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The tumor suppressor proteins ASPP1 and ASPP2 interact with C-Nap1 and regulate centrosome linker reassembly

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ABSTRACT

Centrosome linker tethers interphase centrosomes together allowing them to function as a single microtubule organization center. The centrosome linker is disrupted at the onset of mitosis to ensure timely centrosome disjunction and bipolar spindle formation and is reassembled at the end of mitosis. While the mechanism controlling centrosome linker disassembly at early mitosis has been well explored, little is known about how the linker is subsequently reassembled before mitotic exit. Here we report that ASPP1 and ASPP2, two members of the apoptosis stimulating proteins of p53 (ASPP) family, are involved in centrosome linker reassembly. We showed that ASPP1/2 interacted with centrosome linker protein C-Nap1. Co-depletion of ASPP1 and ASPP2 inhibited re-association of C-Nap1 with centrosome at the end of mitosis. Moreover, ASPP1/2 facilitated the interaction between C-Nap1 and PP1α, and this interaction was significantly reduced by co-depletion of ASPP1/2. ASPP1/2 antagonized the NEK2A-mediated C-Nap1 Ser2417/2421 phosphorylation in a PP1-dependent manner. Co-depletion of ASPP1 and ASPP2 inhibited dephosphorylation of C-Nap1 (Ser2417/2421) at the end of mitosis. Based on these findings, we propose that ASPP1/2 act as PP1-targeting subunits to facilitate C-Nap1 dephosphorylation and centrosome linker reassembly at the end of mitosis.

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1. Introduction

Centrosome, the primary microtubule-organizing center (MTOC) in mammalian cells, determines the geometry of microtubule arrays throughout the cell cycle, and thus is crucial for mitotic spindle formation and cell division [1]. At the core of a typical interphase centrosome are two centrioles, which recruit a matrix of associated pericentriolar materials (PCM) [2]. From G1 to late G2, the cell's two centrosomes are physically connected by a flexible, loosely organized, and dynamic centrosome linker named "G1–G2 tether" [3]. To facilitate centrosome separation at mitotic entry, this linker is disassembled in a process known as centrosome disjunction [3]. At the end of mitosis, the centrosome linker is reassembled to ensure normal interphase centrosome function as a single microtubule-organization center, and to prepare for next cycle of centrosome duplication [4].

Several proteins have been identified as the components of centrosome linker, including C-Nap1 [5,6], rootletin [7,8], CEP68 and CEP215 [9], conductin [10], and LRRC45 [11]. Depletion of any of these proteins results in loss of centrosome cohesion and premature centrosome separation in interphase [6,7,9]. Among them, C-Nap1 is localized to the proximal ends of centrioles and function as a docking site to attach rootletin, Cep68, LRCC45 and centlein to the rootlet emanating from the parental centriole. Linker disassembly and centrosome disjunction is tightly regulated by phosphorylation [4]. At the onset of mitosis, C-Nap1 and rootletin are phosphorylated and displaced from centrosome by NEK2A kinase, thus triggering linker disassembly and centrosome disjunction [7,12,13]. However, the mechanism controlling centrosome linker reassembly remains largely unknown.

ASPP1 and ASPP2 are two members of the ASPP (Apoptosis Stimulating Proteins of p53) family proteins. The best-known function of ASPP is to regulate the apoptosis function of p53 and

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other p53 family member p63 and p73 [14]. ASPP1 and ASPP2 enhance, while iASPP inhibits p53 (or p63, p73)-mediated apoptosis. There is also evidence indicating that ASPP1 and ASPP2 have p53-independent cellular functions. For example, ASPP2 has been shown to contribute to the maintenance of polarity [15] and promote oncogene-induced senescence [16].

Our study identified ASPP1/2 as potential interactors of C-Nap1 and verified both ASPP1 and ASPP2 interacted with C-Nap1 in vivo. Further studies demonstrated ASPP1/2 were required for C-Nap1 re-accumulation at centrosome to ensure linker reassembly. Moreover, we showed that ASPP1/2 recruited PP1α to dephosphorylate C-Nap1 and were required for C-Nap1 dephosphorylation at the end of mitosis.

2. Materials and methods

2.1. Cell culture

293T and HeLa cells were obtained from the American Type Culture Collection (ATCC) and cultured in Dulbeco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) Fetal Bovine Serum (FBS) at 37 °C with 5% CO_2.

2.2. Plasmid construction

The ASPP2 cDNA was kindly provided by Dr. Xin Lu (University of Oxford). The ASPP1 cDNA was obtained from Genecopoeia Inc. The GFP-C-Nap1 cDNA was kindly provided by Dr. Erich A. Nigg (Max-Planck-Institute for Biochemistry). ASPP1 and ASPP2 were subcloned into pCMV-Flag or pCMV-HA vectors using ClonExpress TM II One Step Cloning Kit (Vazyme). The PP1α cDNAs was kindly provided by Dr. Qunyin Lei (Fudan University) and subcloned into pCMV-Myc vectors.

2.3. RNA interference

For siRNA treatments, cells were transfected with 0.05 μM siRNA oligos using Lipofectamine RNAiMAX (Invitrogen). The siRNA oligos against ASPP1 and ASPP2 have been reported previously [17]: siASPP1 (5-GCUCAUGGAAGAUCCAAAU-3), siASPP2 (5-UAUGCAGAGACGUGGUGGA-3), siControl (5-ACAGACUUCGGAGUACCUG-3).

2.4. Immunoprecipitation and western blotting

Transfected cells were lysed in BC100 lysis buffer 24 h after transfection. The cell lysates were incubated with antibody at 4 °C overnight, and thereafter with protein A/G beads for additional 2 h. After five washes with BC100 buffer, the pellets were resuspended in sample buffer. For western blotting, cell lysates or immunoprecipitates were subjected to SDS-PAGE and proteins were transferred to nitrocellulose membranes. The membrane was blocked in Tris-buffered saline (TBS, pH 7.4) containing 5% non-fat milk and 0.1% Tween-20, incubated with primary antibody for 2 h and followed by secondary antibody for 1 h at room temperature. The proteins of interest were visualized using ECL chemiluminescence system (Santa Cruz).

2.5. Antibodies

Commercially available antibodies for WB were as follows: ASPP1 (ab137537; Abcam), ASPP2 (611354; BD Biosciences), C-Nap1 (14498; Proteintech), PCM-1 (5259; Cell Signaling), Centrin (12794; Proteintech), α-Tubulin (1878-s; epitomics), β-Tubulin (05-661; Millipore), PP1α (1950-1; epitomics), p-H3(Ser10) (Sc-8656-R; Santa Cruz), GFP (50430; proteintech), Myc (9E10; Sigma), Flag (M2; Sigma), HA (MM5-101R; Millipore) and Actin (AC-74; Sigma).

Phospho-specific C-Nap1 antibodies were raised against the phosphorylated peptides in rabbits. Antiserum were first pre-absorbed with the nonphosphorylated peptide followed by purification with the phosphorylated peptide coupled to Sepharose beads.

2.6. Immunofluorescence and confocal microscopy

For immunofluorescence, cells were plated on chamber slides, fixed either with methanol at −20 °C for 5 min or with 4% paraformaldehyde at 37 °C for 15 min depending on the antibodies used. After fixation, cells were permeabilized with 0.2% Triton for 5 min, preincubated with centrifuged (14,000 rpm) supernatant of 5% FBS and 5% goat serum in PBS and then incubated with primary antibodies at 4 °C overnight. Slides were washed, incubated with fluorescence-tagged secondary antibodies (Alexa Fluor 488, 568, Molecular probes, Invitrogen) and counterstained with DAPI (Vector Labs) for 1 h at 4 °C. Cells were visualized and imaged using a Zeiss LSM710 confocal microscope equipped with a 60× objective. All immunofluorescence experiments were conducted at least three times. Images of proteins of interest were acquired using identical imaging settings. Image processing and figures were made using PhotoShop CS (Adobe). Statistical analyses were performed by student's t-test. Values with $^*p < 0.01$ are considered statistically significant.

3. Results

3.1. ASPP1/2 interact with C-Nap1 in vivo

In our study aimed at identifying novel ASPP1/2-associated proteins, we isolated ASPP1 or ASPP2 complex from HeLa cell by Tandem Affinity Purification (TAP). Centrosome linker protein C-Nap1 was identified in both complex by mass spectrometric analyses (data not shown). C-Nap1 is a centrosome-localized protein required for cell cycle-regulated centrosome cohesion [6]. Given the interaction between ASPP1/2 and centrosome components has not been reported previously in the literature, we investigated the potential role of ASPP1/2 in regulating centrosome cohesion.

We first examined whether ASPP1 and ASPP2 interacted with C-Nap1 by co-immunoprecipitation. When either Flag-ASPP1 or Flag-ASPP2 was immunoprecipitated with anti-Flag antibody, GFP-C-Nap1 co-precipitated with them (Fig. 1A, B). To test whether endogenous ASPP1/2 could also interact with endogenous C-Nap1, immunoprecipitation using anti-C-Nap1 antibody was performed using cell lysates prepared from HeLa cells. ASPP1 and ASPP2 were detected in C-Nap1 immunoprecipitates by western blotting (WB). In contrast, another ASPP family member, iASPP was not detected in the anti-C-Nap1 immunoprecipitates, indicating C-Nap1 specifically interacted with ASPP1/2 (Fig. 1C). In addition, co-immunoprecipitation experiments using anti-ASPP1 or ASPP2 antibody also identified C-Nap1 in the immunoprecipitates (Fig. 1D, E). Together, these results showed that ASPP1 and ASPP2 interacted with C-Nap1 protein in vivo.

3.2. ASPP1/2 co-depletion inhibits centrosome linker reassembly at the end of mitosis

In order to explore whether ASPP1/2 play roles in regulating centrosome cohesion, we examined the effect of ASPP1/2 co-depletion on C-Nap1 recruitment to centrosome. In control cells (Fig. 2A, left two panels), C-Nap1 was localized to the centrosomes

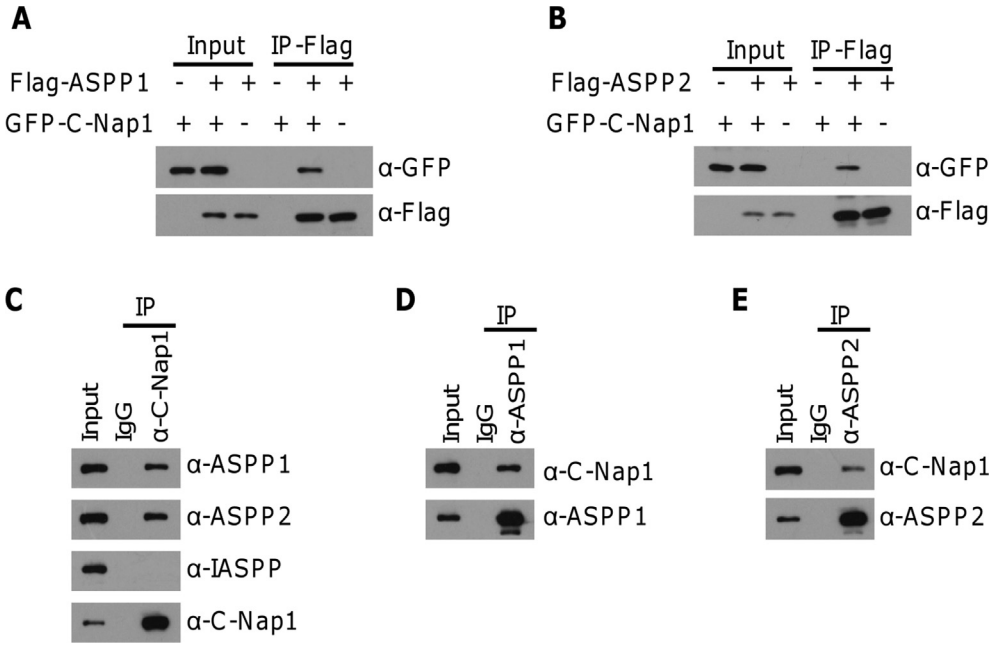

Fig. 1. ASPP1/2 interact with C-Nap1 in vivo. (A–B) 293T cells were co-transfected with GFP-C-Nap1 and Flag-ASPP1 or ASPP2 constructs. Cell lysates were prepared and subjected to immunoprecipitation with anti-Flag antibody. The immunoprecipitates were analyzed by western blotting (WB) with indicated antibodies. (C) Endogenous C-Nap1 interacts with ASPP1, ASPP2 but not iASPP. Endogenous C-Nap1 was immunoprecipitated from Hela cell using anti-C-Nap1 antibodies. An equal amount of non-specific rabbit IgG was used as a control. (D–E) Endogenous ASPP1/2 interact with C-Nap1. Immunoprecipitation using anti-ASPP1 or ASPP2 antibodies were performed using cell lysates prepared from HeLa cells. The presence of C-Nap1 in the immunoprecipitates was detected by WB analyses.

at interphase, but it dissociated from centrosome at the onset of mitosis. Re-association with centrosome then occurred in late telophase or at the onset of G1 phase, when daughter cells are still connected by the post-mitotic bridge. This cell-cycle regulated change of C-Nap1 localization reflects the disruption and reassembly cycle of centrosome linker, which was consistent with previous studies [6]. ASPP1/2 co-depletion did not affect C-Nap1 localization at interphase centrosome, but inhibited C-Nap1 re-accumulation at centrosomes in late telophase (Fig. 2A, right two panels). Quantified results showed that re-association of C-Nap1 to centrosome were compromised in nearly half of the late telophase cells (Fig. 2C). Since C-Nap1 is an essential component of centrosome linker and is required for centrosome cohesion, its failure to reassociate with centrosome thus suggests a defect in centrosome linker reassembly in ASPP1/2 co-depleted cells.

In line with the defect in C-Nap1 re-accumulation at centrosome, the re-localization of pericentrial protein PCM-1 to centrosome at the end of mitosis was also significantly inhibited. PCM-1, a component of pericentriolar matrix (PCM) [18], is an important player for centrosome linker [4]. In control cells (Fig. 2B, left two panels), it concentrated at the centrosome to form PCM-1 granules during interphase, whereas, during mitotic phase, PCM-1 granules became mostly undetectable. At the end of mitosis, the PCM-1 aggregates grew again to their original size. The disassembly and reassembly of PCM-1 granules, reflecting the state of centrosome linker, is cell-cycle regulated [19]. In ASPP1/2 co-depleted cells (Fig. 2B, right two panels), PCM-1 granules reassembly at centrosome in late mitosis was severely inhibited (Fig. 2C). In contrast, Centrin, a core component of the centriole, remained localized to the centrosome during the cell cycle, unaffected by ASPP1/2 co-depletion (Fig. S1, A, B). Western analysis showed that ASPP1 and ASPP2 protein levels were decreased to 10% of those of control cells when ASPP1 and ASPP2 were co-depleted in these experiments (Fig. 2D). It need to be mentioned that depletion of ASPP1 or ASPP2 individually didn't cause obviously change in centrosome linker reassembly (data not shown), suggesting these two protein played compensatory roles in this process. Defect of C-Nap1 re-accumulation and PCM-1 granule reformation at centrosome suggest that ASPP/2 are required for centrosome linker reassembly.

3.3. ASPP1/2 facilitate the interaction between PP1α and C-Nap1

Next, we investigated the underlying mechanism of ASPP1/2 in controlling C-Nap1 re-association with centrosome, which is the prerequisite for centrosome linker reassembly at the end of mitosis. Previous studies demonstrate that the cell cycle-regulated localization of C-Nap1 in centrosome cohesion is regulated by its phosphorylation status [4]. C-Nap1 disassociates from centrosome during early mitosis in response to phosphorylation by NEK2A kinase [12,13], resulting in centrosome linker disruption. At the end of mitosis, C-Nap1 was dephosphorylated and relocalized to centrosome to ensure the reassembly of centrosome linker [6]. A previous study showed C-Nap1 is a substrate for protein phosphatase 1 (PP1) in vitro and in cell extracts [20]. Interestingly, ASPP2 was found to facilitate the interaction between PP1 and its substrate transcriptional factor TAZ to promote substrate dephosphorylation and nuclear localization [21]. Thus, we speculated ASPP1/2 might facilitate the interaction between C-Nap1 and PP1 to promote C-Nap1 dephosphorylation at the end of mitosis.

To test this hypothesis, we investigated whether ASPP1/2 act as molecular adaptors to facilitate interaction between PP1 and C-Nap1. As expected, co-immunoprecipitation assay showed that co-expression of ASPP1 or ASPP2 markedly increased the interaction between c-Nap1 and PP1α (Fig. 3A). ASPP1/2 have a conserved PP1-binding motif (RVXF) near the central region [22]. To test whether the interaction with PP1 is important for the role of ASPP1/2 in the enhancement of C-Nap1-PP1α interaction, we made ASPP1/2 mRVXF mutants that carrying three substitutions in each of the conserved motifs (RVXF-AAxA). As shown in Fig. 3A, the

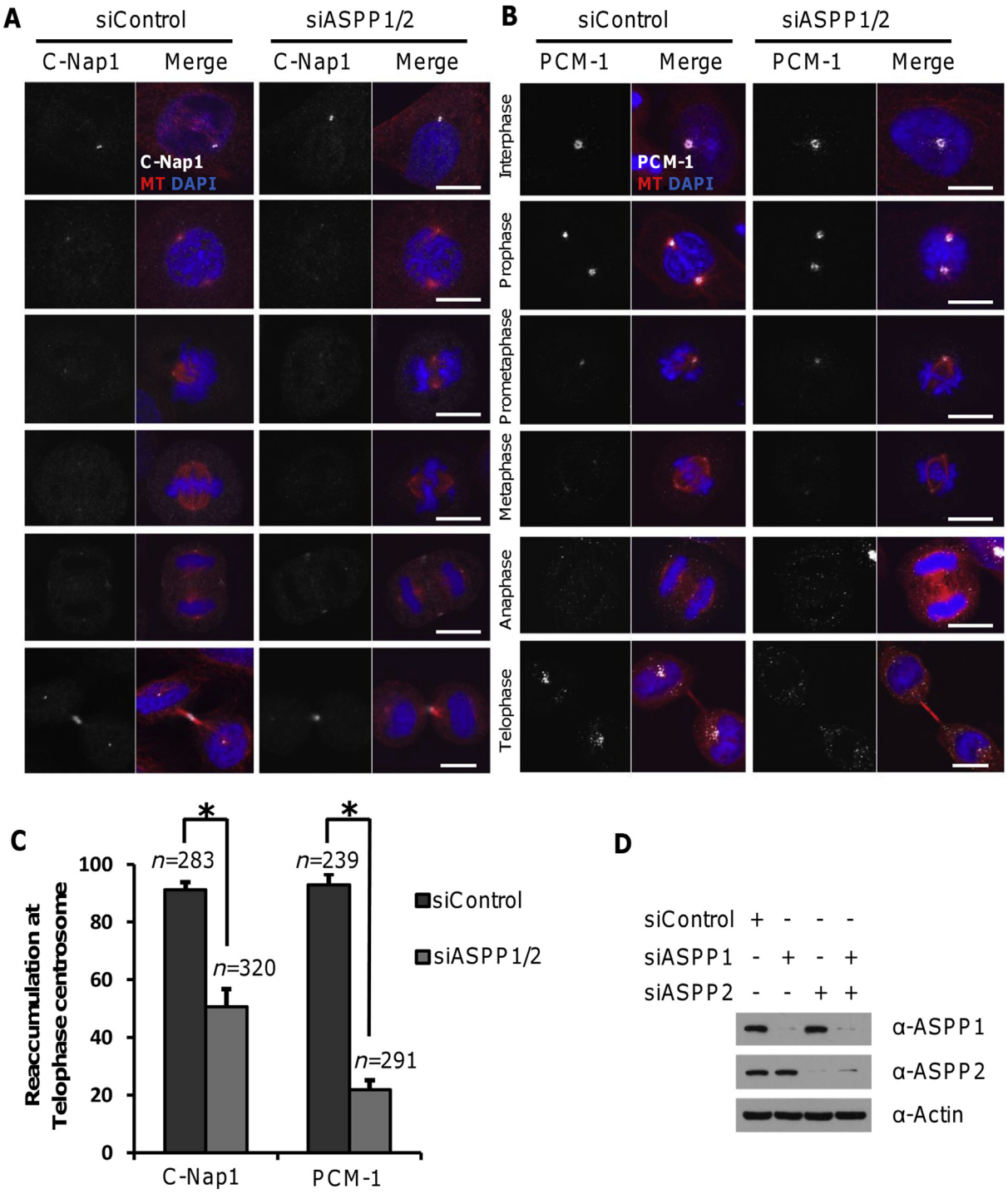

Fig. 2. ASPP1/2 co-depletion inhibits C-Nap1 and PCM1 re-accumulation on centrosome at the end of mitosis. (A)ASPP1/2 co-depletion inhibits C-Nap1 re-accumulation at the centrosome upon exit from mitosis. HeLa cells were transfected with control or ASPP1/2 siRNAs. After 48 h, cells were fixed and stained with anti-C-Nap1 (white) antibody, anti-β-tubulin (red) antibody and DAPI (blue). (B) ASPP1/2 co-depletion inhibits PCM-1 granules reassembly at the end of mitotic phase. Hela cells were treated as above, and stained with anti-PCM-1 (white) antibody, anti-β-tubulin (red) antibody and DAPI (blue). Scale bar = 10 μm. (C) Percentage of cells at late telophase with normal centrosome linker protein re-accumulation. (D) WB analysis of ASPP1/2 protein after siRNA treatment in Hela cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ASPP1 or ASPP2 mRVXF mutants lost their ability to enhance C-Nap1-PP1α interaction as their WT countparts. In agreement with above findings, ASPP1/2 co-depletion significantly reduced the interaction between endogenous C-Nap1 and PP1α (Fig. 3B). Collectively, these results suggest ASPP1/2 facilitate the interaction between C-Nap1 and PP1α in a PP1-binding dependent manner.

3.4. ASPP1/2-PP1α complex dephosphorylates mitotic C-Nap1 at S2417/S2421

Based on our hypothesis that ASPP1/2 mediated C-Nap1 and PP1α interaction may promote C-Nap1 dephosphorylation, we investigated whether ASPP1/2 modulate the mitotic

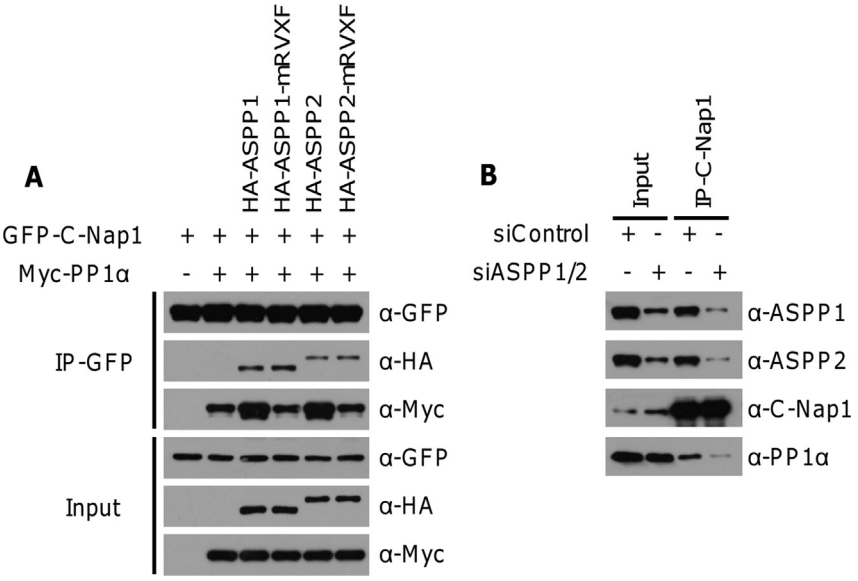

Fig. 3. ASPP1/2 facilitate the interaction between C-Nap1 and PP1α. (A) ASPP1/2 facilitate the interaction between c-Nap1 and PP1α in a PP1-binding dependent manner. 293T cells were co-transfected with indicated constructs. After 24 h, cell lysates were prepared for immunoprecipitation with anti-GFP antibody and WB analyses using indicated antibodies. (B) ASPP1/2 co-depletion reduces the endogenous interaction between C-Nap1 and PP1α. HeLa cells were transfected with the control or ASPP1/2 siRNAs. After 48 h, cell lysates were prepared for immunoprecipitation with anti-C-Nap1 antibody and WB analyses using indicated antibodies.

phosphorylation of C-Nap1 in cellular contexts. Firstly, we raised antibodies specific to C-Nap1 phosphorylated at two serine residues (Ser2417 and Ser2421), which were reported to be phosphorylated by NEK2A [23]. To test whether ASPP1/2 regulate the dephosphorylation of C-Nap1 phospho-Ser2417/2421, ASPP1/2 (WT or mRVXF), C-Nap1 and NEK2A constructs were co-expressed in 293T cells. C-Nap1 was immunoprecipitated and phosphor-C-Nap1 (Ser2417/2421) signal was detected by western blotting using the phosphorylation specific antibody. As shown in Fig. 4A, co-expression of ASPP1-WT or ASPP2-WT dramatically reduced the NEK2A-mediated C-Nap1 Ser2417/2421 phosphorylation, whereas these effects were not observed by the co-expression with ASPP1 or ASPP2-mRVXF mutants. Thus, these results suggested ASPP1/2 can antagonize NEK2A-mediated C-Nap1 Ser2417/2421 phosphorylation in a PP1 binding-dependent manner.

To investigate whether ASPP1/2 co-depletion affected dephosphorylation of phospho-C-Nap1 (Ser2417/2421) during mitosis, cell lysates were prepared from HeLa cells synchronized in prometaphase and then released to fresh media to allow the completion of mitosis. As shown in Fig. 4B, the phospho-C-Nap (Ser2417/2421) signal was gradually decreased to nearly undetectable levels 2 h after nocodazole release in control cells, whereas it remains almost unchanged in ASPP1/2 co-depleted cells. Moreover, the proportion of mitotic cells in telophase is increased in ASPP1/2 co-depleted cells (Fig. S1, C), suggesting that ASPP1/2 are indispensable for C-Nap1 dephosphorylation as well as mitotic exit. In summary, our results suggested that ASPP1/2 promote C-Nap1 dephosphorylation, at least at Ser2417/2421, at the end of mitosis.

4. Discussion

Recent studies have revealed that C-Nap1 is phosphorylated by NEK2A at the onset of mitosis, triggering its disassociation from centrosome and subsequently disruption of centrosome linker [11,12]. It remains unknown how C-Nap1 is re-accumulated to centrosome at the end of mitosis to ensure centrosome linker reassembly. Data from our study suggested a novel role of ASPP1/2 as centrosome-associated PP1 targeting subunits to facilitate C-Nap1 dephosphorylation and centrosome linker reassembly at the end of mitosis. We present several lines of evidence in support of this conclusion. First, ASPP1/2 interacted with C-Nap1 in vivo. Second, ASPP1/2 depletion inhibited C-Nap1 and PCM-1 granule re-accumulation on centrosome at the end of mitosis, reflecting impaired linker reassembly. Third, co-expression of ASPP1/2 enhanced the interaction between C-Nap1 and PP1α and reduced NEK2A mediated C-Nap1 Ser2417/2421 phosphorylation, while co-depletion inhibited C-Nap1 dephosphorylation at the end of mitosis.

Kinase has long been characterized as the director of mitosis, yet the involvement of phosphatase in mitotic processes is only now become evident [24]. The inactivation of mitotic kinase and the reversal of the phosphorylation of its substrate are the keys to the exit from the mitosis [25]. Previous reports suggest the phosphorylation state of C-Nap1 is regulated by an antagonism between NEK2A kinase and PP1 activity [26]. Based on our findings as well as the previous studies, we proposed a model on centrosome linker disassembly-reassembly in cell cycle [Fig. 4C], in which delicate interplay between kinases and phosphatase act to ensure the timely execution of mitotic events. At the onset of mitosis when PP1 activity is inhibited [27], NEK2A is expected to prevail and phosphorylate C-Nap1 thus triggering centrosome linker disassembly and centrosome disjunction to ensure bipolar spindle formation. At late mitosis, the drop in kinase activity shifts the NEK2A/PP1 toward PP1 [28], resulting in PP1 full activation and consequent de-phosphorylation of C-Nap1. Dephosphorylated C-Nap1 then re-accumulated on centrosome to facilitate centrosome linker reassembly upon mitotic exit.

The centrosome is duplicated and separated once per cycle under strict control so that the centrosome cycle is coordinated with the cell division cycle. Perturbations in the centrosome cycle can have catastrophic consequence, such as chromosome instability, a hallmark of cancer [4]. The expression of ASPP1 and ASPP2 have been reported to be down-regulated in various type of human tumors, including breast cancer, hepatocellular carcinomas, non-small cell lung carcinoma, as well as hematopoietic neoplasms [29]. Further investigations are required to determine whether ASPP1/2 under-expression may promote chromosome instability by disturbing the centrosome cycle.

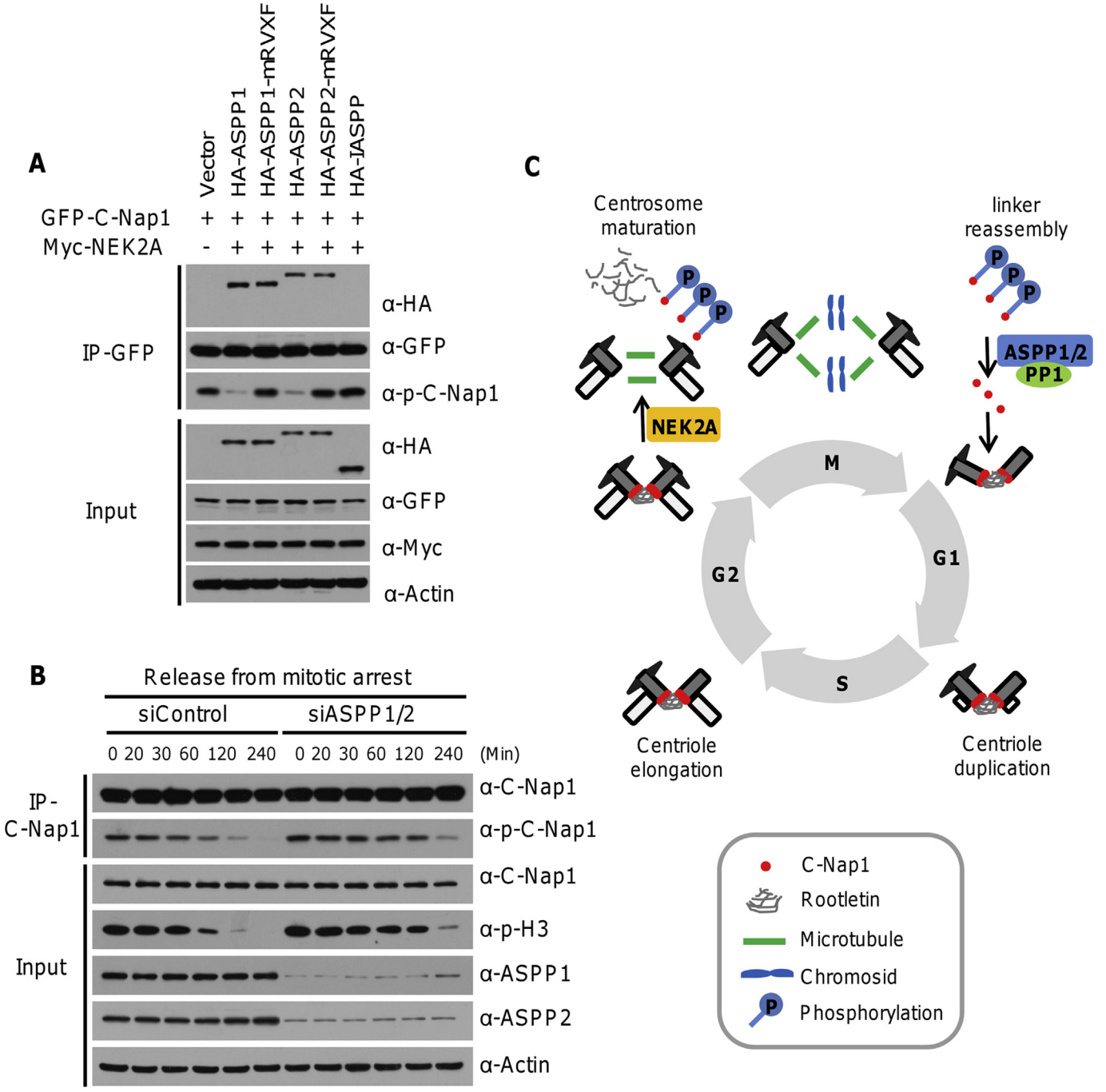

Fig. 4. ASPP1/2-PP1 complex dephosphorylates mitotic C-Nap1 at Ser2417/2421. (A) ASPP1/2 antagonize NEK2A-mediated C-Nap1 Ser2417/2421 phosphorylation. 293T cells were co-transfected with indicated constructs. After 24 h, cell lysates were prepared for immunoprecipitation with anti-GFP antibody and WB analyses using indicated antibodies. (B) HeLa cells were transfected with control or ASPP1/2 siRNAs. After 24 h, HeLa cells were arrested by a sequential thymidine-nocodazole block, collected by shake-off (0 min), and then released from a prometaphase arrest by incubation in drug-free media for the indicated times (20–240 min). The cell lysates were prepared for immunoprecipation with anti-C-Nap1 antibody and WB analyses with indicated antibodies. (C) Model on centrosome linker disassembly-reassembly in cell division cycle.

Conflict of interest

None.

Acknowledgments

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.01.136.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.01.136.

References

[1] J. Luders, T. Stearns, Microtubule-organizing centres: a re-evaluation, Nat. Rev. Mol. Cell. Biol. 8 (2007) 161–167.
[2] M. Bettencourt-Dias, D.M. Glover, Centrosome biogenesis and function: centrosomics brings new understanding, Nat. Rev. Mol. Cell. Biol. 8 (2007) 451–463.
[3] E.A. Nigg, T. Stearns, The centrosome cycle: centriole biogenesis, duplication and inherent asymmetries, Nat. Cell. Biol. 13 (2011) 1154–1160.
[4] B.R. Mardin, E. Schiebel, Breaking the ties that bind: new advances in centrosome biology, J. Cell. Biol. 197 (2012) 11–18.
[5] A.M. Fry, T. Mayor, P. Meraldi, et al., 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.
[6] T. Mayor, Y.D. Stierhof, K. Tanaka, et al., The centrosomal protein C-Nap1 is required for cell cycle-regulated centrosome cohesion, J. Cell. Biol. 151 (2000) 837–846.
[7] S. Bahe, Y.D. Stierhof, C.J. Wilkinson, et al., Rootletin forms centriole-associated filaments and functions in centrosome cohesion, J. Cell. Biol. 171 (2005) 27–33.
[8] J. Yang, M. Adamian, T. Li, Rootletin interacts with C-Nap1 and may function as a physical linker between the pair of centrioles/basal bodies in cells, Mol. Biol. Cell. 17 (2006) 1033–1040.
[9] S. Graser, Y.D. Stierhof, E.A. Nigg, Cep68 and Cep215 (Cdk5rap2) are required for centrosome cohesion, J. Cell. Sci. 120 (2007) 4321–4331.
[10] M.V. Hadjihannas, M. Bruckner, J. Behrens, Conductin/axin2 and Wnt signalling regulates centrosome cohesion, EMBO Rep. 11 (2010) 317–324.
[11] R. He, N. Huang, Y. Bao, et al., LRRC45 is a centrosome linker component required for centrosome cohesion, Cell. Rep. 4 (2013) 1100–1107.
[12] T. Mayor, U. Hacker, Y.D. Stierhof, et al., The mechanism regulating the dissociation of the centrosomal protein C-Nap1 from mitotic spindle poles, J. Cell. Sci. 115 (2002) 3275–3284.
[13] 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.
[14] D. Bergamaschi, Y. Samuels, B. Jin, et al., ASPP1 and ASPP2: common activators of p53 family members, Mol. Cell. Biol. 24 (2004) 1341–1350.
[15] R. Sottocornola, C. Royer, V. Vives, et al., ASPP2 binds Par-3 and controls the polarity and proliferation of neural progenitors during CNS development, Dev. Cell. 19 (2010) 126–137.
[16] Z. Wang, Y. Liu, M. Takahashi, et al., N terminus of ASPP2 binds to Ras and enhances Ras/Raf/MEK/ERK activation to promote oncogene-induced senescence, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 312–317.
[17] Z. Zhu, J. Ramos, K. Kampa, et al., Control of ASPP2/(53BP2L) protein levels by proteasomal degradation modulates p53 apoptotic function, J. Biol. Chem. 280 (2005) 34473–34480.
[18] M. Bornens, Centrosome composition and microtubule anchoring mechanisms, Curr. Opin. Cell. Biol. 14 (2002) 25–34.
[19] A. Kubo, S. Tsukita, Non-membranous granular organelle consisting of PCM-1: subcellular distribution and cell-cycle-dependent assembly/disassembly, J. Cell. Sci. 116 (2003) 919–928.
[20] N.R. Helps, X. Luo, H.M. Barker, et al., NIMA-related kinase 2 (Nek2), a cell-cycle-regulated protein kinase localized to centrosomes, is complexed to protein phosphatase 1, Biochem. J. 349 (2000) 509–518.
[21] C.Y. Liu, X. Lv, T. Li, et al., PP1 cooperates with ASPP2 to dephosphorylate and activate TAZ, J. Biol. Chem. 286 (2011) 5558–5566.
[22] M.P. Egloff, D.F. Johnson, G. Moorhead, et al., Structural basis for the recognition of regulatory subunits by the catalytic subunit of protein phosphatase 1, EMBO J. 16 (1997) 1876–1887.
[23] B.R. Mardin, C. Lange, J.E. Baxter, et al., Components of the Hippo pathway cooperate with Nek2 kinase to regulate centrosome disjunction, Nat. Cell. Biol. 12 (2010) 1166–1176.
[24] P. De Wulf, F. Montani, R. Visintin, Protein phosphatases take the mitotic stage, Curr. Opin. Cell. Biol. 21 (2009) 806–815.
[25] G.B. Moorhead, L. Trinkle-Mulcahy, A. Ulke-Lemee, Emerging roles of nuclear protein phosphatases, Nat. Rev. Mol. Cell. Biol. 8 (2007) 234–244.
[26] P. Meraldi, E.A. Nigg, Centrosome cohesion is regulated by a balance of kinase and phosphatase activities, J. Cell. Sci. 114 (2001) 3749–3757.
[27] F. Puntoni, E. Villa-Moruzzi, Protein phosphatase-1 alpha, gamma 1, and delta: changes in phosphorylation and activity in mitotic HeLa cells and in cells released from the mitotic block, Arch. Biochem. Biophys. 340 (1997) 177–184.
[28] J.Q. Wu, J.Y. Guo, W. Tang, et al., PP1-mediated dephosphorylation of phosphoproteins at mitotic exit is controlled by inhibitor-1 and PP1 phosphorylation, Nat. Cell. Biol. 11 (2009) 644–651.
[29] A. Sullivan, X. Lu, ASPP: a new family of oncogenes and tumour suppressor genes, Br. J. Cancer 96 (2007) 196–200.