REVIEW ARTICLE

Regulation Mechanisms of the Hedgehog Pathway in Pancreatic Cancer: A Review

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is the fourth most common cause of death from cancer. Its 5-year survival rate is less than 5%. This poor prognosis is mostly due to the cancer's early invasion and metastasis formation, leading to an initial diagnosis at an advanced incurable stage in the majority of patients. The only potentially curative treatment is radical surgical resection. The effect of current chemotherapeutics or radiotherapy is limited. Novel therapeutic strategies are therefore much needed.

One of the hallmarks of PDAC is its abundant desmoplastic (stromal) reaction. The Hedgehog (Hh) signaling pathway is critical for embryologic development of the pancreas. Aberrant Hh signaling promotes pancreatic carcinogenesis, the maintenance of the tumor microenvironment and stromal growth. The canonical Hh-pathway in the tumor stroma has been targeted widely but has not yet lead to hopeful clinical results. Targeting both the tumor and its surrounding stroma through Hh pathway inhibition by also targeting non-canonical pathways as apparent in the tumor cell may therefore be a novel treatment strategy for PDAC.

INTRODUCTION

Pancreatic Cancer

Pancreatic ductal adenocarcinoma (PDAC) accounts for more than 90% of pancreatic neoplasms. It is the fourth most common cause of death from cancer in the United States. In 2013, there were 45,220 new cases and 38,460 estimated deaths from PDAC [1]. Most patients are incurable by the time they first develop symptoms. Only five to ten percent present with surgically resectable disease, the only curative treatment to date [2]. Untreated metastatic pancreatic cancer has a median survival of three to five months and six to ten months for locally advanced disease [3]. The overall median survival rate is less than six months, with a 5-year survival rate of less than 6% [4, 5].

Similar to colorectal cancer, pancreatic cancer develops through precursor lesions. The lesions display atypical mucinous epithelium replacing the physiological cuboidal epithelium. Developmental stages range from PanIN 1A to PanIN 3 (carcinoma in situ) [6]. These precursor lesions have an increase in p16 and k-ras mutations with more atypia. As described above, ductal adenocarcinoma account for 90-95% of pancreatic tumors and can occur anywhere in the pancreas [7]. Most occur in the pancreatic head. Ductal adenocarcinoma is characterized by abundant fibrosis, termed desmoplasia. Perineural and vascular invasion are both features of invasive carcinoma.

Seven percent of pancreatic cancers are thought to have some genetic background. BRCA2 mutation carriers have a three to five- fold (95%CI 1.9-6.6) increased risk for PDAC development [8]. About 95% of pancreatic cancers display a loss of function mutation of the tumor suppressor gene p16 [9]. Mutations of the Kras gene product that transduces signals to the growth factor receptor are evident in more than 90% of ductal lesions [10]. P53, that usually controls the cell cycle by inhibiting entry into the S-phase, is mutated in over 50 % of cases [11]. The DPC4/MADH4 gene product dpc4 is completely lost in 55% of infiltrating ductal adenocarcinomas and cancers express CEA, mesothelin and p53 [7].

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Abbreviations PDAC Pancreatic ductal adenocarcinoma
HhHedgehog
Smo Smoothened
Ptch Patched
Hhat Hedgehog acyltransferase
Sufu Suppressor of Fused
Hhip Hedgehog inhibiting protein
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THE HEDGEHOG PATHWAY

Origin

The Hedgehog pathway (Hh) was first discovered in Drosophila, where it governs embryological development. Edward B. Lewis, Christiane Nuesslein-Volhard and Eric F. Wieschaus were awarded the Nobel Prize in 1995 for studying gene mutations in the embryogenesis of the fruit fly, which ultimately led to the discovery of the Hh gene [12,

13]. Hedgehog regulates parts of embryonic segmentation and patterning of adult fly appendages. It also specifies cell types in the dorsal epidermis [14].

In mammalian development there are three Hedgehog genes, e.g. Desert, Indian and Sonic Hedgehog (Dhh, Ihh and Shh respectively). Ihh modulates the formation of cartilage in the appendages and functions as a negative regulator of the differentiation of proliferating chondrocytes [15]. Dhh is associated with germ-cell proliferation, the development of germ cells toward the later stages of spermatogenesis, with interactions of nerve-Schwann cells and signaling peripheral nerve encoating [16, 17]. Sonic Hedgehog is the best studied among all three ligands. In vertebrate embryos Shh regulates dorsal-ventral patterning of the neural tube, the anterior- posterior axis of the limb bud and the somites [18]. When Shh components were knocked out in mice, failed development of the musculature, skeleton, brain and GI tract resulted [19-24]. Shh is a secreted factor made by the endoderm as the gut forms [25]. It targets the adjacent mesoderm, which is demonstrated by high expression of the target gene Patched (Ptch) in the visceral mesoderm [26]. In addition, Shh establishes functions of gut-derived tissues. When Shh is ectopically expressed in the developing pancreatic epithelium, it causes the pancreatic mesoderm to develop into smooth muscle and interstitial cells of Cajal (intestinal cells) [27]. However there is no effect on the endoderm of the pancreas, when Shh is misexpressed, supporting the hypothesis that Shh appears to only be a signal transducer from endoderm to mesoderm [26]. The Gli genes, further downstream members of the Hh-pathway, play important roles in limb and craniofacial development like eyes, nose and teeth as well as in murine lung embryology [28]. Section in situ hybridization of the murine lung revealed the highest concentration of Gli expression in the mesoderm a few nanometers away from the endoderm.

Hh Signaling in the Pancreas

Hh signaling in normal pancreas and in PDAC is exclusively paracrine, with expression of Shh limited to epithelium and response restricted to stroma [29-33]. When Smo was genetically silenced in the pancreatic epithelium of PDAC-susceptible mice, development of tumors was not altered, suggesting that Hh signaling does not occur in an autocrine fashion. In paracrine signaling, tumor-derived Hh ligand signals locally to the stroma, and provides a selective growth advantage for the tumor. This paracrine model of Hh signaling has been established in pancreatic carcinoma where Hh signaling is required for tumor growth but the tumor cells themselves are non-responsive to Hh ligand [34]. Yauch *et al*. utilized species-specific expression profiling to show that Hh pathway antagonist treatment resulted in downregulation of Hh target genes only in the stroma compartment but not within the epithelial cancer cells. Similiarly, Smo expression in mesenchymal cells in the pancreas led to Hh pathway activation. Additionally, when recombinant Shh was added, an increase in proliferation and migration in human pancreatic stellate cells (HPSC) was noted, whereas no change was observed in pancreatic cancer cells (Bxpc3 and Panc1), further supporting a paracrine model in the tumor stroma [35].

Of note is that medulloblastoma which in one third of cases is characterized by an overexpression of Shh target genes, tends to also depict desmoplastic histology in 40 % of the time [36]. The tumor cells mainly produce the ligand Shh themselves. Additionally, the stromal cells stimulate the expression of the ligand Shh in the tumor cell NF-κB-dependent [37]. Inhibition of upstream Hh- pathway members, such as Cyclopamine, leads to stromal growth inhibition [38], underlining the importance of the canonical pathway in tumor stroma.

The Hh-pathway promotes metastasis by increasing snail protein expression and reducing E-cadherin and tight junction expression. Also, Shh increases the angiogenic factor angiopoietin-1, decreases angiopoietin- and antiapoptotic genes, and increases Cyclins (D1 and B1) and proapoptotic genes, like Fas [39-41].

PDGFRα was detected strongly in aberrant crypts and moderately in stroma of colorectal cancers that expressed Gli1. Thus, it may imply that Shh-Gli1 pathway in colorectal cancers is activated through increased expression of PDGFRα [42]. The Sonic Hedgehog pathway also plays a role in repopulation of pancreatic cancer cells after radiation therapy as described by Ma *et al*. They showed that irradiated tumor cells with higher Shh and Gli1 expression were associated with stronger tumor cell repopulation. Moreover, the dying cells stimulated living tumor cell growth that could be further enhanced by Shh signaling agonists or recombinant N-terminal fragment of Shh and inhibited by Shh signaling antagonists (Cyclopamine and Gant 61) or knockdown by Gli1shRNA [43].

On the contrary, Lee *et al.* observed that Hh pathway activation caused stromal hyperplasia and reduced epithelial growth whereas pathway inhibition caused accelerated growth of epithelial elements and suppression of desmoplasia in Kras-driven disease in three distinct mouse models of PDAC [33]. The authors concluded that Hedgehog activity controlled the balance between epithelial and mesenchymal growth.

Activation Modes of the Hedgehog Pathway

Studies highlight the existence of the Shh-Ptch-Smo-Gli axis alias canonical Hh pathway as well as growing evidence for non-canonical pathways that differ from the typical route.

Canonical Activation

Canonical activation is defined as a series of repressive interactions which ends in Gli-mediated transcriptional regulation of a variety of cellular processes (Figure 1).

The Hh pathway is activated by secreted ligands, Sonic, Desert and Indian Hh. Shh precursor is a 45 kDa prepeptide, which is cleaved into a 20 kDa N-terminal Shh by autocatalytic activity. The Shh undergoes C-terminal cholesteroylation and N-terminal palmitoylation by Hedgehog acyltrans-

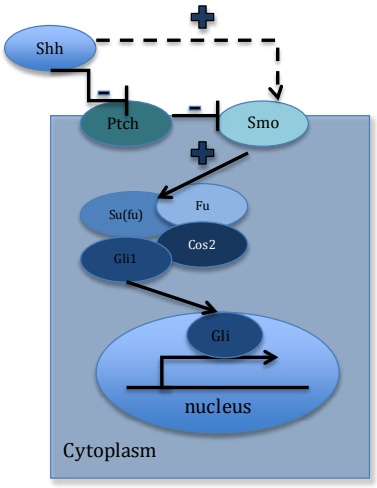

Figure 1. The Hedgehog signaling pathway.
Shh binds to the membrane protein Patched (Ptch). This leads to the indirect activation of Smoothened (Smo) and translocates Gli to the nucleus. Here the sonic hedgehog target genes like Gli, Shh and Ptch are expressed.

ferase (Hhat) for secretion and receptor interaction [44]. This ligand attaches to a transmembrane receptor with 12 membrane-spanning domains and two extracellular loops located on chromosome 9 called Patched1 (Ptch1), a tumor suppressor gene [13]. The binding inhibits the repression of a seven transmembrane G-coupled protein Smoothened (Smo), located on chromosome 7, which is inhibited by the Ptch1 receptor in the absence of the hedgehog ligand by preventing its accumulation in the primary cilium, a single organelle transiently formed during interphase [45]. The former is also inhibited by 3β-hydroxysteroid (Pro-) Vitamin D3 that is moreover pumped by Ptch1 [46].

When the oncogene Smo is released, it is translocated into the cytoplasm where it binds to costal-2, inactivating Suppressor of fused (Sufu) through an unknown mechanism while migrating into the primary cilium [47]. Sufu inhibits transportation of Gli from cytoplasm to nucleus. It stays as a tetra-complex with serine/threonine kinase Fused, the kinestin-like costal-2 and Ci (Gli) [44]. The Sufu gene located on chromosome 10 encodes three different proteins, which all share the same N-terminal [48]. Sufu regulates both the Shh and Wnt signaling, reduces cell proliferation and acts as an oncogene [49]. Within the Wnt pathway Sufu represses β-catenin by shuttling it out of the nucleus, thus repressing β-catenin/Tcf-mediated transcription [49]. An abundant amount of Sufu inhibits Gli1-dependent transcription [50, 51]. The migration of Smo into the primary cilium initiates Sufu's degradation in the proteasomes resulting in the release of Gli2/3 into the nucleus [52, 53]. This leads to the activation of the transcription factor Glioma-associated oncogene homologue 1 (Gli1), a member of the Kruppel family of zinc finger transcription factors, located on chromosome 12 [54-56]. Gli was first identified by Kinzler *et al*. in 1987 [57]. There are three Gli proteins, which are orthologous to Drosophilia cubitus interruptus (ci) that encode both activator and repressor functions [58]. Gli1 acts as a transcriptional activator and oncogene via its C-terminal activator domain, Gli2 is a composite of positive and negative regulatory domains, and Gli3 acts primarily as a transcriptional repressor consistent of an additional N-terminal repressor domain to the usual zinc finger domains and c-terminal activator domain [59].

In the presence of Shh, Gli1 is transcriptionally activated; the phosphorylated and proteolytical ubiquitylation of Gli2 and Gli3 to their truncated repressor forms are inhibited, thus leading to the activation of Hh target genes, such as Gli1, Ptch1 and Hedgehog interacting protein (Hhip), known to diminish ligand diffusion [43, 60]. The inhibiting phosphorylation is performed by protein kinase A (PKA) and glycogen synthase kinase 3 (GSK3). Therefore, the pathway is strictly regulated through a negative feedback mechanism in which activation leads to production of Hhip and Ptch proteins that function to limit Hh signaling [24, 61]. The glycoprotein Hhip is located on chromosome 4 and acts as an antagonist for Shh [60]. It also plays a role in tumor angiogenesis. It is predominantly expressed in endothelial cells. When Notch signaling is upregulated in endothelial cells of vessels during angiogenesis, Hhip is down regulated, which leads to up regulation of Hh-Vascular-endothelial-growth factor (VEGF)-Notch signaling. [62]. Expression is low in gastrointestinal cancers and lung cancers [63]. Hhip inhibits Shh in a similar fashion as Ptch. Hhip, as Ptch, is activated when Shh signaling increases. A frequent loss of heterozygosity is seen in the chromosomal locus of Hhip in pancreatic cancer [60]. Generally it is weakly expressed in pancreatic cancer tissue and absent in many pancreatic cancer cell lines In the majority of pancreatic cancer cell lines the Hhip promoter is hypermethylated which leads to inactivity [64].

Non-Canonical Activation

It is defined as a signaling response that deviates from the canonical paradigm. As seen in colorectal cancer, not all cells express all components of the Hh pathway. Bian *et al.* characterized 25 colorectal adenocarcinoma specimens by in situ hybridization or immunohistochemistry for components of the Hh-pathway (Shh, Ptch1, Gli1 and Hhip). They found that in some cases Ptch1 and Gli1 expression was not in accordance with the expression of Shh suggesting activation by other regulatory mechanisms [42].

Numerous mechanisms have been described (Figure 2):

1) Direct interaction of Hh signaling components with components of other molecular pathways [65]

2) Ligand-independent activation by component mutations

Ad 1) a) Studies have shown a connection between Ptch1 and Cyclin B1 and D1:

Cyclin B acts as a gatekeeper in and out of M-phase during the cell cycle. Cyclin B binds to Cyclin-dependent-kinase1 (Cdk1) in a concentration-dependent manner; the complex is called maturation promoting factor (Mpf). When S-phase is ending, activation of the Mpf occurs via phosphorylation at the activating site of the complex and mitosis is triggered [66]. In vitro evidence by Barnes *et al.* suggested that Ptch1

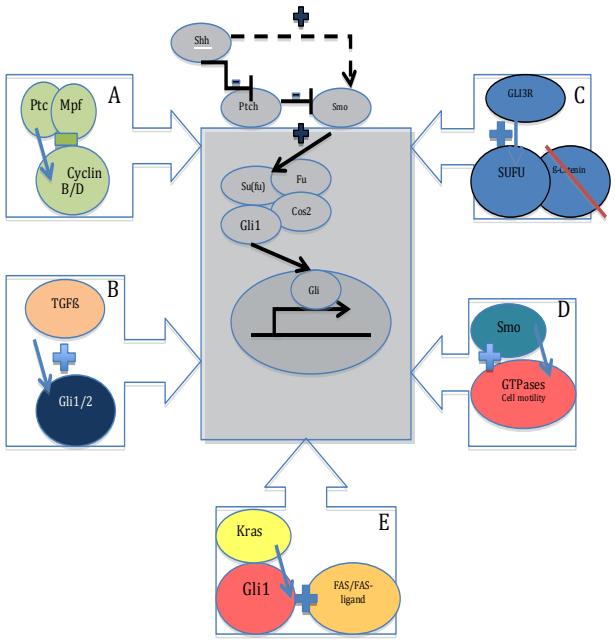

Figure 2. The non- canonical Hedgehog pathway
A) Ptc forms a complex with the maturing promoting factor (Mpf), regulating cell progression into mitosis by inhibiting Cyclin B and D. B) TGF-ß up-regulates Gli1/2 through Smad-3. C) Crosstalks between the Wnt and Hh signaling pathways occur via Gli3-Repressorform (Gli3R), which inhibits ß-Catenin via forming a complex with Sufu.D) Smo directly regulates cell motility via GTPases.E) Gli1 regulates FAS and FAS ligand.

forms a complex with Mpf measured by Immunoprecipitation and therefore directly regulates cell progression into mitosis [67]. Christelle Adolphe *et al.* (2006) generated mice homozygous for a conditional null Ptch1 allele and induced the conditional ablation of Ptch1 in the skin using a specific promoter. Loss of Ptch1 in the basal cell compartment resulted in the development of basal cell carcinoma–like lesions within 4-16 weeks. To clarify the mechanism of Ptch1-induced skin cancer formation, they screened for regulators of the cell cycle (among others) and found a high rate of nuclear expression of Cyclin D1 and B1 in Ptch1-null tumor cells. The authors concluded that constitutive Hh pathway activation promotes the nuclear translocation of cyclin D1 [41]. Katoh and Katoh reported that Gli1 also binds to consensus motifs within the promoter/enhancer motifs MYCN, CCND1 and CCND2 genes upregulating N-Myc, Cyclin D1 and Cyclin D2 and augmenting cell-cycle progression at G1/S and G2/M phases [68].

b) Also, connections between Wnt and Hh signaling have been discovered. In Shh null embryos the Wnt-responsive gene Axin2 was analyzed. Strikingly, compared to controls, Axin2 expression was reduced, suggesting decreased Wnt signaling in the absence of Shh signaling. The authors found that Gli3 was responsible for this effect. When Shh is absent, Gli3 is processed to its repressor form Gli3R. Coelectroporation of Gli3R inhibited the ability of Wnt3 to induce a TCF-luciferase reporter plasmid, indicating an inhibition of the Wnt pathway by Gli3R. Further experiments showed that Gli3R exerted its effect by directly impeding β-catenin by creating a complex with Sufu [69].

c) Furthermore, Kras mutations and upregulated Hh signaling are often found together in pancreatic, lung and colon cancers posing the question of interaction [70-72]. In vitro experiments proved an increase of Gli mediated luciferase activity in Kras-expressing HPDE cells against control. By inhibiting specific targets of RAS downstream effectors Ji *et al.* among others found that intercellular cross talk took place via the RAF/MEK/MAPK pathway [73-76]. Mouse models demonstrated an overexpression of Gli2 when the pancreas harboured an activating Kras mutation [72]. Furthermore, Mills *et al.* identified Gli1 as downstream effector of Kras. Gli1 acts by inducing IL-6 expression, secretion, and promoter activity in pancreatic fibroblasts which triggers STAT3 and tumor initiation [77].

d) Johnson *et al.* suggested a direct regulation of Gli2 expression through Transforming Growth Factor Beta 1 (TGFβ) in mediating breast cancer metastasis to the bone [78]. More specifically Katoh and Katoh described a TGFβ induced Mothers against decapentaplegic homolog 3 (Smad3)-dependent upregulation of Gli1 and Gli2 in human NHDF fibroblasts, HaCaT keratinocytes, and MDA-MB-231 breast cancer cells. The exact mechanism of Smad3- mediated Gli1 upregulation remains unclear. Integrative genomic analyses also demonstrated that Snail/Slug and Notch-HES/HEY signals induce transcriptional downregulation of Hh target genes via E-box and N-box, respectively. Receptor tyrosine kinase (RTK) signals via Phosphatidylinositol-3 Kinase bound to Proteinkinase B (PI3K-AKT) signaling cascade induced stabilization of Gli1 protein, whereas G-protein coupled receptor (GPCR) via Gs-PKA signaling cascade induced degradation of Gli1 protein. So TGFβ and RTK positively regulate Gli1, whereas Notch and GPCR negatively regulate Gli1 [79].

e) Loss of Gli1 accelerates tumor progression through down-regulating FAS and FAS ligand. An in vivo study with a pancreatic cancer mouse model that contained p48 cre-dependent activation of Kras and loss of tumor suppressor p53 and Gli1 resulted in accelerated disease as shown by decreased survival, body weight, fatigue and increased tumor volume. Mills *et al.* concluded the existence of a novel Gli1-FASL/FAS axis [80].

Ad 2) A ligand-independent activation of the Hh-pathway caused by mutations in Ptch1 and Smo results in brain, skin and muscle tumors [81]. Fibroblast migration was mediated through Smo via G_i- protein signaling and activation of Rho family GTPases independent of Gli transcriptional activity. Polizio *et al*. suggested that the regulation of cell motility is a 'prototypical non – canonical response to Shh' [82].

Hh Inhibition as a potential target in cancer

Although the Hh-pathway is a good cancer target in theory, as in vitro studies have demonstrated, there have not been satisfying clinical results to date.

There are a few Hedgehog pathway inhibitors tested in various human cancers. Most frequently targeted has been the Smoothened receptor.

A classic representative is Cyclopamine, a corn lily-derived teratogenic alkaloid that antagonizes Smo. It suppresses the expression of Shh and of Gli1, it also leads to apoptosis in pancreatic cancer cells. In addition, it inhibits tumor growth by decreasing angiogenesis [83]. However, the expectations were not satisfied in pancreatic cancer cell lines that do not show Hh signaling [84]. A number of tumors have been shown to be refractory due to natural and acquired mutations in Smo or amplification of downstream effector Gli2 [78, 85].

Several small molecule inhibitors, such as RU-SKI 43, AZD8542, Gant 58 and 61, MS-0022, IPI-926, GDC-0449 and LDE225 have been developed and studied.

Petrova *et al.* recently published a new therapeutic called RU-SKI 43, a small molecule inhibitor of the Hhat. It targets the enzyme responsible for the attachment of palmitate onto Shh. Palmitoylation plays a pivotal role in determining the signaling potency of Shh in cells. A missing palmitoylation would lessen Shh activity. RU-SKI 43 reduced cancer cell proliferation of the pancreas and Gli-1 activation through Smoothened independent signaling [86]. Petrova *et al.* also established proof for inhibiting the canonical pathway rather than non-canonical signaling by examining a Shh-reporter cell line that produced alkaline phosphatase (AP) in response to Shh. Coculturing with cells expressing Shh and Hhat resulted in AP-production. AP-activity was trimmed down to original level when treated with RU-SKI 43 [87].

Screening of the AstraZeneca compound library using a Gli1 luciferase reporter assay identified AZD8542. Further testing detected effective inhibition of Gli1 expression in HSPC and human prostate stromal cell line (0.25-9.5 fold vs. control, $p<0.05$). In a colon cancer xenograft model, using species-specific primers, strong inhibition of Gli1 expression was discovered only in the mouse stroma but not the human epithelial compartment. Relevant tumor growth inhibition was only seen in combined animal models where tumor cells and fibroblasts were injected [35].

Another target is the Gli-mediated gene transcription via Gant 61 (Gli-ANTagonist 61) and Gant 58 (NSC 136476 and NSC 75503, respectively). Gant 61 was identified from a screen of cells for Gli-inhibitors [85]. Gant 61 is a hexahydropyrimidine derivative, whereas Gant 58 includes a thiopene core with four pyridine rings [85]. They both block Gli-mediated transcription in the nucleus through binding to the 5-zinc finger Gli1 protein between zinc fingers 2 and 3 at sites E119 and E167, independent of the Gli-DNA binding region, and conserved between Gli1 and Gli2 [88]. Gant 61 also blocks Gli1 DNA binding, probably by post-translational modifications like phosphorylation [54]. Fu and colleagues published that Gant 61 inhibited cancer stem cell tumor growth significantly in vitro and in a NOD/SCID/IL2R gamma null mice xenograft model. They also postulated that Gant 61 inhibited EMT by down-regulation of Snail, Slug, Zeb1 and N-cadherin and up-regulation of E-cadherin [89]. Guo *et al*. published an article about Gli-inhibition by transfecting pancreatic cancer cell lines with Gli1-siRNA, which were Gli-positive and Smo-negative in some cases, Smo positive in others. They found inhibitory effects on cell proliferation in all cell lines independent of their Smo-status. The Gli1-siRNA group showed a significant increase in sub- G0/G1 phase cells, indicating a block in cell cycle progression and an induction of apoptosis. Cyclin D2 and BCl-2 were indeed decreased in these cells [90].

Another potential anti-cancer target of the Hh pathway concerning Gli inhibition could be the regulated protein destruction of Gli. In vivo experiments in transgenic mice showed accumulation of Gli1 protein when silencing of degron D_c and D_n (two destruction signals responsible for proteolytic degradation of Gli) was performed. Wild-type Gli1 transgenic mice were born normally without detectable mutated Gli protein and developed BCC-like tumor lesions at 6-8 weeks after birth. The animals with mutant Gli died at birth with shallow skin ulcers throughout the body [91]. This reflects that altered protein accumulation can directly accelerate tumor induction, thus accelerated protein degradation could be a potential target in cancer.

MS-0022 (2-bromo-N-(4-(8-methylimidazo [1,2-a]pyridin-2-yl)phenyl)-benzamide) was identified by Strand *et al.* in 2009. It blocks the translocation of Smo to the cilia. In PANC-1 and SUIT-2 xenograft experiments, MS-0022 treatment led to a partial response, where growth was halted during the first days of treatment compared to the control. Over time, however, both the treated and control xenograft groups reverted to similar growth [53].

Saridgib (IPI-926), is an orally applied Smo-inhibitor. One in vivo study tested whether the delivery and efficacy of gemcitabine, the standard adjuvant and first-line chemotherapeutic in resectable and metastatic pancreatic cancer, could be improved by co-administration of IPI-926. The effects of Smo inhibition were measured in KOC mice after 8-12 days of treatment with IPI-926 or gemcitabine, alone or together. They found depletion of tumor stroma in the IPI-926 treated group, as well as 60% more delivery of gemcitabine into the tumor tissue after 10 days of treatment due to increases angiogenesis in the tumor. However the Smo-inhibitor alone did not show any effects on tumor cell proliferation or apoptosis. Though in combination with gemcibatine, a significant reduction in tumor growth and survival (11 days vs. 25 days (HR 95%CI 0.157 ± 0.458)) was shown. Interestingly, metastasis to the liver was also significantly reduced [92].

A phase II trial for saridegib and gemcitabine had to be stopped prematurely due to an increased mortality in the saridegib group [93].

GDC-0449 alias vismodegib showed a 58% response rate in patients with advanced basal cell carcinoma in a Phase I trial. However, no clinical response was observed during the same trial, in patients with other advanced stage cancers, such as pancreatic cancer [94]. A recent interim analysis of a single-arm phase II study was reported using vismodegib in combination with gemcitabine and nab-pa-

clitaxel. The overall survival was estimated at 10 months for 59 patients versus 8.5 months for patients that were treated with gemcitabine plus nab-paclitaxel [95, 96].

SUMMARY

Aberrant Hh signaling has been reported in many malignancies; among these pancreatic cancer has been a focus for researchers all over the world. Clinical trials of Hh inhibitors are under way in many different types of cancers. Despite encouraging results in in-vitro studies and mouse models, clinical trials have been disappointing. The misregulation of the Hh- pathway has been established in many different tumor types. Loss-of function, gene amplification and transcriptional upregulation of Shh, Ptch, and Gli1 among others are mechanisms for carcinogenesis in pancreatic cancer, medulloblastoma, glioma and lymphoma [68]. First the Hh-pathway was only described in cancer cells, but later the significance of the tumor stroma gained importance. The tumor –stroma interaction is complex. When Shh is missing, the stroma dies, while Gli is important for tumor cell growth. As said before, assuming the presence of the canonical pathway in cancer cells, targeting upstream effectors has not lead to the expected results. The non-canonical pathways that have been or have not been discovered could present an answer. Therefore an aim of future studies should be to elucidate non-canonical Hh –pathways in pancreatic cancer to find potential anticancer targets that target both, the stroma and the cancer cells.

Conflicting Interest

Authors have no conflicts of interest.

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