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Pancreatic cancer: molecular pathogenesis and new therapeutic targets

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Abstract

Patients with pancreatic cancer normally present with advanced disease that is lethal and notoriously difficult to treat. Survival has not improved dramatically, despite routine use of chemotherapy and radiotherapy; this situation signifies an urgent need for novel therapeutic approaches. Over the past decade, a large number of studies that aimed to target the molecular abnormalities implicated in pancreatic tumor growth, invasion, metastasis, angiogenesis and resistance to apoptosis have been published. This research is of particular importance, as recent data suggest that a large number of genetic alterations affect only a few major signaling pathways and processes involved in pancreatic tumorigenesis. Although laboratory results of targeted therapies have been impressive, until now only erlotinib, an epidermal growth-factor receptor tyrosine kinase inhibitor, has demonstrated modest survival benefit in combination with gemcitabine in a phase III clinical trial. Whilst the failures of targeted therapies in the clinical setting are discouraging, lessons have been learnt and new therapeutic targets that hold promise for the future management of the disease are continuously emerging. This Review describes some of the important developments and targeted agents for pancreatic cancer that have been tested in clinical trials.

Introduction

Pancreatic cancer remains an important health problem. Known risk factors for the disease include cigarette smoking, chronic and hereditary pancreatitis, late-onset diabetes mellitus and familial cancer syndromes. Pancreatic cancer is one of the most difficult conditions to treat, although it only accounts for 3% of all cancers; 5-year survival is about 5% in patients with the disease and this figure has remained relatively unchanged over the past 25 years.¹ The majority of patients present with locally advanced or metastatic disease, and such individuals have a grim median survival of 6–10 months, and 3–6 months, respectively.² Although 10–15% of patients have potentially resectable tumors, many experience recurrence of disease following surgery. Gemcitabine is the standard chemotherapeutic drug for patients with advanced pancreatic cancer after a phase III trial in 1997 demonstrated a modest survival advantage of this agent over 5-fluorouracil (median survival 5.65 months versus 4.41 months, respectively $P=0.0025$), and improved alleviation of disease-related symptoms.³ Given the limited effect of conventional therapies, however, a desperate need

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for improved diagnostic and treatment modalities remains. Considerable resources have been channeled to the development of novel therapies that target the molecular aberrations of the disease (Table 1). These targeted therapies are designed to disable the cellular pathways that are essential for cancer to survive. Targeted therapies could also be used in a multimodal treatment regimen in combination with standard radiotherapy and chemotherapy to improve outcomes and overcome drug resistance. In 2008, detailed, global, genomic analyses found that a large number of genetic alterations (an average of 63) affect only a core set of 12 signaling pathways and processes that are each genetically altered in 67–100% of cases of pancreatic cancer.⁴ These data suggest that treatments for pancreatic cancer should target these complex and overlapping signaling pathways, rather than just the products of a single gene (Figure 1). This Review describes some of the important developments in therapies for pancreatic cancer that have been tested both in the laboratory and, most importantly, in subsequent clinical trials.

Targeted therapies in clinical trials

Signal-transduction pathways

The Ras Pathway—*KRAS* is a member of the *Ras* family of genes, which encode membrane-bound GTP-binding proteins. When activated by signaling partners, such as the epidermal growth factor receptor (EGFR), Ras proteins release GDP in exchange for GTP, which converts the Ras protein to the ‘on’ state and activates downstream signaling events, such as the Raf, MAP2K, MAPK and the PI3K–Akt cascades (Figure 2). These events are usually short-lived by virtue of the intrinsic GTPase activity of Ras proteins, which switches these proteins’ effects ‘off’. Mutations of *KRAS*, mostly at codon 12 but also sometimes at codons 13 and 61, are exceptionally frequent in patients with pancreatic cancer.⁵ Mutations in *KRAS* result in impaired GTPase function, which causes KRas to be locked in the GTP-bound ‘on’ state. This malfunction triggers a variety of cellular processes, including transcription, translation, cell-cycle progression, enhanced cell survival and motility. Oncogenic *KRAS* is involved in the initiation or early phase of pancreatic tumorigenesis.

A peptide vaccine that aims to stimulate immunity against cancer cells with mutant Ras proteins has been tested as an adjuvant treatment in patients with pancreatic cancer.⁶ An extension to this research investigated the effects of combination therapy with mutant Ras peptide plus granulocyte-macrophage colony-stimulating factor⁷ or interleukin 2.⁸ Outcomes seemed to be favorable in these phase I–II trials, albeit only in individuals who mounted an immune response (about half of the patients).

For Ras to function, it must undergo post-translational modification so that it can attach to the cell membrane. One essential step involves the addition of a 15-carbon isoprenoid chain, mediated by farnesyltransferase. The therapeutic use of tipifarnib, a farnesyltransferase inhibitor (FTI), in combination with gemcitabine was disappointing in a phase III trial (Table 2).⁹ This finding could be partly explained by the fact that KRas can be alternatively prenylated by the addition of a 20-carbon isoprenoid moiety mediated by the enzyme geranylgeranyltransferase. Moreover, FTIs work largely by inhibition of the cell cycle, but gemcitabine needs cell-cycle progression to be effective. To this end, a dual inhibitor of farnesyltransferase and geranylgeranyltransferase (L-778,123) was tested in a phase I trial in combination with radiotherapy for locally advanced pancreatic cancer.¹⁰ Inhibition of farnesylation and sensitivity to radiotherapy was demonstrated in a patient-derived cell line. Further development of this drug was, nevertheless, halted owing to adverse cardiac effects. Other compounds that are in early phases of clinical testing after yielding promising laboratory results include romidepsin, a histone deacetylase inhibitor that inhibits Ras-mediated signal transduction and thus causes cell-cycle arrest,¹¹ and farnesylthiosalicylic acid (salirasib), which disrupts Ras from its membrane-binding site.¹² These compounds

seem to have clinical activity in combination with gemcitabine and further studies are warranted.

Other strategies that target the Ras signaling pathway include the use of RNA-directed gene-silencing strategies, such as antisense therapy and RNA interference. Antisense therapy involves the use of oligonucleotides that have sequences complementary to a specific target messenger RNA (mRNA), which, therefore, block its translation to protein. In a phase II trial of patients with locally advanced and metastatic pancreatic cancers, the antisense inhibitor of another member of the Ras family (HRas), ISIS 2503, showed a response rate of 10.4% and a median survival of 6.6 months in combination with gemcitabine.¹³ However, initial enthusiasm for this approach is diminishing following the failures of antisense inhibitors such as ISIS 3521 and oblimersen in lung cancer and melanoma, respectively. An alternative method is RNA interference, which involves the manufacture of small interfering RNAs (siRNAs) that are specific for a particular target mRNA. These siRNAs bind to a complex of several proteins, including endoribonucleases, which is then termed the RNA-induced silencing complex. This complex identifies complementary mRNA and effects its cleavage or translational block. This technology is highly specific but has yet to enter clinical trials, although *in vitro* and *in vivo* studies have been promising.^{14,15}

MAP2K, the principal downstream component of Ras signaling, has also been the subject of targeted inhibition. In a phase II trial, the inhibitor CI-1040 (PD184532) did not demonstrate enough antitumor activity to justify further development.¹⁶ Nevertheless, combined inhibition of MAP2K and other kinases (such as EGFR) has been effective in preclinical studies, which suggests that this approach might still have a role in therapy for pancreatic cancer.^{17,18}

The epidermal growth factor receptor pathway—EGFR is a transmembrane receptor tyrosine kinase of the ErbB family. Upon binding to its ligands, homodimerization or heterodimerization with other members of the ErbB family occurs, which leads to phosphorylation of tyrosine residues in its intracellular domain. This process recruits intracellular proteins that cause downstream signaling events through MAPK, PI3K–Akt, and the STAT family of proteins (Figure 2). STAT proteins have roles in cell proliferation, survival, motility, invasion and adhesion. Mechanisms that lead to inappropriate activation of EGFR include receptor overexpression, activating mutations, overexpression of receptor ligands, and/or loss of their negative regulatory pathways. Overexpression of EGFR and its ligands EGF and TGF- α are frequently observed in pancreatic cancer.^{19,20}

In a phase III trial in combination with gemcitabine, erlotinib, an orally active small molecule that binds to the ATP-binding site of EGFR, has demonstrated a small but statistically significant increase in the survival of patients with advanced pancreatic cancer (Table 2).²¹ In 2005, erlotinib was the first targeted therapy approved by the FDA for pancreatic cancer. However, its clinical significance has been criticized and its cost-effectiveness has been questioned.²² Other EGFR tyrosine kinase inhibitors that have been tested in early-phase clinical trials include gefitinib^{23–25} and lapatinib.^{26,27}

Although EGFR inhibitors have shown promising results, inhibition of EGFR using the monoclonal antibody cetuximab was ineffective in a phase III trial in patients with locally advanced and metastatic pancreatic cancers (Table 2).²⁸ No objective responses were seen in phase II trials of cetuximab in combination with gemcitabine and intensity-modulated radiotherapy or cisplatin.^{29,30} A phase II trial of cetuximab in combination with docetaxel and irinotecan is ongoing.³¹

Gastrin and cholecystokinin receptor pathway—The peptide hormone gastrin is secreted by G cells in the gastric antrum and duodenum, and it can act as a growth factor for gastric, colonic and pancreatic cancers. CCK-BR (the gastrin and cholecystokinin receptor), gastrin precursors and the fully amidated gastrin are expressed in 95%, 55–91% and 23% of pancreatic cancers, respectively.³² A selective CCK-BR antagonist, gastrazole, was tested in two small, randomized, controlled trials in patients with advanced pancreatic cancer (Table 2).³³ Gastrazole was superior to placebo, but not to 5-fluorouracil. Another inhibitor, the orally active Z-360, has demonstrated promising laboratory results³⁴ and is tolerated well by patients in combination with gemcitabine.³⁵ A phase III trial of Z-360 is being planned. An alternative approach to blockade of this pathway involves the use of gastrimmune, an immunogen that stimulates the formation of antibodies against gastrin 17 and its precursors. This agent was, however, not successful in a phase III trial (Table 2).³⁶

Angiogenesis

Angiogenesis is essential for solid tumor growth, and is principally mediated by the vascular endothelial growth factor (VEGF) family of proteins and receptors (Figure 2). Stimuli that upregulate VEGF expression include hypoxia, other growth factors and oncogenic proteins (for example, TGF- β , EGF and Ras). VEGF is overexpressed in >90% of pancreatic cancers³⁷ and is, therefore, an appealing target for therapy.

Bevacizumab is a humanized antibody against VEGF and is approved for use in patients with colorectal cancer. However, a phase III trial in advanced pancreatic cancer failed to show any survival benefit for bevacizumab in combination with gemcitabine (Table 2).³⁸ The AVITA (BO17706) phase III study of patients with metastatic pancreatic cancer reported that the addition of bevacizumab to gemcitabine and erlotinib did not significantly prolong overall survival, although a significant improvement in progression-free survival was seen (Table 2).³⁹ A number of other trials are being conducted to examine bevacizumab in combination with other agents or treatment modalities for pancreatic cancer; however, this agent seems unlikely to confer sufficient benefit to justify its licensing for this condition.

The failure of bevacizumab in therapeutic trials for pancreatic cancer highlighted the need for angiogenic inhibitors that could target other non-VEGF pathways and have better access to the tumor environment than an antibody. Sorafenib is a multitargeted kinase inhibitor that inhibits the VEGF receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), SCFR (formerly c-KIT), Raf1 and FLT3, which are all implicated in tumor growth and angiogenesis. Sorafenib was approved in 2005 for the treatment of advanced renal-cell carcinoma. However, a phase II study concluded that, although well-tolerated, it was inactive in patients with advanced pancreatic cancer.⁴⁰ Axitinib is an orally active inhibitor of both VEGFR and related tyrosine kinase receptors at high concentrations. A median survival of 6.9 months was reported for axitinib combined with gemcitabine compared with 5.6 months for gemcitabine alone in a phase II trial in patients with advanced pancreatic cancer, but this finding was not statistically significant.⁴¹ Phase III trials of axitinib combined with gemcitabine are currently in progress. Aflibercept, a recombinant fusion protein that functions as a soluble decoy receptor and thereby inhibits VEGF, is another novel agent being tested in a phase III trial of patients treated with gemcitabine for metastatic pancreatic cancer.

Integrin receptors on the cell surface interact with the extracellular matrix and mediate various signaling pathways (Figure 2). These receptors are involved in many neoplastic processes, including tumor survival, invasion and metastasis. The $\alpha^V\beta^3$ and $\alpha^V\beta^5$ integrins induce angiogenesis, principally via basic fibroblast growth factor and VEGF, respectively. Cilengitide inhibits these integrins, but in a phase II trial in patients with advanced

pancreatic cancer it did not show significant benefit compared to gemcitabine alone.⁴² Other anti-integrin agents, including an antibody against $\alpha^5\beta^1$ (volociximab)⁴³ and an inhibitor of α^2 (E7820),⁴⁴ are in early-phase clinical trials.

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteolytic enzymes that degrade the extracellular matrix and are essential for tumor spread and neovascularization. We should not, therefore, be surprised that imbalance between MMPs and their natural inhibitors is a frequent event in pancreatic cancer. Despite promising laboratory results, MMP inhibitors have failed to live up to their initial therapeutic expectation in three phase III clinical trials (Table 2),^{45–47} although critics argued that the trials included a large number of patients with metastatic disease, which contradicts the rationale of exploiting the cytostatic effect of MMP inhibitors.

Other potential therapeutic targets

Signal-transduction pathways

The PI3K and Akt Pathway—Upon activation by Ras or EGFR, PI3K activates Akt, which in turn has multiple downstream targets, including the mammalian target of rapamycin (mTOR) and the transcription factor NF κ B (Figure 2). mTOR and NF κ B have a variety of roles in cell proliferation, survival, resistance to apoptosis, angiogenesis and invasion. *AKT2* is amplified and the PI3K–Akt pathway is activated in 20% and 59% of pancreatic cancers, respectively.^{48,49} Deregulation of this pathway through aberrant expression of PTEN (phosphatase and tensin homolog, a natural antagonist of PI3K) is frequently observed in pancreatic cancer.⁵⁰ Furthermore, an architectural transcription factor, HMGA1, is overexpressed in pancreatic cancer.⁵¹ This transcription factor activates PI3K–Akt signaling and seems to mediate resistance to gemcitabine,⁵² which, therefore, provides another target for inhibition therapy.^{53,54}

Temsirolimus is an mTOR inhibitor approved for the treatment of renal-cell carcinoma, but use of this agent in pancreatic cancer has been limited.⁵⁵ Other agents, including everolimus and sirolimus, are currently in phase II clinical trials.⁵⁶ A combination of an mTOR inhibitor with other standard or targeted therapies might be needed,^{57,58} as mTOR expression does not correlate with survival of patients.⁵⁹

Curcumin, which is derived from the spice turmeric, can inhibit NF κ B and, therefore, the expression of regulated gene products such as Bcl2, BclXL, COX2, cyclin D1 and survivin, which all have a role in the survival of pancreatic cancer cells.^{60,61} Curcumin can also alter the expression of miRNAs (microRNAs, see below) in pancreatic cancer cells.⁶² Phase II trials of curcumin with and without gemcitabine showed that curcumin was well-tolerated and might have some biological activity in patients with pancreatic cancer.^{63,64} The oral inhibitor of NF κ B–STAT3, RTA 402, is being examined in a phase I–II trial. Bortezomib is a proteasome inhibitor that prevents the degradation of I κ B β , which in turn is an endogenous inhibitor of NF κ B (Figure 2). Bortezomib is licensed for the treatment of refractory multiple myeloma, but unfortunately it failed to show any benefit—either alone or in combination with gemcitabine—in a phase II trial.⁶⁵ This finding could be related to the fact that proteasome inhibition paradoxically activates other antiapoptotic and mitogenic signaling pathways in pancreatic cancer.⁶⁶

The cyclo-oxygenase pathway—The COX enzymes have a principal role in the conversion of arachidonic acid into prostaglandins. COX1 is constitutively expressed and has a homeostatic role. COX2 is inducible by growth factors, cytokines and tumor

promoters, and its expression is upregulated in 90% of pancreatic cancers.⁶⁷ The mechanisms of COX-mediated and prostaglandin-mediated pancreatic-cancer development are complex; they involve multiple mitogenic signaling pathways and molecules that mediate resistance to apoptosis, cell migration, invasion, angiogenesis, immunosuppression, the production of free radicals and peroxidation of procarcinogens to carcinogens.⁶⁸ Inhibition of COX2 by NSAIDs suppressed proliferation of pancreatic cancer cells and angiogenesis, both *in vitro* and *in vivo*.^{68,69} Interestingly, Chuang and colleagues reported in 2008 that the antitumor activity of celecoxib does not correlate with its inhibition of COX2, which suggests the involvement of alternative mechanisms.⁷⁰ Nonetheless, phase II trials of gemcitabine in combination with celecoxib 400 mg twice daily have been conducted, but results were inconclusive. For 20 evaluable patients with metastatic pancreatic cancer, the reported median survival was 6.2 months and the 3-month survival was 72%.⁷¹ For patients with locally advanced or metastatic disease, one study showed a median survival of 9.1 months and overall clinical response of 54.7%,⁷² but another study concluded that the addition of celecoxib had no significant benefit.⁷³ The combination of celecoxib, gemcitabine and irinotecan resulted in a median survival of 13 months and 1-year survival of 64%, and was associated with improvement of pain and quality of life.⁷⁴ A phase III trial of gemcitabine, celecoxib and curcumin is in progress.

The TGF- β and SMAD4 pathway—TGF- β is a cytokine secreted by epithelial, endothelial, hematopoietic and mesenchymal cells. Binding of TGF- β forms a heteromeric complex with the type I and type II TGF- β receptors that triggers the phosphorylation of cytoplasmic SMAD2 and SMAD3. In turn, these SMAD proteins form a complex with SMAD4, which translocates into the nucleus to activate gene transcription (Figure 2). TGF- β can also signal via SMAD-independent pathways that involve Ras, PI3K and MAPK. TGF- β mediates a wide range of physiological processes, such as embryonic development, tissue repair, angiogenesis and immunosuppression. TGF- β also has a complex role in tumorigenesis, as it is tumor-suppressive in epithelial cells, but promotes invasion and metastasis during the late stages of cancer progression. Mutations of the *TGFBR1*, *TGFBR2* and *SMAD4* genes are found in about 1%, 4% and 50% of pancreatic cancers, respectively.⁷⁵ Inactivation of SMAD4 abolishes TGF- β -mediated tumor-suppressive functions while it maintains some tumor-promoting TGF- β responses, such as epithelial–mesenchymal transition, which makes cells migratory and invasive.⁷⁶

TGF- β -based therapeutic strategies are currently in development, including inhibitors of TGFBR1 and TGFBR2.^{77,78} AP 12009, an antisense oligonucleotide specific to TGF- β 2, is currently being tested in a phase I–II study of malignant melanoma, pancreatic cancer and colorectal carcinomas. One patient with advanced pancreatic cancer was still alive 128 weeks after complete regression of liver metastases.⁷⁹

The hepatocyte growth factor receptor pathway—The *MET* oncogene encodes the receptor for hepatocyte growth factor (HGF) and is overexpressed in 78% of pancreatic cancers.⁸⁰ HGF is normally produced by mesenchymal cells and acts on epithelial cells to promote tissue regeneration. In hypoxic conditions, however, tumor-associated fibroblasts produce HGF, which stimulates angiogenesis, tumor growth, enhanced cell motility and extracellular matrix breakdown and leads to invasion and metastasis (Figure 2). Targeting the HGF pathway with use of a synthetic competitive antagonist of HGF^{81,82} and an antibody against the MET receptor⁸³ has yielded encouraging results in the laboratory setting. ARQ 197 is a MET receptor tyrosine kinase inhibitor that is currently being tested in a phase II trial. A phase I study showed that it was tolerated well by patients.⁸⁴

The insulin-like growth factor pathway—The insulin-like growth factor I (IGF-I) receptor, a transmembrane receptor tyrosine kinase, is overexpressed in 64% of pancreatic

cancers.⁸⁵ The IGF-I receptor has antiapoptotic and growth-promoting effects and acts via multiple signaling cascades, including the PI3–Akt, MAPK and STAT pathways (Figure 2). Inhibition of the IGF-I receptor by the tyrosine kinase inhibitor NVP-AEW541, a dominant-negative mutant and RNA interference have all been shown to reduce the growth of pancreatic cancer cells *in vitro* and *in vivo*, and increase chemotherapy-induced or radiation-induced apoptosis.^{86,87} Concomitant inhibition of KRas increases the therapeutic effect of IGF-I receptor antisense oligonucleotide.⁸⁸ Human anti-IGF-I receptor antibodies have been reported to enhance the antitumor effects of gemcitabine and EGFR inhibition *in vivo*.^{89,90} As a result of these findings, phase I–II trials of cixutumumab and MK-0646 with gemcitabine and erlotinib have now commenced for pancreatic cancer.

The focal adhesion kinase pathway—Focal adhesion kinase (FADK) is a cytoplasmic non-receptor tyrosine kinase that mediates functions involved in cell motility and survival and is closely related to the integrin signaling pathway (Figure 2). 48% of pancreatic cancers⁹¹ express FADK and, importantly, it shares a common pathway with IGF-I receptor.⁹² The dual IGF-I receptor–FADK inhibitor NVP-TAE226 has shown significant tumor-suppressive activity *in vivo*.⁹³

The Src pathway—Src is one of nine members of the Src family of non-receptor protein tyrosine kinases. In normal conditions, Src is maintained in a phosphorylated and inactive form, but is activated in a number of malignancies, including in 70% of pancreatic cancers.⁸⁵ Src has diverse roles in cell proliferation, survival, motility, invasiveness, resistance to chemotherapy and angiogenesis. This protein acts via multiple signaling pathways and, therefore, is an ideal target for therapeutic intervention (Figure 2). Src kinase inhibitors have been effective in suppressing pancreatic tumor growth and metastasis *in vivo*.^{94–96} Dasatinib is an orally active multitargeted kinase inhibitor of Src, BCR–ABL, PDGFR, ephrin type A receptor 2 and SCFR, and is licensed for the treatment of chronic myelogenous and acute lymphoblastic leukemias. Dasatinib is being examined in a phase II trial in patients with metastatic pancreatic cancer, as is the related compound saracatinib.

Embryonic signaling pathways

The hedgehog pathway—Three mammalian hedgehog homolog proteins have been identified—DHH, IHH and SHH. These proteins are secreted and specify the organization and structure of many tissues during embryonic development. Activation of the hedgehog signaling pathway is controlled by two transmembrane proteins, the tumor-suppressor PTC1 protein and the oncogenic SMO protein (Figure 2). PTC1 normally suppresses SMO, but mechanisms, such as an inactivating mutation of *PTC1* and the binding of hedgehog proteins to PTC1, relieves this inhibition, which leads to SMO activation of transcriptional responses. SHH is expressed in 70% of human pancreatic adenocarcinomas.⁹⁷ IHH expression is increased 35-fold in pancreatic cancer cells compared with normal tissues.⁹⁸ Mechanisms of tumorigenesis include the effects of hedgehog proteins on the cell-cycle regulators, protection from apoptosis via PI3K–Akt signaling and stabilization of Bcl2 and BclXL and collaboration with activated KRas and angiogenesis. The hedgehog signaling pathway can be inhibited by cyclopamine, which binds to SMO. Laboratory work has demonstrated the effectiveness of cyclopamine in a wide range of digestive-tract tumors, including pancreatic cancer.⁹⁹ Cyclopamine can enhance sensitivity to radiotherapy and chemotherapy and suppress metastatic spread^{100,101} as well as improving antitumor activity when combined with an EGFR inhibitor.¹⁰² A downstream target of the SHH pathway, the transcription factor GLI 1, can also be inhibited by miRNA.¹⁰³

The Notch pathway—The four known human *Notch* genes encode heterodimeric transmembrane receptors, which are important in the development of organs, tissue

proliferation, differentiation and apoptosis. Activation of the Notch signaling pathway leads to proteolytic cleavage of the transmembrane receptors by γ -secretase; the released cytoplasmic domain then migrates to the nucleus and binds to transcription factors, which leads to the expression of a variety of genes (Figure 2). Notch signaling occurs downstream of Ras, EGFR and TGF- α signaling in pancreatic tumorigenesis and promotes tumor vascularization. Downregulation of Notch 1 with siRNA or curcumin (owing to the crosstalk between Notch and NF κ B signaling pathways) can inhibit cell growth and induce apoptosis in pancreatic cancer cell lines *in vitro*.^{104,105} Notch 3 is expressed in around 70% of pancreatic cancers and can be inhibited by siRNA and γ -secretase inhibitor (L-685,458).^{106,107}

The Wnt pathway—19 human *Wnt* genes each encodes a lipid-modified secreted glycoprotein. Wnt signaling is involved in normal embryonic development and homeostatic self-renewal of a number of adult tissues. Three Wnt signaling cascades, namely the canonical Wnt- β -catenin, the planar-cell polarity, and the Wnt-Ca²⁺ pathways. The former is the best known and has been implicated in a variety of cancers including liver, colorectal, breast, prostate, renal and hematological malignancies. Normally β -catenin is phosphorylated and targeted for degradation. However, binding of Wnt proteins results in activation of intracellular pathways that cause β -catenin to enter the nucleus, where its interaction with the T-cell factor (TCF) and lymphoid enhancer factor (LEF) families of transcription factors leads to targeted gene expression (Figure 2). Any gain-of-function mutation of activators or loss-of-function mutation of inhibitors of Wnt signaling could lead to aberrant activation of these signaling pathways, which could result in carcinogenesis and progression. Aberrant activation occurs in 65% of pancreatic cancers.¹⁰⁸ Inhibition of Wnt signaling to reduce proliferation and increase apoptosis of pancreatic cancer cells has been achieved in the laboratory setting by a variety of methods, including the use of β -catenin-interacting protein 1, a dominant-negative mutant of LEF-1, and siRNA against β -catenin or extracellular sulfatases.^{109,110} Wnt signaling is positively regulated by the hedgehog and SMAD4 signaling pathways,^{109,111} which could be targets for a combined inhibitory therapeutic strategy.

The chemokine receptor 4 (CXCR 4) and its ligand, SDF-1 have a role in tumor growth, angiogenesis and, in particular, metastatic spread. *In vitro* blockade of CXCR 4 could inhibit pancreatic cancer growth through inhibition of the canonical Wnt pathway.¹¹² Furthermore, plerixafor, an antagonist of CXCR 4, reduces metastasis by pancreatic cancer cells that are positive for the markers CXCR 4 and CD133 (the latter is a marker of pancreatic cancer stem cells) *in vivo*.¹¹³

Telomerase

The telomeres located at the end of chromosomes normally shrink with each cell division and thereby impose a finite lifespan on the cell. Most malignant cells have detectable activity of telomerase, a reverse transcriptase that contains an RNA template and acts to elongate telomeres. Telomerase is overexpressed in 95% of pancreatic cancers¹¹⁴ which provides a rationale for the development of antitelomerase agents. GV1001 is a telomerase peptide vaccine that has shown some promising results in phase I/II studies.^{115,116} This vaccine is being tested in the large (>1,000 patients), phase III, TeloVac trial with gemcitabine and capecitabine in locally advanced and metastatic pancreatic cancers.

MicroRNAs

The miRNAs are small, endogenous, noncoding RNA molecules that regulate gene expression and are important for developmental and physiological processes. These molecules all negatively regulate gene expression post-transcriptionally and can be either

oncogenic or tumor-suppressive, depending on their target mRNAs.¹¹⁷ Expression profiling showed that at least 100 miRNA precursors are aberrantly expressed in pancreatic cancer or desmoplasia.^{118,119} Anticancer miRNA-based therapy has the theoretical advantage of having multiple targets that are controlled by an individual miRNA by virtue of its post-transcriptional modulation. Therapeutic strategies include the reconstitution of tumor-suppressive miRNAs and the knockdown of oncogenic miRNAs by coding vectors or anti-miRNA oligonucleotides. Studies of these treatment approaches have been limited in pancreatic cancer but have yielded promising results in breast cancer and glioma.

Cancer stem cells

Cancer stem cells possess important properties associated with their normal counterparts, namely the ability for self-renewal and differentiation. Pancreatic-cancer stem cells are identified by their surface markers, such as CD133, CD44, CD24 and flotillin 2 epithelial specific-antigen. Evidence suggests that such cells form a small subset in the heterogeneous tumor population, and contribute to neoplastic progression, metastasis and resistance to chemotherapy and radiotherapy.^{113,120} For this reason, cancer stem cells are thought to be responsible for relapse of disease after clinical remission. Dysregulation of various signaling cascades, including the PTEN, SHH, Notch and Wnt pathways, are frequently observed in cancer stem cells, which provides further rationale for use of these pathways as a target for therapeutic purposes. Further studies are still needed to understand the genetic and biological properties of cancer stem cells for the development of effective treatment modalities.

Conclusions

Although targeted therapies for pancreatic cancer have yielded encouraging results *in vitro* and in animal models, these findings have not been translated to improved outcomes in clinical trials. Reasons for this failure might include an incomplete understanding of the biology of pancreatic cancer, the selection of poor active agents, problems with trial design (such as inappropriate therapeutic end points or patient selection) and the rapidity with which agents move into randomized, controlled trials without the extensive early testing necessary to optimize treatment regimens. Furthermore, preclinical studies performed on mouse models do not always recapitulate the human condition, which is a particular problem with human pancreatic cancer xenografts in immunodeficient mice. Despite these setbacks, lessons have been learnt, and our collective effort has generated a substantial platform of knowledge from which further work could spring. Genetically engineered immunocompetent mice, such as those with *KRAS* or *TP53* mutations, have been developed and they hold promise for the future studies of the disease.¹²¹ The bioavailability of compounds such as antisense oligonucleotides and siRNAs in humans remains a big hurdle, which will require further improvement of gene-delivery strategies.

The individualization of therapy for patients is possible if factors that predict treatment response, such as biological markers, could be determined accurately. Alternatively, resected tumors could be grown in laboratory mice and treated with a series of drugs, and the most effective agent subsequently administered to the patient. This concept is currently being tested in a phase II trial at Johns Hopkins Hospital, MD, US. Until this strategy is proven effective in the clinical setting, multimodal approaches will remain the mainstay of treatment for advanced pancreatic cancer. These approaches are likely to comprise a mixture of targeted agents in combination with conventional chemotherapy and radiotherapy. For a clinically significant effect to be achieved, treatment strategies should either be in the form of a 'horizontal' approach, in which several oncogenic pathways are inhibited, or a 'vertical' approach, whereby multiple levels of a major pathway are targeted. One example currently being investigated in a phase III trial is the treatment combination of celecoxib, curcumin

and gemcitabine for advanced pancreatic cancer. Besides the synergistic antiproliferative and proapoptotic effects of curcumin and celecoxib,¹²² these agents also potentiate the antitumor activity of gemcitabine.^{123,124} Combination therapies, together with improved diagnostic tools and predictive markers, are ultimately hoped to improve the bleak outlook for patients diagnosed with pancreatic cancer. For now, the results of a number of phase III trials are eagerly awaited (Table 3).

Review criteria

PubMed was searched in November 2008 for English-language publications using the terms “pancreatic”, “pancreas”, “carcinoma”, “cancer”, “therapy”, “treatment” and those listed in the article subheadings. Published reports and abstracts from the American Society of Clinical Oncology and the American Association for Cancer Research meetings, were also searched. No exclusion criteria were used. Articles were selected on the basis of relevance and additional papers were identified from their reference lists. The National Cancer Institute website was searched for ongoing clinical trials.

Key points

Pancreatic cancer is a disease that has high morbidity and mortality and is resistant to conventional treatment; therefore, an unmet need for novel therapeutic approaches exists

Important molecular pathways and components involved in pancreatic carcinogenesis have been targeted with therapeutic intent, including Ras, EGFR, VEGF, gastrin and matrix metalloproteinases

Good results from novel therapies have been demonstrated *in vitro* and in animal models, but results from the limited number of clinical trials are less encouraging

Erlotinib, an EGFR tyrosine kinase inhibitor, is the only agent so far that has shown a significant (albeit small) survival benefit in a phase III clinical trial

Potential therapeutic targets that warrant further investigation include other signal-transduction and embryonic pathways, telomerase, microRNAs and cancer stem cells

Future development of targeted treatments should focus on inhibition of multiple signaling pathways, or blockade of one signaling pathway at multiple levels

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Figure 1.

The complex and overlapping pathways and processes involved in pancreatic carcinogenesis. Entities involved in these signal transduction pathways have diverse roles in the promotion of tumor growth, resistance to apoptosis, invasion, metastasis and angiogenesis. Reactivation of physiological, embryonic development pathways is also commonly observed in pancreatic cancer. MMPs are important for tumor invasion and neovascularization. Telomerase is involved in the maintenance of telomeres and is activated in the majority of pancreatic cancers. The miRNAs regulate gene expression post-transcriptionally and can be either oncogenic or tumor-suppressive. Cancer stem cells have been implicated in tumor progression, resistance to chemotherapy and radiotherapy and in disease relapse. Abbreviations: miRNAs, microRNAs; MMP, matrix metalloproteinase.

Figure 2.

A simplified representation of oncogenic signaling cascades in pancreatic cancer. a | Binding of ligands to receptors for integrins, VEGF, EGFR, CCK-B/gastrin, HGF and IGF-I activates signaling cascades including the PI3K–Akt and Ras pathways, which affect downstream targets such as NF κ B, mTOR and MAPK. FADK binds to integrin receptors and Src to growth factor receptors. FADK–Src interaction increases the activity of FADK. PTEN has the opposite effect to PI3K and inhibits the Akt pathway. Proteasomes degrade I κ B, which normally inhibits NF κ B. b | Binding of TGF- β forms a complex with TGFBR1 and TGFBR2, which leads to phosphorylation of SMAD2 and SMAD3. These proteins form a complex with SMAD4, which migrates to the nucleus to activate gene transcription. c | The embryonic signaling pathways. Binding of hedgehog proteins to PTC1 releases inhibition of SMO, which leads to activation of downstream targets such as GLI 1. Activation of Notch by its ligands Delta and Jagged leads to its proteolytic cleavage by γ -secretase, and releases the cytoplasmic domain which translocates to the nucleus and binds to transcription factors such as CSL. β -catenin is normally destined for proteasomal degradation. In the canonical Wnt– β -catenin pathway, binding of Wnt proteins stabilizes β -catenin and induces its translocation to the nucleus. β -catenin forms a complex with the TCF–LEF transcription factors to initiate gene expression. Abbreviation: CD, cytoplasmic domain.

Table 1

Important therapeutic targets in pancreatic cancer

Target	Frequency of mutation or expression in pancreatic cancer (%)
Cholecystokinin-B and gastrin receptor ³²	95
<i>KRAS</i> ⁵	95
Telomerase ¹¹⁴	95
Vascular endothelial growth factor ³⁷	93
Gastrin precursors and gastrin ³²	23–91
Cyclo-oxygenase-2 ⁶⁷	90
Hepatocyte growth factor receptor ⁸⁰	78
Notch ^{3106, 107}	70
<i>SHH</i> ⁹⁷	70
Src ⁸⁵	70
Epidermal growth factor receptor ²⁰	69
β -catenin ¹⁰⁸	65
Insulin-like growth factor-I receptor ⁸⁵	64
Activated Akt ⁴⁹	59
<i>SMAD4</i> ⁷⁵	50
Focal adhesion kinase ⁹¹	48
<i>AKT2</i> ⁴⁸	20
<i>TGFBR2</i> (transforming growth factor- β receptor II) ⁷⁵	4
<i>TGFBR1</i> (transforming growth factor - β receptor I) ⁷⁵	1

Table 2

Completed phase III clinical trials of targeted therapies for pancreatic cancer

Trial	Disease stage	Number of patients	Treatments investigated	Mechanism of treatment	Median survival (months)	1-year survival (%)	PFS (mont hs)	CR + P R (%)	Patients with stable disease (%)
Bramhall <i>et al.</i> (2001)45	Unresectable	414	Marimastat vs Gemcitabine	MMPI	3.4–4.1	14.0–20.0	1.8–1.9*	2.8	n/a
					5.5	19.0	3.8*	25.8	n/a
Bramhall <i>et al.</i> (2002)46	Unresectable	239	Marimastat + gemcitabine vs Gemcitabine	MMPI	5.4	18.0	3.0	11.0	50.0
					5.4	17.0	3.1	16.0	56.0
NCIC CTG (2003)47	Advanced	277	Tanomastat vs Gemcitabine	MMPI	3.74*	10.0	1.68*	0.9	28.7
					6.59*	25.0	3.5*	5.2	53.9
Van Cutsem <i>et al.</i> (2004)9	Advanced	688	Tipifarnib + g emcitabine vs Gemcitabine	RAS FTI	6.3	27.0	3.7	6.0	53.0
					6.0	24.0	3.6	8.0	52.0
Shapiro <i>et al.</i> (2005)36	Advanced	394	G17DT + ge mcitabine vs Gemcitabine	Induction of antibodies against gastrin-17	5.8	n/a	3.9	32.0	n/a
					6.6	n/a	3.9	36.0	n/a
Chau <i>et al.</i> (2006)33	Advanced	Trial A: 18	Gastrazole vs Placebo	Gastrin receptor antagonist	7.9*	33.0*	n/a	n/a	n/a
		Trial B: 98	Gastrazole vs 5-fluorouracil		4.5*	11.0*	n/a	n/a	n/a
					3.6	13.2	2.3	0.0	28.3
					4.2	26.2	2.7	4.8	28.6
CALGB 80303 (2007)38	Advanced	602	Bevacizuma b + gemcitab ine vs Gemcitabine	Anti-VEGF antibody	5.7	n/a	4.8	13.1	40.7
					6.0	n/a	4.3	11.3	35.7

Trial	Disease stage	Number of patients	Treatments investigated	Mechanism of treatment	Median survival (months)	1-year survival (%)	PFS (months)	CR + PR (%)	Patients with stable disease (%)
NCIC CTG (2007)21	Advanced	569	Erlotinib + gemcitabine vs Gemcitabine	EGFR tyrosine kinase inhibitor	6.24 *	23.0 *	3.75 *	8.6	48.9
					5.91 *	17.0 *	3.55 *	8.0	41.2
SWOG S0205 (2007)28	Advanced	766	Cetuximab + gemcitabine vs Gemcitabine	Anti-EGFR antibody	6.5	n/a	3.5	12.0	n/a
					6.0	n/a	3.0	14.0	n/a
AVITA (BO17706) (2008)39	Metastatic	607	Bevacizumab + erlotinib + gemcitabine vs Erlotinib + gemcitabine	Anti-VEGF antibody and EGFR tyrosine kinase inhibitor	7.1	n/a	4.6 *	13.5	n/a
					6.0	n/a	3.6 *	8.6	n/a

Abbreviations: CALGB, Cancer and Leukemia Group B; CR + PR, complete and partial responses; EGFR, epithelial growth factor receptor; FTI, farnesyltransferase inhibitor; MMPI, matrix metalloproteinase inhibitor; n/a, not applicable; NCIC CTG, National Cancer Institute of Canada Clinical Trials Group; PFS, progression-free survival; SWOG, Southwest Oncology Group; VEGF, vascular endothelial growth factor.

* Statistically significant

Table 3

Ongoing phase III clinical trials of targeted therapies for pancreatic cancer

Treatment	Target	Disease stage
Erlotinib, capecitabine and gemcitabine	EGFR	Locally advanced or metastatic
Curcumin, celecoxib and gemcitabine	NFκB and COX2	Locally advanced or metastatic
Axitinib and gemcitabine	VEGF receptor and other tyrosine kinases	Locally advanced or metastatic
Sorafenib and gemcitabine	VEGF receptor and other tyrosine kinases	Locally advanced or metastatic
GV1001, capecitabine and gemcitabine	Telomerase	Locally advanced or metastatic
Aflibercept and gemcitabine	VEGF	Metastatic