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The Cilium-Dependent Hedgehog Signaling in Mammals

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Understanding the molecular signaling network that translates the presence of extracellular signal proteins into the multitude of cellular responses, both transcriptional and non-transcriptional, is critical for our understanding of development and physiology of animals and pathogenesis of numerous human diseases.

The most common study is the Hedgehog (Hh) signaling pathway, which plays well-recognized roles in both development and various types of cancers [1]. Earlier this year, the US Food and Drug Administration has approved Vismodegib, the first drug specifically targeting the Hh pathway, for the treatment of basal cell carcinoma, proving that targeting a specific signaling pathway can be a viable strategy for fighting human diseases [2]. However, the quick emergence of cancers resistant to this drug also attests to the need of additional research aimed at better understanding of the critical events in this pathway [3].

The framework of Hh signal transduction in target cells was first established in the model system *Drosophila melanogaster* [1]. Briefly, the Hh protein binds a receptor complex on the cell surface, of which the main signaling component is a 12-span transmembrane protein named Patched (Ptc). The binding between Hh and Ptc relieves Ptc's inhibitory effect on a serpentine receptor-like protein Smoothened (Smo). This allows the activation of Smo, which transforms a dual functional transcription factor, Cubitus interruptus (Ci), into a transcriptional activator. In the absence of Hh, Smo is inhibited by Ptc, and Ci is processed into a transcriptional repressor.

The importance of Hh signaling in mammalian development was obvious upon the cloning of the mammalian Hh homologues, Shh, Ihh, and Dhh, which are expressed in tissues with well-known organizing activities, such as the zone of polarizing activity in the limbs, the embryonic node, notochord and the floor plate of the spinal cord [4]. Subsequent characterization of the mouse mutants for *Shh, Ptch1* (one of the Ptc homologues in mammals) and *Smo* confirmed that Hh signaling is indeed critical for mammalian development and cancer formation [5-7]. The conserved function of these key pathway regulators between insects and mammals appeared to outline an evolutionary conserved mechanism of Hh signal transduction.

This was changed completely in 2003, when three proteins, Ift88, Ift172 and Kif3a, previously known to be involved in building the flagella in ciliated algae and the cilia in animals, were found to play essential roles in Hh signaling in mammals [8]. The loss of cilia in mice leads to abnormal patterning of the ventral spinal cord and somites, as well as a downregulation of Hh target genes such as *Gli1* and *Ptch1*, suggesting a failure in Gli activation. Interestingly, the same mutant mice also exhibit polydactyly and other morphological characteristics suggesting a possible loss of Gli repressor activities [9].

Genetic epistasis analyses offer great insight into how the cilium affects Hh signaling. The loss of Ptch1 and Rab23, two cell-autonomous negative regulators of the Hh target cell response, fails to activate Hh signaling in the absence of the cilium, suggesting that cilium is involved in the intracellular transduction of the Hh signal [8]. Further analysis showed that Suppressor of Fused (Sufu), an essential negative regulator of the Gli proteins, inhibits Gli activation in the absence of the cilium [10,11]. These studies, strengthened by subsequent cell biology studies revealing the ciliary localization of Ptch1, Smo, Gli and Sufu, suggest a model in which a bulk of the Hh signal transduction occurs in the cilium [12-14] (Figure 1).

Smo is localized to the cilium upon Hh pathway activation through an Arrestin-dependent process [12,15]. Ptch1 inhibits Smo ciliary localization, but the molecular details of this inhibition are not clear [14]. Mutations that prevent Smo ciliary localization appear to dampen the cell's response to Hh; whereas Smo proteins carrying mutations that render them constitutively active are coincidentally localized to

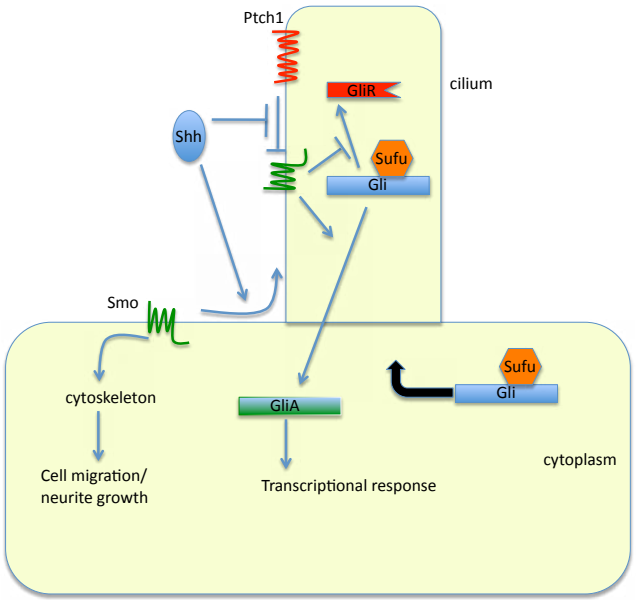

Figure 1: The primary cilium and the Hh signaling. The interaction between Shh and Ptch1 leads to the ciliary localization of Smo, which is otherwise inhibited by Ptch1. A Gli-Sufu complex is also localized to the cilium and this localization is enhanced by Shh. It is believed that Gli proteins are proteolytically processed inside or at the base of the cilium in the absence of Shh. In the presence of Shh, activated Smo promotes the dissociation of Gli proteins from Sufu and turns Gli proteins into transcriptional activators. Smo outside the cilium appears to regulate cell migration and neurite growth in response to Shh.

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the cilium independent of Hh [12]. These results suggest that ciliary localization is an important part of Smo activation. However, ciliary localization per se does not appear to be sufficient for the activation of the pathway because in certain context constitutive ciliary localization of Smo can be associated with decreased Hh pathway activation [16-18]. Obviously, many questions remain on how Smo localization to the cilium is regulated by Hh and Ptch1; why ciliary localization is important for Smo activation and what is needed to activate Smo once it arrives in the cilium.

Although Smo is structurally related to serpentine receptors and it is likely that the second messenger cAMP may play a role in Hh signal transduction, studies in *Drosophila* suggest that direct interaction between Smo and a downstream multi-protein complex containing Ci is a crucial step of the Hh signaling [19-22]. If an analogy can be drawn in vertebrates, then Gli protein localization to the cilium has to occur to allow physical interaction with activated Smo. Some indirect evidence appears to point to such a possibility. For example, cAMP-dependent protein kinase A inhibits Gli protein activation and Gli ciliary localization [23,24]. In addition, disruption of cytoplasmic but not ciliary microtubules blocks the ciliary localization of Gli2 and a coincidental decrease in Hh signaling activity [25]. Although these results are consistent with the requirement of Gli ciliary localization for Hh pathway activation, a solid connection between Gli ciliary localization and their activation needs to be established through more stringent tests.

Although it is clear that the cilium plays a critical role in Gli protein activation as well as their proteolytic processing, some evidence points to the existence of a cilium-independent mechanism of Hh pathway activation. First, double mutant analysis shows that the loss of cilium does not completely rescue the *Ptch1* mutant phenotype, suggesting that at least part of Hh pathway downstream of *Ptch1* is through a cilium-independent route (our unpublished data). Moreover, two recent studies showed that although the primary cilium is required for the induction of brain and skin tumors harboring activating mutations in Smo, it plays an opposite role in the induction of tumors harboring activating mutations in Gli2 [26,27]. This "negative role" of the cilium in Gli2-mediated Hh pathway activation and tumor induction can be partly explained by the loss of Gli3 repressor in the absence of the cilium. It is also possible that cilium may be required for most, but not all Gli activator in this context.

In addition to the transcriptional response mediated by the Gli family proteins, Hh proteins also elicits non-transcriptional responses, such as cell migration and neurite growth in their target cells through a Smo-dependent pathway [28]. Interestingly, this non-canonical Hh response appears to be enhanced in the absence of the cilium, suggesting that the cilium may represent a binary switch between two different pathways downstream of Smo [29].

In summary, the connection between the cilium and Hh signaling is as fascinating to science as it is important to treating various human diseases. The existing evidence shows that the roles of the cilium in Hh signal transduction are complex and deserve more investigation before cilium-based therapeutic strategy can be applied to human patients. We will definitely see more exciting progress in this front for the coming years.

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