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IFT46 plays an essential role in cilia development

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ABSTRACT

Cilia are microtubule-based structures that project into the extracellular space. Ciliary defects are associated with several human diseases, including polycystic kidney disease, primary ciliary dyskinesia, left–right axis patterning, hydrocephalus and retinal degeneration. However, the genetic and cellular biological control of ciliogenesis remains poorly understood. The IFT46 is one of the highly conserved intraflagellar transport complex B proteins. In zebrafish, *ift46* is expressed in various ciliated tissues such as Kupffer's vesicle, pronephric ducts, ears and spinal cord. We show that *ift46* is localized to the basal body. Knockdown of *ift46* gene results in multiple phenotypes associated with various ciliopathies including kidney cysts, pericardial edema and ventral axis curvature. In *ift46* morphants, cilia in kidney and spinal canal are shortened and abnormal. Similar ciliary defects are observed in otic vesicles, lateral line hair cells, olfactory pits, but not in Kupffer's vesicle. To explore the functions of *Ift46* during mouse development, we have generated *Ift46* knock-out mice. The *Ift46* mutants have developmental defects in brain, neural tube and heart. In particular *Ift46*(−/−) homozygotes displays randomization of the embryo heart looping, which is a hallmark of defective left–right (L/R) axis patterning. Taken together, our results demonstrated that IFT46 has an essential role in vertebrate ciliary development.

Introduction

The cilia are microtubule-based organelles evolutionarily conserved from protozoans to vertebrates. Based on their motility, cilia are generally categorized as motile cilia or primary (sensory, non-motile) cilia. A single primary cilium is found on most types of cells, but the number of motile cilia varies in different cells: some, such as those of the embryonic node, have a single motile cilium, while others (ependymal cells in the adult brain and the bronchial epithelium) have multiple motile cilia (Eggenschwiler and Anderson, 2007; Goetz and Anderson, 2010). The importance of primary and motile cilia in embryonic development and in adult physiological processes is underscored by a broad class of human genetic diseases collectively known as "Ciliopathy", which manifest a broad range of phenotypic abnormalities due to ciliary dysfunction, including obesity, diabetes, skeletal defects, *situs inversus*, hydrocephalus and polycystic kidney disease (PKD) (Badano et al., 2006; Fliegauf et al., 2007). Recent investigation into the role of cilia during early vertebrate development has connected cilia to multiple signaling pathways and many developmental processes ranging from left–right patterning to kidney cystogenesis (Hamada et al., 2002; Hildebrandt and Otto, 2005; Raya et al., 2006).

Cilia can be structurally divided into sub-compartments including basal body, transition zone, axoneme, ciliary membrane and the ciliary tip (Nigg and Raff, 2009). During ciliary growth, the axoneme is assembled by the addition of new axonemal subunits to its distal tip. Since cilia lack the machinery necessary for protein synthesis, the site of assembly of the axoneme is far from the site

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of synthesis of axonemal proteins in the cell body (Cole and Snell, 2009; Silverman and Leroux, 2009). This demands the delivery of new axonemal building blocks to their assembly site through the intraflagellar transport (IFT), a conserved process in eukaryotes that assembles, maintains, and disassembles cilia, as well as transduces of cilium-generated signaling. IFT is essential for the development and maintenance of both motile and non-motile sensory cilia. IFT trafficking from the base to the tip of the cilium depends on the microtubules and is associated with two IFT protein complexes, termed IFT-A and IFT-B, which consist of at least 6 and 13 subunits respectively. These IFT proteins are highly conserved across species and are all localized to the cilium, basal body and centrosomes (Rosenbaum and Witman, 2002). In mice, IFT-B is essential for anterograde trafficking, whereas IFT-A is required for retrograde trafficking. Disruption of the kinesin-2 motor or IFT-B blocks cilia formation, while perturbation of retrograde trafficking by disrupting the dynein motor or IFT-A results in short and bulged cilia. These highlight that IFT is indispensable for normal ciliogenesis and maintenance (Scholey, 2003). In addition, IFT has recently attracted intense research interest owing to its association with human disease and developmental abnormalities, including polycystic kidney disease (PKD), hepatic and pancreatic defects, blindness and obesity, skeletal patterning abnormalities as well as *situs inversus* (Pazour and Rosenbaum, 2002; Pazour, 2004).

IFT46 is a core component of the intraflagellar transport machinery and is required for formation of all cilia. As a mammalian homolog of DYF-6 in Caenorhabditis elegans (C. elegans), which was reported to be an IFT-B subunit in Chlamydomonas reinhardtii (C. reinhardtii), it is specifically required for transporting outer dynein arms into the flagella (Hou et al., 2007). The IFT46 mutants of C. reinhardtii and C. elegans are incapable of assembling cilia, demonstrating that IFT46 plays an essential role in ciliogenesis. IFT46 forms a stable trimetric sub-complex within the IFT-B core complex together with IFT52 and IFT88 (Lucker, 2010; Richey and Qin, 2012).

Here we report the characterization of *IFT46* by using two model systems, zebrafish and mouse, to elucidate the expression and function of *IFT46* during vertebrate development. We find enriched expression of *ift46* in ciliated organs during zebrafish embryonic development. In addition, we show that knockdown of *ift46* in zebrafish embryos leads to loss of cilia in various tissues. We also demonstrate that *ift46*, like other IFT subunits, is localized to the basal body in ciliated cells. We have generated knock-out mouse of *Ift46*, which are embryonic lethal at E10.5 and exhibit neural tube defects, cardiac edema and randomized heart looping due to the lack of cilia at the node. Taken together, our results indicate the essential role of IFT46 in vertebrate development.

Results

Overexpression of IFT46 induces apoptotic cell death

We originally isolated a new cDNA clone C11orf60 in a large-scale expression screening of human genes in zebrafish embryos. Subsequent sequencing revealed this cDNA encodes the human ortholog of C. reinhardtii intraflagellar transport protein 46 (IFT46).

When human *IFT46* was overexpressed following the injection of synthetic mRNA of *IFT46* into one cell stage zebrafish at 100 to 200 pg per embryo, we observed increase of apoptosis in the central nervous system of injected embryos in a dose-dependent manner, as determined by acridine orange (AO) staining (Fig. S1A and B). Previous reports have shown that IFT46 in C. reinhardtii is specifically required for transporting outer dynein arms into the flagella (Hou et al., 2007). To better characterize and clarify roles of

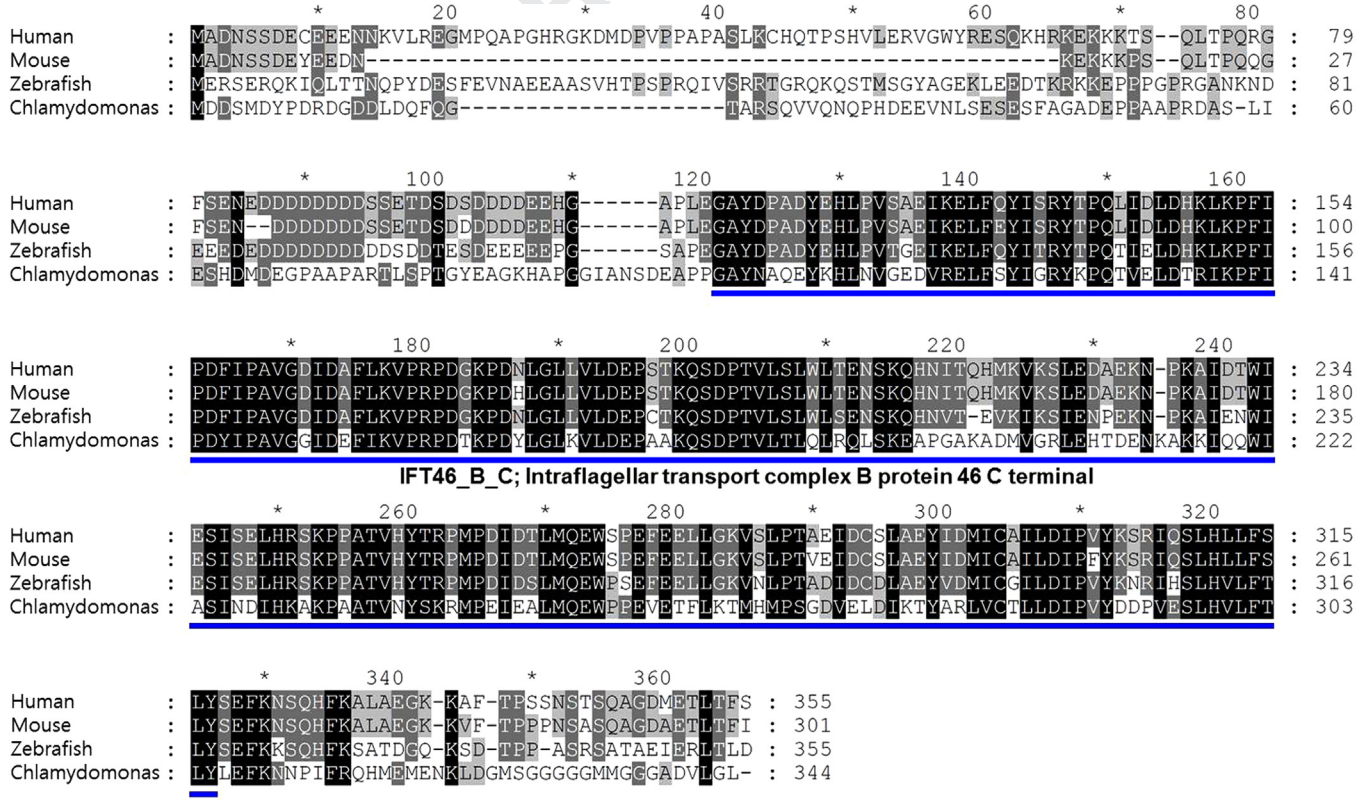

Fig. 1. Alignment of IFT46 protein sequences using GeneDoc. The IFT46 protein sequences from human, mouse, zebrafish and Chlamydomonas are aligned using GeneDoc program. The intraflagellar transport complex B protein 46 C terminal domain is marked with blue bar, The zebrafish ift46 has 59%, 69% similarity to human IFT46 and mouse Ift46 and 69% similarity to Chlamydomonas, respectively.