

## Some More News on the Metastatic Pathway in Pancreatic Cancer

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Pancreatic cancer (PK) is a highly aggressive type of malignancy and the prognosis for this condition which typically presents at a late stage is extremely poor. Invasiveness is an early phenomenon, and extensive local/distant metastasis represents the rule in clinical practice. In this context, a comprehensive understanding of the metastatic pathway(s) seems to be basic for the improvement of the limited therapeutic weapons currently at our disposal. Very recently, some studies have addressed this subject, giving new and important information on the metastatic mechanisms in PK (e.g., motility of cancer cells, growth factors and angiogenic mechanisms).

Tsutsumi *et al.* from the Department of General Surgical Science, Gunma University Graduate School of Medicine, Maebashi, Japan [1] reported the results of an interesting study on the role of the autocrine motility factor (AMF) in the PK metastatic process. AMF/phosphoglucose isomerase (PGI) is a ubiquitous cytosolic enzyme which plays a key role in glycolysis and is also a multifunctional protein which acts in the extracellular milieu as a potent mitogen/cytokine. Increased expression of AMF/PGI and its receptor has been found in a wide spectrum of malignancies and is associated with cancer progression and metastasis. In their study, the authors assessed whether or not overexpression of AMF in human pancreatic cancer cells enhances liver metastasis using an orthotopic mouse tumor model and they also investigated the

intracellular signal transduction pathways of AMF in human pancreatic cancer cell lines. The results showed that: i) overexpression of AMF stimulated the *in vitro* invasion of MIA PaCa-2 cells; ii) *in vivo*, after orthotopic implantation into the pancreas of nude mice, parental and empty vector-transfected MIA PaCa-2 cells produced locally relatively small tumors with no evidence of liver metastasis, whereas AMF-transfected MIA PaCa-2 cells produced large tumors and liver metastases; iii) over-expression of AMF leads to the down-regulation of E-cadherin expression. The authors conclude that AMF expression significantly contributes to the aggressive phenotype of human PK and may thus provide a novel prognostic and therapeutic target. A similar issue was investigated by Shimamura *et al.* from the National Cancer Center Research Institute, Tokyo, Japan [2] who reported an interesting experimental study on the role of dysadherin in PK. Dysadherin is a membrane glycoprotein strongly expressed in several human cancers; overexpression of this molecule in tumor cells is closely associated with a malignant phenotype (e.g., metastasis) and a poor prognosis. The authors, analyzing six PK cell lines, found a positive correlation between dysadherin expression and cell motility. Introduction of small interfering RNA (*si*-RNA) against dysadherin into the Panc-1 cell line caused reduction of dysadherin expression and suppression of cell motility. In contrast, stable transfection of a dysadherin expression vector into the Capan-1 cell line

increased cell motility. In vivo, the metastatic potential of orthotopically transplanted Capan-1 tumor cells in severe combined immunodeficient mice was increased by dysadherin overexpression. Cell morphology and actin organization were also influenced by modulation of dysadherin expression. Cells transfected with dysadherin *si*-RNA tended to have a relatively larger, more spread-out shape and increased number of transverse actin stress fibers as compared to parent cells and cells transfected with control *si*-RNA. The study suggests that dysadherin is able to modulate actin structures, stimulate cell motility, and contribute directly to the metastatic potential of human PK cells.

Starting from the evidence that PK frequently invades and migrates along neural tissue, Okada *et al.* from the Department of Gastrointestinal Surgery, David Geffen School of Medicine at UCLA, Los Angeles, California, USA [3] investigated the role of the nerve growth factor (NGF) in PK metastatic invasion. The relevance of this speculation is important as clinical experience shows that, although the exact mechanisms are unknown, perineural invasion negatively impacts the prognosis for PK patients. The authors hypothesized that NGF released from neural tissue increases the invasive properties of PK cells and they investigated the effect of NGF on the expression and activity of matrix metalloproteinases (MMPs) in human PK cells (previous studies showed that MMPs are overexpressed in PK and are associated with a poor prognosis). The results showed that the NGF dose dependently increased the matrix-MMP-2 protein in the culture medium and stimulated MMP-2 gelatinolytic activity. This effect was mediated by the specific binding of NGF to its receptor *trk A*, which was detected on all pancreatic cancer cells, with the subsequent activation of the p44/42 MAPK signaling pathway. The NGF-induced increase in MMP-2 expression and activity leads to an enhanced invasion *in vitro*. These findings support the hypothesis that neurotrophic factors (e.g., NGF) are critically involved in mediating the perineural invasion of pancreatic cancer, opening a *scenario* of

new molecular targeted therapy. A similar issue was also recently addressed by Crnic *et al.* from the Institute of Biochemistry and Genetics, Department of Clinical-Biological Sciences, University of Basel, Basel, Switzerland [4]. Reduced expression of the neural cell adhesion molecule (NCAM) has been implicated in the progression to tumor malignancy in cancer patients; the authors have previously reported that the loss of NCAM function causes the formation of lymph node metastasis in a transgenic mouse model of pancreatic beta cell carcinogenesis (*Rip1Tag2*). In the aforementioned study, the authors showed that tumors of NCAM-deficient *Rip1Tag2* transgenic mice exhibit up-regulated expression of the lymphangiogenic factors vascular endothelial growth factor (VEGF)-C and -D (17% in wild-type *versus* 60% in NCAM-deficient *Rip1Tag2* mice) and, with it, increased lymphangiogenesis (0% in wild-type *versus* 19% in NCAM-deficient *Rip1Tag2* mice). Repression of VEGF-C and -D function by adenoviral expression of a soluble form of their cognate receptor, VEGF receptor-3, results in reduced tumor lymphangiogenesis (56% *versus* 28% in control *versus* treated mice) and lymph node metastasis (36% *versus* 8% in control *versus* treated mice). The results indicate that the loss of NCAM function causes lymph node metastasis *via* VEGF-C- and VEGF-D-mediated lymphangiogenesis.

Another interesting article was recently published concerning the role of matrix-MMP in metastatic progression of PK from the Department of Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA [5]. The authors tested the hypothesis that the cyclooxygenase-2 (COX-2) product prostaglandin E(2) (PGE(2)) increases cellular invasive potential by inducing matrix-MMP-2 expression and activity through an extracellular signal-regulated kinase (*ERK*)/*Ets-1*-dependent mechanism in PK. PANC-1 and MIAPaCa-2 PK cells were treated with PGE(2) or rofecoxib, a selective COX-2 inhibitor. MMP-2 expression and activity were assayed using

Western blot analysis and zymography, respectively. MMP-2 promoter activity was analyzed with a luciferase-based assay. *Ets-1* activity was analyzed using gel shift assay. *Ets-1* expression was specifically silenced using RNA interference. Cellular invasive and migratory potentials were determined using a Boyden chamber assay with or without matrigel, respectively. Exogenous PGE(2) induced MMP-2 expression and activity and increased *ERK1/2* phosphorylation, *Ets-1* binding activity, and MMP-2 promoter activity. PGE(2) also increased cellular migratory and invasive potentials. The mitogen-activated protein kinase inhibitor PD98059 and *Ets-1* silencing each abolished PGE(2)-induced increases in MMP-2 expression. PD98059 and *Ets-1* silencing each abrogated the effect of PGE(2) on cellular invasive potential but not on cellular migratory potential. Rofecoxib suppressed MMP-2 expression and activity, *Ets-1* binding activity, MMP-2 promoter activity, and cellular migratory and invasive potentials. These results suggest that PGE(2) mediates PK cellular invasiveness through an *ERK/Ets-1*-dependent induction of MMP-2 expression and activity; in addition, one can hypothesize that COX-2 inhibition could represent a strategy to inhibit invasive potential in PK.

Hartel *et al.* from the Department of General Surgery, University of Heidelberg, Germany [6] recently addressed the issue of the growth factor in PK, in particular of the connective tissue growth factor (CTGF) which has recently been implicated in the pathogenesis of fibrotic diseases and tumor stroma. Inasmuch as generation of desmoplastic tissue is characteristic for PK, it is not known whether it gives PK cells a growth advantage or if it is a reaction of the body to inhibit cancer cell progression. The authors analyzed the expression and localization of CTGF and evaluated whether it influences the prognosis of PK. Tissue samples were obtained from 25 individuals (6 women, 19 men) undergoing pancreatic resection for pancreatic cancer. Tissue samples from 13 previously healthy organ donors (5 women, 8 men) served as controls. Expression of CTGF was studied by

Northern blot analysis. *In situ* hybridization and immunohistochemistry localized the respective mRNA moieties and proteins in the tissue samples. Northern blot analysis revealed that PK tissue samples exhibited a 46-fold increase in CTGF mRNA expression ( $P < 0.001$ ) over that of normal controls. *In vitro* studies confirmed that pancreatic stellate cells are the major source of CTGF mRNA expression and revealed a large variance in basal and transforming growth factor- $\alpha$  (TGF $\alpha$ )-induced CTGF expression in cultured pancreatic cancer cells (synthesis of CTGF is regulated by TGF $\alpha$ ). This could also be confirmed by *in situ* hybridization, indicating that CTGF mRNA signals were located principally in fibroblasts, with only weak signals in the cancer cells. High CTGF mRNA levels in the tissue samples correlated with better tumor differentiation ( $P < 0.03$ ). In addition, patients whose tumors exhibited high CTGF mRNA levels (greater than onefold increase above normal controls) lived significantly longer than those whose tumors expressed low CTGF mRNA levels (none to onefold) ( $P < 0.04$ , multivariate analysis). These results indicate that CTGF, as a downstream mediator of TGF $\alpha$ , is overexpressed in connective tissue cells and, to a lesser extent in PK cells. Because patients with high CTGF mRNA expression levels have a better prognosis, these findings indicate that the desmoplastic reaction provides a growth disadvantage for PK cells. Another group (coming from the same University Department of Surgery, University of Heidelberg, Germany) produced very interesting data on the angiogenic mechanism in PK [7]. Starting from the evidence that a low vessel density is a common feature of malignant tumors, the authors suggested that the expansion of the vessel diameter might reconstitute the oxygen and nutrient supply in this situation. The authors compared the number and the diameter of the blood vessels in pancreatic and liver carcinoma with normal tissue using animal models of pancreatic (DSL6A) or hepatocellular (Morris-hepatoma) carcinoma in male Lewis (PK) and ACI (hepatoma) rats

by an orthotopic inoculation of solid tumor fragments (PK) or tumor cells (hepatoma). Six weeks (PK) or 12 days (hepatoma) after tumor implantation, the tumor microvasculature as well as normal pancreatic or liver blood vessels were investigated by intravital microscopy. The number of perfused blood vessels in tumor and healthy tissue was assessed by computer-assisted image analysis. The vessel density in healthy pancreases ( $565 \pm 89$  n/mm<sup>2</sup>) was significantly higher compared to PK ( $116 \pm 36$  n/mm<sup>2</sup>) ( $P < 0.001$ ). Healthy livers also showed a significantly higher vessel density ( $689 \pm 36$  n/mm<sup>2</sup>) as compared to livers affected by a carcinoma ( $286 \pm 32$  n/mm<sup>2</sup>) ( $P < 0.01$ ). The comparison of diameter frequency showed a significant increase of vessel diameter in both malignant tumors compared to normal tissue ( $P < 0.05$ ). The authors concluded that the expansion of endothelial cells during tumor angiogenesis is accompanied, to a large extent, by an increase of vessel diameter rather than by formation of new blood vessels. This may be a possible adaptive mechanism by which experimental pancreatic and hepatocellular cancers expand the diffusion surface of the endothelium to compensate for inadequate neoangiogenesis. We all know that, nowadays, only few patients suffering from PK are truly and finally cured as there are no valid screening tests, and therefore the possibility of diagnosis continues to rely on the recognition of symptoms. Metastasis represents an early phenomenon, even in patients with small tumors, because the metastatic process is characterized by aggressive tumor growth and cell migration, occurring not only through vascular and lymphatic pathways but also along perineural channels. The knowledge of the biological basis for this dismal behavior is the only way to gain some therapeutic advantages in the near future.

*“Tu ne cede malis, sed contra audentior ito”.*  
Vergilius, *Aeneides*, (70 b.C.n.)

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**Abbreviations** AMF: autocrine motility factor; COX-2: cyclooxygenase-2; CTGF: connective tissue growth factor; *ERK*: extracellular signal-regulated kinase; MMPs: matrix metalloproteinases; NCAM: neural cell adhesion molecule; NGF: nerve growth factor; PGE(2): product prostaglandin E(2); PGI: phosphoglucose isomerase; PK: pancreatic cancer; *si*-RNA: small interfering RNA; TGF $\alpha$ s: transforming growth factor- $\alpha$ s; VEGF; vascular endothelial growth factor

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