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ORIGINAL ARTICLE

# Tissue-specific DNA damage response in Mouse Whole-body irradiation

Seon-Gyeong Lee[1,2] · Namwoo Kim[1,2] · In Bae Park[1,5] · Jun Hong Park[3] · Kyungjae Myung[2,4]

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## Abstract

**Background** Genomic instability is a hallmark of various cancers, and DNA repair is an essential process for maintaining genomic integrity. Mammalian cells have developed various DNA repair mechanisms in response to DNA damage. Compared to the cellular response to DNA damage, the in vivo DNA damage response (DDR) of specific tissues has not been studied extensively.

**Objective** In this study, mice were exposed to whole-body gamma (γ)-irradiation to evaluate the specific DDR of various tissues. We treated male C57BL6/J mice with γ-irradiation at different doses, and the DDR protein levels in different tissues were analyzed.

**Results** The level of gamma-H2A histone family member X (γH2AX) increased in most organs after exposure to γ-irradiation. In particular, the liver, lung, and kidney tissues showed higher γH2AX induction upon DNA damage, compared to that in the brain, muscle, and testis tissues. RAD51 was highly expressed in the testis, irrespective of irradiation. The levels of proliferating cell nuclear antigen (PCNA) and ubiquitinated PCNA increased in lung tissues upon irradiation, suggesting that the post-replication repair may mainly operate in the lungs in response to γ-irradiation.

**Conclusion** These results suggest that each tissue has a preferable repair mechanism in response to γ-irradiation. Therefore, the understanding and application of tissue-specific DNA damage responses could improve the clinical approach of radiotherapy for treating specific cancers.

**Keywords** Whole-body irradiation · DNA damage response · γH2AX · RAD51 · PCNA

## Introduction

In mammals, including humans, the genome is continuously damaged by exogenous or endogenous factors during various biological processes such as replication and transcription. This could result in genomic instability if not repaired. Maintenance of genomic stability is important for preventing the differentiation of normal cells into premalignant cells as well as for maintaining normal function (Jackson 2009; Lindahl and Barnes 2000). The loss of genomic stability due to exposure to DNA-damaging agents can trigger the development of various types of cancers and cell death. As an example, ultraviolet radiation, a natural DNA-damaging agent, induces skin cancer (Yu and Lee 2017).

Seon-Gyeong Lee and Namwoo Kim have contributed equally to this work.

✉ Jun Hong Park
jhpark@kiom.re.kr

✉ Kyungjae Myung
kmyung@ibs.re.kr

Seon-Gyeong Lee
seongyeong@unist.ac.kr

Namwoo Kim
camphortreekim@unist.ac.kr

In Bae Park
tdolbae@ibs.re.kr

[1] Department of Biological Sciences, Ulsan National Institute of Science and Technology, Ulsan, Korea

[2] Center for Genomic Integrity, Institute for Basic Science, Ulsan, Korea

[3] Herbal Medicine Resources Research Center, Korea Institute of Oriental Medicine, Naju, Korea

[4] Department of Biomedical Engineering, Ulsan National Institute of Science and Technology, Ulsan, Korea

[5] Korea Mouse Phenotyping Center (KMPC), Collage of Veterinary Medicine, Seoul National University, Seoul, Korea

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In addition, in therapeutic radiation, a pervasive artificial DNA-damaging agent induces cell death in normal cells as a side effect (Armstrong and Kricker 2001; Kim et al. 2014). Radiotherapy involves the application of ionizing radiation to kill malignant cells by inducing DNA damage. More than 50% of patients with cancer undergo various types of radiotherapy as mono- or combination therapies (Baskar et al. 2012). Radiotherapy is applied to various cancers; however, certain cancers respond well to radiation, while others do not. Moreover, radiotherapy induces many adverse effects including cell death in normal tissue (Hauer-Jensen et al. 2014; Ward 1988). Despite active research on the function of repair proteins in in vitro systems, the responses of specific tissues to γ-irradiation in vivo have not been studied extensively.

One of the key tissue responses to irradiation involves a change in protein levels, through induction of gene expression and change in protein stability caused by DNA damage response (DDR). To characterize the DDR in a specific tissue, we selected four DDR proteins that indicate the degree of DNA damage and the integrity of the repair mechanism. The first protein is phosphorylated gamma-H2A histone family member X (γH2AX, H2AX ser139 phosphorylation), which is the most well-known biomarker of DNA damage (Seong et al. 2010). DNA double-strand break, single-strand break, and replication stress induce the phosphorylation of histone H2AX mainly through two phosphatidylinositol 3-kinase-related kinases, ATM and ATR (Kuo and Yang 2008). The second protein is phosphorylated replication protein A2 (pRPA2), which is phosphorylated in response to replication stress and also during end resection of DNA double-strand break repairs (Wang et al. 2001). The phosphorylation of RPA2 at serine 4 and serine 8 is mainly mediated by DNA-PK, whereas serine 33 phosphorylation requires ATR activity (Liaw et al. 2011). The phosphorylation pattern of RPA2 mediates downstream DDR signaling and controls the repair progress. The third protein is RAD51, which plays a central role in homologous recombination (HR) repair (Kawabata et al. 2005). RAD51 levels are altered by numerous stimuli and cellular status such as cell cycle and hypoxic stress. The regulation of RAD51 levels in response to exogenous or endogenous stimulus, is tissue-specific (Gasparini et al. 2014). For example, a low dose of irradiation increases the RAD51 levels by downregulating miR-193b-3p in hepatocytes (Lee et al. 2016). The fourth protein is proliferating cell nuclear antigen (PCNA), a DNA-sliding clamp that plays an essential role in DNA replication (Moldovan et al. 2007). Additionally, PCNA is involved in post-replication repair (PRR) that includes translesion synthesis (TLS) and template switching (Essers et al. 2005). PCNA is post-transcriptionally modified by ubiquitin following DNA damage, and its modification regulates PRR (Hoege et al. 2002). The unloading of PCNA from DNA and de-ubiquitination is regulated by ATPase family AAA domain-containing protein 5 (ATAD5), a yeast Elg1 homologue (Kubota et al. 2015; Lee et al. 2010, 2013).

In this study, we found that various tissues differentially responded to whole-body γ-irradiation (WBI) in a mouse model. Notably, RAD51 and PCNA levels were regulated differently in different tissues in response to γ-irradiation. Studying the irradiation-induced DDR in mammalian tissues and cells is important to expand our knowledge on the DNA damage repair mechanisms. Our study provides a rationale for the appropriate application of radiotherapy and improves the knowledge on the tissue specificity of DDR at the organism level.

## Results

### Tissue-differential response to whole-body γ-irradiation

We measured the level of phosphorylated H2AX protein (γH2AX) in various tissues before and after γ-irradiation. The level of γH2AX was increased in most tissues following irradiation (Figs. 1, 2, and S1). γH2AX was clearly upregulated in all targeted tissues exposed to high-dose irradiation; however, a relatively higher induction of γH2AX in response to low-dose irradiation was observed in the liver, lung and kidney tissues (Fig. 1). This suggests that DDR signaling following γ-irradiation is more intensive in these tissues. In contrast, the induction of γH2AX in other tissues including the brain, muscle, and testis was negligible, when exposed to low-dose irradiation; however, there was a higher induction of γH2AX at high-dose irradiation (5 and 10 Gy, Fig. 2). Furthermore, the basal level of γH2AX was moderately higher in the liver, brain, and testis compared to that in other tissues. Relatively higher level of γH2AX in liver and brain tissue may reflect high basal DNA damage in those tissues. Mouse testis tissues are exposed to constant DNA-damaging stress, during the meiotic recombination-associated double-strand break (Fernandez-Capetillo et al. 2003). The other tissues (skin, pancreas, and heart) also showed an increase in the level of γH2AX in a dose-dependent manner, in response to irradiation (Fig. S1).

To evaluate the activation of the DDR in other ways, we examined the level of phosphorylated RPA2 (pRPA2) at S4/S8 via western blotting. In the absence of γ-irradiation (0 Gy), the level of pRPA2 S4/S8 in the liver, muscle, and testis tissues was higher than that in the lung, kidney, and brain tissues (Figs. 1 and 2). These results suggest that the signaling pathway related to replication fork stress and HR-mediated end resection for endogenous DNA damage may be downregulated in lung, kidney, and brain tissues. We could not observe significant increase of pRPA2 at S4/S8

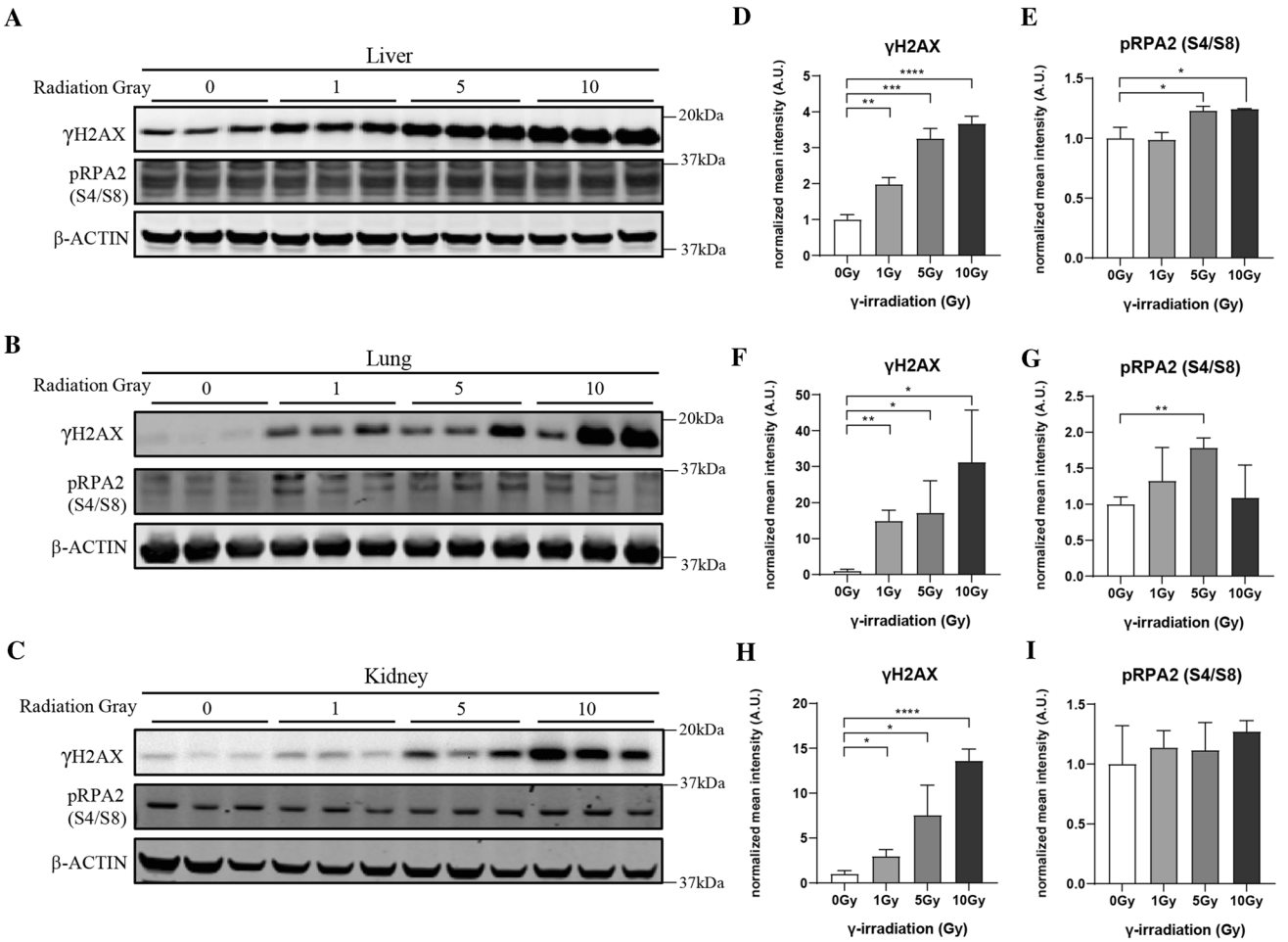


**Fig. 1** DNA damage response to γ-irradiation in the mouse liver, lung, and kidney tissues. Western blot analysis of liver (**A**), lung (**B**), and kidney tissues (**C**) for γH2AX, pRPA2, and β-ACTIN levels following γ-irradiation ($n=3$). **D**, **E** quantification of (**A**). **F**, **G** quantification of (**B**). **H**, **I** quantification of (**C**). Relative amount of proteins were calculated based on target protein/b-Actin density. For a clear comparison, the protein levels were normalized to the values from the 0 Gy (control) group. *γH2AX* gamma-H2A histone family member X, *pRPA2* phosphorylated replication protein A 2

following irradiation, in most tissues; however, we observed a low, but statistically significant increase in liver and lung tissues (Figs. 1 and 2). Therefore, pRPA2 S4/S8 is not a representative marker for γ–irradiation-mediated DDR in most tissues. There is a common response among the organs following γ-irradiation mediated DNA damage; however, the exact extent of the response differs between each organ.

## γ-Irradiation altered the expression level of RAD51 in various tissues

γ-irradiation induces DNA double-strand break in various tissues and activates two main DNA double-strand break repair pathways: HR and NHEJ. To get a perspective about the main repair system for DNA double-strand break, we analyzed the effect of γ-irradiation on the level of RAD51 protein, which functions in the HR repair pathway. RAD51 recombinase promotes strand invasion of damaged DNA to intact sister chromatids during HR, normal meiotic recombination, and fork reversal, to protect the replication fork under replication stress. We found that RAD51 exists in considerable amounts in the testis, irrespective of γ-irradiation (Fig. 3A, Fig. S2A). To determine the pattern of RAD51 expression in various tissues, with or without genotoxic stress, we examined RAD51 protein levels in other tissues following irradiation. The level of RAD51 was not influenced by the dose of irradiation; however, RAD51 was highly upregulated in the testis, compared to that in the other tissues (Fig. S2A). The main function of the testis is spermatogenesis; therefore, the high expression level of RAD51 in the testis may be due to the high necessity of meiotic recombination and the high level of replication stress during spermatogenesis, which is accompanied by meiosis (Petronczki et al. 2003; Srivastava and

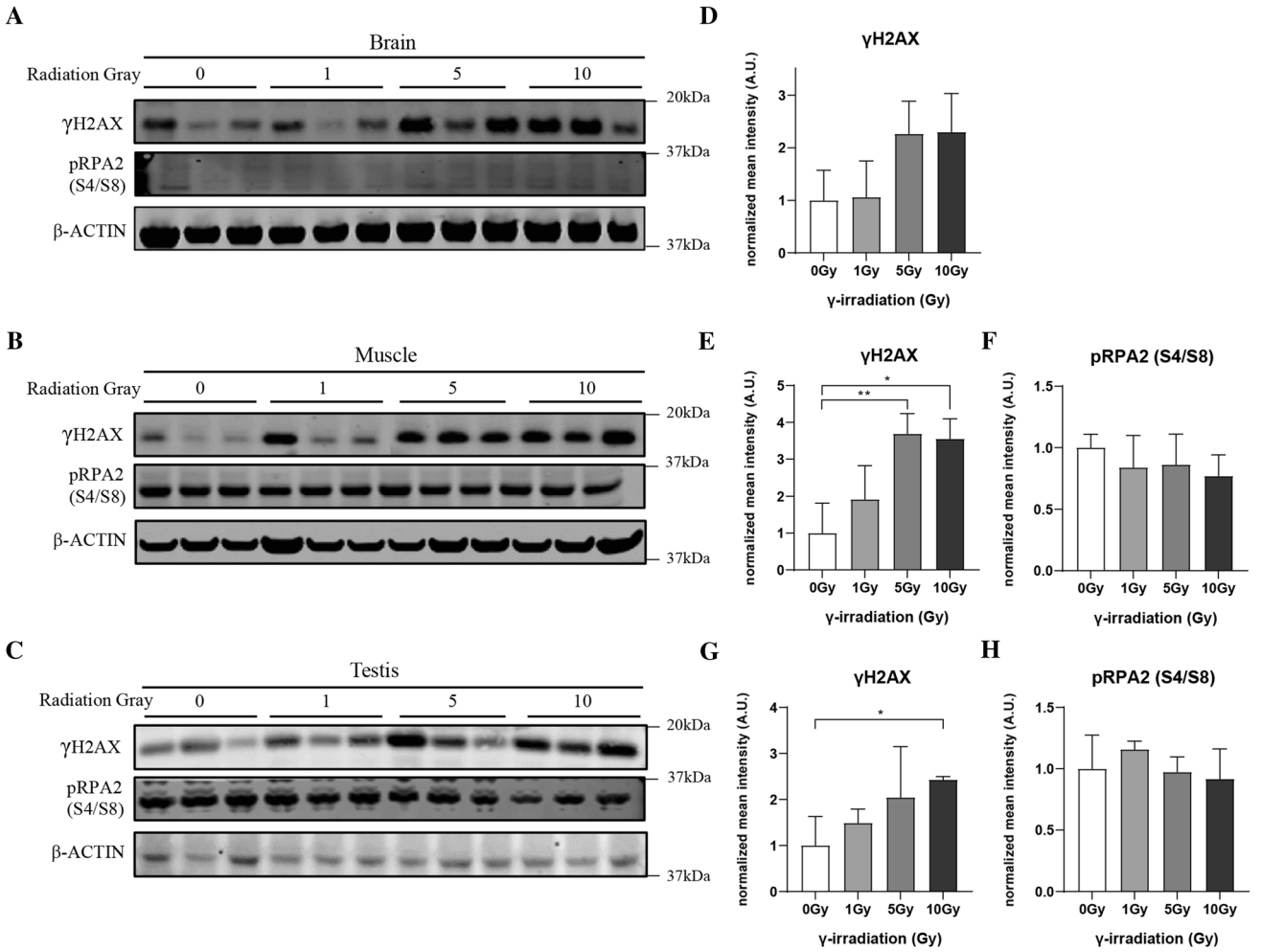


**Fig. 2** DNA damage response to γ-irradiation in the mouse brain, muscle, and testis tissues. Immunoblotting analysis of the brain (**A**), muscle (**B**), and testis tissues (**C**) for γH2AX, pRPA2, and β-ACTIN levels following γ-irradiation ($n=3$). **D** quantification of (**A**). **E**, **F** quantification of (**B**). **G**, **H** quantification of (**C**). *γH2AX* gamma-H2A histone family member X, *pRPA2* phosphorylated replication protein A 2

Raman 2007; Walter et al. 1998). Therefore, high levels of replication stress and meiotic recombination during spermatogenesis results in high levels of RAD51 in the testis, with or without DDR.

The level of RAD51 was significantly reduced in the kidney tissues following exposure to γ-irradiation (1 Gy) (Fig. 3B). The reduction of RAD51 level could not be reversed by treatment with a relatively higher dose of irradiation (10 Gy). This phenomenon was observed only in the kidney tissues, among the tissues tested (Fig. S2A). When we analyzed the levels of other proteins in the kidney tissues, there was no significant reduction like that observed in the levels of RAD51. PCNA levels were not affected by γ-irradiation (Fig. 3B) and the total level of γH2AX was increased in the kidneys following treatment with 5 Gy irradiation (Fig. 1). Therefore, RAD51 was specifically downregulated in the kidney tissues in response to γ-irradiation. This could be attributed to the unique environment and cellular features of the kidney tissues.

## Increase in PCNA levels in the lung and liver following irradiation

A unique pattern of response to γ-irradiation was observed in the lung and liver tissues. The PCNA protein levels increased in the lung and liver following irradiation (Fig. 4), while similar changes were not observed in the other tissues (Fig. 3B, Fig S2B). PCNA plays important roles in DDR including PRR, repair DNA synthesis, in addition to its major role in DNA replication. Additionally, PCNA ubiquitination is an important marker of PRR. PCNA proteins were actively ubiquitylated in the lung tissues (band > 37 kDa size), while this was not observed in the kidney and liver samples, following irradiation. Therefore, PRR expression

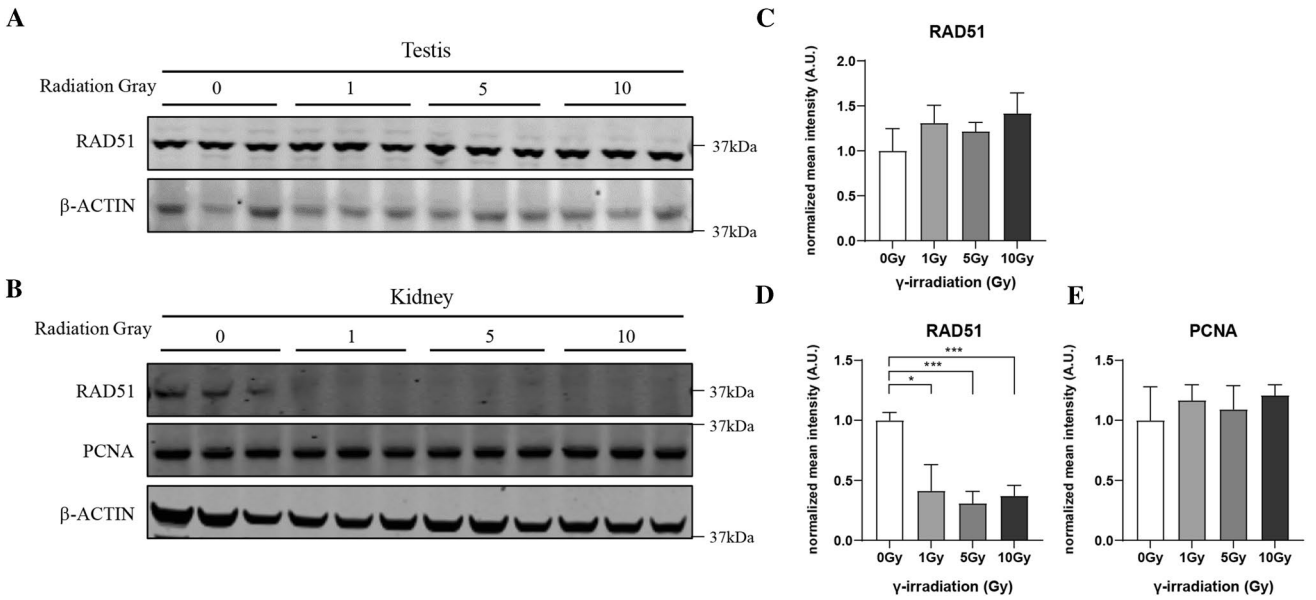


**Fig. 3** Expression of RAD51 in the testis and kidney tissues. **A** Western blot analysis of testis tissues for RAD51 levels in the presence or absence of γ-irradiation ($n=3$). **B** Western blot analysis of kidney tissues for RAD51 and PCNA levels after γ-irradiation ($n=3$). **C** Quantification of (**A**). **D**, **E** Quantification of (**B**). PCNA, proliferating cell nuclear antigen

could be a dominant response only in the lung tissues, following the exposure to γ-irradiation. Taken together, these results suggest that lung and liver tissues could involve a considerably high usage of PCNA mediated repair in response to irradiation-related DNA damage, but the specific mechanism could differ, because the increase in Ub-PCNA levels is observed only in the lung tissue. Therefore, considering RAD51 and PCNA, the tissues of specific organs have specific DDR systems that respond to γ-irradiation.

## Discussion

Ionizing radiation carries a sufficient amount of energy to create various ionized molecules that can damage DNA. Ionizing radiation is used in various fields including research, medicine, manufacturing, construction, and nuclear weapons. In the medical field, ionizing radiation is used in cancer therapy. Ionizing radiation kills malignant cells by inducing DNA damage to block the proliferation of cancer cells (Ward 1988). However, the unrestricted exposure of the other parts of the body creates adverse effects such as tissue damage, mutation, additional carcinogenesis, and cell death (Hauer-Jensen et al. 2014; Hazard et al. 2008; Liebel et al. 2012). Different tissues and cancers respond differently to exogenous stimuli; however, the in vivo response of each tissue to irradiation has not been studied extensively.

To investigate the DDR in various tissues, we treated mice with γ-irradiation. A major finding of this study was the difference in DNA damage levels and DDR to irradiation in various tissues. The liver, lung, and kidney tissues showed an upregulation of γH2AX, compared to that in the brain, muscle, and testis tissues following irradiation. pRPA2 S4/S8 was highly expressed in the liver, muscle, and testis tissues, compared to that in the lung, kidney, and brain tissues. However, pRPA2 S4/S8 expression was high in the liver, muscle, and testis tissues, irrespective of irradiation; this suggests that each tissue responds differently to irradiation. pRPA2 S4/S8 was not induced considerably in the brain tissue following irradiation. The irradiation does not generally reach the brain tissue, because of the depth of the brain and the resistance of neuronal cells to irradiation. Therefore, this result may not reflect the DDR to irradiation in the brain (Ramanan et al. 2009; Suman et al. 2013). The brain is mainly composed of post-mitotic cells; and therefore, pRPA2, which is induced during DNA replication, may not be induced in brain tissues (Giglia-Mari et al. 2011). Each tissue responds differently to DNA damage depending on the characteristics of the tissues such as function, proliferation rate, and developmental status.

RAD51, which plays an important role in HR, was highly expressed in the testis tissue, irrespective of irradiation. The testis contains germ cells and sperms. These cells undergo meiosis, which requires HR for mediating crossover events. Therefore, it is possible that the cells in the testis repair DNA damage via HR by default. Unlike in the testis, RAD51 expression was decreased in the kidney tissues following γ-irradiation. This could be attributed to specific environmental conditions and cell types in the kidneys, such as (1) a change in the micro RNA (miRNA) expression, (2)

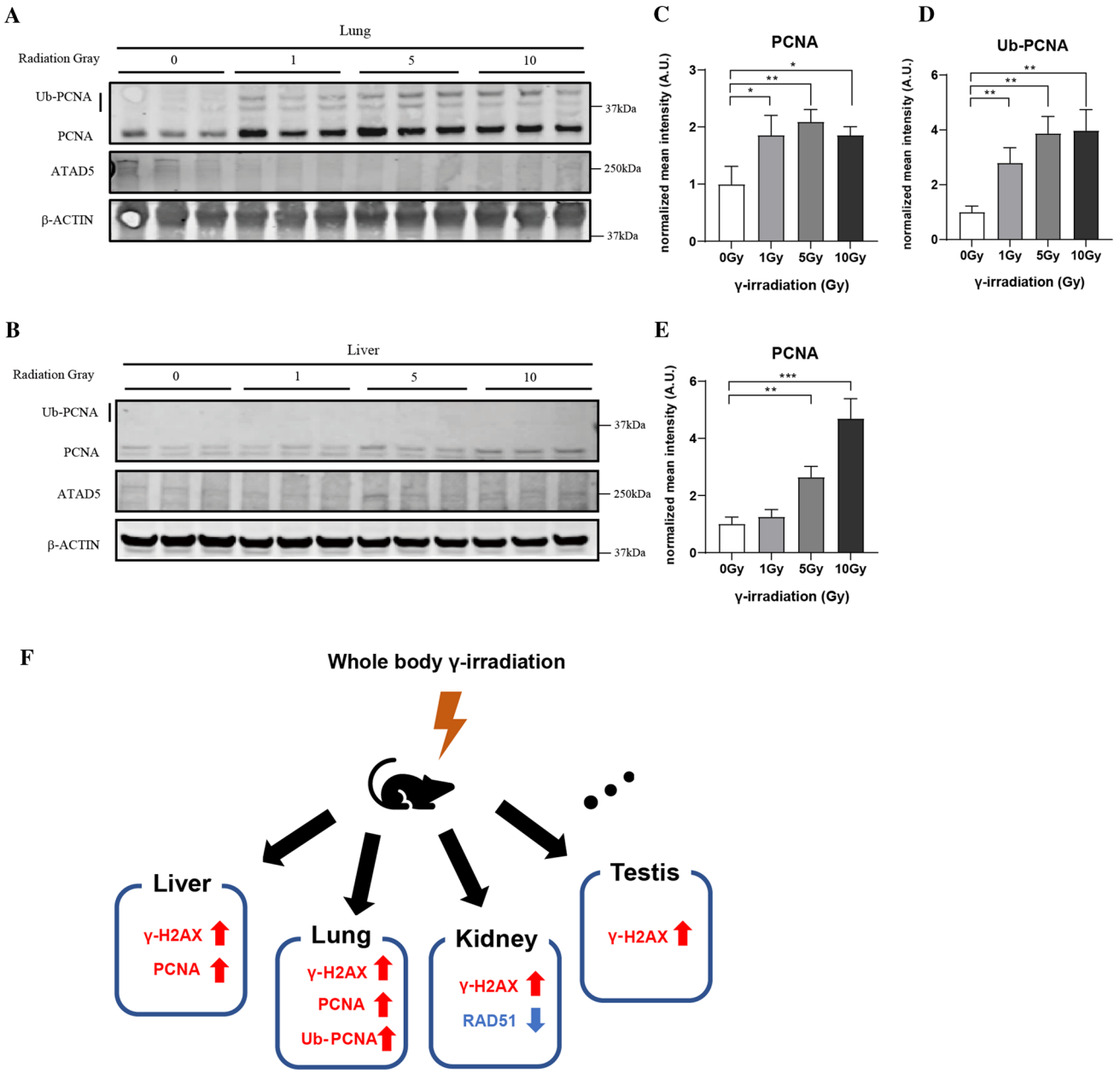


**Fig. 4** Expression of PCNA and ATAD5 in the lung and liver tissues. Western blot analysis of lung (**A**) and liver tissues (**B**) for PCNA and ATAD5 levels following γ-irradiation ($n=3$). **C**–**E** quantification of (**A**). **F**, **G** quantification of (**B**). **F** Schematic explanation of this project. *PCNA* proliferating cell nuclear antigen, *Ub-PCNA* Ubiquitinated PCNA, *ATAD5* ATPase family AAA domain-containing protein 5

formation of variant proteins of RAD51 in the kidney following irradiation, or (3) characteristics of the kidney associated with its function and proliferation rate. RAD51 expression in response to DNA damage is often regulated via miRNA expression (Gasparini et al. 2014; Lee et al. 2016). miR-155 in breast cancer targets RAD51 and impairs HR following irradiation (Gasparini et al. 2014). RAD51 is downregulated in hepatocytes in response to low-dose irradiation (0.01 Gy), via downregulation of miR-193b-3p (Lee et al. 2016). Therefore, it is possible that irradiation influences the expression of miRNA that regulates RAD51 expression, promoting the downregulation of RAD51 in the kidneys. Alternatively, RAD51 variants could form following irradiation; RAD51 variant proteins in human kidney tumors induce DNA strand-exchange defects (Silva et al. 2016). The influence of irradiation on the expression of miRNA or RAD51 variants in the kidneys is unknown. The kidney is a slow growing or non-proliferative organ similar to the brain;

and therefore, the kidneys may utilize the NHEJ pathway for the repair of double-stranded DNA breaks caused by irradiation. The activity of NHEJ is higher in kidney fibroblasts derived from young mice following double-stranded DNA breaks (Vaidya et al. 2014).

ATAD5 plays an important role in PCNA unloading and ubiquitination. Its expression in the liver and lung tissues remained stable; the level of PCNA increased in the lung tissues following irradiation (Fig. 4). PCNA functions mainly during DNA replication as a clamp for DNA replication polymerases and it is also involved in PRR that includes TLS and template switching. Therefore, the lungs could use active PRR mechanisms in response to irradiation; the levels of PCNA and ubiquitinated PCNA reflect the usage of PRR in lung tissues (Fig. 4A).

Each mouse tissue exhibits a different DDR pattern to γ-irradiation and each may have a preferable repair mechanism for DNA double-strand break-induced via irradiation. The liver, lung, and kidney are sensitive to DNA damage caused by irradiation; therefore, these tissues require a controlled treatment involving radiotherapy. The PRR mechanism could be activated in the lung tissues following irradiation. The brain, muscle, and testis tissues are resistant to low-dose irradiation; the testis could possess active HR mechanisms in response to double-stranded DNA breaks. The relation between these mechanisms and the susceptibility of each tissue to damage is not known. It is possible that a combination therapy using an NHEJ inhibitor (such as, KU inhibitors) and radiotherapy could improve the efficacy in treating brain or kidney tumors, because these tissues had a low level of pRPA2 and RAD51 expression, following irradiation. Further studies are necessary for a conclusive determination of the DNA repair capacities of different tissues; however, this study provides a new perspective for improving the application of radiotherapy to different tumors and for reducing the side effects in different tissues.

## Methods

### Mice

Male C57BL/6 J mice were purchased from Orient-Bio (Gyeonggi-do, Korea). All animal procedures were approved and performed according to the guidelines of the Institutional Animal Care and Use Committee of the Ulsan National Institute of Science and Technology, Ulsan, South Korea.

### Irradiation treatment

To investigate the tissue-specific response to γ-irradiation, we exposed C57BL6/J male mice (10 weeks old, $n = 3$) to γ-irradiation in a dose-dependent manner (0, 1, 5, and 10 Gy). The target tissues (brain, liver, lung, testis, muscle, and kidney) were harvested 1 h after irradiation and the levels of proteins related to DDR were measured using western blotting. First, to confirm the DNA damage levels, Mice were treated via γ-irradiation using a Gammacell 3000 Elan from MDS Nordion (Ottawa, Canada), at different strengths (0, 1, 5, and 10 Gy). Subsequently, the mice were euthanized at 1 h after exposure to γ-irradiation.

### Immunoblot analysis

Mice tissues were lysed in radioimmunoprecipitation acid buffer (150 Mm sodium chloride, 1.0% NP-40 or Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate (SDS), 50 mM Tris pH 8.0, 10 mM sodium fluoride, 1 mM sodium orthovanadate, and protease inhibitor (Roche, Basel, Switzerland) (Park et al. 2013). The lysed samples were mixed with a 2 × Laemmli sample buffer and boiled for 10 min at 100 °C. The samples were electrophoresed on an SDS–polyacrylamide gel and transferred to a nitrocellulose membrane (Bio-Rad, Hercules, California, USA). The membranes were incubated with the primary antibodies overnight at 4 °C, followed by the incubation with the secondary antibodies at room temperature (23–28 °C) for 1 h. Images were analyzed using the Odyssey imaging system from LI-COR (Lincoln, NE, USA).

### Antibodies

The following antibodies were used: mouse monoclonal IgG2a PCNA (sc-56; Santa Cruz Biotechnology, Dallas, TX, USA), mouse monoclonal phosphorylated-histone H2AX (Ser139) (#05-636; Millipore, Burlington, MA, USA), rabbit monoclonal RAD51 (D4B10; Cell Signaling Technology, Danvers, MA, USA), rabbit polyclonal phosphorylated-RPA32(S4/S8) (A300-245A; Bethyl, Montgomery, TX, USA) and mouse monoclonal β-actin (MA5-15,739; Thermo Fisher Science, Waltham, MA, USA). The anti-rabbit ATAD5 antibody was prepared as described previously (Park et al. 2019).

### Quantification and statistical analysis

The mean intensity of the protein bands in western blots was quantified using Image J software. Relative intensity of each protein band was calculated (target protein mean intensity)/(β-Actin mean intensity), and then normalized against the values from the 0 Gy group. Bar graph was drawn using Prism8 program (Graphpad). Statistical tests were performed using the student's *t* test embedded in Prism8 program.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s13273-021-00195-w.

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**Author contributions** Conceptualization, JHP and KJM; funding acquisition, KJM; investigation, SGL, NWK, IBP, and JHP; writing—original draft, SGL, NWK, and JHP; Writing—review and editing, JHP and KJM.

## Declarations

**Conflict of interest** Seon-gyeong Lee declares that she has no conflict of interest.
Namwoo Kim declares that he has no conflict of interest.
In Bae Park declares that he has no conflict of interest.
Jun Hong Park declares that he has no conflict of interest.
Kyungjae Myung declares that he has no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants performed by any of the authors. The Animal studies were approved and performed according to the guidelines of the Institutional Animal Care and Use Committee of the Ulsan National Institute of Science and Technology. This study performed following institutional and national guidelines.



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