HHS Public Access

Author manuscript

Dev Cell. Author manuscript; available in PMC 2017 August 22.

Published in final edited form as:

Dev Cell. 2016 August 22; 38(4): 333–344. doi:10.1016/j.devcel.2016.07.026.

Hedgehog signal transduction: key players, oncogenic drivers, and cancer therapy

Ekaterina Pak[1] and **Rosalind A. Segal**[1]

[1]Department of Neurobiology, Harvard Medical School, Boston, MA 02115, USA; Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA 02215, USA.

Summary

The Hedgehog (Hh) signaling pathway governs complex developmental processes, including proliferation and patterning within diverse tissues. These activities rely on a tightly-regulated transduction system that converts graded Hh input signals into specific levels of pathway activity. Uncontrolled activation of Hh signaling drives tumor initiation and maintenance. However, recent entry of pathway-specific inhibitors into the clinic reveals mixed patient responses and thus prompts further exploration of pathway activation and inhibition. In this review, we share emerging insights on regulated and oncogenic Hh signaling, supplemented with updates on the development and use of Hh pathway-targeted therapies.

The evolutionarily conserved Hedgehog (Hh) pathway serves fundamental morphogenic and mitogenic roles in tissue development, homeostasis, and repair. Disruption of Hh signaling underlies a variety of developmental disorders affecting multiple organ systems. Holoprosencephaly and cyclopia, as well as dramatic limb abnormalities, are characteristic of impaired Hh signaling during development. Moreover, ectopic activation of Hh signaling is implicated in a wide range of tumors, including medulloblastoma (MB), basal cell carcinoma (BCC), and many others. Thus, Hh signaling is an area of intense study in both developmental and cancer biology. Here, we provide updates on vertebrate Hh signal transduction and the molecular drivers of Hh pathway-dependent MB and BCC. Additionally, we discuss the application of clinical and preclinical, targeted therapies to treat Hh-dependent tumors.

Hh signal transduction

In mammals, the Hh signaling cascade is initiated by one of three spatiotemporally confined ligands: Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh)

Rosalind_segal@dfci.harvard.edu.

Segal ETOC

The Hedgehog (Hh) signaling pathway governs complex developmental processes including proliferation and patterning of multiple tissues, and its inappropriate activation contributes to many cancers. This Review from Pak and Segal discusses emerging concepts in regulated and oncogenic Hh signaling, and provides an update on the development and use of Hh-targeted therapies.

(reviewed in Ingham and McMahon 2001). Secreted Hh ligands control developmental outcomes in a concentration- and duration-dependent manner. Consequently, the reception and signal transduction system for Hh ligands must convert different levels of signal into specific levels of pathway output. Ultimately, signal transduction results in expression of a transcriptional program mediated by activator and repressor forms of the Gli transcription factors. The ability of this cascade to initiate distinct developmental outcomes in cells exposed to an Hh ligand at different concentrations or for different lengths of time is critical for Hh-dependent establishment of the dorsal-ventral axis during early neural development and formation of the proximal-distal axis in developing limbs. Here, we summarize the vertebrate components of Hh signal transduction and focus on recent updates in this field that contribute to our current understanding of Hh signaling in development and cancer (Figure 1). For the remainder of this review, we will refer to the Hh ligands for general concepts and Shh ligands for specific reports.

Ptch1

The primary receptor for Hh ligands is the twelve-pass transmembrane protein, Patched 1 (Ptch1) (Marigo et al., 1996; Stone et al., 1996). In the absence of ligand, Ptch1 blocks pathway activity. When Hh ligand binds Ptch1, both ligand and receptor are internalized and degraded (Chen and Struhl, 1996; Incardona et al., 2000). Thus, ligand binding not only removes pathway repression by Ptch1, but also limits the half-life of the ligand. The mechanisms by which Ptch1 is removed from the cell surface upon ligand binding are not fully understood. Recently, Shh ligands were shown to induce accumulation of Ptch1 and the E3 ubiquitin ligases Smurf1 and Smurf2 in lipid rafts, which are molecularly distinct domains of the cell membrane (Yue et al., 2014). The ensuing ubiquitination of Ptch1 promoted Ptch1 endosomal trafficking to lysosomes for degradation and was important for Ptch1 clearance and graded pathway activation.

Besides Ptch1, other receptors for Hh ligands modulate pathway activation (reviewed in Beachy et al., 2010). Positive co-receptors include Cdo, Boc, and the vertebrate specific Gas1. Another vertebrate specific receptor, Hhip, acts as a negative regulator of Hh signaling. Many positive co-receptors (Cdo, Boc, and Gas1) are transcriptionally repressed, while negative receptors (Ptch1 and Hhip) are activated following Hh pathway induction. Additionally, proteoglycans function as co-receptors with either positive or negative effects on Hh signaling depending on their unique protein and sugar composition. The resulting network of receptors and feedback loops helps cells properly interpret the duration and graded level of Hh signaling.

Smo

In the absence of Hh ligand, Ptch1 inhibits Smoothened (Smo), a seven-pass transmembrane protein that functions as a potent pathway activator (Murone et al., 1999). The mechanisms by which Ptch1 inhibits Smo are unknown. The current consensus is that Ptch1 does not physically interact with Smo, but rather regulates the transport, synthesis, and/or access of a small molecule (or molecules) that affect Smo activity (Taipale et al., 2002).

Lipid metabolites are popular candidates for the endogenous regulators of Smo activity. The lipophilic secosteroid vitamin D3 was previously proposed to function as a Ptch1-regulated direct inhibitor of Smo (Bijlsma et al., 2006). More recently, a group of cholesterol derivatives called oxysterols were shown to activate Smo by binding to its N-terminal, extracellular cysteine-rich domain (CRD) (Myers et al., 2013; Nachtergaele et al., 2013; Nedelcu et al., 2013). CRD mutants fail to fully respond to Hh stimulation, but also exhibit a higher basal level of signaling compared to wild-type Smo, indicating that the CRD domain suppresses basal Smo activity. Importantly, CRD mutants are still subject to inhibition by Ptch1. Thus, oxysterol binding may be required for maximal Smo activity but not for mediating Ptch1-dependent inhibition of Smo. Additional lipid-based Smo modulators include endocannabinoids from lipoprotein particles that can bind and inhibit Smo activity (Khaliullina et al., 2015). Despite such recent insights on lipophilic regulators of Smo activity, the mechanisms by which Ptch1 represses Smo and how Hh ligand removes this repression remain major questions in the field.

Upon Hh ligand binding and Ptch1 degradation, Smo becomes phosphorylated by casein kinase 1 (CK1) and G-protein coupled receptor kinase 2 (GRK2), moves into the primary cilium (PC), and assumes an activated conformation (Chen et al., 2011a). Differential phosphorylation of Smo may help interpret Hh gradients through a Smo phosphorylation code (Chen and Jiang, 2013). The relay of Hh signaling downstream of activated Smo is not yet completely understood. However, Smo activates both G-protein dependent and independent signals to regulate Gli transcription factors, calcium flux, and metabolic pathways (Arensdorf et al., 2016). Here, we will focus on signals that impinge on Gli transcription factors.

Sufu

The negative pathway regulator, Supressor of fused (Sufu), functions between Smo and the Gli transcription factors (Pearse II et al., 1999; Stone et al., 1999). Sufu directly interacts with and sequesters full-length Gli in the cytoplasm. Sequestration of Gli prevents its nuclear translocation and promotes phosphorylation and processing of full-length Gli into a truncated repressor (Humke et al., 2010). Sequestration also stabilizes full-length Gli2 and Gli3, protecting them from proteasomal degradation and thus maintaining a pool of available Gli proteins for Shh signal transduction (Chen et al., 2009; Wang et al., 2010). Challenging the traditional cytoplasm-centric roles of Sufu, some have suggested that Sufu can regulate Gli activity in the nucleus (Lin et al., 2014).

Kif7

The kinesin protein, Kif7, is also an evolutionarily conserved component of Hh signaling that modulates Gli function downstream of Smo (Cheung et al., 2009; Tay et al., 2005). Kif7 interacts with Gli proteins and exerts both positive and negative regulatory roles in Hh signaling (Endoh-Yamagami et al., 2009; Liem et al., 2009). Kif7 localizes at the base of the primary cilium (PC) in the absence of Hh ligand, but moves into the PC and is important for Gli2 and Gli3 accumulation at the cilium tip when the pathway is stimulated. A recently proposed model incorporates Kif7 phosphorylation with these earlier observations of Kif7 signal transduction (Liu et al., 2014). When the Hh pathway is inactive, Kif7 is

phosphorylated and enriched at the base of the PC, while trafficking of Kif7 and Gli into the cilium is limited. When the pathway is activated, the scaffolding protein PPFIA1 and the phosphatase PP2A are recruited to and dephosphorylate Kif7, leading to increased localization of Kif7 and Gli proteins at the PC tip, Sufu dissociation from Gli proteins, and Gli activation. An intriguing addendum to this model comes from the Anderson group, who recently proposed that a major role for Kif7 in Hh signaling is to control cilium length and architecture (He et al., 2014a). In this role, Kif7 ensures that a single cilium tip compartment is established where Gli and Sufu can localize for signal transduction. As discussed below, functional primary cilia are crucial for proper Hh signal transduction. Accordingly, understanding the roles of Kif7 in Hh signaling requires integrating the direct impact of Kif7 on pathway components with additional contributions of Kif7 activity in cilium assembly.

Gli transcription factors

Graded levels of Hh signaling trigger the expression of different sets of response genes, depending on the ratio of Gli activator (GliA) and Gli repressor (GliR) forms (reviewed in Hui and Angers, 2011). In vertebrates, there are three Gli gene family members: Gli1, Gli2, and Gli3. Gli1 is a Hh response gene that exists only as a transcriptional activator and functions in a positive feedback loop upon pathway activation. Gli2 functions primarily as a transcriptional activator, while Gli3 serves as the primary transcriptional repressor. Multiple mechanisms control GliA and GliR functions, including the regulation by Sufu and Kif7 described above. Post-translational modifications of Gli proteins, including phosphorylation, acetylation, ubiquitination, and sumoylation, also affect Gli output. Here, we review our current understanding of the Gli phosphorylation code.

In the absence of Hh ligand, full-length Gli (GliFL) is phosphorylated by protein kinase A (PKA), glycogen synthase kinase 3 (GSK3), and CK1 (Pan et al., 2006; Pan et al., 2009; Tempé et al., 2006). Hyperphosphorylated GliFL is bound by the adaptor protein β-TrCP, and the resulting complex is ubiquitinated by a Cul1-based E3 ligase and targeted for proteasomal processing to form a truncated transcriptional repressor (GliR) (Wang and Li, 2006). Alternatively, GliFL may also be completely degraded by the proteasome, facilitated by Spop-mediated Cul3-based E3 ligase ubiquitination (Wang et al., 2010). Gli2FL phosphorylation predominantly induces complete proteasomal degradation, while phosphorylated Gli3FL is more efficiently processed into Gli3R (Pan and Wang, 2007). Smo activation blocks Gli proteolysis and simultaneously promotes Gli activator function.

The unique patterns of Gli2/3 phosphorylation may be important for converting differences in Hh signal strength into discrete states of Gli activity. PKA phosphorylation of six conserved serine residues (P1-6) on Gli2/3 drives GliR and inhibits GliA formation (Niewiadomski et al., 2014). Interestingly, selective phosphorylation of the first four PKA sites (P1-4) is sufficient to target processing of full-length Gli into GliR, while inhibition of GliA requires phosphorylation of all six PKA sites. Smo activation reduces phosphorylation at P1-6, which allows PKA-independent Gli phosphorylation at a different cluster of serine/threonine sites (Pc-g) and results in full transcriptional activation of Gli2/3.

While Gli1 is not subject to PKA-mediated proteasomal processing, several other kinases have been shown to alter Gli1 function. Phosphorylation of Gli1 by atypical protein kinase C

ι/λ (aPKC-ι/λ) promotes maximal Gli1 DNA binding and transcriptional activation (Atwood et al., 2013). Gli1 is also phosphorylated by AMP-activated protein kinase (AMPK), which induces Gli1 degradation (Di Magno et al., 2016; Li et al., 2015). Importantly, regulation of Gli1 by the energy sensor AMPK links cellular metabolic state to Hh transcriptional output, which may be crucial for the developmental roles of this pathway.

cAMP and PKA

Cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) is a master negative regulator of the Hh pathway. Both Sufu and Gli2/3 transcription factors are phosphorylated by PKA. Sequential phosphorylation of Sufu by PKA and GSK3 stabilizes Sufu in a complex with Gli2/3 that moves into the primary cilium (PC) in response to Hh ligand (Chen et al., 2011b). The roles of PKA in Gli2/3 phosphorylation and proteolysis were described above.

Many inputs modulate PKA activity and thereby affect Hh pathway output. For example, production of cAMP by adenylyl cyclase and degradation of cAMP by phosphodiesterases can promote and attenuate PKA activity, respectively. Recently, degradation of cAMP was mechanistically linked to signaling by transmembrane neuropilin (Nrp) receptors (Ge et al., 2015). The Nrp ligand Semaphorin3 (Sema3) promotes interaction of phosphodiesterase 4D (PDE4D) with the Nrp cytoplasmic domain. Sema3-Nrp mediated translocation of PDE4D to the plasma membrane brings this phosphodiesterase close to the site of cAMP production and thus permits efficient hydrolysis of cAMP. While Sema3 alone cannot stimulate Hh signal transduction, the Sema3-Nrp-PDE4D axis enhances signaling that has been activated by Shh. Importantly, Shh pathway activation functions in a positive feedback loop to increase *Nrp1* expression, although *Nrp1* is not a direct target of Gli transcription factors (Hillman et al., 2011). Instead, in many cellular contexts, Hh-dependent *Nrp1* expression is positively regulated by the Eya1 phosphatase and the Six1 transcription factor (Eisner et al., 2015). Interestingly, while Eya1, Six1, and Nrp promote Gli2-dependent transcription, they do not alter Shh-dependent inhibition of Gli3R.

In a pivotal study, production of cAMP was mechanistically coupled to Hh signaling via the orphan GPCR, Gpr161 (Mukhopadhyay et al., 2013). In the absence of Shh ligand, Gpr161 localizes to the PC and promotes increased levels of cAMP, probably via $G\alpha_s$-mediated activation of adenylyl cyclase. In the presence of Shh ligand, Gpr161 is removed from cilia, preventing cAMP production and thus promoting pathway activation. A recent update on Gpr161 further elucidates the molecular mechanisms by which it is removed from the PC (Pal et al., 2016). Importantly, Gpr161 unifies many components of Shh signaling: ligand stimulation, PKA regulation, and roles of the PC, which are reviewed in more detail below.

The primary cilium (PC) in Hh signaling

The core components of vertebrate Hh signaling, including Ptch1, Smo, Sufu, Kif7, and Gli proteins, dynamically localize to the PC (reviewed in Nozawa et al., 2013; Goetz and Anderson, 2010). Upon Hh ligand binding, Ptch1 exits the base of the PC, while Smo accumulates within the cilium. Pathway activation also promotes Sufu-Gli complex movement into the PC where they dissociate, resulting in concurrent enrichment of Gli in

the distal tip of the cilium and Gli translocation to the nucleus. Smo translocation and Gli dissociation demonstrate positive regulation of Hh signaling via the PC. Cilia also participate in pathway inhibition by mediating the proteolytic processing of GliFL into GliR that turns off target genes. Thus, mice with defective or absent cilia display functional loss or altered ratios of GliA/R (Goetz and Anderson, 2010).

Precise localization of proteins within the PC is important for signal transduction. For example, the EvC zone, named after Ellis-van Creveld Syndrome, is located at the base of the PC and defines a distinct compartment where Smo accumulates in response to Hh ligands by binding to the proteins EVC and EVC2. Anchoring of the EVC-EVC2 complex to the EvC zone is required for activation of Gli2 but not for regulating levels of Gli3R (Pusapati et al., 2014). This type of GliA/GliR signaling bifurcation downstream of Smo has also been demonstrated for the ciliary basal body-localized protein Dlg5 (Discs large, homolog 5) (Chong et al., 2015). Upon ligand stimulation, Dlg5 interacts with Smo to promote Kif7 and Gli2 ciliary accumulation and Gli2 activation, but Dlg5 is not required for suppression of GliR formation. Subciliary localization of Hh signaling regulators such as Dlg5 and EvC complex proteins may help coordinate the contributions of GliA/GliR functions in response to different levels of pathway activation.

Recent work has highlighted how the lipid composition of the PC contributes to trafficking and signaling of Hh pathway components. The phosphoinositide PI(4)P is enriched in the ciliary membrane, whereas both PI(4)P and $PI(4,5)P_2$ are found in the plasma membrane (Chavez et al., 2015; Garcia-Gonzalo et al., 2015). This distinct lipid composition of the PC is maintained by the cilium-localized phosphatase, Inpp5e, which dephosphorylates ciliary $PI(4,5)P_2$ into PI(4)P. Loss of Inpp5e results in $PI(4,5)P_2$ accumulation at the ciliary membrane, which in turn recruits and maintains Tubby-like protein 3 (Tulp3) and its interacting intraflagellar transport (IFT-A) proteins in the PC. Importantly, the negative Shh regulator Gpr161, discussed above, uses Tulp3 and IFT-A complex to traffic into cilia and therefore also accumulates in the PC upon Inpp5e loss. Ciliary accumulation of Gpr161 in Inpp5e mutants is accompanied by increased cAMP levels and hindered Shh target gene activation. Inpp5e loss also impairs Gli3 accumulation at the ciliary tip following pathway stimulation, but does not affect Smo trafficking. Thus, Inpp5e establishes a distinct phosphoinositide composition in the PC, which limits ciliary accumulation of negative Shh regulators and therefore permits ligand-induced Shh signal transduction.

Studies on the phosphoinositide PI(3)P provide further evidence linking localized lipid dynamics with PC function (Franco et al., 2014). The lipid kinase, PI3K-C2α, is enriched at the ciliary base, where it regulates the generation of a localized pool of PI(3)P. This pool of PI(3)P is necessary for pericentriolar localization and activation of Rab11, which triggers Rab8-dependent protein cargo entry into cilia and is necessary for proper ciliary elongation. Loss of PI3K-C2α causes reduced Smo ciliary accumulation and reduced pathway activation in response to ligand. In these and other studies that disrupt ciliary composition, it is important to note that the observed effects on Hh signaling can at times be difficult to isolate in the context of general PC dysfunction.

Drivers of Hh-dependent tumors: basal cell carcinoma and medulloblastoma

An early link between Hh signaling and cancer was the discovery that inherited loss-of-function mutations in *PTCH1* are responsible for nevoid basal cell carcinoma syndrome (NBCCS), also called Gorlin syndrome (Hahn et al., 1996; Johnson et al., 1996). This autosomal dominant disease is characterized by predisposition to basal cell carcinomas (BCCs) in the skin, as well as higher incidence of other neoplasms, especially medulloblastoma (MB). Although Gorlin syndrome is rare, sporadic BCC is the most common human cancer. Sporadic BCC is associated with UV exposure and is also driven by aberrant activation of Hh signaling.

Another connection between Hh signaling and cancer is evident in the brain tumor MB. Approximately 30% of all MB cases are characterized by a SHH molecular signature (Taylor et al., 2012). The role of Shh signaling in MB formation is in some ways not surprising given the mitogenic role of Shh ligand in driving massive proliferation of granule neuron progenitors (GNPs) in the developing cerebellum (Wechsler-Reya and Scott, 1999). If the Shh pathway is constitutively activated in GNPs, proliferation persists beyond the normal developmental period and can lead to formation of MB (Goodrich et al., 1997; Hatton et al., 2008).

Besides BCC and MB, Hh pathway aberrations and/or activation have been detected in many other cancers and include ligand-dependent and independent, as well as non-canonical Hh signaling (reviewed in Amakye et al., 2013; Barakat et al., 2010; Teglund and Toftgard, 2010). In some instances, tumor initiation and/or survival directly require Hh signaling (BCC, MB, and possibly rhabdomyosarcoma). Other cancers may not exhibit mutations that activate Hh signaling and drive oncogenesis but Hh signaling may instead modulate tumor growth and malignant behavior. This is the case in pancreatic ductal adenocarcinoma (PDAC), where Hh signaling is active in the tumor stroma and may promote or limit tumor growth depending on the level of signaling (Mathew et al., 2014; Tian et al., 2009). Furthermore, pathway activation does not always correlate with favorable treatment potential of Hh pathway inhibitors, as has been demonstrated in studies examining PDAC (Lee et al., 2014a). Studies and controversies surrounding the contributions of Hh signaling to several cancers have been reviewed elsewhere (see Barakat et al., 2010; Teglund and Toftgard, 2010). Here, we focus on the contributions of reported ligand-independent Hh pathway drivers in BCC and MB (Figure 2).

Receptors: Ptch1, Boc, and Smo

As discussed above, Ptch1 suppresses Hh signaling in the absence of ligand whereas Smo activates the pathway in response to ligand binding to Ptch1. Functional loss of *Ptch1* or activating mutations in *Smo* drive tumorigenesis in BCC and MB mouse models, demonstrating the causative roles of these genes in tumor onset (Goodrich et al., 1997; Hatton et al., 2008; Nitzki et al., 2012; Xie et al., 1998). High frequencies of somatic mutations in *PTCH1* (~70-90%), and to a lesser extent *SMO* (~10-20%), are reported in human BCCs (Bonilla et al., 2016; Sekulic and Von Hoff, 2016). *PTCH1* mutations (~45%)

and frequent chromosomal loss of the *PTCH1* locus are also found in SHH-MB, whereas *SMO* mutations (~14%) are less common and are highly enriched in adult versus pediatric patients (Kool et al., 2014).

Another Hh receptor, Boc, was recently implicated in potentiating the progression of early tumorigenic lesions in $Ptch1^{+/-}$ mice into advanced MB (Mille et al., 2014). Since Boc is a transcriptional target of Gli1, this early progression to advanced MB seems to rely on a positive feedback loop in which Shh stimulated *Boc* expression further potentiates Shh signaling, proliferation, and DNA damage for MB progression. The functions of Ptch1, Smo, and Boc require signaling through the primary cilium both during normal development and in tumors.

Negative regulators: Sufu and PKA

As described above, Sufu opposes Hh-dependent gene transcription by sequestering Gli proteins in the cytoplasm. Therefore, loss of Sufu function is expected to drive tumorigenesis. Interestingly, targeted *Sufu* inactivation in mouse skin results in G2/M cell cycle arrest and little or no formation of BCCs (Li et al., 2014). Moreover, $Sufu^{+/-}$ mice are not tumor prone, but $Sufu^{+/-}p53^{-/-}$ mice develop MB with *Sufu* loss of heterozygosity (Lee et al., 2007). Although these murine models suggest that *Sufu* loss alone is not sufficient for tumorigenesis, germline *SUFU* mutations in both BCC and MB patients strongly indicate that it is indeed an authentic tumor suppressor gene (Kool et al., 2014; Smith et al., 2014). In BCC, *SUFU* mutations are rare (<10% somatic) (Bonilla et al., 2016; Sekulic and Von Hoff, 2016). In SHH-MB, *SUFU* mutations occur (~14%), with the majority of these found in infants (0-3 years old) (Kool et al., 2014).

Reduced activity of the master negative regulator PKA also drives oncogenic Hh signaling. This has been well demonstrated by recent studies on the tumor suppressor *GNAS*, which encodes the G protein Gαs that can promote cAMP-dependent PKA activity. *Gnas* loss in murine cerebellar or brainstem progenitors induced formation of MBs with a SHH gene signature (He et al., 2014b). Similarly, epidermal deletion of *Gnas* induced rapid formation of BCC-like lesions (Iglesias-Bartolome et al., 2015). While both studies attribute oncogenesis to reduced PKA activity, other downstream effects of Gαs loss such as YAP1 activation (Iglesias-Bartolome et al., 2015) or PKA-independent effects on Hh signaling (He et al., 2014b) may contribute to tumor formation. Importantly, *GNAS* mutations have been reported in human MB (Huh et al., 2014; Kool et al., 2014) and low expression of *GNAS* defines a particularly aggressive subset of SHH-MB (He et al., 2014b).

Nuclear regulators: Transcription factors and epigenetic modifiers

We discussed above the upstream signaling mechanisms that modulate the activity of Gli transcription factors to affect Hh pathway output. Aberrant activation of Gli-mediated transcription can bypass such tightly-regulated control and promote Hh-driven tumor growth independent of upstream modulators and the primary cilium. For instance, expression and activation of the previously mentioned positive Gli1 regulator, aPKC-ι/λ, is upregulated in mouse and human BCCs and is necessary for BCC cell growth (Atwood et al., 2013). Moreover, increased activity of aPKC-ι/λ may be a mode of resistance to upstream targeting

with Smo inhibitors. Amplification of the MYCN transcription factor, itself a target of Hh signaling, is also implicated in driving MB formation and mediating Smo inhibitor resistance (Hatton et al., 2006; Kenney et al., 2003; Kool et al., 2014). Similarly, amplification of *GLI2* can drive oncogenesis and resistance. About 8% of BCC and SHH-MB have *GLI2* amplification (Bonilla et al., 2016; Kool et al., 2014). In MB, *GLI2* amplifications were identified in children ages 4-17 and predominantly co-occured with *TP53* mutations.

TP53 is one of the most frequently mutated genes in cancer overall and is often mutated in BCC and SHH-MB. This gene codes for the p53 protein, which is important for maintaining genomic stability both via its roles as a transcription factor and via nuclear-independent roles. Between 30-70% of BCCs (Bonilla et al., 2016; Jayaraman et al., 2014) and 10-20% of SHH-MB (Kool et al., 2014; Zhukova et al., 2013) are reported to have mutations in *TP53*. SHH-MB patients with mutant *TP53* are considered at increased risk for adverse outcome, with five-year survival reduced by almost half for these patients (Zhukova et al., 2013). Also, *TP53*-mutated MBs have a high overall mutation rate and some undergo chromothrypsis, massive chromosomal rearrangements that are typically associated with poor survival in multiple cancers (Rausch et al., 2012).

Besides genetic alterations, epigenetic mechanisms also contribute to Hh signaling and MB. For instance, Shh ligand can induce an epigenetic cofactor switch at target genes (Shi et al., 2014). Before induction, poised target genes are marked by an active H3K4me3 mark and a repressive H3K27me3 mark that is maintained by the H3K27 methyltransferase polycomb repressive complex 2 (PRC2). Shh activation recruits the Jmjd3 complex, which displaces PRC2, removes H3K27me3, and enlists the Set1/MLL H3K4 methyltransferase complex to activate gene expression. Importantly, Jmjd3 promotes Shh-dependent cerebellar precursor proliferation and is required for Shh-subtype MB cell growth. Beyond this specific example, genomic data indicates a high prevalence of somatic alterations for chromatin-modifying genes in MB (Batora et al., 2014). Thus, broad interrogation of the MB epigenetic landscape, such as recently reported by Lin et al., may reveal additional epigenomic contributions to the underlying tumor biology (Lin et al., 2016).

Targeting Hh activation in tumors

Hh signaling can be targeted at many levels, from blocking Hh ligands with antibodies to chemically inhibiting Gli function (Figure 2) (Amakye et al., 2013). The largest and most clinically advanced group of Hh signaling inhibitors antagonizes the functions of Smo. Cyclopamine and jervine were the first identified Smo inhibitors. These compounds were initially isolated from corn lilies and verified as the teratogens responsible for various birth defects in the offspring of sheep who ingested the poisonous plants (reviewed in Lee et al., 2014b). Some Smo inhibitors are derivatives of cyclopamine with improved pharmacologic properties. High throughput, *in vitro* screening has led to the identification of other, more potent Smo inhibitors like vismodegib (see Mas and Ruiz i Altaba, 2010; Scales and de Sauvage, 2009). In 2012, vismodegib was the first-in-class US Food and Drug Administration (FDA)-approved Smo inhibitor for the treatment of locally advanced, unresectable, and metastatic BCC. In 2015, sonidegib was also approved for locally

advanced BCC. These and other Smo inhibitors are currently in clinical trials for other cancers, including MB.

Clinical studies with Smo inhibitors

Phase I clinical trials with vismodegib for advanced solid tumors, including BCC and MB, offered initial indications that Smo inhibition was tolerable and in some cases effective for Hh-dependent tumors. These reports led to the ERIVANCE BCC phase II trial that secured vismodegib FDA approval (Sekulic et al., 2012). A 12-month follow-up to the primary ERIVANCE analysis was published last year, confirming efficacy and safety for vismodegib, with demonstrated durability of response (Sekulic et al., 2015). Also published last year was an interim analysis of the STEVIE trial, the largest vismodegib-treated patient series with advanced BCC reported to date (Basset-Seguin et al., 2015). Based on the collective data, overall vismodegib response rates for locally advanced and metastatic BCC are ~45-70% and ~30-40%, respectively. Common adverse events include muscle spasms, alopecia, and taste disturbance. These symptoms are consistent with the expected consequences of reducing Hh signaling in tissues that require this pathway in adulthood. Less frequent, but more serious adverse events and treatment discontinuation due to adverse events are also reported for vismodegib. Furthermore, efficacy can be transient due to outgrowths of resistant tumors. Clinical trials with other Smo inhibitors, including sonidegib and IPI-926, consistently cite similar anti-tumor activity and toxicity profiles (Jimeno et al., 2013; Migden et al., 2015; Rodon et al., 2014). Preclinical models have also revealed developmental bone and dental toxicities after Smo inhibition and continued monitoring of pediatric patients for such toxicities will be needed (Kimura et al., 2008; Robinson et al., 2015).

Clinical trials in SHH-MB also indicate varied rates and durations of responses. A Phase I study of vismodegib in children with recurrent or refractory MB demonstrated complete but transient response in 1 out of 3 SHH-MB patients and no responses in any of the 13 non-SHH patients (Gajjar et al., 2013). In follow-up Phase II trials, 43 adult and pediatric patients with recurrent MB from all four subgroups were treated with vismodegib (Robinson et al., 2015). Decreased tumor size was observed only in SHH-MB patients, and progression-free survival was longer in patients with SHH-MB versus those with non-SHH-MB. Unfortunately, all responses in SHH-MB patients were transient (~3-16 months before disease progression). Analysis of samples either pre- or post-treatment identified some likely causes of the observed resistance, including 3 patients with *SUFU* mutations and 3 patients with *GLI2* amplifications.

Mechanisms of clinical resistance to Smo inhibitors

The first functionally characterized case of clinically acquired resistance to Smo inhibition was reported in a patient with widespread, metastatic MB who initially showed dramatic tumor regression with vismodegib (Rudin et al., 2009). The patient rapidly relapsed due to a *SMO* mutation that abrogated drug binding (Yauch et al., 2009). Beyond this case and the clinical trials data summarized above, there is limited information on resistance in MB due to the low numbers of patients treated with Smo inhibitors. Although advanced BCC is also rare, there is relatively more information on resistance for these patients.

Recently, two studies reported extensive genomic and functional analyses that reveal key concepts of Smo inhibitor resistance in BCC (Atwood et al., 2015; Sharpe et al., 2015). First, reactivation of Shh signaling is the predominant form of resistance, solidifying BCC addiction to the Hh pathway. This observation contrasts with other cancers that can hijack oncogenic signaling from multiple pathways. Second, the most common resistance mutations in BCCs involve the drug target Smo (~50-69% of resistant BCCs). Third, there are two classes of *SMO* mutations—those in proximity to the SMO drug binding pocket and those located distally. Finally, some resistant tumors harbor mutations in other Hh pathway genes, including regulatory units of the cAMP/PKA signaling axis, *GLI2* amplification, and homozygous *SUFU* mutations.

Overall, studies to date indicate that Hh signaling activation downstream of Smo inhibition is a common mechanism of resistance in both BCC and SHH-MB. This may signify a preferred dependence on the Hh pathway in these tumors and/or a limited range of mechanisms that can confer survival advantage under the selective pressure of Smo antagonists. This hypothesis could also explain the observed prevalence of *SMO* mutations in resistant BCCs. Similarly, mutations in the drug target are frequently observed as a major resistance mechanism in other targeted cancer therapies (Pagliarini et al., 2015). Moreover, Atwood et al. suggest that the overall proportion of resistant *SMO* mutations in BCCs is high because only one copy of *SMO* is required to elicit pathway activity (Atwood et al., 2015).

Since BCC is a relatively slow-growing cancer and is also one of the most mutated human cancers (Atwood et al., 2015; Bonilla et al., 2016), more time and larger patient populations may reveal novel insights on tumor biology and drug resistance. For example, a recent report on a large cohort of BCCs identified novel candidate tumor drivers beyond Hh pathway genes, which may explain some of the clinically different responses to Smo inhibition (Bonilla et al., 2016). Likewise, transcriptome analyses and whole genome sequencing have shown extensive heterogeneity within SHH-MB (Kool et al., 2014; Northcott et al., 2011). Identifying and classifying this heterogeneity may prove useful for predicting and understanding resistance.

Preclinical studies on resistance to Smo inhibitors

Clinical efficacy is the ultimate test for Smo inhibitors. Nonetheless, preclinical models are valuable for predicting the tumor-specific limitations of these inhibitors and possible mechanisms of therapeutic resistance. Early preclinical studies confirmed *Smo* mutations and *Gli2* amplifications in murine models of MB resistance (Buonamici et al., 2010). They also indicated that PI3K/AKT signaling, which regulates processes like cell growth and proliferation, is associated with resistance. Combined treatment with a PI3K inhibitor and a Smo inhibitor delayed or prevented the emergence of resistance *in vivo*. Furthermore, genomic loss of *Pten*, a negative regulator in PI3K/AKT signaling, can contribute to vismodegib resistance in Hh-driven models of MB (Metcalfe et al., 2013). Recently, studies with murine tumors and patient-derived xenografts suggested that elevated PI3K signaling may help support treatment-resistant CD15+ cancer stem cells residing as a subpopulation within SHH-MBs (Singh et al., 2016). Therefore, PI3K/AKT signaling could compromise

treatment with Smo inhibition. Importantly, these studies have clinical relevance since patients with SHH-MB harbor recurrent mutations in *PTEN*, *PIK3CA*, and *PIK3C2G* and analysis of SHH-MB tumors shows functional activation of PI3K signaling in >10% of samples (n=155) (Kool et al., 2014). Thus, combination or sequential treatment with Hh and PI3K inhibitors may improve prognosis for some SHH-MB patients.

We recently demonstrated that activation of the RAS/MAPK pathway can also cause resistance to Smo inhibitors by suppressing Shh signaling and shifting tumor cell dependence to Ras signaling (Zhao et al., 2015). RAS/MAPK-mediated resistance was characterized by increased metastatic potential of tumor cells. Notably, RAS/MAPK activation in a metastatic MB lesion and in three resistant BCC tumors provides clinical relevance for these findings.

Another type of resistance reported in SHH-MB is mediated by rare, quiescent $Sox2^+$ cells that have the potential to re-populate the tumor via self-renewal and differentiation (Vanner et al., 2014). Expression of the Sox2 transcription factor has previously been associated with SHH-MB and shown to drive tumor cell proliferation (Ahlfeld et al., 2013). $Sox2^+$ cell populations can be enriched following treatment with either Smo inhibitors or other anti-proliferative agents.

Targeting Hh signaling beyond Smo inhibition

Since resistance to Smo inhibitors can occur, other options for treating Hh-dependent tumors are needed. Two FDA-approved drugs have been identified as inhibitors of the Shh pathway: the antifungal agent itraconazole and arsenic trioxide (ATO), which is used for treatment of acute promyelocytic leukemia (Kim et al., 2010b; Kim et al., 2010a). Itraconazole is thought to inhibit Smo at a site distinct from the critical site bound by cyclopamine, vismodegib, or sonidegib. ATO inhibits Gli2 ciliary accumulation and promotes its degradation. Either single or combination treatment with itraconazole and ATO inhibits Hh signaling and tumor growth in sensitive and resistant tumors (Kim et al., 2013). However, for some *SMO* mutant tumors, these drugs showed only partial inhibition or eventual tumor regrowth. Both itraconazole and ATO have entered clinical trials for BCC.

Recently, inhibition of BET bromodomain proteins (BRD2-BRD4 and BRDT) was evaluated for Shh-dependent tumors (Long et al., 2014; Tang et al., 2014). BET proteins facilitate gene transcription by binding to specific chromatin sites and interacting with transcription elongation factors and RNA polymerase II. Tang et al. showed that Brd4 occupancy at Gli promoters was increased with Hh pathway activation and blocked by the BET protein inhibitor JQ1 (Tang et al., 2014). Either *Brd4* knock-down or addition of JQ1 reduced cell viability, proliferation, and Hh activity in patient-derived tumor cells that were resistant to Smo inhibitors. JQ1 also reduced tumor growth and increased animal survival in MB and BCC tumor allograft models. Additional studies found that JQ1 was effective at reducing cell proliferation and tumor growth of MYC-amplified MBs that were not of the SHH subgroup (Bandopadhayay et al., 2014). Thus, BET inhibitors may be applicable for targeting several oncogenic pathways in MB tumors.

Several other inhibitors that work downstream of Smo offer alternatives for resistant tumors. Small molecules that block Gli function either directly or by blocking Gli interaction with co-activators have been proposed (Bosco-Clement et al., 2014; Lauth et al., 2007). As previously discussed, aPKC-ι/λ promotes BCC growth and may mediate Smo inhibitor resistance. Importantly, preclinical data indicates that treatment with a myristoylated aPKC peptide inhibitor (PSI) is effective against sensitive and resistant BCCs (Atwood et al., 2013). Targeting Hh signaling at the cAMP-dependent PKA node is another strategy. For instance, phosphodiesterase inhibitors may block cAMP degradation and thereby increase activity of the negative Hh pathway regulator PKA. *In vivo* phosphodiesterase inhibition was shown to oppose the growth of Smo inhibitor resistant MB (Ge et al., 2015) as well as Shh-MB driven by loss of *Gnas* (He et al., 2014b). In considering these alternatives, demonstration of target specificity and/or specificity for Shh-dependent tumors will be important.

Conclusions

Here, we reviewed the framework of Hh signal transduction. Recent work has elucidated many molecular mechanisms of pathway modulation: ligand-induced Ptch1 removal from the cell surface, lipid effects on Smo activity, Kif7 phosphorylation and contributions to cilium assembly, regulation of the cAMP/PKA node, and protein localization and trafficking in the primary cilium. We also reviewed components of the pathway that are implicated in driving Hh-dependent tumors, specifically BCC and MB.

Profiling of human tumors has helped efforts to understand the roles of Hh signaling in oncogenesis. Importantly, such work has indicated that there is vast heterogeneity among tumor drivers and behavior in SHH-MB. For instance, as discussed, age correlates with certain types of mutations: young children have *SUFU* mutations while adults harbor *SMO* mutations. This may in part reflect germline mutations in *SUFU* that confer a hereditary predisposition for MBs to present early in life. Other factors that may contribute to age-associated tumor heterogeneity include differences in cells of origin and the contribution of the microenvironment at different developmental stages. Dependence on other pathways and co-existing mutations in other genes like *TP53* also contribute to tumor differences across and within the same age groups.

Entry of Smo inhibitors into the clinic has also enriched our understanding of Hh signaling in cancer. Smo inhibitors constitute the first type of targeted therapy for Hh-dependent tumors and can elicit robust responses in some tumors. However reports of adverse events also suggest that treatment with Smo inhibitors is not a sustainable, long-term solution for tumor management. This reasoning is further emphasized by evidence for both primary and acquired resistance. Reactivation of Hh signaling either by mutation of the drug target Smo or downstream pathway activation is the most common form of resistance. Thus, downstream inhibitors of Hh signaling are being explored. Preclinical models have also implicated other mechanisms of resistance, including PI3K/AKT and RAS/MAPK signaling, and thus encourage evaluation of combination therapies targeting multiple pathways. Given the heterogeneity of MB and high mutation rate of BCCs, in time we may discover that other pathways contribute to Hh-driven oncogenesis and resistance. Additionally, a new

generation of Hh pathway inhibitors that builds on the lessons learned from Smo inhibitors and focuses on nuclear events in the pathway will likely provide further insights on Hh signaling regulation and enable new cures.

Acknowledgement

We thank members of the Segal lab for comments. We apologize to colleagues whose contributions to the field of Hh signaling were not cited due to space limitations. This work was supported by US NIH grants (CA142536 to R.A.S. and F31CA183145 to E.P.) and Alex's Lemonade Stand Foundation.

REFERENCES

Ahlfeld J, Favaro R, Pagella P, Kretzschmar HA, Nicolis S, Schuller U. Sox2 requirement in sonic hedgehog-associated medulloblastoma. Cancer Res. 2013; 73:3796–3807. [PubMed: 23596255]

Amakye D, Jagani Z, Dorsch M. Unraveling the therapeutic potential of the Hedgehog pathway in cancer. Nat Med. 2013; 19:1410–1422. [PubMed: 24202394]

Arensdorf AM, Marada S, Ogden SK. Smoothened Regulation: A Tale of Two Signals. Trends Pharmacol Sci. 2016; 37:62–72. [PubMed: 26432668]

Atwood SX, Li M, Lee A, Tang JY, Oro AE. GLI activation by atypical protein kinase C iota/lambda regulates the growth of basal cell carcinomas. Nature. 2013; 494:484–488. [PubMed: 23446420]

Atwood SX, Sarin KY, Whitson RJ, Li JR, Kim G, Rezaee M, Ally MS, Kim J, Yao C, Chang AL, et al. Smoothened variants explain the majority of drug resistance in basal cell carcinoma. Cancer Cell. 2015; 27:342–353. [PubMed: 25759020]

Bandopadhayay P, Bergthold G, Nguyen B, Schubert S, Gholamin S, Tang Y, Bolin S, Schumacher SE, Zeid R, Masoud S, et al. BET bromodomain inhibition of MYC-amplified medulloblastoma. Clin Cancer Res. 2014; 20:912–925. [PubMed: 24297863]

Barakat MT, Humke EW, Scott MP. Learning from Jekyll to control Hyde: Hedgehog signaling in development and cancer. Trends in Molecular Medicine. 2010; 16:337–348. [PubMed: 20696410]

Basset-Seguin N, Hauschild A, Grob J-J, Kunstfeld R, Dréno B, Mortier L, Ascierto PA, Licitra L, Dutriaux C, Thomas L, et al. Vismodegib in patients with advanced basal cell carcinoma (STEVIE): a pre-planned interim analysis of an international, open-label trial. The Lancet Oncology. 2015; 16:729–736. [PubMed: 25981813]

Batora NV, Sturm D, Jones DT, Kool M, Pfister SM, Northcott PA. Transitioning from genotypes to epigenotypes: why the time has come for medulloblastoma epigenomics. Neuroscience. 2014; 264:171–185. [PubMed: 23876321]

Beachy PA, Hymowitz SG, Lazarus RA, Leahy DJ, Siebold C. Interactions between Hedgehog proteins and their binding partners come into view. Genes Dev. 2010; 24:2001–2012. [PubMed: 20844013]

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. [PubMed: 16895439]

Bonilla X, Parmentier L, King B, Bezrukov F, Kaya G, Zoete V, Seplyarskiy VB, Sharpe HJ, McKee T, Letourneau A, et al. Genomic analysis identifies new drivers and progression pathways in skin basal cell carcinoma. Nat Genet. 2016

Bosco-Clement G, Zhang F, Chen Z, Zhou HM, Li H, Mikami I, Hirata T, Yagui-Beltran A, Lui N, Do HT, et al. Targeting Gli transcription activation by small molecule suppresses tumor growth. Oncogene. 2014; 33:2087–2097. [PubMed: 23686308]

Buonamici S, Williams J, Morrissey M, Wang A, Guo R, Vattay A, Hsiao K, Yuan J, Green J, Ospina B, et al. Interfering with resistance to smoothened antagonists by inhibition of the PI3K pathway in medulloblastoma. Sci Transl Med. 2010; 2:51ra70.

Chavez M, Ena S, Van Sande J, de Kerchove d'Exaerde A, Schurmans S, Schiffmann SN. Modulation of Ciliary Phosphoinositide Content Regulates Trafficking and Sonic Hedgehog Signaling Output. Dev Cell. 2015; 34:338–350. [PubMed: 26190144]

Chen MH, Wilson CW, Li YJ, Law KK, Lu CS, Gacayan R, Zhang X, Hui CC, Chuang PT. Cilium-independent regulation of Gli protein function by Sufu in Hedgehog signaling is evolutionarily conserved. Genes Dev. 2009; 23:1910–1928. [PubMed: 19684112]

Chen Y, Jiang J. Decoding the phosphorylation code in Hedgehog signal transduction. Cell Res. 2013; 23:186–200. [PubMed: 23337587]

Chen Y, Sasai N, Ma G, Yue T, Jia J, Briscoe J, Jiang J. Sonic Hedgehog dependent phosphorylation by CK1alpha and GRK2 is required for ciliary accumulation and activation of smoothened. PLoS Biol. 2011a; 9:e1001083. [PubMed: 21695114]

Chen Y, Struhl G. Dual roles for patched in sequestering and transducing hedgehog. Cell. 1996; 87:553–563. [PubMed: 8898207]

Chen Y, Yue S, Xie L, Pu XH, Jin T, Cheng SY. Dual Phosphorylation of suppressor of fused (Sufu) by PKA and GSK3beta regulates its stability and localization in the primary cilium. J Biol Chem. 2011b; 286:13502–13511. [PubMed: 21317289]

Cheung HOL, Zhang X, Ribeiro A, Mo R, Makino S, Puviindran V, Law KKL, Briscoe J, Hui CC. The kinesin protein Kif7 is a critical regulator of gli transcription factors in mammalian hedgehog signaling. Sci Signal. 2009; 2:ra29. [PubMed: 19549984]

Chong YC, Mann RK, Zhao C, Kato M, Beachy PA. Bifurcating action of Smoothened in Hedgehog signaling is mediated by Dlg5. Genes Dev. 2015; 29:262–276. [PubMed: 25644602]

Di Magno L, Basile A, Coni S, Manni S, Sdruscia G, D'Amico D, Antonucci L, Infante P, De Smaele E, Cucchi D, et al. The energy sensor AMPK regulates Hedgehog signaling in human cells through a unique Gli1 metabolic checkpoint. Oncotarget. 2016; 5

Eisner A, Pazyra-Murphy MF, Durresi E, Zhou P, Zhao X, Chadwick EC, Xu PX, Hillman RT, Scott MP, Greenberg ME, et al. The Eya1 phosphatase promotes Shh signaling during hindbrain development and oncogenesis. Dev Cell. 2015; 33:22–35. [PubMed: 25816987]

Endoh-Yamagami S, Evangelista M, Wilson D, Wen X, Theunissen JW, Phamluong K, Davis M, Scales SJ, Solloway MJ, de Sauvage FJ, et al. The mammalian Cos2 homolog Kif7 plays an essential role in modulating Hh signal transduction during development. Curr Biol. 2009; 19:1320–1326. [PubMed: 19592253]

Franco I, Gulluni F, Campa CC, Costa C, Margaria JP, Ciraolo E, Martini M, Monteyne D, De Luca E, Germena G, et al. PI3K class II alpha controls spatially restricted endosomal PtdIns3P and Rab11 activation to promote primary cilium function. Dev Cell. 2014; 28:647–658. [PubMed: 24697898]

Gajjar A, Stewart CF, Ellison DW, Kaste S, Kun LE, Packer RJ, Goldman S, Chintagumpala M, Wallace D, Takebe N, et al. Phase I study of vismodegib in children with recurrent or refractory medulloblastoma: a pediatric brain tumor consortium study. Clin Cancer Res. 2013; 19:6305–6312. [PubMed: 24077351]

Garcia-Gonzalo FR, Phua SC, Roberson EC, Garcia G 3rd, Abedin M, Schurmans S, Inoue T, Reiter JF. Phosphoinositides Regulate Ciliary Protein Trafficking to Modulate Hedgehog Signaling. Dev Cell. 2015; 34:400–409. [PubMed: 26305592]

Ge X, Milenkovic L, Suyama K, Hartl T, Purzner T, Winans A, Meyer T, Scott MP. Phosphodiesterase 4D acts downstream of neuropilin to control hedgehog signal transduction and the growth of medulloblastoma. Elife. 2015; 4

Goetz SC, Anderson KV. The primary cilium: a signalling centre during vertebrate development. Nat Rev Genet. 2010; 11:331–344. [PubMed: 20395968]

Goodrich LV, Milenkovic L, Higgins KM, Scott MP. Altered neural cell fates and medulloblastoma in mouse patched mutants. Science. 1997; 277:1109–1113. [PubMed: 9262482]

Hahn H, Wicking C, Zaphiropoulous PG, Gailani MR, Shanley S, Chidambaram A, Vorechovsky I, Holmberg E, Unden AB, Gillies S, et al. Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. Cell. 1996; 85:841–851. [PubMed: 8681379]

Hatton BA, Knoepfler PS, Kenney AM, Rowitch DH, de Alboran IM, Olson JM, Eisenman RN. N-myc is an essential downstream effector of Shh signaling during both normal and neoplastic cerebellar growth. Cancer Res. 2006; 66:8655–8661. [PubMed: 16951180]

Hatton BA, Villavicencio EH, Tsuchiya KD, Pritchard JI, Ditzler S, Pullar B, Hansen S, Knoblaugh SE, Lee D, Eberhart CG, et al. The Smo/Smo model: hedgehog-induced medulloblastoma with

90% incidence and leptomeningeal spread. Cancer Res. 2008; 68:1768–1776. [PubMed: 18339857]

He M, Subramanian R, Bangs F, Omelchenko T, Liem KF Jr. Kapoor TM, Anderson KV. The kinesin-4 protein Kif7 regulates mammalian Hedgehog signalling by organizing the cilium tip compartment. Nat Cell Biol. 2014a; 16:663–672. [PubMed: 24952464]

He X, Zhang L, Chen Y, Remke M, Shih D, Lu F, Wang H, Deng Y, Yu Y, Xia Y, et al. The G protein alpha subunit Galphas is a tumor suppressor in Sonic hedgehog-driven medulloblastoma. Nat Med. 2014b; 20:1035–1042. [PubMed: 25150496]

Hillman RT, Feng BY, Ni J, Woo WM, Milenkovic L, Hayden Gephart MG, Teruel MN, Oro AE, Chen JK, Scott MP. Neuropilins are positive regulators of Hedgehog signal transduction. Genes Dev. 2011; 25:2333–2346. [PubMed: 22051878]

Huh JY, Kwon MJ, Seo KY, Kim MK, Chae KY, Kim SH, Ki CS, Yoon MS, Kim DH. Novel nonsense GNAS mutation in a 14-month-old boy with plate-like osteoma cutis and medulloblastoma. J Dermatol. 2014; 41:319–321. [PubMed: 24517547]

Hui CC, Angers S. Gli proteins in development and disease. Annu Rev Cell Dev Biol. 2011; 27:513–537. [PubMed: 21801010]

Humke EW, Dorn KV, Milenkovic L, Scott MP, Rohatgi R. The output of Hedgehog signaling is controlled by the dynamic association between Suppressor of Fused and the Gli proteins. Genes Dev. 2010; 24:670–682. [PubMed: 20360384]

Iglesias-Bartolome R, Torres D, Marone R, Feng X, Martin D, Simaan M, Chen M, Weinstein LS, Taylor SS, Molinolo AA, et al. Inactivation of a Galpha(s)-PKA tumour suppressor pathway in skin stem cells initiates basal-cell carcinogenesis. Nat Cell Biol. 2015; 17:793–803. [PubMed: 25961504]

Incardona JP, Lee JH, Robertson CP, Enga K, Kapur RP, Roelink H. Receptor-mediated endocytosis of soluble and membrane-tethered Sonic hedgehog by Patched-1. Proc Natl Acad Sci U S A. 2000; 97:12044–12049. [PubMed: 11027307]

Ingham PW, Mcmahon AP. Hedgehog signaling in animal development: paradigms and principles. Genes & Development. 2001; 15:3059–3087. [PubMed: 11731473]

Jayaraman SS, Rayhan DJ, Hazany S, Kolodney MS. Mutational landscape of basal cell carcinomas by whole-exome sequencing. J Invest Dermatol. 2014; 134:213–220. [PubMed: 23774526]

Jimeno A, Weiss GJ, Miller WH Jr. Gettinger S, Eigl BJ, Chang AL, Dunbar J, Devens S, Faia K, Skliris G, et al. Phase I study of the Hedgehog pathway inhibitor IPI-926 in adult patients with solid tumors. Clin Cancer Res. 2013; 19:2766–2774. [PubMed: 23575478]

Johnson RL, Rothman AL, Xie J, Goodrich LV, Bare JW, Bonifas JM, Quinn AG, Myers RM, Cox DR, Epstein EH Jr. et al. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. Science. 1996; 272:1668–1671. [PubMed: 8658145]

Kenney AM, Cole MD, Rowitch DH. Nmyc upregulation by sonic hedgehog signaling promotes proliferation in developing cerebellar granule neuron precursors. Development. 2003; 130:15–28. [PubMed: 12441288]

Khaliullina H, Bilgin M, Sampaio JL, Shevchenko A, Eaton S. Endocannabinoids are conserved inhibitors of the Hedgehog pathway. Proc Natl Acad Sci U S A. 2015; 112:3415–3420. [PubMed: 25733905]

Kim J, Aftab BT, Tang JY, Kim D, Lee AH, Rezaee M, Kim J, Chen B, King EM, Borodovsky A, et al. Itraconazole and arsenic trioxide inhibit Hedgehog pathway activation and tumor growth associated with acquired resistance to smoothened antagonists. Cancer Cell. 2013; 23:23–34. [PubMed: 23291299]

Kim J, Lee JJ, Kim J, Gardner D, Beachy PA. Arsenic antagonizes the Hedgehog pathway by preventing ciliary accumulation and reducing stability of the Gli2 transcriptional effector. Proc Natl Acad Sci USA. 2010b; 107:13432–13437. [PubMed: 20624968]

Kim J, Tang JY, Gong R, Kim J, Lee JJ, Clemons KV, Chong CR, Chang KS, Fereshteh M, Gardner D, et al. Itraconazole, a commonly used antifungal that inhibits Hedgehog pathway activity and cancer growth. Cancer Cell. 2010a; 17:388–399. [PubMed: 20385363]

Kimura H, Ng JM, Curran T. Transient inhibition of the Hedgehog pathway in young mice causes permanent defects in bone structure. Cancer Cell. 2008; 13:249–260. [PubMed: 18328428]

Kool M, Jones DT, Jager N, Northcott PA, Pugh TJ, Hovestadt V, Piro RM, Esparza LA, Markant SL, Remke M, et al. Genome sequencing of SHH medulloblastoma predicts genotype-related response to smoothened inhibition. Cancer Cell. 2014; 25:393–405. [PubMed: 24651015]

Lauth M, Bergstrom A, Shimokawa T, Toftgard R. Inhibition of GLI-mediated transcription and tumor cell growth by small-molecule antagonists. Proc Natl Acad Sci U S A. 2007; 104:8455–8460. [PubMed: 17494766]

Lee JJ, Perera RM, Wang H, Wu DC, Liu XS, Han S, Fitamant J, Jones PD, Ghanta KS, Kawano S, et al. Stromal response to Hedgehog signaling restrains pancreatic cancer progression. Proc Natl Acad Sci U S A. 2014a; 111:E3091–E3100. [PubMed: 25024225]

Lee ST, Welch KD, Panter KE, Gardner DR, Garrossian M, Chang CW. Cyclopamine: from cyclops lambs to cancer treatment. J Agric Food Chem. 2014b; 62:7355–7362. [PubMed: 24754790]

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. [PubMed: 17452975]

Li YH, Luo J, Mosley YY, Hedrick VE, Paul LN, Chang J, Zhang G, Wang YK, Banko MR, Brunet A, et al. AMP-Activated Protein Kinase Directly Phosphorylates and Destabilizes Hedgehog Pathway Transcription Factor GLI1 in Medulloblastoma. Cell Rep. 2015; 12:599–609. [PubMed: 26190112]

Li ZJ, Mack SC, Mak TH, Angers S, Taylor MD, Hui CC. Evasion of p53 and G2/M checkpoints are characteristic of Hh-driven basal cell carcinoma. Oncogene. 2014; 33:2674–2680. [PubMed: 23752195]

Liem KF Jr. He M, Ocbina PJ, Anderson KV. Mouse Kif7/Costal2 is a cilia-associated protein that regulates Sonic hedgehog signaling. Proc Natl Acad Sci U S A. 2009; 106:13377–13382. [PubMed: 19666503]

Lin C, Yao E, Wang K, Nozawa Y, Shimizu H, Johnson JR, Chen JN, Krogan NJ, Chuang PT. Regulation of Sufu activity by p66beta and Mycbp provides new insight into vertebrate Hedgehog signaling. Genes Dev. 2014; 28:2547–2563. [PubMed: 25403183]

Lin CY, Erkek S, Tong Y, Yin L, Federation AJ, Zapatka M, Haldipur P, Kawauchi D, Risch T, Warnatz HJ, et al. Active medulloblastoma enhancers reveal subgroup-specific cellular origins. Nature. 2016; 530:57–62. [PubMed: 26814967]

Liu YC, Couzens AL, Deshwar AR, McBroom-Cerajewski LDB, Zhang X, Puviindran V, Scott IC, Gingras AC, Hui CC, Angers S. The PPFIA1-PP2A protein complex promotes trafficking of Kif7 to the ciliary tip and Hedgehog signaling. Sci Signal. 2014; 7

Long J, Li B, Rodriguez-Blanco J, Pastori C, Volmar CH, Wahlestedt C, Capobianco A, Bai F, Pei XH, Ayad NG, et al. The BET bromodomain inhibitor I-BET151 acts downstream of smoothened protein to abrogate the growth of hedgehog protein-driven cancers. J Biol Chem. 2014; 289:35494–35502. [PubMed: 25355313]

Marigo V, Davey RA, Zuo Y, Cunningham JM, Tabin CJ. Biochemical evidence that patched is the hedgehog receptor. Nature. 1996; 384:176–179. [PubMed: 8906794]

Mas C, Ruiz i Altaba A. Small molecule modulation of HH-GLI signaling: current leads, trials and tribulations. Biochem Pharmacol. 2010; 80:712–723. [PubMed: 20412786]

Mathew E, Zhang Y, Holtz AM, Kane KT, Song JY, Allen BL, Pasca di Magliano M. Dosage-dependent regulation of pancreatic cancer growth and angiogenesis by hedgehog signaling. Cell Rep. 2014; 9:484–494. [PubMed: 25310976]

Metcalfe C, Alicke B, Crow A, Lamoureux M, Dijkgraaf GJ, Peale F, Gould SE, de Sauvage FJ. PTEN loss mitigates the response of medulloblastoma to Hedgehog pathway inhibition. Cancer Res. 2013; 73:7034–7042. [PubMed: 24154871]

Migden MR, Guminski A, Gutzmer R, Dirix L, Lewis KD, Combemale P, Herd RM, Kudchadkar R, Trefzer U, Gogov S, et al. Treatment with two different doses of sonidegib in patients with locally advanced or metastatic basal cell carcinoma (BOLT): a multicentre, randomised, double-blind phase 2 trial. The Lancet Oncology. 2015; 16:716–728. [PubMed: 25981810]

Mille F, Tamayo-Orrego L, Levesque M, Remke M, Korshunov A, Cardin J, Bouchard N, Izzi L, Kool M, Northcott PA, et al. The Shh receptor Boc promotes progression of early medulloblastoma to advanced tumors. Dev Cell. 2014; 31:34–47. [PubMed: 25263791]

Mukhopadhyay S, Wen X, Ratti N, Loktev A, Rangell L, Scales SJ, Jackson PK. The ciliary G-protein-coupled receptor Gpr161 negatively regulates the Sonic hedgehog pathway via cAMP signaling. Cell. 2013; 152:210–223. [PubMed: 23332756]

Murone M, Rosenthal A, de Sauvage FJ. Sonic hedgehog signaling by the patched–smoothened receptor complex. Curr Biol. 1999; 9:76–84. [PubMed: 10021362]

Myers BR, Sever N, Chong YC, Kim J, Belani JD, Rychnovsky S, Bazan JF, Beachy PA. Hedgehog pathway modulation by multiple lipid binding sites on the smoothened effector of signal response. Dev Cell. 2013; 26:346–357. [PubMed: 23954590]

Nachtergaele S, Whalen DM, Mydock LK, Zhao Z, Malinauskas T, Krishnan K, Ingham PW, Covey DF, Siebold C, Rohatgi R. Structure and function of the Smoothened extracellular domain in vertebrate Hedgehog signaling. Elife. 2013; 2:e01340. [PubMed: 24171105]

Nedelcu D, Liu J, Xu Y, Jao C, Salic A. Oxysterol binding to the extracellular domain of Smoothened in Hedgehog signaling. Nat Chem Biol. 2013; 9:557–564. [PubMed: 23831757]

Niewiadomski P, Kong JH, Ahrends R, Ma Y, Humke EW, Khan S, Teruel MN, Novitch BG, Rohatgi R. Gli protein activity is controlled by multisite phosphorylation in vertebrate Hedgehog signaling. Cell Rep. 2014; 6:168–181. [PubMed: 24373970]

Nitzki F, Becker M, Frommhold A, Schulz-Schaeffer W, Hahn H. Patched knockout mouse models of Basal cell carcinoma. J Skin Cancer. 2012; 2012:907543. [PubMed: 23024864]

Northcott PA, Hielscher T, Dubuc A, Mack S, Shih D, Remke M, Al-Halabi H, Albrecht S, Jabado N, Eberhart CG, et al. Pediatric and adult sonic hedgehog medulloblastomas are clinically and molecularly distinct. Acta Neuropathol. 2011; 122:231–240. [PubMed: 21681522]

Nozawa YI, Lin C, Chuang PT. Hedgehog signaling from the primary cilium to the nucleus: an emerging picture of ciliary localization, trafficking and transduction. Curr Opin Genet Dev. 2013; 23:429–437. [PubMed: 23725801]

Pagliarini R, Shao W, Sellers WR. Oncogene addiction: pathways of therapeutic response, resistance, and road maps toward a cure. EMBO Rep. 2015; 16:280–296. [PubMed: 25680965]

Pal K, Hwang SH, Somatilaka B, Badgandi H, Jackson PK, DeFea K, Mukhopadhyay S. Smoothened determines beta-arrestin-mediated removal of the G protein-coupled receptor Gpr161 from the primary cilium. J Cell Biol. 2016

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. [PubMed: 16611981]

Pan Y, Wang B. A novel protein-processing domain in Gli2 and Gli3 differentially blocks complete protein degradation by the proteasome. J Biol Chem. 2007; 282:10846–10852. [PubMed: 17283082]

Pan Y, Wang C, Wang B. Phosphorylation of Gli2 by protein kinase A is required for Gli2 processing and degradation and the Sonic Hedgehog-regulated mouse development. Dev Biol. 2009; 326:177–189. [PubMed: 19056373]

Pearse RV II, Collier LS, Scott MP, Tabin CJ. Vertebrate homologs of Drosophila suppressor of fused Interact with the gli family of transcriptional regulators. Dev Biol. 1999; 212:323–336. [PubMed: 10433824]

Pusapati GV, Hughes CE, Dorn KV, Zhang D, Sugianto P, Aravind L, Rohatgi R. EFCAB7 and IQCE regulate hedgehog signaling by tethering the EVC-EVC2 complex to the base of primary cilia. Dev Cell. 2014; 28:483–496. [PubMed: 24582806]

Rausch T, Jones DT, Zapatka M, Stutz AM, Zichner T, Weischenfeldt J, Jager N, Remke M, Shih D, Northcott PA, et al. Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. Cell. 2012; 148:59–71. [PubMed: 22265402]

Robinson GW, Orr BA, Wu G, Gururangan S, Lin T, Qaddoumi I, Packer RJ, Goldman S, Prados MD, Desjardins A, et al. Vismodegib Exerts Targeted Efficacy Against Recurrent Sonic Hedgehog-Subgroup Medulloblastoma: Results From Phase II Pediatric Brain Tumor Consortium Studies PBTC-025B and PBTC-032. J Clin Oncol. 2015; 33:2646–2654. [PubMed: 26169613]

Rodon J, Tawbi HA, Thomas AL, Stoller RG, Turtschi CP, Baselga J, Sarantopoulos J, Mahalingam D, Shou Y, Moles MA, et al. A phase I, multicenter, open-label, first-in-human, dose-escalation study

of the oral smoothened inhibitor Sonidegib (LDE225) in patients with advanced solid tumors. Clin Cancer Res. 2014; 20:1900–1909. [PubMed: 24523439]

Romer JT, Kimura H, Magdaleno S, Sasai K, Fuller C, Baines H, Connelly M, Stewart CF, Gould S, Rubin LL, et al. Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in Ptc1(+/−)p53(−/−) mice. Cancer Cell. 2004; 6:229–240. [PubMed: 15380514]

Rudin CM, Hann CL, Laterra J, Yauch RL, Callahan CA, Fu L, Holcomb T, Stinson J, Gould SE, Coleman B, et al. Treatment of Medulloblastoma with Hedgehog Pathway Inhibitor GDC-0449. N Engl J Med. 2009; 361:1173–1178. [PubMed: 19726761]

Scales SJ, de Sauvage FJ. Mechanisms of Hedgehog pathway activation in cancer and implications for therapy. Trends Pharmacol Sci. 2009; 30:303–312. [PubMed: 19443052]

Sekulic A, Migden MR, Lewis K, Hainsworth JD, Solomon JA, Yoo S, Arron ST, Friedlander PA, Marmur E, Rudin CM, et al. Pivotal ERIVANCE basal cell carcinoma (BCC) study: 12-month update of efficacy and safety of vismodegib in advanced BCC. J Am Acad Dermatol. 2015; 72:1021–1026. e1028. [PubMed: 25981002]

Sekulic A, Migden MR, Oro AE, Dirix L, Lewis KD, Hainsworth JD, Solomon JA, Yoo S, Arron ST, Friedlander PA, et al. Efficacy and Safety of Vismodegib in Advanced Basal-Cell Carcinoma. N Engl J Med. 2012; 366:2171–2179. [PubMed: 22670903]

Sekulic A, Von Hoff D. Hedgehog Pathway Inhibition. Cell. 2016; 164:831. [PubMed: 26919418]

Sharpe HJ, Pau G, Dijkgraaf GJ, Basset-Seguin N, Modrusan Z, Januario T, Tsui V, Durham AB, Dlugosz AA, Haverty PM, et al. Genomic analysis of smoothened inhibitor resistance in basal cell carcinoma. Cancer Cell. 2015; 27:327–341. [PubMed: 25759019]

Shi X, Zhang Z, Zhan X, Cao M, Satoh T, Akira S, Shpargel K, Magnuson T, Li Q, Wang R, et al. An epigenetic switch induced by Shh signalling regulates gene activation during development and medulloblastoma growth. Nat Commun. 2014; 5:5425. [PubMed: 25370275]

Singh AR, Joshi S, Zulcic M, Alcaraz M, Garlich JR, Morales GA, Cho YJ, Bao L, Levy ML, Newbury R, et al. PI-3K Inhibitors Preferentially Target CD15+ Cancer Stem Cell Population in SHH Driven Medulloblastoma. PLoS One. 2016; 11:e0150836. [PubMed: 26938241]

Smith MJ, Beetz C, Williams SG, Bhaskar SS, O'Sullivan J, Anderson B, Daly SB, Urquhart JE, Bholah Z, Oudit D, et al. Germline mutations in SUFU cause Gorlin syndrome-associated childhood medulloblastoma and redefine the risk associated with PTCH1 mutations. J Clin Oncol. 2014; 32:4155–4161. [PubMed: 25403219]

Stone DM, Hynes M, Armanini M, Swanson TA, Gu Q, L. J.R. Scott MP, Pennica D, Goddard A, Phillips H, et al. The tumour-suppressor gene patched encodes a candidate receptor for sonic hedgehog. Nature. 1996; 384:129–134. [PubMed: 8906787]

Stone DM, Murone M, Luoh S, Ye W, Armanini MP, Gurney A, Phillips H, Brush J, Goddard A, de Sauvage FJ, et al. Characterization of the human suppressor of fused, a negative regulator of the zinc-finger transcription factor Gli. J Cell Sci. 1999; 112:4437–4448. [PubMed: 10564661]

Taipale J, Cooper MK, Maiti T, Beachy PA. Patched acts catalytically to suppress the activity of Smoothened. Nature. 2002; 418:892–897. [PubMed: 12192414]

Tang Y, Gholamin S, Schubert S, Willardson MI, Lee A, Bandopadhayay P, Bergthold G, Masoud S, Nguyen B, Vue N, et al. Epigenetic targeting of Hedgehog pathway transcriptional output through BET bromodomain inhibition. Nat Med. 2014; 20:732–740. [PubMed: 24973920]

Tay SY, Ingham PW, Roy S. A homologue of the Drosophila kinesin-like protein Costal2 regulates Hedgehog signal transduction in the vertebrate embryo. Development. 2005; 132:625–634. [PubMed: 15647323]

Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, Eberhart CG, Parsons DW, Rutkowski S, Gajjar A, et al. Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol. 2012; 123:465–472. [PubMed: 22134537]

Teglund S, Toftgard R. Hedgehog beyond medulloblastoma and basal cell carcinoma. Biochim Biophys Acta. 2010; 1805:181–208. [PubMed: 20085802]

Tempé D, Casas M, Karaz S, Blanchet-Tournier MF, Concordet JP. Multisite protein kinase A and glycogen synthase kinase 3beta phosphorylation leads to Gli3 ubiquitination by SCFbetaTrCP. Mol Cell Biol. 2006; 26:4316–4326. [PubMed: 16705181]

Tian H, Callahan CA, DuPree KJ, Darbonne WC, Ahn CP, Scales SJ, de Sauvage FJ. Hedgehog signaling is restricted to the stromal compartment during pancreatic carcinogenesis. Proc Natl Acad Sci U S A. 2009; 106:4254–4259. [PubMed: 19246386]

Vanner RJ, Remke M, Gallo M, Selvadurai HJ, Coutinho F, Lee L, Kushida M, Head R, Morrissy S, Zhu X, et al. Quiescent sox2(+) cells drive hierarchical growth and relapse in sonic hedgehog subgroup medulloblastoma. Cancer Cell. 2014; 26:33–47. [PubMed: 24954133]

Wang B, Li Y. Evidence for the direct involvement of {beta}TrCP in Gli3 protein processing. Proc Natl Acad Sci U S A. 2006; 103:33–38. [PubMed: 16371461]

Wang C, Pan Y, Wang B. Suppressor of fused and Spop regulate the stability, processing and function of Gli2 and Gli3 full-length activators but not their repressors. Development. 2010; 137:2001–2009. [PubMed: 20463034]

Wechsler-Reya RJ, Scott MP. Control of neuronal precursor proliferation in the cerebellum by sonic hedgehog. Neuron. 1999; 22:103–114. [PubMed: 10027293]

Xie J, Murone M, Luoh SM, Ryan A, Gu Q, Zhang C, Bonifas JM, Lam CW, Hynes M, Goddard A, et al. Activating Smoothened mutations in sporadic basal-cell carcinoma. Nature. 1998; 391

Yauch RL, Dijkgraaf GJ, Alicke B, Januario T, Ahn CP, Holcomb T, Pujara K, Stinson J, Callahan CA, Tang T, et al. Smoothened mutation confers resistance to a Hedgehog pathway inhibitor in medulloblastoma. Science. 2009; 326:572–574. [PubMed: 19726788]

Yue S, Tang L, Tang Y, Tang Y, Shen Q, Ding J, Chen Y, Zhang Z, Yu T, Zhang YE, et al. Requirement of Smurf-mediated endocytosis of patched1 in sonic hedgehog signal reception. Elife. 2014; 3:e02555.

Zhao X, Ponomaryov T, Ornell KJ, Zhou P, Dabral SK, Pak E, Li W, Atwood SX, Whitson RJ, Chang AL, et al. RAS/MAPK Activation Drives Resistance to Smo Inhibition, Metastasis, and Tumor Evolution in Shh Pathway-Dependent Tumors. Cancer Res. 2015; 75:3623–3635. [PubMed: 26130651]

Zhukova N, Ramaswamy V, Remke M, Pfaff E, Shih DJ, Martin DC, Castelo-Branco P, Baskin B, Ray PN, Bouffet E, et al. Subgroup-specific prognostic implications of TP53 mutation in medulloblastoma. J Clin Oncol. 2013; 31:2927–2935. [PubMed: 23835706]

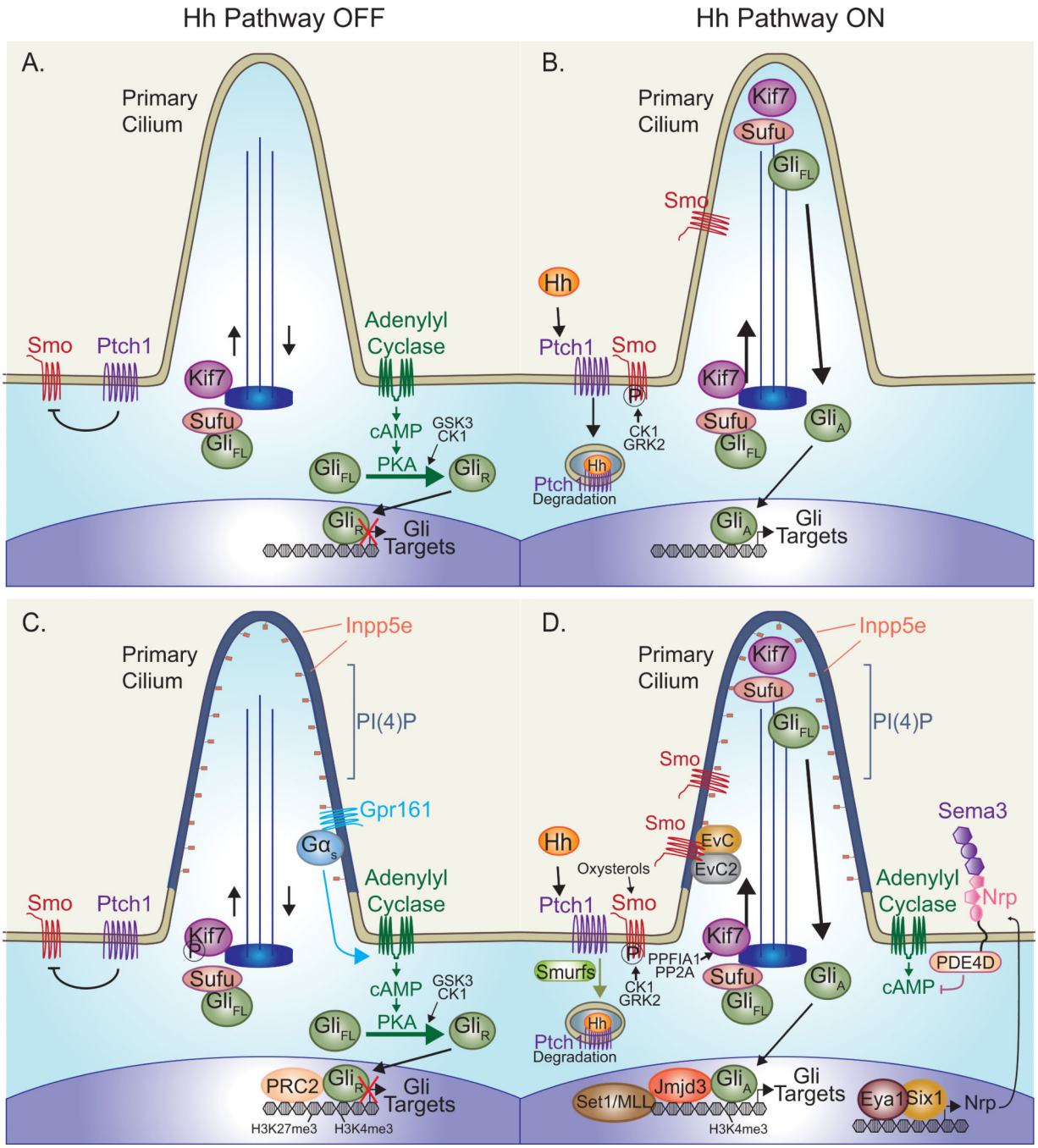
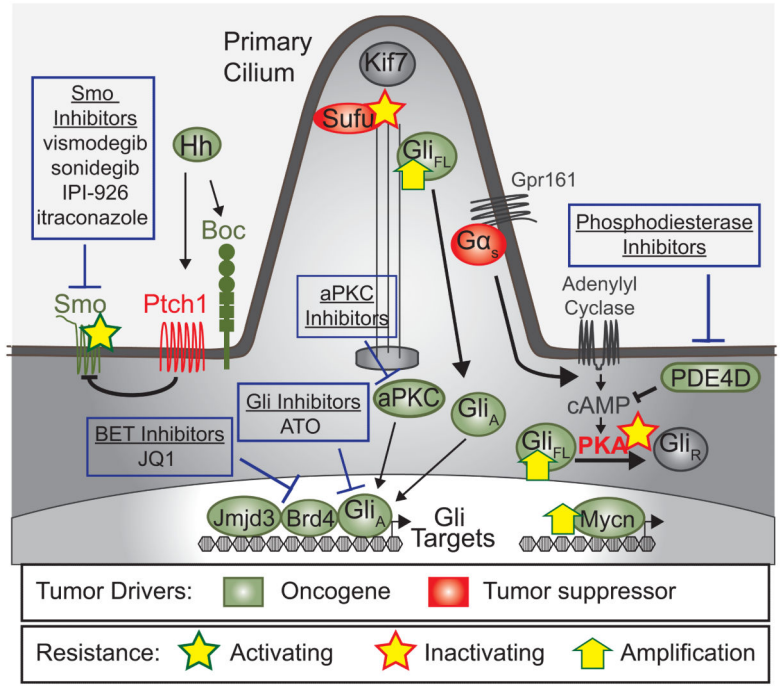


Figure 1. The evolving complexity of Hedgehog (Hh) signal transduction

A simplistic view of Hh signal transduction is portrayed in the upper panels. Recent updates on Hh signaling are depicted in the lower panels. **(A)** In the absence of Hh ligand, Ptch1 inhibits Smo activation and ciliary localization. Low levels of Kif7, Sufu, and full-length Gli (GliFL) enter the primary cilium (PC), which promotes GliFL processing into a repressor form (GliR) after phosphorylation by PKA, GSK3, and CK1. GliR blocks transcription of Hh target genes. **(B)** When Hh ligand binds Ptch1, both ligand and receptor are internalized and degraded. Smo is phosphorylated by CK1 and GRK2, assumes an active conformation,

and moves into the primary cilium (PC). Kif7, Sufu, and Gli also accumulate in the PC, where activated Smo promotes Sufu-Gli dissociation and activation of Gli (GliA). GliA shuttles into the nucleus and induces target gene transcription. **(C)** The PC-localized phosphatase, Inpp5e, maintains a PC lipid composition enriched with the phosphoinositide PI(4)P, which regulates ciliary localization of Hh pathway modulators such as the orphan GPCR, Gpr161. In the absence of Hh ligand, Gpr161 localizes to the PC and promotes production of cAMP, likely via Gαs-mediated activation of adenylyl cyclase, thereby repressing Hh signaling. In the nucleus, the PRC2 complex maintains repressive H3K27me3 to block target gene expression. **(D)** Hh ligand binding to Ptch1 promotes Smurf-mediated ubiquitination, endocytosis, and degradation of Ptch1. Smo becomes activated and its activity can be enhanced by lipid-based modulators, such as oxysterols, which bind to an extracellular domain in Smo. Activated Smo translocates to the PC and can localize at a specialized compartment called the EvC zone; from here Smo transmits signals to activate Gli. Hh stimulation also promotes the formation of a Kif7 complex with the scaffolding protein PPFIA1 and the phosphatase PP2A, resulting in Kif7 dephosphorylation and translocation to the tip of the PC. In the nucleus, Hh activation recruits Jmjd3, which activates target genes by displacing PRC2, enzymatically removing H3K27me3, and recruiting the Set1/MLL H3K4 methyltransferase complex. Also, active Hh signaling induces Eya1/Six1-mediated transcription of Nrp. The Nrp ligand Sema3 promotes recruitment of the phosphodiesterase PDE4D to the Nrp cytoplasmic domain, where it degrades cAMP and therefore reduces PKA levels.

A.



B.

	Preclinical	Clinical	References
Drivers of BCC or MB	Ptch1, Boc, Smo, Sufu, Gli2, Gnas, Mycn, Tp53, aPKC-ι/λ, Jmjd3, Brd4, phosphodiesterase	PTCH1, SMO, SUFU, GLI2, GNAS, MYCN, TP53	Goodrich et al., 1997; Xie et al., 1998; Atwood et al., 2013; Kool et al., 2014; He et al., 2014b; Shi et al., 2014; Tang et al., 2014; Bonilla et al., 2016
Response rates with Smo inhibitors	depends on genetic background and dose (up to 100% in some models)	30-70% in locally advanced and metastatic BCC; variable for MB	Romer et al., 2004; Basset-Seguin et al., 2015; Sekulic and Von Hoff, 2016
Toxicity with Smo inhibitors	weight loss, bone and dental toxicities	muscle spasms, alopecia, dysgeusia, weight loss, fatigue, nausea	Kimura et al., 2008; Sekulic et al., 2012, 2015; Basset-Seguin et al., 2015
Resistance mechanisms with Smo inhibitors	Smo GOF, Sufu LOF, Gli2 amplification, Mycn amplification, PI3K/AKT activation, RAS activation, high Sox2 expression	SMO GOF, SUFU LOF, Gli2 amplification, MYCN amplification	Yauch et al., 2009; Buonamici et al., 2010; Kool et al., 2014; Vanner et al., 2014; Sharpe et al., 2015; Atwood et al., 2015; Zhao et al., 2015
Drug targets	Smo, Gli, Brd4, phosphodiesterase, aPKC-ι/λ	Smo is the only Hh target with FDA approval for BCC	Sekulic et al., 2012; Atwood et al., 2013; Kim et al., 2013; He et al., 2014b Long et al., 2014; Tang et al., 2014

Figure 2. Drivers, drug targets, and resistance mechanisms in oncogenic Hh signaling

(A) Tumor suppressors (red) and oncogenes (green) that have been reported in preclinical and some clinical settings to cause or maintain basal cell carcinoma (BCC) and medulloblastoma (MB) are shown. Stars (activating or inactivating mutations) and arrows (genomic amplifications) indicate pathway components implicated in resistance to Smo inhibitors. Examples of Hh pathway-targeted therapies described in this review are indicated in white boxes. **(B)** Summary of preclinical and clinical evidence for Hh pathway oncogenic drivers in BCC and MB; Smo inhibitor efficacy, toxicity, and resistance; and alternate drug

targets under development. GOF, gain-of-function; LOF, loss-of-function. References are an abridged selection (see text for a more comprehensive list of references).