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Partial liver irradiation in rats induces the hypertrophy of nonirradiated liver lobes through hepatocyte proliferation†

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ABSTRACT

Irradiation of the liver induces a regenerative response in the nonirradiated part of the liver. It is unclear whether this leads to actual liver enlargement. The aim of this study was to evaluate the weight of compensatory hypertrophy that occurs in nonirradiated livers and to clarify the mechanism of hypertrophy from the viewpoint of hepatocyte proliferation. The anterior liver lobes (anterior lobes) were irradiated with 60 Gy of X-rays (X60 Gy) under opening laparotomy. Body weights and liver lobe weights were measured before and at 1, 4, 8 and 12 weeks after irradiation, and serum and liver tissue samples were analyzed at each time point. The anterior lobes atrophied progressively, whereas the posterior liver lobes (posterior lobes) hypertrophied in the X-ray irradiated (X-irradiated) group. Although temporary liver damage was observed after irradiation, liver function did not decrease at any time point. Hepatocyte degeneration and loss were observed in the anterior lobes of the X-irradiated group, and significant fibrosis developed 8 weeks postirradiation. Following irradiation, the proportion of Ki-67-positive cells in the anterior lobes decreased markedly in the early postirradiation period, whereas the proportion of positive cells in the posterior lobes increased, peaking at 4 weeks postirradiation ($P < 0.05$). Increased tumor necrosis factor-α expression was observed only in the anterior liver lobes of the X-irradiated group at 1 and 4 weeks postirradiation. Partial liver irradiation with X60 Gy induced compensatory hypertrophy of nonirradiated liver lobes. This study suggests that liver hypertrophy after partial liver irradiation is caused by increased hepatocyte mitosis.

Keywords: radiation-induced liver disease; partial liver irradiation; X-ray; rat; liver hypertrophy; hepatocyte proliferation

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, and it is treated using various therapies such as liver resection, liver transplantation and local ablation including radiofrequency ablation [1–3]. Radiation therapy was previously an adjunct for the treatment of HCC, but its usefulness has improved in recent years with advances in irradiation technology. For example, stereotactic body radiotherapy was reported to have high therapeutic efficacy comparable with that of radiofrequency ablation and is expected to be a treatment option for patients for whom other treatment methods are unsuitable [4, 5]. Radiation therapy for HCC may be developed further in the future.

However, radiation-induced liver disease (RILD) is a well-known complication of radiotherapy of the liver, although its mechanism remains unclear and treatment for this complication has not been established [6, 7]. Multiple cells such as sinusoidal endothelial cells

and Kupffer cells are involved in the pathogenesis of RILD, and tumor necrosis factor-alpha (TNF-α) and transforming growth factor-beta promote hepatocyte apoptosis and liver fibrosis; however, most of the mechanisms involved are unresolved [7]. Elucidation of the mechanism of RILD and development of treatment methods are essential to expand the indications of radiotherapy for HCC. To date, RILD-related studies have focused on changes in the irradiated areas of the liver. However, the compensatory response in nonirradiated areas of the liver is also important. Because the liver has a high regenerative capacity [8], fully exploiting its potential could lead to the avoidance or treatment of RILD. Although active research in the field of surgery has focused on the regenerative ability of the liver, there have been few studies on the regenerative response after irradiation. In 2009, Zhao *et al.* reported the first data on liver regeneration after partial liver X-ray irradiation (X-irradiation) in rats [9]. Several similar studies have since been reported, all showing that partial liver irradiation of rats with X-rays promotes hepatocyte proliferation in nonirradiated areas [10, 11]. These studies have several problems. First, changes in liver volume were not evaluated. The proliferative response of hepatocytes was evaluated using cell proliferation markers including proliferating cell nucleus antigen. Second, the regenerative capacity of the nonirradiated liver area may have been underestimated. Namely, cell proliferation occurs in the irradiated liver, which may suppress the regenerative capacity of the nonirradiated area. Third, it is difficult to make comparisons between partial hepatectomy (PH) models or portal vein ligation/embolization (PVL/PVE) models because irradiation of specific liver lobes has not been performed.

To solve these problems, we used a model in which the anterior lobes are irradiated using an intraoperative technique and studied the weight change in each liver lobe, as well as liver function and hepatocyte proliferation when the regenerative response occurs only in the nonirradiated lobes.

MATERIALS AND METHODS

Animals

Six-week-old male Wistar rats weighing 120–140 g were purchased from Japan SLC (Shizuoka, Japan). They were kept in a temperature-controlled environment with a 12-h light/dark cycle. Standard laboratory food (CE-2, CLEA Japan, Inc, Tokyo, Japan) and water were provided *ad libitum*. The animal study was reviewed and approved by the Animal Care and Experimentation Committee of our facility (No. 19-051).

Partial liver X-irradiation and sham irradiation

To prepare irradiated and nonirradiated liver lobes in the same rat, we irradiated only the anterior liver lobes [anterior lobes: median lobe (ML) + left lateral lobe (LLL)]. To avoid any damage to the posterior liver lobes [posterior lobes: right lateral lobe (RLL) + caudate lobe (CL)] and the gastrointestinal tract, a shield was inserted in front of the posterior lobes by laparotomy, and the liver was irradiated directly in that condition. The out-of-field leakage dose was 1.88 Gy in the posterior lobes. This is an application of the irradiation method developed by Imaeda *et al.* [12]

Initially, 7-week-old rats were anesthetized by the intraperitoneal injection of ketamine (100 mg/body weight kg) and xylazine (10 mg/body weight kg). After median laparotomy, a 3-mm-thick high-density tungsten sheet (density: 12, Nippon Tungsten Co., Ltd, Fukuoka, Japan) was inserted between the anterior and posterior lobes (Fig. 1). Single doses of 0 (sham) or 60 Gy were delivered by a high-power X-ray generator (TITAN-225S, Shimadzu Industrial Systems Co., Ltd, Shiga, Japan) operating at 200 kVp, 14.6 mA, with a 0.5 mm aluminum plus 0.5 mm copper filter. At a distance of 373.7 mm, the dose rate was 2.22 Gy/min. The abdominal cavity was immediately closed after irradiation. Rats were weighed once a week and kept for 1 to 12 weeks postirradiation.

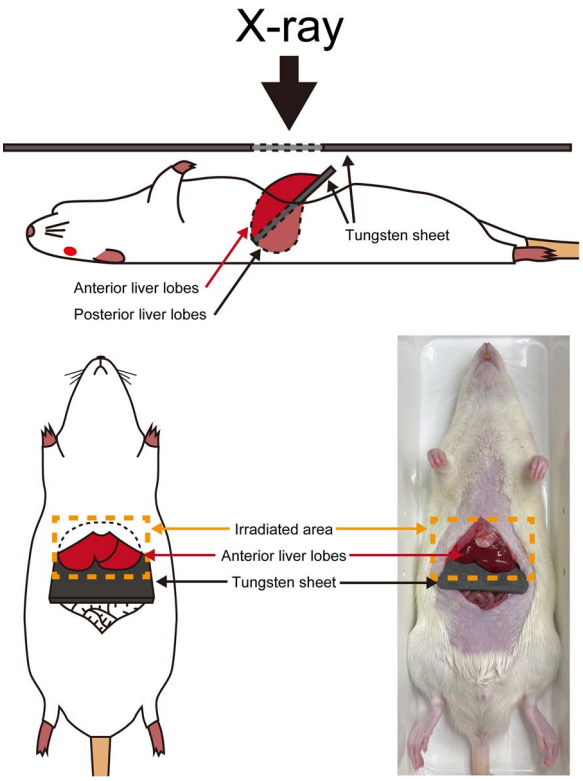

Fig. 1. Schema for the partial liver irradiation technique used in this study.

Liver tissue and blood sampling

Three rats each were sacrificed for sampling at 1, 4, 8 and 12 weeks after sham- and X-irradiation, and at 0 weeks before irradiation for baseline values. Rats were anesthetized and laparotomized using the same procedures as for irradiation, and then blood samples were obtained from the inferior vena cava. Rats were sacrificed by exsanguination, livers were removed, each liver lobe was weighed and the ratio of each wet liver lobe weight to body weight [liver index (LI)] was calculated and expressed as follows: liver weight (g)/100 g body weight. Each liver lobe was divided into two segments and processed for histopathologic evaluation as follows. One of each divided liver lobe was fixed in 4% paraformaldehyde for 48 h, then embedded in paraffin and sectioned (4 μm thickness). The other of each liver lobe was immersed in the embedding medium (Tissue-Tek O.C.T. Compound, Sakura Finetek Japan Co., Ltd, Tokyo, Japan), then frozen and sectioned (7 μm thickness).

Blood tests

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels, markers of liver damage and serum albumin (ALB) and total bilirubin (T-BIL) levels, markers of liver function, were measured by the Oriental Yeast Industry Co., Ltd (Tokyo, Japan). The measurement method for each parameter was as follows; AST, ALT and ALP: Japan Society of Clinical Chemistry transferable method, ALB: bromcresol green method, T-BIL: bilirubin oxidase method. Prealbumin (PA), a sensitive marker of liver function, was also measured using an ELISA kit (OKIA00159, Aviva System Biology Corporation, San Diego, CA, USA) according to the manufacturer's instructions.

Histopathological and immunohistochemical analysis

Paraffin-embedded tissue sections were used to perform hematoxylin and eosin staining, Masson trichrome staining or Ki-67 immunostaining. The frozen tissue sections were used to perform TNF-α immunostaining.

To quantify liver fibrosis, each Masson trichrome-stained section was observed in a 100× field of view, and 20 fields were randomly selected. Fields of view containing giant vascular vessels, long-axis sliced vascular vessels and liver surfaces were excluded. Image analysis software (WinROOF2018, Mitani Corporation, Fukui, Japan) was used to identify fibrotic areas and to calculate the proportion of fibrotic areas [liver fibrosis index (LFI)] in the entire field of view.

Immunostaining for Ki-67 was performed to evaluate the proliferative status of hepatocytes. Anti-Ki-67 polyclonal antibody (1 μg/ml, ab15580, Abcam, Cambridge, UK) was used as the primary antibody. We used Takara POD Conjugate Anti Rabbit for Tissue (MK205, Takara Bio Inc, Shiga, Japan) as the ready-to-use peroxidase-labeled secondary antibody. The peroxidase activity was visualized using a diaminobenzidine kit (Takara DAB Substrate, MK210, Takara Bio Inc). Each stained section was observed in a 200× field of view, and five fields centered on the midpoint between the central vein and the portal vein were randomly selected. In that field of view, ~1000 hepatocytes per section were counted to calculate the proportion of positive cells (proliferation index: PI) and to evaluate hepatocyte proliferation in each liver lobe.

Immunostaining for TNF-α was performed to assess the state of inflammation in the liver. TNF-α antibody (sc-52746, Santa Cruz Biotechnology, Dallas, TX, USA) was used as the primary antibody. We used Takara POD Conjugate Anti Mouse for Tissue (MK204, Takara Bio Inc, Shiga, Japan) as the ready-to-use peroxidase-labeled secondary antibody. The peroxidase activity was visualized using a diaminobenzidine kit (Takara DAB Substrate, MK210).

Statistical analysis

The data are presented as the means ± standard deviations (SDs). $P < 0.05$ was considered statistically significant. The significance of differences between the two different groups was determined by Welch's *t*-test, and the three different groups were determined by one-way ANOVA and Tukey's post hoc test using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [13], which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Survival and body weight of rats after partial liver irradiation

After the establishment of the partial liver irradiation model, no surgery-related and irradiation-related deaths were observed during the experiment. Rat body weights increased over time in all groups, but the X-ray irradiated (X-irradiated) group showed slower weight gain than the sham-irradiated group (Supplementary Fig. 1).

Macroscopic appearance and LI after partial liver irradiation

Changes in the macroscopic appearance were observed in both liver lobes of the X-irradiated group. Irradiated anterior lobes were atrophied later in the observation period, and their coloration was the same as that of the nonirradiated liver lobes. In addition, nonirradiated liver lobes became hypertrophied over time. The surface pattern of the liver lobes was enlarged compared with that of the nonirradiated liver lobes, and the contours of the central veins were easily identified (Fig. 2A).

In the X-irradiated group, the anterior lobes progressively atrophied over time and showed significant weight loss at 8 weeks postirradiation ($P < 0.05$). The LI at 8 weeks postirradiation was 1.8 ± 0.1 and 2.7 ± 0.2 for the X-irradiated and sham-irradiated groups, respectively, and at 12 weeks it was 1.2 ± 0.1 and 2.6 ± 0.1, respectively. However, the posterior lobes of the X-irradiated group were significantly hypertrophied at 4 weeks postirradiation ($P < 0.05$). The LI at 4 weeks postirradiation was 1.4 ± 0.1 and 1.1 ± 0.0, respectively; at 8 weeks, it was 2.1 ± 0.1 and 1.1 ± 0.1, respectively; and at 12 weeks, it was 2.4 ± 0.1 and 1.1 ± 0.0, respectively. The LI of the whole liver was comparable with that of the sham-irradiated group at all time points (Fig. 2B).

Liver injury and function after partial liver irradiation

Blood tests were performed to evaluate the degree of liver damage caused by irradiation and total liver function (Fig. 3). In the X-irradiated group, significant increases in serum ALP were observed at 4 and 12 weeks postirradiation ($P < 0.05$). In the first week after irradiation, serum AST was low in the irradiated group. Serum AST and ALT tended to be elevated at 8 weeks postirradiation, but the change was not significant ($P = 0.0502$ and $P = 0.0612$, respectively). Liver functions indicated by serum T-Bil, ALB and PA did not differ significantly between groups at any time point.

Histopathologic changes after partial liver irradiation

The X-irradiated group showed degenerated hepatocytes with cytoplasm vacuolization in hepatocytes, loss of liver parenchymal cells and disorganized arrangement of sinusoids in the anterior lobes at 4, 8 and 12 weeks postirradiation. In the posterior lobes of the X-irradiated group, no pathologic injury was observed (Supplementary Fig. 2).

Liver fibrosis after partial liver irradiation

Masson trichrome staining was performed to assess liver fibrosis caused by irradiation, and the fibrosis ratio (LFI) was quantified. Significant fibrosis developed in the anterior lobes after 8 weeks in the X-irradiated group ($P < 0.05$) (Fig. 4A). There was no evidence of fibrosis progression in the posterior lobes. Fibrosis of the liver occurred

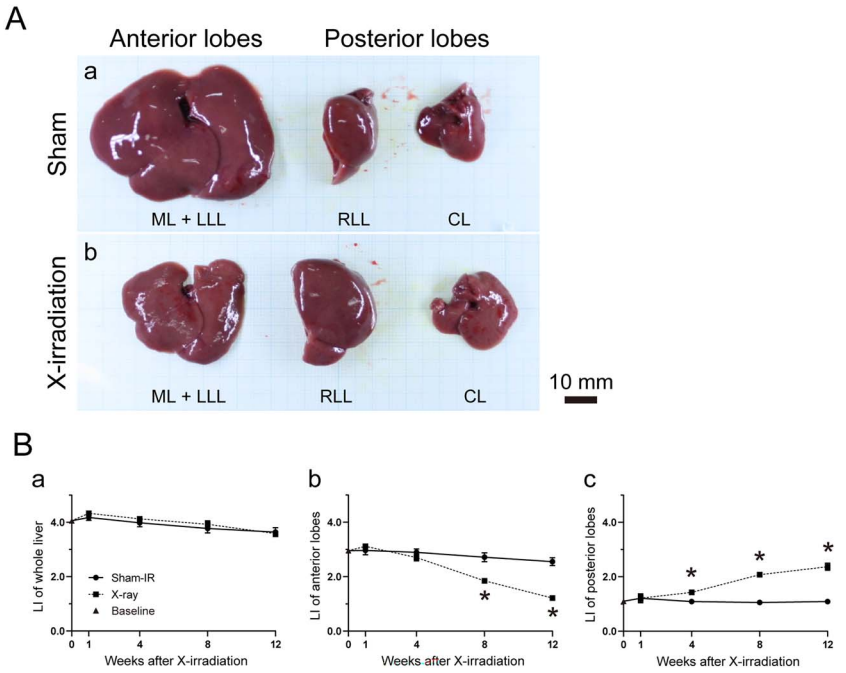

Fig. 2. Macroscopic appearance and weight changes of the liver lobes after partial liver X-irradiation of 60 Gy. (A) Liver lobes at 12 weeks after treatment. Atrophy of ML + LLL and hypertrophy of RLL and CL are observed in the X-irradiated group. (B) Changes in the liver lobe/body weight ratio levels (LI) after partial liver irradiation. Irradiation induced atrophy of the irradiated liver and hypertrophy of the nonirradiated liver. Mean ± SD. $n = 3$. $^*P < 0.05$ vs the sham-irradiated group.

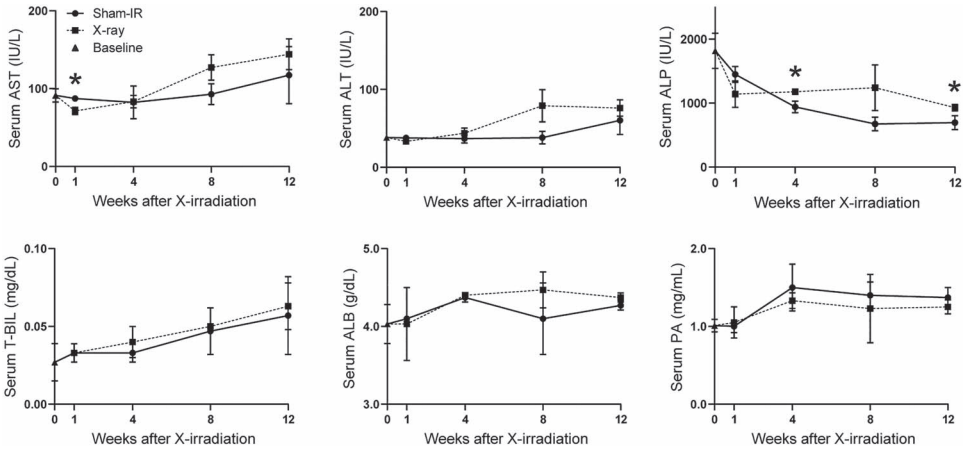

Fig. 3. Changes in blood test values for liver injury and liver function after partial liver irradiation. Partial liver X-irradiation of 60 Gy induced liver damage, but did not cause a loss of liver function. Mean ± SD. $n = 3$. $^*P < 0.05$ vs the sham-irradiated group.

around the portal vein and the central vein and surrounding sinusoids (Fig. 4B).

Liver proliferation after partial liver irradiation

To assess the regenerative response of hepatocytes, immunostaining of Ki-67, a marker of cell proliferation, was performed and its positivity (PI) was quantified (Fig. 5). In the anterior lobes of the X-irradiated group, the proportion of Ki-67-positive cells decreased markedly early after irradiation. This proportion was significantly lower than that of the sham-irradiated group at 1 week postirradiation ($P < 0.05$). Thereafter, PI in the X-irradiated group tended to be lower. PI in the posterior lobes of the X-irradiated group increased significantly and reached a peak at 4 weeks postirradiation ($P < 0.05$).

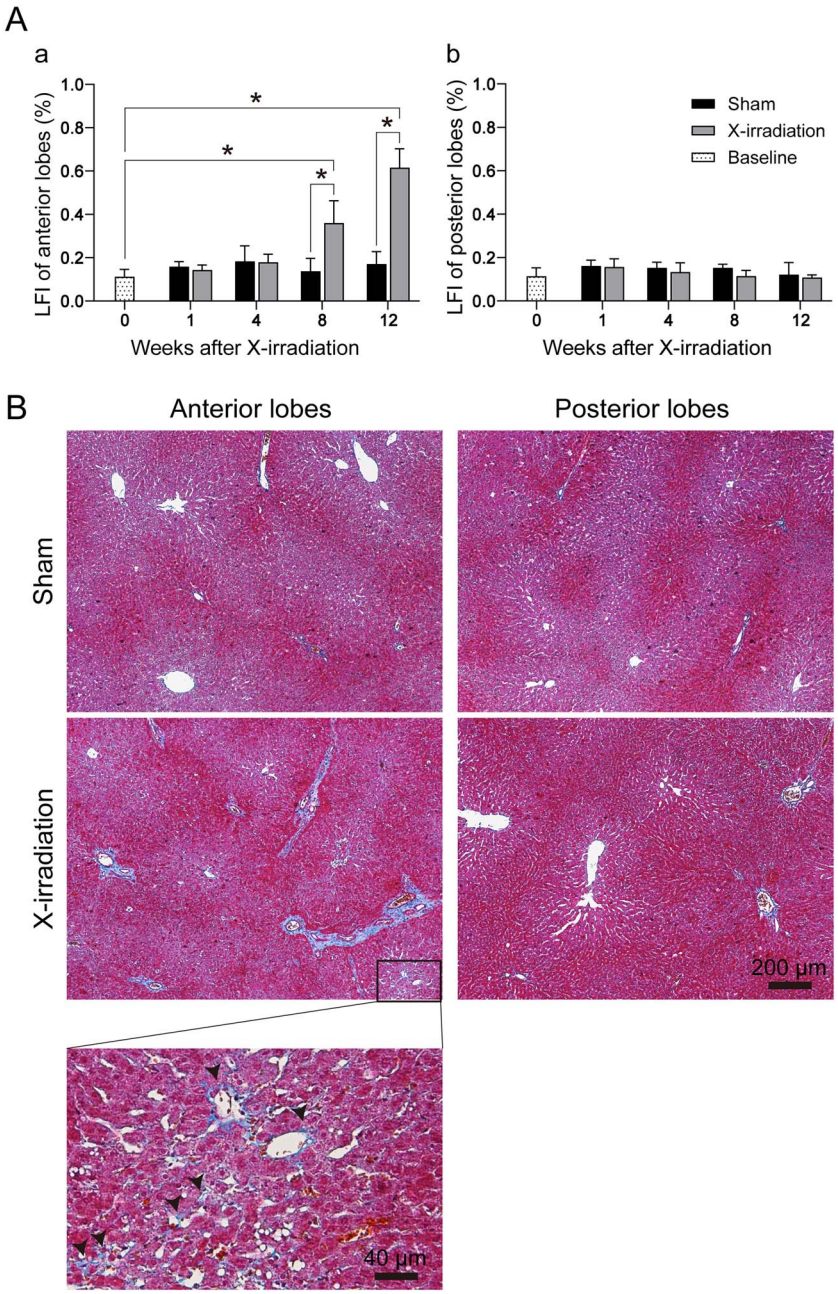

Fig. 4. Liver fibrosis after partial liver X-irradiation of 60 Gy. (A) Liver fibrosis rate (LFI) at each time point. Radiation-induced fibrosis of the irradiated area occurred 8 weeks postirradiation. Statistical analysis was performed by one-way ANOVA. (B) Masson Trichrome staining at 12 weeks after partial liver irradiation (original magnification ×40). Fibrosis is observed around the central vein where hepatocyte damage is common as well as around the portal vein in the anterior lobes of the irradiated group (arrowheads). Mean ± SD. $n = 3$. $^{*}P < 0.05$.

Expression of TNF-α after partial liver irradiation

To investigate the involvement of cytokines in radiation-induced liver injury, TNF-α expression was examined (Fig. 6). In the X-irradiated group, spot TNF-α expression was observed in the anterior lobes 1 week after irradiation. It appeared extensively after 4 weeks and did not occur in the posterior lobes.

DISCUSSION

It has already been reported that partial rat liver irradiation with X-rays induces a proliferative response in hepatocytes in nonirradiated areas [9–11]. Indicators of proliferation used in those studies included Mitotic index, proliferating cell nuclear antigen and cyclin D1. However, it has not been clarified whether liver volume ultimately increases

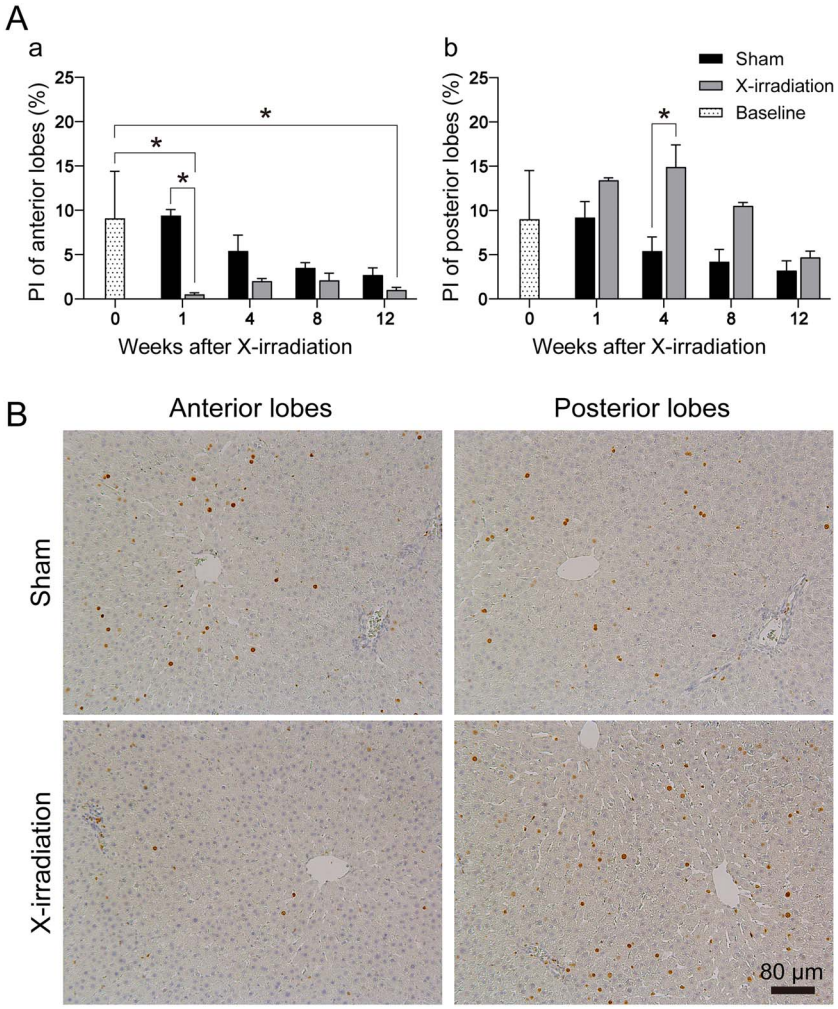

Fig. 5. Hepatocyte proliferation after partial liver X-irradiation of 60 Gy. (A) The proportion of Ki-67-positive cells (PI) at each time point. X-irradiation decreased Ki-67 expression in the irradiated liver lobes and increased expression in the nonirradiated liver lobes. Statistical analysis was performed by one-way ANOVA. (B) Ki-67 immunostaining at 4 weeks after partial liver irradiation (original magnification ×100). Positive cells are increased in the posterior lobes of the irradiated group. Mean ± SD. $n = 3$. $^{*}P < 0.05$.

after hepatocyte proliferation. In our study, we have confirmed for the first time that liver hypertrophy occurs in the nonirradiated liver lobes after partial liver irradiation, in addition to the hepatocyte proliferative response. This is a useful result that compensates for the shortcomings of the previous report. In a previous report, when half of the liver of SD rats were irradiated with 25 Gy of X-rays, the proliferative response of the nonirradiated liver peaked 60 days after irradiation [9, 11]. Nevertheless, in our study, the peak proportion of Ki-67-positive hepatocytes occurred at 4 weeks postirradiation. This difference may be due to the higher irradiation dose, and the X-rays strongly suppressed the proliferative response of the irradiated lobes. Hypertrophy of nonirradiated lobes began around 4 weeks postirradiation and continued until 12 weeks. The changes were most pronounced at weeks 4–8, coinciding with the peak in the proportion of Ki-67-positive hepatocytes. These results strongly suggest that compensatory liver hypertrophy after irradiation is the result of hepatocyte proliferation.

It was previously reported that partial rat liver irradiation with X-rays induced a regenerative response in the irradiated area as well as in the nonirradiated area of the liver [11]. In our study, to evaluate only the regenerative response of the nonirradiated area, it was necessary to administer a sufficient dose that suppressed the regenerative response of the irradiated area. Another study reported that the proliferative response of hepatocytes was markedly suppressed after the single 60 Gy X-irradiation of the whole liver of SD rats [14]. In our preliminary experiments, single irradiation with 30 Gy of X-ray to the anterior lobes of Wistar rats resulted in low hypertrophy in the nonirradiated lobes. For these reasons, we decided to irradiate with 60 Gy of X-rays (X60Gy) to the anterior lobes. The proportion of Ki-67-positive hepatocytes in irradiated lobes was already strongly suppressed in the

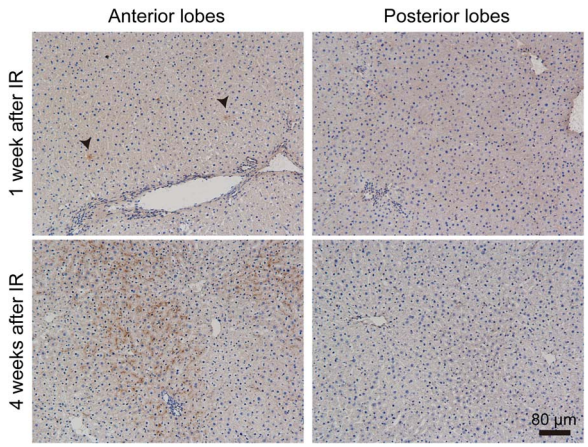

Fig. 6. TNF-α expression of the irradiated anterior lobe and the nonirradiated posterior lobe at 1 and 4 weeks after partial liver X-irradiation of 60 Gy. Arrowheads, spot-like expressions. IR = irradiation.

1-week postirradiation. The results showed that, as expected, irradiation with X60Gy suppressed the proliferative response in irradiated lobes and produced a faster compensatory response in the nonirradiated lobes than previously reported. According to our histological evaluation, hepatocyte degeneration and loss became more pronounced, and fibrosis progressed in the X-irradiated lobes after 8 weeks postirradiation. Since X-irradiation has been shown to induce apoptosis of hepatocytes [7], it may have reduced the number of hepatocytes, resulting in liver atrophy of the irradiated lobes. The proliferative response observed in nonirradiated lobes probably occurred to compensate for such changes in irradiated lobes.

Using the conventional external beam irradiation model, high doses of X-rays kill rats associated with severe gastrointestinal tract damage, making it difficult to perform long-term experiments. In this study, to avoid such problems, we used the irradiation model devised by Imaeda *et al.* [12] The primary advantage of this irradiation model is that minimizing radiation exposure to the posterior lobes and gastrointestinal tract is possible because of the presence of an intrahepatic shielding plate. In practice, no radiation injury to the surrounding organs was observed in the irradiated rats, and no surgical or radiation-related deaths occurred during the observation period, indicating that the irradiation model is a safe and useful irradiation method. By separating the anterior and posterior lobes with a shielding plate as shown in Fig. 1, 70% of the total liver volume was irradiated when radiation was administered from the anterior side. Such a division of the liver lobes is similar to the common experimental model of PH and PVL/PVE [15–17]. Thus, this model can be compared with PH and PVL/PVE models.

In a 70% PVL model with ligated ML and LLL portal veins, atrophy of the ligated lobe and compensatory hypertrophy of the non-ligated lobe were observed 7 days after treatment, with the whole liver weight index remaining nearly constant [16]. In that model, the number of Ki-67-positive hepatocytes was maximal at 48 h after treatment [18]. In a 70% PH model with resection of the ML and LLL, the weight of the non-resected lobes recovered to 86% of the preoperative total liver weight 5 days postoperatively, and the number of Ki-67 positive hepatocytes peaked 3 days postoperatively [15]. The results of the current study, in which the proportion of Ki-67-positive hepatocytes in non-treated lobes increased after treatment and compensatory hypertrophy occurred, were the same as those previously reported. As with 70% PVL, it is interesting that the LI of the whole liver was maintained at a nearly constant level, suggesting a regulatory mechanism prevents the liver from becoming overly enlarged. Although the observation period was short in the present study, it is expected that weight changes in the liver lobes will continue to progress after 12 weeks postirradiation. However, the major difference between the present results and those of the PVL and PH models is the time required for hypertrophy. The LI at 12 weeks after irradiation corresponded to ~3 days after 70% PVL treatment and ~2 days after 70% PH [15, 16]. Why it takes such a long time for compensatory hypertrophy to occur after irradiation might be related to the slow atrophy of the irradiated liver lobes. Many studies have reported on compensatory liver hypertrophy after surgical procedures, and these regulatory mechanisms are gradually being elucidated. Shear stress of sinusoidal endothelial cells following increased portal blood flow is thought to be an important trigger that induces liver regeneration after PVL and PH [8, 16, 19]. Sinusoidal endothelial cells sense fluctuations as mechanical stimuli and cooperate with networks of cytokines, such as transforming growth factor-beta 1 and hepatocyte growth factor to regulate the initiation and termination of liver regeneration [19]. The trigger that causes liver hypertrophy after irradiation is still unknown, but the same mechanism may be involved as described above. The hypothesis is that the atrophy of irradiated liver lobes is accompanied by an increase in blood inflow to nonirradiated liver lobes, which in turn increases the shear stress on sinusoidal endothelial cells and initiates hypertrophy. Future evaluation is needed to determine whether this mechanism applies to postirradiation periods, which, unlike post-liver resection, progress slowly.

TNF-α, one of the inflammatory cytokines, has been found to be deeply involved in various liver diseases [20]. It is upregulated by irradiation and is thought to act in a proactive manner against subsequent hepatocyte apoptosis and liver fibrosis [7, 21]. In this study, we found that expression of TNF-α was increased only in irradiated areas. It had already begun in the first week after irradiation. This result suggests that the inflammatory response in liver tissue potentially occurs even before it is histologically and serologically evident. Increased TNF-α expression in only irradiated lobes may be associated with subsequent liver damage and fibrosis in irradiated lobes.

Previous reports have shown that AST and ALT, indicators of hepatocellular damage, gradually increase after X-ray partial liver irradiation [9–11]. Although not significant, we also observed an increase in those values like those reported. In histological evaluation, we confirmed that hepatocellular damage caused by 60 Gy X-rays occurred mainly in the irradiated lobes, not in the nonirradiated lobes (Supplementary Fig. 2). It was thought that liver function would decline in the X-irradiated lobes, but blood tests such as T-BIL, ALB and PA showed preservation of liver function. This suggests that hypertrophied posterior lobes without hepatocellular damage may have compensated for the reduced function of the anterior lobes caused by the decreased number of hepatocytes.

Various cytokines and drugs, including hepatocyte growth factor, have been investigated for the treatment of RILD [6, 7, 14, 22]. Some of

them have been shown to reduce liver damage in animal experiments, but they have not yet been put into clinical use due to insufficient safety evaluation and other reasons. Our results suggest that functional enhancement and hypertrophy of the nonirradiated liver may be an effective treatment for RILD as an alternative to these agents. Further elucidation of the mechanism by which radiation hypertrophies nonirradiated liver lobes may lead to the development of drugs that promote liver hypertrophy in the future.

There were several study limitations. First, the rodent model does not fully reproduce human RILD [7], and the present results may not be directly applicable to humans. Second, the irradiation treatment in this study was single high-dose irradiation, whereas fractionated irradiation is common in actual clinical practice. This is because the present model required open surgery. It is difficult to perform laparotomy for each irradiation, and the inability to perform fractionated irradiation is a disadvantage of this model. Finally, the livers used in this study were healthy, not cirrhotic. Because the livers of patients who require radiotherapy in clinical practice often have reached cirrhosis, the hypertrophic capacity of the nonirradiated liver might be weak. Gu *et al*. reported that a liver regeneration response also occurred in cirrhotic rats [23], but the actual extent of the compensatory hypertrophy is unknown and requires further evaluation. For clinical application, these issues must be addressed by future research. Our results may be useful as basic data for conducting such studies.

CONCLUSION

A single X60Gy irradiation to 70% of liver volume caused liver atrophy in the irradiated lobes and hypertrophy in the nonirradiated lobes. It was also suggested that the hypertrophy was due to increased hepatocyte mitosis. Overall liver weight was nearly constant and function was maintained. Further elucidation of the changes that occur in the nonirradiated liver lobes may reveal new methods to protect the liver from the adverse effects of radiation.

SUPPLEMENTARY DATA

Supplementary data is available at *Journal of Radiation Research* online.

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CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

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DATA AVAILABILITY

All data are available from the corresponding author upon reasonable request.

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