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Myosin heavy chain 10 (MYH10) is required for centriole migration during the biogenesis of primary cilia

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ABSTRACT

The actin cytoskeleton has been implicated in the assembly of cilia, but roles of actin-dependent motor proteins in ciliogenesis remain unclear. Myosin heavy chain 10 (MYH10), one of the isoforms of non-muscle myosin II, is known to mediate centrosome reorientation during cell migration. Here we show that MYH10 is required for centriole migration to the apical plasma membrane, which occurs at the onset of ciliogenesis. Knockdown of MYH10 in RPE1 cells caused a reduction in the levels of cortical filamentous actin (F-actin) and its binding protein EZRIN. Moreover, both centriole migration and subsequent cilium assembly were defective in MYH10 depleted cells. We further found that MYH10 influences centrosomal recruitment of IFT88, which is required for the transport of building blocks to the ciliary tip. The role of MYH10 in IFT88 recruitment appears to be indirect in that there is a correlation between centriolar IFT88 levels and centriolar positions along the apical-basal axis during ciliogenesis. Our results indicate that MYH10 contributes to ciliogenesis in RPE1 cells by promoting cortical actin-dependent centriole migration.

1. Introduction

Primary cilia are microtubule-based organelles that extend like an antenna and transduce sensory stimuli to the cell body [1,2]. Recent studies have identified large numbers of molecules that are involved in the biogenesis of primary cilia [3,4], yet detailed molecular mechanisms underlying each step of ciliogenesis remain incompletely understood. During early phase of ciliogenesis, the mother centriole migrates close to the apical plasma membrane and serves as a template for the axoneme [5]. Centriole migration is necessary for ciliogenesis, and defects in centriole migration have been implicated in the pathogenesis of Meckel–Gruber syndrome, which is one of the inherited diseases caused by dysfunction of primary cilia [6,7]. However, molecular motors that mediate the migration of centrioles are unknown.

The locomotion of the centriole in migrating cells depends on non-muscle myosin II, the motor protein of the actin cytoskeleton [8,9]. The involvement of the actin cytoskeleton in centriole migration during ciliogenesis has been suggested by the fact that meckelin, Meckel–Gruber syndrome protein required for centriole migration, functions with actin-binding protein filamin A [7]. Moreover, in multi-ciliated cells of mammalian oviducts, suppression of myosin II function interferes with centriole migration [10]. However, roles of non-muscle myosin II in centriole migration for the assembly of primary cilia have not been addressed yet.

A previous RNAi library screen identified MYH10 as a positive factor for ciliogenesis in RPE1 cell [3]. MYH10 is one of the isoforms of non-muscle myosin II, and is known to regulate the orientation of both the centriole and the Golgi apparatus during cell migration [11,12]. A recent study showed that MYH10 regulates ciliogenesis by interacting with microtubule acetyltransferase Mec17 [13]. Mec17 appears to upregulate MYH10 expression in quiescent cells, and MYH10 accumulation facilitates the formation of the pericentrosomal membranous compartment that supplies materials for cilium growth [13]. Here, we demonstrate that MYH10 contributes to ciliogenesis through regulation of cortical actin dynamics and centriole migration. In addition, we report that MYH10-dependent centriole migration affects centriolar recruitment of IFT88, a key molecule in the intraflagellar transport supporting the assembly and maintenance of cilia.

2. Materials and methods

2.1. Cell culture, siRNA, and transfection

Human RPE1 cells were cultured in Dulbecco's modified Eagle's medium/F12 (DMEM/F12), supplemented with 10% (v/v) fetal

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bovine serum, 100 units/ml of penicillin and 100 μg/ml of streptomycin. RPE1 cells were transfected with 10 nM of siRNAs using Lipofectamine RNAiMAX (Invitrogen) in accordance with the manufacturer's reverse transfection protocol. RPE1 cells were transfected for 2 days and cultured for additional 1 day in the absence of serum to induce ciliogenesis. The following siRNAs were used: MYH10 si1: GACTCGUCAGGAACUGGAA, MYH10 si2: UAUUCUCAGAGUAAAUUGG, MYH10 si3 GCACUUGUCUCUCUCAUUU, and CEP164 si: GAGUGAAGGUGUAUCGCUU. For MYH10 overexpression, we used GFP tagged MYH10 vector (Addgene #11348), and pEGFP-C3 vector was used as a negative control. RPE1 cells were transfected with 200 ng of plasmid DNA using Lipofectamine LTX (Invitrogen) in accordance with the manufacturer's forward transfection protocol.

2.2. Western blot

Cells were extracted 48 h after transfection with RIPA lysis buffer [50 mM Tris pH 8.0, 150 mM NaCl, 10 mM EDTA, 1% (v/v) NP40, 1% (v/v) deoxycholic acid, and 0.1% (w/v) SDS] supplemented with protease and phosphatase inhibitors (Sigma–Aldrich). Samples were separated by 6% poly-acrylamide gel and transferred onto nitrocellulose membrane. Membrane were blocked with 5% (w/v) skim milk for 30 min and incubated with primary antibodies diluted in blocking solution overnight at 4 °C. Bound antibodies were visualized with Peroxidase-coupled secondary antibody (Santa Cruz Biotechnology) and ECL solution (Neuronex).

2.3. Immunofluorescence

Cells were grown on Lab-Tek II chamber slides (Thermo Fisher Scientific Inc.) and either fixed in −20 °C methanol for 5 min or in 4% Paraformaldehyde (PFA) for 7 min. Cells fixed with methanol were incubated at RT for 10 min with blocking solution (PBS with 10% FBS and 0.02% (w/v) NaN_3), and 4% PFA treated cells were blocked for 10 min with blocking solution containing 0.1% Triton X-100. After blocking, cells were incubated with primary antibodies diluted with blocking solution for 1 h at RT. Alexa Fluor-conjugated secondary antibodies were used to detect primary antibodies for 1 h at RT. Coverslips were mounted on glass slides in mounting solution composed of 75% glycerol, 0.02% (w/v) NaN_3, 0.5 DAPI μg/ml, and 20 nM Tris pH 8.8. Primary antibodies used are: rabbit anti-MYH10 (Cell signaling), rabbit anti-ARL13B (Proteintech), rabbit anti-CP110 (Proteintech), rabbit anti-IFT88 (Proteintech), rabbit anti-TCTN1 (Proteintech), mouse anti-γ tubulin (Abcam), rabbit anti-EZRIN (Cell signaling), and mouse anti-GT335 (Adipogen).

2.4. Microscopy and image analysis

Fluorescence microscopy was performed on an inverted Delta Vision fluorescence microscope equipped with a cooled CCD camera (Applied precision). Images were taken using a 60X objective (1.42 NA, Plan-Achromat, Olympus). For Fig. 3A, images were stacked with a z-step size of 0.5 μm and processed by volume rendering tool. The inverted confocal microscope (LSM780, Zeiss) was used in study of apical, basal actin structure shown in Fig. 2A and C. In confocal microscopy, images were taken using a 40X objective (LD C-Apochromat, 1.1 W Corr), with water immersion. To quantitate IFT88 immunofluorescence intensity in RPE1 cells, images were captured without auto-intensity adjustment and pixel intensities were measured using Image J software (National Institutes of Health). We conformed to Stefanie Kuhns and his colleague's method for the intensity analysis [14]. Images were sized and placed in figures using Adobe Photoshop CS6.

3. Results

3.1. MYH10 is required for ciliogenesis

To confirm if MYH10 is involved in primary cilium biogenesis, we depleted MYH10 in RPE1 cells using two independent siRNAs and assessed ciliogenic potential of the cells. Cells were serum deprived for final 24 h and primary cilia were marked by immunofluorescence using antibodies raised against ARL13B, a known cilium-specific protein. As shown in Fig. 1A and B, the number of ciliated cells was significantly decreased after MYH10 knockdown. Although ciliated cell numbers decreased consistently, depletion of MYH10 did not cause a complete suppression of ciliogenesis. Thus we were able to examine the effect of MYH10 knockdown on the length of cilia. Cilia from MYH10 depleted cells failed to acquire normal length (Fig. 1C). These results indicate that MYH10 is required for both ciliogenesis and cilium elongation.

Next, we performed a rescue experiment to confirm the specificity of the knockdown phenotypes. We transfected cells with siRNAs which target 5′UTR region of MYH10 gene for 48 h, and then transfected the cells again with MYH10 expression vector for 24 h. Exogenously introduced MYH10 partially rescued ciliogenesis and ciliary elongation (Fig. 1D and E). In addition, efficient MYH10 knockdown was confirmed by immunoblotting (Fig. 1F).

3.2. MYH10 depletion affects the apical enrichment of actin filaments and EZRIN

It has been shown that MYH10 co-localizes with F-actin and influences actin dynamics [12]. We confirmed that MYH10 co-localized with F-actin in serum deprived RPE1 cells (Fig. 2A). Remarkably, cortical actin was co-stained with MYH10 antibodies and MYH10 was also detected in the vicinity of centrioles (Fig. 2B). Apical actin dynamics is thought to play an important role in the initiation of ciliogenesis because centriole migration and basal body docking to the apical membrane involves the cortical actin complex [15]. Localization patterns of MYH10 suggest its potential roles in apical actin dynamics and centriole migration.

To investigate if MYH10 is required for cortical actin complex formation, we examined the expression of EZRIN after MYH10 knockdown. EZRIN is the actin-binding protein that links cytoplasmic membrane to the actin cytoskeleton in polarized epithelial cells. A recent study showed that EZRIN is required for basal body migration and docking in multiciliated cells [16]. As shown in Fig. 2C, EZRIN immunofluorescence intensities were decreased in the apical region of MYH10 depleted cells. Moreover, apical actin staining was also noticeably reduced when compared with control. However, MYH10 knockdown did not affect protein levels of EZRIN (Fig. 2D). These results indicate that MYH10 plays a role in the establishment of the cortical actin structure in RPE1 cells.

3.3. Centriole migration requires MYH10 function

Centriole migration to the apical region of the cell is one of the earliest steps of ciliogenesis and centriole localization is thought be mediated by both actin cytoskeleton and actin-based motor proteins [5]. Previously it has been shown that MYH10 localizes to the vicinity of centrioles in ependymal cells [17], and we also observed similar localization (Fig. 2B). Moreover, MYH10 depletion was shown to induce mislocalization of the Golgi and centrioles during cell migration [11]. Thus, we investigated whether MYH10 is involved in centriole migration to the apical plasma membrane during the biogenesis of primary cilia. Cells were transfected for 48 h with siRNAs and serum-starved for additional 24 h. The position of the centriole/basal body was measured by vertical optical

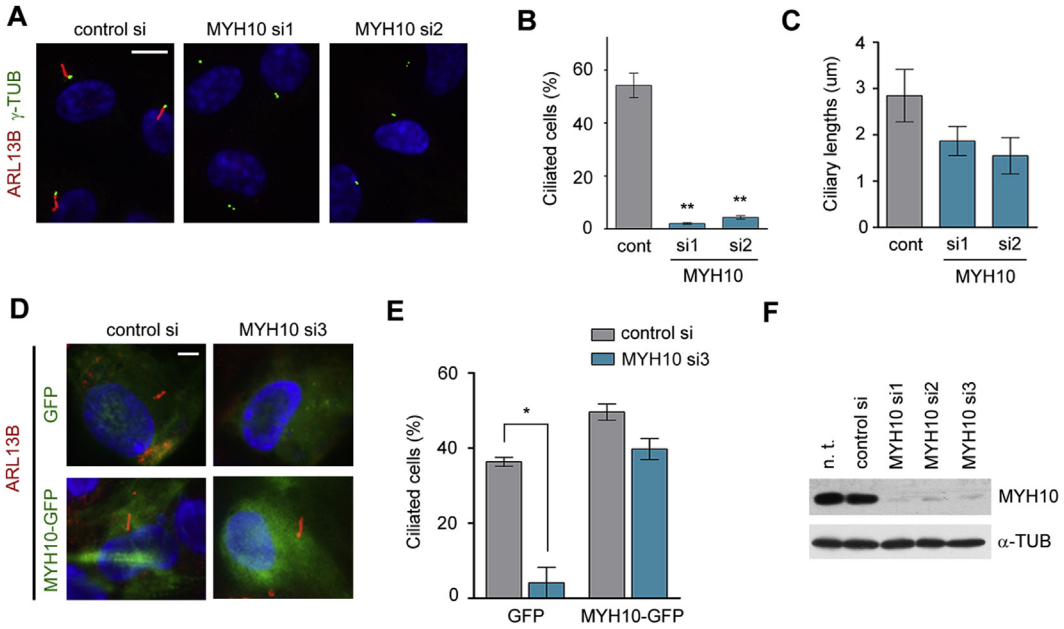

Fig. 1. MYH10 is required for the assembly and elongation of primary cilia. (A) RPE1 cells were transfected with the indicated siRNAs for 48 h, and culture for additional 24 h in serum-free medium. Primary cilia were labeled with anti-ARL13B antibodies, the centriole were labeled with anti-γ tubulin antibodies. The nuclei were visualized by DAPI staining. Quantification of the result shown in (B) and the length of primary cilia was compared between control and MYH10 depleted cells (C). (D) RPE1 cells were transfected with 5′UTR targeted siMYH10 for 48 h, and then GFP tagged MYH10 was overexpressed for 24 h. Empty GFP vector was used as a control. Cilia were immunostained with anti-ARL13B antibodies and quantification of the result is shown in (E). (F) Efficient MYH10 knockdown was confirmed by immunoblotting. All quantification data are mean ± SD from two independent experiments. *P < 0.05 and **P < 0.01 (t-test). Scale bars: 5 μm.

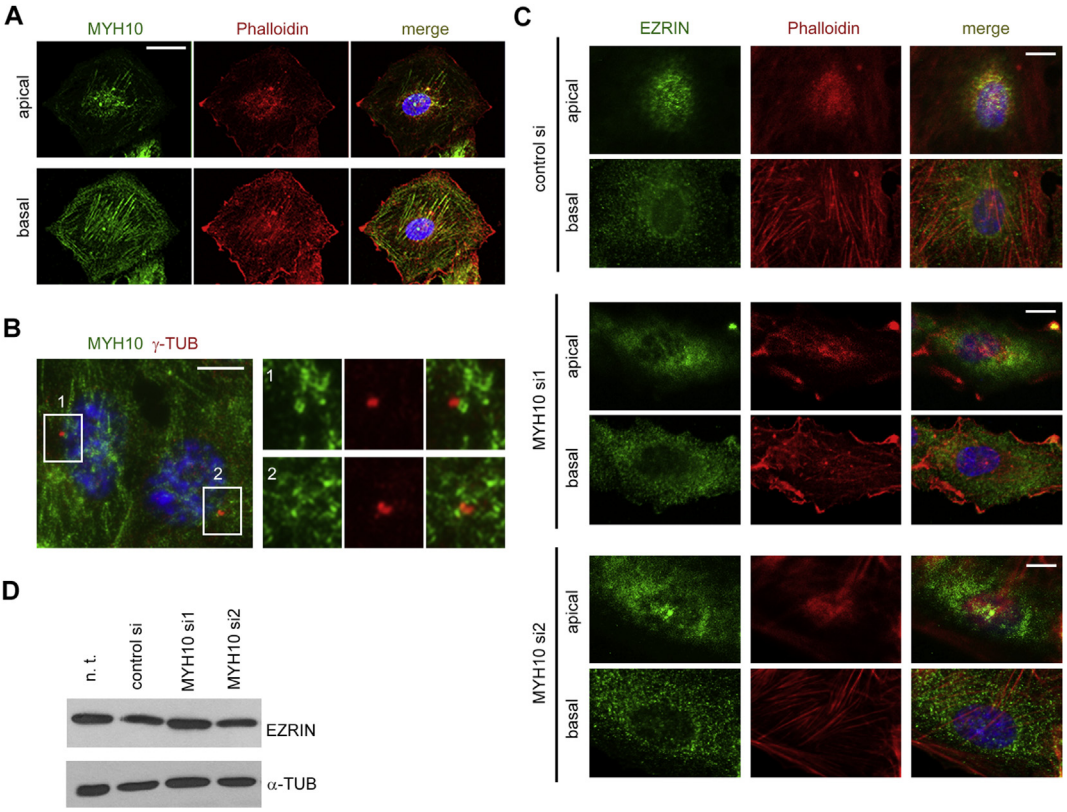

Fig. 2. MYH10 is required for the enrichment of actin filaments and EZRIN at the apical membrane. (A) Apical-basal immunofluorescence images of endogenous MYH10 in RPE1 cells. RPE1 cells were serum-starved for 24 h before fixation. The actin cytoskeleton was visualized by Alexa 594-conjugated Phalloidin. (B) Centrioles labeled with anti-γ tubulin antibodies were co-stained with endogenous MYH10 in serum deprived RPE1 cells. (C) EZRIN expression pattern was visualized by anti-EZRIN immunofluorescence. (D) Western blot showing that MYH10 knockdown does not affect total EZRIN expression levels. Scale bars: 15 μm.

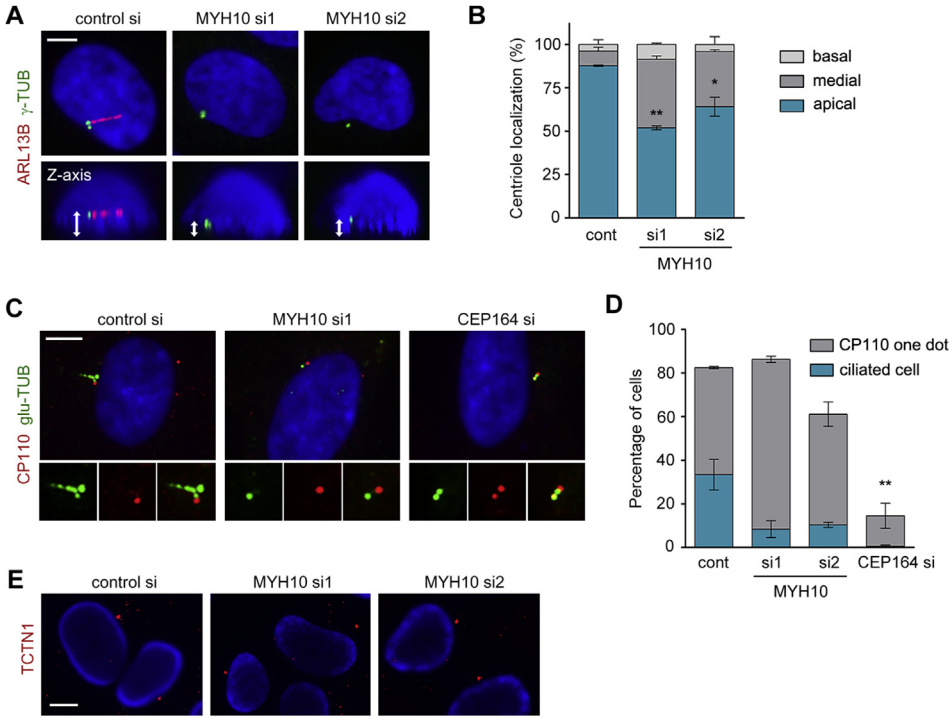

Fig. 3. MYH10 plays a role in centriole migration, but is dispensable for the removal CP110 cap from the mother centriole. (A) RPE1 cells were transfected with the indicated siRNAs for 72 h, and serum-starved for additional 24 h. Location of the centrioles was accessed by immunofluorescence staining of anti-γ tubulin antibodies. Cilia were labeled by anti-ARL13B antibodies and the nuclei were visualized by DAPI. Lower panels are reconstructed fluorescence images showing z-axis view. Double-headed arrows indicate the height of centrioles from the basal layer of the cell. (B) Quantitation of centriole localization. Centriole positions were classified as basal (0.0–1.5 μm), medial (1.5–3.0 μm), and apical (3.0–4.5 μm). (C) RPE1 cells were transfected with the indicated siRNAs for 48 h and cultured for additional 24 h in serum-free medium. The centrioles and cilia were labeled with anti-poly-glutamylated Tubulin antibodies (GT335), and CP110 cap was labeled with anti-CP110 antibodies. (D) Quantification of cells exhibiting single CP110 cap from each centriole pair. (E) Immunofluorescence images showing TCTN1 localization in MYH10 depleted RPE1 cells. All data are mean ± SD from two independent experiments. *$P < 0.05$ and **$P < 0.01$ (t-test). Scale bars: 5 μm.

sections of cells stained with anti-γ-Tubulin antibody. In ciliated control cells, the centrioles/basal bodies were detected within characteristic heights (3.0–4.5 μm) from the base of the cells (Fig. 3A and B). By contrast, a fraction of cells depleted of MYH10 failed to migrate to the apical region. This result indicates that MYH10 is required for centriole migration during ciliogenesis as well as apical actin cortex formation.

Next, we examined the involvement of MYH10 in other early steps in ciliogenesis. CP110 is a major component of the cap structure which localizes to the distal end of both centrioles [18]. It has been shown that CP110 cap suppresses both centriole elongation and axoneme assembly, and is specifically removed from the mother centriole at the onset of ciliogenesis [18]. We examined the removal of CP110 cap from the mother centriole in response to serum starvation after MYH10 knockdown. We double stained cells with anti-CP110 and anti-poly-glutamylated Tubulin antibodies. Depletion of distal appendage protein CEP164 inhibited CP110 removal as previously reported [19] and completely blocked ciliogenesis (Fig. 3C). By contrast, CP110 cap was normally removed in MYH10 depleted cells. Moreover, transition zone protein TCTN1 was properly localized to the centriolar region in MYH10 depleted cells (Fig. 3E). These results show that MYH10 is not involved in CP110 cap removal or transition zone maturation. It is also noteworthy that centriole migration mediated by MYH10 is independent of CP110 cap removal.

3.4. Centriolar IFT88 recruitment is correlated with centriole migration

The assembly of axoneme requires the transport system known as 'intraflagellar transport (IFT)' that is mediated by multiprotein IFT complexes and microtubule-based motor proteins [20,21]. IFT88, one of the IFT complex components, localizes to both the centriole and the intraciliary space, and is involved in material transports to the ciliary tip [22,23]. To test if MYH10 is required for the recruitment of IFT88 to the centriole, we measured fluorescence intensity of anti-IFT88 antibody staining. Remarkably, centriolar intensity of IFT88 staining was significantly reduced in MYH10 depleted cells (Fig. 4A and B). In control cells, IFT88 was recruited to the centrosome even before the assembly of the axoneme, suggesting that loss of centriolar IFT88 in MYH10 depleted cells was not a secondary consequence of ciliogenesis defect (Fig. 4A). The reduction of IFT88 levels in MYH10 depleted cells was partially rescued by an overexpression of MYH10 (Fig. 4C). The transition zone, an intermediate region between the basal body and the cilium, is known to facilitate the docking of IFT molecules [24,25]. As shown in Fig. 3E, normal levels of transition zone protein TCTN1 were observed in the centriolar region of MYH10 depleted cells, suggesting that defects in IFT88 recruitment is not ascribed to a loss of the transition zone.

Interestingly, we found that centriolar levels of IFT88 are correlated with the position of centriole along the apical-basal axis in cells undergoing ciliogenesis (Fig 4D). IFT88 fluorescence intensities of the apical centrioles were higher than those of the basal centrioles. Therefore, MYH10 may indirectly affect IFT88 localization through its role in centriole migration along the vertical axis.

4. Discussion

In the present study, we demonstrate that actin-dependent motor MYH10 is required for the assembly of primary cilia,

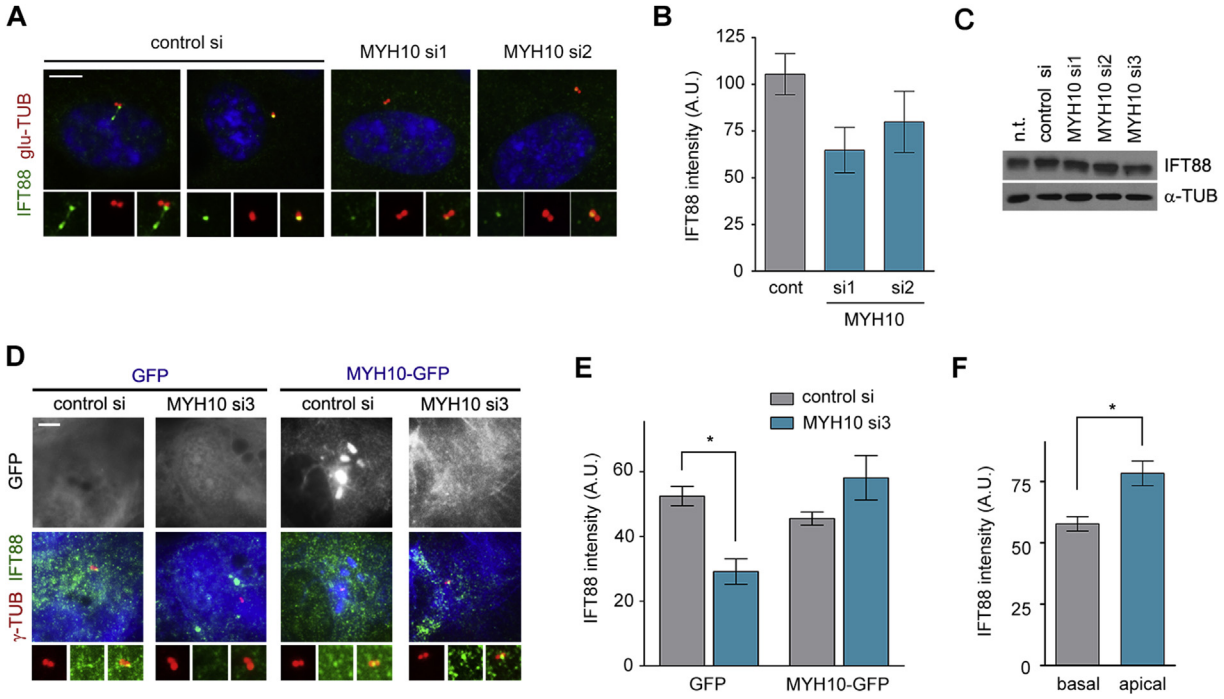

Fig. 4. MYH10 knockdown affects centriolar recruitment of IFT88, which is correlated with centriole migration. (A) Immunofluorescence images showing IFT88 localization. RPE1 cells were transfected with the indicated siRNAs. Insets are magnified views of the centrosomal area. (B) Quantification of centrosomal immunofluorescence intensity of IFT88 (arbitrary unit; a.u.). (C) Western blot showing that MYH10 knockdown does not affect total IFT88 expression levels. (D) RPE1 cells were transfected with 5′UTR targeted MYH10 siRNA for 48 h, and then GFP tagged MYH10 was overexpressed for 24 h. The reduction in centrosomal IFT88 recruitment was rescued by exogenous MYH10. (E) Quantification of centrosomal immunofluorescence intensity of IFT88 (arbitrary unit; a.u.). (F) Normal RPE1 cells were serum-starved for 24 h before fixation. Centrosomal apical-basal positions and IFT88 levels were measured. All quantification data are mean ± SD from two independent experiments. *$P < 0.05$ (t-test). Scale bars: 5 μm.

mediating centriole migration to the apical region of the cell. Previously, branched filamentous actin was recognized as a negative regulator of ciliogenesis in mammalian cells because treatment with cytochalasin D, an actin destabilizer, induces rapid cilium assembly and stimulates axoneme elongation by 2-fold [3,26]. Moreover, actin-associated molecules that are involved in the nucleation of branched actin such as components of ARP2/3 complex, suppress ciliogenesis [3]. However, we found that MYH10, which contributes cell contractility by maintaining stability of stress fibers and filamentous actin [27], is a positive factor for ciliogenesis in RPE1 cells. Recent report also refers its role in ciliogenesis as an interaction partner of microtubule acetyltransferase Mec17 [13]. We observed that MYH10 localizes to both cortical actin networks as well as stress fibers, and plays a role in the assembly of cortical actin and EZRIN. Because, the cortical actin complex appears to be critical for the migration and docking of the centriole, defective centriole migration observed in MYH10 depleted cells may be due to structural defects in the cortical actin complex. However, our results do not exclude the possibility that centrosomally localized MYH10 is involved in the migration.

Both centriole migration and CP110 cap removal are considered to be essential for the initiation of cilium assembly [5]. However, potential causal relationship and temporal order of the two processes have not been addressed. Interestingly, we found that MYH10 is not required for CP110 cap removal from the mother centriole, while it plays a key role in centriole migration. This result suggests that the removal of CP110 cap occurs in response to serum starvation independent of MYH10-mediated centriole migration. Recent studies have shown that CP110 cap removal requires the recruitment of serine–threonine kinase TTBK2 to the centrosome, and CEP164, a distal appendage protein, is essential for TTBK2 recruitment [19]. Thus, it can be also inferred that the activation of CEP164-TTBK2 interaction can occur independent of centriole migration.

The assembly of the axoneme was blocked or incomplete in MYH10 depleted cells. This observation corresponds well with the finding that MYH10 depletion causes a decrease in IFT88 levels from the centrosome. IFT88 reduction does not seem to be caused by dramatic structural defects of the basal body in that TCTN1 is normally localizes to the presumptive transition zone in MYH10 depleted cells. IFT88 is one of the best-studied IFT molecules, moving to the ciliary tip with the microtubule-based motor protein kinesin and other ciliary cargoes [23]. Intact IFT88 is essential for ciliogenesis, and mutations of mouse IFT88 gene (Tg737) causes embryonic lethality and randomized left-right body axis as well as neural tube defects [22,28]. Thus it is likely that the reduction of IFT88 levels in MYH10 depleted cells is responsible for the defects in ciliogenesis and cilium elongation. Remarkably, in normal cells initiating ciliogenesis, levels of IFT88 gradually increased as centrioles migrate to the apical region of the cell. Thus, we suggest that MYH10 indirectly affects the centriolar recruitment of IFT88 through its impact on centriole migration during ciliogenesis.

In conclusion, we demonstrate that MYH10 is required for efficient ciliogenesis, and mediates the link between the apical-basal polarity and microtubule-dependent movements of ciliary components. Future studies that can associate MYH10-dependent actin dynamics with other ciliogenic events such as ciliary vesicle trafficking and docking will further explicate key mechanisms of ciliogenesis.

Conflict of interest

None.

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Transparency document

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References

[1] V. Singla, J.F. Reiter, The primary cilium as the cell's antenna: signaling at a sensory organelle, Science 313 (2006) 629–633.
[2] S.C. Goetz, K.V. Anderson, The primary cilium: a signalling centre during vertebrate development, Nat. Rev. Genet. 11 (2010) 331–344.
[3] J. Kim, J.E. Lee, S. Heynen-Genel, E. Suyama, K. Ono, K. Lee, T. Ideker, P. Aza-Blanc, J.G. Gleeson, Functional genomic screen for modulators of ciliogenesis and cilium length, Nature 464 (2010) 1048–1051.
[4] L.C. Keller, E.P. Romijn, I. Zamora, J.R. Yates III, W.F. Marshall, Proteomic analysis of isolated *Chlamydomonas* centrioles reveals orthologs of ciliary-disease genes, Curr. Biol. 15 (2005) 1090–1098.
[5] H.R. Dawe, H. Farr, K. Gull, Centriole/basal body morphogenesis and migration during ciliogenesis in animal cells, J. Cell. Sci. 120 (2007) 7–15.
[6] H.R. Dawe, U.M. Smith, A.R. Cullinane, D. Gerrelli, P. Cox, J.L. Badano, S. Blair-Reid, N. Sriram, N. Katsanis, T. Attie-Bitach, The Meckel–Gruber syndrome proteins MKS1 and meckelin interact and are required for primary cilium formation, Hum. Mol. Genet. 16 (2007) 173–186.
[7] M. Adams, R.J. Simms, Z. Abdelhamed, H.R. Dawe, K. Szymanska, C.V. Logan, G. Wheway, E. Pitt, K. Gull, M.A. Knowles, A meckelin–filamin A interaction mediates ciliogenesis, Hum. Mol. Genet. 21 (2012) 1272–1286.
[8] C.-M. Lo, D.B. Buxton, G.C. Chua, M. Dembo, R.S. Adelstein, Y.-L. Wang, Non-muscle myosin IIb is involved in the guidance of fibroblast migration, Mol. Biol. Cell. 15 (2004) 982–989.
[9] E.R. Gomes, S. Jani, G.G. Gundersen, Nuclear movement regulated by Cdc42, MRCK, myosin, and actin flow establishes MTOC polarization in migrating cells, Cell 121 (2005) 451–463.
[10] E. Boisvieux-Ulrich, M.-C. Lainé, D. Sandoz, Cytochalasin D inhibits basal body migration and ciliary elongation in quail oviduct epithelium, Cell. Tissue Res. 259 (1990) 443–454.
[11] M. Vicente-Manzanares, J. Zareno, L. Whitmore, C.K. Choi, A.F. Horwitz, Regulation of protrusion, adhesion dynamics, and polarity by myosins IIA and IIB in migrating cells, J. Cell. Biol. 176 (2007) 573–580.
[12] M. Vicente-Manzanares, X. Ma, R.S. Adelstein, A.R. Horwitz, Non-muscle myosin II takes centre stage in cell adhesion and migration, Nat. Rev. Mol. Cell. Biol. 10 (2009) 778–790.
[13] Y. Rao, R. Hao, B. Wang, T.-P. Yao, A Mec17-myosin II effector axis coordinates microtubule acetylation and actin dynamics to control primary cilium biogenesis, PloS One 9 (2014) e114087.
[14] S. Kuhns, K.N. Schmidt, J. Reymann, D.F. Gilbert, A. Neuner, B. Hub, R. Carvalho, P. Wiedemann, H. Zentgraf, H. Erfle, The microtubule affinity regulating kinase MARK4 promotes axoneme extension during early ciliogenesis, J. Cell. Biol. 200 (2013) 505–522.
[15] M.E. Werner, P. Hwang, F. Huisman, P. Taborek, C.Y. Clare, B.J. Mitchell, Actin and microtubules drive differential aspects of planar cell polarity in multiciliated cells, J. Cell Biol. 195 (2011) 19–26.
[16] D. Epting, K. Slanchev, C. Boehlke, S. Hoff, N.T. Loges, T. Yasunaga, L. Indorf, S. Nestel, S.S. Lienkamp, H. Omran, The Rac1 regulator ELMO controls basal body migration and docking in multiciliated cells through interaction with Ezrin, Development 142 (2015) 174–184.
[17] Y. Hirota, A. Meunier, S. Huang, T. Shimozawa, O. Yamada, Y.S. Kida, M. Inoue, T. Ito, H. Kato, M. Sakaguchi, Planar polarity of multiciliated ependymal cells involves the anterior migration of basal bodies regulated by non-muscle myosin II, Development 137 (2010) 3037–3046.
[18] T.I. Schmidt, J. Kleylein-Sohn, J. Westendorf, M. Le Clech, S.B. Lavoie, Y.-D. Stierhof, E.A. Nigg, Control of centriole length by CPAP and CP110, Curr. Biol. 19 (2009) 1005–1011.
[19] L. Čajánek, E.A. Nigg, Cep164 triggers ciliogenesis by recruiting Tau tubulin kinase 2 to the mother centriole, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) E2841–E2850.
[20] J.M. Scholey, Intraflagellar transport motors in cilia: moving along the cell's antenna, J. Cell. Biol. 180 (2008) 23–29.
[21] M. Taschner, S. Bhogaraju, E. Lorentzen, Architecture and function of IFT complex proteins in ciliogenesis, Differentiation 83 (2012) S12–S22.
[22] G.J. Pazour, B.L. Dickert, Y. Vucica, E.S. Seeley, J.L. Rosenbaum, G.B. Witman, D.G. Cole, *Chlamydomonas* IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella, J. Cell. Biol. 151 (2000) 709–718.
[23] C.-H. Sung, M.R. Leroux, The roles of evolutionarily conserved functional modules in cilia-related trafficking, Nat. Cell. Biol. 15 (2013) 1387–1397.
[24] J.A. Deane, D.G. Cole, E.S. Seeley, D.R. Diener, J.L. Rosenbaum, Localization of intraflagellar transport protein IFT52 identifies basal body transitional fibers as the docking site for IFT particles, Curr. Biol. 11 (2001) 1586–1590.
[25] C.L. Williams, C. Li, K. Kida, P.N. Inglis, S. Mohan, L. Semenec, N.J. Bialas, R.M. Stupay, N. Chen, O.E. Blacque, MKS and NPHP modules cooperate to establish basal body/transition zone membrane associations and ciliary gate function during ciliogenesis, J. Cell. Biol. 192 (2011) 1023–1041.
[26] J. Cao, Y. Shen, L. Zhu, Y. Xu, Y. Zhou, Z. Wu, Y. Li, X. Yan, X. Zhu, miR-129-3p controls cilia assembly by regulating CP110 and actin dynamics, Nat. Cell. Biol. 14 (2012) 697–706.
[27] S. Even-Ram, A.D. Doyle, M.A. Conti, K. Matsumoto, R.S. Adelstein, K.M. Yamada, Myosin IIA regulates cell motility and actomyosin–microtubule crosstalk, Nat. Cell. Biol. 9 (2007) 299–309.
[28] N.S. Murcia, W.G. Richards, B.K. Yoder, M.L. Mucenski, J.R. Dunlap, R.P. Woychik, The oak ridge polycystic kidney (orpk) disease gene is required for left-right axis determination, Development 127 (2000) 2347–2355.