Cilia in the nervous system: linking cilia function and neurodevelopmental disorders

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Purpose of review

Ciliopathies are genetic disorders caused by defects of primary ciliary structure and/or function and are characterized by pleiotropic clinical features. The ciliopathies include several partially overlapping syndromes such as Joubert syndrome, Bardet–Biedl syndrome and Meckel–Gruber syndrome, all of which have pronounced neurodevelopmental features. Here we focus on potential roles of cilia in central nervous system function, to explore how impairments may cause brain malformation and neurodevelopmental disease.

Recent findings

Cilia have long been considered as 'sensory cellular antennae', responding as chemo-sensors, mechano-sensors and thermo-sensors, although their roles in development were not well understood until recently. The surprising finding that disparate syndromes are all due to defects of the primary cilia, along with the recent advances in genetics, has helped elucidate further roles of primary cilia beyond sensory functions. Several molecules that are associated with key signaling pathways have been discovered in primary cilia. These include sonic hedgehog, wingless, planar cell polarity and fibroblast growth factor, which are essential for many cellular processes. Additionally, mutations in 'ciliome' genes have largely shown developmental defects such as abnormal body axis and brain malformation, implying disrupted cilia-related signaling pathways. Accordingly, the emerging theme is that primary cilia may play roles as modulators of signal transduction to help shape cellular responses within the environmental context during both development and homeostasis.

Summary

The link between cilia and signal pathways has become a framework for understanding the pathogenesis of ciliopathies. Despite recent progress in ciliary biology, fundamental questions remain about how cilia regulate neuronal function in the central nervous system. Therefore, investigation of ciliary function in the nervous system may reveal cilia-modulating mechanisms in neurodevelopmental processes, as well as suggest new treatments for disease.

Keywords

brain, central nervous system, cilia, ciliopathy, Joubert syndrome, neuron, signaling pathways

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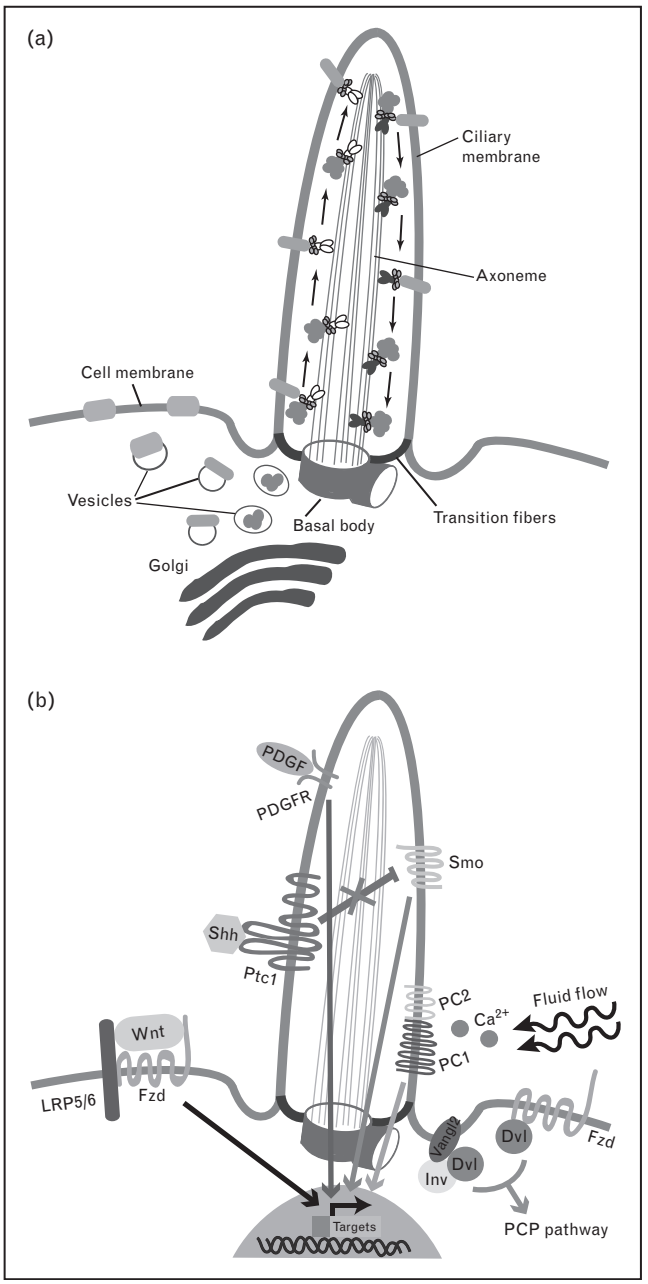
Introduction

The mechanisms associated with ciliopathies have been extensively studied in recent years and the research advances have identified many genes and molecular signals involved in ciliary formation and function. The range of clinical features of ciliopathies includes early fetal lethality, respiratory dysfunction, reproductive sterility, skeletal dysplasia including polydactyly, rib and bone defects, cardiac defects, cystic kidney, hepatic fibrosis, obesity, diabetes and deafness, accounting for almost every organ in the body. Notably, neurological deficits including hydrocephalus and structural brain defects are common features in Joubert syndrome (JBTS), Bardet–Biedl syndrome (BBS), Meckel–Gruber syndrome (MKS) and nephronophthisis (NPHP) [1–3]. Although critical in central nervous system (CNS) development, as well as neurogenesis [4–6], the role of cilia in brain development and neuronal function still remains largely unknown. Moreover, the multitude of affected organs and the variability in clinical presentation of these disorders challenges our understanding of the context-dependent role that cilia play in human health.

Figure 1 Intraflagellar transporting and signaling pathways within the primary cilium



(a) The protein cargos are transported from the Golgi to the basal body by vesicle trafficking in the cellular cytoplasm. In order to move ciliary cytosolic and membrane proteins, kinesin 2 and dynein 2, motor proteins associating with intraflagellar transport (IFT) complex, travel along the axoneme between the base and the tip of the cilium in an anterograde and retrograde direction respectively. (b) The primary cilium modulates several signal transduction pathways. Sonic hedgehog (Shh) signaling is activated by binding of Shh to patched (Ptc1), releasing smoothened (Smo) and the downstream target gene transcription from inhibition. Canonical wingless (Wnt) signaling through low-density lipoprotein receptor-related protein (LRP)5/6 and Frizzled (Fzd) is inhibited by the primary cilium, whereas noncanonical Wnt signaling [planar cell polarity (PCP) pathway] requires the primary cilium to be activated by Dishevelled (Dvl) interacting with either Fzd or Inversin (Inv)/other PCP components such as Vangl2. Platelet-derived growth factor (PDGF) signaling through PDGF receptor (PDGFR) requires ciliary localization of the receptor to activate downstream targets. In response to fluid flow, calcium signaling is activated through mechanosensing by polycystin-1 (PC1) and polycystin-2 (PC2) localized in the ciliary membrane. ▭, ciliary membrane proteins; ●, ciliary proteins; ⊶, IFT complex; ⊂, kinesin 2; ◀, dynein 2; ▭, nonciliary proteins.

Key points

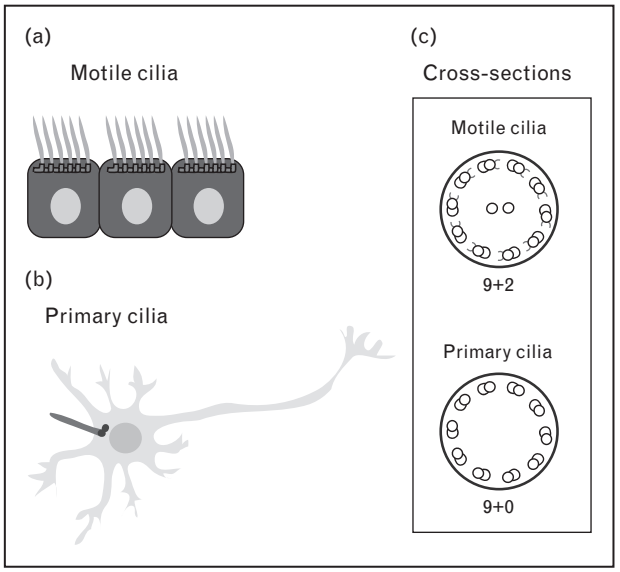
- Defective primary cilia give rise to neurodevelopmental disorders now termed as ciliopathies, including Joubert syndrome.
- Primary cilia in the central nervous system function as mediators of signal transduction during development.
- Signaling pathways such as planar cell polarity involve the function of both motile and primary cilia in brain development.

Cilia are hair-like extensions protruding from the surface from most types of cells including progenitor and differentiated cells. The cilium is composed of a microtubule cytoskeleton (the axoneme) and anchored to the cell by the basal body that is derived from the mother centriole (Fig. 1). There are two types of cilia: motile and nonmotile (primary). Although there are some exceptions, motile cilia have nine doublet microtubules containing inner and outer dynein arms, as well as two central microtubules (9 + 2 axoneme). Primary cilia, on the contrary, have the nine doublet microtubules, but lack the two central microtubules and dynein arms (9 + 0 axoneme) (Fig. 2). In the brain, motile cilia are present only in specific type of cells: ependymal cells lining the ventricle and some choroid plexus (ChP) cells. On the contrary, primary cilia are present in most brain cells: some ChP cells, neural stem cells, neurons and astrocytes within the brain parenchyma. It was initially assumed that primary cilia were simply sensory organelles, responding to flow and receiving external signals during brain development. However, recent studies have demonstrated diverse roles of primary cilia, which include regulation of signal transduction that goes beyond their sensory roles [7••,8•]. Here, we review the predominant neurodevelopmental defects in ciliopathies and speculate on the relationship between ciliary functions and signaling pathways by highlighting newly identified roles of cilia in brain development.

Characteristics of ciliopathies

Many syndromes characterized as ciliopathies display important neurological features, most commonly mental retardation and a variety of structural deficits (Table 1). Apart from the phenotypic characterization of the disorders, identification of causative genes together with studies on gene function have provided new insight to connect ciliary function with key signaling pathways that

Figure 2 Cilia in various types of cells in the brain



(a) Motile cilia anchored to the basal body are present in the ependymal cells lining the ventricle. (b) Neurons contain primary cilia projecting from basal bodies, but the cilium appears buried among the dendrites and axons of adjacent neurons. (c) Schematic diagram of the cilia axoneme cross-section. Motile cilia have 9 + 2 microtubule pair ultrastructure, with inner and outer dynein arms, whereas primary cilia have 9 + 0 microtubule pair ultrastructure, without dynein arms.

are essential in development of the CNS. Here, we first focus on a prototypical ciliopathy, JBTS, as a canonical human neurodevelopmental ciliopathy.

Joubert syndrome

JBTS is a genetically and phenotypically heterogeneous ciliopathy, and like the majority of these diseases, inherited in an autosomal recessive fashion. The key feature on axial brain MRI sections is the 'molar tooth' sign, a distinctive mid-hindbrain malformation useful to delineate JBTS from other cerebellar defects. The key neurological phenotypes include intellectual disability together with oculomotor apraxia and neonatal dysregulations of breathing patterns. It is characterized by not only neurological phenotypes but also other various symptoms: cystic kidney, liver fibrosis, polymicrogyria and polydactyly [9], characteristics overlapping with other ciliopathies (particularly with MKS), thus suggesting a common pathogenesis.

The inclusion of JBTS as a ciliopathy came after the realization that the genes mutated in JBTS patients encode proteins localized predominantly to the primary cilia. In fact, all of the causative JBTS genes identified to date, *INPP5E*, *TMEM216*, *AHI1*, *NPHP1*, *CEP290*, *TMEM67*, *RPGRIP1L*, *ARL13B*, *CC2D2A* and *OFD1*, encode proteins that are localized to the base or the axoneme of the cilium. Further, several of these proteins have been implicated in the modulation of signaling pathways, such as sonic hedgehog (Shh), wingless (Wnt) and phosphatidylinositol [10–13]. Although some genes make greater contribution to the overall disease burden than others, still genetic diagnosis is possible in less than half of patients. Some genotype–phenotype correlations have emerged: we now know that *CEP290* mutations are present in about half of patients displaying disease involving brain, eye and kidney, whereas *AHI1* mutations are present in about 20% of patients with brain and eye pathology and about 10% of patients with only brain involvement. On the contrary, several genes make minor contributions to disease, such as the *ARL13B* and the *RPGRIP1L* genes [9,14].

Table 1 Summary of the ciliopathies

Disorders	CNS defects	Other defects	Signal pathways
JBTS	Cerebellar malformation	Cystic kidney	Shh, Wnt, PI, PCP
	Oculomotor apraxia	Polydactyly	
	Encephalocele	Retinitis pigmentosa	
	Hydrocephalus	Ataxia, hypotonia	
	Mental retardation	Cardiac defects	
MKS	Encephalocele	Cystic kidney	Shh, PCP
	Hydrocephalus	Polydactyly	
	Mental retardation	Retinal degeneration	
		Hepatic fibrosis	
		Cardiac defects	
NPHP	Cerebellar malformation	Hepatic fibrosis	Shh, Wnt, PCP
	Oculomotor apraxia	Situs inversus	
	Mental retardation	Retinitis pigmentosa	
BBS	Mental retardation	Obestiy, diabetes	Shh, Wnt, PCP
		Cystic kidney	
		Polydactyly	
		Retinal degeneration	
OFD1	Cerebellar malformation	Craniofacial malformation	PCP
	Hydrocephalus	Polydactyly	
	Mental retardation	Cystic kidney	

CNS, central nervous system; JBTS, Joubert syndrome; MKS, Meckel–Gruber syndrome; NPHP, nephronophthisis; OFD1, oral–facial–digital syndrome type 1; PCP, planar cell polarity; PI, phosphatidylinositol; Shh, sonic hedgehog; Wnt, wingless.

The mechanism inducing malformed cerebellum and brainstem in JBTS has not been delineated, although some mutant mice of the causative genes (*INPP5E*, *CEP290*, *AHI1* and *ARL13B*) are available now to begin to address this question. Knockout mice for *Inpp5e* are lethal at birth with encephaloceles as a major pathological feature, whereas *Arl13b* mutants die at mid-gestation with neural tube defects (NTDs) [13,15]. Both of these probably represent complete null alleles because either it was engineered as a null (in the case of *Inpp5e*) or it phenocopies a null allele (in the case of the *Arl13b*). It is interesting that in humans with JBTS, only missense mutations in *INPP5E* and *ARL13B* have been reported, which probably represent partially inactive alleles [10,16]. Therefore, the specific brain phenotype of JBTS might result from partially disabled protein activity, rather than loss of protein for INPP5E and ARL13B. However, for other genes like *CEP290* and *AHI1*, almost all JBTS mutations are null truncating mutations. Both *Cep290* and *Ahi1* knockout mice generally display features consistent with ciliopathies such as retinal degeneration and cystic kidney [12,17], but the severity does not correlate with the human disease, as neither mouse mutant has a striking cerebellar phenotype (unpublished observation). Importantly, there are no obvious differences in sequence conservation among these various genes across the mammalian lineage. Thus, the differences might relate to a background/modifier effect, might be due to differences between human and mouse brain, or might represent different requirements for gene function during development. Although it may be difficult to study JBTS using animal models for these reasons, the generation of cerebellar-specific conditional or double/triple-knockout mice might help overcome some of these hurdles.

Despite lack of striking phenotypes in the cerebellum of mutant mice, many JBTS-causing genes are expressed in the CNS, suggesting their potential functions during development. *INPP5E*, encoding inositol polyphosphate-5-phosphatase E, is expressed in the nervous system including brain, retina and spinal cord, and is localized in the primary cilia [10,15]. Furthermore, it has been suggested that *INPP5E* is involved in cilia stability by controlling phosphatidylinositol signaling [10,15]. *AHI1* is also strongly expressed in the brain and retina [17], and plays a role as a positive regulator of canonical Wnt signaling [12]. *ARL13B*, a member of Ras GTPase family, is specifically localized in the cilia of cerebellar granule neurons and in the retinal photoreceptor connecting cilia [16]. *ARL13B* mutant mice have impaired neural tube patterning [13], which is also observed in the mutant mice for *OFD1* [18], a JBTS gene coupled to Shh signaling. These molecular signaling pathways control diverse cellular processes such as cell proliferation, differentiation and migration, all of which are important aspects of neurodevelopment. These new data focused on studying JBTS disease mechanisms complement previous basic studies on primary cilia, supporting the crucial role of primary cilia in the regulation of signaling pathways during CNS development. Investigations of the JBTS causative genes in modulating mechanisms controlling signal transduction will shed light on the specified ciliary roles in the developing nervous system.

The role of cilia in the central nervous system

Consistent with various neurological symptoms detected in ciliopathy patients, most cells in the brain (neurons including neural progenitors and mature neurons, glial cells/astrocytes and ependymal cells) have primary cilia. However, the role of neuronal primary cilia has been largely ignored. Only now have studies started to explore the specific roles of the tiny and mysterious organelles in the nervous system.

The primary cilia in brain development

Brain patterning is controlled by morphogens such as Shh, Wnt and Fgf, which control key transcription factors, to progressively subdivide discrete germinal domains along the dorsal/ventral and anterior/posterior axes. Requirement of morphogen-mediated signaling in this process has implicated a pivotal role of primary cilia during brain patterning. Correspondingly, mutant mice for several intraflagellar transport (IFT) components including IFT88, IFT139 and IFT172, which are essential for ciliogenesis [7••,19–21], have shown malformed brains, revealing the relevance of primary cilia function in brain morphogenesis [21–23].

Shh signaling, among several signal pathways, has been extensively studied in both ciliary function and CNS development. The role of cilia-mediated Shh signaling, especially, was clearly demonstrated in development of specific CNS regions. The hippocampal dentate gyrus, one site where new neurons are continuously produced throughout life and controlling circuit plasticity, learning and memory [24], is under primary cilia-mediated Shh control. Notably, when the ciliary genes *kif3a*, *ift88*, *ftm* or *stumpy* are removed, not only is there a loss of cilia but also there are defects in hippocampal neurogenesis such as decreased proliferation of granule neuron precursors (GNPs) and absence of radial astrocytes in the dentate gyrus [4,5,25]. This suggests a possible connection between Shh signaling, which acts as a mitogen in the GNPs, and ciliary function during hippocampal neurogenesis. Indeed, mutant mice of these ciliary genes appear altered in responsiveness to Shh signal [4]. Interestingly, loss of either cilia or Shh signal (loss of *Smo*) has no effect on generation of other types of cells such as astrocytes in the hippocampus [4], suggesting that the role of cilia-mediated Shh signaling may depend on the cell type and brain region. In the future, it might

be interesting to look for mutations in 'ciliary' genes in patients with cognitive or behavioral defects.

Shh signaling plays a critical role in the development and neurogenesis of the cerebellum. Similar to dentate gyrus granule neurons, the majority of cerebellar granule neurons are produced from cerebellar GNPs (CGNPs) at postnatal ages, under the control of Shh. The link between cilia-mediated Shh signaling and cerebellar development can be extended to the cerebellar hypoplasia observed in many ciliopathies such as JBTS. Accordingly, the conditional removal of ciliary genes (e.g. *kif3a*, *ift88* or *stumpy*) in CGNPs gives rise to striking dysgenesis and abnormal foliation of the cerebellum, as well as decreased expression of Shh target genes and proliferation of CGNPs [5,6,26], indicating that cilia are essential for Shh-dependent proliferation of CGNPs during cerebellar development. Studies focusing on mutations of other ciliary genes (*fantom*, *ofd1* and *smo*) have also shown cerebellar defects [6,18,27]. Here we have highlighted the role of Shh signaling in order to link ciliary function during brain development. However, we also speculate the potential roles of Wnt and Fgf signaling that may be modulated by cilia both in the cerebellum as well as in other regions of the brain.

Defects in axonal tract decussation within the brain, including a complete absence of the pyramidal decussation, have been frequently observed in JBTS patients [28,29]. These axonal deficits may underlie defective functional brain organization, which is associated with sensorimotor and cerebellar cortical activation [30], and underlie some of the motor and cognitive defects in ciliopathies. Concurrently, mental retardation is a fairly common neurological feature among various ciliopathies (Table 1). Although these phenotypes suggest the potential role of neuronal primary cilia in axonal growth and guidance, little is known about the relationship between ciliary and axonal behavior; thus, several questions remain unanswered. First, is the neuronal primary cilium essential for axonal growth or guidance? Second, how can the neuronal cilia regulate axonal behavior? Third, how can axonal defects link to motor and cognitive deficits of ciliopathies? Fourth, how can the tiny neuronal primary cilia lying buried near the cell body (Fig. 2) have such a pronounced effect on the much longer axon? Fifth, how are the spatial constraints overcome? It is tempting to speculate a role of the primary cilia in determining CNS laterality given the role of motile cilia in determining visceral laterality, but there is currently lack of direct evidence of a mechanism for the axonal defects observed in JBTS.

Roles of cilia in the brain: beyond neuronal function

Apart from neuronal primary cilia, motile cilia are evident on ependymal cells lining the lateral ventricular surface in the brain. These cells are polarized in the epithelial plane, and this planar polarity is essential for propelling cerebrospinal fluid (CSF) flow [31]. Defects in ependymal ciliary motility results in an excessive accumulation of CSF, thereby causing hydrocephalus [32–34], and an altered migration of neuroblasts toward the olfactory bulb [35]. Accordingly, hydrocephalus, one of the most common primary cilia-associated anomalies of the CNS [36], has been detected in several ciliopathies (Table 1). However, the cellular and molecular mechanisms for the planar polarity and coordinated ciliary movement have not been fully explored. Recently, several studies have provided insight into the mechanisms underlying planar polarity and the directionality of CSF flow [37•,38,39••]: the precursor cells of ependyma, termed radial glia, already establish orientation of cilia prior to the generation of ependymal cells in the ventricular wall [38]; a combination of planar polarity signaling and hydrodynamic forces (by the emerging fluid flow) regulates docking and rotational orientation of the ependymal motile cilia [39••]; and core planar polarity components such as Celsr1, 2 and 3 and Vangl2 are essential for ciliary orientation and function [37•]. Despite these remarkable findings, there are still unanswered questions. Guirao *et al.* [39••] suggest that existing CSF flow determines the directionality of ependymal cilia, but it is unclear what determines the initial fluid flow direction in the brain lateral ventricle, if this is purely random, or a response to pressure differences. Tissir *et al.* [37•] suggest the lack or dysfunction of ependymal motile cilia could be a contributing factor in the cause of hydrocephalus. They demonstrate that hydrocephalus is probably not due to a lack of ependymal cilia, as there is evidence of pathology prior to the development of motile cilia on these cells. Rather, they suggest that loss of cilia leads to altered function of the ChP epithelium, resulting in hydrocephalus [32].

Potential roles of cilia in the central nervous system: opportunities and challenges

As the cilium has emerged as a key organelle in development of the nervous system, many studies have focused on uncovering hidden mechanisms. By uncovering multifunctional roles and structure–function relationships, some answers have emerged, as well as further mysteries.

Reciprocal function between signaling and cilia

Linking ciliary function in the nervous system with signaling pathways such as planar cell polarity (PCP) and Shh has provided new insight to understanding the mechanism of neural tube closure. However, we still do not fully understand how cilia modulate signaling pathways and many open questions remain: first, which signaling pathways required for ciliogenesis? Disruption of the PCP effector proteins such as Inturned and Fuzzy

resulted in loss of cilia, as well as NTDs, suggesting involvement of cilia in PCP-dependent neural tube closure [40]. Additionally, some mutant mice of Dishevelled (DVL), one of the PCP core proteins, demonstrated rostral NTDs, which are similar to those in IFT or Shh signaling-disrupted mice [41]. Although this suggests the requirement of PCP signaling in ciliogenesis, disruption of most PCP core proteins including DVL, Vangl, Frizzled and Flamingo has not demonstrated ciliary defects. Therefore, PCP proteins may regulate ciliogenesis and signal transduction in parallel. Studies determining genetic relationship between PCP proteins and ciliary proteins may address this possibility. Second, which pathways require the cilium for signal transduction? The interaction between PCP proteins and cilia-localized proteins such as Inversin, BBS4 and BBS10 [42–44] imply that the cilium plays important roles in signal transduction. In the kidney, ciliary protein polycystin 1 is involved in gene transcription to activate two signaling pathways: mTOR (mammalian target of rapamycin) and STAT6 (signal transducer and activator of transcription 6) [45]. However, it is still argued whether cilia themselves or ciliary proteins are necessary. It is noteworthy that zebrafish without cilia exhibit Shh signaling defects, but display normal Wnt and PCP signaling [46]. This suggests that the above two hypotheses may be differently applied in each signal pathway, implicating unique ciliary functions dependent on signaling.

Function of the neuronal primary cilia as sensors

Several sensory neurons in the olfactory, acousticovestibular and visual systems display highly modified specialized cilia. The symptoms of blindness and deafness in ciliopathy patients and models have prompted the question, 'do primary cilia also play roles in sensory signal transduction?' Interestingly, several G protein-coupled receptors such as somatostatin receptor type 3, serotonin receptor 6 (HTR6), melanin-concentrating hormone receptor 1, a subset of dopamine receptors are localized to the primary cilia of neurons [47–50]. These data suggest that neuronal primary cilia may be essential for activating signaling pathways responsible for several sensory inputs. Concurrently, a recent study has suggested that hypothalamic neuronal primary cilia may be responsible for obesity phenotype by controlling leptin signaling [51], although the ciliary localization of leptin receptors remains to be determined. Future studies aimed at disrupting either neuronal primary cilia or signaling molecules in the nervous system will be required to determine direct connection between neuronal primary cilia and sensory transductions.

Tubulin posttranslational modification of cilia in the nervous system

Microtubules, composed of heterodimers of α-tubulin and β-tubulin, are subject to diverse posttranslational modifications (PTMs), including acetylation, tyrosination/detyrosination, polyglutamylation, polyglycylation, palmitoylation and phosphorylation in the highly conserved C-terminal regions [52–54]. The existence of several PTMs has been demonstrated in neuronal and nonneuronal cells, since the first implication of a PTM in brain tubulin [55]. However, the properties of modified microtubules, as well as the connection to neuronal cilia, have remained unclear until now. Recently, several studies have suggested that these PTMs occur at the cilium and play roles in ciliary function [56–60], and have identified various PTM enzymes at the cilium including tubulin tyrosine ligase, polyglutamylase and depolyglutamylase [61–63]. It has been generally accepted that the modified microtubules may play a role in neurite outgrowth and maturation, as well as in maintaining neuronal morphology [64,65]. We thus speculate that the role of ciliary PTMs may be linked to the modified microtubule-based neuronal functions in helping the cell respond to extracellular signaling and/or in microtubule-associated protein-mediated intracellular signal transduction. Therefore, it will be essential to determine: whether the different distributions of tubulin modification enzymes and their regulators depend on cell types or subcellular organelles; how PTMs regulate the cytoskeletal network and dynamics including changes of neuronal morphology and motility; and whether depletion of ciliary genes causes tubulin PTM defects, thereby disrupting neuronal functions.

Conclusion

Dysplasia and dysfunction of primary cilia has been shown to cause defects in the CNS, as well as a number of organs such as heart and kidney in the ciliopathy spectrum. Primary cilia play critical roles in controlling neuronal development, modulating various signal transduction pathways. Despite the enormous amount of progress we have made studying signaling pathways influenced by primary cilia and related genes in the nervous system, we are still far from understanding their roles in classical neuronal behaviors such as neuronal migration and axonal guidance. It is possible that primary cilia in neurons may also act as mediators in signal transduction. Alternatively, they may be involved in cell–cell communication and/or cytoskeleton-associated processes. The field of ciliary biology still has many mysteries yet to be solved. The presence of severe structural multiorgan abnormalities suggests that treatment options in children may be limited. However, at the same time the involvement of ongoing signaling may contribute to the intellectual disabilities and other deficits, which might be amendable to treatments. The search for cilia-modulating pathways or mechanisms to bypass the requirement for cilia in specific pathways might represent one avenue of investigation.

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- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 183).

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