Decreased DAB2IP gene expression, which could be induced by fractionated irradiation, is associated with resistance to γ-rays and α-particles in prostate cancer cells

CHEN YANG[1], HUI HE[1], TINGTING ZHANG[1], YING CHEN[2] and ZHAOLU KONG[1]

[1]Department of Radiobiology, The Institute of Radiation Medicine, Fudan University, Shanghai 200032; [2]Division of Children's Health, Huai'an Women and Children Hospital, Huai'an, Jiangsu 223002, P.R. China

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Abstract. External beam radiation therapy, alone or combined with androgen deprivation, is a well-established treatment for prostate cancer (PCa). However, not all patients benefit from radiotherapy due to congenital or acquired radioresistance. The preliminary results of the present study indicated that the loss of disabled homolog 2 interactive protein (DAB2IP) expression in PCa and normal prostate epithelia results in the resistance to γ-rays. To further explore the association between DAB2IP and ionizing radiation (IR), PCa cells were fractionally irradiated 12 times with 2 Gy of γ-rays and the change in DAB2IP mRNA expression was monitored. Notably, along with a continuous reduction of DAB2IP expression levels, increased expression levels of ataxia-telangiectasia mutated (ATM) was observed in IR-treated cells. In order to improve the sensitivity of DAB2IP-deficient cells to IR, α-particles, a type of high linear energy transfer radiation and KU55933, an ATM inhibitor, were used in the current study. It was determined that α-particle irradiations were more effective than γ-rays on cells expressing expected and decreased levels of DAB2IP. However, cells with a dysfunctional DAB2IP gene were resistant to α-particle irradiation. Treatment with KU55933 did not enhance cell sensitivity to α-irradiation. Therefore, this suggested that DAB2IP downregulation induced by radiotherapy may be associated with acquired radioresistance in patients with PCa.

Correspondence to: Ms. Zhaolu Kong, Department of Radiobiology, The Institute of Radiation Medicine, Fudan University, 2094 Xietu Road, Shanghai 200032, P.R. China
E-mail: kongzhaolu@fudan.edu.cn

Abbreviations: DAB2IP, disabled homolog 2 interactive protein; PCa, prostate cancer; LET, linear energy transfer; SF_2, surviving fraction at 2 Gy; IR, ionizing radiation; ATM, ataxia-telangiectasia mutated; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; ATR, ataxia-telangiectasia and Rad3 related protein; CFA, colony formation assay; DDR, DNA damage response

Key words: irradiation, DAB2IP, radio-resistance, α-particles, ATM, ATR

Introduction

Disabled homolog 2 interactive protein (DAB2IP) is a novel member of the Ras-GTPase-activating protein family, involved in cell proliferation and apoptosis (1). Downregulation of DAB2IP is often detected in high-grade and metastatic prostate cancer (PCa) specimens (2). Kong *et al* (3) determined that PCa cells with DAB2IP deficiency were also resistant to γ radiation and exhibited increased clonogenic survival, robust G_2-M checkpoint control and resistance to ionizing radiation (IR)-induced apoptosis. Wu *et al* (4) reported that loss of DAB2IP expression in PCa indicated chemoresistance via increased expression of the secretory form of clusterin. Recently, Yun *et al* (5) observed that DAB2IP was important in modulating cancer stem cell properties via the CD117-mediated zinc finger E-box binding homeobox 1 signaling pathway. All these previous studies suggest that loss of DAB2IP may complicate PCa treatment as the tumor cells become resistant to conventional radiotherapy and chemotherapy.

α-particles are heavy particulate emissions that travel a short linear distance but deposit a large quantity of energy. This high linear energy transfer (LET) radiation is characterized by enhanced ability to induce cell death, ability to overcome the radioresistance to hypoxia, low-LET radiotherapies and not relying on dose rate (6). The applications of α-particle therapy, also termed targeted α-particle therapy, in anticancer therapeutic strategies have been widely investigated (7,8). The US Food and Drug Administration approved α-particle therapy against PCa in 2013 (9). Novel methods have combined α-particles and effective radiosensitizers in order to target malignant cells without harming normal cells (10,11). In the present study, the radiation strengths of α-particles were utilized to overcome the radioresistance induced by the downregulation of DAB2IP in PCa cells. In addition, the methods of enhancing cellular sensitivity to irradiation were also investigated.

Materials and methods

Cell culture. PC3 human PCa cell line-derivative lines [PC3 short hairpin (sh)DAB2IP and PC3 shVector] were generated using an shRNA-lentiviral system as described previously (1) and maintained in T medium supplemented with 5% fetal calf

serum (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), 100 U/ml penicillin, 100 μg/ml streptomycin, 900 μg/ml G418 and 700 ng/ml puromycin (Invitrogen; Thermo Fisher Scientific, Waltham, MA, USA) in a humidified atmosphere with 5% CO_2 at 37˚C.

Cell irradiation. Cells were irradiated at room temperature in ambient air using a ^{137}Cs source (γ-rays; Nordion, Inc., Toronto, Canada) at a dose rate of 0.79 Gy/min (12) or a ^{241}Am plate source (α-particle; Atom High Tech Co. Ltd., Beijing, China) at a dose rate of 0.25 Gy/min (13). For the fractionated irradiation study, PC3 cells were plated in T25 tissue culture flasks on day 0 and exposed to γ-irradiation with daily dose of 2 Gy from day 1 to day 3. Following a 2 day recovery, cells were exposed to 2 Gy γ-rays for an additional 3 days as indicated in Fig. 1A. The total RNA was harvested for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay on days 0, 4, 9, 14 and 19.

Clonogenic survival. The radiosensitivity of cells was determined using a colony formation assay (CFA). KU55933 or NU7026 (Tocris Bioscience, Ellisville, MO, USA) was added to the medium 1 h prior to IR to a final concentration of 10 μM. The logarithmic-phase cells were treated with increasing doses of γ-rays (0, 2, 4, 6, 8 Gy) or α-radiation (0, 0.2, 0.4, 0.6, 0.8, 2 Gy) and cultured in 60 mm dishes. Subsequent to a 14 day incubation, the colonies were rinsed twice with phosphate-buffered saline (PBS; Beyotime Institute of Biotechnology, Haimen, China), then fixed with methanol (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) for 30 min. Next, 0.1% crystal violet solution (Sangon Biotech Co., Ltd., Shanghai, China) was used to stain the colonies. Finally, colonies containing a minimum of 50 viable cells were counted using an inverted microscope (37XAE; Shanghai Optical Instrument Factory Co., Ltd., Shanghai, China). The surviving fraction curve $S=e^{-(\alpha D+\beta D2)}$ was fitted to the experimental data with a least square fit algorithm using SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA, USA). The surviving fraction at 2 Gy (SF_2) was calculated to compare the sensitivity of cells to IR.

RT-qPCR analysis. Cells were subjected to 0.2 Gy α-particles or 2 Gy γ-rays, respectively. Total RNA was extracted from irradiated-cells or control cells using RNAsimple Total RNA kit (cat. no. DP419; Tiangen Biotech Co. Ltd., Beijing, China) 24 h after IR according to the manufacturer's protocol. The RNA was incubated with DNase I (2.5 μl; cat. no. RT411; Tiangen Biotech Co. Ltd.) in order to eliminate any genomic DNA contamination. The total RNA was then reverse-transcribed using the ReverTra Ace qPCR RT kit (cat. no. FSQ-101; Toyobo Co. Ltd., Osaka, Japan). The temperature protocol for the RT was as follows: 65˚C for 5 min, followed by 42˚C for 60 min and 70˚C for 5 min. cDNA was analyzed by qPCR using 2.5 ng cDNA in a 20 μl reaction volume containing primers and Accupower 2X Greenstar Master mix (cat. no. K6251; Bioneer Corporation, Daejeon, Korea). The program conditions included 95˚C for 5 min, and 40 cycles of 95˚C for 30 sec and 60˚C for 45 sec. 18s rRNA served as the housekeeping control gene. The primers were designed by GenScript (Nanjing, China), as follows: Sense, 5'-TCGTGGAAGGACTCATGACC-3' and antisense, 5'-TCCACCACCCTGTTGCTGTA-3' for DAB2IP; sense, 5'-TTAAGGTGGACCACACAGGA-3' and antisense, 5'-GGCCCTTAACAAGCTGTCTC-3' for ataxia-telangiectasia mutated (ATM); sense, 5'-GTACAAGCCCTGAGGCTTTC-3' and antisense, 5'-GCTGATGCATATCAGAGCGT-3' for DNA-dependent protein kinase catalytic subunit (DNA-PKcs); sense, 5'-AATGTGAGTGGAAGCCATGA-3' and antisense, 5'-TCCGCAGAAGTCTCGTTATG-3' for ataxia-telangiectasia and Rad3 related protein (ATR);sense, 5'-GGAATTGACGGAAGGGCACCACC-3' and antisense, 5'-GTGCAGCCCCGGACATCTAAGG-3' for 18s RNA. The qPCR was performed using MyGo Pro RealTime PCR system (IT-IS International, Ltd., Middlesbrough, UK). Each sample was examined in triplicate and the product quantity was normalized relative to 18s rRNA. The $2^{-\Delta\Delta Cq}$ method was applied to calculate gene expression as described previously (12).

Western blot assay. Lysates from cells irradiated with γ-rays and α-particles were extracted with radioimmunoprecipitation assay lysis buffer (cat. no. P0013B; Beyotime Institute of Biotechnology) mixed with 10 mM phenylmethylsulfonyl fluoride (cat. no. ST506; Beyotime Institute of Biotechnology) 30 min after IR, and the supernatant was collected following centrifugation at 12,000 x g for 10 min at 4˚C. The protein concentration was quantified by Micro BCA Protein assay kit (cat. no. SK3061, Sangon Biotech Co., Ltd.) according to the manufacturer's protocol. An aliquot of total protein (40 μg) was subjected to 6% (for the high-molecular-weight proteins, p-ATM and P-DNA PKcs) or 8% [for phospho-checkpoint kinase 2 (p-CHK2), DAB2IP and β-actin] sodium dodecyl sulfate-polyacrylamide gel electrophoresis (100 V for 2 h) and transferred onto polyvinylidene difluoride membranes (0.45 μm, EMD Millipore, Billerica, MA, USA). The membranes were blocked with 5% bovine serum albumin (cat. no. AR2440, Sangon Biotech Co. Ltd.) for 1 h at room temperature. The membranes were then incubated with primary antibodies at 4˚C overnight. Primary antibodies are listed as follows: Polyclonal rabbit anti-DAB2IP (1:650; received from Professor Hsieh, UT Southwestern Medical Center, Dallas, TX, USA) (1); monoclonal rabbit anti-phospho-ATM (pS1981; 1:1,000; cat. no. 2152-1; Epitomics; Abcam, Cambridge, MA, USA); monoclonal rabbit anti-phospho-DNA-PKcs (pS2056; 1:1,000; cat. no. 3892-1, Epitomics; Abcam); monoclonal rabbit anti-p-CHK2 (pT68; 1:1,000; cat. no. 1538-1, Epitomics; Abcam); monoclonal mouse anti-β-actin as an internal loading control (1:1,000, cat. no. AA128, Beyotime Institute of Biotechnology). The membranes were washed with PBS (pH 7.4) three times and then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies goat anti-rabbit (1:1,000, cat. no. A0208, Beyotime Institute of Biotechnology) and goat anti-mouse (1:1,000, cat. no. A0216, Beyotime Institute of Biotechnology) for 1 h at room temperature. The membranes were washed with PBS (pH 7.4) three times prior to visualization of protein bands by chemiluminescence (BeyoECL Plus, cat. no. P0018, Beyotime Institute of Biotechnology) and detected with the ChemiDoc XRS+ system (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Statistical analysis. The data are presented as the mean ± standard error of the mean of at least three independent experiments. The different groups were compared using the

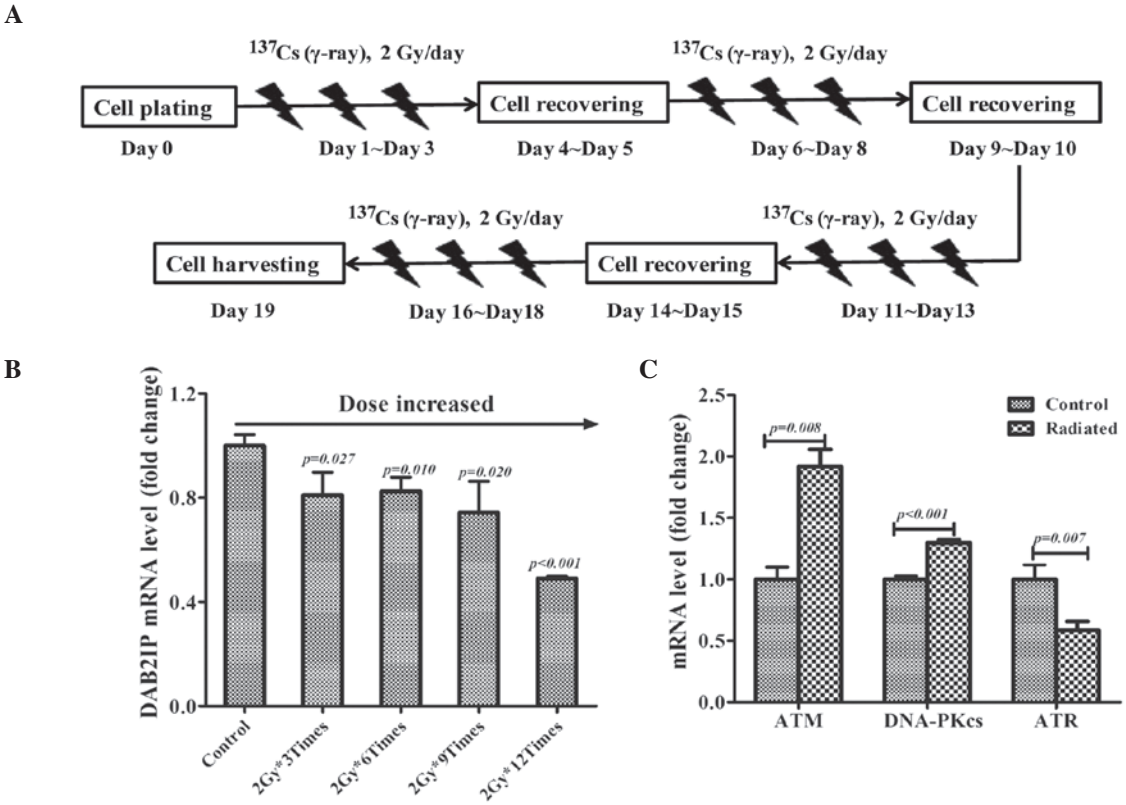

Figure 1. Continuous irradiation decreased DAB2IP mRNA expression in PC3 cells. (A) Cells were exposed to 2 Gy of γ-rays as indicated. Total RNA was harvested 24 h post radiation. (B) Relative levels of mRNA expression of DAB2IP following γ-irradiation and (C) DNA damage response molecules, ATM, DNA-PKcs and ATR in PC3 cells following the last irradiation were detected by reverse transcription-quantitative polymerase chain reaction with untreated cells serving as the control. P-values are presented vs. the control group. The results are presented as the mean of three experiments ± standard error of the mean. DAB2IP, disabled homolog 2 interactive protein; ATM, ataxia-telangiectasia mutated; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; ATR, ataxia-telangiectasia and Rad3 related protein.

unpaired Student's t-test. Statistical analyses were performed with SPSS 18.0 statistics software (SPSS, Inc., Chicago, IL, USA) and P<0.05 was considered to indicate a statistically significant difference.

Results

Fractionated irradiation decreases DAB2IP mRNA expression levels. PC3 cells were fractionally irradiated with 2 Gy/day and the expression of DAB2IP mRNA was monitored by RT-qPCR assay. Compared with non-irradiated cells, the expression of DAB2IP gene was gradually decreased in irradiated cells with the accumulative dose increase (Fig. 1B). It was also determined that the gene expression of three established DNA damage response (DDR) molecules: ATM, DNA-PKcs and ATR in cells irradiated 12 times with 2 Gy. It was observed that fractionated irradiation induced the overexpression of ATM (P=0.008) and DNA-PKcs (P<0.001) mRNA levels, whereas ATR (P=0.007) was downregulated in IR-treated cells (Fig. 1C).

DAB2IP modulates the radiosensitivity of PC3 cells. In the current study, endogenous DAB2IP expression in PC3 was knocked down with DAB2IP shRNA plasmid transfection. Western blot and RT-qPCR analysis indicated that DAB2IP mRNA levels were significantly decreased in PC3 shDAB2IP cells compared with PC3 shVector (P<0.05; Fig. 2A and B). The sensitivity of cells to γ-irradiation was evaluated by CFA. DAB2IP-negative PCa cells exhibited higher levels of clonogenic survival than DAB2IP-positive cells (Fig. 2C). In order to overcome cellular radioresistance of DAB2IP-negative cells, PC3 shVector and PC3 shDAB2IP cells were exposed to a ^{241}Am α-particle plate source and the cell lines exhibited higher sensitivity to α-particles than that to γ-rays, as indicated by the lower level of survival (Fig. 2C). However, DAB2IP-negative cells retained a significantly higher SF_2 value compared with DAB2IP-positive cells (P<0.001; Fig. 2D). Thus, downregulation of DAB2IP produced cells resistant to γ-ray and α-particle irradiation.

DAB2IP influences DDR signaling pathways in response to γ-irradiation. To elucidate the underlying mechanism of DAB2IP loss resulting in radioresistance, the effect of IR on the expression of relevant genes, including ATM, DNA-PKcs and ATR, was investigated and compared. Our previous studies demonstrated that downregulation of DAB2IP gene may induce elevated expression of ATM (12,14). There was no difference between the mRNA expression levels of ATM, DNA-PKcs and ATR in PC3 shVector cells regardless of IR exposure (Fig. 3A). However, significantly higher mRNA expression levels of ATM were detected in PC3 shDAB2IP cells exposed to 2 Gy of γ-rays (P=0.001; Fig. 3B). When ATM phosphorylation was inhibited with 10 μM KU55933 (Fig. 3C), shVector and shDAB2IP cells exhibited enhanced sensitivity

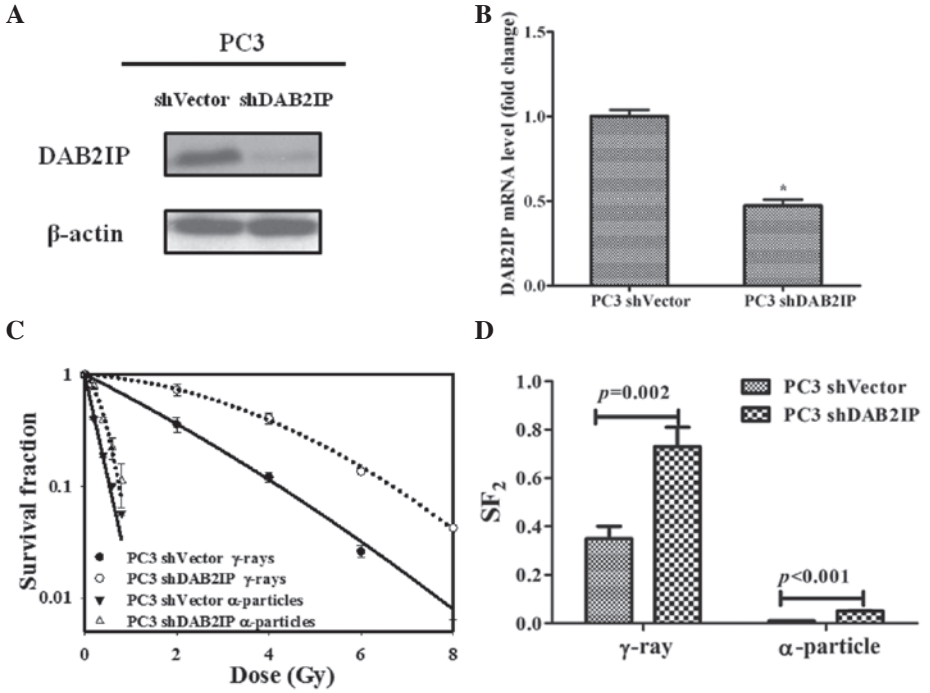

Figure 2. DAB2IP knockdown decreased sensitivity of PC3 cells to γ-rays and α-particles. DAB2IP (A) protein and (B) mRNA expression levels of PC3 shVector and PC3 shDAB2IP cells was determined by western blotting and reverse transcription-quantitative polymerase chain reaction assay, respectively. β-actin served as an internal loading control. *P<0.05 vs. the PC3 shVector cells. (C) Sensitivity of PC3 shVector and PC3 shDAB2IP cells to γ-ray (0, 2, 4, 6, 8 Gy) and α-particle (0, 0.2, 0.4, 0.6, 0.8 Gy) irradiation were detected by a colony formation assay. (D) SF_2 was calculated to compare the sensitivity of cells to γ-rays and α-particles. P-values are presented above the error bars vs. the control group. The results are presented as the mean of three experiments ± standard error of the mean. shDAB2IP, short haripin disabled homolog 2 interactive protein; shVector, short hairpin vector; SF_2, surviving fraction at 2 Gy.

to IR, particularly when treated with higher dose (Fig. 3D and E). On the other hand, NU7026 (12), which limits the activation of DNA-PKcs and CHK2 phosphorylation in response to IR (Fig. 3C), did not significantly affect the survival of the PC3 shVector and PC3 shDAB2IP cells when exposed to γ-rays (Fig. 3D and E).

DAB2IP-deficient cells become resistant to the combined treatment of KU55933 and α-particles. When ^{137}Cs sourced γ-rays were used as reference radiation, the RBE of α-particles was estimated at 9.2 and 11 for 10% survival of PC3 shVector and PC3 shDAB2IP cells, respectively. Compared with the control, PC3 shVector and shDAB2IP cells subjected to 0.2 Gy of α-particle exhibited lower ATM (P<0.001; Fig. 4A and P=0.001; Fig, 4B) and higher ATR (P<0.001; Fig. 4A and P=0.016; Fig. 4B) mRNA expression levels. In addition, there was no significant difference in DNA-PKcs mRNA level detected between untreated and irradiated cells (Fig. 4A and B). The effect of KU55933 and NU7026 on cellular sensitivity to α-particles was determined using CFA. PC3 shVector cells were radiosensitized by KU55933 (Fig. 4C); however, the survival curve of α-irradiated PC3 shDAB2IP cells was not affected by NU7026 and KU55933 (Fig. 4C and D). Therefore, neither the ATM nor the DNA-PKcs inhibitor increased α-particle-induced lethality in DAB2IP-deficient cells.

Discussion

PCa is one of the leading causes of cancer-associated mortality in males. External beam radiation therapy alone, or combined with androgen deprivation, with daily fraction of 1.8~2 Gy over a 7~9-week period is a standard treatment option for organ-confined and regionally advanced PCa patients (15). However, for a significant proportion of high-risk patients with PCa this therapy will fail and metastasis will develop, for which, currently, no curative treatment exists (16). Tumor cells evading IR-induced cell death may lead to disease progression, cancer relapse and acquired radioresistance. A previous study by Ghisolfi *et al* (17) partly elucidated why IR-treated patients with tumor recurrence are often resistant to conventional radiotherapy: Irradiation may induce and accumulate tumor stem cells, which are resistant to antineoplastic therapeutic agents and IR. Currently, numerous studies have presented that DAB2IP is important in associating PCa cells stemness (5), radio/chemosensitivity and the DDR signaling pathway (3-5,12). Previous clinical data indicated that loss of DAB2IP function may be a potential biomarker indicating an unfavorable outcome despite the use of radiotherapy in high-risk patients with PCa (18).

In the present study, clinical fractioned irradiation was mimicked and the effect of IR on mRNA expression levels of DAB2IP, ATM, DNA-PKcs and ATR was investigated by RT-qPCR. ATM, DNA-PKcs and ATR have been established as important signal proteins mediating DDR by homologous recombination and non-homologous end joining (19). Elevated expression of those DDR signal molecules is often associated with enhanced cell viability from irradiation (20). As the IR dose was increased, a reduction of DAB2IP mRNA expression levels was detected in irradiated cells accompanied with elevated mRNA expression of ATM and DNA-PKcs. ATR,

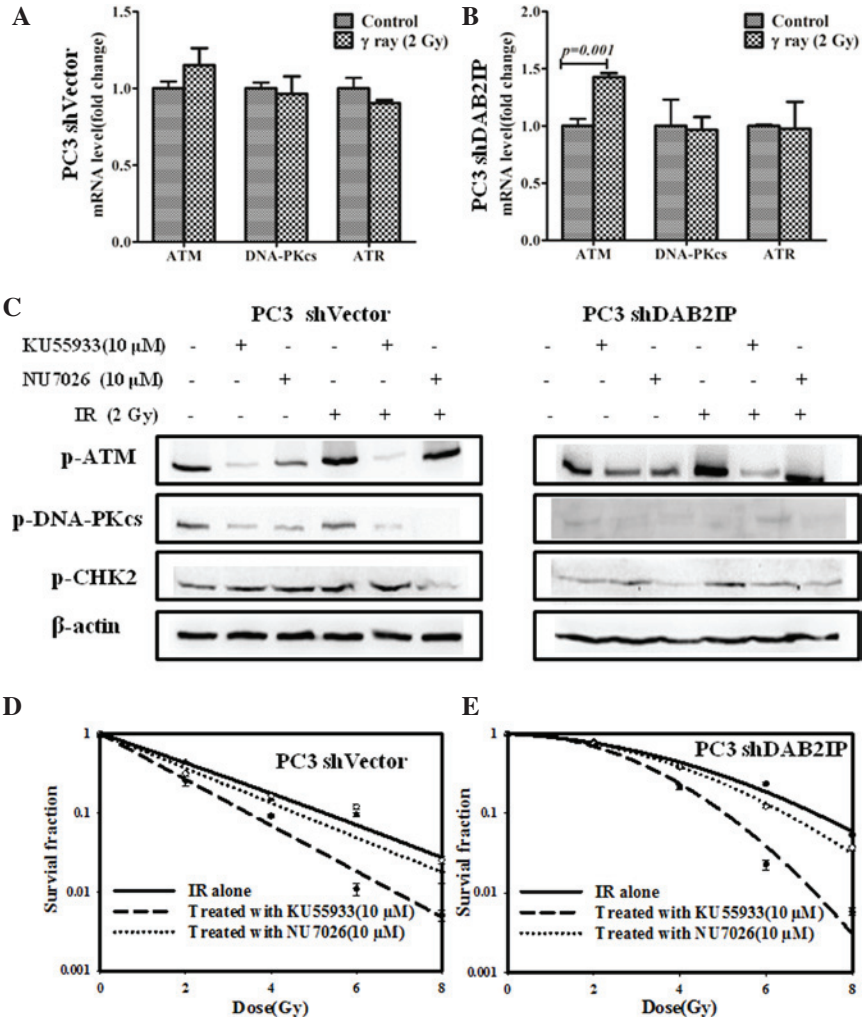

Figure 3. Increased ATM mRNA expression levels were observed in PC3 shDAB2IP cells in response to 2 Gy γ-ray irradiation. Relative mRNA levels of ATM, DNA-PKcs and ATR in (A) PC3 shVector cells and (B) PC3 shDAB2IP cells 24 h after 2 Gy γ-ray irradiation were determined by reverse transcription-quantitative polymerase chain reaction vs. the control. (C) Whole cell lysates were collected 30 min after irradiation and analyzed by western blotting for ATM, DNA-PKcs, CHK2 proteins. Radiosensitivity of (D) PC3 shVector cells and (E) PC3 shDAB2IP cells to irradiation combined with KU55933 or NU7026 were measured by colony formation assay. KU55933 or NU7026 were added to the medium 1 h prior to irradiation to a final concentration of 10 μM. P-values are presented above the error bars vs. control group. The results are presented as the mean of three experiments ± standard error of the mean. shDAB2IP, short haripin disabled homolog 2 interactive protein; shVector, short hairpin vector; IR, ionizing radiation; p-ATM, phospho-ataxia-telangiectasia mutated; p-DNA-PKcs, phospho-DNA-dependent protein kinase catalytic subunit; ATR, ataxia-telangiectasia and Rad3 related protein; p-CHK2, checkpoint kinase.

which usually initiates IR-induced DDR in ATM deficient cells (19), was downregulated in IR-treated PC3 cells. This negative association between DAB2IP and ATM expression was also observed in our previous studies (12,14). DAB2IP is normally distributed in the cytoplasm, whereas ATM is located in the nucleus. The association between DAB2IP and ATM remains to be elucidated. Di Minin *et al* (21) reported that the tumor necrosis factor-dependent transcriptional profile via the nuclear factor-κB and mitogen-activated protein kinase 8 signaling pathway may be induced by the combination of DAB2IP and mutant p53 in the cytoplasm. It is also suggested that DAB2IP may impact ATM expression by feedback regulation, including depleting the substrates of ATM (p53 for example). In addition, tumor cells surviving from fractionated irradiation are usually resistant to IR (22). Therefore, it is possible that DAB2IP deficiency due to long-term exposure to IR may be one of the reasons for the occurrence of acquired radioresistance. It is of note, that at the beginning of the current study, the clinical radiotherapy plan was followed and cells were exposed to 2 Gy for 5 days at 2 day intervals. It was observed that when the accumulated dose >12 Gy was applied, PC3 cells were severely damaged and >10 days were required for cells to recover and continue to proliferate. However, when an IR period of 3 days was selected, along with a 2 day recovery period described in Fig. 1A, cells required a shorter recovery time. Therefore, the fractionated-irradiation schedule does not match the clinical plan.

In order to demonstrate the effect of DAB2IP on the DDR signaling pathway, endogenous DAB2IP of PC3 cells was knocked down using shRNA-lentiviral system and it was observed that low expression of DAB2IP resulted in cells resistance to irradiation by γ-rays and α-particles. Compared with sparely IR of γ-rays, high-LET particles (α-particle) have a higher relative biological effectiveness (RBE) as they lead to more severe and complex damage to DNA, which is more difficult to repair (23). When ^{137}Cs sourced γ-rays were used as reference radiation, the RBE of α-particles was estimated

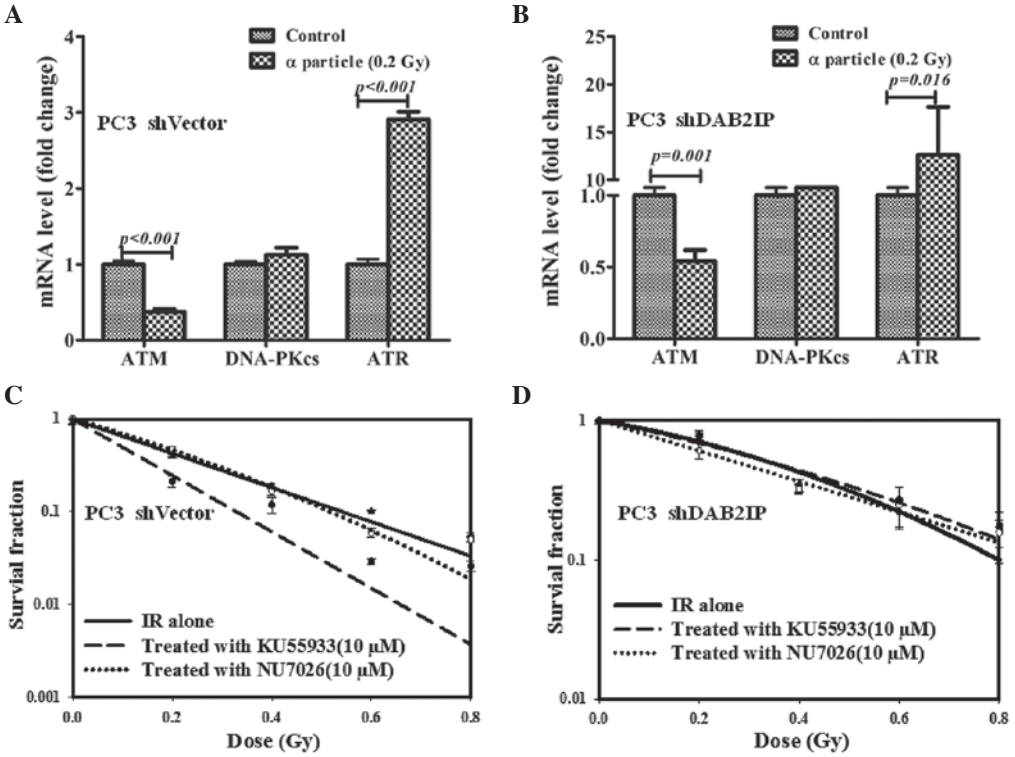

Figure 4. KU55933 did not influence the sensitivity of DAB2IP-deficient cells to α-particles. Relative mRNA levels of ATM, DNA-PKcs and ATR in (A) PC3 shVector cells and (B) PC3 shDAB2IP cells 24 h post 0.2 Gy irradiation of α-particle were determined by reverse transcription-quantitative polymerase chain reaction vs. control group. Radiosensitivity of (C) PC3 shVector cells and (D) PC3 shDAB2IP cells to α-particle combined with KU55933 or NU7026 were determined by colony formation assay. KU55933 or NU7026 was added to the medium 1 h prior to irradiation to a final concentration of 10 μM. P-values are presented above the error bars vs. control group. The results are presented as the mean of three experiments ± standard error of the mean. shDAB2IP, short haripin disabled homolog 2 interactive protein; shVector, short hairpin vector; IR, ionizing radiation; ATM, ataxia-telangiectasia mutated; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; ATR, ataxia-telangiectasia and Rad3 related protein.

at 9.2 and 11 for 10% survival of PC3 shVector and PC3 shDAB2IP cells, respectively. When comparing the cellular response at biologically equivalent doses, it was observed that the change of cells DDR signaling pathway upon γ-rays at 2 Gy with α-particles at 0.2 Gy, which demonstrate similar cell death rates in the present study. It was determined that ATM mRNA expression in PC3 shDAB2IP cells was significantly upregulated in γ-ray-treated cells; however, it was significantly reduced in cells exposed to α-particles. It is of note that a significant increase in ATR mRNA expression was observed in α-particles-irradiated DAB2IP-deficient cells (>15 fold vs. the control group). This trend of ATM and ATR activation following IR was also reported by Xue *et al* (24). Their work also supported that ATR is important for checkpoint regulation following heavy ion beams compared with low-LET radiation. It suggested that ATR may be more important in conferring cellular response to high-LET irradiation. In addition, ATR inhibitors, including VE281 and VE822, may be used to enhance the sensitivity of DAB2IP-deficient cells to high-LET radiation.

ATM phosphorylation is a conserved response to IR across multiple tumor types. KU55933, an ATM inhibitor, may reduce ATM activation in response to low and high-LET radiation (12,25), it attenuated the survival of PC3 shDAB2IP and PC3 shVector cells in response to γ-rays in the current study. However, pretreatment with KU55933 prior to IR did not impact the sensitivity of shDAB2IP cells to α-particles. In addition, NU7026, a DNA-PKcs inhibitor, was used as a negative control for the current study as DNA-PKcs mRNA expression was not affected by γ-rays or α-particles. As expected, inhibition of DNA-PKcs did not influence the radiosensitivity of cells to the two types of radiation.

In conclusion, the results of the present study indicate that DAB2IP may be involved in forming acquired radioresistance in PC3 cells. DAB2IP-deficient cells are resistant to low and high-LET radiation using different mechanisms. DAB2IP-deficient cells are resistant to both γ-rays and α-particles. ATM could be the key molecule mediating the cells' response to low-LET irradiation, whereas the ATR signaling pathway is involved in the resistance to high-LET radiation. Inhibited ATM activation did not enhance the sensitivity of DAB2IP-negative cells to high-LET radiation, which may be due to the increased ATR mRNA expression in the cells.

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