Hedgehog Signaling Pathway: Development of Antagonists for Cancer Therapy

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The hedgehog pathway, initially discovered in *Drosophila* by two Nobel laureates, Dr. Eric Wieschaus and Dr. Christiane Nüsslein-Volhard, is a major regulator for cell differentiation, tissue polarity, and cell proliferation. Studies from many laboratories—including ours—reveal activation of this pathway in most basal cell carcinomas and nearly one third of extracutaneous human cancers, including medulloblastomas and gastrointestinal and prostate cancers. Even more exciting is the discovery and synthesis of specific signaling antagonists for the hedgehog pathway, which have significant clinical implications in novel cancer therapeutics. This review discusses the current understanding of the hedgehog signaling pathway and its activation in human cancers. It also discusses putative and confirmed signaling antagonists and their perspectives in therapeutic applications.

Introduction

The hedgehog (Hh) gene was identified by two Nobel laureates through genetic analysis of the fly segmentation [1]. In the early 1990s, three homologues of the Hh gene were identified in vertebrates. As an essential developmental signaling pathway, the Hh pathway is critical for maintaining tissue polarity and stem cell population. Inactivation of this pathway causes developmental defects such as holoprosencephaly [2], whereas hyperactivation of this pathway is found in most basal cell carcinomas (BCCs) and many extracutaneous cancers [3]. Thus, development of Hh signaling antagonists has significant implications in human cancer therapy.

Overall, the general signaling mechanisms of the Hh pathway are conserved from flies to humans. The seven transmembrane domain–containing protein Smoothened (SMO) serves as the key player for signal transduction of this pathway, whose function is inhibited by another transmembrane protein, Patched (PTC), in the absence of Hh ligands. In the presence of active Hh ligands, binding of Hh to its receptor PTC releases this inhibition, allowing SMO to signal downstream, eventually to Gli transcription factors. As transcription factors, Gli molecules can regulate target gene expression by direct association with a specific consensus sequence located in the promoter region of the target genes. Figure 1 shows the simplified diagram for Hh signaling activation.

Current Understanding of Hedgehog Signaling Mechanisms

Hh proteins (one Hh in flies and three in vertebrates: Sonic hedgehog [Shh], Indian hedgehog, and Desert hedgehog) are secreted molecules, functioning both on nearby and distant cells in developing tissues. Following translation, Hh proteins enter the secretory pathway and undergo autoprocessing and lipid-modification reactions that produce a signaling peptide modified at both its ends by palmitoyl (N-terminus) and cholesteryl (C-terminus) adducts [4]. The movement of Hh proteins is regulated by several molecules, such as the transmembrane transporter–like protein Dispatched (for release of Hh from secreting cells), the heparan sulfate proteoglycans Dally-like and Dally (for extracellular transport of Hh protein), and enzymes such as Sulfateless and Tout-velu (for heparan sulfate biosynthesis) [4].

PTC (one PTC in flies and two in vertebrates: PTCH1 and PTCH2) is the major receptor for Hh proteins [4]. Recent studies indicate that two additional molecules, Cdo and GAS1, are also required for Hh binding [5•–11•]. It is still not entirely clear how binding of Hh proteins results in the pathway activation. One hypothesis is that the function of SMO is normally inhibited by PTC in the absence of Hh. Binding of Hh proteins to the receptor PTC releases PTC-mediated inhibition on SMO; thus, SMO can signal to downstream molecules.

Very little is known about signaling events immediately downstream of SMO. Accumulating evidence from several groups indicates that the primary cilia found on most vertebrate cells play an important but undefined role

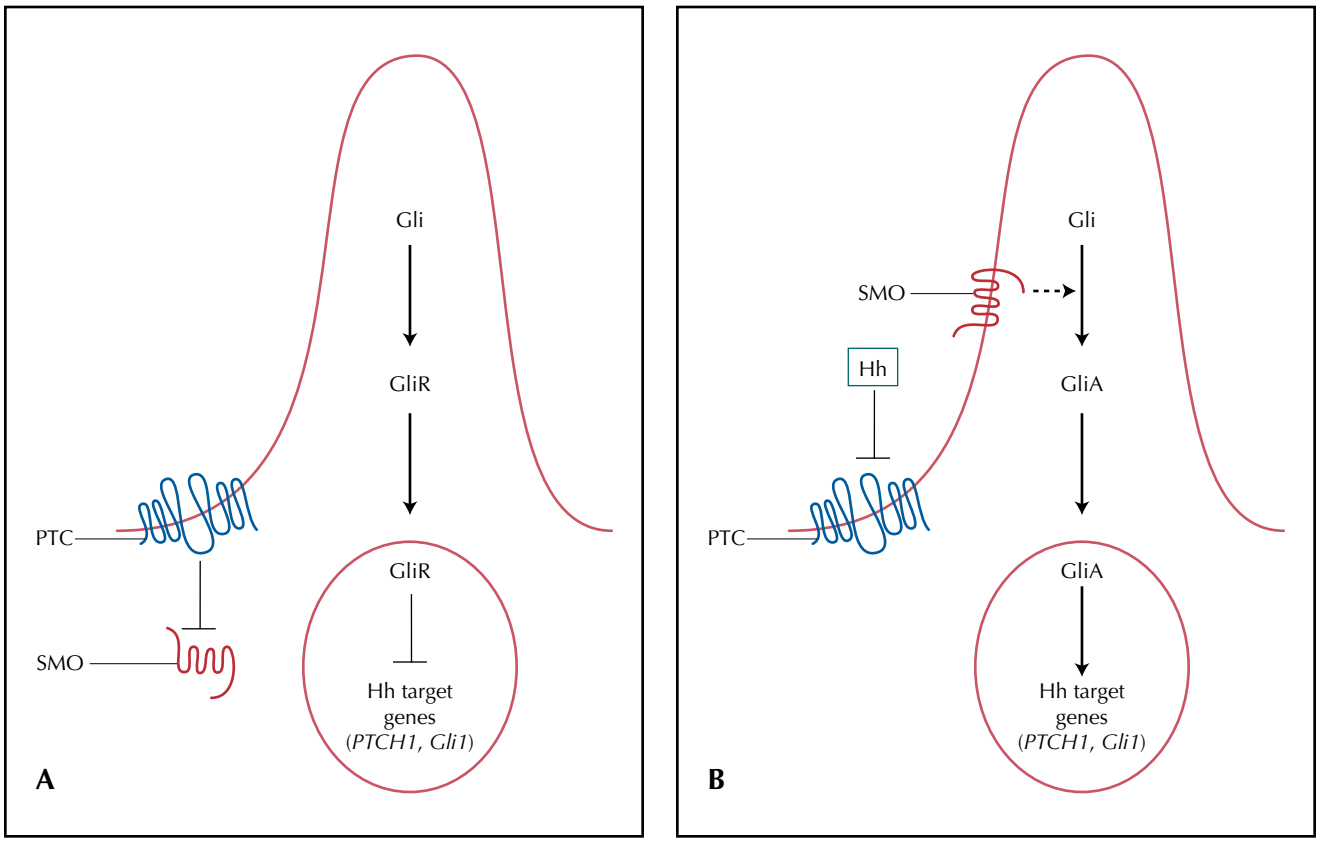

Figure 1. Simplified diagram of hedgehog (Hh) signaling. Smoothened (SMO) is the key signal transducer in the Hh pathway. **(A)** In the absence of Hh, the Hh receptor Patched (PTC) can suppress SMO activity and repressor forms of Gli (GliR) are generated, leading to down-regulation of Hh target genes. **(B)** In the presence of Hh ligands (Sonic, Indian, or Desert hedgehog), PTC is unable to affect SMO signaling. SMO somehow can promote formation of activated forms of Gli (GliA), resulting in upregulation of Hh target genes. Gene mutations (PTC, SMO) or abnormal overexpression of Hh ligands can lead to elevated expression of Hh target genes.

in the Hh pathway [12••,13]. The function of primary cilium is regulated by large protein complexes involved in intraflagellar transport (IFT), which functions in retrograde and anterograde movement of cargo within the primary cilia [13]. A number of mutations encoding IFT proteins involved in primary cilium anterograde IFT have been described, resulting in mice with Hh loss-of-function phenotypes [14]. Several Hh components, including SMO and Gli molecules, are also present at the primary cilium upon Hh stimulation [15••]. A SMO mutant lacking ciliary translocation blocks Hh signaling [12••]. Gli3 processing is significantly affected by IFT mutants [13,16•], suggesting that SMO activates downstream molecules at the cilium. However, it is not clear how SMO is transported to the cilium in response to Hh signaling and how SMO activates downstream effectors. Evidence suggests that SMO is endocytosed and can be degraded in the lysosomes. In cultured mammalian cells, both SMO and PTCH1 are internalized and localized to endosomes, and Hh induces segregation of SMO-containing vehicles from Hh–PTCH1 complexes destined for lysosomal degradation [17]. It is not clear how SMO endocytosis is regulated.

Several molecules from *Drosophila* studies are genetically downstream of SMO signaling, including Cos2 and Fused, but the functions of their vertebrate homologues in Hh signaling remain to be established. Inactivation of vertebrate homologues of Cos2, KIF27, and KIF7 do not affect Hh signaling in cultured mammalian cells [18], suggesting that KIF7 and KIF27 may not be required for Hh signaling in cultured mammalian cells. Because the homology between Cos2 and KIFs is very limited, it is possible that the function of Cos2 in vertebrates is replaced by a few molecules. Alternatively, SMO signaling in vertebrates may utilize a distinct mechanism. Additional evidence from knockout mice with each of these KIF genes should provide the in vivo roles of these Cos2 homologues. Another surprise is that knock out of the vertebrate homologue of Fused can survive for up to 2 weeks but die of hydrocephalus [19•,20•]. No changes of Hh signaling are seen in these knockout mice, suggesting that Fused is not critical for Hh signaling in early embryonic development. Based on these studies, however, one cannot rule out the possibility that Fused is only partially involved in Hh signaling.

Several novel cytoplasmic regulators of Hh signaling have been uniquely identified in mammalian cells, including Rab23 [21] and Tectonic [22]. Both Rab23 and Tectonic are negative regulators of Hh signaling downstream of SMO, but the exact interacting partners are

not clear. Unlike many Rab proteins, Rab23 expresses both in the nucleus and cytoplasm (unpublished data), suggesting that Rab23 may have other functions besides membrane trafficking.

On the other hand, the negatively regulatory functions of suppressor of Fused (Su[Fu]) in vertebrates are enhanced in mammals. Su(Fu) was originally identified genetically in *Drosophila* by its ability to suppress active *fused* mutations, but is not itself required for pathway activity. Several recent studies suggest that Su(Fu) plays a key negative regulatory role in Hh signaling. Su(Fu)-null mouse mutants not only fail to repress the pathway [23••], but have similar phenotypes as inactivation of the other key negative regulator acting upstream, *PTCH1*. Moreover, Su(Fu)-null mouse embryo fibroblasts and wild-type cells treated with Su(Fu) siRNAs display Hh pathway activation, supporting a central role in pathway repression [23••]. The skin phenotype of $Su(Fu)^{+/-}$ mice is as severe as the $Ptch1^{+/-}$ mice; the latter is a classic model for tumor suppressor function in the Hh pathway. At the molecular level, Su(Fu) is shown to associate directly with and to inhibit Gli molecules, although the details remain to be revealed [24].

Ultimately, Hh signaling is transduced to downstream Gli transcription factors, which can regulate target gene expression by direct association with a consensus binding site (5'-tgggtggtc-3') located in the promoter region of the target genes [25]. There are several ways to regulate the activity of Gli transcription factors. First, nuclear-cytoplasmic shuttling of Gli molecules is tightly regulated. For example, protein kinase A is shown to retain Gli1 proteins in the cytoplasm (through a protein kinase A site in the nuclear localization signal peptide) [26], whereas active Ras signaling promotes Gli nuclear localization [27]. Second, ubiquitination and protein degradation of Gli molecules is also regulated by several distinct mechanisms, including βTrCP-, cul3/BTB-, and numb/Itch-mediated Gli ubiquitination. In addition to protein degradation, Gli3 and Gli2 can be processed—to a lesser extent—into transcriptional repressors, which may be mediated by the βTrCP E3 ligase [28]. Fourth, transcriptional activity of Gli molecules is also tightly regulated. It is reported that epidermal growth factor can synergize with Gli transcription factors to regulate target gene expression [29••]. Su(Fu) not only prevents nuclear translocation of Gli molecules, but also inhibits Gli1-mediated transcriptional activity [30].

Several feedback regulatory loops exist in this pathway. PTCH1, HIP, GAS1, and Gli1, which are components of this pathway, are also the target genes. PTCH1 and HIP provide negative feedback mechanisms to maintain the pathway activity at an appropriate level in a given cell. In contrast, Gli1 forms a positive regulatory loop. GAS1, on the other hand, is downregulated by the Hh pathway, although it is positively involved in Hh signaling. Alteration of these loops is expected to result in abnormal signaling of this pathway, such as loss of PTCH1 in BCCs.

Activation of the Hedgehog Pathway in Human Cancers

The major breakthrough in our understanding of Hh signaling in human cancers came from the discovery that mutations of human homologue of the *Drosophila PTCH1* gene are associated with a rare hereditary form of BCC: basal cell nevus syndrome [2]. *PTCH1* is the receptor for Hh proteins, and previous studies have indicated that it mainly functions in embryonic development.

Mutations of *PTCH1* in basal cell nevus syndrome

Loss-of-function mutations of *PTCH1* are the cause of basal cell nevus syndrome. This autosomal dominant disorder is characterized by development of benign and malignant tumors (including multiple BCCs, medulloblastomas, and ovarian fibromas and, less frequently, fibrosarcomas, meningiomas, rhabdomyosarcomas, and cardiac fibromas) as well as developmental defects (eg, pits of the palms and soles; keratocysts of the jaw and other dental malformations; cleft palate; calcification of the falx cerebri; spina bifida occulta and other spine anomalies; bifid ribs and other rib anomalies) [2]. The clinical features of this syndrome were carefully characterized by Dr. Robert Gorlin; therefore, this syndrome is also named the "Gorlin syndrome."

Analysis of the distribution of BCCs in affected individuals from multiple families suggested that the underlying defect might be a mutation in a tumor suppressor gene. The gene was later mapped to chromosome 9q22–31, which is also frequently deleted in sporadic BCCs [2]. Positional cloning and candidate gene approaches identified the human homologue of *Drosophila PTCH1* as a candidate gene [2]. Vertebrate PTCH1 was known to function in the development of the organs, with abnormalities in basal cell nevus syndrome such as neural tube, somites, and limb buds, making *PTCH1* a good candidate gene for this syndrome. Screening of the *PTCH1* coding region revealed a wide spectrum of mutations in Gorlin syndrome patients, with the majority predicted to result in premature protein truncation. *PTCH1* mutations are mainly clustered into the predicted two large extracellular loops and the large intracellular loop [31]. Different kindreds with identical mutations differ dramatically in the extent of clinical features, suggesting that genetic background or environmental factors may have an important role in modifying the spectrum of both developmental and neoplastic traits [2].

The tumor suppressor role of *PTCH1* has been further demonstrated in mice. Mice heterozygous for a *PTCH1*-null mutation exhibit the essential features in basal cell nevus syndrome patients, such as tumor development

(eg, medulloblastomas, rhabdomyosarcomas and BCCs) and developmental defects (eg, spina bifida occulta) [4]. The mouse studies confirm that *PTCH1* functions as a tumor suppressor.

Activation of the hedgehog pathway in sporadic BCCs

BCC, the most common human cancer, consistently has abnormalities of the Hh pathway and often has lost the function of *PTCH1* via point mutations and loss of the remaining allele. Most *PTCH1* mutations lead to loss of the protein function. Mice heterozygous for a *PTCH1*-null mutation develop BCCs following ultraviolet (UV) irradiation or ion radiation. Currently, *Ptch1*$^{+/-}$ mice represent the most practical model of UV-mediated BCC formation [32].

The *PTCH1* gene region is lost in more than 50% human sporadic BCCs whereas the Hh pathway is activated in almost all BCCs, suggesting alteration of additional genes in the Hh pathway in this type of skin cancer. Indeed, mutations of SMO are found in about 10% of sporadic BCCs [3]. Unlike wild-type SMO, expression of activated SMO molecules in mouse skin results in formation of BCC-like tumors [3]. These findings provide additional insights into the role of the Hh pathway in human cancer. It is also reported that Su(Fu) is mutated in some BCCs [33]. Unlike *PTCH1*, no loss of heterozygosity in the *Su(Fu)* gene region is detected in sporadic BCCs, suggesting that Su(Fu) loss is not a major somatic change. Taking all the mutation data into account, there are still about 30% of BCCs without the underlying molecular basis for the activated Hh signaling. Thus, we predict that mutations of additional genes in the Hh pathway are yet to be discovered in sporadic BCCs.

We have shown that activated Hh signaling in BCCs leads to cell proliferation through elevated expression of platelet-derived growth factor receptor-α [3], whereas targeted inhibition of Hh signaling causes apoptosis via Fas induction [34].

Activation of hedgehog signaling in extracutaneous tumors

Recent studies indicate that Hh signaling is activated in many types of extracutaneous tumors, including brain tumors, and gastrointestinal, prostate, lung, and breast cancers. Unlike the situation in BCCs, overexpression of Hh ligands is believed to be responsible for activated Hh signaling in some of these extracutaneous tumors [35,36]. In pancreatic, esophageal, and liver cancers, activation of this pathway is found in both early tumors and metastatic cancer [37•,38], suggesting that Hh signaling may be a major trigger for carcinogenesis. In support of these findings, transgenic mice with pancreatic-specific expression of Shh or Gli2 develop pancreatic tumors [39,40]. In other tumors, such as gastric and prostate cancers, Hh signaling activation is associated with cancer progression. Consistent with these findings, inhibition of Hh signaling in prostate and gastric cancer cells reduces cell invasiveness [32]. Recently, reports have suggested that Hh signaling is required for development and progression of melanoma and gliomas [27,41].

Different results regarding Hh signaling activation in different tumor types have been reported, with sometimes contradictory results. There are several reasons for this discrepancy. First, it is possible that the involvement of Hh signaling in human cancers may be context dependent, occurring in some tissues or cell lines but not in others. Evidence suggests that Hh signaling may be involved in maintaining cancer stem cell proliferation [32]. Second, tumor heterogeneity is a major factor in analysis of Hh target gene expression by real-time polymerase chain reaction. For example, we identified activation of the Hh pathway in prostate cancer from transurethral resection of the prostate specimens more frequently than those from prostatectomy specimens [42]. Third, different standards have been used to define Hh signaling activation. Some groups use elevated expression of *Gli1* as a read-out of Hh signaling activation [27] whereas others assess expression of several Hh target genes, such as *Gli1*, *PTCH1*, *sFRP1*, and *HIP* [38,39,43]. Similarly, some groups use only immunohistochemistry to detect Hh signaling activation [44], while most researchers use multiple approaches. Therefore, there is an urgent need to have a unified standard for detecting Hh signaling activation in human cancer. As the research in this area progresses, we will see a better picture about Hh signaling activation in human cancers.

Small Molecule Modulators of Hedgehog Signaling

Cyclopamine

Cyclopamine, a plant-derived steroidal alkaloid, binds directly to the transmembrane helices of SMO and inhibits Hh signaling [4]. The discovery of small molecule antagonists of SMO such as cyclopamine has opened up exciting new prospects for molecularly targeted therapy and prevention for human cancers associated with Hh signaling.

Oral cyclopamine can block the growth of UV-induced BCCs in *Ptch1*$^{+/-}$ mice by 50%, perhaps by increasing Fas-induced apoptosis [34]. Furthermore, cyclopamine treatment in this mouse model prevents formation of additional microscopic BCCs, implying a potential use of cyclopamine for BCC prevention. Cyclopamine administration reduced BCCs, but not squamous cell carcinomas or fibrosarcomas in these mice, highlighting the specificity of cyclopamine for the Hh pathway [34]. Using murine BCC cell lines derived from this mouse model, cyclopamine is shown to inhibit cell proliferation, possibly through downregulation of platelet-derived growth factor receptor-α. Similarly, cyclopamine is effective in reducing

Table 1. Summary of hedgehog signaling inhibitors

Study	Name	IC_{50}	In vitro/in vivo studies
Taipale et al. [4]	Jervine	500 nM	In vitro and in cultured embryos
Rubin and de Sauvage [32]	Compound 5	< 100 nM	In vitro
Rubin and de Sauvage [32]	Compound Z	< 1 nM	In vitro
Rubin and de Sauvage [32]	2-amino-thiazole	30 nM	In vitro
Athar et al. [34]	Cyclopamine	300 nM	In vivo and in vitro
Ma et al. [38]	KAAD-cyclopamine	20 nM	In vitro cultured cells
Frank-Kamenetsky et al. [47]	CUR61414	200 nM	In vitro animal studies and human clinical trial phase 1
Chen et al. [48]	Sant-1	20 nM	In vitro
Chen et al. [48]	Sant-2	30 nM	In vitro
Chen et al. [48]	Sant-3	100 nM	In vitro
Chen et al. [48]	Sant-4	200 nM	In vitro
Lauth et al. [49•]	Gant-58	5 μM	In vitro and in vivo
Lauth et al. [49•]	Gant-61	5 μM	In vitro and in vivo
Bijlsma et al. [50•]	Vitamin D_3	100 μM	In vitro

IC_{50}—median inhibitory concentration.

medulloblastoma development in *Ptch1*$^{+/-}$ mice [45] as well as tumor growth of many cancer cell lines in *nu/nu* mice.

Synthetic SMO antagonists

Other synthetic SMO antagonists, such as CUR61414 (Curis, Cambridge, MA, and Genentech, South San Francisco, CA), have also been found to be effective in reducing BCCs from *Ptch1*$^{+/-}$ mice. Using an ex vivo model of BCC, CUR61414 causes the regression of UV-induced basaltic lesions in punch biopsies taken from *Ptch1*$^{+/-}$ mice [46]. Since then, a topical formulation of this compound was tested against sporadic BCCs in a phase 1 clinical trial. However, this clinical trial failed to show effects on Hh target gene expression by the compound; the reason is not clear. In addition, several other synthetic compounds have been identified to bind directly to SMO but with no structural similarity to cyclopamine [47,48].

Other hedgehog signaling modulators

A few small molecule inhibitors for Gli1 functions are identified through chemical library screening. Two such inhibitors act in the nucleus to block Gli1 function, and one of them interferes with Gli1 DNA binding in living cells [49•]. Importantly, the discovered compounds efficiently inhibited in vitro tumor cell proliferation in a Gli1-dependent manner and successfully blocked cell growth in an in vivo xenograft model using human prostate cancer cells harboring downstream activation of the Hh pathway [49•]. The growth of these tumors cannot be inhibited by cyclopamine or its analogues, raising the possibility that these Hh antagonists may have broad uses in cancer therapeutics. However, clinical application of these compounds awaits additional preclinical studies in defined tumor models.

Recent studies indicate that vitamin D_3, the secretion of which can be facilitated by PTCH1, can inhibit SMO signaling through direct binding to SMO. This finding raises a possibility to treat BCCs with nutrition supplements [50•]. Because abnormal expression of Shh is very common in several human cancer types, neutralizing antibodies for Shh show effectiveness in reducing cell proliferation in cancer cells with activated Hh signaling [37•]. Future clinical application of Shh-neutralizing antibodies will require additional preclinical studies.

In addition, several synthetic SMO agonists are available for functional studies of Hh signaling in human cancer [47]. With appropriate optimization, it is possible that these Hh agonists may be used to treat human conditions with reduced Hh signaling, such as holoprosencephaly. Table 1 shows the current small molecule inhibitors of Hh signaling.

Conclusions

In summary, rapid advances in our understanding of Hh signaling have provided great opportunities for developing novel therapeutic strategies to treat human cancers with altered Hh signaling. Identification of Hh signaling antagonists will make the novel cancer therapy feasible. The challenges for therapeutic application of Hh signaling inhibitors include identification of the right tumors for therapeutic application, reliable and reproducible animal models for testing these compounds, and optimization of drug dosages to minimize the side effects.

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References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Nüsslein-Volhard C, Wieschaus E: **Mutations affecting segment number and polarity in Drosophila.** *Nature* 1980, **287:**795–801.
2. Bale AE: **Hedgehog signaling and human disease.** *Annu Rev Genomics Hum Genet* 2002, **3:**47–65.
3. Xie J: **Hedgehog signaling in prostate cancer.** *Future Oncol* 2005, **1:**331–338.
4. Taipale J, Chen JK, Cooper MK, et al.: **Effects of oncogenic mutations in Smoothened and Patched can be reversed by cyclopamine.** *Nature* 2000, **406:**1005–1009.

5.• Martinelli DC, Fan CM: **Gas1 extends the range of Hedgehog action by facilitating its signaling.** *Genes Dev* 2007, **21:**1231–1243.

One of the first reports on GAS1 in mediating Hh signaling.

6.• Seppala M, Depew MJ, Martinelli DC, et al.: **Gas1 is a modifier for holoprosencephaly and genetically interacts with sonic hedgehog.** *J Clin Invest* 2007, **117:**1575–1584.

One of the first reports on the role of GAS1 in mediating Hh signaling.

7.• Allen BL, Tenzen T, McMahon AP: **The Hedgehog-binding proteins Gas1 and Cdo cooperate to positively regulate Shh signaling during mouse development.** *Genes Dev* 2007, **21:**1244–1257.

An important report on GAS1's function in mediating Hh signaling.

8.• Okada A, Charron F, Morin S, et al.: **Boc is a receptor for Sonic hedgehog in the guidance of commissural axons.** *Nature* 2006, **444:**369–373.

One of the first reports on Boc in mediating Hh signaling.

9.• Tenzen T, Allen BL, Cole F, et al.: **The cell surface membrane proteins Cdo and Boc are components and targets of the Hedgehog signaling pathway and feedback network in mice.** *Dev Cell* 2006, **10:**647–656.

An important report on Boc's functions in mediating Hh signaling.

10.• Zhang W, Kang JS, Cole F, et al.: **Cdo functions at multiple points in the Sonic Hedgehog pathway, and Cdo-deficient mice accurately model human holoprosencephaly.** *Dev Cell* 2006, **10:**657–665.

An important model for holoprosencephaly.

11.• Yao S, Lum L, Beachy P: **The ihog cell-surface proteins bind Hedgehog and mediate pathway activation.** *Cell* 2006, **125:**343–357.

The first report on Boc in mediating Hh signaling.

12.•• Corbit KC, Aanstad P, Singla V, et al.: **Vertebrate Smoothened functions at the primary cilium.** *Nature* 2005, **437:**1018–1021.

The first report on SMO localization to cilium.

13. Huangfu D, Anderson KV: **Cilia and Hedgehog responsiveness in the mouse.** *Proc Natl Acad Sci U S A* 2005, **102:**11325–11330.
14. Huangfu D, Liu A, Rakeman AS, et al.: **Hedgehog signalling in the mouse requires intraflagellar transport proteins.** *Nature* 2003, **426:**83–87.

15.•• Haycraft CJ, Banizs B, Aydin-Son Y, et al.: **Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function.** *PLoS Genet* 2005, **1:**e53.

The first report on Gli1 and Gli3 localization to cilium.

16.• May SR, Ashique AM, Karlen M, et al.: **Loss of the retrograde motor for IFT disrupts localization of SMO to cilia and prevents the expression of both activator and repressor functions of Gli.** *Dev Biol* 2005, **287:**378–389.

The first report on IFT effects on ciliary localization of SMO.

17. Incardona JP, Gruenberg J, Roelink H: **Sonic Hedgehog induces the segregation of Patched and Smoothened in endosomes.** *Curr Biol* 2002, **12:**983–995.
18. Varjosalo M, Li SP, Taipale J: **Divergence of Hedgehog signal transduction mechanism between Drosophila and mammals.** *Dev Cell* 2006, **10:**177–186.

19.• Merchant M, Evangelista M, Luoh SM, et al.: **Loss of the serine/threonine kinase fused results in postnatal growth defects and lethality due to progressive hydrocephalus.** *Mol Cell Biol* 2005, **25:**7054–7068.

One of the first reports on Fused knockout mice.

20.• Chen MH, Gao N, Kawakami T, Chuang PT: **Mice deficient in the fused homolog do not exhibit phenotypes indicative of perturbed hedgehog signaling during embryonic development.** *Mol Cell Biol* 2005, **25:**7042–7053.

A major report on Fused knockout mice.

21. Eggenschwiler JT, Espinoza E, Anderson KV: **Rab23 is an essential negative regulator of the mouse Sonic Hedgehog signalling pathway.** *Nature* 2001, **412:**194–198.
22. Reiter JF, Skarnes WC: **Tectonic, a novel regulator of the Hedgehog pathway required for both activation and inhibition.** *Genes Dev* 2006, **20:**22–27.

23.•• Svärd J, Henricson KH, Persson-Lek M, et al.: **Genetic elimination of Suppressor of fused reveals an essential repressor function in the mammalian Hedgehog signaling pathway.** *Dev Cell* 2006, **10:**187–197.

The first report on the tumor suppressor role of Su(Fu).

24. Barnfield PC, Zhang X, Thanabalasingham V, et al.: **Negative regulation of Gli1 and Gli2 activator function by Suppressor of fused through multiple mechanisms.** *Differentiation* 2005, **73:**397–405.
25. Sasaki H, Hui C, Nakafuku M, Kondoh H: **A binding site for Gli proteins is essential for HNF-3beta floor plate enhancer activity in transgenics and can respond to Shh in vitro.** *Development* 1997, **124:**1313–1322.
26. Sheng T, Chi S, Zhang X, Xie J: **Regulation of Gli1 localization by the cAMP/protein kinase A signaling axis through a site near the nuclear localization signal.** *J Biol Chem* 2006, **281:**9–12.
27. Stecca B, Mas C, Clement V, et al.: **Melanomas require Hedgehog-GLI signaling regulated by interactions between GLI1 and the RAS-MEK/AKT pathways.** *Proc Natl Acad Sci U S A* 2007, **104:**5895–5900.
28. Wang B, Li Y: **Evidence for the direct involvement of {beta}TrCP in Gli3 protein processing.** *Proc Natl Acad Sci U S A,* 2006, **103:**33–38.

29.•• Kasper M, Schnidar H, Neill GW, et al.: **Selective modulation of Hedgehog/GLI target gene expression by epidermal growth factor signaling in human keratinocytes.** *Mol Cell Biol* 2006, **26:**6283–6298.

The first report on synergy between epidermal growth factor and Hh signaling.

30. Cheng SY, Bishop JM: **Suppressor of Fused represses Gli-mediated transcription by recruiting the SAP18-mSin3 corepressor complex.** *Proc Natl Acad Sci U S A* 2002, **99:**5442–5447.
31. Lindstrom E, Shimokawa T, Toftgard R, Zaphiropoulos PG: **PTCH mutations: distribution and analyses.** *Hum Mutat* 2006, **27:**215–219.
32. Rubin LL, de Sauvage FJ: **Targeting the Hedgehog pathway in cancer.** *Nat Rev Drug Discov* 2006, **5:**1026–1033.
33. Reifenberger J, Wolter M, Knobbe CB, et al.: **Somatic mutations in the PTCH, SMOH, SUFUH and TP53 genes in sporadic basal cell carcinomas.** *Br J Dermatol* 2005, **152:**43–51.

34. Athar M, Li C, Tang X, et al.: **Inhibition of Smoothened signaling prevents ultraviolet B-induced basal cell carcinomas through regulation of Fas expression and apoptosis.** *Cancer Res* 2004, **64:**7545–7552.
35. Berman DM, Karhadkar SS, Maitra A, et al.: **Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours.** *Nature* 2003, **425:**846–851.
36. Watkins DN, Berman DM, Burkholder SG, et al.: **Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer.** *Nature* 2003, **422:**313–317.
37.• Huang S, He J, Zhang X, et al.: **Activation of the hedgehog pathway in human hepatocellular carcinomas.** *Carcinogenesis* 2006, **27:**1334–1340.
A major report on Hh signaling activation in liver cancer.
38. Ma X, Chen K, Huang S, et al.: **Frequent activation of the hedgehog pathway in advanced gastric adenocarcinomas.** *Carcinogenesis* 2005, **26:**1698–1705.
39. Thayer SP, di Magliano MP, Heiser PW, et al.: **Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis.** *Nature* 2003, **425:**851–856.
40. Pasca di Magliano M, Sekine S, Ermilov A, et al.: **Hedgehog/Ras interactions regulate early stages of pancreatic cancer.** *Genes Dev* 2006, **20:**3161–3173.
41. Clement V, Sanchez P, de Tribolet N, et al.: **HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity.** *Curr Biol* 2007, **17:**165–172.
42. Sheng T, Li C, Zhang X, et al.: **Activation of the hedgehog pathway in advanced prostate cancer.** *Mol Cancer* 2004, **3:**29.
43. Karhadkar SS, Bova GS, Abdallah N, et al.: **Hedgehog signalling in prostate regeneration, neoplasia and metastasis.** *Nature* 2004, **431:**707–712.
44. Kubo M, Nakamura M, Tasaki A, et al.: **Hedgehog signaling pathway is a new therapeutic target for patients with breast cancer.** *Cancer Res* 2004, **64:**6071–6074.
45. Sanchez P, Ruiz i Altaba A: **In vivo inhibition of endogenous brain tumors through systemic interference of Hedgehog signaling in mice.** *Mech Dev* 2005, **122:**223–230.
46. Williams JA, Guicherit OM, Zaharian BI, et al.: **Identification of a small molecule inhibitor of the hedgehog signaling pathway: effects on basal cell carcinoma-like lesions.** *Proc Natl Acad Sci U S A* 2003, **100:**4616–4621.
47. Frank-Kamenetsky M, Zhang XM, Bottega S, et al.: **Small-molecule modulators of Hedgehog signaling: identification and characterization of Smoothened agonists and antagonists.** *J Biol* 2002, **1:**10.
48. Chen JK, Taipale J, Young KE, et al.: **Small molecule modulation of Smoothened activity.** *Proc Natl Acad Sci U S A* 2002, **99:**14071–14076.
49.• Lauth M, Bergstrom A, Shimokawa T, Toftgard R: **Inhibition of GLI-mediated transcription and tumor cell growth by small-molecule antagonists.** *Proc Natl Acad Sci U S A* 2007, **104:**8455–8460.
The first report on Gli1 antagonists.
50.• Bijlsma MF, Spek CA, Zivkovic D, et al.: **Repression of smoothened by patched-dependent (pro-)vitamin D3 secretion.** *PLoS Biol,* 2006, **4:**e232.
The first report on vitamin D_3 effects on Hh signaling.