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**Matsuu-Matsuyama, M., Nakashima, M., Shichijo, K., Okaichi, K., Nakayama, T. and Sekine, I. Basic Fibroblast Growth Factor Suppresses Radiation-Induced Apoptosis and TP53 Pathway in Rat Small Intestine. *Radiat. Res.* 174, 52–61 (2010).**

**The effect of basic fibroblast growth factor (bFGF) was studied in radiation-induced apoptosis in rat jejunal crypt cells. Six-week-old male Wistar rats were administered 4 mg/kg bFGF intraperitoneally 25 h before receiving 8 Gy whole-body X rays. The jejunum was removed for analysis from time 0 to 120 h after irradiation. Villus length in control rats declined steadily until 72 h, while in bFGF-treated rats the villi were longer than in the controls until 48 h. Crypt lengths were similar to villi. bFGF treatment increased Ki-67-positive cells in the jejunal crypt at 0, 24 and 48 h. The treatment with bFGF reduced the number of apoptotic cells per jejunal crypt to 23% and 10% of the control values at 3 and 6 h, respectively, and increased numbers of mitotic cells significantly at 48 and 72 h. bFGF decreased the levels of TP53, CDKN1A, Puma and Cleaved caspase 3 at 3 h as detected by Western blot analyses. Our results suggest that bFGF protected against acute radiation-induced injury by suppressing the crypt apoptotic cells including the stem cells and promoted crypt cell proliferation. The inhibition of apoptosis thus might be related to suppression of the TP53 pathway.** 

## INTRODUCTION

High doses of ionizing radiation damage intestinal crypt cells and result in functional changes. These deleterious effects, including malabsorption, are seen clinically as acute small bowel reactions. Crypt disturbance results from cell loss by apoptosis and destruction of the stem cells that are responsible for repopulating the lining of the gut. Cells in the stem cell region of the gut crypt are arranged hierarchically and are rapidly proliferating populations (*1*). Exposure to ionizing radiation results in rapid apoptosis of the stem cells in the jejunal crypt (*2, 3*). Apoptosis is a programmed process of active cell death that involves gross morphological alterations including condensation of nuclear chromatin, compaction of cytoplasmic organelles, membrane blebbing, and cellular fragmentation into apoptotic bodies (*1, 4*).

*TP53* (formerly known as p53) is a tumor suppressor gene and is one of the primary cellular factors determining the nature of growth arrest and/or cell death after exposure to ionizing radiation (*5–8*). *TP53* plays an important role in damage surveillance and as such has been dubbed the guardian of the genome (*9*). Upon DNA damage, TP53 accumulation is increased and translocated to the nucleus where it binds to DNA, acting to regulate the transcription of a number of genes including *CDKN1A* (formerly known as $p21^{WAF1/CIP1}$) and *PUMA*. CDKN1A is a cyclin-dependent kinase (CDK) inhibitory protein that appears to be a critical component of $G_1$ arrest (*10, 11*). PUMA, which is induced by TP53, is a key mediator in the TP53-dependent apoptosis (*12–14*). *PUMA* encodes a BH3-only protein member of the Bcl-2 family and binds Bcl-2 and Bcl-$_x$L, localizes to the mitochondria, and promotes cytochrome c release and apoptosis. Depending on the cellular context, the outcome of a TP53 response results in either cell cycle arrest or apoptosis. Caspase 3 is one of the primary executioners of apoptosis and is necessary for the cleavage of a large number of proteins and margination of apoptosis-associated chromatin, DNA fragmentation, and nuclear collapse during apoptosis (*15*).

Basic fibroblast growth factor (bFGF, also known as FGF-2) was initially regarded as a potent angiogenic factor because it induces endothelial cell proliferation, migration and smooth muscle cell proliferation (*16, 17*). bFGF also improved wound healing in patients with superficial or deep second-degree burns and healing of pressure ulcers in clinical trials (*18, 19*). In the gastrointestinal tract, bFGF enhances epithelial cell proliferation and restitution as well as stem cell survival

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after radiation injury to the intestine (*20, 21*). Okunieff *et al.* suggested that the $LD_{50/30}$ (7.6 Gy, bone marrow syndrome end point) and $LD_{50/6}$ (12.5 Gy, gastrointestinal syndrome) of C3H mice after whole-body irradiation were increased by pretreatment with FGF2 (*22*). Paris *et al.* also suggested that bFGF protected C57BL/6 mice from death by the GI syndrome caused by whole-body radiation doses up to 18 Gy (*23*). It was also suggested that bFGF exerted a beneficial effect on radiation-induced enterocolitis (*24*). Pretreatment with FGF-2 has been reported to protect against radiation-induced apoptosis of crypt cells (*22*), although its mechanism of effect was not determined.

We reported previously that agents such as fermented milk kefir (*25*), sucralfate (*26*) and polaprezinc (*27*) can serve as radioprotectors when administered prior to irradiation to suppress radiation-induced apoptosis in rat colon or small intestine. Sucralfate, an aluminum hydroxide complex of sulfated sucrose (*26*), and polaprezinc, a chelate compound consisting of zinc ion and L-carnosine (*27*), are anti-ulcer agents with a protective effect against radiation-induced injury in the rat colon or small intestine after decreases in TP53 accumulation, CDKN1A expression and BAX/BCL-2 ratio or BAX expression.

In this study the effect of bFGF on acute radiation-induced injury was studied in a rat model in which apoptosis was induced by 8 Gy X rays in jejunal crypt cells. To clarify the mechanism of bFGF in radiation-induced apoptosis, the location of the cells displaying Cleaved caspase 3 expression was examined by immunohistochemical analysis, and the effect of bFGF on TP53, CDKN1A, PUMA and Cleaved caspase 3 was examined by Western blot analysis.

## MATERIALS AND METHODS

### *Animals and Treatment of bFGF*

Six-week-old male Wistar rats (200–250 g) were purchased from Charles River Japan (Atsugi, Japan). The rats were housed in groups of two per cage in an air-conditioned room at 24°C (lights on from 7 a.m. to 9 p.m.) and were allowed free access to food (laboratory chow F2, Japan CLEA, Tokyo, Japan) and tap water at the Laboratory Animal Center of Nagasaki University. All animals were kept in a specific-pathogen-free facility at the Animal Center in accordance with the rules and regulations of the Institutional Animal Care and Use Committee.

bFGF (gift of Kaken Pharmaceutical Co. Ltd., Tokyo, Japan) was diluted with distilled water to a concentration of 0.5 mg/ml immediately before injection. bFGF was given intraperitoneally to rats at a dose of 4 mg/kg 25 h before total-body irradiation. The control rats were injected only with saline. In the bFGF dose–response studies, 1 mg/kg and 2 mg/kg were administered intraperitoneally 25 h before irradiation. Three to six animals were used in each group.

### *Irradiation*

Irradiation was performed between 9:00 a.m. and 12:00 p.m. Rats received whole-body X irradiation from a Toshiba ISOVOLT TITAN 32 X-ray, 200 kV, 15 mA apparatus with 0.5-mm aluminum + 5-mm aluminum filters at a dose rate of 0.8903 Gy/min. The radiation exposure was unilateral. Two rats were treated simultaneously while they were allowed to move freely in a cardboard box. A single dose of 8 Gy was given. Nonirradiated rats (time 0 samples) were handled identically.

### *Histological Methods and Assessment of Apoptosis and Mitosis*

To obtain samples, rats were killed humanely by deep anesthesia at 3, 6, 24, 48, 72 and 120 h after 8 Gy irradiation. Six-centimeter segments of proximal jejunum were obtained from the ligament of Trietz. Tissue samples were fixed overnight in 4% neutral buffered formaldehyde. After fixation, the jejunum was cut longitudinally and processed for embedding in paraffin blocks. Thereafter, 3-μm sections were cut and stained with hematoxylin and eosin (H&E). Three to six animals were used for each group and each data point.

Villus length, defined as the length from the apex of the brush border to the base of the crypt in the jejunum, and crypt depth (along the long axis of the elliptical crypt) were measured using a 100× magnification stage micrometer. The lengths of more than five random villi or crypts were measured, and the measurements were averaged.

Scoring of apoptosis and mitosis was restricted to good longitudinal sections of the crypt in which the base of the crypt was aligned with all of the other crypt bases and showed the crypt lumen. More than 30 good longitudinal sections were selected per animal, and apoptotic cells and mitotic cells were scored in each crypt in H&E-stained sections at 400× magnification by light microscopy as described previously (*25–27*).

### *TUNEL Staining*

Identification of apoptosis was confirmed using a TUNEL technique (Apoptag Peroxidase *In Situ* Apoptosis Detection Kit, Chemicon, Temecula, CA) that detects apoptosis-associated DNA fragmentation by labeling of 3′-OH termini with digoxigenin nucleotides using terminal deoxynucleotidyl transferase (*28*). Sections were deparaffinized in xylene, rehydrated in decreasing concentrations of ethanol, and digested in proteinase K (36 μg/ml) for 15 min at room temperature. Endogenous peroxidase was blocked with 3% hydrogen peroxide. Terminal deoxynucleotidyl transferase (TdT) in reaction buffer (containing a fixed concentration of digoxigenin-labeled nucleotides) was applied to sections for 1 h at 37°C followed by placement of slides in Stop/Wash buffer for 30 min. Apoptotic cells were detected after incubation in the 3,3′-diaminobenzidine (DAB) chromogen (Dako, Carpinteria, CA) for approximately 6 min and counterstaining with Methyl Green (Sigma, St. Louis, MO).

### *Immunohistochemistry*

Immunohistochemical staining was performed for Ki-67 and Cleaved caspase 3. Paraffin sections were prepared and deparaffinized. The sections were placed in 0.01 mol/liter citrate buffer (pH 6.0) and pretreated with microwave heating for antigen retrieval. The sections were reacted with 0.3% $H_2O_2$ in deionized water for 30 min to inhibit endogenous peroxidase activity. The sections were incubated with anti-rat Ki-67 monoclonal antibody (MIB-5) (Dako Cytomation Denmark A/S, Denmark) and Cleaved caspase 3 (Cell Signaling Technology Inc.) diluted 1:25 and 1:200, respectively, in ChemMate™ antibody diluent (Dako) over night at 4°C. After washing with PBS, the sections were incubated with biotinylated anti-rabbit and anti-mouse immunoglobulins for 30 min and then reacted with streptavidin conjugated to horseradish peroxidase for 30 min using an LSAB-2 system-HRP for use on rat specimens (Dako) according to the manufacturer's instructions. Antibody binding was visualized by incubation with 3,3′-diaminobenzidine (DAB) chromogen (Dako). Hematoxylin was used for nuclear counterstaining.

Cells showing immunoreactivity for Cleaved caspase 3 were scored on a cell location basis within the half-crypts of the jejunum according to the method of Wilson *et al.* (*29*) at 400× magnification using light microscopy. A minimum of 30 half-crypts were counted from three rats in every group. The cell numbers in the half-crypts were also determined.

*Western Blotting*

The jejunum tissues of control and bFGF-treated rats were removed at 3, 6 and 24 h after 8 Gy irradiation and frozen immediately. The tissues were then suspended in RIPA buffer (50 m*M* Tris, 150 m*M* NaCl, 1% sodium deoxycholate and 0.05% SDS, pH 7.4), pulverized on ice, and subjected to three freeze-thaw cycles (*30*). The insoluble cell debris was removed by centrifugation. Supernatants were collected and the protein concentrations were determined using a protein assay reagent (Bio-Rad Laboratories, Hercules, CA). Protein samples (30 μg) were subjected to 10% or 15% SDS-polyacrylamide gel electrophoresis and then transferred electrophoretically to Hybond ECL Nitrocellulose Membranes (Amersham, Arlington Heights, IL). Membranes were incubated with mouse monoclonal anti-TP53 (Pab421) antibody (Oncogene Science Inc., Uniondale, NY), rabbit polyclonal anti-CDKN1A (Santa Cruz Biotechnology, Santa Cruz, CA), Puma (Cell Signaling Technology Inc., Beverly, MA) and Cleaved caspase 3 (Cell Signaling Technology Inc.) antibody or rabbit polyclonal anti-actin antibody (Sigma). This was followed with a horseradish peroxidase-conjugated anti-mouse IgG antibody (Zymed Labs. Inc., San Francisco, CA) or a horseradish peroxidase-conjugated anti-rabbit IgG (Amersham). Chemiluminescence (ECL Plus, Amersham) was used to analyze the levels of protein according to the manufacturer's protocol. Blots were exposed to Hyperfilm ECL (Amersham). NIH Image 1.61 software was used in measuring the densities of each of the protein bands. The level of protein after irradiation was determined using the level of actin as a standard.

*Statistical Evaluation of Data*

All values were expressed as the means ± SEM of results obtained from three to six animals per data point. Differences between groups were examined using Student's *t* test. A $P < 0.05$ was considered to be statistically significant.

## RESULTS

### *Effects of bFGF on the Length of Villi and Crypts and Proliferation in Irradiated and Nonirradiated Rats*

To evaluate the effect of bFGF on radiation-induced injury in the rat small intestine, the lengths of villi and crypts were measured in samples taken from 0 to 120 h after irradiation. The length of villi in control rats decreased from 0 to 72 h and then showed an increase at 120 h (Fig. 1A). The length of crypts in control rats showed decreases at 24 h and 48 h and then increased by 120 h (Fig. 1B). For rats treated with bFGF, the villi were significantly longer than controls at 0, 6 and 48 h (Fig. 1A). The crypts of bFGF-treated rats were significantly longer than controls at 0, 3, 6 and 24 h (Fig. 1B). These results indicate that bFGF can protect against acute radiation-induced injury in the rat jejunum.

To investigate the effect of bFGF on proliferation in the jejunum after irradiation, immunohistochemical staining for the Ki-67 antigen was performed. The Ki-67-positive cells in the jejunal crypts of rats treated with

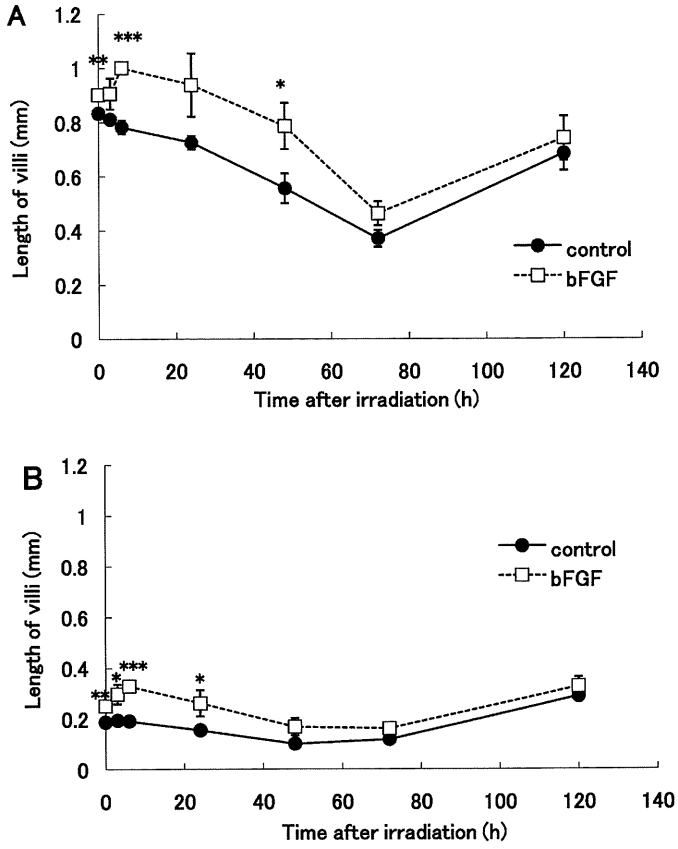


**FIG. 1.** Comparison of the lengths of villi (panel A) and crypts (panel B) after 8 Gy irradiation in rats treated or untreated with bFGF. Data are the means ± SEM of three to six rats per data point. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared to control rats.

bFGF (Fig. 2F) were higher in number than in control rats at 0 h (Fig. 2A) and continued to be higher at 24 (Fig. 2G) and 48 h (Fig. 2H). At the same time, the Ki-67-positive cells in the jejunum of control rats decreased at 24 h (Fig. 2B), and only a few positive cells were seen at 48 h (Fig. 2C). Regenerated crypt cells appeared at 72 h (Fig. 2D). At 72 and 120 h, there was no difference between control and bFGF-treated rats (Fig. 2D and I, E and J).

### *Effects of bFGF on Radiation-Induced Apoptosis and Mitosis of Crypt Cells*

Figure 3A shows histological sections of jejunal crypts from control and bFGF-treated rats stained with TUNEL. At 3 h, a large number of apoptotic cells were observed in the jejunal crypts of control rats. In contrast, only a small number of apoptotic cells were seen in the jejunal crypts of bFGF-treated rats.

Panels B and C of Fig. 3 compare the apoptotic and mitotic indices in the jejunum detected by H&E staining in control and bFGF-treated rats up to 120 h. The apoptosis index in the jejunum peaked in control rats by 3 h and then declined precipitously within 24 h. Pretreatment with bFGF significantly reduced the number of apoptotic jejunal crypt cells at 3 and 6 h to

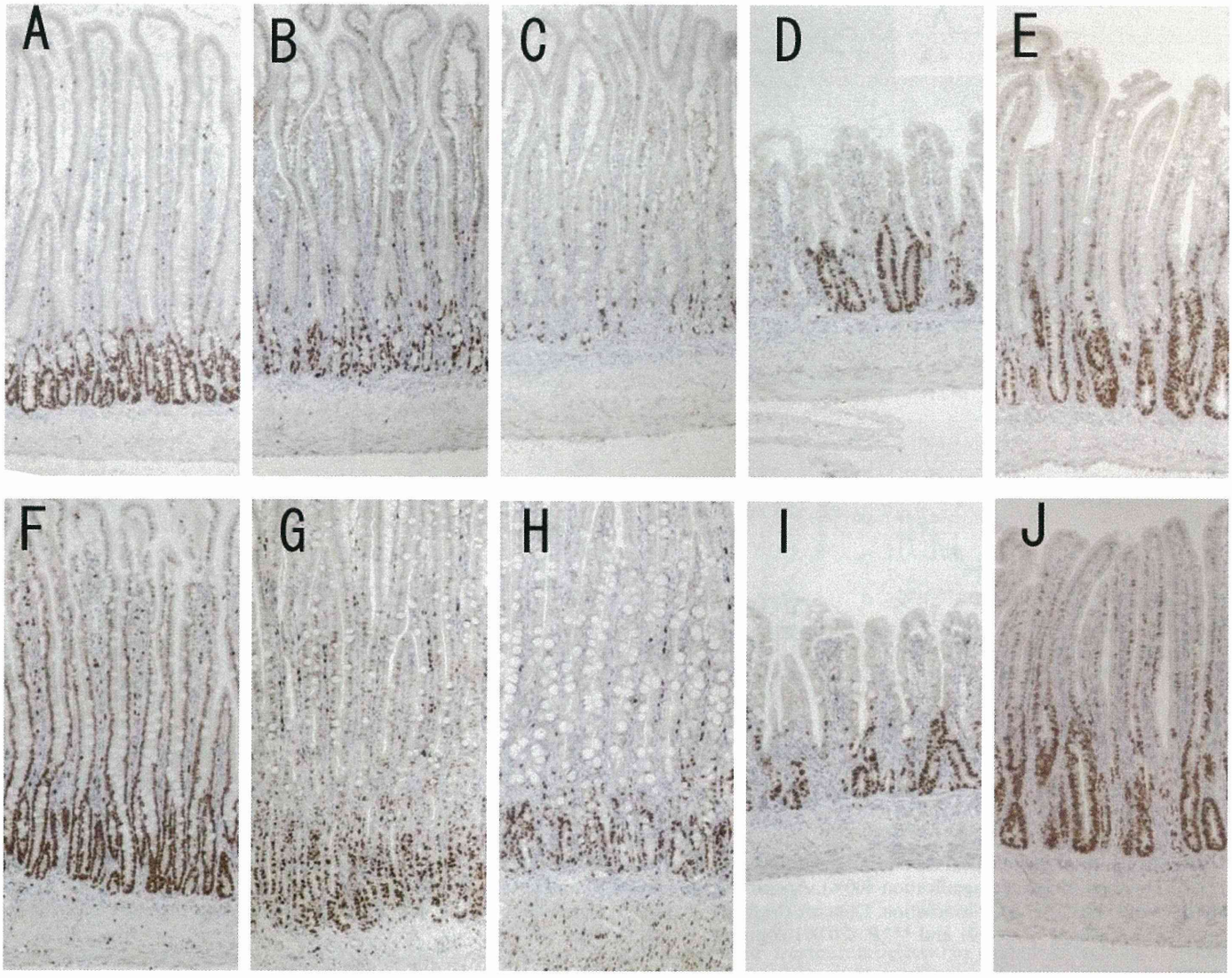


**FIG. 2.** Immunohistochemical staining with anti-Ki-67 monoclonal antibody. Jejunum of control (upper row) and bFGF (lower row)-treated rat at 0 (panels A and F), 24 (panels B and G), 48 (panels C and H), 72 (panels D and I) and 120 h (panels E and J) after 8 Gy irradiation. Original magnification 100×.

22.6% and 10% of the levels in control rats, respectively. There was no difference in the apoptosis index between control and bFGF-treated rats from 24 h to 120 h. There was no difference in the background (0 h) levels of apoptosis in the two groups (Fig. 3B).

The mitotic index in the jejunum of control rats decreased sharply at 3 and 6 h, in contrast to the apoptosis index. This was followed by a gradual increase at 72 h that then continued until 120 h. The mitotic index of bFGF-treated rats was higher than that of control rats at and after 48 h. Significant differences in the mitotic index were noted between control and bFGF-treated rats at 48 and 72 h (Fig. 3C).

*Immunohistochemical Results for Cleaved Caspase 3*

The expression of Cleaved caspase 3 in jejunal crypt cells of control and bFGF-treated rats detected by immunohistochemical staining is shown in Fig. 4. The distribution of positive cell locations is shown in Fig. 4A–D. The time courses of the frequencies of Cleaved caspase 3-positive cells in half-crypts of control and bFGF-treated rats are shown in Fig. 4E. Cleaved caspase 3 expression was localized to the nucleus of the crypt cells after irradiation. There was no expression of Cleaved caspase 3 in the nonirradiated control or bFGF-treated rats. The expression of Cleaved caspase 3 in the irradiated jejunum was increased strongly at 3 h (Fig. 4B), decreased slightly at 6 h (Fig. 4C), and then declined at 24 h (Fig. 4D) in the bottom half of the crypt, while that of bFGF-treated rats showed no increase at any time after irradiation. There was a significant difference at 3 and 6 h ($P < 0.05$) between non-bFGF-treated and bFGF-treated rats (Fig. 4E).

The half-crypt cell numbers of control and bFGF-treated rats are shown in Table 1. The half-crypt cell

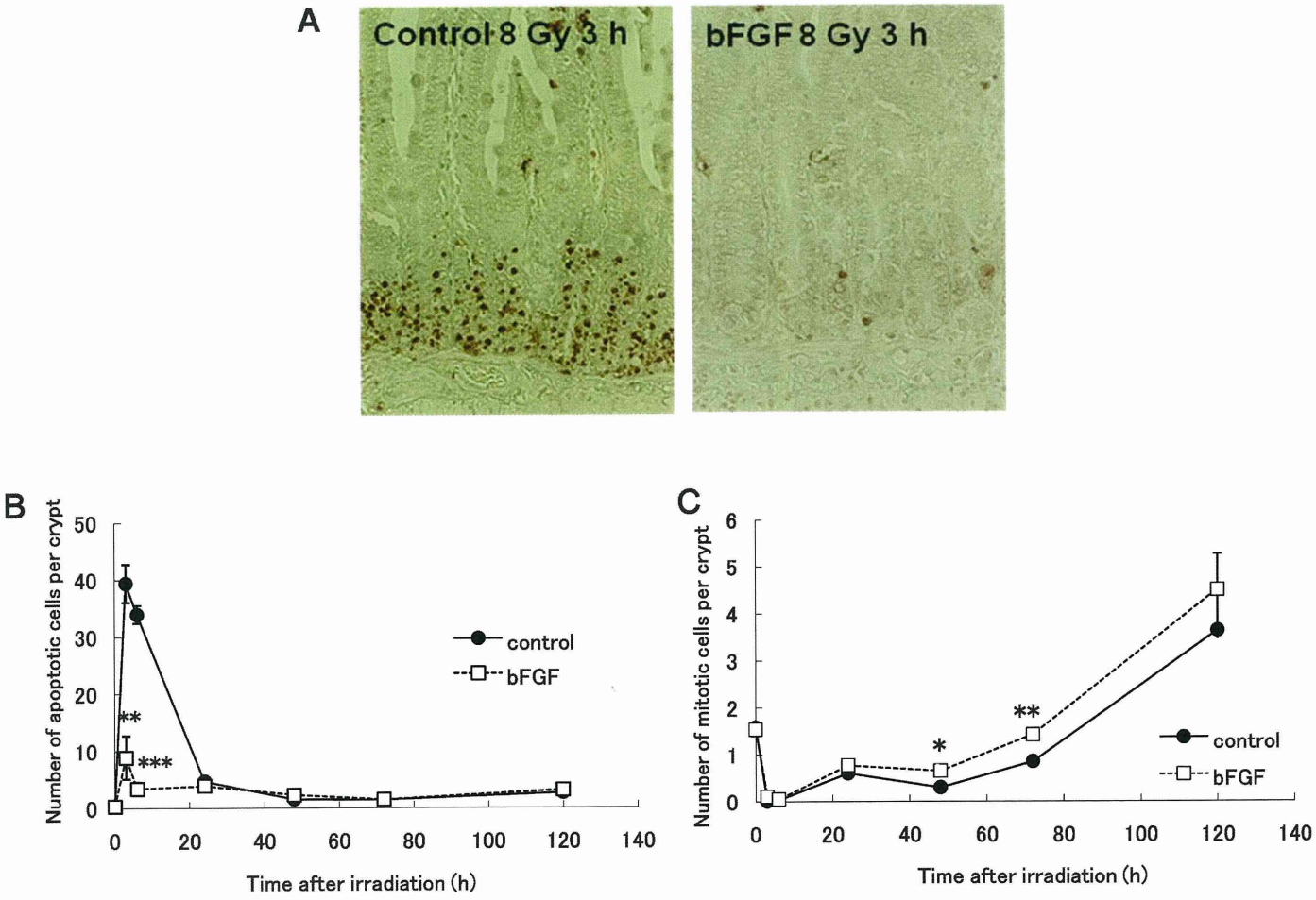


**FIG. 3.** Panel A: Histological sections of small intestine in control rat and bFGF-treated rat at 3 h after 8 Gy irradiation stained by the TUNEL method (original magnification 400×). Apoptosis index (panel B) and mitotic index (panel C) of jejunal crypt cells in control rats and bFGF-treated rats after 8 Gy irradiation. Data are the means ± SEM of three to five rats per data point. More than 30 crypts were analyzed per animal. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared to control rats.

numbers of bFGF-treated rats were significantly higher compared to those of non-bFGF-treated rats at each time after irradiation.

### *Dependence of Radioprotection of Intestinal Crypts on Dose of bFGF*

Figure 5 shows the apoptosis index at 6 h after 8 Gy irradiation in control rats and in rats treated with 1, 2 and 4 mg/kg of bFGF. There was no difference between control rats and bFGF-treated rats at 1 mg/kg. However, suppression of the apoptosis index was clearly shown in rats treated with 2 and 4 mg/kg bFGF.

### *Western Blotting for TP53, CDKN1A, Puma and Cleaved Caspase 3*

To determine how bFGF treatment was interfering with the pathways leading to radiation-induced apoptosis in the rat jejunum, the levels of TP53, CDKN1A, Puma and Cleaved caspase 3 were examined by Western blot analyses; kinetic diagrams are shown in Fig. 6. TP53 accumulation in control rats increased 2.3-fold at 3 h and then decreased at 6 and 24 h. A significant difference was observed between control and bFGF-treated rats, at 3 h ($P < 0.05$) (Fig. 6A). The expression of CDKN1A in control rats increased at 3, 6 and 24 h. There was a significant difference between control and bFGF-treated rats at 3 h ($P < 0.05$) (Fig. 6B). The expression of Puma in control rats increased at 3 and 6 h and then decreased at 24 h. A significant differences were noted between control and bFGF-treated rats at 3 h ($P < 0.01$) (Fig. 5C). The amount of Cleaved caspase 3 in control rats increased at both 3 and 6 h and then decreased to 0.7-fold at 24 h. A significant difference was noted between control and bFGF-treated rats at 3 h ($P < 0.05$) (Fig. 6D). Taken together, these results indicated that bFGF pretreatment inhibited radiation-induced TP53 accumulation and its downstream induction of CDKN1A, Puma and Cleaved caspase 3; this was consistently seen at 3 h.

## DISCUSSION

Radiation enteritis occurs during radiotherapy for many pelvic cancers and intra-abdominal organs such as

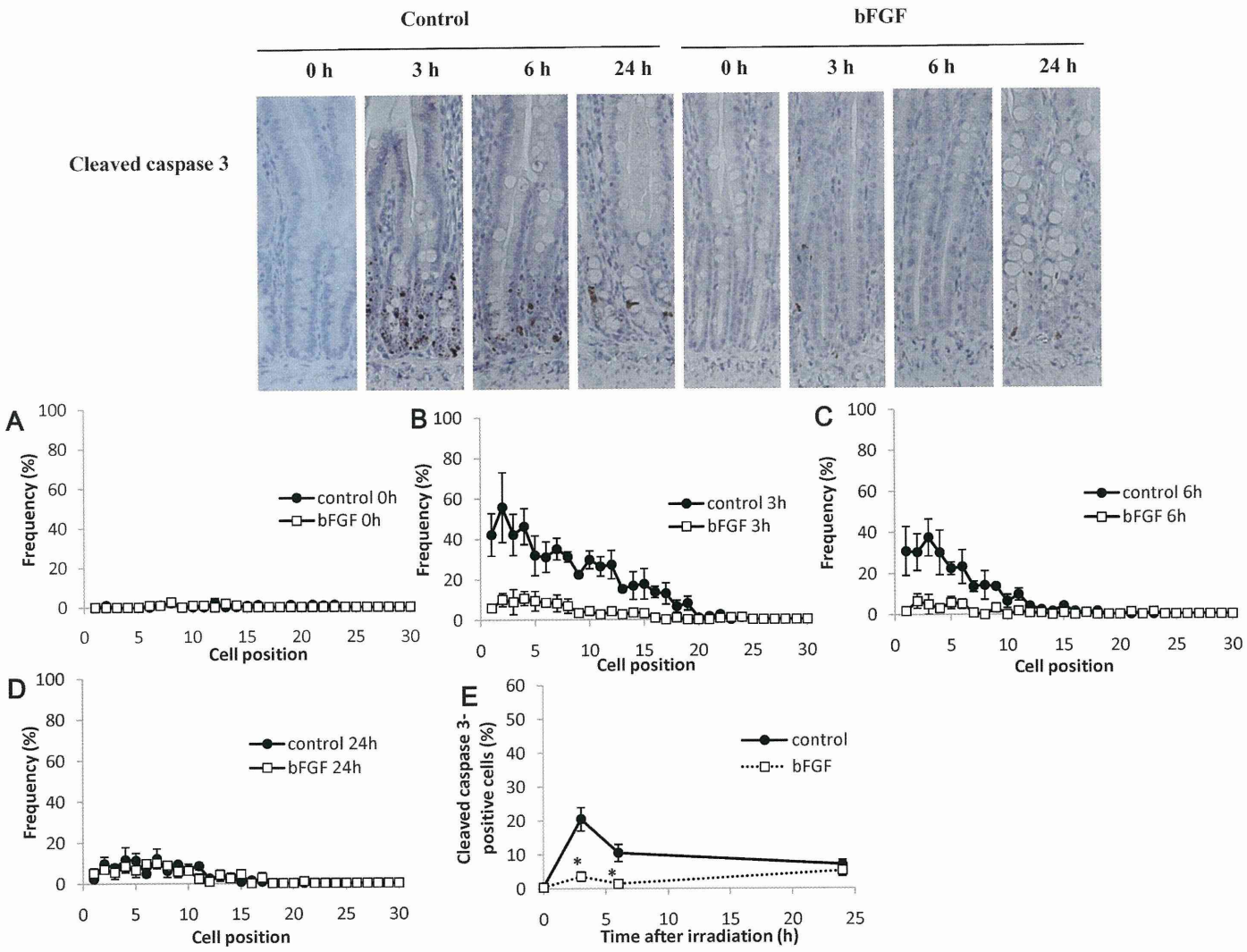


**FIG. 4.** The expression of Cleaved caspase 3 in jejunal crypt cells of control and bFGF-treated rats detected by immunohistochemical staining after exposure to 8 Gy radiation (original magnification 200×). The distribution of the locations of Cleaved caspase 3-positive cells in half-crypts of control and bFGF-treated rats after exposure to 8 Gy is shown in panels A–D. The time course of the percentage of Cleaved caspase 3-positive cells in half-crypts of control and bFGF-treated rats is shown in panel E. Data are means ± SEM for three rats per experimental group. At least 30 half-crypts were scored from each rat. $*P < 0.05$ compared to control rats.

cervix, endometrium, ovary, bladder, prostate and rectum. Although ionizing radiation affects other intra-abdominal organs, the most radiosensitive is the small intestine. Ionizing radiation causes mucosal

**TABLE 1**
**The Half-Crypt Cell Numbers after Irradiation**

| Time after irradiation | Control | bFGF |
|---|---|---|
| 0 h | 25.9 ± 0.5 | 28.3 ± 0.5[b] |
| 3 h | 24.7 ± 0.9 | 28.1 ± 1.1[a] |
| 6 h | 23.4 ± 0.4 | 28.4 ± 1.1[c] |
| 24 h | 14.1 ± 0.3 | 18.3 ± 1.6[a] |

*Notes.* Data are means ± SEM from pooled data of three counts from each of three rats per experimental group. At least 30 half-crypts were scored from each rat. [a]$P < 0.05$, [b]$P < 0.01$ and [c]$P < 0.001$ compared to control rats.

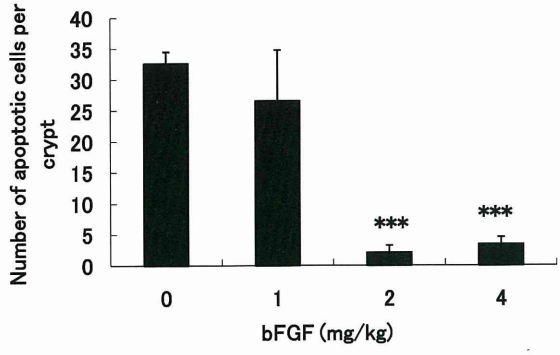


**FIG. 5.** Effect of the dose of bFGF on apoptosis index of jejunal crypt cells 6 h after 8 Gy irradiation. Data are the means ± SEM of three or four rats per data point. More than 30 crypts were selected for analysis per animal. $***P < 0.001$ compared to control rats.

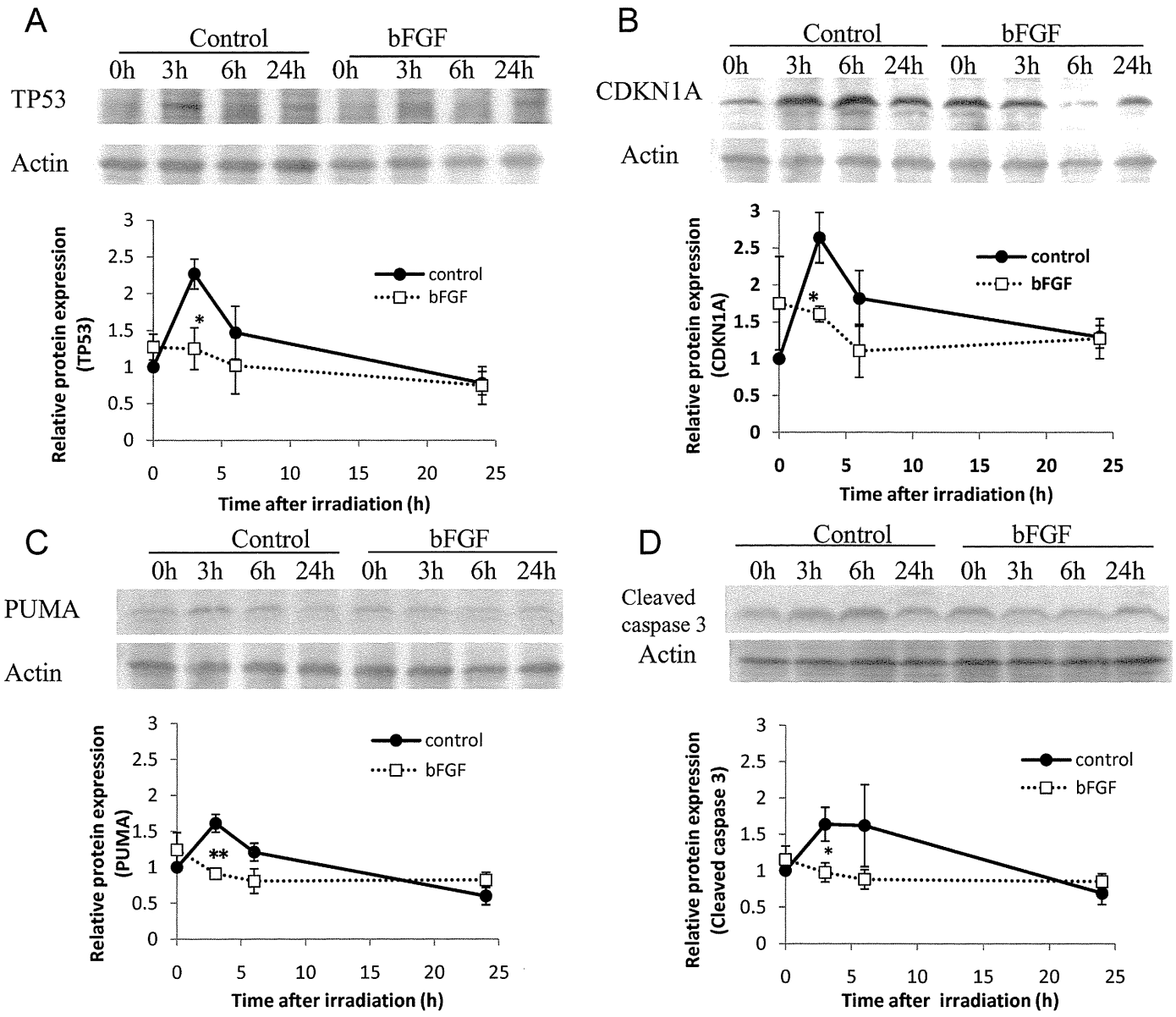


**FIG. 6.** Western blot analysis of the amounts of TP53, CDKN1A, PUMA and Cleaved caspase 3 in the jejunum of control and bFGF-treated rats. The levels of TP53 (panel A), CDKN1A (panel B), PUMA (panel C) and Cleaved caspase 3 (panel D) were quantified by densitometric analysis. Each of the protein levels is expressed as a ratio of nonirradiated rats (0 h) to control rats. Data are means ± SEM of three to four separate experiments with two rats per data point. $*P < 0.05$ and $**P < 0.01$ compared to control rats.

damage in the gastrointestinal epithelium comprised of destruction of crypt cells occurring by the induction of apoptosis, decrease in villus height and number, ulceration and necrosis (*31*). In the previous study, we noted that losses of weight and appetite in spontaneously hypertensive rats were greater than in Wistar-Kyoto rats after 7.5 Gy X irradiation and suggested that this radiation sickness may be due to injury to the gastrointestinal epithelium (*32*). The protection of radiation-induced apoptosis of intestine may lead to the improvement of radiation sickness.

Houchen *et al.* suggested that the expression of FGF-2 mRNA and protein began to increase at 12 h after γ irradiation, and peak levels were observed in the small intestine from 48 to 120 h at doses above 8 Gy, which produced moderate to severe injury to the gastrointestinal epithelium, resulting in progressive loss of viable regenerative crypts and compromising the epithelial barrier (*21*). FGF-2 markedly enhanced survival of crypt stem cells when mice received a single dose of rhFGF-2 (4 μg/g) 25 h before γ irradiation at doses 13.8 Gy. They suggested that FGF-2 could cause cell cycle arrest, allowing time for repair to occur in otherwise replicating cell populations, and that FGF-2 might enhance crypt survival by protecting stem cells from undergoing apoptosis. Okunieff *et al.* reported that bFGF given 24 h before whole-body irradiation with 7–18 Gy protects bowel crypts and a possible intestinal syndrome

(*22*). The protective mechanism may lie in inhibition of radiation-induced apoptosis of crypt cells and may include radioprotection of the vasculature as well. In this study it was clearly demonstrated that bFGF treatment can protect the rat small intestine from radiation-induced injury at an X-ray dose of 8 Gy, which is above the $LD_{50/30}$ (5 Gy) of Wistar rats and is the lowest dose that produced injury to the gastrointestinal epithelium. Our results showed that pretreatment with bFGF inhibited radiation-induced apoptosis of crypt cells, promoted crypt cell proliferation, and decreased the level of TP53 and the downstream proteins after irradiation.

Our study showed that crypt depth and villus length were significantly increased in irradiated rats by bFGF treatment (Fig. 1A, B). Ki-67-positive cells in the jejunal crypt were increased by bFGF treatment (Fig. 2F) compared with their nonirradiated controls at time 0 (Fig. 2A). This indicated that bFGF treatment promoted crypt proliferation. The increase of proliferating crypt cells after bFGF treatment may be related to a change in radiation sensitivity. The numbers of Ki-67-positive cells in the jejunal crypt of bFGF-treated rats were much larger than those of control rats at 24 and 48 h after irradiation (Fig. 2G, H), suggesting that bFGF treatment may promote proliferation of the surviving cells after irradiation. The treatment with bFGF significantly reduced the apoptosis index at 3 and 6 h (Fig. 3A, B). However, the mitotic index of bFGF-treated rats increased from 48 h after irradiation (Fig. 3C). The radioprotective effect of bFGF may involve a suppression of the crypt apoptotic cells including the stem cells and promotion of crypts cell proliferation.

To clarify the locations of the changes in Cleaved caspase 3, an immunohistochemical staining with Cleaved caspase 3 was performed (Fig. 4). bFGF treatment inhibited the expression of Cleaved caspase 3, which in control rats is expressed in the cells near the base of the crypt, especially around cell positions 4–6, in the putative stem cell region (*33*). The number of half-crypt cells in bFGF-treated rats was significantly higher compared to controls at each time after irradiation (Table 1). The increase of cell numbers in the crypt by bFGF treatment may result in the increase of the length of jejunal crypts and villi.

The greatest suppression of radiation-induced apoptosis was observed when rats received a single dose of 2 or 4 mg/kg bFGF. The dose of 4 mg/kg bFGF was the same dose used by Houchen *et al.* (*21*). Both 2 and 4 mg/kg of bFGF had the same effect, but 1 mg/kg bFGF had no protective effect against radiation-induced apoptosis (Fig. 5).

Western blot analysis showed that bFGF pretreatment resulted in a decrease in the accumulation of TP53 as well as a decrease in the induction of the CDKN1A, Puma and Cleaved caspase 3 relative to the control irradiated rat jejunum (Fig. 6). We also demonstrated that bFGF treatment inhibited the expression of Cleaved caspase 3 in the crypt, including stem cell region, with our immunohistochemical findings (Fig. 4). The results for Cleaved caspase 3 in the Western blot analysis (Fig. 6D) concurred with the results of time course frequency detected by immunohistochemical analysis (Fig. 4E). Merritt *et al.* observed a rapid (4.5 h) elevation of TP53 protein in the proliferative compartment of the crypts where cells underwent death by apoptosis after 8 Gy irradiation. They found a total repression of apoptosis in the small intestinal crypt epithelia of TP53-deficient mice 4.5 h after 8 Gy irradiation (*34, 35*). Komarova *et al.* showed that pifithrin-alpha, a chemical inhibitor of TP53, blocked radiation-induced apoptosis of the gastrointestinal epithelium, but it did not alter lethality from the gastrointestinal syndrome (*36, 37*). In our study, the suppression of the TP53 pathway by bFGF treatment may have been a result of the inhibition of radiation-induced apoptosis. Our analysis, however, was restricted to 8 Gy. Further studies are needed to determine whether the same mechanisms occur at higher doses of radiation (i.e., at or above the $LD_{50/6}$). The expression of CDKN1A at 0 h showed no difference between control and bFGF-treated rats (Fig. 6B). These data provide no evidence to support the hypothesis that bFGF modified radiosensitivity through $G_1$ arrest as reported previously by Houchen *et al.* (*21*).

In conclusion, our results suggest that bFGF protects against acute radiation-induced injury in the rat jejunum by suppressing the apoptotic cells in the crypt, including the stem cells, and by accelerating crypt cell proliferation. The mechanism of the protective effect of bFGF against radiation-induced apoptosis may be dependent on suppression of the TP53 pathway. However, further studies of the mechanism are needed. bFGF may reduce gastrointestinal tract damage during abdominal radiation therapy for gastrointestinal, genitourinary and gynecological tumors. Therapeutic effects have been reported for the treatment of experimental dextran sulfate sodium (DSS)-induced colitis and trinitrobenzene sulfonic acid (TNBS)-induced colitis by the rectal administration of bFGF (*38*). Similarly, topical administration of bFGF by enema may be more effective and less hazardous for the treatment of radiation colitis. Further studies are needed to investigate the therapeutic efficacy of such an approach.

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