The Role of the Hedgehog Signaling Pathway in the Development of Basal Cell Carcinoma and Opportunities for Treatment

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Abstract

The hedgehog (Hh) signaling pathway plays an important role in embryogenesis across multiple species. Its activity is reduced or absent in adult organisms. However, activation of the pathway has been shown to be a factor in the development of a number of human malignancies and inhibition of the pathway is being investigated as a potential treatment for multiple cancers. The most extensively investigated and best characterized is basal cell carcinoma (BCC), which occurs in both an inherited form (basal cell nevus syndrome or Gorlin's syndrome) and a sporadic form. Sporadic BCCs are the most common human malignancy. There is recent data available on the use of a small molecule inhibitor of the pathway in BCC.

Background

The hedgehog (Hh) signaling pathway is a critical element regulating both cellular growth and differentiation during embryonic development. It operates in both time- and position-dependent mechanisms to allow developing tissues to attain their correct size, location, and cellular content. The pathway was originally identified in the fruit fly, *Drosophila melanogaster*. Mutation of the single *Hh* gene that is present in this species gives rise to a fruit fly embryo that is covered in spiky processes, suggesting the "hedgehog" name. Genes encoding Hh proteins have subsequently been characterized in a large variety of species (1).

The Hh proteins are hydrophobic secreted proteins. The pathway is generally well conserved between different species. Three homologs have been identified in vertebrates: Sonic hedgehog (SHH), named for the video game character Sonic the Hedgehog; Indian hedgehog (IHH) and Desert hedgehog (DHH), both named for species of hedgehogs. In the absence of Hh, Patched 1 (PTCH1), a 12-transmembrane domain protein located on the cell membrane, suppresses the activation of Smoothened (SMO), a 7-transmembrane G-protein-coupled-like receptor (GPCR) located on the membrane of intracellular endosomes. This suppression inhibits the activation of the Hh signaling pathway (Fig. 1).

Signaling in the pathway is initiated by the Hh ligand binding to PTCH1, which releases the inhibition of SMO (Fig. 2). The signal is then transmitted via a number of interacting proteins leading to activation of the Gli family of zinc finger transcription factors (glioma-associated oncogene) and including Gli1, Gli2, and Gli3. It has recently been shown that Hh pathway signaling in vertebrates is dependent on the primary cilium (2), a cell organelle present on most mammalian cells. PTCH is located on the cell membrane at the base of the primary cilium and, in the absence of Hh, suppresses the activation of SMO by blocking it from entering the cilium. When the Hh pathway is activated by Hh ligand binding to PTCH, this suppresses PTCH and removes its inhibition of SMO. SMO migrates from the intracellular endosome to the cell membrane of the cilium. SMO is activated within the cilium and promotes the activation of Gli proteins from repressor (GliR) to activated (GliA) forms. These enter the nucleus and promote the transcription of the target genes (2). The bound Hh/PTCH complex is internalized from the cell surface into the interior of the cell and is destabilized or degraded (3). In vertebrates, the nonmobile primary cilia are present on most cells and correlate with Hh responsiveness (4). Suppressor of fused (Sufu) acts as a negative regulator of the pathway by binding to Gli, preventing activation of Hh target genes. This has recently been shown to be cilium-independent (5). With activation of the pathway, this regulation is removed.

Hh signaling and activation of the pathway seems to be significantly reduced or absent in adults. However, under certain circumstances, Hh signaling may be of importance in adults, e.g., the proliferation of stem cells in the hematopoietic system, the neural system, and mammary glands (6). However, it has become apparent that aberrant activation caused by activating mutations of the Hh signaling pathway plays an important role in initiating certain cancers (3). These so-called Type I cancers are independent of Hh ligand and include basal cell carcinoma (BCC) and medulloblastoma.

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BCC is the most common human cancer and most occur sporadically. However, there is a rare inherited condition in which individuals develop many BCCs from a relatively early age. This autosomal dominant disorder is the basal cell nevus syndrome (BCNS) or Gorlin's syndrome (7). Other than multiple BCCs, individuals may also develop jaw cysts, palmo-plantar pits, medulloblastomas, ovarian fibromas, rhabdomyosarcomas, meningiomas, and cardiac fibromas. There is often a characteristic facies and calcification of the falx cerebri and bony abnormalities may occur (bifid ribs, spina bifida occulta). The mutant gene in BCNS has been mapped, using genetic linkage studies, to human chromosome 9q22 and then to the PTCH1 gene (8, 9). With loss of function of PTCH1, SMO is no longer suppressed and the Hh pathway is activated. PTCH1 sequences identified it as the homolog of the Hh signaling inhibitor in Drosophila and it functions as a tumor suppressor gene. The inheritance of inactivating mutations of PTCH1 leads to constitutive upregulation of Hh signaling and the development of the BCNS with multiple skin tumors. It has subsequently been shown that approximately 90% of sporadic BCCs have mutations in PTCH1 and approximately 10% have activating mutations of SMO (10, 11). A number of mouse models of the Hh signaling pathway have, furthermore, shown the development of BCC-like tumors in the animals (10).

Medulloblastoma, a malignant tumor arising in the cerebellum, is thought to derive from stem cells or progenitor cells in this portion of the brain. Cerebellar maturation during embryonic development seems to be regulated by activation of the Hh pathway. Inactivating mutations of PTCH, leading to constitutive activation of the Hh signaling pathway, have been shown in approximately 30% of human medulloblastomas. Furthermore, approximately 5% of patients with Gorlin's syndrome develop these tumors (12, 13).

Aberrant activation of the Hh pathway in cancer may also be ligand dependent. Tumor types such as pancreatic and colorectal cancers secrete higher amounts of Hh ligand than the correlative normal tissue types. The Hh ligand from these cell types has been shown to activate the Hh pathway in neighboring stromal cells, which seem to be of myofibroblast lineage (14, 15). This paracrine activation of stromal cells may support the tumor microenvironment.

In yet another variation (reverse paracrine, Type IIIb), the stromal cells may release Hh, activating the pathway in tumor cells (2, 11).

**Clinical-Translational Advances**

It has been recognized for some time that blocking of the Hh pathway in embryogenesis may lead to
Holoprosencephaly, which includes incomplete cleavage of the forebrain, cyclopia, midline clefting of the lip, palate, and nose. Holoprosencephaly is a genetically and phenotypically heterogeneous disorder. One of the gene loci, HPE3, maps to the terminal band of chromosome 7. Extensive physical mapping studies have established a critical interval for HPE3, and subsequently identified the SHH gene as the prime candidate for the disorder (16).

If upregulation of Hh signaling leads to BCC, both sporadic and inherited, could inhibition of Hh signaling be useful as a treatment for these cancers? In 1971, a report was published describing an epidemic of cyclopia in lambs traced to pregnant ewes ingesting Veratrum californicum (California corn lily; ref. 17). The responsible compounds were identified and named cyclopamine and jervine. It is now known that the teratogenic effects of these compounds are due to their specific inhibition of Hh signaling (18), as suggested by similarities between the vertarum-induced holoprosencephaly-like abnormalities associated with loss of Shh function (19). It was shown that cyclopamine inhibits Hh pathway activation by binding directly to SMO (20), leading to its teratogenic effects and established that SMO could be inhibited pharmacologically as a prospective target to treat Hh-related cancers, such as BCC and medulloblastoma. It was shown that cyclopamine or synthetic derivatives with similar activity and with improved potency block activation of the Hh response pathway and abnormal cell growth associated with both types of oncogenic mutations (activating SMO and inactivating PTCH; refs. 21, 22).

Both BCC and medulloblastoma are Hh-ligand independent, mutation-driven cancers. Consequently, clinically effective inhibitors of the pathway need to have a site of action that is at the level of SMO or below. Blocking at the level of PTCH1 will not block the pathway in these tumors (11).

Novel SMO inhibitors have been discovered by high throughput screening of a library of small-molecule compounds and subsequent optimization through medicinal chemistry. One such molecule, GDC-0449 (Genentech), is a selective Hh pathway inhibitor with greater potency and more favorable pharmaceutical properties than cyclopamine (23). It has been reported to have antitumor activity in a clinical trial in locally advanced and metastatic BCC (24) and in a single patient with medulloblastoma (12); although a later SMO mutation conferred resistance (25).

In the BCC phase 1 study, 33 patients 18 years of age and older with biopsy confirmed, locally advanced, or metastatic BCC were treated with GDC-0449. All patients

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**Fig. 2.** Signaling in the pathway is initiated by the Hh ligand binding to PTCH, which releases the inhibition of SMO. SMO migrates from the intracellular endosome to the cell membrane of the cilium. SMO is activated within the cilium and promotes the activation of Gli proteins (GliA). These enter the nucleus and promote the transcription of the Hh target genes. The bound Hh/PTCH complex is internalized from the cell surface into the interior of the cell and is destabilized or degraded.

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were considered by the investigator to have refractory disease. The tumors were evaluated by physical examination or imaging. For patients with radiologically measurable disease, the Response Evaluation Criteria in Solid Tumors (RECIST) was used to determine response. For patients with locally advanced tumors (and no RECIST-measurable criteria) response was assessed by physical exam. The median duration of study treatment was 9.8 months. At data cutoff, all 33 patients had at least one follow-up assessment. Of the 33 patients, 18 had an objective response to GDC-0449, according to assessment on imaging (7 patients), physical examination (10 patients), or both (1 patient). Of the patients who had a response, 2 had a complete response and 16 had a partial response. The other 15 patients had either stable disease (11 patients) or progressive disease (4 patients). Of the 18 patients with metastatic disease, the overall response rate was 50%. For the locally advanced group (15 patients), the overall response rate was 60% (24). There were eight grade 3 adverse events possibly related to the study drug in six patients. These were fatigue, hyponatremia, muscle spasm, and atrial fibrillation. There was one grade 4 adverse event of asymptomatic hyponatremia (deemed unrelated to study drug). One patient withdrew from the study because of adverse events. There were high levels of GLI1 mRNA expression in tumors from the patients, similar to the levels in the more common less advanced BCCs and consistent with constitutive activation of the Hh pathway.

Evidence of activity was also seen in a phase 1 study with another SMO antagonist, BMS-833923, in which a patient with multiple skin BCCs had tumor shrinkage (26).

Future Developments

At this time, clinical activity has only been established for mutation-driven tumors, specifically BCC and medulloblastoma. A phase 2 study in inoperable locally advanced BCC (laBCC) and metastatic BCC (mBCC) was subsequently initiated with GDC-0449.1 Patients in this open label study have a histologically confirmed diagnosis of advanced BCC (either metastatic or locally advanced BCC). Patients with mBCC must have histologic confirmation of a distant BCC metastasis (e.g., lung, liver, lymph nodes, or bone). Patients with laBCC must have disease that is considered inoperable or to have a medical contraindication to surgery. The study is evaluating overall response rate, and if positive, may be the first therapy specifically for advanced BCC.

An investigator-initiated study of GDC-0449 is also ongoing in Gorlin's syndrome. This placebo-controlled study will evaluate the efficacy of this compound in reducing new surgically eligible BCCs in BCNS patients during months 3 through 18, and also in reducing the total number of BCCs of diameter 5 mm or greater on the upper back.2 In addition, a topical Hh pathway inhibitor, LDE225, is being evaluated in patients with Gorlin's syndrome,3 and in sporadic superficial BCCs.4 Ongoing phase 1 and phase 2 studies are also being conducted in medulloblastoma.5

In order to determine whether inhibiting the Hh pathway in tumors with ligand-dependent signaling may be of clinical benefit, a number of additional clinical trials are also being conducted. Phase 2 placebo-controlled studies of GDC-0449 in patients with a variety of tumor types such as colorectal cancer, ovarian cancer, pancreatic cancer, and gastric cancer, and other phase 2 studies in other tumor types, with and without other concurrent therapies, are ongoing and can be found on ClinicalTrials.gov.6 There are a number of other ongoing phase 1b studies with a number of other SMO antagonists such as BMS-833923 (Bristol-Myers Squibb), IPI-926 (Infinity) LDE-225, and PF-04449913 (Pfizer).

Inhibition of the Hh signaling pathway poses challenges for the treatment of less advanced BCC. Although the mutation-driven nature of the tumor seems to be similar in advanced and commonly treated BCC, the current standard of care for these tumors is primarily surgery with a very high cure rate. Current clinical research will determine whether BCC remains a surgical disease, or whether targeted therapy can turn this disease into a medically managed disease, and provide an alternative to surgery.

The delineation of the Hh signaling pathway, the recognition that aberrant Hh signaling may lead to certain cancers, and the understanding of inhibition of the pathway have led to the development of potential therapeutic agents as targeted therapy in cancer. Because the Hh signaling pathway plays such a central role in the pathogenesis of nearly all BCC, targeting this pathway in this tumor type has led to dramatic responses and indications of compelling efficacy. Further development of Hh pathway inhibitors for BCC could convert this disease from a primarily surgically treated disease to a medically treated disease, creating more options for patients.

Disclosure of Potential Conflicts of Interest

Both authors are employees of Genentech, a member of the Roche Group, and hold stock options in Roche.

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References

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