The role of NBS1 in normal cell cycle progression during mitosis

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조선대학교 대학원

의과학과

김 유 리

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지도교수 이 정 희

이 논문을 석사학위신청 논문으로 제출함

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조선대학교 대학원

의과학과

김 유 리

김유리의 석사학위논문을 인준함

위원장 조선대학교 교 수 장인엽 (인)

위 원 조선대학교 조교수 박선주 (인)

위 원 조선대학교 조교수 이정희 (인)

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조선대학교 대학원

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NBS1의 세포분열단계에서의 세포주기 조절기전 연구

김 유 리

지도교수: 이 정 희

조선대학교 일반대학원

의과학과

정상적인 세포주기의 진행을 위해 활성화되는 DNA 손상 반응 체크포인트와 세포분열 체크포인트는 세포의 유전체 안정성 유지에 중요한 역할을 수행한다. 최근 몇몇 연구들은 이 두 체크포인트간의 상호연관성을 증명하였다. DNA 손상반응 단백질 중 하나인 NBS1은 DNA 손상에 의해 유도되는 복구 경로에 중요한 역할을 수행하는 단백질로 알려져 왔다. NBS1은 이런 DNA 손상 복구 경로와 세포주기 검사점의

활성화에 관여하는 중요한 단백질로써 알려져 있다. 본 연구는 yeast-two hybrid screening을 통해 NBS1과 결합하는 SPAG5단백질을 동정하였다. SPAG5은 유사분열의 진행에서 염색체의 온전한 상태를 위해 자매염색분체 결합과 차후 염색분체 분리에 중요한 역할을 수행한다고 밝혀져 있다. 따라서 우리는 NBS1이 SPAG5과의 결합을 통해 유사분열의 진행에 관여하는 기전을 밝히고자 한다. NBS1이 유사분열의 진행에 미치는 영향을 확인하기 위해 세포 내 NBS1의 결핍을 통해 NBS1의 기능을 Western blot과 immunostaining을 통해 조사한 결과 염색체가 방추사의 적도판에 일렬로 배열되지 못하였으며 두 개 이상의 중심체를 형성함으로써 유사분열의 진행을 지연시킴을 확인하였다. 연구결과에 따라 NBS1은 DNA 손상 복구뿐만 아니라 유사분열 진행에도 관여하는 중요한 단백질임을 시사한다.

INTRODUCTION

DNA damage response is a complex signaling process involving the orchestration of a variety of cellular events that rapidly activate in response to DNA damage. DNA damage is generated by endogenous factor such as reactive oxygen species as well as exogenous factor such as ultraviolet radiation, ionizing radiation (IR) and radiomimetic drugs [1]. DNA damage repair is essential to maintain genome integrity by preventing the DNA damage response. Failure to DNA repair will lead to mutation and repair causes genomic instability result in tumorigenesis and other age-related disease [2, 3]. When interphase cells are generated the cytotoxic lesions on DNA produced by IR and radiomimetic drugs, DNA-damage response (DDR) proteins were recruited to DNA lesion site for DNA repair. DNA damage response activate through cellular pathway that sense, signal and repair DNA lesions; initial sensing of DNA breaks followed by downstream events leading to cell cycle arrest, DNA damage repair, and subsequent

cell cycle resumption [1]. Ataxia telangiectasia mutated (ATM), DNA-dependent protein kinase (DNA-PK), phosphorylation-histone γH2AX, DNA damage checkpoint 1 (MDC1), and the Mre11-Rad50-Nbs1 (MRN) complex is play role in early sensors [2, 4]. These DNA repair pathways include a two distinct and complementary Homologous recombination (HR) and non-Homologous end-joining (NHEJ) [1].

The NBS1 (Nijmegen breakage syndrome) is known as DNA repair protein and is characterized by extremely radiation sensitivity and chromosomal instability [5]. NBS1 is one of the MRN complex containing Rad50 and Mre11. This complex performs to DNA double-strand break repair, cell cycle checkpoint, and telomere length maintenance [5, 6]. The NBS1 include a forkhead-associated domain (FHA) and a breast cancer carboxy-terminal domain (BRCT) in the N-terminal region [7]. Both FHA and BRCT domains play crucial role for both binding to histone and recruitment of MRN complex on DNA damage site [6, 8,

9]. In addition, NBS1 interact with γH2AX at DNA damage site. It is essential that NBS1 is required for DNA-damage dependent phosphorylation of Mre11 [7]. This phosphorylation is required for proper nuclear localization of the MRN complex to sites of DNA double-strand breaks [6, 10]. Therefore NBS1 is important for DNA damage repair. More recently, NBS1 localize at centrosomes via an interaction with γ-tubulin [11] and MRE11, one of the MRN complex, are required for accurate chromosome alignment during mitosis [12]. In this study, we find that NBS1 interacts with the centrosomal and microtubule-associated protein SPAG5 yeast two-hybrid screening. These results suggest that NBS1 may have a role in mitotic checkpoint signaling as well as DNA damage checkpoint signaling.

The SPAG5 is a non-motor spindle-associated protein essential for cell cycle progression especially during mitosis and include globular domain in the N-terminal and an extended coiled-coil domain in the C-terminal [13]. Mitosis has

five stages prophase, prometaphase, metaphase, anaphase, telophase and cyto-kines. Prophase occur breakdown of nuclear membrane, spindle fibers appear-ance, chromosomes condense. In prometaphase, spindle fiber attach to chromo-some. At metaphase, chromosomes align to middle of two spindle fibers. In ana-phase, centromeres division is effected by sister chromatids moving to opposite poles. In telophase, chromosomes are decondensed, nuclear membrane is re-formed, and spindle fibers disappear. In cytokines, cytoplasm divides and parent cell becomes two daughter cells including identical genetic information. We fo-calize on metaphase and anaphase. Two opposing kinetochores of a chromosome attach to microtubules emanating from opposite spindle poles [14]. This biopolar kinetochore-microtubule attachment induces chromosome alignments also ena-bles all sister chromatids to divide at anaphase [15]. The APC/C (anaphase-promoting complex or cyclosome) as regulator of chromosome segregation[16] activates the protease separase through triggering the ubiquitination and degra-

dation of securin and cyclinB1, separase inhibitors [17]. Activated separase cleaves cohesion, leading to chromosome separation. Errant chromosome separation causes aneuploidy and its associated disease [18]. Its error is prevented by spindle assembly checkpoint [19]. The spindle assembly checkpoint (SAC) is essential for accurate chromosome separation by delaying anaphase until all chromosomes are attached to a mitotic spindle during mitosis [17, 20, 21]. Spindle assembly checkpoint is important mechanism for accurate chromosome segregation [21-23]. SPAG5 is essential for proper spindle formation and is related to the dynamic and functional regulation of mitotic spindles [24]. It is localized specifically to centorosome and kinetochore from prophase though anaphase during mitosis [13, 25]. These localization plays a crucial role in sister chromatid cohesion and subsequent chromatid separation and is an important regulator of chromosome integrity in human mitosis [13, 26-28]. In mitosis, SPAG5 was phosphorylated by Aurora-A for regulation of spindle assembly checkpoint [26].

Deletion of SPAG5 cause spindle disorganization, chromosome misalignment, leading to spindle assembly checkpoint, aneuploidy, and apoptosis therefore SPAG is important to mitotic progression for normal spindle duplication and normal separate chromosome. Here, we describe interaction of NBS1 and SPAG5 during mitosis and demonstrate on mitotic function of NBS1 through the interaction with SPAG5 during mitosis.

Hence, we show whether NBS1 as DNA damage proteins regulate the cell cycle progression during mitosis. Here we have investigated a connection between DNA damage proteins and spindle assembly checkpoint. We found that NBS1 have relevance to mitotic progression. Depletion of NBS1 causes delay on mitotic progression and chromosome misalignment. Our data suggest that NBS1 plays a crucial role in proper mitosis progression, independent of DNA damage response.

MATERIALS AND METHODS

1. Cell culture and treatments

HeLa and HEK293T cells were purchased from ATCC. They were cultured in Dulbecco's Modified Eagle Medium complemented with 10% fetal bovine serum (Biowest) and streptomycin (0.1 mg/ml), penicillin (100 units/ml) (Gibco) at 37℃ in a 5% CO_2 incubator. For cell cyclesynchronization, HeLa cells were blocked by double thymidine treatment and released into the fresh medium. Cells were grown in the presence of 2mM thymidine (Sigma) for 18hours, followed by removing thymidine for another 17hours and then washed and released into fresh medium for different times as indicated. For mitotic cell synchronization, cells were treated with 100ng/ml nocodazole for 16hours.

2. siRNA transfection and establishment of stable cell lines

HeLa cells were transfected with siRNA oligonuleotide duplexes against NBS1 using Lipofectamine RNAimax (Invitrogen) according to the

maunfacturer's instructions. The siRNA sequences targeting NBS1 (NBS1 siRNA-1; 5′-GGCGUGUCAGUUGAUGAAA-3′ NBS1 siRNA-2; 5′-GUA CGUUGUUGGAAGGAAAUU-3′) were designed and synthesized for transient transfection. AssuTarget Control siRNA (Bioneer) was used as negative control. For shRNA, oligonucleotides encoding the target sequence for NBS1 were an-nealed and cloned into psilencer2.1-U6 vector (Ambion). Stable cell lines were obtained after transfection with Turbofect (Thermo) of a control and NBS1 shRNA-psilencer2.1-U6 construct, and selected in the presence of 500ng/ml G418 for 3weeks.

3. Immunoprecipitation assay and Western blot analysis

The whole cell lysates were prepared by extracting with immunopricipitaion assay buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 0.1% sodium deoxycholate, 0.5% TritonX-100) with protease inhibitors (Roche Diagnostic Corp). The lysates were added to anti-SPAG5 antibody

(Santa Cruz) at 4℃ for 24hours. Protein-A plus agarose beads (Santa Cruz Biotechnology) were added to the lysates, and lysates and beads mixtures were incubated at 4℃ for 4hours with shaking. The beads were washed five times in Lysis buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 0.1% sodium deoxycholate, 0.5% TritonX-100 with protease inhibitors) without protease inhibitors, and resuspended in equal volume of 2X SDS sample buffer. The samples were extracted from the bead by boiling at 95℃ for 5min and then analyzed by western blotting using the appropriate antibodies.

Cells were lysed in lysis buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 0.1% sodium deoxycholate, 0.5% TritonX-100) with protease inhibitors (Roche Diagnostic Corp). Cell lysates were collected by centrifugation at 13,000rpm for 10min. Protein concentrations were measured using the Bradford assay (Bio-Rad). Equal amounts of protein were separated by 6-15% SDS PAGE and then transferred onto a polyvinylidene difluoride mem-

brane (Millipore, Bedford, MA, USA). The membranes were blocked for 1 hr with TBS-T (10 mM Tris-HCl (pH 7.4), 150 mM NaCl and 0.1% Tween-20) containing 5% skim milk and then incubated at 4℃ using following antibodies; anti-NBS1 (Santa Cruz), anti-SPAG5 (Santa Cruz), Securin (Abcam), β-Actin (Santa Cruz biotechnology), CyclineB1 (Santa Cruz biotechnology), BubR1 (BD), Phospho-Histone H3(ser10) (Cell Signaling), Aurora-A (Cell Signaling), α-Tubulin (Sigma), γ-Tubulin (Ab FRONTIER).

The membranes were washed with 0.1% Tween20 containing TBS-T and then incubated for 2 hr with peroxidase-conjugated secondary antibodies (1:5000). The membranes were washed four more times and detected using an enhanced chemiluminescence detection system (ECL; GE Healthcare, Bucking-hamshire, UK).

4. Immunofluorescence microscopy and Live-cell analysis

Cells were grown in 12-well plates is placed on the coverslips. Cells were washed with phosphate-buffered saline and fixed with 4% paraformaldehyde in phosphate-buffered saline for 10mins at room temperature followed by ice-cold methanol for 5min. The fixed cells were permeabilized with 0.3% Triton X-100 in phosphate-buffered saline for 10min and stained with anti-α-Tubulin, anti-γ-Tubulin, anti-NBS1, anti-SPAG5, and anti-Aurora-A. Immunofluorescent images were observed by confocal microscopy (Zeiss LSM510 Meta, Carl zeiss)

For live-cell imaging analysis, control and NBS1 shRNA HeLa cells were transfected with GFP-tagged H2B-pcDNA3 followed by time lapse microscopy using Axio Observer microscope (Carl zeiss) maintained at 37℃ in 5% CO_2. Phase contrast images were acquired at 3-5min intervals for 12hours and analyzed using Zen pro software (Carl zeiss).

5. FACS (Flow cytometry) analysis

The G1-S phase synchronized HeLa cells were collected and washed once with ice-cold PBS, followed by fixing in 70% cold ethanol. The cells were then washed with PBS and stained with propidium iodide (50 ug/ml) containing RNase A (50 ug/ml) at 37℃ for 30min. The DNA content of the HeLa cells was analyzed by a FACScan flowcytometer (BD Biosciences). At least 10,000 events were analyzed, and the percentage of cells in sub-G1 population was calculated. Aggregates of cell debris at the origin of histogram were excluded from the sub-G1 cells.

RESULT

1. Knockdown of NBS1 lead to chromosome misalignment and centrosome defects.

Recently, studies report that MRE11, one of the MRN complexes, are required for accurate chromosome alignment during mitosis [12]. We identified whether another MRN complexes, NBS1 is also related to mitosis function. HeLa was transfected to siRNA-control and siRNA-NBS1-1, 2, A with Lipofectamin RNAimax in opti-MEM. Knockdown of NBS1expression in HeLa cells was confirmed by western blotting. The expression of NBS1 was reduced by more than 90% in cell line transfecting with NBS1 siRNA, compared with that of control siRNA-transfected cells (Fig 1A). Next, we examined NBS1-siRNA (silencing RNA) transfected cells for their effect to chromosomes in metaphase. Normal chromosome in Metaphase was condensed and aligned at the equatorial plane of the cell. It was measured the width of chromosome in metaphase using immuno-

fluorescence microscopy. The chromosomes of metaphase in the cell which is transiently downregulated NBS1 expression showed the misaligned chromosome compared to control cells. Aberrant alignment of chromosome at metaphase plate was closely related with the centrosome amplification. Centrosome is duplicated in prophase for normal chromosome separation. Deletion of NBS1 causes centrosome overduplication, indicating that NBS1 was concerned in mitosis (Fig 1B and C). We presented increased percentage of centrosome overduplication in NBS1-depleted cells (Fig 1D). These results suggest that depletion of NBS1 have influence on mitotic progression of cell cycle.

2. NBS1 is required for centrosome duplication and aberrant chromosome alignment.

To understand the mitotic role of NBS1 in normal cell, we created the stably knockdown of NBS1 expression using integrated NBS1-targeting shRNA (small hairpin RNA) expression vector. It were selected some of the efficient shRNA-

NBS1 expressing cell. Selected NBS1-shRNA expressing cells were tested by Western blot using. Western blot confirmed that expression of NBS1 was decreased by more than 90% in both cell line stably transfecting with NBS1-shRNA, compared with that of control cell (Fig 2A). To identify the mitotic event in NBS1-depleted cells, we performed the immunofluorescence microscopy using α-Tubulin, γ-Tubulin antibodies. (Fig 2B, C). We showed increased percentage of centrosome overduplication in depletion of NBS1 (Fig 2D). This reexamination convinces that NBS1 was important to regulation of chromosome alignment and centrosome duplication.

3. NBS1 behaves as a regulatory protein in mitotic progression.

To evaluate role of NBS1 in normal cell cycle progression, we assessed whether NBS1 was involved in cell cycle progression using sustained expression of NBS1-shRNA (Fig 3A). The identification of NBS1 in cell cycle progression was based on FACS analysis with PI-staining after G1/S phase synchronization

by double-thymidine block. FACS analysis was showed that NBS1-depleted cells caused a delay in escape the mitosis compared to the control cells (Fig 3B). Next, we reconfirm the role of NBS1 in cell cycle progression using live-cell microscopy. Stable NBS1-depletion HeLa cells were transfected with GFP-taggied histon H2B and performed the time-lapse microscopy. Live-cell microscopy observed the mitosis progression of cells expressing GFP-tagged histone H2B and typical examples of image sequences are given (Fig. 3C). Similar to previous result, the mitotic delay in NBS1 depletion was also showed in time-lapse microscopy. To evaluate the cellular events of the mitotic delay, we performed Western blotting analysis in the cells derived from the thymidine block and released (Fig. 3D). Here, we show that cell cycle progression was delayed in mitosis exit of NBS1 depleted cells, compared with control cells. These results demonstrate that NBS1 is required for normal cell cycle progression.

4. NBS1 interacts with SPAG5 at centrosome.

To search for unknown proteins that interaction with NBS1, we performed yeast two-hybrid screening, using human NBS1 as bait. A positive clones isolated from the transformants encodes the SPAG5. To identify the interaction between NBS1 and SPAG5 along cell cycle, we performed the immunofluorescence microscopy to monitor the subcellular localization of both proteins in cells. SPAG5 is mainly known to exist in the centrosome and kinetochores during mitosis, as shown in (Fig 4A). NBS1 also located in the centrosome with SPAG5. More detail, we confirmed whether NBS1directly interaction with SPAG5 in human cells. The cell cycle phases were identified by two ways. First, HeLa cell was grown in serum-free DMEM media during 48hours for G1 phase synchronization, Double-Thymidine (2 mM) block released time from synchronization for G2 phase (0h) and S phase (7.5h), and Nocodazole (100ng) for M phase. The other, HeLa was synchronized by Double-Thymidine(2 mM) block and released

indicated times. Synchronized cells were performed the immunoprecipitation as-say. The cell lysates were immunoprecipitated with SPAG5 antibody, and normal rabbit IgG was used as negative control. Immunoprecipitates were subject to western blotting with NBS1 antibody. On the other hand, amount of NBS1 was equally existed in cell cycle progression. SPAG5 expression was increased dur-ing mitosis. Furthermore, SPAG5 was phosphorylated during mitosis (Fig 4B and C). These results suggest that NBS1 play a role in cell cycle progression during mitosis through interact with SPAG5.

5. NBS1 regulates SPAG5 at centrosome during mitosis.

Some studies reported that SPAG5 localized at kinetochores and centrosome during mitosis [24]. We identify whether NBS1 is able to regulate the SPAG5. To do this, the expression level of SPAG5 in NBS1-depleted cells was deter-mined by immunofluorescence microscopy. Control and NBS1 depleted cells were stained with NBS1 and SPAG5 antibodies. The NBS1-depleted cells

showed that localization of SPAG5 at centrosome has been weakened, compared with control cells (Fig 5A). However, the kinetochore localization of SPAG5 did not affect in the depletion of NBS1. This finding is thought to be due to interacting directly between both proteins at the centrosome. These result that NBS1 can regulate to localization of SPAG5 at centrosome. SPAG5 behaves as phosphoprotein in metaphase during mitosis [26]. In addition, we performed Western blot for checking about alternation of phosphorylation of SPAG5 according to depletion of NBS1. It was not effect. In other words, NBS cannot control to phosphorylation of SPAG5 in metaphase (Fig 5B). Also, SPAG5 was known to conduct as upstream of Aurora-A [25]. We confirmed whether knockdown of NBS1 influence Aurora-A localization at centrosome. It was confirmed by immunofluorescence microscopy using Aurora-A and α-Tubulin antibodies. In depletion of NBS1, Aurora-A localization has been weakened, compared with control cells. SPAG5-depleted cell presents as positive control to comparing

with NBS1 depletion (Fig 6A). Finally, our data suggest that. Cell cycle progres-sion obstructed because NBS1 postponed onset of mitosis. SPAG5 has influence on localization at centrosome through interaction with NBS1, and NBS1 may be upstream of Aurora-A similar to SPAG5. Therefore, NBS1 play additional role cell cycle progression during mitosis.

Figure 1

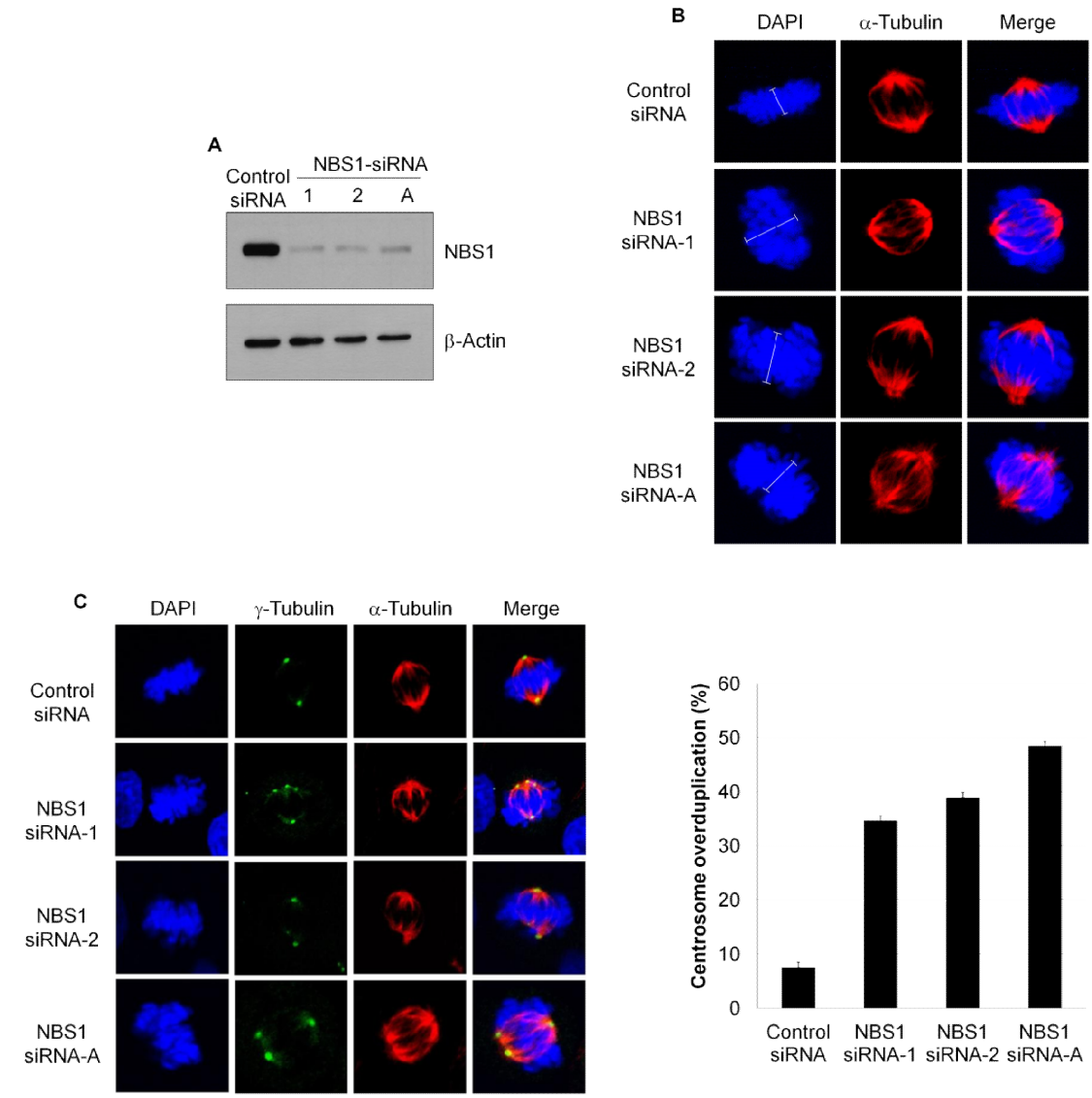


Figure 1. Depletion of NBS1 in HeLa cells causes chromosome misalignment and centrosome overduplication.

(A) HeLa cells were tranfected with siRNA targeting NBS1 with Lipofectamin RNAimax reagent in opti-MEM for 48hours. expression of NBS1 was confirmed by Western blot analysis using NBS1 antibody. β-Actin used as loading control. (B) HeLa cells were grown in 12well plates on coverslips and transfected with control and NBS1 siRNAs after 48hours. The cell was fixed with 4% paraformaldehyde in PBS, and stained with α-Tubulin for microtubule (Red), DAPI for chromatin (Blue) to monitor the chromosome alignment. (C) Transiently, siRNA-NBS1 was immunofluorescence microscopy. These cells were stained with γ-Tubulin for centrosome (green), α-Tubulin for microtu-bule (Red), and DAPI for chromatin (Blue). Quantification of mitosis was deter-mined by confocal microscopy. Error bars represented as mean ±SD

Figure 2

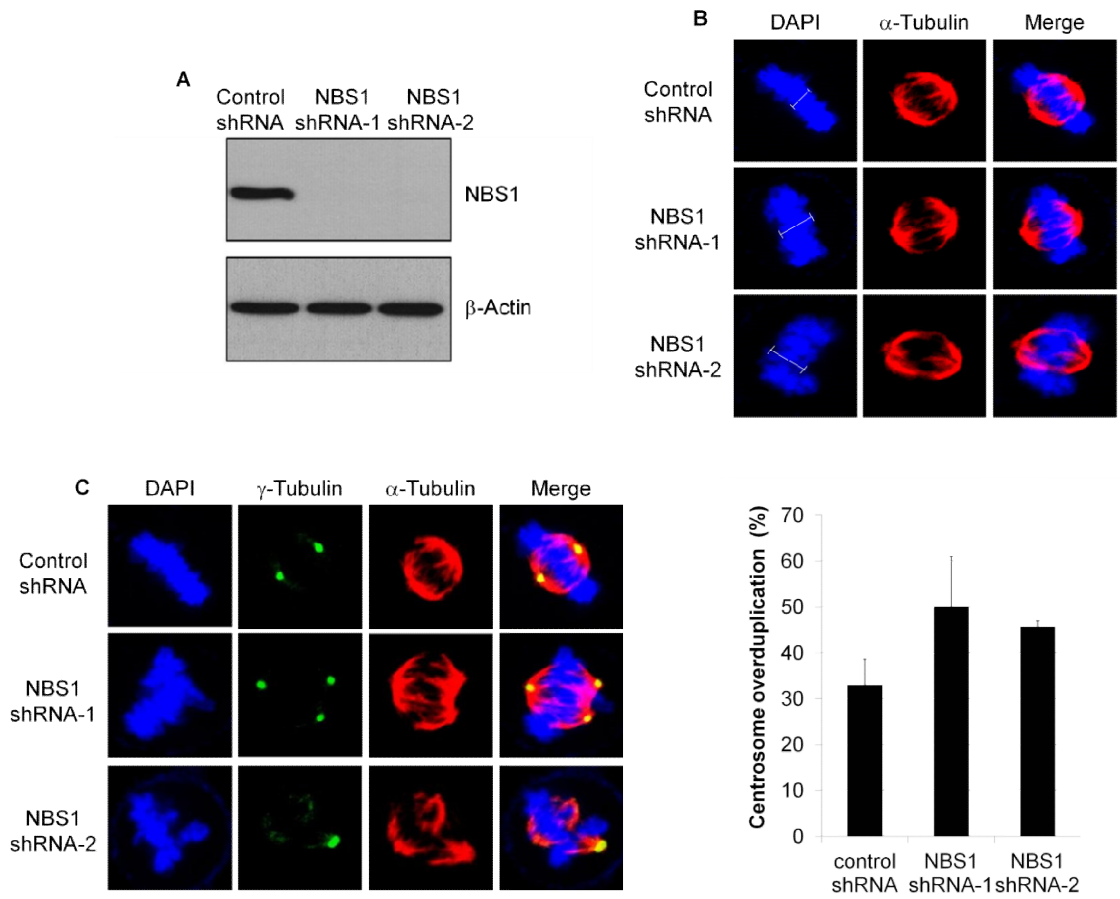
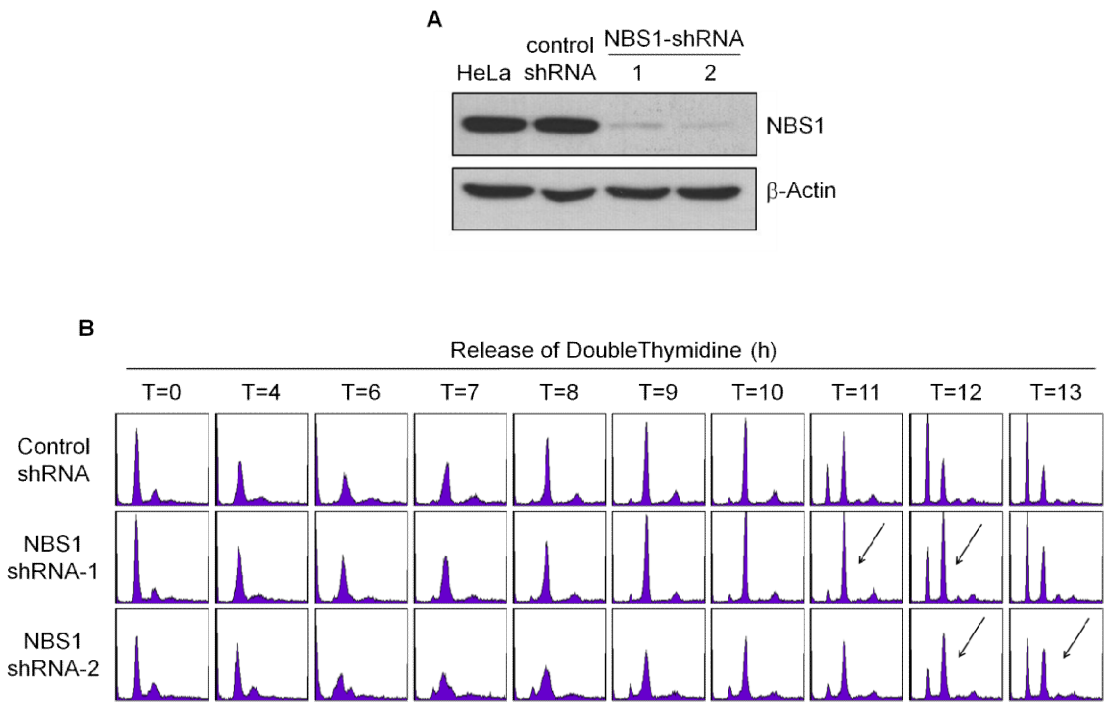
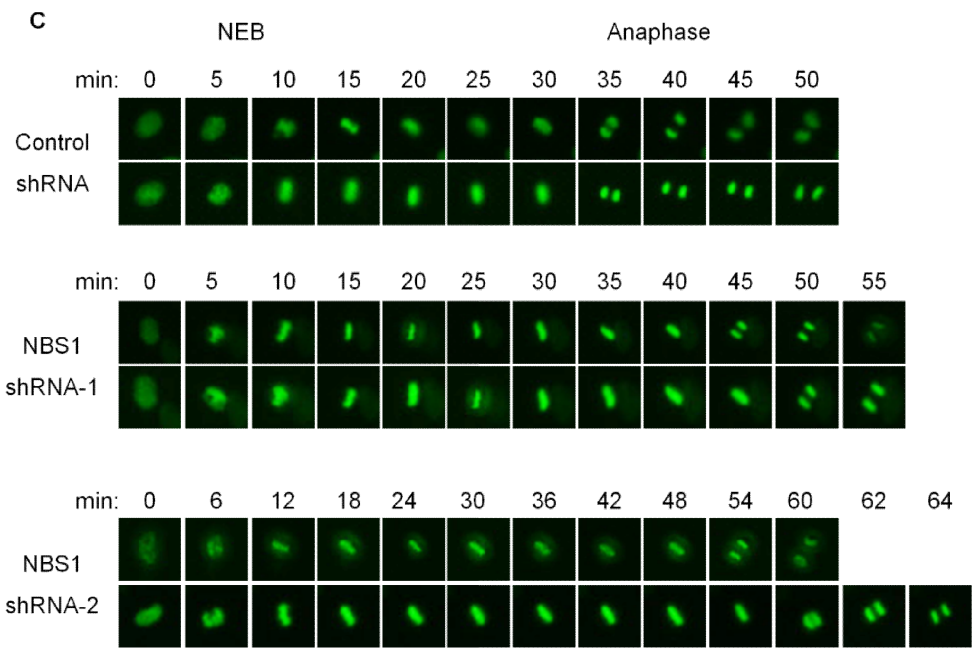


Figure 2. Chromosome misalignment and centrosome overduplication were caused by knockdown NBS1.

NBS1-depleted cells were cultured in DMEM media with G418 (400ng/ml). It were selected some of the efficient shRNA-NBS1 expressing cell. (A) Knockdown of NBS1 was confirmed by Western blot analysis. β-Actin used as loading control. (B) Endogenously, stable cell was fixed by 4% paraformaldehyde in PEM buffer. NBS1-depleted cells have identified chromosome alignment by Immunofluorescence which staining of α-Tubulin (Red) for microtubule, DAPI (Blue) for chromatin. (C) Sustained expression of NBS1-shRNA was confirmed centrosome duplication by immnufluorescence and stained by γ-Tublulin (Green) for spindle, α-Tubulin (Red) for microtubule, DAPI (Blue) for chromatin. Quan-tification of mitosis was determined by confocal microscopy. Error bars repre-sented as mean ±SD.

Figure 3





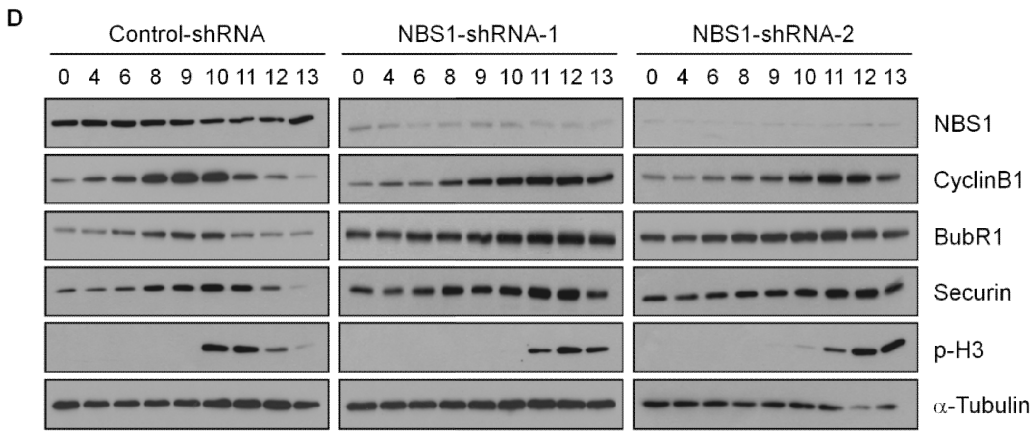


Figure 3. Depletion of NBS1 causes abnormal mitotic progression.

(A) Western blot analysis reveals the downregulation of NBS1 expression level in NBS1-depleted cell. β-Actin used as loading control. (B) Control and NBS1-depleted cells were synchronized with a double-thymidine block and released from this block as indicated times. At the time point cells were stained with PI solution and analyzed the cell cycle progression by FACS analysis. The arrows indicate the mitotic cells which were delayed the mitosis exit compared to control cells. (C) The mitotic progression of cell cycle in NBS1 loss was observed by time-lapse microscopy. The fluorescent images were taken every five minutes from early prophase showing a nuclear envelope breakdown (NEB) to cytokinesis. (D) Knockdown of NBS1 cells were synchronized in G1/S phase by double-thymidine (2 mM). Western blot analysis was carried out specific antibodies against NBS1, CyclinB1, BubR1, Securin, pH3 for metaphase and α-Tubulin as loading control.

Figure 4

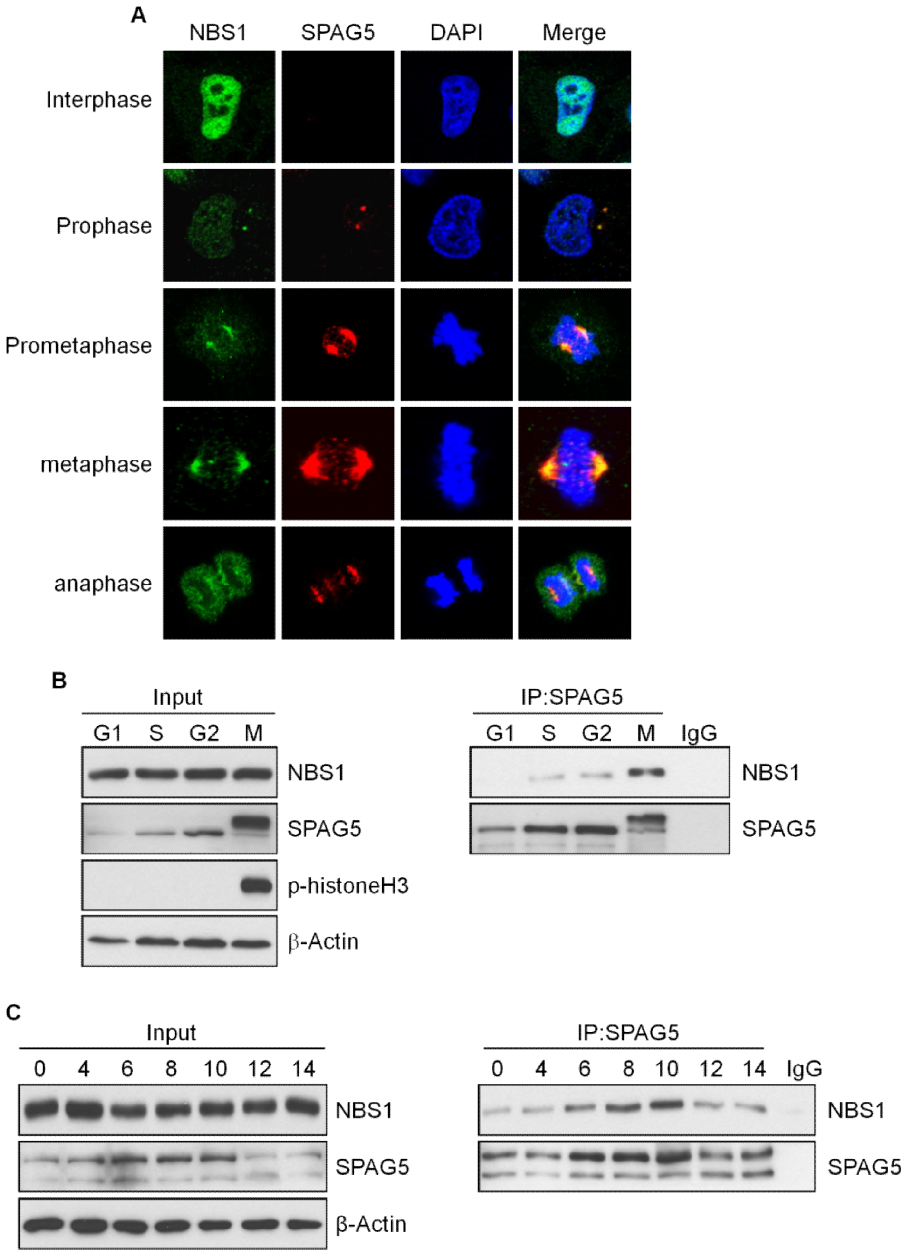


Figure 4. NBS1 interact with SPAG5 and co-localized at the centrosome during mitosis.

HeLa cells were cultured in DMEM media. (A)Immunofluorescence microscopy was performed to identify the cellular localization of NBS1 SPAG5 in growing cells. Asynchronous HeLa cells were fixed with 4% paraformaldehyde in PEM buffer and stained with anti-NBS1 (Green) and anti-SPAG5 (Red) antibodies. (B), (C) HeLa cells were synchronized at cell cycle phase according to material and methods. Synchronized cells were lysed to RIPA buffer, and lysates were immunoprecipitated using anti-SPAG5 antibody. Normal rabbit IgG was used for negative control of immunoprecipitation. Immunoprecipitates were detected using antibodies against SPAG5 and NBS1 by western blot analysis. β-Actin used as loading control.

Figure 5

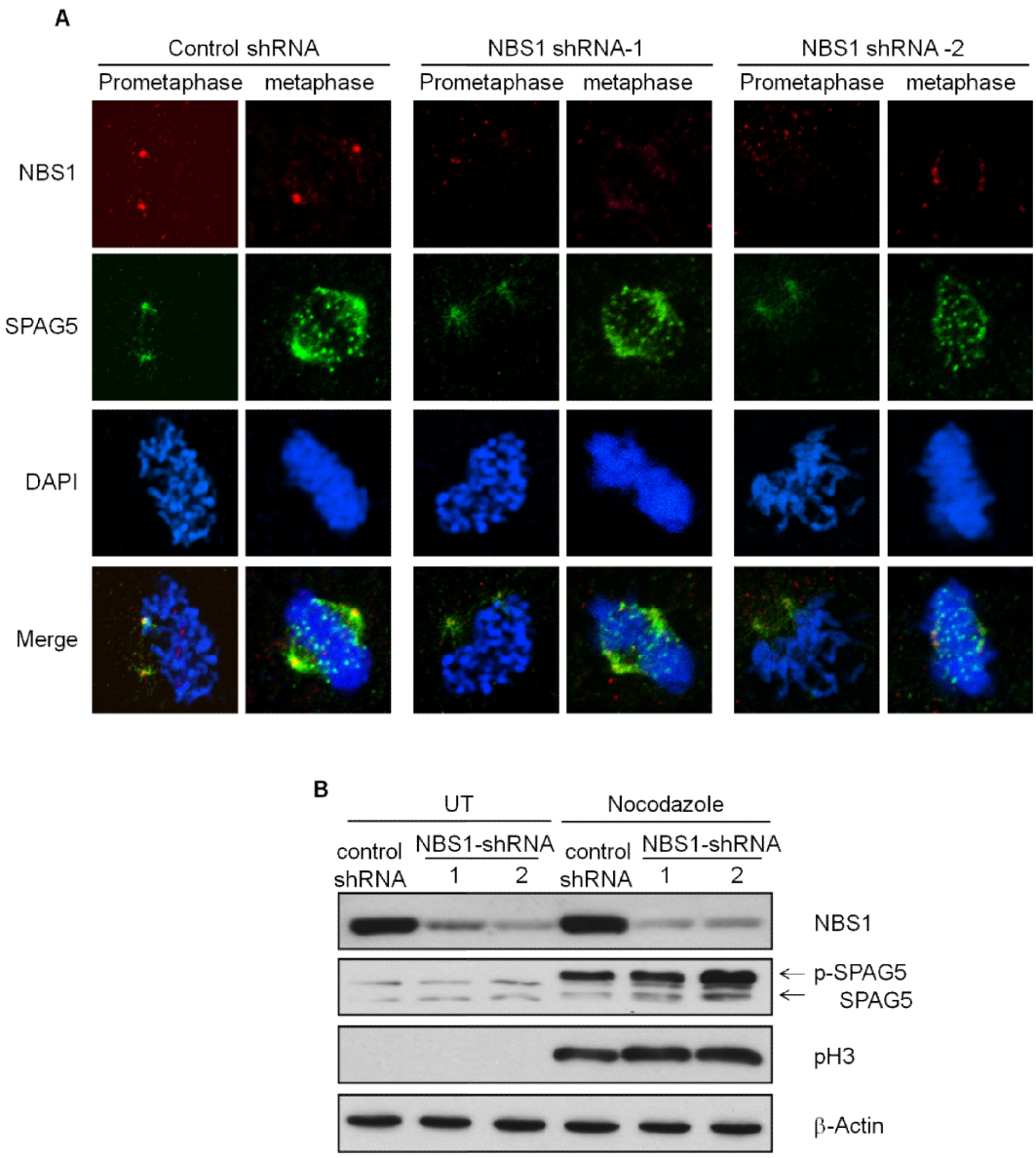


Figure 5. NBS1 is required for localization of SPAG5.

(A) Control and NBS1-depleted cells were fixed and analyzed by immunofluorescence microscopy. Cells were stained for anti-NBS1 and anti-SPAG5 antibodies. Respectly, images were obtained at the indicated cell cycle stages, prometaphase and metaphase, and merged together with DAPI for DNA staining. (B) Control and NBS1-depleted cells were treated with nocodazole for mitotic cells. These cells lysed by RIPA buffer and implemented by western blot analysis using antibodies NBS1, SPAG5. Phosphos-histone H3 is marker as metaphase and β-Actin is a loading control.

Figure 6

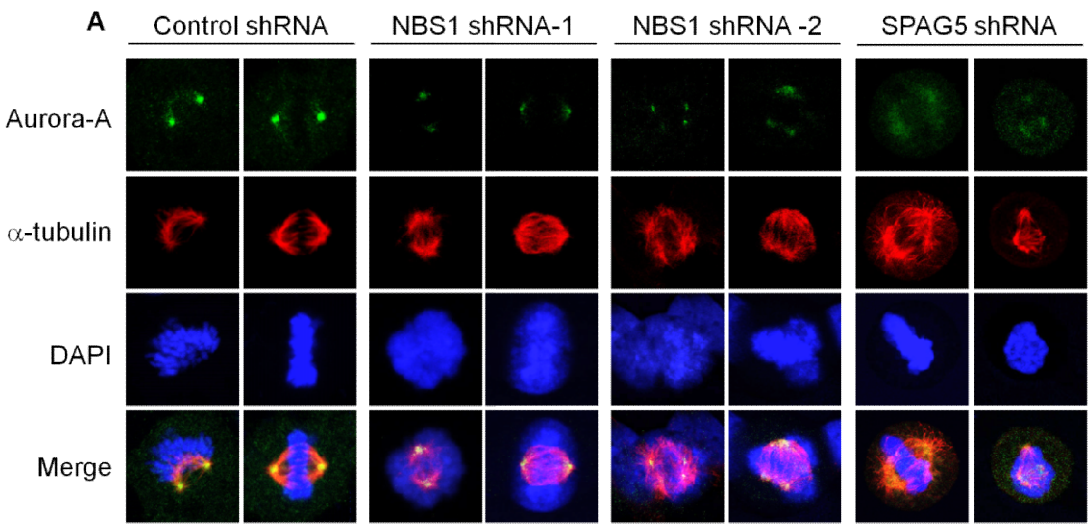


Figure 6. Deletion of NBS1 affects the delocalization of Aurora-A.

(A) Control and NBS1-depleted HeLa cells seeded on coverslips were fixed and stained. Mitotic spindle localization was confirmed by immunofluorescence staining. After cells fixing, deletion of NBS1 was stained using specific antibodies Aurora-A (Green), α-Tubulin (Red) for microtubule and DAPI (Blue) for chro-matin indicating prometaphase and metaphase

DISCUSSION

In this study, we demonstrate a mitotic function of NBS1 in cell cycle progression. NBS1 is a major regulatory protein of DNA damage repair upon ionizing radiation (IR) and radiomimetic drugs [5, 7]. Recently, NBS1 has been reported to localize at centrosome through an interaction with γ-tubulin [11] and loss of NBS1 induces supernumerary centrosome. The MRE11, one of the MRN complexes, are required for accurate chromosome alignment during mitosis [12]. Thus, functional studies of the role of NBS1 in mitosis may be required.

Here, we have found evidence that NBS1 serves as a regulatory protein in cell cycle progression. The absence of NBS1 causes chromosome misalignment and aberrant centrosome duplication in metaphase. Moreover, NBS1-depleted cells caused a delay in escape the mitosis. Furthermore, we conducted yeast two-hybrid and identified interaction between NBS1 and SPAG5. In mammalian cell, we showed NBS1 directly interaction with SPAG5 through endogenous

immunoprecipitation assay. To support for data, we confirmed co-localization both NBS1 and SPAG5 on centrosome. This newly discovery is compatible with the function of NBS1 that localize at centrosomes via an interaction with γ-tubulin [11] and SPAG5 regulate to chromosome alignment among spindle poles in mitosis [24, 29]. In NBS1-depleted cells, we have identified that SPAG5 localization weakens in mitosis. According to report, SPAG5 regulates localization of Aurora-A at centrosome [25]. Aurora-A kinase is a member of the serin/threonine protein kinase family, and is involved in mitotic progression for G2 phase to M phase transition [30]. Thus, we show whether NBS1 also regulate Aurora-A in mitosis. As a result similarly, the absence of NBS1 interfered the centrosome localization of Aurora-A. This finding indicate that NBS1 regulate localization of Aurora-A at centrosome in mitosis.

Finally, our experiments demonstrate an important role of NBS1 in cell cycle progression during mitosis. This is important discovery which have uncovered

for the first time. Many genetic studies of spindle assembly checkpoint support the important role of normal cell cycle progression. Also, we show that depletion of NBS1 affect protein function of cyclinB1, BubR1, and securin during cell cycle. Therefore, we should research on more detail mechanism about mitotic progres-sion of NBS1.

ABSTRACT

The role of NBS1 in normal cell cycle progression during mitosis.

Yu Ri Kim

Advisor: Prof. Jung-Hee Lee, Ph.D.

Department of Biomedical sciences,

Graduate school of Chosun University

To genome maintaining, DNA damage checkpoint and spindle assembly checkpoint plays an important role in mammalian cells. Cell cycle Checkpoint protein can control response to malignant cancers. Recent studies report that DNA damage checkpoint proteins are related to spindle assembly checkpoint.

The NBS1 protein is essential to DNA damage response that maintains genome integrity to prevent cancer and developmental disorders. We find that NBS1 interaction with SPAG5 identified by yeast two-hybrid. The SPAG5 is required to regulation of mitosis spindle. We examined immunofluorescence microscopy, NBS1 foci is correctly located in centrosome in mitosis. Depletion of NBS1 in mammalian cells cause multi-spindle pole with a delay in mitotic progression. These finding demonstrate that Independent of DNA damage response, the NBS1 play a vital role in mitosis progression.

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