Genome-Wide Screen of Human Bromodomain-Containing Proteins Identifies Cecr2 as a Novel DNA Damage Response Protein

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The formation of γ-H2AX foci after DNA double strand breaks (DSBs) is crucial for the cellular response to this lethal DNA damage. We previously have shown that BRG1, a chromatin remodeling enzyme, facilitates DSB repair by stimulating γ-H2AX formation, and this function of BRG1 requires the binding of BRGI to acetylated histone H3 on γ-H2AX-containing nucleosomes using its bromodomain (BRD), a protein module that specifically recognizes acetyl-Lys moieties. We also have shown that the BRD of BRG1, when ectopically expressed in cells, functions as a dominant negative inhibitor of the BRG1 activity to stimulate γ-H2AX and DSB repair. Here, we found that BRDs from a select group of proteins have no such activity, suggesting that the γ-H2AX inhibition activity of BRG1 BRD is specific. This finding led us to search for more BRDs that exhibit γ-H2AX inhibition activity in the hope of finding additional BRD-containing proteins involved in DNA damage responses. We screened a total of 52 individual BRDs present in 38 human BRD-containing proteins, comprising 93% of all human BRDs. We identified the BRD of cat eye syndrome chromosome region candidate 2 (Cecr2), which recently was shown to form a novel chromatin remodeling complex with unknown cellular functions, as having a strong γ-H2AX inhibition activity. This activity of Cecr2 BRD is specific because it depends on the chromatin binding affinity of Cecr2 BRD. Small interfering RNA knockdown experiments showed that Cecr2 is important for γ-H2AX formation and DSB repair. Therefore, our genome-wide screen identifies Cecr2 as a novel DNA damage response protein.

INTRODUCTION

If left unrepaired after being generated by genotoxic agents, such as ionizing radiation (IR), DNA double strand breaks (DSBs) can cause genomic instability and the development of human diseases, including cancer. Cells respond to DSBs by activating the signaling pathways for DNA repair, cell cycle checkpoints and sometimes cell death in a highly coordinated fashion, which is collectively known as DNA damage response (DDR). Crucial to the DDR is the formation of so-called IR-induced foci (IRIF) at the nuclear region containing DSBs, which involves the recruitment of many DDR proteins to the sites of DSBs (Ciccia and Elledge, 2010; Jackson and Bartek, 2009). Recent studies show that ATP-dependent nucleosome remodeling and specific post-translational modifications of histones are important for DDR protein recruitment and IRIF formation (Bao and Shen, 2007; Lukas et al., 2011).

Phosphorylation of histone H2AX at Ser 139 is central to DDR. Immediately after DNA damage, H2AX is phosphorylated by ATM kinase at DSB-surrounding chromatin, and phosphorylated H2AX (γ-H2AX) initially recruits MDC1 to DSBs. In addition to recruiting ATM to DSBs for γ-H2AX amplification, MDC1 recruits many other DDR proteins including RNF8, RNF168, RAP80 and 53BP1 through DDR signaling cascades, which requires other types of histone modifications, such as acetylation, methylation and ubiquitination. The ordered recruitment of DDR proteins to DSBs ultimately leads to the stable organization of IRIF for efficient DSB repair and checkpoint activation (Bonner et al., 2008; van Attikum and Gasser, 2009).

ATP-dependent chromatin remodeling complexes such as SWI/SNF and INO80 have been directly implicated in DDR in yeast and mammals (Bao and Shen, 2007). In mammalian cells, we have shown that BRG1, the catalytic ATPase of SWI/SNF complexes, facilitates DSB repair by enhancing γ-H2AX following DNA damage and binds to γ-H2AX-containing nucleosomes in a manner that is dependent on the Ser-139 phosphorylation (Park et al., 2006; 2009). In a follow-up study, we revealed the mechanism underlying these seemingly paradoxical observations; BRG1 binds to γ-H2AX and stimulates the formation of γ-H2AX (Lee et al., 2010). The cooperative activation loop mechanism suggests that BRG1 binds to acetylated H3 within γ-H2AX-containing nucleosomes using its bromodomain (BRD). BRD is a protein module that specifically recognizes acetyl-Lys moieties in the context of surrounding sequences (Mujtaba et al., 2007), which leads to the stimulation of ATM-induced phosphorylation of H2AX, presumably by nucleo-

some remodeling. Histone acetyltransferase (HAT) Gcn5 is then recruited to γ-H2AX nucleosomes and increases the levels of H3 acetylation, which leads to increased recruitment of BRG1 to γ-H2AX nucleosomes. In this way, BRG1, together with Gcn5, mediates the bidirectional histone crosstalk between γ-H2AX and H3 acetylation, establishing the high levels of these histone modifications at DSBs for efficient DNA repair (Lee et al., 2010).

During the aforementioned investigation, we found that BRG1 BRD alone can bind to acetylated H3 on γ-H2AX nucleosomes, and when BRG1 BRD is ectopically expressed in cells, it inhibits the ability of BRG1 to stimulate γ-H2AX and DSB repair. We have suggested that this function of BRG1 BRD is exerted by its dominant negative effect on BRG1 binding to γ-H2AX nucleosomes, which blocks the cooperative activation loop (Lee et al., 2010). In the present study, we observed that BRDs from a select group of proteins do not exhibit such inhibitory activity, suggesting that γ-H2AX inhibition by BRG1 BRD is due to its specific activity. On the basis of these results, we conducted a genome-wide screen for more BRDs that have a BRG1-BRD-like activity in order to identify novel BRD-containing proteins involved in DDR.

MATERIALS AND METHODS

Antibody
The antibodies against Cecr2, Myc, α-tubulin, GFP, and 53BP1 were purchased from Santa Cruz. γ-H2AX and H2A were purchased from Upstate. GAPDH was purchased from AbFrontier.

Plasmid construction
The expression vector for Myc-BRG1-BRD has been described previously (Lee et al., 2010). Similarly, the expression vectors for Myc-PCAF-BRD, Myc-Gcn5-BRD and Myc-p300-BRD were generated by cloning the sequences encoding each BRD, PCR-amplified from corresponding human cDNAs, into the *Xho*I and *Not*I sites of pCMV/myc/nuc (Invitrogen). The PCR primer sequences are as follows: GCN5, 5′gctcctcgagatgcagctctacacaacc3′ and 5′gcgcgcggccgcctccttgagcttgaag3′; P300, 5′gctcctcgagatgctacgacaggcactg3′ and 5′gcgcgcggccgccttggagcagtatttg3′; PCAF, 5′gctcctcgagatggagcccagagaccctg3′ and 5′gcgcgcggccgcagcttccttaattttac3′.

All individual human BRDs were collected by searching the human genome database by subjecting BRG1 BRD to the basic protein BLAST program (http://blast.ncbi.nlm.nih.gov) as well as searching literature (Supplementary Table S1). To generate the vectors expressing each of the 52 human BRDs tagged with GFP, the pEGFP-N1 vector (Clontech) was modified so that it contained three copies of the nuclear localization signals (NLS) plus the Myc sequence from pCMV/myc/nuc. The sequences encoding BRD and its flanking 10 amino acids were PCR-amplified from a HeLa cDNA library and cloned into the *Nhe*I and *Bgl*II sites of the modified pEGFP-N1 vector. The sequences of the PCR primers for the 52 BRDs are shown in Supplementary Table S1. The vector expressing the Cecr2 dimer was generated by cloning the blunt-end ligation product of the PCR-amplified Cecr2-BRD sequence into the modified pEGFP-N1.

The vectors expressing Cecr2-specific siRNAs were constructed by inserting annealed oligonucleotides into the *Bgl*II-*Hind*III sites of pSuper (Oligoengine). The oligonucleotide sequences are as follows: si-Cecr2-1, 5′gatccccgagcgtcagattcttctagttcaagagactagaagaatctgacgctcttttta3′ and 5′agcttaaaaagagcgtcagattcttctagtctcttgaactagaagaatctgacgctcggg3′; si-Cecr2-

2, 5′gatccccgtacctgaatcgagtacacttcaagagagtgtactcgattcaggtacttttta3′ and 5′agcttaaaaagtacctgaatcgagtacactctcttgaagtgtactcgattcaggtacggg3′; si-Cecr2, 5′gatccccgtctgccggacatcggttattcaagagataaccgatgtccggcagacttttta3′ and 5′agcttaaaaagtctgccggacatcggttatctcttgaataaccgatgtccggcagacgggg3′. Sequences of all plasmid constructs were verified by sequencing.

Chromatin fractionation
Biochemical fractionation was performed as previously described with minor modifications (Park et al., 2006). Briefly, approximately 2×10^5 293T cells were suspended in 50 μl of fractionation buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, and protease inhibitors (5 μg/ml each of pepstatin, leupeptin and aprotinin), and 10 mM NaF) for 60 min on ice and spun down by centrifugation at $16,000 \times g$ for 20 min to separate the supernatant, containing cytoplasmic and soluble nuclear proteins, from the pellet, containing insoluble chromatin-bound proteins.

Other methods
Transfections, immunohistochemistry, histone immunoblots, comet assays and colony formation assays were performed as previously described (Lee et al., 2010; Park et al., 2006).

RESULTS

The γ-H2AX inhibition activity of BRG1 BRD is specific
To determine whether the γ-H2AX inhibition activity of BRG1 BRD is specific or attributed to some general property of BRD, we tested whether the BRDs of other proteins exhibited such activity. We chose the BRDs from PCAF, Gcn5 and p300 for this study because these HATs have been shown to be involved in DDRs (Chao et al., 2006; Das et al., 2009; Lee et al., 2010; Tjeertes et al., 2009). As previously shown (Lee et al., 2010), when BRG1 BRD was ectopically expressed in 293T cells, it efficiently inhibited the formation of γ-H2AX after IR (Fig. 1A). However, none of the three HAT BRDs showed such activity at significant levels (Fig. 1A). Immunofluorescence microscopy showed that, in contrast to BRG1 BRD, none of these BRDs exhibited a significant ability to inhibit IR-induced γ-H2AX foci (Figs. 1B and 1C). Consistent with these results, 293T cells expressing BRG1 BRD were hypersensitive to IR, which was previously shown (Lee et al., 2010), but cells expressing the HAT BRDs exhibited similar IR sensitivities as the control cells harboring the empty vector (Fig. 1D). To check the possibility that the inability of the HAT BRDs to inhibit γ-H2AX was due to inefficient binding of the HAT BRDs to chromatin, we performed biochemical fractionation experiments. The three HAT BRDs were all found in the insoluble chromatin fractions at levels similar to that of BRG1 BRD, indicating that the chromatin binding affinities of these four BRDs are not significantly different. Therefore, these results show that not all BRDs can inhibit γ-H2AX, suggesting that the γ-H2AX inhibition by BRG1 BRD is likely attributed to its specific activity.

Genome-wide screen for human BRDs having γ-H2AX inhibition activity
The aforementioned results have led us to hypothesize that there could be additional BRDs in the human genome that exhibit a BRG1-BRD-like activity, and finding such BRDs would potentially give rise to the identification of novel BRD-containing proteins involved in DDRs. The human genome is known to encode 42 proteins that contain at least one BRD, and the total number of unique individual human BRDs is 56 (Sanchez and
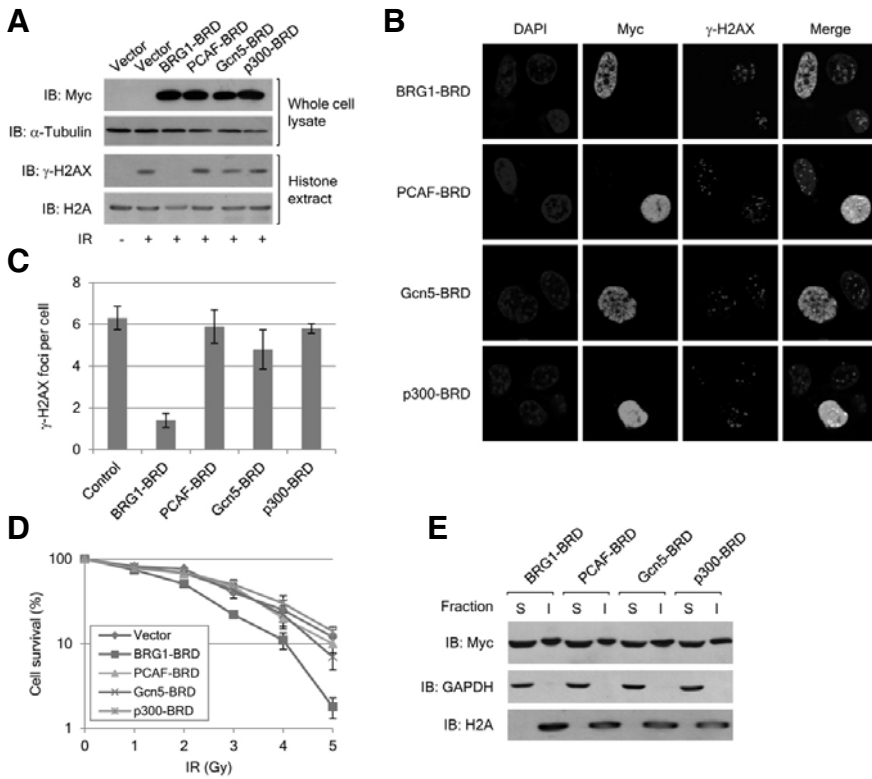
A

B

C

D

E

Fig. 1. The inhibitory activity of BRG1 BRD for γ-H2AX formation is specific. (A) 293T cells were transfected with an empty vector or the indicated expression vectors and left untreated or irradiated by 10 Gy. Cells were collected after 1 h and divided into two for preparations of whole cell lysates and histone extracts, which were subjected to immunoblotting as indicated. The expression of α-tubulin and H2A was analyzed for loading control. (B) 293T cells were transfected with indicated expression vectors, irradiated by 5 Gy and fixed after 1 h for dual stain with anti-Myc and anti-γ-H2AX antibodies in the presence of DAPI. Representative confocal images, capturing both transfected and non-transfected cells, are shown. (C) The average number of γ-H2AX foci per cells was obtained by counting at least 50 cells, both non-transfected (control) and transfected, in each experiments of (B). Error bar indicates mean ± s.d. of three independent experiments. (D) After transfection with indicated vectors, 293T cells were left untreated (0 Gy) or irradiated by 1-5 Gy and subjected to colony forming assays. (E) 293T cells, after transfection with indicated vectors, were subjected to biochemical fractionation using detergent, and soluble (S, cytoplasmic and soluble nuclear proteins) and insoluble (I, chromatin-bound proteins) fractions were analyzed by immunoblotting. GAPDH and H2A were used as indicators of S and I fractions, respectively.
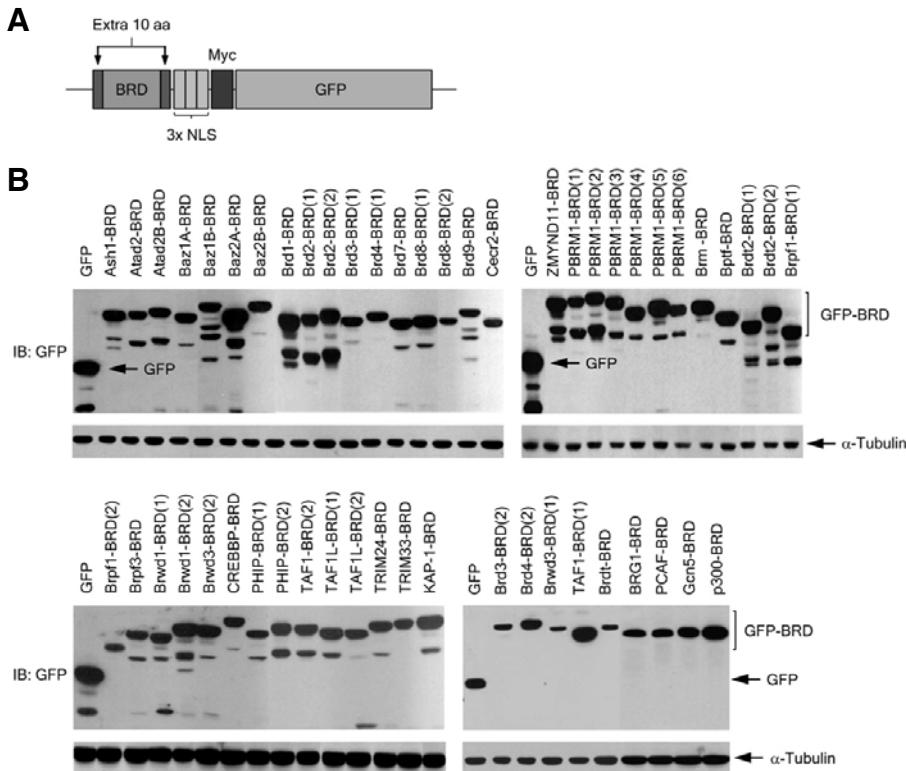
A

B

Fig. 2. Expression of 52 individual human BRDs in 293T cells. (A) The map of the plasmid vector expressing GFP-BRD is shown. Each of the 52 BRDs contains 10 flanking amino acids on either side and a NLS, nuclear localization signal. (B) 293T cells were transfected with vectors expressing GFP or each of the 52 GFP-BRD. Expression of the GFP proteins was analyzed by immunoblotting using anti-GFP and anti-α-tubulin (loading control) antibodies as indicated.
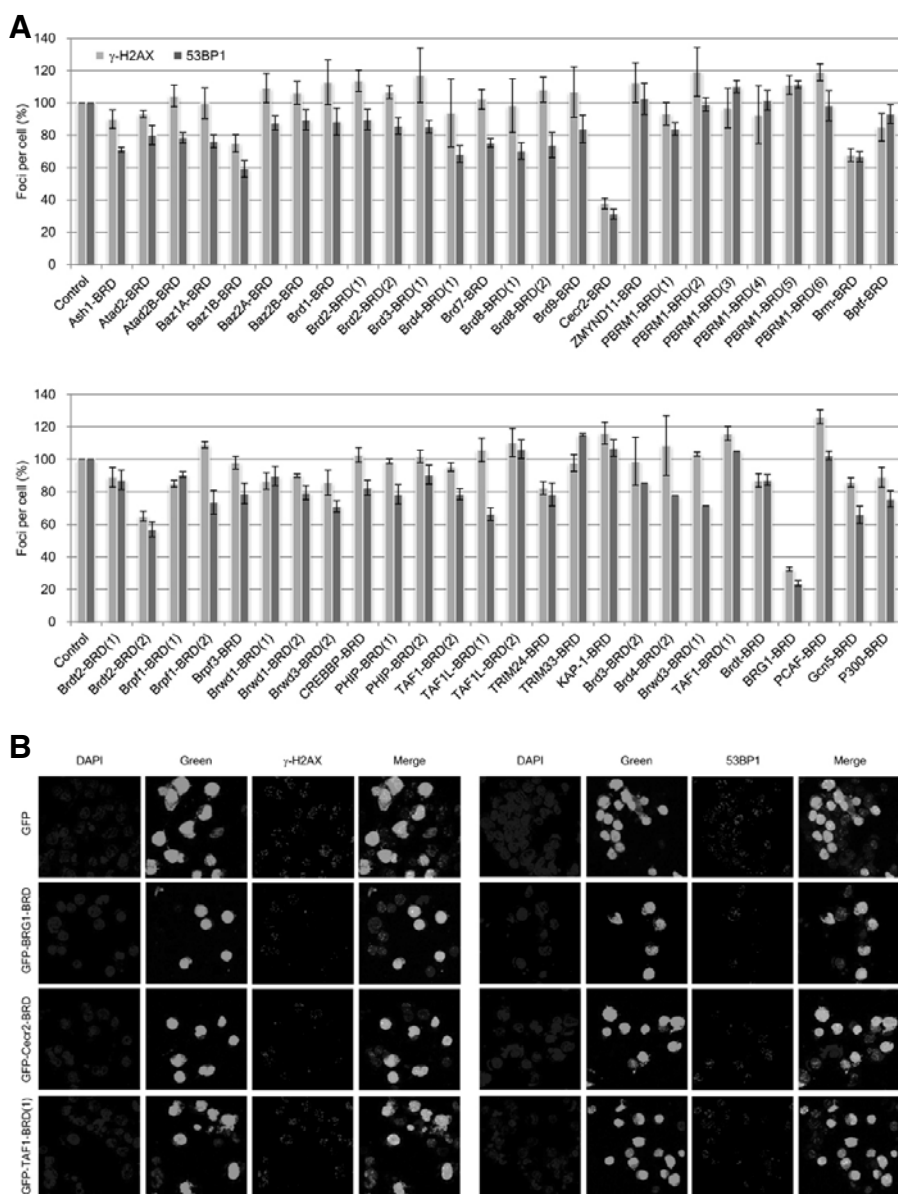
A

B

Fig. 3. Genome-wide screen for human BRDs having γ-H2AX inhibition activity. (A) 293T cells were transfected with vectors expressing the indicated GFP-BRDs, irradiated by 5 Gy and fixed after 1 h for dual stain with anti-γ-H2AX and anti-53BP1 antibodies before confocal images were captured. The average number of γ-H2AX and 53BP1 foci per cell was obtained by counting at least 200 GFP-positive cells, and data are presented as percentages relative to foci in cells expressing GFP only. Error bars indicate mean $\pm$ s.d. of three to six independent experiments. (B) Representative confocal images from the experiments described in (A) are shown.

Zhou, 2009). We were able to clone the sequences encoding each of the 52 BRDs present in 38 human BRD-containing proteins into a mammalian expression vector, which allows us to express each BRD linked to three copies of a nuclear localization signal and a Myc-tagged green fluorescence protein (GFP) (Fig. 2A and Supplementary Table S1). When these vectors were transfected into 293T cells, the 52 GFP-BRD proteins were all expressed efficiently, and their overall expression levels were similar with only minor variations (Fig. 2B).

We then determined the effects of each GFP-BRD on the formation of γ-H2AX foci after IR by immunofluorescence microscopy. To make the assay accurate, we also determined the effects of each GFP-BRD on 53BP1 foci formation, which is downstream of γ-H2AX and requires γ-H2AX for their own formation. Foci were only counted for GFP-positive cells to eliminate variations in transfection efficiency between samples. The

results are shown in Figs. 3A and 3B. As a positive control, BRG1 BRD showed approximately 76% inhibition of γ-H2AX compared to the GFP control. Among the tested BRDs, the BRD of cat eye syndrome chromosome region candidate 2 (Cecr2), which forms a novel chromatin remodeling complex with Snf2L (Banting et al., 2005), exhibited similar levels of γ-H2AX inhibition (approximately 69% inhibition) to BRG1 BRD. Three BRDs, the C-terminal BRD of Brdt2 (Brdt2-BRD(2)) and each BRD of Baz1B and Brm, showed moderate inhibition of γ-H2AX (between 25 and 45%). The remaining BRDs slightly inhibited or even stimulated γ-H2AX at the range of less than 20%. The effects of each BRD on 53BP1 foci correlated with the effects of each BRD on γ-H2AX inhibition, verifying the validity of our screening assay.
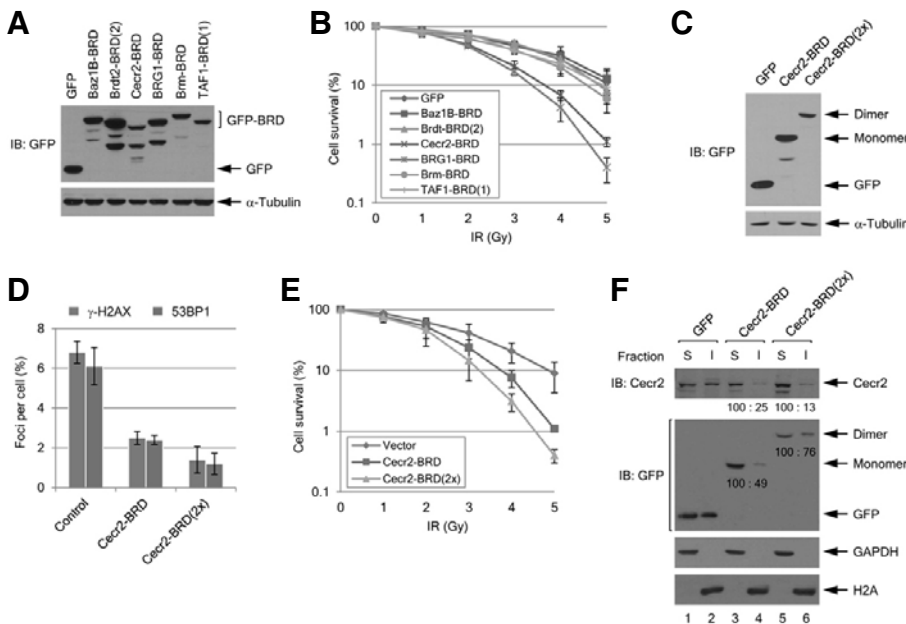
Fig. 4. Cecr2 BRD specifically inhibits γ-H2AX formation and cell survival after DNA damage. (A) The 293T cells were transfected with indicated expression vectors and whole cell lysates were subjected to immunoblotting. (B) The 293T cells, transfected as described in (A), were subjected to colony forming assays after irradiation by various doses of IR. Error bars indicate mean ± s.d. of three independent experiments. (C) 293T cells, transfected with indicated expression vectors, were divided into two groups 1 h after irradiation by 5 Gy. One group was used to prepare whole cell lysates for immunoblotting. (D) The other group of cells from (C) were fixed and dually stained by anti-γ-H2AX and anti-53BP1 antibodies. The average number of γ-H2AX and 53BP1 foci per cell was obtained by counting at least 60 GFP-positive cells and depicted as a graph. Error bars indicate mean ± s.d. of three independent experiments. Control indicates cells expressing GFP only. (E) The 293T cells transfected with indicated expression vectors and subjected to colony forming assays after irradiation by various doses of IR. Error bars indicate mean ± s.d. of three independent experiments. (F) The 293T cells were transfected with indicated expression vectors and subjected to detergent-mediated fractionation as described in Fig. 1E. Soluble and insoluble fractions were analyzed by immunoblotting. Relative band intensity of Cecr2 as well as monomer and dimer Cecr2 BRDs in S and I fractions were determined by densitometer and shown below corresponding bands.

Cecr2 BRD specifically inhibits γ-H2AX and cell survival after DNA damage

Having found that Cecr2 BRD has a strong γ-H2AX inhibition activity, we further investigated this BRD. First, we performed colony forming assays to determine whether Cecr2 BRD can inhibit cell survival following DNA damage. Included in this experiment were the three BRDs that exhibited a moderate γ-H2AX inhibition activity as well as BRG1 BRD and TAF1 BRD(1), which served as positive and negative controls, respectively. Both Cecr2 BRD and BRG1 BRD largely decreased cell survival after IR, whereas the three BRDs and TAF1 BRD(1) showed no significant effect on cell survival as compared to GFP alone (Figs. 4A and 4B). These results confirm the γ-H2AX inhibition activity of Cecr2 BRD and indicate that the moderate γ-H2AX inhibition activities of Baz1B BRD, Brdt2 BRD(2) and Brm BRD may have not been sufficient to harm cell survival after DNA damage.

Next, to ensure the specificity of Cecr2 BRD in γ-H2AX inhibition and cell survival after DNA damage, we generated an additional vector expressing two copies of Cecr2 BRD linked to GFP as previously described (Fig. 4C). We reasoned that the randomly repeating dimer form of Cecr2 BRD should increase chromatin binding when compared to Cecr2 BRD monomers because the dimer form is expected to have an increased affinity to acetylated histones (see below). When the chromatin binding of Cecr2 BRD dimers was compared to the chromatin binding of Cecr2 BRD monomers in 293T cells, the dimer form of Cecr2 BRD more effectively suppressed the formation of γ-H2AX and 53BP1 foci after IR as compared to the monomer (Fig. 4D). Consistently, cells expressing the dimer form of Cecr2 BRD were more sensitive to IR than those expressing the monomer form of Cecr2 BRD (Fig. 4E). As we predicted,

biochemical fractionation using detergent showed that the dimer form of Cecr2 BRD more effectively bound chromatin as compared with the monomer form (Fig. 4F, compare lanes 3 and 4 with lanes 5 and 6). Notably, both forms of Cecr2 BRD dissociated Cecr2 from the chromatin with the dimer showing increased activity compared with the monomer (Fig. 4F, compare lanes 3 and 4 with lanes 5 and 6), suggesting that Cecr2 BRD exerts the γ-H2AX inhibition activity by preventing Cecr2 from binding to chromatin. These results, showing a dose dependency of Cecr2 BRD in γ-H2AX inhibition and cell survival after DNA damage, suggest that the inhibition activities of Cecr2 BRD in these processes are specific.

Cecr2 is important for γ-H2AX formation and DSB repair

The results thus far raised the possibility that Cecr2 is involved in DDRs. To test this possibility, we depleted Cecr2 from 293T cells by transfection with plasmid vectors expressing three different small interfering RNAs (siRNAs), each targeting different sequences of Cecr2 (Fig. 5A). Strikingly, Cecr2 knockdown resulted in large defects in γ-H2AX and 53BP1 foci formation after IR (Figs. 5B and 5C). Neutral comet assays, which specifically measure DSBs, showed that Cecr2 knockdown largely decreased the efficiency of DSB repair (Figs. 5D and 5E), indicating that Cecr2 is important for DSB repair. Further supporting the importance of Cecr2 in DSB repair, siRNA knockdown of Cecr2 decreased cell survival after IR (Fig. 5F).

DISCUSSION

In the present study, we developed a novel strategy to identify BRD-containing DDR proteins that have a dominant-negative inhibitory effect on DDR function of this protein. We screened
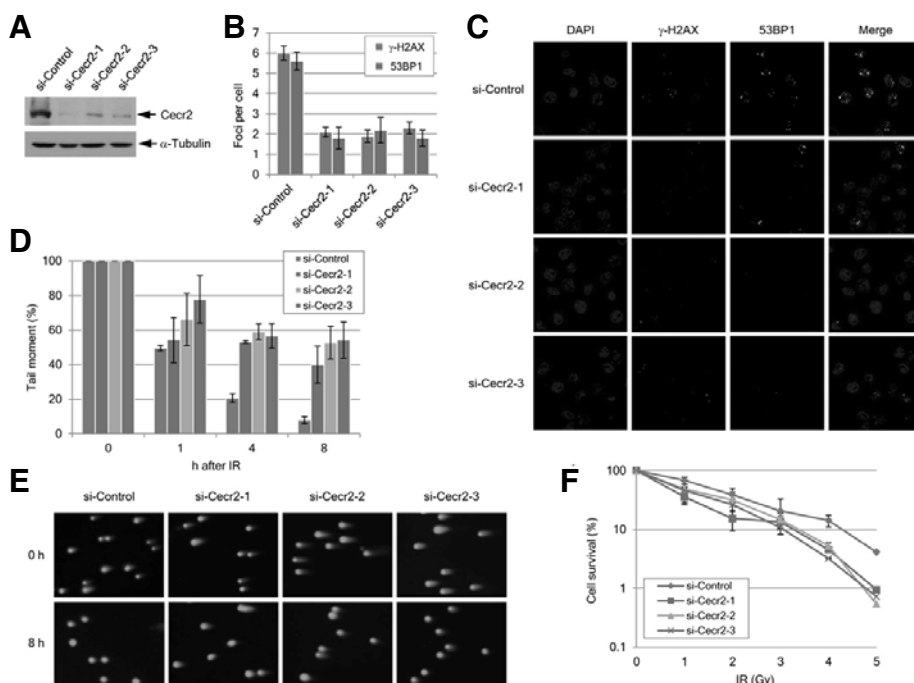
Fig. 5. Cecr2 is important for γ-H2AX formation and DSB repair. (A) The 293T cells were transfected with pSuper vectors expressing non-specific or three different Cecr2-specific siRNAs and divided into three groups. One group was used to analyze Cecr2 knockdown by immunoblotting. (B) The second group of cells was irradiated by 5 Gy and fixed for dual stain with anti-γ-H2AX and anti-53BP1 antibodies before confocal images were captured. The average number of γ-H2AX and 53BP1 foci per cell was obtained by counting at least 100 cells and depicted as a graph. Error bars indicate mean ± s.d. of three independent experiments. (C) Representative confocal images from the experiments in (B) are shown. (D) The last group of cells was irradiated by 20 Gy, collected immediately (0 h) or after various lengths of recovery time, and subjected to neutral comet assay to monitor progress of DSB repair. Average tail moments per cell, representing remaining unrepaired DSBs, were obtained by counting at least 300 comet images and depicted as graph. Error bars indicate mean ± s.d. of three independent experiments. (E) Representative comet images of the experiments in (C) are shown. (F) 293T cells were transfected as described in (A) and subjected to colony forming assays. Error bars indicate mean ± s.d. of three independent experiments.

52 individual BRDs, found in 38 human BRD-containing proteins and comprising 93% of all human BRDs, to identify BRDs that inhibit the formation of γ-H2AX following DNA damage. We were able to select the BRD of Cecr2 as a single prominent candidate that has a strong γ-H2AX inhibition activity and confirmed that this activity of Cecr2 BRD is specific. Importantly, Cecr2 knockdown resulted in defective γ-H2AX and DSB repair, demonstrating that Cecr2 is important for these processes. Our work therefore identified Cecr2 as a novel DDR protein.

The Cecr2 gene encodes a polypeptide of 1484 amino acids containing a single BRD, and it was originally identified as a putative gene present in the chromosome 22q11 region that was duplicated in the human disease cat eye syndrome (Footz et al., 2001). Studies have shown that Cecr2 forms a complex with either SNF2L or SNF2H, two members of the ISWI family of ATP-dependent chromatin remodeling enzymes, and plays a role in neurulation and inner ear development during embryogenesis and spermatogenesis in adult tissues, respectively (Banting et al., 2005; Dawe et al., 2011; Fairbridge et al., 2010; Thompson et al., 2012). Although Cecr2 is thought to function as a transcription factor in the form of chromatin remodeling complex, its exact cellular functions is unknown. In this paper, our work unexpectedly discovered the involvement of Cecr2 in DSB repair. We currently do not know whether Cecr2 contributes to DSB repair by direct mechanisms or indirectly via transcription. Our data, showing that Cecr2 BRD dissociates Cecr2 from chromatin (Fig. 4F), suggest that Cecr2 BRD exerts its γ-H2AX inhibition activity by preventing Cecr2 from binding to chromatin. Thus, Cecr2 may directly participate in stimulating γ-H2AX formation in a manner similar to BRG1. The mechanisms by which Cecr2 stimulates γ-H2AX and DSB repair remain to

be investigated.

BRD is known to bind acetyl-Lys, in a sequence specific manner, with selectivity for different sites in histones (Mujtaba et al., 2007; Taverna et al., 2007; Zhang et al., 2010), which serves as the logical basis of our screening strategy. For example, in combination with peptide binding and mutagenesis analyses, the structural studies have shown that BRG1 BRD uses the H3 sequences, containing acetyl-Lys at the position 14, as a dominant substrate over acetyl-Lys at different positions or in different histones (Shen et al., 2007; Singh et al., 2007). Therefore, it is expected that Cecr2 exerts its DDR function by binding to specific sequences of acetylated histone using its BRD. In this regard, it will be of great interest to identify the acetyl-Lys in histones that Cecr2 BRD specifically recognizes. Such histone acetylation is expected to play an important role in the function of Cecr2 in DDRs.

Because our screening strategy proved to be successful in identifying a novel BRD-containing DDR protein, this strategy can be used to search for DDR proteins that contain chromatin-binding modules recognizing other types of histone modifications, such as methylation. The protein modules recognizing methyl-Lys in histones include chromodomains, tudor domains and PHD fingers (Bienz, 2006; Martin and Zhang, 2005). Several proteins containing one or more of these modules have been shown to be involved in DDRs. For example, 53BP1, involved in DNA damage checkpoint activation, binds to methyl-Lys-79 of H3 at DSBs using its tudor domain (Huyen et al., 2004). Therefore, it will be of interest to determine whether applying our screening strategy to these methyl-Lys binding domains would give rise to the discovery of novel DDR proteins. Furthermore, our strategy is not necessarily limited to the

screening of DDR proteins because it could be used to identify chromatin binding proteins that function in other biological processes, such as cell division and proliferation. Supporting this idea is our observation that some of the BRDs that we analyzed in this study exhibit the ability to suppress cell proliferation under normal culture conditions.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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