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Review

Implications of hedgehog signaling antagonists for cancer therapy

Jingwu Xie*

Department of Pharmacology and Toxicology, Sealy Center for Cancer Cell Biology, University of Texas at Galveston, Galveston, Texas 77555-1048, USA

The hedgehog (Hh) pathway, initially discovered in *Drosophila* by two Nobel laureates, Dr. Eric Wieschaus and Dr. Christiane Nusslein-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 in approximately 30% of extracutaneous human cancers, including medulloblastomas, gastrointestinal, lung, breast and prostate cancers. Thus, it is believed that targeted inhibition of Hh signaling may be effective in treating and preventing many types of human cancers. Even more exciting is the discovery and synthesis of specific signaling antagonists for the Hh pathway, which have significant clinical implications in novel cancer therapeutics. This review discusses the major advances in the current understanding of Hh signaling activation in different types of human cancers, the molecular basis of Hh signaling activation, the major antagonists for Hh signaling inhibition and their potential clinical application in human cancer therapy.

Keywords hedgehog; smoothened; PTCH1; human cancer therapy; basal cell carcinoma; antagonist

The hedgehog (*Hh*) gene was identified by two Nobel laureates through genetic analysis of segmentation of fruit fly *Drosophila* [1]. In the early 1990s, three homologs of the *Hh* gene were identified in vertebrates [2–6]. 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 [7]. Hyperactivation of this pathway is found in most basal cell carcinomas (BCCs) and many extracutaneous cancers [8–10]. The emerging role of Hh signaling in human cancer further emphasizes the importance of studying this pathway.

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*Corresponding author: Tel, 1-409-747-1845; Fax, 1-409-747-1938; E-mail, jinxie@utmb.edu

Current Understanding of Hh Signaling Mechanisms

Overall, the general signaling mechanisms of the Hh pathway is conserved from fly to human [11]. The seven transmembrane domain containing the protein smoothened (SMO) serves as the key player for signal transduction of this pathway. However, the pathway's 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 to Gli transcription factors. As transcription factors, Gli molecules can regulate target gene expression by directly associating with a specific consensus sequence located in the promoter region of the target genes [12,13]. **Fig. 1** shows the simplified diagram of Hh signaling in the presence or absence of Hh.

Hh proteins [one Hh in *Drosophila* and three Hhs in vertebrates: sonic hedgehog (Shh), Indian hedgehog (Ihh) and desert hedgehog (Dhh)] 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 ends by palmitoyl (N-terminus) and cholesteryl (C-terminus) adducts [14–16]. The movement of Hh proteins is regulated by several molecules: Dispatched (Disp), the transmembrane transporter-like protein for release of Hh from secreting cells [11–14]; Dally-like (Dlp) and Dally, heparan sulfate proteoglycans for extracellular transport of Hh protein [15]; and enzymes, such as sulfateless and tout-velu, for heparan sulfate biosynthesis [17–19].

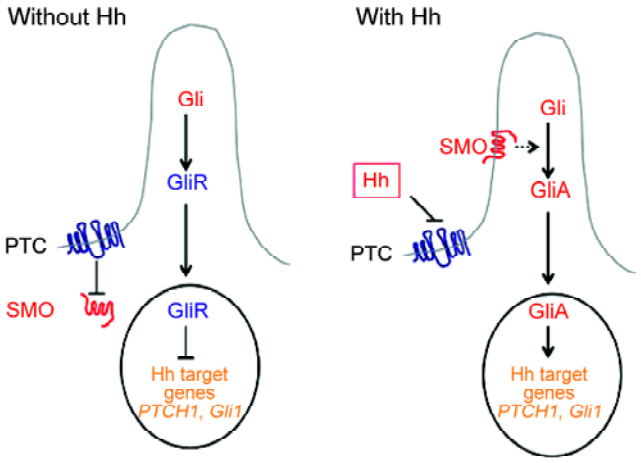

Fig. 1 Simplified diagram of Hh signaling Smoothened (SMO) is the key signal transducer in the hedgehog (Hh) pathway. In the absence of Hh, the Hh receptor patched (PTC) can suppress SMO activity and generate repressor forms of Gli (GliR), leading to down-regulation of Hh target genes. In the presence of Hh ligands, sonic hedgehog (Shh), Indian hedgehog (Ihh) and desert hedgehog (Dhh), PTC is unable to affect SMO signaling. SMO somehow can promote formation of activated forms of Gli (GliA), resulting in up-regulation of Hh target genes. Gene mutations (PTC, SMO) or abnormal over-expression of Hh ligands all can lead to elevated expression of Hh target genes.

PTC [one PTC in flies and two PTCs in vertebrates: patched homolog 1 (PTCH1) and patched homolog 2 (PTCH2)] is the major receptor for Hh proteins [20]. Several molecules are involved in regulating Hh reception. Hh-interacting protein (HIP) can compete with PTC in binding Hh, thus preventing Hh signaling [21]. Recent studies indicate that two additional molecules, Cdo and Gas1, are also required for Hh binding [22–28]. It is still not entirely clear how binding of Hh proteins results in pathway activation. One hypothesis is that, in the absence of Hh, PTC normally inhibits the function of SMO. 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. In *Drosophila*, several laboratories have shown that SMO accumulation is promoted through protein phosphorylation at the C-terminus by protein kinase A (PKA) and casein kinase I [29,30]. SMO mutants lacking these phosphorylation sites are defective in Hh signaling. However, these phosphorylation sites are not conserved in vertebrate SMO, indicating a different mechanism for SMO signaling in higher organisms [30].

Accumulated evidence from several groups indicates that the primary cilia found on most vertebrate cells play an important but undefined role in the Hh pathway [31–35]. Functions of the 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 [36]. A number of mutations encoding IFT proteins involved in the primary cilium anterograde IFT have been described, resulting in mice with Hh loss of function phenotypes [32]. Several Hh components, including SMO and Gli molecules, are also present at the primary cilium upon Hh stimulation [37]. A SMO mutant lacking ciliary translocation blocks Hh signaling [31]. Gli3 processing is significantly affected by IFT mutants [33,34], 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 [38]. 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 [38]. It is not clear how SMO endocytosis is regulated.

Based on studies of *Drosophila*, there are several molecules, including COS2 and Fused, genetically downstream of SMO signaling, but the functions of their vertebrate homologs in Hh signaling remains to be established. Inactivation of vertebrate homologs of COS2, KIF27 and KIF7, do not affect Hh signaling in cultured mammalian cells [39], which suggests that KIF7 and KIF27 may not be required for Hh signaling. Because the homology between COS2 and KIFs is very low, it is possible that a few molecules replace the function of COS2 in vertebrates. Alternatively, SMO signaling in vertebrates may utilize a distinct mechanism. Additional evidence from knockout mice with each of these *KIF* genes should provide insight into the *in vivo* roles of these COS2 homologs. Another surprise is that knockout of the vertebrate homolog of Fused can survive for up to two weeks but die of hydrocephalus [40,41]. No change of Hh signaling is seen in these knockout mice, suggesting that Fused is not critical for Hh signaling in early embryonic development. Based on these studies, however, one can not ignore the possibility that Fused is only partially involved in Hh signaling.

Several novel cytoplasmic regulators of Hh signaling, including Rab23 and tectonic [42,43], have been identified as being unique to mammalian cells. 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 (our unpublished observation), suggesting that Rab23 may have other functions besides membrane trafficking.

The negative regulatory functions of suppressor of Fused [Su(Fu)] in vertebrates, in contrast, are enhanced in mammals. Su(Fu) in *Drosophila* was originally identified genetically by its ability to suppress active Fused mutations, but it 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 [44], but have similar phenotypes as inactivation of the other key negative regulator acting upstream, *PTCH1*. Moreover, Su(Fu) null MEFs and wild-type cells treated with Su(Fu) short interfering RNAs display Hh pathway activation, supporting a central role in pathway repression [44]. The skin phenotype of $Su(Fu)^{+/-}$ mice is as severe as the $PTCH^{+/-}$ 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, though the details are unclear [45].

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 [12,13,46,47]. There are several ways to regulate the activity of Gli transcription factors. First, nuclear-cytoplasmic shuttling of Gli molecules is tightly regulated [45,48–50]. For example, PKA is shown to retain Gli1 proteins in the cytoplasm (through a PKA site in the nuclear localization signal peptide) [48], whereas active Ras signaling promotes Gli nuclear localization [50]. Second, ubiquitination and protein degradation of Gli molecules is also regulated by several distinct mechanisms, including TrCP, Cul3/BTB and Numb/Itch [51–55]. In addition to protein degradation, Gli3 and Gli2, to a lesser extent, can be processed into transcriptional repressors, which may be mediated by the TrCP E3 ligase [53]. Defects in the retrograde motor for IFT are also shown to affect Gli3 processing [56]. Fourth, transcriptional activity of Gli molecules is also tightly regulated. It is reported that EGF can synergize with Gli transcription factors to regulate target gene expression [57]. Su(Fu) not only prevents nuclear translocation of Gli molecules, but it also inhibits Gli1-mediated transcriptional activity [58]. **Table 1** summarizes the major components of the Hh pathway in vertebrates.

There are several feedback regulatory loops in this pathway. PTC, Hh-interacting protein (HIP), Gas1 and Gli1, which are components of this pathway, are also the target genes. PTC 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 is down-regulated by the Hh pathway, but it is positively involved in Hh signaling. Alteration of these loops, such as loss of PTCH1 in BCCs, likely results in abnormal signaling of this pathway.

Activation of the Hh Pathway in Human Cancers

The major breakthrough in our understanding of Hh signaling in human cancers came from the discovery that mutations of the human homolog of the *Drosophila* patched gene (*PTCH1*) are associated with a rare hereditary form of BCC: basal cell nevus syndrome (also called Gorlin syndrome) [59–61]. PTCH1 is the receptor for Hh proteins, and previous studies have indicated that PTCH1 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, the clinical features of which were originally identified by Dr. Robert Gorlin. This autosomal dominant disorder is distinguished by the development of benign and malignant tumors, including multiple BCCs, medulloblastomas and ovarian fibromas, and less frequently fibrosarcomas, meningiomas, rhabdomyosarcomas and cardiac fibromas. The disorder is also characterized by developmental defects such as 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, and bifid ribs and other rib anomalies [62–64].

Analysis of the distribution of BCCs in affected individuals in multiple families suggests that the underlying defect might be a mutation in a tumor suppressor gene. This gene was later mapped to chromosome 9q22-31, which is also frequently deleted in sporadic BCCs [65]. Positional cloning and candidate gene approaches identified the human homolog of *Drosophila patched* as a candidate gene for therapeutic strategies [59,60,66]. Making *PTCH1* a good candidate gene for basal cell nevus syndrome, vertebrate *patched* was known to function in the development of organs, such as neural tube, somites and limb buds [67], with abnormalities. Screening of the *patched* coding region in basal cell nevus syndrome patients revealed a wide spectrum of mutations, the majority of which were predicted to result in premature protein truncation. *PTCH* mutations are mainly clustered into two large extracellular loops and a large intracellular loop [68]. Kindreds with

Table 1 The major components of the hedgehog (Hh) pathway in vertebrates

	Function	Vertebrate gene	Knockout mouse	References
Ligand	Ligand	Sonic hedgehog	Embryonic lethal	[105]
		Indian hedgehog	Embryonic lethal	[106]
		Desert hedgehog	Male infertile	[107]
Hh regulator	Hh inhibitor	Hh-interacting protein	Embryonic lethal	[21]
Receptor	Receptor	Patched homolog (PTCH) 1	Embryonic lethal, cancer	[70,71]
		PTCH2	Viable/cancer prone	[108,109]
Co-receptors	Receptor	Cdo	Embryonic lethal	[110]
		Gas1	Embryonic lethal	[22–24]
Signal transducer	Signal transducer	Smoothened (SMO)	Embryonic lethal	[111]
Signaling intermediates	Homologs of signaling	KIF7	Not available	–
	protein COS2	KIF27	Not available	–
	Ciliary transport	IFT88	Embryonic lethal	[32]
		IFT172	Embryonic lethal	[32]
	Homolog of fly Fu	Fused	Embryonic lethal	[40,41]
	Unknown function	Rab23	Embryonic lethal	[42]
		Tectonic	Embryonic lethal	[43]
	Signaling protein	Suppressor of Fused	Embryonic lethal, cancer	[44]
Transcription factor	Transcriptional factor	Gli1	Viable	[112]
		Gli2	Embryonic lethal	[113]
		Gli3	Embryonic lethal	[113]
Thr/Ser kinase	Gli, SMO phosphorylation	Protein kinase A (4 subunits)	Viable	[114,115]
		GSK3	Embryonic lethal	[116]
		Casein kinase I (many isoforms)	Viable	–
E3 ligase for Gli	E3 ligase	β-TrCP1	Viable	[117]
		Cul3/BTB	Not available	[118]
		Numb/Itch	Embryonic lethal	[119]

identical mutations differ dramatically in the extent of their clinical features, suggesting that genetic background or environmental factors may have an important role in modifying the spectrum of both developmental and neoplastic traits [69].

The tumor suppressor role of *PTCH1* has been further demonstrated in mice. Mice heterozygous for a *PTCH1* null mutation exhibit the same essential features, such as tumor development (*eg* medulloblastomas, rhabdomyosarcomas and BCCs) and developmental defects (*eg* pina bifida occulta), as basal cell nevus syndrome patients [70, 72]. The mouse studies confirm that *PTCH1* functions as a tumor suppressor.

Activation of the Hh pathway in sporadic BCCs

BCC, the most common human cancer, consistently has abnormalities of the Hh pathway and often loses *PTCH1* function due to point mutations and the 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 UV irradiation or ion radiation. Currently, $PTCH^{+/-}$ mice represent the most practical model for UV-mediated BCC formation [72].

The *PTCH1* gene region is lost in more than 50% of 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 [73–77]. Unlike wild-type SMO, expression of activated SMO molecules in mouse skin results in formation of BCC-like tumors [73]. These findings provide additional insight into the role of the Hh pathway in human cancer. It has also been reported that Su(Fu) is mutated in some BCCs [75]. LOH are not detected in the *Su(Fu)* gene region, unlike in the *PTCH1* region, in sporadic BCCs, suggesting that Su(Fu) loss is not a major somatic change.

Taking all the mutation data into account, the underlying molecular basis for the activated Hh signaling still remains unknown in approximately 30% of BCCs. 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 PDGFR [78], whereas targeted inhibition of Hh signaling causes apoptosis via Fas induction [79].

Activation of Hh signaling in extracutaneous tumors

Recent studies indicate that Hh signaling is activated in many types of extracutaneous tumors, including brain, gastrointestinal, prostate, lung and breast cancers. Unlike with BCCs, overexpression of Hh ligands is believed to be responsible for activating Hh signaling in some of these tumors [80,81]. In pancreatic, esophageal and liver cancers, activation of this pathway is found in both early tumors and metastatic cancer [82–84], 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 [85,86]. In other tumors, such as gastric and prostate cancers, Hh signaling activation is associated with cancer progression [82,87–90]. Consistent with these findings, inhibition of Hh signaling in prostate and gastric cancer cells reduces cell invasiveness (our unpublished observation)[88]. Recently, reports have suggested that Hh signaling is required for the development and progression of melanoma, gliomas and B-cell lymphomas [91,92].

Different, and sometimes contradictory results have been reported regarding Hh signaling activation in different tumor types. There are several reasons for this. 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 [93,94]. Second, tumor heterogeneity is a major factor in the analysis of Hh target gene expression by real-time polymerase chain reaction. For example, we identified activation of the Hh pathway in prostate cancer more frequently from transurethral resection of the prostate specimens than from prostatectomy specimens [88]. Third, different standards have been used to define Hh signaling activation. Some studies have used elevated expression of Gli1 as a read-out of Hh signaling activation [50], whereas others have assessed expression of several Hh target genes, such as *Gli1*, *PTCH1*, *sFRP1* and *HIP* [82,83,85,90,95]. Similarly, though most studies have used multiple approaches, some have only involved immunohistochemistry to detect Hh signaling activation [96]. Therefore, it is imperative to establish a unified standard for detecting Hh signaling activation in human cancer. As the research in this area progresses, we will gain a clearer picture about Hh signaling activation in human cancers. **Table 2** provides a summary of current data on Hh signaling activation in human cancers.

Small Molecule Modulators of Hh Signaling

Cyclopamine

Cyclopamine, a plant-derived steroidal alkaloid, binds directly to the transmembrane helices of SMO and inhibits Hh signaling [97]. The discovery of small molecule antagonists of SMO such as cyclopamine has opened up exciting new prospects for molecularly targeted therapy for and prevention of 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 [79]. Furthermore, cyclopamine treatment in this mouse model prevents the formation of additional microscopic BCCs, implying a potential use of cyclopamine in BCC prevention. Cyclopamine administration reduced BCCs, but not SCCs or fibrosarcomas, in these mice, highlighting the specificity of cyclopamine for the Hh pathway [79]. Using murine BCC cell lines derived from this mouse model, cyclopamine is shown to inhibit cell proliferation, possibly through down-regulation of growth factor receptor PDGFR. Similarly, cyclopamine is effective in reducing medulloblastoma development in PTCH1$^{+/-}$ mice as well as tumor growth of many cancer cell lines in nu/nu mice [50,85,90,98,99].

Synthetic SMO antagonists

Other synthetic SMO antagonists, such as CUR61414 from Curis/Genentech, have also been found to be effective in reducing BCCs in PTCH1$^{+/-}$ mice. Using an *ex vivo* model of BCC, CUR61414 caused the regression of UV-induced basaltic lesions in punch biopsies taken from PTCH1$^{+/-}$ mice [100]. Since that study, a topical formulation of this compound has been tested against sporadic BCCs in a phase I clinical trial. However, for unknown reasons, the compound did not appear to affect Hh target gene expression in this clinical trial. Additionally, several other synthetic compounds differing structurally from cyclopamine have been identified for their ability to bind directly to SMO [101,102].

Table 2 Summary of hedgehog (Hh) signaling activation in human cancers

Tumor type	Tumor subtype	Hh signaling activation frequency	Abnormality in Hh pathway	References
Skin cancers	Basal cell carcinomas (BCC)	Almost all BCCs	Patched homolog 1 (PTCH1) down, smoothened (SMO) up or suppressor of Fused [Su(Fu)] down	[59,60,66,73,75–77, 120]
	Melanomas	11 out of 11	Sonic hedgehog (Shh) up	[50]
Brain tumors	Medulloblastomas	30%–100%	PTCH1 down, Su(Fu) down, or REN loss	[121–124]
	Gliomas	35%–80%	Shh up	[91,125]
Prostate cancer	–	70% in advanced/ metastatic tumor	Shh up	[88–90]
Endometrial carcinomas	–	Around 30%	Shh up	[126]
Breast cancer	–	10%–100%	PTCH1 down or Shh up	[96,127–129]
Upper GI tract	Esophageal cancer	Over 50%	Shh or Gli1 up	[80,84,130]
	Gastric cancer	Over 50%	Shh up	[80,82,131–133]
Pancreatic cancer	PDA	Over 80%	Shh up	[80,85,86,134–142]
Liver cancer	Hepatocellular carcinoma	Over 60%	Shh up	[143–147]
Lymphomas	B-cell lymphoma	Over 60%	Eμ-myc lymphoma model	[92,148]
Multiple myelomas	–	Most	Stem cell	[149]

Other Hh 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 Gli function, and one of them interferes with Gli1 DNA binding in living cells [103]. Importantly, the discovered compounds efficiently inhibited *in vitro* tumor cell proliferation in a Gli-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 [103]. The growth of these tumors can not be inhibited by cyclopamine or its analogs, raising the possibility that these Hh antagonists may have broad uses in cancer therapeutics. Clinical application of these compounds, however, awaits additional preclinical studies in defined tumor models.

Recent studies indicate that vitamin D3, the secretion of which can be facilitated by PTCH1, can inhibit SMO signaling through direct binding to SMO. This finding raises the possibility that BCCs may be treated with nutritional supplements [104].

Since abnormal expression of Shh is very common in several human cancer types, neutralizing antibodies for Shh have demonstrated effectiveness in reducing cell proliferation in cancer cells with activated Hh signaling [83]. 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 [101]. 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 3** shows currently known small molecule inhibitors of Hh signaling.

Summary

In summary, rapid advances in our understanding of Hh signaling have provided great opportunities for developing novel therapeutic strategies for human conditions with altered Hh signaling, particularly cancer. Optimized use of Hh signaling antagonists will make these therapies 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.

Table 3 Summary of hedgehog (Hh) signaling inhibitors

Names	EC_{50} (nM)	*In vitro*/*in vivo* studies	References
Cyclopamine	300	*In vivo* and *in vitro* studies	Selected references [79,98,99]
KAAD-cyclopamine	20	*In vitro* cultured cells	Selected references [82,83,97]
Jervine	500	*In vitro* and in cultured embryos	[150–152]
CUR-61414	200	*In vitro*, animal studies and human clinical trial phase I	[101]
Sant-1	20	*In vitro* studies	[102]
Sant-2	30	*In vitro* studies	[102]
Sant-3	100	*In vitro* studies	[102]
Sant-4	200	*In vitro* studies	[102]
Compound 5	<100	*In vitro* studies	[153]
Compound Z	<1	*In vitro* studies	[153]
2-amino-thiazole	30	*In vitro* studies	[94]
Gant-58	5×10^{9}	*In vitro* and *in vivo* studies	[103]
Gant-61	5×10^{9}	*In vitro* and *in vivo* studies	[94]
Vitamin D3	1×10^{11}	*In vitro* studies	[104]

References

1 Nusslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in *Drosophila*. Nature 1980, 287: 795–801

2 Krauss S, Concordet JP, Ingham PW. A functionally conserved homolog of the *Drosophila* segment polarity gene *Hh* is expressed in tissues with polarizing activity in zebrafish embryos. Cell 1993, 75: 1431–1444

3 Echelard Y, Epstein DJ, St-Jacques B, Shen L, Mohler J, McMahon JA, McMahon AP. Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. Cell 1993, 75: 1417–1430

4 Riddle RD, Johnson RL, Laufer E, Tabin C. Sonic hedgehog mediates the polarizing activity of the ZPA. Cell 1993, 75: 1401–1416

5 Chang DT, Lopez A, von Kessler DP, Chiang C, Simandl BK, Zhao R, Seldin MF *et al*. Products, genetic linkage and limb patterning activity of a murine hedgehog gene. Development 1994, 120: 3339–3353

6 Roelink H, Augsburger A, Heemskerk J, Korzh V, Norlin S, Ruiz i Altaba A, Tanabe Y *et al*. Floor plate and motor neuron induction by Vhh-1, a vertebrate homolog of hedgehog expressed by the notochord. Cell 1994, 76: 761–775

7 Bale AE. Hedgehog signaling and human disease. Annu Rev Genomics Hum Genet 2002, 3: 47–65

8 Xie J. Hedgehog signaling in prostate cancer. Future Oncol 2005, 1: 331–338

9 Xie J. Hedgehog signaling pathway: development of antagonists for cancer therapy. Curr Oncol Rep 2008, 10: 107–113

10 Xie J. Molecular biology of basal and squamous cell carcinomas. Adv Exp Med Biol 2008, 624: 241–251

11 Ingham PW, Placzek M. Orchestrating ontogenesis: variations on a theme by sonic hedgehog. Nat Rev Genet 2006, 7: 841–850

12 Sasaki H, Hui C, Nakafuku M, Kondoh H. A binding site for Gli proteins is essential for HNF-3β floor plate enhancer activity in transgenics and can respond to Shh *in vitro*. Development 1997, 124: 1313–1322

13 Kinzler KW, Vogelstein B. The *Gli* gene encodes a nuclear protein which binds specific sequences in the human genome. Mol Cell Biol 1990, 10: 634–642

14 Lee JJ, Ekker SC, von Kessler DP, Porter JA, Sun BI, Beachy PA. Autoproteolysis in hedgehog protein biogenesis. Science 1994, 266: 1528–1537

15 Porter JA, Young KE, Beachy PA. Cholesterol modification of hedgehog signaling proteins in animal development. Science 1996, 274: 255–259

16 Porter JA, von Kessler DP, Ekker SC, Young KE, Lee JJ, Moses K, Beachy PA. The product of hedgehog autoproteolytic cleavage active in local and long-range signaling. Nature 1995, 374: 363–366

17 Toyoda H, Kinoshita-Toyoda A, Fox B, Selleck SB. Structural analysis of glycosaminoglycans in animals bearing mutations in sugarless, sulfateless, and tout-velu. *Drosophila* homologues of vertebrate genes encoding glycosaminoglycan biosynthetic enzymes. J Biol Chem 2000, 275: 21856–21861

18 Bellaiche Y, The I, Perrimon N. Tout-velu is a *Drosophila* homologue of the putative tumor suppressor EXT1 and is needed for Hh diffusion. Nature 1998, 394: 85–88

19 Koziel L, Kunath M, Kelly OG, Vortkamp A. EXT1-dependent heparan sulfate regulates the range of Ihh signaling during endochondral ossification. Dev Cell 2004, 6: 801–813

20 Stone DM, Hynes M, Armanini M, Swanson TA, Gu Q, Johnson RL, Scott MP *et al*. The tumor suppressor gene patched encodes a candidate receptor for sonic hedgehog. Nature 1996, 384: 129–134

21 Chuang PT, McMahon AP. Vertebrate hedgehog signaling modulated by induction of a hedgehog-binding protein. Nature 1999,

397: 617–621
22 Martinelli DC, Fan CM. Gas1 extends the range of hedgehog action by facilitating its signaling. Genes Dev 2007, 21: 1231–1243
23 Seppala M, Depew MJ, Martinelli DC, Fan CM, Sharpe PT, Cobourne MT. Gas1 is a modifier for holoprosencephaly and genetically interacts with sonic hedgehog. J Clin Invest 2007, 117: 1575–1584
24 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
25 Okada A, Charron F, Morin S, Shin DS, Wong K, Fabre PJ, Tessier-Lavigne M *et al*. Boc is a receptor for sonic hedgehog in the guidance of commissural axons. Nature 2006, 444: 369–373
26 Tenzen T, Allen BL, Cole F, Kang JS, Krauss RS, McMahon AP. 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
27 Zhang W, Kang JS, Cole F, Yi MJ, Krauss RS. Cdo functions at multiple points in the sonic hedgehog pathway, and Cdo-deficient mice accurately model human holoprosencephaly. Dev Cell 2006, 10: 657–665
28 Yao S, Lum L, Beachy P. The ihog cell-surface proteins bind hedgehog and mediate pathway activation. Cell 2006, 125: 343–357
29 Jia J, Tong C, Wang B, Luo L, Jiang J. Hedgehog signaling activity of smoothened requires phosphorylation by protein kinase A and casein kinase I. Nature 2004, 432: 1045–1050
30 Zhang C, Williams EH, Guo Y, Lum L, Beachy PA. Extensive phosphorylation of smoothened in hedgehog pathway activation. Proc Natl Acad Sci USA 2004, 101: 17900–17907
31 Corbit KC, Aanstad P, Singla V, Norman AR, Stainier DY, Reiter JF. Vertebrate smoothened functions at the primary cilium. Nature 2005, 437: 1018–1021
32 Huangfu D, Liu A, Rakeman AS, Murcia NS, Niswander L, Anderson KV. Hedgehog signaling in the mouse requires intraflagellar transport proteins. Nature 2003, 426: 83–87
33 May SR, Ashique AM, Karlen M, Wang B, Shen Y, Zarbalis K, Reiter J *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
34 Huangfu D, Anderson KV. Cilia and hedgehog responsiveness in the mouse. Proc Natl Acad Sci USA 2005, 102: 11325–11330
35 Zhang Q, Davenport JR, Croyle MJ, Haycraft CJ, Yoder BK. Disruption of IFT results in both exocrine and endocrine abnormalities in the pancreas of Tg737(orpk) mutant mice. Lab Invest 2005, 85: 45–64
36 Scholey JM, Anderson KV. Intraflagellar transport and cilium-based signaling. Cell 2006, 125: 439–442
37 Haycraft CJ, Banizs B, Aydin-Son Y, Zhang Q, Michaud EJ, Yoder BK. Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function. PLoS Genet 2005, 1: e53
38 Incardona JP, Gruenberg J, Roelink H. Sonic hedgehog induces the segregation of patched and smoothened in endosomes. Curr Biol 2002, 12: 983–995
39 Varjosalo M, Li SP, Taipale J. Divergence of hedgehog signal transduction mechanism between *Drosophila* and mammals. Dev Cell 2006, 10: 177–186
40 Merchant M, Evangelista M, Luoh SM, Frantz GD, Chalasani S, Carano RA, van Hoy M *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
41 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
42 Eggenschwiler JT, Espinoza E, Anderson KV. Rab23 is an essential negative regulator of the mouse sonic hedgehog signaling pathway. Nature 2001, 412: 194–198
43 Reiter JF, Skarnes WC. Tectonic, a novel regulator of the hedgehog pathway required for both activation and inhibition. Genes Dev 2006, 20: 22–27
44 Svard J, Henricson KH, Persson-Lek M, Rozell B, Lauth M, Bergstrom A, Ericson J *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
45 Barnfield PC, Zhang X, Thanabalasingham V, Yoshida M, Hui CC. Negative regulation of Gli1 and Gli2 activator function by suppressor of fused through multiple mechanisms. Differentiation 2005, 73: 397–405
46 Kinzler KW, Ruppert JM, Bigner SH, Vogelstein B. The *Gli* gene is a member of the Krüppel family of zinc finger proteins. Nature 1988, 332: 371–374
47 Ruppert JM, Kinzler KW, Wong AJ, Bigner SH, Kao FT, Law ML, Seuanez HN *et al*. The Gli-Krüppel family of human genes. Mol Cell Biol 1988, 8: 3104–3113
48 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
49 Kogerman P, Grimm T, Kogerman L, Krause D, Unden AB, Sandstedt B, Toftgard R *et al*. Mammalian Suppressor-of-Fused modulates nuclear-cytoplasmic shuttling of Gli1. Nat Cell Biol 1999, 1: 312–319
50 Stecca B, Mas C, Clement V, Zbinden M, Correa R, Piguet V, Beermann F, Ruiz IAA. Melanomas require hedgehog-Gli signaling regulated by interactions between Gli1 and the RAS-MEK/AKT pathways. Proc Natl Acad Sci USA 2007, 104: 5895–5900
51 Pan Y, Bai CB, Joyner AL, Wang B. Sonic hedgehog signaling regulates Gli2 transcriptional activity by suppressing its processing and degradation. Mol Cell Biol 2006, 26: 3365–3377
52 Huntzicker EG, Estay IS, Zhen H, Lokteva LA, Jackson PK, Oro AE. Dual degradation signals control Gli protein stability and tumor formation. Genes Dev 2006, 20: 276–281
53 Wang B, Li Y. Evidence for the direct involvement of β-TrCP in Gli3 protein processing. Proc Natl Acad Sci USA 2006, 103: 33–38
54 Di Marcotullio L, Ferretti E, Greco A, De Smaele E, Po A, Sico MA, Alimandi M *et al*. Numb is a suppressor of hedgehog signaling and targets Gli1 for Itch-dependent ubiquitination. Nat Cell Biol 2006, 8: 1415–1423
55 Jiang J. Regulation of Hh/Gli signaling by dual ubiquitin pathways. Cell Cycle 2006, 5: 2457–2463
56 Huangfu D, Anderson KV. Signaling from SMO to Ci/Gli: conservation and divergence of hedgehog pathways from *Drosophila* to vertebrates. Development 2006, 133: 3–14
57 Kasper M, Schnidar H, Neill GW, Hanneder M, Klingler S, Blaas L, Schmid C *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

58 Cheng SY, Bishop JM. Suppressor of Fused represses Gli-mediated transcription by recruiting the SAP18-mSin3 corepressor complex. Proc Natl Acad Sci USA 2002, 99: 5442–5447
59 Hahn H, Wicking C, Zaphiropoulous PG, Gailani MR, Shanley S, Chidambaram A, Vorechovsky I *et al*. Mutations of the human homolog of *Drosophila* patched in the nevoid basal cell carcinoma syndrome. Cell 1996, 85: 841–851
60 Johnson RL, Rothman AL, Xie J, Goodrich LV, Bare JW, Bonifas JM, Quinn AG *et al*. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. Science 1996, 272: 1668–1671
61 Epstein E Jr. Genetic determinants of basal cell carcinoma risk. Med Pediatr Oncol 2001, 36: 555–558
62 Gorlin RJ. Nevoid basal-cell carcinoma syndrome. Medicine (Baltimore) 1987, 66: 98–113
63 Gorlin RJ. Living history-biography: from oral pathology to craniofacial genetics. Am J Med Genet 1993, 46: 317–334
64 Gorlin RJ, Goltz RW. Multiple nevoid basal-cell epithelioma, jaw cysts and bifid rib. A syndrome. N Engl J Med 1960, 262: 908–912
65 Gailani MR, Bale SJ, Leffell DJ, DiGiovanna JJ, Peck GL, Poliak S, Drum MA *et al*. Developmental defects in Gorlin syndrome related to a putative tumor suppressor gene on chromosome 9. Cell 1992, 69: 111–117
66 Gailani MR, Stahle-Backdahl M, Leffell DJ, Glynn M, Zaphiropoulos PG, Pressman C, Unden AB *et al*. The role of the human homologue of *Drosophila* patched in sporadic basal cell carcinomas. Nat Genet 1996, 14: 78–81
67 Goodrich LV, Johnson RL, Milenkovic L, McMahon JA, Scott MP. Conservation of the hedgehog/patched signaling pathway from flies to mice: induction of a mouse patched gene by hedgehog. Genes Dev 1996, 10: 301–312
68 Lindstrom E, Shimokawa T, Toftgard R, Zaphiropoulos PG. PTCH mutations: distribution and analyses. Hum Mutat 2006, 27: 215–219
69 Bale AE, Yu KP. The hedgehog pathway and basal cell carcinomas. Hum Mol Genet 2001, 10: 757–762
70 Goodrich LV, Milenkovic L, Higgins KM, Scott MP. Altered neural cell fates and medulloblastoma in mouse patched mutants. Science 1997, 277: 1109–1113
71 Hahn H, Wojnowski L, Zimmer AM, Hall J, Miller G, Zimmer A. Rhabdomyosarcomas and radiation hypersensitivity in a mouse model of Gorlin syndrome. Nat Med 1998, 4: 619–622
72 Aszterbaum M, Beech J, Epstein EH Jr. Ultraviolet radiation mutagenesis of hedgehog pathway genes in basal cell carcinomas. J Investig Dermatol Symp Proc 1999, 4: 41–45
73 Xie J, Murone M, Luoh SM, Ryan A, Gu Q, Zhang C, Bonifas JM *et al*. Activating smoothened mutations in sporadic basal-cell carcinoma. Nature 1998, 391: 90–92
74 Lam CW, Xie J, To KF, Ng HK, Lee KC, Yuen NW, Lim PL *et al*. A frequent activated smoothened mutation in sporadic basal cell carcinomas. Oncogene 1999, 18: 833–836
75 Reifenberger J, Wolter M, Knobbe CB, Kohler B, Schonicke A, Scharwachter C, Kumar K *et al*. Somatic mutations in the *PTCH*, *SMOH*, *SUFUH* and *TP53* genes in sporadic basal cell carcinomas. Br J Dermatol 2005, 152: 43–51
76 Reifenberger J, Wolter M, Weber RG, Megahed M, Ruzicka T, Lichter P, Reifenberger G. Missense mutations in *SMOH* in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system. Cancer Res 1998, 58: 1798–1803
77 Couve-Privat S, Bouadjar B, Avril MF, Sarasin A, Daya-Grosjean L. Significantly high levels of ultraviolet-specific mutations in the smoothened gene in basal cell carcinomas from DNA repair-deficient xeroderma pigmentosum patients. Cancer Res 2002, 62: 7186–7189
78 Xie J, Aszterbaum M, Zhang X, Bonifas JM, Zachary C, Epstein E, McCormick F. A role of PDGFRα in basal cell carcinoma proliferation. Proc Natl Acad Sci USA 2001, 98: 9255–9259
79 Athar M, Li C, Tang X, Chi S, Zhang X, Kim AL, Tyring SK *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
80 Berman DM, Karhadkar SS, Maitra A, Montes De Oca R, Gerstenblith MR, Briggs K, Parker AR *et al*. Widespread requirement for hedgehog ligand stimulation in growth of digestive tract tumors. Nature 2003, 425: 846–851
81 Watkins DN, Berman DM, Burkholder SG, Wang B, Beachy PA, Baylin SB. Hedgehog signaling within airway epithelial progenitors and in small-cell lung cancer. Nature 2003, 422: 313–317
82 Ma X, Chen K, Huang S, Zhang X, Adegboyega PA, Evers BM, Zhang H *et al*. Frequent activation of the hedgehog pathway in advanced gastric adenocarcinomas. Carcinogenesis 2005, 26: 1698–1705
83 Huang S, He J, Zhang X, Bian X, Yang L, Xie G, Zhang K *et al*. Activation of the hedgehog pathway in human hepatocellular carcinomas. Carcinogenesis 2006, 27: 1334–1340
84 Ma X, Sheng T, Zhang Y, Zhang X, He J, Huang S, Chen K *et al*. Hedgehog signaling is activated in subsets of esophageal cancers. Int J Cancer 2006, 118: 139–148
85 Thayer SP, Pasca di Magliano M, Heiser PW, Nielsen CM, Roberts DJ, Lauwers GY, Qi YP *et al*. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature 2003, 425: 851–856
86 Pasca di Magliano M, Sekine S, Ermilov A, Ferris J, Dlugosz AA, Hebrok M. Hedgehog/Ras interactions regulate early stages of pancreatic cancer. Genes Dev 2006, 20: 3161–3173
87 Fan L, Pepicelli CV, Dibble CC, Catbagan W, Zarycki JL, Laciak R, Gipp J *et al*. Hedgehog signaling promotes prostate xenograft tumor growth. Endocrinology 2004, 145: 3961–3970
88 Sheng T, Li C, Zhang X, Chi S, He N, Chen K, McCormick F *et al*. Activation of the hedgehog pathway in advanced prostate cancer. Mol Cancer 2004, 3: 29
89 Sanchez P, Hernandez AM, Stecca B, Kahler AJ, DeGueme AM, Barrett A, Beyna M *et al*. Inhibition of prostate cancer proliferation by interference with sonic hedgehog-Gli1 signaling. Proc Natl Acad Sci USA 2004, 101: 12561–12566
90 Karhadkar SS, Bova GS, Abdallah N, Dhara S, Gardner D, Maitra A, Isaacs JT *et al*. Hedgehog signaling in prostate regeneration, neoplasia and metastasis. Nature 2004, 431: 707–712
91 Ehtesham M, Sarangi A, Valadez JG, Chanthaphaychith S, Becher MW, Abel TW, Thompson RC *et al*. Ligand-dependent activation of the hedgehog pathway in glioma progenitor cells. Oncogene 2007, 26: 5752–5761
92 Lindemann RK. Stroma-initiated hedgehog signaling takes center stage in B-cell lymphoma. Cancer Res 2008, 68: 961–964
93 Liu S, Dontu G, Wicha MS. Mammary stem cells, self-renewal pathways, and carcinogenesis. Breast Cancer Res 2005, 7: 86–95
94 Rubin LL, de Sauvage FJ. Targeting the hedgehog pathway in cancer. Nat Rev Drug Discov 2006, 5: 1026–1033
95 Lee Y, Kawagoe R, Sasai K, Li Y, Russell HR, Curran T, McKinnon PJ. Loss of Suppressor-of-Fused function promotes tumorigenesis.

Oncogene 2007, 26: 6442–6447
96 Kubo M, Nakamura M, Tasaki A, Yamanaka N, Nakashima H, Nomura M, Kuroki S *et al*. Hedgehog signaling pathway is a new therapeutic target for patients with breast cancer. Cancer Res 2004, 64: 6071–6074
97 Chen JK, Taipale J, Cooper MK, Beachy PA. Inhibition of hedgehog signaling by direct binding of cyclopamine to smoothened. Genes Dev 2002, 16: 2743–2748
98 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
99 Berman DM, Karhadkar SS, Hallahan AR, Pritchard JI, Eberhart CG, Watkins DN, Chen JK *et al*. Medulloblastoma growth inhibition by hedgehog pathway blockade. Science 2002, 297: 1559–1561
100 Williams JA, Guicherit OM, Zaharian BI, Xu Y, Chai L, Wichterle H, Kon C *et al*. Identification of a small molecule inhibitor of the hedgehog signaling pathway: effects on basal cell carcinoma-like lesions. Proc Natl Acad Sci USA 2003, 100: 4616–4621
101 Frank-Kamenetsky M, Zhang XM, Bottega S, Guicherit O, Wichterle H, Dudek H, Bumcrot D *et al*. Small-molecule modulators of hedgehog signaling: identification and characterization of smoothened agonists and antagonists. J Biol 2002, 1: 10
102 Chen JK, Taipale J, Young KE, Maiti T, Beachy PA. Small molecule modulation of smoothened activity. Proc Natl Acad Sci USA 2002, 99: 14071–14076
103 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 USA 2007, 104: 8455–8460
104 Bijlsma MF, Spek CA, Zivkovic D, van de Water S, Rezaee F, Peppelenbosch MP. Repression of smoothened by patched-dependent (pro-)vitamin D3 secretion. PLoS Biol 2006, 4: e232
105 Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, Westphal H, Beachy PA. Cyclopia and defective axial patterning in mice lacking sonic hedgehog gene function. Nature 1996, 383: 407–413
106 St-Jacques B, Hammerschmidt M, McMahon AP. Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. Genes Dev 1999, 13: 2072–2086.
107 Bitgood MJ, Shen L, McMahon AP. Sertoli cell signaling by desert hedgehog regulates the male germline. Curr Biol 1996, 6: 298–304
108 Nieuwenhuis E, Motoyama J, Barnfield PC, Yoshikawa Y, Zhang X, Mo R, Crackower MA *et al*. Mice with a targeted mutation of *patched2* are viable but develop alopecia and epidermal hyperplasia. Mol Cell Biol 2006, 26: 6609–6622
109 Lee Y, Miller HL, Russell HR, Boyd K, Curran T, McKinnon PJ. Patched2 modulates tumorigenesis in patched1 heterozygous mice. Cancer Res 2006, 66: 6964–6971
110 Zhang W, Yi MJ, Chen X, Cole F, Krauss RS, Kang JS. Cortical thinning and hydrocephalus in mice lacking the immunoglobulin superfamily member Cdo. Mol Cell Biol 2006, 26: 3764–3772
111 Zhang XM, Ramalho-Santos M, McMahon AP. Smoothened mutants reveal redundant roles for Shh and Ihh signaling including regulation of L/R asymmetry by the mouse node. Cell 2001, 105: 781–792
112 Park HL, Bai C, Platt KA, Matise MP, Beeghly A, Hui CC, Nakashima M *et al*. Mouse Gli1 mutants are viable but have defects in Shh signaling in combination with a Gli2 mutation. Development 2000, 127: 1593–1605
113 Motoyama J, Liu J, Mo R, Ding Q, Post M, Hui CC. Essential function of Gli2 and Gli3 in the formation of lung, trachea and oesophagus. Nat Genet 1998, 20: 54–57
114 Qi M, Zhuo M, Skalhegg BS, Brandon EP, Kandel ER, McKnight GS, Idzerda RL. Impaired hippocampal plasticity in mice lacking the Cβ1 catalytic subunit of cAMP-dependent protein kinase. Proc Natl Acad Sci USA 1996, 93: 1571–1576
115 Cummings DE, Brandon EP, Planas JV, Motamed K, Idzerda RL, McKnight GS. Genetically lean mice result from targeted disruption of the RII β subunit of protein kinase A. Nature 1996, 382: 622–626
116 Hoeflich KP, Luo J, Rubie EA, Tsao MS, Jin O, Woodgett JR. Requirement for glycogen synthase kinase-3β in cell survival and NF-κB activation. Nature 2000, 406: 86–90
117 Guardavaccaro D, Kudo Y, Boulaire J, Barchi M, Busino L, Donzelli M, Margottin-Goguet F *et al*. Control of meiotic and mitotic progression by the F box protein beta-Trcp1 *in vivo*. Dev Cell 2003, 4: 799–812
118 Singer JD, Gurian-West M, Clurman B, Roberts JM. Cullin-3 targets cyclin E for ubiquitination and controls S phase in mammalian cells. Genes Dev 1999, 13: 2375–2387
119 Petersen PH, Zou K, Hwang JK, Jan YN, Zhong W. Progenitor cell maintenance requires Numb and Numblike during mouse neurogenesis. Nature 2002, 419: 929–934
120 O'Driscoll L, McMorrow J, Doolan P, McKiernan E, Mehta JP, Ryan E, Gammell P *et al*. Investigation of the molecular profile of basal cell carcinoma using whole genome microarrays. Mol Cancer 2006, 5: 74
121 Raffel C, Jenkins RB, Frederick L, Hebrink D, Alderete B, Fults DW, James CD. Sporadic medulloblastomas contain *PTCH* mutations. Cancer Res 1997, 57: 842–845
122 Xie J, Johnson RL, Zhang X, Bare JW, Waldman FM, Cogen PH, Menon AG *et al*. Mutations of the *patched* gene in several types of sporadic extracutaneous tumors. Cancer Res 1997, 57: 2369–2372
123 Taylor MD, Liu L, Raffel C, Hui CC, Mainprize TG, Zhang X, Agatep R *et al*. Mutations in Su(Fu) predispose to medulloblastoma. Nat Genet 2002, 31: 306–310
124 Di Marcotullio L, Ferretti E, De Smaele E, Argenti B, Mincione C, Zazzeroni F, Gallo R *et al*. REN(KCTD11) is a suppressor of hedgehog signaling and is deleted in human medulloblastoma. Proc Natl Acad Sci USA 2004, 101: 10833–10838
125 Clement V, Sanchez P, de Tribolet N, Radovanovic I, Ruiz i Altaba A. Hedgehog-Gli1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. Curr Biol 2007, 17: 165–172
126 Feng YZ, Shiozawa T, Miyamoto T, Kashima H, Kurai M, Suzuki A, Ying-Song J *et al*. Over-expression of hedgehog signaling molecules and its involvement in the proliferation of endometrial carcinoma cells. Clin Cancer Res 2007, 13: 1389–1398
127 Liu S, Dontu G, Mantle ID, Patel S, Ahn NS, Jackson KW, Suri P *et al*. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. Cancer Res 2006, 66: 6063–6071
128 Wolf I, Bose S, Desmond JC, Lin BT, Williamson EA, Karlan BY, Koeffler HP. Unmasking of epigenetically silenced genes reveals DNA promoter methylation and reduced expression of PTCH in breast cancer. Breast Cancer Res Treat 2007, 105: 139–155
129 Mukherjee S, Frolova N, Sadlonova A, Novak Z, Steg A, Page GP, Welch DR *et al*. Hedgehog signaling and response to cyclopamine differ in epithelial and stromal cells in benign breast and breast cancer. Cancer Biol Ther 2006, 5: 674–683

130 Mori Y, Okumura T, Tsunoda S, Sakai Y, Shimada Y. Gli1 expression is associated with lymph node metastasis and tumor progression in esophageal squamous cell carcinoma. Oncology 2006, 70: 378–389
131 Lee SY, Han HS, Lee KY, Hwang TS, Kim JH, Sung IK, Park HS *et al*. Sonic hedgehog expression in gastric cancer and gastric adenoma. Oncol Rep 2007, 17: 1051–1055
132 Ma XL, Sun HJ, Wang YS, Huang SH, Xie JW, Zhang HW. Study of sonic hedgehog signaling pathway related molecules in gastric carcinoma. World J Gastroenterol 2006, 12: 3965–3969
133 Fukaya M, Isohata N, Ohta H, Aoyagi K, Ochiya T, Saeki N, Yanagihara K *et al*. Hedgehog signal activation in gastric pit cell and in diffuse-type gastric cancer. Gastroenterology 2006, 131: 14–29
134 Morton JP, Mongeau ME, Klimstra DS, Morris JP, Lee YC, Kawaguchi Y, Wright CV *et al*. Sonic hedgehog acts at multiple stages during pancreatic tumorigenesis. Proc Natl Acad Sci USA 2007, 104: 5103–5108
135 Liu MS, Yang PY, Yeh TS. Sonic hedgehog signaling pathway in pancreatic cystic neoplasms and ductal adenocarcinoma. Pancreas 2007, 34: 340–346
136 Feldmann G, Dhara S, Fendrich V, Bedja D, Beaty R, Mullendore M, Karikari C *et al*. Blockade of hedgehog signaling inhibits pancreatic cancer invasion and metastases: a new paradigm for combination therapy in solid cancers. Cancer Res 2007, 67: 2187–2196
137 Gao J, Li Z, Chen Z, Shao J, Zhang L, Xu G, Tu Z *et al*. Antisense SMO under the control of the PTCH1 promoter delivered by an adenoviral vector inhibits the growth of human pancreatic cancer. Gene Ther 2006, 13: 1587–1594
138 Ohuchida K, Mizumoto K, Fujita H, Yamaguchi H, Konomi H, Nagai E, Yamaguchi K *et al*. Sonic hedgehog is an early developmental marker of intraductal papillary mucinous neoplasms: clinical implications of mRNA levels in pancreatic juice. J Pathol 2006, 210: 42–48
139 Kayed H, Kleeff J, Osman T, Keleg S, Buchler MW, Friess H. Hedgehog signaling in the normal and diseased pancreas. Pancreas 2006, 32: 119–129
140 Martin ST, Sato N, Dhara S, Chang R, Hustinx SR, Abe T, Maitra A *et al*. Aberrant methylation of the human hedgehog-interacting protein (*HHIP*) gene in pancreatic neoplasms. Cancer Biol Ther 2005, 4: 728–733
141 Kayed H, Kleeff J, Esposito I, Giese T, Keleg S, Giese N, Buchler MW *et al*. Localization of the human hedgehog-interacting protein (HIP) in the normal and diseased pancreas. Mol Carcinog 2005, 42: 183–192
142 Olsen CL, Hsu PP, Glienke J, Rubanyi GM, Brooks AR. Hedgehog-interacting protein is highly expressed in endothelial cells but down-regulated during angiogenesis and in several human tumors. BMC Cancer 2004, 4: 43
143 Huang S, He J, Zhang X, Bian Y, Yang L, Xie G, Zhang K, Tang W, Stelter AA, Wang Q *et al*. Activation of the hedgehog pathway in human hepatocellular carcinomas. Carcinogenesis 2006, 27: 1334–1340
144 Sicklick JK, Li YX, Jayaraman A, Kannangai R, Qi Y, Vivekanandan P, Ludlow JW *et al*. Dysregulation of the hedgehog pathway in human hepatocarcinogenesis. Carcinogenesis 2006, 27: 748–757
145 Liu YJ, Wang Q, Li W, Huang XH, Zhen MC, Huang SH, Chen LZ *et al*. Rab23 is a potential biological target for treating hepatocellular carcinoma. World J Gastroenterol 2007, 13: 1010–1017
146 Villanueva A, Newell P, Chiang DY, Friedman SL, Llovet JM. Genomics and signaling pathways in hepatocellular carcinoma. Semin Liver Dis 2007, 27: 55–76
147 Patil MA, Zhang J, Ho C, Cheung ST, Fan ST, Chen X. Hedgehog signaling in human hepatocellular carcinoma. Cancer Biol Ther 2006, 5: 111–117
148 Dierks C, Grbic J, Zirlik K, Beigi R, Englund NP, Guo GR, Veelken H *et al*. Essential role of stromally induced hedgehog signaling in B-cell malignancies. Nat Med 2007, 13: 944–951
149 Peacock CD, Wang Q, Gesell GS, Corcoran-Schwartz IM, Jones E, Kim J, Devereux WL *et al*. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. Proc Natl Acad Sci USA 2007, 104: 4048–4053
150 Hu D, Helms JA. The role of sonic hedgehog in normal and abnormal craniofacial morphogenesis. Development 1999, 126: 4873–4884
151 Cooper MK, Porter JA, Young KE, Beachy PA. Teratogen-mediated inhibition of target tissue response to Shh signaling. Science 1998, 280: 1603–1607
152 Mistretta CM, Liu HX, Gaffield W, MacCallum DK. Cyclopamine and jervine in embryonic rat tongue cultures demonstrate a role for Shh signaling in taste papilla development and patterning: fungiform papillae double in number and form in novel locations in dorsal lingual epithelium. Dev Biol 2003, 254: 1–18
153 Borzillo GV, Lippa B. The hedgehog signaling pathway as a target for anticancer drug discovery. Curr Top Med Chem 2005, 5: 147–157