Int J Clin Exp Pathol 2017;10(2):1611-1618
www.ijcep.com /ISSN:1936-2625/IJCEP0036821

Original Article

Minichromosome maintenance complex facilitates the recruitment of BRCA1 onto chromatin and foci formation in A549 cells

Yushang Cui*, Xiaohui Xu*, Cheng Huang, Yingzhi Qin, Naixin Liang, Hongsheng Liu, Shanqing Li

*Department of Thoracic Surgery, Peking Union Medical College Hospital, Beijing, China. *Equal contributors.*

Received July 28, 2016; Accepted October 31, 2016; Epub February 1, 2017; Published February 15, 2017

Abstract: Lung cancer is the most frequent cancer and the leading cause of cancer death worldwide. Therefore, a better understanding of DNA damage repair in cells might be helpful to treat cancers. The present study was aimed to investigate the potential interaction between breast cancer 1 (BRCA1) and minichromosome maintenance proteins (MCMs) during DNA damage in lung carcinoma A549 cells. The recombinant vectors of BRCA1 and MCMs with different tags were constructed and transfected into A549 cells. Immunoprecipitation (IP), immunoblot (IB), and mass spectrometry were performed to screen the possible interactions between BRCA1 and MCMs. In addition, the expression of MCM2 and MCM6 was knocked down by specific short hairpin RNAs (shRNAs). The cells were incubated with camptothecin (CPT) or bleomycin to induce DNA damage, and then the interaction between MCM2 and BRCA1, chromatin fraction, and foci formation of BRCA1 were examined. The results showed that MCM2/3/5/6 was immunoprecipitated against the hemaglutinin (HA)-BRCA1 in A549 nuclei. Upon DNA damage, the interaction between MCM2 and BRCA1 was reduced. Moreover, downregulation of MCM2 or MCM6 could increase the non-chromatin level of BRCA1, but decrease the chromatin level of BRCA1. Knockdown of MCM2 or MCM6 could statistically inhibit foci formation of BRCA1 in A549 nuclei upon bleomycin-induced DNA damage ($P < 0.05$). Our results suggest that there is an interaction between BRCA1 and MCMs in A549 cell nuclei. Down-regulation of MCMs could prevent chromatin fraction and foci formation of BRCA1 upon DNA damage.

Keywords: Lung cancer, breast cancer 1 (BRCA1), minichromosome maintenance protein (MCM), chromatin fraction, foci formation

Introduction

Lung cancer is the most frequent cancer and the leading cause of cancer death among males, and while it is the third most common cancer and the second leading cause of cancer death among females [1]. It has been reported that more than 1.6 million patients are diagnosed with lung cancer and 1.3 million patients die each year [2]. Although significant progress has been achieved in both prevention and treatment of lung cancer in recent years, the mortality is found to rise due to the uncertain molecular pathogenesis. The 5-year survival rate ranges from 4% to 52% depending on the stage at diagnosis [3]. Therefore there is an urgent to better understand the molecular and cellular pathogenesis of lung cancer.

Breast cancer 1 (BRCA1) is a ~220 KDa protein that plays significant roles in a variety of cellular processes including DNA damage repair, cell cycle checkpoint control, cellular proliferation, transcription, centrosome duplication, ubiquitination, and centrosome duplication [4-6]. It is known to be critical in maintaining genetic stability [7]. BRCA1 has been regarded as a tumor suppressor gene [8], and mutations of the *BRCA1* gene cause an increased risk of cancer, such as breast cancer and ovarian cancer [9]. Recently, the functional role of BRCA1 in lung cancer has been extensively studied. It has been reported that low mRNA expression of *BRCA1* is considered as potential predictive marker in lung cancer [10, 11]. However, how exactly BRCA1 exerting its DNA damage repair is not fully understood.

The minichromosome maintenance (MCM) complex is an important replicative helicase in archaea and eukarya [12, 13]. The complex is composed of six related subunits (MCM2 to MCM7), which is necessary for chromosome DNA replication [14]. Thus, this complex is required for DNA damage repair [15]. Here we investigated the potential mechanism with respect to BRCA1 and MCMs during DNA damage in human lung carcinoma A549 cells. Immunoprecipitation (IP), immunoblot (IB), and mass spectrometry were performed to disclose the possible interactions between BRCA1 and MCMs in cell nuclei. Further, the expression of MCMs was knocked down and then the effects of MCMs in regulation of BRCA1 upon DNA damage were explored. Our study might provide a new insight into the possible interactions between BRCA1 and MCMs and a new therapeutic target for lung cancer.

Materials and methods

Cell culture

Human lung carcinoma A549 cells were obtained from the Chinese Academy of Sciences Type Culture Collection (Shanghai, China). The cells were maintained Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% (v/v) fetal bovine serum (FBS, Invitrogen), 1% non-essential amino acids (GE Healthcare Life Sciences, UK), and 1% penicillin-streptomycin solution (GE Healthcare) in a 5% CO_2 incubator at 37°C.

Plasmid construction

The complementary DNA (cDNAs) encoding MCM2, MCM3, MCM5 and MCM6 was amplified by standard polymerase chain reaction (PCR) and confirmed by sequencing. The Flag and hemaglutinin (HA) tag sequences were added to the 3' end of BRCA1 and MCMs (MCM2, MCM3, MCM5 and MCM6) by PCR. Expression plasmid containing pcDNA3.1-HA-BRCA1, pcDNA3.1-Flag-MCM2, pcDNA3.1-Flag-MCM3, pcDNA3.1-Flag-MCM5, and pcDNA3.1-Flag-MCM6 was constructed and purified by Plasmid Purification Kit (Qiagen, Valencia, CA) according to the manufacturer's instruction.

Cell transfection and treatment

The A549 cells were transfected with the above plasmids in serum-free DMEM by using Lipofectamine 2000 (Invitrogen) following to the manufacturer's protocol. When the cells reached 70-80% confluency on 24-well plates, the plasmids (1 μg) were added to each well and incubated for 48 h. The cells were then harvested for further analysis. Moreover, the cells stably expressing HA-tagged BRCA1 were treated with 500 nM camptothecin (CPT) for 2 h to induce DNA damage.

In addition, the cells were transfected with specific short hairpin RNA (shRNA) for MCM2 and MCM6 or negative control and incubated for 48 h. The shRNA plasmid was purchased from GeneChem Inc (GeneChem, Shanghai). Thereafter, bleomycin (2 μM) was performed to induce DNA damage in A549 cells. After transfection, the supernatants were collected for further analysis.

Chromatin fraction

Cell fractionation was carried out according to a previous study [16]. In brief, A549 cells stably expressing HA-tagged BRCA1 were lysed in buffer A (100 μL) (10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 0.34 mM sucrose, and 10% glycerol) supplemented with 0.2% Triton X-100, 1 mM dithiothreitol (DTT), and Protease Inhibitor Cocktail (Sigma-Aldrich, St Louis, MO) for 8 min. After centrifugation, the cytoplasmic fraction was prepared from the supernatant. Then the pellet was resuspended in buffer B (100 μL) which contains 3 mM ethylene diamine tetraacetic acid (EDTA), 0.2 mM ethylene glycol tetraacetic acid (EGTA), 1 mM DTT, and Protease Inhibitor Cocktail on ice for 30 min. After centrifugation, the supernatant was harvested again. The supernatant was now mixed the cytoplasmic fraction with the non-chromatin fraction. Thereafter, the pellet was resuspended in buffer C (150 μL) containing 50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 1% NP-40, 1 mM DTT, and Protease Inhibitor Cocktail, placed on ice, sonicated, centrifuged, and collected as the chromatin fraction. The proteins were quantitated by using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE).

IP and IB analysis

IP and IB analysis were performed to analyze BRCA1 and its potential interactive proteins. Immunoprecipitation and immunoblot analysis were both carried out in nucleoprotein and the whole cell extracts (WCE). The nucleoprotein

Table 1. Mass spectrometry data for MCMs in A549 nuclei

Protein	Peptides	Recovery
MCM2	13	13%
MCM3	12	16%
MCM5	12	18%
MCM6	11	8%

MCM, minichromosome maintenance protein.

samples were extracted from the A549 cells stably expressing HA-tagged BRCA1 by using EpiQuik Nuclear Extraction Kit (Epigentek Group, Farmingdale, NY, USA). Briefly, the A549 cells were washed with cold phosphate-buffered saline (PBS) and then lysed in cold IP lysate buffer, and centrifuged. Thereafter, the cell lysates were immunoprecipitated with anti-MCM2 antibody (Santa Cruz Biotechnology), or anti-BRCA1 antibody (Santa Cruz Biotechnology), or anti-HA antibody (Abcam, Cambridge, UK) and incubated overnight at 4°C. SDS-PAGE was finally performed to analyze the protein levels.

Immunofluorescence microscopy

Immunofluorescence was conducted to analyze foci forming of BRCA1 in A549 cell nuclei. After transfection with MCM2 or MCM6 shRNA and incubation with bleomycin, the cells were fixed with 4% paraformaldehyde, permeabilized in 0.2% Triton X-100, and maintained in specific primary antibody against BRCA1 (Santa Cruz Biotechnology) for 2 h at room temperature. Thereafter, the cells were washed with PBS, and were incubated with appropriate secondary antibody (Santa Cruz Biotechnology) for 2 h at room temperature. The nuclei were stained with 4', 6-diamidino-2-phenylindole (DAPI; Life Technologies, Grand Island, NY). Ten fields were randomly photographed by a fluorescence microscope (Olympus IX51, Tokyo, Japan). The immunofluorescence positive signals were analyzed by NIH ImageJ 1.49 V imaging analysis software (National Institutes of Health, Bethesda, MD).

Mass spectrometry

A549 cells stably expressing HA-BRCA1 was fractionated into cytoplasmic and nuclear compartments. BRCA1-interacting proteins were denatured with 8 M urea, reduced with 10 mM tris-(2-carboxyethyl)-phosphine hydrochloride (TCEP; Sigma-Aldrich), alkylated with 55 mM iodoacetamide (Sigma-Aldrich), and digested overnight with trypsin (Sigma-Aldrich). Then the protein samples were pressure-loaded onto fused silica capillary columns (250 μm; Rancho Corodova, CA, USA) and connected to a silica capillary analytical C18 column (100 μm). After that, the samples were desalted, eluted by linear ion trap (LTQ) mass spectrometer (Thermo Scientific), and chromatographed by Agilent 1200 High Performance Liquid Chromatography (HPLC; Agilent, Waldbronn, Germany). Raw data were collected by RawXtract [17].

Western blot

Total protein was extracted from A549 cells after transfection or incubation by using Protein Extraction Reagent (Thermo Fischer Scientific, Waltham, MA). The protein samples were subjected to SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes. After wash with PBS, the membranes were incubated with the following antibodies: anti-MCM2 antibody (Santa Cruz Biotechnology), anti-MCM6 antibody (Santa Cruz Biotechnology), anti-origin recognition complex 2 (Orc2) antibody (Abcam), anti-PLCγ-1 antibody (Abcam), or GAPDH (Abcam) overnight at 4°C. Thereafter, the membranes were incubated with goat horse radish peroxidase (HRP)-conjugated secondary antibodies (Abcam) for 2 h at room temperature. Positive signals were visualized by enhanced chemiluminescence (ECL) (Beyotime, China).

Statistical analysis

All the experiments were carried out in triplicate and the data were represented as the mean ± standard deviation (SD). Comparisons between groups were conducted by one-way analysis of variance (ANOVA) and t test with Statistical Analysis System (SAS) (version 9.3, SAS Institute, Cary, NC). $P < 0.05$ was considered as statistically significant differences.

Results

BRCA1 interacts with MCMs

To better understand the functional roles of BRCA1 in A549 cells, we tried to identify specific BRCA1-interacting proteins in the nucleus of A549 cells. The transfected cells were immu-

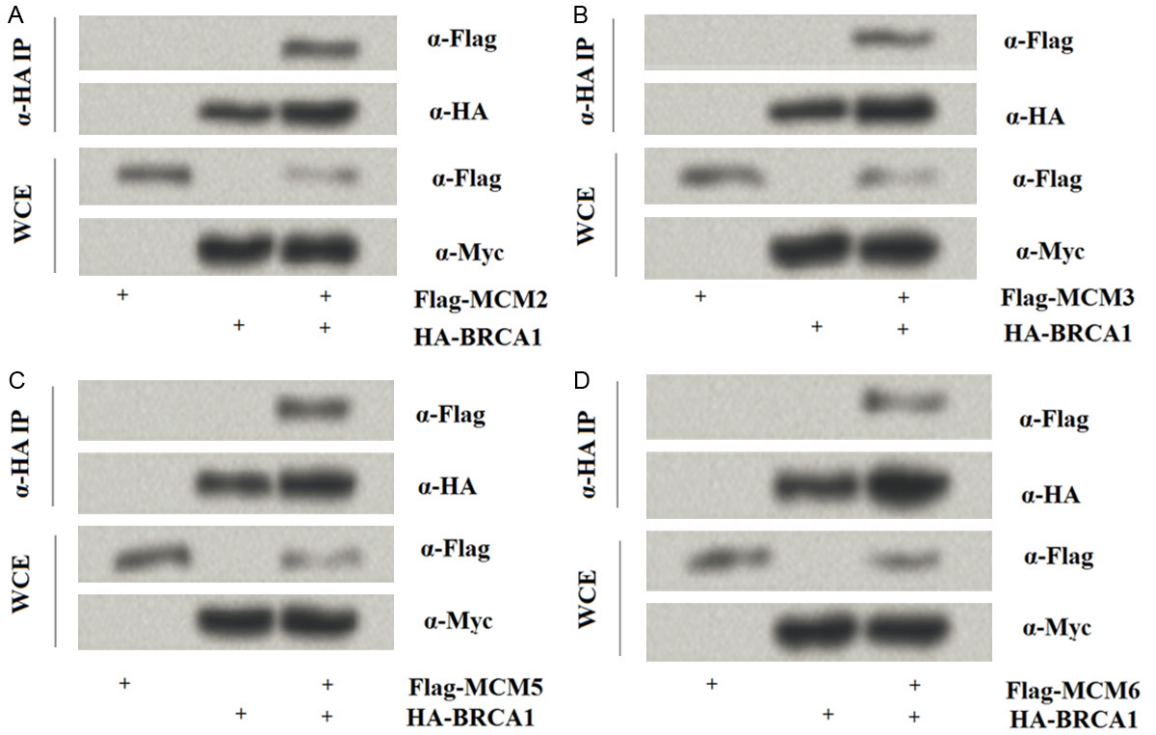

Figure 1. BRCA1 interacts with MCMs. HA-tagged BRCA1 and four MCMs (MCM2, MCM3, MCM5 and MCM6) with Flag tag were overexpressed in A549 cells, and Western blot was carried out to detect BRCA1 or MCM2 (A), MCM3 (B), MCM5 (C), and MCM6 (D). (MCM, minichromosome maintenance protein; BRCA1, breast cancer 1, HA, hemaglutinin; IP, immunoprecipitation; WCE, whole cellular extract).

noprecipitated with the HA tag of BRCA1 in the chromatin fraction, and then subjected to mass spectrometric analysis. The results showed that the peptide recovery of BRCA1 was 13%, 16%, 18%, and 8%, respectively (**Table 1**). Our data of mass spectrometric analysis showed that four MCMs (MCM2, MCM3, MCM5 and MCM6) were identified in IP of BRCA1. These MCMs might interact with BRCA1 in A549 cells. The results of mass spectrometry were confirmed by Western blot. The A549 cells overexpressing HA-tagged BRCA1 and four Flag-tagged MCMs were respectively incubated with antibodies against HA and Flag to detect the presence of these proteins. As shown in **Figure 1A-D**, HA-tagged BRCA1 or Flag-tagged MCM2, MCM3, MCM5, or MCM6 could be detected in WCE of A549 cells, indicating that these proteins were successfully overexpressed. Besides, for the nuclear IP, Flag-tagged MCM2 MCM3, MCM5, or MCM6 and HA-tagged BRCA1 only could be observed in the cells which overexpressed both fusion proteins, but could not be found in nuclei of cells which only expressed one fusion protein. Therefore, the results indicated that BRCA1 interacted with the four MCMs in A549 cell nuclei.

DNA damage reduces the interaction between BRCA1 and MCM2

To test whether the interaction was effected by DNA damage, A549 cells were or were not treated with 500 nM CPT for 2 h to induce DNA damage, the interaction between BRCA1 and MCM2 were analyzed again. IP and IB results showed that compared to the cells not treated with CPT, the interaction between BRCA1 and MCM2 were significantly decreased by CPT. The results demonstrated that the interaction between BRCA1 and MCM2 could be reduced by DNA damage (**Figure 2**).

Downregulation of MCMs reduces BRCA1 chromatin fraction

Based on the above results, we speculated that MCMs might be involved in the function of BRCA1. To confirm the speculation, the expression of MCM2 and MCM6 was downregulated by specific shRNAs, and then the chromatin

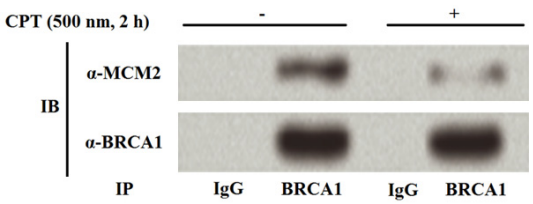

Figure 2. DNA damage reduces the interaction between BRCA1 and MCM2. A549 cells were or were not treated with 500 nM CPT to induce DNA damage, the interaction between BRCA1 and MCM2 were then analyzed by IP and IB. (MCM, minichromosome maintenance protein; BRCA1, breast cancer 1; IP, immunoprecipitation; IB, immunoblot; CPT, camptothecin).

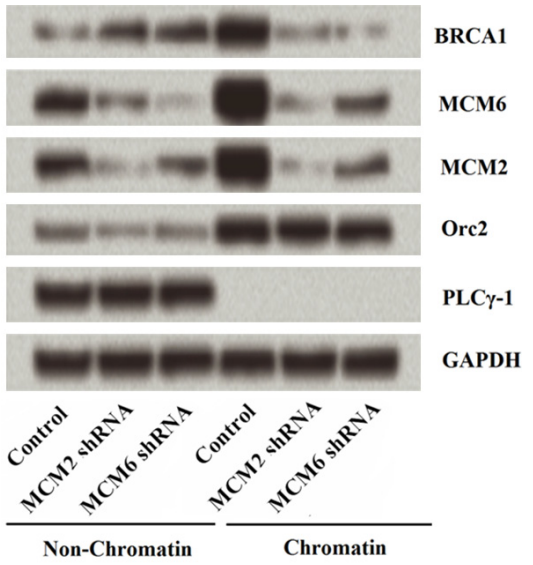

Figure 3. Downregulation of MCMs reduces BRCA1 chromatin fraction. A549 cells were transfected with specific shRNAs against MCM2 or MCM6, and then the protein levels of BRCA1 in non-chromatin and chromatin fractions of A549 cells were determined. Orc2 and PLCγ-1 are respectively marker proteins for chromatin and non-chromatin fractions. (MCM, minichromosome maintenance protein; BRCA1, breast cancer 1; Orc2, Origin recognition complex 2; shRNA, short hairpin RNA).

fraction of BRCA1 was analyzed. As demonstrated in **Figure 3**, the results showed that both the expression levels of MCM2 and MCM6 were successfully downregulated by specific shRNAs in both non-chromatin and chromatin fractions of A549 cells. In addition, we found that downregulation of MCM2 or MCM6 could significantly increase the non-chromatin level of BRCA1, but decrease the chromatin level of BRCA1. These results implied that downregulation of MCMs could reduce BRCA1 chromatin fraction.

Downregulation of MCMs reduces BRCA1 foci formation

We then analyzed the foci forming of BRCA1 under the condition of DNA damage. Bleomycin was performed to induce DNA damage. The expression of MCM2 or MCM6 was confirmed by Western blot before treatment with bleomycin (**Figure 4A**). As expected, the expression of MCM2 and MCM6 was successfully downregulated by specific shRNAs. After induction of DNA damage, the foci formation of BRCA1 in A549 cell nuclei was identified by immunofluorescence. As shown in **Figure 4B**, the results showed that knockdown of MCM2 or MCM6 significantly decreased the foci formation of BRCA1, and there were significant differences (both $P < 0.05$) (**Figure 4C**). The results suggested that knockdown of MCMs might also reduce foci formation of BRCA1 in response to DNA damage.

Discussion

In the present study, we demonstrate that there is an interaction between BRCA1 and MCM complex in A549 cells. BRCA1 interacts with MCM2, MCM3, MCM5, and MCM6 in A549 cell nucleus. But the interaction between MCM2 and BRCA1 is reduced during DNA damage. Downregulation of MCM2 and MCM6 could decrease the chromatin fraction of BRCA1, as well as foci formation of BRCA1 in response to DNA damage. These results suggest that the interaction MCM complex is essential for BRCA1 chromatin fraction and foci formation.

BRCA1 is a human tumor suppressor gene, specifically a caretaker gene [18, 19]. BRCA1, together with BRCA2 has been the most significant findings in human cancer genetics [20]. An increasing number of studies have suggested that BRCA1 is implicated in several cellular and molecular biological events in response to DNA damage including activation of cell cycle checkpoint and repair of DNA double-strand breaks (DSBs) [21-24]. Besides, it has been reported that BRCA1 is involved in the repair of chromosomal damage [25]. It has been well demonstrated that proteins play their roles through interaction with other proteins. BRCA1 is no exception, and BRCA1 has been reported to be interacted with numerous proteins. For example, BRCA1 protein can interact with RAD51, playing significant roles in repair of DNA DSBs [26]. Wang *et al.* found that BRCA1 protein

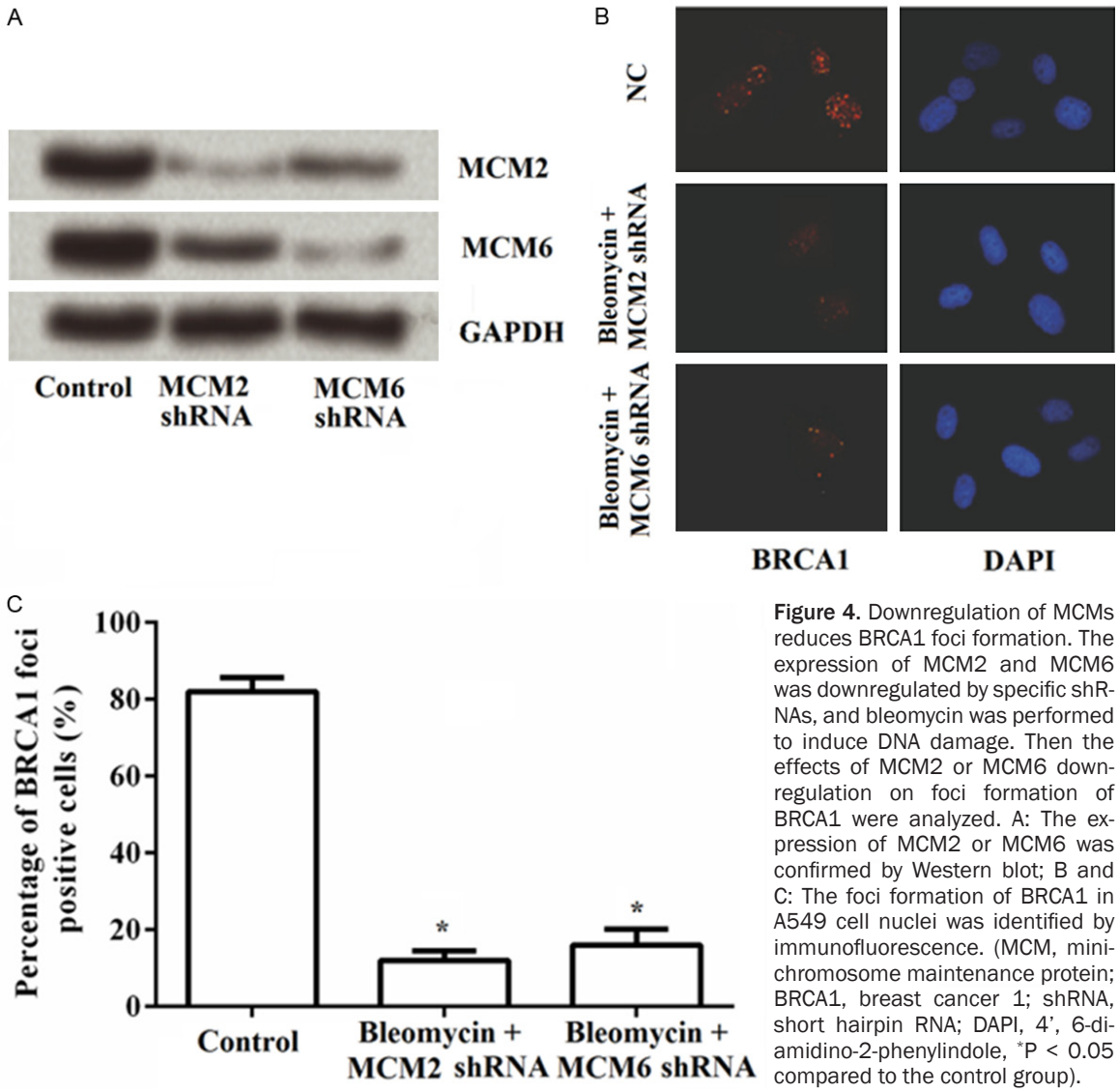

Figure 4. Downregulation of MCMs reduces BRCA1 foci formation. The expression of MCM2 and MCM6 was downregulated by specific shRNAs, and bleomycin was performed to induce DNA damage. Then the effects of MCM2 or MCM6 downregulation on foci formation of BRCA1 were analyzed. A: The expression of MCM2 or MCM6 was confirmed by Western blot; B and C: The foci formation of BRCA1 in A549 cell nuclei was identified by immunofluorescence. (MCM, minichromosome maintenance protein; BRCA1, breast cancer 1; shRNA, short hairpin RNA; DAPI, 4', 6-diamidino-2-phenylindole, $^{*}P < 0.05$ compared to the control group).

could interact with DNA mismatch repair (MMR) gene product MutS Homolog (MSH) 2 *in vitro* and *in vivo* [27]. Additionally, some other proteins that is involved in single-strand repair, such as MSH6 and Poly (ADP-Ribose) Polymerase (PARP), are also reported to be associated with BRCA1-deficient mammary tumors [28]. However, there is a critical question that how BRCA1 exactly is recruited onto chromatin and promotes foci formation. Previous studies have confirmed that MCM complex is essential for chromosome DNA replication; thus, this complex plays important roles in DNA damage repair [15, 29, 30]. Therefore, we speculated that there might be an interaction between BRCA1 and MCM complex.

To confirm the speculation, IP and IB were performed in the nuclear protein of A549 cells followed by mass spectrometry. We observed that MCM2, MCM3, MCM5, and MCM6 were immunoprecipitated by antibodies against the HA-BRCA1 in A549 nuclei. It was noticed that MCM4 and MCM7, however, were not immunoprecipitated and showed low recovery in the mass spectrometry results. Among the subunits, MCM2 has been shown the ability of initiating eukaryotic genome replication. It can be formed a complex with MCM3, 5, and 6, and is responsible for regulating the helicase activity of the complex [31-33]. Therefore, it is possible that BRCA1 interacts with MCM complex by interaction with MCM2/3/5/6. We further confirmed the interaction between MCM2 and

BRCA1 when the cell experienced DNA damage. CPT was added to the A549 cells to induce the DNA damage, and then the interaction was analyzed. The results showed that the interaction between MCM2 and BRCA1 was reduced during DNA damage.

Further, the expression of MCM2 or MCM6 was knocked down by respectively transfection with sh-MCM2 or sh-MCM6 in A549 cells. The levels of BRCA1, MCM2, and MCM6 in both non-chromatin and chromatin were determined. We found that transfection with sh-MCM2 or sh-MCM6 alone could inhibit the protein levels of both MCM2 and MCM6 in both non-chromatin and chromatin, which was in line with a previous study [15]. It was noteworthy that sh-MCM2 or sh-MCM6 could increase the levels of BRCA1 in non-chromatin but decrease the levels of BRCA1 in chromatin. Our results implied that knockdown of MCM protein levels may inhibit the level of MCM complex and its interactive protein BRCA1, subsequently leading to the decreased chromatin fraction of BRCA1 in A549 cells. In addition to the above results, we also explored the effects of knockdown of MCM2 and MCM6 on foci formation of BRCA1 in A549 cell nucleus upon DNA damage. Similarly, as the results of chromatin fraction, the results of immunofluorescence showed that suppression of MCM2 and MCM6 decreased the foci formation of BRCA1 in A549 cell nucleus. The ability of inhibitory chromatin fraction and foci formation by suppressing MCM complex indicated the possibility to regulate DNA damage repair in A549 cells by suppression of BRCA1 and its modulators such as MCMs, thus improving the therapeutic effects of drugs or other treatments.

In conclusion, this study demonstrates that BRCA1 interacts with MCM2/3/5/6, and that suppression of MCMs decreases the chromatin fraction and foci formation of BRCA1 in response to DNA damage in A549 cells. The possible interactions between BRCA1 and MCMs might provide a new insight into the therapeutic target for lung cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Shanqing Li, Department of Thoracic Surgery, Peking Union Medical College Hospital (East), 1 Shuaifuyuan, Dongcheng District, Beijing 100730, China. E-mail: lishanqing100@126.com

References

[1] Mao Y, Yang D, He J and Krasna MJ. Epidemiology of lung cancer. Surg Oncol Clin N Am 2016; 25: 439-445.

[2] Tsao AS, Scagliotti GV, Bunn PA, Carbone DP, Warren GW, Bai C, de Koning HJ, Yousaf-Khan AU, McWilliams A, Adusumilli PS, Rami-Porta R, Asamura H, Van Schil PE, Darling GE, Ramalingam SS, Gomez DR, Rosenzweig KE, Zimmermann S, Peters S, Ignatius Ou SH, Reungwetwattana T, Jänne PA, Mok TS, Wakelee HA, Pirker R, Mazières J, Brahmer JR, Zhou Y, Herbst RS, Papadimitrakopoulou VA, Redman MW, Wynes MW, Gandara DR, Kelly RJ, Hirsch FR, Pass HI and Tsao MS. Scientific advances in lung cancer 2015. J Thoracic Oncol 2016; 11: 613-638.

[3] Howlader N, Noone A, Krapcho M, Neyman N, Aminou R, Waldron W, Altekruse S, Kosary C, Ruhl J and Tatalovich Z. SEER Cancer Statistics Review, 1975-2008. Bethesda, MD: National Cancer Institute; 2011. pp. 1.

[4] Nathanson KN, Wooster R and Weber BL. Breast cancer genetics: what we know and what we need. Nat Med 2001; 7: 552-556.

[5] Wang B, Matsuoka S, Ballif BA, Zhang D, Smogorzewska A, Gygi SP and Elledge SJ. Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. Science 2007; 316: 1194-1198.

[6] Hakem R, de la Pompa JL, Sirard C, Mo R, Woo M, Hakem A, Wakeham A, Potter J, Reitmair A and Billia F. The tumor suppressor gene Brca1 is required for embryonic cellular proliferation in the mouse. Cell 1996; 85: 1009-1023.

[7] Deng CX and Scott F. Role of the tumor suppressor gene Brca1 in genetic stability and mammary gland tumor formation. Oncogene 2000; 19: 1059-64.

[8] McPherson JP, Hande MP, Poonepalli A, Lemmers B, Zablocki E, Migon E, Shehabeldin A, Porras A, Karaskova J and Vukovic B. A role for Brca1 in chromosome end maintenance. Hum Mol Genet 2006; 15: 831-838.

[9] King MC, Marks JH and Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003; 302: 643-646.

[10] Taron M, Rosell R, Felip E, Mendez P, Souglakos J, Ronco MS, Queralt C, Majo J, Sanchez JM and Sanchez JJ. BRCA1 mRNA expression levels as an indicator of chemoresistance in lung cancer. Hum Mol Genet 2004; 13: 2443-2449.

[11] Rosell R, Skrzypski M, Jassem E, Taron M, Bartolucci R, Sanchez JJ, Mendez P, Chaib I, Perez-Roca L and Szymanowska A. BRCA1: a novel prognostic factor in resected non-small-cell lung cancer. PLoS One 2007; 2: e1129.
[12] Kelman Z and Hurwitz J. Structural lessons in DNA replication from the third domain of life. Nat Struct Mol Biol 2003; 10: 148-150.
[13] Kelman Z and White MF. Archaeal DNA replication and repair. Curr Opin Microbiol 2005; 8: 669-676.
[14] Masai H, Matsumoto S, You Z, Yoshizawa-Sugata N and Oda M. Eukaryotic chromosome DNA replication: where, when, and how? Biochemistry 2010; 79: 89.
[15] Han X, Aslanian A, Fu K, Tsuji T and Zhang Y. The interaction between checkpoint kinase 1 (Chk1) and the minichromosome maintenance (MCM) complex is required for DNA damage-induced Chk1 phosphorylation. J Biol Chem 2014; 289: 24716-24723.
[16] Méndez J and Stillman B. Chromatin association of human origin recognition complex, cdc6, and minichromosome maintenance proteins during the cell cycle: assembly of prereplication complexes in late mitosis. Mol Cell Biol 2000; 20: 8602-8612.
[17] McDonald WH, Tabb DL, Sadygov RG, MacCoss MJ, Venable J, Graumann J, Johnson JR, Cociorva D and Yates JR. MS1, MS2, and SQT-three unified, compact, and easily parsed file formats for the storage of shotgun proteomic spectra and identifications. Rapid Commun Mass Spectrom 2004; 18: 2162-2168.
[18] Yoshida K and Miki Y. Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. Cancer Sci 2004; 95: 866-871.
[19] Skolnick MH. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994; 266: 66-71.
[20] Foulkes WD and Shuen AY. In brief: BRCA1 and BRCA2. J Pathol 2013; 230: 347-349.
[21] Huen MS, Sy SM and Chen J. BRCA1 and its toolbox for the maintenance of genome integrity. Nat Rev Mol Cell Biol 2010; 11: 138-148.
[22] Roy R, Chun J and Powell SN. BRCA1 and BRCA2: different roles in a common pathway of genome protection. Nat Rev Cancer 2012; 12: 68-78.
[23] Scully R, Chen J, Ochs RL, Keegan K, Hoekstra M, Feunteun J and Livingston DM. Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage. Cell 1997; 90: 425-435.
[24] Scully R and Livingston DM. In search of the tumour-suppressor functions of BRCA1 and BRCA2. Nature 2000; 408: 429-432.
[25] Friedenson B. The BRCA1/2 pathway prevents hematologic cancers in addition to breast and ovarian cancers. BMC Cancer 2007; 7: 1.
[26] Boulton S. Cellular functions of the BRCA tumour-suppressor proteins. Biochem Soc Trans 2006; 34: 633-645.
[27] Wang Q, Zhang H, Guerrette S, Chen J, Mazurek A, Wilson T, Slupianek A, Skorski T, Fishel R and Greene MI. Adenosine nucleotide modulates the physical interaction between hMSH2 and BRCA1. Oncogene 2001; 20: 4640-4649.
[28] Warmoes M, Jaspers JE, Pham TV, Piersma SR, Oudgenoeg G, Massink MP, Waisfisz Q, Rottenberg S, Boven E and Jonkers J. Proteomics of mouse BRCA1-deficient mammary tumors identifies DNA repair proteins with potential diagnostic and prognostic value in human breast cancer. Mol Cell Proteomics 2012; 11: M111.013334.
[29] Bell SD and Botchan MR. The minichromosome maintenance replicative helicase. Cold Spring Harb Perspect Biol 2013; 5: a012807.
[30] Maiorano D, Lemaître JM and Méchali M. Stepwise regulated chromatin assembly of MCM2-7 proteins. J Biol Chem 2000; 275: 8426-8431.
[31] Kneissl M, Pütter V, Szalay AA and Grummt F. Interaction and assembly of murine pre-replicative complex proteins in yeast and mouse cells. J Mol Biol 2003; 327: 111-128.
[32] DaFonseca CJ, Shu F and Zhang JJ. Identification of two residues in MCM5 critical for the assembly of MCM complexes and Stat1-mediated transcription activation in response to IFN-γ. Proc Natl Acad Sci 2001; 98: 3034-3039.
[33] Yabuta N, Kajimura N, Mayanagi K, Sato M, Gotow T, Uchiyama Y, Ishimi Y and Nojima H. Mammalian Mcm2/4/6/7 complex forms a toroidal structure. Genes Cells 2003; 8: 413-421.