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Original Article

Recombinant cell-detecting RaDR-GFP in mice reveals an association between genomic instability and radiation-induced-thymic lymphoma

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Abstract: In this study, we aimed to investigate how homologous recombinant (HR)-related genomic instability is involved in ionizing radiation (IR)-induced thymic lymphoma in mice. We divided five-week-old Rosa26 Direct Repeat-GFP (RaDR-GFP) transgenic mice into non-IR control and IR groups and exposed the mice in the IR group to a 7.2 Gy dose of γ-rays, delivered in 1.8 Gy fractions, once a week for four weeks. We then estimated mouse survival and recorded their body, thymus, and spleen weights. The frequency of HR events in the chromosomes of the thymus, bone marrow, and spleen cells and the phenotype of thymic lymphoma cells were analyzed using fluorescence-activated cell sorting (FACS). We found that most mice in the IR group developed thymic lymphoma, their survival rate decreasing to 20% after 180 days of IR exposure, whereas no mice died in the non-IR control group until day 400. The thymus and spleen weighed significantly more in the IR-4-month group than that in the non-IR group; however, we observed no significant differences between the body weights of the control and IR mice. FACS analysis indicated that the frequency of HR events significantly increased at two and four months after the last IR dose in the bone marrow and thymus cells, but not in the spleen cells of RaDR-GFP transgenic mice, suggesting that recombinant cells accumulated in the thymus upon IR exposure. This suggests that IR induces genome instability, revealed as increased HR, that drives the development of thymic lymphoma. Additionally, phenotypic analysis of lymphoma cells showed an increase in the $CD4^-/CD8^+$ (CD8SP) cell population and a decrease in the $CD4^+/CD8^-$ (CD4SP) cell population in the IR-4-month group compared to that in the non-IR group, indicating that IR induces an aberrant cell phenotype characteristic of lymphoma. In conclusion, we observed a significant increase in HR events and abnormal phenotype in thymic lymphoma cells at two and four months after IR exposure in both the thymus and bone marrow tissues, suggesting that genomic instability is involved in the early stages of thymic lymphomagenesis. Our study indicates that HR-visualizing RaDR-GFP transgenic mice can help explore the links between the molecular mechanisms of genome instability and IR-induced tumorigenesis.

Keywords: Radiation, lymphoma, genome instability, homologous recombination (replication stress)

Introduction

Ionizing radiation (IR)-induced DNA double-strand breaks (DSBs) are the most damaging types of DNA damage and are generally repaired by either the homologous recombination (HR) or non-homologous end-joining (NHEJ) pathway [1-5]. HR maintains genomic stability by repairing complex DNA damage such as DSBs and inter-strand crosslinks [6-8], which would otherwise result in altered DNA sequences (mutations) and altered genome structures

(translocations, amplifications, inversions, and other complex rearrangement), destabilizing the genome and initiating tumorigenesis [9-12].

Genome instability can be characterized by reduced subcloning efficiency, karyotype heterogeneity, chromosome modification, micronucleus formation, altered mutation rates, gene amplification, and carcinogenesis-related microsatellite instability [13-15]. Destabilization of the genome is widely accepted as one of the most important steps in carcinogenesis [16-18]. Genome instability is a dynamic process that continuously modifies the genome of tumor cells over time [19-23]. The induction of thymic lymphoma in mice using IR was first performed more than 70 years ago [24]. Kaplan et al. determined the optimal dose fraction period [25] widely used as a typical radiation-induced animal carcinogenesis model. C57BL mice, one of the most commonly used mouse strains, and its substrains, such as BALB/c and NFS mice, are susceptible to developing thymic lymphoma upon IR exposure [26-30]. The molecular mechanisms of IR-induced thymic lymphoma include upregulation of methyltransferase SET domain-containing protein 4 (SETD4), miR-21 overexpression, KRAS and p53 mutations, 5'-deletion of Notch1 gene, and stable DNA hypomethylation [31-37].

Recently, Engelward Laboratory developed the RaDR-GFP (Rosa26 Direct Repeat-GFP) mouse model, which enables visualization and quantification of HR DNA damage repair events *in vivo* [38, 39]. In the present study, we used this RaDR-GFP transgenic mouse model to examine HR-mediated genome instability during the development of radiation-induced thymic lymphoma.

Materials and methods

Animals and irradiation

RaDR-GFP transgenic mice on a C57BL/6J background were originally made in Engelward Lab (Massachusetts Institute of Technology, USA). A direct repeat HR substrate in the mouse genome is targeted to the ubiquitously expressed Rosa26 locus, and homologous recombination between the two truncated EGFP expression cassettes yields a fluorescent signal. We used homozygous RaDR-GFP mice generated by an intercross between heterozygotes [38]. The mice were maintained in a pathogen-free (SPF) animal facility with autoclaved cages and sterilized wood chips and fed standard laboratory chow (MB-1, Funabashi Farm Co., Japan) and acidified water *ad libitum*. We divided five-week-old male RaDR-GFP transgenic mice into non-IR control (n=5) and IR groups (n=15) for survival rate analysis. In addition, a total 17 non-IR control and 20 IR mice were used for FACS analysis. IR group mice were exposed to a 7.2 Gy dose of γ-rays delivered in 1.8 Gy fractions (Cesium 137, dose rate 1 cGy/min) once a week for 4 weeks. The mice were held in acrylic containers and exposed to total body irradiation (TBI) at room temperature, and their survival rate and body weight changes were calculated. Some mice were euthanized at 1, 2, 4-month after the last IR dose, and the thymus and spleen weights were estimated. We performed the histopathological analysis of IR-induced thymic lymphoma and other tissues such as the spleen, pancreas, and liver using hematoxylin and eosin (HE) staining. All animal studies were conducted in accordance with the QST's Institutional Animal Welfare Guidelines and were approved by the Animal Experiments Ethics Committee (Approval number: No. 14-1014-2).

Analysis of recombinant cells in the thymus, bone marrow, and spleen tissues

In order to observe HR events (GFP-positive cells) using a fluorescence microscope in the thymic and pancreatic tissues of RaDR-GFP transgenic mice with or without TBI. The visual detection of recombinant cells was done in a blinded fashion. Then we detected and analyzed the frequency of HR events in the chromosomes of the thymus, bone marrow, and spleen cells of RaDR-GFP transgenic mice using flow cytometry. We treated the bone marrow, spleen, and thymus tissues with Tris-buffered ammonium chloride to lyse the RBCs. The tissue suspensions were then washed with phosphate buffered saline (PBS) to prepare single-cell suspensions, which were filtered through a 40-mm cell strainer (Corning Inc, Corning, New York) and fixed with 1% paraformaldehyde PBS (Wako Pure Chemical Industries) at 4°C. The cells were washed once again with PBS, resuspended in Opti-MEM (Life Technologies, Carlsbad, CA, USA), filtered through a 35-mm cell strainer (Corning Inc),

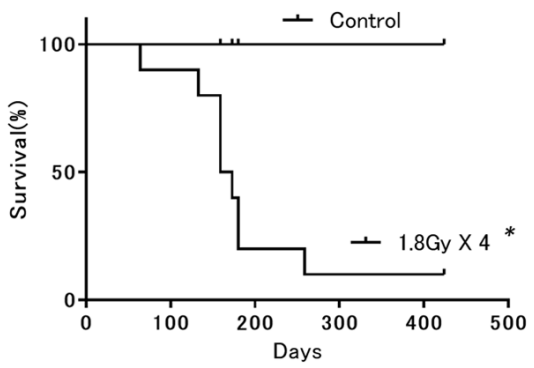


Figure 1. The overall survival rate of RaDR-GFP transgenic mice with or without TBI. Mice in the IR group were exposed to 1.8 Gy of γ-rays once a week for 4 weeks. *$P<0.05$ compared to the control.

and then subjected to FACS (Becton Dickinson, Franklin Lakes, NJ, USA). We used the cells from C57BL/6-Tg (CAG-EGFP) mice (SLC, Inc., Japan) constitutively expressing EGFP and wild-type C57BL/6J mice with no EGFP expression as positive and negative controls [40, 41]. The RaDR-GFP-positive cells showed higher fluorescence at 530 nm than at 580 nm, reflecting HR events in the cells. We analyzed at least 1 million cells from 3 mice for each point. The frequency of recombinant cells was expressed as the number of GFP-positive cell per million nucleated cells.

Phenotype analysis of lymphoma cells

Normal thymocytes are composed of subpopulations of $CD4^-/CD8^-$ (DN), $CD4^+/CD8^+$ (DP), $CD4^+/CD8^-$ (CD4SP), and $CD4^-/CD8^+$ (CD8SP) cells [42]. In general, the DN cells are the least developed, which differentiate into DP cells via intermediate CD8SP cells [43]. Only a small fraction of DP cells differentiate into either CD4SP or CD8SP cells after thymic selection and migrate to peripheral lymphoid organs [44, 45]. For analysis of thymocytes in lymphoma and control thymus, single cell suspensions were mechanically prepared. Cells were incubated with anti-Fcγ III/II receptor (2.4G2) (Biolegend) to block Fc receptors and then stained with PE-Cy7 conjugated anti-CD4 (RM4-5) (Biolegend) and APC-Cy7 conjugated CD8a (53-6.7) (Biolegend) in 2% FBS containing PBS. Dead cells were excluded by staining with 7-aminoactinomycin D (Wako). Stained cells were analyzed with FACS Canto II (BD Bioscience).

Statistical analysis

We used the StatView software (SAS Institute, Inc., Cary, NC, USA) to perform one-way analysis of variance and Mann-Whitney U test for differences in the mean between groups. *Statistical significance was set at* $P<0.05$.

Results

Survival rate of RaDR-GFP transgenic mice after IR exposure

To investigate IR-induced tumorigenesis, we irradiated RaDR-GFP transgenic mice with a 7.2 Gy dose of γ-rays delivered in 1.8 Gy fractions, once a week for 4 weeks. Most mice developed thymic lymphoma, and the survival rate decreased to 20% at 180 days in the IR group, whereas no mice died until 400 days in the non-IR control group (**Figure 1**).

Morphological and histopathological confirmation of thymic lymphoma and its metastasis after IR exposure

We performed macroscopic and histopathological analyses to examine IR-induced thymic lymphoma. We did not observe significant differences in the body weights of the non-IR control and IR mice (**Figure 2A**). However, the thymus (**Figure 2B**) and spleen weights (**Figure 2C**) in IR mice were significantly increased compared to those in the non-IR control group at 4 months after the last IR dose. The histopathological analysis revealed most of the enlarged thymus to be thymic lymphoma (**Figure 2D**), with a tumor incidence (4 months after the last IR) of up to 90% in the IR group. No tumor was induced in the non-IR control group (**Figure 2D**). We observed metastasis in many organs, including the liver (**Figure 2E**), pancreas (**Figure 2F**), lungs (**Figure 2G**), and kidneys (**Figure 2H**), suggesting that IR-induced thymic lymphoma is aggressive and has a high potential to cause distant metastases.

Visible HR events in tissues of non-IR control and IR-induced thymic lymphoma

To confirm the visible HR events occurring after IR exposure in RaDR-GFP transgenic mice, we prepared tissues of non-IR control thymus, pancreas, and IR-induced thymic lymphoma in 4 mm frozen sections stained with DAPI. GFP-

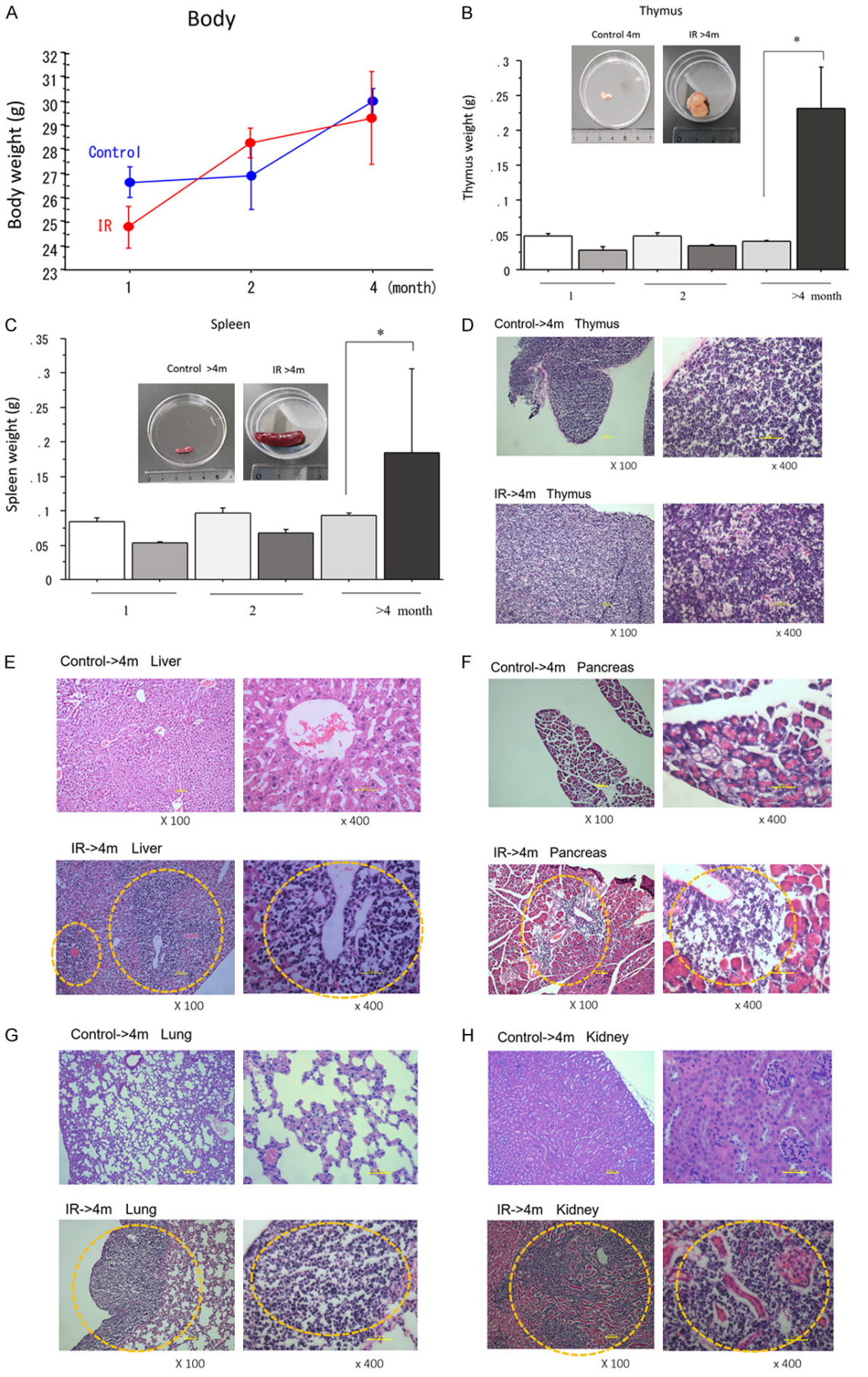

A
Body
Body weight (g)
Control
IR
4 (month)
B
Thymus
Control 4m
IR >4m
Thymus weight (g)
>4 month
C
Spleen
Control >4m
IR >4m
Spleen weight (g)
>4 month
D
Control->4m Thymus
IR->4m Thymus
E
Control->4m Liver
IR->4m Liver
F
Control->4m Pancreas
IR->4m Pancreas
G
Control->4m Lung
IR->4m Lung
H
Control->4m Kidney
IR->4m Kidney
X 100
x 400

Figure 2. (A) Body weight changes in RaDR-GFP transgenic mice 1, 2, and 4 months after TBI. (B) Morphological and weight changes of thymus in RaDR-GFP transgenic mice 1, 2, and 4 months after TBI. (C) Morphological and weight changes of spleen in RaDR-GFP transgenic mice 1, 2, and 4 months after TBI. (D) Histopathological changes in the thymus of non-IR and IR mice. Histopathological changes in the liver (E), pancreas (F), lung (G) and kidney (H) of non-IR and IR mice. Metastases were only seen in the liver, pancreas, lung and kidney of IR mice. Dotted circles indicate the metastasis sites in the various tissues. $^{*}P<0.05$ compared to the control.

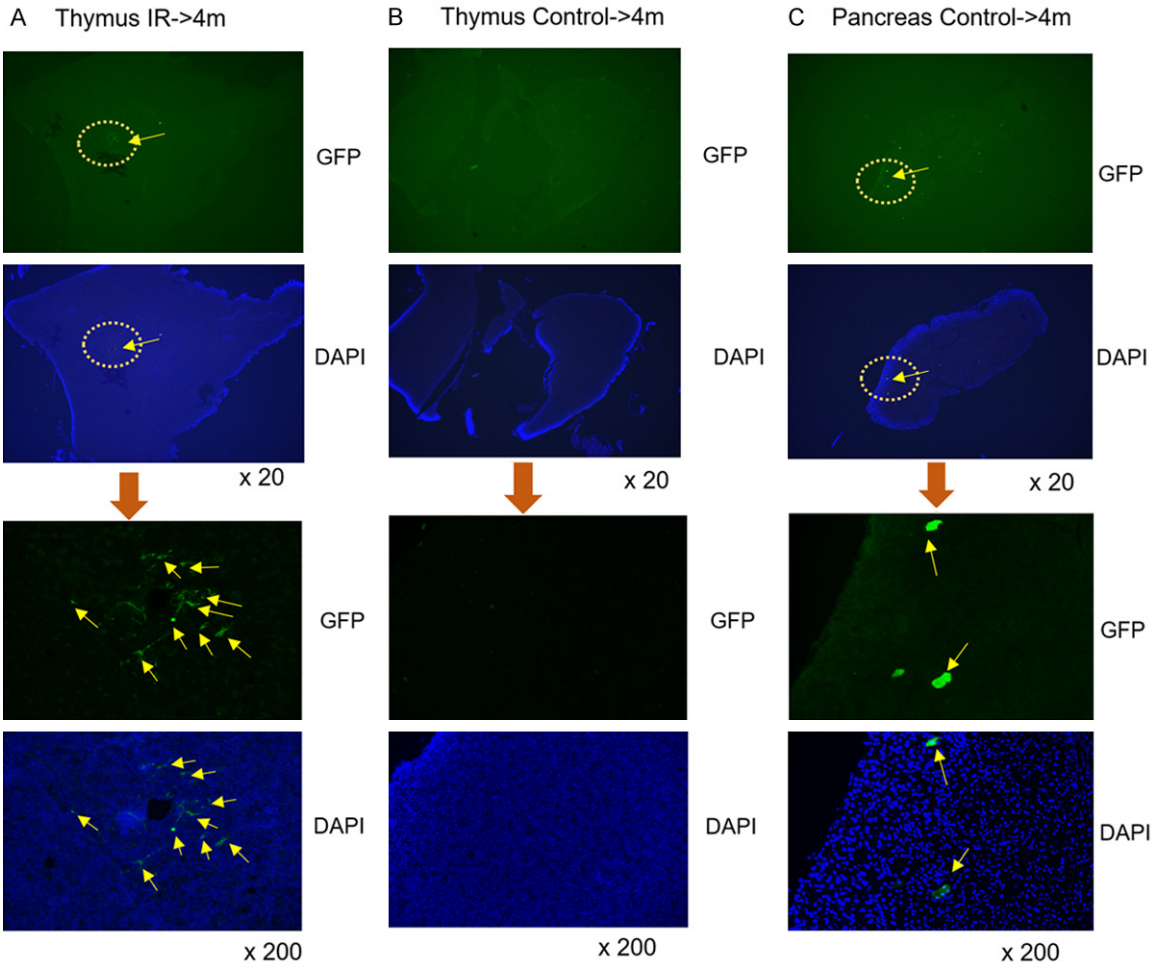


Figure 3. Representative photos of HR events (GFP-positive cells) as observed using a fluorescence microscope in the thymus of IR mice (A), non-IR control (B), and the pancreas of non-IR control (C) RaDR-GFP transgenic mice. Arrows indicate GFP-positive cells in the thymic and pancreatic tissues of RaDR-GFP transgenic mice.

positive cells were observed using fluorescence microscopy in the IR-induced thymic lymphoma tissues (**Figure 3A**) but not in the non-IR control thymus tissues (**Figure 3B**), indicating that IR promotes HR events in murine thymus tissue. Interestingly, we observed few GFP-positive cells in the pancreas of non-IR control mice (**Figure 3C**).

HR frequency in the thymus, bone marrow, and spleen tissue after IR exposure

We performed FACS analysis to quantify HR events in hematopoietic tissues, including thymocytes, bone marrow, and splenocytes. A significant increase in the frequency of HR events was observed 2 to 4 months after the last IR dose in the thymus (**Figure 4A**) and bone marrow (**Figure 4B**), and an increasing tendency of HR events in the spleen (**Figure 4C**) of the RaDR-GFP transgenic mice, suggesting that recombinant cells accumulated in the first two tissues upon IR exposure and that genomic instability was involved in the development mouse thymic lymphoma. We see quite a bit of variation among mice, so it isn't surprising that the results I spleen are not statistically significant.

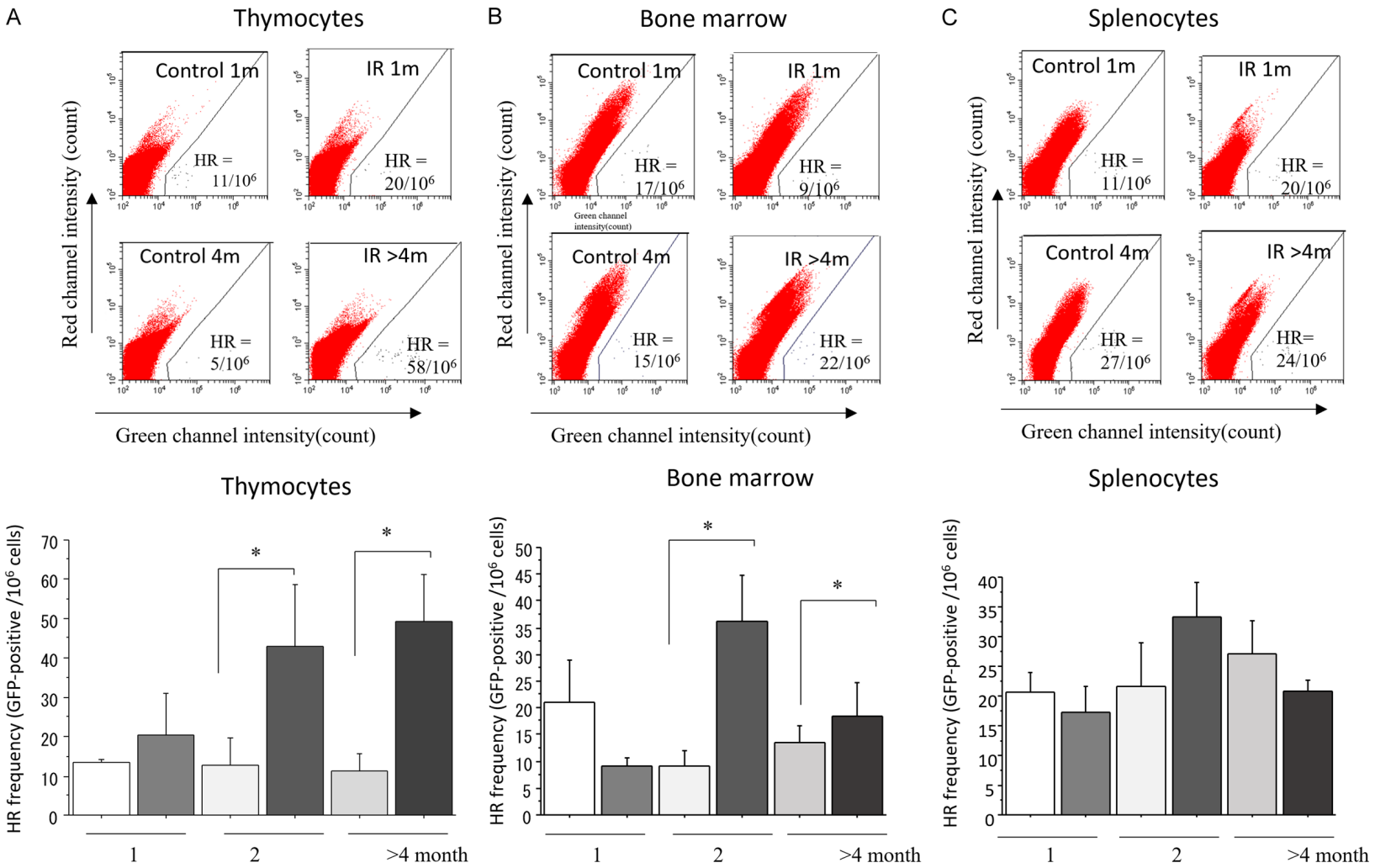


Figure 4. HR frequency analysis using FACS in the thymus, bone marrow, and spleen tissues of RaDR-GFP transgenic mice with or without TBI. For each of three experiments, there were three technical repeats. Light bars represent control samples and dark bars represent IR-treated samples. *P<0.05 compared to the control.

Phenotypic changes in lymphoma cells after IR exposure

To identify phenotypic changes in thymic lymphoma cells, FACS analysis was performed using PE-Cy7 conjugated anti-CD4 (RM4-5) and APC-Cy7 conjugated anti-CD8a antibodies. A representative flow cytometry plots showing $CD4^-/CD8^-$ (DN), $CD4^+/CD8^+$ (DP), $CD4^+/CD8^-$ (CD4SP), and $CD4^-/CD8^+$ (CD8SP) cells is shown in **Figure 5A**. We found that the $CD4^-/CD8^+$ (CD8SP) (**Figure 5B**) cell population increased, while that of the $CD4^+/CD8^-$ (CD4SP) (**Figure 5C**) decreased significantly 4 months after IR exposure compared to that in the non-IR group. In contrast, the $CD4^+/CD8^+$ (DP) (**Figure 5D**) cell population increased, and that of the $CD4^-/CD8^-$ (DN) (**Figure 5E**) decreased significantly 2 months after IR exposure compared to that in the non-IR group.

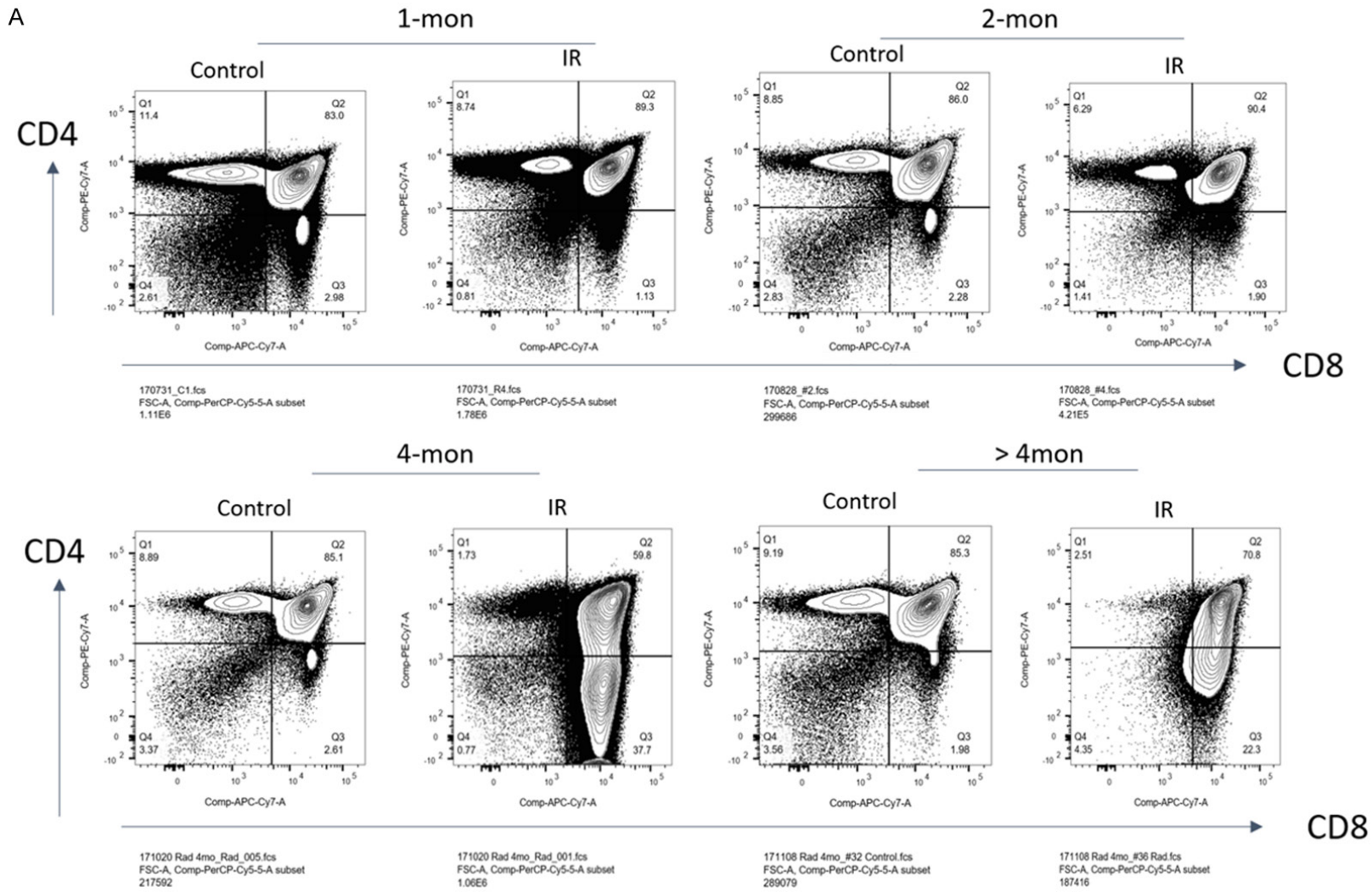
Discussion

In the present study, we found that 4×1.8 Gy of γ-ray irradiation could induce thymic lymphoma in more than 90% of RaDR-GFP transgenic mice 180 days after the last exposure, decreasing their survival rate to 20%. In contrast, the thymus was not enlarged, and the mice did not die in the non-IR mice group until day 400. Additionally, the thymus and spleen from IR mice weighed significantly more than those from the non-IR mice. However, the body weights of IR and non-IR mice were similar. The pathological analysis revealed the enlarged thymus to be thymic lymphoma. IR-induced thymic lymphoma caused distant metastases in multiple organs, including lungs, liver, pancreas, and kidneys. This is in line with previous reports that total-body IR frequently induces thymic lymphomas in certain strains of mice, such as C57BL, BALB/c, and NFS mice [24-30].

Genomic instability is a hallmark of tumorigenesis. IR can destabilize the genome, resulting in mutations and chromosomal aberrations in cells due to misrepair of DNA damage [9-15, 46, 47]. HR is an evolutionarily conserved basic process that plays a major role in maintaining genomic stability through DNA repair and protection. Erroneous HR destabilizes the genome, inducing tumorigenesis [6-9, 43]. Recently, Park et al. reported that partial loss of function of HR gene BRCA1 causes the development of T-lymphoblastic lymphoma using mouse model [48]. However, there is a lack of in vivo data on how genomic instability initiated by the HR process consequently develops into cancer. The present study utilizes the RaDR-GFP transgenic mouse model to monitor HR events in many different tissues [38-40]. We directly visualized GFP-positive cells in IR-induced thymic lymphoma via fluorescence microscopy and observed a significant increase in the frequency of HR events in the bone marrow and thymus 2 to 4 months after IR. No such events were found in non-IR control mice. This finding suggests that the genomic instability caused by HR is involved in IR-induced thymic lymphoma. We have also examined GFP-positive cell distributions in the pancreas, liver and kidney of non-IR control mice in this study. We found GFP-positive cells in the pancreas and very a few in the liver tissues, but none in kidney. This is in line with previous report that GFP-positive cells can be found in 11 different tissues including pancreas and liver of RaDR-GFP transgenic mice [38].

In this study, phenotypic analysis of thymic lymphoma cells showed an increased DP cell population and a decreased DN cell population 2 months after the last IR dose and an increased CD8SP cell population and a decreased CD4SP cell population 4 months after the last IR dose in the IR mice compared to that in the non-IR mice. An important question is the mechanism underlying the IR-inducing onset of thymic lymphoma. Interestingly, a previous study showed that, in the absence of recombinase, IR induced differentiation of DN into DP without T cell receptor beta (TCRb) rearrangement [49]. Moreover, other studies showed that by-passing β-selection spontaneously developed CD8SP lymphoma in the thymus [50, 51]. Overall, one possible mechanism of the lymphoma onset is that IR may induces an abnormal conversion of a small population of DN into DP without β-selection, leading to development of CD8SP thymoma, which may be gradually increased in the thymus.

In summary, we observed prominent HR events 2 and 4 months after the last dose IR in both the thymus and bone marrow tissues, suggesting HR-mediated genomic instability is involved in the development of thymic lymphoma. Our study indicates that HR-visualizing RaDR-GFP


A
1-mon
2-mon
Control
IR
Control
IR
CD4
Q1 11.4
Q2 83.0
Q4 2.61
Q3 2.98
Q1 8.74
Q2 89.3
Q4 0.81
Q3 1.13
Q1 8.85
Q2 86.0
Q4 2.83
Q3 2.28
Q1 6.29
Q2 90.4
Q4 1.41
Q3 1.90
Comp-PE-Cy7-A
Comp-APC-Cy7-A
CD8
170731_C1.fcs
FSC-A, Comp-PerCP-Cy5-5-A subset
1.11E6
170731_R4.fcs
FSC-A, Comp-PerCP-Cy5-5-A subset
1.78E6
170828_#2.fcs
FSC-A, Comp-PerCP-Cy5-5-A subset
299686
170828_#4.fcs
FSC-A, Comp-PerCP-Cy5-5-A subset
4.21E5
4-mon
> 4mon
Control
IR
Control
IR
CD4
Q1 8.89
Q2 85.1
Q4 3.37
Q3 2.61
Q1 1.73
Q2 59.8
Q4 0.77
Q3 37.7
Q1 9.19
Q2 85.3
Q4 3.56
Q3 1.98
Q1 2.51
Q2 70.8
Q4 4.35
Q3 22.3
Comp-PE-Cy7-A
Comp-APC-Cy7-A
CD8
171020 Rad 4mo_Rad_005.fcs
FSC-A, Comp-PerCP-Cy5-5-A subset
217592
171020 Rad 4mo_Rad_001.fcs
FSC-A, Comp-PerCP-Cy5-5-A subset
1.06E6
171108 Rad 4mo_#32 Control.fcs
FSC-A, Comp-PerCP-Cy5-5-A subset
289079
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187416

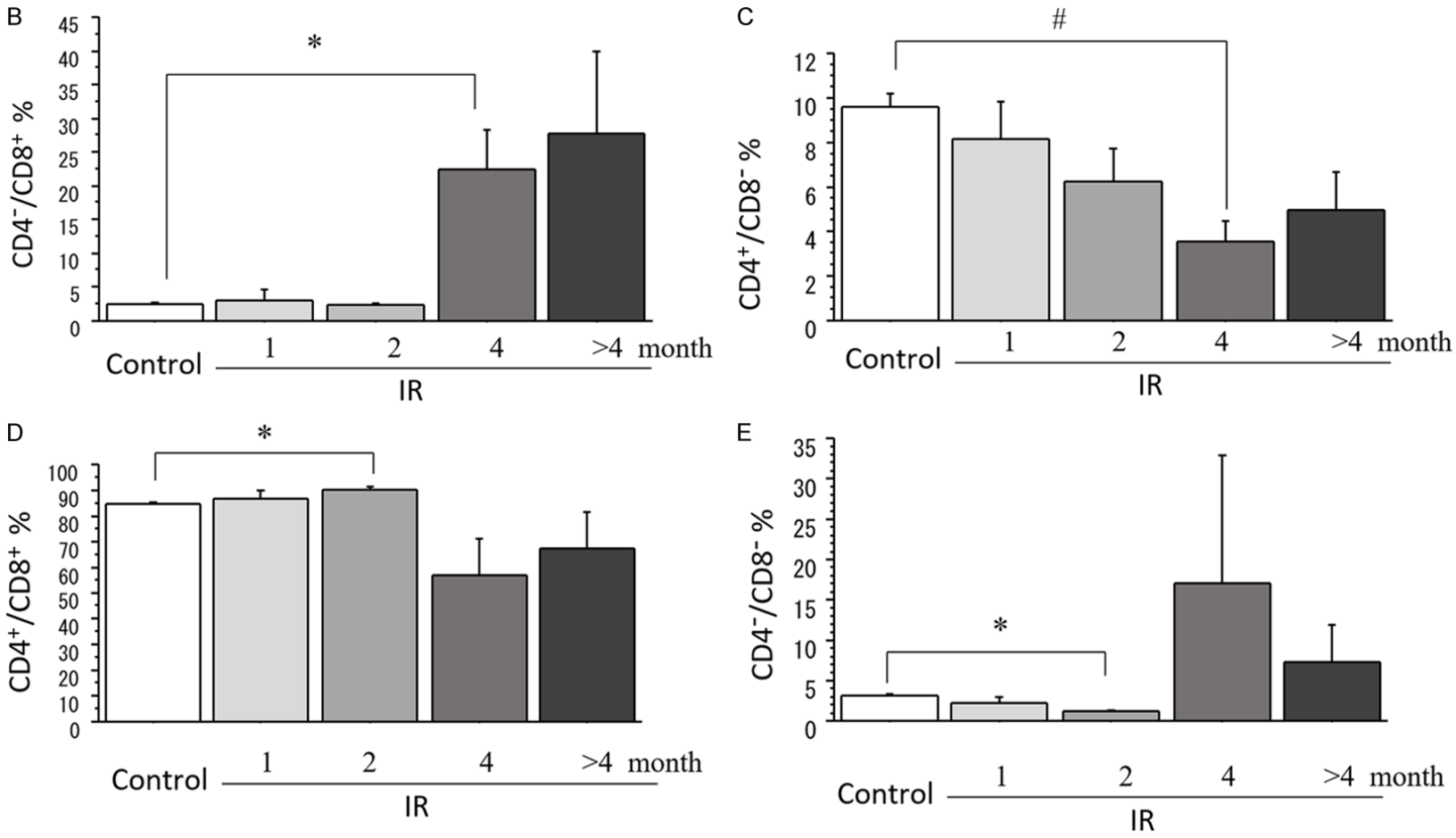

Figure 5. Phenotypic analysis of lymphoma cells using FACS in thymic tissues of RaDR-GFP transgenic mice with or without TBI. (A) Representative flow cytometry charts illustrating $CD4^-/CD8^-$ (DN), $CD4^+/CD8^+$ (DP), $CD4^+/CD8^-$ (CD4SP), and $CD4^-/CD8^+$ (CD8SP) cells. Percentage changes of $CD4^-/CD8^+$ (B), $CD4^+/CD8^-$ (C), $CD4^+/CD8^+$ (D), and $CD4^-/CD8^-$ (E) cells in the thymus of non-IR and IR mice. For each of three experiments, there were three technical repeats. Light bars represent control samples and dark bars represent IR-treated samples. $^*P<0.05$, $^\#P<0.01$ compared to the control.

transgenic mice can be used to explore the molecular mechanisms of genome instability as it relates to IR-induced tumorigenesis.

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Disclosure of conflict of interest

None.

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References

[1] Shrivastav M, De Haro LP and Nickoloff JA. Regulation of DNA double-strand break repair pathway choice. Cell Res 2008; 18: 134-47.
[2] Vignard J, Mirey G and Salles B. Ionizing-radiation induced DNA double-strand breaks: a direct and indirect lighting up. Radiother Oncol 2013; 108: 362-9.
[3] Wang C and Lees-Miller SP. Detection and repair of ionizing radiation-induced DNA double strand breaks: new developments in nonhomologous end joining. Int J Radiat Oncol Biol Phys 2013; 86: 440-9.
[4] Santivasi WL and Xia F. Ionizing radiation-induced DNA damage, response, and repair. Antioxid Redox Signal 2014; 21: 251-9.
[5] Aparicio T, Baer R and Gautier J. DNA double-strand break repair pathway choice and cancer. DNA Repair (Amst) 2014; 19: 169-75.
[6] Bishop AJ and Schiestl RH. Homologous recombination as a mechanism for genome rearrangements: environmental and genetic effects. Hum Mol Genet 2000; 9: 2427-334.
[7] Wright WD, Shah SS and Heyer WD. Homologous recombination and the repair of DNA double-strand breaks. J Biol Chem 2018; 293: 10524-10535.
[8] Bishop AJ and Schiestl RH. Homologous recombination and its role in carcinogenesis. J Biomed Biotechnol 2002; 2: 75-85.
[9] Nickoloff JA, Sharma N, Allen CP, Taylor L, Allen SJ, Jaiswal AS and Hromas R. Roles of homologous recombination in response to ionizing radiation-induced DNA damage. Int J Radiat Biol 2021; 1-12. [Epub ahead of print].
[10] Huang L, Snyder AR and Morgan WF. Radiation-induced genomic instability and its implications for radiation carcinogenesis. Oncogene 2003; 22: 5848-54.
[11] Little JB. Radiation carcinogenesis. Carcinogenesis 2000; 21: 397-404.
[12] Suzuki K, Ojima M, Kodama S and Watanabe M. Radiation-induced DNA damage and delayed induced genomic instability. Oncogene 2003; 22: 6988-93.
[13] Lord CJ and Ashworth A. The DNA damage response and cancer therapy. Nature 2012; 481: 287-94.
[14] Andor N, Maley CC and Ji HP. Genomic instability in cancer: teetering on the limit of tolerance. Cancer Res 2017; 77: 2179-2185.
[15] Jeggo PA, Pearl LH and Carr AM. DNA repair, genome stability and cancer: a historical perspective. Nat Rev Cancer 2016; 16: 35-42.
[16] Ui A, Chiba N and Yasui A. Relationship among DNA double-strand break (DSB), DSB repair, and transcription prevents genome instability and cancer. Cancer Sci 2020; 111: 1443-1451.
[17] Morgan WF. Radiation-induced genomic instability. Health Phys 2011; 100: 280-1.
[18] Baulch JE. Radiation-induced genomic instability, epigenetic mechanisms and the mitochondria: a dysfunctional menage a trois? Int J Radiat Biol 2019; 95: 516-525.
[19] Keszenman DJ, Kolodiuk L and Baulch JE. DNA damage in cells exhibiting radiation-induced genomic instability. Mutagenesis 2015; 30: 451-8.
[20] Mavragani IV, Nikitaki Z, Souli MP, Aziz A, Nowsheen S, Aziz K, Rogakou E and Georgakilas AG. Complex DNA damage: a route to radiation-induced genomic instability and carcinogenesis. Cancers (Basel) 2017; 9: 91.
[21] Yahyapour R, Salajegheh A, Safari A, Amini P, Rezaeyan A, Amraee A and Najafi M. Radiation-induced non-targeted effect and carcinogenesis; implications in clinical radiotherapy. J Biomed Phys Eng 2018; 8: 435-446.
[22] Limoli CL, Ponnaiya B, Corcoran JJ, Giedzinski E, Kaplan MI, Hartmann A and Morgan WF. Genomic instability induced by high and low LET ionizing radiation. Adv Space Res 2000; 25: 2107-17.
[23] Sacher GA and Brues AM. Analysis of lymphoma induction by X rays in mice. Cancer Res 1949; 9: 620.
[24] Kaplan HS and Brown MB. A quantitative dose-response study of lymphoid-tumor development in irradiated C57 black mice. J Natl Cancer Inst 1952; 13: 185-208.
[25] Newcomb EW, Steinberg JJ and Pellicer A. Ras oncogenes and phenotypic staging in N-methylnitrosourea- and gamma-irradiation-induced

thymic lymphomas in C57BL/6J mice. Cancer Res 1988; 48: 5514-5521.
[26] Humblet C, Greimers R, Boniver J and Defrense MP. Stages in the development of radiation-induced thymic lymphomas in C57 BL/Ka mice: preleukemic cells become progressively resistant to the tumor preventing effects of a bone marrow graft. Exp Hematol 1997; 25: 109-113.
[27] Mori N and Takamori Y. Development of non-thymic lymphomas in thymectomized NFS mice exposed to split-dose X-irradiation. J Radiat Res 1990; 31: 389-395.
[28] Liu C, Li B, Cheng Y, Lin J, Hao J, Zhang S, Mitchel RE, Sun D, Ni J, Zhao L, Gao F and Cai J. MiR-21 plays an important role in radiation induced carcinogenesis in BALB/c mice by directly targeting the tumor suppressor gene Bigh3. Int J Biol Sci 2011; 7: 347-363.
[29] Okumoto M, Nishikawa R, Iwai M, Iwai Y, Takamori Y, Niwa O and Yokoro K. Lack of evidence for the involvement of type-C and type-B retroviruses in radiation leukemogenesis of NFS mice. Radiat Res 1990; 121: 267-73.
[30] Takabatake T, Kakinuma S, Hirouchi T, Nakamura MM, Fujikawa K, Nishimura M, Oghiso Y, Shimada Y and Tanaka K. Analysis of changes in DNA copy number in radiation-induced thymic lymphomas of susceptible C57BL/6, resistant C3H and hybrid F1 mice. Radiat Res 2008; 169: 426-36.
[31] Feng X, Lu H, Yue J, Schneider N, Liu J, Denzin LK, Chan CS, De S and Shen Z. Loss of Setd4 delays radiation-induced thymic lymphoma in mice. DNA Repair (Amst) 2020; 86: 102754.
[32] Mao JH, Li J, Jiang T, Li Q, Wu D, Perez-Losada J, DelRosario R, Peterson L, Balmain A and Cai WW. Genomic instability in radiation-induced mouse lymphoma from p53 heterozygous mice. Oncogene 2005; 24: 7924-34.
[33] Kominami R and Niwa O. Radiation carcinogenesis in mouse thymic lymphomas. Cancer Sci 2006; 97: 575-81.
[34] Pogribny I, Koturbash I, Tryndyak V, Hudson D, Stevenson SM, Sedelnikova O, Bonner W and Kovalchuk O. Fractionated low-dose radiation exposure leads to accumulation of DNA damage and profound alterations in DNA and histone methylation in the murine thymus. Mol Cancer Res 2005; 3: 553-61.
[35] Shimada Y, Nishimura M, Kakinuma S, Ogiu T, Fujimoto H, Kubo A, Nagai J, Kobayash K, Tano K, Yoshinaga S and Bhakat KK. Genetic susceptibility to thymic lymphomas and K-ras gene mutation in mice after exposure to X-rays and N-ethyl-N-nitrosourea. Int J Radiat Biol 2003; 79: 423-30.
[36] Koturbash I, Pogribny I and Kovalchuk O. Stable loss of global DNA methylation in the radiation-target tissue--a possible mechanism contributing to radiation carcinogenesis? Biochem Biophys Res Commun 2005; 337: 526-33.
[37] Tsuji H, Ishii-Ohba H, Katsube T, Ukai H, Aizawa S, Doi M, Hioki K and Ogiu T. Involvement of illegitimate V(D)J recombination or microhomology-mediated nonhomologous end-joining in the formation of intragenic deletions of the Notch1 gene in mouse thymic lymphomas. Cancer Res 2004; 64: 8882-90.
[38] Sukup-Jackson MR, Kiraly O, Kay JE, Na L, Rowland EA, Winther KE, Chow DN, Kimoto T, Matsuguchi T, Jonnalagadda VS, Maklakova VI, Singh VR, Wadduwage DN, Rajapakse J, So PT, Collier LS and Engelward BP. Rosa26-GFP direct repeat (RaDR-GFP) mice reveal tissue- and age-dependence of homologous recombination in mammals in vivo. PLoS Genet 2014; 10: e1004299.
[39] Wadduwage DN, Kay J, Singh VR, Kiraly O, Sukup-Jackson MR, Rajapakse J, Engelward BP and So PTC. Automated fluorescence intensity and gradient analysis enables detection of rare fluorescent mutant cells deep within the tissue of RaDR mice. Sci Rep 2018; 8: 12108.
[40] Liu C, Hirakawa H, Tanaka K, Mohd Saaya F, Nenoi M, Fujimori A and Wang B. Reduction of delayed homologous recombination by induction of radioadaptive response in RaDR-GFP mice (yonezawa effect): an old player with a new role. Dose Response 2019; 17: 1559325819833840.
[41] Liu C, Tanaka K, Katsube T, Varès G, Maruyama K, Ninomiya Y, Fardous Z, Sun C, Fujimori A, Moreno SG, Nenoi M and Wang B. Altered Response to total body irradiation of C57BL/6-Tg (CAG-EGFP) mice. Dose Response 2020; 18: 1559325820951332.
[42] Robert PA, Kunze-Schumacher H, Greiff V and Krueger A. Modeling the dynamics of T-cell development in the thymus. Entropy (Basel) 2021; 23: 437.
[43] Matos-Rodrigues G, Martini E and Lopez BS. Mouse models for deciphering the impact of homologous recombination on tumorigenesis. Cancers (Basel) 2021; 13: 2083.
[44] Kurd N and Robey EA. T-cell selection in the thymus: a spatial and temporal perspective. Immunol Rev 2016; 271: 114-26.
[45] Xing Y, Wang X, Jameson SC and Hogquist KA. Late stages of T cell maturation in the thymus involve NF-kappaB and tonic type I interferon signaling. Nat Immunol 2016; 17: 565-73.
[46] Saito Y, Ochiai Y, Kodama Y, Tamura Y, Togashi T, Kosugi-Okano H, Miyazawa T, Wakabayashi Y, Hatakeyama K, Wakana S, Niwa O and Kominami R. Genetic loci controlling susceptibility to gamma-ray-induced thymic lymphoma. Oncogene 2001; 20: 5243-7.

[47] Rivina L, Davoren MJ and Schiestl RH. Mouse models for radiation-induced cancers. Mutagenesis 2016; 31: 491-509.
[48] Park D, Bergin SM, Jones D, Ru P, Koivisto CS, Jeon YJ, Sizemore GM, Kladney RD, Hadjis A, Shakya R and Ludwig T. Ablation of the Brca1-Palb2 interaction phenocopies fanconi anemia in mice. Cancer Res 2020; 80: 4172-4184.
[49] Zúñiga-Pflücker JC, Jiang D, Schwartzberg PL and Lenardo MJ. Sublethal gamma-radiation induces differentiation of CD4-/CD8- into CD4+/CD8+ thymocytes without T cell receptor beta rearrangement in recombinase activation gene 2-/- mice. J Exp Med 1994; 180: 1517-21.
[50] Hodson DJ, Janas ML, Galloway A, Bell SE, Andrews S, Li CM, Pannell R, Siebel CW, MacDonald HR, De Keersmaecker K, Ferrando AA, Grutz G and Turner M. Deletion of the RNA-binding proteins ZFP36L1 and ZFP36L2 leads to perturbed thymic development and T lymphoblastic leukemia. Nat Immunol 2010; 11: 717-24.
[51] Vogel KU, Bell LS, Galloway A, Ahlfors H and Turner M. The RNA-binding proteins Zfp36l1 and Zfp36l2 enforce the thymic beta-selection checkpoint by limiting DNA damage response signaling and cell cycle progression. J Immunol 2016; 197: 2673-2685.