Hedgehog signaling and therapeutics in pancreatic cancer

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Objective. To conduct a systematic review of the role that the hedgehog signaling pathway has in pancreatic cancer tumorigenesis. Methods. PubMed search (2000–2010) and literature based references. Results. Firstly, in 2009 a genetic analysis of pancreatic cancers found that a core set of 12 cellular signaling pathways including hedgehog were genetically altered in 67–100% of cases. Secondly, in vitro and in vivo studies of treatment with cyclopamine (a naturally occurring antagonist of the hedgehog signaling pathway component; Smoothened) has shown that inhibition of hedgehog can abrogate pancreatic cancer metastasis. Thirdly, experimental evidence has demonstrated that sonic hedgehog (Shh) is correlated with desmplasia in pancreatic cancer. This is important because targeting the Shh pathway potentially may facilitate chemotherapeutic drug delivery as pancreatic cancers tend to have a dense fibrotic stroma that extrinsically compresses the tumor vasculature leading to a hypoperfusing intratumoral circulation. It is probable that patients with locally advanced pancreatic cancer will derive the greatest benefit from treatment with Smoothened antagonists. Fourthly, it has been found that ligand-dependent activation by hedgehog occurs in the tumor stromal microenvironment in pancreatic cancer, a paracrine effect on tumorigenesis. Finally, in pancreatic cancer, cells with the CD44+/CD24−/ESA+ immunophenotype select a population enriched for cancer initiating stem cells. Shh is increased 46-fold in CD44+/CD24−/ESA+ cells compared with normal pancreatic epithelial cells. Medications that destruct pancreatic cancer initiating stem cells are a potentially novel strategy in cancer treatment. Conclusions. Aberrant hedgehog signaling occurs in pancreatic cancer tumorigenesis and therapeutics that target the transmembrane receptor Smoothened abrogate hedgehog signaling and may improve the outcomes of patients with pancreatic cancer.

Introduction

The Nobel Prize in Medicine was awarded in 1995 for discoveries concerning ‘the genetic control of early embryonic development’. Two of the German prize recipients in Heidelberg identified genes that control the segmentation pattern of Drosophila melanogaster (fruit fly) embryos. Their genetic screens found that loss of a gene function later to be called hedgehog caused a mutant embryonic phenotype such that the embryos were covered with projections similar in appearance to a hedgehog. There are three hedgehog genes designated by the prefixes sonic, desert and Indian. Sonic hedgehog (Shh) is a developmental morphogen in humans such that one function of Shh expression is that it forms a gradient that is primarily responsible for setting up the anterior–posterior axis in the developing limb (1). More recently, aberrant hedgehog signaling has been implicated in tumorigenesis and hedgehog-directed therapeutics have a potential role in diseases other than cancer such as neurodegeneration and stroke.

The hedgehog pathway: structure, cancer and therapeutics

The hedgehog pathway is activated by secreted hedgehog molecules. These hedgehog ligands bind to a transmembrane protein called Patched (Pch). This ligand receptor binding inhibits the repression of Smoothened another transmembrane receptor that is tonically repressed by the Patched receptor in the absence of hedgehog ligand. Therefore, Smoothened functions only when Hedgehog (Hh) is present. Smoothened transduces the signal intracellularly via Gli zinc finger transcription factors (2) Figure 1.

The hedgehog pathway is most frequently pharmacologically inhibited by targeting the Smoothened receptor. The classic inhibitor is cyclopamine, a naturally occurring teratogenic alkaloid and more recently small molecule inhibitors have been discovered such as GDC-0449, IPI-926 and XL139. The latter medication XL139 (BMS-833923) has shown phase I tumor efficacy against basal cell carcinomas (BCCs) (3). GDC-0449 has also shown efficacy in BCCs. Additionally a phase 1 study of IPI-926 of patients with advanced or metastatic solid tumors found partial responses in patients with BCC and three non-BCC patients had stable disease for at least 6 months (adenocystic carcinoma of the nasopharynx, a neuroendocrine tumor and a case of chondrosarcoma; European Society of Medical Oncology meeting, Milan Italy, October 2010, C.M. Rudin et al.).

Another theoretical mechanism to inhibit hedgehog signalling is to target Gli-mediated gene transcription using candidate medicinal compounds such as the GANT inhibitors; GANT 58 and GANT 61 (see Figure 1). Finally, an important intracellullar compound is suppressor of fused (SUFU). It is not usually targeted by medicinal compounds but is clinically important as it is a tumor suppressor gene and mutations in SUFU can lead to the formation of multiple tumors.

In cancer treatment, much of the evidence for the benefit of inhibiting the hedgehog pathway has been accrued from preclinical model systems. The effect that inhibition of the hedgehog pathway has on medulloblastomas has been evaluated by investigators at St Jude Children’s Research Hospital in Memphis, Tennessee. They demonstrated that in a Ptcl(+/−)/p53(−/−) mouse model, 100% of which are destined to develop medulloblastoma, treatment with a hedgehog antagonist resulted in complete tumor eradication (4). The Hedgehog pathway may be upregulated in tumor cells because of intrinsic mutations in the hedgehog pathway such as is found in medulloblastomas leading to ‘oncogene addiction’. The Hedgehog pathway may also be upregulated because of increased hedgehog ligand. This occurs in stomach and pancreatic cancer (5). The exact mechanism how Hh acts in this later group of tumors is uncertain but may be due to autocrine signaling, selective activation of cancer initiating stem cells or paracrine signaling. Furthermore in pancreatic cancer, mutant KRAS activates Gli1 (6–8) and Gli activity in pancreatic cancer may be modulated by altering the activity of pathways that interact with hedgehog signaling via intercellular cross talk such as with the RAF/MEK/mitogen-activated protein kinase pathway.

It may also be desirable to target different aspects of hedgehog signaling or to be more selective by undertaking localized tissue delivery of hedgehog antagonists to obviate deleterious systemic side effects. For instance, it has been found that transient inhibition of hedgehog signaling in young mice cause permanent structural bone deficits. This is of importance if inhibitors of hedgehog signaling are to be used to treat medulloblastomas a tumor that affects children and young adults (9). Lastly, PF-04449913 is an inhibitor of Hedgehog signaling. A phase II study involving this drug in myeloid malignancies exemplified the risk of contaminating studies with clinically similar tumor
that have differing patterns of Hedgehog pathway activity. This is usually detrimental to study findings. In that study, it was notable that Hedgehog signaling including intact Smoothened expression is required for the >95% of cases of chronic myeloid leukemia that have the Breakpoint cluster region - Abelson leukaemia chimeric fusion gene but not leukemogenesis due to the mixed lineage leukemia: AF9 chimeric fusion gene (10). This is an ever present danger in contemporary trial design.

**Methods**


**Inferences from tumors derived from ectodermal tissue**

Gorlin’s syndrome, a disorder arising from inherited germ line mutations in the Patched gene is a unifying phenotypic expression of the role Hedgehog signaling has in endodermally and ectodermally derived tumors. It is associated with an increased predisposition to the development of multiple BCCs, medulloblastoma and rhabdomyosarcoma as well as prostate, breast and pancreatic cancer. Two of the tumors that this disorder confers an increased risk of; BCC and medulloblastoma were the subject of landmark papers involving GDC-0449 in September 2009 (11,12). Loss of function mutations occur in the gene encoding Patched 1 in BCCs and 30% of medulloblastomas particularly the desmoplastic variant (13–15). Firstly, in patients with metastatic or locally advanced BCC (n = 33), 55% of patients had an objective response (2 complete response and 16 partial response) (11). Of the other 15 patients, 11 had stable disease for up to 10.8 months and four experienced progressive diseases. Hedgehog also has a critical role in cerebellar development in directing the spatial and temporal migration of granule cell neural precursors from the external to the internal granule cell layer. In the second study which was a clinical case report, a patient with treatment refractory metastatic medulloblastoma received GDC-0449 and experienced a rapid but transient tumor response (12). In this case, the patient’s medulloblastoma had loss of heterozygosity and a somatic mutation of the Patched 1 gene. Prior to treatment with GDC-0449 molecular profiling revealed a somatic mutation in Patched 1 (PTCH1-W844C) and upregulation of hedgehog target genes. The PTCH1-W844C mutant was not capable of suppression of smoothened activity in a Hh responsive, Gli-luciferase reporter cell line (16). The loss of a functional PTCH transmembrane receptor causes a loss of the normal tonic repression of the smoothened receptor by the PTCH receptor with consequent tumor growth attributable to hedgehog pathway activation.

**Management of acquired molecular resistance to hedgehog-directed therapeutics**

In the mentioned case of metastatic medulloblastoma, the transient improvement with GDC-0449 in the tumor extent did not persist and a biopsy or recrudescent tumor confirmed the previously detected PTCH-W844C mutation, accompanied by loss of heterozygosity of PTCH leading to constitutive activation of the hedgehog pathway due to a lack of functional PTCH.

In general, mutations in Smoothened account for acquired molecular resistance to GDC-0449 in medulloblastoma. In an evaluating of the mechanism of acquired resistance to GDC-0449 of the aforementioned patient’s medulloblastoma, an amino acid substitution of a conserved aspartic acid residue disrupted GDC-0499 binding to the serpentine Smoothened receptor (16). The investigators identified a heterozygous guanine to cytosine (G to C) missense mutation at position 1697 in the Smoothened locus which was predicted to change codon 473 from Asp to His (D473H) in the GDC-499 resistant progressive medulloblastoma, a change that was not detected in the initial tumor tissue. This mutant allele was found in the recurrent tumor tissue and not in the patient’s initial primary/metastatic tumor or skin biopsy. An analogous well-established scenario is that of chronic myelogenous leukemia that is refractory or develops resistance to imatinib but is responsive to dasatinib. This type of chronic myelogenous leukemia is resistant in vitro and in vivo to dasatinib when it acquires the T315I mutation (17).

In a mouse model interrogating the mechanism for GDC-0449 resistance in medulloblastoma (a drug resistant subcutaneous allograft of medulloblastoma in Pch1 +/-; p53-/- mice selected for GDC-0449 resistance), three separate GDC-0449 resistant tumor lines were established. In one arising drug resistant tumor cell line model, Smoothened...
was sequenced and a heterozygous missense mutation at 1944 causing an aspartic acid-477 to glycine (D477G) change was found that was not in the parental model. In fasciulating evidence, the corresponding residue in human Smoothened is aspartic acid at position 473, the affected locus that was found in the case of the aforementioned relapsed GDC-449 resistant medulloblastoma (16). Predictive modeling concluded that the Asp-473 residue is at the C-terminal end of the sixth transmembrane segment, near the extracellular lip of the heptahelical transmembrane Smoothened receptor.

A correlative example of acquired molecular resistance to targeted therapeutics in cellular components other than those of the hedgehog pathway was described recently in a patient with echinoderm microtubule-associated protein-like 4–anaplastic lymphoma kinase (EML4–ALK) subtype, non-small cell lung cancer (18). Two secondary mutations arose within the kinase domain of the protein product arising from the EML4–ALK fusion gene in tumor cells isolated from a patient during the relapse phase after 5 months of treatment with the ALK inhibitor, crizotinib. These alterations were, G→A and C→A changes at positions corresponding to nucleotides 4374 and 4493 of wild-type ALK complementary DNA. These corresponded to C1156Y and L1196M mutants; the cysteine at position 1156 resides close to the upper edge of the adenosine triphosphate-binding pocket and leucine at position 1196 resided within the kinase domain. Interestingly, L1196 of ALK corresponds to the threonine at position 315 in ABL and at position 790 in the epidermal growth factor receptor, each of which is the site of the most frequently acquired mutations that confer resistance to tyrosine kinase inhibitors in these kinases. Though these are fascinating examples from other molecularly targeted tumor components, it is to be recalled that the evidence from other targeted pathways is predominated by altered kinase function whereas Smoothened is a transmembrane receptor proto-oncogene that relies on heterotrimeric G proteins for signal transduction and can incur molecular mutations but is not a tyrosine kinase or serine threonine kinase.

Ultimately, it may be inferred from preclinical experiments and correlative evidence from other targeted therapeutic model systems that resistance to Smoothened antagonists may be due to molecular mutations in the Smoothened receptor. This resistance is more probably to emerge in tumor subclones rather than homogeneously within tumor masses and the practical implications of this is that withdrawal of a Smoothened antagonist in the absence of an alternative medication could theoretically lead to a rebound tumor flare in the repressed hedgehog reliant residual component of the tumor.

The scenario of aberrant activation of the hedgehog pathway intra-cellular to Smoothened where Smoothened antagonists are ineffective has been described in cases of medulloblastoma, gliomas, pericytomas and prostate cancer (19). Smoothened independent mechanisms for hedgehog pathway activation include loss of heterozygosity of the tumor suppressor gene SUFU, amplification or chromosomal translocation of the Gli genes or stabilization of the translated Gli protein. In the particular case of pancreatic cancer, resistance to Smoothened antagonists may arise by subversion of the pathway by cross talk from the RAS/Raf/MEK pathway. Cyclopamine resistant Gli1(+ ) cell lines derived from adenocarcinoma of the pancreas have previously been reported (20). Candidate screened small molecule inhibitors to tackle the difficulty of Hedgehog activation independent of Smoothened and downstream of SUFU has given rise to the GANT compounds 58 and 61. They both act in the nucleus to block Gli-mediated transcription and GANT 61 also blocks Gli1 DNA binding (19).

Preclinical studies of hedgehog and pancreatic cancer

In considering the clinical implications of experimental evidence for hedgehog pathway inhibition in pancreatic cancer, it is important to appreciate if the evidence is from pancreatic cell lines, tumor xenografts or mouse models that more truly replicate ductal adenocarcinoma of the pancreas. The latter is demonstrated in KPC mouse models that conditionally express mutant KRAS and p53 in pancreatic cells and have the fibroinflammatory tumor investing tissue that occurs in human pancreatic cancer. The dense desmoplastic stromal reaction of pancreatic cancer is an established impediment to delivery of chemotherapy and pancreatic cancers have a hyperperfusing vasculature. Experimental evidence has demonstrated that Shh is correlated with desmplasia in pancreatic cancer (21). Therefore, targeting the Shh pathway potentially could facilitate the delivery of chemotherapeutic drugs by diminishing the desmoplastic stromal reaction. This concept was investigated by a multicentre group using KPC mice. KPC mice demonstrated resistance to gemcitabine compared with transplantation mouse models and Olive et al. in a series of experiments showed that impaired drug delivery was the probable cause. IPI-926, a semisynthetic derivative of cyclopamine that inhibits Smoothened was studied with gemcitabine. Combination treatment with IPI-249/gemcitabine extended median survival of KPC mice from 11 to 25 days (P = 0.001; log-rank test), most tumors decreased in size and IPI-926/gemcitabine treatment significantly decreased metastasis to the liver (P = 0.015; Fischer’s exact test). Treatment benefits were transient, however. Theoretically, combination treatment with a hedgehog antagonist and gemcitabine may increase the tumors bioavailability of the cytotoxic drug (22). Furthermore, in a separate study Feldmann et al. (23), in a genetically engineered mouse model of pancreatic cancer found that hedgehog inhibition with cyclopamine prolonged survival and abrogates systemic metastasis. That study from Johns Hopkins found that the Hedgehog pathway is activated in Pdx1-Cre; Lsl-Kras (G12D); Ink4a/Arf (fox/lox) transgenic mice models of pancreatic cancer. Hedgehog inhibition with cyclopamine significantly prolonged median survival from 61 to 67 days, P = 0.02. In vitro data indicates that the Hedgehog activation is at least partly attributable to activation of KRAS signaling similar to medulloblastoma studies. Finally, hedgehog antagonists may be therapeutically effective in pancreatic cancer because pancreatic cancer has increased activation of the hedgehog pathway. A study of digestive tract tumors found that tumors originating from the pancreas have increased Hh pathway activity suppressible by a Hh antagonist (5).

A second mechanism by which hedgehog antagonism may be therapeutically efficacious in pancreatic cancer is through a paracrine effects on the tumors stromal cells. Yauch et al. have demonstrated that ligand dependent activation by hedgehog occurs in the tumor stromal environment. Exploiting interspecies differences, it was shown that inhibition of hedgehog signaling using an anti Hh antibody or genetic deletion of Smoothened in mouse stroma resulted in tumor growth inhibition in xenograft tumor models (16). Furthermore when hedgehog antagonists were tested in 125 cell lines, no correlation was seen between the extent of basal Hh pathway activity and cellular sensitivity to Hh Antag. Mechanistically, it appears that hedgehog secreted by tumors acts on Smoothened in the stromal cells leading to changes in the extracellular matrix, structural support to tumor growth and stromal cytokine release such as Wnt and insulin like growth factor (IGF) that leads to tumor growth.

Pancreatic cancer stem cells and hedgehog

Cancer stem cells have the properties of self-renewal and multilineage differentiation. They are at the apex of tumor biology architecture. Fluorescent-activated cell sorting and tumor xenograft models in immuno-compromised mice have been used to identify cancer stem cells. In pancreatic cancer, cells with the CD44+CD24–ESA+ cancer stem cells that are essential for tumor metastasis (25). It was found that pancreatic cancer tissue contains these cells that are tumorigenic and highly resistant to standard chemotherapy. CD133+CXCR4+ cells occupy the invasive front at
the tumor host interface of pancreatic tumors and determine the individual tumors, metastatic phenotype. Depletion of the cancer stem cell pool for these migrating cancer stem cells virtually abrogated the metastatic phenotype of pancreatic tumors without affecting their tumorigenic potential. A majority of patients with pancreatic cancer present with locally advanced or metastatic disease and the life expectancies for non-metastatic disease is 6–10 months and 3–6 months for metastatic disease, respectively (26). This emphasizes the importance of effectively targeting the subpopulation of cells in pancreatic cancer responsible for distant disease. If Shh signaling was found to be an important intracellular pathway for the stem cell subcategory defined by CD133(+)CXCR4(+) immunophenotypic positivity that would be a potent theoretical advance in targeted therapeutics and pancreatic cancer. Experimental evidence remains lacking in this specific regard; however, it is probably that upregulation of the hedgehog pathway occurs in CD133(+)CXCR4(+) pancreatic cancer stem cells as it is increased in pancreatic cancer stem cells defined by the CD44+CD24−ESA+ immunophenotype.

In general, therapeutics directed against pancreatic cancer stem cells offer new strategies to treat the disease. As stem cell utilise self-renewal pathways including PTEN, WNT, BMI-1 and Notch in addition to hedgehog pathways including PTEN, WNT, BMI-1 and Notch in addition to the hedgehog pathway involved in apoptosis, DNA damage control, regulation in G1/S phase transition, homophilic cell adhesion, integrin signaling, c-Jun N-terminal kinase signaling, Kras signaling, regulation of invasion, small guanosine triphosphatase dependent signaling, transforming growth factor-beta signaling and Wnt/Notch signaling (27) Table I. Firstly; considering localized and therefore resectable disease. There is an existing phase III evidence supporting adjuvant treatment with gemcitabine of pancreatic cancer in the CONKO-001 trial (31). *In vitro* and *in vivo* studies of treatment with cyclophosphamide has shown that inhibition of hedgehog can abrogate pancreatic cancer metastasis. Treatment of pancreatic cancer cell lines inhibited epithelial to mesenchymal transition by upregulating E-cadherin and downregulating snail (a transcription regulator that downregulates the expression of E-cadherin). Also overexpression of GLI1 *in vitro* cell line studies lead to marked tumor invasion. Also in murine orthoptic xenograft models, cyclophosphamide inhibited metastatic spread and synergized with gemcitabine such that metastasis were completely eliminated (32). The accruing evidence suggests that there may be a future trial of a hedgehog inhibitor incorporated into established treatment paradigms for the adjuvant treatment of pancreatic cancer (ref. Table II).

What of locally advanced pancreatic cancer (LAPC)? In the USA, 40% of patients diagnosed with pancreatic cancer have locally advanced disease (33). This disease is deemed unresectable because of local invasion into retroperitoneal vessels without detectable metastasis. In a retrospective cohort study of data from the Surveillance Epidemiology and End Results program conducted by the Dana Farber Cancer Institute, only 24% of patients with LAPC received what was considered the standard of care; radiotherapy with concomitant S-fluorouracil (34). As Smoothened antagonists decrease the desmoplastic stoma of pancreatic cancer, LAPC represents a treatment group with the promise of important therapeutic improvements if hedgehog inhibitors are incorporated onto the relevant treatment algorithms.

A number of clinical trials have been designed to target the desmoplastic stoma that invests pancreatic cancer cells. This is of importance as depletion of the fibroblastic stroma that contributes to the hypoperfusing tumor vasculature may improve the bioavailability of administered chemotherapeutics. Additionally diminished desmoplasia may remove involvement or encasement of the superior mesenteric artery or superior mesenteric vein portal venous confluence traditional contraindications to pancreaticoduodenectomy. A caution to note is that a study of 593 patients found that the need for vascular resection did not adversely affect postoperative morbidity, mortality or overall survival post pancreaticoduodenectomy (35).

Targeting the fibroblastic tumor stroma of pancreatic cancer is not the exclusive domain of Smoothened antagonists. Nab-paclitaxel (Abraxane later called Abraxix) is a chemotherapeutic agent that was developed to obtivate Cremophor from paclitaxel preparations and consists of albumin bound nanoparticles. The albumin in nab-paclitaxel binds to blood vessels in the tumor microenvironment and to a protein entitled secreted protein acid rich in cysteine (SPARC), that is highly expressed in pancreatic cancer. This has the effect of concentrating the drug within the tumor. A phase II/II study of 63 patients by Von Hoff et al. evaluating the treatment efficacy and toxicity of the combination of gemcitabine and nab-paclitaxel in metastatic pancreatic cancer was reported at the American Society of Clinical Oncology annual meeting in 2009. In that study, serial positron emission tomography scans of 53 patients showed a 23% complete responses rate, 55% partial response rate and 8% stable disease. Of 49 patients evaluated by RECIST criteria, 1 (2%) had a complete response, 12 (24%) had a partial response and 20 (41%) had stable disease. The median survival was 9 months at time of assessment. Patients that were SPARC positive (8/27) were more probably to be responders (75%) than patients who were SPARC negative (26%), P = 0.03. It also appears that a positive SPARC status in these patients treated with nab-paclitaxel and gemcitabine was associated with a longer progression free survival (36). Of relevance, a phase II trial NCT01088815 being lead by The Sidney Kimmel Comprehensive Cancer Centre at Johns Hopkins, Baltimore, Stand up to Cancer and industry sponsors is listed by the National Cancer Institute (NCI) as involving the treatment of metastatic adenocarcinoma of the pancreas with GDC-0449 in combination with chemotherapy (gemcitabine and nab-paclitaxel).

The remarkable efficacy of the nab-paclitaxel gemcitabine combination in metastatic pancreatic cancer has been shown to be due to

### Hedgehog and human adenocarcinoma of the pancreas

Pancreatic adenocarcinoma arises from precursor lesions, pancreatic intratapleial neoplasia (PanIN) that progress from PanIN 1 to 3. PanIN-1 lesions have Kras mutations and overexpression of Her2. In PanIN-2, p16 mutations occur. In PanIN-3 DPC4, P53 and BRCA2 mutations predominate. Shh expression is both a feature of PanIN and pancreatic adenocarcinoma implicating hedgehog as an early and late mediator of pancreatic cancer tumorigenesis. Recurrently altered signaling pathways in pancreatic cancers were determined by Jones et al. by a comprehensive genetic analysis of 24 pancreatic cancers. They sequenced pancreatic cancers transcripts and searched for homozygous deletions and amplifications by microarrays containing probes for ~1 million single nucleotide polymorphisms. It was found that pancreatic cancers contain an average of 63 genetic alterations, the majority of which are point mutations. A core set of 12 cellular signaling pathways one of which was the Hedgehog pathway were identified and they were each genetically altered in 67–100% of the tumors. In the Hedgehog pathway,19 altered genes were identified and 100% of tumors had alterations in at least one of the hedgehog pathway genes. Representative genes in the hedgehog pathway included SOX3, LRP2, TBX 5, GLI1, GLI3, BOC, BMP2 and CREBBP. Of the other altered pathways, they were involved in apoptosis, DNA damage control, regulation in G1/S phase transition, homophilic cell adhesion, integrin signaling, c-Jun N-terminal kinase signaling, Kras signaling, regulation of invasion, small guanosine triphosphatase dependent signaling, transforming growth factor-beta signaling and Wnt/Notch signaling (27) Table I. Therapeutics in pancreatic cancer exploiting the hedgehog pathway

Pancreatic cancer can be divided into localized, locally advanced and metastatic disease. Each subcategory has differing management algorithm and prognosis. Integration of inhibitors or hedgehog signaling also confers different management and outcome possibilities in each subcategory.

In general, therapeutics directed against pancreatic cancer stem cells offer new strategies to treat the disease. As stem cell utilise self-renewal pathways including PTEN, WNT, BMI-1 and Notch in addition to hedgehog, therapeutics directed against these pathways are particularly selective for stem cells. As stem cells frequently evade cytoreductive chemotherapy because they are relatively quiescent and divide infrequently, therapeutics directed against stem cells may decrease tumor repopulation post treatment. Also as CD133(+)CXCR4(+) determines the metastatic phenotype of medications that antagonize the hedgehog pathway into adjuvant treatment paradigms may prove to be particularly efficacious. However, preclinical work remains to be done as it has not been definitively shown that CD133(+)CXCR4(+) cells have increased levels of Shh pathway activity compared with other pancreatic cancer cells.
Increased bioavailability of the chemotherapeutic medications in a mouse xenograft tumor model due to stromal collapse by A. Maitra and M. Hidalgo (36). Further experimental data has been accrued on tumor cell; stromal interactions in pancreatic cancer. A recent study found that SmoM2 is upregulated in pancreatic cancer-associated fibroblasts relative to control fibroblasts and that cancer-associated fibroblasts transduce the Shh ligand downstream intracellular signal to activate expression of Gli1. In microarray analysis of primary pancreatic cancers, intraductal papillary mucinous neoplasms and chronic pancreatitis tissue samples, stromal fibroblasts in chronic pancreatitis were weakly positive for SmoM2 expression in 73.0% of cases, SmoM2 expression in intraductal papillary mucinous neoplasm was 80.0% through weaker than in pancreatic cancers. SmoM2 was heterogeneously expressed in stromal fibroblasts in 96.2% of pancreatic cancers and silent RNA knockdown of SMO blocked the ability of cancer-associated fibroblasts to induce Gli1 expression (37).

GEMCIBATINE is the currently accepted standard of care in advanced pancreatic cancer. Efforts to improve the efficacy of gemcitabine have been mainly unsuccessful. A study by the NCI of Canada Clinical Trials Group found a survival benefit for the combination of gemcitabine and erlotinib over gemcitabine alone of 191 days compared with 177 days (hazard ratio for death 0.82; \( P = 0.02 \)) (38). Though statistically significant the incremental gain in overall survival was not of appreciable clinical importance; however, the paradigm of small molecule-targeted therapeutics having efficacy in this disease was established. Presently, there are six ongoing studies of SmoM2 antagonists (GDC-0449 and IPI-926) incorporation into treatment paradigms for pancreatic cancer being sponsored by the US NCI (see Table II).

### Table II. Studies implicating Shh in pancreatic cancer

<table>
<thead>
<tr>
<th>Principle author</th>
<th>Study Title</th>
<th>Study Design</th>
<th>Study findings</th>
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<tr>
<td>Thayer et al. (20), Massachusetts General Hospital</td>
<td>Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis</td>
<td>Transgenic mice models; screening human adenocarcinoma cell lines for expression of Hh components; Gli-luciferase reporter construct, cell line transfection</td>
<td>Hedgehog may have an early and critical role in pancreatic carcinogenesis aberrant proliferation and tumorigenesis</td>
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<td>Berman et al. (5), Howard Hughes Medical Institute, Johns Hopkins</td>
<td>Widespread requirement for hedgehog ligand stimulation in growth of digestive tract tumors</td>
<td>Cell line Shh, Ihh messenger RNA; Hh-inducible Gli-luciferase reporter; pancreatic carcinoma xenografts; Cyclopamine, 5E1 monoclonal Ab studies</td>
<td>Digestive tract tumors: esophagus, stomach, biliary tract and pancreas but not colon display increased Hh pathway activity</td>
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<tr>
<td>Jones et al. (27), Johns Hopkins</td>
<td>Core signaling pathways in human pancreatic carcinomas revealed by global genomic analysis</td>
<td>Genetic analysis of 24 pancreatic cancers. Determined sequences of 23 219 transcripts representing 20 661 protein-coding genes.</td>
<td>Core set of 12 signaling pathways altered in 67–100% of tumors. Included Hedgehog pathway</td>
</tr>
<tr>
<td>Bailey et al. (28), Eppley Institute, University of Nebraska, Morton et al. (29), University of Mass, PNAS</td>
<td>Sonic hedgehog promotes desmoplasia in pancreatic cancer</td>
<td>Experienced Shh in transformed cell line from human pancreas; inhibited Shh activity in vivo in mice</td>
<td>Shh contributes to the formation of desmoplasia</td>
</tr>
<tr>
<td>Bailey et al. (28), Eppley Institute</td>
<td>Sonic hedgehog paracrine signaling regulates metastasis and lymphangiogenesis in pancreatic cancer</td>
<td>Orthopedic transplantation of cell lines expressing Shh; treatment with 5E1</td>
<td>Enhances proliferation of pancreatic ductal epithelial cells and protects against apoptosis. Shh co-operates with activated Kras and reduces dependence on sustained activation of mitogen-activated protein kinase and phosphatidylinositol 3Kinase/Akt/mammalian target of rapamycin</td>
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<td>Tian et al. (30), Genentech, PNAS</td>
<td>Hedgehog signaling is restricted to the stromal compartment during pancreatic carcinogenesis</td>
<td>An oncogenic allele of Smoothened SmoM2 was expressed to autonomously activate Hh in mouse pancreas</td>
<td>Hedgehog pathway was not activated by expression of SmoM2 in epithelial cells but was activated by expression in mesenchymal cells</td>
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**Inhibition of hedgehog signaling: a therapeutic advance**

Hedgehog directed therapeutics offer new hope in the treatment of pancreatic cancer. It potentially may cause tumor regression by intrinsic inhibition of the hedgehog pathway, decrease the cancer stem population, have a paracrine effect on the tumor stromal cells and improve the therapeutic efficacy of cytotoxic drugs by affecting the desmoplastic fibrotic response and improving chemotherapeutic drug bioavailability. The NCI sponsored trial of GDC-0449 and erlotinib hydrochloride in metastatic pancreatic cancer or unresectable solid tumor is one potentially important advance. Trials with Smoothened antagonists are most probably to have positive findings when the study design involves a Smoothened antagonist given as part of a therapeutic combination. A study in prostate cancer showed that cytotoxicity was induced when cyclopamine and gefitinib a small molecule inhibitor of epidermal growth factor receptor were combined (39,40). Studies have also shown that the phosphoinositide 3-kinase/AKT pathway is essential for Shh signaling (41). Combination treatment with PI3-kinase, mammalian target of rapamycin inhibitors and a Smoothened antagonist is a potentially important combination in treatment of malignant diseases in which hedgehog is implicated. However, it should be noted with regard to the specific case of pancreatic cancer KRAS has been shown to activate hedgehog in pancreatic ductal adenocarcinoma cells by the RAF/MEK/mitogen-activated protein kinase pathway and not the PI3K/AKT pathway. A clinical trial with combined treatment with a MEK inhibitor and Smoothened antagonist may arise in the future (41,42). It is also notable that an analysis of RAS and Hedgehog signaling found that oncogenic RAS induces Shh ligand expression and inhibits the canonical (HH-Ptch-SmoM2-initiated) Hedgehog pathway. It furthermore modulates the (transforming growth factor-\(b\)-initiated) Hedgehog pathway. All these molecular consequences are done by activating DYRK1B. The implication of these findings is that mutant KRAS induces hedgehog signaling in neighboring cells and simultaneously blocks hedgehog signaling in Shh producing tumor cells (43). Yauch et al. in their experiments establishing a paracrine requirement for hedgehog signaling in tumorigenesis found that inhibition of
Hedgehog inhibition for pancreatic ductal adenocarcinoma in the preparative setting  

Phase II, OCRD 201014, NCT01096732  

Operable localized pancreatic cancer treated with GDC-0449 for ~2 weeks from diagnosis to surgery. Initial biopsy and surgical samples  

To detect a change in Hedgehog signaling in peritumoral pancreatic tissue

Hedgehog inhibitors for metastatic adenocarcinoma of the pancreas  

Phase II, J1013, NA 00032683, NCT01088815  

Metastatic adenocarcinoma of the pancreas treated with GDC-0449 in combination with chemotherapy (gemcitabine and nab-Paclitaxel). Open label single arm, multicenter trial  

Progression-free survival

Pilot study of the effect of Hedgehog antagonist GDC-0449 and gemcitabine hydrochloride on cancer stem cells in patients with advanced pancreatic cancer  

No phase specified, UMCC-2010-003 2010-003, 8417, NCT01143 415  

To evaluate the number and percentage of pancreatic cancer stem cells before and after treatment with GDC-0449 in patients with advanced pancreatic cancer.  

Proportion of pancreatic cancer stem cells before and after treatment with GDC-0449. Number of circulating tumor cells at 0, 15 and 43 days.

Phase II randomized study of gemcitabine hydrochloride with versus without Hedgehog antagonist GDC-0449 in patients with recurrent or metastatic pancreatic cancer  

Phase II, UCCRC-84188418, NCI NCT01064622  

Patients with recurrent or metastatic pancreatic cancer treated with gemcitabine hydrochloride with versus without hedgehog antagonist GDC-0449.  

Compare progression free survival of patients with recurrent or metastatic pancreatic cancer treated with gemcitabine hydrochloride with versus without hedgehog antagonist GDC-0449.  

Comparative study of IPI 926 in combination with gemcitabine in patients with metastatic pancreatic cancer

Phase I study of Hedgehog antagonist GDC-0449 and erlotinib hydrochloride with or without gemcitabine hydrochloride in patients with metastatic pancreatic cancer or unrespectable solid tumors  

Phase I, MAYO-MC0811MC0811, 8231, NCT00878163  

GDC-0449 and erlotinib hydrochloride with or without gemcitabine hydrochloride in treating patients with metastatic pancreatic cancer or solid tumors that cannot be removed by surgery  

To determine the maximum tolerated dose of erlotinib hydrochloride and GDC-0449 with or without gemcitabine hydrochloride in patients with unrespectable solid tumors  

Evaluation of IPI 926 in combination with gemcitabine

A study evaluating IPI-926 in combination with gemcitabine in patients with metastatic pancreatic cancer  

Phase II, phase I, IPI-926-03NCT01130142  

To assess the effect of treatment with GDC-0449 alone and in combination with gemcitabine hydrochloride on total circulating tumor cells  

To detect a change in Hedgehog signaling in peritumoral pancreatic tissue

Search performed November 2010

Hh signaling in the stroma can also modulate factors, such as IGF and Wnt signaling pathway components (44). Strands of evidence exist implicating both wnt and IGF in pancreatic cancer, re-emphasizing the potential therapeutic importance of inhibition of the hedgehog pathway. In a study of human pancreatic ductal adenocarcinoma cell lines and their xenografts the following was discovered: (i) hedgehog promotes neovascularisation in pancreatic cancers by regulating Ang-1 and IGF-1 expression in bone marrow-derived pro-angiogenic cells and (ii) host-derived Ang-1 and IGF-1 messenger RNA levels were downregulated by cyclopamine in tumor xenografts. Regarding wnt; in vitro and in vivo studies in human pancreatic tumor cell lines and ectopic mouse model attempted to resensitize gemcitabine refractory pancreatic cells with masitinib (a multitargeted tyrosine kinase inhibitor). Masitinib was found not to have single agent activity but it sensitized Mia Paca2 cells to gemcitabine with a 400 fold reduction in the IC50. Interestingly in transcriptional analysis, the Wnt/beta-catenin signaling pathway was downregulated in the cell lines resensitized by the masitinib/gemcitabine combination.

Greater treatment efficacy in pancreatic cancer is probably to emerge in clinical trials of chemotherapeutics when Smoothened antagonists which target both tumor and stromal cells are used. Pancreatic cancer tumor stromal cells are responsible for hypovascular tumor perfusion due to desmoplasia. Locally advanced and localized pancreatic cancers offer the most favorable theoretical opportunity in this respect. Furthermore, the benefit of selective targeting of pancreatic cancer initiating stem cells, tumor cell proliferation and growth offer additional potential therapeutic benefit.

Conclusion

The 5 year survival rate for pancreatic cancer is 4% compared with the 5 years survival of an American patient diagnosed with breast cancer of 86% and 97% for prostate cancer (45). Preclinical and clinical evidence exists for the important role hedgehog signaling has in pancreatic cancer tumorigenesis with overexpression of Hedgehog ligand occurring in 70% of cases. LAPC representing 30–40% of pancreatic cancers is the most probable subset to derive benefit from inhibition of Hedgehog signaling by targeting Smootherned. A NCI phase II study of GDC-0449 and erlotinib in pancreatic cancer and solid tumors is one potentially important therapeutic advance and further NCI trials involving Smoothened antagonists are accruing or planned. A randomized phase II trial of gemcitabine +/- GDC-0449 for metastatic
pancreatic cancer is also currently ongoing. Hedgehog inhibition incorporated onto treatment paradigms hopefully will offer improved outcomes in this comparatively chemoresistant and radio-resistant malignancy.

Conflict of Interest Statement: None declared.

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