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Primary cilia and forebrain development

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ABSTRACT

With a microtubule-based axoneme supporting its plasma membrane-ensheathed projection from the basal body of almost all cell types in the human body, and present in only one copy per cell, the primary cilium can be considered an organelle *sui generis*. Although it was first observed and recorded in histological studies from the late 19th century, the tiny structure was essentially forgotten for many decades. In the past ten years, however, scientists have turned their eyes once again upon primary cilia and realized that they are very important for the development of almost all organs in the mammalian body, especially those dependent upon the signaling from members Hedgehog family, such as Indian and Sonic hedgehog. In this review, we outline the roles that primary cilia play in forebrain development, not just in the crucial transduction of Sonic hedgehog signaling, but also new results showing that cilia are important for cell cycle progression in proliferating neural precursors. We will focus upon cerebral cortex development but will also discuss the importance of cilia for the embryonic hippocampus, olfactory bulb, and diencephalon.

1. Introduction

Cilia are 1–3 μm membrane-bound, microtubule-based structures found on many cells in the vertebrate body (Davis et al., 2006). Cilia can be classified into two groups regarding their ultrastructure and function; both groups share at their base a centrosome-derived basal body that anchors the cilium within the cytoplasm. The axoneme in motile cilia is organized in a '9 + 2' arrangement, where the double microtubule core in the middle is surrounded by nine microtubule doublets arranged in a circular pattern. Motility is achieved through the protein–protein interactions between the central core and the surrounding doublets. Non-motile cilia, also known as primary cilia, show a '9 + 0' arrangement lacking the central microtubule core. Even though primary cilia have been considered to be completely non-motile, it has been reported that primary cilia at the embryonic node can generate a rhythmic movement, though not in the whiplike fashion of motile cilia (Nonaka et al., 1998). Both classes of cilia are maintained by the transport of protein cargo along the axoneme mediated by a microtubule-based transport, namely intraflagellar transport (IFT) (Scholey and Anderson, 2006). IFT utilizes kinesin-II- and dynein-based motors to facilitate anterograde and retrograde transport, respectively. Interaction between protein cargo and the transport motors occurs through the IFT scaffolding proteins complexes IFTA and IFTB, which in turn are composed of IFTX proteins, in which X denotes the molecular weight of the given protein.

Even though cilia in the past have been undervalued, recent studies of Hedgehog (Hh) signaling reveal striking evidence of the importance of cilia in signal transduction. Hh signaling has a critical role during development because of its implication in patterning events (Dessaud et al., 2008). There are three vertebrate ligands for Hh signaling, Sonic (Shh), Indian and Desert, that all bind to the specific receptor for Hh-family ligands Patched (Ptch1). In the absence of any ligand, Ptch1 prevents Smoothened (Smo), a G-coupled transmembrane protein, to associate with the membrane, thus

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repressing signal transduction. In the presence of a ligand, Ptch1 moves out of the cilium, thereby relieving the inhibition of Smo activity (Corbit et al., 2005; Rohatgi et al., 2007). Smo moves into the primary cilium (Corbit et al., 2005) and transduces the Hh signal through its effects upon the transcription factors Gli2 and Gli3, which also localize to primary cilia (Haycraft et al., 2005). Both transcription factors are activated upon association with cilia, while Gli3 is proteolytically converted from a full-length form to a shorter repressor form (Gli3R) in a cilia-dependent fashion. Dozens of recent papers have proven the dependence upon primary cilia for Shh signaling in the development of such disparate organs as the spinal cord, heart, mammary gland, kidneys, ovaries, and pancreas, while Indian hedgehog has been shown to signal through primary cilia in skeletal development (for a thorough review, see (Tasouri and Tucker, 2011)).

More controversial has been the involvement of primary cilia in Wnt signaling. Although *in vitro* analysis indicated that primary cilia constrain canonical Wnt signaling (Corbit et al., 2008), the evidence from *in vivo* studies is far from clear. Comprehensive analysis of Wnt-dependent developmental processes in zebrafish and the mouse revealed no role for primary cilia (Huang and Schier, 2009; Ocbina et al., 2009), but perturbations in Wnt signaling have been recorded in the later development of many organ systems, including the forebrain, cerebellum, kidney, pancreas, mammary gland, and long bones (reviewed in (Tasouri and Tucker, 2011)). Whether changes in Wnt signaling reflect a secondary effect downstream of a Hh-based perturbation, or in fact indicate a direct role of primary cilia in later Wnt-dependent developmental processes is still unresolved. This review focuses upon the relevance of primary cilia for the development of the forebrain, with an emphasis upon the largest portion of the forebrain, the cerebral cortex. The following results are also summarized in Table 1.

2. Primary cilia and cerebral cortex development

Using a hypomorphic allele of the *Ift88* gene, *cobblestone*, uncovered by ENU mutagenesis, Willaredt et al. (2008) were the first to examine the role that primary cilia have in cortical morphogenesis. They demonstrated that in *cobblestone* mutants both mRNA and protein levels of Ift88 are reduced by 70–80%. *Cobblestone* mutants not only displayed severe malformations of the hippocampus, choroid plexus and cortical hem, but additionally the dorsal–ventral boundary between the pallium and subpallium was not properly determined, although the dorsal and ventral domains were not shifted, as seen in other cilia mutants (Besse et al., 2011; Stottmann et al., 2009). Surprisingly, transmission and scanning electron microscopy presented in this study showed that primary cilia were still present and appeared intact at the ultrastructural level. These various phenotypes are comparable to the ones observed in the *Gli3* deletion mutant *Extra toes* (Xt^J) (Fotaki et al., 2006; Theil et al., 1999; Tole et al., 2000), which prompted the investigation of Gli3 proteolytic processing in *cobblestone* mutants. The authors observed an increase of the full-length form of Gli3. A number of phenotypes seen in the *cobblestone* mutants, such as the formation of rosette-shaped heterotopias in the dorsal cortex or the defects in the determination of tissue of the dorsal telencephalon mirror ones observed in Xt^J/Xt^J embryos. Further analysis of Shh signaling targets, such as *Ptch1* and *Gli1*, showed an increase in their expression in the *cobblestone* mutants (Willaredt et al., 2008). Examination of changes in Wnt signaling also revealed an upregulation of canonical Wnt signaling, as reflected in the increased and ectopic expression of *Wnt7b*, *Wnt8b*, and *Axin2*.

Like Ift88, the protein encoded by the *Ift172* gene is a member of the IFTB complex involved in anterograde ciliary traffic. Inactivation of this gene in the mouse resulted in phenotypes reminiscent of *cobblestone*, in that the mice displayed holoprosencephaly and exencephaly, but in addition the mutants suffered from craniofacial defects (Gorivodsky et al., 2009). Of great interest was their finding that *nodal* expression was eliminated in embryonic day 7.5 (E7.5) embryos, which is important for the subsequent induction of anterior mesendoderm that will go on to express *Shh*, *Foxa2*, and *Gsc* (Vincent et al., 2003). This may contribute to the forebrain phenotypes seen in other ciliary mutants. It is important to point out that the two aforementioned mutants (*cobblestone*, *Ift172*) were in IFTB complex proteins, which would imply that anterograde IFT would be compromised in these mutants. As seen directly below, one might not expect the same phenotype from a mutant in retrograde IFT.

In contrast to Ift88 and Ift172, the protein Ift139, encoded by the gene *Ttc21b*, is a member of the IFTA complex that is involved in retrograde IFT. A mouse mutant for this gene showed a correspondingly different phenotype, in which IFT proteins and their cargo accumulated within the cilium (Stottmann et al., 2009; Tran et al., 2008). This resulted in an increase in Shh signaling, as evidenced by a pronounced ventralization of the forebrain and an upregulation of downstream components of the Shh signaling pathway, including *Shh*, *Ptch1* and *Foxa2*. By crossing the *Ttc21b* mutant to a *Shh* mutant, the resulting phenotype was milder than the *Ttc21b* mutant alone, allowing the authors to conclude that from a genetic perspective, Ttc21b inhibits Shh signaling, presumably by promoting the exit of activated Smo from the cilium. With respect to Wnt signaling, the authors examined a Wnt-sensitive *lacZ* transgene and observed a downregulation, whereas beta-catenin levels showed no change (Stottmann et al., 2009).

Ftm encodes a protein that localizes to the basal body of cilia. The human ortholog of Ftm is called RPGRIP1L, and this gene is mutated in certain patients suffering from Joubert or Meckel syndrome, two well-characterized heritable ciliopathies (Arts et al., 2007; Delous et al., 2007). The mouse mutant for Ftm has been examined for its forebrain phenotype. In the forebrain of the targeted Ftm mutant (Vierkotten et al., 2007), a ventralization phenotype could be recorded, in that two standard markers identifying ventral forebrain, Dlx2 and Gsx2, were enlarged in their expression domains, while the expression domains of the dorsal cortical markers Ngn2 and Pax6 were diminished (Besse et al., 2011). However, this ventralization was limited to the most anterior part of the telencephalon. Unusually, expression of Shh and two downstream targets, Gli1 and Nkx2.1, were unaffected in the ventral forebrain, where they are normally to be found, in midgestation