Molecular Pathways: Novel Approaches for Improved Therapeutic Targeting of Hedgehog Signaling in Cancer Stem Cells

Verline Justilien and Alan P. Fields

Abstract

The Hedgehog (Hh) signaling pathway is critical for embryonic development. In adult tissues, Hh signaling is relatively quiescent with the exception of roles in tissue maintenance and repair. Aberrant activation of Hh signaling is implicated in multiple aspects of transformation, including the maintenance of the cancer stem cell (CSC) phenotype. Preclinical studies indicate that CSCs from many tumor types are sensitive to Hh pathway inhibition and that Hh-targeted therapeutics block many aspects of transformation attributed to CSCs, including drug resistance, relapse, and metastasis. However, to date, Hh inhibitors, specifically those targeting Smoothened [such as vismodegib, BMS-833923, saridegib (IPI-926), sonidegib/erismodegib (LDE225), PF-04449913, LY2940680, LEQ 506, and TAK-441], have demonstrated good efficacy as monotherapy in patients with basal cell carcinoma and medulloblastoma, but have shown limited activity in other tumor types. This lack of success is likely due to many factors, including a lack of patient stratification in early trials, cross-talk between Hh and other oncogenic signaling pathways that can modulate therapeutic response, and a limited knowledge of Hh pathway activation mechanisms in CSCs from most tumor types. Here, we discuss Hh signaling mechanisms in the context of human cancer, particularly in the maintenance of the CSC phenotype, and consider new therapeutic strategies that hold the potential to expand considerably the scope and therapeutic efficacy of Hh-directed anticancer therapy.

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Learning Objectives

Upon completion of this activity, the participant should have a better understanding of Hedgehog signaling mechanisms in cancer stem cells and the biologic rationale for evaluating combined Hedgehog and PKC inhibition for treatment of patients whose tumors harbor 3q26 amplification.

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Background

Hedgehog (Hh) is a highly conserved developmental pathway involved in organogenesis, stem cell maintenance, and tissue repair/regeneration. Aberrant Hh pathway activation controls multiple aspects of tumorigenesis, including initiation, progression, and relapse, at least in part, by driving a cancer stem cell (CSC) phenotype. Mutational Hh pathway activation drives tumor formation in several tumor types, and many other tumors exhibit epigenetic Hh pathway activation. Small-molecule Hh inhibitors have been used as monotherapy and in combined modalities for cancer treatment. To date, however, Hh inhibitors have enjoyed limited success clinically. Here, we discuss oncogenic Hh signaling mechanisms and highlight new therapeutic strategies that may enhance the clinical efficacy and expand the effective use of Hh inhibitors to new tumor types.

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The canonical Hh signaling pathway

Core Hh signaling components include the Hh ligands [sonic Hh (Shh), Indian Hh (Ihh), and Desert Hh (Dhh)], the transmembrane receptor proteins Patched 1 and 2 (PTCH1 and PTCH2), the G-protein-coupled receptor-like protein smoothed (SMO), and the glioma-associated oncogene transcription factors 1 to 3 (GLI1, GLI2, and GLI3; reviewed in ref. 1; Fig. 1). Primary cilia localize these components to activate or repress signaling (2). Canonical Hh signaling is activated when Hh ligand binds PTCH to relieve PTCH-mediated SMO inhibition at the base of the primary cilium (3). SMO then translocates to the cilium tip (4), driving a signaling cascade that results in nuclear GLI transcription and activation. GLI activates transcription of context-specific genes regulating self-renewal, cell fate, survival, angiogenesis, epithelial-to-mesenchymal transition, and cell invasion (reviewed in ref. 5). As Hh transcriptional targets, GLI1 and PTCH1 establish a feedback loop that regulates Hh signaling (6).

Several auxiliary proteins promote or suppress Hh pathway activity (Fig. 1). Hh ligands are synthesized as precursors that undergo autocatalytic cleavage, addition of a carboxy-terminal cholesterol moiety, and amino-terminal palmitoylation mediated by Skinny Hh/Hh acyltransferase (Ski/HHAT) to produce mature ligand, the secretion of which is facilitated by the transmembrane transporter-like protein dispatched (Disp; ref. 1). Growth arrest-senescence 1 (GAS1), CAM-related/downregulated by oncogenes (CDO), brother of CDO (BOC), and glypican-3 are coreceptors that facilitate ligand binding to PTCH (1), whereas Hedgehog interacting peptide represses signaling by sequestering Hh ligand (7). Protein kinase A (PKA), glycogen synthase 3β (GSK3β), casein kinase 1 (CK1), Skip–Gullin–Fbox protein, βtransducin repeat containing protein (βTrCP), and a suppressor complex composed of fused kinase (Fu), suppressor of fused (SUFU), and costal2 (Cos2) regulate GLI expression, stability, and localization (reviewed in ref. 1). Alterations in one or more of these modulatory mechanisms can lead to pathway deregulation and cancer.

Hh signaling in cancer

Both ligand-dependent and ligand-independent mechanisms result in aberrant Hh pathway activation in cancer. Germline or somatic loss-of-function PTCH1 or SUFU, and gain-of-function SMO, mutations constitutively activate ligand-independent Hh signaling and drive basal cell carcinoma (BCC), medulloblastoma (MB), rhabdomyosarcoma, and meningioma tumor development (8–11). GLI1 amplification occurs in glioblastoma and rhabdomyosarcoma, and activating mutations in GLI1 and GLI3 are evident in pancreatic adenocarcinomas (12–14), although the function of these mutations is not fully explored. Pallister–Hall syndrome, characterized by formation of benign hypothalamic hamartomas, is caused by a frameshift GLI3 mutation that generates a C-terminal truncated protein resembling physiologically generated GLI3 repressor (15).

Hh signaling can also drive the transformed phenotype through autocrine or paracrine ligand-dependent mechanisms. Autocrine activation ensues when Hh ligand produced by tumor cells activates Hh signaling in the same or neighboring tumor cells to stimulate survival and tumor growth. Autocrine Hh pathway activation occurs in lung, pancreas, stomach, colon, skin, prostate, breast, and brain cancers (16–23). In these tumors, SMO inhibitors block tumor cell growth in the absence of stromal cells. Paracrine Hh pathway activation occurs when tumor cells secrete Hh ligands that induce Hh activation in stromal cells, which then promote tumor growth by producing angiogenic factors (i.e., IGF and VEGF) and H6 and Wnt signaling activation (24–27). Para-crine Hh signaling occurs in pancreatic, lung, esophageal, gastric, colon, lymphomas, multiple myelomas, and prostate cancers (25, 27–34). Reverse paracrine Hh signaling has also been described in lymphomas and multiple myelomas, in which Hh ligand produced in bone marrow stroma activates Hh signaling in adjacent tumor cells (35).

Hh pathway activation in cancer stem cells

Lineage tracing studies have demonstrated the existence of a subpopulation of tumor cells exhibiting stem-like properties (36–38). These tumor-initiating cells or CSCs exhibit self-renewal, enhanced tumor initiation, and differentiation into transiently amplifying cells that populate the bulk tumor. These cells function in tumor maintenance, metastasis, relapse, and chemoresistance. Hh signaling drives CSC maintenance in lung, breast, pancreas, and colon cancers; glioblastoma; multiple myeloma; and chronic myelogenous leukemia (CML; refs. 16, 18, 20, 22, 39–42). Hh signaling is selectively activated in CSCs compared with bulk tumor cells from these tumor types (18, 20, 22, 41, 42), and directly drives the CSC phenotype by regulating expression of CSC markers aldehyde dehydrogenase, BM1, WNT2, and CD44 (20, 27, 43). Pharmacologic or genetic Hh inhibition in these tumor types decreases self-renewal, tumor growth, and metastasis (16, 18, 20, 22, 39–42). Hh signaling also regulates ABCG2 and MDR expression, suggesting a role in the chemoresistance characteristic of CSCs (44–48).

Clinical-Translational Advances

Hh pathway inhibitors

Four major modes of Hh inhibition have been exploited therapeutically: (i) SMO inhibition; (ii) receptor-ligand disruption; (iii) inhibition of ligand processing; and (iv) GLI inhibition (Fig. 1). Cyclopamine, a naturally occurring SMO inhibitor, established Hh as a viable therapeutic target (49, 50). Although cyclopamine is not clinically useful due to its low potency and bioavailability, more potent and specific SMO inhibitors vismodegib, BMS-833923, saridegib (IPI-926), sonidegib (erismodgib) (LDE225), PF-04449913, LY2940680, LEQ 506, and TAK-441 (Fig. 1) have been developed and evaluated clinically (Table 1, Clinicaltrials.gov). SMO inhibitors are particularly effective against MBs and BCCs harboring SMO or PTCH mutations, and FDA approval of vismodegib for advanced BCC solidified Hh as a bona fide therapeutic target (49, 50).

Hh signaling has also been blocked by disrupting Hh ligand–PTCH interactions (Fig. 1). The Hh ligand monoclonal antibody 5E1, and the macrocyclic small-molecule robustiniakinib, inhibit Hh:PTCH interactions and exhibit antitumor activity (51, 52). Small-molecule inhibitors of Ski/HHAT, an enzyme that catalyzes a key step in Hh ligand processing, have recently been developed. HHAT inhibitors block Hh palmitoylation and prevent pathway activation (53). Agents such as GANT58/GANT61 and HPI 1–4 act by blocking GLI processing, activation, and/or transcriptional activity (54, 55). These agents may be particularly useful in treating tumors exhibiting ligand-independent Hh pathway activation. Although proof-of-concept has been demonstrated with 5E1, robotnikinin, HHAT, and GLI inhibitors in vitro, further testing is required before these agents can be clinically evaluated.
Figure 1. Schematic of Hh signaling in vertebrates. A, in the absence of Hh ligand, PTCH prevents SMO localization to the primary cilium and GLI is suppressed by a protein complex composed of Cos2, Fu, SUFU that promotes PKA, GSK3, and CK1-mediated GLI phosphorylation and partial proteosomal processing of GLI into a repressor form that inhibits expression of Hh target genes. B, Hh ligand requires N-terminal palmitoylation mediated by HHAT to be activated and released into the extracellular space by Disp. The Hh pathway is activated upon Hh ligand binding to PTCH and the coreceptors GAS1, CDO, BOC, which relieves PTCH-mediated inhibition of SMO. Upon activation, SMO translocates to the primary cilia, where it disrupts the repressor protein complex, resulting in GLI translocation to the nucleus and activation of GLI-mediated transcription of gene targets that maintain a CSC phenotype. The Hh pathway can be therapeutically targeted by: (i) SMO inhibition (vismodegib, BMS-833923, IPI-926, LDE225, PF-04449913, LY2940680, LEQ 506, TAK-441 and cyclopamine; (ii) receptor-ligand disruption (5E1 anti-Hh ligand antibody and robotnikinin); (iii) inhibition of ligand processing (HHAT inhibitor RU-SKI 43); or (iv) inhibition of GLI activity (GANT58, GANT61 and HPI 1-4).
Targeting Hh in CSCs

CSCs are emerging therapeutic targets whose efficient elimination may offer longer lasting, potentially curative outcomes in patients with cancer. Hh pathway inhibition is a promising approach to therapeutically target CSCs. Cyclopamine preferentially inhibits pancreatic CSCs but not bulk tumor cells (41), and GLI or SMO gene silencing, or cyclopamine, decreases glioblastoma CSC proliferation, survival, and self-renewal (16, 39).

Table 1. Preclinical and clinical response to SMO inhibition in various tumor types and their chromosome 3q26 amplification status

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Response preclinically</th>
<th>Response clinically clinicaltrials.gov NCT #</th>
<th>3q26 status % with copy-number gains</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCC</td>
<td>Suppressed proliferation, induced apoptosis and regression of lesions</td>
<td>Antitumor activity in metastatic and locally advanced BCC; vismodegib FDA approved for advanced BCC</td>
<td>(74, 75)</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>Decreased proliferation, tumor growth and metastasis</td>
<td>NCT01576666; recruiting patients</td>
<td>25% (TCGA)</td>
<td>(31, 76)</td>
</tr>
<tr>
<td>Cervical</td>
<td>Decreased cell proliferation and survival</td>
<td>NCT01456676; recruiting</td>
<td>77% (TCGA)</td>
<td>(77)</td>
</tr>
<tr>
<td>Chronic</td>
<td>Sensitized cells to chemotherapy; prolonged survival in leukemia mouse model; decreased</td>
<td>NCT01357655; ongoing, not recruiting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>myelogenous</td>
<td>tumorigenic potential of leukemic stem cell population.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>Blocked cell growth in vitro and growth of xenograft tumors in vivo; decreased</td>
<td>NCT00636610; vismodegib does not add to the efficacy of FOLFOX, FOLFIRI, or bevacizumab</td>
<td>11% (TCGA)</td>
<td>(22, 27, 80, 81)</td>
</tr>
<tr>
<td>Esophageal</td>
<td>Decreased cell growth and induced apoptosis</td>
<td>NCT01909402; completed, no results reported</td>
<td>53%</td>
<td>(82, 83)</td>
</tr>
<tr>
<td>Gastric</td>
<td>Decreased cell growth and induced apoptosis</td>
<td>NCT00982592; addition of vismodegib to FOLFOX did not improve PFS in an unselected population</td>
<td>35%</td>
<td>(84, 85)</td>
</tr>
<tr>
<td>Gliomas</td>
<td>Decreased self-renewal of CSCs and potentiated the antiproliferative effect of conventional chemotherapy</td>
<td>NCT01576666; recruiting patients</td>
<td>14% (TCGA)</td>
<td>(39)</td>
</tr>
<tr>
<td>Head and neck</td>
<td>Decreased colony formation in primary tumor cells ex vivo</td>
<td>NCT01576666; recruiting patients</td>
<td>74% (TCGA)</td>
<td>(86)</td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>Blocked proliferation and invasion in vitro and xenograft tumors in vivo</td>
<td>NCT01576666; recruiting patients</td>
<td>17% (TCGA)</td>
<td>(87)</td>
</tr>
<tr>
<td>Kidney</td>
<td>Decreased cell growth and caused tumor regression in vivo</td>
<td>NCT01576666; recruiting patients</td>
<td>15% (TCGA)</td>
<td>(88)</td>
</tr>
<tr>
<td>Lung</td>
<td>Suppressed growth of small-cell lung cancer cells in vitro and in vivo; prevented small-cell lung cancer tumor recurrence after chemotherapy treatment. Inhibited growth of LSCC CSCs</td>
<td>NCT01579929; recruiting</td>
<td>84% LS (TCGA)</td>
<td>(18, 23, 89)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>Induced apoptosis and inhibits growth of cancer cells in mice</td>
<td>NCT01722292; recruiting</td>
<td>32% LAC (TCGA)</td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>Antitumor activity in mouse models</td>
<td>NCT00939484; sustained response in 15% of patients</td>
<td>29% (TCGA)</td>
<td>(29)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Reduced proliferation, prevented recurrence, and lung metastasis</td>
<td>NCT01722292; recruiting</td>
<td>20%</td>
<td>(21)</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>Reduced proliferation, induced apoptosis, and blocked tumorigenicity</td>
<td>NCT01576666; recruiting patients</td>
<td></td>
<td>(93, 94)</td>
</tr>
<tr>
<td>Neuro-endocrine</td>
<td>Blocked cell growth in vitro</td>
<td>NCT00739661; no clinically meaningful improvement in progression-free survival for vismodegib vs. placebo</td>
<td>83%, serous (TCGA)</td>
<td>(96-99)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>Decreased proliferation, mobility, and invasiveness; induced cancer cell dedifferentiation and apoptosis in vitro and decreased tumor growth in vivo.</td>
<td>NCT0096732; terminated</td>
<td>83%, serous (TCGA)</td>
<td>(96-99)</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Blocked growth, migration, invasion, colony formation, the CSC population, tumor growth, and metastasis.</td>
<td>NCT00878163; active, not recruiting</td>
<td>20% (TCGA)</td>
<td>(100, 101)</td>
</tr>
<tr>
<td>Prostate</td>
<td>Prevented tumor growth in xenograft model.</td>
<td>NCT0163084; ongoing, not recruiting</td>
<td>15% (TCGA)</td>
<td>(54)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>Decreased rhabdosarcoma cell proliferation, induced apoptosis, and blocked tumor growth in vivo; decreased osteosarcoma cell growth in vitro and tumor growth in vivo.</td>
<td>NCT0154452; recruiting</td>
<td>13% (TCGA)</td>
<td>(102, 103)</td>
</tr>
<tr>
<td>Uterine</td>
<td>Decreased cell growth in vitro</td>
<td>NCT01576666; recruiting patients</td>
<td>64% (TCGA)</td>
<td>(104)</td>
</tr>
</tbody>
</table>

Abbreviations: LAC, lung adenocarcinoma; LSCC, lung squamous cell carcinoma; SCLC, small-cell lung cancer; TCGA, The Cancer Genome Atlas.
shRNA-mediated knockdown of HHAT or GLI1, or treatment with SMO inhibitor LDE225, blocks growth of lung squamous cell carcinoma (LSCC) CSCs in vitro and tumor formation in vivo (18). Cyclopamine or SE1 antibody reduces multiple myeloma CSC self-renewal and induces terminal differentiation (56). Likewise, Smo inhibition reduces expansion of Bcr-Abl–positive leukemic stem cells in vitro and delays relapse in a mouse CML model (40). Interestingly, inhibition is independent of Bcr-Abl mutation status, indicating that imatinib-resistant leukemic stem cells may retain responsiveness to Hh inhibition.

**Drug combinations therapeutically target Hh in CSCs**

SMO inhibitors are extremely effective against BCC and MB tumors that harbor driver Hh pathway mutations (Table 1). However, despite promising preclinical results, SMO inhibitors have yielded little or no clinical benefit in tumors not harboring pathway mutations (Table 1). The poor clinical performance of SMO inhibitors beyond BCC and MB may be due, at least in part, to cross-talk between Hh and EGFR, RAS–MEK–ERK, PI3K–AKT–mTOR, NOTCH, and/or WNT oncogenic signaling pathways (reviewed in ref. 57). Hh and EGFR pathways can activate each other, and cooperate to induce GLI1 transcriptional targets and promote tumor growth (58). Oncogenic KRAS–MEK–ERK signaling promotes tumorigenesis through paracrine, SMO-independent regulation of GLI1 expression, phosphorylation, degradation, nuclear localization, and activation (59). The PI3K–AKT–mTOR pathway also regulates Hh signaling through GLI1 phosphorylation, nuclear localization, and activation (60). In addition, Shh mediates epithelial-to-mesenchymal transition and metastasis through PI3K/AKT/mTOR activation (61), whereas GLI1 appears to suppress the WNT pathway in colon cancer cells (62). Thus, cross-talk between Hh and other oncogenic signaling pathways may significantly alter clinical response to Hh pathway inhibition and limit efficacy.

Clinical efficacy of Hh inhibitors may be significantly enhanced through rational patient stratification based on advanced knowledge of Hh signaling mechanisms in specific subsets of tumors. For instance, tumors in which Hh signaling is active in CSCs, but not bulk tumor cells, are unlikely to respond effectively to Hh inhibitors as monotherapy. Furthermore, the emergence of drug-resistant SMO mutations is a key factor limiting the efficacy of SMO inhibitors as monotherapy (63–65). Given these limitations, current strategies for therapeutically targeting the Hh pathway are being reevaluated to include strategic combinations of SMO inhibitors with other therapeutic modalities.

Several preclinical studies report success combining SMO inhibitors and conventional cytotoxic antitumor agents. For instance, combined SMO inhibitor, IPI-926 and gemcitabine, blocks tumor growth in a mouse pancreatic cancer model through gemcitabine-mediated cytotoxicity and IPI-926–mediated CSC inhibition (45). In glioblastoma CSC xenografts, SMO inhibitors enhance the effects of temozolomide (66), and in a mouse CML model, cyclopamine enhances the effects of Bcr-Abl inhibitor nilotinib and increases survival by targeting leukemic stem cells (40). Combined docetaxel, NOTCH inhibitor, and cycloamine inhibits growth of docetaxel-resistant prostate CSCs (67), and combined EGFR and SMO inhibition has proved effective in preclinical prostate cancer, BCC, and glioblastoma models (57). Several clinical trials of SMO inhibition combined with other therapeutics are currently under way (Table 1). Results from these trials will provide a key indication of whether use of SMO inhibitors can be effectively extended beyond BCC and MB through use of strategic drug combinations.

Emerging insights into Hh pathway regulation in CSCs may lead to even more effective combination strategies for targeting Hh signaling in these cells. In this regard, the atypical protein kinase C iota (PKCζ) is an oncogene (refs. 68, 69; reviewed in refs. 70, 71) that has emerged as a major regulator of Hh pathway activity in BCC and LSCC (18). In BCC, PKCζ regulates GLI1 in a SMO-independent fashion to promote BCC tumor growth (71), suggesting that PKCζ inhibition may be an alternative approach to treating BCC tumors with acquired resistance to SMO inhibitors. In LSCC, PKCζ and SOX2, both of which are lineage-specific lung oncocenes, cooperate to drive a CSC phenotype through Hh pathway activation (18). Amplification of chromosome 3q26, which occurs in approximately 70% of LSCCs, results in the coamplification and cooverexpression of PKCζ and SOX2, which cooperate to drive cell-autonomous Hh signaling in LSCC CSCs (ref. 18; Fig. 2A). Mechanistically, PKCζ phosphorylates SOX2, a transcription factor that functions in stem cell maintenance, and controls SOX2–mediated transcriptional activation of HhHAT, resulting in increased levels of mature, palmitoylated Hh ligand, and Hh pathway activation that drives the LSCC CSC phenotype (18). PKCζ also activates Rac1–MEK–ERK signaling in LSCC cells to transcriptionally regulate matrix metalloproteinase 10 (MMP10). This PKCζ–Rac1–MEK–ERK–MMP10 signaling axis is required for both CSC maintenance and transformed growth of bulk LSCC cells (Fig. 2B; reviewed in ref. 72). Thus, PKCζ drives both CSC and bulk cell growth, suggesting that combined PKCζ and Hh inhibition may be a particularly effective therapeutic intervention strategy in LSCC. Indeed, combined treatment with the selective PKCζ inhibitor auranozin (ANF; ref. 18) and the SMO inhibitor LDE225 causes synergistic inhibition of LSCC CSC expansion and viability (Fig. 2B). ANF inhibits expression of PKCζ-dependent transcriptional targets HHTAT, GLI1, and MMP10 (18, 72), whereas LDE225 causes decreased GLI1, consistent with off-target effects of these agents. Combined ANF and LDE225 caused a more pronounced inhibition of downstream effectors when compared with either agent alone (Fig. 2C), consistent with the observed synergistic growth inhibition. Because the PKCζ–SOX2–Hh signaling axis is driven by chromosome 3q26 amplification, combined PKCζ and SMO inhibitor may represent a particularly effective treatment strategy for LSCCs harboring chromosome 3q26 amplification. These results have implications well beyond LSCC because many other tumor types harbor this genetic alteration. Indeed, chromosome 3q26 amplification is the most prevalent genetic copy-number gain alteration in human cancers, occurring in approximately 15% of human tumors (73). As a result, PKCζ and SOX2 coamplification and cooverexpression are observed in significant percentages of bladder, breast, cervical, esophageal, head and neck, kidney, lung adenocarcinoma, LSCC, serous ovarian, stomach, and uterine cancers (Table 1; reviewed in ref. 70). Thus, a large patient population, identifiable by tumor-specific 3q26 amplification, is likely to exhibit active PKCζ–SOX2–Hh signaling, a stem-like phenotype driven by Hh pathway activation, and responsiveness to combined ANF/SMO inhibitor treatment. Early-phase clinical trials are being actively pursued to evaluate this novel therapeutic strategy.
Perspective

Hh inhibitors have been successfully used as monotherapy for BCC and MB tumors harboring Hh pathway mutations. However, therapeutic response in these tumors may be limited by the challenge of acquired SMO resistance. SMO inhibitors have been less successful in other tumor types, probably due to many complicating factors, including a lack of patient stratification in early-phase trials, cross-talk between Hh and other signaling pathways, the complexity of Hh signaling in CSCs, bulk tumor cells and tumor microenvironment, and a lack of in-depth knowledge of Hh pathway activation mechanisms in specific tumor subtypes. Tumors not harboring Hh pathway mutations are unlikely to respond to Hh inhibitors alone. However, combining Hh inhibitors with chemotherapeutics or other targeted agents...
coupled with appropriate patient stratification paradigms provide new opportunities for more effective Hh-based therapy. One promising strategy involves combined Hh and PKC inhibitor therapy. PKCi activates a novel PKC–SOX2–Hh signaling axis in CSCs from LSCC tumors harboring chromosome 3q26 amplification, and these cells exhibit synergistic response to combined SMO/PKCi inhibition. The high prevalence of chromosome 3q26 copy-number gains, and the resulting coamplification of PKCi and SOX2, in many tumor types (~15% of human tumors) raises the exciting possibility that combined Hh and PKCi inhibitor therapy will prove effective in the large target patient population whose tumors harbor chromosome 3q26 copy-number gains and a CSC phenotype driven by PKC–SOX2–Hh pathway activation.

**Disclaimer**

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Writing, review, and/or revision of the manuscript: V. Justilien, A.P. Fields

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.P. Fields

Study supervision: A.P. Fields

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Molecular Pathways: Novel Approaches for Improved Therapeutic Targeting of Hedgehog Signaling in Cancer Stem Cells

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