International Journal of Radiation Oncology biology • physics

www.redjournal.org

Biology Contribution

# Identification and Characterization of a Small Inhibitory Peptide That Can Target DNA-PKcs Autophosphorylation and Increase Tumor Radiosensitivity

**Xiaonan Sun, MD, PhD,[*,*] Chunying Yang, PhD,[†,*] Hai Liu, PhD,[*] Qi Wang, MSc,[*] Shi-Xiu Wu, MD,[‡] Xia Li, PhD,[§] Tian Xie, PhD,[§] Kathryn L. Brinkman, BS,[†] Bin S. Teh, MD,[†] E. Brian Butler, MD,[†] Bo Xu, MD, PhD,[†] and Shu Zheng, MD[‖]**

**Department of Radiation Oncology, Sir Run Run Shaw Hospital, Sir Run Run Shaw Institute of Clinical Medicine of Zhejiang University, Hangzhou, China; [†]Department of Radiation Oncology, The Methodist Hospital Research Institute, Weill Cornell Medical College, Houston, TX; [‡]Department of Radiation Oncology, The First Affiliated Hospital of Wenzhou Medical College, Wenzhou, China; [§]Research Center of Biomedicine and Health, Hangzhou Normal University, Hangzhou, China; and [‖]Cancer Institute, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China*

Received Aug 23, 2011, and in revised form Jan 18, 2012. Accepted for publication Jan 31, 2012

**Summary**

Inhibition of the DNA protein kinase catalytic subunit (DNA-PKcs) leads to hypersensitivity to ionizing radiation. Here we report development of small inhibitory peptides targeting DNA-PKcs autophosphorylation. We demonstrate that the BTW3 peptide is capable of inhibiting DNA-PKcs autophosphorylation and inducing cellular radiosensitization.

**Purpose:** The DNA protein kinase catalytic subunit (DNA-PKcs) is one of the critical elements involved in the DNA damage repair process. Inhibition of DNA-PKcs results in hypersensitivity to ionizing radiation (IR); therefore, this approach has been explored to develop molecular targeted radiosensitizers. Here, we aimed to develop small inhibitory peptides that could specifically target DNA-PKcs autophosphorylation, a critical step for the enzymatic activation of the kinase in response to IR.

**Methods and Materials:** We generated several small fusion peptides consisting of 2 functional domains, 1 an internalization domain and the other a DNA-PKcs autophosphorylation inhibitory domain. We characterized the internalization, toxicity, and radiosensitization activities of the fusion peptides. Furthermore, we studied the mechanisms of the inhibitory peptides on DNA-PKcs autophosphorylation and DNA repair.

**Results:** We found that among several peptides, the biotin-labeled peptide 3 (BTW3) peptide, which targets DNA-PKcs threonine 2647 autophosphorylation, can abrogate IR-induced DNA-PKcs activation and cause prolonged γ-H2AX focus formation. We demonstrated that BTW3 exposure led to hypersensitivity to IR in DNA-PKcs-proficient cells but not in DNA-PKcs-deficient cells.

**Conclusions:** The small inhibitory peptide BTW3 can specifically target DNA-PKcs autophosphorylation and enhance radiosensitivity; therefore, it can be further developed as a novel class of radiosensitizer. © 2012 Elsevier Inc.

Reprint requests to: Bo Xu, MD, PhD, Department of Radiation Oncology, The Methodist Hospital Research Institute, 6565 Fannin St, Houston, TX 77030. Tel: (713) 441-3996; Fax: (713) 441-4493; E-mail: bxu@tmhs.org or Shu Zheng, PhD, The Second Affiliated Hospital Zhejiang University School of Medicine Hangzhou, China. Tel: +86-571-87214404; Fax: +86-571-87214404; E-mail: zhengshu@zju.edu.cn

This work was supported in part by Scientific and Technological Program grant Y2100139 of Zhejiang Province, China, to X.S., by the Cancer Fighters (to B.T.) and the Oshman Foundation (to B.X.), and by Department of Defense grant W81XWH-05-1-0018 to B.X.

Conflict of interest: none.

Supplementary material for this article can be found at www.redjournal.org.

[*] Xiaonan Sun and Chunying Yang contributed equally to the manuscript.

Int J Radiation Oncol Biol Phys, Vol. 84, No. 5, pp. 1212–1219, 2012
0360-3016/$ - see front matter 
doi:10.1016/j.ijrobp.2012.01.092

**Keywords:** DNA-PKcs, Peptides, Radiosensitivity

## Introduction

Radiation therapy is considered one of the most important treatments for cancer patients. Approximately 50%-70% of all cancer patients will receive radiotherapy during their treatment. Many factors, such as location, size, hypoxic condition, and genetic factors, influence the cellular radiosensitivity of an individual. Recently, molecular targeted approaches to radiosensitization have generated considerable interest in the development of tumor radiosensitizers.

Essential elements for DNA damage repair are appealing radiosensitization targets. Two predominant repair pathways, including homologous recombination and nonhomologous end-joining (NHEJ), are required for the repair of ionizing radiation (IR)-induced DNA double-strand breaks (DSBs) (1). NHEJ, which brings broken ends together and rejoins them without reference to a second template, is thought to be the prevailing pathway during the G0 and G1 phases of the cell cycle (2). DNA-dependent protein kinase (DNA-PK) is the core of NHEJ machinery, which consists of the Ku70/80 heterodimer and the catalytic subunit (DNA-PKcs) (3). Along with XRCC4, ligase IV, and the endonuclease Artemis, DNA-PK plays an essential role in NHEJ repair. Cells deficient in any component of the NHEJ pathway are defective in DSB repair and are highly sensitive to IR (3).

DNA-PKcs is a member of the phosphatidylinositol-3 kinase (PI3K)-related protein kinase family. Because DNA-PKcs has a critical role in DNA repair, specific inhibitors of DNA-PKcs have been developed and tested as radiosensitizers (4-6). One of the concerns about inhibitors effecting the enzymatic activity of the kinase is its negative effect on immune function, as the kinase is essential for Variable, Diversity and Joining (V[D]J) recombination. Therefore, developing other means of inhibiting DNA-PKcs should be explored. DNA-PKcs is activated by autophosphorylation in two regions: the ABCDE (Thr2609, Ser2612, Thr2620, Ser2624, Thr2638, and Thr2647) and the PQR (Ser2023, Ser2029, Ser2041, Ser2051, Ser2053 and Ser2056) clusters (7-10). The autophosphorylation of DNA-PKcs in response to DNA damage is an early and critical event for proper DSB repair. Previous studies have shown that autophosphorylation of DNA-PKcs in the ABCDE cluster leads to a conformational change that facilitates Artemis nuclease activity (10-12). Mutations in the ABCDE cluster cause a failure to complement the radiosensitivity or DSB repair defect of DNA-PKcs mutant cells, indicating DNA-PKcs autophosphorylation on this cluster is a critical step within NHEJ *in vivo* (7, 8, 13, 14). Therefore, we hypothesized that inhibition of DNA-PKcs autophosphorylation may impair subsequent steps in the DNA repair process and confer cellular radiosensitization.

In this study, we characterized several small peptides containing the ABCDE autophosphorylation cluster sequence of DNA-PKcs and found that 1 peptide, BTW3, targeting DNA-PKcs Thr-2647 phosphorylation, showed a robust radiosensitization effect in a DNA-PKcs-dependent manner. Our data demonstrate proof of principal that targeting DNA-PKcs autophosphorylation can be a novel approach to radiosensitization.

## Methods and Materials

### Cell lines and culture

The human colon cancer RKO cell line, HeLa cervical cancer cell line, and glioblastoma cell line MO59J (ATCC, Manassas, VA, USA) were used in this study. RKO cells were maintained in a humidified atmosphere with 5% $CO_2$ in RPMI 1640 medium containing 10% fetal bovine serum and were supplemented with 4 mM L-glutamine and 100 μg/mL penicillin/streptomycin (all from HyClone Laboratories Inc, South Logan, Utah, USA). HeLa and MO59J cells were grown in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and 100 μg/mL penicillin/streptomycin (Hyclone Laboratories Inc, South Logan, Utah, USA). Generation of stable cell lines was initiated by transfecting DNA-PKcs small hairpin RNA (shRNA) or control shRNA plasmids (Santa Cruz Biotechnology, Santa Cruz, CA) into HeLa cells, using FuGENE HD transfection reagent (Roche, Indianapolis, IN). Cells were divided into 10-cm culture dishes and selected in DMEM containing 4 μg/mL Puromycin (HyClone Laboratories Inc, South Logan, Utah, USA) 48 h later. Puromycin-resistant colonies were individually picked up and detected by Western blotting. Stable cell clones were maintained in DMEM supplemented with 1 μg/mL Puromycin.

### Peptide synthesis

Fusion peptides were synthesized in Hangzhou HuaAn Biotechnology Co Ltd (Hangzhou, China). All peptides had a purity of >98%. Peptides were dissolved in dimethyl sulfoxide (DMSO), stored at −80°C, and diluted in normal growth medium before use.

### Chemicals and irradiation

Cisplatin was purchased from Sigma Chemical Co (St. Louis, MO). A linear accelerator (PRIMUS-M; Siemens) was used to generate 6-MV radiographs at a dose rate of 2 Gy/min. All IR procedures were carried out under normal atmospheric pressure and temperature conditions.

### Colony formation assays

The colony formation assay was performed to determine radiosensitivity. Cells were plated in 6-well plates and allowed to adhere overnight. Cells were treated with various peptides for 1 h and exposed to a range of IR doses (0-6 Gy). After IR, fresh peptides were added every 12 h until 48 h had elapsed, and then the medium was replaced with peptide-free medium. After incubation for 10-14 days, the cells were stained with 0.5% crystal violet in methanol. Colonies (a population of >50 cells) were then counted using microscopy. The survival curves were plotted by linear regression analyses, and the radiosensitivity parameter, $D_0$, was calculated using Sigma Plot software. $D_0$ represents the mean

lethal dose required for 37% survival and is a measure of the intrinsic radiosensitivity of the cell. The sensitizing enhancement ratio (SER) was calculated as previously described (15).

## MTT assay

Cells were plated in 0.1 mL of RPMI 1640 medium in 96-well plates and incubated overnight. On the following day, cells were exposed to peptides (0, 5, 10, 20, 40, 60, and 80 μM) or to cisplatin (0, 5, 10, 20, 50, 60 and 80 μM) as a positive control at the final volume of 0.2 mL per well. At the indicated times, 20 μl of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution in phosphate-buffered saline (PBS) was added to each well for 4 h. After medium was removed, 170 μl of DMSO was added to each well to dissolve the formazan crystals. Absorbance at 490 nm was determined using a Biokinetics plate reader (Bio-Tek Instruments, Inc, Winooski, VT). Triplicate wells were assayed for each condition.

## Immunoblotting

Cells were treated with or without peptides for 1 h, followed by fresh medium, and then exposed to IR (6 Gy). Sixty minutes later, cells were harvested, and proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. For immunoblotting, the DNA-PKcs antibody was purchased from Santa Cruz Biotechnology. Phospho-specific antibodies against DNA-PKcs Thr2647 and Thr 2609 were purchased from Abcam (Cambridge, MA). γ-H2AX (Ser139p) was obtained from Epitomics, Inc (Burlingame, CA), and β-actin was purchased from Cell Signaling Technology, Inc (Beverly, MA). Polyclonal antibodies against Artemis, phospho-specific antibodies against Artemis Ser516 and DNA-PKcs Thr2620 were generated by Hangzhou HuaAn Biotechnology. Peptide sequences synthesized for Artemis, Artemis Ser516, and DNA-PKcs Thr2620 phosphorylation antibody development were LSTNADSQSSSD, TVAGGS(p)QSPKLFS, and GTLQTRT(p)QEGSL, respectively.

## γ-H2AX foci assay

To investigate the effect of peptides on IR-induced focus formation, cells were incubated with or without the peptides for 1 h, followed by IR. A total of 200,000 cells were seeded on sterile coverslips in 6-well plates and incubated for 24 h before treatment. After treatment with the peptides and IR, cells were washed with cold PBS 3 times and fixed for 15 min with 4% paraformaldehyde at room temperature. Cells were then permeabilized for 5 min with 0.5% Triton X-100 in PBS and blocked with 5% bovine serum albumin in PBS for 30 min at room temperature. The cells were incubated with a 1:200 dilution of γ-H2AX antibody (Epitomics) at 4°C overnight, followed by incubation with goat anti-rabbit secondary antibody conjugated with fluorescein isothiocyanate (Santa Cruz Biotechnology) for 1 h at room temperature. Finally, nuclei were counterstained with 4, 6-diamidino-2-phenylindole (Sigma) for 2 min. Coverslips were mounted using HARLECO Krystalon mounting medium (EMD). All coverslips were imaged with an LSM710 model inverted confocal microscope (Zeiss). For each time point, 40 cells were analyzed for number of foci per cell. Experiments were repeated 3 times.

## Statistics

To establish statistical significance, the Student unpaired *t* test was used when 2 treatment groups were compared or the one-way analysis of variance was used when all pair-wise comparisons

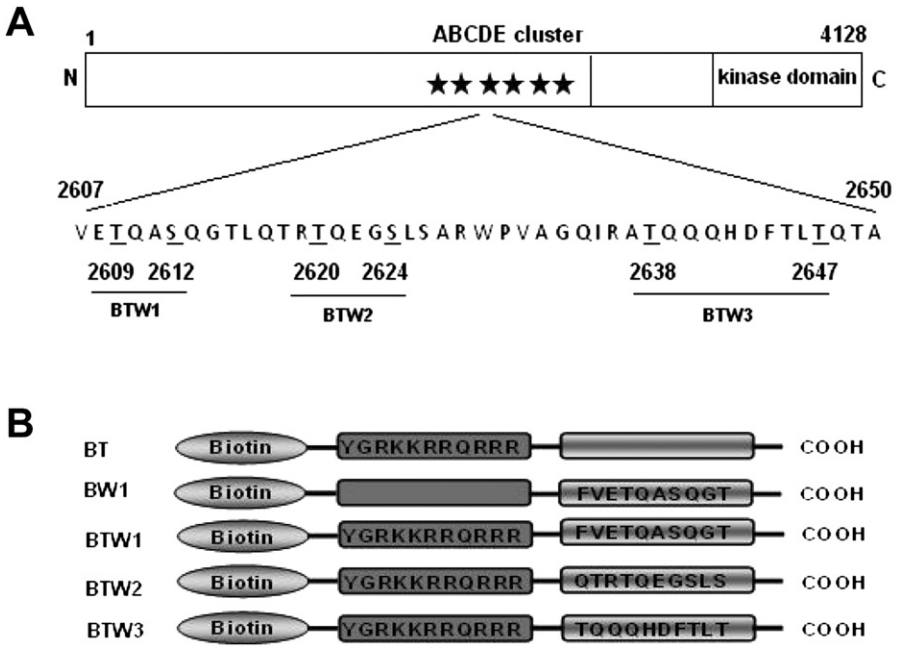


**Fig. 1.** Generation of fusion peptides targeting DNA-PKcs autophosphorylation. (A) Schematic illustration of the DNA-PKcs autophosphorylation clusters and the corresponding peptides BTW1, BTW2, and BTW3. (B) Amino acid sequences of the fusion peptides generated.

among the different treatment groups were performed. *P* values of $<.05$ were considered significant.

## Results

### Generation of the DNA-PKcs inhibitory peptides

DNA-PKcs autophosphorylation at the ABCDE major cluster sites in response to IR is required for efficient end processing in DNA DSB repair (7). It has been shown that mutation of these sites leads to radiation sensitivity (8). Therefore, we hypothesized that blocking the ABCDE cluster autophosphorylation of DNA-PKcs might inhibit recruitment of DNA repair proteins, thereby sensitizing tumor cells to IR. To test this hypothesis, we developed several small fusion peptides containing the AE, B, and CD sequences, respectively (Fig. 1A). Three DNA-PKcs inhibitory peptides representing amino acids 2606-2615(AE sites, BTW1), 2617-2626 (B sites, BTW2), and 2638-2647 (CD sites, BTW3) were synthesized (Fig. 1B). To effectively deliver the peptides to cells, an HIV1 protein transduction domain, TAT, was fused to the target peptides. All these peptides were labeled with a biotin tag at the N terminus for detection. We initially used the TAT-only sequence (BT) and AE sites without TAT sequences (BW1) as controls. Immunofluorescence microscopy analysis found that the peptides were transferred into cells effectively and were found in both the cytoplasm and nucleus (Supplementary Fig E1A). Furthermore, we found that the fluorescence signal gradually decreased after 12 h (Supplementary Fig E1B). However, BW1 did not show cellular localization. The MTT assay showed that there was limited toxicity for up to 80 μM peptide exposure of 12 h (Supplementary Fig E1C).

### BTW3 blocks IR-induced DNA-PKcs autophosphorylation

We then investigated whether the peptides could abrogate DNA-PKcs autophosphorylation by using 3 phospho-specific antibodies (T2647p, T2609p, and T2620p). As shown in Fig. 2, we found that the BTW1 peptide (which corresponds to Thr 2609 and Ser2612) abrogated Thr2609 phosphorylation but did not affect Thr2620 and Thr2647 phosphorylation in response to IR in RKO cells. BTW2 (which corresponds to Thr2620 and Ser2624) diminished Thr 2620 phosphorylation, whereas it partially affected Thr2609 phosphorylation. However, BTW2 had no effect on Thr2647 phosphorylation. Interestingly, BTW3 (which corresponds to Thr 2638 and Thr2647) totally ablated Thr2609, Thr2620, and Thr2647 phosphorylation, indicating that BTW3 possesses a superior inhibitory effect on DNA-PKcs autophosphorylation.

To further determine whether BTW3 specifically inhibits DNA-PKcs autophosphorylation, we synthesized a scrambled sequence peptide named BTS (SYGYHQDEER) by using a random sequence generator. We found phosphorylation on Thr2647 was not affected after BTS treatment (Fig. 3A) in RKO cells. Therefore, BTS was used as a negative control for BTW3. We then tested for phosphorylation of Artemis, a known substrate of DNA-PKcs (16), to further study the effect of the peptides on DNA-PK activity. We found that IR-induced Artemis Ser516 phosphorylation was diminished in the presence of BTW3 but not BTS (Fig. 3B) in RKO cells. Taken together, these data demonstrate that BTW3 can inhibit DNA-PKcs autophosphorylation on the ABCDE cluster, leading to inhibition of DNA-PKcs activation in response to IR.

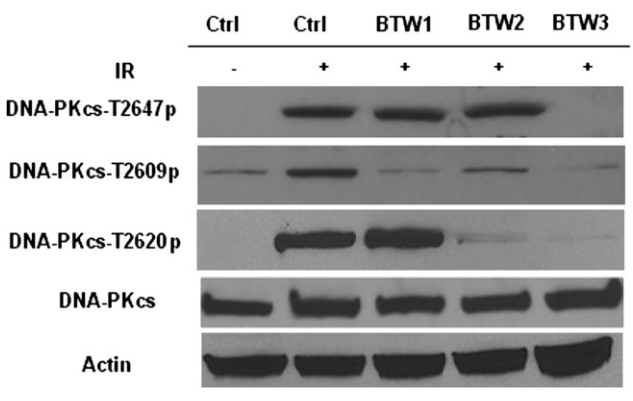


**Fig. 2.** Inhibitory effects of the fusion peptides on DNA-PKcs autophosphorylation. Exponentially growing RKO cells were treated with 10 μM of the fusion peptides for 1 h before they were irradiated (0 or 6 Gy). Total cell lysates were obtained 2 h after IR, and Western blotting was performed with the indicated antibodies.

### BTW3 enhances cellular radiosensitivity

Because DNA-PK activity is required for cell survival in response to DNA damage, we tested whether treatment of cells with BTW

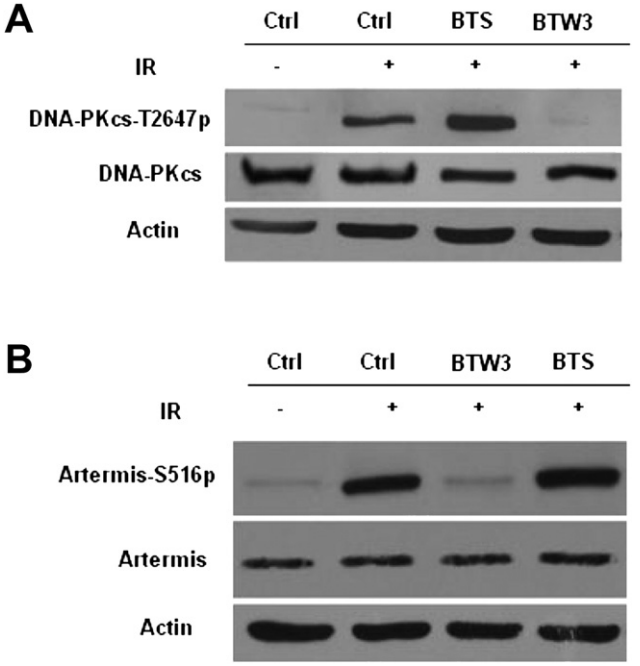


**Fig. 3.** The BTW3 peptide inhibits DNA-PKcs autophosphorylation and Artemis Ser516 phosphorylation in response to IR. Exponentially growing RKO cells were treated with BTW3 and a control peptide, BTS, at 10 μM for 1 h before IR. Following treatment, cells were irradiated (0 or 6 Gy). Total cell lysates were obtained 2 h after IR, and Western blotting was performed with indicated antibodies. (A) DNA-PKcs Thr2647 autophosphorylation. (B) Artemis Ser516 phosphorylation.

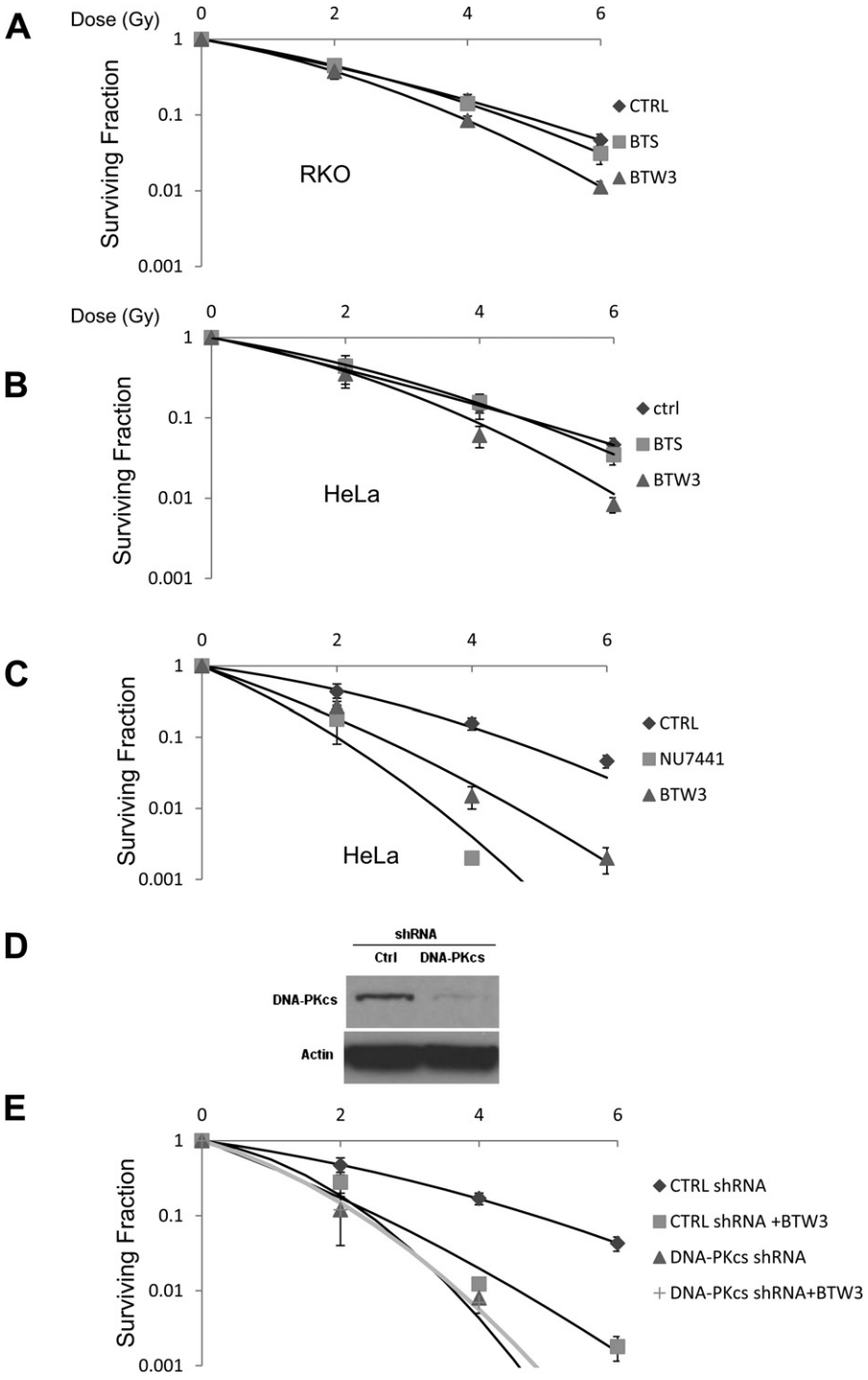


**Fig. 4.** BTW3 enhances cellular radiosensitivity in a DNA-PKcs-dependent manner. RKO (A), HeLa (B and C), or HeLa cells stably transfected with control or DNA-PKcs shRNA (E) cells were seeded in 6-well plates in dilutions suited for colony formation. At 24 h after initial seeding, cells were treated with 10 μM of the fusion peptides 1 h before IR and continuously exposed to the peptides for a total of 48 h. (C) Cells were also treated with 1 μM of NU7441 1 h before IR. Surviving colonies were stained with crystal violet. Survival curves after indicated doses of IR are shown. Error bars represent ± SD. Means from 3 independent experiments are shown. (D) Western blot analysis of using indicated antibodies in cells stably transfected with control shRNA or DNA-PKcs shRNA.

peptides conferred radiosensitization by using the colony formation assay. We found that in human cancer lines RKO (Fig. 4A) and HeLa (Fig. 4B) cells, the radiosensitization parameter $D_0$ for cells treated with BTW3 was 1.83 Gy (for RKO) and 1.23 Gy (for HeLa) compared with 2.68 Gy (RKO) and 2.05 Gy (HeLa) for cells treated with BTS. Significant differences ($P \leq .05$) in clonogenic survival were observed between cells treated with BTW3 and those treated with BTS or control. The SER was 1.46 for

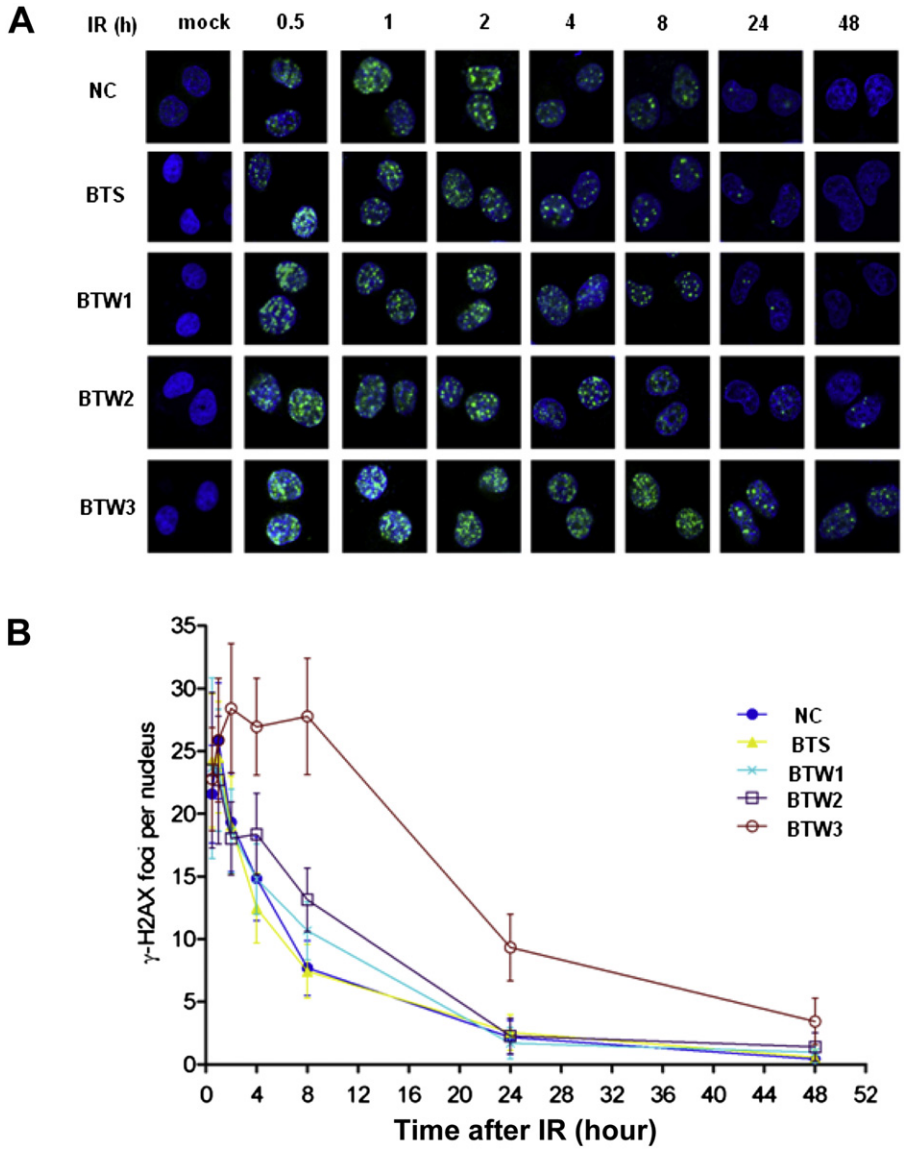


**Fig. 5.** The BTW3 peptide prolongs the retention of IR-induced γ-H2AX foci. (A) Exponentially growing RKO cells were treated with fusion peptides (10 μM) for 1 h, irradiated with 0 (Mock) or 6 Gy, and harvested at indicated time points before immunofluorescence microscopy was used to detect IR-induced γ-H2AX focus formation. (B) Mean γ-H2AX nuclear foci per nucleus were counted and expressed in arbitrary units. Error bars represent ± SD. Means from 3 independent experiments are shown.

RKO and 1.67 for HeLa cells. We also tested BTW1 and BTW2 for their effects on radiosensitivity. However, we did not observe any radiosensitization effect by the two peptides (Supplementary Fig E2). Because NU7441 is a well-studied inhibitor of DNA-PKcs, we also compared the BTW3 peptide with NU7441. We found that a moderate radiosensitization effect could be achieved (Fig. 4C) by BTW3 (SER, 1.72) compared to that with NU7441 (SER, 2.12). To test whether BTW3 specifically inhibited DNA-PKcs to induce radiosensitization, we generated isogenic HeLa cell lines stably expressing either control shRNA or DNA-PKcs shRNA (Fig. 4D). We found that DNA-PKcs-depleted HeLa cells showed an increase in radiosensitivity that could not be further enhanced by the BTW3 peptide. The control group, consisting of HeLa cells transfected with a control shRNA, showed a moderate radiosensitivity but could be sensitized by BTW3 (Fig 4E). Therefore, these observations demonstrated that the BTW3 peptide radiosensitizes tumor cells in a DNA-PKcs-dependent manner.

## BTW3 prolongs the existence of γH2AX foci induced by IR

To further study the biological effect of the BTW3 peptides on the DNA damage response, we tested the peptides' effect on IR-induced formation of γ-H2AX foci at DSB sites. We examined the kinetics of γ-H2AX phosphorylation foci in RKO cells after 6-Gy IR in the presence of the BTW3 or BTS peptide. Neither BTW3 nor BTS alone could induce γ-H2AX focus formation in the absence of IR. We found that exposure

to 6 Gy alone induced 20+ foci per cell at 30 min after IR. The number of foci decreased rapidly, and at 4 h post-IR, there were only 6 foci per cell. Similar γ-H2AX foci numbers were observed in cells treated with the control peptide BTS, in which γ-H2AX foci also declined rapidly and nearly disappeared after 24 h. However, in the presence of BTW3, not only did the amount of IR-induced foci increase significantly (28+ foci per cell), but also the disappearance of foci was significantly retarded. At 48 h after IR, we still observed 5+ foci per cell (Fig. 5A and B). Retention of γ-H2AX foci in cells treated with BTW3 indicates a significant delay of IR-induced DNA damage repair.

## Discussion

DNA-PKcs, which is activated by DNA DSBs upon IR, is a critical component of the NHEJ DNA repair pathway. It has been reported that cells showing resistance to DNA-damaging agents have increased levels of DNA-PKcs activity and that cells deficient in DNA-PKcs have enhanced sensitivity to DNA-damaging agents. Due to its importance in radiosensitivity and DNA repair, DNA-PK has become an appealing target for the development of drugs designed to improve tumor sensitivity to chemotherapy and radiotherapy. Small-molecule inhibitors of DNA-PKcs kinase can increase radiosensitivity of cells to IR. Among these, wortmannin, LY294002, and NU7441 are well-characterized inhibitors of DNA-PKcs, which have been reported to retard DSB repair and enhance the cytotoxicity of IR and etoposide (4, 17-20). In this study, we developed peptides blocking DNA-PKcs autophosphorylation, the critical step in the NHEJ pathway, to selectively inhibit the function of DNA-PK in DNA repair. We developed 3 DNA-PKcs inhibitory peptides, which may bind to the ABCDE sites and block autophosphorylation of DNA-PKcs. Among the inhibitory peptides, we identified BTW3 as a powerful inhibitor of DNA-PKcs autophosphorylation and radiosensitizer. We also found that a slightly smaller yet-to-be-significant radiosensitization effect can be achieved by BTW3 compared to that with the specific DNA-PKcs inhibitor NU7441.

It is interesting to note that individual abrogation of DNA-PKcs Thr2609 by BTW1 and Thr2620 by BTW2 autophosphorylation is not sufficient to produce a radiosensitization effect. However, abrogation of Thr2647 leads to diminished Thr2609 and Thr2620 phosphorylation, indicating Thr2647 is required for the other autophosphorylation events. More importantly, interfering with Thr2647 autophosphorylation leads to hypersensitivity to IR.

BTW3 not only blocks autophosphorylation of DNA-PKcs but also affects the subsequent steps of DNA-PKcs activation (eg, Artemis phosphorylation). This is consistent with recent reports showing that DNA-PKcs autophosphorylation plays an important role in activation of Artemis (11). Loss of Artemis activation could cause incorrect or absent processing of DNA ends, which in turn may affect ligation efficiency. However, the exact mechanisms of the BTW peptide on DNA-PKcs autophosphorylation remain to be elucidated. It is likely that BTW3 may bind to the autophosphorylation sequence site or compete with the substrates of DNA-PKcs, thus blocking the access of kinase. Because DNA-PKcs autophosphorylation may potentially lead to dissociation of the DNA-PK complex, resulting in conformational changes in DNA-PKcs to facilitate subsequent repair steps or creation of docking sites for other DNA repair proteins, it is possible that BTW3 might influence all these processes.

## Conclusions

In summary, we identified the DNA-PKcs inhibitory peptide BTW3, which can abrogate DNA-PKcs autophosphorylation in response to IR. Cells exposed to BTW3 showed an increased radiosensitivity and delayed DNA repair, demonstrating the proof of concept that DNA-PKcs autophosphorylation is an ideal target for development of novel classes of radiosensitizers.

## References


1. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. *Nature* 2009;461:1071-1078.
2. Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu Rev Biochem* 2010; 79:181-211.
3. Finnie NJ, Gottlieb TM, Blunt T, *et al*. DNA-dependent protein kinase defects are linked to deficiencies in DNA repair and V(D)J recombination. *Philos Trans R Soc Lond B Biol Sci* 1996;351: 173-179.
4. Ismail IH, Martensson S, Moshinsky D, *et al*. SU11752 inhibits the DNA-dependent protein kinase and DNA double-strand break repair resulting in ionizing radiation sensitization. *Oncogene* 2004; 23:873-882.
5. Zhao Y, Thomas HD, Batey MA, *et al*. Preclinical evaluation of a potent novel DNA-dependent protein kinase inhibitor NU7441. *Cancer Res* 2006;66:5354-5362.
6. Tavecchio M, Munck JM, Cano C, *et al*. Further characterisation of the cellular activity of the DNA-PK inhibitor, NU7441, reveals potential cross-talk with homologous recombination. *Cancer Chemother Pharmacol* 2012;69:155-164.
7. Ding Q, Reddy YV, Wang W, *et al*. Autophosphorylation of the catalytic subunit of the DNA-dependent protein kinase is required for efficient end processing during DNA double-strand break repair. *Mol Cell Biol* 2003;23:5836-5848.
8. Chan DW, Chen BP, Prithivirajsingh S, *et al*. Autophosphorylation of the DNA-dependent protein kinase catalytic subunit is required for rejoining of DNA double-strand breaks. *Genes Dev* 2002;16: 2333-2338.
9. Douglas P, Sapkota GP, Morrice N, *et al*. Identification of in vitro and in vivo phosphorylation sites in the catalytic subunit of the DNA-dependent protein kinase. *Biochem J* 2002; 368:243-251.
10. Cui X, Yu Y, Gupta S, *et al*. Autophosphorylation of DNA-dependent protein kinase regulates DNA end processing and may also alter double-strand break repair pathway choice. *Mol Cell Biol* 2005;25: 10842-10852.
11. Goodarzi AA, Yu Y, Riballo E, *et al*. DNA-PK autophosphorylation facilitates Artemis endonuclease activity. *EMBO J* 2006; 25:3880-3889.
12. Meek K, Douglas P, Cui X, *et al*. Trans autophosphorylation at DNA-dependent protein kinase's two major autophosphorylation site clusters facilitates end processing but not end joining. *Mol Cell Biol* 2007; 27:3881-3890.
13. Uematsu N, Weterings E, Yano K, *et al*. Autophosphorylation of DNA-PKCS regulates its dynamics at DNA double-strand breaks. *J Cell Biol* 2007;177:219-229.
14. Soubeyrand S, Pope L, Pakuts B, *et al*. Threonines 2638/2647 in DNA-PK are essential for cellular resistance to ionizing radiation. *Cancer Res* 2003;63:1198-1201.
15. Cariveau MJ, Tang X, Cui XL, *et al*. Characterization of an NBS1 C-terminal peptide that can inhibit ataxia telangiectasia mutated (ATM)-mediated DNA damage responses and enhance radiosensitivity. *Mol Pharmacol* 2007;72:320-326.

16. Moshous D, Callebaut I, de Chasseval R, *et al*. Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell* 2001; 105:177-186.
17. Hashimoto M, Rao S, Tokuno O, *et al*. DNA-PK: the major target for wortmannin-mediated radiosensitization by the inhibition of DSB repair via NHEJ pathway. *J Radiat Res (Tokyo)* 2003;44:151-159.
18. Collis SJ, Swartz MJ, Nelson WG, *et al*. Enhanced radiation and chemotherapy-mediated cell killing of human cancer cells by small inhibitory RNA silencing of DNA repair factors. *Cancer Res* 2003;63: 1550-1554.
19. Fukuchi K, Watanabe H, Tomoyasu S, *et al*. Phosphatidylinositol 3-kinase inhibitors, Wortmannin or LY294002, inhibited accumulation of p21 protein after gamma-irradiation by stabilization of the protein. *Biochim Biophys Acta* 2000;1496:207-220.
20. Sarkaria JN, Tibbetts RS, Busby EC, *et al*. Inhibition of phosphoinositide 3-kinase related kinases by the radiosensitizing agent wortmannin. *Cancer Res* 1998;58:4375-4382.