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REVIEW

Zebrafish as a Model for Human Ciliopathies

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ABSTRACT

Cilia, microtubule-based structures found on the surface of almost all vertebrate cells, play an array of diverse biological functions. Abnormal ciliary axonemal structure and function can result in a class of genetic disorders that are collectively termed ciliopathies. Model organisms, including *Chlamydomonas reinhardtii* and *Caenorhabditis elegans* have been widely used to study the complex genetic basis of ciliopathies. Here, we review the advantages of the zebrafish as a vertebrate model for human ciliopathies. We summarize the features of zebrafish cilia, and the major findings and contributions of the zebrafish model in recent studies of human ciliopathies. We also discuss the new genome editing approaches being efficiently used in zebrafish, and the exciting prospects of these approaches in modeling human ciliopathies.

KEYWORDS: Cilia; Zebrafish; Ciliopathy; Disease model

INTRODUCTION

Cilia, or flagella, are evolutionarily conserved organelles existing in diverse organisms from protozoa to primates. In vertebrates, cilia are present in nearly every cell type, and perform diverse biological functions. For example, motile cilia generate force for sperm cell motility and also for fluid flow over the surface of epithelial cells. In contrast, non-motile primary cilia function as a cellular "antenna" for sensing extracellular matrix molecules and growth factors (Ishikawa and Marshall, 2011). Defects in the formation and/or function of cilia can result in a wide spectrum of human genetic ciliopathy disorders, consisting of patients that manifest a constellation of features including retinal degeneration, renal disease, cerebral anomalies, *situs inversus* and other defects (Hildebrandt et al., 2011).

The genetic basis of ciliopathies is highly complex. Ciliopathy proteins localize not only in the axonemal region, but also in the transition zone, the basal body or the centrosome, and in the cytoplasm (Fig. 1). The dynamic and widespread expression patterns of certain ciliopathy proteins have further confounded definitive localization of these proteins. In addition, while mutations of different ciliary genes can result in the same ciliopathy phenotype (genetic heterogeneity), it is true that mutations in the same gene can result in different phenotypic manifestations (pleiotropy). A typical example is the ciliary protein CEP290/NPHP6, which normally localizes to the centrosome complex, the cilium transition zone and the mitotic spindle. Mutations in *CEP290* can result in human ciliopathies ranging from severe, early-onset developmental disorders (Meckel-Gruber syndrome, MKS), to mild, late-onset, degenerative disorders (nephronophthisis, NPHP). Other disorders related to defects in CEP290 include Bardet-Biedl syndrome (BBS), Joubert syndrome (JBTS), Senior-Løken syndrome (SLSN) and Leber congenital amaurosis (LCA) (Sayer et al., 2006; Coppieters et al., 2010; Garcia-Gonzalo et al., 2011; Waters and Beales, 2011). These characteristics of ciliary proteins add to the complexity of studying genetic ciliopathy disorders (Waters and Beales, 2011).

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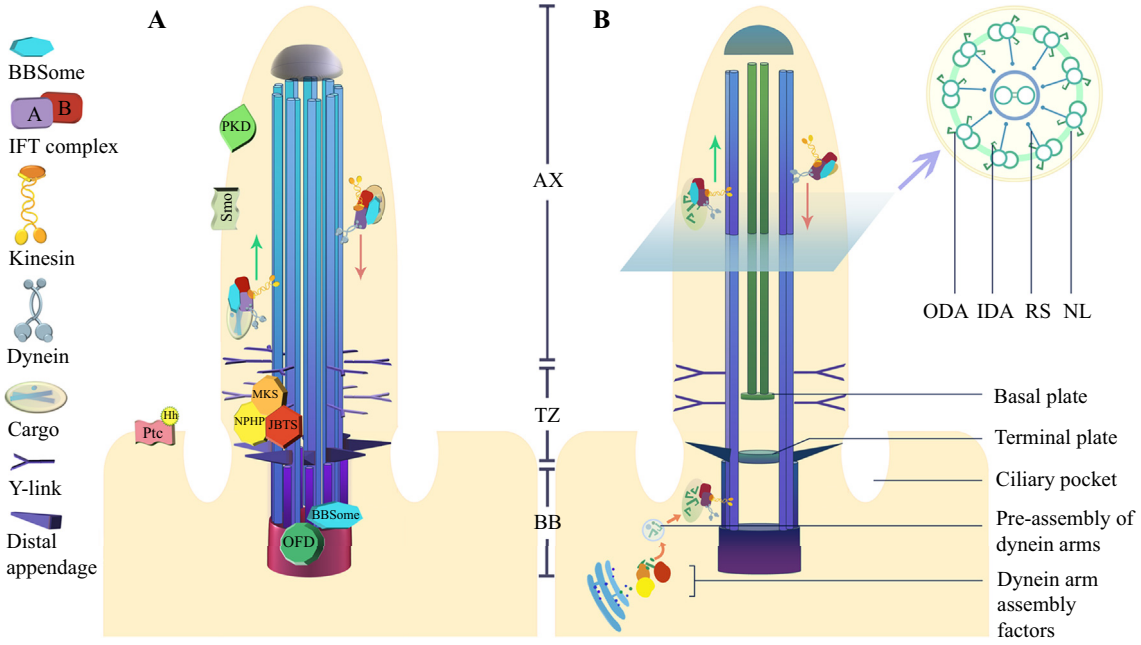

Fig. 1. Schematic diagrams of primary and motile cilia.
A: Diagram of a classical "9 + 0" primary cilium. Cargo proteins are transported by IFT complexes powered by kinesin-2 (anterograde) and cytoplasmic dynein (retrograde) motors. Proteins mutated in human NPHP, MKS and JBTS ciliopathies cluster in large complexes in the transition zone or basal body of the cilium. BBSome proteins localize to the basal body and the ciliary axoneme, where they are associated with IFT. The Smoothened (Smo) protein is translocated to the ciliary membrane upon activation by the Hedgehog (Hh) signaling pathway. **B:** Diagram of a "9 + 2" motile cilium. The structures of motile and primary cilia are similar, with the addition of axonemal dynein arm structures. Components of the outer dynein arms (ODA) and inner dynein arms (IDA) are thought to be pre-assembled by assembly factors in the cytoplasm, and transported into the cilia *via* IFT. Disruption of this process is the major cause of cilia motility defects in human PCD. Abbreviations: AX, axoneme; TZ, transition zone; BB, basal body; RS, radial spoke; NL, nexin links.

Deciphering the mechanisms of human ciliopathies can only be performed through detailed study of the diversity of cilium structure, composition and function. Although studies of *in vitro* cultured cells or tissues have greatly improved our knowledge of cilia function, the use of *in vivo* model systems is vital in order to fully understand the mechanisms underlying ciliopathies. Luckily, due to the highly conserved nature of cilia structure and components, the molecular mechanisms of human cilia development and disease can be learned from simple organisms. Many major discoveries in cilia and flagella, including ciliary intraflagellar transport (IFT), were *de facto* made in *Chlamydomonas reinhardtii* and *Caenorhabditis elegans*. Although being highly informative models, the fact that these invertebrates lack the organs that are affected in human ciliopathy patients, such as kidneys and eyes, renders them incapable of evaluating the relationship between cilia malfunction and organogenesis defects. In recent decades, vertebrate models such as mouse, zebrafish and *Xenopus* have demonstrated great advantages for studying ciliopathies, particularly with respect to the abnormal organogenesis observed in many human ciliopathy disorders. In this review, we describe the features of zebrafish cilia and major contributions of zebrafish as a vertebrate model for the study of human ciliopathies. We also introduce new experimental techniques developed in zebrafish, which have furthered our understanding of roles for ciliary proteins in human development and disease.

CILIA AND IFT

As an evolutionarily conserved organelle, all eukaryotic cilia are virtually similar in their organization. Cilia exhibit a nine-fold symmetric microtubule-based axoneme, a structure derived from the centriole, and emanate from the surface of cells. The majority of vertebrate cilia are classified as either motile cilia (9 + 2), which contain nine doublet microtubules surrounding a central pair of singlet microtubules, or non-motile primary cilia (9 + 0), which only consist of nine doublet microtubules (Satir and Christensen, 2007) (Fig. 1). Other classifications do exist, such as "9 + 0" motile cilia, which exist in the mouse embryonic node (Caspary et al., 2007), and "9 + 2" non-motile kinocilia, which exist in zebrafish hair cells (Yu et al., 2011).

Both motile and primary cilia are assembled and maintained by a dynamic process called IFT. IFT moves cytoplasmic proteins such as tubulins into the cilia, termed anterograde transport, while retrograde IFT particles transport axonemal turnover products from the tip to the base of the cilia (Hao et al., 2011; Taschner et al., 2012; Craft et al., 2015). IFT particles are composed of at least 20 proteins, consisting of a 6-subunit IFT-A complex and a ~14-subunit IFT-B complex (Cole and Snell, 2009; Taschner et al., 2012). Cytoplasmic proteins are thought to be transported into the cilia by binding to the anterograde IFT complex, which is powered by plus-end kinesin 2 motors (Scholey, 2008; Kardon and Vale, 2009)