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 represents an important milestone. On the other hand, Hh pathway antagonists have failed to show 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 limits the activity of currently available pathway antagonists. Further basic and correlative studies to better understand Hh signaling in human tumors and validate putative antitumor mechanisms in the clinical setting may ultimately improve the success of Hh pathway inhibition to other tumor types.

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 3 soluble and lipid modified HH ligands (Sonic, Indian, or Desert HH) found in vertebrates to the 12-pass transmembrane receptor Patched (PTCH1, Fig. 1). In the absence of ligand, PTCH1 represses the 7-pass transmembrane G-protein–coupled receptor (GPCR)-like protein Smoothened (SMO). Ligand binding relieves this inhibition and allows SMO to modulate a cytoplasmic complex containing Suppressor of Fused (SUFU) that modifies the 3 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 posttranscriptional and posttranslational processing events (4). Vertebrate Hh signaling is further regulated by the translocation of signaling components among 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% to 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 overexpression 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 (CSC) 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

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Figure 1. The Hh 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 transcriptional repressors (GLIr). B, HH ligand binding to PTCH depresses SMO and generates activated GLI factors (GLIa) that induce the expression of Hh target genes. Clinical and preclinical inhibitors of pathway signaling are listed at their sites of pathway activity. HPI, hedgehog pathway inhibitor.
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, noncanonical effects should be further studied in human cancers and, along with variations in the mode of canonical pathway activation, must be considered in development of 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 *Veratum 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; ref. 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, and TAK-441; refs. 34–37). All of these novel agents have initiated clinical testing.

SMO inhibitors: early success

SMO inhibitors have been studied as anticancer agents in more than 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 patients 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) showed 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 U.S. Food and Drug Administration 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 representing 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 response.

Mechanisms of clinical resistance have also been reported in a patient with metastatic medulloblastoma who became unresponsive to vismodegib after 3 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

Although 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 5.6 months for patients receiving placebo versus 7.5 months in the vismodegib arm [HR, 0.79; 95% confidence interval (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 with placebo, the addition of vismodegib to FOLFIRI (5-fluorouracil, leucovorin, and oxaliplatin) + bevacizumab or FOLFIRI (5-fluorouracil, leucovorin, and irinotecan) + bevacizumab failed to significantly improve PFS compared with 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 antitumor effects of SMO inhibition, clinical trial designs, the broad
applicability of pathway inhibition in a specific tumor type, and aberrancies in Hh signal transduction. Because clinical activity is detected through endpoints that are dependent on specific antitumor 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 affects 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. Because 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 is not the endpoint that optimally reflects the actual clinical antitumor 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 affect 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 affect the utility of SMO antagonists, alternative targeting strategies have emerged that may ultimately be useful as anticancer agents (Fig. 1). To this end, several preclinical strategies have been developed that target Hh ligand–patched interactions (robotnikinin), the intracellular processing and translocation of pathway components [hedgehog pathway inhibitors (HPI), arsenic and irtraconazole], GLI1 function (GANT-61), or primary cilia formation (HPI; refs. 52–56). Further studies using relevant models in 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 HPIs 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 raise the question of whether Hh pathway inhibition will actually be effective in tumors that typically lack activating mutations. Aberrant Hh signaling has been found to affect 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 examination of 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 use of pathway antagonists beyond BCC.

Disclosure of Potential Conflicts of Interest

W. Matsui holds patent applications with Infinity Pharmaceuticals and is a consultant and advisory board member for Bristol-Myers Squibb and Pfizer. No potential conflicts of interest were disclosed by the other author.
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