The Cytoskeleton Protein Filamin-A Is Required for an Efficient Recombinational DNA Double Strand Break Repair

Jingyin Yue,1 Qin Wang,2 Huimei Lu,1 Mark Brenneman,3 Feiyan Fan,2 and Zhiyuan Shen1

1Department of Radiation Oncology, The Cancer Institute of New Jersey, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, New Brunswick, New Jersey; 2Institute of Radiation Medicine, Peking Union Medical College, Tianjin, China; and 3Department of Genetics, Rutgers University, Piscataway, New Jersey

Abstract

The human actin-binding protein filamin-A (also known as ABP-280) cross-links actin into a dynamic three-dimensional structure. It interacts with >45 proteins of diverse functions, serving as the scaffold in various signaling networks. BRCA2 is a protein that regulates RAD51-dependent recombinational repair of DNA double strand breaks (DSB). Proximate to the COOH terminus of the BRCA2 protein, a conserved and DNA binding domain (BRCA2-DBD) interacts with filamin-A and BCCIP. In this study, we sought to test the hypothesis that filamin-A influences homologous recombinational repair of DSB and the maintenance of genomic stability. We found that lack or reduction of filamin-A sensitizes cells to ionizing radiation, slows the removal of DNA damage–induced γH2AX nuclear foci, reduces RAD51 nuclear focus formation and recruitment to chromatin in response to irradiation, and results in a 2-fold reduction of homologous recombinational repair of DSB. Furthermore, filamin-A–deficient cells have increased frequencies of micronucleus formation after irradiation. Our data illustrate the importance of the cytoskeleton structure in supporting the homologous recombinational DNA repair machinery and genome integrity, and further implicate a potential of filamin-A as a marker for prognosis in DNA damage–based cancer therapy. [Cancer Res 2009;69(20):7978–85]

Introduction

Homologous recombination (HR) plays a critical role in the repair of DNA double strand breaks (DSB). Due to the complexity of HR reactions, a large number of proteins are involved in HR, including enzymes that catalyze the chemical reactions, and accessory proteins that either regulate the activity of the enzymatic proteins or act at the interface between HR and other cellular processes. The key reactions in HR are the search for homology and strand exchange, which are catalyzed by the RecA protein in Escherichia coli and its homologous RAD51 in eukaryotes. The enzyme activities of these proteins are tightly regulated so that HR is coordinated with the cell cycle and other repair processes. In mammalian cells, RAD51 activity is regulated by several accessory proteins such as BRCA2, which was originally implicated as a HR-regulatory protein by its interaction with RAD51 (1–6). Subsequent studies have confirmed a direct role for BRCA2 and its interaction with RAD51 in HR (7–10). Mutations affecting the BRCA2-RAD51 interaction result in HR defects (6, 11). The human BRCA2 encodes a large protein of 3,418 amino acids. Proximate to the BRCA2 COOH terminus, a conserved region encoded by exons 14 to 24 is sometimes called the BRCA2-DNA binding domain (BRCA2-DBD; refs. 12, 13). We previously reported that this BRCA2-DBD interacts with filamin-A and BCCIP (14, 15).

Filamin-A, also known as human actin-binding protein 280 (ABP-280) or filamin-1, is encoded by the X-linked gene FLNa (16, 17). The human filament-A gene encodes a protein of 2,647 amino acids (18). It cross-links cortical actin filaments into a dynamic three-dimensional structure (19). Filamin-A has an elongated homodimeric and Y-shaped structure (18–22). At the NH2 terminus of the monomer, there is an actin-binding domain, followed by 24 tandem repeats of ∼96 amino acids in length. Between repeats 15 and 16, there is a hinge domain, and repeat 24 is separated from repeat 23 by a second hinge domain. The last 65 amino acids of repeat 24 allow filamin-A dimerization to form a Y-shaped structure (18). In addition to filamentous actin, filamin-A interacts with >45 functionally diverse proteins including nuclear proteins, serving as the scaffold in various signaling networks (23, 24). These diverse interactions suggest that filamin-A is a key component of a versatile signaling scaffold.

The Filamin-A and BRCA2 interaction was originally identified by a yeast two-hybrid screen using the conserved BRCA2-DBD as the bait, and then confirmed by an in vitro binding assay using purified recombinant proteins, and an in vivo communoprecipitation (14). Considering that filamin-A is a cytoskeletal protein that mostly associates with cell matrix, and BRCA2 is a critical protein in DNA HR (14), it is possible that filamin-A serves as a scaffold to anchor BRCA2 and/or to assist the assembly of the repair complex, and thus may play a role in DNA repair by HR. Although previous reports have showed that lack of filamin-A sensitizes cells to some DNA damage agents and delays the recovery of G2-cell cycle arrest (14, 25), no direct evidence has been documented for a role of filamin-A in DNA repair. In this report, we test the hypothesis that filamin-A plays a role in recombinational DNA repair and maintenance of genomic stability. We found that lack of filamin-A impairs HR, delays DSB repair, and promotes genomic instability.

Materials and Methods

Cell lines and cell cultures. Human A7, M2 and C8161 melanoma cells were cultured as described previously (18). MDA-MB-231 cell was grown and routinely maintained in DMEM supplemented with 10% calf bovine serum, 2 mol/L glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. HT1080 cells were grown in α-MEM with 100 U/mL penicillin and 100 μg/mL streptomycin. All cells were grown in a 37°C incubator supplied with 5% CO2 and 95% air. The same strategy as previously

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprints: Zhiyuan Shen, Department of Radiation Oncology, The Cancer Institute of New Jersey, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, 195 Little Albany Street, New Brunswick, NJ 08903. Phone: 732-235-6101; Fax: 732-235-7493; E-mail: shenzh@umdnj.edu.

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described (25) was used to knockdown filamin-A in C8161 melanoma cells, MDA-MB-231 breast cancer cells, and HT1080 fibrosarcoma cells.

Construction and expression of filamin-A fragments. Several FLNa fragments (see Results for details) were amplified from pREP4 vector (kindly provided by Dr. T.P. Stossel, Harvard Medical School, Boston, MA) that contains the full length filamin-A cDNA by PCR. After digesting with restrict endonuclease, fragments were inserted into pEYFP-C1 between the HindIII and XbaI sites. This results in the NH₂ terminus fusion of the EYFP protein with the filamin-A fragments, stabilizing the expression of short filamin-A fragments. These vectors were transfected into the HT1080-1885 or M2 cells, and stable clones were selected with 500 μg/mL of G418.

Western blots and extraction of chromatin-bound proteins. Cells were lysed in lysis buffer [50 mmol/L HEPES (pH 7.6), 250 mmol/L NaCl, 5 mmol/L EDTA, 0.1% Nonidet P-40]. The samples were then sonicated and boiled before loading onto 10% SDS-PAGE gel. Filamin-A was detected with an anti–filamin-A antibody (Chemicon International, Inc.). The β-actin was used as a loading control to confirm that an equal amount of proteins was loaded in each sample by probing with anti–β-actin monoclonal antibody (Sigma). RAD51 antibodies (Oncogene Research Products), γH2AX (Serine 139) antibodies (Upstate Biotechnology), and BRCA2 antibodies (Calbiochem) were purchased. To extract chromatin-bound proteins, cells were suspended in hypotonic buffer [10 mmol/L HEPES (pH 7.4), 10 mmol/L KCl, 0.05% NP40, and protease inhibitors] and incubated on ice for 20 min to disrupt cell membranes. Thereafter, nuclei were recovered and treated with 0.2 mol/L HCl on ice for 10 min to extract chromatin-bound protein. Equal volumes of 1 mol/L Tris-HCl (pH 8.0) were used to neutralize the acid-extracted protein solutions.

Radiation survival assay. Log-phase cells were plated on 100-mm culture dishes. The number of cells to be plated for each assay was determined by a pilot experiment so as to yield 50 to 150 surviving colonies per 100-mm dish. The cells were irradiated with Cs-137 γ-rays (dose rate, 0.893 Gy/min) 18 h after the cells were plated. Colonies were grown for 12 to 14 d, after which the colonies were fixed with methanol and stained with 1% crystal violet. The number of colonies was normalized to the number of cells plated to calculate the surviving fraction. Each experiment was performed in triplicate and repeated at least twice.

Immunofluorescent detection of γH2AX and RAD51 nuclear foci. Cells (5 × 10⁵) were plated and grown on glass coverslips for 16 h, then treated or not with γ-rays. The procedures to stain for γH2AX and RAD51 foci have been described previously (26, 27). Immunofluorescent signals were recorded using a Zeiss upright microscope with a UV laser source. RAD51 foci were visualized by the same procedure except that anti-RAD51 (1:1000) primary antibody (Oncogene Research Products) was used.

Measuring DSB-induced HR in HT1080-1885 cells. To assess the role of filamin-A in DSB-induced HR, the HT1080-1885 cell line with a copy of installed HR substrate was used (28). The expression of endogenous
filamin-A in HT1080-1885 was suppressed by RNA interference, using methods reported previously (25). To minimize clonal variation, multiple control and knockdown clones were selected and evaluated for the efficacy of filamin-A knockdown (see Results). We then electroporated or transfected with an equal amount of pCMV-3NLS-I-SceI plasmid that expresses NLS-tagged I-SceI enzyme into each of the clones. Forty-eight hours after transfection, the cells were replated onto 100-mm culture dishes at ~10,000 viable cells per dish, and selected with puromycin. Seven to 10 d after selection in puromycin (1 µg/mL), the cells that underwent I-SceI-induced HR formed colonies and were counted after staining. To determine plating efficiency, a parallel set of plates with appropriate cell dilution was plated to yield ~100 colonies in the absence of G418. The HR frequency was measured as the number of puromycin-resistant colonies per 106 viable cells. The spontaneous HR frequency was measured in cells transfeected with an empty pCMV vector. An average of four to six experiments was analyzed by Student’s t test to determine statistical significance.

**Micronucleus assay.** Log-phase A7 and M2 cells were irradiated with 2 to 10 Gy Cs-137 γ-rays and harvested at indicated times. To assay micronucleus formation, cytchalasin B at 6 µg/mL final concentration was added into culture 24 h after Cs-137 γ-irradiation and maintained for 24 h. Cells were then trypsinized, treated with hypotonic saline, and fixed. Air-dried slides were stained with Giemsa stain. Binucleated cells (~1,000 cells) were scored under light microscopy for the presence or absence of micronuclei. Student’s t test was used for statistical analysis.

**Chromosome break and aberration assays.** To prepare metaphase chromosome spreads, at 15 min, 8, or 24 h after irradiation, cells were blocked at mitosis with colchicine (at 0.08 µg/mL of final concentration) for 4 h, washed with PBS, resuspended in 75 mmol/L KCl for 30 min at 37°C, and fixed twice with freshly made fixing solution (3:1, methanol/acetic acid, v/v) 20 min for each time. Then chromosome spreads were made on glass slides. After staining with Giemsa, 40 metaphase cells were analyzed for each group. Unstable chromosome aberrations (dicentric, acentric ring, chromatid breaks, and acentric fragments) were scored. The formation of dicentric and acentric rings reflects the misjoining of DSB, and the existence of acentric fragments and breaks reflects unrepaired DSB. Statistical analyses for frequency of aberrations were performed using the χ² test, and a P value of <0.05 was considered significant.

**Results**

**Downregulation of filamin-A enhances radiation sensitivity.** In a previous report, we showed that the M2 (filamin-A deficient) cells are more sensitive to γ-radiation than the A7 (filamin-A proficient) cells (14). M2 is a melanoma cell line that has spontaneously lost filamin-A expression, and A7 is a cell line derived from M2 by transfection with a filamin-A-expressing vector (29). These data raised the question of whether depletion of filamin-A in filamin-A-positive cells would sensitize the cells to radiation damage. To answer this question, we constructed filamin-A knockdown cell lines with C8161 melanoma and MDA-MB-231 breast cancer cells (referred to hereafter as MB231). The expression level of filamin-A in these cells was evaluated by Western blot (Fig. 1A). The filamin-A deficiency in these cells did not affect their growth (see Supplementary Fig. S1). Using these cells, we measured their sensitivities to ionizing radiation by colony formation assays (see Materials and Methods for details). As shown in Fig. 1, depletion of filamin-A by RNAi sensitizes cells to γ-rays, to a greater extent for the C8161 cells than the MB231 cells. The less extent of sensitization to radiation in filamin-A–deficient MB231 cells may reflect a cell line–specific dependence on filamin-A to DNA damage. Nevertheless, these data indicate that filamin-A is important for cellular resistance to radiation damage.

**Impaired DSB repair in filamin-A–deficient cells.** Among the many factors that may affect cell sensitivity to DNA damage, DNA repair is the most critical one. The presence of γH2AX nuclear foci...
is widely regarded as a surrogate and sensitive marker for the presence of DSB in chromosomal DNA (30–33), and the number of γH2AX foci is believed to be closely related to the number of DSB in the cells (30, 31, 34). Therefore, a kinetic analysis of the appearance and disappearance of γH2AX foci after irradiation may reveal the DSB repair efficiency in the cells. To assess the role of filamin-A in DSB repair, we performed immunofluorescent staining and counted the number of γH2AX foci in M2 and A7 cells at time points after γ-irradiation. As shown in Fig. 2A, the number of γH2AX foci per cell increased substantially after γ-irradiation. The focus numbers reached similar peak values at 0.5 to 1.0 hour after irradiation in M2 and A7 cells, suggesting that irradiation induces similar levels of initial DNA damage in the two cell lines. Afterwards, γH2AX foci decreased with time as DSBs were repaired. However, the numbers of γH2AX foci in A7 cells decreased significantly faster than those in M2 cells (P < 0.01), especially within the first 4 hours after irradiation. This implies a faster DSB repair in A7 (filamin-A proficient) cells than in M2 (filamin-A deficient). To further confirm the role of filamin-A in DSB repair, we evaluated the effects of filamin-A knockdown on γH2AX protein levels after irradiation in C8161 melanoma and MB231 breast cancer cells. As shown in Fig. 2B, the filamin-A–deficient C8161 and MB231 cells retained higher levels of γH2AX than controls for up to 8 hours after γ-irradiation. These data strongly suggest that lack or reduction of filamin-A slows the repair of DSB. To confirm that filamin-A deficient impairs DSB repair, two additional approaches were used (Fig. 2C and D).

First, incomplete repair of DSB before the cell enters mitosis would increase the level of chromosome fragments and chromatid breaks that are visible on mitotic chromosome spreads. Misjoining of the DSB would increase the appearance of both dicentric and acentric rings on mitotic chromosome spreads. We irradiated A7 and M2 cells with 8 Gy of γ-radiation, at 15 minutes, 8, or 24 hours after the irradiation, mitosis were blocked for 4 hours, and mitotic spreads were prepared. The number of acentric fragments, chromatid breaks, dicentric, and acentric rings were scored in 40 metaphase cells. As shown in Fig. 2C, at 8 and 24 hours after the irradiation, there were significant higher levels of chromosome aberrations in the filamin-A–deficient M2 cells than the filamin-A–proficient A7 cells (P < 0.01). However, there was no significant difference of the initial level of chromosome breaks shortly (15 minutes) after irradiation (P > 0.05), which suggests a similar level of initial DNA damage in these cells.

Second, when the DSB is not repaired or misrepaired before the cells enter mitosis, the abnormal chromosome fragments especially these without functional centromeres, often form micronuclei after the separation of the two daughter nuclei. To verify whether the lack of filamin-A would increase micronucleus formations, cells were irradiated and then immediately blocked by cytochalasin B for 24 hours to arrest the cells at cytokinesis. This allows the nuclear division to complete but prevents the cytoplasm separation, thus forming binuclear cells. Then, the rate of micronuclei in binuclear cells was scored. As shown in Fig. 2D, lack of filamin-A in M2 cells increased the radiation induced micronucleus formation. These data (Fig. 2C and D) firmly support the conclusion of Fig. 2A and B that lack of filamin-A impairs DSB repair, although the percentage of cells with micronuclei do not necessary equal to the survival fraction as measured by colony formation.

Knockdown of filamin-A reduces RAD51 focus formation and recruitment to chromatin in response to irradiation. Within 2 to 6 hours after irradiation, RAD51 redistributes to form nuclear foci (2, 35–41). These DNA damage–induced RAD51 foci are located at single stranded regions where recombination is actively in progress (37). RAD51 focus formation in response to DNA damage is a visual indication for their involvement in HR. Failure or delay in forming RAD51 foci would signal an impaired

![Figure 3. Filamin-A deficiency reduces RAD51 recruitment to DNA damage. Four hours after 2, 4, or 8 Gy of γ-irradiation, the RAD51 nuclear foci and chromatin-bound RAD51 protein were measured. A, Western blot showing that lack of filamin-A in M2 cells does not alter the total RAD51 protein levels either before or after the irradiation. B, the average numbers of RAD51 nuclear foci per cells. C, the percentage of cells with five or more RAD51 foci. A7 and M2 cells in exponential phase were grown on coverslips and irradiated with 0, 2, 4, or 8 Gy of γ-radiation. Cells were fixed 4 h after irradiation and fluorescent immunostaining was performed with anti-RAD51 antibody. RAD51 focus number in individual cells was scored under a fluorescent microscope. At least 250 cells in each experiment were analyzed and data shown are averages of three independent experiments. The P values indicate statistic significance based on t tests. D, the chromatin-bound RAD51 proteins at time points after irradiation. Filamin-A–deficient cells have less chromatin-bound RAD51 protein between 2 and 4 h after irradiation. Histone-2A (H2A) was used as a loading control for chromatin-bound proteins.](Image 300x279 to 548x729)
Knockdown of filamin-A inhibits homologous recombinational repair of DSB. The filamin-A expression in HT1080-1885 cells, which host an HR assay system previously established in the HT1080-1885 fibrosarcoma cell line (28). HT1080-1885 cells with filamin-A knockdown were established using the shRNA approach (see Materials and Methods). We isolated five control clones (Fig. 4A, bottom) and seven knockdown clones (labeled as A7, A8, and A9). To confirm this, we measured the levels of chromatin-bound RAD51 by Western blot. As shown in Fig. 3D, by 4 hours after the irradiation, there was an increase of chromatin-bound RAD51 in the A7 (filamin-A proficient) cells, consistent with the RAD51 nuclear focus formation. However, the chromatin-bound RAD51 in M2 (filamin-A–deficient cells) was reduced compared with the A7 cells. These data also support a role of filamin-A in the recruitment of RAD51 to DNA damage.

Knockdown of filamin-A reduces HR-dependent repair of DSB. After confirming that filamin-A plays a role in RAD51 recruitment to DNA damage, we further tested the hypothesis that lack of filamin-A impairs DSB repair by HR. To do so, we used an in vivo HR assay system previously established in the HT1080-1885 fibrosarcoma cell line (28). HT1080-1885 cells with filamin-A knockdown were established using the shRNA approach (see Materials and Methods). We isolated five control clones (Fig. 4A, 1–5) that express control shRNA, and seven clones express shRNA against filamin-A (Fig. 4A, a–g). As shown in Fig. 4A, the filamin-A shRNA reduced filamin-A expression to varying degrees in individual clones.

HT1080-1885 cells have a single copy of a chromosomal recombination substrate organized as inverted repeats of the puromycin gene (Pur; ref. 28). As shown in Fig. 4B, the recipient copy of pur is not functional due to an 80-bp deletion of coding sequence, into which an 18-bp I-SceI endonuclease recognition site has been inserted. The donor copy of pur is not expressed as it lacks a promoter. When a gene conversion type of recombination between the donor and recipient copies occurs, a functional pur gene will be generated and expressed at the recipient copy, conferring puromycin resistance. Therefore, by determining the frequency of puromycin resistance after I-SceI expression, one can measure the HR frequency. After transfection of I-SceI expression vector into the individual clones (shown in Fig. 4A), the HR frequency was measured. As shown in Fig. 4C, all filamin-A knockdown clones had reduced HR frequencies. The seven knockdown clones had an average of 2-fold HR reduction in comparison with the five control clones (Fig. 4D). These data indicate that filamin-A is required for fully efficient DSB repair by HR.

Filamin-A is a homodimer, and each monomer contains 24 tandem repeats. A hinge-2 domain is located between repeats 23 and 24, which enables flexibility of repeat 24. Our previous report showed that the BRCA2-interacting domain is located between repeats 21 and 24 (14). To confirm that the filamin-A and BRCA2 interaction has direct involvement in the HR regulation, four short filamin-A fragments were constructed (Fig. 5A, bottom): ABD is the actin binding domain; H2/R24 contains hinge-2 and repeat 24; R23/R24 contains the region between repeats 23 and 24; and R21/R24 contains the region between repeats 21 and 24. These fragments

Figure 4. Knockdown of filamin-A inhibits homologous recombinational repair of DSB. The filamin-A expression in HT1080-1885 cells, which host an HR assay substrate, was knocked down by shRNA. Five control clones (1–5) and seven knockdown clones (labeled as a–g) were isolated. Break-induced HR was measured in these clones (see Results for a description of the HR assay). A, the level of filamin-A protein levels in control and knockdown clones. Clones 1 to 5, cells expressing a control shRNA; and clones a to g, cells expressing filamin-A–specific shRNA. HA-I-SceI blot (middle) shows the expression level of the I-SceI enzymes that introduces the site-specific DSB in the HT1080-1885 cells. B, the recombination substrate and the HR mechanism resulting in the reconstitution of a functional pur gene will be generated and expressed at the recipient copy, conferring puromycin resistance. Therefore, by determining the frequency of puromycin resistance after I-SceI expression, one can measure the HR frequency. After transfection of I-SceI expression vector into the individual clones (shown in Fig. 4A), the HR frequency was measured. As shown in Fig. 4C, all filamin-A knockdown clones had reduced HR frequencies. The seven knockdown clones had an average of 2-fold HR reduction in comparison with the five control clones (Fig. 4D). These data indicate that filamin-A is required for fully efficient DSB repair by HR.

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were expressed in the filamin-A wild-type HT1080-1885 cells. As shown in Fig. 5B, expression of the ABD does not inhibit HR. However, other three fragments inhibited the HR at various degrees. The fragment R21/R24 that contains the BRCA2-interacting domain has the strongest inhibition. It is worthwhile to point out that fragment H2/R24 that contains the filamin-A dimerization domain also inhibited the HR. However, this inhibition is not as strong as the R21/R24 fragment (P = 0.025), suggesting that both the interaction between the BRCA2 and self-dimerization of filamin-A are required for efficient HR.

Filamin-A truncations fail to rescue the repair defect in M2 cells. To complement the approach in Fig. 5, we expressed three filamin-A truncation mutants in the filamin-A–deficient M2 cells. These truncated filamin-A lack the COOH terminus dimerization and BRCA2-interacting domain. Because M2 cells do not contain the HR substrate, we measured the γH2AX in response to irradiation to assess the DNA repair capability. As shown in Fig. 6, shortly after the irradiation, all cells have similar level of γH2AX. However, at 2 h and 8 hours after the irradiation, the full-length filamin-A–complemented cells remove γH2AX much more efficiently than the M2 cells. The cells expressing the truncated filamin-A all failed to restore the repair ability, suggesting that the COOH terminus (repeats 21–24) of filamin-A is required for its function in DNA repair. Altogether, Figs. 5 and 6 suggest that the interaction with BRCA2 and self-dimerization of filamin-A are critical for the efficient repair of DSB.

Discussion
In this report, we have provided several lines of evidence for a role of the cytoskeleton protein filamin-A in supporting homologous recombination in mammalian cells.

Figure 5. Inhibition of HR by dominant-negative interference. Four EYFP-fused short filamin-A fragments of filamin-A were expressed in HT1080-1885 cells. The same procedure as Fig. 4 was used to measure ISceI induced HR, except transfection (instead of electroporation) was used to enhance the ISceI transfection. A, the location of four dominant negative fragments of filamin-A. B, the HR frequency. C, the expression of EYFG-tagged filamin-A fragments.

Figure 6. Filamin-A truncations fail to complement the DSB repair defects. Several truncation mutants of filamin-A as illustrated in A (bottom) were expressed in the M2 filamin-A–deficient cells. See text for full description of the truncation mutants. The cell line expressing the full-length filamin-A was used as positive control, and cell line carrying an empty vector was used as negative control. B, the average number of γH2AX foci per cells shortly after irradiation (0.25 h), and after 2 and 8 h of repair for M2-vector (negative control), A7 (M2 cells with full-length filamin-A), and several M2 cells expressing truncated filamin-A. C, Western blots used as an alliterative method to measure the level of γH2AX at various times after irradiation among the same cell lines. Anti-actin blot was used as loading control for γH2AX blot. C, bottom, the confirmed expression of filamin-A truncation mutants.
recombinational repair of DNA damage. These measurements also showed that lack of filamin-A increases the risk of genomic instability after irradiation.

How filamin-A contributes to HR is an issue of discussion. It is known that DSB repair, especially homologous recombinational repair, is a multistep process that requires not only enzymatic proteins but also a large number of accessory proteins. Some of these proteins, such as RAD51, may act as an essential enzyme in the HR reactions, others such as BRCA2 may serve as auxiliary proteins to promote the assembly of the HR enzymes. It is anticipated that the HR machinery may be associated with the cytoskeletal structures to facilitate assembly of the repair complex. It is possible that filamin-A may serve as a nuclear anchor for BRCA2 and HR machinery during HR. In this sense, we suggest that filamin-A is required for an efficient HR repair, but not absolutely required for the HR process. This is reflected in the modest reduction of HR frequency and RAD51 focus formation in filamin-A-deficient cells.

Because filamin-A was originally identified as an actin binding protein that likely to be involved in cytoplasm function, and BRCA2 was originally identified as mainly a nuclear protein, it is worthy to point out that a fraction of filamin-A also resides in the nucleus. This conclusion was reached by several independent groups [14, 43–45]. Furthermore, the majority of a naturally occurred cleavage fragment of filamin-A resides in the nucleus, and participate in other nuclear functions [43, 44].

The identification of a role of filamin-A in homologous recombinational repair not only explains the sensitivity of filamin-A-deficient cells to ionizing radiation, but also explains another previously observed phenotype. We previously reported that the filamin-A-deficient cells had a delayed recovery of G2 arrest after DNA damage (25). Because HR plays a major role in DNA repair during late S and G2 phases, it is understandable that there should be a delayed recovery from G2 arrest after DNA damage for filamin-A-deficient cells due to a delayed repair of DNA damage in S and G2 phase.

Our findings also have a potential clinical implication for cancer therapy, as our data have suggested that whether or not filamin-A is expressed may influence the cell sensitivity to DNA damage. The next question is whether there actually are alterations of filamin-A expression in cancers. If indeed there is a difference of filamin-A expression, then there would be an opportunity to categorize the cancer into two groups based on filamin-A status and treat each group differently. The filamin-A negative cancer would be sensitive to therapeutic DNA damage and would be an ideal candidate for DNA damage-based treatment. We have recently measured the filamin-A expression in a few melanoma tissues, and we found that indeed some melanomas are filamin-A negative (Supplementary Fig. S3). This also raises the issues of whether filamin-A can be used as a target to sensitize filamin-A–positive cancer cells to radiation or other DNA damage–based cancer therapy, and whether the lack of filamin-A expression in cancer can be used as an indicator and prognostic marker for DNA damage–based therapy. As shown in Fig. 1, there is a 30% to 50% increase in sensitivity of filamin-A-deficient cells after 2 Gy of irradiation. This difference has potential significance in radiation therapy to treat cancers with different filamin-A expression status, as a typical course of radiation therapy generally involves multiple doses of 2 Gy irradiation. If each 2 Gy fraction results in 30% to 50% sensitivity, a treatment course of 25 fractions of 2 Gy would significantly affect the outcome.

In summary, our study suggests that the cytoskeleton protein filamin-A is required for a fully efficient homologous recombinational repair of DSBs. Lack of filamin-A may render the cells more sensitive to DNA damage while further increasing the genomic instability. Thus, filamin-A may be useful as a new marker for DNA damage–based cancer therapy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Jingyin Yue, Qin Wang, Huimei Lu, et al.


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