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Review

Cancer Research

Sonic Hedgehog Signaling in Basal Cell Nevus Syndrome

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Abstract

The hedgehog (Hh) signaling pathway is considered to be a major signal transduction pathway during embryonic development, but it usually shuts down after birth. Aberrant Sonic hedgehog (Shh) activation during adulthood leads to neoplastic growth. Basal cell carcinoma (BCC) of the skin is driven by this pathway. Here, we summarize information related to the pathogenesis of this neoplasm, discuss pathways that crosstalk with Shh signaling, and the importance of the primary cilium in this neoplastic process. The identification of the basic/translational components of Shh signaling has led to the discovery of potential mechanism-driven druggable targets and subsequent clinical trials have confirmed their remarkable efficacy in treating BCCs, particularly in patients with nevoid BCC syndrome (NBCCS), an autosomal dominant disorder in which patients inherit a germline mutation in the tumor-suppressor gene *Patched* (*Ptch*). Patients with NBCCS develop dozens to hundreds of BCCs due to derepression of the downstream G-protein–coupled receptor Smoothened (SMO). *Ptch* mutations permit transposition of SMO to the primary cilium followed by enhanced expression of transcription factors Glis that drive cell proliferation and tumor growth. Clinical trials with the SMO inhibitor, vismodegib, showed remarkable efficacy in patients with NBCCS, which finally led to its FDA approval in 2012. *Cancer Res; 74(18); 4967–75. ©2014 AACR.*

Introduction

Skin cancers (basal cell and squamous cell carcinoma and melanoma) are the most common types of human malignancy with approximately 2.8 million new cases diagnosed annually in the United States (1–3). Activated Hedgehog (Hh) signaling driven by mutations in the tumor-suppressor gene *Patched* (*Ptch*) and/or the G-protein–coupled receptor Smoothened (SMO) is known to promote oncogenic signaling and drives the growth of basal cell carcinomas (BCC; ref. 4). Nevoid BCC syndrome (NBCCS), also known as Gorlin syndrome, is an autosomal dominant disorder in which affected individuals develop multiple (dozens to thousands) of microscopic and macroscopic BCCs, various benign hair follicle hamartomas, palmar, and plantar pits in addition to skeletal defects (bifid ribs and syndactyly), central nervous system abnormalities (calcification of the falx cerebri and agenesis of the corpus callosum), craniofacial features (enlarged skull, hypertelorism, and frontal bossing), and benign odontogenic keratocysts of the jaw (4, 5). In addition to BCCs, these patients are at increased risk for other tumors, including medulloblastomas, rhabdomyosarcomas, benign ovarian cysts, cardiac fibromas, and mesenteric cysts linked to aberrant/abnormally increased Sonic hedgehog (Shh) signaling (4).

Disease penetrance in NBCCS is known to be dependent on genetic background. Caucasians with sun-sensitive skin develop far more BCCs as compared with darker skinned individuals (6, 7). For example, the frequency of BCCs development is much higher in patients from the United States, Australia, and the United Kingdom compared with Asians and African-Americans. Similarly, mean age of BCCs onset in Caucasians is much lower than in patients from other Asian or African countries. However, no marked differences were reported in the frequencies of other clinical manifestations in these different populations (6, 7).

Family based linkage analysis identified underlying mutations in the *Patched 1* gene (*Ptch1*) located on chromosome 9 in these tumors (8). PTCH 1 is a highly conserved 12-pass transmembrane protein receptor that negatively regulates (tumor suppressor) the Hh signaling pathway. The Hh ligands namely Shh, Indian hedgehog (Ihh) and Desert hedgehog (Dhh) bind to PTCH 1, thereby releasing the 7-pass transmembrane protein SMO allowing its migration to the tip of the primary cilium in which a multistep process activates Gli transcription factors driving cell proliferation and tumor growth as summarized in Fig. 1. In vertebrates, three isoforms of Glis have been identified. Gli1 and Gli2 are transcription activators whereas Gli3 is generally a transcription repressor (4). Among the three Hh ligands, Shh has been the most widely studied. Shh overexpression in the skin induces epidermal hyperplasia by antagonizing p21-mediated cell-cycle arrest. It also regulates the growth and maturation of the dermal papillae of the hair follicle. It signals via Glis to activate the Brahma/SWI2–related gene 1 (Brg1) in bulge stem cells, which is important for hair

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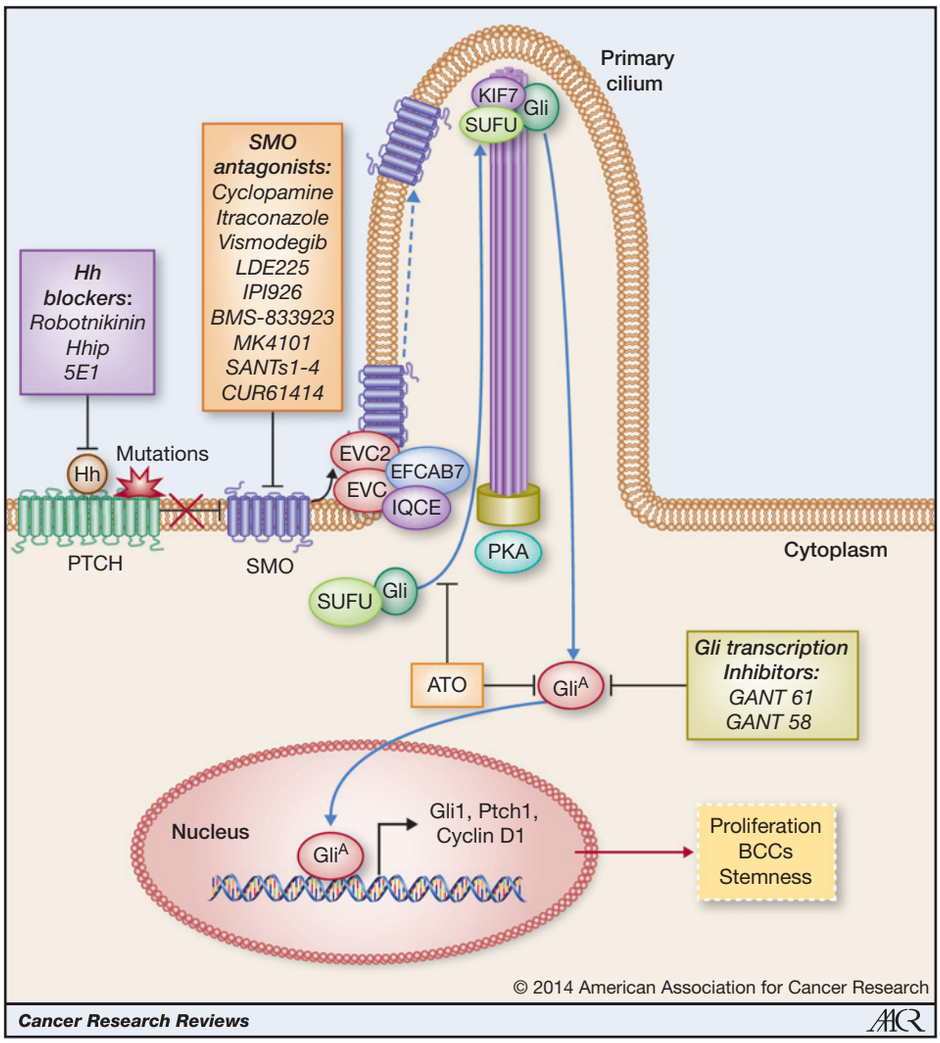

Figure 1. Primary cilium and activation of the Hh signaling pathway. Inactivating mutations in PTCH or binding of Hh ligands to PTCH derepresses SMO, thereby allowing its translocation onto the tip of the primary cilium, leading to transcriptional activation of Gli1/2. Multiple ciliary proteins are involved in processing Hh signal transduction. Ciliary EVC/EVC2 forms a complex with SMO that in turn is tethered by a complex of EFCAB7 and IQCE anchoring the EVC–EVC2 complex in a signaling microdomain at the base of the primary cilium. Activation and nuclear translocation of Gli1/2 involve dissociation of Gli1/2 from its endogenous inhibitor, SuFu. Kif7 cooperates with SuFu in catalyzing dissociation of the Sufu–Gli2 complex. Inhibition of Hh binding to Ptch by robotnikinin or blocking of SMO activation by small molecules such as cyclopamine, itraconazole, vismodegib, LDE225, etc., inhibits Hh signaling and tumor growth. Although ATO blocks translocation of Gli2 to the tip of cilia, GANT61 and GANT58 inhibit Gli1/2 transcriptional activities. Gli^A, Gli activator.

regeneration. Subsequently, Brg1 recruits NFκB required to sustain proliferation in hair matrix cells (9). Dhh overexpression like Shh may lead to marked expansion of epidermal progenitor cells, an increase in basal cell proliferative activity and a delay in basal cell differentiation (10). Ihh is highly expressed in human sebaceous skin tumors and human sebocyte cell lines. Recently, Kakanj and colleagues (9) showed the importance of Ihh in murine skin cancer pathogenesis by controlling proliferation and differentiation. It was also shown to block metastasis (9). Ihh has also been linked to the pathogenesis of Merkel cell carcinoma, a rare but potentially fatal epithelial skin neoplasm (11).

Upregulation of Hh signaling is considered the pivotal abnormality in all BCCs (whether in patients with NBCCS or in sporadic BCCs). Greater than 80% of sporadic BCCs have a loss-of-function mutation in at least one allele of *PTCH1* and the remainders have activating gain-of-function mutations in SMO. These mutations result in aberrant Hh signaling pathway activation. Although aberrant Shh signaling is primarily responsible for the pathogenesis of BCCs, it is also involved in the growth of squamous cell carcinomas (SCC). Both BCCs and SCCs apparently share a common cell of origin, within the interfollicular epidermis, hair follicle bulge, and hair germ because all can give rise to both tumor types, depending on the genetic milieu (for review see refs. 4, 12, 13).

Origin of BCCs

The cellular origin of BCCs has been a source of controversy for over a century. In 1903, BCCs were proposed to originate from the basal layer of the interfollicular epidermis. Other concepts focused on hair follicles or interfollicular epidermis and so-called "follicular germinative cells." Others proposed the involvement of cells from lower portion of the spinous cell layer (14, 15). In 1990, Cotsarelis and colleagues (16) traced a population of presumptive stem cells possessing a quiescent phenotype to the hair follicle bulge both in mouse and humans. On the basis of their long-lived nature, it was proposed that these cells accumulate multiple genetic mutations driving tumor formation (16). Youssef and colleagues (17) generated mice conditionally expressing constitutively active mutant SMO (SmoM2) and showed enhanced Hh signaling in various

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epidermal cellular compartments capable of driving the growth of BCCs. However, SmoM2 activation in bulge stem cells and their transient amplifying progenies did not induce BCCs. Using clonal analysis, these authors showed that BCCs arise from long-term resident progenitor cells of the interfollicular epidermis and the upper infundibulum of the hair follicle, thereby confirming the idea that expression of differentiation markers in tumor cells may not predict the origin of the cancer-initiating cells (17). Subsequently, using cell fate tracking of X-ray–induced BCCs in Ptch1$^{+/-}$ mice, Wang and colleagues (18) showed that these tumors were almost exclusively derived from the keratin 15–expressing stem cells of the bulge. Importantly, conditional p53 loss enhanced BCC growth from both bulge precursors and the interfollicular epidermis, at least in part by enhancing SMO expression (18).

Another remarkable study in this regard was the demonstration that phenotype of Hh-driven skin tumors is regulated by not only the cell of origin but also the tissue context and level of oncogenic signaling. Thus, nodular BCC-like tumors originate from a subset of stem cells localized in the lower bulge and secondary hair germ compartment whereas high-level signaling in the interfollicular epidermis is essential for the growth of superficial BCCs (19).

Mechanisms involving Hh activation

The mechanism of Shh activation may be tumor-specific (4). For BCCs or medulloblastomas, the pathway activation is mutation driven but in other cancers it may be regulated by autocrine or paracrine signaling. Constitutive activation of the Hh signaling pathway in tumors involving lung, stomach, esophagus, pancreas, prostate, breast, liver, and brain without somatic mutations affecting the Hh signaling pathway genes exhibits an autocrine, ligand-dependent activation of Hh signaling. Here, ectopic Hh ligand production in tumor cells or in a small subset of cancer stem cells (CSC) may prolong survival of the tumor cells, thereby contributing to overall tumor growth (20). Aberrant paracrine signaling in prostate and pancreatic models of carcinogenesis alters the tumor microenvironment to enhance aggressive tumor growth (21). In contrast, in hematologic malignancies, including multiple myeloma, lymphoma, and leukemia, Hh is directly secreted by the stromal cells, which are required for the Bcl2-dependent survival of the malignant B cells (22). Noncanonical Hh signaling involves regulation of Hh pathway components independent of downstream Gli-mediated transcription or direct interaction of these proteins with components of other molecular pathways and/or involves atypical interaction of core Hh pathway components with one another. For example, PTCH1 can interact directly with cyclin B1 and caspases. Shh-mediated regulation of cell migration important for tumor invasion and metastasis is independent of downstream components (reviewed in refs. 4, 23). Nonetheless, the role of noncanonical (independent of Gli transcription) Shh signaling in skin tumor growth remains obscure.

Shh signaling crosstalk

Recent studies have highlighted the crosstalk between Hh and other key oncogenic pathways (summarized in Fig. 2), among them include transforming growth factor beta (TGFβ), epidermal growth factor receptor (EGFR), insulin-like growth factor (IGF), tumor necrosis factor (TNF), and Wnt (24–29). We recently showed that the coding region determinant–binding protein (CRD-BP), a direct target of Wnt/β-catenin signaling, binds with Gli1 mRNA and regulates BCC development (30). Wang and colleagues (31) established a key role for crosstalk between the mammalian target of rapamycin (mTOR)/S6 kinase 1(S6K1) and Hh pathways in the pathogenesis of esophageal adenocarcinoma. The activated mTOR/S6K1 signaling promotes Gli1 transcriptional activity through S6K1-mediated Gli1 phosphorylation at Ser84, thereby releasing Gli1 from its endogenous inhibitor, Suppressor of Fused (SuFu). Moreover, inhibition of mTOR pathway signaling by rapamycin blocked S6K1 and augmented the cytocidal effects of the Hh pathway inhibitor. It also blocked Shh signaling, thereby inhibiting the growth of rhabdomyosarcomas in a mouse xenograft assay (32). A role for SuFu and Kif7 has recently been shown in the regulation of Gli2 in the pathogenesis of BCCs (33). Identification of four kinases, unc-51–like kinase 3 (Ulk3), kinesin family member 11 (Kif11), mitogen-activated protein kinase 10 (Map3K10), and dual specificity tyrosine-(Y)-phosphorylation–regulated kinase 2 (Dyrk2) with phenotype similar to Fused (Fu) is interesting but their role in the processing Hh signaling regulatory proteins remains to be defined (34–36).

Aberrant Hh also occurs during malignant progression. Yoo and colleagues (37) showed that Hh signaling enhanced tumor metastasis by driving epithelial–mesenchymal transition (EMT) through activation of the PI3K–Akt pathway and MMP-9. During this process, polarized epithelial cells are transformed into motile mesenchymal cells, thereby facilitating invasiveness and metastasis. Hh signaling also enhances EMT by upregulating the transcription factor Snail and downregulating E-cadherin. The vast majority of BCCs are locally invasive and rarely metastasize (38). De Craene and colleagues (39) showed that *in vivo* expression of Snail results in *de novo* epithelial carcinogenesis (including BCCs) by allowing enhanced survival, expansion of the CSC pool with accumulated DNA damage.

Primary cilium and Hh signaling

The primary cilium is a microtubule-based, membrane-enclosed structure present in all vertebrate cells that is essential for mammalian Hh activation (Fig. 1). Recently, we have verified its importance for skin and hair follicle homeostasis (40). It is known that SMO, and the Glis both localize to primary cilia in a manner gated by Hh pathway activity (41). This translocation suppresses protein kinase A (PKA)–mediated phosphorylation of Gli transcription factors and induces dissociation of Gli proteins from their inhibitor SuFu (42). PKA-mediated phosphorylation of Glis is important in controlling their tissue levels by targeting them to ubiquitination-dependent proteosomal degradation (43).

Ciliopathies is a term used to describe an emerging group of diseases associated with aberrant ciliary function. Ellis–van Creveld syndrome (EVC) or chondroectodermal dysplasia, an autosomal recessive disorder manifests polydactyly,

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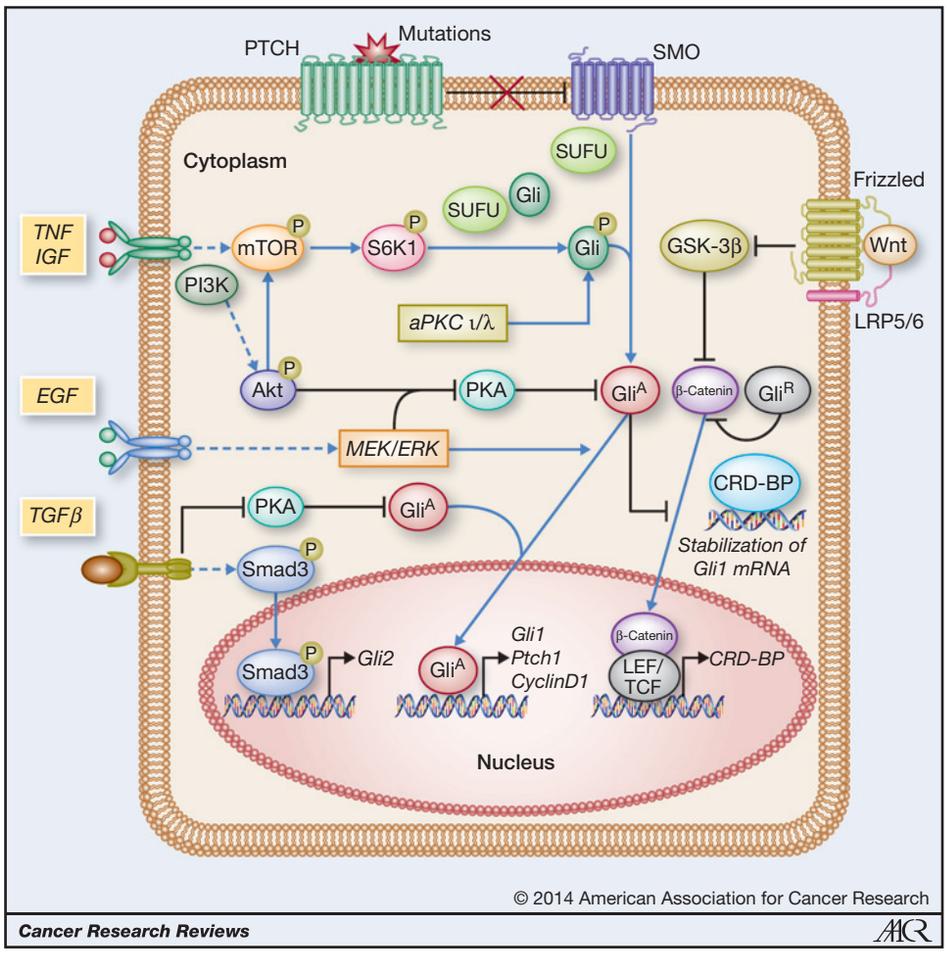

Figure 2. Hh pathway crosstalk with other signaling pathways. The Hh signaling pathway demonstrates complex crosstalk with multiple signaling pathways that modulate cancer pathogenesis. The TNF–mTOR pathway activates Gli1 in an SMO-independent manner. Activated mTOR enhances Gli1 transcriptional activity and oncogenic functions via S6K1-mediated Gli1 phosphorylation at Ser84, which helps to release Gli1 from SUFU. The IGF/PI3K/Akt pathway regulates Gli1/2 activities involving modulation of PKA-dependent phosphorylation of Glis and so are the EGFR/MEK/ERK or TGFβ signaling pathways. In addition, TGFβ can directly induce *Gli2* expression independent of Hh signaling and requires Smad3. Wnt signaling also modulates Hh activity although the underlying cascade remains unclear. CRD-BP, a direct target of Wnt/β-catenin signaling, can bind with Gli1 mRNA promoting tumor growth. Hh signaling can also regulate Wnt signaling through modulation of β-catenin. aPKC-ι/λ acts downstream of SMO to phosphorylate and activate Gli1, resulting in its maximal DNA-binding and transcriptional activation to Gli^A, Gli activator; Gli^R, Gli repressor; TCF, T-cell factor; LEF, lymphoid enhancer–binding factor.

congenital heart defects, and short-limbed dwarfism among other abnormalities (44). A closely related disorder is Weyer acrofacial dysostosis (WAF) that is inherited in an autosomal dominant pattern and is characterized by abnormalities of the teeth and nails, extranummary digits and moderately restricted growth (45, 46). Many of these abnormalities occur in patients with NBCCS. Ciliary dysfunction in EVC has been linked to a mutation in two adjacent genes on chromosome 4 known as *EVC* and *EVC2* that participate in the development of cilia (46). The *EVC* gene encodes for EVC protein, whereas *EVC2* codes for another protein called limbin. *EVC2* mutations also underlie WAF (47).

Following Shh stimulation, Ptch1 suppression of Smo is relieved allowing its translocation to the primary cilium. This translocation of Smo to the primary cilium is essential for Shh-mediated downstream signaling. In mice, intraflagellar transport (IFT) component IFT172 is required for targeting SMO to cilia (48). Dorn and colleagues (47) have identified an SMO–EVC2 signaling complex that localizes to the EvC zone in a distinct portion of the membrane compartment in primary cilia. This is a critical transduction step between SMO and PKA/SuFu (suppression of Gli proteolytic processing), and results in the inhibition of Gli3 repressor formation (recruitment of Gli proteins and SuFu to the tips of cilia) and the induction of activated Gli2 and Gli3 (47). In addition, Yang and colleagues (49) demonstrated that Hh activates SMO by inducing its phosphorylation, which recruits EVC/EVC2 to activate Gli proteins by antagonizing Sufu. More recently, Pusapati and colleagues (50) have identified a complex between two ciliary proteins EF-hand calcium-binding domain 7 (EFCAB7) and IQ domain-containing protein E (IQCE) that positively regulates Hh signaling (Fig. 1). ECFAB7–IQCE anchors the EVC/EVC2 in a signaling microdomain at the base of the primary cilium (50). It is also known that cilia are unique calcium signaling

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organelles regulated by a heteromeric transient receptor potential channel polycystic kidney disease 1–like 1 (PKD1L1)–PKD2L1 that controls ciliary Ca^{2+} concentration and regulates SMO-activated Gli2 translocation and Gli1 activation (51).

Murine models

Murine models of skin carcinogenesis have been major contributors to current understanding of the molecular pathogenesis of nonmelanoma skin cancers, including BCCs and SCCs (12). In particular, murine models of BCCs have clarified the role of Hh signaling in the development of this tumor (reviewed in refs. 4, 12). In a major advance, Oro and colleagues (52) developed *Ptch*$^{+/-}$ knockout mice that have proven extremely useful in expanding knowledge of the pathogenesis and prevention of UVB-induced BCCs. These animals were engineered by deleting exons 1 and 2 and inserting the *Lac Z* reporter gene at the deleted site. These animals spontaneously develop only a few BCCs, similar to the pattern of growth of sporadic BCCs in humans. They also display rarely some phenotypic characteristics of patients with NBCCS. Exposure of these mice to ultraviolet B (UVB) and X-ray greatly augments the growth of BCCs (53).

One example of the utility of these mice is their potential usefulness in defining crosstalk in cancer-relevant signaling pathways. For example, we previously showed that increased expression of ornithine decarboxylase (ODC) enhances the proliferation of BCC cells using *Ptch*$^{+/-}$ mice overexpressing K6-driven ODC. These mice developed a few macroscopic BCC-like lesions spontaneously, and following chronic exposure to UVB developed lesions over the dorsal skin in a pattern similar to that seen in patients with NBCCS (54). An alternate approach for assessing Hh downstream target genes in the pathogenesis of BCCs was the development of transgenic mice overexpressing K5 promoter–driven *Gli1* or *Gli2*. These animals exhibited multiple BCC-like lesions on the ears, tail, trunk, and dorsal aspects of the paws. Albino mice showed characteristics of sporadic human BCCs, whereas pigmented strains exhibited features of pigmented BCCs (55).

Regulation of stemness

Hh signaling can also regulate the self-renewal of CSCs. CSCs are slow-growing cells that often manifest resistance to conventional chemotherapy and radiation protocols. This seems to facilitate cancer relapse after tumor debulking by conventional therapeutic modalities. Thus, Hh inhibition offers a mechanism-driven approach to inhibiting tumor-forming CSCs, ideally in combination with other conventional cancer treatment modalities. Colmont and colleagues (56) isolated BCC cells from human tumors and identified a small subpopulation of $CD200^{+}CD45^{-}$ cells comprising $1.63\% \pm 1.11\%$ of all BCC cells residing at the tumor periphery. In tumor xenografts, tumor-initiating cell (TIC) frequencies approximate one per 1.5 million unsorted BCC cells. These $CD200^{+}CD45^{-}$ BCC cells recreated BCC tumor growth *in vivo* with as few as 10,000 cells whereas $CD200^{-}CD45^{-}$ BCC cells formed no tumors. These data suggest that CSCs exist as subpopulations of BCC TICs that could be targeted to reduce tumor recurrence when Hh inhibitors are discontinued.

Multidrug resistance (MDR) is a major cause of resistance to anticancer therapy. MDR enhances drug efflux from cancer cells mediated by members of the ATP-binding cassette (ABC) transporter family. Sims-Mourtada and colleagues (57) have shown that Hh signaling blockade increases the response of cancer cells to numerous forms of chemotherapy by regulating ABC transporter proteins. These results suggest that the Hh pathway may be a target to overcome MDR, thereby increasing the chemotherapeutic response.

Inhibitors of Shh signaling pathway

Cyclopamine was the first Shh inhibitor identified and has proven useful to complement genetic experiments verifying the growth-promoting/tumorigenic role of Hh signaling (For review please see (4, 12, 58). Cyclopamine was shown to bind with SMO and antagonize its downstream signal transducing functions (59). Using a UVB-induced BCC photocarcinogenesis murine model, our group was the first to show that cyclopamine prevents BCCs (60). Trials assessing its feasibility for human use revealed multiple drawbacks, including low water solubility, weak SMO affinity, poor oral bioavailability and suboptimal pharmacokinetics, and pharmacodynamics, nonspecific cytotoxicity, off-target apoptosis inducing effects with troubling side effects (61–64).

As the role of aberrant Hh signaling in multiple forms of potentially lethal malignancies unfolded, these efforts were redoubled, leading to the discovery of several small molecules (Fig. 1), including SANT1–SANT4, CUR-61414, HhAntag-691, GDC-0449, MK4101, IPI-926, BMS-833923, and others (only a few of them are discussed here due to space constraints; refs. 4, 12). On the basis of preclinical assays, Hh-blocking antibodies that act upstream of SMO such as the 5E1 monoclonal antibody that binds to Shh ligand and disrupts protein binding to the receptor Ptch (Fig. 1) seem promising (4).

Another strategy seeks mimetics of various natural Shh inhibitors such as Hedgehog-interacting protein (Hhip), SUFU, etc., and agents (GANT61 and GANT58) that act on downstream Gli proteins (Fig. 1; ref. 4). We have shown the importance of Hhip in the pathogenesis and prevention of BCCs (65). Robotnikinin, a small molecule that binds with the Shh protein may be an approach to inhibit Shh signaling in tumor stromal cells (Fig. 1; ref. 66). Recently, Atwood and colleagues (67) showed that an atypical protein kinase C ι/λ (aPKC-ι/λ) acts as a novel regulator of Gli (Fig. 2). The concept here is that targeting aPKC-ι/λ in SMO inhibitor–resistant BCCs could be efficacious (67).

In a screen of drugs previously tested in humans, Kim and colleagues (68) identified itraconazole, a systemic imidazole antifungal, as a potent antagonist of the Hh signaling pathway that acts to inhibit SMO by a mechanism distinct from that of other SMO antagonists (Fig. 1). It prevents the ciliary accumulation of SMO that follows Hh stimulation (68). Itraconazole suppressed Hh pathway activity and the growth of Hh-driven medulloblastoma in a mouse allograft model at serum levels comparable with those found in patients undergoing antifungal treatment with the drug. Recently, this drug showed some efficacy in a clinical trial against BCCs in NBCCS (69). Arsenic trioxide (ATO) has been FDA-approved for the treatment of acute promyelocytic leukemia (APL), since 2000. ATO inhibits

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growth of Hh pathway–driven medulloblastoma allografts derived from $Ptch^{+/-}p53^{-/-}$ mice within a range of serum levels comparable with those achieved in treating human APL (70). Similarly, arsenic can block Hh-induced ciliary accumulation of Gli2 (71). In addition, it can also directly bind with Gli1 and can inhibit its activity independent of primary cilia (Fig. 1; ref. 72). Tang and colleagues (73) showed that Vitamin D3 inhibits keratinocyte proliferation and Hh signaling with efficacy matching that of cyclopamine. These Hh inhibitory effects are Vitamin D receptor independent. Topical application of Vitamin D3 to murine BCCs decreased Gli1 and Ki67 staining (73). Interestingly, patients with NBCCS are frequently Vitamin D deficient (74).

Clinical Trials with Small Molecular Weight Inhibitors of Shh Signaling

Over the past 15 years, we have conducted several clinical trials to assess the efficacy and safety of mechanism-driven agents capable of targeting various components of the Hh signaling pathway (Fig. 1) and various Hh-unrelated molecular targets as an approach to inhibit the growth of BCCs in patients with NBCCS (75–78). However, in these studies as well as those done by others, efforts with Hh inhibition proved more effective than blocking other Shh signaling-unrelated molecular targets (75, 76).

Topical formulations

The antitumor efficacy of topically applied cyclopamine was evaluated in patients with BCCs who were scheduled for surgical excision of the tumor (77). All of the cyclopamine-treated tumors regressed clinically and reduced cell proliferation and enhanced differentiation and apoptosis of tumor cells, were confirmed histologically (77). Topically applied CUR61414, an SMO inhibitor, was ineffective (78). Another SMO inhibitor, LDE225 in a double-blind, randomized, vehicle-controlled, intraindividual study in human subject showed limited therapeutic efficacy (79).

Oral agents

Vismodegib (GDC-0449) is structurally related to cyclopamine and a potent Smo inhibitor. Several multicenter trials have been conducted with orally administered vismodegib. In a phase I clinical trial, the safety and pharmacokinetics were tested in patients with metastatic or locally advanced BCCs. The median duration of the study was 9.8 months and 18 of 33 patients had an objective response to drug (80). Another phase I trial in 68 patients with solid tumors refractory to current therapies or for which no standard therapy existed, was conducted. Adverse events, tumor responses, pharmacokinetics, pharmacodynamics, and downmodulation of Gli1 expression in noninvolved skin were assessed. On the basis of this study, the recommended daily dose was 150 mg/d (81). Less frequent administration of the drug showed comparable safety, tolerability, and steady-state levels of total and unbound vismodegib as continuous daily dosing (82).

We conducted a randomized, double-blind, placebo-controlled trial of vismodegib in patients with NBCCS at three clinical centers. The primary end-point was reduction in the incidence of new surgically eligible BCCs (SEB) with vismodegib versus placebo after 3 months; secondary end points included reduction in the size of existing BCCs. In 41 patients followed for a mean of 8 months (range, 1–15) after enrollment, the per patient rate of new SEBs was lower with vismodegib than with placebo (2 vs. 29 cases/group/year, $P < 0.001$), as was the size (the percentage of change from baseline in the sum of the longest diameter) of existing SEBs (-65% vs. -11%, $P = 0.003$). In some patients, all BCCs clinically regressed and no tumors progressed during treatment with vismodegib. Patients receiving vismodegib reported grade 1 or 2 adverse events of dysgeusia (loss of taste), muscle cramps, hair loss, and weight loss. Overall, 54% of patients (14 of 26) receiving vismodegib discontinued drug treatment due to adverse events. At 1 month, vismodegib use had reduced Shh target gene expression in BCCs by 90% ($P < 0.001$) and diminished tumor cell proliferation, but apoptosis was unchanged. No residual BCC tumor was histologically detectable in 83% of biopsy samples taken from sites of clinically regressed BCCs (83).

Another multicenter, international, two-cohort, nonrandomized study of vismodegib, was conducted in patients with metastatic BCC or with locally advanced BCC with inoperable disease or for whom surgery was inappropriate. In 33 patients with metastatic BCC, the independently assessed response rate was 30% whereas in 63 patients with locally advanced BCC, the independently assessed response rate was 43%. The median duration of response was 7.6 months in both cohorts (84).

On the basis of these preclinical and clinical investigations, vismodegib was approved by the FDA for the treatment of recurrent, locally advanced, or metastatic BCCs (85). Several vismodegib-treated patients in these trials developed invasive keratoacanthomas by an unknown mechanism (86, 87). Thus, patients receiving this drug should be monitored carefully for this complication.

In addition to the risk of developing keratoacanthomas, a major challenge with vismodegib is the rapid development of drug resistance that limits its efficacy (88). Induction of new mutations in Smo seems to be responsible for the acquired resistance. Yauch and colleagues (88) identified this mutation as a heterozygous G-to-C missense mutation at position 1697 predicted to change codon 473 from Asp to His. Although this mutant was found to be competent to transduce Hh signaling, it led to loss of SMO binding by vismodegib (88). It was also shown that the PI3K pathway is upregulated in these refractory tumors and PI3K inhibition may significantly delay tumor growth (89).

Summary and Future Prospects

Aberrant Shh signaling is the major driver of both sporadic BCCs and the BCCs that occur in patients with NBCCS as well as many other tumors in which this pathway is activated. Additional Hh signaling modifying proteins seem to be capable of modulating the growth of these tumors in a noncanonical setting. Hh signaling is not considered important in the initiation of other common epidermal cancers such as SCCs but may be involved in the later stages of tumor growth or in

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the development of specific subtypes of SCCs showing altered differentiation patterns. The growth of BCCs may also use crosstalk with other signaling pathways such as mTOR, PI3K–Akt, and/or Wnt. Clarification of the mechanistic basis for such crosstalk may help to identify additional novel targets that could inhibit tumor growth. SMO inhibitors have been shown to have substantial antitumor efficacy in sporadic and NBCCS-associated BCCs. Unfortunately, SMO resistance develops rapidly in many instances (88, 90). In this regard, a search for agents capable of inhibiting SMO by identifying new binding sites could prove useful. Furthermore, targets downstream of SMO such as Gli could also have potential, although none have yet been shown to be clinically effective. Exploration of novel approaches to inhibiting this pathway could prove useful in other epithelial/hematologic neoplasms in which Hh signaling is known to play key roles in tumor development. In this regard, combinational approaches using therapeutic agents targeting other pathways that drive the growth of these tumors should also be explored.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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