinases (MMPs), which are involved in tumor invasion, angiogenesis and regulation of the ECM, respectively.

Hedgehog signaling

In addition to maintaining the CSC compartment, Hh signaling has been implicated in the tumor microenvironment of pancreatic cancer. Although, Hh signaling has not been shown to contribute to normal differentiation of the pancreas, several reports have demonstrated that the Hh pathway is misregulated during the development and progression of pancreatic cancer^[79]. In particular, sonic hedgehog (SHH) is upregulated in pancreatic cancer. Bailey *et al.*^[80] found that SHH expression correlated with the desmoplastic reaction that is characteristic of pancreatic cancer. Overexpression of SHH results in the presence of infiltrating fibroblasts with elongated nuclei that express collagen I and fibronectin. However, tumor-bearing mice treated with the SHH neutralizing antibody 5E1 did not have a desmoplastic response. In particular, they found that SHH acted as a chemoattractant to recruit fibroblasts, which enhances tumor progression. In addition, SHH can induce the differentiation and proliferation of PSCs, which contribute to the desmoplastic reaction that occurs in pancreatic cancer.

As previously mentioned, Olive *et al.*^[75] demonstrated a decrease in the stromal compartment following co-administration of gemcitabine and IPI-926, a Hh antagonist. By decreasing the stromal compartment, they were able to transiently increase the vascularization of the tumor, which correlated with increased levels of gemcitabine within the tumor and resulted in transient stabilization of the disease.

An additional study by Feldmann *et al.*^[76] demonstrated a downregulation of *snail* and an upregulation of *E-cadherin* expression, which was consistent with inhibition of EMT in pancreatic cancer cells following inhibition of Hh signaling with cyclopamine. More importantly, inhibition of EMT in these cells resulted in a noticeable reduction in the invasion capacity of these cells *in vitro*. Conversely, if *Gli1* was overexpressed, the cells no longer expressed *E-cadherin* and were remarkably invasive. Hh inhibition inhibited metastatic spread in an orthotopic xenograph model, and in combination with gemcitabine, significantly reduced the size of the primary implant. In addition, cyclopamine preferentially decreased ALDH positive cells.

Based upon the results of several Hh studies in mice, it appeared that inhibition of the Hh pathway was an effective therapeutic strategy that resulted in not only a decrease in the therapeutically problematic CSCs but also a decrease in the stromal compartment, which resulted in increased tumor vascularization and pharmacodelivery. In addition, inhibition of Hh signaling was shown to reduce the size of the primary tumor as well as inhibit metastatic spread in mice. However, disappointingly, these results were not observed in clinical trials. Infinity pharmaceuticals conducted a double-blind, randomized, placebo-controlled phase 2 clinical trial comparing IPI-926 and gemcitabine to gemcitabine alone in 122 previously untreated patients with metastatic pancreatic cancer and followed patient survival as their primary endpoint. Preliminary analysis showed that the median survival for patients receiving gemcitabine plus IPI-926 was less than the historical median survival for single-agent gemcitabine of approximately six months^[1,81]. While no toxicities were reported, the results of this trial are extremely disappointing given the promising results seen in preclinical studies, and it is still unclear why this trial

was unsuccessful.

Conversely, Kim *et al.*^[82] at the University of Michigan saw promising results using GDC-0449 in treatment-naïve patients with advanced pancreatic cancer. Pancreatic cancer patients were treated with GDC-0449 for three weeks with gemcitabine being added to the treatment on the second week. This group found a 50% progression-free survival at three months with five patients achieving a partial response and another four patients having stable disease. It is possible that this trial was more successful because patients were treated first with a Hh antagonist to disrupt the desmoplastic stroma and improve the subsequent delivery of chemotherapy. However, because of the limited number of patients treated in this trial, it is unclear if these results would be significant when compared with the control arm^[82]. Additional studies treating patients with metastatic pancreatic cancer with LDE225, gemcitabine and nab-Paclitaxel (clinical trial number NCT01431794) or GDC-0449, erlotinib and gemcitabine (clinical trial number NCT00878163) are on-going and the results of these studies are currently unknown^[83,84]. Although all current clinical trials have evaluated the use of Hh antagonists in advanced pancreatic cancer, it may also be interesting to include patients with resectable PDA to see if inhibiting Hh in the primary tumor is able to prevent metastatic spread of the disease.

Extracellular matrix

Activated stromal fibroblasts produce ECM components that alter tumor progression by directly binding with surface proteins on the tumor cells^[85]. Specifically, many MMPs are frequently over expressed in pancreatic cancers and bind to and interact with collagen I to promote invasion of these cells^[86,87,88]. Moreover, hyaluronan (HA), a component of the ECM that is secreted by pancreatic tumor cells^[89], may signal through hyaladherins such as CD44 to regulate receptor tyrosine kinases and small GTPases involved in EMT and angiogenesis^[90]. In addition to promoting invasion of tumor cells, HA sequesters water in the tumor microenvironment leading to increased interstitial fluid pressure (IFP) that, when coupled with inadequate lymphatic drainage, leads to vascular collapse^[91]. This contributes to the chemoresistance that is characteristic of pancreatic cancer. Jacobetz *et al.*^[92] demonstrated that enzymatic targeting of HA improves vascular blood flow and drug delivery in a mouse model of PDA. Thus, targeting HA, or perhaps any component of the ECM, may improve drug delivery in patients with PDA.

Recently, Halozyme developed a PEGylated recombinant human hyaluronidase (PEGPH20) that has shown promising results in patients with stage IV metastatic pancreatic cancer. In preclinical studies, PEGPH20 has been shown to deplete the HA-rich matrix that surrounds pancreatic cancer, possibly enabling better chemotherapy delivery to the tumor. In a recent single-arm Phase 1b trial, 28 stage IV treatment naïve PDA patients were treated with PEGPH20 and gemcitabine. The overall response rate (complete response+partial response) by RECIST 1.1 criteria was 42 percent (95% CI 22-62) for those treated at therapeutic dose levels of PEGPH20 (1.6 and 3.0 µg/kg) as assessed by an independent radiology review ($n=24$). Additionally, in subjects with high levels of HA, the overall response rate was 64%. Because of the promising results, a Phase 2 randomized clinical trial has been initiated in patients with stage IV metastatic pancreatic cancer to compare the efficacy of gemcitabine and nab-paclitaxel either with or without PEGPH20. The primary outcome measure for this study will be progression-free survival^[93].

Immune cells

The immune reaction in PDA is largely immunosuppressive and

pro-tumorigenic, and it begins in the earliest stages of pre-invasive disease and rarely results in an effective response^[94]. Therefore, it is presumed that inflammatory cells help contribute to the progression and development of pancreatic cancer. Support for this presumption comes from extensive evidence suggesting that chronic inflammation of the pancreas, as in the case of pancreatitis, is a risk factor for PDA^[95]. In addition, several pro-inflammatory cytokines such as IL-6, IL-8, IL-10 and IL-1 receptor antagonists are elevated in the serum of patients with PDA, and these cytokines are associated with poor survival^[96]. Interestingly, TAMs are one of the most abundant types of immune cells in the tumor microenvironment^[97]. TAMs are recruited to tumors by various chemokines, VEGF, colony stimulating factor 1 (CSF-1), stromal-derived factor 1 (SDF-1) and thrombospondin-1 (TSP-1)^[98,99,100]. Once activated, TAMs secrete various factors such as PDGF, a potent mediator of PSCs proliferation, into the tumor microenvironment, which contributes to the desmoplastic reaction^[101,102]. In addition, macrophages secrete TGF-β to stimulate the synthesis of type I collagens and fibronectin by PSCs^[103,104]. Thus, the immune reaction within the tumor microenvironment further contributes to the desmoplastic reaction that induces invasion of PDA cells.

Perineural invasion

The normal pancreas is rich in ganglia and myelinated and unmyelinated nerve cells, and invasive cancer cells are able to spread into the nerve sheath as an alternate route for dissemination^[105]. Therefore, pancreatic cancer has one of the highest incidences of perineural invasion among all cancer types^[106]. In PDA patients, the size and density of the pancreatic nerves are increased compared to a normal pancreas. Interestingly, undifferentiated tumors tend to have more perineural invasion^[107]. The exact mechanism by which perineural invasion occurs is unclear, but it appears to be mediated by an interaction between molecules on the cancer cell and peripheral nerves. These include many signaling pathways involved in pain as well as TGF-α, EGFR, the NGF family and their receptors, the GDNF family and their receptors, chemokines and their receptors (CX3CR1, Sema3A, PlxnA1, NRP1 and MMPs) as well as other surface molecules and their receptors^[106]. In particular, it was demonstrated that the neurotrophic factor artemin can influence perineural invasion of PDA cells^[108]. Additionally, norepinephrine has been shown to promote perineural invasion of PDA by increasing levels of phosphorylated STAT3. Inhibition of STAT3 inhibited norepinephrine-induced expression of NGF, MMP2 and MMP9 to decrease invasion of PDA cells^[109]. Given that perineural invasion is an indicator of metastatic spread, patients with perineural invasion have a worse prognosis.

Semaphorins

Axon guidance genes have recently been implicated in perineural invasion of tumor cells during metastatic spread. In particular, the expression of semaphorins, which are primarily involved in axon guidance and blood vessel formation during development, has been shown to influence the invasiveness of many types of cancer. In addition, semaphorins are important in the development of the nervous, immune, cardiovascular, hepatic, gastrointestinal and musculoskeletal systems, but the expression of this family of proteins is dramatically reduced in the adult^[110]. Because of the many roles that semaphorins play during development such as cell adhesion, migration, angiogenesis and immune system development, it is understandable that these pathways can become reactivated in cancer to regulate cell proliferation, invasion and apoptosis.

Emerging evidence suggests that semaphorins and plexins are important mediators of invasion and metastasis of cancer. Recently, the semaphorins have been found to be misregulated in a variety of different cancer types. Semaphorins can be pro-tumorigenic or tumor suppressive depending on the type of semaphorin and the type of cancer.

For example, *Sema3C* has been associated with multi-drug resistance in cancer, and its expression is upregulated following treatment with several chemotherapeutic agents^[111]. In addition, *Sema3C* overexpression has been associated with poor survival in ovarian cancer^[112] and invasion in prostate cancer^[113].

Sema3E expression has been shown to be upregulated in breast, melanoma, colorectal and ovarian cancers. Overexpression of *Sema3E* correlates with increased metastatic ability^[114] and EMT-like characteristics in ovarian cancer^[115]. Additionally, *Sema5A* has been found to be overexpressed in prostate, gastric and pancreatic cancer, and its overexpression correlates with invasion and metastasis regulated by *Plexin-B3* signaling^[116].

Finally, expression *Sema3A* was found to be correlated with poor prognosis in PDA patients^[117] but suppresses tumor growth and metastasis formation in a mouse model of melanoma^[118]. While it is unclear the exact mechanisms by which semaphorins promote or inhibit metastasis of cancer cells, it is clear from their role in development that these proteins are important in cell trafficking.

Although the mechanism is not entirely clear, it is known that semaphorins can influence invasion at several levels. *Sema3E* induces focal adhesion disassembly and integrin endocytosis and reduces cell adhesion^[115,119]. *Sema3E* has also been shown to promote an EMT phenotype in ovarian cancer by inducing the translocation of snail into the nucleus in a *MAPK* and *PI3K*-dependent manner^[120]. *Sema3C* has been shown to decrease *E-cadherin* levels in prostate cancer^[113], but overexpression of *Sema3C* correlates with metastasis of lung adenocarcinoma^[121].

Sema3A-NP1 signaling increases adhesion and decreases metastasis by increasing the expression of a metastatic suppressor, $\alpha 2\beta 1$, through a *GSK-3 β* -dependent pathway^[122]. However, autocrine *Sema3A* signaling can mediate glioma cell migration, invasion and metastasis^[123] through *NP2*, an atypical receptor for *Sema3A*^[124].

One of the better characterized signaling pathways in cancer is *Sema4D-PlexinB1*. When *Sema4D* binds to and induces transphosphorylation of *PlexinB1*, *PlexinB1* recruits *Grb2* and couples with *p190RhoGAP*^[125]. *RhoGAP* then inactivates *RhoA* to inhibit cell migration^[125]. Conversely, *Sema4D-PlexinB1* signaling promotes perineural invasion in a *RhoA/ROCK*-dependent manner. *PlexinB1* overexpression in the tumor causes the tumor cells to be attracted to nerves that express high levels of *Sema4D*. Interestingly, the authors also noted that the nerves were stimulated to produce dendritic like projections toward tumors that overexpressed *PlexinB1*. In addition, tumors that secrete *Sema4D* have a high density of nerves^[126].

Additionally, secretion of *Sema5A* has been shown to be upregulated in pancreatic cancer, and its upregulation is correlated with increased invasiveness and metastatic spread of pancreatic cancer^[110]. In particular, *Sadanandam et al*^[127] found that the extracellular domain of *Sema5A* is secreted from pancreatic tumor cells, which correlates with increased invasion to the liver through enhanced *ERK* phosphorylation. Furthermore, they demonstrated an increase in microvessel density following culture with conditioned media from pancreatic tumor cells that secrete *Sema5A* suggesting that the increase in metastasis to the liver is due to increased angiogenesis, which is mediated through *IL-8* and *VEGF*.

PROSPECTIVE

Pancreatic cancer-related deaths continue to increase annually despite an overall decrease in cancer-related deaths in the United States and in the world. The unique tumor microenvironment of PDA, composed primarily of dense stroma, supports the rapid growth and invasion of PDA cells making it an important target for effective therapies. Although, interactions between tumor cells and stromal cells have been identified in other epithelial-derived cancers, the mechanism by which the stroma mediates invasion of PDA is largely unknown. Investigating invasion-promoting mechanisms within the tumor microenvironment will aid in our understanding of how the tumor microenvironment regulates pancreatic cancer progression and will help to identify novel targets for improving therapies.

ACKNOWLEDGMENTS

This review is part of a thesis dissertation written by Kelly Foley. We would like to thank Dr. Elizabeth Jaffee for her careful reading of this review and helpful suggestions. This work was supported in part by National Institutes of Health Grant K23 CA148964-01 (L.Z.), the NCI SPORE in Gastrointestinal Cancers P50 CA062924-14 (L.Z.), Johns Hopkins School of Medicine Clinical Scientist Award (L.Z.), the National Pancreas Foundation (L.Z.), Lefkofsky Family Foundation (L.Z.), Lustgarten Foundation (L.Z.), Viragh Foundation and the Skip Viragh Pancreatic Cancer Center at Johns Hopkins (L.Z.), and the Sol Goldman Pancreatic Cancer Center (L.Z.).

REFERENCES

- 1 American Cancer Society. Pancreatic cancer. Available from: <http://www.cancer.org/cancer/pancreaticcancer/index>.
- 2 Feig C, Gopinathan A, Neeße A, Chan DS, Cook N, Tuveson DA. The pancreas cancer microenvironment. *Clin Cancer Res* 2012; **18**: 4266-4276
- 3 Hruban RH, Goggins M, Parsons J, Kern SE. Progression model for pancreatic cancer. *Clin Cancer Res* 2000; **6**: 2969-2972
- 4 Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analysis. *Science* 2008; **321**: 1801-1806
- 5 Iacobuzio-Donahue CA, Velculescu VE, Wolfgang CL, Hruban RH. Genetic basis of pancreas cancer development and progression: insights from whole-exome and whole-genome sequencing. *Clin Cancer Res* 2012; **18**: 4257-4265
- 6 Yachida S, Jones S, Bozic I, Antal T, Leary R, Fu B, Kamiyama M, Hruban RH, Eshleman JR, Nowak MA, Velculescu VE, Kinzler KW, Vogelstein B, Iacobuzio-Donahue CA. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* 2010; **467**: 1114-1117
- 7 Friedl P, Brocker E-B. The biology of cell locomotion within three-dimensional extracellular matrix. *Cell Mol Life* 2000; **57**: 41-64
- 8 Cramer LP. Organization and polarity of actin filament networks in cells: implications for the mechanisms of myosin-based cell motility. *Biochem Soc Symp* 1999; **65**: 173-205
- 9 Rohatgi R, Ma L, Miki H, Lopez M, Kirchausen T, Takenawa T, Kirchner MW. The interaction between N-WASP and

- the Arp2/3 complex links Cdc42-dependent signals to actin assembly. *Cell* 1999; **97**: 221-231
- 10 Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell* 2002; **110**: 673-687
 - 11 Mueller SC, Gherzi G, Akiyama SK, Sang QX, Howard L, Pineiro-Sanchez M, Nakahara H, Yeh Y, Chen WT. A novel protease-docking function of integrin at invadopodia. *J Biol Chem* 1999; **274**: 24947-24952
 - 12 Friedl P, Wolf K. Proteolytic and non-proteolytic migration in tumor cells and leukocytes. *Biochem Soc Symp* 2003; **70**: 277-285
 - 13 Ohuchi E, Imai K, Fujii Y, Sato H, Seiki M, Okada Y. Membrane type 1 matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. *J Biol Chem* 1997; **272**: 2446-2451
 - 14 Sameni M, Moin K, Sloane BF. Imaging proteolysis by living human breast cancer cells. *Neoplasia* 2001; **2**: 496-504
 - 15 Kajita M, Itoh Y, Chiba T, Mori H, Okada A, Kinoh H, Seiki M. Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. *J Cell Biol* 2001; **153**: 893-904
 - 16 Sternlicht MD, Lochter A, Sympon CJ, Huey B, Rougier JP, Gray JW, Pinkel D, Bissel MJ, Werb Z. The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. *Cell* 1999; **98**: 137-146
 - 17 Katoh K, Kano Y, Amano M, Onishi H, Kaibuchi K, Fujiwara K. Rho-kinase-mediated contraction of isolated stress fibers. *J Biol Chem* 2001; **153**: 569-584
 - 18 Verkhovskiy AB, Svitkina TM, Borisy GG. Myosin II filament assemblies in the active lamella of fibroblasts: their morphogenesis and role in the formation of actin filament bundles. *J Cell Biol* 1995; **131**: 989-1002
 - 19 Kaibuchi K, Kuroda S, Amano M. Regulation of the cytoskeleton and cell adhesion by the Rho family GTPases in mammalian cells. *Annu Rev Biochem* 1999; **68**: 459-486
 - 20 Pfaff M, Du X, Ginsberg MH. Calpain cleavage of integrin beta cytoplasmic domains. *FEBS Lett* 1999; **460**: 17-22
 - 21 Potter DA, Tirnauer JS, Janssen R, Croall DE, Hughes CN, Fiocco KA, Mier JW, Maki M, Herman IM. Calpain regulates actin remodeling during cell spreading. *J Cell Biol* 1998; **141**: 647-662
 - 22 Moss ML, Lambert MH. Shedding of membrane proteins by ADAM family proteases. *Essays Biochem* 2002; **38**: 141-153
 - 23 Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 2003; **4**: 915-925
 - 24 Kleeff J, Kothari NH, Friess H, Fan H, Korc M. Adenovirus-mediated transfer of a truncated fibroblast growth factor (FGF) type I receptor blocks FGF-2 signaling in multiple pancreatic cancer cell lines. *Pancreas* 2004; **28**: 25-30
 - 25 Nair PN, De Armond DT, Adamo ML, Strodel WE, Freeman JW. 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* 2001; **20**: 8203-8214
 - 26 Matsushita A, Gotze T, Korc M. Hepatocyte growth factor-mediated cell invasion in pancreatic cancer cells is dependent on neuropilin-1. *Cancer Res* 2007; **67**: 10309-10316
 - 27 Bauer TW, Liu W, Fan F, Camp ER, Yang A, Somcio RJ, Bucana CD, Callahan J, Parry GC, Evans DB, Boyd DD, Mazar AP, Ellis LM. Targeting of urokinase plasminogen activator receptor in human pancreatic carcinoma cells inhibits c-Met and insulin-like growth factor-1 receptor-mediated migration and invasion and orthotopic tumor growth in mice. *Cancer Res* 2005; **65**: 7775-7781
 - 28 Baril P, Gangeswaran R, Mahon PC, Caulee K, Kocher HM, Harada T, Zhu M, Kalthoff H, Crnogorac-Jurcevic T, Lemoine NR. Periostin promotes invasiveness and resistance of pancreatic cancer cells to hypoxia-induced cell death: role of the beta4 integrin and the PI3k pathway. *Oncogene* 2007; **26**: 2082-2094
 - 29 Erkan M, Kleeff J, Gorbachevski A, Reiser C, Mitkus T, Esposito I, Giese T, Büchler MW, Giese NA, Friess H. Periostin creates a tumor-supportive microenvironment in the pancreas by sustaining fibrogenic stellate cell activity. *Gastroenterology* 2007; **132**: 1447-1464.
 - 30 Rachagani S, Macha MA, Ponnusamy MP, Haridas D, Kaur S, Jain M, Batra SK. MUC4 potentiates invasion and metastasis of pancreatic cancer cells through stabilization of fibroblast growth factor receptor 1. *Carcinogenesis* 2012; **33**: 1953-1964
 - 31 Thiery JP. Epithelial-mesenchymal transitions in tumour progression. *Nature Rev Cancer* 2002; **2**: 442-454
 - 32 Marchesi F, Monti P, Leone BE, Zerbi A, Vecchi A, Piemonti L, Mantovani A, Allavena P. Increased survival, proliferation, and migration in metastatic human pancreatic tumor cells expressing functional CXCR4. *Cancer Res* 2004; **64**: 8420-8427
 - 33 Wey JS, Fan F, Gray MJ, Bauer TW, McCarty MF, Somcio R, Liu W, Evans DB, Wu Y, Hicklin DJ, Ellis LM. Vascular endothelial growth factor receptor-1 promotes migration and invasion in pancreatic carcinoma cell lines. *Cancer* 2005; **104**: 427-438
 - 34 Pulyaeva H, Bueno J, Polette M, Birembaut P, Sato H, Seiki M, Thompson EW. MT1-MMP correlates with MMP-2 activation potential seen after epithelial to mesenchymal transition in human breast carcinoma cells. *Clin Exp Metastasis* 1997; **15**: 111-120
 - 35 Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 2001; **17**: 463-516
 - 36 Tester AM, Ruangpani N, Anderson RL, Thompson EW. MMP-9 secretion and MMP-2 activation distinguish invasive and metastatic sublines of a mouse mammary carcinoma system showing epithelial-mesenchymal transition traits. *Clin Exp Metastasis* 2000; **18**: 553-560
 - 37 Shih J-Y, Yang P-C. The EMT regulator slug and lung carcinogenesis. *Carcinogenesis* 2011; **32**: 1299-1304
 - 38 Yang MH, Wu MZ, Chiou SH, Chen PM, Chang SY, Liu CJ, Teng SC, Wu KJ. Direct regulation of TWIST by HIF-1alpha promotes metastasis. *Nat Cell Biol* 2008; **10**: 295-305
 - 39 Tian M, Neil JR, Schiemann WP. Transforming growth factor-beta and the hallmarks of cancer. *Cell Signal* 2011; **23**: 951-962
 - 40 Edme N. Ras induces NBT-II epithelial cell scattering through the coordinate activities of Rac and MAPK pathways. *J Cell Sci* 2002; **115**: 2591-2601
 - 41 Jenndahl LE, Isakson P, Baekstrom D. c-erbB2-induced epithelial-mesenchymal transition in mammary epithelial cells is suppressed by cell-cell contact and initiated prior to *E-cadherin* downregulation. *Int J Oncol* 2005; **27**: 439-448
 - 42 Botta GP, Reginato MJ, Reichert M, Rustgi AK, Lelkes PI. Constitutive K-RasG12D activation of ERK2 specifically regulates 3D invasion of human pancreatic cancer cells via MMP-1. *Mol Cancer Res* 2012; **10**: 183-196
 - 43 Cavallaro U, Christofori G. Cell adhesion and signaling by cadherins and Ig-CAMs in cancer. *Nat Rev Cancer* 2004; **4**: 118-132
 - 44 Christofori G. New signals from the invasive front. *Nature* 2006; **441**: 444-450
 - 45 Zheng L, Foley K, Huang L, Leubner A, Mo G, Olino K, Edil BH, Mizuma M, Sharma R, Le DT, Anders RA, Illei PB, Van Eyk JE, Maitra A, Laheru D, Jaffee EM. Tyrosine

- 23 phosphorylation-dependent cell-surface localization of annexin A2 is required for invasion and metastases of pancreatic cancer. *PLoS ONE* 2011; **6**: e19390
- 46 Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Fashid G, Vadas MA, Khew-Goodall Y, Goodall GJ. The miR-200 family and miR-205 regulated epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol* 2008; **10**: 593-601
- 47 Park SM, Gaur AB, Lengyel E, Peter ME. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the *E-cadherin* repressors ZEB1 and ZEB2. *Genes Dev* 2008; **22**: 894-907
- 48 Hamada S, Satoh K, Miura S, Hirota M, Kanno A, Masamune A, Kikuta K, Kume K, Unno J, Egawa S, Motoi F, Unno M, Shimosegawa T. miR-197 induces epithelial-mesenchymal transition in pancreatic cancer cells by targeting p120 catenin. *J Cell Physiol* 2013; **228**: 1255-1263.
- 49 Nagathihalli NS, Merchant NB. Src-mediated regulation of *E-cadherin* and EMT in pancreatic cancer. *Front Biosci* 2012; **17**: 2059-2069.
- 50 Friess H, Yamanaka Y, Buchler M, Ebert M, Beger HG, Gold LL, Korc M. Enhanced expression of transforming growth factor beta isoforms in pancreatic cancer correlates with decreased survival. *Gastroenterology* 1993; **105**: 1846-1856
- 51 Ellenrieder V, Hendler SF, Boeck W, Seufferlein T, Menke A, Ruhland C, Adler G, Gress TM. Transforming growth factor beta1 treatment leads to an epithelial-mesenchymal trans-differentiation of pancreatic cancer cells requiring extracellular signal-related kinase 2 activation. *Cancer Res* 2001; **61**: 4222-4228
- 52 Gordon KJ, Dong M, Chislock EM, Fields TA, Blobel GC. Loss of type III transforming growth factor beta receptor expression increases motility and invasiveness associated with epithelial to mesenchymal transition during pancreatic cancer progression. *Carcinogenesis* 2008; **29**: 252-262
- 53 Cano C, Motto Y, Iovanna JL. Epithelial-to-mesenchymal transition in pancreatic adenocarcinoma. *ScientificWorldJournal* 2010; **10**: 1947-1957
- 54 Gordon KJ, Kirkbride KC, How T, Blobel GC. Bone morphogenetic proteins induce pancreatic cancer cell invasiveness through a Smad1-dependent mechanism that involves matrix metalloproteinase-2. *Carcinogenesis* 2009; **30**: 238-248
- 55 Weaver VM, Fischer AH, Peterson OW, Bissell MJ. The importance of the microenvironment in breast cancer progression: recapitulation of mammary tumorigenesis using a unique human mammary epithelial cell model and a three-dimensional culture assay. *Biochem Cell Biol* 1996; **74**: 833-851
- 56 Santini MT, Rainaldi G. Three-dimensional spheroid model in tumor biology. *Pathobiology* 1999; **67**: 148-157
- 57 Siveen KS, Kuttan G. Role of macrophages in tumour progression. *Immunol Lett* 2009; **123**: 97-102
- 58 Zlotnik A. Chemokines in neoplastic progression. *Semin Cancer Biol* 2004; **14**: 181-185
- 59 Bockhorn M, Jain RK, Munn LL. Active versus passive mechanisms in metastasis: do cancer cells crawl into vessels or are they pushed? *Lancet Oncol* 2007; **8**: 444-448
- 60 Wychoff JB, Jones JG, Condeelis JS, Segall JE. A critical step in metastasis: in vivo analysis of intravasation at the primary tumor. *Cancer Res* 2000; **60**: 2504-2511
- 61 Mishra A, Shiozawa Y, Pienta KJ, Taichman RS. Homing of cancer cells to the bone. *Cancer Microenviron* 2011; **4**: 221-235
- 62 Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, Reichert M, Beatty GL, Rustgi AK, Vonderheide RH, Leach SD, Stanger BZ. EMT and dissemination precede pancreatic tumor formation. *Cell* 2012; **148**: 349-361
- 63 Morris VL, Schmidt EE, MacDonald IC, Groom AC, Chambers AF. Sequential steps in hematogenous metastasis of cancer cells studied by in vivo videomicroscopy. *Invasion Metastasis* 1997; **17**: 281-296
- 64 Leber MF, Efferth T. Molecular principles of cancer invasion and metastasis. *Int J Oncol* 2009; **34**: 881-895
- 65 Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001; **414**: 105-111
- 66 Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ, Heeschen C. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* 2007; **1**: 313-323
- 67 Rasheed ZA, Yang J, Wang Q, Kowalski J, Freed I, Murter C, Hong SM, Koorstra JB, Rajeshkumar NV, He X, Goggins M, Iacobuzio-Donahue C, Berman DM, Laheru D, Jimeno A, Hidalgo M, Maitra A, Matsui W. Prognostic significance of tumorigenic cells with mesenchymal features in pancreatic adenocarcinoma. *J Natl Cancer Inst* 2010; **102**: 340-351
- 68 Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CHM, Jones DL, Visvader J, Weissman IL, Wahl GM. Cancer stem cells—perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res* 2006; **66**: 9339-9344
- 69 Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF, Simeone DM. Identification of pancreatic cancer stem cells. *Cancer Res* 2007; **67**: 1030-1037
- 70 Li C, Wu JJ, Hynes M, Dosch J, Sarkar B, Welling TH, Pasca di Magliano M, Simeone DM. c-Met is a marker of pancreatic cancer stem cells and therapeutic target. *Gastroenterology* 2011; **141**: 2218-2227
- 71 Kim MP, Fleming JB, Wang H, Abbruzzese JL, Choi W, Kopetz S, McConkey DJ, Evans DB, Galllick GE. ALDH activity selectively defines an enhanced tumor-initiating cell population relative to CD133 expression in human pancreatic adenocarcinoma. *PLoS One* 2011; **6**: e20636
- 72 Mani SA, Evans KW, Hollier BG, Guo W, Weinberg RA. Generation of stem-like cells via EMT: a new twist in cancer initiation and progression. In: AACR Education Book. Philadelphia, PA: American Association of Cancer Research, 2009: p. 173.
- 73 Taipale J, Beachy PA. The hedgehog and Wnt signaling pathways in cancer. *Nature* 2001; **411**: 349-354
- 74 Feldmann G, Fendrich V, McGovern K, Bedja D, Bisht S, Alvarez H, Koorstra JB, Habbe N, Karikari, Mullendore M, Gabrielson KL, Sharma R, Matsui W, Maitra A. An orally bioavailable small-molecule inhibitor of hedgehog signaling inhibits tumor initiation and metastasis in pancreatic cancer. *Mol Cancer Ther* 2008; **7**: 2725-2735
- 75 Olive KP, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK, Denicola G, Feig C, Combs C, Winter SP, Ireland-Zecchini H, Reichelt S, Howat WJ, Chang A, Dhara M, Wang L, Ruckert F, Grutzmann R, Pilarsky C, Izeradjene K, Hingorani SR, Huang P, Davies SE, Plunkett W, Egorin M, Hruban RH, Whitebread N, McGovern K, Adams J, Iacobuzio-Donahue C, Griffiths J, Tuveson DA. Inhibition of hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 2009; **324**: 1457-1461
- 76 Feldmann G, Dhara S, Fendrich V, Bedja D, Beaty R, Mullendore M, Karikari C, Alvarez H, Iacobuzio-Donahue C, Jimeno A, Gabrielson KL, Matsui W, Maitra A. Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers. *Cancer Res* 2007; **67**: 2187-2196
- 77 Rasheed ZA, Matsui W, Maitra A. Pathology of pancreatic stroma in PDAC. In: Grippo PJ, Munshi HG, editors. Pancreatic Cancer and Tumor Microenvironment. Trivandrum,

- India: Transworld Research Network, 2012: Chapter 1.
- 78 Sato N, Maehara N, Goggins M. Gene expression profiling of tumor-stromal interactions between pancreatic cancer cells and stromal fibroblasts. *Cancer Res* 2004; **64**: 6950-6956
- 79 Thayer SP, di Magliano MP, Heiser PW, Nielsen CM, Roberts DJ, Lauwers GY, Qi YP, Gysin S, Fernandez-del Castillo C, Yajnik V, Antoniu B, McMahon M, Warshaw AL, Hebrok M. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature* 2003; **425**: 851-856
- 80 Bailey JM, Swanson BJ, Hamada T, Eggers JP, Singh PK, Caffery T, Ouellette MM, Hollingsworth MA. Sonic hedgehog promotes desmoplasia in pancreatic cancer. *Clin Cancer Res* 2008; **14**: 5995-6004
- 81 Infinity Pharmaceuticals. Infinity reports update from Phase 2 study of Saridegib plus Gemcitabine in patients with metastatic pancreatic cancer. Available from: <http://phx.corporate-ir.net/phoenix.zhtml?c=121941&p=irol-newsArticle&ID=1653550&highlight=>.
- 82 Kim EJ. Inhibiting Hedgehog signaling pathway may improve pancreatic cancer treatment. Available from: <http://www.aacr.org/home/public-media/aacr-in-the-news.aspx?d=2830>
- 83 Sidney Kimmel Comprehensive Cancer Center. Gemcitabine+Nab paclitaxel with LDE-225 (Hedgehog inhibitor) as neoadjuvant therapy for pancreatic cancer. Available from: <http://clinicaltrials.gov/show/NCT01431794>.
- 84 National Cancer Institute (NCI). GDC-0449 and erlotinib hydrochloride with or without gemcitabine hydrochloride in treating patients with metastatic pancreatic cancer or solid tumors that cannot be removed by surgery. Available from: <http://clinicaltrials.gov/show/NCT00878163>.
- 85 Vonlaufen A, Phillips PA, Xu Z, Goldstein D, Pirola RC, Wilson JS, Apte MV. Pancreatic stellate cells and pancreatic cancer cells: an unholy alliance. *Cancer Res* 2008; **68**: 7707-7710
- 86 Binker MG, Binker-Cosen AA, Gaisano HY, de Cosen RH, Cosen-Binker Li. TGF-beta1 increases invasiveness of SW1990 cells through Rac1/ROS/NF-kappaB/IL-6/MMP-2. *Biochem Biophys Res Commun* 2011; **405**: 140-145
- 87 Shields MA, Dangi-Garimella S, Krantz SB, Bentrem DJ, Munshi HG. Pancreatic cancer cells respond to type I collagen by inducing snail expression to promote membrane type 1 matrix metalloproteinase-dependent collagen invasion. *J Biol Chem* 2011; **286**: 10495-10504
- 88 Zhang K, Chen D, Jiao Z, Zhang S, Liu X, Cao J, Wu L, Wang D. Slug enhances invasion ability of pancreatic cancer cells through upregulation of matrix metalloproteinase-9 and actin cytoskeleton remodeling. *Lab Invest* 2011; **91**: 426-438
- 89 Mahlbacher V, Sewing A, Elsassner HP, Kern HF. Hyaluronan is a secretory product of human pancreatic adenocarcinoma cells. *Eur J Cell Biol* 1992; **58**: 23-34
- 90 Toole BP, Slomiany MG. Hyaluronan: a constitutive regulator of chemoresistance and malignancy in cancer cells. *Semin Cancer Biol* 2008; **18**: 244-250
- 91 Heldin CH, Rubin K, Pietras K, Ostman A. High interstitial fluid pressure - an obstacle in cancer therapy. *Nat Rev Cancer* 2008; **4**: 806-813
- 92 Jacobetz MA, Chan DS, Neesse A, Bapiro TE, Cook N, Frese KK, Feig C, Nakagawa T, Caldwell ME, Zecchini HI, Lolkema MP, Jiang P, Kultti A, Thompson CB, Maneval DC, Jodrell DI, Frost GI, Shepard HM, Skepper JN, Tuveson DA. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. *Gut* 2013; **62**: 112-120
- 93 Zhu J. A Phase 1b study of gemcitabine plus PEGPH20 (PEGylated recombinant human hyaluronidase) in patients with stage IV previously untreated pancreatic cancer. ECCO-ESMO-ESTRO poster presentation, Amsterdam, September 30, 2013, Abstract #2598.
- 94 Clark CE, Hingorani SR, Mick R, Combs C, Tuveson DA, Vondereide RH. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. *Cancer Res* 2007; **67**: 9518-9527
- 95 Lowenfels AB, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, Dimagno EP, Andren-Sandberg A, Domellof L. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. *N Engl J Med* 1993; **328**: 1433-1437
- 96 Ebrahimi B, Tucker SL, Li D, Abbruzzese JL, Kurzrock R. Cytokines in pancreatic carcinoma: correlation with phenotypic characteristics and prognosis. *Cancer* 2004; **101**: 2727-2736
- 97 Solinas G, Germano G, Mantovani A, Allavena P. Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. *J Leukoc Biol* 2009; **86**: 1065-1073
- 98 Coffelt SB, Hughes R, Lewis CE. Tumor-associated macrophages: effectors of angiogenesis and tumor progression. *Biochim Biophys Acta* 2009; **1769**: 11-18
- 99 Martin-Manso G, Galli S, Ridnour LA, Tsokos M, Wink DA, Roberts DD. Thrombospondin 1 promotes tumor macrophage recruitment and enhances tumor cell cytotoxicity of differentiated U937 cells. *Cancer Res* 2008; **68**: 7090-7099
- 100 Murdoch C, Giannoudis A, Lewis CE. Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. *Blood* 2004; **104**: 2224-2234
- 101 Apte MV, Haber PS, Darby SJ, Rodgers SC, McCaughan GW, Korsten MA, Pirola RC, Wilson JS. Pancreatic stellate cells are activated by proinflammatory cytokines: implications for pancreatic fibrogenesis. *Gut* 1999; **44**: 534-541
- 102 Ross R. Platelet-derived growth factor. *Lancet* 1989; **1**: 1179-1182
- 103 Aoyagi Y, Oda T, Kinoshita T, Nakahashi C, Hasebe T, Ohkohchi N, Ochiai A. Overexpression of TGF-beta by infiltrated granulocytes correlates with the expression of collagen mRNA in pancreatic cancer. *Br J Cancer* 2004; **91**: 1316-1326
- 104 Schmid-Kotsas A, Gross HJ, Menke A, Weidenbach H, Adler G, Siech M, Beger H, Grunert A, Bachem MG. Lipopolysaccharide-activated macrophages stimulate the synthesis of collagen type I and C-fibronectin in cultured pancreatic stellate cells. *Am J Pathol* 1999; **155**: 1749-1758
- 105 Bapat AA, Hostetter G, Von Hoff DD, Han H. Perineural invasion and associated pain in pancreatic cancer. *Nat Rev Cancer* 2011; **11**: 695-707
- 106 Ceyhan GO, Demir IE, Altintas B, Rauch U, Thiel G, Muller MW, Giese NA, Friess H, Schafer K-H. Neural invasion in pancreatic cancer: a mutual tropism between neurons and cancer cells. *Biochem Biophys Res Commun* 2008; **374**: 442-447
- 107 Hirai I, Kimura W, Ozawa K, Kudo S, Suto K, Kuzu H, Fuse A. Perineural invasion in pancreatic cancer. *Pancreas* 2002; **24**: 15-25
- 108 Ceyhan GO, Giese NA, Erkan M, Kerscher AG, Wente MN, Giese T, Büchler MW, Friess H. The neurotrophic factor artemin promotes pancreatic cancer invasion. *Ann Surg* 2006; **244**: 274-281
- 109 Guo K, Ma Q, Li J, Wang Z, Shan T, Li W, Xu Q, Xie K. Interaction of the sympathetic nerve with pancreatic cancer cells promotes perineural invasion through the activation of STAT3 signaling. *Mol Cancer Ther* 2013; **12**: 264-273
- 110 Yazdani U, Terman JR. The semaphorins. *Genome Biol* 2006; **7**: 211
- 111 Yamada T, Endo R, Gotoh M, Hirohashi S. Identification of semaphorin E as a non-MDR drug resistance gene of human cancers. *Proc Natl Acad Sci U S A* 1997; **94**: 14713-14718
- 112 Galani E, Sgouros J, Petropoulou C, Janinis J, Aravantinos G,

- Dionysiou-Asteriou D, Skarlos D, Gonos E. Correlation of MDR-1, nm23-H1 and H sema E gene expression with histopathological findings and clinical outcome in ovarian and breast cancer patients. *Anticancer Res* 2002; **22**: 2275-2280
- 113 Hermann JG, Meadows GG. Increased class 3 semaphorin expression modulates the invasive and adhesive properties of prostate cancer cells. *Int J Oncol* 2007; **30**: 1231-1238
- 114 Christensen C, Ambartsumian N, Gilestro G, Thomsen B, Comoglio P, Tamagnone L, Guldborg P, Lukanidin E. Proteolytic processing converts the repelling signal sema3E into an inducer of invasive growth and lung metastasis. *Cancer Res* 2005; **65**: 6167-6177
- 115 Casazza A, Finisguerra V, Capparuccia L, Camperi A, Swiercz JM, Rizzolio S, Rolny C, Christensen C, Bertotti A, Sarotto I, Risio M, Trusolino L, Weitz J, Schneider M, Mazzone M, Comoglio PM, Tamagnone L. Sema3E-Plexin D1 signaling drives human cancer cell invasiveness and metastatic spreading in mice. *J Clin Invest* 2010; **120**: 2684-2698
- 116 Sadanandam A, Varney ML, Singh S, Ashour AE, Moniaux N, Deb S, Lele SM, Batra SK, Singh RK. High gene expression of semaphorin 5A in pancreatic cancer is associated with tumor growth, invasion and metastasis. *Int J Cancer* 2010; **127**: 1373-1383
- 117 Muller MW, Giese NA, Swiercz JM, Ceyhan GO, Esposito I, Hinz U, Buchler P, Giese T, Buchler MW, Offermann S, Friess H. Association of axon guidance factor semaphorin 3A with poor outcome in pancreatic cancer. *Int J Cancer* 2007; **121**: 2421-2433
- 118 Chakraborty G, Kumar S, Mishra R, Patil TV, Kundu GC. Semaphorin 3A suppresses tumor growth and metastasis in mice melanoma model. *PLoS One* 2012; **7**: e33633
- 119 Sakurai A, Gavard J, Annas-Linhares Y, Basile JR, Amornphimoltham P, Palmby TR, Yagi H, Zhang F, Randazzo PA, Li X, Weigert R, Gutkind JS. Semaphorin 3E initiates antiangiogenic signaling through plexin D1 by regulating Arf6 and R-Ras. *Mol Cell Biol* 2010; **30**: 3086-3098
- 120 Tseng C-H, Murrar KD, Jou M-F, Hsu S-M, Cheng H-J, Huang P-H. Sema3E/plexinD1 mediated epithelial-to-mesenchymal transition in ovarian endometrioid cancer. *PLoS One* 2011; **6**: e19396
- 121 Martin-Satue M, Blanco J. Identification of semaphorin E gene expression in metastatic human lung adenocarcinoma cells by mRNA differential display. *J Surg Oncol* 1999; **72**: 18-23
- 122 Pan H, Wanami LS, Dissanayake TR, Bachelder RE. Autocrine semaphorin 3A stimulates alpha2 beta1 integrin expression/function in breast tumor cells. *Breast Cancer Res Treat* 2009; **118**: 197-205
- 123 Bagci T, Wu JK, Pfannl R, Ilag LL, Jay DG. Autocrine semaphorin 3A signaling promotes glioblastoma dispersal. *Oncogene* 2009; **28**: 3537-3550.
- 124 Nasarre C, Koncina E, Labourdette G, Cremel G, Roussel G, Aunis D, Bagnard D. Neuropilin-2 acts as a modulator of sema3A-dependent glioma cell migration. *Cell Adh Migr* 2009; **3**: 383-389
- 125 Sun T, Krishnan R, Swiercz JM. Grb2 mediates semaphorin-4D-dependent RhoA inactivation. *J Cell Sci* 2012; **125**: 3557-3567
- 126 Binmadi NO, Yang YH, Zhou H, Proia P, Lin YL, De Paula AM, Guimaraes AL, Poswar FO, Sundararajan D, Basile JR. Plexin-B1 and semaphorin 4D cooperate to promote perineural invasion in a RhoA/ROK-dependent manner. *Am J Pathol* 2012; **180**: 1232-1242.
- 127 Sadanandam A, Sidhu SS, Wullschleger S, Singh S, Varney ML, Yang CS, Ashour AE, Batra SK, Singh RK. Secreted semaphorin 5A suppressed pancreatic tumour burden but increased metastasis and endothelial cell proliferation. *Br J Cancer* 2012; **107**: 501-507

Peer reviewers: Kenoki Ohuchida, MD, PhD, Department of Surgery and Oncology, Kyusyu university, 3-1-1 Maedashi Fukuoka, 812-8582, Japan; Kun Chun Chiang, General Surgery Department, Chang Gung Memorial Hospital, 222, Mai-Chin Road, Keelung, Taiwan; Zhou Yuan MD, Department of General Surgery, the 6th Affiliated Hospital of Shanghai Jiaotong University, 600 Yishan St, Shanghai 200233, China.