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# Misactivation of Hedgehog signaling causes inherited and sporadic cancers

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The Hedgehog pathway is critical for the development of diverse organs. Misactivation of the Hedgehog pathway can cause developmental abnormalities and cancers, including medulloblastoma, the most common pediatric brain tumor, and basal cell carcinoma, the most common cancer in the United States. Here, we review how basic, translational, and clinical studies of the Hedgehog pathway have helped reveal how cells communicate, how intercellular communication controls development, how signaling goes awry to cause cancer, and how to use targeted molecular agents to treat both inherited and sporadic cancers.

## Introduction

Cancer is a genetic disease caused by activation of oncogenes, inactivation of tumor suppressors, or a combination thereof (1). The genetic and epigenetic changes underlying cancer can be induced by infectious agents (e.g., human papilloma and hepatitis viruses), environmental carcinogens (e.g., tobacco, asbestos, and ultraviolet radiation), and mistakes in DNA replication or repair. Most cancers are sporadic, meaning they occur in individuals without a family history of cancer or an inherited genetic risk of cancer (2). However, some individuals inherit genetic changes that predispose them to cancer. Inherited mutations can cooperate with acquired mutations to cause malignancies with greater frequency, earlier age of onset, and/or more aggressive clinical behavior than sporadic cancers. For instance, individuals with germline mutations in the retinoblastoma (RB) tumor suppressor may develop multiple aggressive tumors early in life, including ocular retinoblastomas, pineoblastomas, and sarcoma.

Identification of genes and mutations underlying cancer is complicated by the fact that most human malignancies harbor many mutations, only some of which contribute to the cancer phenotype. Functional mutations can be distinguished from passenger mutations by their recurrence in distinct sporadic cancers as well as their ability to dysregulate cell proliferation (3, 4). In contrast to sporadic cancers, inherited cancer predisposition syndromes allow population genetics to identify drivers of malignancy. For example, identifying mutations that segregate with highly penetrant phenotypes in cancer-prone pedigrees provides important clues to the genetic changes that cause cancer. Because the molecular pathways underlying rare inherited cancers and their more common sporadic counterparts are often the same, the identification of inherited cancer-predisposing mutations can illuminate the etiology of sporadic cancers.

As most current cancer treatment modalities (e.g., surgery, cytotoxic chemotherapy, and ionizing radiation) are agnostic to the molecular causes of cancer, much of diagnosis and manage-

ment of cancer proceeds with limited or no investigation into underlying genetic changes. However, improved understanding of cancer genotypes has distinguished subgroups of tumors that display different sensitivities to traditional or targeted therapies, suggesting that genetic profiling of cancers is likely to facilitate more effective and less toxic treatments for cancer patients. Moreover, genetic profiling of normal tissue from cancer patients may provide insights into whether they are at risk of additional cancers. Identification of genetic cancer risk factors may guide medical surveillance, and prophylactic or therapeutic medical interventions. For example, individuals with inherited *BRCA1* or *BRCA2* mutations may opt for regular imaging surveillance or surgical prophylaxis to mitigate their increased risk of breast, ovarian, or prostate cancer.

Here, we review how insights into a pathway involved in inherited cancer predisposition, the Hedgehog (HH) pathway, have informed our knowledge of human malignancy. This curious and ancient pathway is essential for embryonic development and adult tissue homeostasis (5, 6). Molecular studies of the HH pathway have helped reveal how cells communicate, how intercellular communication controls cell growth, how signaling goes awry to cause malignancy, and how to use targeted molecular agents to treat both inherited and sporadic cancers.

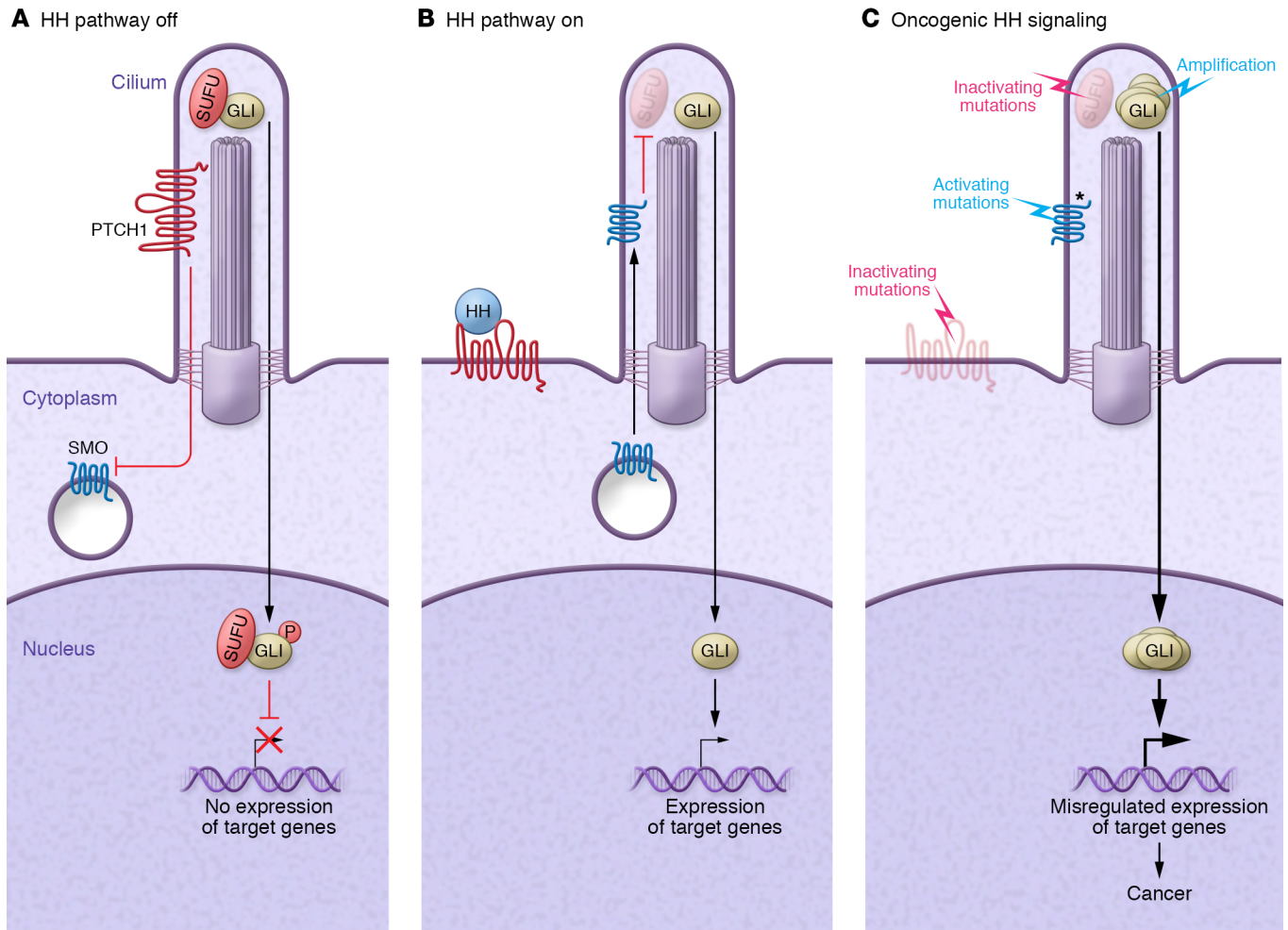
## Hedgehog signaling controls tissue patterning and growth

HH ligands are secreted lipoproteins (7). Mammals have at least three HH genes, the best studied of which encodes Sonic hedgehog (SHH), a crucial regulator of embryonic development and adult tissue homeostasis (8, 9). HH proteins are synthesized, often by regionally defined epithelial cells, as precursors that are activated by autocatalytic cleavage of the C-terminal intein domain (10). Once active, HH ligands stimulate the HH pathway, often in nearby mesenchymal cells, by binding to their transmembrane receptors, Patched 1 (PTCH1) and Patched 2 (PTCH2) (11–13). Vertebrate HH signaling is transduced by the primary cilium, a cellular antenna that projects from the surface of most cells (Figure 1 and refs. 14–16). When HH binds to PTCH proteins at the cilium, a  $\pi$ -cation lock is broken in another transmembrane protein,

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**Figure 1. A model of ciliary Hedgehog signaling.** (A) In the absence of Hedgehog ligands such as SHH, PTCH1 localizes to the primary cilium and, through an unknown mechanism, prevents SMO from entering the cilium. GLI proteins bind SUFU, a negative regulator, and are phosphorylated by kinases, such as PKA, to generate transcriptional repressors that enter the nucleus and silence the Hedgehog transcriptional program. (B) In the presence of SHH, PTCH1 leaves the cilium, allowing SMO to accumulate at the primary cilium membrane. At the cilium, SMO inhibits the formation of GLI3 repressor and activates GLI2, which enters the nucleus to promote transcription of Hedgehog target genes. (C) Inactivating mutations in *PTCH1*, *PTCH2*, or *SUFU*; activating mutations in *SMO* (denoted here as an asterisk); or amplification of *GLI2* can activate expression of Hedgehog target genes in an unregulated way, leading to cancer.

Smoothed (SMO), and SMO accumulates at the cilium to activate the downstream pathway (Figure 1 and refs. 17, 18).

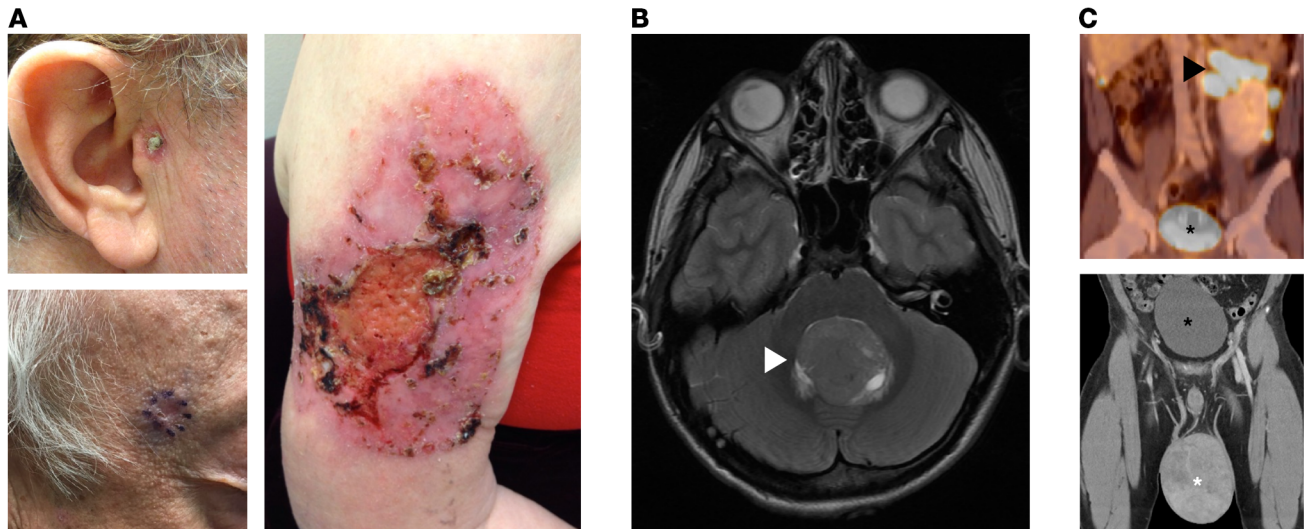
In vertebrates, SMO activates GLI2, the primary activator of the HH transcriptional program (19). Activated SMO also suppresses the formation of a transcriptional repressor form of GLI3, which — through repression of a pathway inhibitor — contributes to the activation of the HH transcriptional program. GLI2 and GLI3 regulate the third member of the GLI family, GLI1, a feed-forward amplifier of transcriptional activation (6). In the absence of HH pathway activation, GLI family members are restrained by binding to Suppressor of Fused (SUFU) (20–22). Upon HH pathway activation, SUFU-GLI complexes accumulate in cilia and dissociate, and the GLI proteins regulate HH target gene expression in the nucleus (23–25).

Despite abiding interest in understanding mechanisms of HH signal transduction, the processes by which HH inhibits PTCH, PTCH inhibits SMO, SMO is activated, and SMO activates GLI

transcription factors, as well as the full diversity of transcriptional targets of HH signaling, remain active areas of investigation. Adding to the difficulty of understanding HH signal transduction, many aspects of the HH pathway display overlapping or tissue-specific behavior. For example, HH signaling adaptation occurs during neural tube patterning, but not in other tissues (26). The tissue-specific aspects of HH signaling during development may help elucidate the different ways in which HH signaling functions in different cancer types.

### Misactivation of the Hedgehog pathway causes cancer

**Basal cell carcinoma.** HH signals are critical for the development of diverse organs, such as the central nervous system, limbs, skeleton, eyes, lungs, muscles, pancreas, testes, and skin (7). In the developing skin, SHH produced by epidermal placodes signals to the underlying dermal condensates to form hair follicles (27, 28). SHH



**Figure 2. Hedgehog pathway-associated cancers.** (A) Basal cell carcinomas (BCCs) of the preauricular space (top left), temple with preoperative markings (bottom left), and upper extremity (right) of adult patients demonstrate the morphologic diversity of BCC, which may present as an open sore, red patch, pearly papule, or scar. (B) T2 magnetic resonance 3-D fast-recovery fast-spin echo cube image of medulloblastoma in the cerebellar vermis (white arrow) of a pediatric patient. (C) Coronal PET (top) and CT (bottom) images of testicular rhabdomyosarcoma (white asterisk) with regional metastases to the left renal hilum (black arrow) in a young adult patient; the bladder in both images is denoted by a black asterisk.

signals promote cell proliferation, and some of the genomic targets of SHH signaling in the skin, such as *E2F2*, are key activators of the cell cycle (29, 30). Consequently, in the absence of SHH, epidermal placodes form but fail to grow down into the dermis (27).

After embryogenesis, the HH pathway maintains tissue homeostasis, in part by regulating stem cells and tissue growth (31–33). In the postnatal skin, hair follicle growth recapitulates much of embryonic hair growth, including the involvement of HH signaling. For example, SHH is expressed during anagen, the hair follicle phase during which there is downward growth of the follicle into the dermis, and is important for maintaining epidermal stem cells and regulating epidermal growth (34–37).

Affecting about 2 million people per year, basal cell carcinoma (BCC) is the most common cancer in the United States (38). BCC is a skin cancer that typically arises in adult patients as a result of ultraviolet radiation (Figure 2A). Ultraviolet radiation causes many genomic mutations, and BCCs typically harbor about 65 mutations per megabase, making them the most mutated human cancer (39). Despite these many mutations, BCCs are unified by a common molecular defect, misactivation of the HH pathway.

The most common HH pathway-activating mutations in BCC include biallelic loss-of-function mutations in genes encoding negative pathway regulators (i.e., *PTCH1*, *PTCH2*, and *SUFU*); amplification of *GLI2*, the gene encoding the principal HH pathway transcriptional activator; and monoallelic activating substitutions in positive pathway regulators, such as *SMO* (Table 1 and refs. 39–42). A frequent activating mutation in *SMO* is W535R, also known as SMOM2, a substitution within the seventh transmembrane domain (42, 43). Consistently, SMOM2 or other activating substitutions in this region activate the unregulated, SHH-independent ciliary accumulation of SMO, emphasizing the intimate association between SMO ciliary accumulation and downstream pathway activation (18).

Despite their extraordinary mutation burden, BCCs are typically slow-growing and often amenable to cure by local excision. However, BCC size, number, or proximity to critical structures such as the eye, lip, or nose can preclude complete excision. In such instances, nonsurgical treatments such as superficial radiotherapy, photodynamic therapy, and topical cytotoxic agents can inhibit the growth of BCC. For patients with localized disease who fail to respond to conventional therapies, or for rare patients with metastatic BCC, systemic therapy with SMO antagonists is superior to traditional systemic chemotherapy (44–46). Although the proportion of tumors that achieve long-term control with SMO antagonists remains to be established, responses to SMO antagonists may be transient (47–49), and the combination of HH pathway inhibition with radiation may increase the durability of molecular therapy for BCC patients (although it may also promote additional resistance mechanisms in recalcitrant BCCs) (50, 51). BCC cells may survive HH pathway inhibition through chromatin remodeling and enhancer reprogramming, but synergy between Wnt and SMO antagonists, or Notch agonists and SMO antagonists, may be a clinically relevant strategy to prevent relapse (52–54).

BCC arises from multiple skin compartments, including hair follicles, the interfollicular epidermis, and mechanosensory niches in the epidermis (55). Many skin cells are ciliated, but as cilia are deconstructed before the cell's entry into mitosis, it might be expected that actively cycling cancer cells would not have cilia. However, many BCC cells do possess cilia (56). During development and cancer alike, cilia are required to transduce HH signals from SMO to GLI transcription factors. In mouse genetic models of BCC, the ability of SMOM2 to trigger oncogenesis depends on the presence of cilia (56, 57). In contrast, oncogenic HH pathway activation downstream of SMO, such as genomic amplification of *GLI2* or loss of *SUFU*, is independent of cilia (56, 57). Thus, HH signal transduction can become deregulated to cause unre-

**Table 1. Cancer-predisposing Hedgehog pathway mutations**

Gene	Germline mutations	Somatic mutations	Notes
<i>PTCH1</i>	Heterozygous inactivation associated with NBCCS	Gene inactivation	Most commonly mutated gene in NBCCS and medulloblastoma
<i>PTCH2</i>	Heterozygous inactivation associated with NBCCS	Gene inactivation	
<i>SMO</i>		Missense hypermorphic mutations	
<i>SUFU</i>	Heterozygous inactivation associated with NBCCS	Gene inactivation	Accounts for a minority of NBCCS; mutation is a poor prognostic factor in medulloblastoma
<i>GLI2</i>		Gene amplification	Amplification is negative prognostic factor in medulloblastoma

NBCCS, nevoid basal cell carcinoma syndrome.

strained growth and cancer in both cilium-dependent and cilium-independent fashions.

**Medulloblastoma.** The functions of HH signaling are perhaps best understood in the patterning of the embryonic central nervous system's dorsoventral axis. During early brain development, SHH produced at the ventral midline helps specify the cerebellum in the hindbrain (58). Later in brain development, SHH is secreted by the choroid plexus into the ventricular space (59), and later still, SHH is produced by Purkinje cells within the developing cerebellum (60, 61). SHH from the choroid plexus and Purkinje cells stimulates the proliferation of cerebellar progenitors in the rhombic lip and the fourth ventricle (59–61). Around birth, the rhombic lip and ventricular progenitors migrate to form the external granular layer and dive into the prospective white matter, where they give birth to a remarkable number of neurons. As in the skin, SHH acts as a mitogen in the developing cerebellum by inducing expression of cell cycle regulators including *N-Myc*, *Ccnd1*, and *Ccnd2* (Figure 3 and ref. 60). At the end of cerebellar development, the number of neurons in the cerebellum dwarfs even the number in the cortex (62); in the mouse, the granule cell precursors in the external granule layer give rise to  $10^8$  cerebellar granule neurons (63, 64).

Consistent with their intimate involvement with HH signaling, primary cilia are also critical for HH-mediated patterning of the central nervous system (65, 66). Defects in HH signaling disrupt cerebellar growth and alter foliation (67). Similarly, inherited defects in ciliary structure can compromise developmental HH signaling and result in defective cerebellar growth as part of Joubert syndrome, a congenital syndrome characterized by agenesis of the cerebellar vermis (68–70).

A few weeks after birth, HH signaling and cerebellar neurogenesis cease (71–73). How HH signaling is turned off at this and other time points during development remains an active area of investigation, but in the cerebellum, cessation of HH signaling may involve feedback from the granule cell precursors to the Purkinje cells or cessation of granule cell response to HH (74).

As in the skin, persistent misactivation of the HH pathway with concomitant loss of control over cell division leads to cancer in the cerebellum (61). Although not every cell that misactivates the HH pathway in the cerebellum generates a tumor, some do give

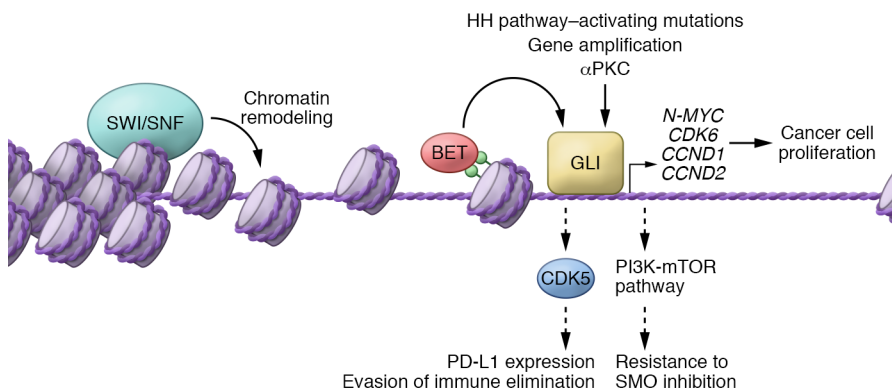
rise to medulloblastoma, the most common malignant brain cancer in children (Figure 2B and ref. 75). Concurrent mutations in other oncogenic pathways or genes, such as p53, may predispose certain cells with misactivated HH signaling in the cerebellum to form tumors (76). The likelihood of these events appears to decrease with age and may be less common in girls, as the incidence of medulloblastoma decreases from 0.53 per 100,000 in children aged 0–4 years, to 0.33 per 100,000 in adolescents aged 15–19 years, and medulloblastoma is more common in boys than in girls (75). Medulloblastomas are mostly treated as a uniform clinical entity, but they constitute molecular subgroups with drastic differences in genomic, demographic, and clinical features (77, 78). Of the 12 or more distinct molecular variants of medulloblastoma, misactivation of the HH pathway underlies approximately one-third of cases (76, 79). In relation to the

other principal subgroups of medulloblastoma tumors, HH pathway-associated medulloblastomas display bimodal distribution, with most cases occurring in infants and adults (80).

The current standard of care for medulloblastoma involves maximal safe resection followed by ionizing radiotherapy to the entire craniospinal axis with concurrent and adjuvant cytotoxic chemotherapy (81). Although this well-established regimen cures approximately 75% of pediatric medulloblastoma patients, long-term survival for some patients within particular medulloblastoma molecular subgroups can be as low as 25% (77, 78). Moreover, survivors are debilitated by significant therapy-associated toxicity, chief among which are neurocognitive deficits, endocrine and growth abnormalities, and an increased risk of secondary malignancy (82–84). To reduce the burden of treatment-associated toxicity of radiotherapy, high-dose chemotherapy with hematopoietic stem cell rescue and other risk-adapted therapeutic strategies have been used, particularly in young patients (85–88). However, outcomes remain suboptimal and patients are frequently afflicted with significant therapy-associated sequelae.

As in BCC, biallelic inactivating *PTCH* and *SUFU* loss-of-function mutations, amplification of *GLI* transcription factors, or activating *SMO* substitutions are common in HH-associated medulloblastoma (Table 1 and ref. 76). Also like BCC, many HH pathway-associated medulloblastoma cells are ciliated, and primary cilia are critical for misactivated *SMO* to drive medulloblastoma formation and growth (57). *GLI2* amplification and, presumably, *SUFU* mutations underlie cilia-independent oncogenic HH pathway activation, and, when they co-occur with mutations compromising p53 function, these *GLI2* and *SUFU* aberrations portend particularly poor prognoses for pediatric medulloblastoma patients (57, 76).

Treatment of HH pathway-associated medulloblastoma with *SMO* antagonists such as vismodegib can dramatically shrink tumors, but recurrence and development of acquired resistance are common and can be rapid (47, 48). Importantly, *SMO* antagonists have only been trialed in medulloblastoma patients with recurrent tumors previously treated with DNA-damaging agents, and prior genotoxic therapy may attenuate the efficacy of *SMO* antagonism. Moreover, only half of pediatric HH pathway-asso-



**Figure 3. A model of the Hedgehog transcriptional program in cancer.** Misactivation of the Hedgehog pathway in cancer through upstream mutations, gene amplification, atypical protein kinase C (αPKC) signaling, or epigenetic misregulation of the SWI/SNF chromatin remodeling complex or BET bromodomain proteins culminates in activation of GLI transcription factors. GLI transcription factors induce the expression of *N-MYC*, *CDK6*, *CCND1*, and *CCND2* to promote cancer cell proliferation and tumor growth. Parallel activation of Cdk5 and PI3K/mTOR signaling may facilitate evasion of immune elimination and resistance to SMO antagonists, respectively.

ciated medulloblastomas respond to SMO inhibition, limiting the proportion of patients who may achieve even a transient benefit from HH pathway inhibition (89). Thus, novel therapies are required to improve outcomes for patients with HH pathway-associated medulloblastoma.

An alternative pharmacologic target of oncogenic HH signaling in medulloblastoma is cyclin-dependent kinase 6 (CDK6), a mitogenic kinase and direct transcriptional target of *GLI2* (Figure 3 and refs. 90, 91). When activated through interaction with cyclin D1, CDK6 induces cell cycle progression by phosphorylating the tumor suppressor RB and relieving repression of E2F transcription factors. Potent small-molecule inhibitors of CDK6 and the related kinase CDK4 have been tested in diverse human malignancies, and are now being investigated in pediatric medulloblastoma patients (92). Another possible pharmacologic target in medulloblastoma is CDK5, a serine-threonine kinase that promotes expression of the immune exhaustion marker programmed death-ligand 1 (PD-L1), thereby allowing tumor cells to evade immune elimination (Figure 3 and ref. 93). The efficacy of CDK6 or CDK5 inhibition for medulloblastoma remains to be established by clinical trials, but intra- and intermedulloblastoma heterogeneity, including divergent clonal selection at the time of recurrence, suggests that molecular monotherapy will not be a durable therapeutic strategy for patients with HH pathway-associated medulloblastoma (94).

Another possible pharmacologic strategy for HH pathway-associated medulloblastoma is to block the formation of lipids that are required for oncogenic HH signaling. The ciliary membrane is enriched in specific lipids, such as phosphatidylinositol 4-phosphate (PtdIns4P) (16). Ciliary PtdIns4P is generated by inositol polyphosphate 5-phosphatase (INPP5E) to restrain the ciliary accumulation of GPR161, an inhibitor of HH signaling (95, 96). In the absence of INPP5E, ciliary PtdIns4P is replaced with phosphatidylinositol 4,5-bisphosphate (PtdIns4,5P), which increases ciliary GPR161 and dampens HH signaling. In medulloblastoma, *INPP5E* constrains oncogenic HH signaling, and *INPP5E* copy number and expression are reduced in HH pathway-associated medullo-

blastoma (97, 98). Thus, the development and implementation of pharmacologic INPP5E inhibitors may increase ciliary GPR161 and restrain oncogenic HH signaling, although, as with SMO inhibition, amplification of *GLI2* or loss of *SUFU* would be expected to circumvent the effect of INPP5E inhibition.

In addition to PtdIns4P, lipids such as oxysterols can be enriched in cilia (16). Sterol biosynthesis is required for vertebrate HH signaling, and both synthetic oxysterols and cholesterol can bind SMO to activate the downstream pathway (17, 99–109). Consistently, statin medications, which inhibit cholesterol biosynthesis, may synergize with HH pathway inhibition for the treatment of medulloblastoma (110). Endogenous, cilia-associated oxysterols activate the HH pathway by interacting with two separate domains of

SMO (111). An enzyme that participates in the production of SMO-activating oxysterols, HSD11β2, is dramatically enriched in HH-associated medulloblastoma, raising the possibility that inhibiting HSD11β2 may also be a strategy for inhibiting HH pathway activity in cancer (111). In support of this hypothesis, genetic inhibition of HSD11β2 in a mouse model of HH-associated medulloblastoma attenuates HH signal transduction and the growth of cancer, and blocking HSD11β2 with an active ingredient from licorice similarly inhibits medulloblastoma growth in mouse models (111). These data suggest that, like INPP5E inhibition, inhibition of ciliary oxysterol biosynthesis may be a tractable alternative means of inhibiting oncogenic HH pathway activation in medulloblastoma.

**Rhabdomyosarcoma.** Rhabdomyosarcoma (RMS), the most common pediatric soft tissue sarcoma, has two major histologic subtypes, alveolar and embryonal (Figure 2C). Genetically, alveolar RMS often harbors reciprocal chromosomal translocations between either of two myogenic transcription factor genes, *PAX3* or *PAX7*, and *FOXO1*, a promitotic transcription factor. Embryonal RMS and alveolar RMS that is not associated with *PAX-FOXO1* translocations are instead characterized by HH pathway misactivation (112–114). In mouse models, HH pathway activity is implicated in RMS cell self-renewal, chemoresistance, motility, and differentiation (115).

Identifying the RMS cell of origin remains an ongoing area of investigation, but RMS may arise from a mesodermal progenitor (116). Many skeletal muscles are derived from somites, paired, segmentally organized blocks of paraxial mesoderm. Subdomains of somites give rise to bone and cartilage (the sclerotome), or skeletal muscle and connective tissue (the epaxial dermomyotome and hypaxial myotome). The epaxial dermomyotome develops into the deep muscles of the back, whereas the hypaxial myotome develops into limb muscles and diaphragm (117, 118). During mouse development, HH signals are critical for the specification of muscle progenitors specifically in the epaxial domain of the somite (119). One possibility is that embryonal RMS arises from persistent epaxial dermomyotome-like progenitors that depend

on HH signals, whereas alveolar RMS arises from HH-independent hypaxial myotome-like progenitors. Multiple origins of RMS may help explain how this cancer can originate throughout the body, even in organs without skeletal muscle such as the urogenital and biliary tracts. Consistently, the effects of SMO inhibitors on RMS cell lines are variable, suggesting that HH pathway inhibition may be efficacious in only a subset of RMS patients (120).

**Nevoid basal cell carcinoma syndrome.** Considering that activating HH pathway mutations cause sporadic BCC and medulloblastoma, it is perhaps unsurprising that inherited mutations in genes encoding negative HH pathway regulators predispose individuals to the same cancers. More specifically, inheritance of inactivating heterozygous mutations in key negative regulators of the HH pathway cause nevoid basal cell carcinoma syndrome (NBCCS), also known as basal cell nevus syndrome or Gorlin syndrome (121–123). Individuals with NBCCS may develop between several and several thousand BCCs (Figure 2A). A subset of individuals with NBCCS are also predisposed to developing medulloblastoma (Figure 2B) or RMS (Figure 2C and refs. 124, 125).

NBCCS is a rare disorder, with an estimated prevalence of 2–4 in 100,000, and with equal incidence in males and females (126). In addition to increased cancer risk, individuals with NBCCS can display developmental abnormalities such as macrocephaly, intracranial calcification, and skeletal changes (i.e., kyphoscoliosis, bifid ribs, craniofacial bossing, hypertelorism, odontogenic jaw keratocysts, and mandibular prognathism). The inheritance pattern for NBCCS is autosomal dominant with complete penetrance, although oncogenesis is caused by a second hit to the wild-type allele, making it recessive at the cellular level. This syndrome probably has affected humans throughout our existence, as ancient Egyptian mummies with NBCCS characteristics have been identified (5).

The cancers and developmental abnormalities that characterize NBCCS can be caused by inactivating mutations in *PTCH1* or *PTCH2*, leading to misactivation of the downstream pathway (12, 122, 123). Inherited mutations in *SUFU* can also cause NBCCS, albeit without apparent odontogenic jaw keratocysts and with possibly higher risk of medulloblastoma (127, 128). The rarity of NBCCS complicates precise estimates of the prevalences of *PTCH1*, *PTCH2*, and *SUFU* mutations in affected individuals, but mutations in one of these three genes are present in all or almost all cases that have been reported (127).

## Hedgehog signaling in other cancers

The increased incidence of BCC, medulloblastoma, and RMS in individuals with NBCCS provides clues about the molecular causes of both the syndromic and the sporadic forms of these tumors. However, beyond these three cancer types, there are limited mechanistic data linking HH pathway mutations to other human malignancies. Consequently, investigations of HH signaling have not translated to new clinical therapies or improved outcomes in prostate, breast, and gastrointestinal cancers — some of the most common cancers — or in lung and pancreatic cancers — some of the deadliest cancers. Thus, it is possible that many cancer-associated mutations affecting HH pathway components are passengers that do not substantively contribute to the cancer phenotype (4). Alternatively, misactivation of aspects of the HH pathway may

contribute to oncogenesis through mechanisms that are distinct from those elucidated in BCC, medulloblastoma, and RMS (129). For example, atypical teratoid rhabdoid tumor (ATRT) is a cancer that may, in part, depend on activation of transcriptional effectors of the HH pathway through a mechanism that does not require activation of the upstream pathway.

**Atypical teratoid rhabdoid tumor.** Only several hundred cases of ATRT are diagnosed in the United States each year, but this enigmatic cancer is the most common malignant brain tumor in children younger than 6 months (130). Survival from ATRT is generally poor, and because of the rarity of diagnosis, a standard of care has yet to be defined. ATRT is associated with inactivating somatic mutations in *SMARCB1* or, rarely, *SMARCA4*, both of which encode essential components of the chromatin remodeling SWI/SNF complex (131, 132). The SWI/SNF complex epigenetically regulates diverse genes involved in lineage specification, stem cell pluripotency, and signal transduction.

Despite sharing underlying mutated genes, ATRT displays three distinct gene expression profiles, one of which is associated with misactivation of the HH pathway (133). The mechanism by which *SMARCB1* or *SMARCA4* mutations cause HH pathway activation occurs via epigenetic derepression of the genes encoding the HH transcriptional effectors *GLI1* and *GLI2* (Figure 3 and refs. 133–135). Activation of a *GLI1*- and *GLI2*-dependent HH transcriptional program in ATRTs therefore circumvents any requirement for HH ligands, SMO, or cilia.

Bromodomain proteins bind to acetylated lysines in histones that mark transcription factor-accessible chromatin. BET bromodomain proteins are critical regulators of *GLI1* and *GLI2* that directly occupy the promoter of both genes (Figure 3 and ref. 136). Inhibiting *GLI* transcription factors by antagonizing BET bromodomain proteins is effective in mouse models of diverse HH-associated cancers, including BCC, medulloblastoma, and ATRT (136). Moreover, BET bromodomain inhibition is effective for HH pathway-associated cancers even in the presence of genetic lesions that confer resistance to SMO inhibition, and therefore represents a promising therapeutic target for other HH-associated cancers. *CDK6* is also enriched in HH-subgroup ATRT tumors, and pharmacologic inhibition of *CDK6* may be similarly useful for treating patients with ATRT (133, 137).

Beyond BCC, medulloblastoma, RMS, and ATRT, aberrant HH signaling has been implicated in diverse human malignancies, but mechanistic connections are often sparsely understood or confusing. For example, in meningioma, the most common intracranial tumor in the United States, rare HH pathway mutations are found in tumors with excellent clinical outcomes (138, 139). Nevertheless, constitutive activation of SMO in the meninges fails to cause meningioma in most mice, suggesting that HH misactivation is not sufficient to induce or drive meningioma growth (140).

**Pancreatic ductal adenocarcinoma.** Pancreatic ductal adenocarcinoma (PDAC) is associated with upregulation of *SHH*, but in mice, SMO is dispensable for PDAC growth, suggesting that misactivation of the HH pathway is not a critical driver of this cancer either (141, 142). Moreover, unlike BCC and medulloblastoma, PDAC is associated with loss of cilia (143, 144), providing ancillary evidence that the normal HH pathway is not hyperactivated in PDAC. However, PDAC stromal cells, such as fibroblasts and endo-

**Table 2. FDA-approved molecular therapies affecting the Hedgehog pathway in cancer**

Agent	Target	Approved indication
Vismodegib (GDC-0049)	SMO	BCC, recurrent medulloblastoma
Sonidegib (LDE225, erismodegib)	SMO	BCC
Arsenic trioxide	GLI	Acute promyelocytic leukemia

thelial cells, respond to SHH produced by tumor cells, suggesting that paracrine signaling may modulate PDAC progression (145–147). SHH and tumor-associated stromal cells induce the development of desmoplastic PDAC (144, 148), suggesting that the HH pathway may function non-cell-autonomously in PDAC. Consistently, depleting PDAC-associated stromal cells accelerates tumor growth, suggesting that pharmacologic inhibition of HH signaling will not be an effective strategy for patients with PDAC (149–152).

The observation that stromal HH signaling restrains PDAC growth suggests that paracrine signaling may modulate the progression of other cancers (147, 150–152). Indeed, HH signaling also restrains bladder cancer progression (153), and HH signals from prostate cancer signal to tumor-associated stroma (154, 155). These data have important implications for the clinical implementation of HH pathway antagonists, and suggest that HH pathway inhibition could accelerate the growth of some cancers. In support of that hypothesis, clinical trials of SMO antagonists for PDAC have failed to demonstrate a benefit or have been halted early for poor outcomes (156). Although a subset of individuals with genetically defined HH pathway-associated PDAC may benefit from targeted therapy, the lessons learned from PDAC preclinical models and clinical testing demonstrate that HH pathway antagonists cannot be broadly applied.

**Lung cancer.** Lung cancer is the leading cause of cancer mortality in the United States, and HH signaling has been implicated in small-cell, non-small-cell, and mesothelioma lung tumors. In pleural mesothelioma, SHH and GLI1 are expressed, and SMO antagonists attenuate growth in vitro and in vivo (157). However, HH pathway mutations in mesothelioma are rare, and whether HH signaling drives growth in human patients is not known (158). GLI1 is expressed in most non-small-cell lung cancers, but responses to SMO antagonists in vitro are heterogeneous, raising the possibility that a subset of these tumors may benefit from HH pathway inhibition (159). In a mouse model of small-cell lung cancer, genetic deletion of *Smo* suppresses tumor initiation and progression, suggesting that misactivation of the HH pathway contributes to tumor growth (160). Thus, given the poor clinical outcomes with the current standard of care for small-cell lung cancer, HH pathway inhibitors such as SMO and BET bromodomain antagonists are of clinical interest (161).

**Prostate cancer.** With the exception of BCC, prostate cancer is the most common malignancy in adult males in the United States. During prostate development, HH signaling regulates epithelial tubule formation (162). HH signaling also mediates regeneration of prostatic epithelial cells, and in prostate cancer, the HH pathway is implicated in dedifferentiation, invasiveness, and metastasis (163).

Consistently, HH pathway mutations are identified in prostate cancer, and SMO inhibitors and *GLI1* RNA interference both block the growth of cultured prostate cancer cells in vitro (164, 165). In prostate cancer, androgen receptors bind GLI2 and GLI3 to inhibit the proteolytic generation of GLI3 repressor and activate the HH transcriptional program (166). Breast cancer, another hormone-driven malignancy, displays a strong correlation between GLI1 expression in the nucleus and estrogen receptor staining; furthermore, elevated SHH expression delineates a subpopulation of breast cancer patients with aggressive pathologic features (167, 168). Despite these preclinical data, and clinical evidence that SMO antagonists suppress HH signaling in localized high-risk and metastatic prostate cancer, whether HH pathway inhibition would be effective in either prostate and breast cancer remains unclear (169).

### Hedgehog pathway-directed therapeutics

The discovery of cyclopamine, a teratogenic plant-derived alkaloid that inhibits HH signal transduction, provided a foundation for developing HH pathway inhibitors for use in cancer therapy (170, 171). Cyclopamine binds to SMO and inhibits the unregulated activity caused by oncogenic mutations (172, 173). Some agents modeled on cyclopamine inhibit HH pathway-associated BCC, but other HH pathway modulators, such as inhibitors of SHH and GLI transcription factors, have not successfully transitioned to clinical use because of short half-life, poor pharmacokinetic or pharmacodynamic properties, or lack of efficacy in human patients (174–177).

The most commonly used HH pathway antagonists in cancer are vismodegib and sonidegib (Table 2). These agents have pharmacokinetics and pharmacodynamics compatible with systemic administration in humans and have been extensively studied in BCC and medulloblastoma (178, 179). Both BCC and medulloblastoma can regress below the threshold of detection by visual, histopathologic, or radiologic examination in response to vismodegib or sonidegib, but a significant proportion of BCC and HH-associated medulloblastoma patients fail to respond to SMO inhibition (44, 45, 180–182). Responses in BCC can be durable, but adult patients receiving vismodegib for BCC report adverse events such as nausea, muscle cramps, loss of taste, weight loss, and alopecia (45, 182). Although typically mild, these symptoms can cause patients to discontinue therapy, which may lead to recurrence of BCC in some patients. Pediatric medulloblastoma patients experience similar symptoms with SMO antagonists, but can suffer premature and irreversible growth plate fusion from on-target inhibition of HH signaling (183).

Among patients with HH pathway-associated cancers that respond to HH pathway antagonists, tumors can acquire resistance to molecular therapy. Despite remarkable clinical responses in metastatic HH-associated medulloblastoma, targeted HH pathway inhibition is associated only with transient tumor regression followed by recurrence of resistant cancers (47–49). One way in which tumors evade SMO inhibition to maintain constitutively high levels of HH pathway activity is through SMO mutations that disrupt drug binding (48, 184). Additionally, genomic amplification of *GLI* transcription factors or HH target genes such as *CCND1*, as well as loss-of-function *SUFU* mutations, can bypass SMO inhibition to maintain uncontrolled growth in both medulloblastoma and BCC (Figure 3 and refs. 49, 185, 186). Other means



of circumventing SMO inhibition in BCC can include increased expression of the transcription factor SRF, which cooperates with its partner MKL1 to increase the transcriptional activity of GLI1 (187). Medulloblastomas that are resistant to SMO inhibitors may also display activation of phosphoinositide-3-kinase and AKT signaling, and inhibiting these pathways may delay the development of resistance (Figure 3 and ref. 49).

Arsenic trioxide, which can inhibit HH signal transduction by preventing ciliary accumulation and DNA binding of GLI transcription factors, as well as destabilizing of GLI2, may provide an approach to blocking the growth of tumors with acquired resistance to SMO antagonists (188–190). Arsenic trioxide also blocks the JNK, NF- $\kappa$ B, thioredoxin reductase, and MAPK pathways, and effects on pathways other than HH may underlie its efficacy in the treatment of promyelocytic leukemia (Table 2 and ref. 191).

Taken together, these data suggest that molecular monotherapy may not be an effective strategy for patients with HH pathway-associated cancers. Rather, concurrent inhibition of (a) pathway activators, such as GLI transcription factors or INPP5E and HSD11 $\beta$ 2, which regulate cilia-associated lipids that modulate HH signaling intensity; (b) pathway mediators, such as BET bro-

modomain proteins, which participate in GLI transcriptional activation; and (c) pathway effectors, such as CDK5 or CDK6, which are involved in suppressing immune clearance and activating the cell cycle, respectively, may improve the durability of clinical responses and decrease acquired resistance. Elucidation of effective molecular antagonists against these targets will likely require a partnership of foundational biology and deep analysis of human tumors, involving the collaborative efforts of basic, translational, and clinical scientists.

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