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REVIEW

Hedgehog-Gli signaling in basal cell carcinoma and other skin cancers: prospects for therapy

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Abstract: The Hedgehog (Hh) signaling pathway is of critical importance during embryonic development, where it directs a number of cellular processes, including cell proliferation, differentiation, and patterning. In normal adult tissues, Hh signaling is mostly involved in stem cell maintenance, tissue repair, and regeneration. Over the last two decades, aberrant activation of Hh signaling has been linked to several types of cancer, including those of the skin. In particular, the critical role of Hh signaling in the development of basal cell carcinoma has been demonstrated by several mouse models and genetic mutation analyses. In addition, several clinical trials using Hh signaling inhibitors have been shown to be effective treatments in basal cell carcinoma. Recent evidence indicates that activation of the Hh pathway plays an important role in other types of human skin cancer, such as melanoma and cutaneous squamous cell carcinoma. In this review, we provide an overview of the roles of Hh pathway in skin cancers, including basal cell carcinoma, melanoma, and squamous cell carcinoma. Finally, we discuss the rapid development of drugs that target the Hh pathway and the implications for skin cancer therapy.

Keywords: Hedgehog, Gli transcription factors, basal cell carcinoma, melanoma, squamous cell carcinoma, small-molecule inhibitors

Introduction

Major progress has been made in our understanding of how the Hedgehog (Hh) pathway operates since the discovery in 1980 of Hh as a segment polarity gene in *Drosophila*.[1] Several years passed before the finding that inactivation of the Sonic Hedgehog gene is responsible for the hereditary developmental disorder holoprosencephaly.[2,3] Since then, Hh signaling has been shown to play a critical role not only in embryonic development, but also in other normal processes, such as regulation of stem cells and maintenance of tissue homeostasis.[4] The initial association between the Hh pathway and human cancers was made as a result of the discovery that loss-of-function mutations in human *Ptch1* are associated with Gorlin syndrome, a rare and hereditary disorder. Patients with Gorlin syndrome show a broad spectrum of developmental defects and have a predisposition to develop basal cell carcinoma (BCC) and medulloblastoma.[5] During the past 15 years, numerous studies have revealed aberrant activation of Hh signaling not only in BCC, but also in medulloblastoma, glioblastoma, leukemia, and gastrointestinal, lung, ovarian, breast, liver, pancreatic, and prostate cancers.[6,7] Recent evidence indicates that activation of Hh signaling plays an important role not only in BCC but also in other types of skin cancer, such as melanoma and squamous cell carcinoma (SCC). Here, we focus on the role of Hh signaling in the development of skin cancer and review what has been learned from experimental mouse models

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carrying genetic modifications and from preclinical studies using specific Hh inhibitors. Lastly, we discuss the rapid development of drugs that target the Hh pathway and the implications for skin cancer therapy.

Hedgehog signaling in vertebrates

Hh signaling is highly conserved from *Drosophila* to humans,[8] although there are significant differences between vertebrates and invertebrates. Ptch and SMO proteins are conserved and maintain their function in mammals, whereas the Hh ligand has diversified into Sonic (Shh), Indian (Ihh), and Desert (Dhh) Hedgehog, and the function of the downstream transcription factor *Cubitus interruptus* (Ci) has multiplied into three Gli proteins (Gli1, Gli2, and Gli3). Figure 1 shows a simplified view of the Hh signaling pathway in vertebrates.

Signal transduction depends on the secretion of Hh ligands from the producing cell. Hh ligands are synthesized as precursors, which undergo autoproteolytic cleavage to form amino-terminal protein fragments. This cleavage is essential for the covalent attachment of a cholesterol molecule at the carboxyl terminus[9] and palmitic acid at the amino terminus[10] by Skinny, the Hh acyltransferase.[11] These lipid modifications are required for the correct movement and reception of the ligands.[12,13] Secretion of mature Hh ligands is mediated by Dispatched (Disp), a 12-transmembrane protein with structural homology to Ptch.[14,15] In addition to Disp, several other proteins are involved in this process, including the proteoglycans Dally and Dally-like,[16–18] Tout-velu, and Sulfateless.[19,20]

The signaling cascade of the Hh pathway is initiated by binding of Hh ligands to the 12-pass transmembrane protein receptor Ptch, which resides in the primary cilium, a non-motile structure that plays an important role in the transduction of Hh signaling.[21,22] Several factors are involved in the binding of Hh ligands to Ptch. The Hh-interacting protein (HHIP) can compete with Ptch for Hh binding, thus acting as a negative regulator of Hh signaling.[23] Conversely, Cdo, Boc, Gas1, and glypican-3 act as coreceptors of Hh.[24–29] Binding of Hh ligands to Ptch leads to relocalization of the ligand/receptor complex from the primary cilium to the endosomal vesicles and induces a conformational change of SMO.[21] Activated SMO then translocates into the cilium[30] and triggers a series of intracellular events that promote the formation of Gli activator forms (Gli-A). Gli2/3-A translocate into the nucleus and induce Hh target genes[31,32] (Figure 1A). In the absence of ligands, Ptch inhibits activation of the pathway

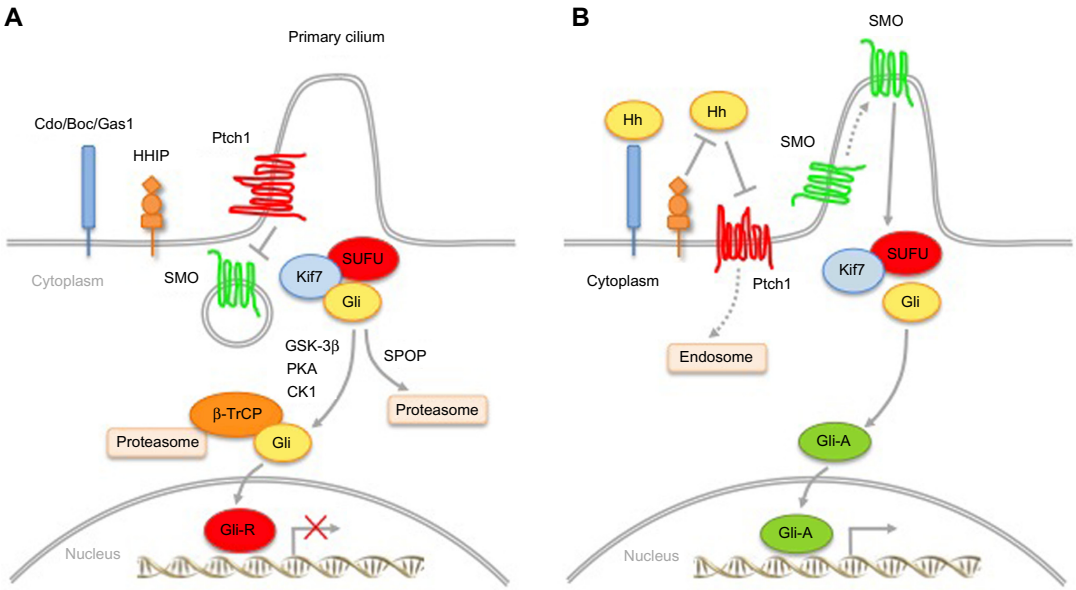

Figure 1 Simplified Hedgehog (Hh) signaling pathway in mammals.

Notes: In the absence of Hh ligands (**A**), Ptch1 receptor at the base of the primary cilium inhibits the function of SMO by preventing its entry into the cilium. Gli3 and, to a lesser extent Gli2, are converted to C-terminally truncated repressor forms (Gli-R) and translocate into the nucleus, where they inhibit the transcription of Hh target genes. Formation of Gli-R is promoted by sequential phosphorylation of full length Gli by a kinase cascade that includes PKA, GSK-3β, and CK1, which creates binding sites for the adapter protein β-TrCP. The Gli/β-TrCP complex is ubiquitinated by Cul1-based E3 ligase, which results in partial Gli degradation by the proteasome and formation of Gli-R. In addition to partial degradation, full-length Gli may be completely degraded by the proteasome through SPOP-mediated Cul3-based E3 ligase ubiquitination. Upon Hh ligand binding (**B**), Ptch is displaced from the cilium, becomes internalized in endosomes, and degraded. SMO relocates from intracellular vesicles to the cilium. Active SMO promotes a signaling cascade that leads to translocation of activated forms of Gli (Gli-A) into the nucleus, where they induce the transcription of Hh target genes, such as Gli1, Ptch1, and HHIP. HHIP competes with binding of the Hh ligands, while the GPI-linked Gas1 and the Ig/Fn repeat-containing surface proteins Cdo and Boc act as coreceptors of Hh.

Abbreviations: Boc, brother of Cdo; CK1, casein kinase 1; Gas1, growth arrest-specific protein 1; GSK-3β, glycogen synthase kinase 3β; Hh, Hedgehog; Kif7, kinesin family member 7; PKA, protein kinase A; Ptch, Patched; SMO, Smoothened; SPOP, speckle-type POZ protein; SUFU, Suppressor of Fused; β-TrCP, β-transducin repeat-containing protein; HHIP, Hh-interacting protein.

by preventing SMO from entering the cilium. This results in phosphorylation and proteasome-mediated carboxyl cleavage of Gli3 and, to a lesser extent, of Gli2 to repressor forms (Gli2/3-R);[33,34] Gli1 is transcriptionally repressed, with consequent silencing of the pathway (Figure 1).

The final effectors downstream of SMO in the mammalian Hh signaling pathway are the Gli transcription factors (Gli1, Gli2, Gli3), all members of the Kruppel family. They share five conserved C_2-H_2 zinc-finger DNA binding domains and a consensus histidine/cysteine linker sequence between zinc fingers. The Gli factors recognize the consensus sequence 5′-GACCACCCA-3′ in the promoter of their target genes,[35] although they can bind to variant Gli binding sites with lower affinity, still leading to strong transcriptional activation.[36]

Gli1 is a potent transcriptional activator and a direct target of Gli2.[37] Being directly regulated by Hh signaling, Gli1 represents the best read-out of Hh pathway activation.[38] Gli2 has a N-terminal repressor domain and a C-terminal activator domain. Gli2 can act as an activator or, in its C-terminal deleted form, as a repressor. Gli3 acts mostly as a repressor in its C-terminal cleaved form, although it can also have positive effects.[39] The activity of the Gli transcription factors is regulated by a number of post-translational modifications, including cytoplasmic-nuclear shuttling, phosphorylation, acetylation, ubiquitination, and protein degradation.

Suppressor of Fused (SUFU) is the main negative regulator of Hh signaling, and controls Gli nuclear localization and transcriptional activity;[40,41] in turn, Hh signaling regulates SUFU activity by inducing its turnover via the ubiquitin-proteasome system.[42] Protein kinase A (PKA) can retain Gli1 in the cytoplasm, inhibiting its transcriptional activity.[43] Gli2 and Gli3 processing is triggered by PKA-dependent phosphorylations, which are required for subsequent casein kinase 1 (CK1) and glycogen synthase kinase 3β (GSK3-beta) phosphorylations and recruitment of the β-transducin repeat-containing protein (β-TrCP) ubiquitin ligase.[33,34,44,45] In this context, Kif7 plays a regulatory role in controlling the efficient relocalization of Gli3 to the cilium in response to Shh and its processing to Gli3-R.[46] The dual specificity Yak-1-related kinases 1 (Dyrk1) and 2 (Dyrk2) modulate the Hh pathway in opposite ways. Dyrk1 increases Gli1 nuclear retention and transcriptional activity,[47] whereas Dyrk2 directly phosphorylates Gli2 and induces its degradation by the ubiquitin-proteasome system.[48] The serine/threonine unc-51-like kinase 3 enhances Gli1 (and Gli2) transcriptional activity.[49] Atypical protein kinase C (aPKC) ι/λ[50] and the downstream effector of the mammalian target of rapamycin (mTOR) pathway ribosomal protein S6 kinase 1 activate Gli1.[51] Deacetylation of Gli1 and Gli2 by histone deacetylase 1 increases their transcriptional activity.[52]

Degradation is also important for Gli1; two degradation sequences, degron N and degron C, mediate recognition by the β-TrCP E3 ubiquitin ligase to allow ubiquitination and degradation by the proteasome.[53] Gli1 is also targeted for proteolysis by Itch, another E3 ubiquitin ligase.[54] Similarly, Ci/Gli can be degraded through the ubiquitin E3 ligase adaptors Roadkill and HIB/SPOP in an Hh-dependent manner,[55,56] the latter being mediated by multiple Ser/Thr-rich degrons.[57] Upon genotoxic stress, p53 induces the acetyltransferase p300/CREB-binding protein (CBP)-associated factor (PCAF), identified as a novel E3 ubiquitin ligase targeting Gli1 for proteasomal degradation.[58]

Skin cancers linked to aberrant Hedgehog pathway activity

Skin cancer is by far the most frequent cancer worldwide and its incidence is increasing every year. Most of the insights into the role of Hh signaling in human cancers came from studies on BCC. However, recent evidence indicates that activation of the Hh pathway plays an important role in other types of human skin cancer, including melanoma and SCC.

Basal cell carcinoma

BCC is the most frequent form of human cancer. The incidence of BCC is strongly associated with exposure to ultraviolet radiation, and it often develops in elderly people with fair skin phototypes, especially on the head and neck. Additional risk factors include ionizing radiation, arsenic exposure, smoking, and immunosuppression.[59,60] Although disfiguring if allowed to grow, BCCs are benign tumors that rarely metastasize beyond the primary tumor site. The most common histological subtypes are nodular and superficial, both showing a less aggressive clinical course. On the other hand, micronodular, infiltrative, and mixed morphological variants are more aggressive. Clinically, BCCs appear as a pearly papule or nodule with overlying telangiectasias and rolled borders, with or without ulceration, and may be pigmented.[59,60]

The first link between Hh signaling and cancer came from the finding that loss-of-function mutations in the *Ptch1* gene on chromosome 9q22 were the cause of the nevoid BCC syndrome (NBCCS) or Gorlin syndrome.[61–63] NBCCS is an autosomal dominant disorder strongly predisposing to the development of BCC at a young age.[5] Moreover, these patients show a broad spectrum of developmental defects

and a high incidence of other neoplasms, particularly medulloblastoma, meningioma, ovarian and heart fibroma, fetal rhabdomyoma, and rhabdomyosarcoma.[64] In BCC from NBCCS patients, it was found that one allele of the *Ptch1* gene is mutated and the other allele is deleted, showing for the first time that Ptch1 behaves as a classical tumor suppressor according to Knudson's two-hit model.[65] More recently, a truncating germline mutation in *SUFU* was found in a family with features of Gorlin syndrome, presenting medulloblastoma but not BCC. Of note, no *Ptch1* mutations were detected in this family, suggesting that mutations in *SUFU* might be another cause of Gorlin syndrome.[66]

Later, it was found that sporadic BCCs also have a high frequency of loss-of-function mutations in *Ptch1* and, to a lesser extent, activating mutations in *SMO* (Table 1). Notably, the downstream effectors of the Hh pathway (Gli1/2/3) are rarely found to be mutated in sporadic BCCs (Table 1). Inactivating mutations in *Ptch1* occur in about 70%–80% of BCCs, and they mostly produce a truncated protein or are frameshift or missense mutations.[62,63,67–71] A nonsense mutation of *Ptch1* (Q688X) produces a truncated form of the protein, which enhances Gli1 activity independent of stimulation with Shh.[72] Recently, three different splice site mutations have been described in sporadic BCCs.[71] In another fraction of BCCs, activation of Hh signaling results from gain-of-function mutations in *SMO* (about 6%–21%),[70,73–75] or less frequently from inactivating mutations of *SUFU*.[70] Mutational analysis identified recurrent mutations in *SMO*, such as the G-to-A transition at base pair 1,685 of exon 10, which produces the amino acid change of Arg to Gln at codon 562 (activating mutation M1)[73] or G-to-T transversion at base pair 1,604 of exon 9, which changes codon 535 from Trp to Leu (activating mutation M2).[73,75] Both hot spot mutations lead to constitutive activation of Hh signaling. Recently, a translocation in *Shh* has been identified in a sporadic case of BCC. The translocation occurs between chromosomes 7 and Y, and fuses the middle of the *Shh* promoter with Y chromosome sequences, leaving 140 kb of regulatory sequences upstream of the *Shh* transcription start site. The authors demonstrated that the mutant promoter drives high expression of Shh protein in the skin,[76] in contrast with the absence of expression of Shh in sporadic BCCs with *Ptch1* and *SMO* mutations.[77]

Mouse models of BCC

Genetic data in patients have established the role of the Hh pathway in BCC; nevertheless, in the last few years, several mouse models using tissue-specific activation of the Hh pathway have provided a powerful tool to investigate the mechanisms of Hh-mediated development of BCC and to identify the BCC cell of origin. All current BCC mouse models target different components of Hh signaling, from the

Table 1 Genetic aberrations in the Hedgehog pathway identified in skin cancers

Gene	Mutation type	Tumor type	Percent mutated samples[a]	Reference
Ptch1	Loss-of-function, nonsense	BCC	40–67	62,63,67–70
	Missense, nonsense, splice site	BCC	75	71
	Missense, nonsense	cSCC	17	150
	Missense, nonsense, homozygous deletion	Melanoma	3–5.5	128,129, TCGA[b]
SMO	Gain-of-function, missense	BCC	9.5–20.6	70,73–75
	Missense	cSCC	7.7	150
	Missense, nonsense, amplification	Melanoma	2.2–8	128,129, TCGA[b]
SUFU	Missense	BCC	4.7	70
	Missense	cSCC	2.6	150
	Missense	Melanoma	0.7–3.3	128,129, TCGA[b]
Shh	Translocation	BCC	One case	76
	Missense, frameshift	cSCC	17.9	150
	Missense, amplification	Melanoma	0–4.7	128,129, TCGA[b]
HHIP	Missense, nonsense, amplification	Melanoma	6.6–9.1	128,129, TCGA[b]
	Missense	cSCC	30.7	150
Gli1	Missense, nonsense, amplification	Melanoma	1.1–7.2	128,129, TCGA[b]
	Missense, nonsense	cSCC	23	150
Gli2	Missense, nonsense, amplification	Melanoma	2.2–12.2	128,129, TCGA[b]
	Missense, nonsense	cSCC	25.6	150
Gli3	Missense, splice site, amplification	Melanoma	3.3–7.2	128,129, TCGA[b]
	Missense, nonsense	cSCC	23	150

Notes: [a]Percentage of patients with mutations (nonsense, missense, frameshift) or other alterations (translocations, amplifications, homozygous deletions). The possible function of missense mutations remains to be verified; [b]results shown here are data generated by the TCGA Research Network at http://cancergenome.nih.gov/.[205]
Abbreviations: BCC, basal cell carcinoma; cSCC, cutaneous squamous cell carcinoma; TCGA, The Cancer Genome Atlas.

Shh ligand, to the transmembrane proteins Ptch and SMO, SUFU, and the Gli transcription factors, and are summarized in Table 2.

Ptch1$^{+/-}$ mice kept under normal conditions rarely develop full-blown BCCs; rather, they show skin proliferation similar to human basaloid follicular hamartoma.[78] BCCs occur only after exposure to ultraviolet ionizing radiation[78,79] similar to development of BCC in NBCCS patients, suggesting that ultraviolet exposure is a very important risk factor for BCC. Because homozygous *Ptch1* knockdown leads to embryonic lethality due to heart and neural tube closure defects,[80–82] skin-specific knockout of *Ptch1* has been generated. Combining conditional *Ptch1* knockout driven by *K6a-Cre*, an inducible *K6a* promoter, *Ptch1*$^{-/-}$ mice developed BCC-like lesions.[83] Similarly, the use of other skin-specific Cre strains to drive homozygous *Ptch1* ablation, such as *K14-cre* and *Mx1-Cre,* or *Ptch*$^{flox/flox}$ mice crossed with *Rosa26CreERT2* mice *(R26-Cre*$^{ERT2+/-}$) produced BCC lesions.[84,85] Interestingly, in the latter model, 100% of animals develop BCCs with features of the nodular subtype, are noninvasive, and are characterized by strong Hh pathway activation, as witnessed by abundant expression of *Gli1* and *Ptch1*.[85] In a similar model, Ptch$^{flox/flox}$ mice were crossed with *K5-Cre*^{ERT} mice, which express the transgene in cells of the basal layer of the skin. All mice develop BCC lesions, but the *K5-Cre*^{ERT} is highly leaky, resulting in formation of BCCs even without Cre activation.[86] In addition to *Ptch1* inactivation, overexpression of Gli transcription factors leads to BCC. Ectopic expression of *Gli1* in the embryonic frog epidermis leads to formation of epidermal tumors[87] and its overexpression in the mouse epidermis drives formation of BCC-like tumors.[88] Likewise, overexpression of *Gli2* in mouse skin leads to development of BCC,[89] and sustained Hh signaling appears to be required for growth of BCC in a mouse model allowing conditional *Gli2* expression, because transgene inactivation leads to BCC regression.[90] Similarly, *K14-Shh* and *K5-SMO-M2* transgenic mice develop BCC-like tumors at an early age.[73,91] However, expression of mutant human SMO under the control of a truncated *Keratin 5 (K5)* promoter (*ΔK5-SMO-M2*) is not sufficient for development of full-blown BCCs.[92] Skin-specific knockin SMO-M2 mice (*CAGGS-Cre; R26-SMO-M2* or *K14-Cre*^{ERT}*; R26-SMO-M2*) develop multiple BCCs.[93,94] *SUFU*$^{+/-}$ mice develop basaloid follicular hamartoma lesions similar to those seen in *Ptch1*$^{+/-}$ mice, but more frequently,[95] and even compound *Ptch1*$^{+/-}$*; SUFU*$^{+/-}$ mice lack signs of full-blown BCC lesions.[96] This suggests that the combined reduction in gene dosage of these two negative regulators is still insufficient to reach the threshold of Gli activity required for development of BCC. The current view is that increasing levels of Hh pathway activation determines various stages in a spectrum ranging from benign hamartomas to malignant BCC lesions. For instance, comparing two different BCC models, Grachtchouk et al found that *K5-Gli2* mice with strong Hh signaling developed full-blown BCCs,[89] while the weaker Hh signal in *ΔK5-SMO-M2* mice results in follicular hamartomas.[92]

As mentioned earlier, primary cilia are an important organelle for Hh signal transduction in mammals. Primary cilia contain multiple components of the Hh pathway, including Shh, Ptch1, SMO, and Gli, and are present in the majority

Table 2 Mouse models of BCC with genetically modified Hedgehog pathway components

Mouse model	Additional treatment	Tumor type
K14-Shh		BCC-like lesions[91]
K5-SMO-M2		BCC-like lesions[73]
Ptch$^{+/-}$ (deleted exons 1,2)		Trichoblastomas[78]
Ptch$^{+/-}$ (deleted exons 1,2)	UV, X-ray	BCC, trichoblastomas[78]
ΔK5-SMO-M2		Basaloid follicular hamartomas[92]
K5-Gli1		BCC-like lesions, trichoepitheliomas, cylindromas, and trichoblastomas[88]
K5-Gli2		BCC[89]
K5-Gli2ΔN2		Trichoblastomas, cylindromas, basaloid follicular hamartomas, and BCC[201]
Ptch1$^{+/-}$ (deleted exons 6,7)		Basaloid hyperproliferation[79]
Ptch1$^{+/-}$ (deleted exons 6,7)	X-ray	BCC (nodular and infiltrative)[79]
SUFU$^{+/-}$		Basaloid follicular hamartomas[95]
SUFU$^{+/-}$*; Ptch1*$^{+/-}$		Basaloid follicular hamartomas[96]
K5-tTA; TRE-Gli2		BCC[90]
K6a-Cre; Ptch1$^{fl/fl}$		BCC-like lesions[83]
K14-Cre; Ptch1$^{fl/fl}$		BCC-like lesions[84]
Mx1-Cre; Ptch1$^{fl/fl}$		BCC-like lesions[84]
R26-Cre^{ERT2}*; Ptch*$^{fl/fl}$		BCC-like lesions[85]
K5-Cre^{ERT}*; Ptch*$^{fl/fl}$		BCC-like lesions (leaky)[86]
CAGGS-Cre^{ER}*; R26-SMO-M2*		BCC-like lesions[93]
K14-Cre^{ERT}*; R26-SMO-M2*		BCC-like lesions[94]
K14-Cre^{ERT}*; R26-SMO-M2*		Inhibition of BCC-like lesions[94]
Kif3a$^{fl/-}$ *or Ift88*$^{fl/-}$		
K14-Cre^{ERT}*; CLEG2*^{cond}		BCC-like lesions[94]
K14-Cre^{ERT}*; CLEG2*^{cond}		Increased BCC-like lesions[94]
Kif3a$^{fl/-}$ *or Ift88*$^{fl/-}$		
CD4-Cre; Ptch$^{fl/fl}$	DMBA/TPA	BCC-like lesions[202]

Abbreviations: BCC, basal cell carcinoma; DMBA, 7,12-dimethylbenz(a)anthracene; TPA, 12-O-tetradecanoylphorbol-13-acetate; UV, ultraviolet light; R26-SMO-M2, Rosa26-flox-STOP-flox-SMOM2-YFP; CLEG2^{cond}, CAG-flox-GFP-flox-Myc-Gli2ΔN.

of human BCCs. The question of whether primary cilia are required for Hh-driven development of BCC has been addressed using a tissue-specific knockout. Genetic disruption of cilia formation by conditional ablation of *Kif3a* and of *Ift88*, both of which required for ciliogenesis, decreases formation of BCC in *K14-Cre^{ERT}*; *R26-SMO-M2* mice, but increases tumor formation in a *Gli2* conditional transgenic model.[94] This apparently paradoxical effect suggests that cilia play a dual role in Hh-mediated tumorigenesis, which could be explained by the fact that cilia are required for formation of both repressor and activator forms of Gli.[97–99]

Cell of origin of BCC

The cellular origin of BCC has been debated for a long time. It has been proposed that BCCs may arise from the outer root sheet or bulge of the hair follicle, and stem and progenitor cells are thought to be the probable source of initiation of BCC due to their long life and self-renewal ability. However, recent studies using Cre-mediated cell-specific targeting both by lineage tracing and specific activation of Hh signaling in distinct skin cell populations gave unexpected results (Table 3). The first of these studies, using cell-specific Cre to activate expression of *R26-SMO-M2* in mice, localized the cell of origin of BCC in the long-term resident progenitor cells of the interfollicular epidermis (IFE) and the upper infundibulum.[100] However, *K15-Cre* and K14-Cre; *R26-SMO-M2* mice, where SMO-M2 is mostly targeted in stem cells and transient amplifying progenitors of the hair follicle (HF), show only basaloid lesions and never BCCs.[100] In contrast, using cell fate tracking in *Ptch1$^{+/-}$* mice exposed to X-ray, Wang et al[101] demonstrated the BCC cell of origin in keratin 15-positive stem cells of the HF bulge. Interestingly, loss of p53 in these mice was associated with enhanced BCC formation, not only from the HF bulge but also from the IFE.[101] Using conditional expression of *SMO-M2* in keratin 15-positive cells, another group showed that wounding recruits HF *SMO-M2*-expressing cells to the wound site (IFE), where they give rise to superficial BCC-like tumors.[102] The same type of tumor was observed upon conditional expression of *SMO-M2* in keratin 14-positive cells, even in the absence of wounding.[102] Conditional overexpression of *Gli1* or homozygous deletion of Ptch1 under the control of the *K5* promoter results in BCC formation, preferentially in the IFE but also in the HF.[103] Lineage tracing of Lgr5+ stem cells showed that HF-associated and IFE-associated lesions had distinct cells of origin and that Lgr5-labeled HF cells were able to give rise to BCCs in the IFE upon wounding,[103] in agreement with Wong and Reiter.[102] Another study revealed that low levels of Gli2ΔN expression in the basal compartment do not lead to nodular BCCs in the HF, but to slow-growing basaloid follicular hamartomas resembling tumors found in *ΔK5-SMO-M2* mice.[104] These studies suggest that BCCs can arise from cells competent to receive Hh signal and able to activate Gli transcription factors.

Table 3 Mouse models used to identify the BCC cell of origin

Mouse model	Additional treatment	Tumor type
Shh-Cre^{ERT}; R26-SMO-M2		No lesions[100]
K15-Cre^{ERT}; R26-SMO-M2		Dysplastic lesions[100]
K19-Cre^{ERT}; R26-SMO-M2		Dysplastic lesions[100]
K15-Cre^{PR1}; Ptch1$^{+/-}$	X-ray, *p53$^{fl/fl}$*	BCC, increased by p53 loss[101]
K14-Cre^{ER2}; Ptch1$^{+/-}$	X-ray, *p53$^{fl/fl}$*	BCC, increased by p53 loss[101]
K5-tTA; TRE-Gli1	± wound	BCC, increased w/wounding[103]
K5-Cre^{PR1}; Ptch1$^{fl/fl}$	± wound	BCC, increased w/wounding[103]
Lgr5-EGFP-Cre^{ERT2}; Ptch1$^{fl/fl}$	± wound	BCC, increased w/wounding[103]
K15-Cre^{PR1}; R26-SMO-M2		Basaloid proliferation[102]
K15-Cre^{PR1}; R26-SMO-M2	Wound	BCC (IFE)[102]
K14-Cre^{ERT}; R26-SMO-M2	± wound	BCC[102]
K14-rtTA/K5-rtTA; TetO-Gli2ΔN		BCC (nodular HF; superficial IFE)[104]
K15-Cre^{PR1}; R26-LSL-rtTA; TetO-Gli2ΔN		Nodular BCC (HF)[104]
Lgr5-EGFP-Cre^{ERT2}; R26-LSL-rtTA; TetO-Gli2ΔN		Nodular BCC (HF)[104]
K5-Cre^{ERT2}; R26-LSL-rtTA; TetO-Gli2ΔN		BCC (nodular HF; superficial IFE)[104]
K5-Cre; R26-LSL-rtTA; TetO-Gli2ΔN		BFH (IFE, HF)[104]
K15-Cre^{PR1}; R26-LSL-rtTA; SMOA1		Hyperplasia (HF)[104]

Abbreviations: BCC, basal cell carcinoma; BFH, basaloid follicular hamartoma; HF, hair follicle; IFE, interfollicular epidermis; R26-SMO-M2, Rosa26-flox-STOP-flox-SMO-M2-YFP; w/wounding, with wounding.

Hh targets and interaction with other pathways in BCC

Both Gli1 and Gli2 are highly expressed in human BCCs.[87,105] In particular, Gli2 directly regulates the expression of Gli1, further activating the Hh pathway.[37] Gli2 silencing reduces growth of BCC xenografts by decreasing vascularization and increasing apoptosis.[106] Activation of the Hh pathway might exert its mitogenic effect on keratinocytes by activation of several targets. For instance, Gli1 can induce expression of platelet-derived growth factor receptor alpha[107] and FOXM1,[108] and Gli2 activates FOXE1.[109] Gli2 has been shown to activate the antiapoptotic factor Bcl-2[110] and to counteract death ligand-mediated apoptosis by inducing expression of

the caspase-8 inhibitor c-FLIP,[111] suggesting an antiapoptotic role in BCC. Hh signaling enhances ribosomal RNA transcription in BCC by increasing basonuclin gene expression.[112] In addition, insulin-like growth factor binding protein 2 is upregulated in both murine and human BCCs and has been shown to play a role in Hh-mediated expansion of epidermal progenitor cells in *K14-Cre; Ptch1$^{fl/fl}$* skin explants.[113]

Hh signaling has been shown to cross-talk with other signaling pathways. The Wnt pathway plays a critical role in development of the HF, and both human and murine BCCs show increased levels of β-catenin.[114] Conditional overexpression of the Wnt pathway antagonist Dkk1 results in inhibition of Hh-driven benign hamartomas.[115] The epidermal growth factor receptor/MEK/ERK pathway has been shown to modulate Gli-dependent transcription in human keratinocytes[116] and to synergize with Hh signaling in inducing oncogenic transformation of human keratinocytes through activation of c-Jun.[117] In accordance with these studies, an increase of c-Jun upon Hh or epidermal growth factor receptor activation inhibits the tumor suppressor miR-203, which in turn represses c-Jun, creating a negative regulatory loop.[118] Interestingly, Hh and epidermal growth factor receptor signaling synergistically activate a number of cooperation response genes, including SOX2, SOX9, JUN, CXCR4, and FGF19, which are required for growth of BCC in vivo.[119] In addition, loss of p53 has been shown to accelerate tumorigenesis of BCC in *Ptch1$^{+/-}$* mice,[101] likely through Gli1 activation.[120]

Melanoma

Melanoma is the most aggressive form of skin cancer and originates from the malignant transformation of melanocytes or neural crest-derived precursors.[121] For the purpose of this review, we focus on malignant melanoma affecting the skin, ie, cutaneous melanoma (herein referred to as melanoma). While melanoma accounts for less than 10% of all skin cancers, it is responsible for more than 75% of skin cancer-related deaths.[122] The overall 5-year survival rate for patients with localized melanoma is about 98%, but it falls to 62% and 16% for patients with lymph nodes and distant metastases, respectively.[123] Cutaneous melanomas harbor mutually exclusive activating mutations in *BRAF* and *NRAS*, occurring respectively in 50% and 15%–20% of cases, and deletion of the *CDKN2A* locus.[124–126] Recent exome sequencing studies have identified a variety of additional alterations, including mutations in *GRIN2A*, *PPP6C*, and *KIT*, and amplification of *TERT*, *CCND1*, *KIT*, and *MITF-M*.[127–129]

The importance of Hh signaling in melanoma tumorigenesis was not revealed until recently (Table 4). Our group was the first to demonstrate that human melanomas express Hh pathway components, and they require active Hh signaling for growth and proliferation. We showed that melanoma cells, but not the surrounding stroma, express *Shh*, *Gli1*, and *Ptch1* and that melanoma cells in vitro respond to inhibition of the Hh pathway through cyclopamine (a SMO antagonist) or silencing of *Gli1* and *Gli2* by drastically decreasing their proliferation. Importantly, treatment with cyclopamine or silencing of *SMO* reduced tumor growth in an orthotopic xenograft model and abolished tumor recurrence, and systemic treatment with cyclopamine prevented metastatic growth in the lungs of mice.[130] These effects appear to be specific, because Gli1 epistatically rescues the inhibitory effect of cyclopamine on cell proliferation, and the latter mimics inhibition of SMO via RNA interference.[130] In addition, we showed that Hh signaling is required also in tumors induced by oncogenic NRAS in a *Tyrosinase-NRAS^{Q61K}; Ink4a$^{-/-}$* mouse model. Our study provided the first evidence that endogenous Ras-MEK and AKT signaling regulate the nuclear localization and transcriptional activity of Gli1 in melanoma cells.[130]

Table 4 Experimental in vitro and in vivo models in which interference with the Hedgehog pathway reduces growth of melanoma cells

Hh pathway inhibition	In vitro model	In vivo model
Cyclopamine, shRNA SMO[130]	Melanoma cells	Xenografts in nude mice *Tyrosinase-NRAS^{Q61K}; Ink4a$^{-/-}$*
Cyclopamine, GANT61, shRNA SMO/Gli1[134]	Melanoma CSCs	ALDH^{high} melanoma CSC in nude mice
Cyclopamine/CCT[137]	Melanoma cells	
LDE-225, cyclopamine[132]	Melanoma cell lines	Xenografts in nude mice
LDE-225, siRNA SMO, cyclopamine[131]	Melanoma cell lines	Xenografts in nude mice
Cyclopamine[144]	Uveal melanoma cell lines	

Abbreviations: shRNA, short hairpin RNA; siRNA, short interfering RNA; CSCs, cancer stem cells; ALDH^{high} cells, cells with high aldehyde dehydrogenase activity; CCT, CCT007093 (WIP1 inhibitor); Hh, Hedgehog.

Two recent studies confirmed and extended our previous findings (Table 4). In one study, it was shown that Hh pathway members are highly expressed in a subset (50%) of melanoma cell lines; in particular *SMO*, *Gli2*, and *Ptch1* are upregulated, while the negative regulators of Hh signaling, *PKA* and *DYRK2*, are downregulated when compared with melanocytes.[131] Interestingly, high Hh pathway activity is associated with decreased post-recurrence survival in patients with metastatic melanoma.[131] The second study found that expression of Gli1 is higher in human primary melanoma

harboring BRAF(V600E) mutation than in those with wild-type BRAF.[132] These studies also showed that LDE-225, a well tolerated oral SMO antagonist currently in Phase II clinical trials for advanced BCC (see later), reduces proliferation of human melanoma cell lines by inducing apoptosis, and decreases human melanoma xenograft growth in nude mice.[131,132] Interestingly, a study reported by O'Reilly et al[131] showed a more dramatic proliferative effect in BRAF mutant cell lines than in wild-type BRAF cells and a modest but significant effect of combining BRAF and Hh inhibitors, suggesting that combined therapy targeting mutant BRAF and Hh could be beneficial in patients with mutated BRAF and activated Hh signaling. Interestingly, inhibition of Hh signaling with LDE-225 leads to upregulation of the programmed cell death mediator *XAF1* and apoptosis in vitro and in vivo.[131]

Multiple lines of evidence indicate that Hh signaling regulates cancer stem cells (CSCs) in several types of cancer,[133] including melanoma. Putative melanoma CSCs with high aldehyde dehydrogenase activity have been shown to require Hh signaling, because pharmacological inhibition of the Hh pathway with cyclopamine or GANT61 (a Gli1/2 inhibitor) and genetic silencing of SMO and Gli1 drastically reduces self-renewal of melanoma CSCs and tumorigenicity in vivo.[134] The critical role of Hh in CSCs is elicited through the subverted regulation of stemness genes, such as Nanog and SOX2,[135,136] which are overexpressed in certain types of cancer. For instance, it was recently shown by our group that both Gli1 and Gli2 bind to the *SOX2* promoter in melanoma cells and that SOX2 function is required for Hh pathway-mediated self-renewal of melanoma CSCs.[136]

Activation of Hh signaling in melanoma can be mediated by the oncogenic phosphatase WIP1, which increases the stability and transcriptional activity of Gli1 in patient-derived primary melanoma cells.[137] Ectopic expression of Gli1 or of an active form of Gli2 (ΔNGli2) in N/TERT human keratinocytes increases their resistance to apoptosis induced by ultraviolet B and DNA-alkylating agents and induces epithelial-to-mesenchymal transition.[138] The Hh pathway is also involved in resistance to treatment with BRAF inhibitors, because it mediates upregulation of platelet-derived growth factor receptor alpha observed in BRAF inhibitor-resistant cells, and inhibition of the Hh pathway restores the sensitivity of the cell to BRAF inhibitors.[139]

Hh signaling also plays a role in the progression of melanoma by contributing to the acquisition of invasive behavior. Melanoma cells with high Gli2 expression are characterized by an invasive and metastatic phenotype associated with loss of E-cadherin and secretion of metalloproteases, and metastasize to bone more quickly than cells with low Gli2 expression.[140] Gli2 represses MITF-M by direct binding to its promoter and contributes to the loss of melanocytic differentiation markers.[141,142] Gli1 directly induces osteopontin, and their high expression levels correlate with tumor progression and metastasis in human melanoma.[143] Likewise, inhibition of Hh signaling in uveal melanoma cell lines decreases cell viability, epithelial-mesenchymal transition, and angiogenesis.[144]

Hh pathway mutations in melanoma have been identified in two whole-exome sequencing studies[128,129] and in The Cancer Genome Atlas Research Network (http://cancergenome.nih.gov/), where 91, 121, and 278 melanoma samples were respectively analyzed. Interestingly, data from The Cancer Genome Atlas indicate that 35% of patients show one alteration (mutation or copy number variation) in at least one component of the Hh pathway, with co-occurrence of amplifications in *SMO* and Shh ($P<0.001$), co-occurrence of mutations in Gli1 and Gli2 ($P<0.005$), and mutations in Ptch1 and *HHIP* ($P=0.014$).[145,146] These alterations are predominantly missense mutations and amplifications, and all occur in the main components of the Hh pathway, from *Ptch1* and *SMO* to the downstream effectors *Gli1/2/3*, in contrast with BCC mutations, which are mostly found in *Ptch1* and *SMO* (Table 1). The relevance of these mutations in melanoma remains to be determined, and only further functional studies will shed light on their impact on the development and progression of melanoma. Unlike in BCC, no genetic mouse models have been established thus far for Hh pathway-mediated development of melanoma. Nevertheless, *K5-Gli2* transgenic mice form hyperpigmented BCC-like tumors,[89] and *ΔK5-SMO-M2* transgenic mice[92] and aging mice lacking one *SUFU* allele[95] develop skin pigmentation. In addition, injection of *Gli1* mRNA into the epidermis/neural crest of Xenopus embryos induces formation of pigmented epithelial tumors expressing high levels of the melanoma marker *Mitf*.[130]

Squamous cell carcinoma

SCC originates from the squamous epithelium of the skin, but can occur also in the lungs, oral mucous membranes, esophagus, cervix, bladder, and genitals. SCCs usually appear in areas exposed to the sun and look like scaly red patches, open sores, elevated growths with a central depression, or warts. SCC is the second most common cancer of the skin after BCC. However, SCC is more aggressive than BCC, because it grows faster and has higher rates of metastasis and mortality.[147]

Although the link between aberrant activation of the Hh pathway and SCC is not as strong as that in BCC, a number

of studies have shown a potential role of the Hh pathway in different types of SCC. However, for the purpose of this review, the focus is on skin SCC. Ping et al[148] provided the first evidence that *Ptch* is mutated in a subset of skin SCC from individuals with a history of multiple BCC. Later, a high prevalence of allelic loss at 9q22.3, including *Ptch*, was demonstrated in skin SCC.[149] A recent exome sequencing analysis in 39 cases of aggressive cutaneous SCCs identified missense mutations in most of the components of Hh signaling, including the *Gli* transcription factors[150] (Table 1). However, these mutations so far are of unknown significance. Evidence of activation of Hh signaling in skin SCC derives mainly from immunochemistry studies, which reveal high expression of major components of Hh signaling in the tumor compared with control tissues.[151,152]

While the role of Ptch1 in BCC tumor suppression is clear, mouse models suggest an opposing function of Ptch1 in SCC. Wakabayashi et al[153] reported that a single polymorphism at the *Ptch1* C-terminus (T1267N) confers increased susceptibility to Ras-induced tumor formation in FVB mice. Overexpression of the $Ptch1^{FVB}$ allele driven by the K14 promoter ($K14\text{-}Ptch1^{FVB}$) is sufficient to drive Ras-induced formation of early post-natal SCC in mice with C57BL/6 background, but is not required for tumor maintenance. A second study from the same group showed that chemically induced development of SCC increased and tumor latency decreased in adult $K14\text{-}Ptch1^{FVB}$ mice compared with their wild-type counterparts, without aberrant activation of the Hh pathway.[154] These authors proposed that $Ptch1^{FVB}$ promotes SCC formation through regulation of Ras-induced apoptosis; high levels of active Ras and inhibition of the tumor suppressor Tid1 by $Ptch1^{FVB}$ results in SCC tumor formation, whereas in the C57BL/6 background, SCC tumor formation is suppressed, because Ptch1 is unable to inhibit Tid1, resulting in an increase in Ras-induced apoptosis.[153,154]

Inhibitors of the Hh pathway and their application in skin cancer

Three major targeting sites for Hh signaling inhibitors have been identified, ie, SMO protein, Gli transcription factors, and other agents that directly or indirectly modulate the Hh pathway. Table 5 lists Hh signaling inhibitors that are in use or have potential clinical use for the treatment of BCC.

SMO inhibitors

The idea of targeting the Hh pathway for treating cancers came from the finding that ingestion of corn lilies (*Veratrum californicum*) by pregnant sheep induced birth defects in their offspring (cyclopia)[155] similar to those observed in mice lacking Shh.[156] The active compound, cyclopamine, was later purified and shown to inhibit Hh signaling[157,158] and to bind SMO.[159] Initial studies showed that oral administration of cyclopamine drastically reduced growth and development of BCCs in $Ptch^{+/-}$ mice exposed to ultraviolet light,[160] and topical application of cyclopamine can induce regression of human BCC.[161] Several groups have confirmed that cyclopamine also decreases growth of many human cancer cell lines in xenotransplantation, including melanoma.[6] However, cyclopamine is not suitable for clinical development because of its poor oral solubility. Subsequent work led to the discovery of a number of new SMO antagonists, including GDC-0449 (vismodegib), the first SMO inhibitor approved by the US Food and Drug Administration for locally advanced and metastatic BCC.[162–164] Other current SMO inhibitors that are in Phase I or Phase II clinical trials to treat locally advanced or metastatic BCC are LDE-225 (erismodegib),[165,166] itraconazole,[167,168] BMS-833923,[169] and LEQ-506.[170] Two SMO inhibitors, IPI-926 (saridegib) and TAK-441, have been discontinued for lack of efficacy.[171,172] A number of additional SMO antagonists have been used in preclinical studies, including Cur-61414 (HhAntag),[173] Sant1-4,[159] and Sant75[174] (Table 5).

Clinical trials showed a heterogeneous response to vismodegib depending on the type of BCC. The most sensitive patients are those with NBCCS, who showed a 100% response rate without signs of resistance during treatment, as described for medulloblastoma.[163] In contrast, in sporadic cases, only 57% of patients with late advanced or metastatic BCC showed tumor regression in Phase I clinical trials,[162,175] and only 30% of metastatic and 43% of late advanced BCCs responded in Phase II clinical trials.[164] These results suggest that tumors with a low mutation rate, such as in patients with NBCCS, are predicted to respond well to SMO inhibition, whereas metastatic BCCs with a high mutation rate have a greater likelihood of developing acquired resistance during treatment.[176]

Little is known about resistance mechanisms in BCC, but studies in other Hh-driven cancers suggest that BCCs can bypass SMO inhibition through Hh-specific genetic alterations or compensatory adaptation.[176] Studies in mice and humans harboring SMO inhibitor-resistant medulloblastoma have shed light on the mechanisms of acquired resistance, which could derive from mutations in human *SMO* (D473H) and the matching mutation in the mouse (D477G),[177] amplification of downstream Hh target genes, such as *Gli2* and *CCND1*,[178,179] and upregulation of other oncogenic pathways, such as that for phosphatidylinositide 3-kinase-AKT.[178]

Table 5 Hedgehog pathway antagonists in use or with potential clinical use for the treatment of BCC

Target	Inhibitor	Study phase	Indications	Clinical trials*
SMO inhibitors				
SMO	GDC-0449 (vismodegib)[162–164]	In clinical use	BCC and BCNS	Several[a]
SMO	LDE-225 (erismodegib)[165,166]	II	BCC and BCNS	Several[b]
SMO	Itraconazole	II	BCC	NCT01108094
SMO	BMS-833923	I	Locally advanced or metastatic BCC	NCT00670189
SMO	LEQ-506	I	Locally advanced or metastatic BCC	NCT01106508
SMO	IPI-926 (saridegib)[203]	I	BCC	–
SMO	TAK-441[204]	I	BCC	NCT01204073
SMO	Cur-61414[173,183]	Preclinical	–	–
SMO	SANT1–4[159]	Preclinical	–	–
SMO	SANT75[174]	Preclinical	–	–
Downstream Hh pathway inhibitors				
Gli	ATO[168]	Pilot	BCC	NCT01791894
Gli	GANT58, 61[184]	Preclinical	–	–
Gli	HPI 1–4[186]	Preclinical	–	–
Gli	Glabrescione B[189]	Preclinical	–	–
Gli	JQ1[190]	Preclinical	–	–
Other agents				
PKA	Imiquimod[191]	In clinical use	Superficial BCC	Several[c]
SMO	Provitamin D3	III	BCC	NCT01358045
RARβ/RARγ	Tazarotene	II	BCC in BCNS	NCT00489086
RARβ/RARγ	Tazarotene	II	BCC in BCNS	NCT00783965
aPKC	PSI[50]	Preclinical	–	–

Notes: *Clinicaltrials.gov NCT identifier. [a]Clinical trials for GDC-0449: NCT00607724, NCT00833417, NCT00957229, NCT00959647, NCT01160250, NCT01201915, NCT01367665, NCT01700049, NCT01815840, NCT01898598, and NCT02067104; [b]clinical trials for LDE-225: NCT00880308, NCT00961896, NCT01033019, NCT01208831, NCT01327053, NCT01350115, and NCT01529450; [c]clinical trials for imiquimod: NCT00066872, NCT00129519, NCT00189241, NCT00189306, NCT00204555, NCT00314756, NCT00581425, NCT01212549, and NCT01212562.

Abbreviations: Hh, Hedgehog; BCC, basal cell carcinoma; BCNS, basal cell nevus syndrome; SMO, Smoothened; PKA, protein kinase A; aPKC, atypical protein kinase C ι/λ; RARβ/RARγ, retinoic acid receptor β/γ; ATO, arsenic trioxide; PSI, specific peptide inhibitor; Gli, Gli transcription factors.

Treatment with vismodegib and other systemic Hh pathway inhibitors is associated with mild to moderate side effects, which include muscle cramps, nausea, diarrhea, alteration in taste perception, weight loss, and alopecia. Most of these side effects cease after patients stopped taking the drug. It is likely that hair loss, altered taste, and diarrhea are related directly to inhibition of the intended target SMO, since Hh signaling is known to be active in the HF, taste buds, and the gastrointestinal tract.[180–182] Therefore, such effects are unlikely to be avoided by modifying the molecular structure of these agents. The location of BCCs makes them ideal for topical use of these inhibitors, thus limiting the side effects associated with systemic treatment. One study using topical LDE-225 for 4 weeks documented an effective reduction in tumor size or clinical clearing, correlated with inhibition of Hh signaling.[165] In another study, the treatment was ineffective, probably because of lack of Hh pathway blockade, despite the fact that the drug (Cur-61414) showed efficacy in mice.[183]

Itraconazole, an antifungal agent approved by the US Food and Drug Administration, has been identified as a potent inhibitor of the Hh pathway by preventing cilial translocation of SMO.[167] Systemic administration of itraconazole has been shown to delay development of BCC in *Ptch1*$^{+/-}$ mice, and it is also active against drug-resistant mutant SMO D477G and Gli2 overexpression.[168] At present, itraconazole is under Phase II investigation as a treatment for BCC (Table 5).

Gli antagonists

The development of molecules able to directly target the Gli transcription factors, the final effectors of the Hh signaling, would be effective in skin tumors with mutations that are downstream of SMO and perhaps overcome anti-SMO drug resistance. Unfortunately, so far, only few molecules acting on Gli proteins have been identified and their use is limited to preclinical studies. Cell-based screening for inhibitors of Gli1-mediated transcription identified two structurally different compounds, GANT61 and GANT58. Both are capable of interfering with Gli1-mediated and Gli2-mediated transcription and inhibit tumor cell growth in a Gli-dependent manner.[184] Screening of natural products identified physalins F and B as inhibitors of Gli-mediated transcriptional activity.[185] More recently, HPI-1/4 were described to act at or downstream of SUFU through various mechanisms, such as

interfering with processing or activation of Gli. In particular, HPI-1 and HPI-4 have been shown to increase the proteolytic cleavage of Gli2 to its repressor form, whereas HPI-4 also decreases Gli1 stability.[186]

One example of a drug found to target the Gli transcription factors is arsenic trioxide,[187] a therapeutic agent already approved for acute promyelocytic leukemia. Mechanistically, arsenic trioxide binds directly to Gli1 protein and inhibits its transcriptional activity[188] and blocks Hh-induced ciliary accumulation of Gli2.[187] The in vivo efficacy of arsenic trioxide was demonstrated in mouse models of medulloblastoma,[187,188] and a pilot clinical study of arsenic trioxide in BCC is ongoing (Table 5).

Recently, the small molecule Glabrescione B was shown to interfere with the interaction between Gli1 and target DNA.[189] Glabrescione B is an isoflavone naturally present in the seeds of *Derris glabrescens*. Remarkably, as a consequence of its strong inhibition of Gli1 activity, Glabrescione B inhibits growth of Hh-dependent BCC and medulloblastoma tumor cells in vitro and in vivo.[189]

Inhibition of BET bromodomain proteins has recently emerged as a novel strategy for epigenetic targeting of the transcriptional output of the Hh pathway.[190] The BET bromodomain protein BRD4 is a critical regulator of *Gli1* and *Gli2* transcription via direct occupancy of their promoter. Interestingly, occupancy of *Gli1* and *Gli2* promoters by BRD4 and transcriptional activation at cancer-specific Gli promoter-binding sites are markedly inhibited by the BET inhibitor JQ1. In *Ptch*-deficient BCC and medulloblastoma mouse models and in patient-derived tumors with constitutive Hh pathway activation, JQ1 decreases proliferation and viability of tumor cells in vitro and in vivo.[190]

Other agents

Other compounds might inhibit Hh signaling by targeting proteins and/or pathways that modulate Gli transcription factors. For instance, imiquimod, a nucleoside analog of the imidazoquinoline family and approved by the US Food and Drug Administration for the treatment of BCC,[191] has been shown to directly repress Hh signaling by inducing PKA-mediated Gli phosphorylation with consequent reduction of Gli activator levels in BCC and medulloblastoma.[192]

Vitamin D3 has been shown to efficiently block Hh signaling in vitro and to mimic the SMO loss-of-function phenotype in a zebrafish model system.[193] Vitamin D3 also inhibits proliferation and Hh signaling in BCC cell lines. These effects seem to be independent of the vitamin D receptor, because its genetic silencing does not abrogate the inhibitory effect of vitamin D3.[194] In addition, it was shown by a different group that application of calcitriol, the physiologically active form of vitamin D3, inhibits proliferation and growth of BCC in *Ptch*-deficient mice in vitro and in vivo, and stimulates differentiation of BCC.[195] Of note, in the same paper, the authors showed that calcitriol inhibits Hh signaling at the level of SMO in a vitamin D receptor-independent manner. At present, a Phase III trial combining application of topical vitamin D3 and treatment with anti-inflammatory agents is ongoing (Table 5).

Topical treatment with the RARβ/RARγ-selective retinoid tazarotene has been shown to reduce the number and size of BCC in irradiated *Ptch1*$^{+/-}$ mice.[196] Tazarotene also inhibited in vitro growth of a murine BCC keratinocyte cell line and downregulated Gli1.[197] Its efficacy in BCC patients is under investigation in Phase II clinical trials (Table 5).

Another potentially interesting therapeutic target recently identified as a novel activator of Gli1 transcriptional activity is aPKC. Loss of aPKC or inhibition with a specific peptide inhibitor suppressed Hh signaling and growth of a mouse BCC cell line. Of note, aPKC activity is increased in sensitive and resistant human BCCs when compared with normal skin, and topical treatment with the specific peptide inhibitor led to reduction of tumor growth in mouse BCC allografts.[50]

Prospects for melanoma and SCC

Recent studies have started to elucidate the role of Hh signaling in melanoma, demonstrating its requirement for melanoma cell growth. In fact, inhibition of the Hh pathway with the SMO antagonist cyclopamine or LDE-225 or with the Gli1/2 inhibitor GANT61, or through genetic silencing of *SMO* and *Gli1* drastically reduces proliferation of human melanoma cells in vitro and in mouse xenografts in vivo.[130–132,134] Interestingly, BRAF mutant melanoma cell lines are more sensitive to SMO inhibition with LDE-225 than melanoma cells with wild-type BRAF.[131] Moreover, combination of LDE-225 with the mutant BRAF inhibitor vemurafenib reduced the proliferation of melanoma cells more efficiently than the single agent alone, suggesting the attractive possibility of a combined therapy targeting both mutant BRAF and Hh signaling in patients with melanoma.[131] From a therapeutic point of view, it would also be interesting to test whether SMO inhibition might be effective in vemurafenib-resistant melanomas with mutant BRAF. In support of this, a recent report showed that upregulation of Hh-mediated platelet-derived growth factor receptor alpha leads to BRAF inhibitor resistance and suggested that blockade of the Hh pathway restores sensitivity of melanoma cells

to BRAF inhibitors.[139] These data represent a rationale for translation of combinatorial treatment using Hh and BRAF inhibitors for melanoma to the clinical setting.

The role of Hh signaling in SCC is less clear. Our knowledge of the involvement of Hh signaling in SCC of the human skin derives mainly from immunochemistry studies, which reveal expression of Hh pathway components in cancer cells, but functional studies in human SCCs are lacking. More importantly, mouse models suggest that Ptch1 mainly promotes development of murine SCC.[153,154] Therefore, inhibition of Hh might have completely opposite effects in BCC and SCC, ie, Hh inhibitors may be therapeutic agents for BCC, but might promote SCC. In support of this notion, recent reports described cases of patients developing SCC soon after initiation of vismodegib for BCC.[198–200] This could be due to appearance of a squamous component within a metatypical BCC or to squamous differentiation of stem cells present in the deep epidermal layer or in the follicular bulge through inhibition of the Hh pathway.[198] Therefore, further studies are needed to investigate the molecular mechanisms by which Hh signaling mediates development of SCC before advocating use of Hh inhibitors in SCC patients.

Conclusion

Recent advances have shed light on the role of Hh signaling in skin cancers, including BCC, melanoma, and SCC. What are the prospects for skin cancer therapy? While recent clinical trials using Hh inhibitors have identified effective treatments for BCC, further basic understanding of the molecular mechanisms by which Hh signaling mediates tumor development in melanoma and especially in SCC is required. In particular, animal models of Hh-mediated tumorigenesis for melanoma have not been established as yet. Another challenging question is the nature of the genetic and molecular events that cooperate with active Hh signaling in melanoma tumorigenesis. Only a clear understanding of the mechanisms leading to Gli activation will allow for selection of the appropriate Hh pathway inhibitors and, in cases of a crosstalk between Hh and other oncogenic pathways, the optimal combinatorial partner. This is particularly relevant for melanomas, a cancer characterized by a non-canonical activation of the Hh pathway.

Another complication is that tumors, in particular melanomas, are heterogeneous, with multiple clones in the same lesion. It is possible that during treatment, drug-resistant clones that are initially present in low numbers become dominant as they gain a growth advantage and sensitive clones die. This suggests that initially targeting multiple pathways at the same time may be more effective that targeting a single one. In addition, given the opposite roles of Ptch1 in BCC and SCC, inhibitors of Hh signaling may be therapeutic agents for BCCs, but might have additional effects in promoting SCCs. Therefore, it will be equally important to monitor the appearance of SCC in BCC patients treated with Hh inhibitors. Future studies will certainly provide answers to these questions, and hopefully what has been learned from treating BCC with Hh pathway inhibitors will open new prospects for the treatment of melanoma, SCC, and possibly other tumors that depend on active Hh signaling.

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