The Hedgehog Signaling Pathway in Cancer

Marie Evangelista, Hua Tian, and Frederic J. de Sauvage

Abstract

The Hedgehog (Hh) pathway is a signaling cascade that directs patterning in most animals and is crucial for proper development. At the molecular level, Hh ligands drive cell proliferation in some cell types while causing others to undergo differentiation. Hh signaling is most active during embryogenesis, and aberrant reactivation of the pathway in adult tissue can lead to the development of cancer. A comprehensive understanding of Hh signaling during development will undoubtedly shed light into the mechanism of Hh in cancer progression and identify potential targets for therapeutic intervention.

Pathway Overview

Hedgehog (Hh) is a morphogen that acts in a short- or long-range fashion on various tissue types (ref to refs. 1 – 3 for a comprehensive review of Hh signaling). In mammals, there are three Hh proteins: Sonic Hh, Indian Hh, and Desert Hh. In cells that make Hh, newly made protein enters the secretory pathway and undergoes autoprocessing and lipid modifications, resulting in the addition of a palmitoyl group to its NH2 terminus and cholesterol to its COOH terminus (4). Subsequent release of Hh depends on Dispatched (Disp), a multi-transmembrane protein, whereas diffusion of Hh requires Tout-velu-dependent synthesis of heparan sulfate proteoglycans (5, 6).

In cells receiving the Hh signal, pathway transmission is regulated at multiple levels. In the absence of Hh, Patched1 (Ptc1), a 12-transmembrane receptor, acts catalytically to suppress the activity of Smoothened (Smo), a seven-transmembrane protein, by preventing its localization to the cell surface (Fig. 1A; refs. 7, 8). Hh pathway activation is initiated when Hh ligand binds to Ptc1, relieving its inhibition on Smo (Fig. 1B; refs. 1, 2, 9). The amount of Hh available to bind Ptc1 is tightly regulated by Hh-binding proteins, such as Hh-interacting protein (Hip; ref. 10) and growth arrest-specific gene (Gas1; ref. 11), which sequester Hh. In contrast, two newly identified single-transmembrane proteins, cell adhesion molecule-related/down-regulated by oncogenes (Cdon/Cdo) and brother of Cdo (Boc), may facilitate Hh ligand binding to Ptc1 (12 – 14).

Surface localization of Smo is thought to initiate a signaling cascade, leading to the activation of the glioma-associated (Gli) family of zinc finger transcription factors. In vertebrates, there are three Gli proteins, with Gli1 and Gli2 thought to activate Hh target genes whereas Gli3 is thought to act mainly as a repressor (15). For the purposes of this review, the three proteins will be referred to as Gli proteins. In the absence of Hh signaling, protein kinases, such as protein kinase A, glycogen synthase kinase 3b, and casein kinase 1a, phosphorylate Gli, leading to proteosome-mediated cleavage of Gli into an NH2-terminal truncated form, which acts as a repressor of Hh target gene expression (Fig. 1A; refs. 16 – 19). Suppressor of fused (Sufu) acts as another negative regulator of the pathway by binding to Gli, both in the cytoplasm and in the nucleus, to prevent it from activating Hh target genes (20 – 23).

In the presence of Hh ligand, the precise mechanism by which the Hh signal is transmitted from Smo to Gli is not well understood in vertebrates. In flies, Hh binding to Ptc leads to the surface localization of Smo along with a complex involving the kinase Fused (Fu) and the kinesin-like molecule Costal 2 (Cos2; refs. 24 – 27). Thus, Fu and Cos2 relay the signal from Smo to cubitus interruptus (Ci), the fly homologue of Gli. However, in vertebrates, this mechanism does not seem to be conserved, as a Cos2 orthologue has not been identified and Fu is not required for transducing the Hh signal (28, 29). In contrast, recent studies have shown that Hh signaling in vertebrates requires the presence of a nonmotile cilium (Fig. 1B; refs. 30 – 32). Genetic studies in mice revealed that disruption of several components of anterograde or retrograde intraflagellar transport leads to neural tube patterning and limb development defects caused by impaired Hh signaling between Smo and Gli (30 – 32). How Smo signals to the Gli proteins is currently an active area of research in the Hh field today.

Hh Signaling in Cancer

Aberrant activation of the Hh pathway in cancers is caused either by mutations in the pathway (ligand independent) or through Hh overexpression (ligand dependent). Connections between the former and cancer were established from observations that mutations in the negative regulator, PTCH1, led to Gorlin syndrome (33, 34), a condition where patients develop numerous basal cell carcinomas (BCC) and are predisposed to medulloblastoma (a rare tumor of cerebellar granule neuron progenitor cells) and rhabdomyosarcoma (a muscle tumor). Inactivating mutations in PTCH1 have been detected in the majority of sporadic BCC, although a small number (~10%) are
caused by a specific SMO mutation that make tumors less sensitive to PTCH1 repression (35, 36). In almost all BCC cases, the Hh pathway is hyperactivated as indicated by up-regulation of Hh target genes (37). Mutations in PTCH1, SMO, or SUFU have also been identified in sporadic medulloblastoma, where one third of the tumors show increased Hh signaling (38, 39).

Although mutations in components of the Hh pathway have not yet been identified in tumors other than those mentioned above, activation of the Hh pathway via ligand overexpression is being documented in a growing number of cancers. Several reports have shown that Hh overexpression, sometimes accompanied by increased expression of Hh target genes, is
Role of the Hh Pathway in Ligand-Overexpressing Tumors

The mechanism by which Hh acts in ligand-overexpressing cancers is still unclear. One model suggests that these tumors rely on autocrine signaling, where Hh ligand produced by tumor cells acts on neighboring tumor cells to stimulate their growth or survival. This model is supported by in vitro data showing that proliferation of tumor cell lines is accelerated by addition of Hh ligand and inhibited by a Hh neutralizing antibody or by addition of cyclopamine, a Hh pathway antagonist. In this model, Hh ligand may directly affect the survival or proliferation of the entire tumor cell (40–44). However, in prostate, Hh pathway activity seems to correlate with higher-grade tumors, and treatment of an aggressive prostate xenograft model with cyclopamine, a pathway antagonist (see below), prevents metastasis to the lung. Furthermore, constitutive activation of the Hh pathway by overexpressing Gli1 in a rarely metastasizing clone, AT2.1, drove tumors to metastasize to the lung through induction of genes involved in epithelial-mesenchymal transition (43). Thus, it has been suggested that overexpression of Hh ligand leading to pathway activation may be important for tumor proliferation, survival, and/or metastasis (40–44).

Alternatively, Hh may be acting on a small fraction of cancer stem cells that are capable of self-renewal and differentiation among multiple lineages. Hh signaling is required for the normal growth and regeneration of organs, such as lung, gastrointestinal tract, prostate, from stem cells, and inappropriate and constitutive activation of Hh pathway during tissue repair and regeneration could promote tumorigenesis. Hh signaling has been indicated in proliferation of tissue stem cells from central nervous system (45–47), mammary gland (48), and hematopoietic stem cell (49). Genetic inhibition of Sonic Hh signaling results in the loss of neural stem cell forming potential from embryonic cortex (50), and activation of Hh signaling was shown to increase the number of mammary stem cell in vitro (51). In addition, pharmacologic inhibition of Hh signaling with cyclopamine in adult mice leads to reduced proliferation of stem cell in the subventricular zone (45). Expression of Hh pathway components has also been detected in mammary gland stem cells and in human breast “cancer stem cells” characterized as CD44+/CD24−/lowLin− (51). In vitro, cyclopamine was able to decrease mammary gland stem cells as measured by their capacity to form mammospheres (48, 51). Therefore, inhibition of the Hh pathway may provide a way to directly target cell populations that ultimately cause tumors.

Finally, Hh may act through a paracrine mechanism in ligand-dependent tumors. This model is more reminiscent of its role in development and organogenesis, where Hh ligand is secreted from the epithelium and signals to the underlying myofibroblast/mesenchyme/stroma compartment. The stromal compartment then signals back to the epithelium to regulate epithelial proliferation and differentiation through the production of factors, such as insulin-like growth factor (52), platelet-derived growth factor (53), fibroblast growth factor (54), bone morphogenetic protein (55), Notch (56), and Wnt (57, 58), which have been identified as Hh target genes in various models. However, to date, few universal target genes have been identified across different systems and much work still needs to be done to determine how Hh overexpression contributes to tumorigenesis. Undoubtedly, to successfully target Hh-overexpressing cancers, it will be important to understand the precise mechanism of how Hh exerts its effects.

However, results derived from cell lines grown in vitro should be interpreted with caution. In some of these experiments, high concentrations of Hh antagonist, 100- to 1,000-fold higher than what is needed to block Hh signaling, were necessary to cause growth inhibition. Interestingly, cell lines established from the Ptch1+/P53−/− medulloblastoma model also become insensitive to Hh antagonists, whereas tumors directly passaged s.c. in mice (i.e., direct allografts) maintained their molecular signature and were reproducibly responsive to Hh antagonists (59). This suggests that cell lines grown in vitro may lose their dependence on the Hh pathway for survival and that the high concentrations of Hh antagonist required to inhibit their growth may be due to nonspecific effects.

Clinical-Translational Advances

The clear link between Hh pathway and human cancers, such as BCC and medulloblastoma, drove the effort to identify small-molecule Hh antagonists to block the pathway. In addition to cyclopamine, a natural Hh antagonist isolated from corn lilies that targets Smo (60), different classes of small-molecule Hh antagonists have been identified through cell-based screens using Hh reporter assays (61, 62). Using cyclopamine or small-molecule Hh antagonists as tools, experiments have shown the potential use of targeting this pathway both in tumors where the pathway is mutated and in tumors where Hh ligand is overexpressed. Elegantly illustrating the former, small-molecule Hh antagonists have been used to treat endogenous medulloblastoma in the Ptch1+/P53−/− mouse model where tumors develop with 100% incidence (63). In addition, studies have shown that cyclopamine can inhibit growth of medulloblastoma tumors in vivo and in vitro (64, 65). Surprisingly, most human medulloblastoma tumors tested seemed to be responsive, suggesting potential broad application of Hh antagonist in treating this particular brain tumor, of which only ~25% harbor mutations in the Hh pathway.

Hh antagonists have also shown promising results for treatment of BCC. In ex vivo models of BCC, CIUR-61414, an aminoproline Hh antagonist, eliminated BCC-like lesions in various mouse models where tumor formation was driven by exposure of skin explant cultures to recombinant Hh or by PTCH1 mutations. This compound effectively down-regulates Hh reporter gene expression and induces apoptosis specifically within BCC-like lesions while leaving normal adjacent cell intact (66).

In xenograft models of Hh-overexpressing tumors, including small cell lung cancer, gastric and upper gastrointestinal tract cancer, pancreatic cancer, and prostate cancer, tumor growth inhibition on treatment with cyclopamine has also been shown (40–44). However, the activity of other pharmacologic agents

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has yet to be reported in these models, and the exact role of the Hh pathway in these types of tumors still needs to be fully investigated to better assess the potential use of Hh pathway antagonist in the clinic.

Conclusion and Outlook

Aberrant Hh pathway activation, driven by either mutation or ligand overexpression, is often associated with human cancers. Inhibition of the Hh pathway may provide a therapeutically important treatment for tumors, such as pancreatic cancer, where few options are available. Effective intervention of the Hh pathway can be achieved at the level of ligand binding to its receptor using anti-Hh antibodies or through inhibition of downstream effector molecules, such as Smo, with small-molecule antagonists. The latter has broader applications in treating both ligand-dependent and mutation-driven cancers.

An important issue that remains to be addressed is the potential toxicity of blocking a critical developmental pathway, such as Hh. Thus far, treatment with Hh antagonists, such as cyclopamine, seems to be well tolerated in animals. However, because the pathway is reactivated during tissue repair and regeneration through stimulation of endogenous stem cell populations, the effect of systemic administration on stem cell populations within the bone marrow, gut, muscle, liver, skin, brain, and others will have to be carefully monitored.

References


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