Biochemical and Biophysical Research Communications 404 (2011) 413–418

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

ATM-mediated NuSAP phosphorylation induces mitotic arrest

Ping Xie [a,b], Lu Li [b], Guichun Xing [b], Chunyan Tian [b], Yuxin Yin [c], Fuchu He [a,b,*], Lingqiang Zhang [b,1]

[a] School of Life Sciences, Tsinghua University, Beijing 100084, China
[b] State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing Institute of Radiation Medicine, Beijing 100850, China
[c] Department of Pathology, School of Basic Medical Sciences, Peking University, Beijing 100191, China

ARTICLE INFO

Article history:
Received 23 November 2010
Available online 3 December 2010

Keywords:
NuSAP
ATM
Spindle assembly
Phosphorylation
G2/M-phase

ABSTRACT

NuSAP is a microtubule-associated protein that plays an important role in spindle assembly. NuSAP deficiency in mice leads to early embryonic lethality. Spindle assembly in NuSAP-deficient cells is highly inefficient and chromosomes remain dispersed in the mitotic cytoplasm. ATM is a key kinase that phosphorylates a series of substrates to mediate G1/S control. However, the role of ATM at the G2/M phase is not well understood. Here we demonstrate that ectopic expression of NuSAP lead to mitotic arrest observably dependent on the kinase activity of ATM. When endogenous ATM was depleted or its kinase activity was inhibited, NuSAP could not cause mitotic arrest. We further show ATM interacts with NuSAP and phosphorylates NuSAP on Ser124. The phosphorylation and interaction occur specifically at G2/M-phase. Collectively, our work has uncovered an ATM-dependent checkpoint pathway that prevents mitotic progression by targeting a microtubule-associated protein, NuSAP.

1. Introduction

One of the most critical steps during cell cycle progression is the correct segregation of sister chromatids during mitosis. This process depends on the reorganization of the interphase microtubules into a highly dynamic bipolar array known as the mitotic spindle. In animal cells, spindle assembly is initiated at the time when centrosomes separate and involves changes in the functional properties of several microtubule-associated proteins [1,2]. NuSAP (nucleolar spindle-associated protein) was identified as an essential microtubule-binding protein and a target of RanGTP to promote the formation of microtubules near chromosomes [3,4]. NuSAP plays a key role in the mitotic RanGTP network, who is actively imported into the nucleus during interphase, and localized to the spindle during mitosis [5]. APC/C ubiquitin E3 ligase complex promotes the ubiquitination and degradation of NuSAP during spindle formation [6,7]. NuSAP expression is highly correlated with cell proliferation during embryogenesis and adult life, making it a reliable marker of proliferating cells. NuSAP deficiency in mice leads to early embryonic lethality [8]. Spindle assembly in NuSAP-deficient cells is highly inefficient and chromosomes remain dispersed in the mitotic cytoplasm. As a result of sustained spindle checkpoint activity, the cells are unable to progress through mitosis, eventually leading to caspase activation and apoptotic cell death.

A–T is a rare, autosomal recessive disorder characterized by progressive cerebellar ataxia, neuro-degeneration, radiosensitivity, cell-cycle checkpoint defects, genome instability, and a predisposition to cancer [9–11]. The gene responsible for the A–T phenotype encodes ATM, which belongs to phosphoinositol-3-kinase (PI3K) family and plays a key role in DNA damage responses and cell-cycle control [12]. Activated ATM phosphorylates a series of substrates on SQ/TQ motif, including p53, MDM2, BRCA1, p95/NBS1, Chk2, RAD9, RAD17, FANCD2, SMC1, at G1, S phase and at the G2/M transition [10–16]. Cell lines derived from AT patients exhibit abnormalities related to DNA damage and repair such as chromosomal instability, cell-cycle checkpoint defects in G1, S and G2/M, sensitivity to ionizing radiation and telomere end-to-end fusions. However, the role of ATM in mitosis arrest control is not well understood.

During mitosis, excessive amount or knockdown of NuSAP could lead to disruption of cell division [8]. The molecular mechanism by which NuSAP regulates the mitosis remains unclear. Here we show that NuSAP regulates the cell cycle progression dependent on the existing of functional ATM kinase. NuSAP was identified as a novel phosphorylation substrate of ATM during mitosis but not G1/S phase. The phosphorylation is required for NuSAP to promote the formation of microtubules.

2. Materials and methods

2.1. Plasmid constructs, antibodies and reagents

Plasmids of NuSAP including its point-mutants were constructed by PCR, followed by subcloning into various vectors.

* Corresponding author at: State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing Institute of Radiation Medicine, Beijing 100850, China.
E-mail addresses: hefc@nic.bmi.ac.cn (F. He), zhanglq@nic.bmi.ac.cn (L. Zhang).
[1] Co-corresponding author.

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.11.135

Flag-ATM was a gift from Michael B. Kastan (Department of Hematology–Oncology, St. Jude Children's Research Hospital). Anti-Myc antibody was purchased from Clontech. Anti-Flag M2 antibody, caffeine, Nocodazole, thymidine and aphidicolin were from Sigma. ATM polyclonal antibody Ab-1 (NB 100–104) was from Novus Biologicals. NuSAP polyclonal antibody Ab-1 (ab-93779) was from Abcam. Antibody against phospho-(Ser/Thr) ATM/ATR substrate, phosphorylated Ser15 of p53, phosphorylated Ser10 of Histone H3 and GFP-antibody were from Cell Signaling Technology. Antibodies to Cyclin B and GAPDH were from Invitrogen and Santa Cruz, respectively.

2.2. Cell culture and treatments

Most cell lines were maintained in DMEM (Hyclone). All cells were supplemented with 10% fetal bovine serum (FBS; Hyclone), penicillin (50 U/ml), and streptomycin (50 μg/ml) (Hyclone). Caffeine was added to the medium at the concentration of 2 mM 4 h prior to transfection as described previously [17]. Cells were incubated in the medium supplemented with 2 mM thymidine and aphidicolin for G1-phase arrest or with 2 mM Nocodazole for M-phase arrest [18].

2.3. Cell cycle distribution analysis

For each cell line, 2×10^5 cells were seeded per well in 6-well plates. After 18 h of incubation, cells were transfected with pEGFP, pEGFP-NuSAP wild-type or mutants. Attached cells were harvested by trypsinized treatment and pooled with detached cells from the growth medium. Cells were pelleted by centrifugation at 2000 rpm for 5 min, washed twice with PBS, and resuspended in 300 μl PBS (containing 30 μl FBS) and fixed in 2 ml ice cold 70% ethanol. Samples were kept at −20 °C until analysis by flow cytometry. Cells were washed and treated with 0.1 mg/ml RNase A (Roche) for 30 min and stained with 40 μg/ml Propidium Iodide (Sigma). DNA content was analyzed by FACS Calibur (B–D USA) [19].

2.4. Confocal microscopy, immuno-fluorescence analysis

For immuno-staining of endogenous tubulin, HEK293T and ATS4 cells transfected with NuSAP-GFP were fixed in 4% PFA (paraformaldehyde) for 10 min. Images of endogenous tubulin during mitosis and interphase were primarily made using anti-tubulin antibody in cells that were fixed in 0.1% PBST (containing 0.5% Triton X-100) for 15 min. Further processing included incubating cells in 5% BSA for 10 min before incubations with primary for 3 h at 37 °C and with secondary antibody for 1 h at room temperature. Secondary antibodies were conjugated to TRITC (Molecular Probes, Inc.). Cells were analyzed in PBS when the nucleus was stained with DAPI. Images of fixed cells were acquired on a confocal microscope using LaserSharp software [20].

2.5. RNA interference and real-time PCR

The short interfering RNA (siRNA) specifically targeting ATM (5′-UGGUGCUAUUUACGGAGCU-3′) and non-targeting control siRNA (5′-UUCUCCGAACGUGUCACGU-3′) were synthesized by Shanghai GenePharm. All siRNA and short hairpin RNA (shRNA) transfections were performed with Lipofectamine 2000 (Invitrogen) and the RNA interfering efficiency was assessed by Western blot analysis. Real-time PCR was performed as described previously [21].

2.6. Immunoprecipitation and immunoblotting

Transfection was performed with Lipofectamine 2000 (Invitrogen). At 24–48 h after transfection, cells were harvested and lysed in Hepes lysis buffer (20 mM Hepes pH 7.2, 50 mM NaCl, 0.5% Triton X-100, 1 mM NaF, 1 mM dithiothreitol) supplemented with protease inhibitor cocktail (Roche, Indianapolis, Indiana, USA). The lysate was incubated with indicated antibody 3 h at 4 °C, then added Protein A/G-plus Agarose (Santa Cruz) and rotated gently more than 8 h at 4 °C. The immunoprecipitates were washed at least three times in lysis buffer, and proteins were recovered by boiling the beads in 2× SDS sample buffer and analyzed by Western blotting followed by detection with the related secondary antibody with a Super Signal chemiluminescence kit (Pierce).

3. Results

3.1. NuSAP overexpression-triggered mitotic arrest requires ATM

To investigate the consequence of dysregulation of NuSAP protein level, we performed a cell-cycle analysis to detect the effect of overexpression of NuSAP on cell cycle progression. Fig. 1A showed that increased mitotic index could be observed in several cells lines transfected with NuSAP-GFP, including COS7, Bel-7402, MCF7, SMMC-7721, HEK293, HEK293T, HCT15 and HCT116 cells, whereas no increase was detected in cells transfected with vector mock. Strikingly, NuSAP could not trigger such G2/M arrest in ATS4 fibroblast cells, which lack ATM but retain ATR (ATM and Rad3-related) function [22]. Consistently, overexpressed NuSAP could induce mitotic microtubes bundling in HEK293T cells, while no significant alteration of microtubule networks was detected in ATS4 fibroblast cells transfected with NuSAP (Fig. 1B).

We ask whether ATM has a significant impact on the function of NuSAP. First, we depleted ATM in COS7 and HEK293T cells using specific siRNA duplexes against ATM and performed a cell-cycle analysis by FACS. ATM depletion induced a significant decrease of G2/M proportion even NuSAP was overexpressed (Fig. 1C), implicating that NuSAP overexpression-triggered mitotic arrest requires the presence of ATM. Second, to explore whether the kinase activity of ATM is required for NuSAP to induce G2/M arrest, caffeine, a potent inhibitor of ATM and ATR to overcome cell-cycle checkpoint responses [23,24] was used. Upon treatment with caffeine, cells overexpressing NuSAP-GFP failed to arrest at G2/M phase, suggesting that the kinase activity of ATM is required for NuSAP function (Fig. 1D).

3.2. ATM-mediated NuSAP phosphorylation on Ser124 is critical for NuSAP-induced mitotic arrest

Considering that ATM is a Ser/Thr kinase, and NuSAP contains five potential ATM phosphorylation sites (i.e. SQ/TQ motif), we asked whether NuSAP is a phosphorylation substrate of ATM. NuSAP was ectopic expressed and immunoprecipitated from HEK293T cell extracts, and the possible phosphorylated form was assessed by immunoblotting with an antibody specifically recognizing the ATM/ATR substrate. As shown in Fig. 2A, NuSAP phosphorylation was easily detected when NuSAP was overexpressed in HEK293T cells (lane 2). Importantly, ATM inhibition by caffeine treatment or depletion by RNAi abolished this phosphorylation of NuSAP (lanes 3 and 4). However, this phosphorylation could not be detectable in ATS4 cells (Fig. 2B, lane 2). Re-introduction of Flag-ATM wild-type but not the kinase-dead (KD) mutant into ATS4 cells restored the NuSAP phosphorylation although the expression level of total NuSAP was not altered (Fig. 2B, lanes 3 and 4). These results indicate that NuSAP is a phosphorylation substrate of ATM kinase in mammalian cells.

Analysis of NuSAP with Scansite software (http://scansite.mit.edu) at high stringency revealed Ser124 as a potential ATM phosphorylation sites. Replacement of Ser124 with alanine completely abolished NuSAP phosphorylation, whereas other four

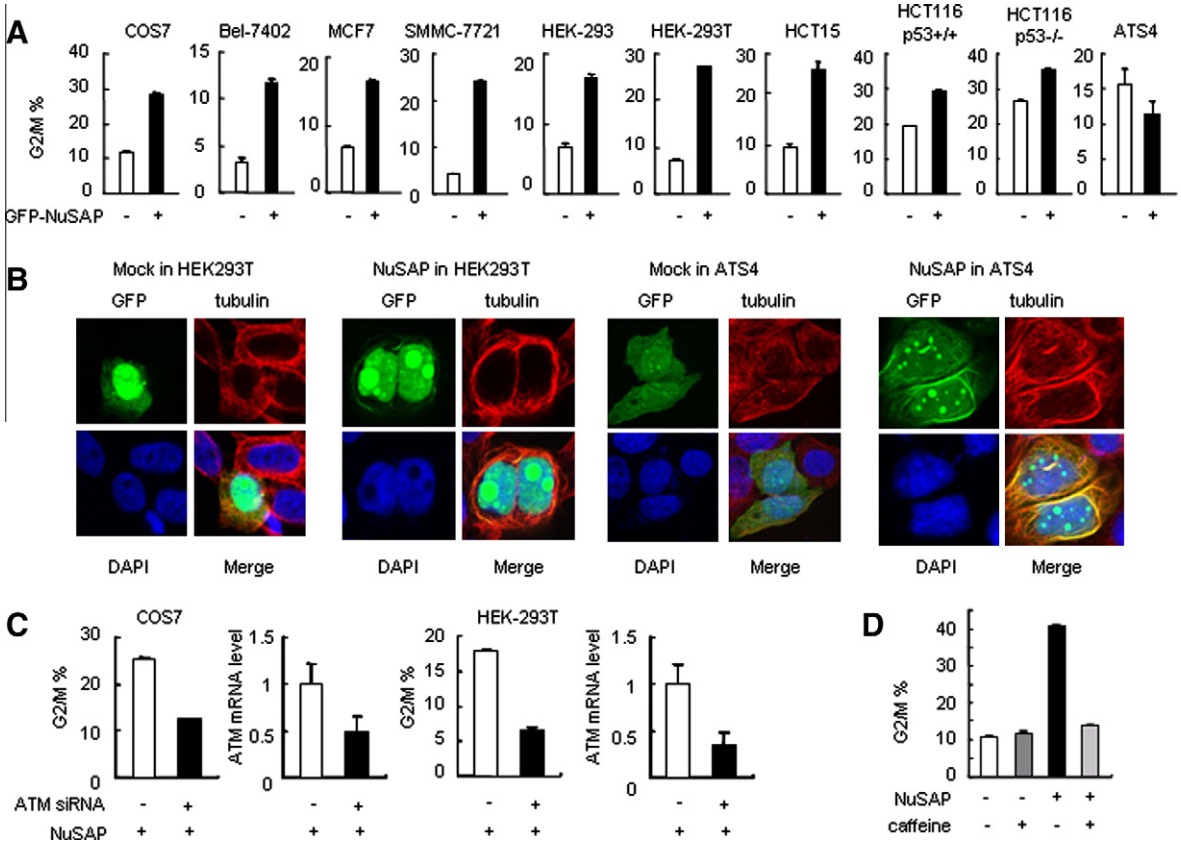

Fig. 1. NuSAP-triggered mitotic arrest requires ATM. (A) GFP-NuSAP or GFP vector was transfected into the indicated cells. Forty-eight hours after transfection, the cell cycle distribution was analyzed by FACS and shown as mean ± SD of three independent experiments. SD, standard derivation. (B) HEK293T and ATS4 cells were each transfected with GFP vector mock or GFP-NuSAP, at 36 h post-transfection, cells were fixed by formaldehyde and incubated with β-tubulin antibody for immuno-fluorescence analysis. Then the cells were visualized with a confocal fluorescence microscope. (C) FACS analysis in COS7 and HEK293T cells transfected with NuSAP together with siRNA against ATM or control siRNA. The efficiency of ATM knockdown was determined by real-time PCR and shown. (D) Caffeine was added to the culture medium at the concentration of 2 mM 4 h prior to transfection with NuSAP-GFP in HEK293T cells. The FACS data are shown.

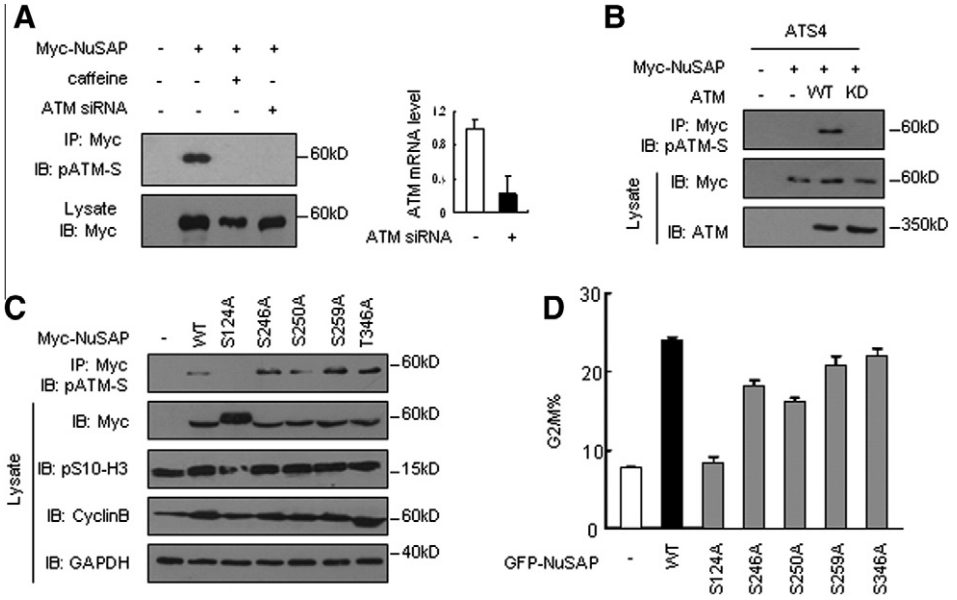

Fig. 2. ATM-mediated NuSAP phosphorylation on Ser124. (A) NuSAP-Myc was transfected into HEK293T cells together with or without ATM siRNA. Four hours prior to transfection, caffeine was added as indicated. NuSAP was immunoprecipitated by anti-Myc antibody and analyzed by Western blot with anti-pATM-substrate. Endogenous ATM was analyzed by real-time PCR to show the efficiency of knockdown. (B) NuSAP-Myc and Flag-tagged wild-type or kinase-dead ATM were transfected into ATS4 cells as indicated. Phosphorylation of immunoprecipitated NuSAP was analyzed by Western blot. (C) Wild-type NuSAP or the indicated Ser/Thr-to-Ala mutant was transfected into HEK293T cells. The immunoprecipitated NuSAP proteins were analyzed to detect ATM-mediated phosphorylation. The total cell lysates were subjected to Western blot with the indicated Myc, H3-pS10, Cyclin B or GAPDH antibody. (D) FACS analysis of HEK293T cells transfected with NuSAP WT or mutants as described in Fig. 1A.

Ser/Thr sites mutation had little or weak effects (Fig. 2C). NuSAP-S124A also lost the ability to induce the mitotic arrest (Fig. 2D) and could not enhance the levels of H3-pSer10 and cyclin B (Fig. 2C, lane 3), two well-defined mitotic markers [25]. These results suggested that overexpression of NuSAP induced mitotic arrest dependent of ATM-mediated phosphorylation of NuSAP on Ser124.

3.3. ATM-mediated NuSAP phosphorylation occurs specifically at G2/M-phase

Most of the identified substrates of ATM regulate cell cycle at G1/S phase. However, substrate of ATM function at G2/M phase is rare, and it is still not clear how ATM regulate mitosis arrest. Considering that NuSAP is selectively expressed in proliferating cell cycle phases, its mRNA and protein levels peak at the transition of G2 to mitosis and abruptly decline after cell division [4]. We ask whether its phosphorylation form also selectively appears in certain phase. To investigate such hypothesis, we used two kinds of drugs, thymidine–aphidicolin double block and nocodazole treatment, to synchronize the cell cycle at G1/S boundary or G2/M phase, respectively [26,27]. Histone H3 Ser10 phosphorylation and Cyclin B were used as mitotic markers. Phosphorylated form of NuSAP was observed robustly in cells synchronized at the G2/M-phase (Fig. 3A, lane 3). By contrast, no phosphorylation of NuSAP was detected in cells synchronized at the G1/S-phase although the ectopic NuSAP was expressed at a high level (lane 4). As expected, when cells were overexpressed NuSAP or treated with nocodazole, both H3 Ser10 phosphorylation and Cyclin B level were up-regulated (Fig. 3A).

To further confirm the specificity of ATM phosphorylation on NuSAP at the G2- or M-phase, we detected the phosphorylation of endogenous NuSAP and performed a time-course analysis to monitor the alteration of NuSAP phosphorylation after nocodazole release. Upon nocodazole release, phosphorylation of endogenous NuSAP was easily detected and maintained to 2.5 h. After 3 h of nocodazole release, it was nearly difficult to detect NuSAP phosphorylation as well as total NuSAP protein (Fig. 3B), which might be due to the degradation of NuSAP by APC/C ubiquitin ligase at the anaphase before mitosis exit. Similar to the variation trend of NuSAP phosphorylation, Histone H3 Ser10 phosphorylation and Cyclin B also decreased with the nocodazole release. These results clearly establish that ATM-mediated NuSAP phosphorylation specifically appears at G2/M-phase, suggesting NuSAP is a novel substrate of ATM at G2/M-phase.

3.4. NuSAP interacts with ATM at G2/M-phase

Next, we went onto investigate the interaction between NuSAP and ATM, and co-immunoprecipitation (Co-IP) assay was performed to detect whether NuSAP protein could associate with ATM in vivo. As shown in Fig. 4A, Myc-tagged NuSAP protein could be detected in ATM immunoprecipitate (lane 6). As a positive control [28], CKIP-1 could be also co-immunoprecipitated with ATM (lane 8). To test if NuSAP is able to interact with ATM under physiological conditions, endogenous Co-IP assay was carried out. Cell

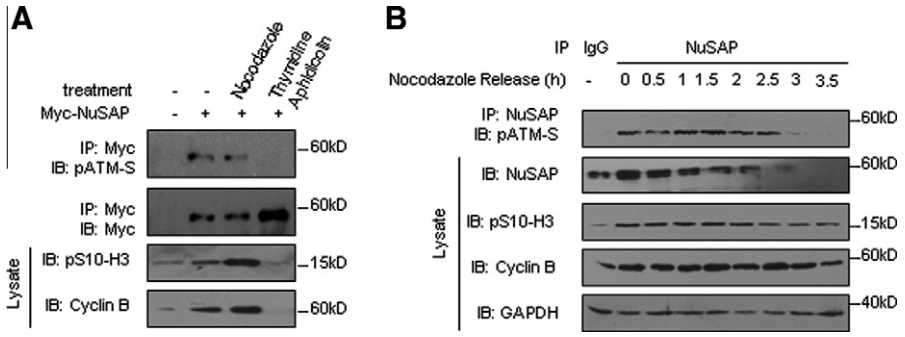

Fig. 3. ATM-mediated NuSAP phosphorylation occurs at G2/M-phase. (A) Myc-NuSAP was transfected into HEK293T cells. One parallel group was treated with 2 mM nocodazole for 12 h, and the other parallel group was treated with 2 mM thymidine–aphidicolin for 12 h. Phosphorylation of NuSAP was analyzed by Western blot. (B) HEK293T cells were treated with 2 mM nocodazole for 12 h. At the indicated time points after nocodazole release, endogenous NuSAP proteins were immunoprecipitated with anti-NuSAP and immunoblotted with the antibody against ATM substrate. The total cell lysates were analyzed with the indicated NuSAP, H3-pS10, Cyclin B or GAPDH antibody.

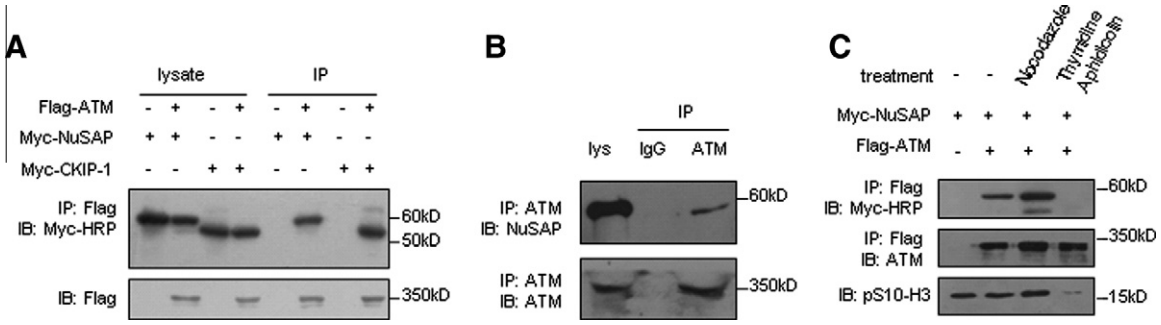

Fig. 4. ATM interacts with NuSAP at G2/M-phase. (A) HEK293T cells were transfected with the indicated expression plasmids. Cell lysates were immunoprecipitated with anti-Flag antibody. The cell lysates and immunoprecipitates were detected by Western blot with anti-Myc-HRP and anti-Flag antibodies as indicated. (B) Co-immunoprecipitation of endogenous NuSAP and endogenous ATM with each other from HepG2 cells. Western blot analysis of whole-cell lysate and the indicated immunoprecipitates with anti-NuSAP and anti-ATM antibody are shown. (C) HEK293T cells were transfected with Myc-NuSAP, Flag-ATM and treated with nocodazole (2 mM) or thymidine–aphidicolin (2 mM) for 12 h. The cell lysates and Flag-immunoprecipitates were analyzed by Western blot with anti-Myc-HRP or ATM. The cell lysate was also analyzed with anti-H3-pS10 antibody to indicate the G2/M arrest.

lysates prepared from HepG2 cells were immunoprecipitated with an anti-ATM antibody or normal IgG as a control, followed by immunoblotting with anti-NuSAP antibody. As shown in Fig. 4B, endogenous NuSAP could be co-immunoprecipitated with endogenous ATM but not the control IgG. Taken together, the results showed that NuSAP could interact with ATM in vivo.

We asked whether the interaction between the two proteins occurs in certain cell cycle phase. Similar co-immunoprecipitation study was performed under the condition of nocodazole or thymidine–aphidicolin treatment. In agreement with the former results, NuSAP could interact with ATM. Importantly, the treatment of nocodazole significantly enhanced the amount of co-immunoprecipitated NuSAP. By contrast, NuSAP could not associate with ATM at the G1/S-phase (Fig. 4C). Taken together, these data suggest that the binding of NuSAP to ATM specifically occurs at G2/M-phase, in conformity with phosphorylation on NuSAP.

4. Discussion

Cell-cycle checkpoints protect mammalian cells from the permanent genetic damage that can lead to transformation and oncogenesis. ATM has been identified to mediate cell-cycle control at different phases. A multitude of DNA repair and checkpoint proteins have been identified as substrates of ATM kinase. For example, the tumor suppressor p53 was shown to play a critical role in the G1/S checkpoint and efficient induction of p53 after ionizing radiation requires ATM phosphorylation [16]. MDM2, RAD9, Chk2 were also known as ATM targets at G1-phase [11]. It has also been shown that the MRN complex and ATM function in a common pathway, and that NBS1 in particular plays a role at S-phase as a substrate of ATM kinase activity [28]. ATM target BRCA1, RAD17 on multiple sites at G2-phase, and these different phosphorylation events elicited different effects on cell cycle progression [11]. Spindle assembly is the result of many balanced processes. Chromatin generates the formation of a gradient of RanGTP bound molecules which promote microtubule polymerization around chromatin. Substrates of checkpoint proteins such as Chk2 take an active role in this process. Chk2 is indeed required to inhibit mitosis and spindle assembly in Drosophila embryos in the presence of DNA damage [15]. In spite of the recent progress in the elucidation of the role of ATM, the effects of ATM activation in cells progressing through mitosis are poorly understood, and substrates of ATM in mitotic arrest cells remain seldom and elusive.

NuSAP induces extensive bundling of spindle microtubules and causes bundled microtubules within spindle-like structures to become longer. Previous studies showed that NuSAP plays an important role in spindle assembly and its depletion by RNAi results in spindle deficiency [4,8]. Our results indicated that NuSAP overexpression could impressively trigger mitotic arrest and induce mitotic microtubes bundling in a series of cell lines. However, NuSAP had a much lower activity in ATS4 fibroblast cells, which lost ATM but retain ATR function. Microtubules appeared less compacted around chromatin and chromosome capture, alignment and segregation were inefficient. Moreover, under ATM knockdown, or ATM kinase inactive conditions, no significant alteration of mitotic arrest was detected even overexpressing NuSAP. We further showed that ATM phosphorylated NuSAP robustly at the G2/M phase. Our primary data may provide the evidence that ATM takes part in the spindle assembly by regulating the function of microtube associate protein NuSAP.

Similar to NuSAP, the centrosomal protein CEP63 has been identified as an ATM and ATR target required for normal spindle assembly. ATM and ATR phosphorylate Xenopus CEP63 (XCEP63) on Ser560 and promote its delocalization from the centrosome. Suppression of ATM and ATR activity or mutation of XCEP63 Ser560 to Ala prevented spindle assembly defects. Consistently, inactivation of the CEP63 gene in avian DT40 cells impaired spindle assembly and prevented ATM- and ATR-dependent effects on mitosis [29,30]. Our study provided that ATM phosphorylates NuSAP on Ser124, whereas the S124A mutant had no effect in mitotic arrest. In addition, ATM-mediated NuSAP phosphorylation occurs at G2/M-phase, suggesting NuSAP is a substrate of ATM at G2/M-phase. Future study should be performed to deeply investigate the role of NuSAP in the effects of ATM on spindle assembly and maintenance of genome stability.

Acknowledgments

We are grateful to Dr. Michael B. Kastan for providing ATM plasmids. This work was supported by the Chinese National Basic Research Programs [2007CB914601 and 2010CB912202], the National Key Technologies R&D Program for New Drugs [2009ZX09503-002 and 2009ZX09301-002].

References

[1] T. Gaglio, M.A. Dionne, D.A. Compton, Mitotic spindle poles are organized by structural and motor proteins in addition to centrosomes, J. Cell Biol. 138 (1997) 1055–1066.
[2] E. Karsenti, I. Vernos, The mitotic spindle: a self-made machine, Science 294 (2001) 543–547.
[3] P. Kalab, R.T. Pu, M. Dasso, The ran GTPase regulates mitotic spindle assembly, Curr. Biol. 9 (1999) 481–484.
[4] T. Raemaekers, K. Ribbeck, J. Beaudouin, W. Annaert, M. Van Camp, I. Stockmans, N. Smets, R. Bouillon, J. Ellenberg, G. Carmeliet, NuSAP, a novel microtubule-associated protein involved in mitotic spindle organization, J. Cell Biol. 162 (2003) 1017–1029.
[5] K. Ribbeck, A.C. Groen, R. Santarella, M.T. Bohnsack, T. Raemaekers, T. Kocher, M. Gentzel, D. Gorlich, M. Wilm, G. Carmeliet, NuSAP, a mitotic RanGTP target that stabilizes and cross-links microtubules, Mol. Biol. Cell 17 (2006) 2646–2660.
[6] L. Li, Y. Zhou, L. Sun, G. Xing, C. Tian, J. Sun, L. Zhang, F. He, NuSAP is degraded by APC/C-Cdh1 and its overexpression results in mitotic arrest dependent of its microtubules' affinity, Cell. Signal. 19 (2007) 2046–2055.
[7] L. Song, M. Rape, Regulated degradation of spindle assembly factors by the anaphase-promoting complex, Mol. Cell 14 (2010) 369–382.
[8] A.V. Bosch, T. Raemaekers, S. Denayer, S. Torrekens, N. Smets, K. Moermans, M. Dewerchin, P. Carmeliet, G. Carmeliet, NuSAP is essential for chromatin-induced spindle formation during early embryogenesis, J. Cell Sci. 123 (2010) 3244–3255.
[9] E. Boder, R.P. Sedgwick, Ataxia–telangiectasia; a familial syndrome of progressive cerebellar ataxia, oculocutaneous telangiectasia and frequent pulmonary infection, Pediatrics 21 (1985) 526–554.
[10] M.B. Kastan, D.S. Lim, The many substrates and functions of ATM, Nat. Rev. 1 (2000) 179–186.
[11] Y. Shiloh, ATM and related protein kinases: safeguarding genome integrity, Nat. Rev. Cancer 3 (2003) 155–168.
[12] M.F. Lavin, Y. Shiloh, The genetic defect in ataxia–telangiectasia, Annu. Rev. Immunol. 15 (1997) 177–202.
[13] K. Savitsky, S. Sfez, D.A. Tagle, A. Sartiel, F.S. Collins, Y. Shiloh, G. Rotman, The complete sequence of the coding region of the ATM gene reveals similarity to cell cycle regulators in different species, Human Mol. Genet. 4 (1995) 2025–2032.
[14] D.S. Lim, S.T. Kim, B. Xu, R.S. Maser, J. Lin, J.H. Petrini, M.B. Kastan, Cell cycle checkpoint signaling through the ATM and ATR kinases, Nature 404 (2000) 613–617.
[15] S. Matsuoka, G. Rotman, A. Ogawa, Y. Shiloh, K. Tamai, S.J. Elledge, Ataxia telangiectasia-mutated phosphorylates Chk2 in vivo and in vitro, Proc. Natl. Acad. Sci. USA 97 (2000) 10389–10394.
[16] R. Maya, M. Balass, S.T. Kim, D. Shkedy, J.F. Leal, O. Shifman, M. Moas, T. Buschmann, Z. Ronai, Y. Shiloh, M.B. Kastan, E. Katzir, M. Oren, ATM-dependent phosphorylation of Mdm2 on serine 395: role in p53 activation by DNA damage, Genes Dev. 15 (2001) 1067–1077.
[17] Y. Zhang, Y. Xiong, A p53 amino-terminal nuclear export signal inhibited by DNA damage-induced phosphorylation, Science 292 (2001) 1910–1915.
[18] X. Wang, D.Y. Jin, H.L. Wong, H. Feng, Y.C. Wong, S.W. Tsao, MAD2-induced sensitization to vincristine is associated with mitotic arrest and Raf/Bcl-2 phosphorylation in nasopharyngeal carcinoma cells, Oncogene 22 (2003) 109–116.
[19] G. Normand, P.G. Hemmati, B. Verdoodt, C. von Haefen, J. Wendt, D. Guner, E. May, B. Dorken, P.T. Daniel, P14ARF induces G2 cell cycle arrest in p53- and p21-deficient cells by down-regulating p34cdc2 kinase activity, J. Biol. Chem. 280 (2005) 7118–7130.

[20] M. Trieselmnann, S. Armstrong, J. Rauw, Ran modulates spindle assembly by regulating a subset of TPX2 and Kid activities including Aurora A activation, J. Cell Sci. 116 (2003) 4791–4798.
[21] P. Xie, C.Y. Tian, L.G. An, J. Nie, K.F. Lu, G.C. Xing, L.Q. Zhang, F.C. He, Histone methyltransferase protein SETD2 interacts with p53 and selectively regulates its downstream genes, Cell. Signal. 20 (2008) 1671–1678.
[22] L. Zhang, Y. Tie, C. Tian, G. Xing, Y. Song, Y. Zhun, Z. Sun, F. He, CKIP-1 recruits nuclear ATM partially to the plasma membrane through interaction with ATM, Cell. Signal. 18 (2006) 1386–1395.
[23] J.N. Sarkaria, E.C. Busby, R.S. Tibbetts, P. Roos, Y. Taya, L.M. Karnitz, R.T. Abraham, Inhibition of ATM and ATR kinase activities by the Radiosensitizing Agent, Caffeine, Cancer Res. 59 (1999) 4375–4382.
[24] D. Cortez, Caffeine inhibits checkpoint responses without inhibiting the Ataxia–Telangiectasia-mutated (ATM) and ATM- and Rad3-related (ATR) protein kinases, J. Biol. Chem. 278 (2003) 37139–37145.
[25] Z. Wang, H. Wei, Y. Yu, J. Sun, Y. Yang, G. Xing, S. Wu, Y. Zhou, Y. Zhu, C. Zhang, T. Zhou, X. Zhao, Q. Sun, F. He, Characterization of Ceap-11 and Ceap-16, two novel splicing-variant-proteins, associated with centrosome, microtubule aggregation and cell proliferation, J. Mol. Biol. 343 (2004) 71–82.
[26] M. Iizuka, T. Matsui, H. Takisawa, M.M. Smith, Regulation of replication licensing by acetyltransferase Hbo1, Mol. Cell. Biol. 26 (2006) 1098–1108.
[27] J. Bothos, M.K. Summers, M. Venere, D.M. Scolnick, T.D. Halazonetis, The Chfr mitotic checkpoint protein functions with Ubc13-Mms2 to form Lys63-linked polyubiquitin chains, Oncogene 22 (2003) 7101–7107.
[28] C.T. Carson, R.A. Schwartz, T.H. Stracker, C.E. Lilley, D.V. Lee, M.D. Weitzman, The Mre11 complex is required for ATM activation and the G2/M checkpoint, EMBO J. 22 (2003) 6610–6620.
[29] E. Smith, D. Dejsuphong, A. Balestrini, M. Hampel, C. Lenz, S. Takeda, A. Vindigni, V. Costanzo, An ATM- and ATR-dependent checkpoint inactivates spindle assembly by targeting CEP63, Nat. Cell Biol. 11 (2009) 278–285.
[30] N. Brown, V. Costanzo, An ATM and ATR dependent pathway targeting centrosome dependent spindle assembly, Cell Cycle 8 (2009) 1997–2001.