

# Pancreatic Cancer: Current Concepts in Invasion and Metastasis

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## 1. Introduction

Pancreatic cancer remains to be one of the most lethal solid tumours of the gastrointestinal tract with a 5-year survival rate lower than 5%. It is characterised by late diagnosis, aggressive local invasion, early systemic dissemination and resistance to chemo- and radiotherapy. At the time of diagnosis more than 85% of patients have already developed metastasis and are therefore not eligible for local treatments with curative intention such as surgery or radiotherapy. Chemotherapy with Gemcitabine is the mainstay of palliative treatment with modest antitumour effects in these patients.

The process of cancer initiation, progression and metastasis remains to be poorly understood. Little is known about the development of metastatic progression and the dissemination of cells from the primary tumour site into distant organs. A better understanding and thorough investigation of the biology behind pancreatic cancer invasion and metastasis is urgently needed. Current concepts and emerging fields of research in pancreatic cancer metastasis shall be discussed in this chapter.

## 2. Mechanisms of metastatic evolution

Metastasis has been conventionally viewed as the last step in a cumulative process of genetic alterations within cells of a primary tumour mass. For metastatic cells to progress, they have to acquire distinct properties such as loss of cell adhesion, acquisition of an invasive potential, ability of intravasation, transport through the circulation, extravasation, formation of micro-metastases, and finally the ability to induce an angiogenic switch to form macro-metastasis (Coghlin & Murray, 2010). This hypothesis has been recently challenged by several groups (Bernards & Weinberg, 2002; Weinberg, 2008; Klein, 2008). An alternative model is proposed that considers the genetic alterations accumulated at the initial stages of

tumour's evolution to be sufficient to promote the metastasis process. Two distinct models arising from this hypothesis are discussed in the following section.

## 2.1 Metastasis: Progression models

### 2.1.1 Late metastasis (linear progression) model

This model is based on Leslie Fould's description of a step-wise progression of morphological abnormalities accompanying cancer (Klein, 2009). It places selection of genetic and epigenetic modifications mostly inside the established primary tumour for competitive fitness. After multiple rounds, the cells may be able to proliferate relatively autonomously at a competitive rate and seed to secondary metastatic niche sites (Fig.1). Late disseminating cells are expected to be genetically identical to the parental cells of the primary tumour and should be subject to similar tumour cell targeting therapeutic modalities (Klein, 2008, 2009; Coghlin & Murray, 2010).

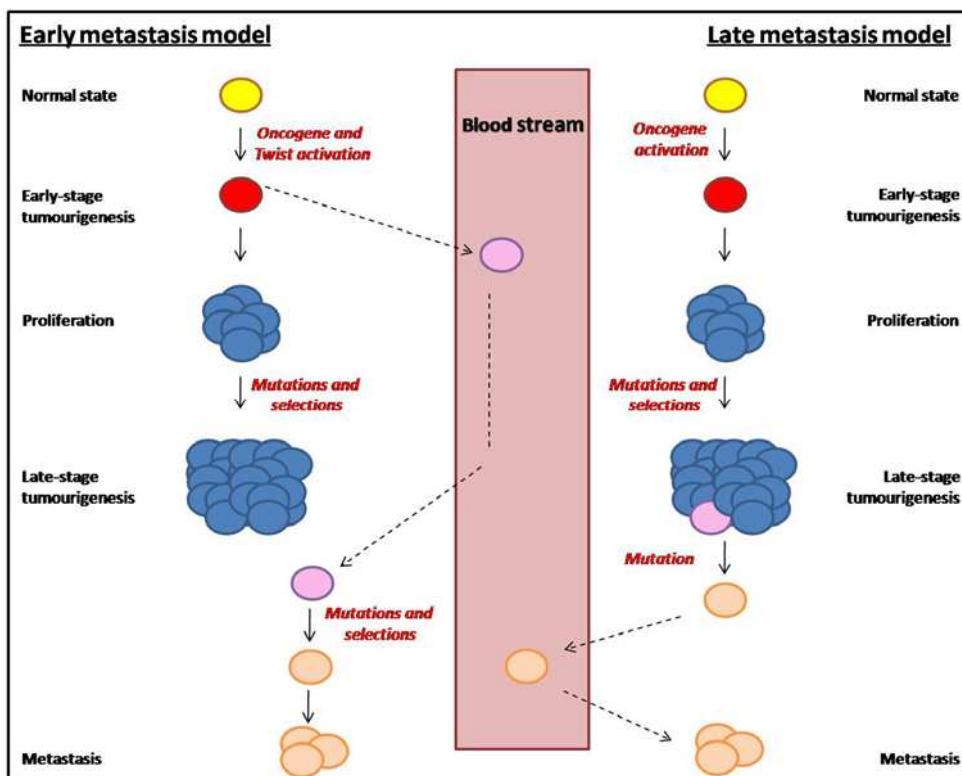


Fig. 1. Models of metastasis evolution: in the late metastasis model, cells acquire genetic and epigenetic modifications mainly in the primary tumour site of the organ. Mutated cells disseminate within the blood stream into the final metastatic niche. However, in the early metastasis model cells accumulate genetic alterations at distant sites and thus diverge from the primary tumours at both genetic and epigenetic levels.

### **2.1.2 Early metastasis (parallel progression) model**

In contrast to the late metastasis model, here tumour cells are believed to leave the primary site quite early up-on activation of factors such as Twist (master regulator of embryonic morphogenesis) with a major role in metastasis during tumourigenesis (Yang et al., 2004) and to diverge genetically at ectopic sites whereby they generate a further cascade of metastatic cells (Fig.1). Owing to the different selection pressures at different niches (metastatic sites) and inherent genetic instability of tumour cells, parallel progression predicts greater variation among metastatic founder and primary tumour cells. Consequently they may respond differently to systemically administered drugs (Klein, 2008, 2009; Coghlin & Murray, 2010).

### **2.2 Progression of metastasis in pancreas cancer**

Whether the dismal prognosis of patients with pancreatic cancer compared to those with other cancer types is a consequence of late diagnosis or early dissemination to distant organs is still debated. In a study performed by Yachida et al., data generated from sequencing of the genomes of seven pancreatic cancer metastases, were used to evaluate any clonal relationship between primary and their corresponding metastatic cancers. After development of the parental clone, clonal evolution continues within the parental site giving rise to distant metastases. They provided evidence that primary pancreatic cancers contain a mix of geographically and genetically distinct sub-clones, each harbouring large numbers of cells that are present within the primary tumour years before the metastases become clinically apparent (Yachida et al., 2010). Additional studies led to estimation of at least three time-scales associated with tumour progression: the time between tumour initiation to establishment of the founder cell of the parental clone (average 10 years, T1); the sojourn time between the arising parental clone and its acquisition of metastatic potential (average 6.8 years, T2); and the time from metastatic dissemination to patient's death (average 2.7 years, T3) (Yachida et al., 2010; Lubeck, 2010). Unfortunately, the vast majority of cancer patients are diagnosed within the last two years of tumour development. The great challenge would rather be the detection of these lesions during or shortly after T1, but before T2-T3 i.e., the seeding of metastases.

## **3. The host and the tumour micro-environment**

Malignant cells do not exist in isolation, but are rather intensely communicating with the surrounding cells in their micro-environment such as microvessel endothelial cells, macrophages, fibroblasts, bone-marrow-derived cells etc. These interactions significantly contribute to tumour proliferation, local invasion and distant metastasis.

### **3.1 Pancreatic stellate cells: Role in pancreatic cancer metastasis**

The transition of the pancreatic stellate cells (PSC) from a quiescent to an "activated" or "myofibroblastic" state plays a key role in various pathogenic disorders of the exocrine pancreas. In quiescent state, PSC express intermediate filament proteins desmin, glial fibrillary acidic protein (GFAP), vimentin and nestin. Up-on activation, induced by various stimuli such as injury, PSC attain other markers such as smooth muscle actin (SMA), and

interstitial collagen type I. Therefore, activated PSC constitute a major source for development of tissue fibrosis attributed to e.g. chronic pancreatitis and pancreatic adenocarcinoma (PDAC) (Omary et al., 2007; Hwang et al., 2008).

Intense fibrotic reaction referred to as tumour desmoplasia is a hallmark of pancreatic cancer (Cruikshank et al., 1986; Hwang et al., 2008; Bachem et al., 2008; He et al., 2007; Erkan et al., 2010) whereby infiltrating carcinoma cells get surrounded by a dense fibrotic stroma consisting mainly of collagen types I and III as well as fibronectin (Imamura et al., 1995; Bachem et al., 2008). PSC, attracted by pancreatic cancer cells, get activated by various paracrine stimulants and growth factors, such as platelet-derived growth factor (PDGF) rendering them motile and proliferative, fibrogenic mediators such as transforming growth factor beta (TGF $\beta$ ) which stimulate matrix synthesis resulting in this desmoplastic reaction, cytokines such as interleukin II-1, II-6, II-8, and tumour necrosis factor alpha (TNF $\alpha$ ) (Omary et al., 2007; Bachem et al., 2008). Among the key implications of the desmoplastic reaction are the promotion of survival and prevention of apoptosis of pancreatic tumour cells through direct interaction with extra-cellular matrix (ECM), increased production of matrix metalloproteases (MMP) and serine proteases such as members of the plasminogen activator system (Vaquero et al., 2003; Edderkaoui et al., 2005).

Additionally, soluble factors produced by PSC themselves stimulate the proliferation and survival of pancreatic cancer cells. Hwang et al. have shown that co-injection of PSC along with pancreatic cancer cells increases tumour incidence, size, and metastasis in an orthotopic model of pancreatic cancer (Hwang et al., 2008). In addition, combined inoculation of human pancreatic cancer and stellate cells in a xenograft model was shown to promote tumour growth and progression as compared to inoculation of tumour cells alone (Bachem et al., 2008). In another study, significantly elevated expression of urokinase plasminogen activator (uPA) as well as fibroblastic uPA receptor (uPAR) was correlated with liver metastasis of human pancreatic cancers, indicating a possible role of stromal fibroblasts in promoting pancreas cancer dissemination. Co-culturing of peri-tumour fibroblasts with metastatic BxPC3 pancreas cancer cells activates matrix metalloprotease-2 (MMP-2) and up-regulates uPAR expression, along with elevated expression of integrin  $\alpha 6 \beta 1$  in BxPC3 cells. This suggests a possible interaction between integrins of cancer cells and the uPAR of the stromal fibroblasts along the uPAR-uPA-MMP-2 cascade (He et al., 2007). Moreover, Buechler et al. demonstrate a *de novo* transcriptional regulation of uPAR mRNA by the hypoxia-inducible factor-1 (HIF-1), accompanied by an increased rate of hypoxia-induced metastasis (Buechler et al., 2009). Taken all together, pancreas cancer growth and progression are accelerated via an orchestrated functional interaction among carcinoma cells and stellate/stromal fibroblast cells.

### 3.2 Tumour-associated macrophages: Role in cancer metastasis

Tumour-associated macrophages (TAM) are bone-marrow-derived cells capable of promoting tumour invasion, angiogenesis, immune evasion, and migration (Allavena et al., 2008; Coghlin & Murray, 2010). In normal tissues, pathogenic challenge or wounding results in the local expression of a variety of growth factors e.g. colony stimulating factor 1 (CSF1) also known as macrophage CSF, granulocyte-macrophage CSF (GM-CSF), TGF $\beta 1$ , in

addition to various chemokines such as CCL2, CCL7, CCL8, CCL3 CCL4. Such a milieu recruits circulating monocytes and stimulates their differentiation into macrophages (Pollard et al., 2004).

At the primary tumour site, hypoxia-related factors and oncogenic induction of pro-inflammatory mediators mentioned above also result in the recruitment of macrophages (TAM) as well as mobilisation of bone-marrow-derived progenitor cells. TAM may thereafter stimulate angiogenesis by expressing factors such as vascular endothelial growth factor (VEGF), and angiopoietin 1/2 (ANG1-ANG2). Moreover, they recruit hematopoietic cells (e.g., mast cells or neutrophils) which exert similar functions (Pollard et al., 2004).

Along with activated stromal cells, TAM act synergistically with malignant cells to degrade the ECM and release growth factors favouring invasiveness. TAM promote invasion by producing proteases that digest the basement membrane and remodel the stromal matrix. Additionally, they produce multiple growth factors which stimulate the growth of the tumour cells themselves (Pollard et al., 2004).

## **4. Local invasion and distant metastasis**

### **4.1 The epithelial-to-mesenchymal transition (EMT)**

Epithelial-to-mesenchymal transition (EMT) describes a biologic process in which polarised epithelial cell under-go various bio-chemical modifications and ultimately gain mesenchymal characteristics i.e., enhanced migration and invasiveness, increased resistance to apoptosis, as well production of ECM components (Kalluri & Neilson, 2003). A proposal has been made which classifies EMT into three different sub-types. Type 1 EMT is associated with implantation and embryonic gastrulation and gives rise to the mesoderm, endoderm and to mobile neural crest cells. On the other hand, type 2 EMT promotes organ fibrosis mediated by inflammatory cells. The last type of EMT, type 3, is associated with cancer progression and metastasis (Kalluri & Weinberg, 2009).

The EMT process is characterised by decreased expression of Epithelial cell-cell junction molecule E-cadherin along with increased expression of non-epithelial cadherins mostly N-cadherin and acquisition of mesenchymal cytoskeleton markers such as vimentin and SMA, accompanied by nuclear beta-catenin accumulation (Shintani et al., 2006; Yang & Weinberg, 2008; Coghlin & Murray, 2010). Tumour cells undergoing EMT are typically seen at the invasive fronts of primary tumours. These cells may eventually enter into subsequent steps of the invasion-metastasis cascade namely intravasation, transport through the circulation, extravasation, formation of micro-metastases, and colonisation (Fig. 2) (Thiery, 2002, 2003; Fidler & Poste, 2008; Brabletz et al., 2001).

EMT is initiated by extra-cellular signals e.g. hepatocyte growth factor (HGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), and TGF $\beta$  which activate multiple EMT-inducing transcription factors notably Snail, Slug, zinc finger E-box binding homoeobox (ZEB1) and Twist (Savagner et al., 2001; Lee et al., 2006; Shintani et al., 2006; Kalluri & Weinberg, 2009). Activation of EMT cascade also involves the disruption of cell-cell adherens junctions and integrin-mediated adhesion in the ECM.

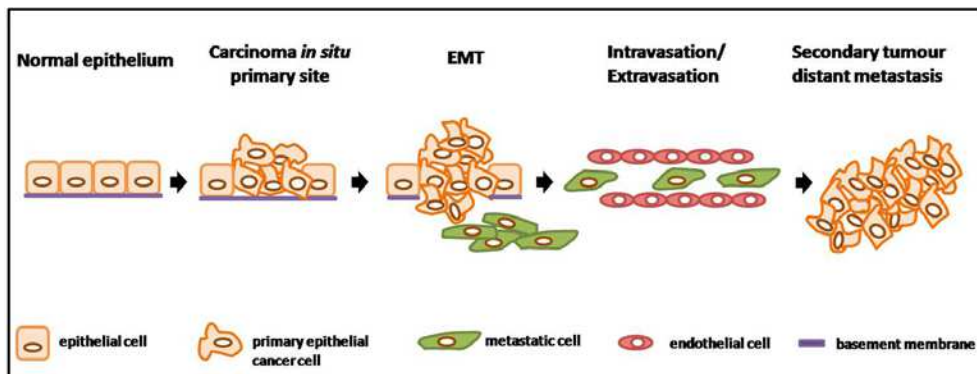


Fig. 2. The role of EMT in cancer metastasis: epithelial tumour cells lose their polarity and detach from the basement membrane. This situation is referred to as *carcinoma in situ*. During the EMT process, primary epithelial tumour cells undergo various modifications whereby they lose their epithelial markers and acquire mesenchymal properties, i.e. digest the basic membrane and invade the surrounding tissue. The onset of angiogenic switch and establishment of abnormal tumour neo-vasculature favours the intravasation of metastatic cells into the blood-stream. Only a small fraction of disseminated tumour cells (DTC) survive in circulation, extravasate into distant organs and form single cell or small tumour colonies also called micro-metastases. DTCs or micro-metastases could remain dormant for a prolonged period of time until they eventually switch to angiogenic macroscopic metastatic lesion and become clinically apparent (Folkman & Kalluri, 2004; Almog et al., 2009). Interestingly, metastatic lesions derived from carcinomas demonstrate the epithelial characteristic of their primary tumour of origin suggesting that according to this model mesenchymal to epithelial transition (MET) occur during tumour colonisation in the distant sites.

#### 4.2 Matrix metalloproteases (MMP)

MMP are a family of enzymes involved in degradation of ECM components e.g. collagen and fibronectin (Jimenez et al., 2000). MMP of either malignant cells or induced fibroblasts at the invasive front are thought to mediate cancer invasion and metastasis via disruption of tumour cell adhesion molecules and degradation of basement membranes and other matrix components.

Immunohistochemical studies have demonstrated over-expression of active MMP-2, MMP-9 (Gress et al., 1995; Koshiba et al., 1998), MMP-7 and MMP-11 (Bramhall et al. 1997) in tumours with lymph node metastasis. The involvement of MMP-1 in promoting angiogenesis and metastatic spread has been recently linked to pancreatic cancer (Abdollahi et al., 2007). Using an orthotopic mouse model of pancreatic cancer, the MMP inhibitor Batimastat was shown to improve the survival and to reduce growth of tumours in mice implanted with HPAC, a moderately differentiated pancreatic cancer cell line (Zevros et al., 1997). Recent work in multiple carcinomas suggests a crucial role of transcriptional regulators Snail and Slug in the regulation of E-Cadherin expression (Gavert & Ben-Ze'ev, 2008). In pancreatic cancer, Slug is shown to promote the invasiveness of tumour cells

through up-regulating ECM proteins such as MMP-9 along with remodeling of the actin cytoskeleton (Zhang et al., 2011).

### 4.3 Growth factor expression patterns in pancreas metastasis

Among its pleiotropic effects TGF $\beta$  was shown to potently induce EMT in different tumours. TGF $\beta$  signalling in pancreatic cancer is often attenuated because of alterations in components of this pathway (Jonson et al., 2001). Using genetic array data, genes responsive to TGF $\beta$  in pancreas cancer cell line PANC-1 are depicted to be involved in ECM remodeling, cell motility, adhesion, angiogenesis, cell cycle, proliferation and apoptosis (Gaspar et al., 2006). Negative regulation of E-cadherin necessary for EMT has been observed in PANC-1 cultures (Halder et al., 2005), and has been associated with lymph node metastasis (Pignatelli et al., 1994). Regulation of *JAG1* by TGF $\beta$  on the array is interesting because its gene product Jagged1 (Notch ligand), is influenced by TGF $\beta$  in cultured epithelial cells. Notch signalling is an important cell signalling pathway involved in regulation of the balance between cell proliferation, differentiation, and apoptosis. Notch-1 has been reported to induce nuclear factor  $\kappa$ B (NF $\kappa$ B) promoter activity (Jang et al., 2004). Down-regulation of Notch-1, and consequently of NF $\kappa$ B and MMP-9 inhibits invasion of pancreatic cancer cells through matrigel (Wang et al., 2006). Inhibition of TGF $\beta$  signalling reduces PDAC growth and invasiveness (Gaspar et al., 2006).

TGF $\beta$  signalling is altered in human pancreas cancer cells with one half of tumours showing allelic deletions or inactivating mutations of the *Smad4* gene. These cells show an enhanced TGF $\beta$ -mediated EMT as determined by increased vimentin expression and decreased beta-catenin and E-cadherin expression. TGF $\beta$ -mediated invasion is suppressed in *Smad4* intact cells *in vitro* which show reduced dissemination in an orthotopic pancreatic cancer model (Zhao et al., 2008). Interestingly, cells with an intact *Smad* pathway reveal reduced activation of signal transducer and activator of transcription (STAT3). STAT3 is constitutively activated in various carcinomas and plays a pivotal role in regulating cell growth, cell survival and angiogenesis (Yu & Jove, 2003; Abdollahi et al., 2004). Inhibition of STAT3 phosphorylation by *Smad4* suppresses TGF $\beta$ -mediated invasion and metastasis of pancreatic cancer cells (Zhao et al., 2008). Therefore, reconstitution of *Smad4* activity or suppression of STAT3 down-stream signalling constitutes attractive targets to inhibit TGF $\beta$ -induced EMT and pro-metastatic effects.

Other factors known to be frequently over-expressed in several human malignancies and to be associated with invasion of tumour cells are the insulin-like growth factor I receptor (IGF-IR) and HGF receptor c-Met. IGF-IR over-expression and excessive activation are associated with malignant transformation, tumour aggressiveness, and protection from apoptosis (Macaulay, 1992; Sell et al., 1995). Over-expression of IGF-IR, reported in pancreatic carcinomas, is regulated by AKT activation thereby promoting invasiveness of human pancreatic cells (Tanno et al., 2001). Furthermore IGF-IR and c-Met co-operate synergistically to induce migration and invasion of human pancreatic carcinoma cells (Bauer et al., 2006).

EGFR is over-expressed in approximately 90% of pancreatic carcinoma and is associated with poor prognosis. Blocking of EGFR signalling in various animal models reduces growth and spread of pancreas carcinoma (Baselga & Arteaga, 2005). Of note, low-molecular weight

tyrosine kinase inhibitors targeting EGFR such as erlotinib are the only class of targeted inhibitors so far demonstrating additional mild benefits when combined with gemcitabine versus gemcitabine mono-therapy in treatment of metastatic PDAC (Moore et al., 2007). Accordingly, erlotinib is the only targeted therapy currently approved for clinical use in PDAC. In extension of this work, dual inhibition of EGFR signalling using erlotinib (alone or in combination with gemcitabine) and of hedgehog signalling with cyclopamine were shown to inhibit tumour growth, increase apoptosis, and suppress the dissemination of pancreatic cancer cells (Feldman et al., 2007; Mimeault et al., 2005, Hu et al., 2007).

#### 4.4 Hypoxia and angiogenesis

The presence of significant hypoxia in pancreatic cancers has been long suspected due to the relatively poor contrast agent enhancement of pancreatic cancer lesions suggestive of hypovascular regions in e.g. computer tomography (CTscans, Megibow, 1992). Koong et al. directly detected hypoxia in pancreas cancer by placement of intra-tumoural needles measuring tissue oxygen levels at the time of resection in seven operable pancreatic cancer patients (Koong et al., 2000). Hypoxia renders tumours more aggressive, and resistant to chemo- and radio-therapy (Garcea et al., 2006, Abdollahi et al. 2005). Therefore, the combination of desmoplastic reaction and strong intra-tumoural hypoxia synergistically contribute to the inherent resistance of pancreas cancer against cancer therapies such as chemotherapy.

For a tumour or any other tissue to grow above the size of  $1\text{mm}^3$ , recruitment of new vessels is required. This process is termed tumour angiogenesis (Folkman, 1971). The “angiogenic switch” is considered a hallmark of cancer and refers to the phenomenon in which the balance of pro- and anti-angiogenic factors is shifted towards the pro-angiogenic state (Hanahan & Folkman, 1996; Abdollahi et al., 2005; Hanahan & Weinberg, 2011). Nevertheless, the hypo-vascular phenotype of pancreatic tumours observed by contrast enhanced non-invasive imaging techniques misled the research in this field to precept that angiogenesis is not playing a key role in development of PDAC. The role of an angiogenic micro-environment in development of pancreatic cancer is only recently reported (Abdollahi et al., 2007). It is shown that the angiogenic state gradually switches from normal pancreas (off) to chronic inflammation (pancreatitis, intermediate) to primary pancreatic tumour and distant metastases (on). These data indicate that aberrant pro-angiogenic micro-environment might contribute to the 19-fold increased cancer risk in patients with chronic pancreatitis (Abdollahi et al., 2007). Although several angiogenic factors have been associated with PDAC, most of the studies have so far focused on VEGF reporting its pivotal role in stimulation of endothelial cell proliferation, migration, gene activation, and apoptosis evasion (Dvorak et al., 1995; Ferrara, 1999, Abdollahi et al. 2005). VEGF is a dimeric cytokine with members including VEGF-A (most common isoform), VEGF-B, VEGF-C, VEGF-D and VEGF-E. VEGF-A exerts its effects on target endothelial cells via binding to its specific transmembrane tyrosine kinase receptors VEGFR-1 and VEGFR-2 (Ferrara, 1999; Dvorak, 2002). Hypoxia has been shown to stimulate VEGF transcription in pancreatic carcinoma cell lines. This requires Src activation and leads to increased steady-state levels of HIF-1 $\alpha$  and increased phosphorylation of STAT3. Expression of VEGF in STAT3 or HIF-1 $\alpha$  dominant negative mutants is significantly reduced. Together, STAT3 and HIF-1 $\alpha$  are both required for maximum transcription of VEGF mRNA following hypoxia (Gray et al., 2005).



#### 4.4.1 Local invasion and lymph-angiogenesis

Tumour cell dissemination may follow several patterns e.g. local invasion, lymphatics, hematogenous spread, or direct seeding of body cavities or surfaces (Rubbia-Brandt et al., 2004). The recent discovery of lymphatic endothelium-specific markers such as VEGFR-3, LYVE-1 and lymph-angiogenic growth factors VEGF-C and VEGF-D has allowed better understanding of tumour-associated *de novo* lymph-angiogenesis – the generation of new lymphatic vessel – in the metastatic process (Kopfstein et al., 2007). The expression of VEGF-C and VEGF-D is reported in a variety of human tumours and is correlated with markers of lymphatic vessel density (LVD), lymph node metastasis and poor prognosis (Sleeman et al., 2009). Tumour-associated lymphatic vessels have been reported to occur both peri- and/or intra-tumourally with the latter case correlating with lymph node metastasis and prognosis. In contrast to most tumours, in pancreatic carcinoma the correlation between lymph-angiogenesis marker and worse prognosis is still controversially debated (Sleeman & Thiele, 2009).

VEGF-C and VEGF-D are the most extensively studied factors that enhance tumour-induced lymph-angiogenesis and lymph node metastasis (Thiele & Sleeman, 2006). VEGF-C is highly expressed in pancreatic cancer tissue and cell lines, while its receptor VEGFR-3 is expressed on cancer stromal cells. Thus, local tumour growth is promoted via paracrine stimulation of VEGFR-3 expressing stromal cells leading to the entry of cancer cells into peritumoural lymphatics (Schneider et al., 2006). Kopfstein et al. have used Rip1VEGF-D transgenic mouse model of pancreatic  $\beta$ -cell carcinogenesis to investigate the functional role of VEGF-D in inducing lymph-angiogenesis and tumour progression. They show that VEGF-D expressing tumours exhibit peri-tumoural lymphangiogenesis along with lymphocyte accumulations and hemorrhages, with frequent lymph node and lung but not hepatic metastases (Kopfstein et al., 2007). Similar to VEGF-D, transgenic expression of VEGF-C in Rip1VEGF-C model induces peri-tumoural, but not intra-tumoural lymph-angiogenesis, and promoting lymph node metastasis without affecting blood vessel angiogenesis (Mandriota et al., 2001).

Additional mechanisms are described to be involved in tumour lymph-angiogenesis e.g. insertion of endothelial cells into the existing lymphatic endothelium. The existence of lymphatic progenitor cells was attributed to the CD34+ CD133+ VEGFR-3+ expressing cells which could differentiate into cells expressing vascular and lymphatic endothelial cell markers (Salven et al., 2003). Moreover, expression of chemokine receptor CCR7 by tumour cells enables them to migrate to lymphatic endothelial cells expressing the cognate ligand CCL21. Interestingly, CCR7+ tumour cells could produce CCR7 ligands and migrate with the lymphatic fluid in a process referred to as autologous chemotaxis (Sleeman et al., 2009)

#### 4.4.2 VEGF and liver metastasis

VEGF expression is closely related with micro-vessel density and seems to be a crucial indicator for liver metastasis and a poor prognosis in pancreatic adenocarcinoma (Seo et al., 2000). Elevated VEGF levels are correlated with tumour size (Itakura et al., 1997) and liver metastasis (Seo et al., 2000). Others have shown that the presence of TGF $\beta$  in the tumour micro-environment plays an important role in enhancing liver metastasis by modulating the capacity of angiogenesis and immunogenicity (Teraoka et al., 2001). Histological studies in

pancreatic cancer patients often show invasion into large veins and dissemination into the liver. This is accompanied by elevated expression of MMP-2 and MMP-9 (Nagakawa et al., 2002). VEGF levels were shown to be markedly elevated in liver metastases as compared to non-tumour bearing liver. However, no correlation was found in VEGF expression between liver metastases and primary pancreatic carcinoma (Tawada et al., 2008).

#### 4.5 Role of chemokines in pancreas metastasis

Chemokines are low molecular-weight peptide ligands involved in the trafficking of leukocytes and other motile cells (Murphy et al., 2000; Mellado et al., 2001). Their receptors are cell-surface, seven trans-membrane G protein-coupled receptors. Many chemokines have more than one ligand and can activate more than one receptor.

##### 4.5.1 Role of the chemokine receptor CXCR4 in pancreas metastasis

CXCR4 has been detected on many leukocytes such as lymphocytes, monocytes, natural killer cells, as well as on vascular smooth muscle cells, endothelial cells, and astrocytes (Caruz et al., 1998; Wegner et al., 1998; Zhang et al., 1998; Balkwill, 2004). The chemokine CXCL12, originally termed stromal derived factor 1 (SDF-1), is a ligand for CXCR4. In normal adult, the interplay between CXCR4 and CXCL12 is critical for homing and retention of hematopoietic progenitor cells in the bone-marrow (Richard & Blay, 2008). High levels of CXCR4 are expressed by these progenitor cells, which in turn get attracted to CXCL12 produced by stromal cells in specialised bone-marrow niches (Aiuti et al., 1997). Stem cells and some other differentiated cells in the pathological contexts of inflammation and tissue regeneration or repair are also influenced by the chemo-attractant potency of CXCL12. It is postulated that metastatic cancer cells subvert the physiologic function of CXCR4/CXCL12 in controlling cell migration and homing.

It has been shown that CXCL12/CXCR4 axis promotes progression and dissemination of various carcinomas. CXCR4 is over-expressed at high levels on cells of solid epithelial cancers including pancreas, and CXCL12 concentrates in fluid-filled cavities through which many cancers disseminate and at tissue sites where metastases develop (Richard & Blay, 2008). Most human pancreatic cancer tissues and more than 50% of pancreatic cell lines stain positively for CXCR4 and express CXCR4 protein, respectively. Chemotaxis induction of human pancreas carcinomas, as well as stimulation of their proliferation and survival is induced by CXCL12 (Figure 3) (Marchesi et al., 2004; Koshiba et al., 2000). Kayali and colleagues have shown in an interferon gamma-non-obese diabetic mouse model that CXCL12 stimulates the phosphorylation of AKT, mitogen-activated protein kinase (MAPK), and Src in pancreatic duct cells, and that it influences ductal cell migration. Blocking the CXCL12/CXCR4 axis in this model leads to a reduced proliferation and increased apoptosis of pancreatic ductal cells (Kayali et al., 2003). Moreover, CXCR4 small molecule antagonists, such as TN14003, were shown to inhibit migration of human pancreatic cancer cells *in vitro* via alteration of MAPK phosphorylation (Mori et al., 2004).

Recently, a study in 30 patients with pancreatic cancer was initiated to evaluate the expression of CXCL12 and CXCR4 in tumour tissues, normal pancreas, and regional lymph nodes. They report low CXCL12 levels in tumour tissues as compared to para-cancerous tissues, normal pancreas, and lymph nodes. On the other hand, levels of CXCR4 in tumour

tissues were markedly higher. Additionally, they depict a significant correlation among the expression of CXCL12/CXCR4 axis and that of lymph node metastases (Cui et al., 2010).

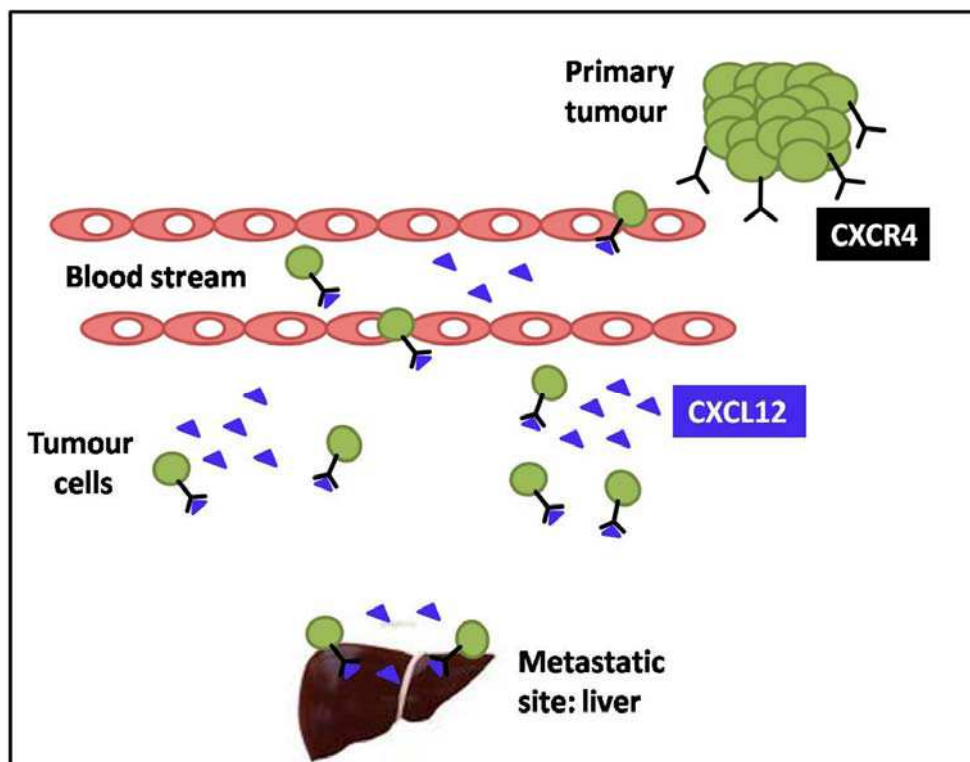


Fig. 3. Dissemination of tumour cells via the CXCL12/CXCR4 axis. Chemokine signalling enhances tumour invasion, tissue remodelling and tumour resistance to apoptotic stimuli. Moreover, pancreatic tumour cells expressing CXCR4 migrate towards the gradient of CXCL12 released by distant organs such as lymph nodes, lung, and liver. Hence, in addition to facilitating tumor invasion, the chemokine guidance plays a critical role in spread of tumour cells from primary sites to form distant metastases.

Several factors regulating the expression of CXCR4 in tumour cells reside within the tumour micro-environment. It has been shown that hypoxia via hypoxia-inducible factor 1 alpha (HIF1 $\alpha$ ) upregulates CXCR4 (Staller et al., 2003). Accordingly, CXCR4 expression was found to be enhanced in CXCR4-positive cell lines cultured under hypoxic conditions (Marchesi et al., 2004). As mentioned above matrix metalloproteases such as MMP-2 and MMP-9 have been associated with haematogenous tumour spreading. Chemokine and MMP activity seems to be intertwined as treatment of tumour cell lines with CXCL12 were shown to activate MMPs and trigger tumour cell invasion (Marchesi et al., 2004). Gao et al. proposed a

crucial role for PSC in promoting the invasion of human pancreatic cancer cells through the CXCL12/CXCR4 axis (Gao et al., 2010). In addition, CXCL12 was shown to protect CXCR4-positive pancreatic tumour cells from serum starvation-induced death or interleukin-1-induced damage via decreasing their rate of apoptosis (Marchesi et al., 2004). Together, these data suggest an important role for chemokine signalling in matrix-remodelling, tumour invasion and enhanced cell survival by evading apoptotic stimuli.

#### 4.6 Influence of PPAR on pancreas metastasis

Peroxisome proliferator-activated receptors (PPAR) are ligand-activated transcription factors belonging to the super-family of nuclear hormone receptors (Isseman & Green, 1990). Three major sub-types have been described so far: PPAR- $\alpha$ , PPAR- $\beta/\delta$ , and PPAR- $\gamma$ . PPAR- $\gamma$  has been recently shown to be over-expressed in pancreatic cancers (Eibl et al., 2004). Troglitazone, a synthetic PPAR- $\gamma$  agonist/ligand, leads to G1 cell cycle accumulation and inhibits cellular proliferation *in vitro* (Itami et al., 2001). Implantation of PANC-1 tumours in nude mice shows significant inhibition of tumour growth treated with pioglitazone, another PPAR- $\gamma$  agonist (Itami et al., 2001). In another study, both ciglitazone and 15d-prostaglandin J2 (15d-PGJ2) were shown to inhibit the growth of four tested pancreatic cancer lines. Treatment with 15d-PGJ2 significantly suppresses pancreatic cancer cell invasiveness which is accompanied by a reduction of MMP-2 and MMP-9 protein levels and activity (Hashimoto et al., 2002). The anti-tumour activity on pancreas cancer cell invasion was in part attributed to the influence of PPAR- $\gamma$  ligands on the serine protease urokinase-type plasminogen activator (uPA) and its receptor (uPAR) (Sawai et al., 2006). In addition, synthetic PPAR- $\gamma$  agonists may impede metastasis formation via interference with chemokine signalling by decreasing CXCR4 expression levels (Richard & Blay, 2008). Of note, data generated with synthetic PPAR ligands known as PPAR “agonists” or “antagonists” are not always matching data generated by genetic knock-down studies suggesting that binding of synthetic ligands may induce additional or different effects, by e.g. release of endogenous ligands (Lee et al., 2003; Plutzky, 2003). In this context, anti-metastatic but not anti-proliferative effects were also reported after treatment of pancreas cancer with a synthetic PPAR- $\gamma$  ligand (T0070907), known to be a specific PPAR- $\gamma$  antagonist (Nakajima et al., 2008). In contrast, PPAR- $\gamma$  agonists were shown to inhibit the growth of pancreas tumours via downregulation of VEGF and thus inhibition of tumour angiogenesis (Dong et al., 2009). This is in line with previous observations suggesting an involvement of PPAR- $\gamma$  signalling in the angiogenesis process (Panigrahy et al. 2002). PPAR- $\alpha$  and PPAR- $\beta/\delta$ , the two other members of this family were also proposed to play a critical role in tumor growth and angiogenesis (Park et al., 2001, Abdollahi et al. 2007; Müller-Brüsselbach et al., 2007; Wang et al., 2006; Kaipainen et al., 2007; Panigrahy et al. 2008). In particular, PPAR- $\beta/\delta$  expression levels were shown to be gradually increased from normal pancreas to chronic pancreatitis to primary tumor and distant metastasis of pancreas cancer (Abdollahi et al. 2007). Moreover, PPAR- $\beta/\delta$  was found to play a central role within a network of genes that govern the angiogenic switch process. Accordingly, targeted removal of PPAR- $\beta/\delta$  in tumor microenvironment via implantation of wt-tumors in PPAR- $\beta/\delta$  knockdown mouse resulted in impaired tumor growth and angiogenesis (Abdollahi et al. 2007). This data are consistent with other studies reporting on impaired wound healing and reduced body fat; both processes known to be angiogenesis dependent (Peters et al., 2000; Michalik et al., 2001).

#### 4.7 Genomic studies

Recent advances in high-throughput sequencing analysis have improved our understanding of genetic alterations in pancreatic cancer. In 2008 Jones et al. reported on sequencing protein-coding exons from 20,735 genes in 24 pancreatic cancers. They found that pancreatic cancer contains an average of 63 genetic alterations, the majority of which were point mutations that could be assigned to a core set of 12 cellular signalling pathways being altered in 67% to 100% percent of pancreatic cancers. These include, apoptosis (100% affected), DNA damage control (83%), regulation of G1/S phase transition (100%), hedgehog signalling (100%), homophilic cell adhesion (79%), integrin signalling (67%), c-Jun N-terminal kinase signalling (96%), K-Ras signalling (100%), regulation of invasion (92%), small GTP-ase Ras-independent signalling (79%), TGF- $\beta$  signalling (100%), and Wnt/Notch signalling (100%). Although these pathways partially overlap in the majority of the patients tested, every individual tumour might reveal variations in the alterations observed in pathway components. This perspective likely applies to most of epithelial cancers, and explains the heterogeneity within individual genes and within individual tumours (Jones et al., 2008).

Shi et al. have established a highly metastatic pancreatic cancer line SW1990HM from intrasplenic injection of SW1990 tumor cells. Gene expression profiles of SW1990HM and SW1990 cells show 40 metastasis-related genes expressed with a 3-fold difference. From the 40 genes 32.5% are assigned to be adhesion and ECM-related genes, namely matrix metalloproteases (*MMP-10*, *MMP-9*, *MMP-7*), E-cadherin tumour suppressor gene (*CDH1*), and the golgi enzyme glycosyltransferase (*MGAT5*). Another 30% are found to be cell-growth and proliferation-related such as insulin growth factor 1 (*IGF1*), interleukin 8 receptor beta (*IL8RB*), integrin A7 (*ITGA7*), murine double minute oncogene (*MDM2*), mesenchymal epithelial transition factor (*MET*), somatostatin receptor 2 (*SSTR2*), and *VEGF* (Shi et al., 2009).

Thakur and colleagues have utilized Ela-c-myc transgenic mice, described previously to develop acinar carcinoma (50%) as well as mixed ductal and acinar cell carcinoma (50%), to show spontaneous metastasis to the liver (Liao et al., 2006, 2007; Thakur et al., 2008). Microarray analyses revealed up-regulation of genes involved in DNA replication, cell proliferation and cell cycle regulation, chromosome organization, and signal transduction. Many genes are related to the maintenance of chromosomal structure and integrity such as mini-chromosome maintenance 2 (*MCM2*), *MCM5*; *MCM10*; structural maintenance of chromosome 211 (*SMC211*), *SMC411*, *SMC511*, *RAD51*, and *BRCA1*.

In alignment with these data expression analysis of two established cell lines (HPAC and PANC1) in terms of their patterns of invasiveness, reveals significant increase in the expression of DNA repair genes. DNA copy number of *BRCA1* and *RAD51* genes is also found to be increased in tissues isolated from metastatic pancreas cancer in comparison to normal tissue from the respective sites (Mathews et al., 2011).

Thakur et al also described elevated expression levels of *IGFBP1* and *Serpin1* in liver metastatic tissues as compared to primary pancreatic tumours and normal pancreas. Both genes are also known to be over-expressed in highly metastatic human pancreatic cell lines (PANC28, CoLo357fg, L3.6pl) in comparison to less metastatic cell lines (PANC1 and BxPC3) (Thakur et al., 2008).

## 5. Peri-neural invasion

Tumour peri-neural invasion (PNI), i.e., the neurotropism of pancreatic tumour cells and their metastasis into the peri-neural space of peripheral nerves constitutes a unique feature of pancreatic ductal adenocarcinoma (PDAC). PNI is associated with poor prognosis in patients due to the fact that tumour cells disseminating along nerve fascicles are spared by surgery and could therefore contribute to the local recurrence of pancreatic cancer (Marchesi et al., 2010; Pour et al., 2003). The human pancreas harbours a large amount of neural tissue and is innervated by the autonomic nervous system through plexi from the celiac and superior mesenteric artery ganglia. In majority of pancreatic cancer patients (~90%) tumor cells infiltrate intra-pancreatic nerves, with involvement of about 70% of extra-pancreatic nerves. Neural infiltration by cancer cells along with the accompanying ultimate nerve damage serve to cause the characteristic severe pain in pancreatic cancer patients (Pour et al., 2003). Morphologic changes at the migration front include characteristic increased neural density and hypertrophy and clustering of malignant cells around the neuritis (Ceyhan et al., 2008; Dai et al., 2007).

### 5.1 Mediators and molecular mechanisms of PNI

Investigation of many pathologic sections reports an increase in the size of nerve fibres in the vicinity of pancreatic tumours, suggesting the necessity of neurotropic factors, growth factors, and axonal guidance molecules as key players in this aspect (Chedotal et al., 2005; Chilton et al., 2006). Major neurotropic factors, such as neurotrophins (NT) which include nerve growth factor (NGF), brain-derived nerve growth factors (BDNF), NT-3, NT-4, and NT-5, are over-expressed in tumour cells and intra-tumoural nerves (Ketterer et al., 2003). Other factors including hematopoietic colony stimulating factors (G-CSF) and their receptors (G-CSFR and GM-CSFR $\alpha$ ) have also been shown to be expressed at high levels in pancreatic tumour micro-environment and to be associated with induction of pain. To this end, injection of anti-sera containing neutralising anti-bodies against G-CSF and GM-CSF receptors in a murine model of tumour-induced bone pain prevents hyperalgesia and reduces the number of nerves branching into the skin surrounding the tumour (Schweizerhof et al., 2009). In addition, myelin associated glycoprotein (MAG or Siglec-4a), expressed by Schwann cells bind to mucin 1 (MUC1) enriched on the surface of pancreas tumour cells (Swanson et al., 2007).

### 5.2 Chemokines and tumour PNI

CX3C chemokine receptor 1 (CX3CR1) also known as the fractalkine receptor or G-protein coupled receptor 13 (GPR13) are known to be involved in leukocyte adhesion and migration. Cells expressing this receptor bind to corresponding ligand CX3CL1 expressed on the surface of neurons, nerve fibres, and activated endothelial cells (Marchesi et al., 2010). In contrast to normal pancreas cells, tumour cells over-express CX3CR1 which in turn stimulates PNI. A large fraction (~90%) of pancreatic cancer biopsies are CX3CR1 positive and high receptor expression is associated with prominent PNI in pancreas cancer (Marchesi et al., 2008). A novel CX3CR1 antagonist has been recently developed and shown to block the cell adhesion along the CX3CL1/CX3CR1 axis (Dorgham et al., 2009). Thus, interference with CX3CL1/CX3CR1 signalling poses an attractive approach in prevention of PNI. Figure 4 illustrates some molecular mechanisms known to be involved in PNI.

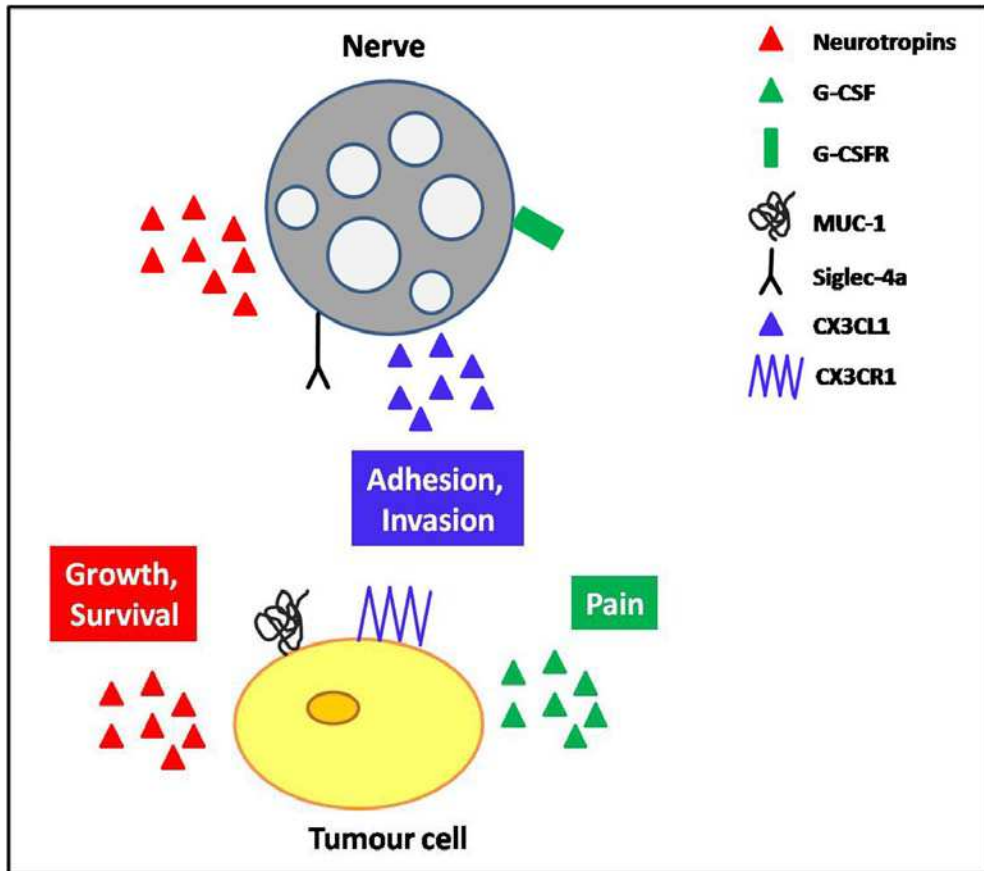


Fig. 4. Molecular mechanisms of PNI: Tumour cells expressing CX3CR1 and MUC-1 infiltrate the peri-neural space and adhere to CX3CL1 and Siglec-4a on the surface of neural cells. Both cell types secrete neurotrophins which are crucial for sustaining growth and survival of both cell types. Pain perception is influenced upon the interaction between CSF secreted by tumour cells and their receptors CSFR on the surface of neural cells.

A novel method has been developed by Abiatari et al. to monitor *ex vivo* PNI of PDAC tumour cells into surgically resected rat vagal nerves. Genome-wide transcriptional analyses deciphered a set of differentially regulated genes in high versus low invasive pancreas tumour cells. Kinesin family member 14 (KIF14) and Rho-GDP dissociation inhibitor  $\beta$  (ARHGDI $\beta$ ) are among two candidate PNI genes identified. Increased expression of both proteins was confined to tumour cells invading the peri-neural niche in pancreatic tumour patients. Finally, functional knock-down of KIF14 and ARHGDI $\beta$  resulted in altered PNI of tumour cells (Abiatari et al., 2009). These data indicate that a better molecular characterization of the PNI process is a prerequisite for development of targeted therapies aiming to inhibit the pancreatic cancer metastasis.

## 6. Pancreatic cancer stem cells

An emerging field of cancer research attempts to identify cellular hierarchies among the tumour cell population. Evidence are provided for tumor cells with self-renewing and stem-cell-like characteristics within solid tumours termed tumour-initiating cells (TICs) or cancer stem cells (CSC) (Reya et al., 2001; Al Hajj et al., 2003; Singh et al., 2004 O'Brien et al., 2007; Ricci-Vitani et al., 2007). It is hypothesised that CSC undergo EMT at the invasive front of primary tumours and migrate to colonise new tissue. The concept of "migrating CSC" describes a cancer stem cell which possess both an element of stemness and mobility (Brabletz et al., 2005). The relationship between CSC and pancreatic cancer progression was investigated by Li et al. (2007). They have chosen cancer stem cell markers based on previous work on breast cancer stem cells. These include the cell surface markers CD44, CD24, and epithelial-specific antigen (ESA). CD44+ CD24+ ESA+ pancreatic CSC demonstrate typical features observed in adult stem cells such as the ability of self-renewal, generation of differentiated progeny, and activation of developmental signalling pathways such as sonic hedgehog (Li et al., 2007). They further reported that only hundred human CD44+ CD24+ ESA+ pancreatic CSC are required to generate subcutaneous tumours in 50% of immuno-compromised SCID mice.

CD133 is yet another potential marker discussed to be characteristic for pancreatic CSC (Hermann et al., 2007). They showed that the capacity of cells to form primary tumours following orthotopic implantation in nude mice was exclusive to the CD133+ sub-population which also demonstrated inherent resistance to gemcitabine chemotherapy. Further studies on highly metastatic pancreatic cancer cell line L3.6pl identified two sub-sets of tumour cells based on the expression of CXCR4 receptor (Miller et al., 2008). Depletion of CXCR4 subset of CD133+ pancreatic CSCs precluded the formation of spontaneous liver metastases. In line with above mentioned data, CXCL12 appears to be the strongest inducer of migration in CD133+ cancer cells *in vitro*. A component of the therapeutic plant *Boswellia serrata*, acetyl-11-keto- $\beta$ -boswellic acid (AKBA), has been shown to down-regulate CXCR4 expression in pancreatic tumour cells and suppress cancer cell invasion (Park et al., 2011). Negative staining of cytokeratin epithelial cell marker in the CD133+ (in contrast to CD133- CSC) indicated EMT phenotype, thereby explaining their invasive potential (Mani et al., 2008). Blocking of the CXCL12/CXCR4 interaction with the CXCR4 non-peptidic antagonist reduces the spread of CD133+/CXCR4+ invasive pancreas cancer cells (Hermann et al., 2007).

Correlation between CD133 expression and lymph node metastasis in pancreatic cancer was investigated by Maeda et al. Immunohistochemical assessment of samples from 80 patients with PDAC after surgery revealed <15% CD133+ tumours cells per tumour in only 60% of specimen (48/80) suggesting a low frequency of these cells in PDAC. However, if CD133+ cells were detected they were cytokeratin negative and were confined to the glandular structures in the periphery of tumours. CD133 expression significantly correlated with clinicopathological parameter including VEGF-C expression, lymphatic invasion and lymph node metastasis (Maeda et al., 2008).

## 7. Conclusion

A better comprehension of the processes governing the formation of metastases is critical towards development of more advanced cancer treatment modalities. Various theories of



metastatic cancer progression have lately emerged. In contrast to late dissemination model early dissemination and parallel evolution of tumours in primary vs. metastatic sites impact current perception of tumour heterogeneity and consequently will impact the development of targeted cancer treatment strategies. The emergence of novel sequencing and high-throughput genetics methods will hopefully assist cancer research in defining the relevance of each model in pancreatic cancer development.

Acquisition of multiple genetic aberrations in the tumour cells is crucial for initiation of cancer. However, the communication of tumour cells with tumour-micro-environment is a prerequisite for successful tumour progression towards metastatic disease. Therefore, a better understanding of molecular mechanism underlying the orchestrated action between tumor cells and its micro-environmental participants such as stellate cells, endothelial cells, pericytes, immune cells and bone-marrow derived cells are urgently needed. The contribution of angiogenesis, EMT, cytokine/chemokine axis, neurotropism, hypoxia and tissue remodelling in development of pancreatic cancer are still in an early stage. Further research is needed to elaborate the molecular characteristics of specific niches such as liver vs. lymph nodes in development of PDAC metastases. In contrast to dynamic models considering tumour cell plasticity as the pivotal force behind its ability to gain specific traits when exposed to e.g. EMT or hypoxia stimuli, the existence of deterministic hierarchies among pancreatic tumour cells as proposed by the emerging tumour stem cell community remain elusive. In conclusion, a concerted multidisciplinary effort is needed to identify novel targets, rationally design therapies and ultimately improve the treatment of this devastating disease.

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## 9. References

- Abdollahi, A; Hahnfeldt, P; Maercker, C; Gröne, HJ; Debus, J; Ansorge, W; Folkman, J; Hlatky, L; & Huber, PE. (2004). Endostatin's antiangiogenic signalling network. *Mol Cell*, 13, Mar 2004, 649-663
- Abdollahi, A; Hlatky, L; & Huber, PE. (2005). Endostatin: the logic of antiangiogenic therapy. *Drug Resist Update*, 8, Feb-Apr 2005, 59-74
- Abdollahi, A; Schwager, C; Kleeff, J; Esposito, I; Domhan, S; Peschke, P; Hauser, K; Hahnfeldt, P; Hlatky, L; Debus, J; Peters, JM; Friess, H; Folkman, J; & Huber, PE. (2007). Transcriptional network governing the angiogenic switch in human pancreatic cancer. *Proc Natl Acad Sci USA.*, 104, Jul 2007, 12890-12895
- Abdollahi, A; Folkman, J. (2010) Evading tumour evasion: current concepts and perspectives of anti-angiogenic cancer therapy. *Drug Resist Update*, 13, Feb-Apr, 16-28.
- Abiatari, I; DeOliveira, T; Kerkadze, V; Schwager, C; Esposito, I; Giese, NA; Huber, P; Bergman, F; Abdollahi, A; Friess, H; & Kleeff, J. (2009). Consensus transcriptome

- signature of perineural invasion in pancreatic carcinoma. *Mol Cancer Ther.*, 8, Jun 2009, 1494-1504
- Aiuti, A; Webb, IJ; Bleul, C; Springer, T, & Gutierrez-Ramos, JC. (1997). The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. *J Exp Med.*, 185, Jan 1997, 111-120
- Al-Hajj, M; Wicha, MS; Benito-Hernandez, A; Morrison, SJ; & Clarke, MF. (2003). Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA.*, 100, Apr 2003, 3983-3988
- Allavena, P; Sica, A; Solinas, G; Porta, C; & Mantovani, A. (2008). The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. *Crit Rev Oncol Hematol.*, 66, Apr 2008, 1-9
- Alomg, N; Ma, L; Raychowdhury, R; Schwager, C; Erber, R; Short, S; Hlatky, L; Vajkoczy, P; Huber, PE; Folkman, J; & Abdollahi, A. (2009). Transcriptional switch of dormant tumors to fast-growing angiogenic phenotype. *Cancer Res.*, 69, Feb 2009, 836-844
- Bachem, MG; Zhou, S; Buck, K; Schneiderhan, W; & Siech, M. (2008). Pancreatic stellate cells--role in pancreas cancer. *Langenbecks Arch Surg*, 393, Nov 2008, 891-900
- Balkwill, F. (2004). The significance of cancer cell expression of the chemokine receptor CXCR4. *Semin. Cancer Biol.*, 14, Jun 2004, 171-179
- Baselga, J; & Arteaga, CL. (2005). Critical update and emerging trends in epidermal growth factor receptor targeting in cancer. *J Clin Oncol.*, 23, Apr 2005, 2445-2459
- Bauer, TW; Somcio, RJ; Fan, F; Liu, W; Johnson, M; Lesslie, DP; Evans, DB; Gallick, GE; & Ellis, LM. (2006). Regulatory role of c-Met in insulin-like growth factor-I receptor-mediated migration and invasion of human pancreatic carcinoma cells. *Mol Cancer Ther.*, 5, Jul 2006, 1676-1682
- Bernards, R. & Weinberg, RA. (2002). A progression puzzle. *Nature*, 418, Aug 2002, 823
- Brabletz, T; Jung, A; Reu, S; Porzner, M; Hlubek, F; Kunz-Schughart, LA; Knuechel, R; & Kirchner, T. (2001). Variable beta-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. *Proc Natl Acad Sci USA*, 98, Aug 2001, 10365-10361
- Brabletz, T; Jung, A; Spaderna, S; Hlubek, F; & Kirchner, T. (2005). Opinion: migrating cancer stem cells - an integrated concept of malignant tumour progression. *Nat Rev Cancer*, 5, Sep 2005, 744-749
- Bramhall, SR; Neoptolemos, JP; Stamp, GW; & Lemoine, NR. (1997). Imbalance of expression of matrix metalloproteinases (MMPs) and tissue inhibitors of the matrix metalloproteinases (TIMPs) in human pancreatic carcinoma. *J Pathol.*, 182, Jul 1997, 347-355
- Büchler, P; Reber, HA; Tomlinson, JS; Hankinson, O, Kallifatidis, G, Friess, H; Herr, I; & Hines, OJ. (2009). Transcriptional regulation of urokinase-type plasminogen activator receptor by hypoxia-inducible factor 1 is crucial for invasion of pancreatic and liver cancer. *Neoplasia*, 11, Feb 2009, 196-206
- Caruz, A; Samsom, M; Alonso, JM; Alcamoi, J; Baleux, F; Virelizier, JL; Parmentier, M; & Arenzana-Seisdedos, F. (1998). Genomic organization and promoter characterization of human CXCR4 gene. *FEBS Lett.*, 426, Apr 1998, 271-278

- Ceyhan, GO; Bergmann, F; Kadihasanoglu, M; Altintas, B; Demir, IE; Hinz, U; Müller, MW; Giese, T; Büchler, MW; Giese, NA; & Friess, H. (2009). Pancreatic neuropathy and neuropathic pain—a comprehensive pathomorphological study of 546 cases. *Gastroenterology*, 136, Jan 2009, 177-186
- Chédotal, A; Kerjan, G; & Moreau-Fauvarque, C. (2005). The brain within the tumor: new roles for axon guidance molecules in cancers. *Cell Death Differ.*, 12, Aug 2005, 1044-1056
- Chilton JK. (2006). Molecular mechanisms of axon guidance. *Dev. Biol.*, 292, Apr 2006, 13-24
- Coghlin, C.; & Murray, GI. (2010). Current and emerging concepts in tumour metastasis. *J Pathol.*, 222, Sep 2010, 1-15
- Cruickshank, AH. (1986). Solid carcinomas of the exocrine pancreas. *Pathology of the pancreas*, Springer London, UK, 155-177
- Cui K, Zhao W, Wang C, Wang A, Zhang B, Zhou W, Yu J, Sun Z, Li S. (2010). *J Surg Res.*, ahead of print, Mar 2010
- Dai, H; Li, R; Wheeler, T; Ozen, M; Ittmann, M; Anderson, M; Wang, Y; Rowley, D; Younes, M; & Ayala, GE. (2007). Enhanced survival in perineural invasion of pancreatic cancer: an in vitro approach. *Hum Pathol.*, 38, Feb 2007, 299-307
- Dong, YW; Wang, XP; & Wu, K. (2009). Suppression of pancreatic carcinoma growth by activating peroxisome proliferator-activated receptor gamma involves angiogenesis inhibition. *World J Gastroenterol.*, 15, Jan 2009, 441-448
- Dorgham, K; Ghadiri, A; Hermand, P; Rodero, M; Poupel, L; Iga, M; Hartley, O; Gorochov, G; Combadière, C; & Deterre, P. (2009). An engineered CX3CR1 antagonist endowed with anti-inflammatory activity. *J Leukoc Biol.*, 86, Oct 2009, 903-911
- Dvorak, HF. (2002). Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. *J Clin Oncol.*, 20, Nov 2002, 4368-4380
- Dvorak, HF; Brown, LF; Detmar, M; & Dvorak, AM. (1995). Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol.*, 146, May 1995, 1029-1039
- Edderkaoui, M; Hong, P; Vaquero, EC; Lee, JK; Fischer, L; Friess, H; Buchler, MW; Lerch, MM; Pandol, SJ; & Gukovskaya, AS. (2005). Extracellular matrix stimulates reactive oxygen species production and increases pancreatic cancer cell survival through 5-lipoxygenase and NADPH oxidase. *Am J Physiol Gastrointest Liver Physiol.*, 289, Dec 2005, G1137-1147
- Eibl, G; Reber, HA; Hines, OJ, & Go, VL. (2004). COX and PPAR: possible interactions in pancreatic cancer. *Pancreas*, 29, Nov 2004, 247-253
- Erkan, M; Weis, N; Pan, Z; Schwager, C; Samkharadze, T; Jiang, X; Wirkner, U; Giese, NA; Ansorge, W; Debus, J; Huber, PE; Friess, H; Abdollahi, A; & Kleeff, J. (2010). Organ-, inflammation- and cancer specific transcriptional fingerprints of pancreatic and hepatic stellate cells. *Mol Cancer*; 9, Apr 2010,
- 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. (2007). Blockade of hedgehog signaling inhibits pancreatic cancer invasion and

- metastases: a new paradigm for combination therapy in solid cancers. *Cancer Res.*, 67, Mar 2007, 2187-2196
- Ferrara, N. (1999). Molecular and biological properties of vascular endothelial growth factor. *J Mol Med (Berl.)*, 77, Jul 1999, 527-543
- Fidler, IJ; & Poste, G. (2008). The "seed and soil" hypothesis revisited. *Lancet Oncol.*, 9, Aug 2008, 808
- Folkman, J. (1971). Tumour angiogenesis: therapeutic implications. *N Engl J Med.*, 285, Nov 1971, 1182-1186
- Folkman, J; & Kalluri, R. (2004). Cancer without disease. *Nature*, 427, Feb 2004, 787
- Gao, Z; Wang, X; Wu, K; Zhao, Y; & 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, May 2010, 186-193
- Garcea, G; Doucas, H; Steward, WP; Dennison, AR; & Berry, DP. (2006). Hypoxia and angiogenesis in pancreatic cancer. *ANZ J Surg.*, 76, Sep 2006, 830-842
- Gaspar, NJ; Li, L; Kapoun, AM; Medicherla, S; Reddy, M; Li, G; O'Young, G; Quon, D; Henson, M; Damm, DL; Muir, GT; Murphy, A; Higgins, LS; Chakravarty, S; & Wong, DH. (2006). Inhibition of transforming growth factor beta signaling reduces pancreatic adenocarcinoma growth and invasiveness. *Mol Pharmacol.*, 72, Jul 2007, 152-161
- Gavert, N; & Ben-Ze'ev, A. (2008). Epithelial-mesenchymal transition and the invasive potential of tumors. *Trends Mol Med.*, 14, May 2008, 199-209
- Gray, MJ; Zhang, J; Ellis, LM; Semenza, GL; Evans, DB; Watowich, SS; & Gallick, GE. (2005). HIF-1 $\alpha$ , STAT3, CBP/p300 and Ref-1/APE are components of a transcriptional complex that regulates Src-dependent hypoxia-induced expression of VEGF in pancreatic and prostate carcinomas. *Oncogene*, 24, Apr 2005, 3110-3120
- Gress, TM; Müller-Pillasch, F; Lerch, MM; Friess, H; Büchler, M; & Adler, G. (1995). Expression and in-situ localization of genes coding for extracellular matrix proteins and extracellular matrix degrading proteases in pancreatic cancer. *Int J Cancer*, 62, Aug 1995, 407-413
- Halder, SK; Beauchamp, RD; & Datta, PK. (2005). A specific inhibitor of TGF-beta receptor kinase, SB-431542, as a potent antitumor agent for human cancers. *Neoplasia*, 7, May 2005, 509-521
- Hanahan, D; & Folkman, J. (1996). Patterns and emerging mechanisms of the angiogenic switch during tumourigenesis. *Cell*, 86, Aug 1996, 353-364
- Hanahan, D; & Weinberg, RA. (2011). Hallmarks of cancer: the next generation. *Cell*, 144, Mar 2011, 646-674
- Hashimoto, K; Ethridge, RT; & Evers, BM. (2002). Peroxisome proliferator-activated receptor gamma ligand inhibits cell growth and invasion of human pancreatic cancer cells. *Int J Gastrointest Cancer*, 32, 2002, 7-22
- He, Y; Liu, XD; Chen, ZY; Zhu, J; Xiong, Y; Li, K; Dong, JH; & Li, X. (2007). Interaction between cancer cells and stromal fibroblasts is required for activation of the uPAR-uPA-MMP-2 cascade in pancreatic cancer metastasis. *Clin Cancer Res*, 13, Jun 2007, 7-38

- Hermann, PC; Huber, SL; Herrler, T; Aicher, A; Ellwart, JW; Guba, M; Bruns, CJ; & Heeschen, C. (2007). Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell*, 1, Sep 2007, 313-323.
- Hu, WG, Liu, T, Xiong, JX, & Wang, CY. (2007). Blockade of sonic hedgehog signal pathway enhances antiproliferative effect of EGFR inhibitor in pancreatic cancer cells. *Acta Pharmacol Sin.*, 28, Aug 2007, 1224-1230
- Hwang, RF; Moore, T; Arumugam, T; Ramachandran, V; Amos, KD; Rivera, A; Ji, B; Evans, DB; & Logsdon, CD. (2008). Cancer-associated stromal fibroblasts promote pancreatic tumor progression. *Cancer Res.*, 68, 918-926
- Imamura, T; Iguchi, H; Manabe, T; Ohshio, G; Yoshimura, T; Wang, ZH; Suwa, H; Ishigami, S; & Imamura, M. (1995). Quantitative analysis of collagen and collagen subtypes I, III, and V in human pancreatic cancer, tumor-associated chronic pancreatitis, and alcoholic chronic pancreatitis. *Pancreas*, 11, Nov 1995, 357-364
- Issemann, I; & Green, S. (1990). Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature*, 347, Oct 1990, 645-650
- Itakura, J; Ishiwata, T; Friess, H; Fujii, H; Matsumoto, Y; Büchler, MW; & Korc, M. (1997). Enhanced expression of vascular endothelial growth factor in human pancreatic cancer correlates with local disease progression. *Clin Cancer Res.*, 3, Aug 1997, 1309-1316
- Itami, A; Watanabe, G; Shimada, Y; Hashimoto, Y; Kawamura, J; Kato, M; Hosotani, R; & Imamura, M. (2001). Ligands for peroxisome proliferator-activated receptor gamma inhibit growth of pancreatic cancers both in vitro and in vivo. *Int J Cancer*, 94, Nov 2001, 370-376
- Jang, MS; Miao, H; Carlesso, N; Shelly, L; Zlobin, A; Darack, N; Qin, JZ; Nickoloff, BJ; & Miele, L. (2004). Notch-1 regulates cell death independently of differentiation in murine erythroleukemia cells through multiple apoptosis and cell cycle pathways. *J cell Physiol.*, 199, Jun 2004, 418-433
- Jimenez, RE; Hartwig, W; Antoniu, BA; Compton, CC; Warshaw, AL; & Fernández-Del Castillo, C. (2000). Effect of matrix metalloproteinase inhibition on pancreatic cancer invasion and metastasis: an additive strategy for cancer control. *Ann Surg.*, 231, May 2000, 644-654
- 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. (2008). Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science*, 321, Sep 2008, 1801-1806
- Jonson, T; Albrechtsson, E; Axelson, J; Heidenblad, M; Gorunova, L; Johansson, B; & Höglund, M. (2001). Altered expression of TGFB receptors and mitogenic effects of TGFB in pancreatic carcinomas. *Int J Oncol.*, 19, Jul 2001, 71-81

- Kaipainen, A, Kieran, MW, Huang, S, Butterfield, C, Bielenberg, D, Mostoslavsky, G, Mulligan, R, Folkman, J, & Panigrahy, D. (2007). PPARalpha deficiency in inflammatory cells suppresses tumor growth. *PLoS One*, 2, Feb 2007, e260
- Kalluri, R; & Neilson, EG. (2003). Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest.*, 112, Dec 2003, 1776-1784
- Kalluri, R; & Weinberg RA. (2009). The basics of epithelial-mesenchymal transition. *J Clin Invest.*, 120, May 2010, 120-125
- Kayali, AG; Van Gunst, K; Campbell, IL; Stotland, A; Kritzik, M; Liu, G; Flodström-Tullberg, M; Zhang, YQ; & Sarvetnick, N. (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, Nov 2003, 859-869
- Ketterer, K; Rao, S; Friess, H; Weiss, J; Büchler, MW; & Korc, M. (2003). Reverse transcription-PCR analysis of laser-captured cells points to potential paracrine and autocrine actions of neurotrophins in pancreatic cancer. *Clin Cancer Res.*, 9, Nov 2003, 5127-5136
- Klein, CA. (2008). Cancer. The metastasis cascade. *Science*, 321, Sep 2008, 1785-1787
- Klein, CA. (2009). Parallel progression of primary tumours and metastases. *Nature*, 9, Apr 2009, 302-312
- Koong, AC; Mehta, VK; Le, QT; Fisher, GA; Terris, DJ; Brown, JM; Bastidas, AJ; & Vierra, M. (2000). Pancreatic tumors show high levels of hypoxia. *Int J Radiat Oncol Biol Phys.*, 48, Nov 2000, 919-922
- Kopfstein, L; Veikkola, T; Djonov, VG; Baeriswyl, V; Schomber, T; Strittmatter, K; Stacker, SA; Achen, MG; Alitalo, K; & Christofori, G. (2007). Distinct roles of vascular endothelial growth factor-D in lymphangiogenesis and metastasis. *Am J Pathol.*, 170, Apr 2007, 1348-1361
- Koshiba, T; Hosotani, R; Miyamoto, Y; Ida, J; Tsuji, S; Nakajima, S; Kawaguchi, M; Kobayashi, H; Doi, R; Hori, T; Fujii, N; & Imamura, M. (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, Sep 2000, 3530-3535
- Koshiba, T; Hosotani, R; Wada, M; Miyamoto, Y; Fujimoto, K; Lee, JU; Doi, R; Aii, S; & Imamura, M. (1998). Involvement of matrix metalloproteinase-2 activity in invasion and metastasis of pancreatic carcinoma. *Cancer*, 82, Feb 1998, 642-650
- Lee, CH; Chawla, A; Urbiztondo, N; Liao, D; Boisvert, WA, Evans, RM, Curtiss, LK. (2003). Transcriptional repression of atherogenic inflammation: modulation by PPARdelta. *Science*, 302, Oct 2003, 453-457
- Lee, JM; Dedhar, S; Kalluri, R; & Thompson, EW. (2006). The epithelial-mesenchymal transition: new insights in signaling, development, and disease. *J Cell Biol.*, 172, Mar 2006, 973-981
- Li, C; Heidt, DG; Dalerba, P; Burant, CF; Zhang, L; Adsay, V; Wicha, M; Clarke, MF; & Simeone, DM. (2007). Identification of pancreatic cancer stem cells. *Cancer Res.*, 67, Feb 2007, 1030-1037

- Liao, DJ; Wang, Y; Wu, J; Adsay, NV; Grignon, D; Khanani, F; & Sarkar, FH. (2006). Characterization of pancreatic lesions from MT-tgf alpha, Ela-myc and MT-tgf alpha/Ela-myc single and double transgenic mice. *J Carcinog.*, 5, Jul 2006
- Liao, DJ; Adsay, NV; Khanani, F; Grignon, D; Thakur, A; & Sarkar, FH. (2007). Histological complexities of pancreatic lesions from transgenic mouse models are consistent with biological and morphological heterogeneity of human pancreatic cancer. *Histol Histopathol.*, 22, Jun 2007 661-676
- Luebeck, E. (2010). Genomic evolution of metastasis. *Nature*, 467, Oct 2010, 1053-1055
- Macaulay, VM. (1992). Insulin-like growth factors and cancer. *Br J Cancer*, 65, Mar 1992, 311-320
- Maeda, S; Shinchu, H; Kurahara, H; Mataka, Y; Maemura, K; Sato, M; Natsugoe, S; Aikou, T; & Takao, S. (2008). CD133 expression is correlated with lymph node metastasis and vascular endothelial growth factor-C expression in pancreatic cancer. *Br J Cancer*, 98, Apr 2008, 1389-1397
- Mandriota, SJ; Jussila, L; Jeltsch, M; Compagni, A; Baetens, D; Prevo, R; Banerji, S; Huarte, J; Montesano, R; Jackson, DG; Orci, L; Alitalo, K; Christofori, G; & Pepper, MS. (2001). Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis. *EMBO J.*, 20, Feb 2001, 672-682
- Mani, SA; Guo, W; Liao, MJ; Eaton, EN; Ayyanan, A; Zhou, AY; Brooks, M; Reinhard, F; Zhang, CC; Shipitsin, M; Campbell, LL; Polyak, K; Brisken, C; Yang, J; & Weinberg RA. (2008). The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell*, 133, May 2008, 704-715
- Marchesi, F; Monti, P; Leone, BE; Zerbi, A; Vecchi, A; Piemonti, L; Mantovani, A; & Allavena, P. (2004). Increased survival, proliferation, and migration in metastatic human pancreatic tumor cells expressing functional CXCR4. *Cancer Res.*, 64, Nov 2004, 8420-8427
- Marchesi, F; Piemonti, L; Fedele, G; Destro, A; Roncalli, M; Albarello, L; Doglioni, C; Anselmo, A; Doni, A; Bianchi, P; Laghi, L; Malesci, A; Cervo, L; Malosio, M; Reni, M; Zerbi, A; Di Carlo, V; Mantovani, A; & Allavena P. (2008). The chemokine receptor CX3CR1 is involved in the neural tropism and malignant behavior of pancreatic ductal adenocarcinoma. *Cancer Res.*, 68, Nov 2008, 9090-9069
- Marchesi, F; Piemonti, L; Mantovani, A; & Allavena, P. (2010). Molecular mechanisms of perineural invasion, a forgotten pathway of dissemination and metastasis. *Cytokine Growth Factor Rev.*, 21, Feb 2010, 77-82
- Mathews, LA; Cabarcas, SM; Hurt, EM; Zhang, X; Jaffee, EM; & Farrar, WL. (2011). Increased expression of DNA repair genes in invasive human pancreatic cancer cells. *Pancreas*, 40, Jul 2011, 730-739
- Megibow, AJ. (1992). Pancreatic adenocarcinoma: designing the examination to evaluate the clinical questions. *Radiology*, 183, May 1992, 297-303
- Mellado, M; Rodríguez-Frade, JM; Mañes, S; & Martínez-A, C. (2001). Chemokine signaling and functional responses: the role of receptor dimerization and TK pathway activation. *Annu Rev Immunol.*, 19, 2001, 397-421
- Michalik, L; Desvergne, B; Tan, NS; Basu-Modak, S; Escher, P; Rieusset, J; Peters, JM; Kaya, G; Gonzalez, FJ; Zakany, J; Metzger, D; Chambon, P; Duboule, D; & Wahli, W.

- (2001). Impaired skin wound healing in peroxisome proliferator-activated receptor (PPAR)alpha and PPARbeta mutant mice. *J Cell Biol.*, 154, Aug 2001, 799-814
- Miller, RJ; Banisadr, G; & Bhattacharyya, BJ. (2008). CXCR4 signaling in the regulation of stem cell migration and development. *J Neuroimmunol.*, 198, Jul 2008, 31-38
- Mimeault, M; Brand, RE; Sasson, AA; & Batra, SK. (2005). Recent advances on the molecular mechanisms involved in pancreatic cancer progression and therapies. *Pancreas*, 31, Nov 2005, 301-316
- Moore, MJ; Goldstein, D; Hamm, J; Figer, A; Hecht, JR; Gallinger, S; Au, HJ; Murawa, P; Walde, D, Wolff, RA, Campos, D; Lim, R; Ding, K; Clark, G; Voskoglou-Nomikos, T; Ptasynski, M; Parulekar, W; & National Cancer Institute of Canada Clinical Trials Group. (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. *Clin Oncol.*, 25, May 2007, 1960-1966
- Mori, T; Doi, R; Koizumi, M; Toyoda, E; Ito, D; Kami, K; Masui, T; Fujimoto, K; Tamamura, H; Hiramatsu, K; Fujii, N; & Imamura, M. (2004). CXCR4 antagonist inhibits stromal cell-derived factor 1-induced migration and invasion of human pancreatic cancer. *Mol Cancer Ther.*, 3, Jan 2004, 29-37
- Müller-Brüsselbach, S; Kömhoff, M; Rieck, M; Meissner, W; Kaddatz, K; Adamkiewicz, J; Keil, B; Klose, KJ; Moll, R; Burdick, AD; Peters, JM; & Müller, R. (2007). Deregulation of tumor angiogenesis and blockade of tumor growth in PPARbeta-deficient mice. *EMBO J.*, 26, Aug 2007, 3686-3698
- Murphy, PM; Baggiolini, M; Charo, IF; Hébert, CA; Horuk, R; Matsushima, K; Miller, LH; Oppenheim, JJ; & Power CA. (2000). International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev.*, 52, Mar 2000, 145-176
- Nagakawa, Y; Aoki, T; Kasuya, K; Tsuchida, A; & Koyanagi, Y. (2002). Histologic features of venous invasion, expression of vascular endothelial growth factor and matrix metalloproteinase-2 and matrix metalloproteinase-9, and the relation with liver metastasis in pancreatic cancer. *Pancreas*, 24, Mar 2002, 169-178
- Nakajima, A; Tomimoto, A; Fujita, K; Sugiyama, M; Takahashi, H; Ikeda, I; Hosono, K; Endo, H; Yoneda, K; Iida, H; Inamori, M; Kubota, K; Saito, S; Nakajima, N; Wada, K; Nagashima, Y; & Nakagama, H. (2008). Inhibition of peroxisome proliferator-activated receptor gamma activity suppresses pancreatic cancer cell motility. *Cancer Sci.*, 99, Oct 2008, 1892-1900
- O'Brien, CA; Pollett, A; Gallinger, S; & Dick, JE. (2007). A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature*, 445, Jan 2007, 106-110
- Omary, MB; Lugea, A; Lowe, AW; & Pandolfi, SJ. (2007). The pancreatic stellate cell: a star on the rise in pancreatic diseases. *J Clin Invest*, 117, 50-59
- Panigrahy, D; Singer, S; Shen, LQ; Butterfield, CE; Freedman, DA; Chen, EJ; Moses, MA; Kilroy, S; Duensing, S; Fletcher, C; Fletcher, JA; Hlatky, L; Hahnfeldt, P; Folkman, J; & Kaipainen, A. (2002). PPARgamma ligands inhibit primary tumor growth and metastasis by inhibiting angiogenesis. *J Clin Invest.*, 110, Oct 2002, 923-932
- Panigrahy D, Kaipainen A, Huang S, Butterfield CE, Barnés CM, Fannon M, Laforme AM, Chaponis DM, Folkman J, Kieran MW. (2008). PPARalpha agonist fenofibrate

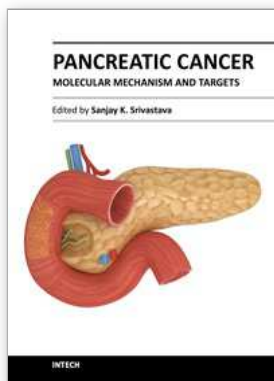


- suppresses tumor growth through direct and indirect angiogenesis inhibition. *Proc Natl Acad Sci USA*, 105, Jan 2008, 985-990
- Park, BH; Vogelstein, B; & Kinzler, KW. (2001). Genetic disruption of PPARdelta decreases the tumorigenicity of human colon cancer cells. *Proc Natl Acad Sci USA.*, 98, Feb 2001, 2598-2603
- Peters, JM; Lee, SS; Li, W; Ward, JM; Gavrilova, O; Everett, C; Reitman, ML; Hudson, LD; & Gonzalez, FJ. (2000). Growth, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor beta(delta). *Mol Cell Biol.*, 20, Jul 2000, 5119-5128
- Pignatelli, M; Ansari, TW; Gunter, P; Liu, D; Hirano, S; Takeichi, M; Klöppel, G; & Lemoine, NR. (1994). Loss of membranous E-cadherin expression in pancreatic cancer: correlation with lymph node metastasis, high grade, and advanced stage. *J Pathol.*, 174, Dec 1994, 243-248
- Plutzky, J. (2003). Medicine PPARs as therapeutic targets: reverse cardiology? *Science*, 302, Oct 2003, 406-407
- Pollard, JW. (2004). Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer*, 4, Jan 2004, 71-78
- Pour, PM; Bell, RH; & Batra, SK. (2003). Neural invasion in the staging of pancreatic cancer. *Pancreas*, 26, May 2003, 322-325
- Reya, T; Morrison, SJ; Clarke, MF; & Weissman, IL. (2001). Stem cells, cancer, and cancer stem cells. *Nature*, 414, Nov 2001, 105-111
- Ricci-Vitani, L; Lombardi, DG; Pillozzi, E; Biffoni, M; Todaro, M; Peschle, C; & De Maria, R. (2007). Identification and expansion of human colon-cancer-initiating cells. *Nature*, 445, Jan 2007, 111-115
- Richard, CL; & Blay, J. (2008). CXCR4 in Cancer and Its Regulation by PPARgamma. *PPAR Res.*, 2008, Jul 2008, 1-19
- Rubbia-Brandt, L; Terris, B; Giostra, E; Dousset, B; Morel, P; & Pepper, MS. (2004). Lymphatic vessel density and vascular endothelial growth factor-C expression correlate with malignant behavior in human pancreatic endocrine tumors. *Clin Cancer Res.*, 10, Oct 2004, 6919-6928
- Salven, P; Mustjoki, S; Alitalo, R; Alitalo, K; & Raffi, S. (2003). VEGFR-3 and CD133 identify a population of CD34+ lymphatic/vascular endothelial precursor cells. *Blood*, 101, Jan 2003, 168-172
- Savagner, P. (2001). Leaving the neighborhood: molecular mechanisms involved during epithelial-mesenchymal transition. *Bioessays*, 10, Oct 2001, 912-923
- Sawai, H; Liu, J; Reber, HA; Hines, OJ; & Eibl, G. (2006). Activation of peroxisome proliferator-activated receptor-gamma decreases pancreatic cancer cell invasion through modulation of the plasminogen activator system. *Mol Cancer Res.*, 4, Mar 2006, 159-167
- Schneider, M; Büchler, P; Giese, N; Giese, T; Wilting, J; Büchler, MW; & Friess, H. (2006). Role of lymphangiogenesis and lymphangiogenic factors during pancreatic cancer progression and lymphatic spread. *Int J Oncol.*, 28, Apr 2006, 883-890
- Schweizerhof, M; Stösser, S; Kurejova, M; Njoo, C; Gangadharan, V; Agarwal, N; Schmelz, M; Bali, KK; Michalski, CW; Brugger, S; Dickenson, A; Simone, DA; & Kuner, R.

- (2009). Hematopoietic colony-stimulating factors mediate tumor-nerve interactions and bone cancer pain. *Nat Med.*, 15, Jul 2009, 802-807
- Sell, C; Baserga, R; & Rubin, R. (1995). Insulin-like growth factor I (IGF-I) and the IGF-I receptor prevent etoposide-induced apoptosis. *Cancer Res.*, 55, Jan 1995, 303-306
- Seo, Y; Baba, H; Fukuda, T; Takashima, M; & Sugimachi, K. (2000). High expression of vascular endothelial growth factor is associated with liver metastasis and a poor prognosis for patients with ductal pancreatic adenocarcinoma. *Cancer*, 88, May 2000, 2239-2245
- Shi, WD; Meng, ZQ; Chen, Z; Lin, JH; Zhou, ZH; & Liu, LM. (2009). Identification of liver metastasis-related genes in a novel human pancreatic carcinoma cell model by microarray analysis. *Cancer Lett.*, 283, Sep 2009, 84-91
- Shintani, Y, Hollingsworth, MA, Wheelock, MJ, & Johnson, KR. (2006). Collagen I promotes metastasis in pancreatic cancer by activating c-Jun NH(2)-terminal kinase 1 and up-regulating N-cadherin expression. *Cancer Res.*, 15, Dec 2006, 11745-11753
- Singh, SK; Hawkins, C; Clarke, ID; Squire, JA; Bayani, J; Hide, T; Henkelman, RM; Cusimano, MD; & Dirks, PB. (2004). Identification of human brain tumour initiating cells. *Nature*, 432, Nov 2004, 396-401
- Sleeman, J; Schmid, A; & Thiele, W. (2009). Tumor lymphatics. *Semin Cancer Biol.*, 19, Oct 2009, 285-297
- Sleeman, JP; & Thiele, W. (2009). Tumor metastasis and the lymphatic vasculature. *Int J Cancer*, 125, Dec 2009, 2747-2756
- Staller, P; Sulitkova, J; Lisztwan, J; Moch, H; Oakeley, EJ; & Krek, W. (2003). Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. *Nature*, 425, Sep 2003, 307-311
- Swanson, BJ; McDermott, KM; Singh, PK; Eggers, JP; Crocker, PR; & Hollingsworth, MA. (2007). MUC1 is a counter-receptor for myelin-associated glycoprotein (Siglec-4a) and their interaction contributes to adhesion in pancreatic cancer perineural invasion. *Cancer Res.*, 67, Nov 2007, 10222-10229
- Tanno, S; Tanno, S; Mitsuuchi, Y; Altomare, DA; Xiao, GH; & Testa, JR. (2001). AKT activation up-regulates insulin-like growth factor I receptor expression and promotes invasiveness of human pancreatic cancer cells. *Cancer Res.*, 61, Jan 2001, 589-593
- Tawada, K; Ishihara, T; Kobayashi, A; Yamaguchi, T; Tsuyuguchi, T; Matsuyama, M; & Yokosuka, O. (2008). Quantitative analysis of vascular endothelial growth factor in liver metastases from pancreatic carcinoma as a predictor of chemotherapeutic effect and prognosis. *Clin Cancer Res.*, 14, Nov 2008, 7438-7443
- Teraoka, H; Sawada, T; Nishihara, T; Yashiro, M; Ohira, M; Ishikawa, T; Nishino, H; & Hirakawa, K. (2001). Enhanced VEGF production and decreased immunogenicity induced by TGF-beta 1 promote liver metastasis of pancreatic cancer. *Br J Cancer*, 85, Aug 2001, 612-617
- Thakur, A; Bollig, A; Wu, J; & Liao, DJ. (2008). Gene expression profiles in primary pancreatic tumors and metastatic lesions of Ela-c-myc transgenic mice. *Mol Cancer*, 7:11, Jan 2008

- Thiele, W; & Sleeman, JP. (2006). Tumor-induced lymphangiogenesis: a target for cancer therapy? *J Biotechnol.*, 124, Jun 2006, 224-241
- Thiery, JP. (2002). Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer*, 2, Jun 2002, 442-454
- Thiery, JP. (2003). Epithelial-mesenchymal transitions in development and pathologies. *Curr Opin Cell Biol.*, 15, Dec 2003, 740-746
- Vaquero, EC; Edderkaoui, M; Nam, KJ; Gukovsky, I; Pandol, SJ; & Gukovskaya, AS. (2003). Extracellular matrix proteins protect pancreatic cancer cells from death via mitochondrial and nonmitochondrial pathways. *Gastroenterology*, 125, Oct 2003, 1188-1202
- Wang, Z; Banerjee, S; Li, Y; Rahman, KM; Zhang, Y; & Sarkar, FH. (2006). Down-regulation of notch-1 inhibits invasion by inactivation of nuclear factor-kappaB, vascular endothelial growth factor, and matrix metalloproteinase-9 in pancreatic cancer cells. *Cancer Res.*, 66, Mar 2006, 2778-2784
- Wang, D, Wang, H, Guo, Y, Ning, W, Katkuri, S, Wahli, W, Desvergne, B, Dey, SK, & DuBois, RN. (2006). Crosstalk between peroxisome proliferator-activated receptor delta and VEGF stimulates cancer progression. *Proc Natl Acad Sci U S A.*, 103, Dec 2006, 19069-1974
- Wegner, SA; Ehrenberg, PK; Chang, G; Dayhoff, DE; Sleeker, AL; & Michael, NL. (1998). Genomic organization and functional characterization of the chemokine receptor CXCR4, a major entry co-receptor for human immunodeficiency virus type 1. *J Biol Chem.*, 273, Feb 1998, 4754-4760
- Weinberg, R. (2008). The many faces of tumour dormancy. *APMIS*, 116, Jul-Aug 2008, 548-551, ISSN 0903-4641
- Yachida, S.; Jones, S; Bozic, I; Antal; T; Leary; R; Fu; B; Kamiyama, M; Hruban, R; Eshelman, J; Nowak, M; Velculescu, V; Kinzler, K; Vogelstein, B; & Iacobuzio-Donhaue, C. (2010). Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature*, 467, Oct 2010, 1114-1117
- Yang, J; & Weinberg, RA. (2008). Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev Cell.*, 14, Jun 2008, 818-829
- Yang, J; Mani, SA; Donaher, JL; Ramaswamy, S; Itzykson, RA; Come, C; Savagner, P; Gitelman, I; Richardson, A; & Weinberg, RA. (2004). Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell*, 117, Jun 2004, 927-939
- Yu, H; & Jove, R. (2003). The STATs of cancer--new molecular targets come of age. *Nat Rev Cancer*, 4, Feb 2004, 97-105
- Zervos, EE; Norman, JG; Gower, WR; Franz, MG; & Rosemurgy AS. (1997). Matrix metalloproteinase inhibition attenuates human pancreatic cancer growth in vitro and decreases mortality and tumorigenesis in vivo. *J Surg Res.*, 69, May 1997, 367-371
- Zhang, K; Chen, D; Jiao, X; Zhang, S; Liu, X; Cao, J; Wu, L; & Wang, D. (2011). Slug enhances invasion ability of pancreatic cancer cells through upregulation of matrix metalloproteinase-9 and actin cytoskeleton remodeling. *Lab Invest.*, 91, Mar 2011, 426-438

- Zhang, L; He, T; Talal, A; Wang, G; Frankel, SS; & Ho, DD. (1998). In vivo distribution of the human immunodeficiency virus/simian immunodeficiency virus coreceptors: CXCR4, CCR3, and CCR5. *J Virol.*, 72, Jun 1998, 5035-5045
- Zhao, S; Venkatasubbarao, K; Lazor, JW; Sperry, J; Jin, C; Cao, L; & Freeman, JW. (2008). Inhibition of STAT3 Tyr705 phosphorylation by Smad4 suppresses transforming growth factor beta-mediated invasion and metastasis in pancreatic cancer cells. *Cancer Res.*, 68, Jun 2008, 4221-4228



## **Pancreatic Cancer - Molecular Mechanism and Targets**

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This book provides the reader with an overall understanding of the biology of pancreatic cancer, hereditary, complex signaling pathways and alternative therapies. The book explains nutrigenomics and epigenetics mechanisms such as DNA methylation, which may explain the etiology or progression of pancreatic cancer. Book also summarizes the molecular control of oncogenic pathways such as K-Ras and KLF4. Since pancreatic cancer metastasizes to vital organs resulting in poor prognosis, special emphasis is given to the mechanism of tumor cell invasion and metastasis. Role of nitric oxide and Syk kinase in tumor metastasis is discussed in detail. Prevention strategies for pancreatic cancer are also described. The molecular mechanisms of the anti-cancer effects of curcumin, benzyl isothiocyanate and vitamin D are discussed in detail. Furthermore, this book covers the basic mechanisms of resistance of pancreatic cancer to chemotherapy drugs such as gemcitabine and 5-fluorouracil.

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