Molecular changes in pancreatic cancer : implications for molecular targeting therapy

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Abstract

Pancreatic ductal adenocarcinoma has a high mortality rate, which is generally related to the initial diagnosis coming at late stage disease combined with a lack of effective treatment options. Gemcitabine has been the most commonly used drug over the past decade and is still the cornerstone of therapy in adjuvant and metastatic settings. Intrinsic or acquired resistance of tumours to gemcitabine is, however, a major clinical problem. New therapeutic strategies are urgently needed whereas we also need to identify new prognostic and predictive biomarkers. This article focuses on gemcitabine resistance, on the role of chemokines and chemokine receptors in pancreatic carcinoma initiation and progression, and on stellate cells as partners in crime with neoplastic epithelial cells. (Acta gastroenterol. belg., **2012**, 75, **210-214**).

Key words : pancreas, adenocarcinoma, chemokines, nucleoside transporters, stellate cells, gemcitabine.

Pancreatic ductal adenocarcinoma (PDAC) is by far the most frequent type of pancreatic cancer accounting for approximately 85% of all pancreatic tumours (1). It is the tenth most common type of cancer in Western countries, but due to its aggressive nature, it ranks fourth in cancer mortality statistics (2).

In Belgium, approximately 1100 new cases are diagnosed each year (3). For all stages, survival at five years is less than 5% (4). Due to the high incidence of metastasis at the time of diagnosis, surgery is, however, rarely a viable option (5). In patients with resectable disease, adjuvant chemotherapy more than doubles the 5 year survival rate, from about 10% with surgery alone to around 25% with post-operative chemotherapy (6-8).

Until recently, gemcitabine (2',2'-difluorodeoxycytidine) was the standard treatment for advanced and metastatic pancreatic cancer patients, since it was shown more than a decade ago to induce clinical benefit and to improve survival when compared to weekly bolus 5-fluorouracil (9). In order to improve patients' outcome many trials have, during the last 10 years, explored the pharmacokinetic modulation of gemcitabine and combination therapies with gemcitabine and other anti-cancer agents with consistent negative results. Only recently, results of the ESPAC-3 trial have shown that 5-fluorouracil based chemotherapy is as efficient as gemcitabine (10), whereas adjuvant radiochemotherapy remains controversial, especially in Europe (7,11).

Gemcitabine has a complicated mechanism of action. Cellular resistance to gemcitabine treatment may be an initial property (intrinsic resistance), but can also be acquired during gemcitabine treatment. Several mechanisms have been described, which are related both to its metabolism and to its targets (12).

New therapeutic strategies are urgently needed whereas we also need to identify new prognostic and predictive biomarkers. This article focuses on gemcitabine resistance, on the role of chemokines and chemokine receptors in PDAC initiation and progression, and on stellate cells as partners in crime with neoplastic epithelial cells.

Molecular determinants of gemcitabine benefit and resistance

Gemcitabine exerts its cytotoxic actions primarily by the incorporation of gemcitabine triphosphate into DNA, leading to masked chain termination (13). Gemcitabine is also incorporated into RNA (14). Permeation of gemcitabine through the plasma membrane requires specialised nucleoside transporter proteins ; the major mediators of gemcitabine uptake into human cells appear to be the human equilibrative nucleoside transporter 1 (hENT1) and, to a lesser degree, the human concentrative nucleoside transporter 3 (hCNT3) (Fig. 1).

Cells lacking hENT1 are highly resistant to gemcitabine, and advanced pancreatic cancer patients with hENT1 expression have significantly longer survival after gemcitabine chemotherapy than patients affected by tumours without detectable hENT1 (15,16). Using immunohistochemistry, we found that patients with low tumour hENT1 and hCNT3 expression have shorter disease-free and overall survival times than patients with high expression of these molecules, after treatment with surgery and adjuvant gemcitabine (17). Similarly, high expression of ribonucleotide reductase M1 (RRM1) and of excision repair cross complementation group 1 (ERCC1) protein levels are associated with sensitivity to gemcitabine (18,19).

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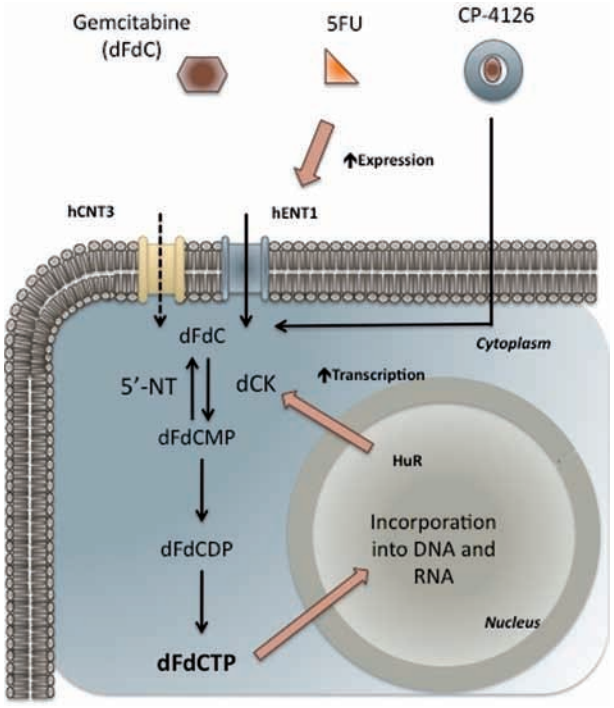

Fig. 1. — Metabolism and mechanisms of action of gemcitabine. dFdC : 2',2'-difluorodeoxycytidine ; 5FU : 5-fluorouracil ; hCNT3 : human concentrative nucleoside transporter 3 ; hENT1 : human equilibrative nucleoside transporter 1 ; 5'-NT : 5'-nucleotidase ; dCK : deoxycytidine kinase ; dFdCMP : difluorodeoxycytidine monophosphate ; dFdCDP : difluorodeoxycytidine diphosphate ; dFdCTP : difluorodeoxycytidine triphosphate ; HuR : Hu antigen R.

Pretreatment with thymidylate synthase inhibitors increases the expression of hENT1, improving the therapeutic benefits of gemcitabine (20). Similar *in vitro* findings with the thymidylate synthase inhibitor 5-fluorouracil (5FU) have led to increased expression of hENT-1, augmenting single agent gemcitabine treatment *in vitro* (21). Another at least partial solution in gemcitabine-resistant PDAC is the facilitation of intracellular entry of gemcitabine through mechanisms not depending on nucleoside transporters, thereby bypassing genetic variability and various grades of hENT1 expression (22). The 5'-elaidic ester of gemcitabine (CP-4126 ; Clavis Pharma ASA, Oslo, Norway) is a fatty acid derivative of gemcitabine that exerts its anti-metabolic action intracellularly but is independent of nucleoside transporters for the intracellular entry in order to exert its cytotoxic effects (23). In contrast to gemcitabine, *in vivo* studies showed that CP-4126 can be administered orally, with a schedule and dose dependent toxicity and antitumour activity (24).

Gemcitabine is a deoxycytidine analog which requires phosphorylation by deoxycytidine kinase (dCK) (Fig. 1). We found that dCK expression was the sole significant prognostic factor in multivariate analysis for both the disease-free survival and overall survival in PDAC patients with an adjuvant gemcitabine-based therapy (25). Deficiency in dCK activity has been associated with intrinsic resistance to gemcitabine (26) and is also common in acquired gemcitabine resistance (27,28). The stress response protein Hu antigen R (HuR), a RNA-binding protein, modulates dCK mRNA expression and is associated with overall survival in resected pancreatic cancer patients who received a gemcitabine-based adjuvant therapy (29,30). Increased HuR levels and activity may facilitate efficient intracellular activation of gemcitabine and confer increased cytotoxicity but, on the other hand, may also facilitate nucleotide synthesis and tumour cell survival (31). Therefore, to prove whether HuR is a predictive assay for benefit from gemcitabine therapy and not simply a marker of prognosis, prospective molecular correlative studies in trials where patients are randomised to either gemcitabine or a non-nucleoside therapy are desirable.

Role of the SDF-1/CXCR4 axis in PDAC and possibility for therapeutic interaction

Chemokines belong to the small molecule chemoattractive cytokine family and are grouped into CXC chemokines and CC chemokines, on the basis of the characteristic presence of four conserved cysteine residues (32-34).

Stromal-derived factor-1 (SDF-1 or CXCL12), a broadly expressed CXC chemokine, was identified as a growth factor for B cell progenitors and a chemotactic factor for T cells and monocytes, and in B-cell lymphopoiesis and bone marrow myelopoiesis (35-37). High levels of SDF-1 are produced in lymph nodes, liver, lung, bone marrow and brain (38) ; these are common sites of PDAC metastases, suggesting that this chemokine accounts for the homing of pancreatic cancer cells to specific organs.

The chemotactic effect of SDF-1 is mediated by the chemokine receptor CXCR4 (36,39), a protein frequently overexpressed on the surface of human tumour cells of epithelial origin (40-42). Most pancreatic cancer cell lines also express CXCR4 ; in such cell lines, SDF-1 not only enhances chemotaxis, transendothelial migration and matrigel invasion, but also stimulates cell proliferation and survival ; moreover, SDF-1 protects pancreatic cancer cells from serum deprivation-induced apoptosis (43-46) (Fig. 2). Most of these effects were abrogated or significantly reduced with the use of an anti-CXCR4 monoclonal antibody (44-46).

Expression of CXCR4 is found in almost all types of tissue-committed stem cells in the body. Indeed, in human, pancreatic cancer tissue contains cancer stem cells defined by CD133 expression that are exclusively tumourigenic and highly resistant to standard chemotherapy. In the invasive front of pancreatic tumours, a distinct subpopulation of $CD133^{+}$ $CXCR4^{+}$ cancer stem cells has been identified, which determines the metastatic phenotype of the individual tumour. Depletion of the

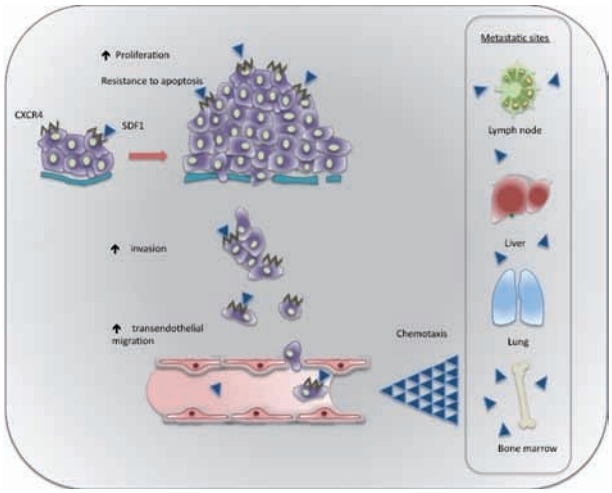

Fig. 2. — The SDF-1/CXCR4 axis in pancreatic ductal adenocarcinoma. Binding of SDF-1 to CXCR4 on cancer cells induces proliferation and resistance to apoptosis, and enhances invasion and transendothelial migration. SDF-1, expressed in lymph nodes, liver, lung, and bone marrow has a chemotactic effect on cancer cells.

cancer stem cell pool for these migrating cancer stem cells significantly reduces the metastatic phenotype of pancreatic tumours without affecting their tumourigenic potential, which indicates that a subpopulation of migrating CD133+ CXCR4+ cancer stem cells is essential for tumour metastasis (47).

SDF-1 also binds another chemokine receptor, CXCR7, which is present on the surface of many different malignant cell types (48), on tumour-associated blood vessels, but not on normal vasculature (49). CXCR7 promotes the survival of tumour cells by preventing apoptosis, increased adhesion properties and dissemination, but does not mediate chemotaxis towards SDF-1 (48). CXCR7 has been detected in pancreatic tissue and PDAC cell lines, and might be implicated in the pathogenesis of PDAC by a proliferative effect on tumour cells and/or stimulating cancer cells dissemination (50,51).

By using tissue microarray and immunohistochemistry, we showed in two series of resected PDAC that CXCR4 expression is an independent and strong worse prognostic marker for overall survival (51,52). No association was found between CXCR7 expression and the patients' outcome (51).

Nowadays concordant and accumulative data suggest that an autocrine/paracrine loop involving the SDF-1/CXCR4 axis may play a major role in PDAC pathogenesis, and CXCR4 appears as an attractive target for therapeutic options. Small molecule CXCR4 anatagonists are currently being tested in phase I/II clinical trials and could be attractive therapeutic candidates to combine with gemcitabine. If confirmed in larger prospective series, CXCR4 immunohistochemistry may assist clinicians to select those patients who require adjuvant treatment.

Stellate cells : partners in crime with neoplastic epithelial cells

The majority of PDACs is characterized by a desmoplastic/stromal reaction consisting of abundant fibrous tissue with extracellular matrix proteins, new blood vessels, and stromal cells (53,54). The cells responsible for the production of the desmoplastic reaction in PDAC are pancreatic stellate cells (PSCs), which are now recognised as key cells in pancreatic fibrogenesis (54-56).

In health, PSCs exist in a quiescent state (Fig. 3a). In diseased states, under the influence of growth factors, cytokines, and oxidant stress, PSCs transform into a myofibroblast-like (α-smooth muscle actin positive) phenotype secreting excess amounts of extracellular matrix as well as matrix degrading enzymes (57,58). Pancreatic cancer cells secrete growth factors such as transforming growth factor-β1 (TGF-β1), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF), all known to induce PSC activation (59). On the other hand, PSCs secrete various factors, including PDGF, stromal-derived factor 1 (SDF-1), and epidermal growth factor (EGF), that mediate effects on tumour growth, invasion, angiogenesis, metastasis and resistance to chemotherapy (58,60) (Fig. 3b). Moreover, recently it has been shown that PSCs also promote radioprotection (61) and epithelial-mesenchymal transition in pancreatic cancer cells (62).

Interestingly, in an orthotopic model of pancreatic cancer human PSCs have been shown in metastatic nodules in the liver of nude mice, suggesting that PSCs might comigrate with cancer cells to distant sites and perhaps aid in the implantation of tumour in the new microenvironment (63) (Fig. 3b). These observation – based on serial sections of liver metastatic nodules stained with antibodies directed against human nuclear antigen and α-smooth muscle actin – implies significant cell-cell interactions between the two cell types. No direct interaction of tumour integrins with adhesion molecules on the surface of PSCs has, however, been reported so far.

The observed growth advantage of pancreatic cancer cells in the presence of PSCs may be mediated by two mechanisms – increased mitosis and decreased programmed cell death (apoptosis) (59). Anyway, since activated PSCs are key players in PDAC promotion and progression, therapeutic targeting of these pathways may provide new avenues for antifibrotic and antineoplastic therapies.

Signaling by PDGF, the most potent mitogenic stimulus for PSC, is one therapeutic approach that has been studied. Administration of the PDGF inhibitor trapidil was found to suppress PDGF-induced ERK activation, leading to decreased PSC proliferation (64). Similarly, treatment with curcumin (deferuloylmethane) results in

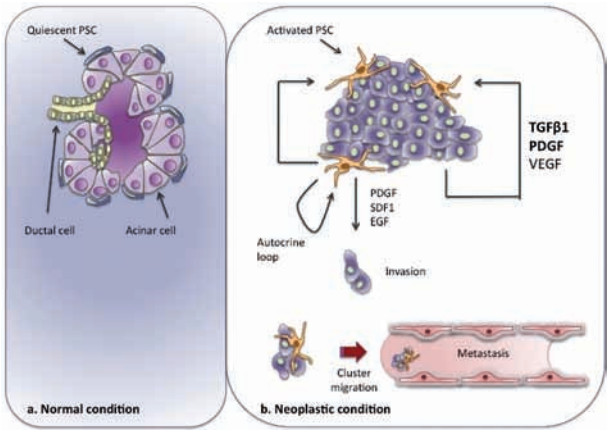

Fig. 3. — Role of pancreatic stellate cells in adenocarcinoma. a. Under normal conditions, pancreatic stellate cells (PSCs) are found around acinar cells. b. Pancreatic cancer cells secrete growth factors that induce PSC activation. On the other hand, PSCs secrete various factors that mediate effects on tumour growth, invasion, and metastasis. PSCs might comigrate with cancer cells to distant sites and perhaps aid in the implantation of tumour in the new microenvironment.

decreased PDGF-induced proliferation of PSCs *in vitro*, along with reduction in α-smooth muscle actin expression (65).

Another potential strategy is to target TGF-β, a major fibrogenic cytokine and activator of PSCs. Although the role of TGF-β□n PDAC is complex, a study examining the effects of SMAD7, an intracellular inhibitor of TGF-β□ignaling, using a transgenic mouse model reported antifibrotic activity and reduced PSC activation (66). Future studies should, however, focus on selective inhibitors that do not involve the tumour-suppressive effects of TGF-β.

Conclusions and prospects

PDAC is an almost universally lethal disease and despite extensive research over the last decades, this has not changed significantly. Nevertheless, much progress has been made in understanding gemcitabine resistance, the role of chemokines, and interactions between pancreatic cancer cells and PSCs, suggesting that different therapeutic strategies based on these new insights are forthcoming. Increasing focus exists on designing the so-called targeted treatment strategies in which the molecular characteristics of a tumour guide therapy. It seems logical that over the next few years multiple small steps, hopefully adding up to significant progress, will be taken on the road to targeted treatment of PDAC.

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