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The regulatory mechanism of NBS1 by LAP2-α

조선대학교 대학원

의과학과

문 주 란

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LAP2-α 에 의한 NBS1 활성 조절 연구

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이 논문을 석사학위신청 논문으로 제출함

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LAP2-α에 의한 NBS1 활성 조절 연구

문 주 란

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의과학과

MRE11/RAD50/NBS1 (MRN) 복합체의 구성 요소 중 하나인 NBS1은 DNA 손상에 대한 반응과 염색체 무결성을 유지하는데 있어 중요한 역할을 하는 단백질이다. 일반적으로 NBS1 단백질 자체는 DNA 손상 복구 및 DNA 손상 신호 전달에 필요한 DNA 결합 및 kinase 활성을 갖고 있지 않지만 DDR에 관여하는 단백질들과 결합하여 DNA DSB의 접근성을 갖고 있으며, 중심 영역에

있는 세린/글루타민 모티프 (SQ motif)는 이온화 방사선에 반응하여 ATM ki-nase에 의해 인산화 되고, 이러한 인산화는 cell cycle checkpoint 컨트롤 및 텔로미어 유지에 있어서 중요한 역할을 한다. 그러나 DNA 손상 복구에서 NBS1의 정확한 기능은 완전히 밝혀지지 않았다. 본 연구에서는 이런 DNA 손상 반응에 중요한 역할을 하는 NBS1의 조절 기전을 좀 더 자세히 밝히고자, Yeast two-hybrid screening을 통해 NBS1과 결합하는 LAP2α (TMPO, Lamina-associated polypeptide (LAP)2α)라는 새로운 단백질을 동정하였다. 본 연구에서 우리는 DNA 손상 복구 반응에서 LAP2α가 어떠한 메커니즘을 통해 NBS1의 활성을 조절하는지에 대해 밝히고자 한다. 먼저 흥미롭게도 세포 내/외에서 방사선 조사 후 NBS1과 LAP2α의 결합이 증가함을 확인하였다. 이온화 방사선을 포함한 DNA 손상을 유도하는 시약 (HU, CPT, Olaparib)을 이용한 clonal survival을 통해 LAP2α의 기능 상실이 특히 이온화 방사선 (IR)에 의한 DNA손상 시 세포 민감도를 증가시킴을 확인하였다. 또한 γ-H2AX foci 염색을 통해 LAP2α의 감소가 DNA 손상 복구에 결함이 있음을 확인하였고 상동

재조합 활성 분석법을 통해서 LAP2α가 NBS1에 의존적인 경로를 통해 상동 재조합 활성을 조절하는 단백질 임을 확인하였다. 그리고 PLA assay를 통해 LAP2α가 DNA damage site로 모집 됨을 보았다. 우리는 LAP2α가 어떠한 메커니즘으로 NBS1과 함께 상호작용하여 DNA 손상 반응에 관여하는지에 알아보고자 몇가지 실험 방법으로 접근해 보았다. 먼저 NBS1과 LAP2α의 도메인 결실 돌연변이를 이용한 면역침강법으로 우리는 LAP2α가 NBS1의 BRCT2 domain과 결합하여 NBS1과 인산화 단백질 사이의 상호작용에 관여함으로써 DDR경로에서 역할을 수행할 수 있는 가능성을 확인하였다. 그리고 NBS1이 choromosome binding domain을 포함한 LAP2α specific region과 상호작용함으로써 DNA에 대한 접근성을 가질 수 있음을 확인하였다. 다른 접근으로 DNA 손상 반응에서 NBS1과 관련된 ATM-CHK2 경로에 LAP2α의 결핍이 어떠한 영향을 주는지에 대해 알아보고자 하였다. 웨스턴블랏 실험을 통해 ATM-CHK2 경로에 관련된 단백질의 발현 양에 대해 확인하였고 LAP2α의 결핍이 NBS1과 관련된 ATM-CHK2 경로에 영향을 주지 않음을

확인하였다. 그리고 단백질 변형과 관련된 메커니즘으로 접근해 보았다. DNA 손상 반응에 관련된 여러 단백질처럼 이온화 방사선에 의해 LAP2α가 인산화 되는지 확인해 보고자 하였다. 먼저 면역침강법을 통해 이온화 방사선으로 DNA 손상을 주었을 때 LAP2α의 인산화 여부를 확인하고자 하였고 LAP2α가 인산화 됨을 확인하였다. 추가로 LAP2α의 시퀀스 분석을 통해 LAP2α에 ATM 인산화 타겟 시퀀스가 있음을 알 수 있었다. 이러한 결과를 바탕으로 LC-MS/MS분석을 진행하였지만 분석결과 이온화 방사선 처리 유무에 LAP2α의 인산화에 변화가 없음을 확인하였다. 따라서, 본 연구는 DNA 손상 반응에 있어서 상동 재조합 활성을 조절하는 새로운 조절 인자 LAP2α를 동정함과 동시에 LAP2α가 관여하는 NBS1의 새로운 조절 기전을 제시하는 바이다.

INTRODUCTION

Each cell in the human body influenced by tens of thousands of DNA lesions daily. If they are incorrectly repaired, these lesions cause mutations or genome aberrations that threaten cell or organism viability. To cope with threats from DNA damage, cells have evolved a mechanism known as DNA damage reactions (DDR) collectively to detect DNA lesions, send presence signals, and promote repair. [1]. DNA damage come from either endogenous sources, such as cellular metabolic processes, or exogenous sources, including environmental factors such as radiation[2]. A lot of proteins and pathways are involved in multiple DNA repair pathways, which correct the different types of DNA damage. If the damage cannot be removed, apoptosis occurs. Therefore, accurate regulation of complex process networks that allow genetic diversity but protect against genetic instability and consequences for tumor formation is necessary for maintaining genomic stability.

Cells have at least five major DNA repair pathway. There are base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR) and non-homologous end joining (NHEJ). These pathways are active throughout different stages of the cell cycle, enabling the cells to repair the DNA damage[3]. Among the various types of DNA lesions, single strand breaks (SSBs) are very common[4], direct attacks of intracellular metabolites and spontaneous DNA spoil occur at tens of thousands of frequencies per cell per day. [5]. Among the DNA repair pathway, base excision repair (BER), nucleotide excision repair (NER) and mismatch repair (MMR) are single-strand break repair pathways (SSBR). On the other hand, DNA double-strand breaks (DSBs) are the most cytotoxic DNA lesions. So their detection, signaling, and repair require a comprehensive cellular response collectively known as the DNA damage response (DDR)[6]. There are two main strategies are employed for DSB repair: homologous recombination (HR) and non-homologous end-joining (NHEJ). Homologous

recombination (HR) maintains genomic integrity by promoting "error-free" repair of DNA double-stranded breaks (DSB) primarily in the phases S and G2 of the mitotic cell cycle, whereas non-homologous end joining (NHEJ) performed throughout the cell cycle, especially preferred pathway for DSB repair in G1 phase. NHEJ is "error-prone" repair because NHEJ directly ligates DNA-ends[4, 7].

The DSB repair pathway can be classified into three main functional components : (1) sensors of DNA damage, (2) signal transducers, and (3) effectors[4]. The Mre11-Rad50-Nbs1 (MRN) complex located in the hub of the eukaryotic DSB response mechanism, has a crucial role in each of these three facets of DSB repair. MRN complex acts as the first DSB sensors, co-activator of DSB-induced cell cycle checkpoint signaling and DSB repair effector in both the HR and NHEJ pathways. In other words, MRN complex organizes DDR response in double-strand break (DSB)[4, 8].

Homologous recombination (HR) pathway : After DSB recognition, MRN complex recognizes the DNA DSBs and that recruits and activates the upstream DDR kinase ataxia telangiectasia mutated (ATM). ATM triggers a cascade of DDR events on the chromatin flanking DSBs. 5′–3′ end resection is initiated by the MRN (Mre11, Rad50, Nbs1) complex and CtIP. At the DSB ends, Exo1, DNA2, and Sgs1 proteins further resects one strand of DNA directionally (5′ −3′) to generate a 3′ single strand DNA (ssDNA) tail. Then, resected ssDNA−ends are coated by rep−lication protein A (RPA), which is subsequently replaced by another protein Rad51 in a BRCA1− and BRCA2−dependent process. Then, the nucleofilament could search for the homologous DNA sequences and invade into the homologous DNA sequences. After invasion, DNA is synthesized using homologous DNA sequences as templates. After DNA synthesis, the DNA ends are connected to the other side of the DSB.[4, 9, 10].

Human NBS1 consists of 754 amino acids that contains two functional regions at the N terminus and the C terminus. The N-terminus contains a forkhead-associated domain (FHA) and tandem BRCA1 C-terminal (BRCT) motifs (BRCT1 and BRCT2), which bind phosphorylated proteins, including CtIP and MDC1. Also NBS1 interact directly with the histone protein H2AX, which is rapidly phosphorylated following DSB induction through FHA/BRCT domain. The C terminus of NBS1 contains at least five regions conserved in vertebrates: an RPA-binding region, an RAD18-binding region, an MRE11-binding region, an RNF20-binding region and an ATM-binding region[11].

In this study, we found that NBS1 interacts with LAP2α by yeast-two hybrid screening. Also, this interaction was confirmed by immunoprecipitation and PLA assay. Our results indicated that LAP2α deficient cells were hypersensitive to IR-induced colony formation and have a major DSB-repair defect, shown by presence of late γ-H2AX foci. And we found that LAP2α is recruited as a DNA

damage site through PLA assay. Furthermore, LAP2α deficient cells showed im-paired Homologous recombination activity. Through IP experiments using domain mutants, we found that LAP2α binds to the BRCT2 domain of NBS1 and NBS1 binds to the specific region of LAP2α. And through western blotting, we found that depletion of LAP2α did not affect NBS1-mediated ATM-CHK2 signaling. Besides, we found that phosphorylation of LAP2α through IP experiments, but it could not be confirmed by LC-MSMS.

Here, we suggest that LAP2α is DSB-responsive-IR-hypersensitive NBS1 binding partner. Furthermore, we tried to find out the regulatory mechanism of NBS1 by LAP2α in several way.

MATERIALS AND METHODS

1. Cell culture and treatment

HeLa, U2OS, and HEK293T cells were obtained from ATCC and grown in DMEM (Dulbecco's modified Eagles's medium, Gibco) supplemented with 10% fetal bovine serum (FBS, YOUNG IN FRONTIER) and antibiotics at 37℃ in a 5% CO2 incubator. HeLa DR-GFP (pHPRT-DRGFP expressing stable cell lines based on HeLa cells) and U2OS DR-GFP (pHPRT-DRGFP expressing stable cell lines based on U2OS cells) cells were grown in DMEM medium supplemented with 10% FBS and antibiotics at 37℃ in a 5% CO2 incubator. In order to induce DNA double strand breaks, cells were irradiated with 10Gy from 137Cs source (Gammar cell 3000 Elan radiograph, Best Theratronics) and recovered several times in a 37℃ incubator.

2. siRNA transfection

HeLa, U2OS, and HEK293T cells were transfected with siRNA oligonucleotide duplexes against LAP2α using RNA IMAX (Invitrogen) according to the manufacture's instruction. The siRNA sequences targeting LAP2α (LAP2α siRNA #1: 5'-GGACTTCTCCAGTGACGAA-3', LAP2α siRNA #2: 5'-TACCTACTGAGTGCTATGT-3') designed and synthesized for transient transfection.

3. Immunoprecipitation assay

For immunoprecipitation assay, cells were lysed in NP-40 buffer [50mM Tris PH 8.0, 150mM NaCl, 1% NP-40, and 5mM EDTA, 1 mM phenylmethylsulfoyl fluoride (PMSF), 1 mM dithiothreitol (DTT), and protease inhibitor cocktail (Roche Diagnostic Corp)] and incubated at 4 ℃ for 30 min. These lysates were cleared by centrifugation and incubated with antibodies, or normal IgG (control) at 4℃ for 24hours. And then, protein A/G plus-agarose beads (Santa Cruz Biotechnology), G-sepharose or A sepharose (GE Healthcare) were added to the lysates,

and beads mixtures were incubated at 4℃ for 4 hours with shaking. The beads were washed five times in NP-40 buffer and then samples were boiled in 2 × SDS loading buffer. Then, samples were analyzed by western blotting using the appro-priate antibodies.

4. Western blot analysis

For immunoblotting analysis, cells were collected and lysed in RIPA buffer [50mM Tris-HCl (pH 7.5), 150mM NaCl, 1% NP-40, 0.5% sodium deoxycho-late(NADOC), 0.1% sodium dodecyl sulfate(SDS), 1 mM phenylmethylsulfoyl flu-oride (PMSF), 1 mM dithiothreitol (DTT), and protease inhibitor cocktail (Roche Diagnostic Corp)]. Protein concentrations were measured using the Bradford as-say (Bio-Rad). The same amounts of protein were separated by 6-15% SDS-PAGE and then transferred to PVDF (polyvinylidene difluoride) membranes (Mil-lipore, Bedford, MA, USA). PVDF membranes were followed by blocking with 5%

milk in TBS-T (10mM Tris-HCl (pH 7.4), 150mM NaCl and 0.1% Tween-20) and incubated overnight at 4℃ with primary antibodies (1:1000). The membranes were washed 5 times for 10min with TBS-T and then incubated for 2 hours with peroxidase-conjugated secondary antibodies (1:4000) at RT. The membranes were washed 5 times for 10min, and developed using an enhanced chemilumines-cence detection system (ECL; GE Healthcare, Buckinghamshire, UK).

5. Clonal survival assay

Control cell and LAP2α depleted cells were treated with various doses of damaging agent (IR, HU, CPT, Olaparib) and split into 60mm dish or 6-well plates. After 10-14 days, viable cells were fixed with 95% methanol and stained with 1% methylene blue in 20% ethanol, and then counted. Each experiment was repeated at least three times.

6. Immunofluorescence microscopy

For immunofluorescence studies, cells were seeded onto glass-coverslips and irradiated with 10 Gy of ionizing radiation (IR). Cells were fixed with 4% para-formaldehyde (PFA) for 10min and ice-cold 98% methanol for 5 min, then per-meabilized with 0.5% Triton-X for 15 min and blocked in 5% bovine serum albumin for 1hour. Cells were incubated with indicated primary antibodies overnight at 4℃, then incubated Alexaconjugated secondary antibodies for 2 hours at room tem-perature. After washing, the cells were mounted using Vectashield mounting me-dium with 4, 6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA, USA). Fluorescence images were taken using a confocal microscopy (Zeiss LSM510 Meta: Carl Zeiss) analyzed with ZEN software.

7. Yease two hybrid screening

Yeast two hybrid screening of NBS1 was performed in yeast PBN204 strain

containing 3 reporters (URA3, lacZ, and ADE2) under the control of different GAL promoters with the use of the PanBioNet protocol (PanBioNet corp., Korea).

8. Homologous recombination assay (DR-GFP assay)

To measure the HR repair, stable cell lines expressing DR-GFP reports were generated by transfection using RNA IMAX (Invitrogen). HeLa-DR-GFP and U2OS-DR-GFP cells were transfected with control or LAP2α siRNA, and then transfected with 0.5 μg of I-SceI-expressing vector. After 48hr, cells were fixed 4 % paraformaldehyde and stained 5μg/ml Hoechst (Sigma) for 1hrs. The images were shown at x20 magnification using an inverted fluorescence microscope (IN Cell Analyzer 2500 HS). The data are presented as the mean ± SD. value in three independent experiments.

9. In situ proximity ligation assay (PLA)

For PLA assay, cells were seeded onto glass-coverslips. The cells fixed with

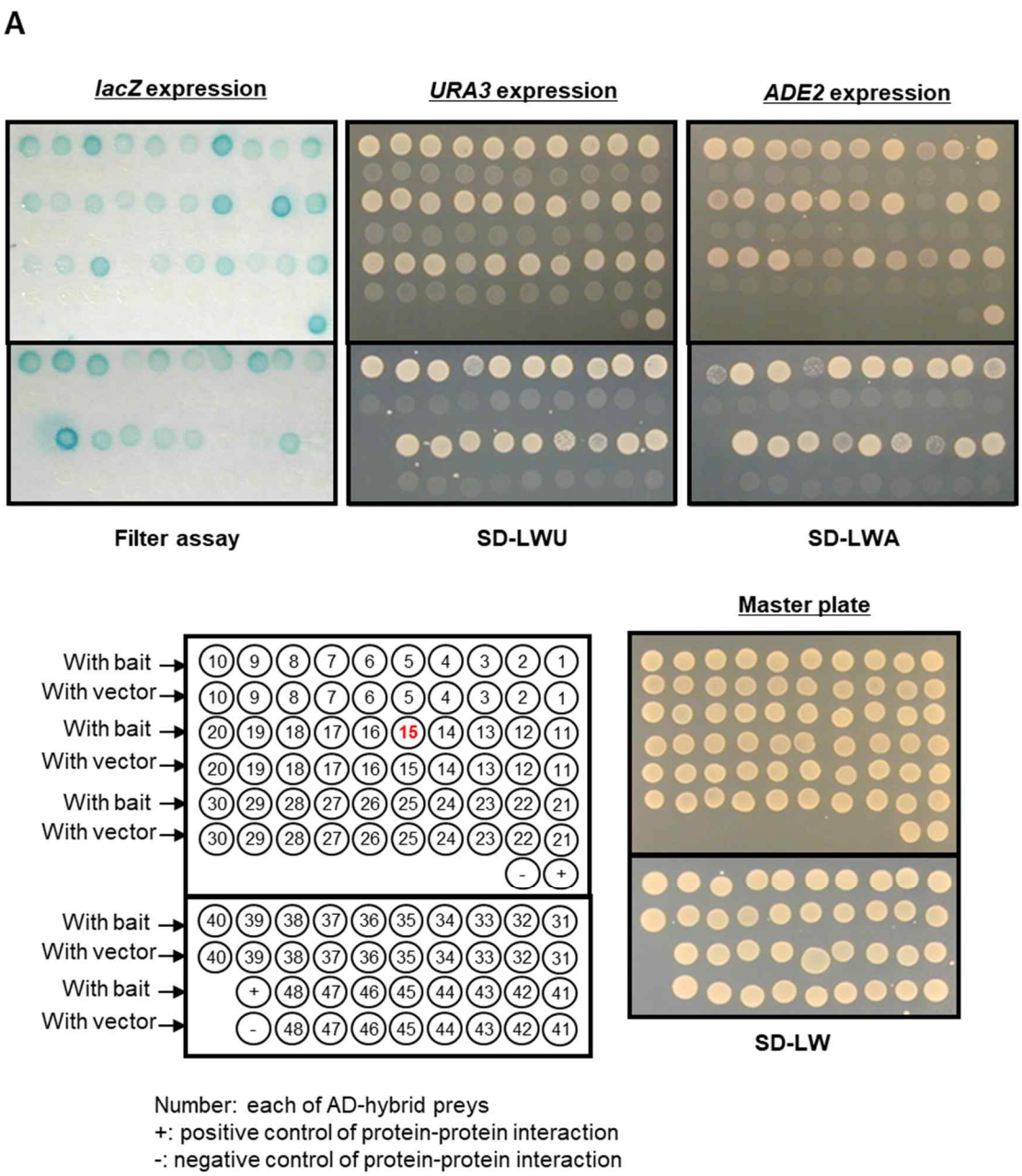
4% formaldehyde, permeabilized with 0.3% Triton X- 100, and then blocked using Duolink blocking buffer for 1hour. Two primary antibody incubation performed overnight at 4℃ with gentle agitation. Samples were washed twice with 0.05% Tween-20 containing PBS , and then incubated with PLA probes MINUS and PLUS (DUO92005, Sigma) for 1hour at 37℃. After washed using buffer A (0.01M Tris, 0.15M NaCl, 0.05% Tween-20, pH7.4), the probes were ligated with two other circle-forming DNA oligonucleotides by ligation-ligase solution for 30min at 37℃. After washed using buffer A, add the amplification-polymerase solution and incubation over 90min at 37℃. After washed using buffer B (0.2M Tris, 0.1M NaCl, pH7.5) for 10min twice, the samples were dried for 10min in the dark RT. Then the cells were mounted using Vectachield mounting medium with 4, 6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA, USA). Fluorescence images were taken using fluorescence microscope (IN Cell Analyzer 2500 HS).

RESULT

1. Identification of LAP2α as a NBS1-associated protein

To identify new regulators of NBS1, we performed the yeast two-hybrid screening. Yeast-two-hybrid (Y2H) is largely used as a strategy to detect protein-protein interactions (PPIs)[12]. We used the full length-NBS1 as bait and HeLa cDNA library as preys for Y2H screening. Yeast transformants were spread on selection medium SD-LWU (SD without leucine, tryptophan and uracil), SD-LWA (SD without leucine, tryptophan and adenosine) and filter assay (Fig 1A). Through this experiment, 48 positive colonies could be obtained, and 11 genes were identified, one of which, is thymopoietin (TMPO) (Fig 1B).

Figure 1



B

Prey ID (AD-hybrid)	Description	Reporter expression		
		lacZ	URA3	ADE2
1-3, 5, 6, 8, 10, 20, 24-25, 33, 40, 46	sperm associated antigen 5 **(SPAG5)**	+	+	+
4, 28	telomeric repeat binding factor (NIMA-interacting) 1 **(TERF1)**	+	+	+
7, 16, 19, 21-23, 29, 32, 34-36, 38, 39, 42, 45, 47, 48	E2F transcription factor 1 **(E2F1)**	+	+	+
9, 11, 14, 30, 31, 37, 43, 44	actinin, alpha 1 **(ACTN1)**	+	+	+
12	ataxin 2 **(ATXN2)**	+	+	+
13	chromosome 15 open reading frame 23 **(C15orf23)**	-	+	-
15	thymopoietin **(TMPO)**	+	+	+
17, 41	karyopherin alpha 2 (RAG cohort 1, importin alpha 1) **(KPNA2)**	+	+	+
18	RAD50 interactor 1 **(RINT1)**	+	+	+
26	translocated promoter region (to activated MET oncogene) **(TPR)**	+	+	-
27	HCLS1 associated protein X-1 **(HAX1)**	-	+	-

Figure 1. Yeast two hybrid screening identifies proteins that bind to NBS1.

(A) Yeast two hybrid screening to identify NBS1 interaction protein. NBS1 WT used as bait and HeLa cDNA library as preys.

(B) A list of proteins identified in the yeast two hybrid screening. The each clone was determined by DNA sequencing and BLAST search. The 15th clone is thymopoietin (TMPO).

2. NBS1 interacts with LAP2α.

To confirm the interaction between LAP2α and NBS1, we performed the co-immunoprecipitation assay. For production of DSBs, HeLa cells were treated with 5Gy of IR, then recovered for 3 hours. Total lysates performed immunoprecipitation using anti-LAP2α antibodies followed by immunoblotting using NBS1 or LAP2α antibodies. Endogenous immunoprecipitation data showed that two protein interacted each other in undamaged cell, and the interaction was increased by IR treatment (Figure 2A). Furthermore, interaction between NBS1 and LAP2α were confirmed by ectopically expressed FLAG-NBS1 and HA-LAP2α co-precipitates (Figure 2B). HEK 293T was transfected with FLAG-NBS1 WT and HA-LAP2α WT. Exogenous co-immunoprecipitation assay was performed by using anti-FLAG antibody (Figure 2B). This result showed a same tendency to endogenous immunoprecipitation assay results. The interaction between the NBS1 and LAP2 α was also confirmed through proximity ligation assay (PLA). Proximity ligation

assay (PLA) is antibody-based detection method for protein-protein interaction. This method allows direct visualization, as well as quantification and subcellular localization of protein-protein interaction/associations in fixed cells. The interacting proteins are targeted by specific antibodies conjugated with oligonucleotides and if in close proximity, ligation of the oligonucleotide moieties creates a DNA sequence that can be amplified exponentially by PCR to obtain powerful signal amplification. In this manner, each protein-protein association generates a fluorescent signal detectable under the fluorescence microscope [13]. As shown Fig 2C and D, we confirmed that the non-irradiated cell shows a weak PLA signal, whereas the irradiated cell shows a stronger PLA signal than the non-irradiated cell. Together, these data showed that LAP2α is new binding partner of NBS1 and interaction of two protein increased under IR-induced DNA damage. So we suggested that NBS1 interacts with LAP2α.

Figure 2

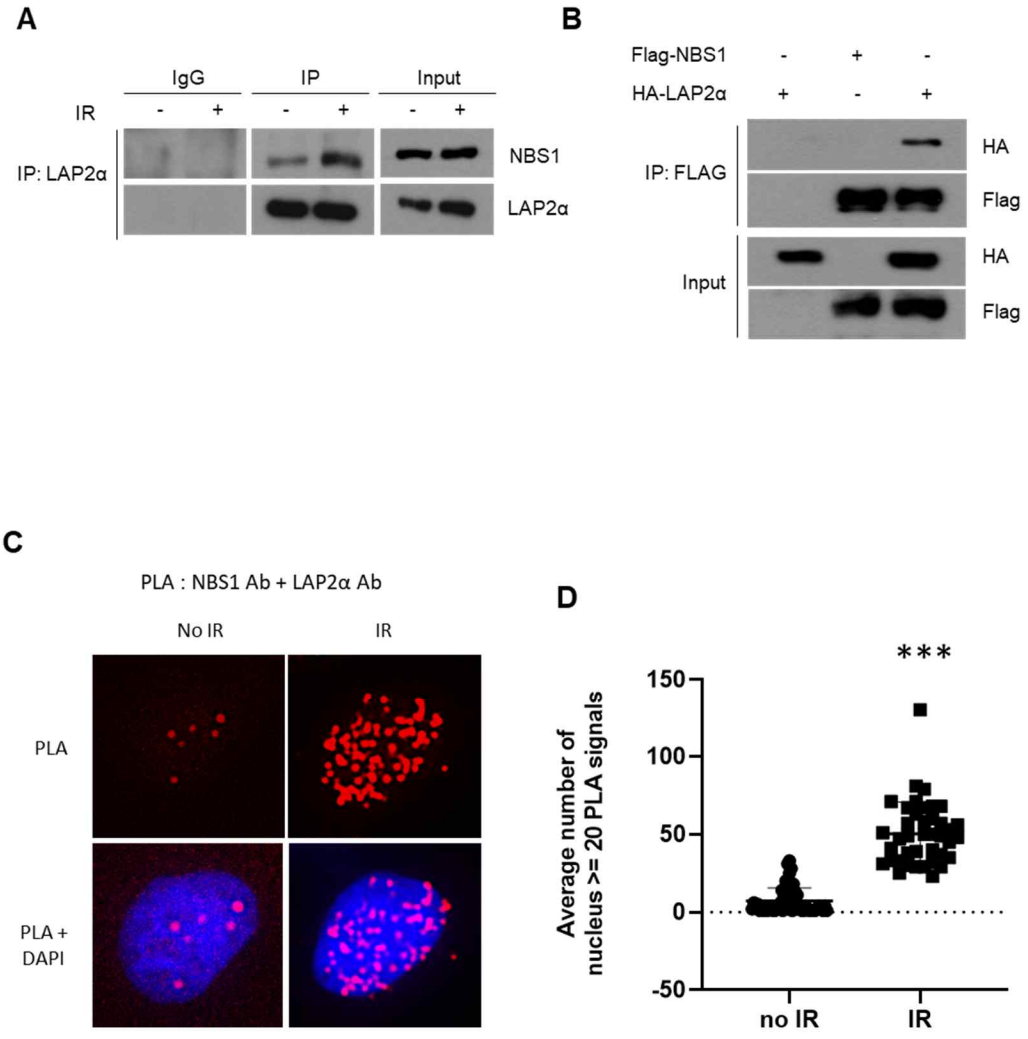


Figure 2. NBS1 interacts with LAP2 α.

(A) Endogenous immunoprecipitation assay (IP). HeLa cells exposed to 10Gy IR and harvested after 3 hours. Total lysates performed immunoprecipitation using anti-LAP2α antibodies followed by immunoblotting using NBS1 or LAP2α anti-bodies. Normal mouse lgG used for negative control

(B) Exogenous immunoprecipitation assay (IP). 293T cells exposed to 10Gy IR and harvested after 3 hours. Total lysates were subjected to immunoprecipitation using anti-FLAG antibody followed by Western blotting using antibodies against either HA or FLAG..

(C) Proximity ligation assay (PLA). HeLa cells exposed to 10Gy IR and fixed after 3 hours. Anti-NBS1 antibody and anti-LAP2α antibody were used for PLA. DNA were counterstained with DAPI.

(D) PLA signals were quantified as foci per nucleus for each dose. Data are pre-sented as means ±SD. (n=3). ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$.

3. LAP2α WT interacts with BRCT2 domain of NBS1 and NBS1 WT interacts with LAP2α specific region.

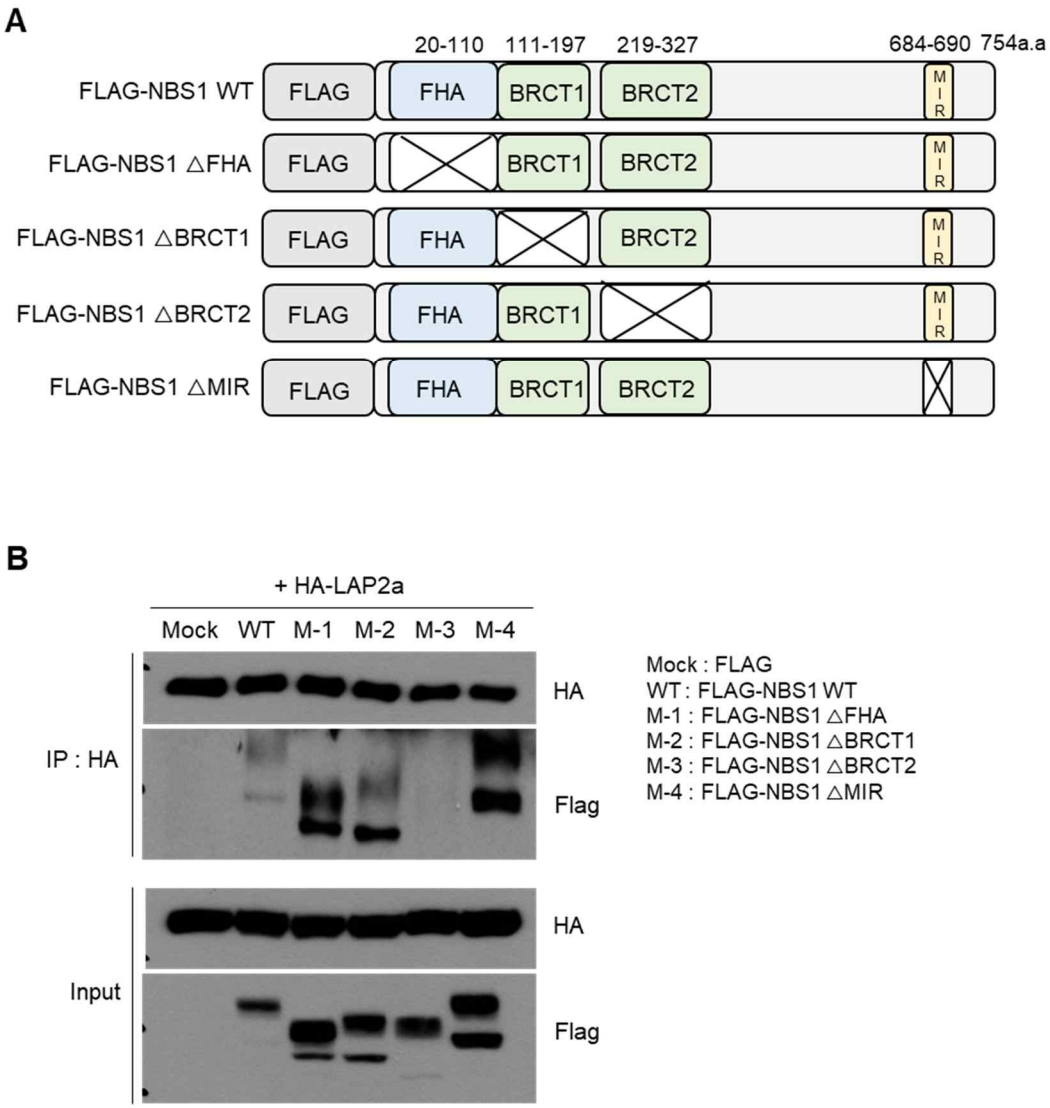
To map the domain of NBS1 involved with binding to LAP2α, we designed NBS1 domain deletion mutant constructs (FLAG-NBS1 △FHA, FLAG-NBS1 △BRCT1, FLAG-NBS1 △BRCT2, FLAG-NBS1 △MIR) (Fig 3A). Using these mutants, we carried out immunoprecipitation assay. HEK 293T was co-transfected with FLAG-NBS1 deletion mutants and HA-LAP2α WT. Exogenous co-immunoprecipitation assay was performed by using anti-HA antibody. In this experiment, we find that HA-LAP2α WT does not bind to FLAG-NBS1△BRCT2 (Fig 3B). This result means LAP2α WT interact to BRCT2 domain of NBS1. The NBS1 N-terminus contains a forkhead-associated domain (FHA) and tandem BRCA1 C-terminal (BRCT) motifs (BRCT1 and BRCT2), which bind phosphorylated proteins, including CtIP and MDC1 [14-16]. So we could think the possibility that LAP2α may play a role in the DDR pathway through interaction BRCT2 domain of NBS1.

Furthermore, we designed LAP2α domain deletion mutant constructs (HA-LAP2α specific, HA-LAP2α common) for map the domain of LAP2α involved with binding to NBS1 (Fig 3C). The N-terminus of all LAP2 isoforms (LAP2 common region) commonly contains LEM (LAP2-Emerin-MAN1)-motif and LEM (LAP2-Emerin-MAN1)-like motif. The LEM-motif interacts with chromatin binding protein BAF (barrier-to-autointegration factor) that enable to interacts with DNA. And LEM-like motif interacts with DNA directly. Unlike other LAP2 isoforms, C-terminus of LAP2α (LAP2α specific region) contains A-type binding region as well as chromosome association domains, an interaction site for the cell cycle and differentiation regulator and retinoblastoma protein (pRb) [17, 18]. Us-ing LAP2α domain deletion mutants, we carried out immunoprecipitation assay. HEK 293T was co-transfected with HA-LAP2α deletion mutants and FLAG-NBS1 WT. Exogenous co-immunoprecipitation assay was performed by using anti-FLAG antibody. In this experiment, we find that FLAG-NBS1 WT binds to

HA-LAP2α specific (Fig 3D).

Based on two IP experiments, LAP2α interacts with BRCT2 domain of NBS1, so we could think about the possibility that LAP2α could play a role in the DDR pathway by engaging in the interaction between NBS1 and phosphorylated protein. In addition, NBS1 interacts with LAP2α specific region, through this result, we could think that NBS1 could have access to DNA by interacting with LAP2α spe-cific region including chromosome binding domain.

Figure 3



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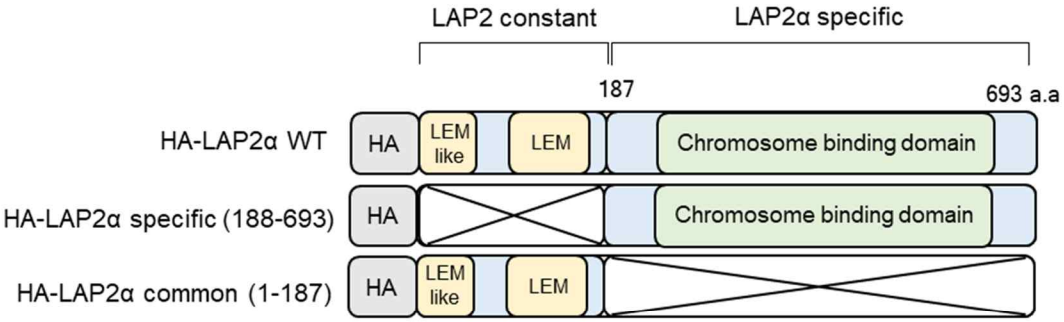

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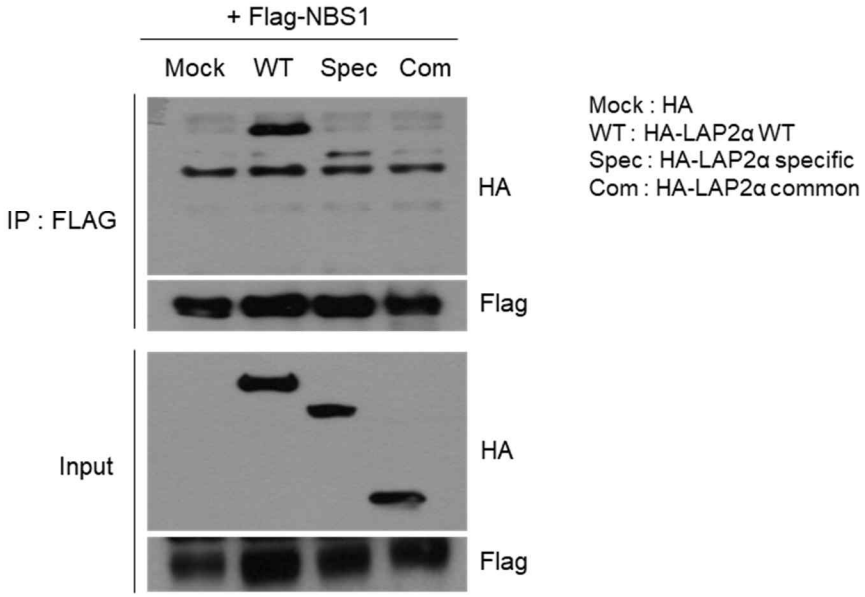


Figure 3. LAP2α WT interacts with BRCT2 domain of NBS1 and NBS1 WT interacts with LAP2α specific region.

(A) Schematic presentation of the NBS1 domain deletion mutant constructs. (FLAG-NBS1 WT : 1-754a.a, FLAG-NBS1 △FHA : 1-19 a.a + 109-754 a.a, FLAG-NBS1 △BRCT1 : 1-110 a.a + 198-754 a.a, FLAG-NBS1 △BRCT2 : 1-218 a.a + 327-754 a.a, FLAG-NBS1 △MIR: 1-681 a.a + 694-754 a.a.)

(B) Exogenous immunoprecipitation assay (IP). HEK 263T cells were transfected with HA-LAP2α WT and FLAG-NBS1 deletion mutants. After 48 hours, total lysates performed immunoprecipitation using anti-HA antibody and analyzed by Western blotting using the HA or FLAG antibodies.

(C) Schematic presentation of the LAP2α deletion mutant constructs. (HA-LAP2α WT : 1-693 a.a, HA- LAP2α specific : 187-693 a.a, HA- LAP2α common : 1-187 a.a)

(D) Exogenous immunoprecipitation assay (IP). HEK 263T cells were transfected with FLAG-NBS1 WT and HA-LAP2α deletion mutants. After 48 hours, total lysates performed immunoprecipitation using anti-FLAG antibody and analyzed by Western blotting using the HA or FLAG antibodies.

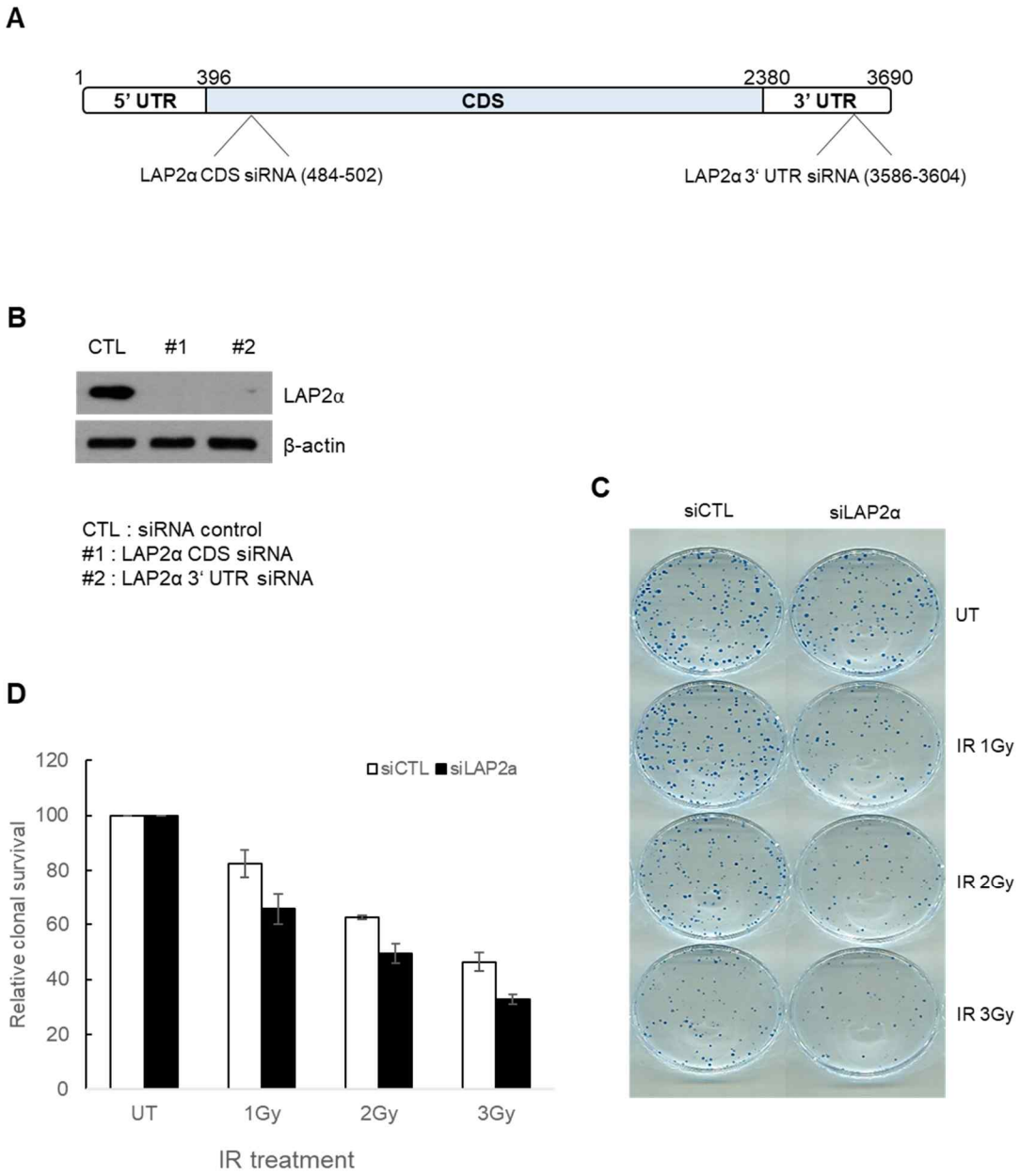
4. Depletion of LAP2α sensitive to ionizing radiation (IR).

We discovered that interaction of NBS1 and LAP2α increase after IR-induced DNA damage. Ionizing radiation (IR) is one of the DNA damage source, so we wondered what affects to depleted LAP2α cells under exposed another DNA damage source. First, we designed siRNA against LAP2α that recognized CDS and 3′UTR sequence (Fig 4A). We named these siRNA to siLAP2α#1 (recognized CDS), siLAP2α#2 (recognized 3′UTR). To inspect how efficient this siRNA inhibited expression of LAP2α gene, we performed western blot assay using LAP2α antibody (Fig 4B). The result showed that expression of LAP2α was effectively decreased by more than 90% in LAP2α siRNA transfected cells, compared with that of control siRNA-treated cells.

To investigate the potential role of in DDR, we performed clonal survival assay with other DNA damage source including IR. In HeLa cells, we transfected control or LAP2α siRNA #2 and treated with each DNA damage sources, such as

Ionizing radiation (IR), Hydroxylurea (HU), Camptothecin (CPT) and Olaparib, then monitored for 10 to 14 days (Fig 4D-G). IR (Ionizing radiation) induces DNA double strand breaks (DSBs) and Hydroxylurea (HU) induces DNA replication stress and Camptothecin (CPT) induces DNA single strand breaks (SSBs). In case of Olaparib, as a PARP1 inhibitor, induces SSBs and increases the number of DSBs. As a result of experiment, in case of HU, CPT and Olaparib, there is no difference in the number of colonies between control cells and LAP2α-depleted cell. But in case of IR, we found that the number of colonies were decreased in LAP2α-depleted cell, compared to control cells. These data indicated that LAP2α-knock-down cells are hypersensitive to IR-induced DNA damage. Therefore, we could suggest that LAP2α regulate IR-induced DNA damage response.

Figure 4



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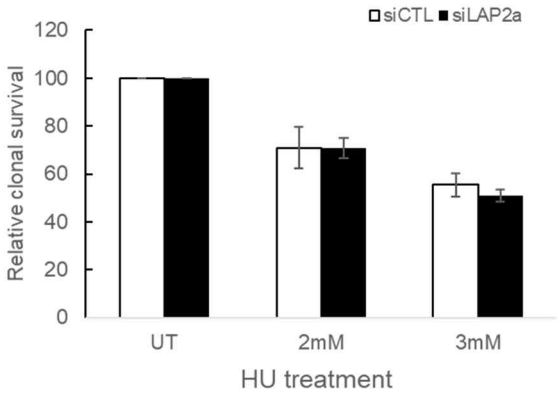

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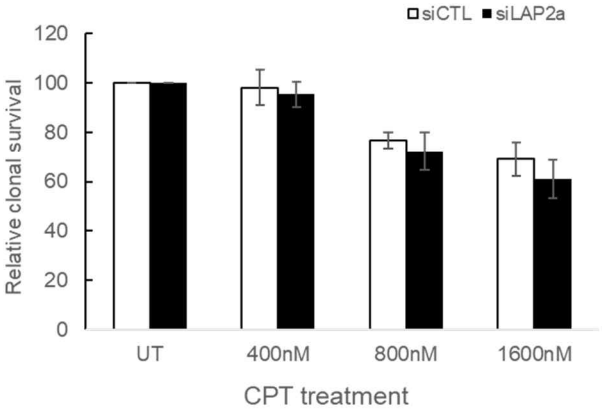

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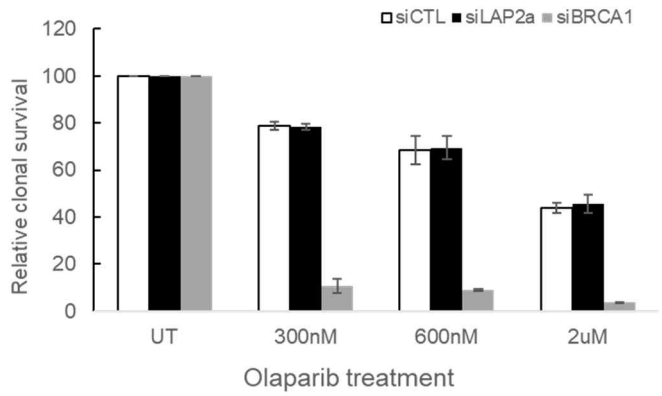


Figure 4. Depletion of LAP2α sensitive to ionizing radiation (IR).

(A) Schematic illustration of the design of LAP2α siRNAs.

(B) HeLa cells were transfected with siRNA control and siLAP2α #1, #2. LAP2α siRNA #1 recognize CDS sequence (484-502), siLAP2α #4 recognize 3'UTR sequence (3586-3604). Each siRNA were transfected into HeLa cells. After 48hrs, the expression level of LAP2α was confirmed by western blotting using anti- LAP2α antibody. β-actin was showed as a loading control.

(C) Clonal survival assay using ionizing radiation (IR). Control and LAP2α depleted Hela cells were untreated or treated with 1, 2, and 3Gy ionizing radiations. After 2 weeks, cells were stained with methylene blue and counted the number of stained colonies.

(D, E, F, G) LAP2α-depleted cells are hypersensitive to IR in HeLa cells. HeLa cells were transfected with siRNA-control or siRNA-LAP2α#2. Control and LAP2α depleted HeLa cells were untreated or treated with IR (D), HU (E), CPT

(F) or Olaparib (G) each doses. After 2 weeks, cells were stained with methylene blue and counted the number of stained colonies.

5. Depletion of LAP2α leads to impaired to DSB repair.

When cells are exposed to ionizing radiation or DNA damaging agents, double strand breaks (DSB) occurs, resulting in rapid phosphorylation of histone H2A variant H2AX. Phosphorylation of H2AX in Ser 139(γ-H2AX) is used as a sensi-tive marker for DNA damage because it is rich and fast and well associated with each DSB. So It can be used to examine the produced DNA damage and subsequent repair of DNA lesions. [19]. Therefore, γ-H2AX is used as an indicator of DSB induction and DNA repair, indicating that cells have not repaired from DNA damage remain in the γ-H2AX foci. We measured remained γ-H2AX formation after DSB in control and LAP2α-depleted HeLa cells. Control and LAP2α-depleted cells were treated with IR, and fixed in different time intervals. The formation of γ-H2AX foci was analyzed by immunofluorescence microscopy. Control cells quickly formed γ-H2AX foci after exposure to IR and resolved almost completely after 24

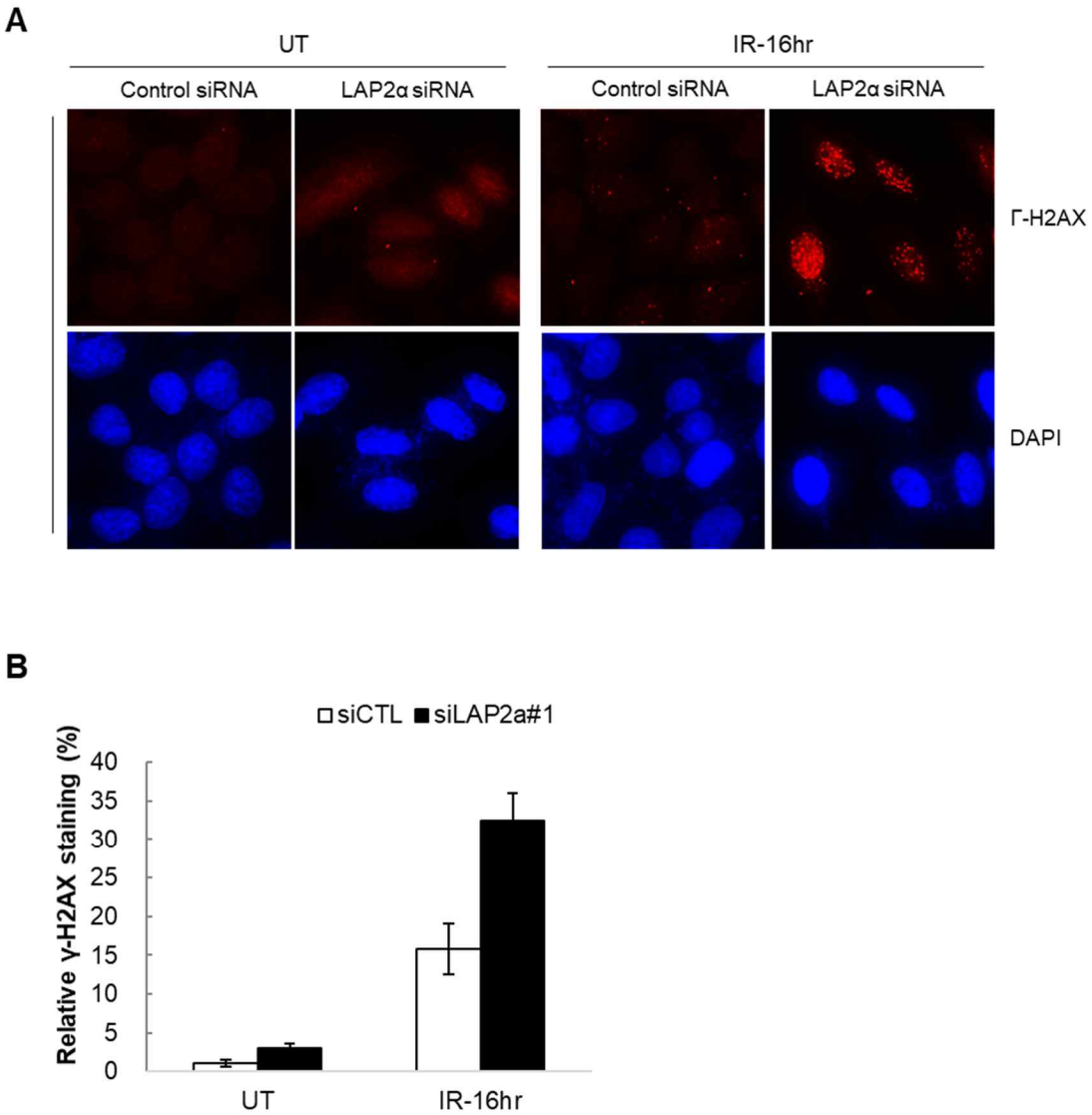
hours. This represents an efficient DNA repair. LAP2α-depleted cells also rapidly formed γ-H2AX foci after IR exposure, but 16 hours later, large amount of foci still remained in LAP2α-depleted cells, indicating defective DNA repair (Fig 5A, B). These results suggest that LAP2α is required for DSB repair.

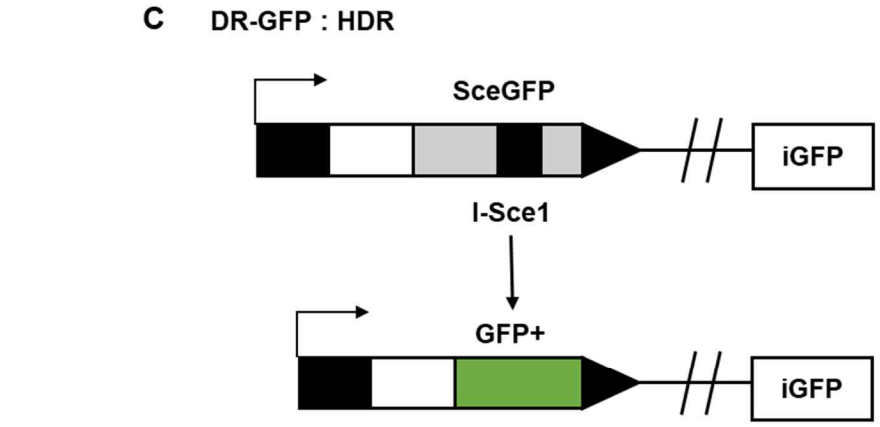
DNA double-strand breaks (DSBs) are the most harmful type of DNA damage. Accordingly, cells have two major pathways for repair of DSBs : homologous recombination (HR) and non-homologous end joining (NHEJ) [20, 21]. As is well known, NBS1 directly mediates DSB repair through HR and in the previous experiment, we confirmed that LAP2α was involved in repair. To demonstrate the role of LAP2α in the HR, we examined whether LAP2α depletion would lead to functional change in HR of DSBs. Several approaches for estimating cellular HR activity have been developed. One example is the direct-repeat GFP (DR-GFP) assay, uses genetically modified cell lines [22-24]. This modified cell lines have two incomplete GFP cassettes are stably integrated into the genome In the first

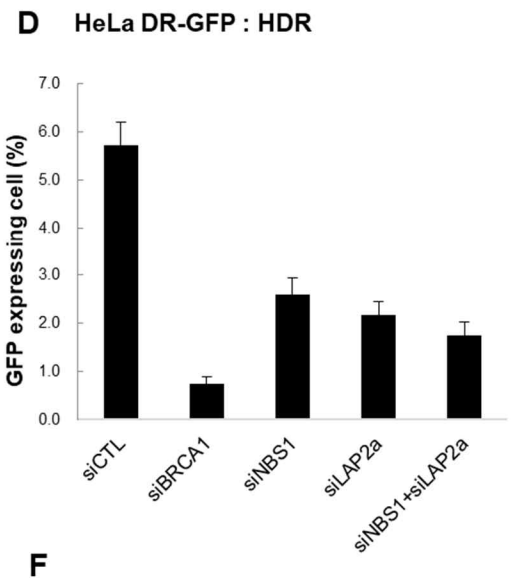
cassette, the GFP gene has a promoter, but does not function because it contains premature stop codon and I-SceI restriction site. The second cassette has a intact coding sequence but no promoter. In HR-efficient cells, the DSB produced by I-SceI in the first cassette is repaired by HR using the second cassette as a template and produces a complete GFP gene with a functional promoter (Fig 5C) [25]. In this system, repair efficiency via HR is monitored by measuring the percentage of cells expressing GFP using INCELL Analyzer 2500HS. To evaluate biological functions of LAP2α in DNA damage repair, we performed DR-GFP assay. As shown in Figure 5D and 5E, BRCA1-depleted cell specifically inhibits HR DSB repair. BRCA1-depleted cell was used as negative control for DR-GFP assay system. We found that LAP2α depleted cells showed reduction of HR repaired GFP positive population compared with control cells. This result suggest that LAP2α depletion lead to defect of HR repair. No additional decrease in HR was showed when NBS1 and LAP2α were depleted simultaneously (Figure 5D). This result

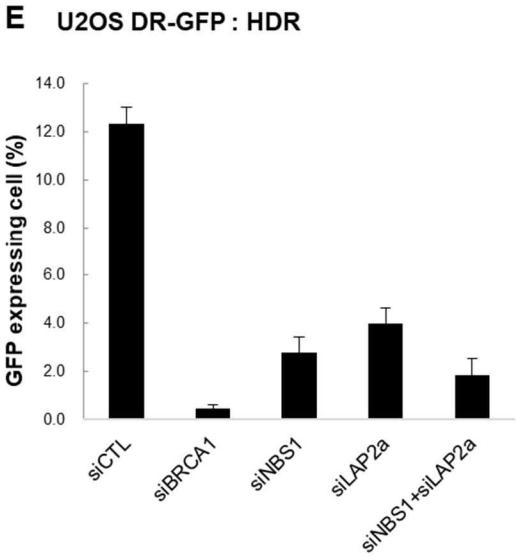
suggest that LAP2α regulate HR repair through regulating NBS1. Same result was obtained in U2OS-DR-GFP cells (Fig 5E). Moreover, HR repair was rescued through the ectopic expression of LAP2α in cells depleted of endogenous LAP2α by an siRNA targeting 3′UTR (Fig 5F). Taken together, these results indicate that LAP2α contributes to HR repair through regulating NBS1.

Figure 5









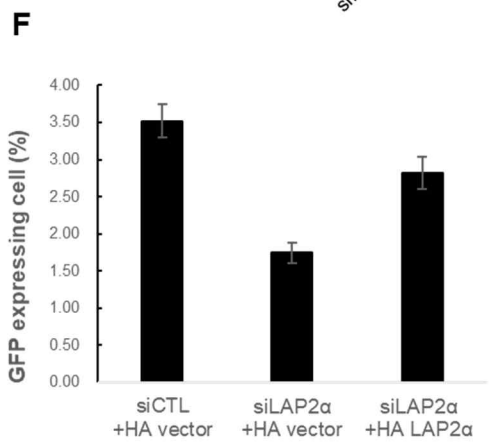


Figure 5. Depletion of LAP2α leads to impaired DSB repair.

(A) HeLa cells were transfected with siRNA and treated 10Gy IR after 48 hours. Then it was fixed by time. Cells were stained with anti-γH2AX antibody and nuclei stained with DAPI.

(B) γ -H2AX foci were quantified as foci per nucleus for each dose. Data are presented as means ±SD (n=3)

(C) A schematic of the DR-GFP reporter system. Direct-repeat GFP (DR-GFP) assay. DR-GFP assay using to monitor HR. DR-GFP assay is shown along with the HDR (Homology-directed repair) product that uses iGFP as the tem-plate for nascent DNA synthesis, which results in the restoration of a GFP ex-pression cassette.

(D, E, F) HR efficiencies in DR-GFP-HeLa cells treated with the indicated siRNAs. Each siRNAs were transfected into DR-GFP-HeLa cell lines for 5 hours and then transfected with I-SceI. After 72 hours, GFP-positive cells were

counted by INCELL Analyzer 2500HS. The values is the percentage of GFP ex-pressing cells determined and represent the mean ± SD of three independent experiments performed in duplicates.

(F) Each siRNAs were transfected into DR-GFP-HeLa cell lines for 5 hours and then transfected with DNA constructs and I-SceI. After 72 hours, GFP-positive cells were counted by INCELL Analyzer 2500HS.

6. LAP2α is recruited at DNA damage site.

Many studies reported several years ago, the generation of DSBs leads to the re-localization of many DNA damage response (DDR) proteins such as MRE11/NBS1/RAD50, MDC1, 53BP1, and BRCA1 to nuclear foci where these proteins co-localize and interact with γH2AX[26]. We wondered that when DNA damage was induced, whether LAP2α localized to site of DNA damage like other DDR proteins. So first we checked whether LAP2α foci formation after DSB induction by IR (Fig 6A). Cells were fixed with 4% paraformaldehyde and immunofluorescence assay performed. However, we couldn't found LAP2α foci formation independently of IR and we experimented again immunofluorescence assay using another anti-LAP2α antibodies and secondary antibodies, but the results were same. So we approached using another experiment to check LAP2α localization, performed proximity ligation assay (PLA). HeLa cells were induced with 10Gy ionizing radiation (IR) for each times (1hr, 6hr) and fixed with 4%

paraformaldehyde. The cells were stained with an anti- LAP2α antibody and anti-γH2AX antibody. The PLA signal indicated interaction with LAP2α and γH2AX. As shown Fig 6B, the PLA signal was increased in IR-induced cells. This result implied that LAP2α may have been recruited to DNA damage site.

Figure 6

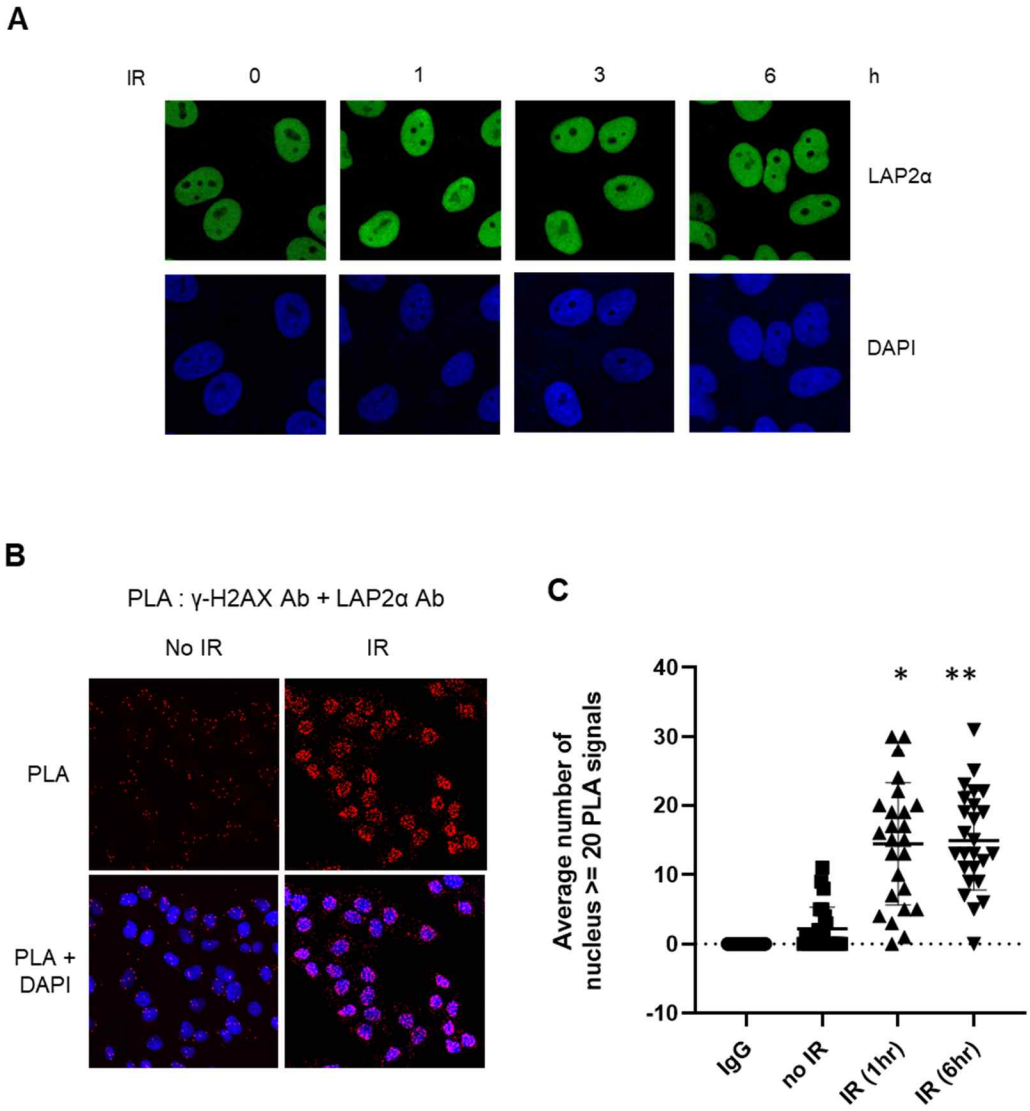


Figure 6. LAP2α is recruited at DNA damage site.

(A) HeLa cells were transfected with anti- LAP2α siRNA and treated 10Gy ionizing radiation (IR) after 48 hours. Then it was fixed by time. Cells were stained with anti- LAP2α antibody and nuclei stained with DAPI.

(B) Proximity ligation assay (PLA). Immunofluorescence PLA signal shows protein-protein interactions between LAP2α and γH2AX in HeLa cell. The cells were stained with an rabbit anti- LAP2α antibody and mouse anti-γH2AX anti-body, then performed using PLUS and MINUS PLA probes (oligo-conjugated secondary antibodies).

(C) PLA signals were quantified as foci per nucleus. Data are presented as means ±SD (n=3). ***, $p \leq 0.001$; **, $p \leq 0.01$; *, $p \leq 0.05$.

7. LAP2α does not affect NBS1-mediated ATM-CHK2 signaling.

Cellular responses to DNA damage are regulated primarily by two kinase signaling cascades activated by DNA double-stranded breaks (DSB) and single-stranded DNA, which ATM-Chk2 and ATR-Chk1 pathways. [27]. These signaling pathway is conserved from the human to the mouse and plays important roles in DNA damage checkpoint responses. In case of ATM-CHK2 pathway, IR first activates ATM, then ATM activate CHK2, a cell cycle checkpoint kinase, most probably by phosphorylation at Thr 68[28], then activated CHK2 phosphorylates CDC25A, another cell cycle signaling kinase at Ser 123, which leads to a phosphorylation of CDK2 at Tyr 15 and Thr 14 and a disruption of cyclin E/CDK2 kinase which causes the intra-S-phase checkpoint[29]. NBS1 has been reported to be required for the activation of ATM and CHK2 after IR[30-35]. The down-regulation of NBS1 by siRNA leads to a decreased activation of CHK2

after exposure to IR[33]. Thus, NBS1 could be involved in regulating intra-S-phase checkpoint control via ATM and CHK2[36].

Based on the previous experiment, the interaction between NBS1 and LAP2α was confirmed, and the effect of LAP2α in DDR was also confirmed, so we experimented whether LAP2α affects ATM-Chk2 pathway. We checked the ATM-NBS1-CHK2 signaling. The ATM kinase was activated by IR and there were induce in the phosphorylation of ATM (S1981), NBS1 (S343) and Chk2 (T68) unlike undamaged cells. But as shown Fig 6, we found that there was no significant difference in the phosphorylation levels of ATM, NBS1, CHK2 protein between the sicontrol-treated cell and siLAP2α-treated cell. As a result of this experiment, we suggest that LAP2α does not affect ATM-CHk2 pathway by NBS1.

Figure 7

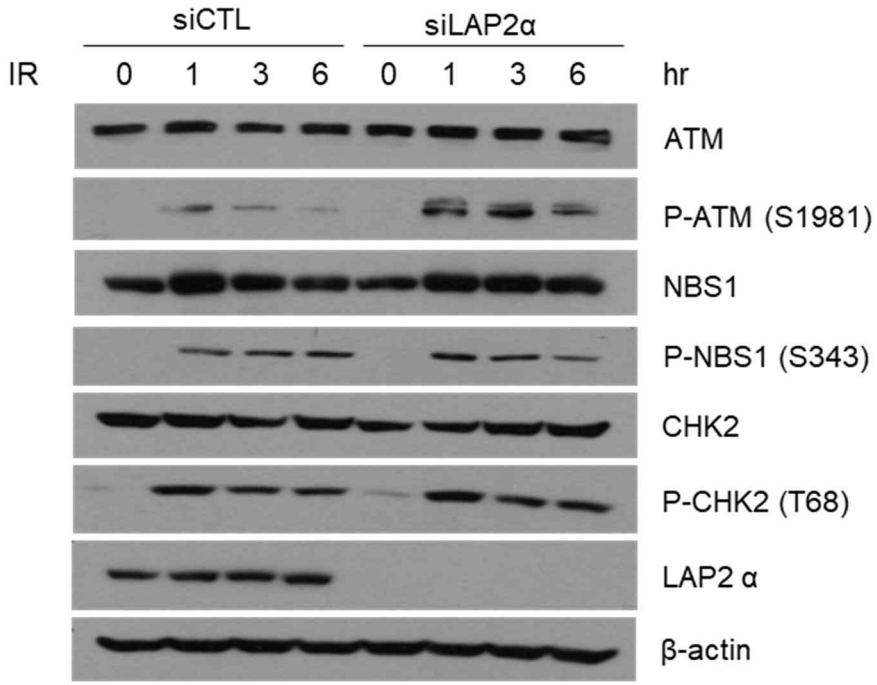


Figure 7. LAP2α does not affect ATM-CHK2 signaling.

HeLa cells were transfected with siRNA-control and siRNA-LAP2α #1. After 48hrs, control and LAP2α depleted HeLa cells were treated with 5Gy ionizing radiations and harvested after 1, 3 and 6 hours. Total lysates performed western blotting using antibodies against ATM, phosphor-ATM (Ser1981), NBS1, phosphor-NBS1 (Ser343), Chk2, phosphor-Chk2 (Thr68). LAP2α and β-actin.

8. LAP2α may be phosphorylated by IR damage.

The DDR proteins provide a molecular switch that regulates DSB repair through PTMs. PTMs involve a series of transient, reversible covalent modifications of amino acidic residues, such as serine and threonine phosphorylation, lysine acetylation and ubiquitylation, and lysine and arginine methylation. PTMs is becoming increasingly important in DDR because it has the ability to change protein activity by attaching small molecules to substrate proteins. PTMs of proteins play an important role in the first stage of DDR by mediating protein-protein interaction and regulating protein trafficking, localization, activity and stability. Among PTMs, phosphorylation is the addition of a phosphate (PO4) group to a protein in order to induce a conformational change that initiates protein activation or deactivation. Phosphorylation of DNA repair proteins generally activates proteins to facilitate DNA repair. The mechanism of reversible phosphorylation in proteins is an important regulatory mechanism for DNA repair pathways.[37, 38]. We first focused

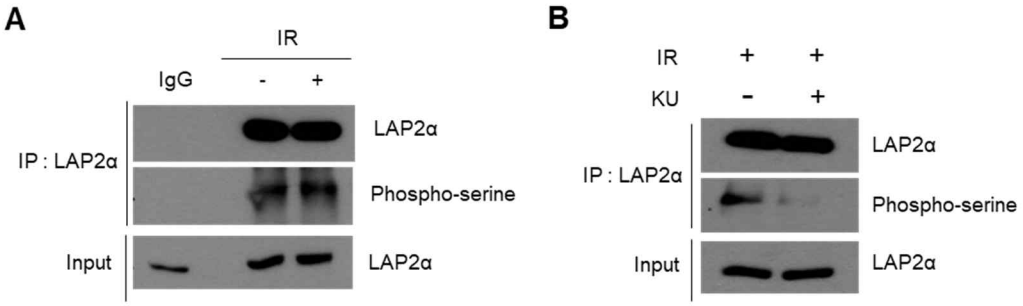
on phosphorylation in PTMs, and tried to find out whether LAP2α is phosphorylated by IR, like other DDR proteins. Previously, it is reported that LAP2α is phosphorylated by mitotic kinases(cdk1) in its chromatin binding domain[39]. So we checked if there was any change in the phosphorylation of LAP2α when IR damage was treated by performing co-immunoprecipitation experiments using antibody that recognize phosphorylated serine. HeLa cells exposed to 10Gy IR and harvested after 3 hours. The whole cell lysates was immunoprecipitated with an anti-LAP2α antibody. Immunoprecipitates were subjected to western blotting with an anti- LAP2α antibody and anti- phosphorylated serine antibody. As result shown in Figure 8A, LAP2α was phosphorylated. However, the levels of phosphorylation was similar between undamaged cells and damaged cell. To rule out of mitosis-related phosphorylation of LAP2α, we performed additional experiments. We performed immunoprecipitation experiments using ATM specific inhibitor (KU 55933) to determine whether phosphorylation of LAP2α was affected by DNA

damage. As shown Fig 8B, it was confirmed that the level of phospho-serine was decreased in cells treated with ATM inhibitor (KU 55933). As a result of the IP experiment, we confirmed the possibility that LAP2α is a new target protein of ATM kinase.

ATM/ATR-like protein kinase mainly responds to SQ or TQ motifs and plays a central role in maintaining genomic stability and phosphorylation of numerous substrates in response to DNA damage. ATM/ATR substrates often contain several closely spaced SQ/TQ motifs in regions called SQ/TQ cluster domains (SCDs). SCDs are now considered a structural hallmark of DNA-damage-response proteins [40]. We checked that LAP2α have five SQ/TQ sequences which are ATM/ATR phospho-target consensus sequences (Fig 8C), and performed additional Group-based pre-diction system (GPS) analysis (Fig 8D). Based on the results of IP experiments and putative amino acid sequence analysis, the possi-

bility of our hypothesis was confirmed. So we performed mass spectrometry analysis to search post-translational modifications (PTM) of LAP2α. Samples prepared immunoprecipitation with anti-LAP2α antibody in HeLa cells and electrophoresis with SDS-PAGE, then stain with Coomassie Blue (Fig 8E). The stained band was analyzed using mass spectrometry. Through PTM analysis, it was not found phosphorylation of LAP2α. However, possibility of phosphorylation of LAP2α still remain.

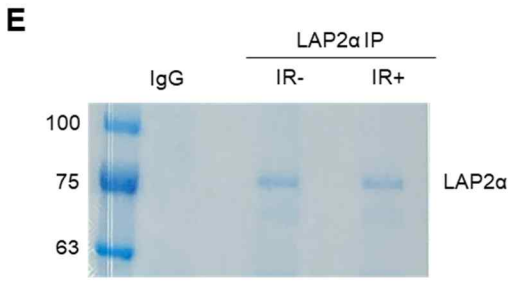
Figure 8



C

S296 SSSSQPEH
S366 SESSQLIS
S480 AKQSQHDK
T528 REATQILS
S598 ADISQAAQ

ATM/ATR phospho-target
consensus sequence : SQ/TQ



D

kinase	code	position	peptide	score
ATM	S	296	EKSSSSSSQPEHSAM	14.472
ATM	S	310	MLVSTAASPSLIKET	13.904
ATM	S	366	ALEESESSQLISPPL	16.134
ATM	S	480	GLEVAKQSQHDKIDA	11.714
ATM	T	528	PVSSREATQILSVPK	14.237
ATM	S	598	KAMQADISQAAQILS	13.078
ATR	S	296	EKSSSSSSQPEHSAM	10.917
ATR	S	310	MLVSTAASPSLIKET	10.113
ATR	S	366	ALEESESSQLISPPL	13.267
ATR	S	480	GLEVAKQSQHDKIDA	10.201
ATR	T	528	PVSSREATQILSVPK	11.846
ATR	S	598	KAMQADISQAAQILS	11.454

Figure 8. LAP2α may be phosphorylated by IR damage but it could not be confirmed through LC-MCMC.

(A) Endogenous immunoprecipitation assay (IP). HeLa cells exposed to 10Gy IR and harvested after 3 hours. Total lysates performed immunoprecipitation using anti-LAP2α antibodies followed by western blotting using LAP2α or phosphor-serine antibodies.

(B) ATM inhibitor (KU-55933) blocks constitutive ATM-dependent phosphorylation. Cells were treated with DMSO carrier or 10 μM KU-55933 for 24 hrs, and exposed to 10Gy IR and harvested after 3 hours. Total lysates performed immunoprecipitation using anti-LAP2α antibodies, and immunoblotted for phosphoserine antibody.

(C) Sequence analysis of SQ and TQ sites of human LAP2α.

(D) Group-based prediction system (GPS) analysis of SQ and TQ motif of human LAP2α. Kinase-specific phosphorylation site prediction analysis was performed

by using the GPS against ATM/ATR kinase.

(E) Sample preparation for PTM (Post-Translational Modifications) analysis using LC-MSMS. HeLa cells exposed to 10Gy IR and harvested after 3 hours. Total lysates performed immunoprecipitation using anti-LAP2α antibodies. Performed the SDS-PAGE and Gel staining using Coomassie Brilliant Blue Staining (CBB staining).

DISCUSSION

Here, we found Lamina-associated polypeptide 2, isoform alpha (TMPO) as new binding partner of NBS1, through yeast two hybrid screening. Lamina-associated polypeptide (LAP) 2α is a LEM (lamina-associated polypeptide emerin MAN1) family protein which interacts with A-type lamins and chromatin in cell cycle dependent. Lamins are intermediate filament proteins in nucleus which build a filamentous protein meshwork with several inner nuclear membrane proteins at the nuclear periphery which called nuclear lamina[41]. Especially, lamins A and C interact with LAP2α. In this study, first, we investigated the relevance of LAP2α for DDR. Depletion of LAP2α resulted in cellular hypersensitivity and impaired DNA damage repair to IR, as detected by colony survival assay and late γ-H2AX foci staining. And we confirmed that depletion of LAP2α impaired HR-mediated repair by DR-GFP assay. We found that LAP2α

depleted cells showed reduction of HR repaired GFP positive population com-pared with control cells. This result indicated that LAP2α regulate HR repair through NBS1-dependent manner. Through PLA assay, we showed that PLA signal between LAP2α and γ-H2AX increased after treatment of IR. This result indicate that LAP2α is recruited to DSB site and it is in line with NBS1 binding to γ-H2AX. Therefore, we found that LAP2α was involved in HR dependent on NBS1 and interacted with γ-H2AX in IR induction, just like NBS1.

The NBS1 protein is a component of the MRN complex that plays a crucial role in the cellular response to DNA damage and the maintenance of chromoso-mal integrity[36]. We showed interaction with NBS1 and LAP2α through en-dogenous/exogenous immunoprecipitation and PLA assay. Also we found that the NBS1/LAP2α interaction was increased when inducing IR. At the molecular level, MRN consist of two meiotic recombination 11 homolog 1 (MRE11) subu-

nits, two ATP-binding cassette (ABC)-ATPase (RAD50) units, and two phos-phopeptide-binding Nijmegen breakage syndrome protein 1 (NBS1) subu-nits[42]. MRE11 required for DNA DSB repair, such as 3′-5′ double-strand DNA exonuclease, single-strand DNA endonuclease, and DNA unwinding activ-ity. RAD50 is a member of the structure maintenance of chromosome (SMC) protein family; it has two ATPase motifs at its N- and C-terminal ends and forms an anti-parallel homodimer with a flexible hinge region that may adopt a "V-like" conformation. The "V-like" structure could serve as a bridge to hold together the broken ends of DSB and prevent them from floating away, as well as restrict the extent of nucleotide degradation of MRE11 to prevent excessive degradation[36]. MRE11 and RAD50, which form a complex with NBS1, also play an important role in DDR, in this respect, it is necessary to confirm the relevance with LAP2α and MRE11/RAD50 which are other components of MRN complex.

We tried to find out which mechanisms between NBS1 and LAP2α are involved in DDR. First, through a co-immunoprecipitation experiment using a NBS1 deletion domain mutants, we found out that LAP2α interacted NBS1 through BRCT2 domain of NBS1. The BRCT2 domain in NBS1 which bind phosphorylated proteins, including CtIP and MDC1 [17-19]. It is indicated that there are possibility that LAP2α could play a role in the DDR pathway by engaging in the interaction between NBS1 and phosphorylated proteins. Therefore, we must have done to further demonstrate with detailed mechanism of this relationship and we have to make sure that co-immunoprecipitation assay because the band of between NBS WT and LAP2α WT is weaker than other bands. Additionally we performed co-immunoprecipitation experiment using a LAP2α deletion domain mutants, we found that NBS1 interacts with LAP2α specific region, so we could think that LAP2α gives DNA accessibility to NBS1, such as γ-H2AX.

Second, we approached ATM-CHK2 signaling related to NBS1. Reportedly,

NBS1 could be involved in regulating cell cycle checkpoint control via ATM and CHK2[36]. As a binding partner of NBS1, we investigated whether LAP2α affects ATM-CHK2 signaling. We performed western blotting and it was found that there was no difference in the expression level of the protein between the siCTL-treated cell and siLAP2α-treated cell when inducing IR. But there is a limitation in that only protein expression were performed through western blotting. Therefore, it is necessary to approach this point through varied experiments.

Third, we focused on LAP2α modification. We experimented whether LAP2α was phosphorylated when IR was induced. Through co-IP experiment, we confirmed the serine phosphorylation of LAP2α and then performed the next experiment. Although there was a phospho-target site of ATM/ATR in LAP2α, LC-MSMS analysis results show that there was no change in phosphorylation of LAP2α according to the presence or absence of IR. We needed to think about the phosphorylation of LAP2α confirmed by the co-IP experiment. Reportedly, the

mitotic cyclin-dependent kinase 1 (cdk1) phosphorylates the C-terminal chromosome association region of LAP2α[39]. These factors may have affected co-IP experiments to confirm the phosphorylation of LAP2α. However, in the co-IP experiment using Ku, which is an ATM inhibitor, there is a change in LAP2α phosphorylation due to the presence or absence of IR, which needs to be confirmed as an additional experiment.

In this report, we have shown that LAP2α is DSB-responsive-IR-hypersensitive NBS1 binding partner. Furthermore, we tried to find out the regulatory mechanism of NBS1 by LAP2α in several way. In the future, we will perform additional experiments related to ATM-CHK2 signaling and LAP2α phosphorylation. Further we will studies about another regulatory mechanism between NBS1 and LAP2α in DDR.

ABSTRACT

The regulatory mechanism of NBS1 by LAP2-α

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NBS1, one of the components of the MRE11 RAD50 NBS1 (MRN) complex, is a protein that plays an important role in responding to DNA damage and maintaining chromosomal integrity. However, the exact function of NBS1 in DNA damage recovery has not been fully identified. Here, we show that LAP2α interact to BRCT2 domain of NBS1. LAP2α (TMPO, Lamina-associated polypeptide (LAP) 2α) is a LEM (Lamina-associated polypeptideEMERIN MAN1) family protein

known as nucleoplastic A-type Lamin and chromosome, but research on DNA damage recovery has not been sufficient. In this report, we show that depletion of LAP2α results in cellular hypersensitivity and impaired homologous recombination to IR, as detected by clonal survival assay, late γ-H2AX foci staining and DR-GFP assay. We also found that LAP2α is recruited to DNA damage sites and phosphorylated after IR treatment. Thus, we suggested that LAP2α is a novel binding partner of NBS1 and a new DNA damage response regulator involved in DNA damage.

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