Activation of Nuclear Factor κB In vivo Selectively Protects the Murine Small Intestine against Ionizing Radiation-Induced Damage

Yong Wang,1 Aimin Meng,1 Hainan Lang,2 Stephen A. Brown,4,5 Jennifer L. Konopa,4 Mark S. Kindy,3 Richard A. Schmiedt,7 John S. Thompson,4,5 and Daohong Zhou1

Departments of 1Pathology and Laboratory Medicine, 2Otolaryngology-Head and Neck Surgery, and 3Physiology and Neuroscience, Medical University of South Carolina, Charleston, South Carolina; 4Department of Medicine, University of Kentucky, Lexington, Kentucky; and 5Veterans Administration Medical Center, Lexington, Kentucky

ABSTRACT

Exposure of mice to total body irradiation induces nuclear factor κB (NFκB) activation in a tissue-specific manner. In addition to the spleen, lymph nodes, and bone marrow, the tissues that exhibit NFκB activation now include the newly identified site of the intestinal epithelial cells. NFκB activated by total body irradiation mainly consists of NFκB p50/RelA heterodimers, and genetically targeted disruption of the NFκB p50 gene in mice significantly decreased the activation. By comparing tissue damage and lethality in wild-type and NFκB p50 knockout (p50−/−) mice after they were exposed to increased doses of total body irradiation, we additionally examined the role of NFκB activation in total body irradiation-induced tissue damage. The results show that p50−/− mice are more sensitive to total body irradiation-induced lethality than wild-type mice (LD50/Day 7: wild-type = 13.12 Gy versus p50−/− = 7.75 Gy and LD50/Day 30: wild-type = 9.31 Gy versus p50−/− = 7.81 Gy). The increased radiosensitivity of p50−/− mice was associated with an elevated level of apoptosis in intestinal epithelial cells and decreased survival of the small intestinal crypts compared with wild-type mice (P < 0.01). In addition, RelA/TNFR1-deficient (RelA/TNFRI−/−) mice also exhibited a significant increase in intestinal epithelial cell apoptosis after they were exposed to total body irradiation as compared with TNFR1-deficient (TNFRI−/−) mice (P < 0.01). In contrast, no significant increase in total body irradiation-induced apoptosis or tissue injury was observed in bone marrow cells, spleen lymphocytes, and the liver, heart, lung, and kidney of p50−/− mice in comparison with wild-type mice. These findings indicate that activation of NFκB selectively protects the small intestine against ionizing radiation-induced damage.

INTRODUCTION

Nuclear factor κB (NFκB) is a dimeric DNA binding protein consisting of members of the NFκB/Rel family, which includes the subunits of NFκB1 (p50), NFκB2 (p52), RelA, RelB, and c-Rel (1–3). Its expression is ubiquitous in mammalian cells. Normally, NFκB resides in the cytoplasm in an inactive form in association with inhibitory proteins. These inhibitory proteins, which belong to a family of proteins named inhibitor of NFκB (4), prevent NFκB nuclear translocation by masking the NFκB nuclear localization signal and thus, inhibit NFκB DNA binding and transactivational function (1–3). Various stimuli activate a large number of distinct signaling pathways that eventually result in the phosphorylation of inhibitor of NFκB and its subsequent degradation by the proteasome or its dissociation from NFκB without additional degradation (1–3). The released NFκB then translocates to the nucleus and binds to κB or κB-like DNA motifs to initiate gene transcription. The putative target genes of NFκB are mainly involved in immune and inflammatory responses (1–3). These genes encode a variety of inflammatory molecules, including various inflammatory cytokines and adhesion molecules. In addition, NFκB also regulates the expression of many genes of which the products are involved in the control of cell proliferation and cell death (4–7).

Tumor cells usually express high levels of constitutive NFκB activity (8, 9). In addition, exposure of these cells to various cytotoxic agents including ionizing radiation increases NFκB activity (7–13). The role of NFκB in tumorigenesis and cellular resistance to tumor therapy has been extensively studied. The majority of reported studies have demonstrated that NFκB activation may give transformed cells a growth and survival advantage and additionally may render tumor cells resistant to ionizing radiation and a variety of cytotoxic agents by induction of antiapoptotic proteins (7–13). Therefore, molecularly targeted inhibition of NFκB has been actively pursued as a potential and novel adjuvant treatment for cancer in conjunction with radiotherapy and chemotherapy (7–13).

The purpose of using NFκB inhibitors as an adjuvant therapy for cancer is to increase the therapeutic index of radiotherapy and chemotherapy. The success of this approach relies on its ability to promote tumor cell killing by ionizing radiation or chemotherapy but to spare normal tissues from enhanced damage. Therefore, it is critical to determine the effects of NFκB inhibition on normal tissue function in response to ionizing radiation, because activation of NFκB by ionizing radiation has been documented not only in various tumor cells but also in different types of cultured normal cells in vitro (14–25). Previously using a mouse model, we investigated the tissue specificity of ionizing radiation-induced NFκB activation in vivo and found that total body irradiation induces NFκB activation in a tissue-specific manner (26). The activation was observed in the bone marrow and the peripheral lymphoid tissues of the spleen and mesenteric lymph nodes shortly after mice were exposed to a lethal dose of total body irradiation but was absent in all of the other tissues examined, including the liver, lung, colon, thymus, and brain (26). Now, we have discovered that exposure of mice to total body irradiation also activates NFκB in intestinal epithelial cells of the small intestine, a prime target of ionizing radiation damage. The NFκB activated by total body irradiation in various tissues mainly consists of NFκB p50/RelA heterodimers and genetically targeted disruption of the NFκB p50 gene in mice significantly decreased the activation. Therefore, in the present study we compared the tissue damage and lethality in wild-type and NFκB p50 knockout (p50−/−) mice after they were exposed to increasing doses of total body irradiation to determine the role of NFκB activation in ionizing radiation-induced normal tissue damage. The results of this study have important clinical implications in cancer therapy using NFκB inhibitors as an adjuvant therapeutic agent in conjunction with ionizing radiation.

MATERIALS AND METHODS

Mice. Normal male C57BL/6J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Inbred B6,129PF2 (p50 wild-type or wild-type), B6,129P-Nfkb1 (p50 knockout or p50−/−), RelA/TNFRI-deficient (RelA/TNFRI−/−), and TNFR1-deficient (TNFRI−/−) mice were bred at the Medical...
Analysis of Bone Marrow-Mononuclear Cell Apoptosis and the Hematopoietic Function.

Analysis of NFκB Activities by Gel Shift and Supershift Assay. The double-stranded oligonucleotides containing a consensus κB sequence (5'-AGTTGAGGAGACTTTCCCAGGC-3'); Integrated DNA Technologies, Inc., Coralville, IA) were labeled using the Biotin 3′ End DNA Labeling kit (Pierce, Rockford, IL) according to the manufacturer's protocol. The gel shift assay was performed using the LightShift Chemiluminescent electrophoretic mobility shift analysis kit (Pierce) following the manufacturer's instructions. Briefly, an aliquot of nuclear extracts containing 5 μg of protein was incubated with 2 μl of electrophoretic mobility shift analysis binding buffer, 20 fmol of biotin-labeled NFκB probe and 1 μl of poly(dI-dC)·poly(dI-dC) (molecular weight, 5000) for 20 min at room temperature. The reaction mixture was separated on a 6% native polyacrylamide gel by electrophoresis and then transferred to Biodyne B Nylon Membrane (Pierce). After the membrane was incubated with LightShift Stabilized Streptavidin-Horseradish Peroxidase Conjugate and the Luminol/Enhancer and Stable Peroxide Solution, the NFκB DNA complexes were detected by exposure of the membrane to X-ray film. The relative nuclear NFκB DNA binding activities were quantified by scanning densitometry. The specificity of the identified NFκB DNA binding activity in the nuclear extracts was confirmed by using 200-fold excess of unlabeled NFκB, mutated NFκB (Santa Cruz Biotechnology, Santa Cruz, CA), or activator protein-1 (Promega, Madison, WI) oligonucleotides. The addition of the excess unlabeled NFκB oligonucleotides into the gel shift reaction resulted in elimination of the relative NFκB DNA binding activities, whereas that of the excess unlabeled mutated NFκB or activator protein-1 oligonucleotides did not affect the assay (Fig. 1C). For gel supershift analysis, extracted nuclear proteins (5 μg) were incubated with 2 μg of the polyclonal antibodies specifically against the p50, p52, RelA, and/or Erg-2 proteins (Santa Cruz Biotechnology) for 20 min before their incubations with biotin-labeled NFκB probe in the gel shift assay described above.

Analysis of NFκB Proteins in Bone Marrow Stems and Progenitors. After blocking endogenous peroxidase with 3% hydrogen peroxide solution for 15 min, the sections were boiled in 10 mM citrate buffer (pH 6.0) for 10 min and then cooled for 20 min to enhance antigen exposure. Nonspecific binding was blocked by incubation of the sections in 10% normal horse serum for 30 min. The slides were incubated with the primary antibodies against RelA and/or Erg-2 proteins (Santa Cruz Biotechnology) for 1 h at room temperature and then incubated with the secondary antibodies for another hour. After washing three times with PBS, the sections were incubated with diaminobenzidine (DAB) for visualization. The slides were counterstained with hematoxylin and mounted.

Intestinal Apoptosis Assay. The small intestine was rapidly removed from the abdomen after mice were euthanized at various times as described in individual experimental plans. The intestinal contents were removed, and then the intestinal tube was flushed with saline and cut into ~10 cm lengths, which were bundled together with microprop tape before fixation with 10% neutral-buffered formalin overnight. The tissues were embedded in paraffin, and sections (5 μm) were cut perpendicular to the long axis of the intestine and stained with hematoxylin and eosin. The number of apoptotic cells per crypt was assessed by morphological criteria in a blind fashion as described previously by Potten et al. (33). Only well-oriented crypts (50 crypts/animal) in longitudinal sections containing Paneth cells, a crypt lumen, and an uninterrupted column of epithelial cells extending to the crypt-villus junction were scored. In addition, ionizing radiation-induced intestinal epithelial cell apoptosis was examined by immunohistochemistry of active caspase 3 and terminal deoxynucleotidyl transferase-mediated nick end labeling assay (34). For active caspase 3 immunohistochemistry, intestinal tissue sections were deparaffinized and rehydrated. After blocking endogenous peroxidase with 0.3% hydrogen peroxide solution for 15 min, the sections were boiled in 10 mM citrate buffer (pH 6.0) for 10 min and then cooled for 20 min to enhance antigen exposure. Nonspecific binding was blocked by incubation of the sections in 10% normal horse serum.
goat serum for 30 min. The sections were incubated with 1:500 rabbit polyclonal anti-caspase 3 antibody (R&D Systems, Minneapolis, MN) for 18 h at 4°C, extensively washed, and then incubated with 1:200 biotinylated goat antirabbit secondary antibody for 45 min at room temperature. The immunostaining was developed using Vectastain ABC reagents (Vector Laboratories, Inc., Burlingame, CA). 3,3′-diaminobenzidine, and hydrogen peroxide. The sections were counterstained with hematoxylin. Terminal deoxynucleotidyl transferase-mediated nick end labeling assay was performed using the ApopTag fluorescent in situ apoptosis detection kit (Serologicals, Norcross, GA). Briefly, deparaffinized and rehydrated intestinal tissue sections were permeabilized with proteinase K (20 μg/ml) for 20 min, washed, and then incubated with digoxigenin-deoxynucleotide triphosphates and terminal deoxynucleotidyl transferase at 37°C for 1 h. The sections were immersed in stop/wash buffer for 10 min to terminate the reaction and then incubated with fluorescent-conjugated antidigoxigenin antibody for 30 min. Antifade mounting medium was used for fluorescence coverslipping.

Crypt Survival Assay. Three days after exposure to ionizing radiation (12 Gy), each mouse received i.p. injection of 120 mg/kg BrdUrd (Sigma, St. Louis, MO) and 12 mg/kg 5-fluoro-2′-deoxyuridine (Sigma) to label the S-phase cells. Two hours after the injection, mice were euthanized, and their proximal jejunum were collected, prepared, fixed, embedded, and sectioned at 5 μm as described above. Cells incorporating bromo-deoxyuridine were detected by mouse antihematoxylin antibody and visualized by immunofluorescence using Texas red-labeled goat antimouse IgG (red) and Hoechst 33342 (blue, for nuclear counter staining). A surviving crypt is defined as one containing ≥5 bromo-deoxyuridine-positive cells as described previously (35).

The number of surviving crypts per cross-section was determined for each mouse by scoring the number of surviving crypts in 10 complete, well-oriented cross-sections in a blind manner and dividing the total by the number of cross-sections scored.

Statistical Analysis. The data were analyzed by analysis of variance. If analysis of variance justified post hoc comparisons between group means, these were conducted using the Student-Newman-Keuls test for multiple comparisons. For experiments in which only single experimental and control groups were used, group differences were examined by unpaired Student’s t test. Differences were considered significant at P < 0.05. All of these analyses were done using GraphPad Prism from GraphPad Software, Inc. (San Diego, CA).

RESULTS

Ionizing Radiation Induces NFκB Activation in Intestinal Epithelial Cells in a Dose- and Time-Dependent Manner. We demonstrated previously that mice responded to total body irradiation with increased NFκB activity in a tissue-specific manner (26). Specifically, the increase in NFκB activity was found in the bone marrow, spleen, and lymph node, but was not seen in the liver, lung, colon, thymus, and brain (26). Now, we demonstrate that exposure of mice to total body irradiation also activated NFκB in intestinal epithelial cells in a dose-dependent manner after mice were exposed to increasing doses of total body irradiation (from 0.5 to 12 Gy; Fig. 1A). In addition, the increase in NFκB activity in intestinal epithelial cells was time dependent, because the increase occurred within 30 minutes, peaked at 2 hours, and then gradually declined thereafter but remained elevated for up to 24 hours after exposure to 8 Gy total body irradiation (Fig. 1B).

The molecular composition of the NFκB activated by ionizing radiation in intestinal epithelial cells was determined by gel supershift assay. As shown in Fig. 1C, a single retarded band that represented the specific NFκB DNA binding activity in the nuclear extracts of irradiated intestinal epithelial cells was abrogated by the addition of excess unlabeled NFκB oligonucleotides but was not affected by that of unlabeled activator protein-1 or mutated NFκB oligonucleotides, demonstrating the specificity of the assay. The retarded band was supershifted by the addition of anti-p50 and/or anti-RelA antibodies, but was not changed by the addition of anti-Erg-1 antibody, indicating that ionizing radiation-activated NFκB in intestinal epithelial cells mainly consisted of p50/RelA heterodimers. A similar molecular composition of ionizing radiation-activated NFκB was also found in the spleen, lymph node, and bone marrow in our studies reported previously (26, 27, 30).

Targeted Disruption of the p50 Gene in Mice Attenuated Ionizing Radiation-Induced NFκB Activation in Intestinal Epithelial Cells. Because the NFκB complex activated by ionizing radiation in murine intestinal epithelial cells mainly consists of p50/RelA heterodimers, we examined whether the gene-targeted disruption of the p50 gene could attenuate ionizing radiation-induced NFκB activation in intestinal epithelial cells. As shown in Fig. 2, intestinal epithelial cells from both unirradiated wild-type and p50−/− mice expressed a barely detectable level of NFκB activity. Exposure of wild-type mice to 8 Gy total body irradiation resulted in a 219-fold increase in intestinal epithelial NFκB activity, whereas only a 43-fold increase in NFκB activity was observed in the intestinal epithelium of p50−/− mice after the same dose of total body irradiation (P < 0.001). The residual NFκB activity activated by ionizing radiation in p50−/− intestinal epithelium mainly consists of p52/RelA heterodimers (Fig. 6242
This finding is in agreement with our previous observations in the spleen, lymph node, and bone marrow (27, 30), demonstrating that the p50 NFκB subunit is an essential component of the NFκB complexes activated by ionizing radiation in vivo and that the lack of p50 cannot be fully replaced by the other members of the NFκB/Rel family.

Activation of NFκB Selectively Protects Intestinal Epithelial Cells of the Small Intestinal Crypts from Ionizing Radiation-Induced Damage. NFκB can induce the expression of many proapoptotic and antiapoptotic proteins that regulate cell survival, and its activation has been implicated in inhibition or promotion of apoptosis in a cell type- and stimulus-dependent manner (4–7). The role of NFκB activation in ionizing radiation-induced normal tissue damage has not been well established. However, our recent studies have shown that activation of NFκB by ionizing radiation has no significant effect on ionizing radiation-induced splenic lymphocyte apoptosis (27). More recently, Egan et al. (36) have also reported that activation NFκB protects intestinal epithelial cells from ionizing radiation-induced apoptosis. Therefore, we additionally examined the role of NFκB in ionizing radiation-induced normal tissue damage in various nonlymphoid tissues. We compared total body irradiation-induced tissue damage and apoptosis in wild-type versus p50−/− mice, because p50−/− mice exhibited a significant reduction in ionizing radiation-induced NFκB activation (Fig. 2; refs. 27, 30).

As shown in Fig. 3A, exposure of wild-type and p50−/− mice to total body irradiation induced bone marrow-mononuclear cell apoptosis in a dose- and time-dependent manner. Similarly, total body irradiation (4 Gy) also significantly decreased the frequency of various day types of cobblestone area-forming cell, probably due to the induction of apoptosis (Fig. 3B; refs. 28, 37). However, there was no significant difference between wild-type animals and p50−/− mice in their response to ionizing radiation-induced bone marrow-mononuclear cell apoptosis and in their decrease in cobblestone area-forming cell frequency (P > 0.05), suggesting that activation of NFκB by ionizing radiation had no significant effect on ionizing radiation-induced bone marrow toxicity.

Exposure of wild-type and p50−/− mice to total body irradiation (8 Gy) also significantly increased intestinal epithelial cell apoptosis in the small intestinal crypts at 6 and 24 hours after ionizing radiation as compared with the baseline levels of apoptosis in unirradiated mice (P < 0.01; Fig. 4A and Fig. 5A). The increase in intestinal epithelial cell apoptosis then declined to insignificant levels at 72 hours after total body irradiation (P > 0.05; Fig. 4A). Although the levels of intestinal epithelial cell apoptosis were not significantly different between wild-type and p50−/− mice at 6 hours after ionizing radiation (apoptotic cell/crypt: wild-type 4.3 ± 0.7 versus p50−/− 3.8 ± 0.4, P > 0.05), the level of intestinal epithelial cell apoptosis in p50−/− mice was 1.4-fold higher than that of wild-type at 24 hours after total body irradiation (apoptotic cell/crypt: wild-type 4.1 ± 0.2 versus p50−/− 5.8 ± 0.5, P < 0.01). The increase in ionizing radiation-induced intestinal epithelial cell apoptosis in p50−/− mice at 24 hours after total body irradiation was associated with an enhanced activation of caspase 3 and DNA fragmentation (terminal deoxynucleotidyl transferase-mediated nick end labeling assay; Fig. 5A). Similarly, RelA/TNFRI−/− mice also showed a 1.5-fold increase in intestinal epithelial cell apoptosis at 24 hours after total body irradiation (8 Gy) as compared with TNFR1−/− mice (apoptotic cell/crypt: TNFR1−/− 3.5 ± 0.2 versus RelA/TNFRI−/− 5.3 ± 0.5, P < 0.01; Fig. 4B). In addition, p50−/− mice exhibited a diminished survival of the intestinal crypts at 3 days after exposure to 12 Gy total body irradiation when compared with the same dose-irradiated wild-type mice (Fig. 4C and Fig. 5B). The number of surviving crypts was significantly lower in irradiated p50−/− mice than that of irradiated wild-type mice (surviving crypts/cross-section: wild-type 28.3 ± 2.3 versus p50−/− 20.3 ± 0.9, P = 0.012; Fig. 4C). In contrast, no significant tissue damage was observed in the liver, heart, lung, and kidney after p50−/− and wild-type mice were exposed to 8 Gy total body irradiation (data not shown). Together, the data presented above suggest that activation of NFκB selectively protects the small intestine from ionizing radiation-induced damage but has no significant effect on irradiating radiation-induced lymphoid and hematopoietic toxicity.

Targeted Disruption of the p50 Gene Sensitizes Mice to Total Body Irradiation-Induced Lethality. To determine whether targeted disruption of the p50 gene not only increases ionizing radiation-induced intestinal epithelial cell damage but also more importantly sensitizes mice to total body irradiation-induced lethality, wild-type and p50−/− mice were exposed to increasing doses of total body irradiation, and their survival was monitored for 30 days. The LD50 values for day 7 (LD50/7) and day 30 (LD50/30) were calculated for both strains of mice and are presented in Table 1. As shown in Table 1, p50−/− mice had much lower LD50/7 and LD50/30 values than those of wild-type mice (LD50/7: p50−/− = 7.75 Gy versus wild-type = 13.12 Gy).
value represents ionizing radiation-induced hematopoietic syndrome (38). This suggestion is in agreement with the observations that p50−/− mice exhibited an increase in intestinal epithelial cell apoptosis and decrease in crypt survival after they were exposed to total body irradiation.

**DISCUSSION**

Molecularly targeted inhibition of NFκB has the potential to be developed as a novel cancer therapy. Particularly, treatment with a combination of NFκB inhibitors and conventional ionizing radiation or chemotherapeutic agents may dramatically improve the antitumor

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**Fig. 4.** Lack of p50 or RelA sensitizes intestinal epithelial cells to ionizing radiation-induced damage. A, wild-type (WT) and p50−/− mice were exposed to 8 Gy total body irradiation or unirradiated (control). Apoptotic intestinal epithelial cells in the small intestinal crypts were determined at 6 (n = 3), 24 (n = 7), and 72 hours (n = 3) after total body irradiation (TBI). The results are expressed as mean; bars, ± SE. A, P < 0.01 versus their respective unirradiated controls (n = 3); b, P < 0.01 versus 24 hours after irradiated wild-type mice. B, TNFR1−/− and RelA/TNFR1−/− mice (n = 4) were exposed to 8 Gy total body irradiation. Apoptotic intestinal epithelial cells in the small intestinal crypts were determined at 24 hours after total body irradiation. The results are expressed as mean; bars, ± SE. C, wild-type and p50−/− mice were exposed to 12 Gy total body irradiation. The number of surviving crypts in the small intestine was determined at 3 days after total body irradiation. The results are expressed as mean; bars, ± SE (n = 7).

Gy: LD50/30; p50−/− = 7.81 Gy versus wild-type = 9.31 Gy), indicating that p50−/− mice were more sensitive to total body irradiation-induced death than wild-type mice. However, the LD50/30 value of p50−/− mice was similar to their LD50/7 value. This suggests that the increased susceptibility of p50−/− mice to total body irradiation-induced lethality is mainly attributable to an enhanced intestinal damage, because it has been well established that the LD50/7 value indicates that ionizing radiation caused intestinal toxicity and LD50/30

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**Table 1**

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<td>WT</td>
<td>13.12</td>
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<td>p50−/−</td>
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<td>(7.17–8.32)†</td>
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<td>DMF†</td>
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NOTE. Groups of WT mice (15–34 per group) were exposed to 8–15 Gy TBI, and groups of p50−/− mice (15–35 per group) were exposed to 6–9.5 Gy TBI. The survival of these mice was recorded during a 30-day observation period after TBI.

Abbreviations: WT, wild-type; DMF, dose modification factor; TBI, total body irradiation.

† 95% confidence intervals.

† DMF = LD50/30 WT/LD50/30 p50−/−.

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**Fig. 5.** Representative photomicrographs of irradiated small intestinal crypts. A, apoptotic cells (arrow-pointed) in the small intestinal crypts of wild-type (WT) and p50−/− mice at 24 hours after exposure to 8 Gy total body irradiation were identified by hematoxylin and eosin (H&E) staining, active caspase 3 immunostaining, and terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assay (×400). B, surviving crypts (arrow-pointed) in wild-type and p50−/− mouse intestines at 3 days after exposure to 12 Gy total body irradiation. The S-phase cells incorporating bromodeoxyuridine were stained red by mouse antiribomodeoxyuridine antibody, and Texas red-labeled goat antimouse IgG and nuclei were stained blue with Hoechst 33342.

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response. However, the effect of NFκB inhibition on the normal tissue response to ionizing radiation- and chemotherapy-induced injury must be evaluated before the clinical application of NFκB inhibition. Therefore, using a mouse model we examined the tissue specificity of ionizing radiation-activated NFκB and the role of NFκB in ionizing radiation-induced normal tissue damage. In addition to the spleen, lymph node, and bone marrow identified in our previous studies (26, 27, 30), we now discovered that exposure of mice to a low dose (≤2 Gy) of total body irradiation also activated NFκB in intestinal epithelial cells of the small intestine. The activation of NFκB in these tissues was in a dose-dependent fashion when mice were exposed to increasing doses (up to 12 Gy) of total body irradiation. In contrast, no significant activation of NFκB was observed in the liver, lung, colon, and brain after mice were exposed to total body irradiation in the same dose range (26, 27, 30). These results suggest that when mice are exposed to a clinically relevant dose (≤2 Gy) of total body irradiation, ionizing radiation activates NFκB in vivo in a tissue-specific manner.

However, when mice were exposed to a super lethal dose (20 Gy) of total body irradiation, a significant activation of NFκB was found in the liver and kidney (39). In addition, a delayed activation of NFκB was also observed in irradiated rat lung after 20 Gy pulmonary irradiation (40). These findings demonstrate that the tissues that are prone to ionizing radiation toxicity, such as the spleen, lymph node, bone marrow, and small intestine, are more sensitive to ionizing radiation-activated NFκB, whereas radiosensitive tissues are less responsive to ionizing radiation for NFκB activation. Thus, it appears that different normal tissues possess diverse sensitivity to ionizing radiation-induced activation of NFκB, which correlates to their susceptibility to ionizing radiation-induced tissue damage.

Such a correlation implies that NFκB may function as a sensor that can detect ionizing radiation-induced tissue damage. In turn, activated NFκB can modulate tissue responses to ionizing radiation damage by stimulating the expression of various genes that are involved in regulation of cell survival, cell proliferation, and tissue inflammation (1–3). Depending on the particular tissue involved, activation of NFκB may confer tissue protection or contribute to tissue injury in response to ionizing radiation (27, 36, 40). In agreement with this hypothesis, we found that down-regulation of NFκB activation by the targeted disruption of the p50 or RelA gene in mice sensitized intestinal epithelial cells to ionizing radiation-induced damage. A similar finding was reported recently by Egan et al. (36) using mice that had genetically targeted ablation of inhibitor of NFκB kinase-β expression in intestinal epithelial cells to specifically block NFκB activation in these cells. These findings indicate that activation of NFκB is radioprotective in the small intestine. Correspondingly, p50−/− mice exhibited a higher sensitivity to ionizing radiation-induced intestinal syndrome and had a much lower LD50 value than wild-type animals after they were exposed to increasing doses of total body irradiation. However, the inhibition of NFκB activation by targeted disruption of the p50 gene had no significant effects on ionizing radiation-induced lymphoid and hematopoietic cell damage nor did it affect the responses of the liver, lung, heart, and kidney to ionizing radiation (27). This suggests that ionizing radiation not only activates NFκB in vivo in a tissue-specific manner, but more importantly the activation also confers tissue-specific protection against ionizing radiation-induced normal tissue damage.

The mechanisms underlying the tissue-specific protection against ionizing radiation by NFκB activation are not clear at the present. It may relate to cross-talk among different transcriptional factors activated by ionizing radiation. For example, it is well known that exposure of mice to total body irradiation also induces tissue-specific activation of p53 (41–43). Transcriptional induction of certain apoptosis proteins by p53 contributes to ionizing radiation-induced apoptosis in various tissues (41–45). NFκB and p53 share the transcriptional coactivator proteins CREB-binding protein and p300 (46–48). Competition between NFκB and p53 for binding to these transcriptional coactivator proteins may dictate the outcome of the responses in different tissues to ionizing radiation and has yet to be investigated.

The discovery that mice respond to total body irradiation with tissue-specific activation of NFκB and that this activation can selectively protect the intestine from ionizing radiation-induced damage is intriguing. It suggests that additional investigations are required for a better understanding of the role of NFκB in radiation biology and cancer therapy to provide guidance to the development of targeted NFκB inhibition as a novel adjuvant therapy for cancer in combination with radiotherapy. Particularly, it has yet to be determined if activation of NFκB by a clinical relevant dose of fractionated irradiation confers a similar protection against ionizing radiation-induced normal tissue damage. These investigations will be important to determine whether irradiation therapy and NFκB inhibition should or should not overlap in sensitive normal tissues to avoid an augmented normal tissue injury. On the basis of our finding, we hypothesize that a combination therapy using a NFκB inhibitor plus ionizing radiation will be useful in treating tumors that are not in close proximity to the small intestine. However, it may carry a significant risk if it is used to treat abdominal tumors, because NFκB inhibition may also increase ionizing radiation-induced intestinal damage. Likewise, these studies suggest that combination of NFκB inhibition with chemotherapy may also carry a substantial risk of normal tissue damage due to the overlap of the two therapies within the body, which has yet to be determined. This underscores the importance of evaluating the potential clinical complications using ionizing radiation and chemotherapy in combination with NFκB inhibitors in cancer treatment.

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NFκB PROTECTS SMALL INTESTINE FROM IRRADIATION DAMAGE


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