Casein kinase 1δ functions at the centrosome and Golgi to promote ciliogenesis

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ABSTRACT Inhibition of casein kinase 1 delta (CK1δ) blocks primary ciliogenesis in human telomerase reverse transcriptase immortalized retinal pigmented epithelial and mouse inner medullary collecting duct cells-3. Mouse embryonic fibroblasts (MEFs) and retinal cells from Csnk1d (CK1δ)-null mice also exhibit ciliogenesis defects. CK1δ catalytic activity and centrosomal localization signal (CLS) are required to rescue cilia formation in MEFs Csnk1d null. Furthermore, expression of a truncated derivative containing the CLS displaces full-length CK1δ from the centrosome and decreases ciliary length in control MEFs, suggesting that centrosomal CK1δ has a role in ciliogenesis. CK1δ inhibition also alters pericentrosomal or ciliary distribution of several proteins involved in ciliary transport, including Ras-like in rat brain-11A, Ras-like in rat brain-8, centrosomal protein of 290 kDa, pericentriolar material protein 1, and polycystin-2, as well as the Golgi distribution of its binding partner, A-kinase anchor protein 450 (AKAP450). As reported for AKAP450, CK1δ was required for microtubule nucleation at the Golgi and maintenance of Golgi integrity. Overexpression of an AKAP450 fragment containing the CK1δ-binding site inhibits Golgi-derived microtubule nucleation, Golgi distribution of intraflagellar transport protein 20 homologue, and ciliogenesis. Our results suggest that CK1δ mediates primary ciliogenesis by multiple mechanisms, one involving its centrosomal function and another dependent on its interaction with AKAP450 at the Golgi, where it is important for maintaining Golgi organization and polarized trafficking of multiple factors that mediate ciliary transport.

INTRODUCTION

The primary cilium is a conserved, microtubule (MT)-based structure that emanates from the basal body, a membrane-docked mother centriole (Satir and Christensen, 2007; Garcia-Gonzalo and Reiter, 2012). It serves important sensory and signaling functions via membrane proteins specifically localized to its surface. Defects in primary ciliogenesis have been implicated in several genetic disorders, including Bardet–Biedl, Joubert, and Meckel–Gruber syndromes and polycystic kidney disease (Marshall, 2008; Gerdes et al., 2009; Kim and Dynlacht, 2013).

Formation of the primary cilium requires polarized protein transport to the basal body and subsequent incorporation of cargo into the ciliary membrane (Gerdes et al., 2009; Nachury et al., 2010). This process depends on MT networks and various effectors that facilitate vesicle trafficking and subsequent assembly of the ciliary membrane (Berbari et al., 2008; Garcia-Gonzalo et al., 2011). The small GTPase Ras-like in rat brain 11 (Rab11), a mediator of transport in the endocytic recycling compartment and trans-Golgi network, has an important role, as it recruits Rabin8 (a Rab8 guanine nucleotide exchange factor) to activate Ras-like in rat brain 8 (Rab8),...
which promotes the fusion of vesicles containing cilia-bound cargo into the nascent ciliary membrane (Knodler et al., 2010; Westlake et al., 2011; Hsiao et al., 2012). The axonemal cytoskeleton of the cilium is assembled via a process called intraflagellar transport that is mediated by large protein complexes known as intraflagellar transport protein particles (Pedersen and Rosenbaum, 2008). Intraflagellar transport protein 20 homologue (IFT20) is a component of IFT particles that also is present in the Golgi complex, where it facilitates the transport of ciliary membrane proteins such as polycystin-2 to the pericentrosomal region (Follit et al., 2006).

Several ciliary proteins—factors required for cilia formation or function—are located at centriolar satellites (Lopes et al., 2011). For instance, centrosomal protein of 290 kDa (CEP290)/NPHP6 functions in ciliary transport and organization of the MT network (Kim et al., 2008; Coppieters et al., 2010). Pericentriolar material protein 1 (PCM1), a CEP290-binding protein, assists in anchoring MTs and recruiting several key centrosomal components (e.g., centrin, pericentrin, and ninein) and various ciliary proteins, such as BBS4 and OFD1, to the centrosomal or pericentrosomal area (Dammersmann and Merdes, 2002; Lopes et al., 2011; Stowe et al., 2012).

Casein kinase 1δ (CK1δ) is an evolutionarily conserved serine/threonine kinase that participates in diverse cellular processes, including vesicle trafficking, DNA repair, chromosome segregation, cell cycle progression, circadian rhythm, Wnt signaling, and neurite outgrowth (Gross and Anderson, 1998; Knippelschild et al., 2005; Bischof et al., 2011; Cheong and Virshup, 2011; Greer and Rubin, 2011). CK1δ localizes to the centrosome, trans-Golgi network (TGN), and cytoplasmic vesicles (Behrendt et al., 2000; Greer and Rubin, 2011; Jakobsen et al., 2011). Its centrosomal location is required for Wnt3a-dependent neuritogenesis in TC-32 Ewing sarcoma cells (Greer and Rubin, 2011). Recently, a CK1δ binding partner, A-kinase anchor protein 450 (AKAP450)/centrosomal Golgi N-kinase anchoring protein, was implicated in ciliogenesis through its regulation of Golgi positioning, integrity, and the nucleation of MTs at the Golgi (Rivero et al., 2009; Hurtado et al., 2011).

Here we report that CK1δ is a mediator of ciliogenesis in several model systems. Its role in ciliogenesis involves functions at both the centrosome and the Golgi, the latter associated with AKAP450, Golgi-derived MT nucleation, Golgi organization, and directed protein trafficking. Inhibition of CK1δ expression or kinase activity disrupted the intracellular distribution of many effectors of ciliogenesis, including Rab11a, Rab8a, CEP290, PCM1, AKAP450, and IFT20. Its effect on ciliogenesis likely depends on a multifaceted regulation of MT networks and vesicle trafficking required for ciliary transport.

RESULTS

CK1δ mediates ciliogenesis in multiple model systems

We initially determined whether CK1δ participates in ciliogenesis by treating human telomerase reverse transcriptase immortalized retinal pigmented epithelial (hTERT-RPE) cells with small interfering RNA (siRNA). After 48 h of serum starvation, 85.0% of cells incubated with negative control luciferase (Luc) siRNA displayed a primary cilium. Only 38.8% of cells exposed to CK1δ negative control luciferase (Luc) siRNA were ciliated, whereas 85.0% of cells incubated with siRNA reagents targeting CK1δ were ciliated, whereas primary cilium was present in >60% of cells, however, ciliary length was shortened (Figure 1E). PF670462 also decreased ciliary length in mouse inner medullary collecting duct cells-3 (mIMCD3; Supplementary Figure S1, C and D), another line commonly used in studies of ciliogenesis.

To ensure that observations made with the siRNA reagents and kinase inhibitor were due to on-target effects, we performed experiments with mouse embryo fibroblasts (MEFs) homozygous for a CK1δ floxed allele (Etchegary et al., 2009). Whereas 77.1% of MEF cells infected with negative control adenovirus expressing GFP contained a primary cilium, only 29.1% of MEF cells treated with adenovirus expressing Cre and lacking CK1δ were ciliated (Figure 2, A–C). Moreover, examination of retinal tissue from late-gestation wild-type (WT) versus Csnk1d null embryos revealed a decline in ciliary length of neural progenitor and RPE cells when CK1δ was absent (Supplementary Figure S2). Taken together, these results demonstrated that CK1δ has a significant role in ciliogenesis.

Structure–function analysis of CK1δ in MEF primary ciliogenesis

CK1δ contains a kinase domain comprising approximately two-thirds of its sequence and a centrosomal localization signal (CLS) located in its C-terminal domain. To investigate the function of these domains in ciliogenesis, we generated various CK1δ constructs, including ones encoding Myc-tagged, full-length WT CK1δ, full-length CK1δ with a K38A substitution that lacks kinase activity (DeMaggio et al., 1992; Fish et al., 1995; Murakami et al., 1999), a truncated derivative missing the C-terminal domain (ΔC), and a derivative containing only the C-terminal domain fused to enhanced GFP (8CT-EGFP; Figure 2, D and E; Greer and Rubin, 2011). Consistent with the data summarized in Figure 2B, cilia were absent or shorter in MEFΔCsnk1d null cells transfected with pDNA3.3 empty vector compared with MEFΔCsnk1d cells (Figure 2F). Transient transfection of CK1δ WT restored cilia to 80% of cells (44/55) and ciliary length to a level matching that of MEFΔCsnk1d cells. In contrast, none of the other derivatives was able to rescue the ciliary defect (Figure 2F). This confirmed that the catalytic activity of CK1δ was required for optimal cilia formation, as implied by the experiments with PF670462. It also indicated that kinase activity was not sufficient for normal cilia formation, as the C-terminal domain containing the CLS also was necessary to restore ciliogenesis.

To investigate a potential requirement for centrosomal CK1δ in ciliogenesis, we transiently transfected MEFΔCsnk1d cells with 8CT-EGFP, which previously was shown to displace full-length CK1δ from the centrosome of TC-32 cells (Greer and Rubin, 2011). Expression of 8CT-EGFP significantly reduced ciliary length, whereas c-EGFP did not (Figure 2, G and H). Consistent with our earlier results with TC-32 cells, when MEFΔCsnk1d cells were cotransfected with full-length Myc-CK1δ WT and either 8CT-EGFP or EGFP, Myc-CK1δ was displaced from the centrosome only in the presence of 8CT-EGFP and not displaced from other subcellular compartments such as the Golgi (Supplemental Figure S3). These observations suggested that the centrosomal localization of CK1δ was critical for ciliogenesis.

CK1δ regulates Rab11a/Rab8a distribution

We tested the hypothesis that disruption of CK1δ expression affected the distribution and function of Rab11a and Rab8a in primary ciliogenesis. GFP-Rab11a stably expressed in hTERT-RPE cells was detected at the base of primary cilia (Figure 3, A and B), consistent
δ promotes ciliogenesis
association rather than increased degradation, as Western blot analysis indicated that the protein level in whole-cell lysates was unchanged (unpublished data). CK1ε siRNA did not affect the pericentrosomal localization of GFP-Rab11a (unpublished data). Furthermore, the ciliary distribution of GFP-Rab8a also was inhibited by knockdown of CK1δ but not CK1ε (Figure 3, C and D).

CEP290 and PCM1 distribution in centriolar satellites is inhibited by CK1δ siRNA
Because CEP290 interacts with PCM1 and is required for Rab8 localization to the primary cilium (Kim et al., 2008), we investigated the effect of CK1δ siRNA on their distribution in hTERT-RPE cells. CEP290 punctae coincided with the centrosomal marker pericentrin and were scattered beyond it in control cells treated with Luc siRNA (Figure 4A). CK1δ knockdown resulted in substantial decrease in the number of punctae near the centrosome, although there still was overlap of CEP290 and pericentrin staining (Figure 4, A and B). CK1ε knockdown did not alter the basic staining pattern of CEP290 (Figure 4, A and B). PCM1 also had a pericentrosomal distribution, which was strikingly dispersed by CK1δ but not CK1ε siRNA (Figure 4, C and D). CEP290 and PCM1 protein levels in whole-cell lysates were similar in the various siRNA treatment groups (Figure 4E), suggesting that the diminished signal intensities associated with CK1δ siRNA were due to intracellular dispersion of the proteins rather than degradation. The pericentrosomal localization of CEP290 and PCM1 also was perturbed in MEF Csnk1d null cells (Supplemental Figure S4). However, PCM1 localization was not disrupted by δCT-EGFP (Supplemental Figure S5A), implying that centrosomal CK1δ was not important for PCM1 subcellular targeting. Thus our results indicate that CK1δ is required for proper positioning of these centriolar satellite proteins, although the mechanism does not involve centrosomal CK1δ.

AKAP450 distribution is dispersed by CK1δ inhibition
We also examined the effect of CK1δ on the intracellular distribution of its binding partner AKAP450 because AKAP450 has a role in ciliogenesis (Hurtado et al., 2011). AKAP450 colocalized with the centrosomal marker γ-tubulin and the cis-Golgi marker GM130 in hTERT-RPE cells (Figure 5A).

The AKAP450 signal was dramatically reduced in cells transfected with CK1δ siRNA (Figure 5B). PF670462 also decreased AKAP450 signal intensity, particularly at the Golgi (Figure 5C). As with CEP290 and PCM1, Western blot analysis indicated that CK1δ with previous reports (Westlake et al., 2007; Knodler et al., 2010). CK1δ siRNA markedly disrupted this pattern, as the GFP signal often was diffuse or undetectable. The diminished GFP staining may result from dispersion of GFP-Rab11a due to impaired membrane

FIGURE 1: CK1δ mediates primary ciliogenesis in hTERT-RPE cells. (A) Representative confocal micrographs of hTERT-RPE cells treated with siRNA reagents targeting luciferase (negative control), CK1δ, or CK1ε. Acetylated tubulin antibody (red) highlights the cilium, γ-tubulin (green) is a centrosomal marker, and DAPI (blue) stains the nucleus. Overlap of acetylated tubulin and γ-tubulin signal is yellow. Bars, 10 μm. (B) Quantitative analysis of data in A. Bar graph and error bars represent mean ± SD from three independent experiments. **p = 0.007 (t test, compared with Luc siRNA). (C) Western blot analysis of CK1δ and CK1ε in hTERT-RPE whole-cell lysates 72 h after transfection with indicated siRNA reagents. Immunoblot of α-tubulin served as a loading control. (D) Dose-dependent effect of CK1ε/δ kinase inhibitor PF670462 on percentage of cells with primary cilium. Sample number N = 316, 117, 247, 78, and 217 cells examined in 0, 0.3, 1.0, 3.0, and 10 μM treatment groups, respectively. (E) Dose-dependent effect of PF670462 on ciliary length. Box represents middle 50% of values for ciliary length; line inside the box indicates median value; and whiskers show upper 25% and lower 25% of values. N = 99, 117, and 98 cells examined in 0, 0.3, and 1.0 μM treatment groups, respectively. One-way ANOVA, p < 0.0001; Tukey’s test, *p = 0.0177, ****p < 0.0001; NS = not significant. See also Supplemental Figure S1.
siRNA did not alter the AKAP450 protein content in whole-cell lysates (Figure 4E), implying that the decline in signal intensity was due to dispersion of AKAP450 in the cell. A similar decrease in signal intensity at the centrosome and Golgi was observed in MEF<sub>Csnk1d null</sub> cells (Figure 5D). These results demonstrate that the normal intracellular distribution of AKAP450 depends on the expression and catalytic activity of CK1δ.

**CK1δ inhibition disrupts cis-Golgi organization, IFT20 distribution, and Golgi-derived MT nucleation**

Because AKAP450 functions at the cis-Golgi to maintain the ribbon structure and location of the Golgi near the centrosome, we investigated the possibility that CK1δ inhibition would alter the architecture and position of the Golgi apparatus. In hTERT-RPE cells transfected with Luc siRNA, GM130 was observed in a highly polarized, ribbon structure around the centrosome (Figure 5E), a pattern that is critical for directional protein transport (Yadav et al., 2009). Strikingly, the polarized ribbon structure of GM130 was lost in hTERT-RPE cells transfected with CK1δ siRNA (Figure 5E). To further characterize this phenotype, we measured the Golgi diameter and distance between the centrosome and the center of a circle circumscribing the Golgi (Hurtado et al., 2011). CK1δ siRNA significantly increased the Golgi diameter and centrosome–Golgi distance (Figure 5, F and G). Similar changes in Golgi positioning and morphology were observed when hTERT-RPE cells were treated with PF670462 and in MEF<sub>Csnk1d null</sub> cells (Figure 5, H and I). Supporting the idea that these changes were independent of centrosomal CK1δ, expression of δCT-EGFP in MEF<sub>Ctl.</sub> cells did not alter the GM130 staining pattern (Supplemental Figure S5B).

IFT20 is primarily associated with the cis- and medial cisternae of the Golgi complex, where it functions in the delivery of ciliary membrane proteins from the Golgi complex to the cilium (Follit et al., 2006). Knockdown of CK1δ in hTERT-RPE cells stably expressing GFP-tagged IFT20 disrupted the intracellular distribution of IFT20 (Figure 6A), consistent with perturbation of the GM130 staining pattern (Figure 6A). Similar results were obtained when mIMCD3 cells expressing IFT20-GFP were treated with PF670462 (Figure 6B). In addition, the kinase inhibitor decreased the accumulation of polycystin-2 at the centrosome of...
mIMCD3 cells (Figure 6, C and D), suggesting a defect in IFT20-dependent transport from the Golgi.

The ribbon structure and positioning of the Golgi are highly dependent on MTs (Thyberg and Moskalewski, 1999). Whereas the centrosome is the major site for MT nucleation, Golgi-derived MTs are required for normal Golgi morphology and polarized vesicle transport (Efimov et al., 2007). Golgi MT nucleation is mediated by AKAP450 (Rivero et al., 2009). Therefore we explored the idea that dispersion of AKAP450 and disruption of Golgi structure and positioning were associated with a defect in MT nucleation at the Golgi. Nocodazole treatment followed by washout enabled us to visualize MT asters arising from Golgi fragments stained with GM130 antibody (Figure 7). In contrast to the hTERT-RPE cells transfected with Luc or CK1ε siRNA, knockdown of CK1δ expression inhibited MT formation at the Golgi (Figure 7). Centrosomal MT nucleation appeared to be only partially inhibited in CK1δ-depleted cells. Similar results were obtained with hTERT-RPE cells pretreated with PF670462 (Supplemental Figure S6). Our results indicate that CK1δ is required for normal AKAP450 and IFT20 localization and MT nucleation at the Golgi.

AKAP450/159-463 blocked Golgi MT growth after nocodazole washout and decreased ciliary length. These results were consistent with previous work (Hurtado et al., 2011), reinforcing the view that AKAP450-GM130 interaction enables MT nucleation at the Golgi and consequently ciliogenesis. AKAP450/3643-3908 blocked neither MT nucleation nor cilogenesis, implying that centrosomal AKAP450 is not required for Golgi-derived MT regrowth or ciliogenesis. Finally, AKAP450/3315-3496 inhibited MT nucleation at the Golgi and sharply reduced ciliary length. These data suggest that direct interaction between CK1δ and AKAP450 is necessary for MT nucleation at the Golgi and ciliogenesis.

**DISCUSSION**

In the present study we demonstrated that CK1δ contributes to ciliogenesis in several models, including cell lines, primary MEF cells, and embryonic tissue from mouse. We originally hypothesized that centrosomal CK1δ would have a role in ciliogenesis because its centrosomal location is critical for Wnt signaling (Greer and Rubin, 2011). Our present findings are consistent with this idea, as a CK1δ construct lacking the CLS was unable to rescue ciliogenesis in MEF\(^{\text{CK1δ null}}\) cells, and another
on CK1δ interaction with AKAP450, which is associated with Golgi-derived MT nucleation and Golgi organization. Disruption of CK1δ expression results in a pattern of protein mislocalization that likely is due to defective trafficking from the Golgi and perhaps elsewhere. We believe that CK1δ functions to coordinate the positioning and activity of multiple ciliary effectors, such as Rab11a and Rab8a, CEP290, PCM1, and IFT20, to mediate transport of polycystin-2 and other membrane cargo from the Golgi to the basal body and nascent cilium (Supplemental Figure S8).

Previous work demonstrated that CK1δ binds to AKAP450 (Sillibourne et al., 2002), but the present study provides the first evidence that their interaction is functionally important. Suppression of CK1δ expression in hTERT-RPE and MEF cells mimicked the effects of AKAP450 knockdown on Golgi organization and Golgi-derived MT nucleation (Rivero et al., 2009), suggesting that CK1δ and AKAP450 are key components of a common mechanism. Knockdown of CK1δ and inhibition of its kinase activity each resulted in dispersion of AKAP450 from its usual polarized perinuclear distribution. This is most likely caused by defects in Golgi positioning, as demonstrated by scattering of the cis-Golgi marker GM130. Additional experiments indicated that association of AKAP450 and GM130 was not affected by CK1δ siRNA (unpublished data). CK1δ siRNA also caused loss of AKAP450 from the centrosome, an effect that was not evident after treatment with the kinase inhibitor PF670462. Although detailed understanding of CK1δ activity in regulating MT nucleation and related Golgi organization requires further investigation, the ability of an AKAP450 fragment containing the CK1δ-binding site to disrupt Golgi-derived MT nucleation, GM130 and IFT20 distribution, and ciliogenesis indicates that the interaction between CK1δ and AKAP450 is important for these processes.

Typically, we only observed partial inhibition of ciliogenesis after blockade of CK1δ expression or kinase activity. This might be due to incomplete suppression of CK1δ expression or catalytic activity or to the presence of redundant mechanisms. CK1α1 has been detected in centrosomal preparations but not linked to cilia formation (Jakobsen et al., 2011). CK1γ was identified as a mediator of Rab11-dependent polarized vesicle trafficking in Drosophila, although there was no indication that it has a role in ciliogenesis (Gault et al., 2012). CK1e activates minus end–directed transport of pigment granules along MTs in Xenopus melanophores (Ikeda et al., 2011). However, despite this function in transport, our results...
FIGURE 5: Intracellular distribution of AKAP450 and GM130 is regulated by CK1δ. (A) AKAP450 distribution in hTERT-RPE cells cultured with normal growth medium. GM130 and γ-tubulin were markers for cis-Golgi and centrosome, respectively. Arrows indicate the centrosome; bars, 5 μm (A–D). (B) AKAP450 distribution in hTERT-RPE cells treated with luciferase or CK1δ siRNA. Cells were maintained in serum-free medium and fixed 72 h after siRNA transfection. (C) AKAP450 distribution in hTERT-RPE cells treated with PF670462 or DMSO. Cells were maintained in serum-free medium with the indicated reagents for 48 h. (D) AKAP450 distribution in MEFCtl. and MEFCK1δ null cells cultured in growth medium and stained as indicated. (E) GM130 localization in hTERT-RPE cells treated with luciferase or CK1δ siRNA. Cells were maintained in serum-free medium and fixed 72 h after siRNA transfection. Arrows indicate the centrosome; bars, 5 μm (E, H, I). (F) Golgi diameter in cells transfected with CK1δ or luciferase siRNA in presence or absence of serum. N = 123, 110, 111, and 155 cells in treatment groups from left to right. One-way ANOVA, p < 0.0001; Tukey’s test, ****p < 0.0001, NS = not significant. (G) Centrosome–Golgi distance in cells treated as described in F. N as in F. Data are presented as mean ± SEM. One-way ANOVA; p < 0.0001; Tukey’s test, ****p < 0.0001, NS = not significant. (H) GM130 distribution in hTERT-RPE cells after PF670462 or dimethyl sulfoxide treatment. Cells were maintained in serum-free medium with the indicated reagents for 48 h. (I) GM130 distribution in MEFCtl. and MEFCK1δ null cells in growth medium and stained as indicated.
documented a sharp contrast between the effects of CK1δ and CK1ε knockout, clearly indicating that CK1ε did not facilitate ciliogenesis in our model systems. This dichotomy of function was consistent with observations previously made regarding the specific role of CK1δ in Wnt3a-dependent neurite outgrowth (Greer and Rubin, 2011).

Numerous studies implicate CK1δ in various forms of polarized transport. The CK1δ orthologue in yeast, Hrr25p, supports the directionality of endoplasmic reticulum–Golgi traffic by phosphorylating the Sec23p/Sec24p coat complex (Lord et al., 2011). Snapin, another CK1δ substrate, is a soluble N-ethylmaleimide–sensitive factor attachment protein receptor–associated protein that is believed to participate in exocytosis of cellular transport vesicles (Wolff et al., 2006). CK1δ was reported to mediate repositioning of the centrosome in T-cells during formation of the immune synapse (Zyss et al., 2011), a process that resembles ciliogenesis (Finetti et al., 2009) and requires Rab11 (Gorska et al., 2009). The mechanism involved CK1δ phosphorylation of end-binding protein 1, a plus end MT–associated protein that also participates in ciliogenesis (Schroder et al., 2007, 2011). Of interest, another CK1δ substrate, Dishevelled (McKay et al., 2001), is essential for apical positioning of the basal body in multiciliated Xenopus embryo epidermal cells (Park et al., 2008), although there is no evidence that phosphorylation by CK1δ is involved. Future study of CK1δ activity is likely to provide insights into ciliogenesis and other processes that rely on polarized trafficking such as cell migration and cell division.

**MATERIALS AND METHODS**

**Cell culture**

hTERT-RPE cells, hTERT-RPE cells stably expressing GFP-Rab8a (Westlake et al., 2011) or GFP-Rab11a, mIMCD3 cells, mIMCD3 cells stably expressing IFT20-GFP (Follit et al., 2006), and MEF cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin in a 5% CO2, humidified, 37°C cell culture incubator. GFP-Rab11a was stably expressed in RPE FRT cell lines using the GLAP1 FRT vector as previously described (Sang et al., 2011). Similarly, IFT20-GFP was stably expressed in RPE FRT cell lines using the GLAP7 FRT vector.

**Isolation of MEF cells from mouse embryo**

Mice carrying a Csnk1d floxed allele (B6.129S4-Csnk1dtm1Drw/J, stock number...
δ promotes ciliogenesis

Isolation of RPE layer and retinal tissue from mouse embryos for immunostaining

Eyes were removed from killed E18.5 embryos and incubated for 20 min in 1× phosphate-buffered saline (PBS) at 4°C to facilitate dissection of the RPE layer from the remaining retinal tissue using a Zeiss Stereo scope (Stemi 2000-c; Zeiss, Jena, Germany). Both specimens were fixed in 4% paraformaldehyde for 30 min at room temperature. After washing with 1× PBS, RPE and retinal tissues were immunostained with the indicated reagents using the standard protocol (see later description), and flat mounts of stained tissues were imaged by confocal microscopy.

Ciliogenesis assays

To induce primary ciliogenesis, hTERT-RPE cells that had been grown on coverslips to 90% confluency were serum starved for 48 h and processed for immunostaining. For siRNA experiments, growth medium was replaced with serum-free DMEM 24 h after transfection, and cells were maintained for 48 h in this medium before processing for confocal microscopy. To examine the effect of PF670462 on ciliogenesis, hTERT-RPE and mIMCD3 cells were cultured in serum-free DMEM containing different concentrations of the kinase inhibitor for 48 h. MEF cells were not subjected to serum starvation in ciliogenesis assays. When experiments involved the use of DNA constructs, cells were processed for Western blotting or immunostaining 48 h after transfection.

Chemicals

See Supplemental Table S1.

siRNA reagents and transfection

For CK1δ knockdown, four different siRNA reagents were used independently. For CK1ε knockdown, two different siRNA reagents were used independently. A luciferase siRNA was synthesized by Dharmacon (Lafayette, CO) and used as negative control. See Supplemental Table S1 for sequence information. siRNA transfection experiments in hTERT-RPE cells were performed with the Amaxa system (Amaxa, Cologne, Germany) according to the manufacturer's protocol, using 200 pmol of siRNA/10^6 cells, with Nucleofector V (VACA-0003), program X-001. The effects of siRNA treatment were analyzed 48–72 h after transfection.

Recombinant DNA and transfection

The pcDNA3.3 6xMyc-mouse CK1δ WT and ΔCT (lacking C-terminal domain), δCT-EGFP, and εCT-EGFP were previously described (Greer et al., 2009). Embryonic day 12.5 (E12.5) embryos were obtained from the Jackson Laboratory (Bar Harbor, ME). Embryonic day 12.5 (E12.5) embryos homozygous for the floxed allele were harvested, and heads and all internal organs were removed. The remaining tissues (trunk and limbs) were digested with 0.25% trypsin for 30 min and subsequently homogenized by titration. Digested tissues were transferred into a flask containing warm DMEM supplemented with 10% FBS, and medium was replaced the next day. Once confluent, the primary MEF cells were collected and stored at −80°C for short-term use or in a liquid nitrogen tank for long-term applications. To generate Csnk1d-null or control MEFs, cells were infected with adenovirus encoding Cre recombinase (Ad-Cre) or GFP (Ad-GFP), respectively.
FIGURE 8: CK1δ-binding AKAP450 fragment inhibited IFT20 Golgi distribution, Golgi-derived MT nucleation and ciliogenesis. (A) Schematic diagram of GFP-labeled AKAP450 fragments. (B) Western blot analysis of AKAP450 fragments and EGFP expressed in MEF\textsuperscript{Ctl.} cells. HSP70 was a loading control. (C) GM130 and IFT20 localization in MEF\textsuperscript{Ctl.} cells expressing AKAP450 fragments or EGFP. Bars, 5 μm. (D) Quantitative analysis of GM130 and IFT20 colocalization illustrated in C. N = 11, 18, 15, and 15 for treatment groups from left to right. One-way ANOVA, p < 0.001; Tukey’s test, *p < 0.05, ***p < 0.001, NS = not significant. (E) Golgi-derived MT nucleation in MEF\textsuperscript{Ctl.} cells transfected with GFP-labeled AKAP450 fragments. At 48 h after DNA transfection, MEF\textsuperscript{Ctl.} cells were incubated with nocodazole (10 μM) for 2 h and then washed and processed as described in the legend to Figure 7. Representative micrographs show cells fixed after 5 min of recovery. Bars, 5 μm. (F) Ciliary length in MEF\textsuperscript{Ctl.} cells transfected with GFP-labeled AKAP450 fragments. At 48 h after DNA transfection, cells were fixed and processed as described in Materials and Methods. N = 49, 50, 52, and 52 cells for treatment groups from left to right. One-way ANOVA, p < 0.0001; Tukey’s test, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, NS = not significant. See also Supplemental Figure S7.
and Rubin, 2011). CK1\(\delta\) K38A, a kinase-inactive form, was generated by site-directed mutagenesis according to manufacturer's protocol (Quik-Change II; Agilent Technologies, Santa Clara, CA). IFT20-GFP was PCR amplified from pEGFP-N1-IFT20 (JAF 2.13; Follit et al., 2006), cloned into pENTR/D-TOPO vector, and subsequently recloned into GLAP7 vector using LR clonase reaction in the Gateway cloning system (Invitrogen, Carlsbad, CA). AKAP450/159-463-GFP (numbering based on National Center for Biotechnology Information [NCBI] reference sequence NP_671714.1) and GFP-AKAP450/3643-3908 (numbering based on GenBank, CAB40713.1) constructs were kind gifts from Irina Kaverina (Vanderbilt University, Nashville, TN) and Sean Munro (MRC Laboratory of Molecular Biology, Cambridge, United Kingdom), respectively. To generate AKAP450/3315-3496-GFP (numbering based on NCBI reference sequence NP_671714.1), the corresponding AKAP450 fragment was amplified from hTERT-RPE cDNA by PCR. Primers contained HindIII and BamHI sites at the 5' and 3' ends, respectively; PCR product was digested with these enzymes and ligated into pEGFP-N1 that was similarly digested. Sequences of new constructs were verified by analysis in the DNA Sequencing MiniCore Facility at the National Cancer Institute. (Bethesda, MD). For DNA transfection in MEF cells, Lipofectamine LTX with Plus reagent (15338030; Invitrogen) was used according to manufacturer’s protocol.

**Antibodies used for Western blotting and immunostaining**

See Supplemental Table S1.

**Immunoblotting**

Eighty to 90% confluent monolayers that had been seeded in 6- or 12-well cell culture plates were rinsed twice with PBS, lysed, and processed for SDS–PAGE and Western blot analysis as previously described (Greer and Rubin, 2011). For immunoblot analysis to verify siRNA knockdown of endogenous proteins, hTERT-RPE cells that had been transfected with siRNA reagents were seeded in 6- or 12-well cell culture plates and harvested 48–72 h after transfection.

**Immunofluorescence analysis**

To visualize cilia, cells cultured on 12-mm-diameter glass coverslips (Fisher, Pittsburgh, PA) were first washed once with PBS and then with PHEM (60 mM Na–1,4-piperazinediethanesulfonic acid, 25 mM Na–4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 10 mM Na–ethylene glycol tetraacetic acid, and 2 mM MgCl\(_2\), pH 6.9), followed by treatment with PHEM containing 0.19 M NaCl, 1% saponin, 10 \(\mu\)M Taxol, and 0.1% dimethyl sulfoxide for 5 min at room temperature to permeabilize cells and stabilize tubulin. Cultures were then immersed in cold 100% MeOH at \(-30^\circ\)C for 10 min, rehydrated by rinsing three times with PBS, and treated with blocking solution for 30 min at \(37^\circ\)C. As blocking solution, 6% BSA in PBS (for secondary antibody/antibodies produced in goat) or donkey serum (for secondary antibody/antibodies produced in donkey) was used. Primary antibody/antibodies was/were added with blocking solution, and samples were incubated for 1 h at \(37^\circ\)C or overnight at \(4^\circ\)C. After washing three times with PBS, samples were incubated with secondary antibody reagent(s) and 4',6-diamidino-2-phenylindole (DAPI) with blocking solution for 45 min at room temperature. After three washes PBS, coverslips were mounted on glass slides (VWR Scientific, Radnor, PA) using ProLong Gold Antifade reagent (P36930; Invitrogen). To perform immunostaining in GFP-expressing cells, formaldehyde/paraformaldehyde fixation was used instead of MeOH to preserve GFP signal (Figures 2, F–H, 3, 6, A–C, and 8F and Supplemental Figures S3, S5, and S7). After washing with PBS and PHEM, cells were fixed with freshly prepared 3.7% formaldehyde and 1% Triton X-100 in PHEM supplemented with Taxol (10 \(\mu\)M) for 10 min at room temperature. Cells were washed and blocked, followed by the same procedure as described after MeOH fixation. For experiments presented in Figures 4, A–C, and 5, A–E and H–I, and Supplemental Figures S1A and S4, cells were fixed with cold 100% MeOH, followed by blocking and antibody treatment. For Supplemental Figure S1B, cells were fixed with 3.7% formaldehyde at room temperature for 15 min and treated with 0.1% Triton X-100 for 10 min, followed by blocking and antibody treatment. In experiments presented in Figure 8C, cells were fixed with 4% paraformaldehyde at room temperature for 15 min and treated with 0.1% Triton X-100 for 10 min, followed by blocking and antibody treatment.

**MT regrowth assays**

Cells grown on coverslips were treated with nocodazole (10 \(\mu\)M) in serum-containing DMEM at \(37^\circ\)C for 2 h; then nocodazole was washed out three times with cold, serum-free DMEM. After warm (room temperature) medium was added, cells were returned to \(37^\circ\)/\(\text{CO}_2\) incubator and harvested at different times (1, 3, 5, 7 min) by applying either cold 100% MeOH (for Figure 7 and Supplemental Figure S6) or cold PHEM (containing 3.7% formaldehyde, 1% Triton X-100, and 10 \(\mu\)M Taxol for Figure 8E) and maintenance at \(-20^\circ\)C for 10 min or on ice for 10 min, respectively. Cells were washed with PBS, blocked, and incubated with primary antibodies (tyrosinated tubulin, γ-tubulin, GM130), followed by incubation with secondary antibodies (and DAPI when needed).

**Cell imaging, measurement of ciliary length, and analysis of Golgi morphology**

Fluorescence images were collected with a laser-scanning confocal microscope (S10 LSCM) and a 63× objective (Carl Zeiss, Jena, Germany). Zeiss LSM image browser, version 4.0.0.157, was used for image processing, and composite figures were prepared with PhotoShop CS3, version 10.0.1 (Adobe Systems, San Jose, CA). Images of cilia were viewed with ImageJ, version 1.47k (http://imagej.nih.gov/ij), and ciliary length was measured using its line tool. Depending on the form of cilia, straight, segmented, or free-hand line was chosen. Golgi apparatus diameter and centrosome–Golgi distance were measured as previously reported (Hurtado et al., 2011), using ImageJ software. Colocalization was examined using Colocalization Finder in ImageJ (http://rsbweb.nih.gov/ij/plugins/colocalization-finder.html).

**Statistical analysis**

The significance of differences in data was determined with Student's \(t\) test or one-way analysis of variance (ANOVA) followed by Tukey’s test or Fisher’s exact test, depending on experiments (see figure legends). The differences were considered to be significant for \(p < 0.05\).

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