

Subject Review

Hedgehog Signaling: Networking to Nurture a Promalignant Tumor Microenvironment

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Abstract

In addition to its role in embryonic development, the Hedgehog pathway has been shown to be an active participant in cancer development, progression, and metastasis. Although this pathway is activated by autocrine signaling by Hedgehog ligands, it can also initiate paracrine signaling with cells in the microenvironment. This creates a network of Hedgehog signaling that determines the malignant behavior of the tumor cells. As a result of paracrine signal transmission, the effects of Hedgehog signaling most profoundly influence the stromal cells that constitute the tumor microenvironment. The stromal cells in turn produce factors that nurture the tumor. Thus, such a resonating cross-talk can amplify Hedgehog signaling, resulting in molecular chatter that overall promotes tumor progression. Inhibitors of Hedgehog signaling have been the subject of intense research. Several of these inhibitors are currently being evaluated in clinical trials. Here, we review the role of the Hedgehog pathway in the signature characteristics of cancer cells that determine tumor development, progression, and metastasis. This review condenses the latest findings on the signaling pathways that are activated and/or regulated by molecules generated from Hedgehog signaling in cancer and cites promising clinical interventions. Finally, we discuss future directions for identifying the appropriate patients for therapy, developing reliable markers of efficacy of treatment, and combating resistance to Hedgehog pathway inhibitors. *Mol Cancer Res*; 9(9); 1165–74. ©2011 AACR.

Introduction

Cancer remains one of the leading causes of mortality and morbidity throughout the world. Although they are 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 deregulated signaling pathways that define a variety of clinical consequences and indications for therapeutic intervention. Cancer cells aberrantly reactivate ontogenic developmental pathways, such as Wnt, Notch, and Hedgehog, to provide growth and survival advantages to the tumor cells.

The Hedgehog 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 glioma-associated oncogene homolog (GLI) transcription factors, resulting in transcriptional activation of target genes. The products of these genes 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 the cancer cells themselves, adjacent noncancer cells, and 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 Hedgehog pathway may hold several opportunities for cancer treatment. This review condenses the latest findings on the signaling pathways that are activated due to molecular cross-talk from Hedgehog signaling in cancer, and focuses on the hallmarks of malignancy.

Hedgehog Signaling: An Overview

Hedgehog signaling has been well characterized in *Drosophila* development. Recent studies have also determined an important role for Hedgehog 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 limbs (5–7). Classical Hedgehog pathway activation requires the binding of any Hedgehog ligand [e.g., Sonic Hedgehog (SHH), Indian Hedgehog (IHH), or Desert Hedgehog (DHH)] to the extracellular domain of the membrane-bound receptor Patched (PTCH; ref. 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, whereas 2 extracellular loops in its tertiary conformation bind Hedgehog ligands (9). Humans have 2 PTCH receptors, PTCH1 and PTCH2, which differ slightly in amino acid composition

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in the N-terminal region (9). Although both receptors have been implicated in a variety of diseases, most cancers in which Hedgehog signaling is activated have been shown to work through PTCH1. More information is emerging about the immediate signaling downstream of Hedgehog ligands binding to PTCH. Binding of Hedgehog ligands to PTCH causes the release of inhibitory actions of PTCH on the vesicle-bound Smoothed (SMO; ref. 10). The PTCH-Hedgehog ligand complex results in the translocation of SMO to primary cilium regions in the cell membrane. At the cell membrane, SMO activates the GLI family of transcription factors through the involvement of intraflagellar transport proteins. These proteins play an active role in the functioning of the cilium, which serves as a signalsome to allow the assembly of essential signaling components (11). The GLI transcription factors are members of the Kruppel family of zinc finger transcription factors. The GLI family is composed of three members (GLI1, GLI2, and GLI3), each of which plays distinct roles in Hedgehog signaling. GLI1 is gene activating, whereas GLI3 is repressive (9). GLI2 displays duality in both activation and repression, depending on the context of the overall Hedgehog signaling (12). Activation of GLI1 and GLI2 leads to 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 posttranscrip-

tionally spliced variants of GLI1, as well as transcripts that differ in their 5' untranslated region (13). Although the GLI1 Δ N variant (which lacks exons 2 and 3 corresponding to aa 1–128) has a transcriptionally weaker activity, truncated GLI1 (tGLI1, which 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

Hedgehog signaling can be constitutive due to (i) loss-of-function mutations in *PTCH*, (ii) gain-of-function mutations in *SMOH*, (iii) upregulated expression of the ligands (14), or (iv) dysregulated *GLI* expression (15). As a result of posttranslational lipid modifications, the Hedgehog ligands can be tethered to the surface membrane of Hedgehog-producing cells (16). They also can be released from the producer cells as monomeric or polymeric morphogens. Typically, Hedgehog ligands tend to be less stable in a monomeric conformation than in a polymeric one, and can result in short- and long-range paracrine signaling, respectively (17). During development, regulation occurs 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 Hedgehog signaling also is amplified when responding cells produce additional growth factors to support tumor growth or survival (Fig. 1).

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

Target gene	Cancer type(s)	Effect/cancer hallmark potentiated	References
Cyclin D1/2	Skin, brain, breast	Proliferation	Yoon et al (27); Fiaschi et al (79)
Osteopontin	Melanoma, breast cancer	Proliferation, invasion/migration	Yoon et al (27); Das et al (30)
Bcl2	Gastric, brain, pancreatic, skin, lymphoma	Antiapoptosis, prosurvival	Xu et al (29); Han et al (35); Bar et al (106); Bigelow et al (37); Singh et al (23)
IGFBP6	Pancreatic	Proliferation	Xu et al (29)
MUC5AC	Pancreatic	EMT/invasion/migration	Inaguma et al (53)
PTCH	BCC, brain	Proliferation	Berman et al (25)
Snai1	Skin, breast	EMT/invasion/migration	Li et al (51, 67); Fiaschi et al (79)
JAG2	Gastric	EMT/invasion/migration	Reviewed in Katoh and Katoh (107)
FOXM1	Basal cell carcinoma	Proliferation	Teh et al (36)
MDM2	Breast	Prosurvival, proliferation	Abe et al (38)
↓P21	Ovarian	Proliferation	Chen et al (24)
↓P27	Ovarian	Proliferation	Chen et al (24)
VEGF	Pancreatic	Angiogenesis	Yamazaki et al (44)
Ang1/2	Pancreatic	Angiogenesis	Yamazaki et al (44)

NOTE: The Hedgehog pathway is known to elicit several signaling cascades due to the variety of molecules generated by its activation. Although some of the targets are direct, several functional changes may be indirect. Hedgehog signaling may increase, decrease, or stabilize signaling molecules that essentially contribute to the promalignant tumor microenvironment.

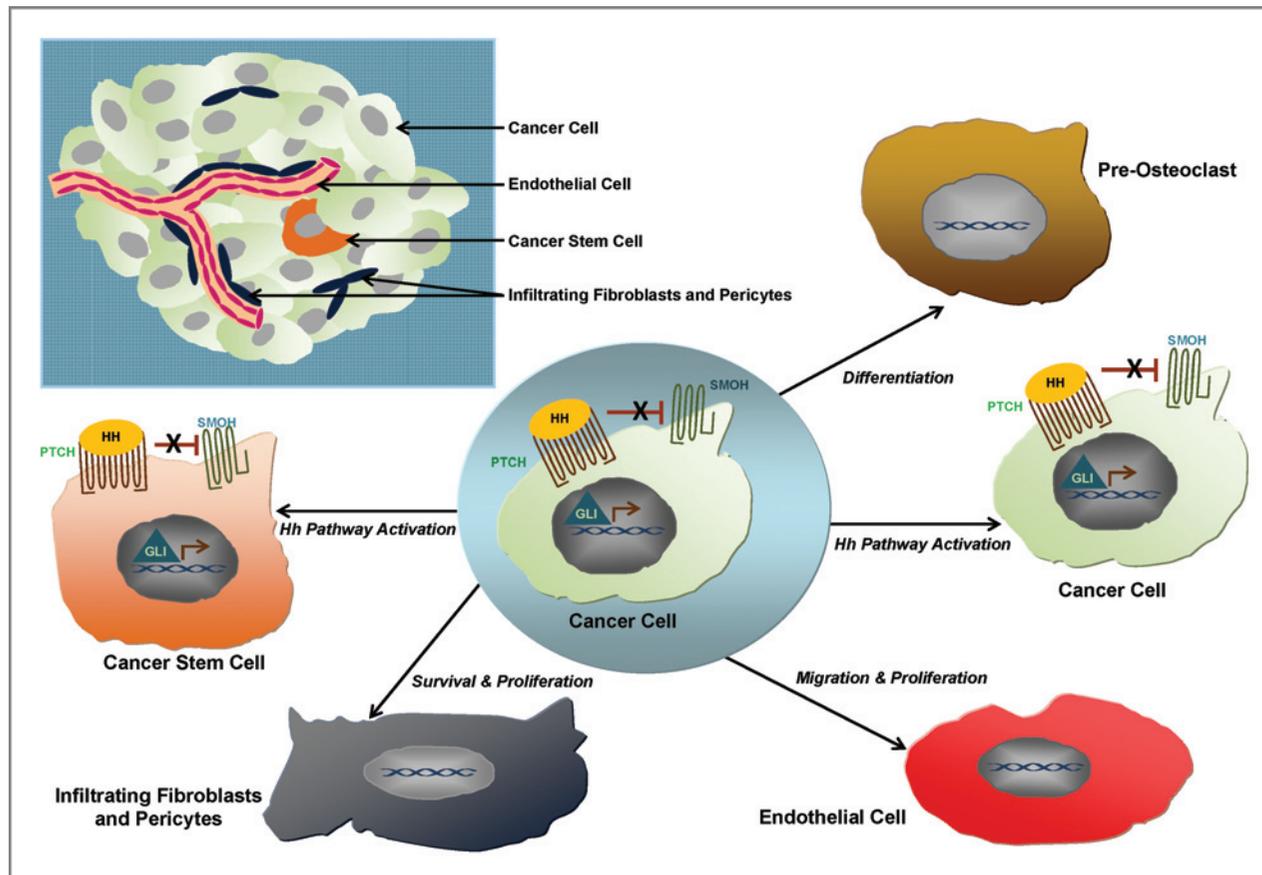


Figure 1. Homotypic and heterotypic Hedgehog signaling. Tumors are heterogeneous masses that are often vascularized (indicated by red vessels). A cancer cell that has constitutively activated Hedgehog signaling (center) produces Hedgehog ligands that can activate other Hedgehog responding cells (epithelial or other), and it also produces a variety of GLI-mediated gene products that may promote an enhanced promalignant tumor microenvironment, including cell growth, survival, motility, and differentiation and perpetuation of autocrine Hedgehog signaling via synthesis of more Hedgehog ligands. Each cell's response may directly or indirectly support promalignant features of the progressing cancer. Cancer cells can produce and secrete Hedgehog ligands that activate Hedgehog signaling in other cancer cells (homotypic signaling). They may also express Hedgehog ligands and/or other signaling molecules that promote signaling in nonepithelial cells (heterotypic/paracrine signaling).

The Hedgehog Pathway Affects Multiple Attributes of Cancer

The multistep 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 antigrowth factors, sustained angiogenesis, and tissue invasion and metastasis. A few more hallmarks, such as genetic instability, metabolic flexibility, immune evasion, inflammation, and the tumor microenvironment, have recently emerged. Each of these hallmarks can be directly or indirectly regulated through signaling cascades initiated by extracellular cues and signaling molecules.

During embryonic development in humans and in several vertebrates, Hedgehog signaling has been reported to promote cell proliferation, angiogenesis, epithelial-mesenchymal transition (EMT), and stem cell renewal/growth under conditions of fetal or hypoxic oxygen tension (2, 20–22).

Because the Hedgehog 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 that is capable of potentiating malignant phenotypes in several types of transformed cells. We address the relationship between Hedgehog signaling and the hallmarks of cancer. We also summarize the effects of Hedgehog signaling on the tumor microenvironment, which in turn can modulate the malignant behavior of tumor cells.

Self-sufficiency of growth signals and limitless replicative potential

One of the more salient features of cancer is uncontrolled cell proliferation. The Hedgehog pathway directly targets several genes that are proproliferative. Furthermore, the diverse growth signals that are generated by Hedgehog signaling influence a wide variety of cells, including the heterogeneous tumor and cells from the microenvironment. Although there are no reports thus far

on the impact of Hedgehog signaling on the length of telomeres or telomerase activity, a role for Hedgehog signaling in promoting proliferation has emerged from findings that inhibition of Hedgehog signaling causes a decrease in the proliferative potential of cancer cells (23–25). Stecca and colleagues (26) reported that GLI-Hedgehog signaling promotes the proliferation and survival of melanoma cancer cells. One of the best-known transcriptional targets of Hedgehog signaling is cyclin D2 (27). Cyclin D2 complexes with CDK4 or CDK6 and promotes G1-to-S cell cycle progression. When Hedgehog signaling is inhibited, glioma cancer cells undergo cell cycle arrest and apoptosis due to downregulation of the cyclins (28).

Hedgehog signaling upregulates the expression of several secreted proteins that render cells in a proproliferative status. GLI1 upregulates insulin-like growth factor binding protein (IGFBP)-6 (27). IGFBP, a secreted molecule, can bind to the IGF receptor and signal to promote cell growth (27, 29). Hedgehog signaling also upregulates another secreted protein, osteopontin (30). In fact, ablation of Hedgehog signaling caused a decrease in osteopontin expression simultaneously with a decrease in the proliferative capacity of the tumor cells, a property that was restored when osteopontin was reexpressed in the cells (30). As such, constitutively activated Hedgehog signaling may be one of the key driving forces for uncontrolled cell proliferation.

Evasion of apoptosis and insensitivity to antigrowth factors

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

Gynecological cancers often present deregulated Hedgehog signaling. Ovarian and endometrial cancers display a correlation between the increased expression of Hedgehog 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, and its inhibition leads to growth suppression and apoptosis (39, 40). Overexpression of Hedgehog signaling molecules is associated with increased proliferation of endometrial carcinoma cells and ovarian cancer systems characterized by downregulation of p21 and p27, both of which are potent inhibitors of cell cycle progression.

Sustained angiogenesis

Hedgehog signaling promotes angiogenesis during fetal development as well as during times of injury (2, 21, 41). In cancer, Hedgehog signaling contributes to increased vascularity and promotes tumor growth and hematogenous metastasis (26). Tumor vasculature generated by Hedgehog signaling displays leaky blood vessel structures with increased vascular permeability (42). Hedgehog signaling promotes the expression of VEGF-A, VEGF-B, VEGF-C, and angiopoietin (Ang)-2. Whereas 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 the 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, stromal derived factor (SDF)-1, and Ang-1 (44). Pancreatic ductal adenocarcinoma-derived SHH induces expression of Ang-1 and IGF-1 in bone marrow-derived proangiogenic cells, and causes an increase in their migration and capillary morphogenesis (45). As such, in addition to its role in development, the Hedgehog pathway enhances angiogenesis in cancers and promotes their malignant behavior.

Tissue invasion and metastasis

Invasion and migration represent clinically relevant features of cancer. Hedgehog signaling enhances invasion and migration in many cancers, including breast, ovarian, skin, melanoma, prostate, and pancreatic cancers (22, 40, 46–49). In addition, GLI1 transcriptionally upregulates chemoattractants, such as IGFBP, fibroblast growth factor, VEGF, and Ang, that act locally and distantly and enhance cell migration and/or invasion (27, 44). Das and colleagues (30) reported that GLI1-Hedgehog signaling transcriptionally upregulates osteopontin in melanoma. As a secreted molecule, osteopontin is a potent chemoattractant with a classic integrin-binding RGD motif that promotes invasive attributes by activating the expression of matrix metalloprotease (MMP) (50). Abolishing Hedgehog signaling by silencing *GLI1* reduced malignant properties, characterized by slower tumor growth and decreased metastasis, concomitantly with a reduced expression of osteopontin, suggesting that osteopontin mediates the promalignant effects of the Hedgehog 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 that are highly motile and invasive. Li and colleagues (51) 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, a transcription factor that regulates several genes associated with cell migration and motility as well as cell-cell adhesions. In gastric cancer cells, Hedgehog 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-5AC (MUC5AC), resulting in decreased membranous E-cadherin, increased nuclear accumulation of β -catenin, and increased pancreatic cancer cell migration and invasion (53). Thus, Hedgehog 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 that is 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 misrepair 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 by exposure to exogenous agents, such as ionizing radiation or radiomimetic chemicals, as well as by 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 Hedgehog signaling (54). Conversely, compromised expression of Ptch (Ptch^{+/-} mice) or upregulated GLI1 expression disrupted checkpoint kinase 1 (Chk1) 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 and colleagues (56) revealed that upregulated expression of GLI1 causes genomic instability, likely due to enhanced expression of the stem cell gene SOX2. Thus, aberrant Hedgehog 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 tumors undergo metabolic switches that enable them to use an anaerobic means of obtaining energy. The Hedgehog pathway plays an important role during embryonic development characterized by low oxygen tensions. This pathway is reactivated in several tumors in regions of hypoxic oxygen tensions similar to fetal (57). Although no published evidence directly implicates Hedgehog signaling in the Warburg phenomenon, analyses of hypoxic versus normoxic conditions clearly indicate the indirect roles of Hedgehog signaling in cancer progression. Souzaki and colleagues (48) reported that hypoxia activates Hedgehog signaling in

pancreatic cancers, leading to the transcription of molecules that enhance invasiveness. It is likely that Hedgehog signaling-enhanced growth potential may be a result of or may lead to an increased ability to uptake and metabolize glucose for proliferative activities (27, 29, 46).

Evading immune surveillance

The immune system of 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 the immune response and escaping from immune surveillance. Over the past few years, Hager-Theodorides and colleagues (58) and Rowbotham and colleagues (59, 60) have published a body of work that elucidates the role played by Hedgehog signaling in T-cell fate and activation and T-cell receptor selection. They determined that the Hedgehog pathway decreases the selection and repertoire of T cells, compromising their activation and proliferation.

Inflammation

The expression of Hedgehog ligands and the activity of Hedgehog signaling are increased under inflammatory conditions (61, 62). Hedgehog inflammation events vary by the cell/tissue type, location, and overall environmental stresses. Hedgehog ligands are upregulated in a variety of inflammatory bowel diseases. Sustained inflammation, which is key to promoting clinical complications associated with several inflammatory bowel diseases, often increases the risk of developing gastrointestinal cancers of the colon, stomach, and small intestine (63, 64). Endogenous Hedgehog 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- κ B signaling pathway, and Shh promotes the proliferation of pancreatic cancer cells in a paracrine manner through Hedgehog signaling (65). With regard to gastric cancer, it has been proposed that, although loss of Shh is essential for the formation of atrophy and metaplasia at early stages in *Helicobacter pylori*-induced gastritis, increased Shh signaling is important for promoting tumor growth and proliferation at later stages. In chronic *H. pylori* infections leading to carcinogenesis, the stromal cells show upregulated expression of GLI1 and the antiapoptotic bcl-2, in addition to elevated NF- κ B activity and IL-8 expression. IL-8 expressed as a consequence of elevated NF- κ B activity promotes the expression of Shh in tumor 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 (63).

As noted above, the Hedgehog pathway plays a role in immunological responses that often overlap with inflammation. CD4⁺ T cells treated with exogenous Shh stimulate production of inflammatory cytokines, such as IL-2, IFN- γ , and IL-10 (64). In some contexts, however, the Hedgehog pathway can be anti-inflammatory (66), which suggests the

possibility that Hedgehog signaling plays 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 Hedgehog pathway has been reported to affect the maintenance of the cancer stem cell population (46, 67), the cell population that self-renews and generates cells that form the bulk of the tumor. Whereas the Hedgehog pathway is active in glioma sphere-forming cells and in CD44⁺ CD24^{-/Low} Lin⁻ breast cancer stem cells, the maintenance and expansion of leukemia and multiple myeloma stem cells depend on Hedgehog signaling (31, 68). Treatment with cyclopamine causes a profound decrease in the hematopoietic stem cell population (69). The regulatory loop between Hedgehog signaling and p53, or rather the delicate balance between them, has also been proposed to regulate the number of cancer stem cells (69). Several studies have suggested that Hedgehog signaling has potent proproliferative effects in cancer stem cell/stem cell-like populations. The role of Hedgehog signaling in enhancing the viability of powerfully resilient stem cell populations was shown in cancers of the colon, breast, brain, and blood (31, 46, 64, 68, 70–75). Hedgehog signaling from glioblastoma stem cells upregulates insulin receptor substrate 1 (34, 76) and enhances survival of brain tumor stem cell populations (77). In mammary epithelial cells and in ovarian cancer, the expression of the self-renewal gene BMI-1 is upregulated due to Hedgehog signaling (78, 79). Given the fact that stem cells confer resistance to chemotherapy and radiotherapy and findings that suggest a role for Hedgehog signaling in chemotherapy and radiotherapy resistance, it is indeed conceivable that this pathway affects cancer stemness.

Tumor microenvironment

The range of Hedgehog signaling is extensive. In a heterogeneous tumor mass, combinations of paracrine, juxtacrine, and autocrine signaling are possible and have been shown to promote tumor progression (80). However, the tumor microenvironment also contains nonepithelial cells that play an equally important role in promoting cancer. Described below are a few stromal compartment members that at any given time may be involved directly or indirectly in cancer progression. These typically nontransformed cells are also sensitive to signaling cues generated by Hedgehog signaling from cancer cells; however, these cells can also generate signaling molecules that can act on the cancer cells, leading to further amplification of Hedgehog signaling (Fig. 1).

Fibroblasts. Fibroblasts are phenotypically mesenchymal cells that 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. Hedgehog 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 (81). 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 may drive epithelial cell proliferation, leading to genetic instability and accumulation of mutations (81). Although Hedgehog signaling is activated in hepatic cancers, it is unclear at what stage this pathway contributes. Stellate cells also appear to play a key role in pancreatic cancer. Hedgehog signaling generated from pancreatic cancer cells regulates several proteins, such as TGF- β , platelet-derived growth factor, SHH, and galectin-3, all of which activate stellate cells to produce proproliferative, promigratory, prosurvival molecules (e.g., platelet-derived growth factor, SDF-1, EGF, IGF, and SDF-1) that promiscuously signal to cancer and stromal cells (82).

Heterotypic paracrine Hedgehog signaling is also evident in the murine melanoma tumor microenvironment. Fibroblasts begin to express Ptch when cocultured with a Shh-producing melanoma cell line, B16F0 (42). This implies that Hedgehog signaling can become activated in cells surrounding nontransformed cells as a consequence of being in close proximity to the cancerous Shh-producing cells.

Endothelial cells. The Hedgehog pathway highly influences the proangiogenic behavior of endothelial cells. Hedgehog ligands have been shown to promote proliferation, invasion, and migration of endothelial cells (22, 83). Recent findings suggest that Hedgehog 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 Hedgehog ligands (22, 83). 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 Hedgehog ligands (22). SHH promotes endothelial cell expression of MMP9 and osteopontin, and it causes Rho-dependent migration (84) and phosphorylation of focal adhesion kinases (85). In fact, SHH present in microparticles released by lymphocytes can correct endothelial cell injury by increasing the expression of nitric oxide (86). The behavior of endothelial cells is largely in response to the ligands expressed by the tumor cells.

Osteoblasts and osteoclasts. Bone presents a rich hypoxic microenvironment for metastatic prostate and breast cancer cells. Metastatic tumor cells initiate a cross-talk with cells in bone, disrupting the homeostasis of the osteoblasts and osteoclasts. Our recent research elucidated the role of tumor cell-initiated Hedgehog signaling in osteoblast and osteoclast differentiation and their mineralization and resorptive activities, respectively. Breast cancer cells express Hedgehog ligands that directly activate

osteoclast precursors to terminally differentiate into osteoclasts that can resorb bone matrix through the MMPs and cathepsin K proteases and tartrate-resistant acid phosphatase (87). GLI2 expression in MDA-MB-231 cells causes increased osteolysis by enhancing the expression of parathyroid hormone-related protein (88). There is also evidence of nonclassical activation of GLI signaling, initiated by Runx2 and TGF- β , leading to enhanced osteolysis (89, 90). Thus, modulation of the metastatic site is facilitated by the cross-activation of Hedgehog signaling initiated by tumor cells.

We have shown that the Hedgehog pathway influences multiple aspects of tumor development, progression, and metastasis. In the following section, we briefly describe the clinical status of various Hedgehog inhibitors.

Therapeutic Targeting of Hedgehog Signaling

Hedgehog inhibitors mainly target the Hedgehog pathway by (i) neutralizing the activity of Hedgehog ligands, (ii) inhibiting SMO, and (iii) inhibiting the activity of GLI transcription factors. Cyclopamine, the first naturally occurring Hedgehog inhibitor to be identified, has shown promise for managing multiple malignancies (47, 91–94).

Although CUR61414 (Curis/Genentech) failed a phase I clinical trial (69), GDC-0449 (Genentech/Roche/Curis), a potent orally bioavailable SMO inhibitor, is in phase II trials (69, 95) and has shown promising outcomes in basal cell carcinoma (96) and medulloblastoma (95, 97, 98). IPI-926 (Infinity Pharmaceuticals) is in phase I clinical trials following a successful preclinical trial (99). BMS833923/XL139 (Bristol Myers Squibb/Exelixis) and LDE225 (Novartis), both orally bioavailable SMO antagonists, are also in clinical trials (95).

Other Hedgehog pathway inhibitors include vitamin D3 (which directly binds SMO), 5E1 Hedgehog ligand neutralizing antibody, curcumin, and arsenic (100). GANT-58 and GANT-61, inhibitors of GLI transcriptional activity, caused tumor regression in preclinical models (101). New research has uncovered 4 Hedgehog inhibitors that are epistatic to Sufu and target GLI processing, GLI activation, and cilium formation (102). These 4 Hedgehog inhibitors are mechanistically distinct from SMO antagonists.

Conclusions

It is now understood that the Hedgehog pathway plays an important role in all aspects of tumor progression. What began as early investigations in basal cell carcinoma and medulloblastoma soon grew to include several solid and hematologic malignancies. The involvement of this pathway in maintaining the cancer stem cell phenotype suggests that it also plays a role in tumor development. The Hedgehog pathway is not exclusive and cross-talks with other signaling pathways (e.g., the EGF, NF- κ B, and Wnt pathways) as well as p53. Signaling via this pathway can be activated in a ligand-dependent manner

and by activating mutations of members of this pathway. The Hedgehog pathway is not confined to autocrine signaling alone; it participates in paracrine signaling between the ligand-producing cells and the surrounding responding cells. The relevance of paracrine Hedgehog signaling was highlighted further when inhibitors of this pathway were shown to have a more profound effect on stromal cells than on the tumor cells themselves in xenograft models. Although various inhibitors have now made their way into both early and advanced clinical trials, researchers are continuing to design more effective, more specific, and less toxic compounds to inhibit Hedgehog signaling. However, one must take care when devising strategies to target the Hedgehog pathway. It is necessary to bear in mind that the targets may not necessarily reside in Hedgehog-producing cancer cells; rather, they may be located in the nontransformed cells in the stromal compartment that respond to the signaling molecules generated via the pathway. The role of non-canonical Hedgehog signaling in activating other pathways, such as Erk (103) and Rho signaling (83), must also be factored into these strategies. The efficacy of a compound is always put to the test in a clinical trial, where the ever-evolving tumor and its microenvironment can develop resistance to that compound 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 (104). Thus, although the search for alternate or complementary strategies to inhibit Hedgehog signaling continues, some promising data have emerged showing that targeting GLI1 directly can resensitize glioma tumors to drug therapies (105). In sum, there is an urgent and unmet need to better define existing modalities and identify novel strategies to inhibit Hedgehog signaling, identify the appropriate patients for therapy, develop reliable markers of efficacy of treatment, and combat resistance to Hedgehog pathway inhibitors by targeting not only the tumor cells but also the paracrine network in the tumor microenvironment that dictates and modulates the malignant behavior of tumor cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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