대한방사선종양학회지 2009;27(4):218~227 DOI: 10.3857/jkstro.2009.27.4.218

Different Expressions of HIF-1α, Bcl-2 and Baxin DU145 Prostate Cancer Cells Transplanted in Nude Mouse between X-Ray and Neutron Irradiation

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Purpose: To investigate the radiobiologic effects of neutron and X-ray irradiation on DU-145 prostate carcinoma cells by identifying the differences of HIF-1α expression and apoptosis.

Materials and Methods: Nude mice were injected with the human prostate cancer cell line, DU-145, and then irradiated with 2 Gy and 10 Gy X-rays, or 0.6 Gy and 3.3 Gy neutrons, respectively. The mice were sacrificed at 24 hours and 120 hours after irradiation. The expression levels of HIF-1α, Bcl-2 and Bax were compared with immunohistochemical staining and western blotting. The apoptotic indexes were compared with the Terminal deoxynucleotidyl biotin-dUTP nick and labeling (TUNEL) assay.

Results: At day 1, HIF-1α and Bcl-2 expression decreased, while Bax expression and the number of TUNEL positive cells increased in neutron irradiated groups for the control and X-ray irradiated groups. The Bcl-2/Bax ratio was significantly lower in the neutron irradiated groups regardless of dose (p=0.001). The same pattern of the differences in the expressions of the HIF-1α, Bcl-2, Bax, Bcl-2/Bax ratio, and apoptotic indexes were indentified at day 5. HIF-1α expression was related with Bcl-2 (p=0.031), Bax (p=0.037) expressions and the apoptotic indexes (p=0.016) at day 5.

Conclusion: Neutron irradiation showed a decrease in HIF-1α, Bcl-2 expression, and Bcl-2/Bax ratio, but increased Bax expression regardless of dose. This study suggests that the differences radiobiological responses between photon and neutron irradiation may be related to different HIF-1α expression and subsequent apoptotic protein expressions.

Key Words: HIF-1α, Bcl-2, Bax, Neutron, Prostate cancer

Introduction

Radiation therapy with high linear energy transfer (LET) beams such as neutrons or heavy ions have been discussed as a promising treatment modality for certain radiation resistant tumors.[1,2] Among them, fast neutrons were the first type of high-LET radiation used clinically, which showed negative results with unacceptable late complications due to suboptimal technical conditions. Nevertheless, a greater benefit from neutrons than from photon radiotherapy was found at several tumor sites including the prostate cancers.[3,4] Based on the fast neutron experience, radiobiological arguments for the potential clinical indications of neutron irradiations for slowly growing and well-differentiated photon-resistant tumors are still suggested. Physically, the benefits of neutrons over photons come from the different mode of interactions within the cell. The neutrons directly interact with nuclei of atoms to produce heavy particles of dense ionizations and subsequently cause more critical DNA damages such as double strand break, which is less repairable. Thus the neutrons are more effective in killing hypoxic tumor cells and less dependent on the cell cycle phase than photons with a greater relative biologic effectiveness (RBE), which is usually in the range of 2 to 5.[5]

Submitted August 20, 2009, accepted November 12, 2009
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This work was supported by Nuclear Research & Development Program of National Research Foundation of Korea (NRF) funded by Ministry of Education, Science & Technology (MEST) (grant code: M20090078063).

Meanwhile, little investigation has been performed to find the molecular parameters implicating the biologic effect of neutron therapy on the hypoxic tumor cells. In this regard, the HIF-1α, which is a transcription factor critical for tumor adaptation to hypoxic microenvironment has been suggested to have a critical role in several tumors. For the prostate cancers, the expression of HIF-1α is known as an early event during their carcinogenesis.[6] But there are only few reports for the relationship between HIF-1α expression and prostate cancers.

Besides the role of HIF-1α in the hypoxic response, a possible role of HIF-1α in the modulation of apoptosis has been suggested. Previous study suggested that HIF-1α could display either a pro-apoptotic or an anti-apoptotic role according to the conditions.[7] Furthermore, neutron itself may induce the modulation of Bcl-2 and Bax resulting in apoptosis in a lymphoma cell line.[8] Thus in this study, the expression status of HIF-1α by X-ray and neutron irradiation and resultant apoptotic protein Bcl-2 and Bax expressions in prostate cancer cells transplanted in nude mouse were investigated.

Materials and Methods

1. Animals and tissue preparations

The human prostate carcinoma cell line DU-145 was purchased from the Korean Cell Line Bank (Seoul, Korea). The cells were cultured in Roswell Park Memorial Institute 1640 (Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco BRL) at 37°C in 5% CO_2, 95% O_2 in a humidified cell incubator. The medium was changed every 2 days. The cultured DU-145 cells were inoculated to the nude mice.

Nude mice weighing 20±1 g (5 weeks in age) were obtained from a commercial breeder (Charles River Technology; Orient Co., Seoul, Korea) for the experiment. The experimental procedures were performed in accordance with the animal care guidelines of National Institute of Health (NIH) and the Korean Academy of Medical Sciences. The animals were housed under specific pathogen-free conditions (20±2°C) lighting from 07:00 to 19:00 and were supplied with autoclaved food and water ad libitum.

Nude mice of tumor-treated groups received subcutaneous injections of approximately 5×10^6 DU-145 prostate cancer cells in 0.1 ml sterile phosphate buffered saline on the back of the mice. The tumors were allowed to grow in the animals for 2 weeks after the initial injection of the tumor cells.

The half of the animals were sacrificed at 24 hours after irradiation (day 1) for protein analysis and the rest of the animals were sacrificed at 120 hours after irradiation (day 5) for tumor weight comparing by cervical dislocation method, and tissue samples were collected. The tumor tissues were immediately frozen with Tissue-Tek® O.C.T compound (Sakura Finetek Inc, Torrance, CA, USA) at −70°C.

2. Irradiation

All animals were irradiated using close-fitting Perspex boxes (22 cm × 11 cm × 4 cm) at 2 weeks after DU-145 cell injection. The X-ray was irradiated at a dose rate of 240 cGy/min with a linear accelerator (Clinac 2100C, Varian Co., Pala Alto, CA, USA). Fast neutron was produced by the bombardment of beryllium by proton $^9Be(p, n)^{10}B$ as a nuclear reaction by the cyclotron (MC-50; Scanditronix, Uppsala, Sweden) located in the Korea Institute of Radiological and Medical Sciences (Seoul, Korea). Then, mean and maximum energy of neutron by this nuclear reaction are 34.4 MeV, 49.3 MeV, respectively. From the pooled data on the radiation sensitivity of the neutron beam of the cyclotron,[9,10] the biologically equivalent doses between X-ray and neutron were determined. The animals were divided into five groups (n=10 in each group): the tumor-treated non-irradiated group as a control, the tumor- treated 2 Gy, the tumor-treated 10 Gy X-ray irradiated group, the tumor-treated 0.6 Gy neutron (biologically equivalent dose to 2 Gy X-ray), the tumor treated 3.3 Gy neutron (biologically equivalent dose to 10 Gy X-ray) irradiated group.

3. Evaluation of relative tumor weight

The size of tumor was measured with digital camera three times during experiments by same observer. The size of tumor growing onto back in nude mice was measured using Image-Pro®Plus image analyzer (Media Cybernetics Inc., Silver Spring, MD, USA). The estimated tumor weight (ETW) was calculated using the formula: ETW mg=L mm×(W mm)^2/2, where L is the length and W is the width of the tumor. The relative tumor weight (RTW) was calculated, for the evaluation of the treatments, as follows: RTW=Wi/Wo, where Wi is the mean ETW at each time point and Wo is the mean ETW

at the start of the treatment.

4. Western blot analysis

The tumor cells were lysed in a ice-cold whole cell lysate buffer containing 50 mM HEPES (4-[2-hydroxyethyl]-1-piperavine-ethane-sulfonic acid) (pH 7.5), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM magnesium chloride hexahydrate, 1 mM ethyleneglycol-bis-(β-aminoethyl ether) -N,N'-tetraacetic acid, 1 mM phenyl-methyl-sulfonyl fluoride, 2 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM sodium ortho vanadate, and 100 mM sodium floride, and the mixture was incubated 30 minutes at 4°C. Cell debris was removed by microcentrifugation, followed by quick freezing of the supernatant. The protein concentration was measured using a Bio-Rad colorimetric protein assay kit (Bio-Rad, Hercules, CA, USA). Protein of 30 μg was separated on SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane (Schleicher & Schuell GmbH, Dassel, Germany). Rabbit polyclonal anti-HIF-1 α, anit-Bcl-2, and anti-Bax antibodies (1 : 500; Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used as the primary antibodies. Horseradish peroxidase-conjugated anti-rabbit antibody (1 : 2000; Vector Laboratories, Burlingame, CA, USA) was used as a secondary antibody. Band detection was performed using the enhanced chemiluminescence detection system (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA).

5. Terminal deoxynucleotidyl biotin-dUTP nick end labeling (TUNEL) assay

For visualizing DNA fragmentation, TUNEL staining was performed using an In Situ Cell Death Detection Kit® (Roche, Mannheim, Germany) according to the manufacturer's protocol. In brief, the sections were fixed in ethanol-acetic acid (2 : 1) and rinsed. The sections were then incubated with 100 μg/ml proteinase K, rinsed, incubated in 3% H_2O_2, permeabilized with 0.5% Triton X-100, rinsed again and incubated in the TUNEL reaction mixture. The sections were rinsed and visualized using Converter-POD with 0.02% 3,3'-diaminobenzidine (DAB). Mayer's hematoxylin (DAKO, Glostrup, Denmark) was used for counter-staining and the sections were finally mounted onto gelatin-coated slides. The slides were air-dried overnight at room temperature, and the coverslips

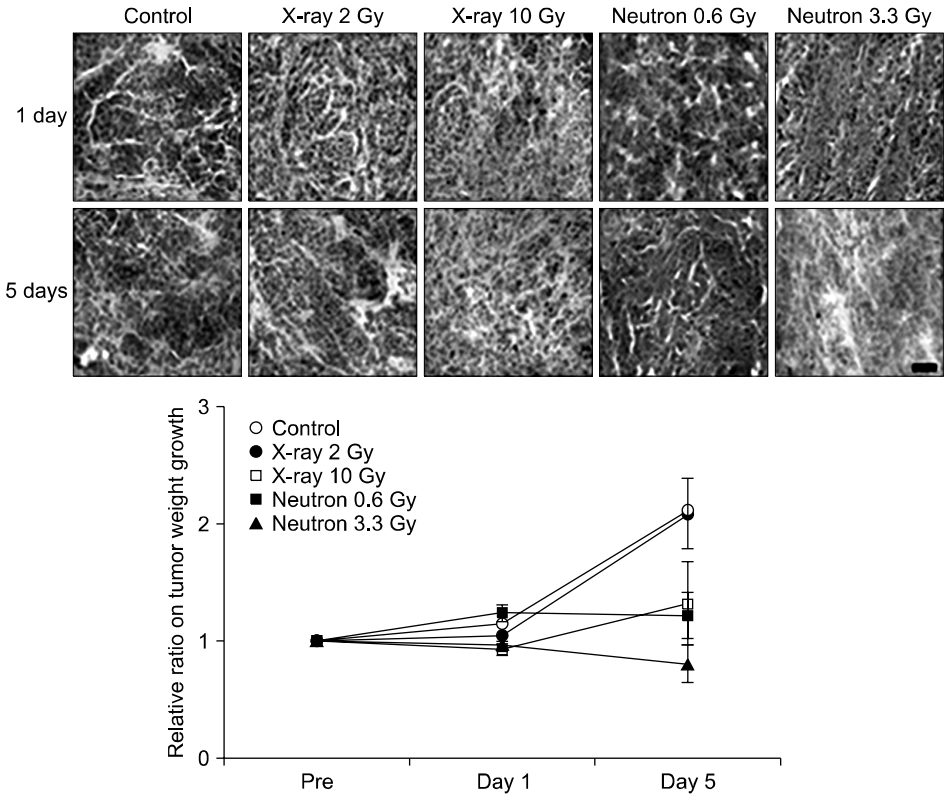

Fig. 1. At day 1, the relative ratio of tumor weight in the control group, 2 Gy X-ray, 10 Gy X-ray, 0.6 Gy neutron and 3.3 Gy neutron irradiated groups were 1.14, 1.05, 0.10, and 1.02, respectively. At day 5, those were 2.13, 2.20, 1.43, 1.32, and 0.90, respectively. At day 5, the relative ratio of tumor weight in the control group and the 2 Gy X-ray irradiated group were significantly increased than others (Upper: H&E stain of tumor [bar: 100 μm]).

were mounted using Permount®. The number of TUNEL-positive cells was expressed as the number of cells per square 100 micrometer.

6. Data analysis

All data were analyzed using the statistical software SPSS ver. 12.0 (SPSS Inc., Chicago, IL, USA). For the comparison between groups, one-way analysis of variance between groups (ANOVA) and Duncan's post-hoc test were performed and differences among groups were considered statistically significant at $p<0.05$.

Results

1. Tumor weight changes

The relative ratio of tumor weight changes after irradiation was quite different among the groups according to the measurement time. The mean relative ratio of tumor weight in the control group and in the 2 Gy X-ray irradiated group were increased to 1.14 and 1.05 at day 1, respectively. At day 5, the mean relative ratio of tumor weight was increased to 2.13 and 2.2, respectively. However, those in 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated groups did not show significant change. At day 1, the mean relative ratio of tumor weight in 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 0.99, 1.27, and 1.02, respectively. And those were 1.43, 1.32, and 0.90 at day 5, respectively. At day 5, the relative ratio of tumor weight in the control group and the 2 Gy X-ray irradiated group were significantly increased than others (p=0.03) (Fig. 1).

2. HIF-1α expression

HIF-1α expressions were decreased in 10 Gy X-ray and neutron irradiated groups in relation with the control and 2 Gy

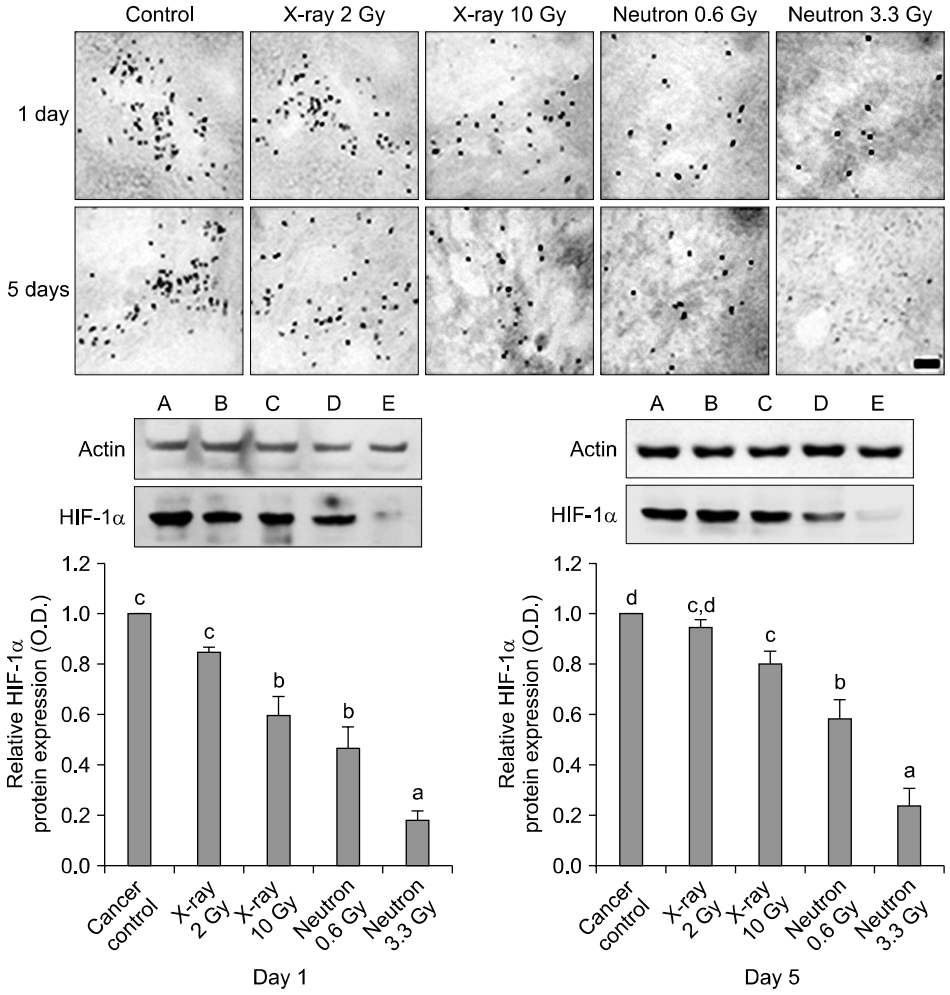

Fig. 2. At day 1, the HIF-1α expression of 2 Gy X-ray, 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 0.85, 0.60, 0.47, and 0.18, respectively. At day 5, those were 0.95, 0.80, 0.59, and 0.24, respectively. 3.3 Gy neutron irradiated group showed significantly decreased HIF-1α expression than other groups (A: Control group, B: 2 Gy X-ray irradiated group, C: 10 Gy X-ray irradiated group, D: 0.6 Gy neutron irradiated group, E: 3.3 Gy neutron irradiated group. a, b, c, d: subsets for alpha= 0.05 in post hoc test).

X-ray irradiated group at day 1. At day 1, the HIF-1α expression of 2 Gy X-ray, 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 0.85, 0.60, 0.47, and 0.18, respectively. Among the irradiated groups, 3.3 Gy neutron irradiated group showed significantly decreased expression in relation with other groups (p=0.000). HIF-1α expressions were significantly decreased in neutron irradiated groups in relation with the control and X-ray irradiated groups at day 5. At day 5, the HIF-1α expression in 2 Gy X-ray, 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 0.95, 0.80, 0.59, and 0.24, respectively. Among the irradiated groups, 3.3 Gy neutron irradiated group showed significantly decreased expression in relation with other groups (p=0.000) (Fig. 2).

3. Bcl-2 expression

Bcl-2 expressions were decreased in 2 Gy X-ray and neutron irradiated groups in relation with the other groups at day1. At day 1, the Bcl-2 expression in 2 Gy X-ray, 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 0.65, 0.84, 0.24, and 0.14, respectively. Among the irradiated groups, neutron irradiated groups showed significantly decreased expression in relation with other groups (p=.000). Bcl-2 expressions were decreased in neutron irradiated groups in relation with the control and X-ray irradiated groups at day 5. At day 5, the Bcl-2 protein expression in 2 Gy X-ray, 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 0.65, 0.58, 0.32, and 0.18, respectively. Among the irradiated groups, 3.3 Gy neutron irradiated group showed significantly decreased expression in relation with other groups (p=0.000) (Fig. 3).

4. Bax expression

Bax expressions also differed between X-ray and neutron

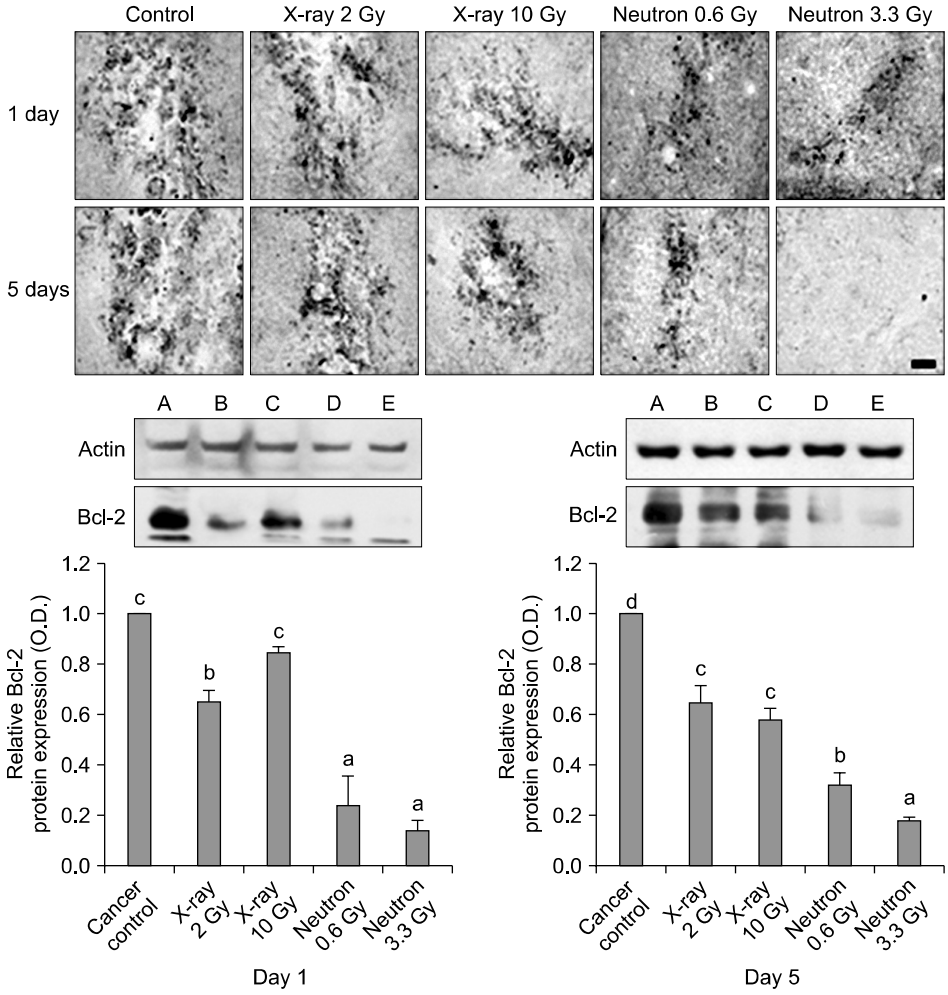

Fig. 3. At day 1, the Bcl-2 expression in 2 Gy X-ray, 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 0.65, 0.84, 0.24, and 0.14, respectively, and at day 5, 0.65, 0.58, 0.32, and 0.18, respectively. Neutron irradiated groups at day 1 and 3.3 Gy neutron irradiated group at day 5 showed significantly decreased Bcl-2 expression than other groups (A: Control group, B: 2 Gy X-ray irradiated group, C: 10 Gy X-ray irradiated group, D: 0.6 Gy neutron irradiated group, E: 3.3 Gy neutron irradiated group. a, b, c, d: subsets for alpha=0.05 in post hoc test).

irradiated groups. At day 1, the Bax protein expression in 2 Gy X-ray, 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 1.83, 1.63, 4.09, and 3.84, respectively. Among the irradiated groups, neutron irradiated groups showed significantly increased expression in relation with other groups (p=0.000). At day 5, the Bax protein expression in 2 Gy X-ray, 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 0.10, 3.60, 17.71, and 17.19, respectively. Among the irradiated groups, neutron irradiated groups showed significantly increased expression in relation with other groups (p=0.016) (Fig. 4).

5. Bcl-2/Bax ratio

Bcl-2/Bax ratio significantly differed among the groups. At day 1, the Bcl-2/Bax ratio in 2 Gy X-ray, 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 0.4024, 0.82, 0.05, and 0.04, respectively. Among the irradiated groups, neutron irradiated groups showed significantly low Bcl-2/Bax ratio in relation with other groups (p=0.001). At day 5, the Bcl-2/Bax ratio in 2 Gy X-ray, 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 0.66, 0.23, 0.05, and 0.02, respectively. Among the irradiated groups, neutron irradiated groups showed significantly low Bcl-2/Bax ratio in relation with other groups (p=0.000).

6. TUNEL

TUNEL expression also differed among the groups. At day 1, the number of TUNEL-positive cells in control group, 2 Gy X-ray, 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 3.60, 4.70, 9.65, 13.35, and 15.05, respectively. Among the irradiated groups, neutron irradiated groups showed significantly increased number of TUNEL-positive cells in relation with other groups (p=0.000) At day 5, the number of TUNEL-positive cells in control group, 2 Gy

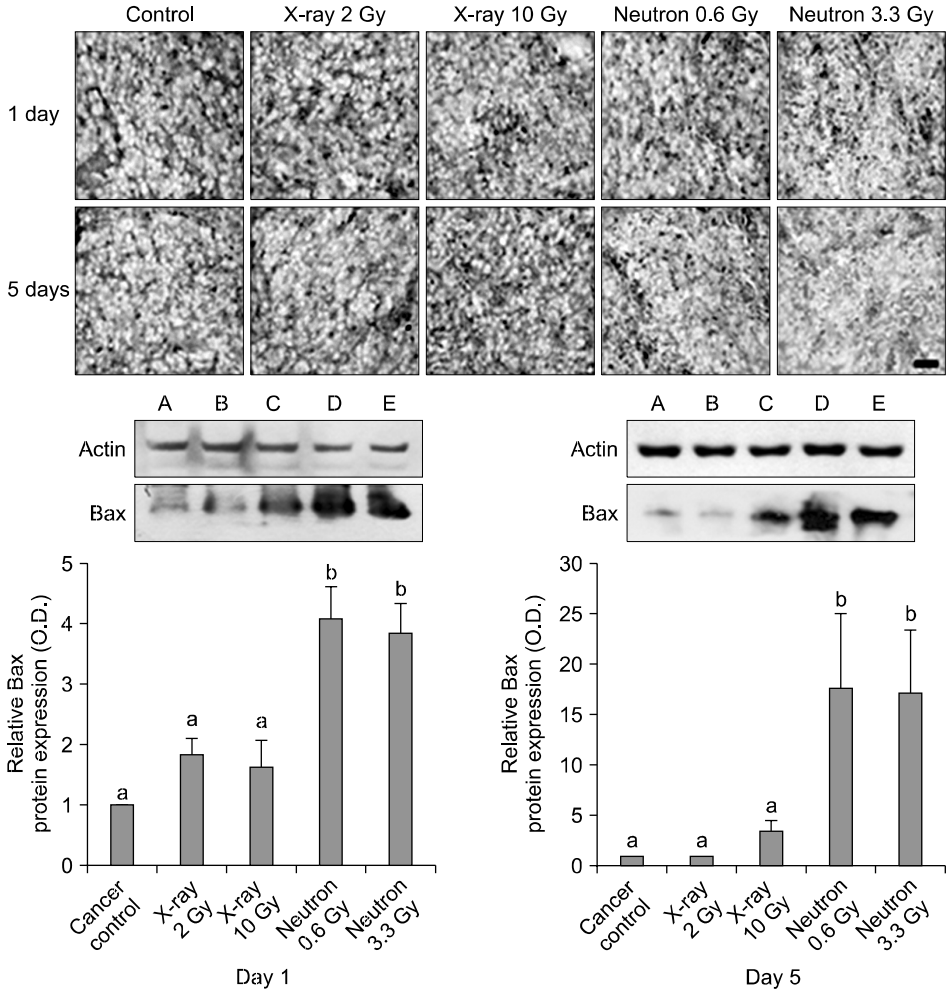

Fig. 4. At day 1, the Bax expression in 2 Gy X-ray, 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 1.83, 1.63, 4.09, and 3.84, respectively, and at day 5, 1.00, 3.60, 17.71, and 17.19, respectively. Neutron irradiated groups showed significantly increased Bax expression than other groups (A: Control group, B: 2 Gy X-ray irradiated group, C: 10 Gy X-ray irradiated group, D: 0.6 Gy neutron irradiated group, E: 3.3 Gy neutron irradiated group. a, b, c, d: subsets for alpha=0.05 in post hoc test).

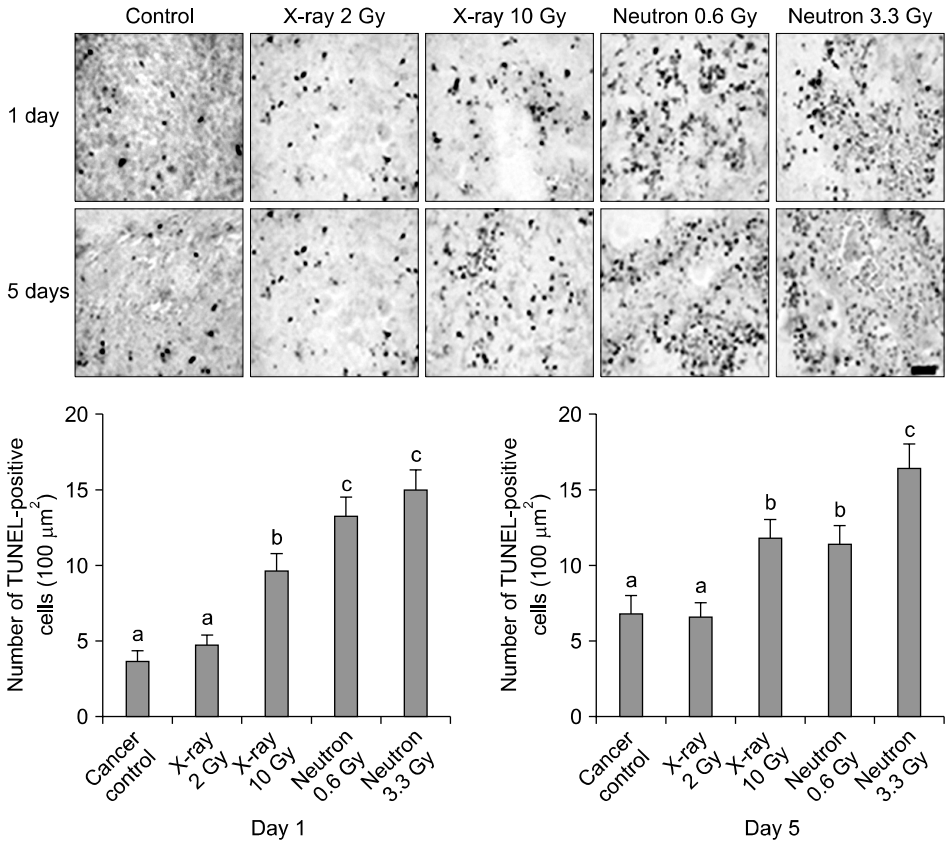

Fig. 5. At day 1, the number of TUNEL-positive cells in control group, 2 Gy X-ray, 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 3.60, 4.70, 9.65, 13.35, and 15.05, respectively, and at day 5, 6.80, 6.60, 11.90, 11.50, and 16.45, respectively. Neutron irradiated groups at day 1 and 3.3 Gy neutron irradiated group at day 5 showed significantly increased number of TUNEL-positive cells than other groups (a, b, c: subsets for alpha=0.05 in post hoc test).

X-ray, 10 Gy X-ray, 0.6 Gy neutron, and 3.3 Gy neutron irradiated group were 6.80, 6.60, 11.90, 11.50, and 16.45, respectively. Among the irradiated groups, 3.3 Gy neutron irradiated group showed significantly increased number of TUNEL-positive cells in relation with other groups (p=0.000) (Fig. 5).

Discussion and Conclusion

Recently, the tumor hypoxia and related microenvironment are being focused as potential prognostic factors in prostate cancers. For the prostate cancers, hypoxia cells may be an even more critical element in carcinogenesis and progression because clinically relevant levels of hypoxia are detected in 30 ~90% of prostate cancers.[11] The prostate tumor cells respond to hypoxic conditions to cause changes in gene expression mediated by the upregulation of HIF-1 α,[12] which is known to occur as an early event of their carcinogenesis.[6] The HIF-1 α maintains cytoprotective defence against hypoxic injury by activation of transcription of numerous genes[13] so that the HIF-1 α and/or its related genes may influence hypoxia and tumor radiosensitivity.[14] It is reported that the radiotherapy induces tumor reoxygenation, which leads to nuclear accumulation of HIF-1 α in response to reactive oxygen. And the increased HIF-1 α activity in tumors causes significant radioprotection of the tumor vasculature and through stimulating endothelial cell survival and redioresistance of the tumors.[15] It is suggested that inhibiting postradiation HIF-1 α activation significantly increases tumor radiosensitivity as a result of enhanced vascular destruction.[16] These reports suggest that HIF-1 α has key role in radiation response and survival of the cells irradiated.

Meanwhile, the fast neutrons are more effective for hypoxic cells because neutrons are less affected by oxygen status and cause more double strand breaks with greater RBE and LET than X-rays. With the improved beam delivery systems and collimation, the neutrons showed superior results to external beam photon radiotherapy in the treatment of prostate tumors.[3,4] But there are little investigations for the difference of HIF-1 α expression induced by photon and neutron in the

prostate cancer cells. In our study, with same biological effective dose of X-rays, the neutron irradiation significantly decreased HIF-1α expression. The decreased HIF-1α expression was found at day 1 which was maintained to day 5. The results suggest the inhibition effect of neutron on HIF-1α expressions and may provide a rationale for using neutron irradiation for HIF-1α suppression. Novel therapeutic strategies using neutron beam therapy to target the hypoxic response and resulting defective DNA repair may therefore be effective to improve clinical outcome.

Some investigations for the difference in the level of apoptosis induced by photon and neutron have been undertaken. In previous reports, the apoptosis and LET level of the radiations showed discrepancies. Holl et al.[17] reported that the effects of high-LET radiations on murine spleen cells using 65 MeV neutrons or 15 MV X-rays at doses ranging from 0.2 to 3 Gy, the level of apoptosis occurring at various times postirradiation was found to be identical for high- and low-LET radiations. Vral et al.[18] reported that the radiosensitivity of lymphocytes and their response to undergo early interphase cell death by apoptosis is largely independent of LET. However, Meijer et al.[19] reported that in the human peripheral G_0 lymphocytes exposed in vitro to doses up to 3 Gy of high or low LET radiations, high LET radiation induced a faster apoptotic response as compared with gamma-photons of low-LET radiation. Wang et al.[20] reported that using Chinese rectal carcinoma cell line HR8348 cells, fast neutron with dose of 0, 0.67, 1.34, 2.01, 2.68, and 3.34 Gy were irradiated which is equivalent to 0, 2, 4, 6, 8, and 10 Gy X-ray, and resulting higher apoptosis with fast neutron than X-ray. So, it is still controversial whether the neutrons cause more apoptosis than photons. This seems to indicate that apoptosis is liable to the influence of many different factors, such as LET spectrum, radiation dose range, cell type, time of evaluation and method of analysis. It is suggested that external as well as cellular factors might differentially modulate the sensitivity of tumor cells to fast neutrons and photons. In our study, HIF-1α expression was significantly related with Bcl-2 expression (p=0.031), Bax expression (p=0.037) and TUNEL (p=0.016) at day 5. This results suggest that HIF-1α and apoptosis are associated in DU-145 prostate cancer cells and there was significant difference of apoptosis between neutron and X-ray.

The HIF-1α expression did not show relationship with any of those gene expressions at day 1. The HIF-1α may precede the expression of Bcl-2, Bax and apoptotic change with time gap. Strowbridge et al.[8] demonstrated that down regulation of Bcl-2 and increased Bax protein expression were seen as early as 24 hours after 0.5 and 1.5 Gy of neutron irradiation. In the report using the intermediate grade non-Hodgkin's lymphoma cell line, WSU-DLCL2, which is resistant to chemotherapy, the authors demonstrated that neutron radiation at doses of 0.5 and 1.5 Gy caused apoptosis as a result of down-regulation of Bcl-2 and increased expression of Bax at 24, 48, 72 hours compared with the control. Thus it is suggested that the HIF-1α expressions preceded the expressions of Bcl-2 and Bax, so significant relationship were shown only at day 5 in our study.

Bcl-2 expression has been known to have a negative effect on the outcome of prostate cancer patients after radiation therapy. Bcl-2 expression is correlated with radiation resistance due to a delay in the onset of radiation-induced apoptosis[21] and thus it affects the treatment outcome of radiation therapy for prostate cancer.[22] It has been reported that the Bcl-2 specific antisense oligonucleotide sensitizes prostate cancer cells to RT independently of p53.[23] Meanwhile, in several researches, the Bcl-2 and Bax both genes are used as potential predictive markers of the therapeutic response to radiotherapy.[24,25] Mackey et al.[24] performed a retrospective review of records from 41 patients who had undergone external beam radiotherapy for prostate cancer, and they suggested that patients with an elevated Bcl-2/Bax ratio are at an increased risk of radiotherapy failure. In another study of a group of more locally advanced patients who had been treated with radiation therapy (Radiation Therapy Oncology Group [RTOG] trial 86-10), neither Bcl-2 nor Bax expression levels were predictive of the outcome.[26] However, more recent RTOG trial 92-02 study suggests that the combination of negative Bcl-2/normal Bax expression is significantly related to a reduced biochemical and clinical failure of prostate cancer after radiation therapy.[25] In our study, the Bcl-2 expression was suppressed in the neutron irradiated groups and Bax expression was increased in the neutron irradiated groups, so the Bcl-2/Bax ratio significantly suppressed in the neutron irradiated groups at day 1 (p=0.001) and days 5 (p=0.000).

In conclusion, the results that the expression of HIF-1α

after neutron treatment was significantly suppressed compared with X-ray may suggest neutron irradiation for prostate cancer patients may have radiobiological benefits through suppression of apoptotic protein expression. Nevertheless, several other factors may contribute to radiobiological advantages of neutron beam therapy and further investigations are necessary.

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국문초록

누드마우스에 주입된 DU-145 전립샘암에서 엑스선과 중성자선에 의한 HIF-1α, Bcl-2, Bax 발현의 차이

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목 적: 전립샘암 세포주 DU 145에서 엑스선과 중성자선에 의한 HIF-1α와 아포프토시스 발현의 차이를 비교함으로써 엑스선과 중성자선의 방사선생물학적 차이의 기전을 알아보고자 한다.

대상 및 방법: 누드 마우스에 DU 145 전립샘암 세포주를 주입한 후 2 Gy 엑스선, 10 Gy 엑스선, 0.6 Gy 중성자선, 3.3 Gy 중성자선을 각각 조사했다. 엑스선을 조사한 군과 중성자선을 조사한 군에서 HIF-1α, Bcl-2, Bax, 아포프토시스 발현 정도를 면역조직화학 염색과 western blotting을 이용하여 비교하였다. 아포토시스의 정도는 terminal deoxynucleotidyl biotin-dUTP nick end labeling (TUNEL) 염색을 이용하여 비교하였다.

결 과: 방사선 조사 1일째, X선을 조사한 군과 비교했을 때, 중성자선을 조사한 군에서 HIF-1α와 Bcl-2의 발현은 감소하였고, Bax와 아포프토시스 세포의 수는 증가하였다. Bcl-2/Bax 비는 중성자선을 조사한 군에서 의미 있게 감소하였다. 이러한 HIF-1α, Bcl-2, Bax, Bcl-2/Bax 비, 아포프토시스 발현의 차이는 방사선 조사 5일째에도 동일하게 유지되어 나타났다. 또한, HIF-1α 발현은 방사선 조사 5일째 Bcl-2 (p=0.031), Bax (p=0.037), TUNEL (p=0.016) 발현과 연관성을 보였다.

결 론: 중성자선 조사한 경우 엑스선에 비해 HIF-1α와 Bcl-2 발현, Bcl-2/Bax 비가 감소하고, Bax 발현은 증가하였다. 중성자선 치료의 광자선과 다른 방사선생물학적인 반응은 HIF-1α와 그로 인한 아포프토시스 관련 단백질 발현의 차이와 연관성이 있을 것으로 생각한다.

핵심용어: HIF-1α, Bcl-2, Bax, 중성자선, 전립샘암