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Learning from Jekyll to control Hyde: Hedgehog Signaling in Development and Cancer

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Abstract

The Hedgehog transduction cascade is a key part of the system that regulates how a single cell is transformed into a complex multicellular organism. This ubiquitous developmental pathway controls cell proliferation, differentiation, and patterning of tissues during embryogenesis but then, in many tissues, is suppressed in the adult. The Hedgehog pathway can become reactivated in cancer. With a single efficient switch, the cancer is able to activate cell-growth pathways, recruit a blood supply, and invade adjacent tissues. In this review, we focus on how and when the normal Hedgehog pathway is turned back on to aid the neoplastic process. Cancers can be categorized based on the role Hh signaling plays in their growth. In the first category, abnormal Hh signaling initiates growth of the tumor. In the second category, Hh signaling is important for maintenance of the tumor. In the third, unclassified, category of tumors, Hh signaling has been implicated but its role is not yet defined.

Introduction

The orderly process of development depends upon well-orchestrated signals. These signals transform a single cell into a complex multicellular organism. Incredibly, in spite of the complex end-result, the transformation employs relatively few types of signal, including Wnt, Notch, TGF β , FGF, and Hedgehog (Hh). These secreted protein signals direct cell proliferation, cell fate determination, epithelium-to-mesenchyme transitions, and rearrangement of cells by motility and adhesion changes. The ability to build organs and tissues during development is highly relevant to cancer. Mounting evidence suggests that tumors hijack normal developmental pathways for their own growth. By activating a single transduction pathway, the cancer is able to turn on growth, recruit a blood supply, and

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invade adjacent tissues. In this review, we first focus on how Hh signaling leads to normal organ development and then describe how the reawakened Hh cascade drives the initiation, growth, invasion, and maintenance of tumors associated with these organ systems.

Hh signaling was first described in the context of cell fate determination and patterning of the fruit fly, *Drosophila melanogaster* [1]. The core components of the Hh pathway have been delineated in the *Drosophila* and are conserved in mammals (Figure A) [2]. The basic signaling cascade exists as a series of repressive interactions, each protein holding the next in check, until the Hh-ligand induces the transcription of a still mostly unknown array of target genes. In the absence of Hh pathway activity, genes are actively repressed. When secreted Hh binds to its receptor Ptc, the inhibition of Smoothened (Smo) by Ptc is relieved. Smo subsequently activates the transcription of target genes (including *ptc* and *gli*) through the Gli family of transcription factors [3]. The resultant genetic program forms and organizes many tissues and organ systems during embryogenesis.

The role Hh plays in the growth of tumors can be classified according to how the pathway is activated (for a review, see [4]). These mechanisms include loss-of-function mutations in inhibitory proteins such as Patched1, gain-of-function mutations in positive regulators such as Smo, over-expression of the Hh ligands leading to activation of the pathway in an autocrine or paracrine fashion, and renewal of cancer stem cells (Figure C). Hh signaling was first linked to cancer when a mutation in the *PTCH* (human *patched1* gene) was found to cause Gorlin syndrome, a rare genetic disorder characterized by tumor formation in the skin (Basal Cell Carcinoma, BCC), cerebellum (medulloblastoma, MB), and soft tissue (Rhabdomyosarcoma, RMS) [5-7]. Loss of one copy of *PTCH* is sufficient to cause the syndrome; germline mutation of both copies is presumably fatal as it is in mice [6-8]. Thus *PTCH* is a tumor suppressor gene; *PTCH* mutations are inherited as autosomal dominant causes of Gorlin syndrome. In the tumors, both copies of the gene are usually inactivated. In the late 1990s, most sporadic BCCs were found to have hyper-activated Hh signaling [7]. Subsequently, mutations in other Hh pathway components, including hyper-activating mutations of *SMO* [9] and loss-of-function mutations in *Suppressor of fused* (*SUFU*), were discovered in BCC [10]. Activating Hh pathway mutations can cause sporadic MB [11]. As is typical in other developmental pathways, activating components of the pathway are potentially proto-oncogenes that promote tumor growth when over-active, while restraining components of the normal pathway are tumor suppressors that can become damaged to allow tumor growth (Figure A). Following this initial indication that the Hh pathway was playing a role in rare cancers, numerous other cancers have been discovered to have aberrant pathway activation (Figure B). It has been speculated that Hh plays a role in over one-third of deaths caused by cancers [12].

Development provides a critical context for understanding tumorigenesis. Just as Hh signaling will play a different role in each tissue where it operates, the effect of damage to Hh signaling may have different implications for tumorigenesis in each type of developing cancer. In this review, we classify tumors by when Hh becomes important for the neoplastic process (Figure D). We describe Hh signaling during normal development of the tissue, followed by how Hh is thought to be important for the tumor arising from that same tissue. The first category includes cancers in which a mutation in the Hh pathway plays an initiating role in tumorigenesis. Examples of this type include MB and BCC. The second category includes cancers where Hh signaling does not initiate tumorigenesis but contributes to maintaining tumor growth. This category includes colon cancer and pancreatic cancer. The final category, the unclassified group, includes cancers where the Hh pathway has been implicated, but its exact role remains a mystery. An example is lymphoma. Finally, we will discuss future challenges in tumor biology as they relate to Hh signaling.

Category 1 cancers: Hh signaling is important for tumor initiation and maintenance

In Gorlin syndrome the mutation was mapped to loss of one functional copy of the human *patched1* (*PTCH*) gene [7]. The discovery led to the engineering of several mouse models of Hh pathway activation. These mouse models revealed that constitutive Hh signaling alone is sufficient to form BCC, RMS, and MB (for reviews [13], [14]). Activating the Hh pathway at any of several levels of the signal transduction cascade, or in conjunction with inactivation of additional tumor suppressor genes, can lead to formation of these tumors [15, 16]. A large proportion of sporadic BCCs and a subset of MBs and RMSs, harbor activating mutations of Hh pathway genes [7, 9, 17, 18]. These findings with sporadic BCC, MB, and RMS implicate activating Hh pathway mutations and the resultant Hh target gene over-activity as initiating events in tumorigenesis.

Skin development and basal cell carcinoma

Skin is the largest organ in the body, acting as a barrier to protect against external insult and to regulate internal temperature and water balance. Skin is composed of stratified layers of epithelia. The deepest stratum consists of a single layer of cells that are continually proliferating to replace outer terminally differentiated cells that are endlessly sloughed off. During embryogenesis, skin is formed when epithelial stem cells are guided by a series of epithelial–mesenchymal interactions to become the epidermis, associated hair follicles, and glandular structures (for a review see [19]).

One signal important for the formation of the hair follicle is Sonic hedgehog (Shh), one of three mammalian Hh ligands. During normal hair development, Shh is expressed in an area called the epithelial placode and its receptor Ptc is expressed in the underlying dermal condensate. Shh acts on Ptc to cause epidermal proliferation and invagination to form a hair follicle. Mice with forced expression of Shh in the skin have abnormal epidermal proliferation, whereas mice lacking Shh fail to develop normal epithelial invaginations during hair follicle development [20–22]. In the adult, hair follicles cycling continuously through periods of active growth (anagen), regression (catagen) and quiescence (telogen) [23]. Reactivation of follicle growth in the adult is regulated by the same signals important for follicle formation during embryogenesis. Cyclic production of Shh drives the hair follicle cycle. Increased *Shh* expression and activation of Hh target genes in the adult acts as a biologic switch to induce resting hair follicles to enter anagen, with consequent hair growth [24]. Once the hair follicle is regenerated, Shh expression is shut down and Hh targets are turned off.

Because of constant exposure to and damage from the environment, skin is the site of the most common cancer, basal cell carcinoma (for a review see [13]). Although the prevalence is high, the death rate is very low because these tumors rarely metastasize. If not treated, BCC can cause significant local tissue destruction and, for those that develop metastatic disease, it can be difficult to cure. Morphologically, the tumor resembles hair follicles and is thought to arise from the abnormal proliferation of the cells that normally form the hair follicle (for a review see [25]).

Aberrant Hh signal transduction is thought to be the pivotal abnormality in all BCCs. Activating mutations in the Hh pathway on multiple levels can lead to BCC. More than 90% of sporadic BCCs have mutations in one allele of *PTCH* and an additional 10% have activating mutations in the gene *SMOOTHENED* (*SMO*), a downstream component of the Hh pathway [13]. Mutations identified in *PTCH* and *SMO* presumably arise from UV radiation and result in Hh pathway over-activity [26, 27]. Mice heterozygous for *PTCH*

develop BCC after the deletion of the wildtype copy [21, 28]. Constitutively active *SMOH* can produce murine BCC [9]. Mice engineered to contain one inactive allele of the Hh pathway negative component *SuFu* can develop BCC [29]. Activating the transcriptional effectors Gli1 or Gli2 can lead to BCC [30, 31]. The magnitude of Hh target gene activation often correlates with the severity of the tumor phenotype in skin [30].

Constitutively active Hh signaling induces Wnt/ β -catenin signaling in BCC, and this induction is required for tumor formation [30, 32]. Like Hh, Wnt/ β -catenin is important for hair follicle development and cycling. The Wnt signaling pathway initiates hair bud formation and is required for the subsequent Hh signaling that results in proliferation of the epithelium to form the mature follicle [6]. Interestingly, in Hh pathway-driven BCC, the relationship is reversed.

While the mainstay of BCC treatment remains surgical, understanding the pathogenesis of BCC has given rise to new pharmacologic therapies (for a review see [33]). One exciting new option employs compounds that bind to and inhibit the Smoothed protein [34]. Some are being used in initial trials with BCC cases that become locally advanced or metastatic. Numerous companies are developing Hh pathway inhibitors. The Genentech-Curis GDC-0449 oral compound has been described most extensively. In a recently reported clinical trial, GDC-0449 had antitumor activity in locally advanced or metastatic BCC that had Hh target gene over-activity. Of 33 patients in the study, 18 had an objective response to GDC-0449, according to assessment by imaging and/or physical examination. Of the patients who had a response, 2 had a complete response and 16 had a partial response. The other 15 patients had either stable disease (11 patients) or progressive disease (4 patients) [34].

The primary cilium, a tiny hair-like projection on the surface of most cells that is important for Hh signaling, has been recently implicated in BCC and MB tumorigenesis [35, 36]. Cilia appear to have both activating and inhibitory roles in modulating Hh pathway activity—a role that depends on the level at which the pathway is mutated. Because of the dual role of primary cilia in these tumors, these micro antennae are not yet clear candidates for pharmacologic manipulation as therapeutic targets. Therapeutic advances will likely come as we develop a better understanding of the role of primary cilia in Hh signaling.

Cerebellum development and medulloblastoma

Normal cerebellum development requires formation and organization of five classes of cerebellar neurons [37-39]. Cerebellar granule neurons (GNs) are the most abundant type of neuron in the brain, with each mouse brain containing approximately 10^8 GNs. In the mouse, immature granule neurons proliferate in the external germinal layer (EGL) of the cerebellum and, in the first three weeks following birth, a large pool of granule neuron precursors (GNPs) is generated on the surface of the developing cerebellum. *Ptc1* restrains proliferation of GNPs until Shh is secreted by Purkinje neurons to oppose the *Ptc1* restraint of target gene activation [40]. Hh pathway activation results in the proliferation of GNPs within the EGL of the cerebellum. This was established by *in vivo* and *in vitro* studies demonstrating that treatment of cultured GNPs with Shh prevents differentiation and maintains proliferation, while blocking Shh function reduces GNP proliferation [40]. Following this period of proliferation, GNPs exit the cell cycle and become mature granule neurons [40, 41]. How GNPs cease proliferating to become mature granule neurons is unclear [14, 42].

MB, a tumor that appears to arise from over-proliferating GNPs, is the most common malignant brain tumor in children [43]. Many children with MB survive, but they often suffer long-term cognitive and psychosocial impairment as a result of the standard

radiotherapy and chemotherapeutic regimen [43]. These consequences of therapy underscore the importance of understanding the process of tumorigenesis in MB to specifically target pathways important for tumor formation.

Mutations that reduce restraints on the Hh pathway in GNPs lead to MB. Reducing the dose of *PTCH* by half results in less opposition to the inductive strength of the Shh signal and a persistent expression of Hh target genes including those that support continued cell division and tumorigenesis. *Ptc1*^{+/-} heterozygous mice that have approximately 50% reduction in *Ptc1* transcript abundance relative to wild-type mice, show reduced Shh target gene expression and unregulated GNP proliferation [44]. Hyperproliferative GNPs form patches of pre-neoplastic lesions, which appear on the surface of *ptc1*^{+/-} mouse cerebellums, but not on the cerebellums of wild-type mice [45, 46]. Most of these pre-neoplastic lesions are transient, with detectable GNP foci persisting only on the cerebellar cortical surfaces of the approximately 15% of mice that are developing or have developed MB tumors by 3 months of age [44].

The full process of neoplastic progression in MB remains incompletely understood. Although constitutive Hh pathway activation in *Ptc1* heterozygous mouse MB lesions is well-documented, *Ptc1* haploinsufficiency itself is not sufficient for neoplastic transformation given the low penetrance of MB in mice heterozygous for a mutation in the *Ptc1* allele [45, 46]. An additional mutation resulting in constitutive Shh target gene activation is required to release GNPs from the anti-proliferative influence *Ptc1*. Given that *Ptc1* is a tumor suppressor gene, one clear candidate for a possible “second hit” to promote tumorigenesis was somatic mutation of the second copy of *ptc1* leading to loss of heterozygosity, as in human basal cell carcinoma. Data support inactivation of the second *Ptc1* allele and ensuing loss of heterozygosity as a mechanism for MB tumorigenesis [47], but other mutations leading to neoplastic progression of early lesions may contribute instead or in addition [15, 16]. The primary cilium, a sub-cellular organelle important for proper Hh pathway signaling has been recently implicated as the “second hit” in MB and BCC tumorigenesis [35, 36].

Our enhanced understanding of the role of Hh in cerebellum development and neoplastic transformation of MB has led to repeated attempts to disrupt Hh signaling in MB using animal models [48]. Inhibiting Smo blocks proliferation in resected human MB tumors and prevents formation of MBs in mouse models of MB. Smo antagonists induce a gene expression profile in GNPs consistent with a reduction in proliferation and an enhancement of differentiation ([49]; for a review, see [50]). Because of the importance of Hh in normal bone development, mice treated with Smo antagonist suffer from irreversible defects in bone structure [51]. Second-generation Hh-pathway inhibitors including a small molecule inhibitor of Gli1 are currently being tested [52].

The partial success of the Hh inhibitor GDC-0449 in BCC has led to tests of its usefulness for human MB. GDC-0449 was recently used to treat a 26-year-old man with metastatic MB that was refractory to treatment [34]. Treatment with GDC-0449 resulted in rapid regression of the tumor and reduction of symptoms, but the effectiveness of the therapy was transient, due to quickly-acquired resistance due to mutation of *SMO* [53]. These clinical results highlight the promising future of rationally designed targeted therapy, as well as its challenges.

Muscle development and rhabdomyosarcoma

The Hh pathway is critical for many epithelial-mesenchymal interactions in development and plays an important role in myogenesis—the formation and specification of muscle [54]. Myogenesis occurs after muscle precursor cells have been specified, in part by expression of

muscle regulatory and myocyte enhancer factors [54]. Hh has been implicated as a regulatory signal for the induction of muscle-specific genes and expansion of muscle progenitor populations during embryogenesis [54]. Normally turned off in adult musculature, Hh is reactivated during regeneration of adult skeletal muscle after an injury [55].

Unrestrained Hh activity, for example after *Ptc1* loss, can lead to rhabdomyosarcoma (RMS). RMS is the most common soft-tissue sarcoma in the pediatric population, accounting for up to 10% of solid pediatric malignancies [56]. RMS morphologically resembles skeletal muscle and is classified into two sub-types with varied tumor locations and ages of presentation. Staging and treatment of RMS depends on tumor size, spread, and effectiveness of resection [56]. While most RMS occurs sporadically, it is sometimes associated with human Gorlin syndrome and the mouse *Ptc1* haploinsufficiency model of Gorlin syndrome (reviewed in [14, 18]). RMS tumors have a variety of genetic and molecular abnormalities, some of them useful for accurate prognosis or identification and targeted therapy of biological subtypes [57]. Most alveolar RMS tumors have translocations involving PAX3-FKHR or Pax7-FKHR fusion genes as well as *PTCH* imbalances [58]. In addition to the Gorlin syndrome model, manipulation of PAX and FOX genes, coupled with combinatorial activation of known oncogenes and inactivation of known tumor suppressor genes, leads to RMS in mouse models [58-60]. Still, little is known about the underlying genetic changes that cause a developing skeletal muscle cell, or mesenchymal progenitor cell derived from the embryonic mesoderm, to transform into invasive malignant RMS.

Category 2 cancers: Hh signaling is important for maintenance not initiation of the tumor

Category 2 includes cancers where Hh signaling does not play a role in initiating tumors, but instead is important for tumor maintenance and growth through constitutive activation of developmental programs. Examples of Category 2 tumors include colon cancer and pancreatic cancer. In both, Hh signals derived from the stroma are important for maintaining proliferation of tumor cells.

Colon cancer

The gastrointestinal tract forms from two germ layers, the endoderm and mesoderm, and is innervated by cells arising from the ectoderm. *Shh* and *Ihh* knockout mice, and *in situ* expression data, reveal that folding the primitive tube into the complex adult organ depends on Hh ligands carrying signals between the epithelial cells and the surrounding mesenchymal cells in a paracrine fashion (for reviews see [36, 61]). As the primitive gut tube forms it develops villi, finger-like projections of the small intestine that project into the lumen, by upward growth of the underlying mesenchyme. In a similar fashion, epithelial folds are formed in the large intestine. Early in development, *Shh* and *Ihh* are found diffusely throughout the endoderm. Which cells express and which cells receive the Hh signal in this tissue is controversial. Experiments to determine this are difficult, and cellular targets of Hh signaling are stage-specific during development. The current hypothesis is that the villi and epithelial folds form when *Shh* expression is restricted to the area that eventually becomes the base of these folds. Eventually, cells differentiate at the base of these intestinal invaginations to become glandular crypts. The restriction of *Shh* expression inhibits epithelial proliferation to allow normal crypt development by growth of the flanking epithelium.

A recent study profiled Hh pathway activity in the mouse from embryonic day 10.5 to adult in murine antrum, pyloric region, small intestine, and colon [62]. These data indicate that

paracrine Hh signaling occurs throughout the gastrointestinal tract during embryonic and adult life [62]. Mice lacking Shh cannot form the invaginated surface of the intestine and instead over-grow the epithelium [63, 64]. *Ihh* knockout mice have decreased numbers of hypoplastic villi as well as a failure to form the enteric nervous system resulting in dilation of their colon [64]. Hh signaling plays an important role in the radial patterning of the developing colon including regulation of smooth muscle formation and proper rotation of the entire system. The adult colon produces a low level of *Shh* mRNA at the base of the crypts [57]. The distal colon accumulates Ihh at the tips of the differentiated colonic enterocytes. *Ptc1* mRNA is detected in the mesenchyme underlying the Ihh-positive cells, suggesting that the direction of signaling in the adult is similar to that of the developing embryo [57]. Recent data implicate Hh signaling in patterning processes such as formation of the pyloric sphincter and active modulation of villus core smooth muscle [62].

The connection between Hh and colon cancer was reported when transcripts of *Shh*, *Patched1*, and *Smoothed* genes were found in human and mouse hyperplastic polyps, adenomas, and adenocarcinomas of the colon [65, 66]. The growth of colon cancer cell lines *in vitro* is inhibited by cyclopamine, an inhibitor of the Hh pathway. Both of these findings suggested that Hh was acting in an autocrine fashion in colon cancer. The cancer may have been producing its own ligand and receptor in a positive feedback for growth. These data has recently been criticized when the original cyclopamine inhibitor studies were redone at inhibitory doses that restrict the drug effects to Smo which did not inhibit the colon cell lines [67]. A new idea was suggested based upon normal development. In this view colon cancer cells over-produce Hh ligands that influence the surrounding non-malignant mesenchyme to release a feedback mitogen, in a paracrine loop. Inhibiting the stroma's ability to activate the Hh pathway with an oral antagonist, an antibody, or genetic deletion inhibits the ability of human colon cancer xenografts to grow [67]. Epithelial cells of human colon carcinomas and their stem cells have Hh target gene activity peaks at the time of metastatic spread [68]. The details of signal interplay between the carcinoma and its surrounding stroma remain controversial, but if the developmental pathway is reactivated, Shh would signal in a paracrine fashion [69].

Hh pathway mutations may drive colon cancer growth indirectly by allowing activation of the Wnt pathway (for a review see [70]). Wnt is important during early gut development when β -catenin promotes the formation of the gut endoderm and its intestinal fate. In concert with Hh, activation of Wnt is required for proper formation and maintenance of the villi and crypts. Accumulation of nuclear β -catenin, a hallmark of active Wnt signaling, occurs in intestinal crypts [71]. Removal of Tcf4, an activator of Wnt target gene transcription, prevents development of intestinal crypt progenitor cells [72]. Over-expression of oncogenic forms of β -catenin, or mutations in a negative Wnt pathway component, Adenomatous polyposis coli (APC), results in hyper-proliferation of the epithelium [73]. The Wnt pathway is active in up to 90% of hereditary and sporadic colon cancers (for a review see [74]).

The Hh pathway can inhibit the Wnt pathway [75]. When either Shh or Ihh is inhibited the Wnt target genes *Engrailed-1* and *BMP-4*, normally restricted to the crypt, become widely expressed throughout the intestinal epithelium and may increase epithelial proliferation. Loss of Hh ligands may activate the Wnt pathway in colon cancer. *Ihh* expression is absent, for example, from adenomatous polyps in patients with familial adenomatous polyposis [76]. Gli1 negatively regulates Wnt signaling by suppressing nuclear accumulation of β -catenin [77]. If Shh suppresses Wnt activation, the reason for the apparent overlap of Shh and nuclear β -catenin expression in the intestinal crypts is not clear. The answer may come with improved immunohistochemistry and imaging studies. The Hh ligand knockout mice do not resolve the relationship with Wnt. While *Shh* knockout mice have intestinal metaplasia, *Ihh* knockout mice have fewer cycling epithelial cells [63, 64].

The role of Hh actions in colon cancer remains controversial, but clinical trials have already begun. Based on preclinical data from xenograft mouse models as well as phase I trials, GDC-0449 is being tested in a phase II clinical trial as therapy for metastatic colon cancer (www.clinicaltrials.gov). Given that Hh appears to be important for the normal regeneration of the intestinal crypts, it will be important to determine what effect inhibiting the Hh pathway has on normal intestinal turnover and response to injury.

Pancreas development and cancer

Hh signaling is crucial for the development of the pancreas (for a review see [61]). About 95% of the pancreas consists of exocrine cells that produce digestive enzymes while the remainder consists of small clusters of endocrine cells important for the production of various hormones including insulin. The adult pancreas is formed by the fusion of two primitive pancreatic buds, a ventral bud that ultimately forms the pancreatic head and uncinate process, and a dorsal bud that ultimately forms the pancreatic tail. Pancreas development is dependent upon signaling between the mesoderm and two regions of ventral and dorsal foregut endoderm. The endoderm uniformly expresses *Shh* and *Ihh* until about E8.5. At that time release of FGF-2 and Activin-B from the notocord stimulates increased expression of the transcription factor *Pdx1* and loss *Patched* and *Shh* expression in the dorsal endoderm. Absence of *Shh* allows formation of the dorsal pancreatic bud, and contributes to forming the ventral pancreatic bud. Forced expression of *Shh* in mouse pancreas precursor cells prevents normal pancreas formation and creates tissue that resembles intestine [78]. *Xenopus* injected with mRNA encoding constitutively active Smoothened completely lack a pancreas [79]. Mouse embryos exposed to cyclopamine, an inhibitor of Smo and therefore of Hh signal transduction, form ectopic pancreatic tissue throughout the intestine [80]. Thus loss of *Shh* promotes pancreatic development and over-expression of *Shh* prevents proper pancreatic development. In the adult human, *Ihh*, *Ptc1*, and Smo are localized in pancreatic endocrine tissue and may foster insulin secretion [81]. *Shh* expression is absent in adult pancreas [82]. Inhibiting Hh signal transduction in hamster pancreatic ductal cells reduces cell growth, suggesting that Hh is important for normal pancreatic regeneration [83].

Pancreatic cancer is the fourth most common malignancy in the United States, with 30,000 new cases diagnosed each year. The bleak landscape of pancreatic cancer therapy is highlighted by the deaths of about this same number of pancreatic cancer patients each year. A large body of literature suggests that activated Hh target genes drive human pancreatic adenocarcinoma [83]. Primary human tumor specimens and tumor cell lines have high levels of mRNA and protein from the target genes *Gli1* and *Ptc1* [65, 66]. Inhibiting Hh signaling using a direct inhibitor of Smo, cyclopamine, can inhibit the growth of a subset of human pancreatic cancer cell lines grown *in vitro* or in xenograft models [65, 66]. Cyclopamine can even inhibit growth of cell lines derived from aggressive, metastatic tumors suggesting that in spite of their aggressive behavior, the tumors are still dependant on proper Hh signal transduction pathway for growth [65]. Over-expression of *Shh* in the mouse pancreas causes “PanIN” lesions similar to early human pancreatic neoplasms in morphology and genetic changes (e.g. Her2/Neu amplification, Ras mutations) [84]. A mouse with inducible activation of a dominant-active Gli2 developed undifferentiated tumors that grew invasively [85]. When dominant active Gli2 was produced in the pancreatic epithelium along with constitutively active Ras, extensive PanIN lesions and reduced latency of onset of invasive pancreatic tumors was observed [85]. This work strongly suggests that a subset of pancreatic cancers are driven by Hh signaling and that this pathway is an attractive target for novel therapeutics.

Hh signaling in pancreatic cancer occurs in a paracrine fashion [67]. Initial studies had demonstrated that pancreatic tumors have a high level of Hh target gene transcription as measured by *Ptc1* mRNA compared with adjacent normal tissue. Blocking pathway activity

with the Smo antagonist cyclopamine, or with an anti-Shh antibody, inhibited tumor growth, suggesting that pancreatic tumor growth is driven by a Shh autocrine loop [65, 66]. This hypothesis has recently been discarded in favor of a paracrine loop that recapitulates normal pancreatic development. The two reasons are, first, the cyclopamine dose used to inhibit pancreatic cell lines was found to exhibit off target effects [67] and, second, the amount of *Gli1* transcription in pancreatic cancer cells, a measure of Hh target gene activity, did not correlate with sensitivity to antagonists of the Hh pathway. Instead, pancreatic tumors increase production of the Shh ligand, which activates the Hh pathway in surrounding non-malignant stromal cells. The activated stromal cells signal back to the pancreatic tumor by an as yet undiscovered pathway to support epithelial growth and invasion. Inhibiting the stroma's ability to activate its Hh pathway with an oral antagonist, an antibody, or targeted genetic deletion inhibits the ability of human colon cancer xenografts to grow [67]. In a recent review, Theunissen and colleagues discuss epithelial cancers, including colon and pancreatic cancers. In these tumors, Hh target gene activation in the stroma provides a favorable environment for tumor growth [69]. The findings underscore two mysteries of Shh paracrine signaling in pancreatic adenocarcinoma. First, what genetic programs drive expression of Shh in pancreatic adenocarcinoma cells and, second, what is the feedback signal from the stroma.

Other theories for how the Hh pathway promotes pancreatic tumor growth include support of cancer stem cells and angiogenesis. Hidalgo and colleagues propose that Hh may stimulate proliferation of cancer stem cells, a proposed small fraction of the mass of tumor cells in epithelial carcinomas [86]. Nakamura proposes that Shh released from the pancreatic tumors may stimulate growth and stabilization of tumor vasculature [87]. They suggest that bone marrow-derived pro-angiogenic cells are targets for Shh signal and play a critical role in tumor neovascularization.

In summary, category 2 tumors include cancers that rely on Hh signaling to provide a proliferative signal once the tumor has formed, but Hh pathway activity does not initiate tumor formation. Now we turn to tumors that may eventually be added to this category.

Category 3 “unclassified” cancers: Hh signaling has been implicated in tumorigenesis but its role is not defined

Category 3 cancers include those where the Hh pathway is active, but its role in the initiation and growth of the tumor is not well defined. Many cancers fall into this category and include lymphoma, breast cancer, ovarian cancer, esophageal cancer, prostate cancer, liver cancer, and lung cancer.

Hematopoiesis and lymphoma

During development Hh ligands play numerous roles in the initiation and growth of hematopoietic cells (for a review see [82]). Ihh released from stromal cells activates hematopoiesis and supports growth of developing hematopoietic cells [88, 89]. In adults, Hh ligands released from bone marrow stroma and received by hematopoietic stem cells is required for their renewal [90, 91]. Shh produced by follicular dendritic cells of the lymph node is important for survival, proliferation, and antibody production by B cells [92, 93]. Shh can regulate proliferation of T-cells, and cytokine release [94, 95].

The ligands Ihh and Shh promote survival of B-cell malignancies such as lymphoma [96, 97]. Unlike solid tumors which themselves produce Hh ligand, Ihh and Shh are produced by surrounding non-malignant cells in the bone marrow, spleen, and lymph nodes to allow growth of lymphoma cells. These tissues serve as the equivalent of a stem cell niche, nourishing the ongoing production of dividing cells. In a c-myc-driven mouse model of

Burkitt's lymphoma, lymphoma cells retrieved from the transgenic mice underwent apoptosis in the absence of their surrounding microenvironment unless they were grown in the presence of Shh or Ihh. This suggests that the stroma provides the tumor cells with a pro-survival signal in the form of Hh ligands or other factors whose production is controlled by Hh ligands. The authors suggest that the consequence of activating Hh target genes in lymphoma may be increased production of pro-survival factors such as Bcl-2 and Bcl-xL. Supporting this idea, inhibition of the Hh pathway with cyclopamine caused apoptosis of cultured primary lymphoma cells and reduced *Bcl-2* transcription. Cyclopamine-induced cell death did not occur with tumor cells that, despite the cyclopamine, over-expressed *Bcl-2*.

Another study documented the importance of Hh signaling in mantle cell lymphoma (MCL), an aggressive subtype of B-cell lymphoma [98]. Transcripts of genes encoding the Hh pathway components Patched, Smoothened, Gli1, and Gli2 were over-produced in primary MCL cells and MCL cell lines. The growth of one of the MCL cell lines in culture was partially responsive to exogenous Shh. The growth of this same cell line was inhibited by addition of cyclopamine in the absence of Shh. Reduction of *Gli1* or *Gli2* mRNA with antisense oligonucleotides decreased the viability of four cell lines and increased their susceptibility to chemotherapy (doxorubicin)-induced cell death, though the effect of the antisense oligonucleotides, with or without chemotherapy, was minimal. MCL cells have increased Bcl-2 and cyclin D1 protein expression. Reduction of *Gli1* or *Gli2* mRNA minimally decreased the amount of Bcl2 and cyclin-D1 transcripts. The authors hypothesize that in MCL over-production of Gli1 and Gli2 proteins may increase Bcl-2 and cyclin D1 protein production and consequently survival. How the *Gli* genes are induced and when in MCL development they increase has not been reported.

Dysregulated Shh signaling may also be important for T-cell malignancies. One group demonstrated that *Shh*, but not *Gli1*, is amplified in anaplastic lymphoma kinase (ALK)-positive anaplastic large cell lymphoma (ALCL) tumors and cell lines [99]. Inhibition of Shh signaling with cyclopamine and Gli1 expression silencing with siRNA decreased viability of these lymphoma cells [99].

Prostate development and cancer

The Hh pathway is important for prostate gland patterning and development. The prostate exocrine gland operates in the male reproductive tract after developing through steps of epithelial branching [100]. Shh and Ihh are made by the fetal urothelium and, as in many viscera, Hh signaling influences proliferation and differentiation, including budding of prostatic ducts and stromal development [101, 102]. In mice, the level of Hh signaling declines as the organ matures and remains at low levels into adulthood [102]. In contrast to the low levels of Hh signaling in adult mice, significant Hh target gene transcription occurs in the adult human prostate [102]. The levels of Hh pathway activity into adulthood directly correlate with the expansion of glands and stroma of the prostate that occurs in benign prostatic hyperplasia and prostatic carcinoma [102, 103].

Whether Hh signaling is important for prostate cancer remains controversial [103-105]. Prostate cancer results from a multistep process that begins with a circumscribed non-invasive low-grade nodule composed of morphologically normal glands and progresses to an infiltrating higher grade adenocarcinoma, composed of atypical glands infiltrating the surrounding prostatic and urothelial tissue. In advanced human prostate cancers, Hh target gene expression is high [52, 103]. Autocrine Hh-signaling by prostate tumor cells has been proposed as a mechanism for their continued proliferation and invasive behavior, but some recent studies found no evidence of autonomous or paracrine signaling in prostate cancer cell lines using luciferase-based assays of target gene activation and analysis of Hh target

gene transcript levels [104, 105]. Hh pathway activation alone is insufficient for development of prostate carcinoma. Constitutive activation of Hh target genes by an activated allele of *Smoothed* in postnatal prostate epithelium did not cause tumor formation or any prostatic abnormality in 12 month-old mice [106]. Hh pathway activity could contribute to sustained growth of prostate carcinoma, but further work is needed to define the role of Hh, if any, in this cancer.

Esophageal development and cancer

Hh is important for the proper formation of the esophagus but its exact role in normal development is controversial (for a review see [61]). Differentiation of the esophageal layers begins at about E15 in the mouse. At E17, the epithelium contains ciliated and squamous cells. The ciliated cells are rapidly lost after birth. Shh is initially expressed throughout the developing endoderm, but becomes restricted to distal endoderm at later stages [63]. *Ptc1* and *Gli* genes are transcribed in the mesoderm [106]. Hh-signaling in normal developing esophagus goes from endoderm to mesoderm. In *Shh*^{-/-} mice, the esophagus fails to separate from the trachea, resulting in numerous tracheoesophageal fistulae [63, 107]. The esophagus of the *Gli2*^{-/-} mouse has a very small lumen with no smooth muscle layer and only a few mesenchymal cells [107]. *Gli2*^{-/-}, *Gli3*^{+/-} mice have only a small proximal esophageal remnant [107]. When *Gli2* and *Gli3* are genetically deleted, the mice fail to form an esophagus [107]. The phenotype results from inadequate mesenchymal growth.

In chronic gastric reflux disease in humans, acid from the stomach damages the mature esophagus and can lead to cellular metaplasia, a preneoplastic lesion, within the esophagus. Reflux is well-established as a risk factor for developing esophageal carcinoma and mouse models of chronic reflux are used to study esophageal carcinoma. Esophageal carcinomas can be divided into adenocarcinoma and squamous cell carcinoma subclasses. Ideas about a role for Hh signaling in esophageal carcinoma are based on correlating expression of Hh pathway components with histopathology of surgical specimens—not a strong basis for any conclusion.

Ihh was found to be over-expressed in cell lines derived from samples of esophageal carcinoma when compared with normal esophageal tissue which had no expression [108]. The expression of *Shh*, high in normal esophageal tissue, was lost in esophageal carcinoma cell lines. Using high doses of cyclopamine to block the Hh pathway; doses that may well affect other processes, slightly inhibited growth of the cell lines. In numerous esophageal squamous cell carcinoma cell lines, transcripts encoding Hh components are increased when compared with normal esophagus [109]. In a xenograft model of esophageal adenocarcinoma, activation of Hh target genes preceded regrowth of tumors after chemoradiation therapy [110]. Inhibiting Hh signaling using cyclopamine or siRNA to *Gli1* resulted in a significant decrease in tumor proliferation. Studies have been published correlating Hh expression levels in human esophageal carcinoma to outcomes. One study suggested that greater than 80% of post-chemotherapy human esophageal tumor biopsies had elevated protein levels of Shh and nuclear *Gli1* [110]. In our own lab we find that immunohistochemical staining on tissue specimens is difficult at best. Using a combination of *in situ* hybridization, real-time PCR, and immunohistochemistry, Hh pathway components were increased in primary human esophageal tumors when compared with normal esophageal tissue [111]. Staining of *Gli1* in esophageal tumors has been correlated with the extent of primary tumor, lymph node metastases and prognosis but the evidence is far from definitive [109]. In one study, detection of nuclear *Gli1* protein was an independent predictor of early relapse and poor prognosis in esophageal squamous cell carcinoma after preoperative chemoradiation therapy but it will be important to perform more controls to ensure that the staining is specific [112].

While Hh signaling clearly plays an important role in esophageal development, all the esophageal cancer findings provide only weak and circumstantial evidence for the relevance of Hh signaling. Further research will need to be conducted using mutations of the Hh pathway in a mouse model of esophageal carcinoma.

Mammary development and cancer

The epithelial component of the mammary gland originates from the ectoderm and a mature mammary gland develops from multiple epithelial-stromal tissue interactions. Many of these epithelial-stromal interactions are Hh pathway-mediated and crucial for proper mammary gland ductal morphogenesis (for a review, see [115]). *Ihh* and *Dhh* expression is low in early development, but rise during maturation of the ducts. The ligands are also elevated in ducts and alveoli during pregnancy and in later stages of involution and gland remodeling. The timing of Hh ligand production in mammary gland suggests that Hh signaling is reproductive-state dependent [116]. *Gli2* is expressed in the stromal compartment during early stages of mammary development, but becomes both epithelial and stromal during lactation and pregnancy [117]. *Ptc1* is expressed in both epithelial and stromal compartments in a developmental stage-dependent manner. The details of Hh during development of the mammary gland are still being elucidated.

Excess Hh signaling results in dysplasias and hyperplasias of mammary gland ducts [117, 118]. Heterozygous *Ptc1* mutations, which allow over-activation of Hh target genes, cause ductal hyperplasias and dysplasias [17, 118]. *In vivo* activation of human Smo under the mouse mammary tumor virus (MMTV) promoter results in increased proliferation, atypical differentiation, and ductal dysplasias distinct from those caused by *Ptc1* heterozygosity [119]. *Gli2* homozygous null mutants, where target gene transcription is reduced, have epithelial hyperplasias and ductal outgrowths [117]. In *Ptc1* and *Gli2* mutants, morphological defects were not observed when mutant epithelium was transplanted into a wild-type stromal background, suggesting that proper Hh signaling within the stroma is important during ductal development [116, 120].

Hereditary and sporadic breast cancers develop through abnormal activation of mammary gland self-renewal pathways. Hh signaling may be important for the maintenance and progression of breast cancer. Heterozygous *PTCH* mutations were identified in approximately a subset of human breast cancers, but their significance is unclear [17]. In human breast tumors, *Gli2* is expressed in pre-neoplastic hyperplastic alveolar nodules, but not in advanced mammary carcinoma, suggesting a role for *Gli2* in initiation but not maintenance of breast cancer. Deletion of the *PTCH* locus is the fourth most common change among tumor suppressor genes in breast cancer: 19% of human breast cancers and 33% of human breast cancer cell lines [17, 121]. Hh target genes, including *Ptc1* and *Gli1*, are elevated in many human invasive breast cancers compared with normal controls [122]. Studies of differential expression of Hh pathway genes showed that *Shh*, *Gli1*, *PTCH*, and *Smo* mRNAs are elevated in tumor samples, and higher levels of these proteins in stroma relative to tumor epithelium (for a review, see [116]). The correlative studies between high Hh target gene expression, often in tumor stroma, and breast cancer, indicate that Hh pathway activity could be important in breast cancers.

Ovarian development and cancer

Early in gestation, large primordial germ cells appear in the endoderm of the yolk sac wall of the primitive hindgut. These germ-cell precursors migrate to the urogenital ridge and form an undifferentiated gonad comprised of a surface epithelium surrounding the internal blastema. The blastema is a primordial mesenchymal cellular mass that in females will

eventually become the ovarian medulla. During mid-gestation, the first evidence of ovarian follicles is seen (for a review, see [123]).

In the mature ovary, follicles produce steroids and oocytes for ovulation. The primordial pool of ovarian follicles is present from birth and follicles are selected from this pool for continued development in preparation for fertilization (for a review, see [123-125]). As in other tissues, follicle development is a balance between proliferation and differentiation. In order for the ovarian follicle to develop, oocytes, granulosa cells, and theca cells must signal properly to other cells within and surrounding the maturing follicle [124]. As a primary follicle develops, granulosa cell architecture changes from flattened to cuboidal and the cells begin to secrete factors that control follicle development [123, 124].

Hh ligands are important for ovarian follicle development, ovulation, and reproduction. Primordial follicles do not have detectable levels of Hh pathway component mRNA [126]. At the primary follicle stage, when granulosa cells change morphology, Indian (Ihh) and Desert Hh (Dhh) secreted from granulosa cells induce target gene expression including *Ptc1*, *Ptc2*, *Gli1* and *Hip1* in theca cells of maturing ovarian follicles. Theca cells, derived from mesenchyme, are capable of producing steroids and are necessary for ovarian follicle development. Russell et al. examined ovarian expression of *Ihh*, *Dhh*, and *Shh* during mouse development [126]. *Ihh* and *Dhh* expression were low at birth, increased by postnatal day 4 when primordial follicles begin developing, and maintained elevated expression in prepubertal and adult mice [126, 127]. *Shh* was highest in ovaries on the day of birth, decreased transiently on postnatal day 4, returned to higher levels in prepubertal mice, then declined in adults. Blocking granulosa cell Hh signaling increased levels of progesterone. While correlative, this finding suggests that lower Hh may cause differentiation of granulosa cells into luteal cells following the LH surge [126, 127]. Oocytes, which express *Ptc1*, position themselves according to Ihh and Dhh signals from granulosa cells [127]. The *Dhh* homozygous null mutant mouse has no ovarian phenotype, indicating either redundancy between *Ihh* and *Dhh*, or *Ihh* as the main follicle development signal [123]. Thus Hh target gene induction occurs in the ovarian mesenchyme and Hh ligands (most likely Ihh) from granulosa cells regulate follicle development, granulosa cell proliferation, and progesterone production in the developing and mature mammalian ovary.

The connection between Hh signaling and ovarian carcinoma remains controversial. Ovarian fibromas occur in human Gorlin syndrome, the disease caused by haploinsufficiency of the *PTCH* tumor suppressor gene [122]. Transcription of the Hh ligand and pathway component genes *Shh*, *Dhh*, *Ptc1*, *Smo*, and *Gli1* was elevated in ovarian carcinoma relative to adjacent ovarian surface epithelium [128]. Expression of these genes increased with the morphological abnormality of malignant cells. Benign cells had the lowest expression, while borderline and malignant cells had progressively higher expression, and expression of target genes correlated with cell proliferation [128]. Other groups have demonstrated epigenetic regulation of *PTCH* in ovarian carcinoma [122]. We still lack a clear understanding of Hh pathway deregulation as an inciting or secondary event in ovarian carcinoma.

Liver development and hepatocellular carcinoma

The role of Hh signaling in liver development remains poorly characterized. BMP, FGF and Hh signals are important for liver organogenesis, implying a role for Hh signaling during early stages of liver development [12]. Most cells of the adult liver, however, have low levels of Hh target gene expression. Hh signaling in the adult liver maintains the hepatic stellate cell population—a population in the liver important for hepatic regeneration, fibrosis, and repair [129, 130]. Cells important for regeneration and repair, such as hepatic stellate cells and epithelial progenitor cells, are capable of Hh production and respond to Hh ligand [131, 132].

The Hh pathway is activated during liver repair and in both subsets of liver cancer, hepatocellular carcinoma (HCC) and cholangiocarcinoma. Chronic repair states, which can lead to HCC, are associated with activated Hh target genes [12, 133]. Liver cancers, including HCC arising from hepatocytes and cholangiocarcinoma arising from bile ducts, are the fourth leading cause of cancer deaths worldwide and the most rapidly increasing cancer sub-type in the U.S. HCC and cholangiocarcinoma produce Hh pathway components and HCC cells express Hh target genes [134]. *SHH* is expressed in about 60% of HCC surgical specimens and elevated Hh target gene expression (*PTCH* and *Gli1*) occurs in >50% of tumors relative to adjacent normal liver [135, 136]. Gorlin's syndrome is not associated with HCC, though a few cases have been reported [137]. At least three HCC cell lines have detectable expression of Hh target genes. In the cell lines, inhibition of Smo with its antagonist cyclopamine reduces expression of Hh target genes and results in apoptosis [135]. Because a similar pro-apoptotic effect was not observed after cyclopamine administration to HCC cell lines without Hh activation, the effect may specifically target the Hh pathway and implicate pathway activation in HCC progression. Though Hh signaling has been implicated in the initiation and progression of liver cancer, the precise role of Hh signaling in tumorigenesis remains unclear.

Lung development and cancer

Lung development begins with budding from the primitive foregut, followed by branching and bronchopulmonary segment formation. The next phase involves branching of ducts into terminal bronchioles and formation of an epithelial-lined airway conducting system, then additional branching of bronchioles and angiogenesis within the mesenchyme. The later terminal sac and alveolar phases of development form the adult lung with fully mature alveoli (for a review, see [63, 138]). In mature lung, respiratory epithelium covers the trachea, bronchi, bronchioles, and distal alveolar units. Multiple cell types, including basal cells, alveolar cells, and neuroendocrine cells, are present in the lung.

During development, all three Hh ligands control patterning of pulmonary airways from bronchi to alveolar sacs [139]. In the pseudo-glandular phase of lung development, Shh is a spatial signal for bronchiole patterning. Shh is produced by the epithelium at the far tips of bronchioles and received by the surrounding mesenchyme [140]. Gli genes are expressed in mesoderm surrounding bronchi and the trachea [140-143]. Disruption of Gli3 or Shh genes causes lung morphology abnormalities [144].

Respiratory epithelium repair, and lung cancer, have been associated with increased activation of Hh pathway target genes. Mature respiratory epithelium is regenerated at a very slow rate, unless injured by toxins or smoking [139, 144]. When injury does occur, it results in repair, proliferation, and partial reconstitution of cell populations that regenerate the respiratory epithelium [145]. Tobacco smoking introduces toxic mutagens into the lung and is responsible for greater than 80% of lung cancer, an extremely common malignancy that carries a very poor prognosis [139]. Because lung damage and consequential repair process lead to expansion of precursor populations, it is likely that repair processes induced by smoking-related damage, coupled with mutagenic effects of smoking, results in a predisposition to lung cancer [139]. Lung injury causes elevated *Shh*, *Gli*, and *Ptc* mRNA in the epithelium [139, 146, 147].

Primary lung cancer is classified as Small Cell (SCLC) or Non-Small Cell (NSCLC). SCLC and NSCLC have both been associated with activated Hh target genes [148]. Both lung cancers are commonly diagnosed late in the course of the disease, underscoring the importance of understanding tumor initiation and developing effective therapeutic options [148]. Approximately 80% of primary lung cancer is NSCLC. In a recent Hh antagonist screen with 60 human lung cancer cell lines, a subset of NSCLC cell lines was found to

over-express Hh and Hh target genes [148]. Some groups have reported that NSCLC cell lines express Hh, as well as key Hh target genes, consistent with constitutive activity and/or Hh activation through an autocrine mechanism [144, 147, 148]. SCLC is highly associated with smoking and comprises approximately 20% of lung carcinomas. Some types of SCLC have morphology reminiscent of a Hh-regulated phase of epithelial differentiation during airway development. Hh regulated genes are active in a subset of human SCLC cell lines. In these lines, inhibition of Smo with cyclopamine suppresses growth *in vitro* and in xenograft models [67]. Others did not identify a correlation between purported Hh antagonist-mediated growth inhibition and Hh pathway target gene expression in human SCLC and NSCLC cell lines [67]. In spite of the unclear role of Hh in lung cancer, clinical trials are in progress using the Hh inhibitor GDC-0449 [149]

Conclusions

In the same way that developmental biology has been unified by near-universal molecules, genes, and signaling pathways, cancer biology has been refined by the recognition of thematic changes that give tumor cells the properties they require to grow. Hh signaling is sufficient to trigger cancer in cells where Hh signaling is normally sufficient to trigger growth, presumably because the mature cells already possess the additional gene activities that are needed—and genes are off that must be off. Category 1 cancers occur in tissues where Hh normally regulates cell division, such as cerebellum and skin. In Category 2 cancers, mutations in the Hh pathway are not sufficient for tumorigenesis. There could be several reasons: In that cell type, Hh target genes may not include genes involved in cell division, or other genes may be active that block mitogenesis by Hh, or critical cofactors needed to act in concert with Hh target genes to stimulate growth may be unexpressed, inactive, or inaccessible.

Despite the inability of Hh to start tumorigenesis in cells of origin for Category 2 tumors, Hh signaling can still make the cancer worse. A recent study of pancreatic, colorectal, brain, and breast tumors employed full-genome sequencing to investigate what genetic changes are in fact observed in the tumors [150]. In pancreatic tumors, the results revealed a dozen pathways or processes tend to be affected. Some are properties like invasiveness and homophilic cell adhesion; others are activation of pathways including Wnt, Notch, TGF β , JNK, and Hh. Presumably the activity of each of these pathways confers on cells advantageous properties with respect to their growth and survival, the worst kind of “evolution” for the afflicted patient.

It will be important and fascinating to learn exactly how Hh target genes add to the malignant properties of the transformed cells. Will the targets be general to all cells where Hh signaling is activated, or to only particular tissues? What properties of normal cells are under the control of Hh signaling, and how does that regulation contribute to events during tumorigenesis? We can look forward to important insights about which target genes are important for the contributions of Hh signaling to each of the various types of cancer. We can also expect to learn how specific Hh targets confer important properties to normal cells, such as migration, surface properties, or production of specialized proteins. The cancer biology will very likely be informative about which target genes underlie which cell properties. Understanding the detailed molecular mechanism of mammalian Hh signaling will lead to the development of novel therapies for these lethal cancers.

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Figure 1.

Activating components of the Hh pathway support normal development of the organs and tissues indicated, but are potential proto-oncogenes that promote tumor growth in those same tissues when overactive. Restraining components of the normal Hh pathway also support development, but function as tumor suppressors that allow tumor growth if they are damaged in adulthood. This schematic depicts the array of tissues in which Hh supports development and can promote tumorigenesis.

Figure 2.

This simplified view of the Hh pathway depicts only those core components most commonly mutated in cancer. The basic signaling cascade consists of a series of repressive interactions. Normally, in the absence of Shh, Gli proteins and target genes are actively repressed. When secreted Shh binds to its receptor Patched (Ptc/PTCH), the inhibition of Smoothened (Smo/SMOH), a G protein-coupled receptor that activates downstream intracellular components of the pathway, by Ptc is relieved.

Figure 3.

Mechanisms for Hh pathway involvement in cancer include the following: loss-of-function mutations in inhibitory proteins such as Ptc1, gain-of-function mutations in positive regulators such as Smo and overexpression of the Hh ligands, leading to autocrine or paracrine activation of the pathway and renewal of cancer stem cells (Adapted from Rubin and de Sauvage. Nature Reviews Drug Discovery 5, 1026-1033. 2006)

Figure 4.

In the first two categories of Hh-associated tumors, Hh pathway deregulation supports different aspects of tumorigenesis. In the final category of Hh-associated tumors, more data are needed to determine the role of Hh in tumor initiation and/or maintenance.