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Hedgehog trafficking, cilia and brain functions

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ABSTRACT

The primary cilium has recently emerged as an important center for transduction of the Sonic Hedgehog (Shh) signal. Genetic studies have shown that Shh signaling at the level of primary cilia is essential for patterning the ventral neural tube and regulating adult stem cells. Some defects observed in human diseases and resulting from mutations affecting the organization of the primary cilium have been attributed to defective Shh signaling. The recent development of Shh pathway inhibitors for treating tumors linked to perturbations of Shh signaling has fostered studies to understand their mechanism of action in Shh receptor complex trafficking at the primary cilium.

1. Introduction

The Sonic Hedgehog (Shh) signaling pathway is well known for its roles in patterning and growth of brain structures during development (Dessaud et al., 2008; Varjosalo and Taipale, 2008). The early discovery of Shh expression throughout the adult rodent brain (Traiffort et al., 2010, 1998) has generated considerable interest and novel functions for this protein have progressively emerged (Borzillo and Lippa, 2005; Traiffort et al., 2010). The importance of Shh signaling in adult brain plasticity is demonstrated by its implication in neural stem cell maintenance in adult neurogenic niches (Han and Alvarez-Buylla, 2010; Suh et al., 2009). Humans affected by the Gorlin syndrome have inactivating mutations in the key Shh receptor Patched (Ptc), characterized as a negative regulator of Shh signaling. They display susceptibility to develop medulloblastoma, one of the most malignant brain tumors in childhood. As a consequence of these mutations, the Ptc-mediated inhibition exerted on the Smoothened (Smo) receptor, the main positive regulator of the pathway, is relieved, leading to the tumorigenic process. Thus, inhibiting the Shh signaling pathway by small molecule inhibitors has generated considerable interest for treating these tumors, and several inhibitors of Smo are currently being evaluated for treating medulloblastomas (Heretsch et al., 2010; Mas and Ruiz i Altaba, 2010; Scales and de Sauvage, 2009). The recent discovery that Shh signaling depends on primary cilia (Huangfu et al., 2003) has fostered studies aimed at characterizing the distribution and the regulation of the pathway at the level of this important signaling center (Goetz and Anderson, 2010; Louvi and Grove, 2011; Simpson et al., 2009). Genetic studies in mice showed that Shh signaling at the level of the primary cilium is essential for patterning of the ventral neural tube in the mouse embryo but also in the regulation of adult stem cells (Han and Alvarez-Buylla, 2010). In addition, some defects in human diseases known as ciliopathies and resulting from mutations affecting the organization of the primary cilia, have been attributed to defective Hedgehog (Hh) signaling (Goetz and Anderson, 2010). Here, we review the role of Hh signaling and trafficking at the primary cilium during brain development and in mature neural tissues. We also highlight its importance for treating brain tumors and understanding the complex traits of several human disorders linked to primary cilia defects.

2. Transduction of Hh signaling at the primary cilium

In vertebrates, the primary cilium is defined as a microtubule-based organelle of about 1–5 μm in length. It extends from the cell surface as a single, non motile, antenna-like structure and is present on most cell types in embryonic and adult tissues (Bettencourt-Dias et al., 2011; Louvi and Grove, 2011). The ciliary basal body is formed from the mother centriole and acts as a docking area for a large number of pericentriolar proteins. The axoneme, constituted by nine doublets of microtubules, extends from the basal body through the cilium. Between the basal body and the axoneme, the transition fibers create a permeable barrier between the cilium and the rest of the cell. Selective import or export of proteins between the cytoplasm and the cilium require intraflagellar transport (IFT) particles forming two complexes B and A, which use the anterograde kinesin-2 (also known as the Kif3 motor complex) and the retrograde dynein motors, respectively (Rosenbaum and Witman, 2002; Taschner et al., 2011). The

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primary cilium differs from the secondary motile cilia. In the latter, the axoneme contains an extra central pair of microtubules linked to the nine outer microtubule pairs. Intense genetic, molecular, biochemical and pharmacological studies have recently been conducted to understand its ultrastructure and its functions in the central nervous system (CNS). Notably, efforts have been made to identify components of signal transduction pathways that are present in this organelle. Trafficking of proteins involved in the Hh signaling pathway up and down the cilium in stem or precursor cells has rapidly emerged as a key step in neural development (Goetz and Anderson, 2010; Louvi and Grove, 2011).

Shh, Indian hedgehog, and Desert Hedgehog genes encode a family of secreted peptides with key roles in tissue patterning during embryogenesis. Their roles in adult neural tissues and the involvement of the associated signaling pathway in brain physiology and in pathologies are just beginning to be explored (Traiffort et al., 2010). These proteins mediate their action via a receptor complex associating two transmembrane proteins: Ptc, the Shh receptor, which displays a transporter-like structure, and Smo, a putative member of the G protein-coupled receptor superfamily, which transduces the Shh signal downstream of Ptc. The repression exerted by Ptc on Smo is relieved when Shh binds Ptc and a complex signaling cascade is initiated leading to the activation of the transcription factors of the Gli family (Gli1-3) and to the transcription of target genes including Ptc and Gli1 themselves (Fig. 1) (Ruiz i Altaba et al., 2007). Activation of the canonical Shh pathway leads to the inhibition of Gli transcription factor processing into their transcriptional repressor forms and to the concomitant accumulation of their activator forms. Gli1 constitutes a convenient readout for pathway activation and amplifies the Hh response. Gli2 and Gli3 function mainly as transcriptional activator and repressor, respectively, even if both can show the opposite activity in specific contexts (Riobo and Manning, 2007; Ruiz i Altaba et al., 2007).

Besides the canonical Shh pathway, a non-canonical pathway has also been described. It induces synchronous Ca^{2+} spikes and IP3 transients at the neuronal primary cilium through the activation of Smo (Belgacem and Borodinsky, 2011). Several additional proteins such as the negative regulator Hedgehog-interacting protein (Hip), which is found in a soluble and membrane associated form in brain regions (Coulombe et al., 2004), the two cell surface immunoglobulin/fibronectin proteins, Boc and Cdo, and the growth arrest-specific 1 protein (Gas1) bind Hh proteins with high affinity, function as Shh coreceptors and promote Hh signaling according to unknown mechanisms (Varjosalo and Taipale, 2008; Allen et al., 2011). The structurally related Boc and Cdo are integral membrane proteins conserved from *Drosophila* to rodent whereas Gas1, a glycosylphosphatidylinositol anchored plasma membrane protein, is specific to Hh signaling in vertebrates. A likely model proposes that Gas1, Cdo and Boc form a physical complex with Ptc and function as essential coreceptors that mediate multiple cellular responses to Hh. However, this requirement of Hh coreceptors depends on the cell type and the stage of development (Allen et al., 2011; Izzi et al., 2011).

Smo, Ptc, Gli1-3 and the negative regulator Sufu have been detected at the primary cilium (Corbit et al., 2005; Haycraft et al., 2005; Huangfu and Anderson, 2006; Rohatgi et al., 2007; Roudaut et al., 2011). Shh has been identified close to the cilium base in target neural progenitors during active Shh signaling in the neural tube (Chamberlain et al., 2008). Ptc is proposed to be localized to the base of the cilium in the absence of its ligand and to inhibit signaling by preventing Smo localization to the cilium (Fig. 1). Upon ligand binding, simultaneous removal of Ptc and localization of Smo to cilia occur. β-arrestins might mediate Smo interaction with the Kif3a kinesin motor protein, regulating Smo localization to primary cilia (Kovacs et al., 2008; Rohatgi et al., 2007). Alternatively, Smo may

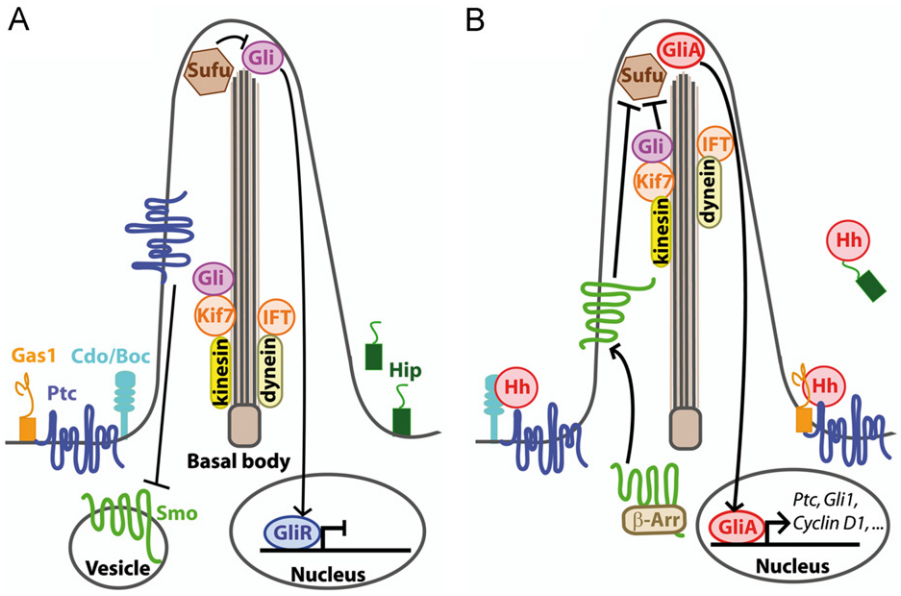

Fig. 1. Hedgehog signaling pathway at the primary cilium. (A) In the absence of Hedgehog (Hh) ligand, the receptor Patched (Ptc, blue) is located in the cilium and represses Smoothened (Smo, green) mostly found outside the cilium, by an as yet unknown mechanism. Full-length Gli transcription factors (pink) present in the cilia in a complex with the anterograde IFT-kinesin motor Kif7. Repressor factors such as Sufu (brown) promote Gli truncation into their repressor forms (GliR, blue). The retrograde IFT-dynein motor allows GliR to reach the nucleus and to inhibit the transcription of the target genes. Hip (green), a negative regulator of the pathway binds Hh and is found as both membrane-associated and soluble forms. Gas1 (orange), Cdo and Boc (blue) are other membrane proteins that bind Hh and are considered as positive regulators of the pathway. They have not yet been identified at the primary cilium. (B) In the presence of Hh ligand (red), Smo inhibition is relieved allowing its translocation and accumulation in the cilium involving its interaction with ß-arrestin (ß-arr, brown). This accumulation relieves the inhibition that Sufu exerts on Gli transcription factors and leads to the conversion of Gli transcription factors into their activator forms (GliA, red). Kif7 translocates to the cilium tip, promoting Gli accumulation at that location, where Kif7 may also block the function of Sufu, resulting in activation of the Gli proteins. GliA reaches the nucleus and activates the transcription of Hh target genes including Ptc and Gli1 themselves.