Targeting BRG1 Chromatin Remodeler via Its Bromodomain for Enhanced Tumor Cell Radiosensitivity In Vitro and In Vivo

Su-Jung Kwon, Seul-Ki Lee, Juri Na, Shin-Ai Lee, Han-Sae Lee, Ji-Hye Park, June-Key Chung, Hyewon Youn, and Jongbum Kwon

Abstract

Radiotherapy treats cancer by inducing DNA double-strand breaks (DSB) in tumor cells using ionizing radiation. However, DNA repair in tumor cells often leads to radioresistance and unsuccessful outcome. Inhibition of DNA repair by targeting repair proteins can increase radiosensitivity of tumor cells. The BRG1 chromatin remodeling enzyme assists DSB repair by stimulating γ-H2AX and DSB repair after irradiation and increased the radiosensitivity in various human cancer cells, including HT29 colon cancer. Dimerization of BRG1-BRD, increasing its chromatin binding affinity, aggravated the defects in γ-H2AX and DSB repair and further enhanced the radiosensitivity. While little affecting the upstream ATM activation, BRG1-BRD in irradiated HT29 cells inhibited the recruitment of 53BP1 to damaged chromatin, the downstream event of γ-H2AX, and compromised the G2–M checkpoint and increased apoptosis. Importantly, in a xenograft mouse model, BRG1-BRD increased the radiosensitivity of HT29 tumors, which was further enhanced by dimerization. These data suggest that BRG1-BRD radiosensitizes tumor cells by a dominant negative activity against BRG1, which disrupts γ-H2AX and its downstream 53BP1 pathways, leading to inefficient DNA repair, G2–M checkpoint defect, and increased apoptosis. This work therefore identifies BRG1-BRD as a novel tumor radiosensitizer and its action mechanism, providing the first example of chromatin remodeler as a target for improving cancer radiotherapy.

Introduction

Radiotherapy, one of the major approaches of treating cancer, uses ionizing radiation (IR) to kill tumor cells by inducing DNA double-strand breaks (DSB), the most lethal DNA damage. The cancer killing activity of radiotherapy relies on the sustained presence of DSBs that directs the cytotoxic activity on tumor cells. However, DNA repair in tumor cells often leads to radioresistance and unsuccessful treatment. Blockade of DSB repair through targeting repair proteins can sensitize tumor cells to IR and increase radiotherapy efficacy. Therefore, identification of novel targets to sensitize tumor cells to IR will provide a window for the development of novel sensitizers for cancer radiotherapy (1–3).

In response to DSBs, cells activate the so-called DNA damage response (DDR), a complex signaling pathway that activates the DNA damage checkpoint, halts cell cycle, and directs DNA repair in a highly coordinated fashion. In case DSBs are left unrepaird, cells undergo apoptosis to prevent their damaged genome from being transmitted to offspring. DDR is initiated by the activation of the PI3K-like kinase ATM, which leads to recruitment of many downstream proteins, such as MDC1 and 53BP1, to DSB sites through a DDR signaling cascade. γ-H2AX (phosphorylated form of histone H2AX), which is generated by ATM, plays a central role in recruiting DDR proteins and thereby forming the so-called IR-induced foci or repair foci in the nucleus. The formation of repair foci following DSB generation is critical for DNA damage checkpoint activation, damage repair, and cell survival (4–6).

The packaging of the eukaryotic genome into nucleosomes and higher order chromatin structure presents a barrier to protein access to target DNA and chromatin modifications therefore play a pivotal role in the DNA-templated nuclear processes such as DNA repair (7, 8). ATP-dependent chromatin remodeling is a prominent mechanism for chromatin modification and is mediated by the multiprotein remodeling complexes that use ATP hydrolysis energy to slide, evict, or restructure nucleosomes. Recent studies have shown that the chromatin remodeling complexes such as SWI/SNF and INO80 are involved in DDR (9–14).

We previously have shown that BRG1, the catalytic ATPase of the SWI/SNF complex (15), assists DSB repair by stimulating the...
formation of γ-H2AX and prevents the cell death via apoptosis after DNA damage (12, 16). The subsequent study has revealed that the so-called cooperative activation loop mechanism is responsible for the BRG1 stimulation of γ-H2AX (17). Briefly, BRG1 binds to γ-H2AX–containing nucleosomes by interacting with acetylated H3 using bromodomain (BRD), the protein module that recognizes acetyl-Lys moieties (18), and stimulates ATM-mediated γ-H2AX formation through nucleosome remodeling activity. The γ-H2AX then recruits GCN5 histone acetyltransferase to increase H3 acetylation, which leads to further recruitment of BRG1 to γ-H2AX nucleosomes. This cooperative action of BRG1, γ-H2AX, and acetylated H3 provides an efficient way to accumulate γ-H2AX and acetylated H3 within the sites of DSB. Given the role for BRG1 in DSBR repair, we sought to investigate whether BRG1 could be a target for enhancing the response to radiotherapy in cancer treatment.

Materials and Methods

Cell lines and antibodies

The three cancer cell lines, HT29 (ATCC HTB-38), MDA-MB-231 (ATCC HTB-26), and A549 (ATCC CCL-185) were purchased from ATCC and cultured in McCoy’s 5A medium (ATCC HTB-26), and A549 (ATCC CCL-185) were purchased from EMD Calbiochem; anti-p-p53 and anti-p-p53 from AbFrontier; anti-ATM and anti-p21 antibodies from EMD Millipore; anti-GAPDH antibody from Cell Signaling Technology; anti-p16 antibody from Santa Cruz Biototechnology; anti-γ-H2AX, anti-H2A, and anti-p-ATM(S1981) antibodies from EMD Millipore; anti-GAPDH antibody from AbFrontier; anti-ATM and anti-p21 antibodies from EMD Calbiochem; anti-p-CHK1(S345) and anti-p-p53 (S15) from Cell Signaling Technology; anti-p16 antibody from Abcam; and anti-myc antibody (mouse IgG) from Enzo Life Sciences.

Vector construction

The pCMV/myc/nuc (Invitrogen)-based plasmid vector-expressing BRD(m) has been described previously (17). The expression vector for BRD(d) was generated by cloning the blunt-end ligation product of the PCR-amplified BRG1-BRD sequences into pCMV/myc/nuc. The retroviral vector-expressing BRD(m) and BRD(d) were generated by cloning the PCR products, amplified from pCMV/myc/nuc-BRG1-BRD(m) and pCMV/myc/nuc-BRG1-BRD(d), respectively, into the BamHI-NotI sites of the pMX-puro vector such that the encoded proteins contained three copies of BRD(d), respectively, into the BamHI-NotI sites of the pMX-puro vector for BRD(d) was generated by cloning the blunt-end ligation vector such that the encoded proteins contained three copies of

Transfection and viral infection

Plasmid DNA transfection was performed using the calcium phosphate method. For retroviral infection, the pMX vectors were transfected into Plat-E cells with polybrene 10 μg/mL for 24 hours at 37°C. The pMX vectors were maintained in 0.4 μg/mL of puromycin. The HT29-luc clones harboring empty vector or expressing Myc-BRD (m) or Myc-BRD(d) were established by puromycin selection of single clones following infection with the corresponding pMX-puro viruses.

Counting repair foci

The immunofluorescence microscopy for γ-H2AX, p-ATM, MDC1, and 53BP1 foci measurement was performed as previously described (19, 20). For the sake of convenience and consistency, a single optical section of confocal images was captured instead of capturing multiple sections, and only foci with certain size and brightness which were considered to reside on a same plane were counted. This method gave a consistent and quantitative result that is well correlated with that obtained by whole foci counting.

Senescence-associated β-galactosidase assay

HT29 cells were washed three times with PBS and fixed in 4% paraformaldehyde for 5 minutes. After wash with PBS three times, the cells were incubated with freshly made β-galactosidase staining solution [1 mg/mL of 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal), 150 mmol/L sodium chloride, 5 mmol/L potassium ferricyanide, 5 mmol/L potassium ferrocyanide, 2 mmol/L magnesium chloride] for 24 hours at 37°C (without CO2). The staining solution was titrated with 0.1 mol/L NaPi buffer to pH 6.0, and the stained cells were observed under microscope (Zeiss).

Tumor implantation and IR exposure

HT29-luc cells (3 × 10⁶) were subcutaneously injected with 40 μL of Matrigel to dorsal part of male BALB/c nu/nu nude mice. After 7 days, the grafted tumors were exposed to 9-Gy IR with high-energy X-ray Linear Accelerator (Clinac 4/100, Varian Medical Systems). All animal experiments described in this study were performed under approval from the Seoul National University Institutional Animal Care and Use Committee (Seoul, Korea).

In vivo bioluminescence imaging

IVIS was performed using the IVIS 100 imaging system with a charge-coupled device camera (Caliper Life Sciences). The mice were kept on the imaging stage under anesthesia with 1.5% isoflurane gas in oxygen at a flow rate of 1.5 L/minute. D-luciferin (Molecular Probes, Invitrogen) was given 150 mg/kg body weight by intraperitoneal injection and bioluminescence signals were collected at 10 to 30 minutes with maximum intensity. The mice were positioned prone for in vivo bioluminescence image of xenograft tumors.

Analysis of in vivo bioluminescence data

The signal intensities of emitted light from xenograft tumors were presented as pseudocolor images ranging from red (maximum) to blue (minimum) based on their number. Gray-scale photographs and corresponding pseudocolor images were superimposed with XENON image analysis software. Signals emitted by regions of interest (ROI) were measured and data were expressed as photon fluence (photon/s/cm²/steradian), which refers to the photons emitted from a unit solid angle of a sphere. The background signal intensity was subtracted electronically for normalization both from the images and from the measurements of photon flux. Relative intensities of ROI were calculated and plotted with the mean ± SEM.

Measurement of tumor volume by caliper

The length and width of tumors were measured by a caliper before bioluminescence imaging analysis and the tumor volumes
were calculated according to the method by Tomayko and Reynolds (tumor volume = 1/2(length × width²; ref. 21).

Statistical analysis
The significance of differences between measurements was evaluated by Student t test using Microsoft Excel. A P value <0.05 was deemed to indicate statistical significance. IC₅₀ was calculated using SigmaPlot 8.0 software.

Other methods
Clonogenic assays, biochemical fractionation experiments, immunoblot analysis, neutral comet assay, acid extraction and immunoblot analysis of histones, and IHC of paraffin-embedded frozen tissue were performed as previously described (17, 19, 22). Propidium iodide (PI) and Annexin V staining for FACS analysis was performed as previously described (16).

Results
BRG1-BRD inhibits DSB repair via blockade of BRG1 chromatin binding
Consistent with the cooperative activation loop model, ectopic expression of BRG1-BRD inhibits γ-H2AX formation and DSB repair in 293T human embryonic kidney cells (17). We reasoned that BRG1-BRD exerts the DNA repair inhibitory activity through a dominant negative function to compete against BRG1 in binding the acetylated histones on γ-H2AX nucleosomes (Supplementary Fig. S1). To test this hypothesis, we expressed Myc-tagged BRG1-BRD (designated as BRD(m); m for monomer; Fig. 1A) in 293T cells and separated chromatin-bound and -unbound fractions by biochemical fractionation. BRD(m) expression (Fig. 1B, lane 2) resulted in a decrease of BRG1 level in the chromatin-bound fraction (Fig. 1C, compare lanes 1 and 2 with lanes 3 and 4), showing that BRG1-BRD indeed inhibits BRG1 binding to chromatin.

As dimerization increases chromatin binding affinity of the BRD of CECR2 (19), we tested whether such effect also would be seen for BRG1-BRD. We generated the plasmid vector-expressing tandemly linked dimeric Myc-tagged BRG1-BRD (designated as BRD(d); d for dimer; Fig. 1A). When this vector was transfected into 293T cells, BRD(d) was expressed at the similar level as BRD(m; Fig. 1B). BRD(d) bound to chromatin with a higher affinity and inhibited BRG1 chromatin binding more strongly than BRD(m; Fig. 1C, compare lanes 3 and 4 with lanes 5 and 6). These results led us to test whether BRD(d) would have stronger inhibitory activity against γ-H2AX than BRD(m). Indeed, BRD(d) inhibited the γ-H2AX induction after irradiation more effectively than BRD(m; Fig. 1D–F). In addition, BRD(m) inhibited DSB repair and BRD(d) showed a stronger effect than BRD(m; Fig. 1G and H). Consistent with these results, the cells expressing BRD(d) showed higher sensitivity to irradiation than the cells expressing BRD(m; Fig. 1I). The results thus far collectively suggest that BRG1-BRD inhibits DSB repair through direct blockade of BRG1 chromatin binding and this activity of BRG1-BRD is enhanced by increasing its chromatin binding affinity by dimerization.

BRG1-BRD radiosensitizes various human cancer cells, including HT29, by inhibiting γ-H2AX and DSB repair
Next, we investigated whether BRG1-BRD radiosensitizes human cancer cells. We selected the three cancer cell lines, HT29, MDA-MB-231, and A549, typically used for studying the radioresistance problem, as representatives of colon, breast, and lung tumors, respectively. All these cells expressed BRG1 at the compatible levels with 293T cells (Fig. 2A), making them suitable for our study. When the cancer cells were infected with the retroviral vectors expressing BRD(m) or BRD(d), the two proteins were expressed at the similar levels (Fig. 2B and Supplementary Fig. S2A and B). BRD(m) expression increased the sensitivity of the cancer cells to irradiation and the magnitudes of the sensitizing effects were similar among the three cancer types; the ED50s were reduced by 13% to 15% with the dose enhancement factors (DEF) being 1.15 to 1.17 as compared with empty vector (Fig. 2C and Supplementary Fig. S2C and S2D). Notably, BRD(d) further increased the radiosensitivity of the cancer cells by the significant levels beyond those obtained by BRD(m); the ED50s were reduced by approximately 22% to 30% with the DEIs being 1.28 to 1.44 as compared with empty vector (Fig. 2C and Supplementary Fig. S2C and S2D). Therefore, BRG1-BRD has a radiosensitizing activity on human cancer cells and this activity of BRG1-BRD is enhanced by dimerization.

To determine whether the increased radiosensitivity of the cancer cells expressing BRG1-BRD was attributed to defective DSB repair, we conducted the series of the similar experiments as previously described for 293T cells. First, we examined HT29 cells. BRG1-BRD inhibited BRG1 binding to chromatin (Fig. 2D, lanes 3 and 4) and this activity of BRG1-BRD was increased by dimerization (Fig. 2D, lanes 5 and 6). The monomeric and dimeric BRG1-BRDs increasingly inhibited the γ-H2AX formation (Fig. 2E–G) and DSB repair after irradiation (Fig. 2H and I). When we examined MDA-MB-231 and A549 cells with respect to the effects of BRD(m) and BRD(d) on γ-H2AX and DSB repair, we obtained the similar results (Supplementary Figs. S3A–S3E and S4A–S4E). The viability of 293T and the three cancer cells was not significantly affected by the expression of BRD(d) in the absence of irradiation (Supplementary Fig. S5A and S5B), indicating that the radiosensitizing activity of BRG1-BRD is specific to DNA damage. All these data suggest that BRG1-BRD increases the radiosensitivity of cancer cells by inhibiting DSB repair and the radiosensitizing activity of BRG1-BRD can be enhanced by increasing its chromatin binding affinity via dimerization.

BRG1-BRD in irradiated HT29 cells inhibits 53BP1 recruitment to DSB sites without affecting ATM, CHK2, and p53 activations
We then investigated what other aspects of DDR than γ-H2AX BRG1-BRD affect in cancer cells. We addressed this issue by focusing on HT29 cells using BRD(d). ATM phosphorylation at Ser-1981, indicative of the activation state (23), increased after irradiation in both control and BRD(d)-transfected cells (Fig. 3A), and phospho-ATM (Fig. 3B and C) and MDC1 foci (Fig. 3D and E), which promote each other in a feedback activation process, normally formed in the irradiated BRD(d)-expressing cells, indicating that BRG1-BRD has no effect on ATM activation and recruitment to damaged chromatin in HT29 cells. Consistent with these results, ATM-mediated Ser-15 phosphorylation and stabilization of p53 normally occurred in the irradiated HT29 cells regardless of BRD(d) expression (Fig. 3A). Unlike in normal cells, the activated p53 in these cells was sustained throughout the time course analyzed up to 24 hours (data not shown).

Checkpoint kinase 2 (CHK2) is one of the major targets of ATM during DDR and its activation is initiated by phosphorylation at Thr-68, leading the G2–M cell-cycle arrest and in some times DNA damage-induced apoptosis (DDIA; ref. 24). CHK2 activation after
Figure 1.

BRG1-BRD inhibits DSB repair via blockade of BRG1 chromatin binding. A, map of BRG1, monomeric (m), and dimeric (d) BRG1-BRDs. The helicase/SANT-associated (HSA) and ATPase domains of BRG1 are also indicated. B, after transfection with the plasmid vectors expressing no protein, Myc-tagged BRD(m) or BRD(d), 293T cells were subjected to immunoblotting. C, after transfection as in B, 293T cells were subjected to detergent fractionation to separate chromatin-unbound (U) and chromatin-bound fractions (B) followed by immunoblotting. The expression of GAPDH and H2A was analyzed as markers of the U and B fractions, respectively. The BRG1 and BRG1-BRD bands were quantitated by a densitometer and the relative distribution of the proteins between the two fractions are shown. D, after transfection as in B, 293T cells were collected before and 1 hour after irradiation (10 Gy). The whole-cell lysates (WCL) and histone extracts were analyzed for the expression of Myc-tagged proteins and γ-H2AX by immunoblotting, respectively. E, after transfection as in B, 293T cells were fixed before and 1 hour after irradiation (5 Gy) to determine γ-H2AX foci by immunofluorescence microscopy. Average number of γ-H2AX foci per cell was obtained by counting at least 100 cells. Data, mean ± SD of three independent experiments. F, representative confocal images for E. G, 293T cells transfected as in B were harvested immediately (0 hour), 2, 6, or 12 hours after irradiation (20 Gy) before being subjected to neutral comet assay, specifically detecting DSBs. The average tail moments were obtained by measuring at least 300 cells and graphed by setting the value at 0 hour as 100%. Data, mean ± SD of three independent experiments. H, representative comet images for G. I, 293T cells transfected as in B were exposed to various doses of IR and subjected to clonogenic assay. EDS0s and DEFs are summarized in the table. Data, mean ± SD of three independent experiments performed in triplicate. *P for the comparisons of vector with BRD(m) and BRD(m) with BRD(d). *P < 0.05; **P < 0.01; ***P < 0.001.
irradiation normally occurred in both control and BRD(d)-transfected HT29 cells (Fig. 3F). CHK1, the kinase that is phosphorylated by both ATM and the related kinase ATR after DNA damage, was also activated in the irradiated HT29 cells regardless of BRD(d) expression (Fig. 3F). Therefore, BRG1-BRD does not affect the activation of CHK1/2 after DNA damage in HT29 cells. Given that BRG1-BRD has no effect on the upstream pathway of γ-H2AX, we checked whether it inhibits 53BP1 recruitment to DSB sites, the downstream event of γ-H2AX. Indeed, the formation of 53BP1 foci after irradiation was greatly reduced by BRD(d) expression (Fig. 3G and H). In conclusion, BRG1-BRD in irradiated HT29 cells inhibits γ-H2AX formation and 53BP1 recruitment to damaged chromatin without affecting the ATM-CHK2 pathway or p53 activation.

BRG1-BRD in irradiated HT29 cells compromises G2-M checkpoint and increases apoptosis

The previous study showed that inefficient DNA repair in BRG1 knockdown cells leads to defective G2-M checkpoint and increased apoptosis in mouse fibroblast cells (16). We examined whether BRG1-BRD expression would have such effects on HT29 cells. In the absence of irradiation, the BRD(d)-transfected cells showed a normal cell-cycle profile similar to the control cells (Fig. 4A). After irradiation, both cells showed G2-M arrest, however, the extent of...
the arrest was approximately 23% less in the BRD(d)-transfected cells compared with the control cells (Fig. 4A), indicating that BRG1-BRD compromised the G2→M checkpoint after DNA damage. Next, we determined the impact of BRG1-BRD on DDIA. Although the control and BRD(d)-transfected cells showed a similar level of basal apoptosis before irradiation, apoptosis largely increased after irradiation with the levels approximately two times higher in the BRD(d)-transfected cells than the control cells (Fig. 4B), indicating that BRG1-BRD increased DDIA in HT29 cells.

As senescence is another important mechanism to stop cell proliferation after DNA damage (25), we investigated whether BRG1-BRD influences senescence of HT29 cells after irradiation. The control and BRD(d)-transfected cells were compared before and after irradiation for the β-galactosidase activity, the specific marker for senescent cells. In the both cells, senescence was detected at low levels before irradiation, which remained almost unchanged until 3 days and highly increased at day 4 after irradiation. There was no difference in the levels of senescence
between the two cell types in any conditions (Fig. 4C and D and data not shown). Consistent with these results, the senescence markers, such as p53, p21, and p16, were detected at similar levels from the control and BRD(d)-transfected cells at 2 days after irradiation (Fig. 4E). These data show that BRG1-BRD has little effect on the senescence of HT29 cells after DNA damage.

BRG1-BRD radiosensitizes HT29 tumors in vivo

Having found the radiosensitizing activity of BRG1-BRD on cancer cells in vitro, we determined whether it functions in vivo. To this end, we used mouse xenograft cancer model combined with the in vivo bioluminescence imaging system (IVIS) that permits to visualize and quantify growth of transplanted tumors without sacrificing animals (26, 27). We established the HT29-luc cells stably expressing the luciferase gene. The HT29-luc cells were further engineered by viral infection to generate the clones harboring empty vector or expressing BRD(m) or BRD(d; Fig. 5A), which showed the increased radiosensitivity depending on monomeric and dimeric BRG1-BRDs like their parental HT29 cells (data not shown).
We transplanted each of the three types of the aforementioned HT29-luc cells in the athymic nude mice and allowed the cells to grow for 7 days to form tumors with a certain size. The tumors were then irradiated by 9 Gy and their growth was monitored by IVIS at various times up to 28 days (Fig. 5B). The tumor growth was also determined by measuring the actual tumor volumes by caliper to confirm the results by IVIS. The three HT29 tumors were not significantly different in size 7 days after transplantation (day 0, before irradiation; Fig. 5C and D), indicating that BRG1-BRD did not affect the growth of untreated tumors. After irradiation, the tumors harboring empty vector continued to grow until day 28 by average 34-fold (by IVIS) or 19-folds (by volume; Fig. 5C, E and F). The tumors expressing BRD(m) also continually grew until day 28, however, the growth of these tumors increased by average only about 14-fold (by IVIS) or 7-folds (by volume; Fig. 5C, E and F), indicating that the BRD(m) expression largely retarded the tumor growth after irradiation. Notably, the tumors expressing BRD(d) showed approximately three (by IVIS) or four times (by volume) less growth than the tumors expressing BRD(m) on day 28 (Fig. 5C, E and F). At necropsy, Myc-tagged proteins were detected in the nuclei of the tumor cells expressing BRD(m) or BRD(d) but not the tumor cells harboring vector (Supplementary Fig. S6), confirming that the BRG1-BRD expression was sustained until day 28. These results show that BRG1-BRD sensitizes HT29 tumors to radiotherapy in vivo and
BRG1-BRD enhances the sensitivity of HT29 cells to DSB-generating chemotherapeutic drugs

Finally, we wondered whether BRG1-BRD would also sensitize cancer cells to chemotherapeutic drugs. We tested three commonly used chemical drugs, doxorubicin, etoposide, and cisplatin. We observed that BRG1-BRD enhanced the sensitivity of HT29 cells to doxorubicin and etoposide, albeit moderately but reproducibly, but not to cisplatin (Fig. 6A). Considering that doxorubicin and etoposide generate DSBs like IR, whereas cisplatin induces DNA strand cross-link, the sensitizing activity of BRG1-BRD on HT29 cells may be specific to the damage of DSB generation.

Discussion

Our previous studies have shown that BRG1 assists DSB repair by stimulating γ-H2AX formation and BRG1 binding to acetylated histones on DSB-surrounding chromatin via BRD is critical for this activity. On the basis of these findings, we hypothesized that BRG1-BRD may sensitize tumor cells to radiotherapy through a dominant negative activity against BRG1. In the present study, using clonogenic assay as well as xenograft mouse cancer model, dominant negative activity against BRG1. In the present study, BRG1-BRD has no effect on ATM/MDC activation and recruitment to DSB sites or activation of the ATM targets Chk2 and p53. Instead, BRG1-BRD inhibits the formation of γ-H2AX and its downstream event of 53BP1 recruitment to damaged chromatin, which leads to G2–M checkpoint defect and inefficient DNA repair probably due to a failure to recruit additional downstream DDR proteins, eventually resulting in increased apoptosis.

Recent studies have highlighted the importance of histone modifications and chromatin remodeling in DNA repair, suggesting that the proteins responsible for such epigenetic modifications could be targets for improving cancer radiotherapy. It has been known that compounds that inhibit the activity of histone deacetylases (HDAC), which are responsible for removing acetyl groups from lysine residues on histones, enhance radiosensitivity of cancer cells in vitro and xenograft tumors in vivo (29, 30). Although the exact mechanisms by which HDAC inhibitors enhance cancer cell killing are difficult to define because of a wide range of HDAC downstream targets involved in numerous cellular processes, there are evidence suggesting that HDAC inhibitors exert the cell killing activity via modulation of DNA repair. For example, the HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA) cause a delay in downregulation of γ-H2AX after induction by irradiation, indicative of defective DSB repair (31–34). Our work, validating BRG1 as a target for cancer radiosensitization, therefore further substantiates the feasibility of the approaches to enhance cancer radiotherapy by modulating the epigenetic control of DNA repair.

Our results, showing that the chromatin-binding affinity of BRG1-BRD is parallel with the inhibitory activity against BRG1 chromatin binding, suggest that BRG1-BRD blocks BRG1 chromatin binding directly and specifically. In this regard, it will be of theoretical importance to develop BRG1-BRD as an anticancer agent.
interest to test whether higher-order multimerization (more than dimer) would even further enhance the radiosensitizing activity of BRG1-BRD. These results also emphasize that tumor cell radiosensitivity can be achieved by blocking a specific step of the BRG1-assisted DSB repair process without depleting or inactivating the BRG1 protein itself. This strategy may likely provide better opportunity to inhibit DNA repair with minimal effects on the BRG1’s other cellular functions such as transcription and cell-cycle control (15).

Although BRG1-BRD itself could be used as a radiosensitizer for cancer treatment provided with an appropriate gene delivery system, small-molecule drugs that specifically inhibit the interaction between BRG1-BRD and acetylated histone would be more useful for clinical applications. It has been known that developing small-molecule drugs that target protein–protein interactions is extremely difficult in general because their interacting surfaces are usually wide and shallow (35). However, all the BRD modules, including BRG1-BRD, share a conserved structural feature in that they have a central deep and narrow hydrophobic cavity and recognize acetyl-Lys in a sequence-dependent manner using this pocket (36–38), suggesting the possibility to design small-molecule inhibitors of certain BRDs. Indeed, recent studies have reported the small-molecule compounds called JQ1 and I-BET that specifically fit into the hydrophobic pocket of the BRD of bromodomain and extra-terminal (BET) family proteins and function as potent inhibitors against the cellular function of these proteins (39–41). Thus, it may be likely possible to develop small-molecule inhibitors that specifically target BRG1-BRD (42, 43). In this regard, our work provides the experimental basis for the validity of developing specific BRG1-BRD inhibitors as radiosensitizers for cancer treatment.

In summary, we have established the BRD of BRG1 chromatin remodeling enzyme as a novel radiosensitizer for human tumor cells. To our knowledge, this is the first evidence that ATPase chromatin remodeler can be a target for improving cancer radiotherapy. In addition, our strategy to use the dominant negativity of BRG1-BRD as a means to radiosensitize tumor cells is based on the defined molecular mechanisms by which BRG1 assists DSB repair, which therefore provides an excellent example for the mechanism-based approaches to cancer radiotherapeutics. It should be noted, however, that, as the radiosensitizing activity of BRG1-BRD on cancer cells is not robust, rational combination therapy with commonly used chemotherapeutic drugs would promise more efficient cancer treatment. Further investigations will allow one to fully understand how BRG1-BRD radiosensitizes tumor cells and help to maximize its sensitizing effects, which may eventually lead to improvements of the therapeutic outcome for cancers that are difficult to control due to radioresistance.

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

Authors’ Contributions
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.-J. Kwon, S.-K. Lee, J. Na, S.-A. Lee, H. Youn
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.-J. Kwon, S. K. Lee, J. Na, H. Youn, J. Kwon
Writing, review, and/or revision of the manuscript: J. Na, S.-A. Lee, H. Youn, J. Kwon
Study supervision: H. Youn, J. Kwon

Grant Support
S.-J. Kwon, S.-K. Lee, S.-A. Lee, H.-S. Lee, J.-H. Park, and J. Kwon were supported by the grants of the Korean Health Technology R&D Project (A101709) and the National Research Foundation (NRF) of Korea (2012R1A2A2A01003744). S.-J. Kwon, S. K. Lee, H.-S. Lee, and J. Kwon were also supported by NRF-2012R1A5A1048236, J. Na, J. K. Chung, and H. Youn were supported by NRF-2011-0030680. J.-H. Park was also supported by RPF Grant 2009 of Ewha Womans University.

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Received May 2, 2014; revised November 17, 2014; accepted November 19, 2014; published OnlineFirst December 12, 2014.


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Su-Jung Kwon, Seul-Ki Lee, Juri Na, et al.