AHI1, a ciliopathy-causing gene, is involved in the formation and function of primary cilia through the regulation of intracellular vesicular trafficking

by

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ABSTRACT

The primary cilium is a polarized cellular structure composed of microtubule-bundles projecting from the apical surface of almost every cell, and defects in this structure have been implicated in several human diseases, now termed ciliopathies, such as in retinal degeneration, polycystic kidney disease, CNS malformation, and obesity. The crucial role for primary cilia as a signaling center for cells in embryonic development and tissue homeostasis has been revealed through genetic and functional studies of ciliary proteins. However, the molecular mechanisms underlying the pathogenesis of ciliopathies remain to be elucidated. In this thesis, we demonstrated that the *Abelson-helper integration site 1 (AHI1)* gene, mutations in which cause the ciliopathy, Joubert syndrome (JBTS, an inherited neurodevelopmental disorder), encodes for a ciliary protein involved in the formation of primary cilia, since defective cilium formation is observed in Ahi1 deficient cells. This lack of cilia formation is likely due to disrupted intracellular vesicular trafficking. Moreover, a role for Ahi1 in cilia formation and function was identified through studies showing that the stabilization and recruitment of the master ciliary trafficking modulator, Rab8, to the basal body of primary cilia requires Ahi1. Additionally, *Ahi1*$^{-/-}$ mice develop early onset retinal degeneration resulting from disrupted rhodopsin trafficking to photoreceptor outer segments, a specialized primary cilium, due to decreased levels of Rab8 in *Ahi1*$^{-/-}$ retinas. Together, these results suggest a regulatory role for Ahi1 in Rab8-mediated ciliary protein transport. Enriched expression of receptors and signaling molecules at the cilia make primary cilia a specialized structure for cell signaling. However, it is unclear how primary cilia mediate signal transduction in mature neurons, in which signal transmission is predominantly mediated by synapses. Deletions of *Ahi1*, not only reduce formation of primary cilia in neurons, but also disrupt ciliary localization of receptors, namely the melanin concentrating hormone receptor (MchR1). Decreased ciliary MchR1 expression in *Ahi1*$^{-/-}$ neurons appears to be a result of disrupted trafficking from the cell body surface to the ciliary membrane, since comparable levels of surface MchR1 were found in *Ahi1*$^{-/-}$ and control neurons. Wildtype neurons display a translocation of MchR1 from primary cilia upon melanin concentrating hormone (MCH) stimulation and subsequently reduce forskolin-induced cAMP production. In *Ahi1*$^{-/-}$ neurons that express MchR1 normally on

cell bodies, but lose MchR1 only at primary cilia, these cells are unresponsiveness to MCH-induced changes in cAMP levels. These results clearly demonstrate a critical role for primary cilia as a requisite location for receptors important for receiving and transducing extracellular stimuli-induced signaling. In addition to inactivation of Ahi1 via genetic deletion or RNAi knockdown, characterization of the effects of the JBTS associated missense mutations in *AHI1* on its functions could also help to elucidate the molecular mechanisms underlying the phenotypes observed in JBTS. The JBTS missense mutation V443D in AHI1 that occurs in the region with no known protein binding motifs appears to alter protein function given its aberrant localization and protein interactions, as well as decreased protein expression likely due to protein structure instability. Absence of expressed AHI1-V443D at the basal body of primary cilia and at cell junctions in mouse IMCD3 cells suggests a critical function for AHI1 at primary cilia and for cell polarity establishment, In further support, expression of AHI1-V443D results in a decrease in binding to a known AHI1 interactor, NPHP1 (another JBTS-causing protein important for cell polarity). Conversely, Huntingtin-associated protein 1 (HAP1, a regulatory protein in intracellular trafficking and receptor endocytosis) displays increased binding with AHI1-V443D. A JBTS-like phenotype is reported in *Hap1* deficient mice; however, disrupting the interaction of Ahi1 with Hap1 appears not to be necessary for cilia function upon growth factor stimulation and TrkB receptor endocytosis. This implicates another unknown pathway involving an Ahi1/Hap1 interaction that is required for neuron development. Together, this thesis provides insight into a regulatory mechanism for cilia formation and function through studying the roles for the ciliopathy associated gene, *AHI1*, in ciliary trafficking, in addition to providing a foundation for further study of this protein in cilia formation and cilia-mediated signaling. Moreover, the crucial role for primary cilia in neuronal signal transduction is further supported in this study. This work will help to elucidate the molecular mechanisms of how dysfunctional primary cilia lead to the CNS phenotypes observed in JBTS and identify the regulatory mechanism for signal transduction mediated by primary cilia.