Pancreatic cancer: Translational research aspects and clinical implications

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Abstract
Despite improvements in surgical techniques and adjuvant chemotherapy, the overall mortality rates in pancreatic cancer have generally remained relatively unchanged and the 5-year survival rate is actually below 2%. This paper will address the importance of achieving an early diagnosis and identifying markers for prognosis and response to therapy such as genes, proteins, microRNAs or epigenetic modifications. However, there are still major hurdles when translating investigational biomarkers into routine clinical practice. Furthermore, novel ways of secondary screening in high-risk individuals, such as artificial neural networks and modern imaging, will be discussed. Drug resistance is ubiquitous in pancreatic cancer. Several mechanisms of drug resistance have already been revealed, including human equilibrative nucleoside transporter-1 status, multidrug resistance proteins, aberrant signaling pathways, microRNAs, stromal influence, epithelial-mesenchymal transition-type cells and recently the presence of cancer stem cells/cancer-initiating cells. These factors must be considered when developing more customized types of intervention ("personalized medicine"). In the future, multifunctional nanoparticles that combine a specific targeting agent, an imaging probe, a cell-penetrating agent, a biocompatible polymer and an anti-cancer drug may become valuable for the management of patients with pancreatic cancer.

INTRODUCTION
Pancreatic cancer has an approximate incidence of 11.4/100,000 inhabitants per year, and is recognized as the fourth cause of cancer-related death, with an overall 5-year survival of less than 1%-2%[1-3]. Total costs, including care-related costs and loss of production (due especially to premature death) related to pancreatic cancer in Sweden in the year 2009 were 86-93 million euros (population 9.1 million), corresponding to a society cost in the West of up to 10 million euros per 1 million inhabitants per year[4]. Smoking and also family history (in about 5%-10% of cases) are established risk factors for the development of pancreatic cancer[5]. There is a weaker positive associa-
tion for other factors including obesity, diabetes mellitus, chronic pancreatitis, ABO genotype, race, periodontal disease, occupational exposures, dietary factors, *Helicobacter pylori* and gallstones[8,9]. It is to be stated that the median age at diagnosis is in general 66-68 years[7], though early onset pancreatic cancer, i.e., occurring prior to 50 years of age, accounts for less than 6% of patients and is associated with more advanced disease at presentation and a tendency for shorter overall survival[6]. Gender-specific differences in the incidence of pancreatic cancer have been observed, including higher rates in males[6].

Chemotherapy and to a lesser extent, radiotherapy, have emerged as valuable adjuncts to the management of pancreatic cancer. A few studies reported that "marginally resectable" pancreatic tumors shrink after radiochemotherapy and may become resectable[10-12]. Neoadjuvant treatment of resectable pancreatic cancer is associated with fewer positive lymph nodes and increased survival (median 34 mo vs 19 mo, *P = 0.03*)[13]. In the ESPAC-1 study, 6 mo of postoperative 5-fluorouracil (5-FU) and folinic acid (FA) increased median survival from 14 mo to 19.7 mo, but there was no effect provided by radiochemotherapy[14]. Long-term follow-up after adjuvant chemotherapy demonstrated even better results with a median 21-23 mo survival following adjuvant chemotherapy vs 8-16 mo for observation[15,16]. The validity of gemcitabine as an adjuvant agent has been confirmed[17]. The ESPAC-3 study reported similar outcomes between 5-FU and FA vs gemcitabine (*n = 1088*)[18]. In unresectable pancreatic cancer, most regimens are also gemcitabine-based. The use of gemcitabine has increased median survival from 3-4 mo to 5.5-7 mo[19,20]. Recently, FOLFIRINOX (oxaliplatin, irinotecan, leucovorin, fluorouracil) surpassed the effectiveness of gemcitabine by showing longer survival (11.1 mo vs 6.8 mo; *P < 0.001*)[21]. The utilization of molecular targeted treatment in pancreatic cancer outside of clinical trials has been limited. Erlotinib provided a modest survival benefit in advanced pancreatic cancer showing other cells to be the cancer-initiating cells. Differenced acinar cells have been shown to cause PanIN and pancreatic cancer following activation of KRAS in *vivo*[22-24]. Moreover, insulin-positive endocrine cells and PDX1-expressing cells have been demonstrated to induce PDAC[25]. It should be noted that the cell of origin, in which tumorigenesis is initiated, could be different from the cancer stem cell, which propagates the tumor[26]. Identification of cells of origin in PDAC may allow earlier detection of malignancy and better preventive and treatment tools.

**Crosstalk tumor-stroma**

Desmoplasia is a characteristic feature of pancreatic cancer and the stromal compartment has been considered to be a physical barrier for drug delivery[27]. The pancreatic stellate cell (PSC) has a key role in stroma formation. In addition to endogenous quiescent PSCs, bone marrow may also contribute to the population of activated PSCs[28]. PSCs are involved in tumor growth, invasion, metastasis and resistance to radiochemotherapy[29,30]. Furthermore, PSCs accompany cancer cells to distant metastatic sites, stimulate angiogenesis and have the capacity to migrate over the endothelial barrier to and from blood vessels[31]. A limited number of studies have attempted to block PSC activity in the setting of pancreatic cancer. For example, halofuginone, a smad3-phosphorylation-inhibitor, reduces PSC activation and prevents pancreatic xenograft tumor development[32]. Retinoic acid can also inhibit PSC activity and reduces wnt-β-catenin signaling in cancer cells and their invasive ability[33]. Key signaling pathways between PSCs and cancer cells have been identified and involve e.g., sonic hedgehog, galecints, endothelins and platelet-derived growth factor[34], which thereby represent potential therapeutic targets.

**Cancer stem cells and epithelial-mesenchymal transition**

Pancreatic cancer stem cells constitute a minority of cancer cells (1%-5%) and have the ability to self-renew, and of the tumor-suppressor genes *CDKN2A, TP53, DPC4* and *BRCA2*, as well as chromosomal losses, gene amplifications and telomere shortening have also been observed[35]. Reactivation of developmental pathways, such as hedgehog, notch and wnt/β-catenin, may be crucial for the development of PDAC[23]. In addition to genetic alterations, many lines of evidence indicate that epigenetic changes play a role in pancreatic carcinogenesis. DNA methylation and histone modification frequently alter gene function without changing the DNA sequence, and have the potential to be used as diagnostic markers in pancreatic cancer[26]. MicroRNAs are non-coding segments of RNA that can regulate gene expression. Aberrant expression of microRNAs contributes to tumor progression and has been associated with drug resistance[36].

Because all three precursor lesions of PDAC possess ductal characteristics, it has been suggested that the lesions develop from ductal cells. However, the study of mouse models of pancreatic cancer has broadened the current understanding of pancreatic carcinogenesis by showing other cells to be the cancer-initiating cells. Differentiated acinar cells have been shown to cause PanIN and pancreatic cancer following activation of KRAS in *vivo*[22-24]. Moreover, insulin-positive endocrine cells and PDX1-expressing cells have been demonstrated to induce PDAC[25]. It should be noted that the cell of origin, in which tumorigenesis is initiated, could be different from the cancer stem cell, which propagates the tumor[32]. Identification of cells of origin in PDAC may allow earlier detection of malignancy and better preventive and treatment tools.
are resistant to chemotherapy and radiation\[43\]. They are characterized by several surface markers including CD44, CD24, epithelial specific antigen, aldehyde dehydrogenase, CD133 and CXCR4\[48\]. Furthermore, it has been observed that pancreatic cancer cells that were cultured in gemcitabine demonstrated characteristics of epithelial-mesenchymal transition (EMT)\[43\]. They also showed increased expression of cell surface proteins associated with cancer stem cells. In pancreatic cancer xenografts, radiation or gemcitabine therapy leads to enrichment of the EMT cells\[44\]. Wnt, notch and hedgehog are important signaling events in cancer stem cells, and can become novel therapeutic targets\[41\]. Ongoing clinical trials are currently investigating PRI-724 (inhibitor of wnt), MK-0752 (inhibitor of notch) and GDC-0449 (inhibitor of hedgehog) in patients with advanced pancreatic cancer (www.clinicaltrials.gov). Future therapeutic strategies may need to combine targeting of cancer stem cells and EMT cells with the targeting of other cells in the microenvironment, e.g., stromal cells, in order to achieve maximal benefit.

**Pro-inflammatory response**

Inflammation is closely related to the development and progression of pancreatic cancer, and molecular factors such as STAT3 have been suggested to play a key role in creating a pro-inflammatory tumor microenvironment\[48\]. Clinical studies have shown that a pro-inflammatory response is both prognostically negative and promotes tumor proliferation\[46\]. Inflammatory factors may also contribute to the profound weight loss and cancer cachexia frequently seen in pancreatic cancer\[45\]. Elucidation of the mechanisms underlying the crosstalk between inflammation, cancer and stroma may improve the management of pancreatic cancer, as a frequent desmoplastic reaction is noted.

**Chemoresistance**

Gemcitabine has represented the first-line of chemotherapeutic agents in pancreatic cancer. A frequent problem, though, is drug resistance and lack of response to therapy given. Nucleoside transporters, such as human equilibrative nucleoside transporter-1 (hENT-1), appear to regulate the intracellular uptake of gemcitabine\[48\]. One of the proposed mechanisms of chemoresistance is a reduction in hENT-1 expression. Determination of hENT-1 status at the time of cancer diagnosis, and also modifications of gemcitabine in order to bypass the nucleoside receptor, may represent novel types of targeted approaches in the management of patients with pancreatic cancer\[48\]. Multidrug resistance (MDR) proteins including ABC-transporters have also been implicated in drug resistance in pancreatic cancer and limit the efficacy of gemcitabine\[49\]. Another mechanism that contributes to chemoresistance is the tumor microenvironment surrounding the cancer cells, including cancer stem cells, EMT cells and stellate cells. Furthermore, the hypoxic stroma could be a physical barrier preventing chemotherapeutic drugs from reaching pancreatic cancer cells, and depletion of the stroma could enhance cancer drug delivery\[53\]. Aberrant signaling pathways also have a role in drug resistance. The PI3K/Akt signaling pathway is commonly overactive in pancreatic cancer. PI3K stimulates proliferation and confers chemoresistance\[50\]. MicroRNAs have received increased attention in recent years. Targeting of microRNAs may help overcome drug resistance in pancreatic cancer and improve clinical outcome\[27\].

**BIOMARKERS**

Biomarkers can be applied in several areas of disease management including diagnosis, prognosis, staging and prediction and monitoring of therapeutic response. The different types of biomarkers include genes, proteins, metabolites, microRNAs and epigenetic modifications. CA 19-9 has some value for detection of recurrent disease\[51\], but so far no other biomarker is recommended for routine clinical use in pancreatic cancer. Recently, a seven-gene panel was identified as being differentially expressed between pancreatic cancer ($n = 36$) and normal samples ($n = 19$)\[52\]. Validation using two blood-based biomarkers from this panel, tenascin C and tissue factor pathway inhibitor, yielded a combined area under the curve (AUC) of 0.88 and, with addition of CA19-9, a combined AUC for the three-gene panel of 0.99 with 100% specificity at 90% sensitivity and 97% sensitivity at 90% specificity.

Proteomic profiling of pancreatic cancer serum has been promising. Most studies have used surface enhanced laser desorption (SELDI) or matrix assisted laser desorption/ionization (MALDI) yielding a sensitivity in the range of 78% to 100% and a specificity between 74% and 100%\[53\]. Immunohistochemistry (IHC) is the most practical method for evaluating protein expression changes in histopathology. It can be combined with tissue microarray technology to allow rapid testing of immunohistochemical markers on many tumors in a single experiment. During the past decade, a multitude of immunohistochemical biomarkers that are potentially involved in pancreatic carcinogenesis and drug responsiveness have been studied for their prognostic and predictive value, but none of them have yet proved to be sufficiently useful for use in routine clinical practice\[54\]. Apart from the tumor compartment, stromal tissue may also be analyzed and it has been discovered that stromal secreted protein acidic and rich in cysteine has been associated with outcome in pancreatic cancer\[55\]. A panel of IHC markers may prove clinically valuable in the future. Furthermore, metabolomic studies of pancreatic cancer are promising and may be useful in identifying benign from malignant conditions\[56-58\]. MicroRNA is a new class of biomarkers. Aberrant expression of miRNA-21 and miRNA-34a has been associated with survival in resectable pancreatic cancer\[59\]. Epigenetic changes, such as histone modification, may be used as novel biomarkers in pancreatic cancer\[60\].

Although a multitude of investigational biomarkers have been identified, translation into routine clinical
practice has been difficult. To improve methodological reporting several guidelines have been developed. For diagnostic biomarkers, the STAndards for Reporting Diagnostic accuracy (STARD) guidelines are available. For prognostic studies, the REporting recommendations for tumor MARKer prognostic studies (REMARK) guidelines are available. The process of translating biomarkers is complex and the path from discovery to clinical application may be long and arduous. The effective demonstration of clinical utility of the biomarker will remain the key to its gaining widespread acceptance, but regulatory issues and budgetary constraints of the biomarker industry remain major challenges.

**IMAGING**

The detection of precursor lesions of pancreatic cancer would be a key factor in improving the prognosis. Non-invasive imaging techniques such as ultrasound, computed tomography and magnetic resonance imaging do not accurately identify PanINs. Positron emission tomography (PET) is a functional imaging modality that utilizes the principle that metabolic alterations in tumors occur prior to notable morphological alterations. The radioactive tracer $^{18}F$-fluorodeoxyglucose (FDG) has been used extensively for PET imaging of malignant tumors. Malignant tissue has increased glucose metabolism as compared to its surrounding tissue, which leads to focal FDG-uptake visualized by PET. PET/CT has come to play an increasing role in pancreatic cancer, due to the ability to accurately detect small primary pancreatic lesions and distant metastases, as well as recurrences following surgery. It has been shown that an elevated glucose metabolism occurs already in precursor lesions of pancreatic cancer, with the opportunity of detecting these changes with PET/CT, and thus improving diagnosis and outcome. Eser et al. recently described a technique that could improve diagnosis and also grading of PanINs using in vivo molecular imaging based on cathepsins.

**PANCREATIC CANCER AND DIABETES MELLITUS**

Up to 80% of patients with pancreatic cancer have diabetes mellitus or pathologic glucose tolerance test at diagnosis. Long-standing type II diabetes is a predisposing factor for pancreatic cancer, while new-onset diabetes may indicate subclinical cancer. The molecular mechanisms linking long-standing diabetes to pancreatic cancer are incompletely understood. Diabetes may promote the neoplastic process by several mechanisms including hyperinsulinemia (endogenous or exogenous), hyperglycemia and chronic inflammation. The insulin and insulin-like growth factor (IGF) receptors are frequently expressed in pancreatic cancer and contribute to neoplastic growth and progression. The administration of the anti-diabetic agent metformin may reduce the incidence of pancreatic cancer in patients with type II diabetes. In xenograft models, metformin inhibits the growth of pancreatic cancer cells by interfering with insulin/IGF-1 receptor and G-protein-coupled receptor signaling. In addition, metformin can inhibit tumor growth by inactivating cancer stem cell-like cells. Studies have sought to elucidate molecular alterations that link diabetes and cancer, and one such molecular connection could be TCF7L2 (T-cell factor 7-like 2) and p53. In a recently published case-control study, rs780094 was selected as one of 10 diabetes-associated single-nucleotide polymorphisms related to increased pancreatic cancer risk. However, diabetes in pancreatic cancer is mostly new-onset, i.e., occurring 24 mo prior to cancer diagnosis, and is likely related to secondary effects from the tumor, which is supported by the observation that glucose metabolism is improved following tumor resection. Although the exact mechanisms behind pancreatic cancer-induced diabetes are yet to be disclosed, there is ample evidence for a tumor-derived influence on glucose metabolism, leading to disturbed β-cell function, peripheral insulin resistance, hyperglycemia and finally diabetes mellitus.

**SCREENING**

Pancreatic cancer develops over a long time span, providing a strong rationale for developing techniques for early detection. It may take ten years or more between the initial mutation and first non-metastatic tumor cell, and another five years for the development of metastatic capacity and death after an additional two years. This implies a therapeutic window of opportunity for both early diagnosis and treatment. Chromothripsis is a new concept that involves the simultaneous acquisition of multiple mutations in a single catastrophic event. This phenomenon may be present in 2%-3% of all human cancers, but the incidence may be higher in certain tumors, such as osteosarcomas and chordomas.

Patients with pancreatic cancer usually have generic symptoms and are often difficult to diagnose at an early stage. There are several risk groups where secondary screening for pancreatic cancer may be appropriate, e.g., patients with heredity, IPMN, or new-onset diabetes mellitus. Distinguishing pancreatic cancer-associated diabetes from the more common general type 2 diabetes may identify individuals with a potentially resectable pancreatic cancer. Huang et al. identified vanin-1 and matrix metalloproteinase 9 as useful biomarkers for the discrimination of pancreatic cancer-associated diabetes from type II diabetes.

Artificial neural networks represent non-linear pattern recognition techniques that simulate the analytic processes of the human brain. They have been utilized in complex medical decision-making, including diagnosis, prognosis and risk stratification. A major benefit of these networks is the ability to recognize complex relationships between input and output data that may be hidden to conventional statistical methods. Initial reports on the use of artificial neural networks combined with proteomic
data have provided promising results concerning the detection of pancreatic cancer\(^{[68]}\). The future application of artificial neural networks based on parameters including age, smoking, heredity, chronic pancreatitis, new-onset diabetes mellitus, biomarkers and imaging findings imply promise for early detection of pancreatic cancer, and may be used as screening tools.

**NANOMEDICINE**

Nanomedicine is defined as the application of nanotechnology to medicine. Nanoparticles are in the range of 1-100 nm. Examples of nanoparticles include liposomes (phospholipid vesicles), dendrimers (synthetic polymers), carbon nanotubes (fullerene), quantum dots (colloidal fluorescent semiconductor nanocrystals), magnetic nanoparticles (spherical nanocrystals with a Fe\(^{3+}\) and Fe\(^{3+}\) core) and gold nanoparticles (metallic nanoparticles). The application of nanoparticles in medicine include e.g., diagnostics, imaging and drug delivery.

Nanoparticles enable refined diagnostics at the level of single cells and molecules. For example, magnetic nanoparticles have been coupled with molecular targeting ligands to improve imaging of early pancreatic tumors in vivo\(^{[69]}\). Quantum dots conjugated with RGD peptides have been reported for in vivo imaging of pancreatic tumor vasculature\(^{[66]}\). Drug resistance is a recognized challenge in pancreatic cancer. Gemcitabine-squalene obtained by covalently coupling gemcitabine at the 4-amino group with squalene, a natural lipid, has been shown to make tumor cells more sensitive to gemcitabine\(^{[91]}\). Recently, polymeric nanoparticles encapsulating hedgehog-inhibitors or curcumin have been produced that inhibit the growth of orthotopic pancreatic cancer xenografts\(^{[63,90]}\). Gold nanoparticles have been utilized to induce intracellular hyperthermia in a murine model of pancreatic cancer after radiofrequency field exposure\(^{[84]}\). An ongoing phase I study (NCT00968604) of advanced pancreatic cancer is currently investigating the effects of intravenous injection of the liposome nanoparticle BikDD, which contains a pro-apoptotic agent. Several nanoparticle-based anticancer drugs are already on the market, e.g., Abraxane\(^{®}\) (albumin-bound paclitaxel), Myocet\(^{®}\) (liposomal doxorubicin) and Oncaspar\(^{®}\) (PEG-L-asparaginase)\(^{[88]}\).

While monofunctional nanoparticles only carry out one function, multifunctional nanoparticles have the ability to perform several tasks. Multifunctional nanocarriers using a specific targeting agent, an imaging probe, a cell-penetrating agent such as TAT peptide, a biocompatible polymer such as polyethylene glycol (PEG) and an anticancer drug, may result in effective tumor destruction with minimal toxicity (Figure 1).

**CONCLUSION**

Pancreatic cancer is a condition with an almost total lethal outcome. Despite advancement in surgical techniques and adjuvant treatment, the prognosis has only marginally improved. Novel therapeutic interventions have been tested but with limited effect. Research should continue to focus on biomarkers for early diagnosis, prognosis and prediction and monitoring of therapeutic response. Screening of high-risk individuals using novel approaches such as artificial neural networks could be considered. Mechanisms of chemoresistance have been elucidated, including hENT-1 status, MDR proteins, aberrant signaling pathways, microRNAs and micro-environmental factors, which should underlie future development of targeted therapy. The identification of cancer-initiating cells represents a fundamental shift in our understanding of the intrinsic drug resistance of pancreatic cancer. Multifunctional nanoparticles have the potential to combine imaging, diagnosis and therapy in a single vehicle. It is expected that nanomedicine will have a prominent role in the quest for a successful therapy for this recalcitrant disease.

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![Figure 1 Multifunctional nanoparticle. PEG: Polyethylene glycol.](image)
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