Hedgehog signaling: From basic research to clinical applications

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Studies of the major signaling pathways have revealed a connection between development, regeneration, and cancer, highlighting common signaling networks in these processes. The Hedgehog (Hh) pathway plays a central role in the development of most tissues and organs in mammals. Hh signaling is also required for tissue homeostasis and regeneration in adults, while perturbed Hh signaling is associated with human cancers. A fundamental understanding of Hh signaling will not only enhance our knowledge of how the embryos are patterned but also provide tools to treat diseases related to aberrant Hh signaling. Studies have yielded a basic framework of Hh signaling, which establishes the foundation for addressing unresolved issues of Hh signaling. A detailed characterization of the biochemical interactions between Hh components will help explain the production of graded Hh responses required for tissue patterning. Additional cell biological and genetic studies will offer new insight into the role of Hh signaling in homeostasis and regeneration. Finally, drugs that are capable of manipulating the Hh pathway can be used to treat human diseases caused by disrupted Hh signaling. These investigations will serve as a paradigm for studying signal transduction/integration in homeostasis and disease, and for translating discovery from bench to bedside.

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Introduction

The Hedgehog (Hh) pathway is one of several major signaling pathways that control key steps of embryonic development.1–3 Hh signaling also participates in tissue homeostasis and regeneration4 while perturbation of Hh signaling is associated with several human cancers.5–7 This highlights the versatility of conserved signaling pathways in multiple signaling contexts during the life span of an organism from embryonic development to postnatal physiology and pathology. Elucidating the molecular mechanisms of Hh signaling will increase our fundamental understanding of Hh signaling and serve as a paradigm to illuminate pathway design and evolution. These studies also hold the promise to provide tools for detecting and eventually treating human diseases caused by aberrant Hh signaling.

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The current model of mammalian Hh signal transduction

The basic framework of Hh signaling has been established through extensive studies in cell-based assays and model organisms. Hh signaling adopts multiple control steps at different subcellular locales that engage various regulatory mechanisms, some of which seem to be unique to Hh signaling. This important property ensures the production of graded responses in Hh-responsive cells. Moreover, such a pathway design enables tight regulation of Hh signaling in a temporally and spatially specific manner, a key requirement for tissue patterning and homeostasis.

Hedgehog ligand biogenesis and release

Among the three mammalian Hh ligands, Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh), Shh exhibits the widest tissue expression and is also the best characterized. Hh proteins elicit dose-dependent responses and, in several tissues, fulfill the definition of a classical morphogen, for instance, in the developing neural tube and the limb. Thus, elucidating the molecular basis of Hh protein distribution and action will reveal a fundamental mechanism of tissue patterning (see Fig. 1). The Hh ligand undergoes proteolytic cleavage to produce an N-terminal signaling fragment with dual lipid modification. The mature form has a cholesterol adduct at its N-terminus and a palmitoyl moiety at its C-terminus. Cholesterol modification is mediated by the C-terminal fragment of the Hh protein while Skinny hedgehog (Skn/Hhat), an acyltransferase, catalyzes the addition of a palmitoyl group. Lipid modification enhances local Hh protein levels and activity but also restricts its long-range distribution. The lipidated Hh ligand is released from Hh-producing cells, a process that requires the transmembrane protein Dispatched (Disp), and the secreted protein SCUBE2, and is subsequently delivered to Hh-responding cells. Several models have been proposed for Hh distribution, including a soluble Hh protein complex, a lipoprotein particle, exosomes (membrane vesicles), and actin-based specialized filopodia (cytonemes). An important task in this active area of research is to uncover the in vivo relevance of the proposed modes of Hh distribution. This could be challenging because many of these processes involve machinery that also functions in other processes and the machinery that executes each mode of transport has not been fully characterized. Moreover, whether Hh-producing cells utilize a unique method or a combination of mechanisms to distribute its Hh ligand and whether different tissues preferentially rely on a particular mechanism require further studies. Finally, it is unclear if these are universal mechanisms in distributing other signaling molecules.

Hedgehog signal transduction

Hh signaling is initiated through binding of the Hh ligand to its receptor, Patched 1 (Ptch1), a 12-pass transmembrane protein with topological similarity to transporters (see Fig. 2). This process is facilitated by several accessory proteins, including Boc (bioregional Cdon-binding protein), Cdon (cell-adhesion-molecule-related), and Gas1 (growth arrest specific 1). Distinct complexes that are comprised of Ptch1 and one of its coreceptors have been reported, indicating the overlapping and distinct functions of these accessory proteins. However, the molecular basis of how Hh coreceptors enhance Hh signaling remains unknown. Hh binding to Ptch1 relieves Ptch1 repression of the seven-pass transmembrane protein, Smoothened (Smo), and triggers a cascade of signaling events downstream from Smo. Lipidated Hh protein has increased signaling activity and it is unclear how lipid modification confers such an important property.

Similarly, despite extensive studies, the molecular mechanisms by which Ptch1 regulates Smo activity remain an enigma. Because Ptch1 does not directly interact with Smo, it is generally believed that a small molecule (or molecules) regulated by Ptch1 mediates the interaction between Ptch and Smo. The identity of the mediator between Ptch and Smo remains elusive. Such molecules may not be amenable to genetic manipulations and their discovery would be likely to rely on cell-based assays and proteomic approaches. Interestingly, oxysterols bind Smo and promote Smo activity, adding another layer of regulation to Hh signaling and strengthening a link between Hh signaling and lipid metabolism. The purported small molecule mediator of Ptch can possibly influence oxysterols in addition to inhibiting Smo. As discussed below, mutations in Ptch1 or Smo underlie several human tumors, and a mechanistic understanding of how Hh signal is transduced via Ptch/co-receptor/Smo will have a major impact on our fundamental understanding of tumor development and treatment.

Control of Hh target gene expression

Three transcription factors, Gli1–3, mediate Hh target gene expression in the nucleus, and to some extent Gli2 undergo limited proteolysis to generate a transcriptional repressor that inhibits Hh target gene expression in the absence of Hh signaling. Hh signaling not only inhibits Gli repressor formation but also promotes the production of Gli activators (derived from full-length Gli proteins) to activate nuclear Hh targets. Unlike Gli2/3, Gli1 does not undergo limited proteolysis and only functions as an activator. Gli1 transcription is induced by Hh signaling and serves to amplify the Hh signal. Hh target gene expression is controlled by varying levels and combinations of Gli activators and repressors in a given tissue, thus generating Hh outputs appropriate for tissue development. The overlapping function of Gli proteins and the variable expression and requirements of individual Gli proteins in diverse tissues render predictions of Hh responses and phenotypes a challenging task. An important question is to identify and characterize Gli targets in diverse tissues in order to further understand how Hh signaling controls various aspects of tissue development and homeostasis. In this regard, significant progress has been made in the neural tube and the limb. A combination of chromatin immunoprecipitation sequencing (ChIP-Seq), transgenesis and modeling has yielded a network of signaling that serves as a blueprint for further
Extension of these studies to all three Gli proteins and to other tissues would enable a better description of how a combinatorial use of the three Gli proteins can produce graded Hh responses.

A key negative regulator in Hh signaling downstream from Smo is Suppressor of Fused (Sufu). Sufu controls the protein levels, activity and distribution of the three Gli transcription factors and Sufu/Gli constitute a major regulatory hub in Hh signaling downstream from Smo. Sufu can sequester Gli proteins, regulate Gli protein levels, promote the production of Gli repressor and control Gli protein activity. This perhaps endows Hh-responsive cells with the ability to produce complex responses and offers focal points for integrating inputs. Loss of Sufu leads refinement.
to upregulation of Hh signaling that is associated with reduced Gli protein levels. This is consistent with Sufu’s role as a major negative regulator of Hh signaling. Interestingly, maximal Hh signaling in Sufu mutants is not achieved, suggesting that Sufu also exerts positive effects on Hh signaling. This likely results from Sufu regulation of full-length Gli protein levels (and consequently Gli activators). In fact, simultaneous perturbation of Gli activators and repressors in other settings also increases Hh signaling but compromises maximal Hh signaling. Studies on Sufu regulation and function will continue to yield key insight into Hh pathway regulation.

Feedback control of Hh signaling

In addition to signal amplification using a positive feedback loop, Hh signal transduction also employs negative feedback to modulate the Hh signal. Both Ptc1 and Hhip are Hh-binding proteins and are induced by Hh signaling. They can function to sequester the Hh ligand and alter the dynamics of Hh signaling. Interestingly, Boc and Cdon do not participate in Ptc-mediated feedback regulation since their expression is downregulated by Hh signaling although Boc/Cdon facilitate Ptc-mediated Hh reception. Because both signaling strength and duration are important for Hh-responsive cells, feedback control of Hh signaling provides an important means to fine-tune signaling outputs. It is conceivable that feedback control could be utilized to regulate the levels or activity of other Hh pathway components. This would confer new properties to Hh signaling. Modeling would facilitate predictions of properties of Hh responses but these computational models need to be tested in an experimental setting both in vitro and in vivo.

Hh pathway components exhibit transcriptional and post-transcriptional regulations. They are utilized in different subcellular locales and control different steps of Hh signaling. A key issue is to assess the contribution of each type of regulation to essential properties of Hh signaling such as signal amplification, signal output, and signal modulation.
Evolutionary divergence of Hh signaling

While the basic design of the Hh pathway is largely conserved among diverse species, evolutionary divergence has occurred, in particular from fly to mammal. Gene duplication has yielded three Hh ligands, two Ptc receptors, two Kif kinesin proteins, and three Gli transcription factors in mammals. This could have allowed paralogs to diverge in function and trigger corresponding changes in their interacting partners. Consistent with this, Sufu subsumes a central role in negatively regulating Gli activity while the Fu kinase is dispensable in mammals. This is in contrast to a central role of Fu in Drosophila Hh signaling and lack of overt phenotypes in Sufu mutant flies. The powerful technology of next-generation sequencing and genome editing (such as CRISPR/Cas) can be readily applied to diverse organisms. This would open up a new avenue to test experimentally how various Hh pathway components and their interactions emerged, evolved and were even replaced in different species during evolution.

The origin of the Hh pathway is unknown. It has been speculated that it may have evolved from machinery that senses nutrients. This is based on the connection between lipid metabolism and Hh signaling and the function of homologs of Hh pathway components in organisms lacking active Hh signaling and thus predating the Hh pathway. Again, the ability to manipulate the genome with greater ease would allow testing of these hypotheses.

The primary cilium and vertebrate Hedgehog signaling

Another unique aspect of vertebrate Hh signaling is the utilization of the primary cilium for transducing the Hh signal. The primary cilium is a microtubule-based, evolutionarily conserved organelle. Construction of the cilium relies on intraflagellar transport powered by the anterograde kinesin and the retrograde dynein motor. Most vertebrate cells have a single primary cilium. Genetic analysis of mutants defective in ciliogenesis or ciliary function provides strong evidence to support an essential role of the cilium in Hh signal transduction. Consistent with this model, most core Hh components localize to the cilium in a dynamic manner. How primary cilia control various steps of Hh signal transduction is under intensive investigation. A current model suggests that Sufu is recruited to the cilium via Gli proteins. Sufu, Gli, and kinesin Kif7 are enriched at the cilium tip. Sufu-Gli association is proposed to be disrupted upon Hh stimulation perhaps at the cilium tip, allowing the production of Gli activators. Kif7 controls cilium architecture at the tip and this could affect Sufu-Gli activity. New tools are needed in order to establish the function of ciliary localization of Hh pathway components. The connection between Hh signaling and lipid metabolism has long fueled the speculation that changes in membrane lipid contents have an important impact on Hh signaling. Interestingly, the ciliary membrane seems to contain specialized lipid composition and may provide a conducive environment for Hh signal transduction.

Noncanonical Hh signaling

Interestingly, several Hh-dependent functions, such as chemotaxis and pathfinding, appear to be independent of the primary cilium and Gli-mediated transcription. Smo at different subcellular locales was proposed to account for eliciting distinct Hh outputs. Smo on the primary cilium relays the Hh signal to Gli proteins, culminating in transcriptional responses whereas Smo outside the cilium regulates chemotactic responses to Hh. Further studies will shed light on the adaptation of the primary cilium in Hh signaling.

Hh signaling and congenital anomalies

Studies in model organisms indicate that the Hh pathway controls key steps of the developmental processes in many tissues. It is expected that mutations in Hh pathway components will lead to human congenital anomalies. Indeed, mutations in Shh have been detected in human patients with holoprosencephaly whereas mutations in Ihh are associated with brachydactyly and Dhh mutations are found in gonadal dysgenesis. Disruption of the human Hh acyltransferase that catalyzes the transfer of a palmitate moiety to Hh proteins results in 46, XY Disorder of Sex Development. As next-generation sequencing becomes more routinely used in clinical diagnosis, it is anticipated that additional cases of birth defects related to the Hh pathway will be discovered. Severe loss-of-function mutations in Hh pathway components are likely to cause serious developmental defects incompatible with life and the fetuses die in utero. In this case, whole genome sequencing would allow us to determine whether missense mutations produce milder or unexpected phenotypes in living patients, which are in general under-diagnosed. These studies will provide important information on phenotype-genotype correlations. They will not only assist clinical diagnosis but also inform us of the function of Hh signaling in human development. With induced pluripotent stem cell and genome editing technology, mutations corresponding to human mutations can be readily created in cell lines and model organisms to facilitate phenotypic analysis and mechanistic studies.

Mutations that disrupt ciliary function (ciliopathies) are associated with a wide range of human syndromes. A number of disease-causing genes have been identified in ciliopathy patients. Developmental abnormalities with hallmarks of Hh phenotypes in these patients are probably caused by defective Hh signaling. By contrast, it has been difficult to pinpoint the underlying signaling defects in other anomalies, particularly dysfunction of postnatal physiology. In many instances, it is unclear whether and how disruption of Hh signaling contributes to the phenotypic consequences.

Hh signaling and tissue regeneration and repair

Hh signaling has been studied in many adult tissues in the context of progenitor cells and tissue regeneration and repair. In a number of tissues, active Hh signaling can be detected in a small population that possesses progenitor potential, and in many cases Hh signaling is activated upon
tissue injury. It is postulated that activation of the Hh pathway serves to stimulate cell proliferation essential for tissue repair. For instance, Gli-expressing cells contribute to the neural stem cell population located in the subventricular zone of the lateral ventricles and in the subgranular zone of the hippocampal dentate gyrus in the murine nervous system. A major challenge is to establish the functional role of Hh signaling in tissue regeneration in a given tissue. In many instances, it is difficult to unambiguously identify Hh-producing and -responsive cells in adult tissues in homeostasis and following injury. Tools are generally not available to eliminate these cell populations exclusively and efficiently in adults in order to evaluate the effects of perturbed Hh signaling. Moreover, Hh signaling probably interacts with other pathways in the process of tissue repair. Dissecting the signaling cascade and crosstalk and assessing the contribution of Hh signaling in a given tissue is a major effort.

Hh signaling and tumor development

Perturbation of Hh signaling is associated with several human cancers, notably basal cell carcinoma (BCC) and medulloblastoma. Mutations in Ptc1 are found in most human BCCs, suggesting that enhanced Hh signaling probably causes BCC. While it is not possible to establish definitively a causal relationship between mutations in Hh components and human cancers, studies in mice and cell lines have provided strong evidence to support Hh pathway disruption as a major factor in developing human BCC and a subset of medulloblastomas. Several other types of human cancers such as digestive tract tumors have also been associated with perturbed Hh signaling. Even if mutations in Hh components are not required for tumor initiation, it remains possible that these tumors utilize the Hh pathway for growth or survival at certain stages of tumor development. Next-generation sequencing will continue to provide important information on Hh signaling and human cancers, in particular, in uncovering the spectrum of cancers that relies on Hh signaling.

Strikingly, molecules that inhibit Smo activity have been shown to be effective in treating patients with invasive BCC or medulloblastoma related to Hh pathway overactivity. Smo appears to be a very druggable target in the Hh pathway and various Smo inhibitors (such as GDC-0449) have been developed. This illustrates the feasibility of targeting the Hh pathway for treating diseases caused by aberrant Hh signaling. Patients who received GDC-0449 (Vismodegib, Genentech Inc., South San Francisco, CA, USA) treatment initially responded but eventually developed resistance. Genomic analysis, in some cases, revealed mutations in Smo that conferred resistance to GDC-0449. This problem is common in targeted therapies and effective approaches that overcome resistance will serve as a paradigm for understanding drug resistance in targeted therapies. Apart from its immediate side effects, Smo antagonists can inhibit bone growth in animal studies presumably related to Hh function in tissue homeostasis and regeneration/repair. This raises the important question of how long-term use of Hh inhibitors can disturb normal physiology.

Molecules that target other Hh pathway components such as Gli have also been reported. Efforts to develop drugs against other Hh players are underway. In addition to offering tools to probe the mechanisms of Hh signaling, they could provide potential therapies for Hh-related tumors or diseases.

As discussed above, Hh signaling may be utilized at one stage of tumor development for their growth in tumor types other than the well-characterized, Hh-related tumors. In this scenario, the blockade of Hh signaling in combination with chemotherapeutic drugs would still confer therapeutic benefits.

Given tissue complexity, tumors derived from different germ layers seem to respond differently to small molecules that perturb Hh signaling. The aforementioned Smo antagonists reduce the growth of basal cell carcinoma and medulloblastoma, indicating a critical role of Hh signaling in promoting tumor growth. By contrast, Hh agonists cause stromal hyperplasia in the pancreas and subsequently inhibit epithelial growth. Moreover, genetic ablation of Hh responses in murine models can accelerate tumor development in the pancreas or bladder. This suggests that Hh pathway activation could slow tumor development in these cases. These seemingly paradoxical outcomes stem from complex tissue interactions. Nevertheless, they can be understood on the basis of the proliferative effect of Hh signaling and activation of other signaling pathways in a particular tissue.

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References

Hedgehog signaling in development and disease


