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

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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. Pre-clinical 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, crosstalk 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 anti-cancer therapy.

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.

The canonical Hh signaling pathway

Core Hh signaling components include the Hh ligands (sonic Hh (Shh); Indian Hh, (Ihh) and Desert Hh, (Dhh)), the trans-membrane receptor proteins Patched 1 and 2 (PTCH1 and PTCH2), the G-protein-coupled receptor-like protein Smoothened (SMO) and the glioma-associated oncogene transcription factors 1–3 (GLI1, GLI2 and GLI3) (reviewed in (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 ciliumtip (4), driving a signaling cascade that results in nuclear GLI translocation and activation. GLI activates transcription of context-specific genes regulating self-renewal, cell fate, survival, angiogenesis, epithelial-mesenchymal transition and cell invasion (reviewed in (5)). As Hh transcriptional targets, *GLI1* and *PTCH* establish a feedback loop that regulates Hh signaling (6).

Several accessory 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, whose secretion is facilitated by the transmembrane transporter-like protein Dispatched (Disp) (1). Growth Arrest Specific 1 (GAS1), CAM-related/down-regulated by oncogenes (CDO), brother of CDO (BOC) and Glypican-3 (GPC3) are co-receptors that facilitate ligand binding to PTCH (1), whereas Hedgehog Interacting Peptide (HhIP) represses signaling by sequestering Hh ligand (7). Protein kinase A (PKA), glycogen synthase 3β (GSK3β), casein kinase I (CK1), Skip–Cullin–Fbox (SCF) protein, βTransducin repeat Containing Protein (βTrCP), and a suppressor complex comprised of Fused kinase (Fu), Suppressor of Fused (Sufu) and Costal2 (Cos2) regulate GLI expression, stability and localization (reviewed in (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-independent mechanisms result in aberrant Hh pathway activation in cancer. Germline or somatic loss-of-function *PTCH* 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 IL6 and Wnt signaling activation (24-27). Paracrine 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 sub-population of tumor cells exhibiting stem-like properties (36-38). These tumor-initiating or cancer stem cells (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, colon, glioblastoma, multiple myeloma, and chronic myeloid leukemia (CML) (16, 18, 20, 22, 39-42). Hh signaling is selectively activated in CSCs compared to 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, BMI1, 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 chemo-resistance characteristic of CSCs (44-48).

Clinical-Translational Advances

Hh pathway inhibitors

Four major modes of Hh inhibition have been exploited therapeutically: *1)* SMO inhibition; *2)* receptor-ligand disruption; **3)** inhibition of ligand processing; and *4)* GLI inhibition (Fig. 1). Cyclopamine, a naturally-occurring SMO inhibitor, established Hh as a viable therapeutic target (49, 50). Though 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/Erismodegib (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. 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 Robotnikinin, inhibit Hh:PTCH interactions and exhibit anti-tumor 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.

Targeting Hh in CSCs

CSCs are emerging therapeutic targets whose efficient elimination may offer longer lasting, potentially curative outcomes in cancer patients. 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). 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 5E1 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 vivo* 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 crosstalk between Hh and EGFR, RAS/MEK/ERK, PI3K/AKT/mTOR, NOTCH and/or WNT oncogenic signaling pathways (reviewed in (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 GLI 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, crosstalk 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 anti-tumor 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 cyclopamine inhibits growth of docetaxel-resistant prostate CSCs (67), and combined EGFR and SMO inhibition has proven effective in preclinical prostate, BCC and glioblastoma models (57). Several clinical trials of SMO inhibition combined with other therapeutics are currently underway (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 (PKCι) is an oncogene (68, 69) (reviewed in (70) (71) that has emerged as a major regulator of Hh pathway activity in BCC and LSCC (18). In BCC, PKCι regulates GLI1 in a SMO-independent fashion to promote BCC tumor growth (71), suggesting that PKCι inhibition may be an alternative approach to treating BCC tumors with acquired resistance to SMO inhibitors. In LSCC, PKCι and SOX2, both of which are lineage-specific lung oncogenes, cooperate to drive a CSC phenotype through Hh pathway activation (18). Amplification of chromosome 3q26, which occurs in ∼70% of LSCCs, results in the co-amplification and co-overexpression of PKCι and SOX2 which cooperate to drive cell-autonomous Hh signaling in LSCC CSCs (18) (Fig. 2A). Mechanistically, PKCι phosphorylates SOX2, a transcription factor that functions in stem cell maintenance, and controls SOX2-mediated transcriptional activation of HHAT, resulting in increased levels of mature, palmitoylated Hh ligand and Hh pathway activation that drives the LSCC CSC phenotype (18). PKCι also activates Rac1/MEK/ERK signaling in LSCC cells to transcriptionally-regulate Matrix Metalloproteinase 10 (MMP10). This PKCι/ Rac1/MEK/ERK/MMP10 signaling axis is required for both CSC maintenance and transformed growth of bulk LSCC cells (Fig. 2A) (reviewed in (70). Thus, PKCι drives both CSC and bulk tumor cell growth suggesting that combined PKCι and Hh inhibition may be a particularly effective therapeutic intervention strategy in LSCC. Indeed, combined treatment with the selective PKCι inhibitor auranofin (ANF) (18) and the SMO inhibitor LDE225 causes synergistic inhibition of LSCC CSC expansion and viability (Fig. 2B). ANF inhibits expression of PKCι-dependent transcriptional targets HHAT, GLI1 and MMP10 (18, 72), whereas LDE225 causes decreased GLI1, consistent with on-target effects of these agents. Combined ANF and LDE225 caused a more pronounced inhibition of downstream effectors when compared to either agent alone (Fig. 2C), consistent with the observed synergistic growth inhibition. Since the PKCι-SOX2-Hh signaling axis is driven by chromosome 3q26 amplification, combined PKCι 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, PKCι and SOX2 co-amplification and co-overexpression is observed in significant percentages of bladder, breast, cervical, esophageal, head and neck, kidney, lung adenocarcinoma, lung squamous cell carcinoma, serous ovarian, stomach and uterine cancers (Table 1, (reviewed in (70)). Thus, a large patient population, identifiable by tumor-specific 3q26 amplification, is likely to exhibit active PKCι/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 employed 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, crosstalk 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 PKCι inhibitor therapy. PKCι activates a novel PKCι-SOX2-Hh signaling axis in CSCs from LSCC tumors harboring chromosome 3q26 amplification, and these cells exhibit synergistic response to combined SMO/PKCι inhibition. The high prevalence of chromosome 3q26 copy number gains, and the resulting co-amplification of PKCι and SOX2, in many tumor types (∼15% of human tumors) raises the exciting possibility that combined Hh and PKCι 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 PKCXι-SOX2-Hh pathway activation.

Acknowledgments

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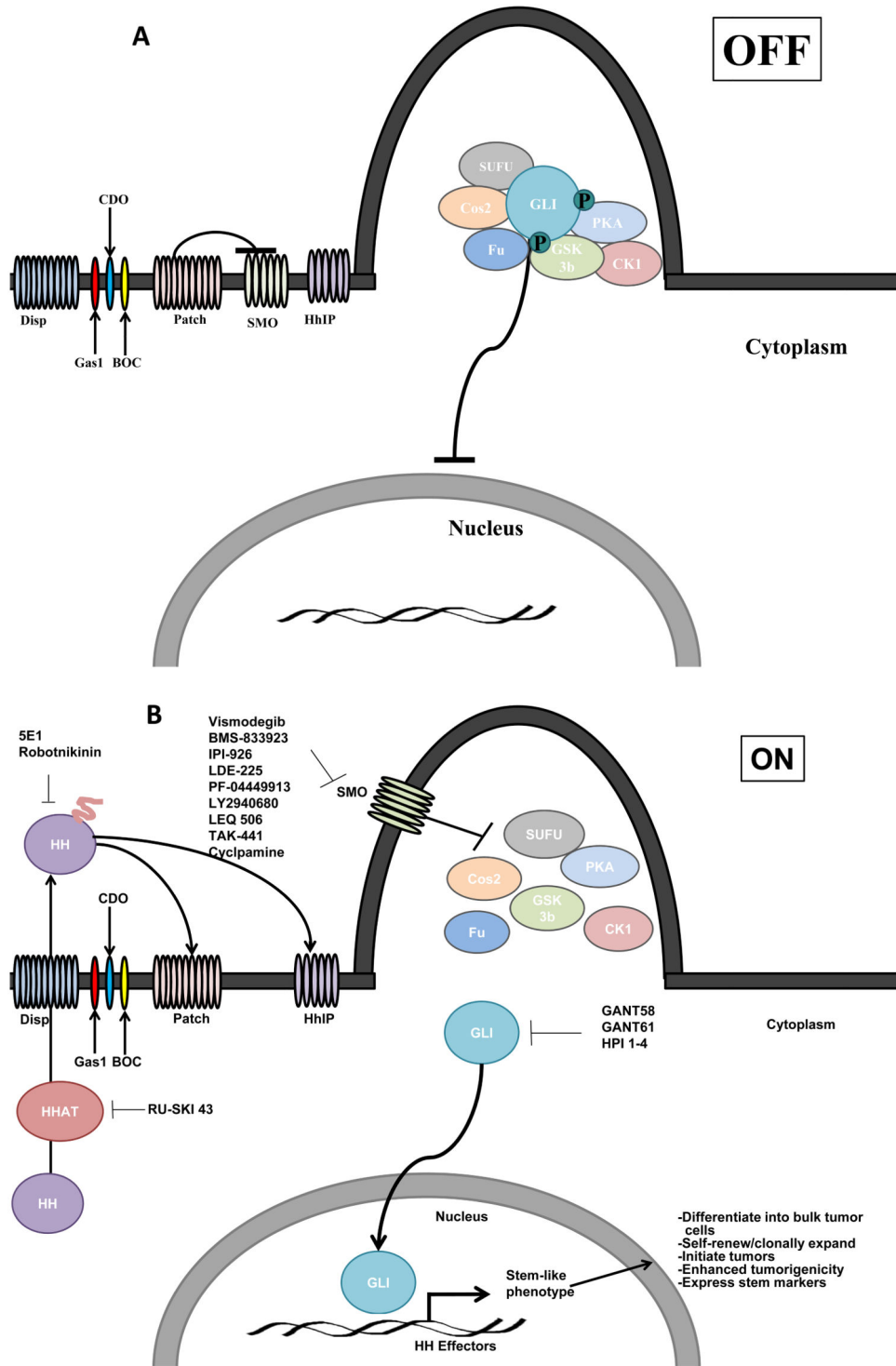
Figure 1.
Schematic of Hedgehog (Hh) signaling in Vertebrates. A) In the absence of Hh ligand, Patched (PTCH) prevents SMO localization to the primary cilium and GLI is suppressed by a protein complex composed of Costal2 (Cos2), Fused (Fu), Suppressor of Fused (SUFU) that promotes Protein Kinase A (PKA), Glycogen Synthase Kinase 3 (GSK3) and Casein

Kinase 1 (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 Hh acyltransferase (HHAT) to be activated and released into the extracellular space by Dispatched (Disp). The Hh pathway is activated upon Hh ligand binding to PTCH and the co-receptors Growth Arrest Specific 1 (GAS1), CAM-related/down-regulated by oncogenes (CDO), brother of CDO (BOC) which relieves PTCH-mediated inhibition of SMO. Upon activation, SMO translocations 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: **1)** SMO inhibition (Vismodegib, BMS-833923, IPI-926, LDE225, PF-04449913, LY2940680, LEQ 506, TAK-441 and cyclopamine; **2)** receptor-ligand disruption (5E1 anti-Hh ligand antibody and robotnikinin); **3)** inhibition of ligand processing (HHAT inhibitor RU-SKI 43); or **4)** inhibition of GLI activity (GANT58, GANT61 and HPI 1-4).
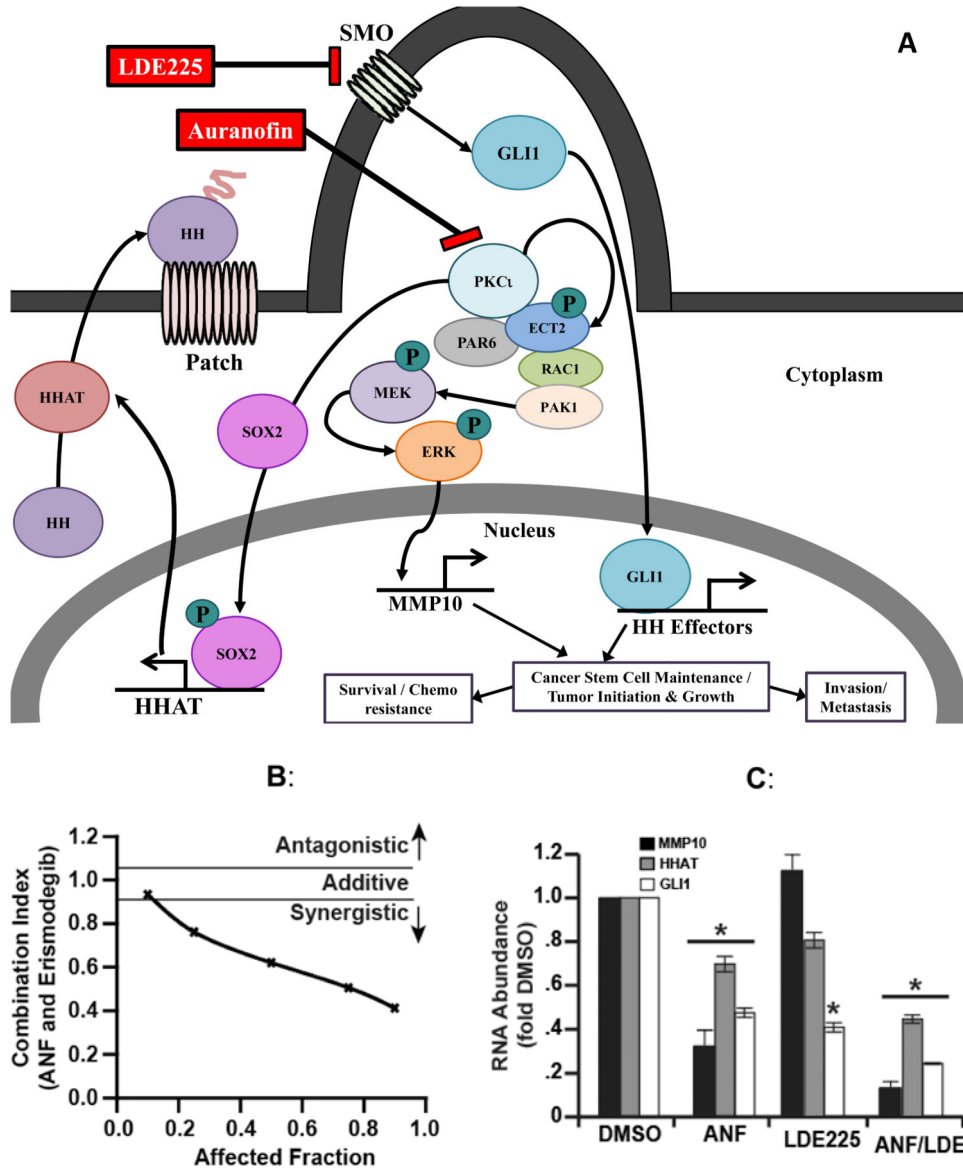
Figure 2.
Combined inhibition of PKCι and Hh signaling inhibits the CSC phenotype. A) PKCι regulates multiple signaling pathways that maintain both CSC and bulk tumor cells and drives tumor initiation, growth, survival, invasion, metastasis and chemoresistance. In lung squamous cell carcinoma (LSCC) cells harboring 3q26 amplification, PKCι phosphorylates SOX2 and regulates SOX2-mediated transcriptional activation of HHAT leading to autocrine Hh pathway activation. This PKCι/SOX2/Hh signaling axis is required to maintain a CSC phenotype. PKCι also forms an oncogenic complex with the polarity protein PAR6 and the guanine nucleotide exchange factor Epithelial Cell Transforming Sequence 2 (ECT2) that functions to drive a RAC1/PAK/MEK/ERK signaling cascade that transcriptional upregulates Matrix Metalloproteinase 10 (MMP10) to promote tumorigenicity of both bulk tumor and cancer stem cells. B) The PKCι inhibitor, Auranofin and the SMO inhibitor, LDE225 synergistically block the growth of LSCC CSCs. C) PKCι

and Hh inhibition transcriptionally downregulate their respective transcriptional targets HHAT, GLI1 and MMP10 and combined inhibition of PKCι and Hh further downregulates expression of these gene targets. DMSO, dimethyl sulfoxide.

Table 1

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

Cancer type	Response pre-clinically	Response Clinically Clinicaltrials.gov NCT #	3q26 status % with copy # gains	Reference
Basal cell carcinoma	Suppressed proliferation, induced apoptosis and regression of lesions.	Antitumor activity in metastatic and locally advanced BCC; Vismodegib FDA approved for advanced BCC		(74, 75)
Breast	Decreased proliferation, tumor growth and metastasis.	NCT01576666; recruiting patients	25% (TCGA)	(31, 76)
Cervical	Decreased cell proliferation and survival.		77% (TCGA)	(77)
Chronic myelogenous leukemia	Sensitized cells to chemotherapy. Prolonged survival in leukemia mouse model. Decreased tumorigenic potential of leukemic stem cell population.	NCT01456676; recruiting NCT01218477; completed, no results reported NCT01357655; ongoing, not recruiting		(40, 78, 79)
Colorectal	Blocked cell growth *in vitro* and growth of xenograft tumors *in vivo*. Decreased recurrence and metastases.	NTC00636610; Vismodegib does not add to the efficacy of FOLFOX, FOLFIRI, or bevacizumab NCT01576666; recruiting patients	11% (TCGA)	(22, 27, 80, 81)
Esophageal	Decreased cell growth and induced apoptosis.	NCT00909402; completed, no results reported	53%	(82, 83)
Gastric	Decreased cell growth and induced apoptosis.	NCT00982592; Addition of Vismodegib to FOLFOX did not improve PFS in an unselected population NCT00909402; completed, no results reported NCT01576666; recruiting patients	35% (TCGA)	(84, 85)
Gliomas	Decreased self-renewal of CSCs and potentiated the anti-proliferative effect of conventional chemotherapy.	NCT01576666; recruiting patients	14% (TCGA)	(39)
Head and Neck	Decreased colony formation in primary tumor cells *ex vivo*.		74% (TCGA)	(86)
Hepatocellular	Blocked proliferation and invasion *in vitro* and xenograft tumors *in vivo*.		17% (TCGA)	(87)
Kidney	Decreased cell growth and caused tumor regression in vivo.		15% (TCGA)	(88)
Lung	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 lung squamous cell carcinoma CSCs.	NCT01579929; recruiting NCT01722292; recruiting	84% LSCC (TCGA) 32% LAC (TCGA) 27% SCLC	(18, 23, 89)
Lymphoma	Induced apoptosis and inhibits growth of cancer cells in mice.		29% (TCGA)	(29)

Cancer type	Response pre-clinically	Response Clinically Clinicaltrials.gov NCT #	3q26 status % with copy # gains	Reference
Medulloblastoma	Antitumor activity in mouse models.	NCT00939484, sustained response in 15% of patients		(74, 90-92)
Melanoma	Reduced proliferation, prevented recurrence and lung metastasis.		20%	(21)
Neuroblastoma	Reduced proliferation, induced apoptosis and blocked tumorigenicity.			(93, 94)
Neuro-endocrine	Blocked cell growth *in vitro*.			(95)
Ovarian	Decreased proliferation, mobility and invasiveness. Induced cancer cell dedifferentiation and apoptosis *in vitro* and decreased tumor growth *in vivo*.	NTC00739661; No clinically meaningful improvement in progression-free survival for vismodegib versus placebo	83%, serous (TCGA)	(96-99)
Pancreatic	Blocked growth, migration, invasion, colony formation, the cancer stem cell population, tumor growth and metastasis.	NCT01096732; terminated NCT00878163; Active, not recruiting NCT01576666; recruiting NCT01537107; recruiting	20% (TCGA)	(100, 101)
Prostate	Prevented tumour growth in xenograft model.	NCT01163084; ongoing, not recruiting	15% (TCGA)	(54)
Sarcoma	Decreased rhabdosarcoma cell proliferation, induced apoptosis and blocked tumor growth in vivo. Decreased osteocarcinoma cell growth *in vitro* and tumor growth *in vivo*.	NCT01154452; recruiting	13% (TCGA)	(102, 103)
Uterine	Decreased cell growth *in vitro*.		64% (TCGA)	(104)