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Plk1-dependent and -independent roles of an ODF2 splice variant, hCenexin1, at the centrosome of somatic cells

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Abstract

Outer Dense Fiber 2 (ODF2) was initially identified as a major component of the sperm tail cytoskeleton, and was later suggested to be localized to somatic centrosomes and required for the formation of primary cilia. Here, we showed that a splice variant of hODF2 called hCenexin1, but not hODF2 itself, efficiently localizes to somatic centrosomes via an isoform-specific C-terminal extension and recruits Plk1 through a Cdc2-dependent phospho-S796 motif within the extension. This interaction and Plk1 activity were important for proper recruitment of pericentrin and γ-tubulin, and, ultimately, for formation of normal bipolar spindles. Earlier in the cell cycle, hCenexin1, but again not hODF2, also contributed to centrosomal recruitment of ninein and primary cilia formation independent of Plk1 interaction. These findings provide a unique example of how a splice-generated C-terminal extension of a sperm tail-associating protein mediates unanticipated centrosomal events at distinct stages of the somatic cell cycle.

Keywords

hCenexin1; polo-like kinase 1; centrosome; mitosis; ciliogenesis

Introduction

ODF2 belongs to a conserved family of proteins (610 – 659 residues long for ODF2 homologs in human, mouse, rat, and chicken) that was initially identified as a major component of the sperm tail cytoskeleton. Later, ODF2 was proposed to be a scaffold protein at the centrosomes in non-sperm cells, largely based on the observation that an antibody that specifically

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recognizes an *ODF2*-encoded protein decorated the distal/subdistal appendages of the mother centriole early in the cell cycle and also the daughter centriole at the later stages after centrosome duplication (Ishikawa et al., 2005; Nakagawa et al., 2001). Alternatively, these observations hint that an anti-ODF2-immunoreactive protein(s) other than ODF2 may localize to the somatic centrosomes and play a role in proper centrosome function. In support of the latter view, a splicing variant of hODF2 called hCenexin1 (calculated size of a 93-kDa protein) that possesses a unique C-terminal extension has been isolated from HeLa cells (Soung et al., 2006). How hCenexin1 differentially localizes to the somatic centrosomes and whether it plays a role distinct from that of ODF2 during cell proliferation are not known.

The centrosome is the main microtubule-organizing center (MTOC) of the cell. It is surrounded with pericentriolar material (PCM) that functions as a scaffold to recruit various proteins critical for microtubule nucleation (Doxsey et al., 2005). Among the proteins recruited to the centrosome is polo-like kinase 1 (Plk1), a conserved Ser/Thr protein kinase critical for proper centrosome maturation (Barr et al., 2004). Studies show that the polo-box domain (PBD) present in the C-terminal non-catalytic region of Plk1 plays an essential role in targeting its catalytic activity to specific subcellular locations (Lee et al., 1998). The PBD forms a phosphoepitope-binding module and specifically interacts with a phosphorylated motif within its binding target (Elia et al., 2003a), suggesting that phospho-dependent PBD interaction is critical for proper Plk1 localization. However, components critical for Plk1 recruitment to the centrosomes and mechanisms underlying this event are largely elusive at present.

Aside from the role of centrosomes in microtubule nucleation, the mother centriole in quiescent cells plays a critical role in primary cilium formation by forming the basal body. Primary cilia (9+0 axoneme) formed on the surface of most mammalian cells are crucial sensory organelles for various biological processes and defects in ciliary function are associated with a wide range of pathologies, including developmental disorders and cancer (Fliegauf et al., 2007). Deletion of the *ODF2* locus in a mouse embryonic carcinoma cell line disrupts ninein localization and impairs primary cilia formation (Ishikawa et al., 2005), raising the possibility that this locus-encoded protein(s) is required for proper ciliogenesis.

In this study, we demonstrated that Cenexin1, a 93-kDa ODF2-splicing variant, is abundantly expressed in various somatic cells and tissues and associates with centrosomes, whereas ODF2 was richly expressed in adult testis and appeared to be largely associated with sperm tails. Unlike ODF2, Cenexin1 possesses a unique C-terminal extension that plays multiple roles at distinct stages of the cell cycle. Early in the cell cycle, hCenexin1 is required for normal ninein recruitment and primary cilia formation in a Plk1-independent manner. During late G2 and M phases, Cdc2 generates a PBD-docking site within the C-terminal extension of hCenexin1, thereby allowing proper Plk1 recruitment to the centrosomes and normal mitotic progression. We propose that the alternative splicing-generated C-terminal extension of hCenexin1 plays a critical role in targeting the protein to the mother centriole, thus promoting both Plk1-dependent mitotic functions and Plk1-independent ninein recruitment and ciliogenesis at distinct stages of the somatic cell cycle.

Results

Plk1 binds to the C-terminal region of hCenexin1 during the late stages of the cell cycle

Since hCenexin1 and hODF2 are splicing variants expressed from the same genomic locus (Soung et al., 2006), we closely investigated their expression patterns in various cultured cells and mouse tissues (Fig. S1A). Examination of various human cultured cells revealed that hCenexin1 (which is sensitive to both si-781 and si-2066) was the major variant in all the cells examined, whereas hODF2 (which is sensitive to si-781 but not to si-2066) was detectable at a low level (Fig. S1B-C). Treatment of mouse cultured lines with si-781 also established

mCenexin1 as the major variant that distinctly localizes to the mother centrioles (Fig. S1D-E). The level of mODF2 was beyond the detection limit (Fig. S1D) (si-2066 was not used because it fails to target the mCenexin1 mRNA). Consistent with these observations, various mouse somatic tissues expressed mCenexin1 as the major variant (Fig. S2A). However, examination of tissues obtained from mouse testis revealed that mODF2 was expressed at a very high level in adult testis, but not in testis before puberty (P7 and P14) (Fig. S2A). Immunostaining of seminiferous tubules from adult mice showed that mODF2 was heavily present along the length of sperm tails, but was not detectably associated with the centrioles (Fig. S2B). These observations suggest that mODF2 expression is largely confined to adult testis and that mODF2 is the major variant associated with sperm tails but not significantly with centrioles.

A widespread expression of hCenexin1, but not hODF2, in various somatic cells and tissues suggest that hCenexin1 is likely important for proper centrosome functions. Consistent with this view, EGFP-fused hCenexin1 strongly localized to mother centrioles and weakly to daughter centrioles, whereas hODF2 diffusely localized to nucleoplasm and cytosol with occasional centrosome-localized signals (Fig. 1A). Interestingly, affinity-purification of a ZZ (IgG-binding motif of protein A)-tagged hCenexin1, but not hODF2, co-purified Plk1 (Fig. 1B), suggesting that hCenexin1 specifically interacts with Plk1 at the centrosomes.

Since the PBD plays a critical role for subcellular localization of Plk1, we next examined whether the PBD is sufficient to interact with hCenexin1. Precipitation of GST-PBD co-precipitated hCenexin1, but not hODF2 or hCenexin1(1–613) lacking the unique C-terminal extension (Fig. 1C). Since PBD binds to a phosphoepitope, these findings suggest that the C-terminal extension of hCenexin1 contains a phosphoepitope(s) critical for PBD binding. In support of this argument, PBD, but not the corresponding PBD(H538A K540M) phosphate pincer mutant {PBD(AM)}, interacted with endogenous hCenexin1 under the conditions where it interacted with phospho-Cdc25C (p-Cdc25C) (Fig. 1D), a mitotic phosphatase reported to bind to the PBD (Elia et al., 2003b). Analyses of the samples released from a G1/S block showed that the PBD interacts with hCenexin1 concurrently with the PBD-Cdc25C interaction at late G2 and early M phase of the cell cycle (Fig. 1E). Furthermore, a prolonged separation of mitotic lysates revealed a λ phosphatase-sensitive, slow-migrating, hCenexin1 form (Fig. 1F) and treatment of mitotic lysates with λ phosphatase greatly diminished the PBD-hCenexin1 interaction (Fig. S3A). These results suggest that hCenexin1 is phosphorylated during the late stages of the cell cycle and that this event is critical for PBD binding.

Phosphorylation of hCenexin1 at S796 is critical for the hCenexin1-Plk1 interaction

To determine whether the C-terminal region of hCenexin1 is sufficient for the PBD binding, HeLa cells were infected with adenoviruses expressing the full-length or various hCenexin1 truncations (T1 – T7) or transfected with the T8 truncation (we failed to generate adenovirus expressing T8). Cells were then treated with nocodazole to enrich the mitotic population and subjected to PBD pull-downs. Bead-bound GST-PBD precipitated the T5, T6, or T7 truncations as efficiently as the full-length hCenexin1, while it precipitated T8 somewhat less efficiently (partly due to the aggregation of the transfected T8 form) (Fig. 2A). By contrast, GST-PBD bound to T1, T2, T3, or T4 only marginally (Fig. 2A). PBD, but not the PBD(AM) mutant, bound to T8 (Fig. 2B), suggesting that T8 bears a phosphoepitope that is sufficient to bind to PBD. In line with this argument, mass spectrometry analyses for the mitotic EGFP-T7 immunoprecipitates identified a phospho-S796 (p-S796)-containing peptide that resides within the T8 sequence (Fig. 2C). In addition, a mutation of S796 to A was sufficient to eliminate the mitosis-specific, slow-migrating, hCenexin1 form (Fig. S3B). Thus, hCenexin1 is phosphorylated at S796 *in vivo*.

Next, we tested whether the S796 residue is critical for Plk1 interaction by performing PBD pull-downs with HeLa cell lysates expressing various hCenexin1 forms. The PBD bound to

exogenously expressed hCenexin1 but not the S796A, S796E, and S796D mutants, while it bound to endogenous hCenexin1 present in all of the respective samples (Fig. 2D). Inability of the phospho-mimicking S796E or S796D mutants to interact with PBD suggests that the phosphorylated S796 residue is absolutely required for this event. As expected if the p-S796-containing motif were sufficient for the PBD interaction, bead-bound p-S796 peptide, but not the corresponding non-phosphopeptide, precipitated Plk1 (Fig. 2E). Moreover, provision of the p-S796 peptide, but not the non-phospho-form, interfered with the PBD-hCenexin1 interaction (Fig. 2F) and greatly diminished the level of co-precipitating Plk1 (Fig. 2G). These observations suggest that the p-S796-dependent hCenexin1-Plk1 PBD interaction is the major interaction between these two proteins.

Cdc2-dependent phosphorylation of hCenexin1 at S796 is sufficient to interact with Plk1

Plk1 is targeted to specific subcellular locations through binding of the PBD to a protein that is frequently phosphorylated by Pro-directed kinases (Lowery et al., 2005). Consistently, the S796 motif falls into a consensus phosphorylation sequence for Cdc2 (S/T-P-X-R/K; X is any amino acid residue). To investigate whether Cdc2 or other Pro-directed kinases such as Mps1 and MAPK could phosphorylate S796 and generate a PBD-docking site, we first examined whether treatment of mitotic HeLa cells with previously characterized kinase inhibitors influences the level of PBD binding. Acute inhibition of Cdc2 activity by BMI-1026 (Seong et al., 2003) for 30 min drastically diminished the PBD-hCenexin1 interaction, whereas inhibition of Mps1 by SB600125 (Schmidt et al., 2005) or the ERK1/2 upstream kinase MEK1/2 by U0126 (Favata et al., 1998) did not (Fig. 3A).

However, since inhibition of Cdc2 activity induces an exit from mitosis, the reduced level of the PBD-hCenexin1 interaction could also be a direct consequence of hCenexin1 dephosphorylation following mitotic exit. To eliminate this possibility, we reexamined the effect of Cdc2 inhibition on the PBD-hCenexin1 interaction in late G2. To this end, cells expressing EGFP-T8 were synchronously arrested with etoposide, a topoisomerase II inhibitor that induces DNA-damage-dependent G2 arrest (Montecucco and Biamonti, 2007), and then the effect of Cdc2 inhibition on the level of S796 phosphorylation was monitored by immunoblotting with an anti-p-S796 antibody (Fig. S4). Although the level of p-S796 hCenexin1 in G2 phase was several-fold lower than that in M phase, inhibition of Cdc2 by BMI-1026 further diminished the overall level of the p-S796 epitope in the total lysates (Fig. 3B, input). Likewise, the BMI-1026 treatment greatly impaired the PBD-EGFP-T8 interaction under the conditions that disrupt the PBD-p-Cdc25C interaction (Fig. 3B). The PBD(AM) mutant failed to interact with p-S796 hCenexin1 or p-Cdc25C (Fig. 3B). Thus, Cdc2 is likely responsible for the generation of the p-S796 epitope that is critical for the hCenexin1-PBD interaction. The low level of the hCenexin1-PBD interaction even after 2 h of BMI-1026 treatment could be due to an ineffective elimination of the p-S796 epitope under these conditions. Alternatively, Pro-directed kinase(s) other than Cdc2 may contribute to the generation of the p-S796 epitope. However, treatment of cells with small molecule compounds that inhibit various Pro-directed kinases such as Mps1, ERK1/2, and GSK-3 failed to significantly influence the level of the p-S796 epitope (Fig. S5).

Consistent with Fig. 3B, overexpression of Cdc2 and Cyclin B1 in cells arrested at the G1/S boundary to minimize the endogenous p-S796 level greatly enhanced the level of the p-S796 epitope (Fig. 3C). In a second experiment, purified Cdc2/Cyclin B1 complex was able to generate the p-S796 epitope on a C-terminal fragment of hCenexin1, which, in turn, precipitated Plk1, but not Plk2 or Plk3, from transfected HeLa cell lysates (Fig. 3D). Interestingly, examination of the p-S796 levels in G2-arrested cells revealed that depletion of Cyclin B1 did not significantly influence the level of S796 phosphorylation. However, depletion of Cyclin B2, which can functionally compensate for the downregulated Cyclin B1

(Bellanger et al., 2007), greatly diminished the level of the p-S796 epitope, while co-depletion of both Cyclin B1 and B2 further lowered the p-S796 level (Fig. S6). These observations suggest that B-type cyclin (e.g., Cyclin B2)-associated Cdc2 phosphorylates hCenexin1 at S796 and that this step is crucial for the generation of a docking site specific to the PBD of Plk1.

S796-dependent ectopic hCenexin1-Plk1 assemblies frequently serve as microtubule organizing centers (MTOCs)

Overexpression of EGFP-hCenexin1-sil or EGFP-hCenexin1(S796A)-sil bearing the sh-781-, sh-1235-insensitive silent mutations {for simplicity, hCenexin1 or hCeneixn1(S796A), respectively, hereafter} resulted in generation of multiple ectopic green fluorescent dots in the cytoplasm. To investigate the significance of the hCenexin1-Plk1 interaction, we examined whether hCenexin1 overexpression recruited Plk1 in a S796-dependent manner in cells silenced for endogenous hCenexin1 and hODF2 (sh-781). Since Plk1 is abundantly expressed in late G2 and early M phase of the cell cycle, cells were enriched in these stages by double thymidine block and release for 10 h (DT 10 h). Greater than 95% of the cells with ectopic hCenexin1 dots displayed colocalized Plk1 signals, whereas less than 2% of cells with ectopic hCenexin1(S796A) dots exhibited Plk1 colocalization (Fig. 4A–B). Further highlighting the importance of the mitotic Cdc2 activity in promoting the hCenexin1-Plk1 interaction, the efficiency of Plk1 recruitment to the ectopic hCenexin1 dots was much greater during early mitosis (Pro) than during S or G2 (Fig. 4C).

We then examined whether hCenexin1 phosphorylation at S796 is important for proper recruitment of Plk1 to the native centrosomes by quantifying the centrosomal Plk1 intensities in sh-781 cells expressing either EGFP-hCenexin1 or the EGFP-hCenexin1(S796A) mutant. Both EGFP-hCenexin1 and EGFP-hCenexin1(S796A) proficiently localized to the centrosomes (Fig. S7). In S/G2 and prophase cells, EGFP-hCenexin1-associating centrosomes efficiently recruited Plk1 to the centrosomes with a preference to mother centriole, whereas EGFP-hCenexin1(S796A)-associating centrosomes recruited Plk1 only weakly (Fig. 4D and Fig. S8A). However, in metaphase cells, Plk1 also localized to the centrosomes that associated with EGFP-hCenexin1(S796A), albeit less efficiently than the EGFP-hCenexin1-associating centrosomes (Fig. 4D). These observations suggest that, while the p-S796-dependent hCenexin1 function is vitally required for Plk1 recruitment to the late interphase centrosomes, it may not be the only mechanism for targeting Plk1 to the matured mitotic centrosomes. In contrast to these findings at the native centrosomes, ectopic hCenexin1 dots strictly required the p-S796 motif to recruit Plk1 at all stages of the cell cycle. Thus, we examined the ectopic hCenexin1 dots in detail to further understand the significance of the hCenexin1-Plk1 interaction in the recruitment of other centrosomal components.

Analyses of the ectopic hCenexin1 dots revealed that ~25% of the DT 10 h cells with the ectopic hCenexin1-Plk1 assemblies displayed γ-tubulin signals recruited to the assemblies (Fig. 4E-F and Fig. S8B). hCenexin1 dots that did not contain a detectable level of recruited Plk1 failed to display a detectable level of γ-tubulin signal (Fig. S8B). By contrast, ectopic hCenexin1 (S796A) dots were not able to significantly recruit γ-tubulin. Consistently, ~9% of the cells containing ectopic hCenexin1 dots exhibited multiple microtubule nucleation activities, whereas less than 2% of cells with ectopic hCenexin1(S796A) dots displayed this activity (Fig. 4G–H). These observations hint that formation of the hCenexin1-Plk1 assemblies could be an important step for the recruitment of γ-tubulin and subsequent microtubule nucleation.

Recruitment of pericentrin and γ-tubulin by ectopic hCenexin1-Plk1 assemblies

Since the hCenexin1-Plk1 assemblies were capable of recruiting γ-tubulin, we next examined whether these assemblies contain a centrosomal component(s) critical for the recruitment of

the γ-TuRC complex. Immunostaining analyses for various centrosomal proteins revealed that pericentrin, a major component of the PCM important for the recruitment of γ-TuRC and therefore microtubule nucleation (Dictenberg et al., 1998; Takahashi et al., 2002; Zimmerman et al., 2004), was recruited to the ectopic hCenexin1-Plk1 assemblies in 32% of late G2 and early mitotic cells with multiple hCenexin1 dots. Under the same conditions, only weak pericentrin signals co-localized with the Plk1-binding defective hCenexin1(S796A) dots (Fig. 5A–B and Fig. S8C). Like Plk1, pericentrin preferentially localized to mother centrioles (Fig. S8D–E). Two other less-characterized centrosomal proteins, Cep131 and CG-Nap, were also recruited to the assemblies, but in a manner independent of the hCenexin1-Plk1 interaction (Fig. 5A).

We then examined whether Plk1 is required for proper localization of pericentrin and γ-tubulin. Acute inhibition of Plk1 activity by BI2536 treatment impaired both pericentrin and γ-tubulin recruitment to the ectopic dots. Cells depleted of Plk1 by RNAi also exhibited greatly diminished levels of pericentrin and γ-tubulin signals at the ectopic dots, whereas a fraction of Plk1-positive cells (cells undepleted of Plk1) among the Plk1 RNAi cells retained these signals (Fig. 5C). Depletion of Plk1 did not alter the levels of hCenexin1, pericentrin, and γ-tubulin expression (data not shown). Analyses on the native centrosomes also showed that inhibition of Plk1 activity or depletion of Plk1 significantly diminished the levels of pericentrin and γ-tubulin at the mitotic centrosomes (Fig. 5D). Interphase centrosomes, however, did not require Plk1 activity for pericentrin and γ-tubulin recruitment (Fig. 5E). Collectively, these observations suggest that the initial formation of the hCenexin1-Plk1 complex at late G2 or early mitotic centrosomes plays an important role in proper recruitment of pericentrin that, in turn, leads to γ-tubulin recruitment and formation of MTOCs.

The S796-dependent hCenexin1-Plk1 interaction is critical for mitotic progression, but not for normal ninein recruitment

It has been shown that silencing of hCenexin1 and hODF2 by RNAi leads to a defect in normal mitotic progression (Soung et al., 2006). To investigate whether this defect is due to the loss of p-S796-dependent hCenexin1-Plk1 interaction, we generated HeLa cell lines expressing control vector or the indicated hCenexin1/hODF2 alleles bearing appropriate silent mutations, depleted of endogenous hCenexin1 and hODF2 by RNAi, and then examined (Fig. 6A–C). We observed that ~19% of the mitotic cells expressing control vector exhibited misaligned chromosomes with weakened bipolar spindles or unfocused spindle poles (Fig. 6B–C and Fig. S9A–B). Presumably as a result of a compromised spindle checkpoint after a pre-anaphase delay, a low but significant fraction of cells with lagging chromosomes and multipolar spindles was also observed. Expression of wild-type hCenexin1 rescued these defects, whereas expression of either hCenexin1(S796A), hODF2, or hCenexin1(1–613) did not (Fig. 6B–C). Close examination of the hCenexin1(S796A)-expressing cells by time-lapse microscopy revealed that these cells were significantly delayed during pre-anaphase progression (Fig. 6D and Fig. S9). These data strongly suggest that the p-S796-dependent hCenexin1-Plk1 interaction is required for proper bipolar spindle formation and mitotic progression.

Next, since deletion of the hCenexin1/hODF2 locus disrupts mother centriole appendages and eliminates two appendage-associated ninein dots (thus resulting in the 1+1 configuration, instead of the normal 3+1 configuration) (Ishikawa et al., 2005), we examined whether the S796-dependent hCenexin1-Plk1 interaction is important for normal recruitment of ninein to the appendages of mother centrioles. Expression of either hCenexin1 or hCenexin1(S796A) rescued the ninein recruitment defect associated with the depletion of hCenexin1 and hODF2, whereas expression of hODF2 or hCenexin1(1–613) did not (Fig. 6E). These results suggest that ninein recruitment to the mother centriole appendages is independent of the hCenexin1-Plk1 interaction. In addition, since hODF2 and hCenexin1(1–613) did not efficiently localize

to the centrosomes, failure of these constructs to recruit ninein to the mother centriole appendages could be attributable to their inability to localize to this site.

S796-independent hCenexin1 function is required for proper primary cilia formation

Previous reports suggest that ODF2 plays an important role in ciliogenesis (Ishikawa et al., 2005; Yoshimura et al., 2007). However, since Cenexin1, but not ODF2, is abundantly expressed in somatic cells and the localization patterns of Cenexin1 and ODF2 appear to be distinct, we investigated the requirement of these two proteins during primary cilium formation. Two days after serum starvation, hTERT-RPE cells silenced for control luciferase (sh-Luc) exhibited primary cilia in greater than 92% of the population with an average length of 4.7 μm (Fig. 6F). In contrast, cells silenced for both hCenexin1 and hODF2 by sh-781 exhibited much shorter (an average length of 0.9 μm) primary cilia in ~30% of the population. Surprisingly, sh-2066 cells depleted of hCenexin1, but not hODF2, displayed primary cilia in ~3% of the population with an average length of only 0.2 μm (Fig. 6F). The degrees of primary cilia formation in the control sh-Luc, sh-781, or sh-2066 cells closely correlated with the levels of localized hCenexin1 signals in the respective samples (Fig. 6F).

Next, we observed that expression of either hCenexin1 or hCenexin1(S796A) was sufficient to rescue the ciliogenesis defect in cells depleted of both hCenexin1 and hODF2, whereas expression of hODF2 or hCenexin1(1–613) rescued this defect only marginally (Fig. 6G–H). These observations suggest that, unlike the previous observations (Ishikawa et al., 2005; Yoshimura et al., 2007), hCenexin1, but not hODF2, is primarily required for proper ciliogenesis, and that hCenexin1 contributes to this event in a manner independent of the hCenexin1-Plk1 interaction. Notably, while hCenexin1, hCenexin1(S796A), and hCenexin1 (1–613) all localized to the centrosomes, hODF2 distinctly localized along the axoneme of cilia (Fig. 6G) as observed previously (Yoshimura et al., 2007), hinting that the role of hODF2 during primary cilia formation could be distinct from that of hCenexin1.

Discussion

Distinct expression and function of Cenexin1 and ODF2

ODF2 was originally identified as a major component of the sperm tail cytoskeleton that is thought to provide structural and mechanical support for long sperm tails (Brohmann et al., 1997; Hoyer-Fender et al., 1998). Subsequent studies suggested that ODF2 functions as a widespread centrosomal protein (Nakagawa et al., 2001) and plays a role during primary cilia biogenesis (Ishikawa et al., 2005; Yoshimura et al., 2007). However, the results provided here demonstrated that ODF2 is primarily expressed in testis after puberty and associates along the axoneme of sperm tails. In stark contrast, Cenexin1, a splicing variant of ODF2, is abundantly expressed in somatic cells and tissues with preferential localization to mother centrioles. These observations suggest that the anti-ODF2 antigen previously observed at the centrosomes of various somatic cells (Nakagawa et al., 2001) is likely Cenexin1.

Comparison of the primary amino acid sequences between hCenexin1 and hODF2 revealed that hCenexin1 bears a unique C-terminal extension expressed from exons 21 to 24 (Fig. S1A). Close examination of the localization patterns of various hCenexin1 truncations suggests that the C-terminal extension is important for efficient localization to mother centrioles (Fig. S7). Thus, the C-terminal extension provides Cenexin1 the ability to distinctly localize to mother centriole and the absence of this extension permits ODF2 to associate with sperm tail. Underlying the importance of the C-terminal extension in specifying the centriole localization, hCenexin1, but not hODF2, plays a critical role in various biochemical and cellular events such as phospho-dependent Plk1 recruitment and bipolar spindle formation and phospho-independent ninein recruitment and ciliogenesis. A search of the NCBI database revealed that

an uncharacterized locus (GenBank accession number, XP_001184343.1) in purple sea urchin, *S. Purpuratus*, encodes a hCenexin1 homolog (31% identity with the full-length hCenexin1 and 44% identity with the C-terminal extension) with a potential PBD-binding motif (S-S/T-P) analogous to the hCenexin1 S796 site, suggesting that the function of the C-terminal extension is likely conserved throughout evolution.

Plk1-dependent centrosome maturation pathway

Although Plk1 has been shown to play a critical role in centrosome maturation, it has been difficult to delineate the downstream events mediated by Plk1 due to the presence of many Plk1-binding proteins at the centrosomes. Our results suggest that the formation of the hCenexin1-Plk1 complex is an important step in proper recruitment of pericentrin and γ-tubulin to the centrosomes. Since pericentrin has been shown to play an important role in anchoring the γ-tubulin ring complex and thereby providing microtubule nucleation sites (Dictenberg et al., 1998; Takahashi et al., 2002; Zimmerman et al., 2004), we propose that pericentrin links the hCenexin1-Plk1 complex to the γ-tubulin ring complex. Consistent with this notion, depletion of hCenexin1 impaired the localization of Plk1 and pericentrin, and weakened the recruitment of γ-tubulin (Fig. S10A–B). Overexpression of pericentrin B/kendrin, the major variant in HeLa cells (Fig. S11A), did not significantly alter hCenexin1 and Plk1 localization, but greatly increased the degree of γ-tubulin recruitment (Fig. S11B–C). Conversely, silencing of pericentrin significantly diminished the level of γ-tubulin at centrosomes (Fig. S11D).

Notably, mutations in the *PCNT* locus, which cripple both of the pericentrin splicing variants (the full-length protein, pericentrin B/kendrin, and a C-terminally truncated variant, pericentrin A), have been shown to be associated with the development of microcephaly and primordial dwarfism as a result of disorganized mitotic spindles and chromosome missegregation (Griffith et al., 2008; Rauch et al., 2008). Thus, we hypothesize that proper regulation of pericentrin by the hCenexin1-Plk1 complex is crucial for normal mitotic progression and cellular proliferation. Intriguingly, Plk1 activity was required for proper phosphorylation of pericentrin *in vivo* (Jung-Eun Park and K. S. Lee, unpublished), although the importance of this phosphorylation event for pericentrin recruitment or function has not yet been determined. Nevertheless, additional components are likely required for efficient recruitment of pericentrin to the hCenexin1-Plk1 complex. Testing of various centrosomal components revealed that co-expression of Cep131 greatly augmented the level of pericentrin and γ-tubulin at the hCenexin1-Plk1 assemblies (Jung-Eun Park and K. S. Lee, unpublished), although underlying mechanisms on this event remain to be further investigated. It is also noteworthy that depletion of hCenexin1 resulted in delocalization of a mother centriole appendage protein, centriolin (Gromley et al., 2003) (Fig. S12), raising the possibility that hCenexin1 may have a much broader function in the organization of the mother centriole.

Physiological significance of the S796-dependent hCenexin1-Plk1 interaction in proper bipolar spindle formation

Plk1 association with the centrosome appears to be important for microtubule nucleation and mitotic spindle formation (Casenghi et al., 2003; de Cárcer et al., 2001). Yet, the mechanism underlying this event has been elusive. Our results provided here demonstrate that hCenexin1, but not hODF2, serves as a platform for the recruitment of Plk1 to the late interphase and early mitotic centrosomes via Cdc2-dependent hCenexin1 phosphorylation at S796. Highlighting the importance of this event, loss of S796-dependent hCenexin1-Plk1 interaction impaired Plk1 localization to the centrosomes and induced defects in proper bipolar spindle formation and chromosome segregation. In line with these observations, cells expressing a dominant-negative PBD, which induces mislocalization of endogenous Plk1, also exhibit a spindle checkpoint-dependent mitotic arrest with a defect in chromosome congression (Hanisch et al., 2006; Seong et al., 2002). The finding that the formation of the hCenexin1-Plk1 complex is important for

the recruitment of pericentrin and γ-tubulin to the centrosomes helps explain why the loss of the hCenexin1-Plk1 interaction ultimately leads to improper bipolar spindle formation and chromosome missegregation as observed in hCenexin1(S796A)-expressing cells. In contrast to these observations, however, ninein, which is critical for microtubule nucleation and anchoring at the interphase centrosomes (Delgehyr et al., 2005; Mogensen et al., 2000), was normally recruited to both the hCenexin1- and hCenexin1(S796A)-localized interphase centrosomes (Fig. 6D) and dissociated from the respective mitotic centrosomes (data not shown). These results suggest that the S796-dependent hCenexin1-Plk1 interaction facilitates proper mitotic progression independently of hCenexin1-dependent ninein recruitment to the interphase centrosomes.

The S796-independent hCenexin1 functions in ciliogenesis

A previous report showed that mouse embryonic carcinoma cells deleted of the mCenexin1/mODF2 locus fail to generate primary cilia, whereas provision of mODF2 into these cells restores primary cilia formation (Ishikawa et al., 2005). Subsequent studies demonstrated that the C-terminal 20 amino acid region uniquely found in hODF2 (shortened for Outer Dense Fiber of sperm tails 2 isoform 2; GenBank accession number, NP_702915; 1–638 residues) and hODF2 isoform 3 (shortened for Outer Dense Fiber of sperm tails 2 isoform 3; GenBank accession number, AAP83847; 1–657 residues) (see Fig. S1A) is critical for interacting with one of the Rab GTPase membrane trafficking regulators, Rab8a, and that this interaction is important for proper ciliogenesis (Yoshimura et al., 2007). Interestingly, our results demonstrated that depletion of hCenexin1 alone was sufficient to severely impair primary cilia formation (we failed to generate a hODF2-specific siRNA to examine the effect of hODF2 depletion). In addition, expression of the full-length hCenexin1, but not hODF2, efficiently induced primary cilia formation in cells depleted of both hCenexin1 and hODF2. These observations suggest that hCenexin1, but not hODF2, plays a major role in primary cilia formation, although we can not exclude a unique role of hODF2 in this process. In line with these observations, hCenexin1, but not hODF2, is abundantly expressed in somatic cells. Also, culturing the cells under starvation conditions that promote primary cilia formation failed to elevate the level of hODF2 expression (Fig. S13). However, it is noteworthy that hODF2 localizes along the primary cilia (Yoshimura et al., 2007), whereas hCenexin1 localization is confined to mother centrioles, hinting that Cenexin1 and ODF2 have different roles during ciliogenesis.

Our results demonstrated that the C-terminal extension of hCenexin1 is crucial for proper ciliogenesis. However, the S796-dependent hCenexin1-Plk1 interaction was not required for this process. Similarly, hCenexin1-dependent ninein recruitment to the mother centriole appendages required the C-terminal extension of hCenexin1, but not the hCenexin1-Plk1 interaction. Depletion of ninein has been shown to impair primary cilia formation (Graser et al., 2007). Thus, the C-terminal extension-dependent ninein recruitment could be important for Plk1-independent ciliogenesis early in the cell cycle rather than for Plk1-dependent microtubule function late in mitosis (Fig. 7). Consistent with this argument, Plk1 was not detectably expressed during the quiescent stage of the cell cycle (Fig. S14A). Furthermore, depletion or inhibition of Plk1 did not significantly influence the level of primary cilia formation (Fig. S14A–C). Since ciliogenesis occurs in nondividing cells arrested in G0 and G1, whereas the hCenexin1-Plk1 interaction takes place during the late stage of the cell cycle (G2 and M), we propose that the C-terminal extension of hCenexin1 functions as two distinct scaffolds in a temporally regulated manner – one as a phospho-independent scaffold to promote ninein recruitment and ciliogenesis and the other as a phospho-dependent scaffold to promote Plk1-dependent microtubule function (Fig. 7).

The centrosome associates with various cellular proteins and plays a critical role in diverse cellular processes including bipolar spindle formation and ciliogenesis. Loss of proper centrosome function leads to mitotic infidelity that results in chromosome missegregation and cell death. Our results showed that Cenexin1 localizes to the centrosomes through the function of its unique C-terminal extension, whereas ODF2 localizes to sperm tails and structurally-related axonemes of primary cilia. Cenexin1, but not ODF2, contributes to proper ninein recruitment, ciliogenesis, and microtubule nucleation by localizing to the mother centriole via its C-terminal extension. Thus, the alternative splicing-generated C-terminal extension of hCenexin1 dramatically alters the characteristic trait of a sperm tail/primary cilia-associating protein and allows hCenexin1 to carry out unexpected functions at different stages of the somatic cell cycle. In this regard, identification of additional proteins that specifically interact with the C-terminal extension, but not with ODF2, will likely be critical in better understanding how hCenexin1 can function as a multi-faceted mediator for both Plk1-dependent and –independent cellular processes at the somatic centrosomes.

Experimental procedures

Plasmid construction, cell culture, and virus generation

Detailed information on the construction of various plasmids, cell culture, and production of lentivirus and adenovirus are described in Supplemental Online Materials. All the siRNA target sequences used in this study are listed in Table S1.

GST-PBD, S796 peptide, and GST-hCenexin1(647–805) pull-down assays

Preparation of ligands and detailed information on pull-down assays with GST-PBD, S796 peptide, and GST-hCenexin1(647–805) are described in Supplemental Online Materials.

Antibody production and immunoblotting analyses

Anti-hCenexin1/hODF2 antibody that targets the residues 250–632 of hCenexin1 (residues 255 to 618 of hOdf2) has been described previously (Soung et al., 2006). This antibody recognizes both hCenexin1 and hODF2 and their murine homologs. Anti-p-S796 antibody was generated using a synthetic peptide, Ac-PYSTFLTSpSPIRSRSPPA-NH2 (p-S796 is underlined), and then was affinity-purified (Rockland Immunologicals Inc., Gilbertsville, PA). To detect specific p-S796 signals, immunoblotting analysis was carried out in the presence of 4 μg/ml of the p-S796 epitope peptide or the corresponding non-phosphopeptide. Immunoblotting analyses were performed using the enhanced chemiluminescence (ECL) detection system (Pierce, Rockford, IL). All antibodies used in this study are listed in Table S2.

ZZ affinity purification, immunoprecipitation, and kinase assays

Detailed information on a ZZ-based affinity chromatography, immunoprecipitation, and *in vitro* kinase assays with purified Cyclin B1/Cdc2 complex are described in the Supplemental Online Materials.

Mass spectrometry analysis

EGFP-hCenexin1-T7 was immunoprecipitated from mitotic HeLa cells and then subjected to mass spectrometry analysis. Further details are described in the Supplemental Online Materials.

Indirect immunofluorescence microscopy and quantification

Indirect immunostaining was carried out as described previously (Seong et al., 2002). Confocal microscopy, quantification of fluorescence intensities, and measurement of primary cilia length are described in detail in the Supplemental Online Materials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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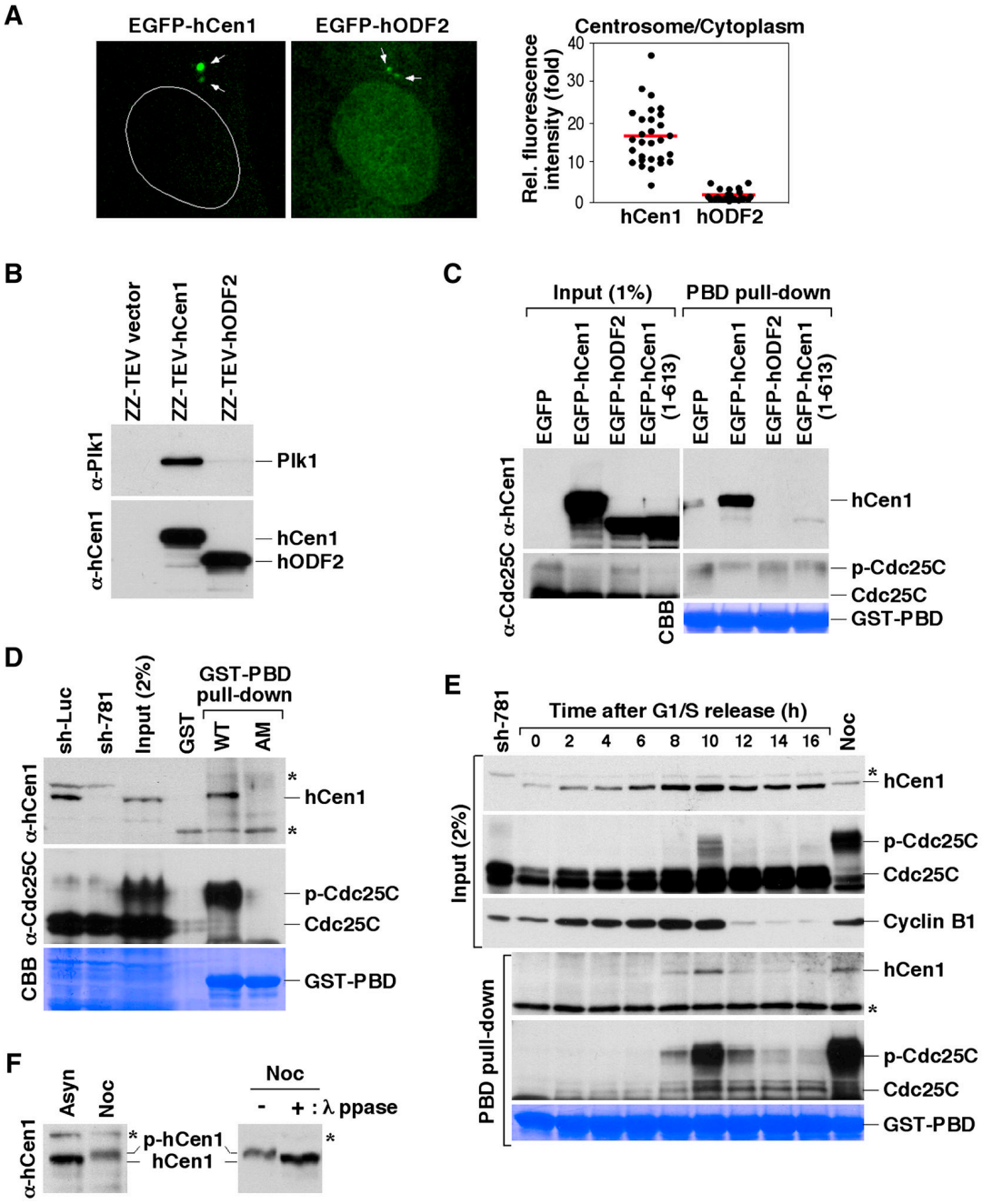

Fig. 1.

Plk1 binds to the C-terminal region of hCenexin1 during the late stages of the cell cycle. **(A)**, HeLa cells were infected with adenovirus expressing either EGFP-hCenexin1 or EGFP-ODF2 and then subjected to confocal microscopy **(Left)**. Arrows indicate centrioles. Because of a low background signals for EGFP-hCenexin1, a circle was drawn to indicate the position of nuclear membrane. Relative fluorescence intensities for centrosome-localized EGFP-hCenexin1 (n = 29 cells) and EGFP-ODF2 (n = 23 cells) signals over cytoplasmic background were quantified **(Right)**. Bars (red), the averages of relative fluorescence intensities. **(B)**, Total cellular lysates from mitotic HeLa cells were fractionated using a ZZ tag-affinity column. Proteins associated with hCenexin1 or hODF2 were eluted after TEV digestion, and then

analyzed. **(C)**, Total cellular lysates were prepared from HeLa cells infected with the indicated adenoviruses and treated with nocodazole for 16 h. Samples were subjected to PBD pull-downs as described in the Experimental procedures. After immunoblotting, the membrane was stained with Coomassie (CBB) to determine the levels of ligands precipitated. **(D)**, Total lysates prepared from mitotic HeLa cells were subjected to PBD pull-downs with bead-bound wild-type GST-PBD (WT) or the GST-PBD(H538A K540M) mutant (AM). Asterisks, non-specific cross-reacting proteins. **(E)**, HeLa cells released from double thymidine block into fresh medium were harvested for PBD pull-downs. Cells silenced for hCenexin1 (sh-781) or treated with nocodazole (Noc) were included for comparison. Asterisks, cross-reacting proteins. **(F)**, Total cellular proteins were prepared from HeLa cells growing asynchronously (Asyn) or treated with nocodazole for 16 h (Noc). To reveal a slow-migrating hCenexin1 form, samples were long separated by 7.5% low-bis (96:1 acrylamide:bis-acrylamide) SDS-PAGE and immunoblotted **(Left)**. Mitotic HeLa lysates (50 μg) were either treated with λ-phosphatase or left untreated and analyzed **(Right)**. Asterisks, cross-reacting proteins.

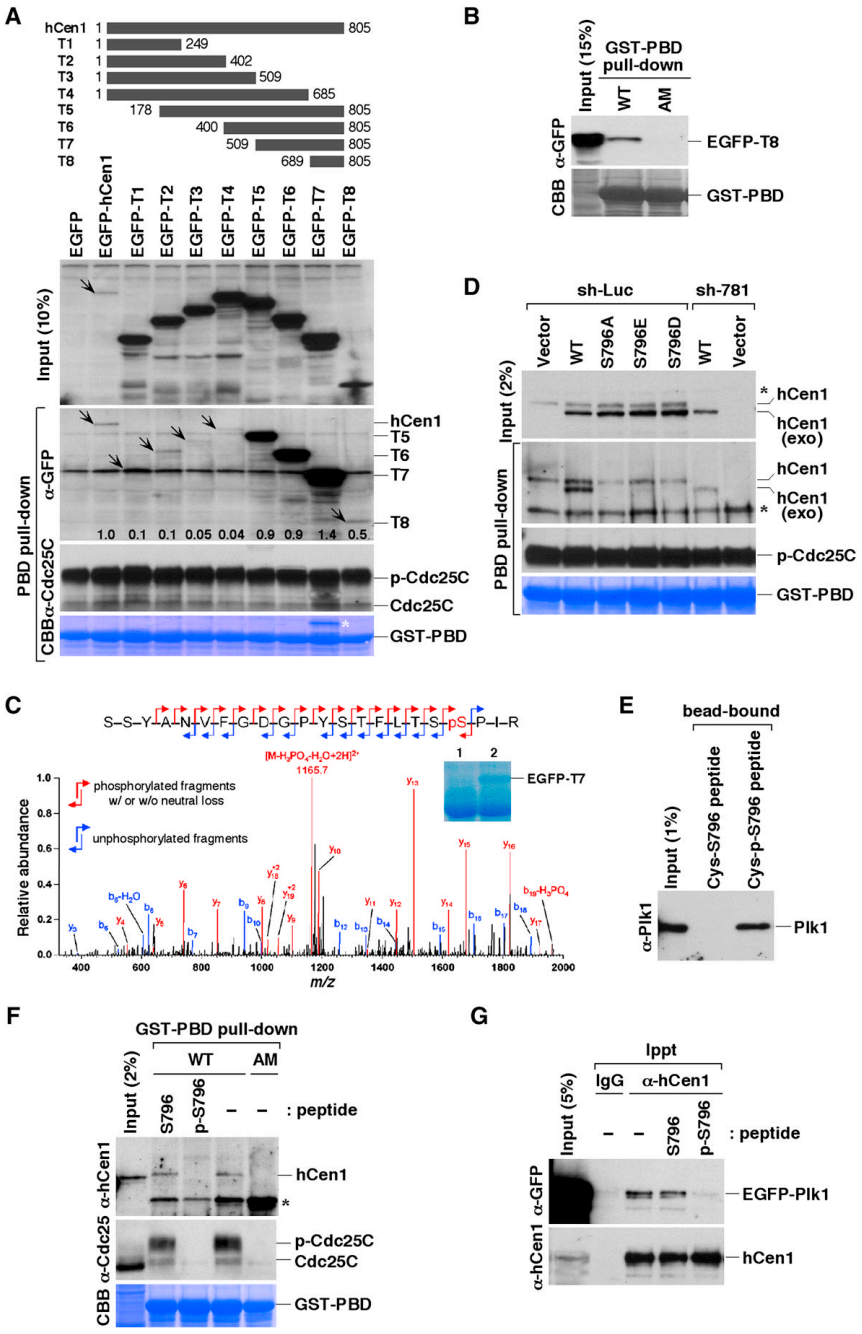

Fig. 2.

Phosphorylation of hCenexin1 at S796 is critical for the hCenexin1-Plk1 interaction. **(A)**, HeLa cells expressing various EGFP-hCenexin1 forms were treated with nocodazole for 16 h, harvested, and then subjected to PBD pull-downs. Numbers indicate relative efficiencies of PBD-binding in comparison to the levels of input. Arrows, weakly detectable hCenexin1 proteins; Asterisk, EGFP-T7 stained with Coomassie. **(B)**, Mitotic HeLa lysates expressing EGFP-T8 were incubated with wild-type GST-PBD (WT) or the GST-PBD(H538A K540M) mutant (AM), and then analyzed. **(C)**, To determine *in vivo* phosphorylation site(s) on the C-terminal region of hCenexin1, EGFP-T7 immunoprecipitated from nocodazole-treated HeLa cells (inset gel) was subjected to mass spectrometry analysis as described in the Experimental

procedures. The phosphorylation site was determined by the phosphorylated (red) fragment ions with (w/) or without (w/o) neutral loss of phosphate and unphosphorylated (blue) fragment ions. **(D)**, Mitotic HeLa cells expressing various hCenexin1 forms were subjected to PBD pull-downs. To mark the positions of the endogenous and exogenous hCenexin1 proteins, samples expressing wild-type hCenexin1 (WT) or vector but depleted of endogenous hCenexin1 (sh-781) (lanes 6 and 7) were included. Due to a cloning variation at the N-terminal region, the exogenous hCenexin1 migrated about 5-kDa faster than the endogenous hCenexin1. Asterisks, non-specific cross-reacting proteins. **(E)**, A pair of short N-terminal Cys-containing non-phospho- and phospho-S796 peptide was cross-linked to the beads and then incubated with mitotic HeLa lysates. The resulting peptide-associated cellular proteins were analyzed. **(F)**, Mitotic HeLa lysates were preincubated with buffer (−), non-phospho-, or phospho-S796 peptide for 20 min, and then subjected to PBD pull-downs. Likely due to phosphorylation, migration of the PBD-bound hCenexin1 was often distinguishably slower than that of hCenexin1 in the input. Asterisk, a cross-reacting protein. **(G)**, Mitotic HeLa cells expressing EGFP-Plk1 were preincubated with buffer (−) or the indicated peptide prior to immunoprecipitation.

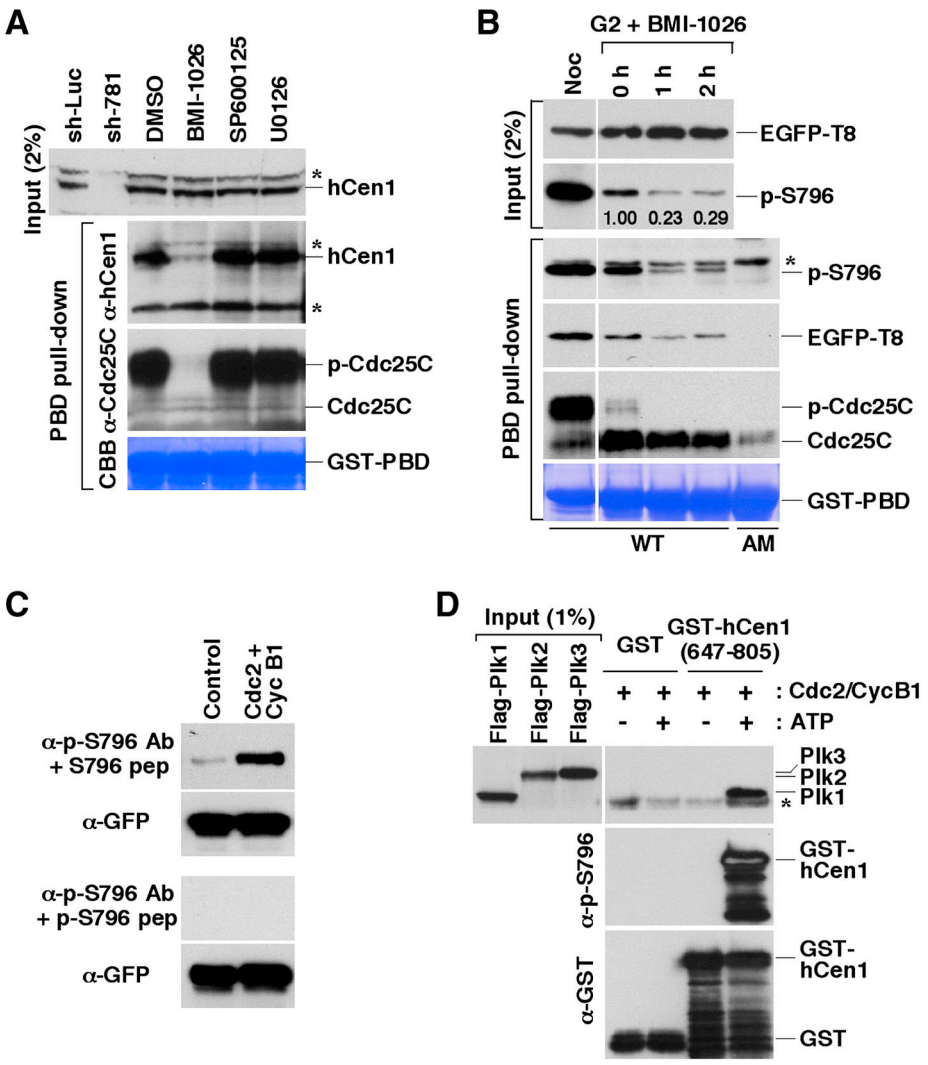

Fig. 3.

Cdc2-dependent phosphorylation of hCenexin1 at S796 is sufficient to induce the hCenexin1-Plk1 interaction. **(A)**, Mitotic HeLa cells treated with the indicated inhibitors for 30 min were harvested and subjected to PBD pull-downs. Asterisks, non-specific cross-reacting proteins. **(B)**, HeLa cells stably expressing EGFP-T8(689–805) were arrested at the G1/S boundary by double thymidine treatment, released into fresh medium for 3 h, and then treated with etoposide for 7 h to re-arrest the cells in G2. BMI-1026 was added into the etoposide-containing medium 1 h or 2 h before harvest. Nocodazole-arrested cells (Noc) were included for comparison. To determine the level of phospho-independent binding, nocodazole-treated lysates from lane 1 were also pulled down with the PBD(H538A K540M) (AM) mutant. Numbers, the level of p-S796 EGFP-T8 over total EGFP-T8 relative to the 0 h sample. Asterisk, a cross-reacting protein. **(C)**, HeLa cells expressing EGFP-T8 were infected with adenoviruses encoding Cdc2 and Cyclin B1, and then arrested at the G1/S boundary before harvest. Samples were immunoblotted with anti-p-S796 antibody preincubated with the indicated peptides or with anti-GFP antibody to determine the level of total EGFP-T8. **(D)**, Bead-bound control GST or GST-hCenexin1(647–805) were reacted with purified Cdc2/Cyclin B1 complex in the presence or absence of ATP. After washing, the resulting ligands were incubated with a 1:1:1 mixture

of transfected lysates for Flag-Plk1, Flag-Plk2, and Flag-Plk3, and then the ligand-associating proteins were analyzed. Asterisk, a cross-reacting protein.

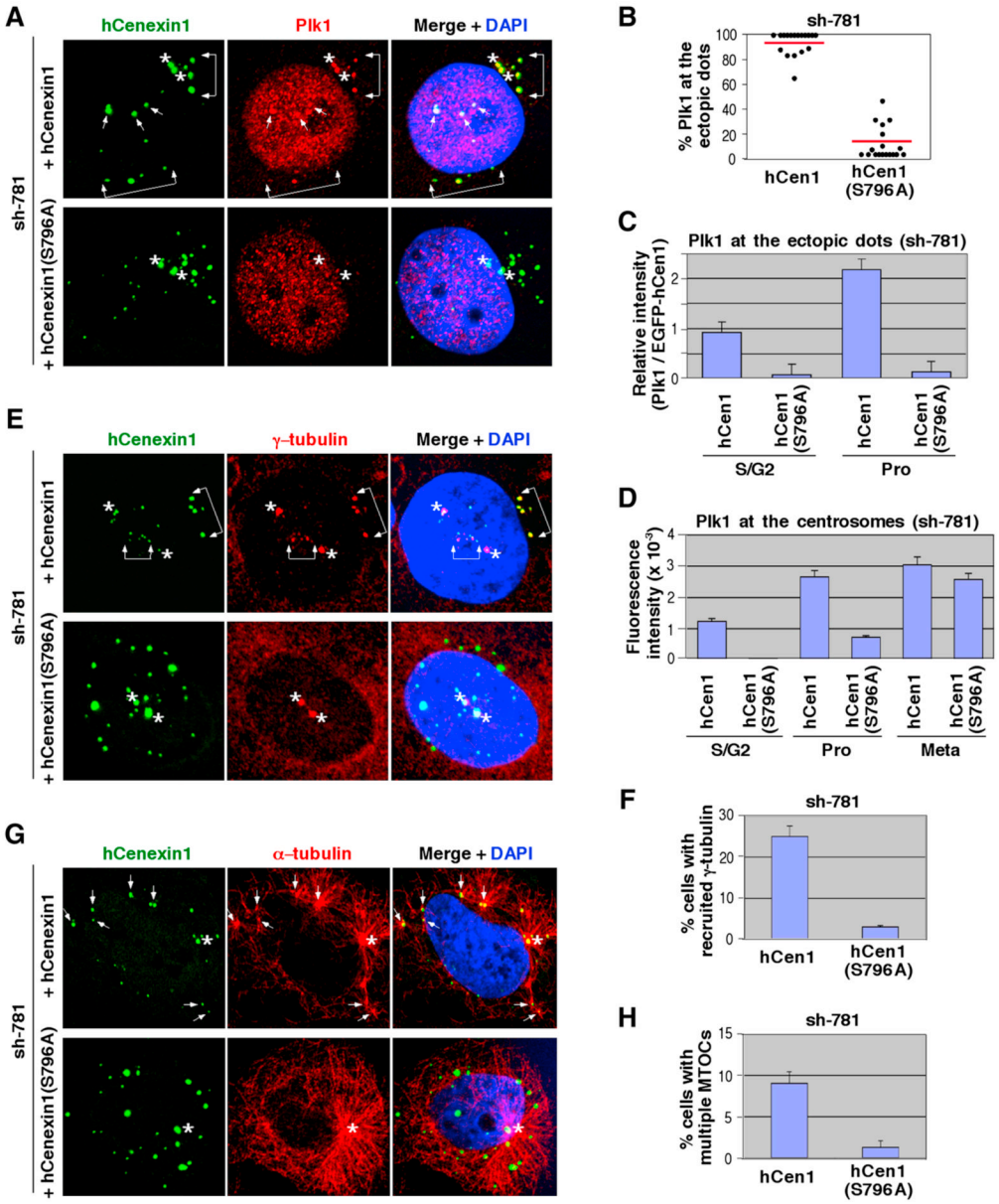

Fig. 4.

Overexpressed hCenexin1, but not hCenexin1(S796A), forms hCenexin1-Plk1 assemblies, recruits γ-tubulin, and generates ectopic microtubule organizing centers. **(A–F)**, HeLa cells silenced for hCenexin1 (sh-781) were arrested by double thymidine block and released for 10 h (DT 10 h) to enrich the G2 population. Upon releasing from the block, cells were infected with adenovirus expressing EGFP-hCenexin1 or the respective S796A mutant. **(A and E)**, The resulting cells were fixed and immunostained. Arrows and arrowed brackets indicate multiple Plk1 and γ-tubulin signals recruited to the ectopic hCenexin1 dots. **(B)**, Cells (n = 18 for each group) exhibiting multiple dots of either hCenexin1 or hCenexin1(S796A) in **(A)** were quantified to determine the percentage of Plk1 colocalization with the hCenexin1 dots. Bars (red), the averages of Plk1 recruitment to the ectopic hCenexin1 dots. **(C–D)**, Using the samples in **(A)**, the fluorescence intensities of Plk1 recruited to the ectopic hCenexin1 dots **(C)** or the endogenous centrosomes **(D)** were quantified at different stages of the cell cycle. Greater than

150 ectopic dots **(C)** or 30 centrosomes **(D)** for each group were analyzed. Bars, standard deviation. **(F)**, Cells (n = ~200) with γ-tubulin signals recruited to the ectopic hCenexin1 dots were quantified. Bars, standard deviation. **(G-H),** To examine microtubule nucleation activity of the hCenexin1-Plk1 assemblies, cells were prepared similarly as in (**A)** except that they were released into fresh media for 7 h, treated with nocodazole (330 nM) for 3 h, and then released into fresh medium for 6 min before fixation. After immunostaining with anti-α-tubulin antibody **(G)**, cells (n > 300) with ectopic MTOCs were quantified. **(H)**. Bars, standard deviation.

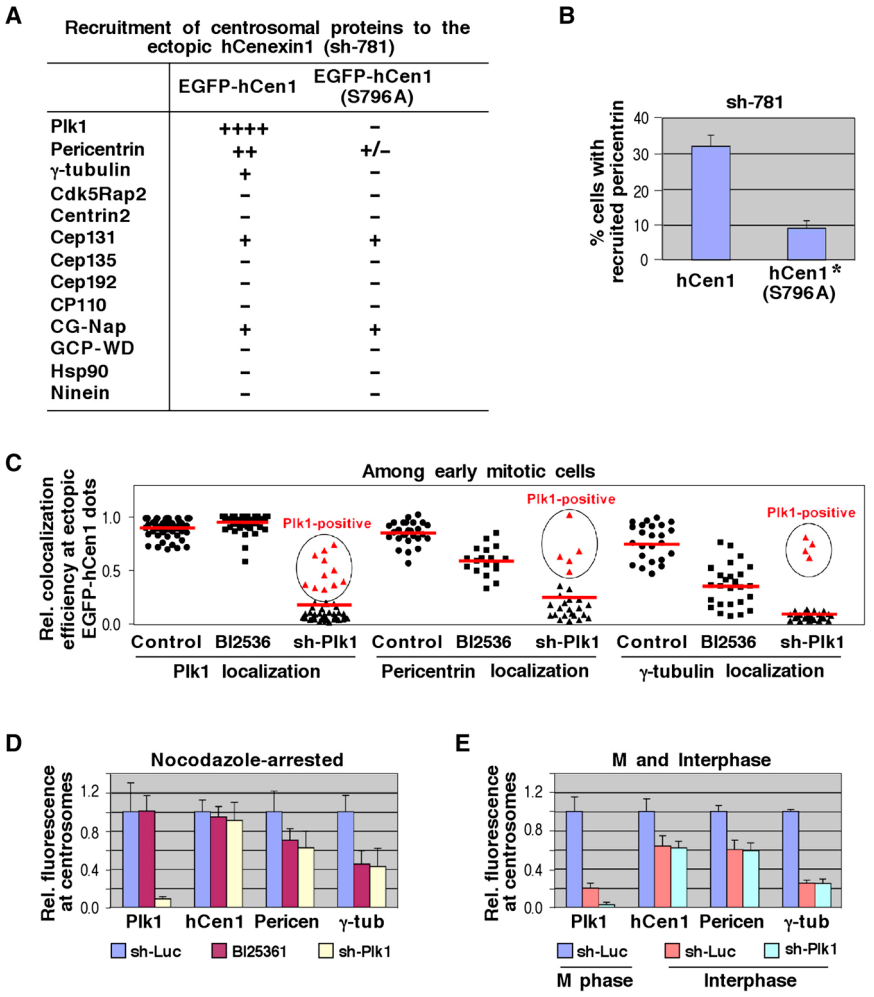

Fig. 5.

Proper recruitment of pericentrin and γ-tubulin by the hCenexin1-Plk1 complex requires Plk1 function. **(A–C)**, The DT 10 h cells prepared as in Fig. 4A were stained with the indicated antibodies. Recruitment of various centrosomal components to the ectopic hCenexin1 dots were examined among the cells in late G2 or early M phase, the stages where Plk1 is abundantly expressed. Intensities of the recruited signals were categorized from strong (++++) to undetectable (−) **(A)**. The percentage of pericentrin colocalization with the ectopic hCenexin1 dots was quantified in > 300 cells **(B)**. Asterisk denotes weakly recruited pericentrin signals. Bars, standard deviation. **(C)**, EGFP-hCenexin1-expressing HeLa cells or the EGFP-hCenexin1 cells silenced for Plk1 (sh-Plk1) were released from a G1/S block into fresh medium for 10 h. A fraction of the unsilenced cells were treated with BI2536 for 3 h before harvest. To minimize an indirect effect of prolonged Plk1 depletion or inhibition, the total period of release was kept for 10 h and only the prophase cells with condensed chromosomes were analyzed. Bars (red), the averages of colocalization efficiency. Plk1-positive cells (marked in red) indicate a fraction of the sh-Plk1 cells with yet detectable Plk1 signals. **(D–E)**, To examine the effect of Plk1 inhibition or depletion on the localization of hCenexin1, pericentrin, and γ-tubulin to the centrosomes, HeLa cells infected with control sh-Luc or sh-Plk1 lentivirus for 1 day were first arrested by double thymidine treatment. Cells were then released into either nocodazole-containing medium for 12 h to trap the cells in prometaphase **(D)** or fresh medium

for 6 h to enrich the cells in S/G2 **(E)**. BI2536 treatment was carried out 3 h prior to harvest. Bars, standard deviation.

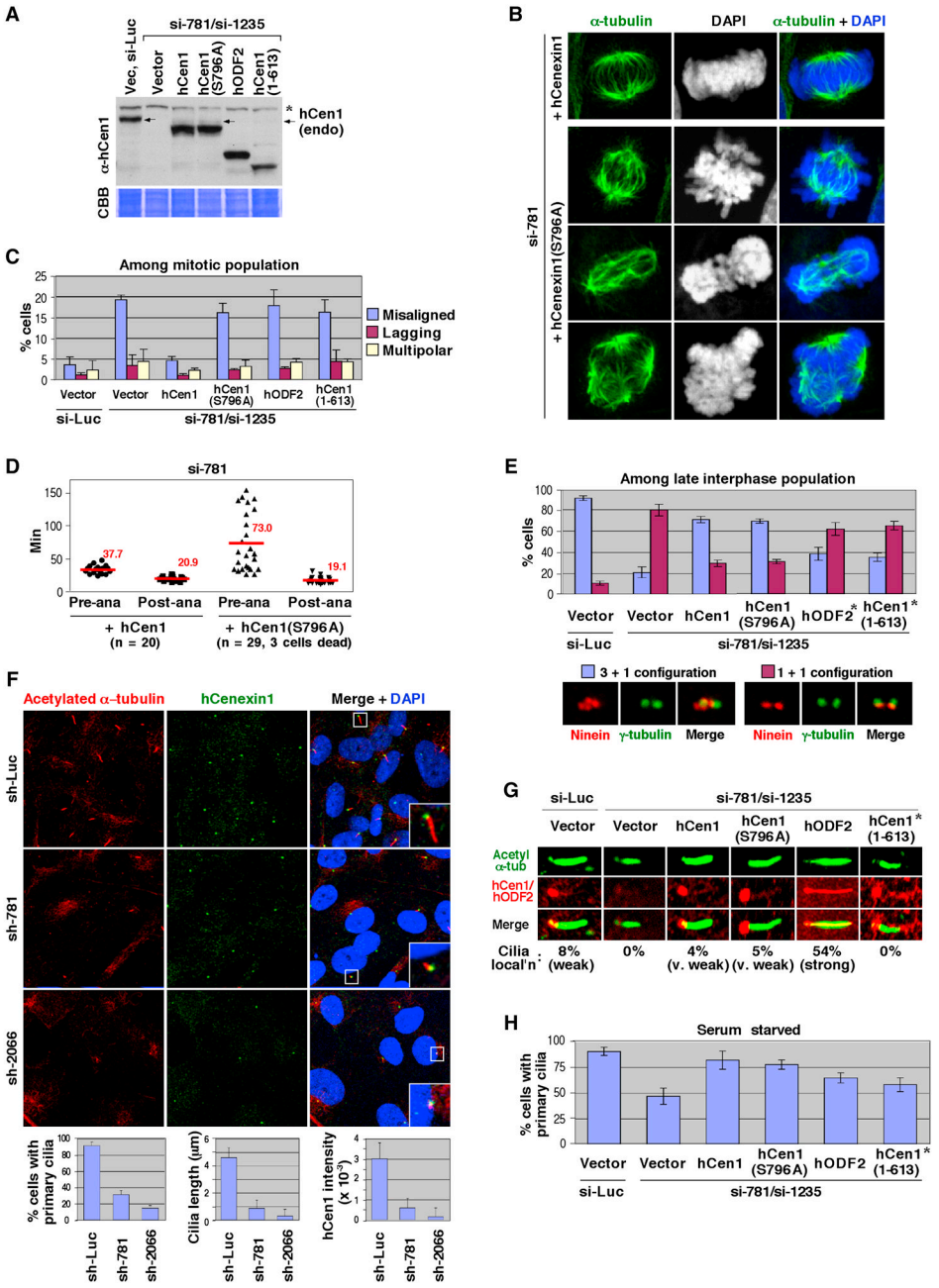

Fig. 6.

S796-dependent hCenexin1 function is important for proper mitotic progression, but not for ninein localization and ciliogenesis. **(A–C)**, HeLa cells expressing the indicated hCenexin1 or hODF2 construct were depleted of endogenous hCenexin1 and hODF2 by si-781 or si-1235. The cells were released from a G1/S block for 10 h and subjected to immunoblotting **(A)** and immunostaining **(B)** analyses. Asterisk in **(A)**, a non-specific cross-reacting protein. **(C)**, The cells in **(B)** exhibiting abnormal chromosome segregation or multipolar spindles were quantified. **(D)**, HeLa cells expressing hCenexin1 or hCenexin1(S796A) were depleted of endogenous hCenexin1 and hODF2. Cells releasing from a G1/S block were subjected to time-lapse microscopy (see Fig. S9). The length of time required for pre- or post-anaphase

progression was quantified. Red bars with numbers, the averages of time-length in minute. **(E)**, Asynchronously growing HeLa cells expressing the indicated constructs were depleted of endogenous hCenexin1 and hODF2 as in **(A)**, and then subjected to immunostaining analyses. Since ninein delocalizes from the mitotic centrosomes, ninein configuration was examined among the interphase cells (n > 400). **(Right)**, Representative images of ninein localization with 3+1 or 1+1 configuration are shown. Asterisks indicate the 3+1 configuration with poorly separated ninein dots. **(F)**, hTERT-RPE cells were infected with lentivirus expressing control luciferase (sh-Luc), sh-718 (for depletion of both hCenexin1 and hODF2), or sh-2066 (for depletion of hCenexin1 only), cultured under serum starvation for 2 days, and then immunostained. Samples were quantified to determine the percentages of primary cilia formation (> 400 cells) **(Left)**, the length of primary cilia (> 50 cells) **(Middle)**, and the fluorescence intensities of hCenexin1 signals (> 50 cells) **(Right)**. Error bars, standard deviation. **(G–H)**, hTERT-RPE cells expressing the indicated hCenexin1 or hODF2 constructs were depleted of endogenous hCenexin1 and hODF2 by si-781 or si-1235. Two days after serum starvation, cells were immunostained **(G)** and quantified in over 500 cells **(H)**. Percentages in **(G)**, efficiency of localization along the axoneme of cilia; Bars in **(H)**, standard deviation; Asterisks in **(G)** and **(H)**, hCenexin1(1–613) exhibits significantly shorter primary cilia than other constructs.

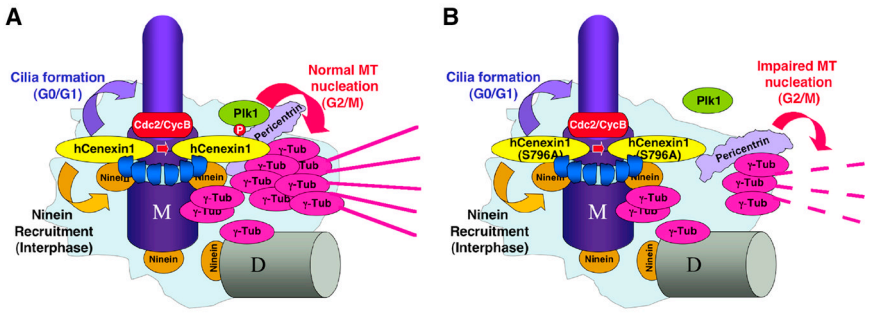

Fig. 7.

A model illustrating multiple functions of hCenexin1 in both Plk1-independent ninein recruitment and ciliogenesis and Plk1-dependent microtubule nucleation at distinct stages of the cell cycle. **(A)**, In G0/G1 stage of the cell cycle, hCenexin1 plays a critical role in the formation of primary cilia (vertical cylinder). Throughout interphase, hCenexin1 is also required for proper recruitment of ninein to the distal/subdistal appendages of mother centrioles (M), an event that may contribute to proper ciliogenesis. Consistent with a low level of Plk1 expression during interphase, both ninein recruitment and primary cilia formation occur in a Plk1-independent manner. During the late stages of the cell cycle (late G2 and early M), Cdc2 phosphorylates hCenexin1 at S796 and generates a PBD-docking site crucial for Plk1 recruitment to the centrosomes. The formation of the hCenexin1-Plk1 complex is important for proper recruitment of pericentrin, which in turn facilitates γ-tubulin accumulation and centrosome maturation, thus promoting microtubule nucleation. **(B)**, Absence of Cdc2-dependent hCenexin1 phosphorylation at S796 impairs Plk1 recruitment to the centrosomes, which results in reduced levels of recruited pericentrin and γ-tubulin and, therefore, improper microtubule nucleation. Both ninein localization and primary cilia formation at the interphase centrosomes occur normally under these conditions. Cdc2/CycB, B-type cyclin-associated Cdc2 activity; M, mother centrioles, D, daughter centrioles.