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NEK2 phosphorylation antagonizes the microtubule stabilizing activity of centrobin

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ABSTRACT

Centrobin was initially identified as a centrosome protein for centriole duplication. Centrobin is also detected outside the centrosome and involved in other cellular functions, such as spindle assembly. We previously reported that centrobin is a substrate of both NEK2 and PLK1, but it is not clear what functional properties of centrobin are regulated by two kinases. Here, we report that centrobin is involved in cell spreading, migration and microtubule stabilization in interphase cells. The NEK2-depleted cells looked spread with well-developed microtubule networks and migrated faster than the control cells. The microtubule stability in NEK2-depleted cells was higher than the control cells. However, the opposite was the case in centrobin-depleted cells. The opposite outcomes in NEK2- and centrobin-depleted cells suggest that NEK2 antagonizes biological functions of centrobin. We identified NEK2 phosphorylation sites within centrobin, which is distinct from the PLK1 phosphorylation sites. In fact, the phospho-resistant mutant of centrobin against NEK2 stabilized microtubule networks in vivo. Based on the results, we propose that NEK2 phosphorylation antagonizes the microtubule stabilizing activity of centrobin. Centrobin is a novel example that NEK2 and PLK1 independently phosphorylate a substrate and result in opposite outcomes in substrate function.

1. Introduction

An interphase cell includes a mixed population of microtubules. Some microtubules show dynamic instability with a short half-life, while the other microtubules are stable with a long half-life [1]. Stable microtubules are frequently modified by acetylation, and become resistant to depolymerizing agents such as nocodazole [2]. A significant change in microtubule dynamics is accompanied when a cell enters mitosis [3,4]. Most microtubules in mitotic cells are short and dynamic to function as spindles. Phosphorylation is one of key regulatory mechanisms for microtubule dynamics during the cell cycle [5].

NEK2 is a serine/threonine kinase whose activity oscillates during the cell cycle [6]. The kinase activity of NEK2 is high at S and G2 phase and low at M and G1 phase [7]. An augmented activity of NEK2 at G2 phase contributes to centrosome separation by phosphorylating C-NAP1 and rootletin, components of inter-centriolar linker proteins [8–10]. Nek2 proteins are also localized in both the nucleus and cytoplasm throughout the cell cycle, and exhibited dynamic changes in distribution, depending on the cell cycle stage [11]. Nek2 is associated with chromosomes from prophase to metaphase and then is dissociated upon entering into anaphase [11,12]. This dynamic distribution of NEK2 implies its involvement in multiple cellular functions during the cell cycle [11]. For example, NEK2 is located at the kinetochore of mitotic cells and regulates signaling of the spindle assembly checkpoint through the HEC1 phosphorylation or interaction with MAD1 [13–15]. NEK2 is also critical for bipolar spindle pole formation in acentrosomal mouse oocytes and early embryos [16,17]. Currently available data consistently support that NEK2 as well as other NEK kinases coordinates microtubule-dependent processes in both inside and outside the centrosome [6].

Centrobin was originally identified as a daughter centriole-associated protein [18]. Depletion of centrobin results in centrosomes with one or no centriole, demonstrating that centrobin is required for centriole duplication [18]. It was proposed that centrobin facilitates the elongation and stability of centrioles via its interaction with tubulins [19]. Centrobin is also detected outside the centrosome and involved in other cellular functions [20]. Centrobin-depleted cells show a range of spindle abnormalities including unfocused poles that are not associated with centrosomes, S-shaped spindles and mini spindles [20,21]. In fact, centrobin is associated with microtubules and promotes microtubule polymerization and stabilization in vitro [22].

We previously observed that centrobin is a substrate of both NEK2 and PLK1 [20,22]. However, it is not clear what functional properties of centrobin are regulated by two kinases. In this study, we elucidate biological functions of centrobin in cell spreading and migration. Furthermore, we investigated how NEK2 regulates the microtubule stabilizing functions of centrobin.

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2. Materials and methods

2.1. Antibodies, transfection and RNA interference

α-Tubulin (Sigma), acetylated α-tubulin (Sigma), γ-tubulin (Sigma), HA (Sigma), FLAG (Sigma) and NEK2 (BD bioscience) antibodies were used according to the manufacturer's instruction. Centrobin antibodies were used as previously described [20]. Transient transfection of plasmid DNA was performed using Lipofectamine Plus reagent (Invitrogen) according to the manufacturer's instruction. For RNA interference, siRNAs specific to *NEK2* (5′-GGCAAATTCAGGCGAATTC-3′), *NEK2*-3′UTR (5′-GCTGTAGTGTTGAATACTT-3′) and *centrobin* (5′-GGATGGTTCTAAGCATATC-3′) were purchased from ST Pharm and transfected into the cells using Lipofectamine RNAi max (Invitrogen) according to the manufacturer′s instruction. Non-specific control siRNA (5′-AAGTAGCCGAGCTTCGATTGC-3′) was also used.

2.2. Cell culture and stable cell lines

293T, HeLa and tet-on HeLa cells were cultured in Dulbecco's modified Eagles's medium (DMEM) supplemented with 10% FBS. U2OS cells were cultured in McCoy's 5A media supplemented with 10% FBS. RPE1 cells were cultured in DMEM F12 supplemented with 10% FBS. Stable tet-on HeLa cell lines were generated with Lenti-X HT packaging system (Clontech) according to manufacturer's instruction using pLVX-IRES-Puro vector which is substituted its original promoter with tet-responsive promoter.

2.3. Immunoblot and immunoprecipitation

For immunoblot analysis, protein samples were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred onto a nitrocellulose membrane. The membrane was incubated with a primary antibody for 2 h after blocking with 5% skim milk in 0.1% TBST (Tris-buffered saline TBS with 0.1% Triton X-100) for 30 min, and incubated with a horseradish peroxidase-conjugated secondary antibody for 30 min after washing three times with 0.1% TBST. And then, the membrane was incubated with the ECL solution after washing three times with 0.1% TBST, and exposed to an X-ray film. For immunoprecipitation, the cells were lysed with the NP40 buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% NP40) with protease inhibitors for 20 min on ice and centrifuged with 15,000*g* for 20 min at 4 °C. The supernatant was incubated with specific antibodies for 2 h, followed by protein A Sepharose (Amersham Pharmacia) for 2 h at 4 °C.

2.4. Immunocytochemistry and image processing

For immunochemistry, the cells cultured on coverslip were fixed with cold methanol for 10 min after washing with phosphate-buffered saline (PBS). The fixed cells were blocked with 5% bovine serum albumin (BSA) in 0.1% PBST (PBS with 0.1% Triton X-100), incubated with the primary antibodies for 1 h, washed with 0.1% PBST three times, and incubated with secondary antibodies for 30 min. And then, the cells were washed three times with 0.1% PBST, incubated with DAPI solution to stain DNA, and the coverslip was mounted on a glass slide. The immunostained cells were observed using fluorescence microscope with a CCD (Qicam Fast 1394; Qimaging) camera and processed with ImagePro 5.0 (Media Cybernetics, Inc.) software.

2.5. Nocodazole-resistance assay

Nocodazole-resistance assay was performed as previously described with a slight modification [23]. In brief, the cells cultured on coverslip were treated with 2 mM thymidine for 16 h to synchronize cell cycle and incubated with 2 μM nocodazole for the last 30–60 min at 37 °C. And then, the cells were rinsed twice in PEM buffer (100 mM PIPES [pH 6.9], 1 mM EGTA, 2 mM $MgCl_2$), incubated 1 min at 37 °C with 0.2% Triton X-100 in PEM to remove monomeric tubulin, rinsed again twice in PEM buffer, and fixed with cold methanol. After fixation, the cells were subjected to immunocytochemistry using acetylated α-tubulin antibody. To quantitative analysis, the cells were scored for the presence of more than 10 acetylated microtubules per cell.

2.6. In vitro kinase assay

For the preparation of NEK2 kinases, 293T cells transfected with wild-type NEK2 (*pNEK2RHA1*) or kinase-dead NEK2 (*pNEK2KHA5*) expression vectors were lysed with NP40 lysis buffer and subjected to immunoprecipitation with an antibody against the HA tag. The immunoprecipitates were washed twice with lysis buffer and once with kinase buffer (50 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM Na_3VO_4, 1 g/ml heparin). The centrobin substrates were prepared from bacterially expressed fusion proteins. Kinase reactions were carried out for 30 min at 30 °C in kinase buffer supplemented with 5 μM ATP, 1 mM dithiothreitol and 5 μCi [γ-^{32}P]ATP in a total volume of 20 μM. The reactions were stopped by adding 2× SDS sample buffer and heating for 5 min at 95 °C. Protein samples were subjected to SDS–PAGE and transferred onto a polyvinylidene fluoride (PVDF) membrane. The membrane was exposed to a BAS plate or an X-ray film to obtain an autoradiograph image, and then stained with Coomassie brilliant blue solution.

2.7. Cell migration and cell spreading

For cell migration assay, cells were grown to subconfluency and treated with 10 μg/ml Mitomycin C (Sigma) for 3 h to arrest cell proliferation. And then, a wound track was introduced by scraping the cell monolayer with a yellow pipette tip. After rinsing with PBS, the cells were cultured in growth medium for a further 24 h and the recovery area was measured. For cell spreading assay, cells were treated with 2 mM thymidine for 16 h to synchronize cell cycle, and the cell area was measured. For measurement of the area, phase-contrast images were analyzed using ImagePro 5.0 software.

3. Results

3.1. NEK2 antagonizes centrobin functions in cell spreading and migration

We previously reported that centrobin is a substrate of NEK2 [20]. To have an insight into the functional outcomes of NEK2 phosphorylation on centrobin, we compared the knockdown phenotypes of NEK2 and centrobin in cell morphology. The results showed that the NEK2-depleted HeLa, U2OS and RPE1 cells looked spread with well-developed microtubule networks (Fig. 1A). On the other hand, the centrobin-depleted cells appeared shrunk with disrupted microtubule networks (Fig. 1A).

Next, we examined the cell migration ability of the NEK2- or centrobin-depleted cells. The results showed that the NEK2-depleted cells migrated faster than the control cells but the opposite was the case in centrobin-depleted cells (Fig. 1B). The opposite outcomes in NEK2- and centrobin-depleted cells suggest that NEK2 antagonizes biological functions of centrobin.

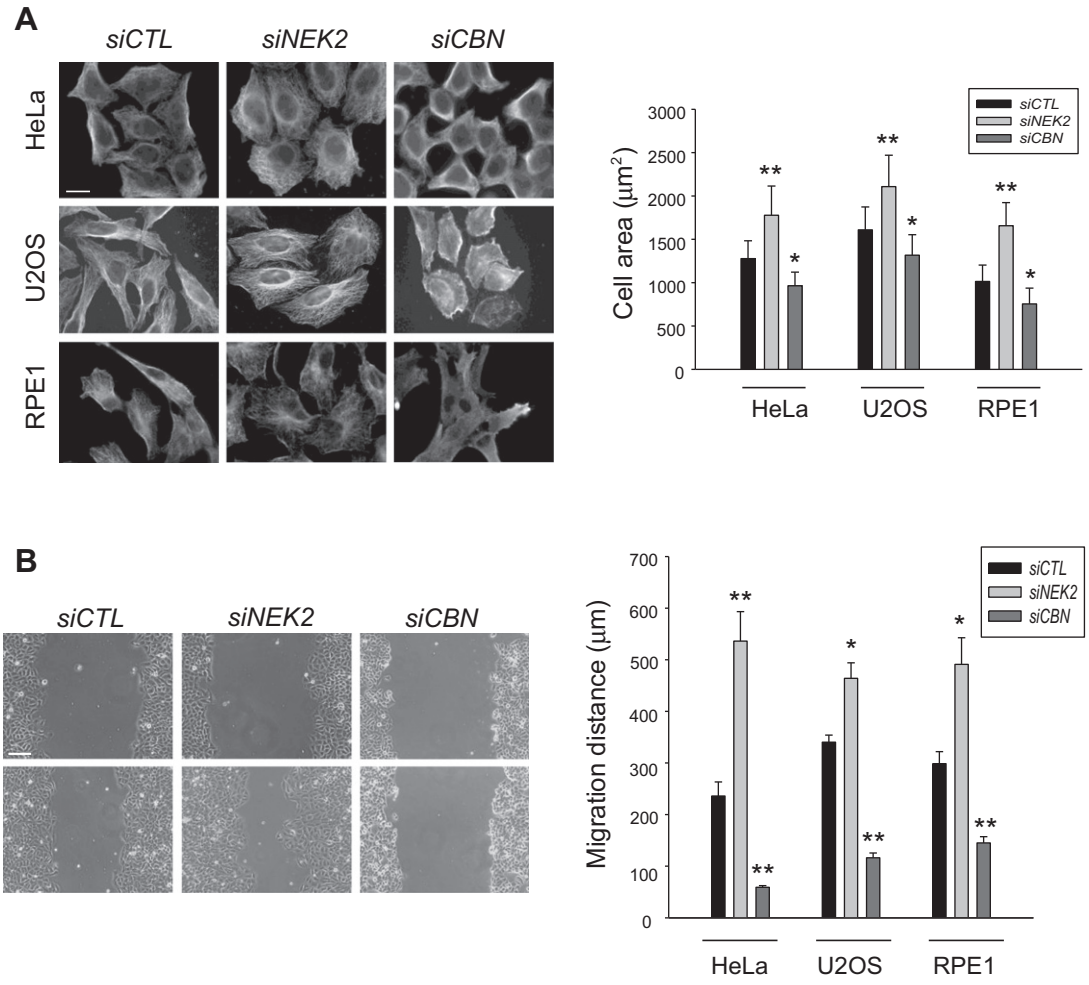

Fig. 1. Cell spreading and migration in NEK2- and centrobin-depleted cells. (A) Endogenous NEK2 and centrobin in HeLa, U2OS or RPE1 cells were depleted with siRNA transfection. The cells were than treated with 2 mM thymidine for 16 h to arrest the cell cycle at S phase and immunostained with the antibody specific to α-tubulin. Scale bar, 20 μm. The cell area was measured using the Image-Pro software. Over 300 cells per experimental group were measured in three independent experiments. (B) The cell cycle-arrested cells migrated into the gaps for 24 h and the distance was measured. Scale bar, 200 μm. Values are means and standard errors with three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$, in comparison to the control.

3.2. NEK2 reduces the microtubule stabilizing activity of centrobin

It is known that acetylated microtubules are stable and therefore resistant to microtubule destabilizers such as nocodazole [2]. We observed that a significant amount of acetylated microtubules still remained in NEK2-depleted cells after 2 μM nocodazole treatment (Fig. 2A). On the other hand, many of the centrobin-depleted cells were absent of acetylated microtubules after the nocodazole treatment (Fig. 2A). To quantify the stability of microtubules, we determined the number of cells with acetylated microtubules after nocodazole treatment for 0.5 and 1 h [23]. The result showed that the microtubule stability in NEK2-depleted cells was higher by twofold than the control cells (Fig. 2B). On the other hand, the microtubule stability in centrobin-depleted cells was significantly reduced (Fig. 2B). Since co-depletion of both NEK2 and centrobin revealed reduction in microtubule stability, it is likely that centrobin is essential for microtubule stabilization and NEK2 antagonizes the centrobin function (Fig. 2B).

We performed rescue experiments with inducible cell lines in which wild-type or kinase-dead NEK2 is stably expressed. Immunoblot analyses confirmed that doxycycline enhanced the ectopic NEK2 levels significantly, but a considerable amount of ectopic NEK2 was also detected even without induction (Fig. 2C). In these conditions, the microtubule stability was significantly reduced by ectopic expression of wild type NEK2 but not of kinase-dead NEK2 (Fig. 2C). These results indicate that the kinase activity of NEK2 is necessary for regulation of microtubule stability in interphase cells. We also performed rescue experiments with the centrobin-expressing HeLa cells. The results showed that ectopic centrobin effectively rescued the centrobin depletion with a significant increase in microtubule stability (Fig. 2D). Collectively, these results suggest that NEK2 phosphorylation suppresses the microtubule stabilizing activity of centrobin.

3.3. Centrobin is specifically phosphorylated by NEK2

We performed in vitro kinase assays to pinpoint NEK2 phosphorylation sites within the centrobin protein. A previous study of ours revealed that NEK2 phosphorylation sites are located within 1–193 residues of centrobin [20]. Using truncated centrobin fusion proteins as substrates, we limited specific NEK2 phosphorylation sites within 30–56 residues of centrobin, which are distinct from PLK1 phosphorylation sites at 1–29 residues of centrobin (Fig. 3A) [22].

We prepared GST-CBN$^{30-56}$ in which each of the serine and threonine residues is substituted with alanine and used them as

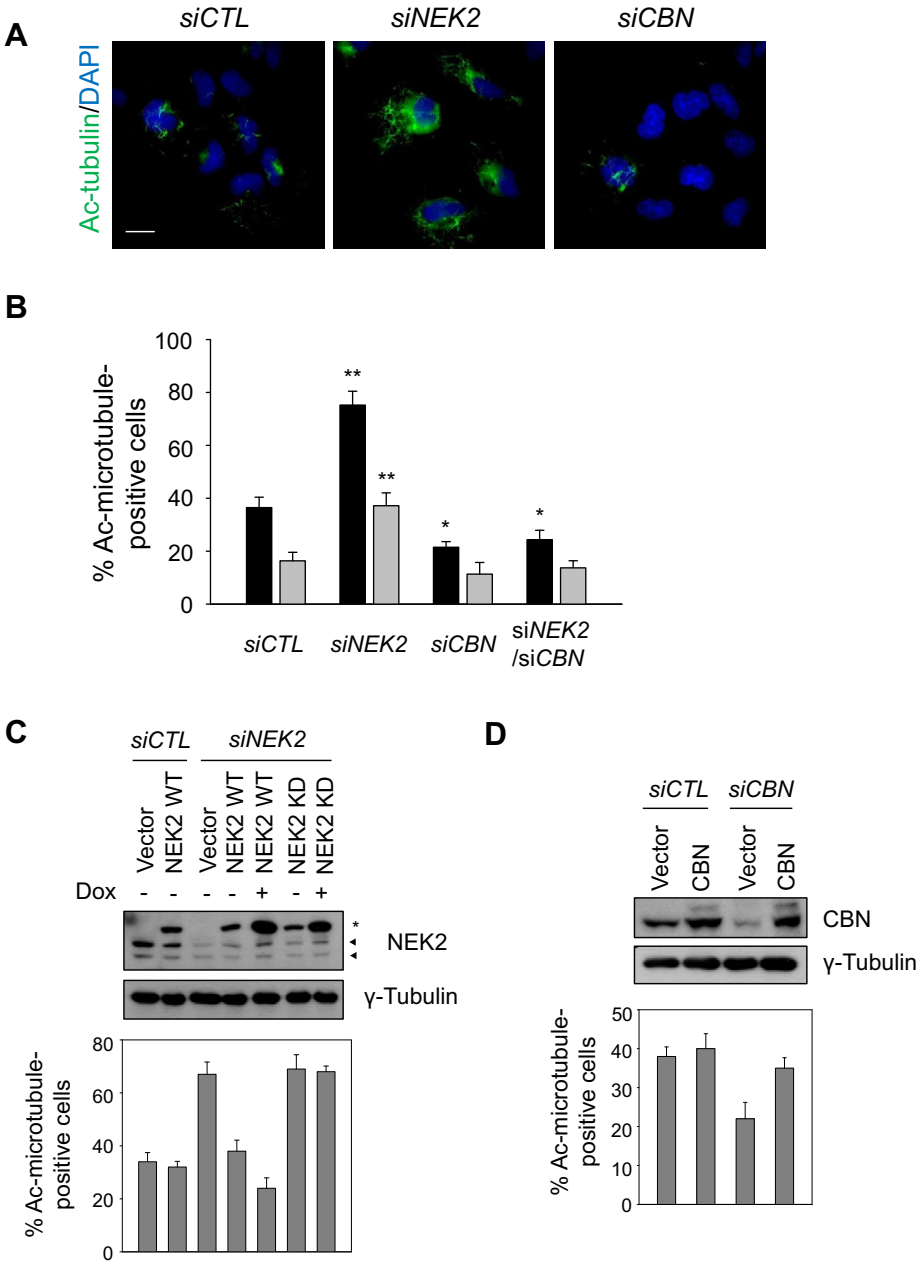

Fig. 2. Microtubule stability in NEK2- and centrobin-depleted cells. (A) NEK2- and centrobin-depleted HeLa cells were treated with 2 μM nocodazole for 0.5 h and subjected to immunostaining with the acetylated α-tubulin antibody (green). Nuclei were stained with DAPI (blue). Scale bar, 10 μm. (B) The NEK2- and/or centrobin-depleted cells were treated with nocodazole for 0.5 h (black bar) or 1 h (gray bar), and the number of cells with residual acetylated tubulins was counted. (C) Endogenous NEK2 was depleted in inducible HeLa cell lines in which wild-type (WT) or kinase-dead (KD) FLAG-NEK2 was stably expressed. Immunoblot analysis was performed to determine the endogenous (arrowheads) and ectopic (asterisk) NEK2 protein along with γ-tubulin. The same set of cells was subjected to the nocodazole-resistant assays. (D) The centrobin-depleted cells were rescued with ectopic centrobin and were subjected to immunoblot analysis with the centrobin and γ-tubulin antibodies. The nocodazole-resistant assay was performed with the same set of cells. Over 300 cells per experimental group were counted in three independent experiments. Values are means and standard errors. $^{*}P < 0.05$; $^{**}P < 0.005$, in comparison to the control. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

substrates. The results showed that the alanine-substituted mutants of GST-CBN$^{30–56}$ at T35, S36, S41 and S45 were not effectively phosphorylated by NEK2, suggesting that these four residues of centrobin are candidate phosphorylation sites of NEK2 (Fig. 3B).

We examined in vivo phosphorylation of the ectopic centrobin protein with co-transfection of wild-type or kinase-dead FLAG-NEK2 into 293T cells. The mobility-shifted bands of FLAG-CBN were observed in cells co-transfected with NEK2 but not with the kinase-dead mutant, suggesting that they are the phosphorylated forms of centrobin (Fig. 3C). We also observed that the mobility-shifted band intensities were significantly reduced in FLAG-CBN^{T35,S36,41,45A}-expressing cells. It is interesting that ectopic NEK2 effectively phosphorylated FLAG-CBN^{T3,S4,21,22A} which is the phospho-resistant form against PLK1 (Fig. 3C) [22]. These results suggest that NEK2 phosphorylates specific sites of centrobin, which are distinct from the PLK1 phosphorylation sites.

3.4. The phospho-resistant centrobin stabilizes microtubules

We generated inducible HeLa cell lines in which wild-type or phospho-resistant mutant (T35A, S36A, S41A and S45A) of centrobin (FLAG-CBN) is stably expressed. The immunoblot analysis re-

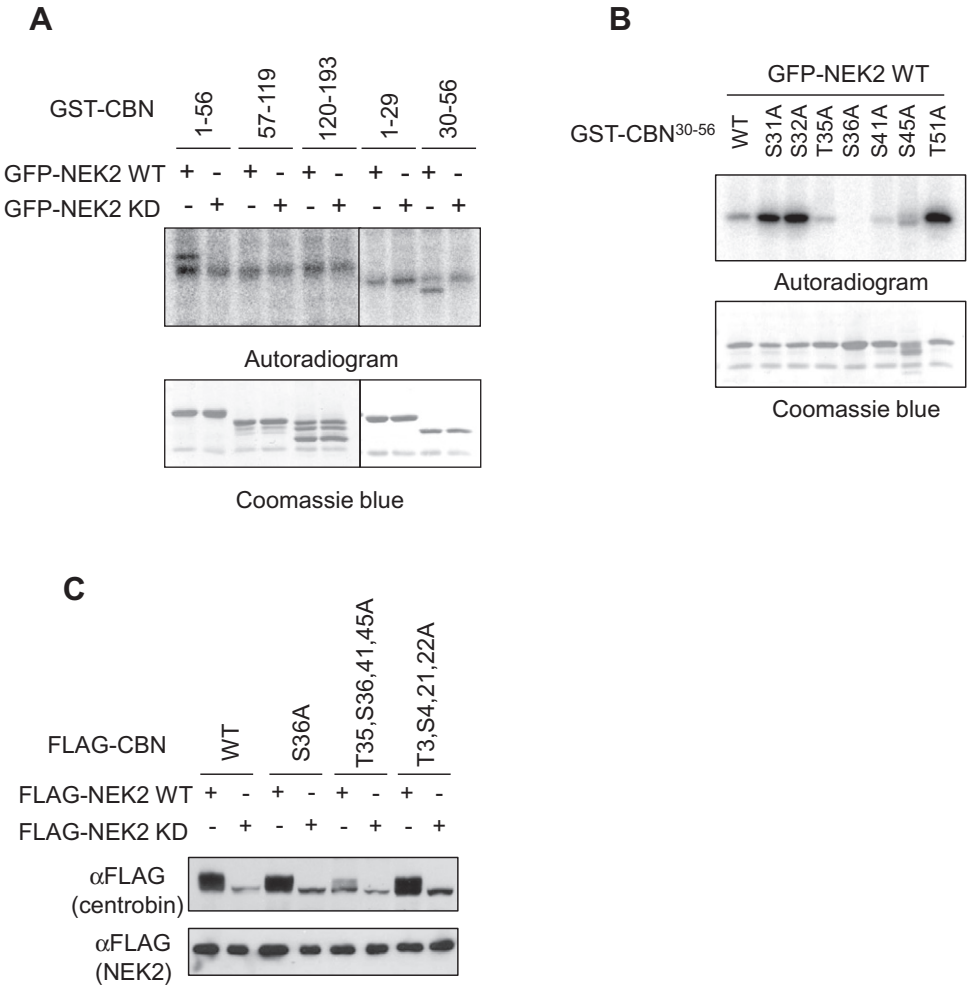

Fig. 3. NEK2 phosphorylation of centrobin in vitro and in vivo. (A) In vitro kinase assay was performed with wild type (WT) or kinase-dead (KD) form of GFP-NEK2 which was expressed in 293T cells. As substrates, truncated mutants of GST-centrobin (GST-CBN) were purified from the bacterial lysates. The amount of GST-CBN substrates was determined with Coomassie Blue staining. The phosphorylation activity of the GST-centrobin proteins was visualized by autoradiography. (B) The phospho-resistant point mutants of GST-CBN$^{30-56}$ in which each of all the serines and threonines was substituted with alanine were used as substrates. (C) Wild-type or point mutants of FLAG-CBN were expressed in 293T cells, along with wild-type or kinase-dead form of FLAG-NEK2. The mutants of FLAG-CBN include alanine substitutes at a single (S36) or four candidate phosphorylation sites for NEK2 (T35, S36, S41 and S45) and another four candidate phosphorylation sites for PLK1 (T3, S4, S21 and S22). The cell lysates were subjected to immunoblot analysis with FLAG antibody for detection of both centrobin and NEK2.

vealed that the basal levels of FLAG-CBN are comparable to those of endogenous centrobin (Fig. 4A). Furthermore, doxycycline induced the FLAG-CBN levels by twentyfold further (Fig. 4A).

We observed that the cell area of FLAG-CBN^{4A}-expressing cells significantly increased in comparison to that of FLAG-CBN-expressing cells (Fig. 4B). In addition, the cell migration ability of the FLAG-CBN^{4A}-expressing cells was enhanced by twofold in comparison to the controls (Fig. 4C). We also performed nocodazole-resistant assays with the phospho-resistant centrobin mutant. The results showed that the nocodazole-resistance of FLAG-CBN^{4A}-expressing cells increased by twofold in comparison to the control cells (Fig. 4D). These results indicate the phospho-resistant centrobin mutant stabilizes microtubule networks.

4. Discussion

Our knockdown experiments revealed that centrobin is required for cell spreading, motility and microtubule stabilization. The knockdown phenotypes of NEK2 are quite opposite to those of centrobin, suggesting that NEK2 phosphorylation antagonizes the centrobin functions. In support to this view, the phospho-resistant mutant of centrobin enhances the cell spreading, motility and microtubule stabilization.

It is interesting that centrobin is also phosphorylated by PLK1 [22]. However, unlike NEK2, PLK1 phosphorylation enhances the microtubule stabilizing activity of centrobin [22]. NLP, which functions for microtubule nucleation and anchoring in interphase cells, is also known to be phosphorylated by both NEK2 and PLK1 [24,25]. In this case, NEK2 functions as a priming kinase for PLK1 phosphorylation and helps the PLK1 action for suppression of NLP function [25]. We do not believe that NEK2 functions as a priming kinase for PLK1 in case of centrobin, because PLK1 effectively phosphorylates centrobin even in absence of active NEK2 [22]. Therefore, centrobin is a novel example that NEK2 and PLK1 independently phosphorylate a substrate and result in opposite outcomes in terms of the substrate function.

Physiological significance of NEK2 phosphorylation of centrobin remains to be investigated. Since the NEK2 activity is highest at G2 phase, NEK2 may control centrobin functions before mitosis, possibly for reorganization of microtubule networks prior to mitosis (Fig. 4E). Once cells enter mitosis, PLK1 becomes active and phos-

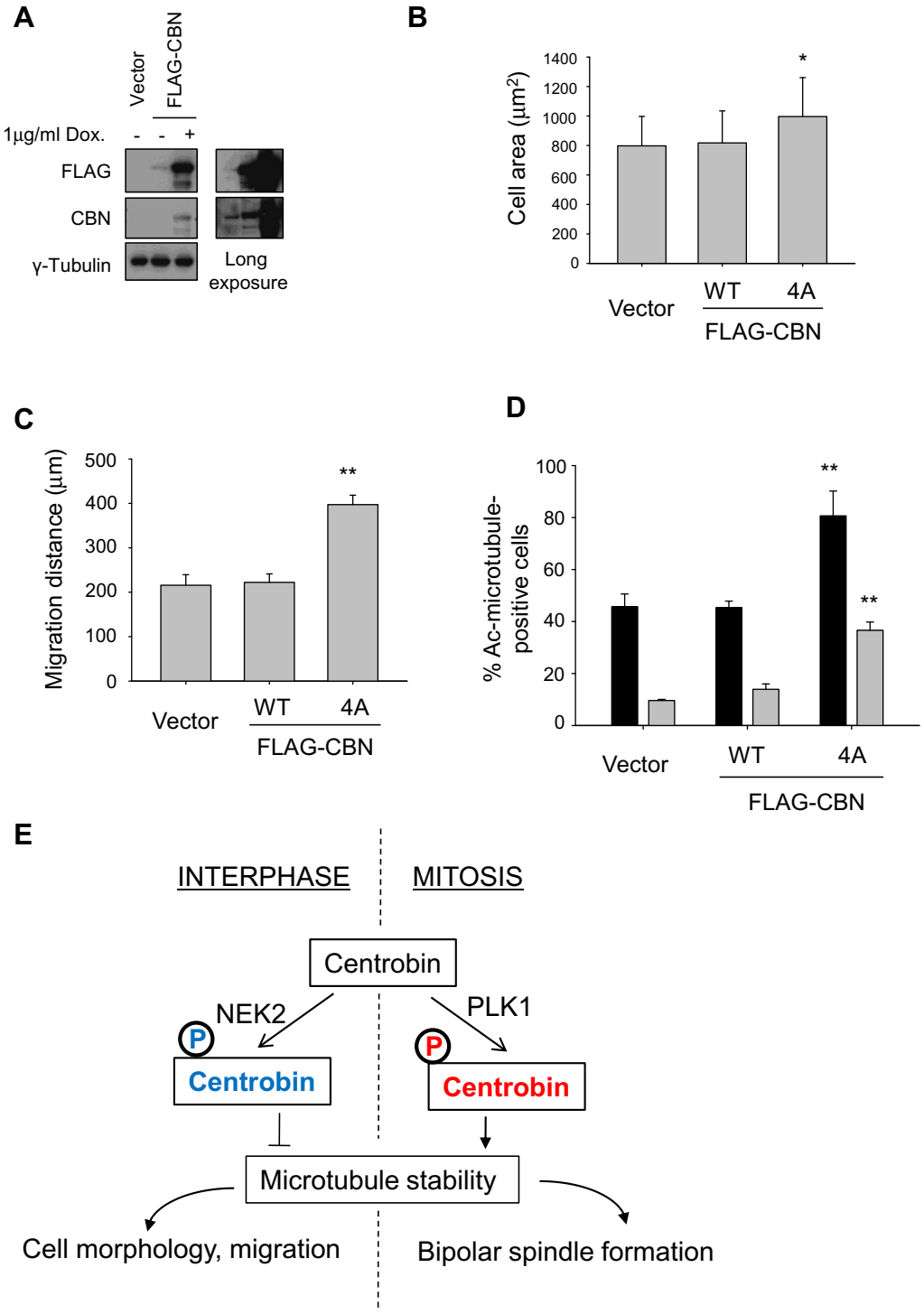

Fig. 4. Cell spreading, migration and microtubule stability in the phospho-resistant centrobin-expressing cells. (A) The tet-on HeLa cells stably expressing FLAG-CBN were subjected to immunoblot analysis with the antibodies specific to centrobin, FLAG and γ-tubulin. (B) Cell area of the FLAG-CBN-expressing cells was determined using the Image-Pro software. Over 300 cells per experimental group were measured in three independent experiments. (C) Migration of the FLAG-CBN-expressing cells was determined during 24 h period. (D) The FLAG-CBN-expressing cells were treated with 2 μM nocodazole for 0.5 h (black bar) or 1 h (gray bar), and the number of cells with residual acetylated tubulin was counted. Values are means and standard errors. $^{*}P < 0.05$; $^{**}P < 0.01$, in comparison to the control. (E) Model. In interphase cells, NEK2 phosphorylation suppresses centrobin functions for cell spreading and migration. In mitotic cells, PLK1 phosphorylation enhances centrobin functions for spindle formation.

phorylates centrobin. PLK1 phosphorylation of centrobin is critical for bipolar spindle formation (Fig. 4E). That is, centrobin may be a microtubule stabilizer whose activity is controlled by two different kinases in cell cycle stage-specific manners. However, we do not rule out the possibility that NEK2 phosphorylation of centrobin is involved in the other cellular processes than cell cycle regulation.

Acknowledgments

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References

[1] E. Schulze, M. Kirschner, Dynamic and stable populations of microtubules in cells, J. Cell Biol. 104 (1987) 277–288.
[2] G. Piperno, M. LeDizet, X.J. Chang, Microtubules containing acetylated alpha-tubulin in mammalian cells in culture, J. Cell Biol. 104 (1987) 289–302.
[3] Y. Zhai, P.J. Kronebusch, P.M. Simon, G.G. Borisy, Microtubule dynamics at the G2/M transition: abrupt breakdown of cytoplasmic microtubules at nuclear envelope breakdown and implications for spindle morphogenesis, J. Cell Biol. 135 (1996) 201–214.
[4] L. Cassimeris, Accessory protein regulation of microtubule dynamics throughout the cell cycle, Curr. Opin. Cell Biol. 11 (1999) 134–141.
[5] B. Howell, D.J. Odde, L. Cassimeris, Kinase and phosphatase inhibitors cause rapid alterations in microtubule dynamic instability in living cells, Cell Motil. Cytoskeleton 38 (1997) 201–214.
[6] A.M. Fry, L. O'Regan, S.R. Sabir, R. Bayliss, Cell cycle regulation by the NEK family of protein kinases, J. Cell Sci. 125 (2012) 4423–4433.
[7] A.M. Fry, S.J. Schultz, J. Bartek, E.A. Nigg, Substrate specificity and cell cycle regulation of the Nek2 protein kinase, a potential human homolog of the mitotic regulator NIMA of Aspergillus nidulans, J. Biol. Chem. 270 (1995) 12899–12905.
[8] A.M. Fry, T. Mayor, P. Meraldi, Y.D. Stierhof, K. Tanaka, E.A. Nigg, C-Nap1, a novel centrosomal coiled-coil protein and candidate substrate of the cell cycle-regulated protein kinase Nek2, J. Cell Biol. 141 (1998) 1563–1574.
[9] A.J. Faragher, A.M. Fry, Nek2A kinase stimulates centrosome disjunction and is required for formation of bipolar mitotic spindles, Mol. Biol. Cell 14 (2003) 2876–2889.
[10] S. Bahe, Y.D. Stierhof, C.J. Wilkinson, F. Leiss, E.A. Nigg, Rootletin forms centriole-associated filaments and functions in centrosome cohesion, J. Cell Biol. 171 (2005) 27–33.
[11] Y.H. Kim, J.Y. Choi, Y. Jeong, D.J. Wolgemuth, K. Rhee, Nek2 localizes to multiple sites in mitotic cells, suggesting its involvement in multiple cellular functions during the cell cycle, Biochem. Biophys. Res. Commun. 290 (2002) 730–736.
[12] K. Rhee, D.J. Wolgemuth, The NIMA-related kinase 2, Nek2, is expressed in specific stages of the meiotic cell cycle and associates with meiotic chromosomes, Development 124 (1997) 2167–2177.
[13] Y. Lou, J. Yao, A. Zereshki, Z. Dou, K. Ahmed, H. Wang, J. Hu, Y. Wang, X. Yao, NEK2A interacts with MAD1 and possibly functions as a novel integrator of the spindle checkpoint signaling, J. Biol. Chem. 279 (2004) 20049–20057.
[14] J. Du, X. Cai, J. Yao, X. Ding, Q. Wu, S. Pei, K. Jiang, Y. Zhang, W. Wang, Y. Shi, Y. Lai, J. Shen, M. Teng, H. Huang, Q. Fei, E.S. Reddy, J. Zhu, C. Jin, X. Yao, The mitotic checkpoint kinase NEK2A regulates kinetochore microtubule attachment stability, Oncogene 27 (2008) 4107–4114.
[15] R. Wei, B. Ngo, G. Wu, W.H. Lee, Phosphorylation of the Ndc80 complex protein, HEC1, by Nek2 kinase modulates chromosome alignment and signaling of the spindle assembly checkpoint, Mol. Biol. Cell 22 (2011) 3584–3594.
[16] S. Sonn, I. Khang, K. Kim, K. Rhee, Suppression of Nek2A in mouse early embryos confirms its requirement for chromosome segregation, J. Cell Sci. 117 (2004) 5557–5566.
[17] S. Sonn, G.T. Oh, K. Rhee, Nek2 and its substrate, centrobin/Nip2, are required for proper meiotic spindle formation of the mouse oocytes, Zygote 19 (2011) 15–20.
[18] C. Zou, J. Li, Y. Bai, W.T. Gunning, D.E. Wazer, V. Band, Q. Gao, Centrobin: a novel daughter centriole-associated protein that is required for centriole duplication, J. Cell Biol. 171 (2005) 437–445.
[19] R. Gudi, C. Zou, J. Li, Q. Gao, Centrobin–tubulin interaction is required for centriole elongation and stability, J. Cell Biol. 193 (2011) 711–725.
[20] Y. Jeong, J. Lee, K. Kim, J.C. Yoo, K. Rhee, Characterization of NIP2/centrobin, a novel substrate of Nek2, and its potential role in microtubule stabilization, J. Cell Sci. 120 (2007) 2106–2116.
[21] J.M. Jeffery, A.J. Urquhart, V.N. Subramaniam, R.G. Parton, K.K. Khanna, Centrobin regulates the assembly of functional mitotic spindles, Oncogene 29 (2010) 2649–2658.
[22] J. Lee, Y. Jeong, S. Jeong, K. Rhee, Centrobin/NIP2 is a microtubule stabilizer whose activity is enhanced by PLK1 phosphorylation during mitosis, J. Biol. Chem. 285 (2010) 25476–25484.
[23] S. Khawaja, G.G. Gundersen, J.C. Bulinski, Enhanced stability of microtubules enriched in detyrosinated tubulin is not a direct function of detyrosination level, J. Cell Biol. 106 (1988) 141–149.
[24] M. Casenghi, P. Meraldi, U. Weinhart, P.I. Duncan, R. Körner, E.A. Nigg, Polo-like kinase 1 regulates Nlp, a centrosome protein involved in microtubule nucleation, Dev. Cell 5 (2003) 113–125.
[25] J. Rapley, J.E. Baxter, J. Blot, S.L. Wattam, M. Casenghi, P. Meraldi, E.A. Nigg, A.M. Fry, Coordinate regulation of the mother centriole component nlp by nek2 and plk1 protein kinases, Mol. Cell Biol. 25 (2005) 1309–1324.