The association of changes in RAD51 and survivin expression levels with the proton beam sensitivity of Capan-1 and Panc-1 human pancreatic cancer cells

MIN-GU LEE, KYU-SHIK LEE and KYUNG-SOO NAM

Department of Pharmacology and Intractable Disease Research Center, School of Medicine, Dongguk University, Gyeongju, Gyeongsanbuk-do 38066, Republic of Korea

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Abstract. Fewer than 20% of patients diagnosed with pancreatic cancer can be treated with surgical resection. The effects of proton beam irradiation were evaluated on the cell viabilities in Panc-1 and Capan-1 pancreatic cancer cells. The cells were irradiated with proton beams at the center of Bragg peaks with a 6-cm width using a proton accelerator. Cell proliferation was assessed with the MTT assay, gene expression was analyzed with semi-quantitative or quantitative reverse transcription-polymerase chain reaction analyses and protein expression was evaluated by western blotting. The results demonstrated that Capan-1 cells had lower cell viability than Panc-1 cells at 72 h after proton beam irradiation. Furthermore, the cleaved poly (ADP-ribose) polymerase protein level was increased by irradiation in Capan-1 cells, but not in Panc-1 cells. Additionally, it was determined that histone H2AX phosphorylation in the two cell lines was increased by irradiation. Although a 16 Gy proton beam was only slightly up-regulated cyclin-dependent kinase inhibitor 1 (p21) protein expression in Capan-1 cells, p21 expression levels in Capan-1 and Panc-1 cells were significantly increased at 72 h after irradiation. Furthermore, it was observed that the expression of DNA repair protein RAD51 homolog 1 (RAD51), a homogenous repair enzyme, was decreased in what appeared to be a dose-dependent manner by irradiation in Capan-1 cells. Contrastingly, the transcription of survivin in Panc-1 was significantly enhanced. The results suggest that RAD51 and survivin are potent markers that determine the therapeutic efficacy of proton beam therapy in patients with pancreatic cancer.

Correspondence to: Professor Kyung-Soo Nam or Dr Kyu-Shik Lee, Department of Pharmacology and Intractable Disease Research Center, School of Medicine, Dongguk University, 123 Dongdaero, Gyeongju, Gyeongsanbuk-do 38066, Republic of Korea
E-mail: namks@dongguk.ac.kr
E-mail: there1@dongguk.ac.kr

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Introduction

Pancreatic cancer is an intractable disease with a 5-year survival rate of <5% (1). Additionally, even if pancreatic cancer tumors are identified at an early stage without symptoms, the prognosis of the patients and recurrence of the tumors tend to be worse and higher, respectively, than other cancers (2). Surgical resection is considered in 15-20% of patients due to late-stage diagnosis (3). Therefore, chemotherapy and radiation therapy are treatment modalities used for patients with pancreatic cancer who cannot undergo surgical resection (4). However, these therapeutic strategies do not fully improve the survival rate and prognosis in pancreatic patients (2). In spite of its limitations, radiotherapy is still being used for the treatment of patients with pancreatic cancer (1).

In recent years, high-energy radiation therapies using X-rays, gamma rays and proton beams are being used to improve the therapeutic efficiency of various cancer patients (5). Among these, X-ray radiation therapy has been demonstrated to be the most common tool to treat cancer patients (5). However, physical and molecular biological damage in normal adjacent tissues close to tumor tissues are further induced by X-ray radiation (6,7). Therefore, X-ray radiation may not be a useful therapeutic tool to treat pancreatic cancer with high dose radiation. In contrast, proton beam (PB) therapy is a newly proposed radiation therapeutic tool that can irradiate the target tumors with high-dose energy, minimizing injuries to normal adjacent tissues (8). Clinically, PB therapy has been used to treat intractable cancers, including brain, eye, neck and liver cancers (9,10).

During radiation therapy, cell death occurs in tumor tissues via cell cycle arrest induced by radiation-mediated DNA damage (11). However, cell death is determined by radiosensitivity, which depends on the type of cancer and cancer cell (12). Radiosensitivity is an important measurement in radiation therapy to determine treatment efficiency (13). However, to date, there is no diagnostic tool for evaluating radiosensitivity.

Several studies have revealed that the expression of DNA repair protein RAD51 homolog 1 (RAD51), a DNA repair

protein, and survivin, a member of protein family responsible for the inhibition of apoptosis, affect radioresistance (14,15). RAD51 is a key protein that repairs double-stranded DNA damage; increased RAD51 expression has been observed in various cancers (16). A number of studies have suggested that RAD51 is a potent target for cancer therapy, demonstrating that radiation- and chemo-sensitivities were induced by the inhibition of RAD51 expression (16-21). Survivin serves a role in regulating cell cycle, cell protection and cell division, inhibiting apoptosis by blocking caspase (22,23), and has been reported as a radioresistance factor in pancreatic cancer (24). In addition, several investigations have suggested that survivin may be a potent target for cancer treatment due to its overexpression in various human tumors (25,26).

In the current study, the effects of PB irradiation were observed on the cell survival of two human pancreatic cell lines: Capan-1, which has been demonstrated to express high levels of cyclooxygenase (COX)-2, and Panc-1, which has been revealed to express low levels of COX-2 (27). Several investigations determined an association between radiosensitivity and COX-2 expression (28,29). Therefore, the effects of PB on cell survival were assessed in the two pancreatic cancer cell lines to confirm whether COX-2 expression level correlated with radiosensitivity. Additionally, the regulation of survivin and RAD51 expression by PB therapy was investigated, as they are factors that influence radioresistance.

Materials and methods

Cell culture. Human pancreatic cancer cell lines, Capan-1 and Panc-1, were purchased from the Korean Cell Line Bank (Seoul, Korea). Capan-1 and Panc-1 cells were cultured in Roswell Park Memorial Institute-1640 medium and Dulbecco's modified Eagle's medium supplemented with 1% antimycotic/antibiotic solution (all Welgene, Inc., Gyeongsan, Korea) and 10% heat-inactivated fetal bovine serum (American Type Culture Collection, Manassas, VA, USA) at 37˚C in a 5% CO_2 atmosphere.

PB irradiation. PB irradiation was performed using a 100 MeV proton accelerator in Korea Multi-Purpose Accelerator Complex at Korean Atomic Energy Research Institute (Gyeongju, Korea). The cells were irradiated with PB at a dose of 2, 4, 8 or 16 Gy with Bragg peaks width of 6 cm (30).

Cell cytotoxicity assay. A total of 5,000 cells/well were seeded onto 96-well plates. The cells were further cultured for 72 h at 37˚C after PB irradiation. Then, 20 μl of 5 mg/ml MTT reagent (Sigma Aldrich; Merck KGaA, Darmstadt, Germany) was added in each well and further incubated at 37˚C for 4 h to generate formazan. Insoluble formazan was dissolved with dimethyl sulfate (Duksan Pure Chemicals Co., Ltd., Ansan, Korea) and was colorimetrically analyzed by measuring optical density at 540 nm using Spectramax M2 (Molecular Devices, LLC, Sunnyvale, CA, USA).

Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) and RT- quantitative (q)PCR. The cells were irradiated with a PB and cultured for an additional 72 h. Then, the cells were trypsinized and collected by centrifugation at room temperature for 3 min with 1,000 x g. Total RNA extraction was performed with the easy-BLUE™ Total RNA extraction kit (iNtRON Biotechnology Inc., Sungnam, Korea) according to the manufacturer's protocol. Total RNA (1 μg) was used for cDNA synthesis using dNTP (final concentration, 0.5 mM), Goscript™ Reverse Transcriptase (both Promega Corporation, Madison, WI, USA) and Random Primer pd(N)9 (Takara Bio, Inc., Otsu, Japan). The reaction was performed in 1X Goscript reaction buffer containing 2 mM $MgCl_2$ (Promega Corporation). Amplification of synthesized cDNA was performed as previously described (31). The primer sequences for target genes were as follows: COX-2, forward 5'-TTCACG CATCAGTTT TTCAA-'3 and reverse 5'-ACAGCAAACCGT AGATGCTC-3' (32); survivin, forward 5'-ATTTGAATCGCG GGACCCGTTG-3' and reverse 5'-TGGCTCGTTCTCAGT GGGGCAGT-3' (33); GAPDH, forward 5'-ATCCCATCACCA TCTTCCAG-3' and reverse 5'-TTCTAGACGGCAGGTCAG GT-3' (34). All PCR primers were synthesized from Bioneer Corporation (Daejeon, Korea). For semi-quantitative RT-PCR analysis, PCR amplicons were synthesized with Dream Taq polymerase (Promega Corporation) and the thermocycling conditions were as follows: For COX-2, pre-heating for 10 min at 95˚C, 35 cycles of 95˚C for 30 sec, 60˚C for 30 sec and 72˚C for 45 sec, and final extension for 10 min at 72˚C; for GAPDH, pre-heating for 10 min at 95˚C, 25 cycles of 95˚C for 30 sec, 60˚C for 30 sec and 72˚C for 30 sec, and final extension for 10 min at 72˚C. The amplicons were then electrophoresed on 1% agarose gels containing EtBr (Amresco, LLC, Solon, OH, USA) and the bands were visualized with a UV transilluminator. Bands densities were analyzed with Scion Image software (Alpha 4.0.3.2; Scion Corporation, Frederick, MD, USA). qPCR analyses were performed with Q Green Sybr Green Master Mix Kit (Cellsafe Co., Ltd., Yongin, Korea) using Eco™ Real-Time PCR (Illumina, Inc., San Diego, CA, USA). The thermocycling conditions were as follows: pre-heating for 5 min at 95˚C, 45 cycles of 95˚C for 10 sec, 60˚C for 15 sec and 72˚C for 20 sec. Relative mRNA expression was automatically determined using Eco™ software v3.1.7 (Illumina, Inc.) via the $2^{-\Delta\Delta Cq}$ method (35). GAPDH was used as the internal control.

Western blot analysis. Cells were lysed with radioimmunoprecipitation assay lysis buffer (Biosesang, Seongnam, Korea) containing protease inhibitor cocktail and phosphatase inhibitor cocktail (GenDEPOT, LLC, Barker, TX, USA). Whole cell lysates were centrifuged at 13,000 x g for 20 min at 4˚C and stored at -80˚C until to use. The protein concentration was measured with the bicinchoninic acid method. Total protein (20 μg/lane) was subjected to SDS-PAGE on 12 or 15% gel and transferred to polyvinylidenefluoride (PVDF) membrane (Pall Life Science, Port Washington, NY, USA). The membranes were blocked with 5% non-fat dry milk in Tris-buffered saline (TBS)-Tween (50 mM Tris-HCl, 150 mM NaCl, 0.1% Tween-20) for 1 h at room temperature. The primary antibodies were diluted at 1:3,000 in 5% non-fat dry milk or 1% bovine serum albumin (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) in TBS-Tween solution and were probed for overnight at 4˚C onto PVDF membrane. Then, secondary antibodies were diluted at 1:5,000 in TBS and the reaction was performed at room temperature for 1 h. The bands for target

proteins were visualized with handmade chemiluminascent substrate [100 mM Tris (pH 8.5; BioShop Canada, Inc., Burlington, ON, Canada), 1.25 mM luminol, 198 μM coumaric acid and 0.01% hydrogen peroxide (all Sigma Aldrich; Merck KGaA)] and photographed using Luminescent Image Analyzer LAS-4000 (Fujifilm Corporation, Tokyo, Japan). The bands were densitometrically analyzed with Scion Image software (Alpha 4.0.3.2). The primary antibodies against RAD51 (cat. no. sc-8349) and β-actin (cat. no. sc-69879) were purchased from Santa Cruz Biotechnology, Inc., and antibodies for histone 2AX (H2A.X; cat. no. 7631), phospho-H2A.X (cat. no. 9718), cyclin-dependent kinase inhibitor 1 (p21; cat. no. 2947), poly (ADP-ribose) polymerase (PARP; cat. no. 9542), caspase-7 (cat. no. 9492), caspase-3 (cat. no. 9662), signal transducer and activator of transcription 3 (STAT3; cat. no. 4904), phospho-STAT3 (cat. no. 9145) and survivin (cat. no. 2808) were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Horseradish peroxidase-conjugated goat anti-rabbit (cat. no. NCI1460KR) and anti-mouse (cat. no. sc-2005) immunoglobulin G antibodies were bought from Thermo Scientific Fisher Scientific, Inc. and Santa Cruz Biotechnology, Inc., respectively.

Statistical analysis. Statistical significance was determined using the Student's t-test or a one-way analysis of variance followed by the Tukey's post hoc test. Statistical analyses were conducted using SPSS V20.0 software (SPSS, Inc., Chicago, IL, USA). Data are presented as the mean ± standard deviation. $P<0.05$ indicated that the difference between groups was statistically significant.

Results

Cell viability decreases in Capan-1 cells following PB irradiation. The inhibition of pancreatic cancer cell growth by a COX-2 inhibitor, celecoxib, was demonstrated in a previous investigation (36). Shin *et al* (29) demonstrated that celecoxib treatment enhanced radiosensitivity in various cancer cells, suggesting that COX-2 expression is closely associated with radiosensitivity. Therefore, the change of cell viability by PB irradiation was assessed in the two pancreatic cancer cells, Capan-1 cells, which expressed high levels of COX-2 mRNA, and Panc-1 cells, which expressed low level of COX-2 mRNA (Fig. 1A). Unexpectedly, it was demonstrated that there was a significantly lower cell viability following PB irradiation in Capan-1 cells compared with control cells (Fig. 1B). No significant differences were identified in the cell viability of Panc-1 cells.

PB irradiation increases the expression of phosphorylation of H2A.X and the expression of p21. Cell death and cell cycle arrest by PB irradiation have been determined to be closely linked with DNA damage in various cancer cells (37). As shown in Fig. 1B, higher cytotoxicity following PB was observed in Capan-1, but not Panc-1 cells. Therefore, the association between cytotoxicity and DNA damage was examined through the change of phosphorylation of H2A histone family, member X (H2A.X), a histone protein phosphorylated by DNA damage. The phosphorylation of H2A.X in Capan-1 and Panc-1 cells was significantly increased by PB irradiation compared

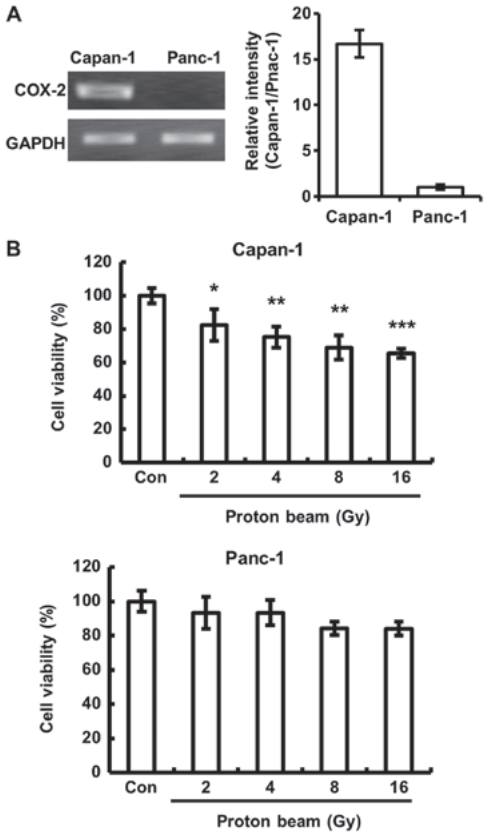

Figure 1. Cell viability decreases in Capan-1 cells following protein beam irradiation. (A) COX-2 mRNA expression level in Panc-1 and Capan-1 human pancreatic cancer cells. mRNA expression was assessed using a semi-quantitative reverse transcription-polymerase chain reaction analysis. (B) Cell viability of Capan-1 and Panc-1 cells measured 72 h after irradiation with 2, 4, 8 or 16 Gy proton beams. Cell viability was assessed using an MTT assay. The experiments were independently performed three times in triplicate. Values are presented as mean ± standard deviation. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. Con. COX, cyclooxygenase; Con, control.

with control cells (Fig. 2A). This result suggests that DNA damage by PB irradiation increased with dosage in Capan-1 and Panc-1 cells. Additionally, the effect of PB irradiation on cell cycle arrest was investigated. p21 protein expression levels in the two cells were increased by PB compared with control cells, indicating the occurrence of cell cycle arrest (Fig. 2B). However, 16 Gy of PB irradiation did not enhance p21 protein expression in Capan-1 cells.

PB induces PARP cleavage in a caspase-3-independent manner. PARP serves an important role in the repair of damaged DNA caused by a variety of cellular stresses (38). Also, cleaved PARP (~89 kDa) is known as a marker for apoptotic cell death (39). Therefore, PARP cleavage by PB irradiation was explored to determine whether Capan-1 cell death by PB was due to apoptosis. Cleaved PARP significantly increased with PB irradiation, while the intact form of PARP was significantly decreased with PB irradiation in Capan-1 cells compared with control cells (Fig. 3A). However, in spite of significant decreases in PARP due to PB irradiation in Panc-1 cells compared with control cells, PARP cleavage was not detected in Panc-1 cells. Cleaved PARP is primarily produced by caspase-3 (40). Therefore, the authors of the current study postulated that the decrease of PARP in Panc-1 cells is caused

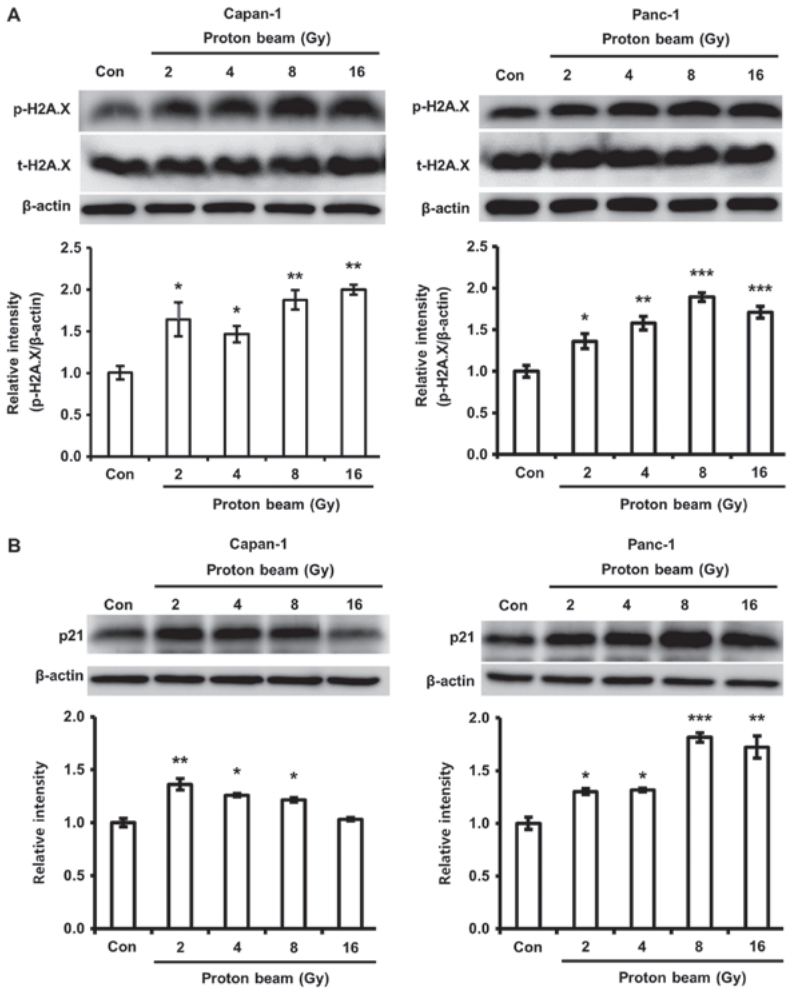

Figure 2. Proton beam irradiation increases the expression of phosphorylation of H2A.X and the expression of p21. Protein expression levels were assessed 72 h after irradiation with 2, 4, 8 or 16 Gy proton beam using western blot analyses. (A) Phosphorylation of H2A.X in Capan-1 and Panc-1 human pancreatic cells. (B) p21 expression in Capan-1 and Panc-1 human pancreatic cells. Values are presented as mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.005 vs. Con. H2A.X, histone H2AX; p21, cyclin-dependent kinase inhibitor 1; p-, phosphorylated; t-, total; Con, control.

by the down-regulation of caspase-3-independent cleavage. Based the results, the effects of PB irradiation on caspase-3 activation was investigated to confirm whether the cleavage of PARP was mediated in a caspase-3-dependent manner. As shown in Fig. 3B, the cleaved forms of caspase-3 and -7 were not detected in either cell line.

PB irradiation decreases STAT3 phosphorylation. The importance of STAT3 on cell cycle arrest and apoptosis in chemotherapy and radiotherapy was reported by previous investigations (41,42). Li *et al* (43) revealed that the down-regulation of STAT3 by short hairpin RNA led to an increase of radiosensitivity. Furthermore, the enhancement of apoptosis and cell cycle arrest by the inhibition of STAT3 signaling was demonstrated in colorectal cancer cells (42). Therefore, the authors of the current study postulated that STAT3 signaling may be associated with cell cycle arrest and/or cell death in Capan-1 and Panc-1 cells. Phosphorylated STAT3 was significantly reduced by PB irradiation in Capan-1 and Panc-1 cells compared with control cells (Fig. 4). The decrease of phosphorylated STAT3 by PB irradiation corresponded with an increase of H2A.X phosphorylation and p21 expression in the Capan-1 and Panc-1 cells.

PB irradiation decreases RAD51 expression in Capan-1 cells. The fate of DNA-damaged cancer cells by chemotherapy and radiotherapy is determined by whether DNA damage is repaired (44,45). Therefore, the activities of DNA repair enzymes have been demonstrated to be closely associated with cell death and cell cycle arrest (45). RAD51, a homologous recombination repair enzyme, serves an important role in the repair of radiation-induced DNA damage and has been implicated as a radiosensitivity determinant (46). The change in RAD51 expression by PB was surveyed. It was demonstrated that a significant decrease of RAD51 protein expression by PB irradiation was identified in Capan-1 cells compared with control cells, but not in Panc-1 cells (Fig. 5). This finding corresponds to the change of cell viability by PB irradiation in Capan-1 and Panc-1 cells.

PB irradiation increases survivin protein and mRNA expression in Panc-1 cells. A variety of survival factors participate in determining whether cells die or survive from chemotherapy and radiotherapy. Survivin is one of the survival factors that has been considered as a determinant of cell sensitivity to radiation in pancreatic cancer cells (27). Therefore, change in survivin expression

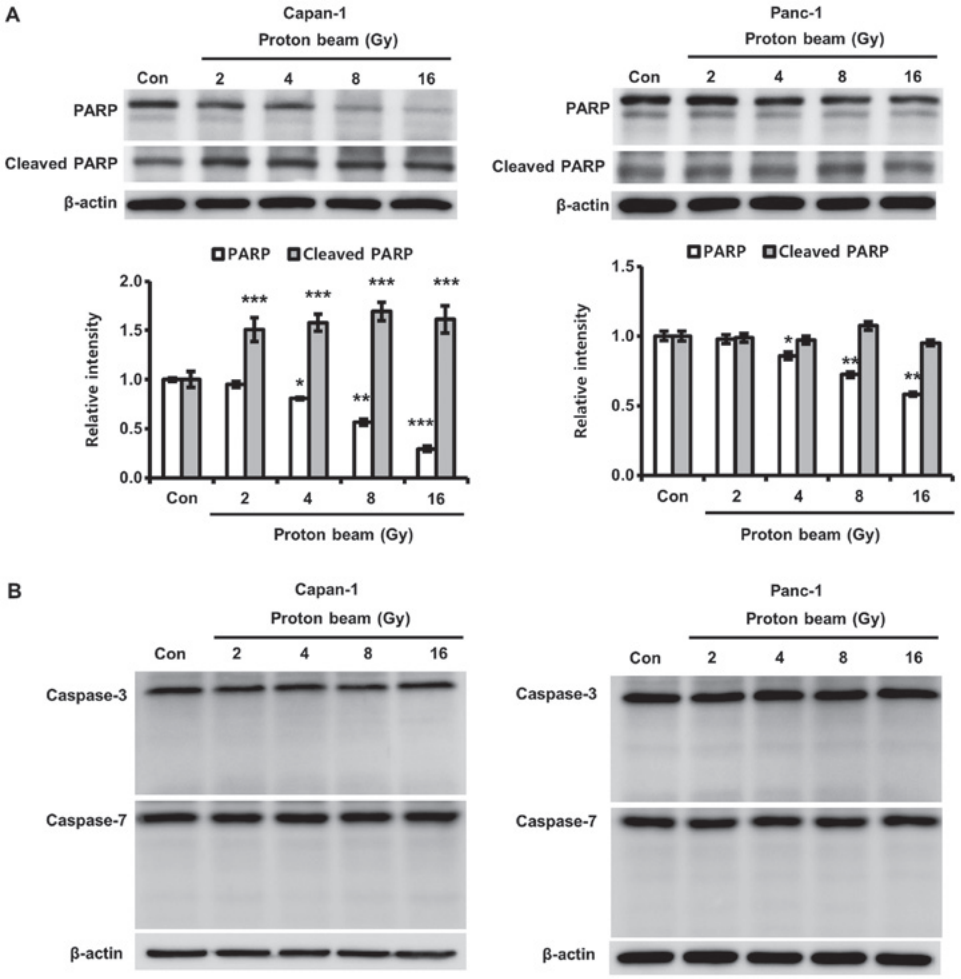

Figure 3. Proton beam induces PARP cleavage in a caspase-3-independent manner in Capan-1 cells. Protein expression levels were assessed 72 h after irradiation with 2, 4, 8 or 16 Gy proton beam using western blot analyses. (A) PARP cleavage in Capan-1 and Panc-1 human pancreatic cells. Values are presented as mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.001 vs. Con. (B) Caspase-3 and caspase-7 cleavage in Panc-1 and Capan-1 cells. PARP, poly (ADP-ribose) polymerase; Con, control.

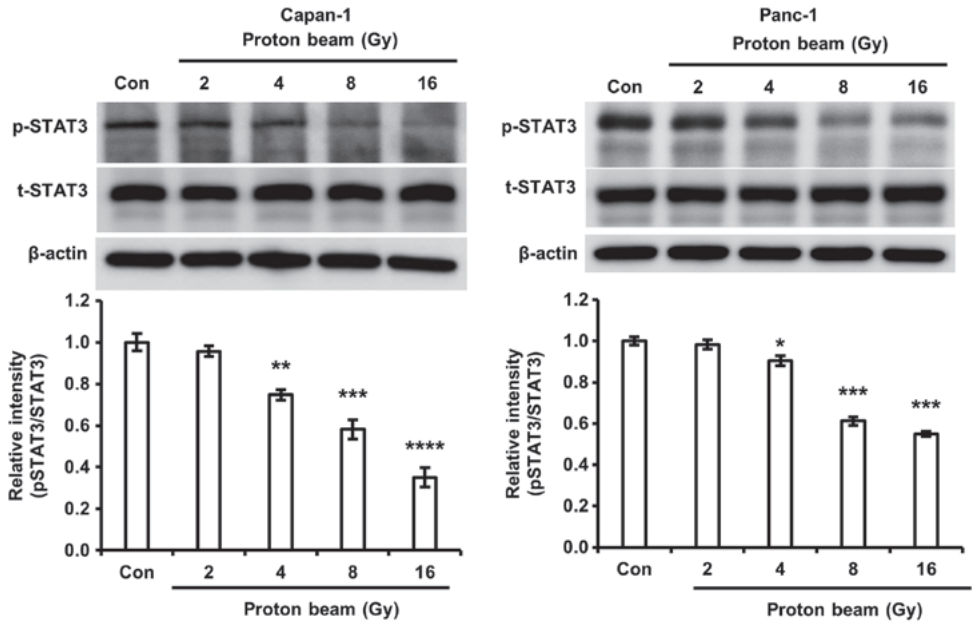

Figure 4. Proton beam irradiation decreases STAT3 phosphorylation. Protein expression levels were assessed 72 h after irradiation with 2, 4, 8 or 16 Gy proton beam using western blot analyses. The phosphorylation of STAT3 was assessed in Capan-1 and Panc-1 human pancreatic cells. Values are presented as mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0005 vs. Con. STAT3, signal transducer and activator of transcription 3; p-, phosphorylated; t-, total; Con, control.

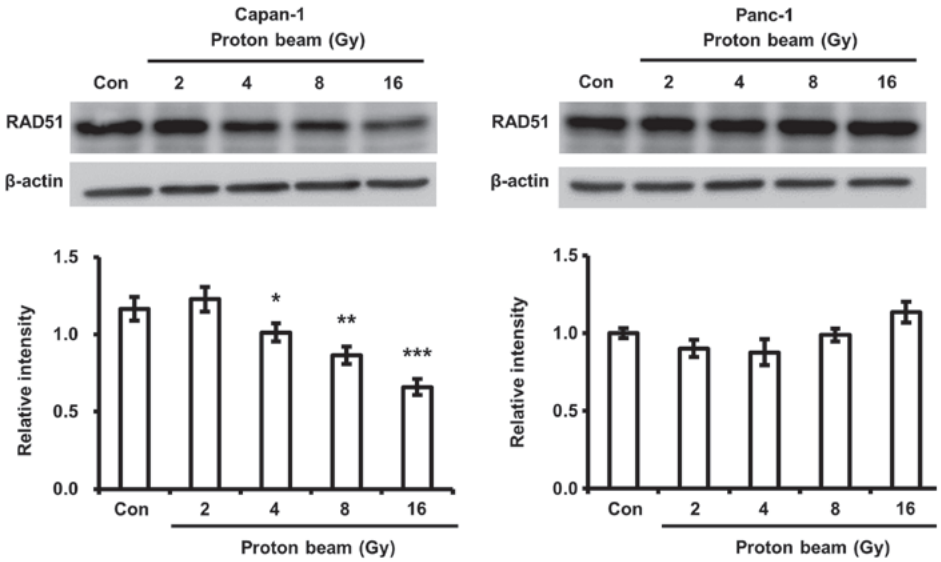

Figure 5. Proton beam irradiation decreases RAD51 expression in Capan-1 cells. The protein expression levels of RAD51 were assessed in Capan-1 and Panc-1 human pancreatic cells 72 h after irradiation with 2, 4, 8 or 16 Gy proton beam using western blot analysis. Values are presented as mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.001 vs. Con. RAD51, DNA repair protein RAD51 homolog 1; Con, control.

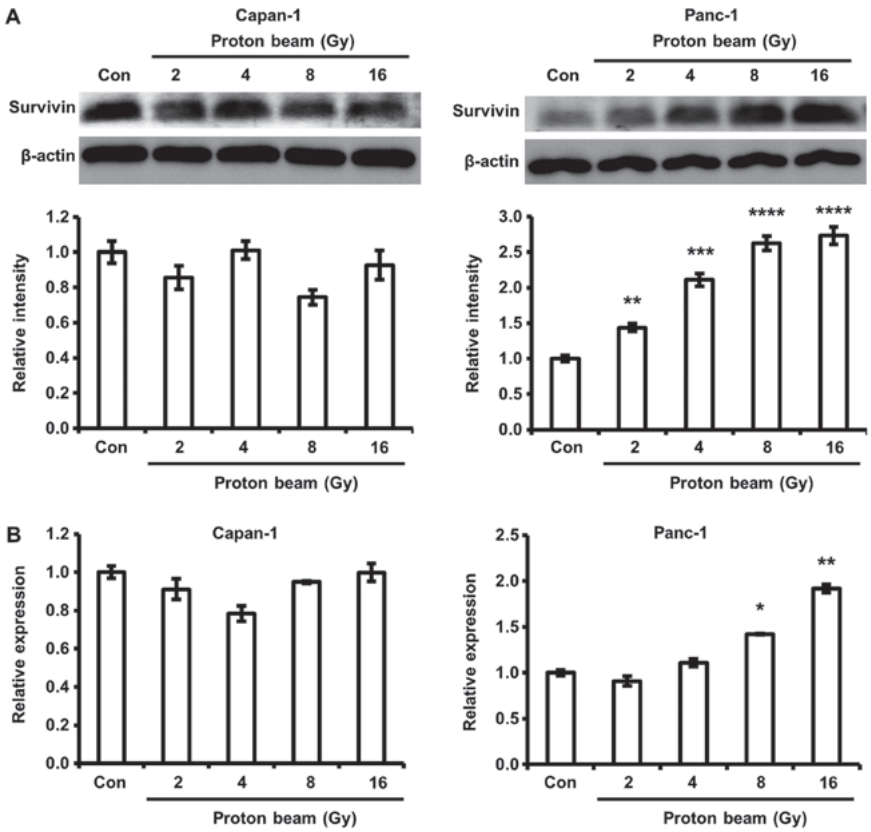

Figure 6. Proton beam irradiation increases survivin protein and mRNA expression in Panc-1 cells. Protein and mRNA expression levels were assessed 72 h after irradiation with 2, 4, 8 or 16 Gy proton beam using western blot and reverse transcription-quantitative polymerase chain reaction analyses, respectively. The (A) protein and (B) mRNA expression levels of survivin in Capan-1 and Panc-1 human pancreatic cells. Values are presented as mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0005 vs. Con. Con, control.

by PB irradiation was investigated in Capan-1 and Panc-1 cells. PB significantly increased survivin gene and protein expression in Panc-1 cells compared with control cells (Fig. 6). However, the survivin gene and protein in Capan-1 cells were not significantly increased by PB irradiation.

Discussion

Previous studies suggested that the radiosensitivities of several cancer cells were enhanced by celecoxib, a selective COX-2 inhibitor (28,29). Additionally, several experiments

demonstrated that COX-2 expression levels were associated with radioresistance in various cancer cells (47-49). These results imply that COX-2 may act as a determinant of radiosensitivity for cancer cells. The present investigation revealed that Capan-1 cells were more sensitive to PB irradiation than Panc-1 cells within 72 h. Also, the expression level of COX-2 in Capan-1 cells was higher than that of Panc-1 cells. Consequentially, this implies that the COX-2 expression level was not associated with radiosensitivity to PB irradiation. Therefore, the present investigation demonstrated, at least to some degree, that COX-2 may not act as a determinant of radiosensitivity for PB irradiation in Capan-1 and Panc-1 human pancreatic cancer cells in the first 72 h after irradiation.

As mentioned earlier, STAT3 signaling has been revealed to be associated with radiosensitivity, cell cycle arrest and apoptosis induced by anti-cancer drugs and radiation (41-43). The current study determined that an increase in H2A.X phosphorylation and a decrease of STAT3 phosphorylation occurred simultaneously when the cells were irradiated with a PB. Chen *et al* (50) revealed that the inhibition of phosphorylated STAT3 was linked with an increase of H2A.X phosphorylation, indicating DNA damage. Furthermore, Wen *et al* revealed that apoptosis was enhanced with the phosphorylation of H2A.X by mammalian STE20-like kinase 1 (51). These findings indicate that apoptotic cell death induced by DNA damage was increase by an induction of H2A.X phosphorylation, suggesting that the cell death of Capan-1 cells by PB irradiation is regulated by STAT3-mediated H2A.X phosphorylation. However, the regulation of STAT3-mediated H2A.X phosphorylation did not affect the viability of Panc-1 cells in the first 72 h after irradiation.

DNA damage-mediated cell death is primarily regulated by caspase-3 dependent apoptosis; the process is accompanied by PARP fragmentation (52). In the present study, the authors observed the induction of PARP cleavage in Capan-1 cells by PB irradiation. However, increases in cleaved caspase-3 and -7 were not detected in Capan-1 cells. The loss of PARP function by fragmentation has been demonstrated to cause cell death (40). Therefore, Capan-1 cell cell death by PB irradiation is mediated by PARP fragmentation in a caspase-3-independent manner.

Additionally, the current study revealed a decrease in PARP expression by PB irradiation in Panc-1 cells without the induction of cell death and PARP fragmentation. Powel *et al* (53) revealed that PARP inhibitors act as tumor-specific radiosensitizers in pre-clinical and clinical studies; implying that radiosensitivity should be increased by a down-regulation of PARP expression. Furthermore, hyperradiosensitivity was elicited by PARP silencing in HeLa cells (54). However, the association between radiosensitivity and a decrease in PARP expression in Capan-1 and Panc-1 was not identified in the present study. Therefore, the present study suggests that PARP may not act as a determinant of radiosensitivity for PB irradiation, at least in Capan-1 and Panc-1 cells in the first 72 h after irradiation.

Yu *et al* (55) previously revealed that a novel STAT3 activation inhibitor induced cell cycle arrest and apoptosis in HL-60 and K562 human myeloid leukemia cell lines. Those results implied that increased p21 expression, a cell cycle arrest marker protein, is correlated with the inhibition of STAT3 phosphorylation. In the current study, the authors observed that increases in P21 expression by PB irradiation were detected in the two cells investigated. The increase corresponded with a decrease of STAT3 phosphorylation. Cell cycle arrest has been demonstrated to be closely associated with DNA damage (52). Therefore, an increase in H2A.X phosphorylation is linked with the induction of p21 expression. A close association between an increase in H2A.X phosphorylation and p21 expression was identified in the current study. Furthermore, the changes were coupled with decreased STAT3 phosphorylation, except 16 Gy PB-irradiated Capan-1 cells. These results suggest that PB irradiation should induce cell death and/or cell cycle arrest through the regulation of STAT3 signaling, regardless of radiosensitivity.

The survival of DNA-damaged cancer cells is determined by whether cancer cells can repair damage to DNA (44,45). The current study revealed that the viability of Capan-1 cells was decreased and not in Panc-1 cells, and the difference was associated with a decrease of RAD51 expression. The result suggests that the weakening of homologous recombination repair activity mediated by the down-regulation of RAD51 expression causes higher cytotoxicity in Capan-1 cells following PB irradiation, but not Panc-1. Furthermore, lower radiosensitivity of Panc-1 was associated with an increase in survivin expression by PB irradiation. RAD51 and survivin are known to be dependent on radiosensitivity. Studies have demonstrated that the expression levels of RAD51 and survivin were associated with radiosensitivity (27,46,55). However, a correlation between radiaosensitivity and changes in RAD51 and survivin expression has not been identified in previous investigations. In the present study, it was revealed that the inhibition of RAD51 protein expression by PB was associated with an increase of cell death in Capan-1 cells. The results demonstrate that radiosensitivities of Capan-1 and Panc-1 cells following PB therapy may be determined by whether a PB leads to the reduction of RAD51 expression and/or the induction of survivin expression.

Taken together, although many previous studies reported the involvement of COX-2, PARP, RAD51, and survivin in radiosensitivity, the current study demonstrated that radiosensitivity in early stages (<72 h) of PB treatment may be predominantly determined by the regulation of RAD51 and/or survivin expression, at least in Capan-1 and Panc-1 cells. However, the regulation of COX-2 and PARP expression levels did not determine radiosensitivity. The current investigation demonstrates that changes in RAD51 and survivin expression are important roles in determining radiosensitivity and implies that a specialized strategy is necessary for improving the efficacy of PB therapy in patients with pancreatic cancer.

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Availability of data and materials

All the original data generated for this manuscript are available upon request.

Authors' contributions

KSL and KSN designed the experiment. MGL and KSL performed experiments. MGL, KSL and KSN analyzed the data and wrote the manuscript. All authors confirmed and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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