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Molecular Pathways: The Hedgehog Signaling Pathway in Cancer

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Abstract

The Hedgehog (Hh) signaling pathway regulates embryonic development and may be aberrantly activated in a wide variety of human cancers. Efforts to target pathogenic Hh signaling have steadily progressed from the laboratory to the clinic, and the recent approval of the Hh pathway inhibitor vismodegib for patients with advanced basal cell carcinoma (BCC) represents an important milestone. On the other hand, Hh pathway antagonists have failed to demonstrate significant clinical activity in other solid tumors. The reasons for these negative results are not precisely understood, but it is possible that the impact of Hh pathway inhibition has not been adequately measured by the clinical endpoints used thus far or that aberrancies in Hh signal transduction limit the activity of currently available pathway antagonists. Further basic and correlative studies to better understand Hh signaling in human tumors and validate putative anti-tumor mechanisms in the clinical setting may ultimately improve the success of Hh pathway inhibition to other tumor types.

Keywords

Hedgehog; Smoothened antagonists; basal cell carcinoma; clinical trials

Background

The Hedgehog (Hh) signaling pathway is highly conserved from flies to humans and is essential for development of the normal embryo (1, 2). In mammals, Hh signaling regulates both patterning and polarity events during early embryogenesis and the morphogenesis of specific organs and tissues. The pathway is subsequently silenced in most adult tissues but can be reactivated following injury to promote repair and regeneration. Furthermore, aberrant Hh signaling has been detected in many human cancers suggesting a broad role in carcinogenesis.

Hh signal transduction

Hh signaling is initiated by binding of one of the three soluble and lipid modified HH ligands (Sonic, Indian, or Desert HH) found in vertebrates to the twelve-pass transmembrane receptor Patched (PTCH1, Figure). In the absence of ligand, PTCH1 represses the seven-pass transmembrane G-protein coupled receptor (GPCR)-like protein Smoothened (SMO).

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Ligand binding relieves this inhibition and allows SMO to modulate a cytoplasmic complex containing Suppressor of Fused (SUFU) that modifies the three Glioma associated (GLI) transcriptional regulators through phosphorylation, sumoylation, and selective proteolysis (3). GLI1 induces and GLI3 represses Hh target genes that include *GLI1*, *PTCH1*, *Cyclin D1, c-Myc*, and *Bcl-2*, whereas GLI2 can act in either a positive or negative manner depending on post-transcriptional and post-translational processing events (4). Vertebrate Hh signaling is further regulated by the translocation of signaling components between the cytoplasm, plasma membrane, nucleus, and primary cilium that acts as a sensor to monitor the extracellular environment (5). PTCH1 is initially located in the primary cilium and SMO within cytoplasmic vesicles. Following ligand binding to PTCH1, SMO moves to the primary cilium where it interacts with the GLI processing complex that eventually results in the nuclear translocation of the GLI transcriptional regulators (6).

The Hh pathway and cancer

A role for Hh signaling in cancer was initially provided by studies in Gorlin syndrome, an autosomal dominant disorder characterized by craniofacial and skeletal abnormalities and a markedly increased risk of advanced basal cell carcinoma (BCC) and medulloblastoma (7, 8). The discovery of *PTCH1* mutations as the cause of Gorlin syndrome suggested that dysregulated Hh pathway activity was responsible for the development of these cancers (9, 10), and these findings were substantiated by the identification of *PTCH1*, *SMO*, and to a lesser extent *SUFU* mutations in approximately 90% and 15–30% of spontaneously arising BCCs and medulloblastomas, respectively (11, 12). Furthermore, the recapitulation of BCC and medulloblastoma in transgenic mouse models has provided definitive proof that *PTCH1* and *SMO* mutations are a causal factor in these tumor types.

Aberrant Hh pathway activity is also a feature of many other human cancers. However, activating mutations in pathway components are uncommon and over-expression of HH ligands is thought to drive increased pathway activity. In these “ligand-dependent” tumors, several types of Hh signaling have been described. Autocrine and juxtacrine signaling in which tumor cells both secrete and respond to HH ligands has been reported in many cancers including small cell lung, pancreas, colorectal, and metastatic prostate carcinomas as well as melanoma and glioblastoma (13–18). Paracrine signaling in which the cells secreting ligands are distinct from those responding with pathway activation has also been described in lymphoma and multiple myeloma in which HH ligands produced by stromal cells in the local microenvironment induce pathway activity in tumor cells (19). Alternatively, studies in epithelial cancers have found that paracrine Hh signaling is reversed with tumor cells secreting HH ligands that activate signaling within stromal cells to produce secondary factors supporting angiogenesis and tumor cell proliferation and survival (20, 21).

The Hh pathway can also regulate cancer stem cells (CSCs) with enhanced tumor initiating and self-renewal potential. In multiple myeloma, Hh pathway activation induces the expansion of CSCs whereas pathway inhibition results in terminal differentiation, loss of self-renewal, and exhaustion of the malignant clone (22). Studies in chronic myeloid leukemia (CML) and breast cancer have similarly found that Hh pathway inhibition limits tumorigenic potential and self-renewal (23–25). Emerging data suggest that CSCs in solid tumors are involved in metastatic disease progression (26), and the Hh pathway has been found to regulate the epithelial-mesenchymal transition and dissemination of CSCs in pancreatic and colorectal carcinoma (15, 27). Therefore, the Hh signaling pathway may specify CSC fate decisions similar to its role in development.

Most studies have focused on canonical Hh signaling events, but GLI-independent effects have been identified in normal cells that may contribute to its pathogenic role in cancer. For example, SMO has been found to activate the RhoA and Rac1 GTPases to induce

cytoskeletal remodeling, fibroblast migration, and endothelial tubulogenesis (28, 29). In addition, PTCH1 has been found to act as a dependence receptor that directly triggers apoptosis in the absence of ligand, whereas ligand binding induces canonical target gene expression (30). Therefore, non-canonical effects should be further studied in human cancers and, along with variations in the mode of canonical pathway activation, must be considered when developing clinical targeting strategies.

Clinical-Translational Advances

The development of strategies targeting the Hh signaling pathway began with the discovery that cyclopamine, a steroidal alkaloid derived from *Veratrum californicum*, inhibits SMO (31, 32). Cyclopamine has been extensively used to study Hh signaling and found to inhibit tumor growth in multiple *in vitro* and *in vivo* models. Efforts to improve the specificity, potency, and pharmacologic profile of cyclopamine have led to the synthesis of novel derivatives (IPI-926) (33). In addition, large-scale chemical library screens have been undertaken to identify inhibitors of Hh signaling and have generated novel SMO antagonists (GDC-0449, LDE225, PF04449913, TAK-441) (34–37). All of these novel agents have initiated clinical testing.

SMO inhibitors: early success

SMO inhibitors have been studied as anti-cancer agents in over 50 clinical trials across a wide range of tumor types (38). The earliest reported clinical data involved a phase I trial of vismodegib (Erivedge, GDC-0449, Genentech and Curis) in refractory solid tumor patients (39). Early activity was observed in patents with locally advanced or metastatic BCC, presumably because of the high incidence of Hh pathway activating mutations, and this study was expanded to specifically study BCC (40). Of 33 advanced BCC patients receiving vismodegib, 55% of patients experienced clinical responses, including 2 complete responses. Serious grade 3 or 4 toxicities were infrequent (21% of patients) and consisted of fatigue, nausea, dysgeusia, and muscle cramps that have been similarly observed with other SMO inhibitors. A subsequent open label, single-arm phase II trial involving 96 BCC patients (ERIVANCE BCC) demonstrated overall response rates of 43% and 30% in patients with locally advanced and metastatic disease, respectively, and a median duration of response of 7.6 months (41). These results led to the approval of vismodegib by the FDA as a treatment for advanced or metastatic BCC in January 2012.

The SMO antagonist PF-04449913 (Pfizer) has also provided encouraging early results in patients with hematologic malignancies (42). In this dose-escalation phase I trial, 32 patients with a variety of diseases including acute myeloid leukemia, myelodysplastic syndrome, myelofibrosis, CML, and chronic myelomonocytic leukemia (CMMoL) received PF-04449913 as a single agent. Responses were observed across all diseases as evidenced by decreased leukemic blast counts and/or improvements in normal hematopoiesis, and a patient with transformed CMMoL achieved a complete remission.

Mechanisms of clinical resistance have also been reported in a patient with metastatic medulloblastoma who became unresponsive to vismodegib after three months of therapy (43). Serial tumor biopsies identified a novel SMO mutation at relapse that interferes with vismodegib binding, and subsequent studies showed that a similar SMO mutation could be generated in mouse medulloblastoma cells gaining *in vivo* resistance (44). Given that the various SMO inhibitors in development are chemically distinct, it is possible that these other agents may not display cross-resistance, but data thus far are limited.

Smo antagonists: negative clinical data

While SMO antagonists are active in BCC, clinical results in other solid tumors have been less encouraging. A phase II, randomized, double-blind, placebo-controlled trial of single-agent vismodegib in ovarian cancer has recently been reported (45). In this trial, 104 women received vismodegib or placebo as maintenance therapy following second or third complete remission. At a median follow-up of 5.7 months and analysis of 57 progression-free survival (PFS) events, the median PFS was 5.8 months for patients receiving placebo vs. 7.5 months in the vismodegib arm (HR=0.79 (95% CI: 0.46, 1.35; p=0.39)). A second phase II, randomized, double-blind, placebo-controlled trial compared the addition of vismodegib or placebo to standard first-line chemotherapy in 199 patients with metastatic colorectal carcinoma (46). Compared to placebo, the addition of vismodegib to FOLFOX (5-fluorouracil, leucovorin, oxaliplatin) + bevacizumab or FOLFIRI (5-fluorouracil, leucovorin, irinotecan) + bevacizumab failed to significantly improve PFS compared to chemotherapy + bevacizumab alone (HR stratified by chemotherapy regimen=1.24 (95% CI: 0.83, 1.87; p=0.30)). In metastatic pancreatic cancer, the cyclopamine analogue saridegib (IPI-926, Infinity Pharmaceuticals) was studied in combination with gemcitabine in a phase II, randomized, placebo-controlled trial. A total of 122 patients were studied, but the trial was halted prematurely after patients in the saridegib + gemcitabine arm were found to have a higher rate of progressive disease and lower median survival than those receiving gemcitabine alone.

Potential explanations and future directions

The reasons for negative results in solid tumors other than BCC are unclear but may include the postulated anti-tumor effects of SMO inhibition, clinical trial designs, the broad applicability of pathway inhibition in a specific tumor type, and aberrancies in Hh signal transduction. Since clinical activity is detected through endpoints that are dependent on specific anti-tumor effects, efficacy may not be observed if either the proposed mechanism of action or the choice of endpoints is incorrect. For example, if Hh pathway inhibition primarily impacts tumor cell proliferation and survival, then response rates that reflect changes in tumor burden or PFS should be affected even with the use of SMO antagonists as single agents as in BCC. Alternatively, if SMO antagonists primarily modulate the microenvironment and sensitize tumors to chemotherapy, positive effects on response rates and PFS should be observed only when they are given concomitantly with other therapies. Finally, if Hh signaling primarily regulates rare CSCs responsible for disease relapse and metastatic progression, significant changes in response rates are unlikely to be observed but relapse or metastasis-free survival may be prolonged. Since multiple effects have been ascribed to the Hh in pancreatic and colorectal cancer in the preclinical setting (14, 15, 20, 27), it is possible that PFS was not the endpoint that optimally reflects the actual clinical anti-tumor effects of SMO inhibition. Correlative studies may be able to determine whether Hh signaling predominantly occurs within a specific cell compartment and what cellular effects actually take place in response to SMO inhibition. These insights would be invaluable in guiding the design of subsequent trials.

It is also possible that the Hh pathway is not uniformly active or pathogenic in all cases of a specific tumor type, and clinical efficacy was limited to a specific subset of patients in these clinical trials. Predictive biomarkers have been identified for a number of therapies that can be used to select patients with enhanced responses, such as overexpression of Her2/neu and treatment with trastuzumab in breast cancer. However, biomarkers of response to SMO antagonists have not been established in any tumor type, but may allow patient selection during clinical testing. Thus far, changes in *GLI1* and/or *PTCH1* expression have been used as pharmacodynamic markers in normal tissues, such as hair follicles, to provide evidence that SMO antagonists actually inhibit Hh signaling *in vivo* (39), but it is not known whether

the expression of these genes within tumors can identify patients with tumors responsive to SMO antagonists. It is likely that the diverse modes of pathway activation and aberrant signaling events in cancer will further complicate the discovery of predictive biomarkers. Moreover, it is possible that these biomarkers will need to be assessed in specific cell compartments, such as stromal cells or CSCs. Given the success of SMO inhibition, further correlative studies in BCC and preclinical studies in other tumor types may identify specific biomarkers that improve patient selection.

Mammalian Hh signal transduction has been largely deciphered by studying normal embryonic fibroblasts, and it is possible that aberrant signaling events within the context of specific cancers impact the efficacy of SMO antagonists. For example, *SUFU* mutations in medulloblastoma or the direct induction of *GLI1* expression by the EWS-FLI fusion protein in Ewing sarcoma may result in pathway activation downstream of SMO (47–49). Similarly, the absence of the primary cilium that is required for Hh signaling in normal cells may lead to tumor formation by activated GLI2 or allow SMO-independent pathway activation through the loss of GLI3 repressor function (50, 51). Although potential aberrancies in Hh signal transduction may prevent the utility of SMO antagonists, alternative targeting strategies have emerged that may ultimately be useful as anti-cancer agents (Figure). To this end, several preclinical strategies have been developed that target Hh ligand-patched interactions (robotnikinin), the intracellular processing and translocation of pathway components (HPIs, arsenic, itraconazole), GLI1 function (GANT-61), or primary cilia formation (HPI) (52–56). Further studies using relevant models and specific tumor types may provide valuable mechanistic data that not only suggest clinical potential but also specify the design of clinical trials.

Summary

The development of Hh pathway inhibitors has been marked by success and failure. The association between *PTCH1* mutations in Gorlin syndrome and aberrant pathway activity in BCC along with the discovery of cyclopamine as a naturally occurring SMO antagonist and the development and approval of vismodegib provides an exceptional example of successful translational research. On the other hand, negative clinical results question whether Hh pathway inhibition will actually be effective in tumors that typically lack activating mutations. Aberrant Hh signaling has been found to impact cancers in multiple and diverse ways, but it is unclear which of these is clinically relevant. However, better understanding these mechanisms in the clinical setting should dictate the choice of clinical trial endpoints. Correlative studies from completed and ongoing trials may provide critical insights in this regard and should include examining the effects of SMO antagonists on tumor cells, stromal cells, and CSCs. Moreover, studies to determine why many advanced BCC patients do not respond to SMO antagonists despite likely harboring activating mutations may reveal specific mechanisms responsible for the lack of efficacy in other tumor types. Finally, continued basic studies of the Hh pathway as a regulator of embryonic development may provide a reference point to further understand aberrancies in signal transduction that occur in cancer and lead to the development of novel targeting strategies as well as define predictive biomarkers capable of identifying responsive cases. Therefore, correlative and basic studies of Hh signaling within the context of human cancers coupled with clinical trial designs and endpoints capable of evaluating its precise role in a specific cancer may expand the utility of pathway antagonists beyond BCC.

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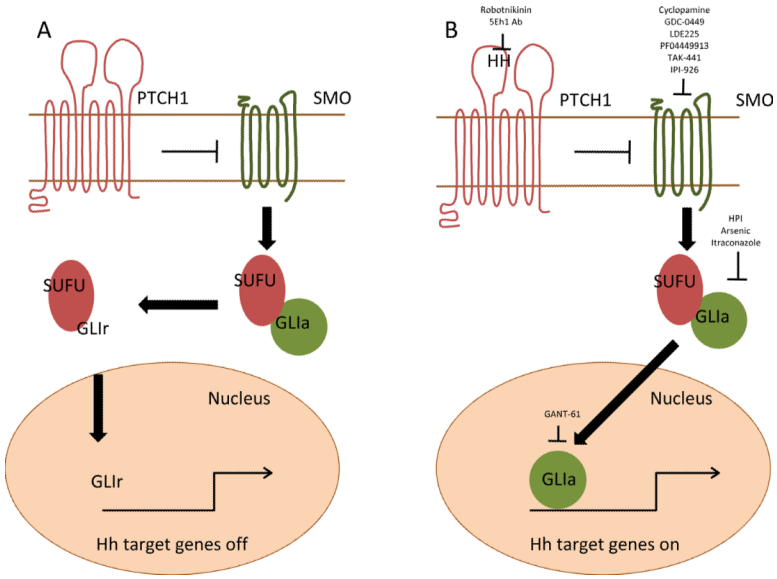

figure 1.

The Hedgehog signaling pathway. Positive and negative regulatory components are depicted in green and red, respectively. A. In the absence of HH ligand, PTCH1 inhibits SMO allowing the GLI processing complex containing SUFU to generate GLIr transcriptional repressors. B. HH ligand binding to PTCH depresses SMO and generates GLIa nuclear factors that induce the expression of Hh target genes. Clinical and preclinical inhibitors of pathway signaling are listed at their sites of pathway activity.