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Inhibition of Radiation-Induced Apoptosis via Overexpression of SMP30 in Smad3-Knockout Mice Liver

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Gamma-radiation/Liver injury/Smad3/Apoptosis/SMP30.

Apoptosis occurs early after irradiation and may be a good indicator of radiation damages. Since elevated levels of TGF-β are associated with radiation-induced inflammation, the null mice of Smad3, a key downstream mediator of TGF-β, show accelerated healing of irradiated injury. In order to evaluate resistance to radiation-induced liver injuries in Smad3-null mice, we determined the occurrence of apoptosis and the expression of senescence marker protein-30 (SMP30), as an anti-apoptotic marker, after irradiation to the liver. The livers of Smad3-mutant mice were exposed to local irradiation of 15 gray, from a ^{60}Co-gamma radiation. One week after irradiation, in Smad3-KO mice, radiation-induced apoptosis was at lower levels compared to those of irradiated WT mice. These findings were well matched with the expression of CYP2E1, which plays a role in hepatic injuries produced by oxidative stress. In addition, antioxidant related protein, the SMP30 levels were reduced by gamma irradiation in both groups. Interestingly, the increased expression of SMP30 expression in Smad3-KO mice liver was preserved at a higher level than that of the WT mice after irradiation. Therefore, these results suggest that the interruption of TGF-β signaling by deletion of Smad3 brings about inhibition of hepatic apoptosis after ionizing irradiation. Moreover, the protective effect to ionizing radiation might be in correlation with the overexpression of SMP30 in the Smad3-null mice, which may act as an anti-apoptotic signaling molecule. The alteration of SMP30 by interruption of Smad3 might be a useful therapeutic target and diagnostic marker for radiation-induced liver damages.

INTRODUCTION

Clinically radiotherapy has played a limited role in the treatment of malignant intrahepatic cancers due to the low tolerance of liver to radiotherapy. Radiation-induced liver damage had been observed in 5–10% of patients, who had received radiation doses exceeding 30 Gy.[1,2] In particular, ionizing irradiation induces multiple biological effects through direct interaction with DNA or by the production of activated free radical species from water molecules. This can lead to oxidative damage and apoptosis in the dividing cells.[3] Apoptosis occurs early after irradiation, which may be a better indicator of radiation damage, yet it needs a tissue biopsy. The most salient feature of apoptosis is the lack of an attendant inflammatory response or tissue damage.[4] Recent evidence, however, has indicated that apoptotic cells may be actively involved in the suppression of inflammatory responses by inducing anti-inflammatory cytokines.[5]

Recently, Conery AR. *et al*. have shown that TGF-β induces apoptosis in intestinal epithelial cells by the overexpression of Smad3 and the sensitivity to TGF-β induced apoptosis is regulated by crosstalk between the Akt/PKB serine/threonine kinase and Smad3 through a mechanism that is independent of Akt kinase activity.[6] In radiation-induced liver damage, however, the induction of apoptosis and its correlation with the TGF-β/smad3 pathway were not elucidated. Vodovotz *et al*. reported that the chronic overexpression of active TGF-β1 is associated with an increase in radio-sensitivity and that this effect may be mediated by the increased sensitivity of bone marrow to the suppressive

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effects of radiation. Since TGF-β1 levels can be greatly elevated in patients with certain tumors, these findings may be significant for radiotherapy.[7] Similar to the mechanisms, Ashcroft *et al*. proposed, regarding to the cause to reduce the expression of TGF-β1 in the granulation tissue after wounding in Smad3-KO mice, we suggest that the impaired autoinduction of TGF-β1 in Smad3-null macrophages and fibroblasts[8] most likely accounts for the significant portion of the difference in the expression of TGF-β1 after irradiation. The null mice for Smad3 showed protective effects against the radiation-induced cutaneous injuries to reduce inflammation and accumulation of a matrix, since the level of TGF-β associated with radiation-induced inflammation and fibrosis was diminished.[8]

Smad3, a major downstream signal transducer in the TGF-β signaling pathway, runs from the cell membrane to the nucleus and regulates the expression of TGF-β target genes.[6] The impairment of the Smad3 pathway results in the escape from growth inhibition and promotes cell proliferation, thereby contributing to carcinogenesis.[9] The characterization of the basal phenotype of Smad3-null mice, as well as in studies from the incision-induced wound healing of these mice, suggest that Smad3 play an important role in both inflammation and fibrosis *in vivo*. The Smad3-null mice died from defects in mucosal immunity, which is suggestive of defects in the neutrophil chemotaxis and is consistent with the observation that the chemotactic response of Smad3-null neutrophils to TGF-β is impaired both *in vivo* and *in vitro.*[10] Since Smad3-null mice show reduced levels of inflammation, TGF-β1, related to radiation-induced inflammation and fibrosis, and matrix accumulation after wounding,[11,12] we proposed that loss of Smad3 would also decrease the severity of inflammation and apoptosis, which are induced by irradiation in the liver tissue.

Senescence marker protein-30 (SMP30) was initially identified as a novel protein that is highly expressed in hepatocytes and in renal tubular epithelia, and its amount decreases with aging.[13] SMP30 has been reported to enhance plasma membrane Ca^{2+}-pump activity in the liver and kidney, thereby protect cells from Ca^{2+} ionophore-induced apoptosis.[14] Moreover, hepatocytes completely lacking SMP30 ($SMP30^{-/-}$) had significantly increased susceptibility to apoptosis induced by tumor necrosis fator-a (TNF-a) plus actinomycin D (Act-D) than $SMP30^{+/+}$ hepatocytes, indicating that SMP30 rescues TNF-a plus Act-D induced apoptosis. These results strongly suggest that SMP30 may play a role as an anti-oxidant and anti-apoptotic protein.[15]

Therefore, we hypothesize that the loss of Smad3 will decrease liver damage induced by irradiation. In order to evaluate resistance to radiation-induced liver injuries in Smad3-null mice, we determine the relation between occurrence of apoptosis and the expression of senescence marker protein-30 (SMP30) in the liver has been irradiated.

MATERIALS AND METHODS

Animals

$Smad3^{ex8/ex8}$-null C57BL/6 mice generated by the targeted disruption of the Smad3 gene by homologous recombination was kindly provided by Dr. Anita B. Roberts of the Laboratory of Cell Regulation and Carcinogenesis (National Cancer Institute, MD, USA). The mutant mice were housed in a room at 22 ± 2°C, with a 12-hour light-dark cycle, and were given food and water *ad libitum*. Experiments were performed in accordance with the NIH guidelines for the Care and Use of Laboratory Animals. Genotypes were determined by a PCR using tail DNA (Fig. 1).[10,12] For PCR analysis, the $Smad3^{+/+}$ allele was detected using primer 1: 5'-CCACTTCATTGCCATATGCCCTG-3' and primer 2: 5'-CCCGAACAGTTGGATTCACACA-3'. This primer pair amplifies a fragment of 431 bp from $Smad3^{+/+}$ mice, but not from $Smad3^{-/-}$ mice. DNA was also amplified using the primer 1 and primer 3: 5'-CCAGACTGCCTTGGGAAAAGC-3', which is located in the pLoxpneo to detect the mutant *Smad3* allele. In this case, a 284 bp fragment was detected for the $Smad3^{-/-}$ allele, whereas no such signal was detected in the wild-type mice.

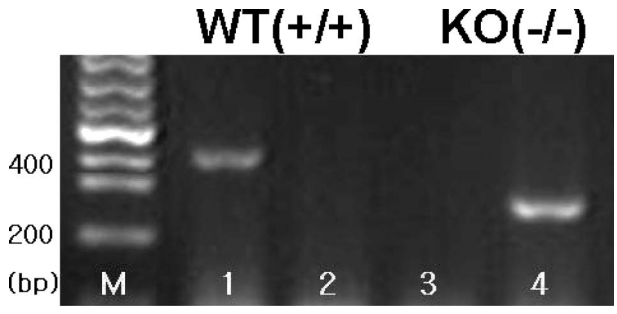

Fig. 1. Confirmation of genotype in Smad3 mutant mice. Ethidium bromide-stained agarose gel showing polymerase chain reaction (PCR) products of tail DNA from Smad3 mutant mice. The 431 bp band (lane 1) indicates the wild-type ($Smad3^{+/+}$) and the 284 bp band (lane 4) represents knockout ($Smad3^{-/-}$) mice. Lane designations: lane M, DNA molecular size marker; lane 1, tail DNA of $Smad3^{+/+}$ mice with primer 1 plus primer 2 mixture; lane 2, tail DNA of $Smad3^{+/+}$ mice with primer 1 plus primer 3 mixture, lane 3, tail DNA of $Smad3^{-/-}$ mice with primer 1 plus primer 2 mixture, lane 4, tail DNA of $Smad3^{-/-}$ mice with primer 1 plus primer 3 mixture.

Animal treatments

Eight-week-old male Smad3-mutant mice were divided according to genotype (5 mice per each group). These wild-type (WT) and homozygous-type (KO) C57BL/6 mice were subdivided into four groups, non-irradiated (NW and NK) and irradiated (RW and RK) groups. The animals were anesthetized using injection of Ketamine (Yuhan Co., Seoul,

Korea 50 mg/kg, i.p.) and Xylazine (Bayer, Ansan, Korea, 5 mg/kg, i.p.) prior to immobilization in the recumbent position on a treatment table. The liver portion was centered in a 2 × 4 cm exposure field with the other abdomen and the body was protected using customized lead shielding. The mice were subjected to a single irradiation of 0 (non-irradiation) and 15 gray (Gy), at a dosage rate of 1.95 Gy/min, from a ^{60}Co-gamma radiation source unit (Theratron 780 Model, AECL, Ontario, Canada).

Serum analysis

All mice were sacrificed one week after irradiation. Blood was collected from the caudal vena cava and separated serum to evaluate aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity, total protein and albumin levels. The activities of serum markers were assayed using a commercial assay kit (Yeongdong Pharmaceutical, Seoul, Korea). Usually, these markers were used as parameters for damages and protein synthetic function of liver.

Histopathological evaluation of the liver tissue

All mice were sacrificed and each irradiation-related change in the adjacent intra-abdominal organs was seen by macroscopic inspection one week after irradiation. Liver pieces were rapidly removed at random and fixed in a 10% neutral buffered formalin, processed routinely, and embedded in paraffin wax. The sections were stained with hematoxylin and eosin (H&E) and immunohistochemically. For immunohistochemistry, liver sections were deparaffinized and incubated in 3% hydrogen peroxide for 30 min. The primary antibodies used were the monoclonal rabbit anti-CYP2E1 antibody (Chemicon International Inc., CA, USA), and the polyclonal rabbit anti-phospho-Smad3 (p-Smad3) antibody (Cell Signaling Technology, MA, USA). The antigen-antibody complex was visualized by an avidin-biotin peroxidase complex solution using an ABC kit (Vector Laboratories, CA, USA) with 3,3-diamino benzidine (Zymed Laboratories Inc., CA, USA).

TUNEL assay

Apoptotic cells were determined by a terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-biotin nick end labeling (TUNEL) method (Roche Diagnostics, IN, USA). The preparation reacted with the proteolytic enzyme (20 μg/mL of proteinase K), and was rinsed with deionized distilled water (DDW). Then, the preparation reacted with 50 mL TdT mixture at 37°C for 1 h, which was combined with reagents 1 and 2, containing: 200 mmol/L potassium cacodylate; 25 mmol/L Tris-HCl (pH 6.5); 0.25 g/L bovine serum albumin; 1 mmol/L $COCl_2$; 5 mmol/L biotin-dUTP; and 100 U/mL TdT. After being rinsed with a PBS, 350 mL of reagent was added at 37°C for 40 min and stained with a NBT/BCIP detection system kit (Promega, WI, USA).

Immunoblotting

Snap-frozen liver tissues were homogenized in a RIPA buffer containing 0.1 mM of Na_3VO_4 and protease inhibitor cocktail tablets (Roche, Mannheim, Germany). The protein concentration was determined by the Bradford method. The protein samples (30 μg per lane) were separated by 10% SDS-polyacrylamide gel electrophoresis. For immunoblotting, the proteins were electro-transferred to a PVDF membrane (Schleicher & Schuell, Dassel, Germany). A goat polyclonal anti-SMP30 antibody (SantaCruz Biothechnology, California, USA) and a rabbit polyclonal p-Smad3 (Cell Signaling Technology, MA, USA) were used as the primary antibodies. All membranes were immunoblotted for the β-actin antibody (SantaCruz Biotechnology, California, USA), as the internal standard protein. Specific binding was detected by using the Super Signal West Dura Extended Duration Substrate (PIERCE, IL, USA) and by the exposure of blots to Medical X-ray Film (Kodak, Tokyo, Japan).

Statistical analysis

All results were expressed as means ± SD and a statistical analysis was performed by using a Student's *t-test*. For statistics of data immunohistochemistry and immunobloting analysis were repeated four times in this study. Differences at $p < 0.005$ (*) and $p < 0.0005$ (**) were considered to be significant.

RESULTS

Biochemical changes of serum parameters

In order to assess the liver damage induced by irradiation, the level of AST and ALT activity, total protein and albumin level was determined from the serum of mice (Table 1). Biochemically, total protein, albumin, ALT and AST are some indicators of hepatic function. Interestingly, levels of total protein and albumin significantly ($p < 0.005$) increased in Smad3-KO mice compared to those levels of Smad3-WT mice. In the irradiated Smad3-WT group, the activity of serum AST showed a significant elevation compared to the non-irradiated WT group (Table 1). These results indicated that liver damage of the Smad3-WT mice was induced more severely than that of the Smad3-KO mice after irradiation.

The expression level of the phospho-Smad3 protein after irradiation

In general, p-Smad3 was expressed during the activation of Smad3 by phosphorylation in the cytoplasm and nucleus. In the irradiated WT mice, dramatic increases in the expression of p-Smad3 could be observed, while the expressions of p-Smad3 not significantly increased in the irradiated Smad3-KO mice. The expression of p-Smad3 in the Smad3-KO mice was caused by using the antibody having cross-reactivity with p-Smad2. The phosphorylation of Smad2 could result in enhanced band of p-Smad3 in irradiated

Table 1. Biochemical changes of serum parameters at one week after irradiation

Parameter Groups[a]	Total protein (g/d*l*)	Albumin (g/d*l*)	AST (IU/*l*)	ALT (IU/*l*)
NW	4.79 ± 0.03	2.75 ± 0.07	44.83 ± 3.29	19.83 ± 0.42
NK	5.63 ± 0.36*	3.04 ± 0.09*	45.65 ± 0.49	20.54 ± 0.03
RW	4.49 ± 0.29	2.50 ± 0.31	72.37 ± 10.67*	23.37 ± 3.43
RK	4.60 ± 0.03	2.76 ± 0.01	48.80 ± 0.79	20.89 ± 1.11

[a], NW, non-irradiated Smad3-WT group; NK, the non-irradiated Smad3-KO group; RW, irradiated Smad3-WT group; RK, irradiated Smad3-KO group. The values are given as the mean of each group ± SD (n = 5). *, Significant difference ($p < 0.005$) is compared to the non-irradiated Smad3-WT group by Student's *t test*.

Smad3-KO mice comparing to non-irradiated (Fig. 2). Immunohistochemical positive reactions for p-Smad3 were significantly expressed in the hepatocytes around the central vein of the irradiated WT (RW) group (data not shown).

Lower occurrence of radiation-induced apoptosis in Smad3-KO mice

The histopathological findings of the irradiated animals were not noticeably different from the control except for the varying amount of congestion and dilatation of the sinusoids and a slight infiltration of the inflammatory cells. At one week after irradiation, brown apoptotic hepatocytes stained with TUNEL assay were observed and these apoptotic cells mostly gathered around microvascular structures in the liver tissue (Fig. 3). In the Smad3-KO mice, the occurrences of apoptotic hepatocytes were detected at a lower level than those of the Smad-WT mice after irradiation. Apoptotic activity was significantly inhibited in the Smad3-KO mice and it was distinguished from radiation-induced apoptosis in the Smad-WT mice.

The effects of irradiation on the expression of cytochrome P450 2E1 (CYP2E1)

The expressions of cytochrome P450 2E1 (CYP2E1) were monitored in the livers of mice one week after 15 Gy of γ-irradiation (Fig. 4). The distribution of hepatic CYP2E1 markedly increased in the hepatocytes around the central veins after irradiation, as compared to the non-irradiated control groups. In the Smad3-KO mice, however, a mild increase in the expression of CYP2E1 could be detected after irradiation. These expressions of CYP2E1 were correlated with inhibition of TUNEL assay in Smad3-KO mice.

Alterations of the SMP30 expression level

In immunoblot analysis, the levels of SMP30 expression dramatically decreased in the wild type after irradiation. However, hepatic expression of SMP30 in the Smad3-KO mice was preserved at a higher level than that of the WT mice after irradiation (Fig. 5). Interestingly in the non-irradiated group, SMP30 protein levels of Smad3-KO mice were significantly higher compared to those of WT mice.

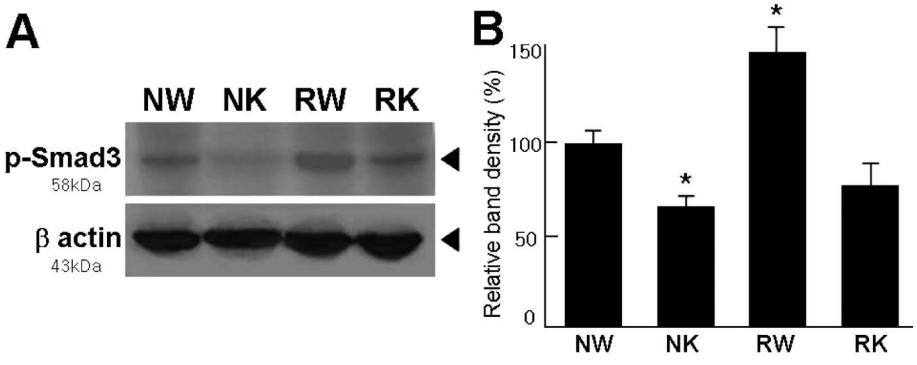

Fig. 2. The expressions of p-Smad3 in Smad3-mutant mice at one week after irradiation. **A,** Liver proteins (50 μg/lane) of Smad3-mutant mice were loaded onto 8% SDS-PAGE gel and followed by immunobloting for p-Smad3 and β-actin (as internal standard) antibodies. **B,** The graph represents relative band density (%) to β-actin. The lanes of Fig. 2A and 2B as are indicated; NW is the non-irradiated WT group, NK is the non-irradiated KO group, RW is the irradiated WT group, and RK is the irradiated KO group of the Smad3-mutant mice. *, Significant difference ($p < 0.005$) is compared to the non-irradiated Smad3-WT group by Student's *t test.*

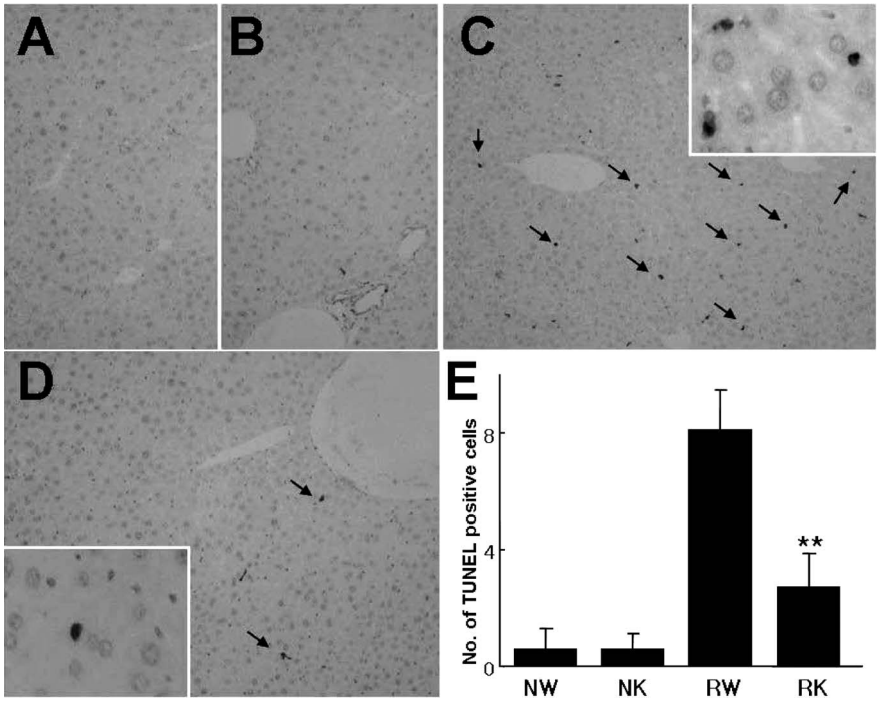

Fig. 3. TUNEL assay in the liver tissue of the Smad3-mutant mice at one week after irradiation. At one week after irradiation, brown apoptotic hepatocytes stained with TUNEL assay increased in the irradiated groups. **A,** Non-irradiated Smad3-WT mice (NW) liver. **B,** Non-irradiated Smad3-KO mice (NK) liver. **C,** Irradiated Smad3-WT mice (RW) liver. **D,** Irradiated Smad3-KO mice (RK) liver. TUNEL staining. Original magnification × 33. Insert figures of Fig. 3C and 3D are the high magnification (× 132) of each figure. **E,** The graph represents positive reactive cells for the TUNEL stain. Values were expressed as mean and SD, and were counted in 10 uniform randomly chosen areas of each sample for a final magnification of × 132. The graphs are indicated; NW is the non-irradiated WT group, NK is the non-irradiated KO group, RW is the irradiated WT group, and RK is the irradiated KO group of Smad3-mutant mice. **, Significant difference ($p < 0.0005$) is compared to positive cell number of the irradiated Smad3-WT group by Student's *t* test.

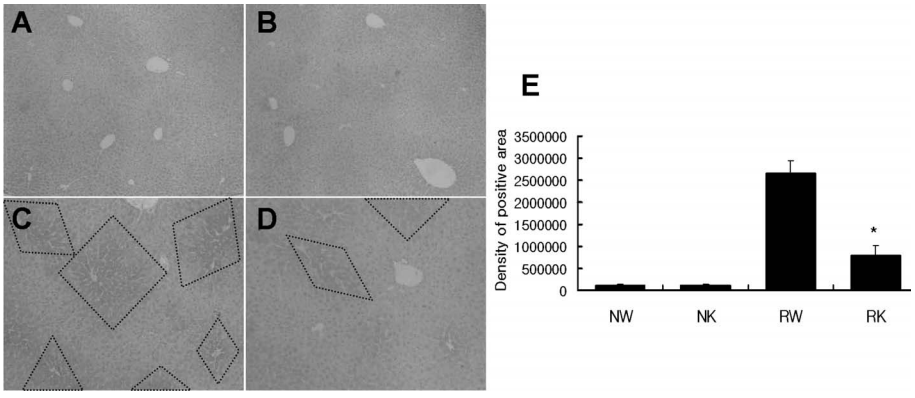

Fig. 4. Immunohistochemial expressions of CYP2E1 in the Smad3-mutant mice liver tissue at one week after irradiation. **A,** Non-irradiated Smad3-WT mice (NW) liver. **B,** Non-irradiated Smad3-KO mice (NK) liver. **C,** Irradiated Smad3-WT mice (RW) liver. **D,** Irradiated Smad3-KO mice (RK) liver. The positive reactions of CYP2E1 (dotted diaper) strongly increased in the hepatocytes around the central veins of the irradiated Smad3-WT mice (RW). However, in the Smad3-KO mice (RK) weak expressions of CYP2E1 detected compared to those of Smad3-WT mice. Immunostain for CYP2E1. Original magnification × 66. **E,** The graph represents the density of immunopositive area in mice liver. The results of immunohistochemical expression of CYP2E1 were quantified by using ImageJ software and values were expressed as mean and SD. The graphs are indicated; NW is the non-irradiated WT group, NK is the non-irradiated KO group, RW is the irradiated WT group, and RK is the irradiated KO group of Smad3-mutant mice. *, Significant difference ($p < 0.005$) is compared to the irradiated Smad3-WT group by Student's *t* test.

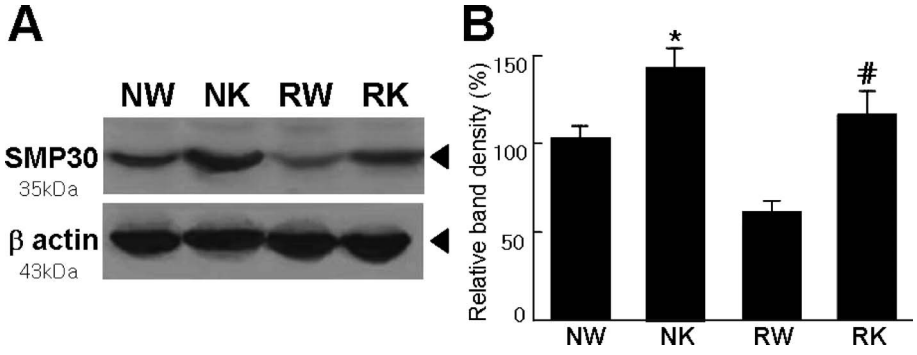

Fig. 5. The expressions of SMP30 in Smad3-mutant mice at one week after irradiation. **A,** Immunoblot analysis for SMP30 and β-actin (as an internal standard) antibodies. **B,** The graph represents relative band density (%) to β-actin. Lanes Fig. 5A and 5B are as indicated; NW is the non-irradiated WT group, NK is the non-irradiated KO group, RW is the irradiated WT group, and RK is the irradiated KO group of the Smad3-mutant mice. *, Significant difference ($p < 0.005$) is compared to the non-irradiated Smad3-WT group by Student's *t test.* #, Significant difference ($p < 0.0005$) is compared to the irradiated Smad3-WT group by Student's *t test.*

DISCUSSION

First, we proposed that the loss of Smad3 would decrease liver damage induced by γ-irradiation. Ionizing radiation-induced damage to living cells is mediated through the generation of oxygen-derived free radicals and hydrogen peroxide, which lead to oxidative damage and apoptosis in the dividing cells.[16] Recent evidence has indicated that apoptotic cells may be actively involved in the suppression of inflammatory responses by inducing anti-inflammatory cytokines.[8]

The irradiation to the skin of mice shows an increase in the expression of TGF-β1 mRNA between 6 hours and 9 months after irradiation.[17] Irradiation of other tissue such as that of lungs,[18] intestines,[19] bladders[20] and liver[21] is also associated with an increase in TGF-β expression. Anscher *et al*. declared that after irradiation the immunohistochemical staining for TGF-β1 strongly appeared in the central lobular hepatocytes.[21] These results suggest that the chronic overexpression of active TGF-β1 is associated with an increase in radio-sensitivity. Exposure to ionizing radiation has been reported to increase the production of both total and active[22] TGF-β form within several hours and that the expression of TGF-β1 can persist for months.[23] It has been reported that TGF-β is an inhibitor of the proliferation of hepatocytes[24] and induces oxidative stress leading to hepatocyte apoptosis at higher concentration.[25,26] Therefore, attenuation of the action of TGF-β by absence of Smad3 might facilitate the process of hepatic regeneration or prevent hepatocyte damage from occurring.

As our results, the significantly increased levels of total protein and albumin in the non-irradiated Smad3-KO mice suggest that the blockade of TGF-β pathway could improve liver function to increase protein synthesis, although there was no significant difference on AST and ALT levels between non-irradiated Smad3-WT and Smad3-KO mice. On the other hand, we also consider that increased uptake of albumin in kidney by inhibition of TGF-β, mediated Smad2/3 pathway, which is produced in the proximal tubular cells and decreases receptor-mediated endocytosis[27] result in increase of albumin in serum. However, the underlying mechanism of elevation in total protein and albumin, correlated to inhibition of Smad3 mediated TGF-β pathway, is not clear.

At one week after irradiation, radiation-induced apoptosis of the Smad3-KO mice was observed at lower levels than that of the WT mice liver. Therefore, these results suggest that the interruption of TGF-β signaling by the deletion of Smad3 bring about hepatic apoptosis after ionizing irradiation. In this study, the inhibition of apoptosis was in correlation with the decreased expression of CYP2E1 in the irradiated Smad3-KO mice, compared to that of the irradiated WT mice. CYP2E1, in particular, is thought to play a role in hepatic damage through the production of ROS. Oxidative stress, created by CYP2E1 overexpression, may promote damage to the liver cell by sensitizing hepatocytes to oxidant-induced damage from Kupffer cell-produced reactive oxygen intermediates (ROI) or cytokines.[28] Generally, in the liver, γ-irradiation induces hepatic glutathione *S*-transferase and microsomal epoxide hydrolase with concomitant increases in the levels of mRNA.[29] The induction of Phase II detoxifying enzymes by γ-irradiation may represent adaptive responses against oxidative stress and it may protect cells against subsequent exposure to radiation.[30] Since CYP2E1-induction is associated with change in energy metabolism, mitochondrial dysfunction after γ-irradiation was in correlation with the expression of CYP2E1, from the other study.[31]

SMP30, antioxidant related protein, levels were dramatically reduced by gamma irradiation in both irradiated mice. The highly increased expression of SMP30 in the liver of

Smad3-KO mice, however, was preserved at a higher level than that of the WT mice after irradiation. The radiation resistance of various mammalian cell lines has been shown to correlate with their SOD activity or glutathione peroxidase activity, suggesting that ionizing radiation and oxidative stress have common cellular effects.[32] Moreover, Reddy *et al*. reported that *Sod2*-deficient cells showed dramatic mitochondrial damage, cytochrome C leakage, caspase 3 activation and increased apoptotic cell death, when they were challenged with O_2^-. Thus, mitochondrial enzyme (*Sod2*) deficiency plays an important role in the initiation of apoptosis in the lens epithelium.[33]

Previously, we observed that CCl_4-induced liver fibrosis in Smad3-KO mice was reduced. In proteomic analysis, expressions of antioxidant related enzymes, such as SMP30 and selenium binding proteins (SeBPs), were elevated compared with those of the Smad3-WT mice. These results suggest that the Smad3 pathway was in correlation with the antioxidant defense system in liver damage (from our unpublished data). Recently, Matsuyama *et al*. suggested that Akt, a serine-threonine kinase known to exert anti-apoptotic effects, may act in the protective effect of SMP30 on apoptosis in hepatocytes.[34] Activation of phosphatidylinositol 3-kinase (PI3K)-mediated Akt pathway can cause increased expression of anti-apoptotic members of Bcl-2 family proteins,[35] which may function at a post-mitochondrial step to prevent caspase-dependent pathway.[36] Akt prevents phophorylation and nuclear translocation of Smad3 and thereby results in inhibition of Smad3-mediated transcription and apoptosis.[6] We suggest, hereby, that the activation of Akt caused by increased SMP30 in Smad3 KO mice play a role to rescue apoptosis of hepatocytes. However, the relationship between SMP30 and Smad3 has been hardly studied yet.

In conclusion, these results strongly suggest that the interruption of TGF-β signaling by the deletion of Smad3 brings about inhibition of hepatic apoptosis after ionizing irradiation. Moreover, the blocking of the Smad3 pathway provoked the overexpression of antioxidant defense systems, such as SMP30 within the liver. Therefore, the protective effect to ionizing radiation might be in correlation with the overexpression of SMP30, acting as an anti-apoptotic signaling molecule, in the Smad3-KO mice in this study. The alteration of SMP30 by the interruption of Smad3 might be a useful therapeutic target and diagnostic marker for radiation-induced liver injury.

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