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## Mini-Review

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# Pancreatic Cancer: Pathobiology, Treatment Options, and Drug Delivery

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**Abstract.** Pancreatic cancer is the fourth leading cause of cancer-related deaths in the USA. The high mortality rate is partly due to lack of effective treatments. This review summarizes the pathobiology and current treatment options for pancreatic cancer. Moreover, the review discusses the opportunities of developing novel therapies for pancreatic cancer provided by the progress in understanding the genetic mutations, tumor microenvironment, cancer stem cells, and drug delivery.

**KEY WORDS:** Cancer stem cells; Drug delivery; Genetic profile; Microenvironment; Pancreatic cancer.

## INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer-related deaths in the USA. In spite of the recent advances in screening, operation, chemotherapy, and radiotherapy, there has been little improvement in the survival of pancreatic cancer patients. The expected incidence of pancreatic cancer in 2009 is 42,470 cases with 35,240 deaths (1). In the USA, the age-adjusted incidence of pancreatic cancer is higher in African Americans than in Caucasians, and it is higher in men than in women. A number of risk factors have been identified, such as age, cigarette smoking, family history, and medical conditions including gestational diabetes, pancreatitis, and diabetes mellitus (2). At the time of diagnosis, 10% to 20% of patients have tumors localized in pancreas, and 40% of patients have locally advanced cancer with tumors extending to adjacent organs. About 40% to 50% of patients have metastatic pancreatic cancer, a rapidly progressing, debilitating disease, characterized by pain, asthenia, anorexia, and cachexia (3). The prognosis for patients with pancreatic cancer is poor, and the 5-year survival rate is less than 5%. The factors contributing to the high mortality rate of pancreatic cancer include lack of early detection methods due to absence of symptoms and effective screening tests, high rate of relapse, and limited effective therapies.

There are several excellent reviews on the clinical experiences and molecular pathogenesis of pancreatic cancer (4–6). The goal of the present review is to capture the key aspects of these diverse subject matters, in order to highlight the unmet needs and the areas ripe for translational research on developing novel therapies. Part 1 of this review summarizes the pathobiology and current treatment options. Part 2 outlines the progress in understanding the genetic mutations, tumor microenvironments, cancer stem cells, and barriers to drug delivery in pancreatic cancer and the emerging research opportunities.

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## PART 1. CURRENT PANCREATIC CANCER THERAPY

Pancreatic cancer is divided into two types according to the origin of tumors, exocrine and endocrine. Endocrine pancreatic cancer, originating from islet cells that make multiple hormones including insulin, accounts for only 2–4% of the incidence. Standard treatment options for endocrine pancreas cancer include surgery, chemotherapy, and hormone therapy; the median survival time is between 2 and 3 years. The prognosis depends on the type of islet cell cancer, the extent of metastasis, and the overall health of patients, whereas factors such as age, sex, and tumor type do not impact on prognosis (7).

About 95% of pancreatic cancer originates in the exocrine pancreas. Infiltrating ductal adenocarcinoma is by far the most common form of malignant pancreatic cancer. More than 80% of these carcinomas are unresectable at the time of diagnosis due to metastasis into adjacent or distant organs, including liver and lung (8). Approximately 19% of pancreatic cancer patients survive 1 year after diagnosis and 4% for 5 years (9), making this disease the most lethal cancer. Treatment options for pancreatic cancer vary with the disease stage and the general health of a patient. The American Joint Committee on Cancer classifies pancreatic cancer into four stages, I through IV, depending on the extent of spreading. For treatment purposes, pancreatic tumors are generally classified as resectable, unresectable locally advanced, or metastatic, each with different treatment options.

### Therapies for Resectable Disease

About 20% of pancreatic cancers are detected at the resectable stage. For these patients, pancreaticoduodenectomy (surgical removal of the pancreatic head, duodenum, gallbladder, and bile duct), with or without the gastric antrum, is the standard of care (10). Due to the high recurrence rate after surgery, adjuvant therapy is advocated and postoperative/preoperative adjuvant therapy has been

used. In the USA, chemoradiotherapy followed by chemotherapy is considered the optimal adjuvant therapy (11). Five randomized phase III trials completed to date involving over 1,600 patients have collectively shown that combinations of 5-fluorouracil or gemcitabine with radiation prolonged the overall survival or disease-free survival intervals and increased the 2-year survival rate from less than 20% to 30–40% even though the median survival time remains at less than 2 years (4).

### Therapies for Locally Advanced Disease

For locally advanced pancreatic cancer, which includes local spread or encasement of critical vascular structures, surgical resection is not an option. There is no consensus on treatment options due to the inconsistent results of clinical trials with chemotherapy, radiotherapy, or their combination. The common practice is 5-fluorouracil-based chemoradiation. This is based on the results of a Gastrointestinal Tumor Study Group trial, showing that chemoradiation following surgery significantly prolonged the median survival time from 11 to 20 months compared to no adjuvant therapy (12). A recent review evaluated the quality and clinical relevance of 21 phase III trials using overall survival time as the primary endpoint and time-to-progression, response rate, toxicities as the secondary endpoints; gemcitabine- and 5-fluorouracil-based chemoradiotherapies were identified as the two main treatment options (13).

### Therapies for Metastatic Advanced Disease

The goal of systemic chemotherapy is to provide symptomatic relief and prolong survival. Metastatic pancreatic tumors are highly chemoresistant; response rates to multiple classes of agents (i.e., antimetabolites, alkylating agents, antibiotics, and anthracyclines), used as single agent and in combination therapy, are less than 20% (9). Gemcitabine has replaced 5-fluorouracil as the standard treatment for metastatic pancreatic cancer due to the longer overall survival time and apparent clinical improvements in the three common debilitating symptoms of pain, functional impairment, and weight loss (14). However, gemcitabine offers only a moderate 6-week increase in median survival, from about 4.5 to about 6 months. Table I summarizes the past and ongoing clinical trials on combining gemcitabine with other chemotherapeutic agents, i.e., antimetabolite (fluorouracil, pemetrexed), topoisomerase I inhibitors (irinotecan, exatecan), platinum (cisplatin, oxaliplatin), and taxanes (paclitaxel, docetaxel). A phase III trial showed that adding oxaliplatin to gemcitabine improved the response rate and progression-free survival and provided clinical benefits but failed to demonstrate a longer overall survival (15). A double-blind phase III trial showed that adding erlotinib (epidermal growth factor receptor 1 (EGFR1) inhibitor) to gemcitabine gives a 1-week-longer overall survival (median 6.24 vs 5.91 months) and a higher 1-year survival rate (23% vs 17%) (16). In spite of these recent advances, patients with metastatic pancreatic cancer continue to have bleak prognosis with a median overall survival time of 3–6 months. The lack of effective treatments remains one of the greatest challenges in clinical oncology, and there is an urgent need to develop efficacious treatments.

Historically, drug development efforts in pancreatic cancer have lagged behind other types of cancer, in part because it is a relatively minor cancer and is highly resistant to cytotoxic agents or molecular targeted agents. Several favorable changes over the last decades, including the explosion of knowledge in the pathogenesis and tumor biology of this disease and the general shift of research emphasis to translational research, have the potential of yielding novel therapeutic approaches.

The remainder of this review provides an overview of the progress in understanding the genetic mutations, tumor microenvironments, cancer stem cells, and barriers to drug delivery in pancreatic cancer and further discusses the pharmaceutical research opportunities offered by these advances.

## PART 2. PHARMACEUTICAL RESEARCH OPPORTUNITIES

### Genetic Profiles of Pancreatic Cancer

*Oncogenes and Tumor Suppressor Genes.* Pancreatic cancer is a genetic disease caused by multiple inherited and somatic genetic mutations accumulated over many years. The earliest recognizable genetic defect is the shortening of telomeres, leading to instability of chromosomes; fluorescence *in situ* hybridization results showed shortened telomeres in 96% of 82 pancreatic intraepithelial neoplasia samples (17). Defects of Fanconi/BRCA2 pathway also lead to genetic instabilities. The BRCA2 protein, *via* its interaction with the recombinase and the DNA repair protein RAD51, is responsible for maintaining genomic integrity by repairing DNA double-strand breaks (18). Germ line mutations in the BRCA2 gene are present in 17% and 5% of patients with familial and sporadic pancreatic cancer, respectively (19).

The genes mutated in pancreatic cancer include Kras, p16, p53, and SMAD4/DPC4. At least three of these genetic alterations are simultaneously present in >75% of all pancreatic cancers (20). Mutations that activate Kras oncogene and loss of the p16/CDKN2A suppressor gene represent the most common genetic abnormality and are present in about 90% of pancreatic cancer (21,22). Activated Kras is considered one of the earliest genetic abnormalities in tumor progression and engages several downstream effector pathways including RAF-mitogen-activated protein kinase, phosphoinositide-3-kinase, and RalGDS, all of which play critical roles in pancreatic carcinogenesis, cell proliferation, and survival (6). p16 is involved in cell cycle progression as an inhibitor of cyclin D/CDK4-6 kinase complex and plays an important role in sporadic pancreatic cancer (22). Inactivation of p53 gene, present in about 50–75% of pancreatic cancers, allows cells to proceed in division in the presence of damaged DNA, thereby leading to accumulation of additional genetic abnormalities. Tumor suppressor gene DPC4 is a mediator of the growth inhibitory effect of TGF- $\beta$  and a positive regulator of angiogenesis inhibitor TSP-1 causing tumor growth suppression (23). Inactivation of DPC4 (SMAD4) is found in up to 55% of pancreatic cancer (24).

Table II summarizes the origins and the mutated oncogenes and tumor suppressor genes in the commonly used pancreatic cancer cell lines (25–27). Among these nine

**Table I.** Chemotherapeutic Agents Used in Pancreatic Cancer Clinical Trials

Mechanisms or categories	Agents	Therapeutic approaches and disease targets	Reference	
Antimetabolite	Gemcitabine	Neoadjuvant chemotherapy: combined with other chemotherapeutic agents	(14,16,86–98)	
		Locally advanced disease: combined with radiation, other chemotherapeutic agents		
		Metastatic disease: single agent (standard); combined with 5-fluorouracil, topoisomerase I inhibitors, platinum, taxanes, or molecularly targeted agents		
		Capecitabine	Locally advanced or metastatic disease: combined with chemotherapeutic agents and radiation	(88,92,99,100)
		s-1	Locally advanced or metastatic pancreatic cancer, combined with radiotherapy or chemotherapy	(101,102)
		5-Fluorouracil	Adjuvant therapy: single agent or in combination with other chemotherapeutic agents	(12,90,103–105)
			Locally advanced disease: combined with radiation	
		Tegafur-uracil (UFT)	Metastatic disease: single agent or combined with gemcitabine	(97)
		Leucovorin	Resected pancreatic cancer: combined with gemcitabine	(106)
		Pemetrexed	Adjuvant therapy: combined with other chemotherapy	(107)
Platinums	Cisplatin	Metastatic disease: combined with gemcitabine	(90,91,93,102,108)	
		Neoadjuvant chemotherapy: combined with gemcitabine; Adjuvant therapy: combined with other chemotherapy		
		Metastatic disease: combined with gemcitabine		
	Oxaliplatin	Metastatic disease: combined with gemcitabine; second line for advanced disease	(15,100)	
Taxanes	Paclitaxel	Metastatic disease: combined with gemcitabine or other chemotherapeutic agents	(109,110)	
Antibiotics	Docetaxel	Metastatic disease: single agent or combined with gemcitabine	(111,112)	
	Doxorubicin	Adjuvant therapy: combined with other chemotherapy	(86)	
	Mitomycin C	Adjuvant therapy: combined with other chemotherapy	(86)	
Topo-I inhibitor	Irinotecan	Metastatic disease: combined with gemcitabine	(95,113)	
	Exatecan	Metastatic disease: single agent or combined with gemcitabine	(114,115)	
Anti-HIV	Nelfinavir	Locally advanced disease: combined with gemcitabine and radiation	(89)	
Antiangiogenesis	Cetuximab	Locally advanced or metastatic disease: combined with gemcitabine	(75,98,116)	
	Bevacizumab	Advanced disease: combined with gemcitabine;	(96,117)	
		Metastatic disease: combined with gemcitabine and erlotinib		
	Erlotinib	Advanced disease: combined with gemcitabine	(16)	
	Gefitinib	Metastatic disease: combined with gemcitabine	(76)	
Sorafenib	Advanced disease: combined with gemcitabine	(77)		

cell lines, six show simultaneous mutation or deletion of Kras, p53, and p16, and five have mutation or deletion of DPC4. In contrast, Capan-1 is the only cell line deficient in BRCA-2.

The commonly mutated genes in pancreatic cancer are genes that control signaling pathways critical for cell cycle regulation, cell survival, and proliferation. The presentation of multiple lesions in pancreatic cancer is a stark contrast to some of the more manageable cancers such as chronic myelogenous leukemia and gastrointestinal stromal tumors that show a single genetic lesion in BCR-ABL and is readily treatable with imatinib mesylate (Gleevec®) (28).

**Gene Mutations that Promote Chemoresistance.** Pancreatic cancer is highly resistant to a broad spectrum of chemotherapeutic agents. Although the underlying reasons are not fully understood, the genetic mutations that trigger proto-oncogene expression or inactivate tumor suppressor genes are believed to contribute to chemoresistance. For example, inactivation of p16 leads to overexpression of pRB that is associated with chemoresistance (29). Pancreatic cancer cells and patient tumors express high levels of known chemoresistance factors such as *mdr1* p-glycoprotein, glutathione,

and the p65/RelA subunit of nuclear factor kappa B (NF-κB) (30–32). Overexpression of NF-κB is selectively found in several pancreatic cell lines and 70% of adenocarcinomas, but not in normal pancreatic tissues. Pancreatic cancer cells also show elevated expression of growth factors and their receptors; some of which (e.g., fibroblast growth factors or FGF) are associated with broad-spectrum chemoresistance (33–35).

**Gene Therapy in Pancreatic Cancer.** Progress in understanding the genetic causes of the pancreatic cancer has led to the consideration of gene therapy. The common approaches for gene therapy are as follows. (a) Use of antisense and RNA interference (RNAi) to inhibit the activated oncogenes. For example, antisense Kras suppressed the growth of cultured pancreatic cancer cells and the growth of intraperitoneal xenograft tumors in nude mice. (b) Replacement of inactivated tumor suppressor genes (e.g., p53, p16, SMAD4, CDK1A). For example, reintroduction or expression of wild-type p53 in pancreatic cancer cell lines inhibited tumor growth and induced tumor apoptosis under *in vitro* and *in vivo* conditions and enhanced the activity of gemcitabine in a subcutaneous tumor model. (c) Targeting angiogenesis. For

**Table II.** Origin and Genetic Profile of Pancreatic Cancer Cell Lines

Cell line	Origin	Kras	P53	P16	DPC4/smad	DCC	BRCA2
Panc-1	Pancreas	+	+	–	Wt	Wt	Wt
MiaPaCa-2		+	+	–	Wt	–	Wt
Capan-2		+	Wt	+	Wt	Wt	Wt
BxPc3		Wt	+	–	–	Wt	Wt
Su.86.86	Liver metastasis	+	+	–	Wt	Wt	Wt
Capan-1		+	+	–	+	Wt	+
CFPAC-1		+	+	+	–	Wt	Wt
Hs766T	Lymph metastasis	Wt	+	+	–	Wt	Wt
AsPC-1	Ascites	+	+	+	+	Wt	Wt

+ mutation, – deletion, *Wt* wild type

example, an adenoviral vector encoding soluble fit-1, which inhibits the activity of vascular endothelial growth factor (VEGF), suppressed the growth of xenograft tumors in mice. (d) Promoting apoptosis. One example is to use RNAi to inhibit the antiapoptotic gene *bcl-2* in order to inhibit cell proliferation and induce apoptosis. (e) Gene-directed pro-drug activation. An example is the use of a replication-deficient adenovirus, carrying the gene encoding the enzyme cytosine deaminase that converts 5-fluorocytosine to 5-fluorouracil, to inhibit the growth of murine Pan02 cells under *in vitro* and *in vivo* conditions. (f) Replication-competent oncolytic adenoviruses, which selectively infect and replicate in tumor cells thus causing cell death (36). An emerging strategy is personalized treatment based on the genetic profiles of patients. An example is to use DNA-intercalating agents such as mitomycin C to treat patients with BRCA2 mutations, in order to reduce the ability of tumor cells to repair the drug-induced DNA double-strand breaks (37).

To date, clinical success using gene therapy is limited. This is partly because there are multiple genetic mutations in patients bearing pancreatic cancer, which makes it unlikely that targeting a single faulty gene will produce substantial therapeutic benefits. For example, inhibition of epidermal growth factor receptor or EGFR was not effective due to mutations in the downstream *Kras* (5). Simultaneously targeting multiple genes may be a more fruitful approach. However, the intersecting and compensatory signaling pathways controlled by the multiple genes mutated in pancreatic cancer make it very difficult to orchestrate the multiprong attacks. An alternative to targeting the upstream genes would be targeting their downstream effectors or lesions, such as DNA repair proteins and transcriptional factors.

### Pancreatic Cancer Stem Cells

The hypothesis of cancer stem cells was first proposed in 1983 by Mackillop (38). This attractive hypothesis is consistent with various experimental observations, such as high tumorigenic potential of certain tumor subpopulations (39). A cancer stem cell is defined by its ability to self-renew and produce differentiated progenies. The cellular origin of cancer stem cells is not fully understood. The facts that several progressive mutations are necessary for a cell to become cancerous and that cancer stem cells can self-renew have led to the hypothesis that cancer stem cells are derived either from normal stem cells or from more differentiated cells with oncogenic mutations permitting self-renewal (40).

The existence of cancer stem cells was first verified in acute myelogenous leukemia and has since been identified in breast, lung, brain, liver, melanoma, colon, prostate, ovarian, and pancreatic cancers (39,41–52).

**Pancreatic Cancer Stem Cell Markers.** Multiple research groups have isolated pancreatic cancer stem cells and characterized the stem cell surface markers. Several lines of evidence have shown that more than one set of specific cell surface markers may be enriched in pancreatic cancer stem cells.

Cell surface markers CD44 and CD24 have been used to identify cancer stem cells under *in vitro* and *in vivo* conditions. Subpopulations of pancreatic adenocarcinoma PANC-1 cells that expressed CD44 and CD24 were isolated using flow cytometry (50). Compared with CD44<sup>–</sup>CD24<sup>–</sup> cells, CD44<sup>+</sup>CD24<sup>+</sup> cells had a lower proliferation rate *in vitro* and a 20-fold higher tumorigenic potential in nude mice. Digestion of xenograft tumors with collagenase and sorting the cell populations with CD44, CD24, and/or epithelial-specific antigen (ESA) stem cell surface marker yielded the CD44<sup>+</sup>CD24<sup>+</sup>ESA<sup>+</sup> subpopulation that was highly tumorigenic and showed the characteristics of stem cells such as self-renewal, ability to produce differentiated progeny, and increased sonic hedgehog expression (51).

CD133 has been used as a marker for stem cells in multiple types of human cancer including pancreatic cancer. ATP-binding cassette (ABC) transporters, which are responsible for drug efflux and may confer chemoresistance, are present in pancreatic cancer stem cells (53). The expression of these two markers (ABCG2 and CD133) in five pancreatic adenocarcinoma cell lines was studied using RT-PCR; all showed significantly higher levels of ABCG2 and two also showed higher CD133 levels, compared to nonmalignant fibroblasts (52). CD133<sup>+</sup> cells in the invasive border zone of patient tumor samples showed high expression of CXCR4, the specific receptor of the cytokine stromal-cell-derived factor 1. The CD133<sup>+</sup>/CXCR4<sup>+</sup> cells determined the metastatic phenotype, as depletion of this subpopulation abrogated the metastatic phenotype of pancreatic tumors without affecting their tumorigenic potential (49).

**Therapeutic Implications of Pancreatic Cancer Stem Cells.** Cancer stem cells are resistant to anticancer therapy, probably because they have accumulated mutations in drug resistance genes, have high levels of DNA repair proteins and are efficient in repairing DNA damages, express a wide

variety of transporters that lower the intracellular drug concentrations, are usually quiescent, and have their own microenvironment (e.g., tumor stroma) that supports self-renewal and survival (53–55). As cancer stem cells can lead to tumor regeneration, treatments targeting the stem cell population may be more likely to produce a cure compared to the standard cytotoxic chemotherapy. On the other hand, the potential similarities and overlap of phenotypes and cell signal transduction pathways between somatic and cancer stem cells indicate that the effectiveness of stem cell therapy hinges on the ability to target the cancer stem cells and to avoid eliciting toxicity to normal stem cells. In this regard, the presence of specific cancer stem cell markers may offer an opportunity for marker-specific delivery.

### Microenvironment in Pancreatic Cancer

Comparison of the *in vitro* chemosensitivity of human cancer cells shows that pancreatic cancer cell lines are about equally responsive as cell lines derived from other cancers. For example, the 50% inhibitory concentrations of paclitaxel in pancreatic, breast, or prostate tumor cells are all in nanomolar range (56). Yet patients with pancreatic cancer show much lower response rate to paclitaxel compared to breast and prostate cancer, suggesting that other tumor-specific factors such as the unique microenvironment in pancreatic cancer play a role in chemoresistance.

Tumor microenvironment plays a critical role in tumor initiation, progression, metastasis, and response to treatment. A solid tumor comprises cells (tumor or nontumor) and extracellular matrix (ECM). Nontumor cells (i.e., stromal tissue) and ECM can affect the biology of tumor cells and/or form barriers to drug delivery (57). The following sections discuss the effects of microenvironment on chemosensitivity of pancreatic cancer and the experimental approaches to improve the treatment outcome.

*Stromal Tissue, Extracellular Matrix Proteins, and Pancreatic Stellate Cells.* Pancreatic cancer has the unique property of a high stromal-to-epithelial ratio, with epithelial cells frequently accounting for <20% of the tumor volume (9). Tumor stroma is composed of cellular components including fibroblasts, macrophage, and stellate cells, plus extracellular component or ECM that comprises proteins that facilitate tumor cell proliferation, migration, and invasion (58). The high proportion of stromal cells in pancreatic cancer is associated with overexpression of several growth factors, e.g., VEGF, EGF, and FGF and their receptors (59). Multiple growth factors and cytokines cause resistance to anticancer drugs in cell culture or animal tumor models and are usually associated with poor chemotherapy response rate or patient prognosis (35). For example, our laboratory has shown that the expression levels of two FGF confer broad-spectrum drug resistance to chemotherapeutic agents with different chemical structures and function mechanisms (33,34).

Stellate cells are stellate-shaped periacinar cells, comprising approximately 4% of the cells in the pancreas (60). Stellate cells are the predominant mesenchymal cells within the stroma and the principal source of ECM (61). Stellate cells are activated by inflammatory cytokines (e.g., interleukin (IL)-1 and IL-6), growth factors (e.g., TGF $\beta$ 1, TNF $\alpha$ , and FGF), ethanol, acetaldehyde, and oxidative stress factors released by inflam-

matory cells. The activated, myofibroblast-like phenotype of stellate cells expresses  $\alpha$ -smooth muscle actin (SMA) filaments and synthesizes excessive amount of ECM, comprising mainly fibrillar collagens (type 1, type 3) and fibronectin (62). Stellate cells also secrete matrix metalloproteinases or MMPs (e.g., MMP2 and MMP9), which can degrade the basement membrane collagen, thus facilitating inflammation, fibrosis, angiogenesis, and tumor invasion. Coimplantation of stellate cells with pancreatic cancer cells increases the take rate of tumor formation and promotes tumor progression (63). In general, the presence of stellate cells provides a favorable microenvironment for pancreatic cancer cells to proliferate and invade, and pancreatic carcinoma shows higher number of SMA-positive cells (64). ECM proteins also confer chemoresistance; coating cell culture plates with ECM proteins or coculture with fibroblast diminishes the sensitivity of pancreatic cancer cell to multiple chemotherapeutic agents *in vitro* (57).

In addition to providing the above-mentioned favorable environments for tumor development, growth, and metastasis, the stromal components in pancreatic cancer also protect tumor cells from chemotherapy by diminishing the drug delivery to tumor cells, as follows.

*Drug Delivery in Pancreatic Cancer.* Chemotherapeutic agents are often administered systemically; thus, drug delivery to a tumor involves several processes including transport within a vessel, transport across the vessel wall into surrounding tissues, and transport through interstitial space within a tumor (65). Each process is affected by both the physicochemical properties of the drug/macromolecules such as binding to extracellular and intracellular components and molecule diffusivity and the biological properties of a solid tumor such as tumor blood flow, lymph flow, vascular pressure and interstitial pressure, angiogenesis, regional vessel distribution, tumor cell density, and the extent of stromal tissues and interstitial space. In addition to these challenges for traditional small-molecule cytotoxic agents, delivery of the newer macromolecular agents such as proteins, peptides, and gene vectors presents additional difficulties such as their rapid systemic elimination, uptake by the reticuloendothelial system, and deposition in normal organs or tissues (66). Due to these multiple and significant barriers, delivering an effective drug at effective concentrations to all cells in a solid tumor remains a formidable challenge.

Among solid tumors, drug delivery to pancreatic tumors is especially difficult due to its hypovascular and poorly perfused nature. The presence of stromal components increases the interstitial fluid pressure, thus preventing drugs from penetrating the tissue interstitium (67). In addition, the network of tumor stroma and ECM proteins imposes a barrier for drug delivery (68,69). In pancreatic cancer, the epithelial cancer cells are surrounded by fibrotic stroma comprising activated fibroblasts, immune cells, blood vessels, and ECM. In contrast to other solid tumors where cancer-associated fibroblasts promote tumor growth and angiogenesis, the fibroblasts and fibrotic stroma in pancreatic tumors inhibit the formation and the function of blood vasculature, resulting in the sparse vasculature that is only partially functional and physically separated from the cancer cells by stroma. This unique microenvironment diminishes the drug delivery *via* the perfusing blood and therefore reduces the effectiveness of systemic chemotherapy that relies on functional vasculature for delivery to tumor cells.

Multiple lines of evidence support that improved vasculature or perfusion enhances drug delivery in pancreatic tumors. First, elimination of fibroblasts using a hedgehog inhibitor to disrupt the tumor–stroma signaling resulted in a transient increase in the mean vessel density in tumors and yielded a 60% higher delivery and a greater antitumor activity of intravenous gemcitabine (68). Second, intra-arterial infusion of angiotensin II, a potent vasoconstrictor that selectively constricts the arterioles in nontumor tissues with no effects on tumor arterioles, increased tumor perfusion and enhanced the delivery of concurrently administered methotrexate and 5-fluorouracil. This approach was evaluated in 32 patients; adding angiotensin II to traditional cytotoxic chemotherapeutics improved the median survival time (13 months compared to the historical value of 5–7 months) (70). Third, knockout of the regulator of G protein signaling 5 (Rgs5), a master gene responsible for the abnormal tumor vascular morphology in mice, reduced the vascular leakiness and normalized the vessels, resulting in an enhanced efficacy of a vaccine against pancreatic tumor antigen in a murine pancreatic islet cell carcinoma (71).

*Pharmaceutical Research Opportunities Offered by the Unique Biology and Microenvironment of Pancreatic Cancer.* The advances in understanding the complex interactions between cancer cells and their microenvironment have led to several approaches to prevent or reverse the malignant transformation of solid tumors in general, and some have been tested in pancreatic cancer. These include targeting the molecules responsible for angiogenesis, the MMPs and molecules involved in tumor–stromal interaction.

Several antiangiogenesis agents have shown activity in solid tumors including lung, colorectal, and breast cancers (72–74). The agents under investigation in pancreatic cancer include (a) monoclonal antibodies against EGFR (e.g., cetuximab) and VEGF (e.g., bevacizumab) and (b) tyrosine kinase inhibitors against EGFR (e.g., erlotinib and gefitinib) and against VEGFR (e.g., sorafenib and sunitinib) (16,75–78). Only erlotinib, when added to gemcitabine, was able to produce a slightly longer median survival time in pancreatic cancer patients (16).

Extensive preclinical and clinical studies of MMP inhibitors have been conducted in several types of cancer. The results are disappointing as several phase II/III trials have shown that these compounds have no discernable activity (79). A possible reason for the failure is that there are multiple MMPs, rendering the inhibition of a single MMP inadequate. In addition, because the activated MMP may have opposite effects in being both protumorigenic and prohomeostatic, MMP inhibition may yield beneficial or undesirable results (80).

Adhesion molecules such as integrins (81) and ICAM-1 (82) that are expressed by stromal tissues and involved in mediating the attachment of tumor cells to ECM, as well as ECM proteins including fibronectins, lamins, and collagens (57), are potential therapeutic targets. These strategies are currently in preclinical investigations.

As discussed throughout this review, pancreatic cancer, due to its pathogenesis, multiple genetic lesions, and unique microenvironment, is highly resistant to therapy. In spite of the many advances in understanding the biology of the disease, none has yet led to meaningful improvement of patient management. The complexity of this disease is such

that it is reasonable to expect significant hurdles before the pathobiological findings, e.g., genetic lesions, can be translated into useful treatments. Hence, additional approaches that do not require successfully correcting the multiple biological lesions are needed. We believe overcoming the drug delivery barriers represents a viable alternative that may yield benefits in the more immediate horizon.

Recent studies have shown that the unique fibrostroma microenvironment in pancreatic tumors leads to impaired vasculature and causes inadequate supply of blood-carried drug to cancer cells (68,70,71). These studies also show that reversal of these vascular effects to the extent that was sufficient to transiently increase the blood flow by 50–150% and to transiently improve the drug supply by 60% was able to improve the therapeutic efficacy of systemic chemotherapy. These data indicate that the chemoresistance of pancreatic cancer can be partly overcome by a relatively minor enhancement of drug delivery and further suggest exploring other treatment approaches that do not depend on blood supply for delivery.

Our laboratory is evaluating loco-regional treatment as an alternative/addition to the conventional intravenous treatment. The majority of pancreatic cancer is unresectable, and the stage III or IV disease show metastases in the retroperitoneal and intraperitoneal space. Hence, loco-regional delivery such as intraperitoneal therapy may be a suitable delivery route. During intraperitoneal therapy, the drug enters a tumor directly from the peritoneal fluid, does not require delivery *via* the vasculature, and therefore is not subjected to the same fibrostroma-related problem as intravenous delivery. Addition of intraperitoneal therapy to intravenous therapy has shown significant survival benefits in advanced ovarian cancer patients; multiple studies have shown significant targeting advantage in patients, with ratios of peritoneal cavity-to-systemic blood drug exposure ranging from 12 for cisplatin to 1,000 for paclitaxel (83,84). But toxicities and other issues have prohibited the widespread use of intraperitoneal therapy.

The toxicities of intraperitoneal therapy are generally related to the procedures for administration and the drugs given, i.e., intraperitoneal administration of the FDA-approved intravenous drug solutions as a bolus dose through an indwelling catheter. The use of catheters is associated with higher risk of infection and fever and occasionally physical damage to peritoneal tissues (e.g., tissue perforation). The bolus presentation of the entire dose all-at-once can lead to local toxicity. Another limitation is the inability to penetrate bulky tumors; studies have shown that intraperitoneal therapy yielded better prognosis and longer survival interval in ovarian cancer patients with smaller tumors ( $\leq 0.5$  cm) compared to larger tumors ( $\geq 2$  cm) (summarized in (85)). In order to overcome these issues, our laboratory has developed a two-component, paclitaxel-loaded, tumor-penetrating microparticles (TPM) that release the drug at pharmacodynamically optimized rates. The first component releases paclitaxel rapidly to induce apoptosis and expand the interstitial space and thereby promote the penetration of the remaining particles. The fractionated dose presentation may reduce the local toxicity. The sustained drug presentation may eliminate the need for repeated catheterization. The fast plus slow drug release may improve control of tumors with heterogeneous growth rates and minimize the failure of animal-to-human translation caused by interspecies differences in tumor growth rates. TPM, due to its size and

polymer composition, is designed to be retained in the abdominal cavity and adhere to tumors and shows greater therapeutic efficacy over the conventional Cremophor formulation of paclitaxel in mice-bearing metastatic tumors in the intraperitoneal and retroperitoneal space (85). Evaluation of TPM in metastatic pancreatic cancer patients is planned.

## CONCLUSIONS

Pancreatic cancer remains one of the most lethal malignancies, and patients with metastatic pancreatic cancer have a bleak prognosis. The high mortality can be attributed to late diagnosis, rapid disease progression, and poor response to chemotherapy or radiotherapy. The poor response of pancreatic cancer patients to systemic treatment is in part due to (a) frequent and multiple genetic alterations, (b) unique tumor microenvironment, and (c) impaired/inefficient drug delivery. A better understanding of the genetic mutations, cellular and molecular signaling mechanisms, tumor microenvironments, and cancer stem cells underlying the development, progression, and metastasis of pancreatic cancer offers the opportunities to develop novel therapeutics in the long term. Advances in developing effective drug delivery systems that do not rely on the vasculature for delivery offer a near-term possibility for improving the efficacy of therapeutics in this disease. These areas represent unmet needs deserving the attention of pharmaceutical researchers.

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