Hedgehog Signaling: Networking to Nurture a Pro-Malignant Tumor Microenvironment

Running title: Hedgehog in tumor microenvironment

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ABSTRACT

In addition to its role in development, the Hedgehog (Hh) pathway has been shown to be an active participant in cancer development, progression, and metastasis. While the pathway is activated by autocrine signaling by Hh ligands, it can also initiate paracrine signaling with cells in the microenvironment. This creates a network of Hh signaling that determines the malignant behavior of the tumor cells. As a result of paracrine signal transmission, the effect of Hh signaling most profoundly impacts the stromal cells that comprise the tumor microenvironment. The stromal cells in turn, produce factors that nurture the tumor. Thus, such a resonating crosstalk can amplify Hh signaling resulting in ‘molecular chatter’ that overall promotes tumor progression.

Hh signaling or rather its inhibition has been the subject of intense research to uncover inhibitors. Several of these inhibitors are currently being evaluated in clinical trials. We have reviewed the role of the Hh pathway in impacting the signature characteristics of cancer cells that determine tumor development, progression and metastasis. This review condenses the latest findings on the signaling pathways activated and/or regulated via molecules generated from Hh signaling in cancer, promising clinical interventions, future directions to identify the appropriate patients for therapy, develop reliable markers of efficacy of treatment and finally combat resistance to Hh pathway inhibitors.
Introduction

Cancer remains one of the leading causes of mortality and morbidity throughout the world. While diverse in their tissues of origin, all cancers share the fundamental feature of propagated genetic mutations to daughter cells. These propagated mutations generally lead to de-regulated signaling pathways that define a variety of clinical consequences and dictations for therapeutic intervention. Cancer cells aberrantly re-activate ontogenic developmental pathways such as Wnt, Notch and Hedgehog (Hh) to provide growth and survival advantage to the tumor cells.

The Hh pathway is normally activated during embryonic development and promotes vascularization as well as digitization (1, 2). Activation of this pathway leads to nuclear translocation of the GLI transcription factors resulting in transcriptional activation of target genes, the products of which either directly or indirectly result in the generation of molecules, some of which are secreted or transported outside the cells. The secreted molecules then create a potent milieu that can stimulate signaling cascades in cancer cells themselves, adjacent non-cancer cells, as well as cells distant to the primary tumor site or condition the secondary site for the arrival of the tumor cells (3). A recent body of scientific and clinical data supports the idea that the Hh pathway may hold several opportunities for cancer treatment. This review condenses the latest findings on the signaling pathways activated due to molecular crosstalk from Hh signaling in cancer and focuses on the hallmarks of malignancy.

Hh signaling: an overview

Hh signaling has been well characterized in Drosophila development. Recent studies also have determined an important role for Hh signaling in human renal development (4), and development and patterning of various vertebrate tissues and organs, including the brain and spinal cord, eye, craniofacial structures, and the limbs (5-7). Classical Hh pathway activation requires the binding of any Hh ligand, Sonic(SHH)/Indian (IHH)/Desert(DHH), to the extracellular domain of the membrane bound receptor Patched (PTCH) (8). PTCH is a 1,500
amino acid protein that spans the cell membranes 12 times. Both, the N and C terminal domains of PTCH are cytoplasmic while two extracellular loops in its tertiary conformation bind Hh ligands (9). There are two PTCH receptors in humans, PTCH1 & PTCH2 that slightly differ in amino acid composition in the N terminal region (9). Although both receptors have been implicated in a variety of diseases, most cancers in which Hh signaling is activated have been documented to work through PTCH1. More information is emerging about the immediate signaling downstream of Hh ligands binding to PTCH. Binding of Hh ligands to PTCH causes the release of inhibitory actions of PTCH on the vesicle bound Smoothed, SMO (10). The PTCH-Hh ligand complex results in the translocation of SMO to primary cilium regions in the cell membrane. At the cell membrane, SMO activates the GLI (Glioma-associated oncogene homolog) family of transcription factors through the involvement of intraflagellar transport proteins that play an active role in the functioning of the cilium that serves as a signalsome to allow assembly of essential signaling components (11). The GLI transcription factors are members of the Kruppel family of zinc finger transcription factors. The GLI family comprises three members GLI1, GLI2, and GLI3, each of which plays distinct roles in Hh signaling. GLI1 is gene-activating, while GLI3 is repressive (9). GLI2 displays duality in both, activation or repression, depending on the context of overall Hh signaling (12). Activation of GLI1 and GLI2 leads to the conversion of the GLI code from a ‘repressor’ state to an ‘activator’ state (12), translocation into the nucleus and transcription of target genes. Upregulated expression of such target genes directly or indirectly leads to the synthesis of signaling molecules, some of which may be secreted and enrich the tumor microenvironment for cancer growth and progression (Table 1). In addition to amplification and polymorphism, recent studies have characterized novel post-transcriptionally spliced variants of GLI1 as well as transcripts that differ in their 5’ untranslated region (13). While the GLI1ΔN variant (lacks exons 2 & 3 corresponding to aa 1-128) has a transcriptionally weaker activity, truncated GLI1 (tGLI1:contains an in-frame deletion
of 123 bases, corresponding to 41 amino acids spanning aa 34-74) is transcriptionally more potent than full-length GLI1 (13).

**Hedgehog Signaling: Autocrine, Juxtacrine, and Paracrine modes**

Hh signaling can be constitutive due to (a) loss-of-function mutations in PTCH or (b) gain-of-function mutations in SMOH or (c) due to upregulated expression of the ligands (14) or (d) due to dysregulated GLI expression (15). The Hh ligands can be tethered to the Hh-producing cells' surface membrane due to post-translational lipid modifications (16). They also can be released from the producer cells as monomeric or polymeric morphogens. Typically monomeric Hh ligands tend to be less stable than when in a polymeric conformation, and can result in short and long range paracrine signaling respectively (17). During development there is regulation at the levels of production, dissemination and presentation of the Shh gradient as the ligand can move many cell diameters from the source of production and regulate development (18). Thus, ligand-induced signaling may be autocrine or juxtacrine (side-by-side) or paracrine. Paracrine Hh signaling also is amplified when responding cells produce additional growth factors to support tumor growth or survival (Figure 1).

**The Hh Pathway impacts multiple attributes of cancer**

The multi-step development of cancer is characterized by the acquisition of the following hallmarks that determine tumor development, progression and metastasis (19): self sufficiency of growth signals and limitless replicative potential, evasion of apoptosis and insensitivity to anti-growth factors, sustained angiogenesis, and tissue invasion and metastasis. A few more hallmarks have recently emerged; these include genetic instability, metabolic flexibility, immune evasion, inflammation, and the tumor microenvironment. Each of the hallmarks can be directly or indirectly regulated through signaling cascades initiated from extracellular cues and signaling molecules.
During embryonic development in humans and in several vertebrates, Hh signaling has been reported to promote cell proliferation, angiogenesis, epithelial to mesenchymal transition (EMT), stem cell renewal/growth under conditions of fetal or hypoxic oxygen tension (2, 20-22). Since the Hh pathway is a key contributor to secreted molecules found in the extracellular matrix during fetal development, deregulated and/or constitutive activation of this pathway in cancer results in a rich microenvironment capable of potentiating malignant phenotypes in several types of transformed cells. The relationship between Hh signaling and its influence on the hallmarks of cancer are addressed below. We have also summarized the effects of Hh signaling on the tumor microenvironment that in turn, can modulate the malignant behavior of the tumor cells.

**Self sufficiency of growth signals & limitless replicative potential**

One of the more salient features of cancer includes uncontrolled cell proliferation. The Hh pathway directly targets several genes that are pro-proliferative. Furthermore, the diversity of the growth signals that are generated via Hh signaling impact a wide variety of cells including the heterogeneous tumor and cells from the microenvironment. While there are no reports thus far on the impact of Hh signaling on the length of telomeres or telomerase activity, a role for Hh signaling in promoting proliferation came about, through findings showing that inhibition of Hh signaling caused a decrease in the proliferative potential of cancer cells (23-25). Stecca et al reported that GLI-Hh signaling promotes proliferation and survival of melanoma cancer cells (26). One of the most well-known transcriptional targets of Hh signaling is cyclin D2 (27). Cyclin D2 complexes with CDK4 or CDK6 and promotes G1 to S cell cycle progression. When Hh signaling is inhibited, glioma cancer cells undergo cell cycle arrest and apoptosis due to downregulation of cyclins (28).

Hh signaling upregulates the expression of several secreted proteins that render cells in a pro-proliferative status. GLI1 upregulates Insulin-like Growth Factor Binding Protein, IGFBP-6 (27).
IGFBP, a secreted molecule, can bind to IGF receptor and signal to promote cell growth (27, 29). Hh signaling also upregulates another secreted protein, osteopontin (30). In fact, ablating Hh signaling causes a decrease in osteopontin expression simultaneous with a decrease in the proliferative capacity of the tumor cells, a property that was restored when osteopontin was re-expressed in the cells (30). As such, constitutively activated Hh signaling may be one of the key driving forces for uncontrolled cell proliferation.

**Evasion of apoptosis & insensitivity to anti-growth factors**

Apoptosis or programmed cell death typically follows cellular insult that compromises genetic integrity beyond repair capabilities. Deregulation of apoptotic process could lead to severe genomic instability and propagation of acquired mutations. Several leukemic cancers show deregulated Hh signaling that has been implicated to play a direct role in pro-survival phenotypes (31, 32). In some cases, the anti-apoptotic features seen in some types of leukemia may actually be due to the down-regulation of Hh signaling molecules (32, 33). This suggests that the molecules generated from Hh signaling may be different in solid versus hematologic cancers even though the outcomes of more malignant phenotypes are still observed. Pro-survival molecules generated via Hh signaling are noted among a host of other malignancies such as brain, gastric and pancreatic and basal cell carcinomas (29, 34-36). A growing body of work has elucidated that the Hh pathway in these cancers specifically targets transcription of the anti-apoptosis molecule, Bcl-2 (35, 37). The Hh pathway appears to regulate the stability and accumulation of p53 in breast cancer cells. Specifically, Hh signaling activates Mdm2 that promotes ubiquitination of p53 resulting in its elimination (38).

Gynecological cancers often present deregulated Hh signaling. Ovarian and endometrial cancers display a correlation between the increased expression of Hh ligands and molecules associated with the pathway and the increased stage of cancer progression. The pathway is activated in ovarian carcinomas, correlating with cell proliferation: its inhibition leads to growth
suppression and apoptosis (39, 40). Overexpression of Hh signaling molecules is associated with increased proliferation of endometrial carcinoma cells and ovarian cancer systems characterized by downregulation of p21 and p27, potent inhibitors of cell cycle progression.

Sustained angiogenesis

Hh signaling promotes angiogenesis during fetal development as well as during times of injury (2, 21, 41). In cancer, Hh signaling contributes to increased vascularity and promotes tumor growth and hematogenous metastasis (26). Tumor vasculature generated via Hh signaling displays “leaky” blood vessel structures that have increased vascular permeability (42). Hh signaling promotes the expression of VEGF-A, VEGF-B, VEGF-C and Ang2. While the VEGFRs play an important role in neoangiogenesis and metastasis, VEGF-C promotes lymphatic metastasis of tumors (43). In pancreatic tumors, SHH-signaling enhances lymphangiogenesis likely by promoting motility of the lymphatic endothelial cells (43). Pancreatic tumor-derived SHH also enhances the angiogenic function of endothelial progenitor cells by upregulating the expression of VEGF, SDF-1 and Ang-1 (44). Pancreatic ductal adenocarcinoma-derived SHH induces expression of Ang-1 and IGF-1 in bone marrow-derived pro-angiogenic cells and causes an increase in their migration and capillary morphogenesis (45). As such, other than in development, the Hh pathway enhances angiogenesis in cancers and promotes their malignant behavior.

Tissue invasion & metastasis

Invasion and migration represent clinically relevant features of cancer. Hh signaling enhances invasion and migration in many cancers including, breast, ovarian, skin, melanomas, prostate and pancreatic cancer (22, 40, 46-49). In addition, GLI1 transcriptionally upregulates chemo-attractants such as IGFBPs, FGFs, VEGF, and angiopoietins that act locally and distantly and enhance cell migration and/or invasion (27, 44). In melanoma, Das et al have reported that
GLI1-Hh signaling transcriptionally upregulates osteopontin (30). As a secreted molecule, osteopontin is a potent chemoattractant with a classic integrin binding RGD motif that promotes invasive attributes by activating expression of MMPs (50). Abolishing Hh signaling by silencing GLI1 reduced malignant properties characterized by slower tumor growth and decreased metastasis, concomitant with the reduced expression of osteopontin, suggesting that osteopontin mediates the pro-malignant effects of the Hh pathway on tumor cells (30). Other studies have shown that nuclear translocation of GLI1 can directly target genes associated with EMT. This transition results in cells which are highly motile and invasive. Li et al. were among the first to report that GLI1 transcriptionally potentiates expression of Snail/Snai1, a transcriptional repressor of E-cadherin expression in basal cell carcinomas and other transformed epithelial skin conditions leading to the nuclear translocation of β-catenin (51), a transcription factor that regulates several genes associated with cell migration and motility as well as cell-cell adhesions. In gastric cancer cells, Hh signaling causes increased invasion and motility associated with the upregulation of TGF-β, increased cell proliferation and activated MMPs (52). GLI1 also upregulates the expression of an extracellular protein, mucin 5 ac, MUC5AC resulting in decreased membranous E-cadherin, increased nuclear accumulation of β-catenin, as well as increased pancreatic cancer cell migration and invasion (53). Thus, Hh signaling may be a good target for treating aggressive cancers that currently have limited therapeutic interventions.

Genomic instability
DNA repair is an essential cellular process required to maintain genomic integrity in the face of potentially lethal genetic damage. Failure to repair a double strand break can trigger cell death, whereas mis-repair of the break can lead to the generation of chromosomal translocations leading to tumor development and/or progression. Double strand breaks in DNA can be induced following exposure to exogenous agents, such as ionizing radiation or radiomimetic chemicals,
as well as naturally occurring intermediates of normal physiological processes. When the ability to repair DNA damage due to double strand breaks was abolished in mice, Ptch1 function was specifically lost and was accompanied by an aberrant activation of Hh signaling (54). Conversely, compromised expression of Ptch (Ptch<sup>−/−</sup> mice) or upregulated GLI1 expression disrupted Chk1 (checkpoint kinase 1) activation by preventing the interaction of Chk1 with Claspin, a Chk1 adaptor protein that is required for Chk1 activation (55). An independent study by Snijders <em>et al</em> reported that upregulated expression of GLI1 caused genomic instability likely due to enhanced expression of the stem cell gene, SOX2 (56). Thus, aberrant Hh signaling can promote tumorigenesis by disabling key signaling pathways that help maintain genomic stability and inhibit tumorigenesis.

**Metabolic flexibility**

Low oxygen availability often dictates the mechanisms by which a cell consumes and processes energy. Many tumor masses undergo metabolic switches where they use anaerobic means to obtain energy. The Hh pathway plays an important role during embryonic development characterized by low oxygen tensions. Interestingly, this pathway is reactivated in several tumors in regions of hypoxic oxygen tensions similar to fetal (57). While no published evidence directly implicates Hh signaling in the Warburg phenomenon, analysis of hypoxic versus normoxic conditions clearly indicate the indirect roles of Hh signaling in cancer progression. Onishi <em>et al</em> reported that hypoxia activates Hh signaling in pancreatic cancers leading to the transcription of molecules that enhance invasiveness (48). It is likely that Hh signaling-enhanced growth potential may be a result of or may lead to increased ability to uptake and metabolize glucose for pro-proliferative activities (27, 29, 46).
Evading immune surveillance

The immune system in a cancer patient may play a pivotal role in the progression of the disease state and/or clinical outcome. Some cancers can circumnavigate inflammatory responses associated with aggressively growing masses; consequently, attenuating immune response and escaping from immune surveillance. Over the past few years Rowbotham et al have published a body of work that elucidates the role Hh signaling plays in T-cell fate and activation and T-cell receptor selection. They have determined that the Hh pathway decreases the selection and repertoire of T-cells compromising their activation and proliferation (58-60).

Inflammation

The expression of Hh ligands as well as the activity of Hh signaling is increased under inflammatory conditions (61, 62). Hh-inflammation events vary by cell/tissue type, location, and overall environmental stresses. Hh ligands are upregulated in a variety of inflammatory bowel diseases (IBDs). Sustained inflammation, key to promoting clinical complications associated with several IBDs, often increase risks for the development of gastro-intestinal cancers of the colon, stomach and small intestine (63, 64). Endogenous Hh signaling is an early mediator of liver injury and inflammation after ischemia reperfusion (61). In pancreatic cancer, inflammation-stimulated monocytes produce Shh through activation of the NF-kappaB signaling pathway; Shh promotes proliferation of the pancreatic cancer cells in a paracrine manner through Hh signaling (65). In gastric cancer, it is proposed that while loss of Shh is essential to the formation of atrophy and metaplasia at early stages in Helicobacter pylori-induced gastritis, increased Shh signaling is important in promoting tumor growth and proliferation at later stages. In chronic Helicobacter pylori infections leading to carcinogenesis, the stromal cells show upregulated expression of GLI1 and the anti-apoptotic bcl-2, in addition to elevated NF-κB activity and IL-8 expression. IL-8 expressed as a consequence of elevated NF-κB activity promotes expression of Shh in the tumors cells. It is also likely that Shh signaling mediates IFN-γ recruitment of
mesenchymal stem cells to the gastric mucosa, where they begin to express Shh and promote cancer progression (66).

As reflected above (immune evasion), the Hh pathway plays a role in immunological responses which often overlap with inflammation. CD4(+)T cells treated with exogenous Shh stimulate production of inflammatory cytokines such as, IL-2, IFN-gamma, and IL-10 (64). In some contexts, however, the Hh pathway can be anti-inflammatory (67) which suggests the possibility of Hh signaling playing dual roles in promoting both cancer inflammation and host tolerance. This duality may explain some aspects of the threshold that exists between tumor growth and immune surveillance evasion in cancer progression.

**Cancer stem cells**

The Hh pathway has been reported to impact the maintenance of the cancer stem cell population (46, 68), the cell population that self-renews, and generates cells that form the bulk of the tumor. While the Hh pathway is active in gliomasphere-forming cells and in CD44+ CD24−/Low Lin− breast cancer stem cells, maintenance and expansion of leukemia and multiple myeloma stem cells depends on Hh signaling (31, 69). Treatment with cyclopamine causes a profound decrease in the hematopoietic stem cell population (70). The regulatory loop between Hh signaling and p53, or rather the delicate balance between them, has also been proposed to regulate the numbers of cancer stem cells (71). Several studies have implicated the potent pro-proliferative effects of Hh signaling in cancer stem cell/stem cell like pollutions. Cancers of the colon, breast, brain, and blood demonstrate the role of Hh signaling in enriching the viability of the powerfully resilient stem cell populations (31, 46, 64, 68, 72-77). Hh signaling from glioblastoma stem cells upregulates IRS1 (insulin receptor substrate 1) (34, 78) and enhances survival of brain tumor stem cell populations (79). In mammary epithelial cells and in ovarian cancer, the expression of the self-renewal gene, BMI-1, is upregulated due to Hh signaling (80, 81). Given the fact that stem cells confer resistance to chemotherapy and radiotherapy and
findings that implicate a role for Hh signaling in chemotherapy and radiotherapy resistance, it is indeed conceivable that this pathway impacts cancer stem-ness.

**The tumor microenvironment**

As stated earlier in this review, the range and extent of Hh signaling is expansive. In a heterogeneous tumor mass, the combinations of paracrine, juxtacrine and autocrine signaling are all possible and have been documented to promote tumor progression (82). However, the tumor microenvironment also contains non-epithelial cells which play an equally important role in promoting cancer. Listed below are a few stromal compartment members, which at any given time may be involved directly or indirectly in cancer progression. These typically non-transformed cells are also sensitive to signaling cues generated via Hh signaling from cancer cells; however these cells can also generate signaling molecules which can act upon the cancer cells leading to further amplification of Hh signaling (Figure 1).

(A) Fibroblasts

Fibroblasts are phenotypically mesenchymal cells which can make up a large part of the tumor stroma. Their primary roles include synthesis, secretion and remodeling of the extracellular matrix. Several biological phenomena such as inflammation and angiogenesis require extracellular remodeling. Hh signaling has long been accepted as one of the key pathways involved in tissue remodeling and repair upon injury and ischemia. Cirrhotic livers frequently develop primary liver cancers as a consequence of disease complications (83). SHH expression is elevated in several cirrhotic livers and may directly target fibroblastic hepatic stellate cells (fibroblasts) to survive and remodel liver stroma raising the possibility that the increased and sustained synthesis of SHH in the stromal compartment could actually be driving epithelial cell proliferation which may lead to genetic instability and accumulation of mutations (83). Although Hh signaling is activated in hepatic cancers, it is unclear at what stage this pathway is contributing. Stellate cells also appear to be playing a key role in pancreatic cancer. Hh
signaling generated from pancreatic cancer cells regulates several proteins such as TGF-β, PDGF, SHH, and galectin-3, all of which activate stellate cells to produce pro-proliferative, pro-migratory, pro-survival molecules such as PDGF, SDF-1, EGF, IGF, and SDF-1 that promiscuously signal to cancer and stromal cells (84).

Heterotypic paracrine Hh signaling is also evident in the murine melanoma tumor microenvironment. Fibroblasts begin expressing Ptch when co-cultured with a Shh-producing melanoma cell line, B16F0 (42). This implies that Hh signaling can become activated in cells surrounding non-transformed cells as a consequence of being within close proximity of the cancerous Shh producing cells.

(B) Endothelial cells
The Hh pathway highly influences the pro-angiogenic behavior of endothelial cells. Hh ligands have been shown to promote proliferation, invasion, and migration of endothelial cells (22, 85). Recent findings suggest that Hh signaling does not occur through the canonical SHH-PTCH binding, but through other unknown mechanisms. Endothelial cells do not appear to inherently express, or produce Hh ligands (22, 85). It is believed that GLI nuclear translocation may be facilitated unconventionally through integrin activation or other focal adhesion related signaling cascades in the presence of Hh ligands (22). SHH promotes endothelial cell expression of MMP9 and osteopontin and causes Rho-dependent migration (86) and phosphorylation of FAKs (87). In fact, SHH present in microparticles released by lymphocytes can correct endothelial cell injury via increase in the expression of nitric oxide (88). The behavior of endothelial cells is largely in response to the ligands expressed by the tumor cells.

(C) Osteoblasts and osteoclasts
The bone presents a rich, hypoxic microenvironment for metastatic prostate and breast cancer cells. The metastatic tumor cells initiate a crosstalk with cells in bone disrupting the homeostasis of the osteoblasts and osteoclasts. Our recent research has elucidated the role of tumor cell-initiated Hh signaling in osteoblast and osteoclast differentiation and their mineralization and
resorptive activities, respectively. Breast cancer cells express Hh ligands that directly activate osteoclast precursors to terminally differentiate into osteoclasts that can resorb bone matrix through the MMPs and cathepsin K proteases and tartarate-resistant acid phosphatase (89). GLI2 expression in the MDA-MB-231 cells causes increased osteolysis by enhancing the expression of PTHrP (90). There is also evidence of non-classical activation of GLI signaling, initiated by Runx2 and TGF-beta leading to enhanced osteolysis (91, 92). Thus, modulation of the metastatic site is facilitated by the cross-activation of Hh signaling initiated by tumor cells.

As presented, the Hh pathway impacts multiple aspects of tumor development, progression and metastasis. The following section will briefly summarize the clinical status of various Hh inhibitors.

**Therapeutic targeting of Hh signaling**

Hh inhibitors mainly target via (a) neutralizing the activity of Hh ligands, (b) inhibiting SMO, and (c) inhibiting activity of the GLI transcription factors. Cyclopamine, the first identified naturally-occurring Hh inhibitor, has shown promise in managing multiple malignancies (47, 93-96).

While CUR61414 (Curis/Genentech) failed a Phase I clinical trial (71), GDC-0449 (Genentech/Roche/Curis), a potent orally bioavailable SMO inhibitor is in Phase II trials (71, 97), and has shown promising outcomes in basal cell carcinoma (98) and medulloblastoma (97, 99, 100). IPI-926 (Infinity Pharmaceuticals) is in Phase I clinical trials following a successful pre-clinical trial (101). BMS833923/XL139 (Bristol Myers Squibb/Exelixis) and LDE225 (Novartis), both orally bioavailable SMO antagonists, are also in clinical trials (97).

Other Hh pathway inhibitors include vitamin D3 (that directly binds SMO), 5E1 Hh ligand neutralizing antibody, curcumin, and arsenic (102). Inhibitors of GLI transcriptional activity, GANT-58 and GANT-61, caused tumor regression in preclinical models (103). New research has uncovered reported 4 Hh inhibitors that are epistatic to Sufu and target GLI processing, GLI activation and cilium formation (104), and are mechanistically distinct from SMO antagonists.
Summary

The functions of the Hh pathway have now been understood to be important in all aspects of tumor progression. What began as early investigations in basal cell carcinoma and medulloblastoma have now transcended to several solid and hematologic malignancies. The involvement in maintaining the cancer stem cell phenotype also attests a role for this pathway in tumor development. Not being exclusive, the Hh pathway crosstalks with other signaling pathways such as the EGF pathway, NF-kB pathway, Wnt pathway and with p53. Signaling via this pathway can be activated by a ligand-dependent manner and also by activating mutations of members of this pathway. Not confined to autocrine signaling alone, the Hh pathway participates in paracrine signaling between the ligand-producing cells and the surrounding responding cells. The relevance of paracrine Hh signaling was highlighted further when inhibitors of this pathway had a more profound effect on the stromal cells rather than the tumor cells themselves in xenograft models. While various inhibitors have now made their way into early and advanced clinical trials, research continues on designing more effective, more specific and less toxic compounds to inhibit Hh signaling. Careful consideration must be taken when devising strategies to target the Hh pathway. It is necessary to bear in mind that targets may not necessarily reside in Hh producing cancer cells, but rather the non-transformed cells in the stromal compartment which are responding to the signaling molecules generated via the pathway. The role of non-canonical Hh signaling in activating other pathways such as Erk (105) and Rho signaling (85) must also be factored in these strategies. The efficacy of the compounds is always put to test in clinical trials where the ever-evolving tumor and its microenvironment can develop resistance to the compounds and become refractory to treatment. This was exemplified by the dramatic initial response to GDC-0449, followed by recurrence of tumor in a patient with medulloblastoma (attributed to a mutation in the target molecule, SMO). The pathway also upregulates expression of genes that confer multidrug resistance (106). Thus, while the search for alternate or complementary strategies to inhibit Hh signaling continues, some promising data
has emerged showing that targeting Gli1 directly, can re-sensitize glioma tumors to drug therapies (107). In sum, there is an urgent and unmet need to better define existing modalities and identify novel strategies to inhibit Hh signaling, identify the appropriate patients for therapy, develop reliable markers of efficacy of treatment and finally combat resistance to Hh pathway inhibitors by targeting not only the tumor cells, but also the paracrine network existing in the tumor microenvironment that dictates and modulates the malignant behavior of the tumor cells.
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>Ang1/2</td>
<td>Angiopoietin 1/2</td>
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<tr>
<td>DHH</td>
<td>Desert Hedgehog</td>
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<tr>
<td>EMT</td>
<td>Epithelial mesenchymal transition</td>
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<td>FAK</td>
<td>Focal adhesion kinase</td>
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<td>Hh</td>
<td>Hedgehog</td>
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<td>IGF</td>
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<td>VEGF</td>
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FIGURE LEGENDS

Figure 1: Homotypic and heterotypic Hh signaling.

Tumors are heterogenous masses that are often vascularized (indicated by red vessels).

A cancer cell that has constitutively activated Hh signaling (centre) produces Hh ligands that can activate other Hh responding cells (epithelial or other), and also produces a variety of GLI-mediated gene products that may promote an enhanced pro-malignant tumor micro-environment, including, cell growth, survival, motility, and differentiation and perpetuation of autocrine Hh signaling via synthesis of more Hh ligands. Each cell’s response may directly or indirectly support pro-malignant features of the progressing cancer.

Cancer cells can produce and secrete Hh ligands that activate Hh signaling in other cancer cells (homotypic signaling). They may also express Hh ligands and/or other signaling molecules which promote signaling in non-epithelial cells (heterotypic/paracrine signaling).

Table 1: Target genes regulated by GLI1/2-mediated activity
References


Table 1: Target genes regulated by GLI1/2-mediated activity

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Cancer Type(s)</th>
<th>Effect/Cancer Hallmark Potiated</th>
<th>References</th>
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<tr>
<td>Cyclin D1 &amp;2</td>
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<td>Proliferation</td>
<td>Yoon et al, 2002; Fiaschi et al, 2009</td>
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<td>Proliferation, Invasion/Migration</td>
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<td>Bcl2</td>
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<td>FOXM1</td>
<td>Basal cell carcinoma</td>
<td>Proliferation</td>
<td>Teh et al, 2002</td>
</tr>
<tr>
<td>MDM2</td>
<td>Breast</td>
<td>Pro-survival, proliferation</td>
<td>Abe et al, 2008</td>
</tr>
<tr>
<td>↓P21</td>
<td>Ovarian</td>
<td>Proliferation</td>
<td>Chen et al, 2007</td>
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<td>↓P27</td>
<td>Ovarian</td>
<td>Proliferation</td>
<td>Chen et al, 2007</td>
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<tr>
<td>VEGF</td>
<td>Pancreatic</td>
<td>Angiogenesis</td>
<td>Yamazaki et al, 2008</td>
</tr>
<tr>
<td>Angiopoetin1/2</td>
<td>Pancreatic</td>
<td>Angiogenesis</td>
<td>Yamazaki et al, 2008</td>
</tr>
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</table>

The Hh pathway is known to elicit several signaling cascades due to the variety of molecules generated from its activation. Although some of the targets are direct, several functional changes may be indirect. Hh signaling may increase, decrease or stabilize signaling molecules that essentially contribute to the pro-malignant tumor microenvironment.
Molecular Cancer Research

Hedgehog Signaling: Networking to Nurture a Pro-Malignant Tumor Microenvironment

Lillianne G Harris, Rajeev S. Samant and Lalita A Shevde

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