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NEK7 is a centrosomal kinase critical for microtubule nucleation

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Abstract

NIMA in *Aspergillus nidulans* is a mitotic kinase for chromosome condensation and segregation. We characterized NEK7, a human homologue of *Aspergillus* NIMA. NEK7 was located at the centrosome throughout the cell cycle. Temporal localization of NEK7 at midbody of the cytokinetic cell was also observed. NEK7 knockdown by RNAi caused a prometaphase arrest of the cell cycle with monopolar or disorganized spindle. We propose that such defects in spindle assembly are resulted from reduction in microtubule nucleation activity at the centrosome. In consistent to the proposal, we observed a decrease in the centrosomal γ-tubulin levels and reduction of the microtubule re-growth activity in the *NEK7*-suppressed cells. In addition, it was evident that NEK7 was directly involved in cytokinesis.

Keywords: NEK7; Mitotic kinases; Centrosome; Spindle assembly; Microtubule nucleation; Cytokinesis

Phosphorylation is one of the key regulatory mechanisms for execution of a series of mitotic events. Once Cdc2 is activated at the onset of mitosis, several other serine/threonine kinases, such as Aurora, NIMA-related (NEK) and Polo-like (PLK) kinases are accompanied for mitotic progression. Such mitotic kinases are often multifunctional. For example, PLK1 is implicated in diverse mitotic functions, including centrosome maturation, bipolar spindle assembly, sister chromatid cohesion, anaphase-promoting complex/cyclosome activation, and cytokinesis [1]. To carry out these functions, PLK1 phosphorylates multiple substrates at the sites of action, including centrosome, kinetochore, and midbody. Furthermore, the mitotic kinases are functionally interactive so that a kinase is activated by phosphorylation with another kinase, or that two kinases share a substrate for an action [2]. Mitotic kinases are often linked to tumorigenesis as their overexpression was frequently observed in solid human tumor tissues [3].

NEK kinases were initially identified as human homologues of the *Aspergillus* NIMA, and so far 11 *NEK*s have been identified in the human genome [4,5]. As *Apsergillus* NIMA was known as a mitotic kinase for chromosome condensation and segregation [6,7], human NEKs have been also suspected to be involved in mitotic events [5]. Perhaps NEK2 is most extensively studied among the NEK family proteins. NEK2 is centrosomal and promotes the splitting of duplicated centrosomes at the onset of mitosis [8]. NEK2 is also present outside of the centrosome, and involved in other mitotic events, such as spindle assembly and checkpoint [9–12]. Other NEK family proteins have been investigated to some extents. Cumulative works suggested that NEK6, NEK7, and NEK9 are involved in mitosis while NEK1 and NEK8 are linked to polycystic kidney disease [13–17].

In this paper, we investigated biological functions of NEK7. The results showed that NEK7 was centrosomal and critical for spindle assembly at prometaphase and for cytokinesis.

Materials and methods

Antibodies. Polyclonal NEK7 antiserum was raised against the GST-NEK7 fusion protein. The NEK7 antibody was affinity purified by incubation of the antiserum with a strip of nitrocellulose membrane blotted with the NEK7 fusion protein and eluted with 100 mM glycine, pH 2.5.

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The antibodies against α-tubulin (Sigma), β-tubulin (Sigma), γ-tubulin (Sigma), the Myc tag (Cell Signaling), GFP (Cell Signaling), Aurora B (BD Biosciences), NEK2 (Transduction Laboratories), PLK1 (Zymed), and phospho-histone H3 (Upstate) were purchased.

Cell culture, transfection, flow cytometry, immunoblot, and immunocytochemistry. HeLa, U2OS, and 293T cells were cultured in DMEM with 10% fetal bovine serum. The cells were treated with 2 mM thymidine and 100 ng/ml nocodazole for S and M phase arrest, respectively. Transient transfection into HeLa or 293T cells was carried out using Lipofectamine Plus reagent (Invitrogen). Flow cytometry, immunoblot and immunocytochemistry were carried out as described previously [18].

Microtubule re-growth assay. Cells were placed on ice for 30 min to disrupt cellular microtubule organization, and then transferred to a 37 °C environment to initiate microtubule growth. Twenty seconds later, the cells were fixed and subjected to immunocytochemistry.

RNA interference. For RNAi suppression, siRNAs specific to *NEK7* (CTCCGACAGTTAGTTAATATT), *NEK2* (AAGCGGGACTTCCGCACATAC), and *PLK1* (GCGGGACTTCCGCACATACTT) were transfected into HeLa cells with oligofectAMINE (Invitrogen) according to the manufacture's instructions. Forty-eight hours after transfection, the cells were fixed for immunocytochemistry or prepared for immunoblot or FACS analysis. Non-specific control siRNA (AAGTAGCCGAGCTTCGATTGC) was also prepared.

Results

Expression and subcellular localization of NEK7

We raised a polyclonal antibody against bacterially expressed GST-NEK7 fusion protein and affinity-purified. Immunoblot analysis was carried out with the HeLa cells in which Myc-NEK7 or GFP-NEK7 was ectopically expressed. The results showed that the NEK7 antibody detected not only the ectopic NEK7 proteins but also the endogenous NEK7 protein of 34 kDa in size (Fig. 1A).

The NEK7 protein levels were determined in the HeLa cells whose cell cycle was arrested at S and M phases with treatment of thymidine and nocodazole, respectively. The results showed that the cellular NEK7 levels were more or less constant throughout the cell cycle (Fig. 1B).

When the NEK7 antibody was used for immunostaining U2OS cells, it detected the centrosome distinctly. NEK7 was present at centrosome throughout the cell cycle (Fig. 1C). In addition, the NEK7 signal was also detected at spindle midzone of the anaphase cells and eventually concentrated at the midbody (Fig. 1C, Supplementary Fig. 1). Presence of NEK7 in cytoplasm was also evident, since its diffused cytoplasmic signal disappeared in the *NEK7*-suppressed cells (Fig. 2A). Such subcellular localization of NEK7 suggests its functional link to the centrosome and mitosis.

NEK7 is required for the microtubule nucleation activity of the centrosome

We transfected siRNAs specific to *NEK7* or other related kinases, such as *PLK1* and *NEK2*, and observed that their expression was suppressed efficiently (Supplementary Fig. 2). At the same time, we observed increases

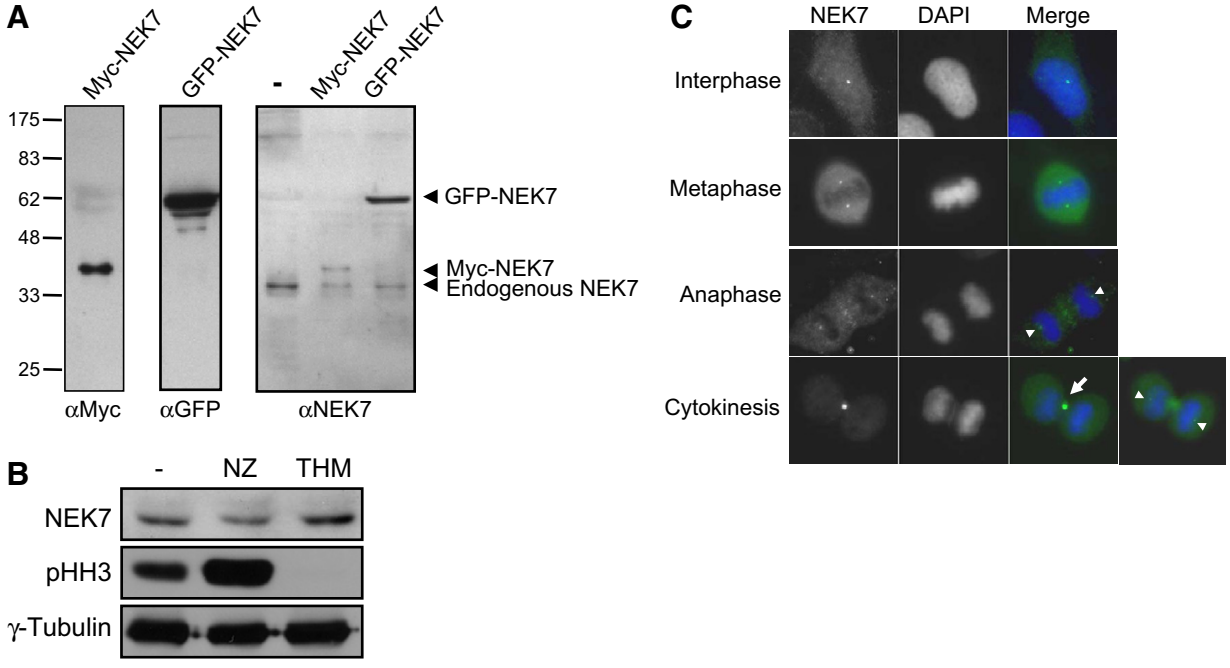

Fig. 1. Expression and subcellular localization of NEK7. (A) HeLa cells were transfected with *pMyc-NEK7* or *pGFP-NEK7*. Twenty-four hours after transfection, the cell lysates were prepared and subjected to immunoblot analysis with antibodies specific to NEK7, GFP, and the Myc tag. The endogenous NEK7 was detected along with the ectopic NEK7 proteins. (B) The HeLa cells were treated with 100 ng/ml nocodazole (NZ) for M phase arrest or with 2 mM thymidine (THM) for S phase arrest. The cell lysates were then prepared and subjected to immunoblot analysis with antibodies specific to NEK7, phospho-histone H3 (pHH3), and γ-tubulin. (C) The U2OS cells were immunostained with the NEK7 antibody (green). DNA was stained with DAPI. Arrowheads and an arrow indicated spindle poles and the spindle midzone, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

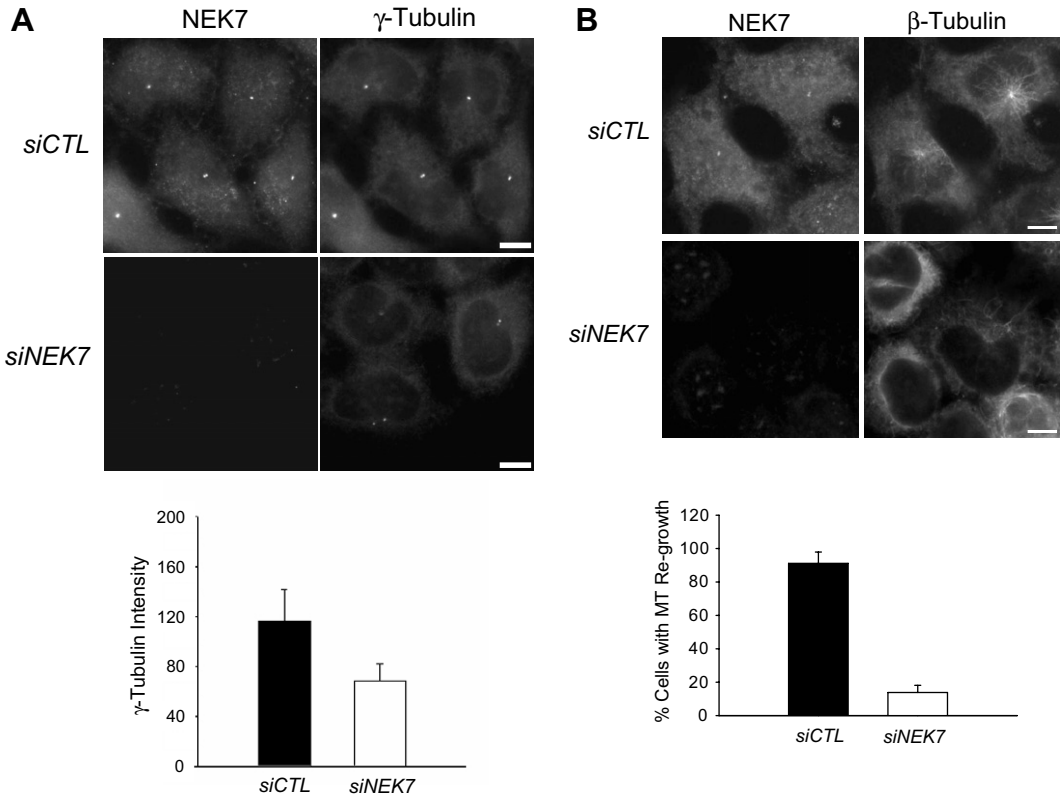

Fig. 2. Reduction of the microtubule nucleation activity in the *NEK7*-suppressed cells. (A) The *NEK7*-suppressed HeLa cells were stained with antibodies specific to NEK7 and γ-tubulin. The relative amount of the centrosomal γ-tubulin was measured by the immunostaining intensities. For statistical analyses, the experiments were repeated three times and more than 20 cells were used per each experimental group. The results were shown as means and standard errors. (B) The microtubule re-growth assay was carried out with the *NEK7*-suppressed HeLa cells. The cells were immunostained with antibodies specific to NEK7 and β-tubulin. For statistical analysis, the assay was repeated three times. Scale bars, 10 μm.

in the phospho-histone H3 (pHH3) levels in the *PLK1*- and *NEK7*-suppressed cells, indicating that the cell cycle of these population is accumulated at M phase.

We co-immunostained the *NEK7*-suppressed cells with antibodies specific to γ-tubulin and NEK7. As expected, the NEK7 signal disappeared below a detection level at the centrosome and cytoplasm of the *NEK7*-suppressed cells (Fig. 2A). At the same time, the centrosomal γ-tubulin levels were reduced by 60% of control, suggesting that NEK7 is involved in γ-tubulin recruitment into the centrosome (Fig. 2A). Next, we examined the microtubule nucleation activity in the *NEK7*-suppressed cells. Results from the microtubule re-growth assay indicated that the microtubule nucleation activity was reduced significantly in the *NEK7*-suppressed cells (Fig. 2B).

NEK7 is required for bipolar spindle formation in mitotic cells

FACS analysis revealed an increase of the 4N population in both *NEK7*- and *PLK1*-suppressed cells (Supplementary Fig. 3A). As indicated by the pHH3-positive cell population as well as by microscopic observation, increases in the M phase cells were also accompanied (Supplementary Fig. 3A and B). These results suggest that both NEK7 and PLK1 are required for M phase progression of the cell cycle.

Majority of the mitotic cells in the *NEK7* knockdown population appeared to be arrested at prometaphase with nuclear envelope breakdown and chromosome condensation (Fig. 3A). We were able to detect a pair of centrosomes at the *NEK7*-suppressed cells at M phase. However, the centrosomes were positioned in the middle of the cells next to each other without visible spindle irradiations. As a result, condensed chromosomes became scattered throughout the cell (Fig. 3A). Such phenotypes were comparable to the *PLK1*-suppressed cells of which the cell cycle was arrested at prometaphase with monopolar and disorganized spindles in an equal odd [19,20] (Fig. 3B). Since both monopolar and disorganized spindles were resulted from reduction in γ-tubulin recruitment and centrosomal spindle irradiation [19,20], it is likely that the *NEK7*- as well as *PLK1*-suppressed cells had defects in microtubule nucleation activity of the centrosome.

Another distinct phenotypes observed in the *NEK7*-suppressed cells are presence of the multinucleated cells (Supplementary Fig. 4). Each cell contains several or more small nuclei with diffused chromatin. The multinucleation is probably a consequence of the aberrant mitotic exit without appropriate chromosome segregation and cytokinesis

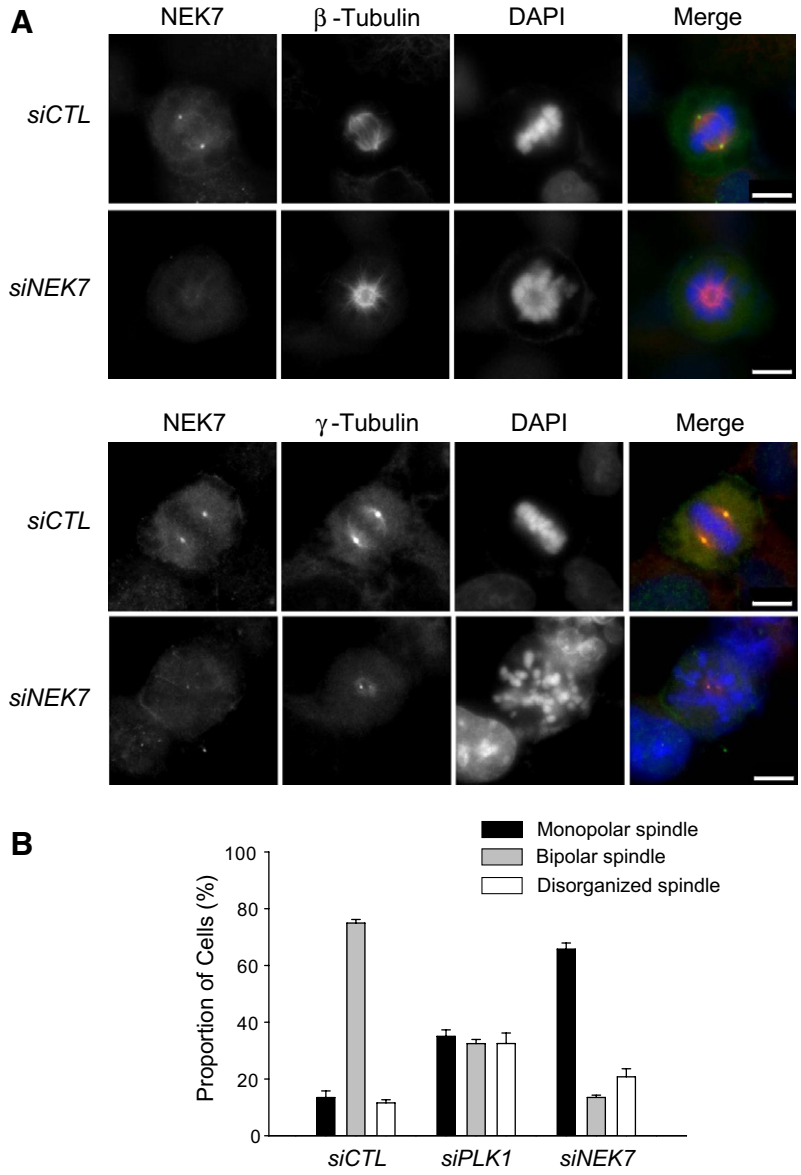

Fig. 3. *NEK7*-suppression induced mitotic arrest with defects in bipolar spindle assembly. (A) The HeLa cells were transfected with *siCTL* or *siNEK7*. Forty-eight hours later, the cells were immunostained with antibodies specific to NEK7 (green) and β- or γ-tubulins (red). DNA was stained with DAPI. Scale bars, 10 μm. (B) Statistical analysis of the phenotypes of the HeLa cells transfected with *siCTL*, *siPLK1* or *siNEK7*. The experiments were repeated three times and more than 50 mitotic cells were used per each experimental group. The results were presented as means and standard errors. Black, gray and blank bars represent monopolar, bipolar, and irregular spindles, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

[21]. Individual mininucleus would contain a misaligned clump of chromosomes. Such multinucleated cells have been observed in cells whose mitosis was arrested with a low concentration of paclitaxel, causing mitotic exit without cytokinesis [21,22]. Therefore, it is suspected that a fraction of the *NEK7*-suppressed cells somehow escaped from the prometaphase arrest and ended up forming the multinuclear cells.

Direct involvement of NEK7 in cytokinesis

In the previous experiments, we observed that *NEK7* knockdown resulted in accumulation of the prometaphase cells, due to the failure in bipolar spindle formation. In order to determine additional phenotypes of the *NEK7*-suppressed cell, we removed the spindle checkpoint by depleting the MAD2 protein simultaneously. As a result, the pHH3 levels were reduced significantly in the *NEK7*/*MAD2* double knockdown cells, indicating that the mitotic cell population was reduced by passing the spindle checkpoint and exiting mitosis (Supplementary Fig. 5A). A similar phenomenon was also observed in the *PLK1*/*MAD2* double knockdown cells [20] (Supplementary Fig. 5A). In consistent, microscopic observations revealed reduction in mitotic cells in the double knockdown populations (Supplementary Fig. 5B). These results suggest that the mitotic

arrest in both *NEK7*- and *PLK1*-suppressed cells were attributed, in part, to spindle checkpoint [20].

Immunocytochemical analysis revealed the presence of abnormal nuclei in the *NEK7*/*MAD2* and *PLK1*/*MAD2* double knockdown cells (Fig. 4A). Irregular shaped nuclei and multiple nuclei were observed frequently in both populations. Abnormal shapes of nuclei were also observed in the *MAD2*-suppressed cells, as reported previously [23]. However, nuclear abnormality was severer and more frequent in the double knockdown cells than in the *MAD2* single knockdown cells, indicating that depletion of NEK7 or PLK1 is responsible for the phenotype to some extents (Fig. 4A).

We determined cell cycle stages of the double knockdown cells. As shown previously, *NEK7*- or *PLK1*-suppression induced accumulation of the 4N cells, probably due to the prometaphase arrest with defects in spindle assembly (Supplementary Fig. 5C). The cell cycle profile of the *MAD2*-suppressed population looked more or less normal [23,24] (Supplementary Fig. 5C). In the *NEK7*/*MAD2* double knockdown population, the number of pHH3-positive cells was reduced to a normal range while that of 4N population was not affected (Supplementary Fig. 5C). Similar results were also observed in the *PLK1*/*MAD2* double knockdown population, suggesting that the double knockdown cells exited the mitosis, but failed cytokinesis.

The *PLK1*/*MAD2* or *NEK7*/*MAD2* double knockdown cells were immunostained with an antibody specific to Aurora B, a marker for the cytokinetic process [25]. The results showed that *NEK7*/*MAD2* double knockdown cells underwent cytokinesis with a much less frequency than the *PLK1*/*MAD2* double knockdown cells did (Fig. 4B). Furthermore, we observed morphological differences between the double knockdown cells. In *PLK1*/*MAD2*-suppressed cells, nuclear division occurred in an asymmetric manner, as reported previously [20] (Fig. 4B). On the other hand, the *NEK7*/*MAD2*-suppressed cells did not enter cytokinesis but formed abnormal nuclei, similar to the *Aurora B*-suppressed cells [26,27]. These results suggest direct involvement of NEK7 in cytokinesis.

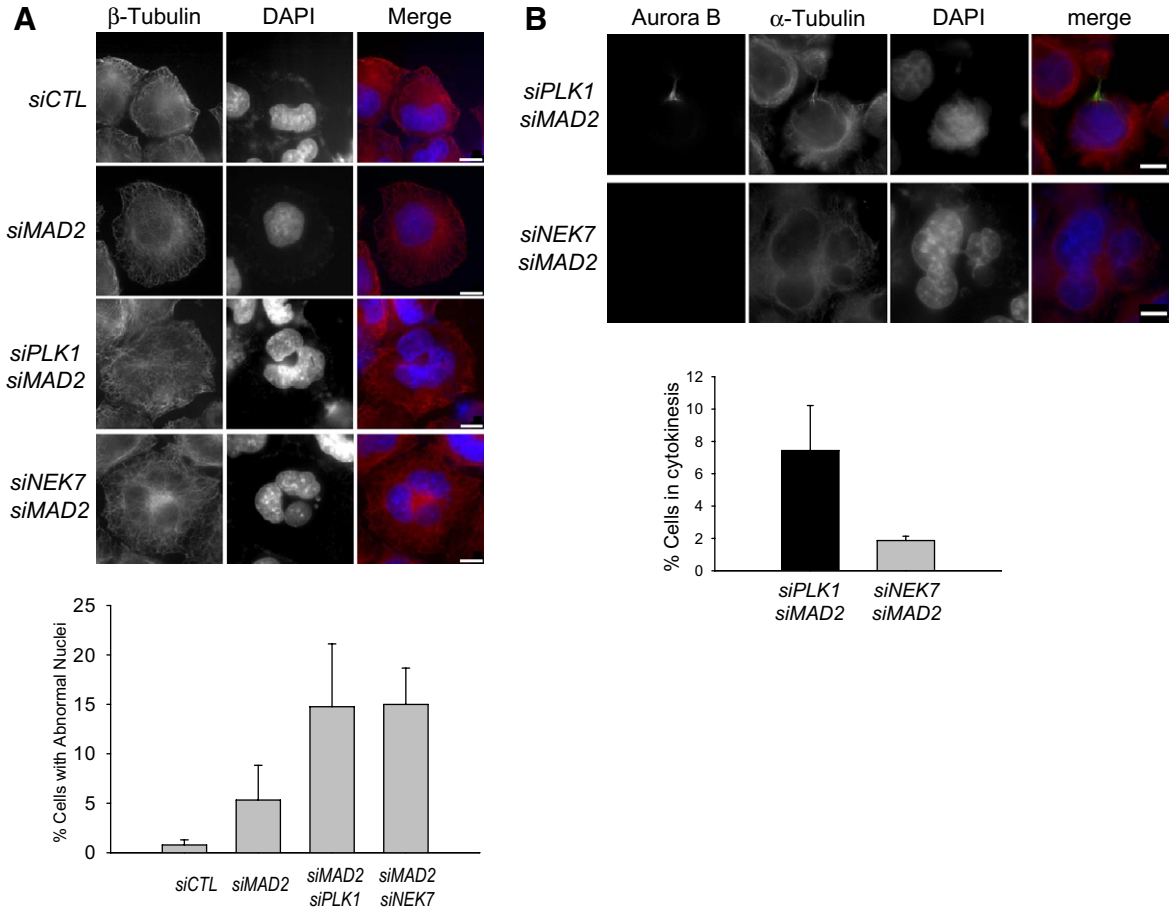

Fig. 4. Direct involvement of NEK7 in cytokinesis. (A) The HeLa cells were transfected with siRNAs specific to *NEK7* or *PLK1*, along with *siMAD2*. Forty-eight hours later, the cells were immunostained with the β-tubulin antibody (red). DNA was stained with DAPI. Scale bars, 10 μm. The cells with abnormal nuclei were counted and analyzed statistically with three independent experiments. The results were presented as means and standard errors. (B) The HeLa cells were transfected with siRNAs specific to *NEK7* or *PLK1*, along with *siMAD2*. Forty-eight hours later, the cells were co-immunostained with antibodies specific to Aurora B and α-tubulin. Number of cells at cytokinesis was counted and compared. For statistical analysis, the experiments were repeated three times and more than 50 cells were used per each experimental group. The results were presented as means and standard errors. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Discussion

In the present study, we observed that NEK7 was located at the centrosome throughout the cell cycle. Temporal localization of NEK7 at the midbody of the cytokinetic cells was also evident. A similar subcellular distribution has been reported in other NEK family proteins. For example, NEK2 exhibited dynamic changes in its distribution during the cell cycle but was detected at the centrosome persistently and also at the midbody of the mitotic cells [8,10]. A phosphorylated form of NEK9 was centrosomal [15]. In fact, *Aspergillus* NIMA, the founding member of the NEK family proteins, was detected at the mitotic spindle and spindle pole bodies [7]. These results suggest that NIMA and NEK family proteins share not only structural homology but also functional resemblance in relation to microtubule organizing and cilia formation [5].

The *NEK7*-suppressed cells were accumulated at prometaphase with monopolar or disorganized spindle. Yissachar et al. also observed a similar phenotype at prometaphase with multipolar spindle in the NEK7-depleted cells [14]. We believe that such defects in spindle assembly are resulted from reduction in microtubule nucleation activity, as evidenced by reduction of the centrosomal γ-tubulin levels [28]. NEK7 is also required for the microtubule nucleation in interphase cells as evidenced by the microtubule re-growth assay. However, the most visible phenotype would be defects in spindle assembly when the highest microtubule nucleation activity was required throughout the cell cycle [29].

So far PLK1 [19,20], Aurora A [30,31], NEK2 [12], and, recently, CDK11 [32] have been known to be located at the centrosome and control the microtubule nucleation and anchoring activity. Depletion of these kinases usually resulted in prometaphase arrest with defects in spindle assembly. Reduction in the centrosomal γ-tubulin levels were typically accompanied. In the present study, we added NEK7 to the list of the centrosomal kinases. It is likely that phosphorylation is an important mechanism in regulating microtubule nucleation and anchoring at the centrosome.

Defects in spindle assembly were also observed when a number of centrosomal protein components were depleted. Therefore, it is likely that centrosomal kinases regulate biological functions of these centrosomal proteins for microtubule nucleation at mitosis. In fact, a limited number of the centrosomal proteins have been identified as substrates of the centrosomal kinases. For example, NLP is involved in microtubule nucleation in interphase cells and it is phosphorylated by NEK2 and PLK1 sequentially [33,34]. TACC3 and Eg5 are known to be substrates of Aurora A and play roles in γ-tubulin recruitment [30,35,36]. Nonetheless, the current information is not sufficient to elucidate whole regulatory mechanisms for microtubule nucleation and anchoring in the centrosome. It is helpful if molecular identity of the NEK7 substrates is discovered.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.05.206.

References

[1] F.A. Barr, H.H. Sillje, E.A. Nigg, Polo-like kinases and the orchestration of cell division, Nat. Rev. Mol. Cell Biol. 5 (2004) 429–440.
[2] J.J. Li, S.A. Li, Mitotic kinases: the key to duplication, segregation, and cytokinesis errors, chromosomal instability, and oncogenesis, Pharmacol. Ther. 111 (2006) 974–984.
[3] S.L. Carter, A.C. Eklund, I.S. Kohane, L.N. Harris, Z. Szallasi, A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers, Nat. Genet. 38 (2006) 1043–1048.
[4] S.J. Schultz, E.A. Nigg, Identification of 21 novel human protein kinases, including 3 members of a family related to the cell cycle regulator *nimA* of *Aspergillus nidulans*, Cell Growth Differ. 4 (1994) 821–830.
[5] L.M. Quarmby, M.R. Mahjoub, Caught Nek-ing: cilia and centrioles, J. Cell Sci. 118 (2005) 5161–5169.
[6] A.H. Osmani, S.L. McGuire, S.A. Osmani, Parallel activation of the NIMA and $p34^{cdc2}$ cell cycle-regulated protein kinases is required to initiate mitosis in *A. nidulans*, Cell 67 (1991) 283–291.
[7] C.P. De Souza, A.H. Osmani, L.P. Wu, J.L. Spotts, S.A. Osmani, Mitotic histone H3 phosphorylation by the NIMA kinase in *Aspergillus nidulans*, Cell 102 (2000) 293–302.
[8] A.M. Fry, T. Mayor, P. Meraldi, E.A. Nigg, A centrosomal function for the human Nek2 protein kinase, a member of the NIMA family of cell cycle regulators, EMBO J. 17 (1998) 470–481.
[9] 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.
[10] 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.
[11] Y. Chen, D.J. Riley, L. Zheng, P.L. Chen, W.H. Lee, Phosphorylation of the mitotic regulator protein Hec1 by Nek2 kinase is essential for faithful chromosome segregation, J. Biol. Chem. 277 (2002) 49408–49416.
[12] 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.
[13] M.J. Yin, L. Shao, D. Voehringer, T. Smeal, B. Jallal, The serine/threonine kinase Nek6 is required for cell cycle progression through mitosis, J. Biol. Chem. 278 (2003) 52454–52460.
[14] N. Yissachar, H. Salem, T. Tennenbaum, B. Motro, Nek7 kinase is enriched at the centrosome, and is required for proper spindle assembly and mitotic progression, FEBS Lett. 580 (2006) 6489–6495.
[15] J. Roig, A. Groen, J. Caldwell, J. Avruch, Active Nercc1 protein kinase concentrates at centrosomes early in mitosis and is necessary for proper spindle assembly, Mol. Biol. Cell 16 (2005) 4827–4840.

[16] P. Upadhya, E.H. Birkenmeier, C.S. Birkenmeier, J.E. Barker, Mutations in a NIMA-related kinase gene, *Nek1*, cause pleiotropic effects including a progressive polycystic kidney disease in mice, Proc. Natl. Acad. Sci. USA 97 (2000) 217–221.
[17] S. Liu, W. Lu, T. Obara, S. Kuida, J. Lehoczky, K. Dewar, I.A. Drummond, D.R. Beier, A defect in a novel Nek-family kinase causes cystic kidney disease in the mouse and in zebrafish, Development 129 (2002) 5839–5846.
[18] 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.
[19] I. Sumara, J.F. Gimenez-Abian, D. Gerlich, T. Hirota, C. Kraft, C. de la Torre, J. Ellenberg, J.M. Peters, Roles of Polo-like kinase 1 in the assembly of functional mitotic spindles, Curr. Biol. 14 (2004) 1712–1722.
[20] M.A.T.M. van Vugt, B.C.M. van de Weerdt, G. Vader, H. Janssen, J. Calafat, R. Klompmaker, R.M.F. Wolthuis, R.H. Madema, Polo-like kinase 1 is required fro bipolar spindle formation but is dispensible for anaphase promoting complex/Cdc20 activation and initiation of cytokinesis, J. Biol. Chem. 279 (2004) 36841–36854.
[21] R. Panvichian, K. Orth, M.L. Day, K.C. Day, M.J. Pilat, K.J. Pienta, Paclitaxel-associated multimininucleation is permitted by the inhibition of caspase activation: A potential early step in drug resistance, Cancer Res. 58 (1998) 4667–4672.
[22] M.V. Blagosklonny, Z. Darzynkiewicz, H.D. Halicka, P. Pozarowski, Z.N. Demidenko, J.J. Barry, K.R. Kamath, R.A. Herrmann, Paclitaxel induces primary and postmitotic G1 arrest in human arterial smooth muscle cells, Cell Cycle 3 (2004) 1050–1056.
[23] G.J. Kops, D.R. Foltz, D.W. Cleveland, Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint, Proc. Natl. Acad. Sci. USA 101 (2004) 8699–8704.
[24] T. Sudo, M. Nitta, H. Saya, N.T. Ueno, Dependence of paclitaxel sensitivity on a functional spindle assembly checkpoint, Cancer Res. 64 (2004) 2502–2508.
[25] M. Carmena, W.C. Earnshaw, The cellular geography of aurora kinases, Nat. Rev. Mol. Cell Biol. 4 (2003) 842–854.
[26] Y. Terada, M. Tatsuka, F. Suzuki, Y. Yasuda, S. Fujita, M. Otsu, AIM-1: a mammalian midbody-associated protein required for cytokinesis, EMBO J. 17 (1998) 667–676.
[27] B. Delaval, A. Ferrand, N. Conte, C. Larroque, D. Hernandez-Verdun, C. Prigent, D. Birnbaum, Aurora B-TACC1 protein complex in cytokinesis, Oncogene 23 (2004) 4516–4522.
[28] J.G. Ault, C.L. Rieder, Centrosome and kinetochore movement during mitosis, Curr. Opin. Cell Biol. 6 (1994) 41–49.
[29] S. Gadde, R. Heald, Mechanisms and molecules of the mitotic spindle, Curr. Biol. 14 (2004) R797–R805.
[30] R. Giet, D. McLean, S. Descamps, M.J. Lee, J.W. Raff, C. Prigent, D.M. Glover, *Drosophila* Aurora A kinase is required to localize D-TACC to centrosomes and to regulate astral microtubules, J. Cell Biol. 156 (2002) 437–451.
[31] Q. Liu, J.V. Ruderman, Aurora A, mitotic entry, and spindle bipolarity, Proc. Natl. Acad. Sci. USA 103 (2006) 5811–5816.
[32] C. Petretti, M. Savoian, E. Montembault, D.M. Glover, C. Prigent, R. Giet, The PITSLRE/CDK11^{p58} protein kinase promotes centrosome maturation and bipolar spindle formation, EMBO Rep. 7 (2006) 418–424.
[33] M. Casenghi, P. Meraldi, U. Weinhart, P.I. Duncan, R. Korner, E.A. Nigg, Polo-like kinase 1 regulates Nlp, a centrosome protein involved in microtubule nucleation, Dev. Cell 5 (2003) 113–125.
[34] 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.
[35] R. Giet, R. Uzbekov, F. Cubizolles, K. Le Guellec, C. Prigent, The *Xenopus laevis* aurora-related protein kinase pEg2 associates with and phosphorylates the kinesin-related protein XlEg5, J. Biol. Chem. 274 (1999) 15005–15013.
[36] K. Kinoshita, T.L. Noetzel, L. Pelletier, K. Mechtler, D.N. Drechsel, A. Schwager, M. Lee, J.W. Raff, A.A. Hyman, Aurora A phosphorylation of TACC3/maskin is required for centrosome-dependent microtubule assembly in mitosis, J. Cell Biol. 170 (2005) 1047–1055.