Chapter 18

Effects of Radioactive Cesium-Containing Water on Mice

The Effect of Low-Dose Internal Exposure of Multigeneration Chronic Oral Intake of ^{137}Cs in Mice Offspring

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Abstract To investigate the transgenerational effect of chronic low-dose-rate internal radiation exposure after the Fukushima Nuclear Power Plant accident in Japan, every generation of mice was maintained in a radioisotope facility with free access to drinking water containing $^{137}\text{CsCl}$ (100 Bq/ml). Descendent mice were assessed for $\gamma\text{-H2AX}$ foci in hepatocytes, the micronucleus test and chromosome aberration analysis in bone marrow cells, DNA mutations in the liver, tumorigenicity in the lung, oxidative stress in blood plasma, and metabolome analysis in the heart.

In this chapter, the author tries to introduce that animal experiments are useful to understand low-dose and low-dose-rate radiation effects.

Keywords Radioactive cesium · Drinking water · Mice · Transgenerational effects · Internal exposure · Low-dose radiation

18.1 Background

18.1.1 *Why Have Low-Dose Radiation Issues Not Been Resolved, Despite the Passage of More Than 90 Years?*

A study report by Muller in 1927 using *Drosophila* illustrated the concerns of the effect of low-dose (LD) radiation in humans on the next generation [1, 2]. Then, in the 1950s these concerns became more pressing with the expected increase in environmental radiation (global fallout) caused by nuclear tests at Bikini Atoll and the

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rise of pronuclear power advocates [3]. And it had been proposed to establish United Nations Scientific Committee on the effects of Atomic Radiation (UNSCEAR) at the UN General Assembly in 1955.

Recessive lethal mutations generated by genetic damage caused by radiation-induced DNA double-strand breaks create latent lethal actions in offspring. In particular, recessive lethal mutations on the X chromosome exert a more lethal effect in males, who have only one X chromosome, than in females, who have two X chromosomes. For this reason, the male population was expected to decrease, presenting an urgent problem for humankind, and there was heated discussion on the establishment of safe ranges for public exposure [4].

There is an increased incidence of cancer in the atomic bomb survivors of Hiroshima and Nagasaki (Hibakusha) [5–8]. Gene mutations are considered as the main cause of carcinogenesis [9]. These further increased the concern over radiation-induced mutagenesis [10–13] and carcinogenesis [14]. Fortunately, there was no reduction in the male population due the effect of radiation even among Hibakusha [15, 16], but concerns over the genetic and the carcinogenic effect of low-dose (LD) radiation exposure on the next generation has remained the central proposition of the discussion [17–23].

However, even in 2018, more than 90 years since these concerns were first raised, there are no firm conclusions on the safe range of LD radiation. The scientific opinion on the effect of LD radiation has been divided extremely as seen in the debate on the effect of LD radiation on the prevalence of childhood thyroid cancer attributed to the Fukushima Daiichi Nuclear Power Plant (FNPP) accident [24, 25]. Dispute of so-called scientists on this topic in front of the general public created blunders resulting in a massive loss of faith in scientists by the general public. Unfortunately, some people misunderstand the dose limit of radiation management guidelines as the dangerous border. Furthermore, the baseless testimony by some scientists adversely affects the consensus formation between the administration and the public, which could be the field of trans-science [26]. The job of scientists is not to decide whether LD radiation is safe or dangerous but to provide evidence-based quantitative data for risk assessment [27] and publicize the data to the public in a fair and easy-to-understand manner.

Why have these issues not been resolved, despite the passage of more than 70 years? However, most of the scientists have virtually focused their research on the use of radiation as a tool, but not mainly implemented on the presence or absence of a safety range of LD radiation. There are a number of reasons for this question such as:

1. Large-scale samples and long-term observations are required to obtain data on the effect of LD radiation.
2. Physical dose measurement is rather easy, but the evaluation of absorbed dose is difficult.
3. The impact of the results could be low in spite of the laborious experiments under limited conditions, which makes scientists have little incentive for this research.

4. Difficulty to quantify confounding factors and to evaluate exposure dose in epidemiological studies.
5. Difficult to verify negative data and, as a result, to acquire research funding constantly.

It is also envisioned that there are a large volume of buried data on the effect of LD radiation that have not been disclosed to the world among research, which is because positive data are publishable but negative ones are not even well-planned and precise as the research is (particularly with animal experiments).

To resolve these issues, it is essential to motivate scientists to acquire data as a legacy for future generations, develop methods able to quantitatively evaluate radiation with a high level of sensitivity even in areas of LD radiation, and accumulate basic data that will form evidence for risk evaluation.

18.1.2 Investigating the Effect of Multigenerational Low-Dose (LD) Internal Exposure on Offspring

Concern of LD radiation exposure as a social problem immediately after the Chernobyl nuclear power plant (CNPP) accident (1986) put emphasis on the carcinogenicity of the radiation-exposed generation and the genetic impact on the next generation. Previous research on the effect of radiation exposure have been reported through the life span study (LSS) and adult health study (AHS) of Hibakusha, industrial radiation exposure [28–33], medical radiation exposure [34–36], high background exposure [37–39] and various animal experiments [40–43]. Recently studies have attempted to establish highly sensitive quantitative detection of biological reactants after exposure to LD radiation [44–46]. However, almost all of these studies focused on carcinogenesis and mutation of the first or the second generation only. It can be said that there has been no research on analyzing the effect of LD and LD-rate (LDR) internal exposure on descendants of multiple generations. Minor variations of each generation need to be accumulated over consecutive generations until the effect of radiation becomes evident, which would take more than several hundred years in humans. Therefore, the author attempted to evaluate the transgenerational effect of LD radiation within a short timeframe using inbred mice with more rapid intergenerational changes than humans. The author believes that a variety of compelling results on biological effects of LD radiation have been obtained from animal experiments in the author's laboratory. In this chapter, the author would like to introduce those results in the hope of helping readers consider the effect of persistent internal LD radiation.

18.2 Experimental Methods and Results

18.2.1 Effects of Radioactive Cesium-Containing Water on Mice

Two littermates were selected from a litter of A/J mouse strain. One group was reared with free drinking of radioactive cesium chloride ($^{137}\text{CsCl}$) containing water (100 Bq/ml) as the LD internal exposure group (^{137}Cs group), and the other group was reared with ^{137}Cs free intake of water as control without radiation. The offspring of these mice produced more than 15 generational changes through sibling mating (equates to approximately 300 years of generational changes in humans), and the following 1–8 experiments were carried out by selected mice from the ^{137}Cs and the control groups with the same ancestor (origin). Since manuscripts of experiments 3 to 8 are in preparation, detailed data of these experiments are not shown.

1. Organ distribution of ^{137}Cs in Mice

Figure 18.1 shows the distribution of ^{137}Cs in the organs of small wild animals collected in a moderately contaminated region of Belarus (Babchin Village) 11 years after the CNPP accident [47, 48].

Figure 18.2 shows the difference of ^{137}Cs concentration in organs of A/J mice that continuously drank $^{137}\text{CsCl}$ water (100 Bq/ml) as drinking water for 8 months in the laboratory [49]. As shown in Figs. 18.1 and 18.2, there was a large accumulation of ^{137}Cs in muscles. Also, despite drinking a constant daily amount of ^{137}Cs , the amount of ^{137}Cs in the organs was constant, reaching a plateau corresponding to the consumption amount and flattening out thereafter.

Fig. 18.1 Distribution of ^{137}Cs in the various organs of wild animals living in the middle-level contaminated areas (Babchin Village) of Belarus in 1997

Fig. 18.2 (a) The accumulation of ^{137}Cs over time in each organ in male mice that had ingested water supplemented with $^{137}\text{CsCl}$ (100 Bq/ml). The estimated intake was 16 Bq/g body weight/day and 440 Bq/mouse/day. (b) Body distribution of ^{137}Cs after 8 months of ad libitum ^{137}Cs water (10 Bq/ml or 100 Bq/ml) (error bar represents 95% CI)

Figure 18.3 shows the overview of the experiment on the transgenerational effect by internal exposure to ^{137}Cs water. Figure 18.4 shows the chronic internal exposure and ^{137}Cs concentration in the liver at all stages of mouse development in this experiment.

Figure 18.5 presents the 18th-generation (8 months old) mice and shows that the amount of ^{137}Cs in all the organs rapidly attenuated after ^{137}Cs was ceased from drinking. ^{137}Cs concentration in muscles declined more slowly than that in other organs [49].

2. Effect on Intergenerational Litter Size and Sex Ratio

Mean sex ratio (Fig. 18.6a) and mean litter size (Fig. 18.6b) were compared between the ^{137}Cs group and the control group over generations 1–18 [49]. If the amount of intake was converted to drinking water in humans, 100 Bq/ml ^{137}Cs water for mice would equate to 100,000 Bq/L for humans. There was no effect on the mouse sex ratio and litter size even after 18 continuous generations of mice continued to drink 100 Bq/ml ^{137}Cs water [49]. In this experiment, it will be the proof that there was no decrease in the male population due to radiation effects of the fallout of nuclear tests.

Fig. 18.3 The overview of experiments on transgenerational effects by internal exposure of ¹³⁷Cs

Fig. 18.4 Chronic internal exposure and ¹³⁷Cs concentration at all stages of mouse development. The graph shows the change of internal ¹³⁷Cs concentration in the liver of mouse from fetus to 15 months age

Fig. 18.5 The decrease of the ^{137}Cs level in 8-month-old female mice in the 18th generation after the mice had started drinking nonradioactive water

Fig. 18.6 (a) The mean sex ratio of all generations (F_1 – F_{18}), (b) the mean litter size of all generations (F_1 – F_{18}) (error bar represents 95% CI)

3. *Induction of DNA Double-Strand Breaks in Hepatocytes*

Measurement of the number of γ -H2AX foci, which is the indicator of the first process for repair of DNA double-strand breaks generated in cells [50–54], was performed with hepatocytes. There were significantly more DNA double-strand breaks per generation (100 days) with approximately 50 mGy (2313 Bq per mouse, mean 93.54 Bq/g body weight, 59.5 Bq per liver) of ^{137}Cs internal exposure compared to the control group.

4. *Chromosomal Abnormalities and the Micronucleus Test in Bone Marrow Cells*

Chromosomal abnormalities common to all cells were detected using the multicolor Fluorescence In Situ Hybridization (FISH) technique, and the micronucleus test was conducted in the tenth-generation mice. No common chromosomal abnormalities were found in any of bone marrow cells in the ^{137}Cs group. There was increased incidence of DNA double-strand breaks, as described in 3 (above), but common chromosomal abnormalities carried over to the next generation were not found after the tenth generation of mice. Similarly, the micronucleus test revealed no significant increase in the ^{137}Cs group.

5. *Effect of Low-Dose (LD) Radiation on Lung Carcinogenesis*

The incidence of lung tumors and the mean tumor mass in 10-month-old mice were assessed. There was no difference in the incidence of spontaneous onset and urethane-induced lung tumors between the ^{137}Cs group and the control group. Interestingly, the growth rate of tumors was significantly inhibited in the ^{137}Cs group.

6. *Oxidative Stress in Blood Plasma*

The balance of oxidative stress and antioxidant capacity in the body was studied. In the ^{137}Cs group, there was a significant elevation of plasma 8-oxodihydroguanine, which is an indicator of oxidative stress, but there was no significant difference from the control group in terms of antioxidant capacity. The generated oxidative stress was considered to be within an allowable biological range.

7. *Metabolome Analysis in the Heart*

The glycolytic pathway in the ^{137}Cs group was inhibited, and there was also a reduction in antioxidants such as glutathione (GSH) and cysteine.

8. *Quantitative Analysis of DNA Base Mutations by Whole Genome Sequencing in the Liver*

Detection of base sequence mutations in noncoding regions, where base mutations generated in germ cells do not affect life support but are expected to accumulate in each subsequent generation, was attempted in male mice of F_{20} and F_{23} origin. There was a higher incidence of single-nucleotide variant (SNV) and insertion/deletion (Indel) per 3 billion bases at intron and intergenic sites than at exon sites by comparing whole genome sequence in each generation. However, even with repeated generations, there was not a large difference in the total base mutation rate between the ^{137}Cs group and the control group.

18.2.2 Detecting Carcinogenesis and Mutations Caused by Oxidative Stress

The fatal effect of radiation is the DNA double-strand break. In the high-dose range of high linear energy transfer (LET) radiation, dominant process of the breaks is caused by direct action, while in low LET radiation such as X-rays, about two-thirds of the biologic damages are caused by indirect action through radicals generated by reaction with water in the cell [55]. In case of LD and LDR radiation, the action exerting the greatest effect is assumed to be oxidative stress caused by reactive oxygen species attributed to radiation exposure. Reports indicate that using genetically modified mice (DNA mismatch repair-deficient mice) that are unable to repair the DNA damage enables highly sensitive detection of DNA damage caused by oxidative stress agents and radiation [56, 57]. Also, these mice show a high incidence of small intestine tumors through oral consumption of an oxidative stress agent potassium bromide (KBr) aqueous solution [58, 59]. The author has started quantitative research on carcinogenesis and mutations caused by oxidative stress using this mouse strain.

18.2.3 Comparison of Internal Exposure and External Exposure

The effect of exposure in humans is based on the absorbed radiation dose in Gy (J/kg). However, this unit is obtained from the energy absorbed per kg of tissue. Internal exposure experiments using mice are different from external exposure ones, so the absorbed radiation dose from the radioactive substance must be considered in terms of the amount accumulated in a body that weighs less than 1 kg (around 0.025 kg). However, most of the γ -rays from a mouse body measuring approximately 3 cm in diameter completely pass out of the body without transferring the energy. In these instances, is it a good idea to evaluate with Gy, which is an absorption dose to the order of kg? This same question can be applied to humans. It is vital to confirm if the effect of internal exposure can be evaluated with a dose conversion factor from Bq to Gy in same way in infants and adults where there are differences in the distribution and size of organs. The dose conversion factor from Bq to Gy for rats is shown by ICRP (pub108), but no information for mice. If the actual internal exposure dose is not accurately evaluated in the mouse experiment, it significantly affects the quality of outcome of the experiment. To determine the dose conversion factor of the mice in these experimental conditions, it can be considered three evaluation methods. Previous evaluation methods for the internal exposure dose include the dose estimated from physical calculations (3.14 $\mu\text{Gy.g/Bq.day}$) and evaluation methods using Monte Carlo analysis such as the EGS5 code system (Electron Gamma Shower Version 5) (3.00 $\mu\text{Gy.g/Bq.day}$, evaluated by Endo D, Rakuno Gakuen Univ.) and the PHITS (Particle and Heavy Ion Transport Code System)

Fig. 18.7 The strategy gap in vivo and in vitro

(3.40 $\mu\text{Gy.g/Bq.day}$: evaluated by Endo S, Hiroshima Univ.). We are continuing research to establish methods for evaluating internal exposure dose with an attempt to adopt new techniques such as quantifying noncoding RNA (ncRNA) specific to the reaction against radiation exposure [45]. If it is possible to detect a dose-dependent reaction with highly sensitive and precise quantification of the biological reactions caused by radiation, then it would be a great help to verify equivalent dose of internal and external exposure.

There are strategy gap between in vivo and in vitro experiments (Fig.18.7). “In vivo” is like a black box, and “in vitro” is like a complex electronic circuit. We want to know what is going on inside the black box, and we want to know the pathway of circuit from the beginning to the end. Particularly in studies of LD radiation effects, bridging the gap between in vivo and in vitro experiments can effectively lead to clear results.

Table 18.1 Homology between mouse and human. Spontaneous mutation rate per generation, base substitution rate per nucleotide per generation and the number of genes in mice are almost the same as humans. This is the reason that the mice can be used as the experimental animal to know hereditary influence in humans. ^aAverage of base substitution rate in references of human [58–68]

	Mutation rate per generation	Base substitution rate per nucleotide per generation	Generation interval (months)	Number of genes	Homology with the human DNA sequence
Human	3.6×10^{-6a}	1.2×10^{-8b}	300	30,000	
Mouse	6.1×10^{-6a}	0.54×10^{-8c}	3	30,000	97%
<i>Drosophila</i> (fruit flies)	1.8×10^{-6a}		0.3	14,000	60%

^a[58]

^b[59–68]

^c[69]

18.3 Conclusion

The experiments with *Drosophila* made people aware of the effect of radiation on human genetics. The mouse is a closer experimental animal to humans. However, the data obtained from inbred mouse strains are the same as those obtained from monozygotic twins, as all the mice have the same genetic background, so the obtained data reflect genetic bias of one person, irrespective of the number of mice used. Therefore, inbred mice may not be suitable for regulatory science research for the general public, where a variety of exposure disorders are envisioned. However, mice have almost the same spontaneous mutation rate per generation, base substitution rate per nucleotide per generation, and number of genes as humans (Table 18.1) [60–71]. Therefore, the inbred mouse experiment is effective for removing confounding factors and for detecting fundamental biological effects, which would be inconceivable in humans.

Obtaining data that contribute to the establishment of the radiation safety range has not necessarily been vigorously implemented for more than 70 years since the nuclear test at the Bikini Atoll. Those data will also be extremely effective to determine if an unpredictable radiation exposure occurs. We hope that many scholars will undertake research on the effect of LD radiation throughout the world.

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References

1. Muller HJ (1927) Artificial transmutation of the gene. *Science* 66:84–87
2. Muller HJ (1928) The measurement of gene mutation rate in *Drosophila*, its high variability, and its dependence upon temperature. *Genetics* 13:279
3. Federation of American Scientist, “Proposal for a United Nations Commission to study the problem of H-bomb test,” February 16, 1955, in folder: “Studies on effect of 1955,” Box 232, Records relating to atomic energy matters, 1948–1962, special assistant for the secretary of state for atomic energy and outer space, Records of the department of state, National Archives at College Park, USA
4. Schull WJ, Neel JV (1958) Radiation and the sex ratio in man. *Science* 128(3320):343–348
5. Oho G (1956) New statistical observation of malignant neoplastic death in A-bomb survivors. *Jpn Med J* 1686:8–18. in Japanese
6. Pierce DA, Shimizu Y, Preston DL et al (1996) Studies of the mortality of atomic bomb survivors. Report 12, Part I. Cancer: 1950–1990. *Radiat Res* 146(1):1–27
7. Preston DL, Ron E, Tokuoka S et al (2007) Solid cancer incidence in atomic bomb survivors: 1958–1998. *Radiat Res* 168(1):1–64
8. Richardson D, Sugiyama H, Nishi N et al (2009) Ionizing radiation and leukemia mortality among Japanese atomic bomb survivors, 1950–2000. *Radiat Res* 172(3):368–382
9. Deman J, Van Larebeke N (2001) Carcinogenesis: mutations and mutagens. *Tumour Biol* 22(3):191–202
10. United Nations Scientific Committee on the effects of atomic radiation. Radiation-induced chromosome aberrations in human cells. UNSCEAR (1969) Reports 1969. United Nations, New York, pp 98–155
11. Sasaki MS, Miyata H (1968) Biological dosimetry in atomic bomb survivors. *Nature* 220:1189–1193
12. Russel WL (1955) Genetic effects of radiation in mice and their bearing on the estimation of human hazards. *Proc Int Conf Peacef Uses Atom Energy* 11:382–383. United Nation, 1956
13. Sankaranarayanan K (1982) Genetic effects of ionizing radiation in multicellular eukaryotes and the assessment of genetic radiation hazards in man. Elsevier, Amsterdam
14. Little JB (2000) Radiation carcinogenesis. *Carcinogenesis* 21(3):397–404
15. Schull WJ, Neel JV, Hashizume A (1966) Some further observations on the sex ratio among infants born to survivors of the atomic bombings of Hiroshima and Nagasaki. *Am J Hum Genet* 18(4):328–338
16. Neel JV, Schull WJ (1991) Children of atomic bomb survivors. A genetic study. National Academy Press, Washington, DC
17. Yoshimoto Y, Neel JV, Schull WJ et al (1990) The frequency of malignant tumors during the first two decades of life in the offspring of atomic bomb survivors, RERF technical report, pp 4–90 (*Am J Hum Genet*, 1990; 46:1041–1052)
18. Furitsu K, Ryo H, Yeliseeva KG et al (2005) Microsatellite mutations show no increases in the children of the Chernobyl liquidators. *Mutat Res* 581:69–82
19. Fujiwara S, Suyama A, Cologne JB et al (2008) Prevalence of adult-onset multifactorial disease among offspring of atomic bomb survivors. *Radiat Res* 170:451–457
20. Kodaira M, Roy H, Kamata N et al (2010) No evidence of increased mutation rates at microsatellite loci in offspring of A-bomb survivors. *Radiat Res* 173:205–213
21. Tatsukawa Y, Cologen JB, Hsu WL et al (2013) Radiation risk of individual multifactorial diseases in offspring of the atomic-bomb survivors: a clinical health study. *J Radiol Prot* 33:281–293
22. Izumi S, Koyama K, Soda M et al (2003) Cancer incidence in children and young adults did not increase relative to parental exposure to atomic bomb. *Br J Cancer* 89:1709–1713
23. Sankaranarayanan K, Chakraborty R (2000) Ionizing radiation and genetic risks XI. The doubling dose estimates from the mid-1950s to the present and the conceptual change to the use

- of human data on spontaneous mutation rates and mouse data on induced mutation rates for doubling dose calculations. *Mutat Res* 453:107–127
24. Tsuda T, Tokinobu A, Yamamoto E et al (2016) Thyroid cancer detection by ultrasound among residents ages 18 years and younger in Fukushima, Japan: 2011 to 2014. *Epidemiology* 27:316–322
 25. Ohira T, Takahashi H, Yasumura S et al (2016) Comparison of childhood thyroid cancer prevalence among 3 areas based on external radiation dose after the Fukushima Daiichi nuclear power plant accident. The Fukushima health management survey. *Medicine* 95(35):e4472. <https://doi.org/10.1097/MD.0000000000004472>
 26. Weinberg AM (1972) Science and trans-science. *Minerva* 10(2):209–222
 27. Sankaranarayanan K, Nikjoo H (2015) Genome-based, mechanism-driven computational modeling of risks of ionizing radiation: the next frontier in genetic risk estimation? *Mutat Res* 764:1–15
 28. Glenn JA Jr, Galindo J, Lawrence CE (1960) Chronic radium poisoning in a dial painter. Case report. *Am J Roentgenol Radium Therapy, Nucl Med* 83:465–473
 29. Woodard HQ, Higinbotham NL (1962) Development of osteogenic sarcoma in a radium dial painter thirty-seven years after the end of exposure. *Am J Med* 32:96–102
 30. Gardner MJ, Snee MP, Hall AJ et al (1990) Results of case-control study of leukaemia and lymphoma among young people near Sellafield nuclear plant in West Cumbria. *BMJ* 300:423–429
 31. Workfold R (2002) Cancer in offspring after paternal preconceptional irradiation. *J Radiol Prot* 22:191–194
 32. Johnson KJ, Alexander BH, Doody MM et al (2008) Childhood cancer in the offspring born in 1921–1984 to US radiologic technologists. *Br J Cancer* 99(3):545–550
 33. Leuraud K, Richardson DB, Cardis E et al (2015) Ionising radiation and risk of death from leukaemia and lymphoma in radiation-monitored workers (INWORKS): an international cohort study. *Lancet Haematol* 2(7):e276–e281
 34. Robison LL, Armstrong GT, Boice JD et al (2009) The childhood cancer survivor study: a National Cancer Institute-supported resource for outcome and intervention research. *J Clin Oncol* 27(14):2308–2318
 35. Madanat-Harjuoja LM, Malila N, Lähteenmäki PM et al (2010) Preterm delivery among female survivors of childhood, adolescent and young adulthood cancer. *Int J Cancer* 127(7):1669–1679
 36. Pearce MS, Salotti JA, Little MP et al (2012) Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a retrospective cohort study. *Lancet* 380(9840):499–505
 37. Jiang T, Hayata I, Wang C et al (2000) Dose-effect relationship of dicentric and ring chromosomes in lymphocytes of individuals living in the high background radiation areas in China. *J Radiat Res* 41(Suppl):63–68
 38. Tao Z, Zha Y, Akiba S et al (2000) Cancer mortality in the high background radiation areas of Yangjiang, China during the period between 1979 and 1995. *J Radiat Res* 41(Suppl):31–41
 39. Zhang W, Wang C, Chen D et al (2003) Imperceptible effect of radiation based on stable type chromosome aberrations accumulated in the lymphocytes of residents in the high background radiation area in China. *J Radiat Res* 44(1):69–74
 40. Ishii K, Hosoi Y, Yamada S et al (1996) Decreased incidence of thymic lymphoma in AKR mice as a result of chronic, fractionate low dose total-body X irradiation. *Radiat Res* 146:582–585
 41. Courtade M, Billote C, Gasset G et al (2002) Life span, cancer and non-cancer disease in mouse exposed to a continuous very low dose of γ -irradiation. *Int J Radiat Biol* 78:845–855
 42. Tanaka S, Tanaka IB, Sasagawa S et al (2003) No lengthening of life span in mice continuously exposed to gamma rays at very low dose rates. *Radiat Res* 160(3):376–379
 43. Tang FR, Loke WK, Khoo BC (2017) Low-dose or low-dose-rate ionizing radiation–induced bioeffects in animal models. *J Radiat Res* 58(2):165–182
 44. Nakajima H, Ohno M, Ishihara H (2018) Quantitative assessment for the effects of chronic low-dose internal Cesium-137 radiation exposure on genomic, carcinogenic and hereditary effects

- in mice. Report for Study (Group) of the Health Effects of Radiation Organized by Ministry of the Environment, Japan, p 143. https://www.env.go.jp/chemi/rhm/reports/h2903e_3.pdf
45. Ishihara H, Tanaka I, Yakumaru H et al (2016) Quantification of damage due to low-dose radiation exposure in mice: construction and application of a biodosimetric model using mRNA indicators in circulating white blood cells. *J Radiat Res* 57(1):25–34
 46. Manens L, Grison S, Bertho JM et al (2016) Chronic exposure of adult, postnatal and in utero rat models to low-dose ¹³⁷Cesium: impact on circulating biomarkers. *J Radiat Res* 57(6):607–619
 47. Nakajima H, Ryo H, Yamaguchi Y et al (2000) Biological concentration of radionuclides in plants and animals after the Chernobyl catastrophe. In: Sato F, Yamada Y, Onodera J (eds) Biological effects of low dose radiation. Institute for Environmental Sciences, Aomori, pp 199–205
 48. Nakajima H, Saito T, Ryo H et al (2008) Ecological decrease and biological concentration of radionuclides in plants and animals after the Chernobyl catastrophe. In: Miura T, Kinoshita N (eds) Proceedings of the eighth workshop on environmental radioactivity. High Energy Accelerator Research Organization (KEK), Proceedings 2007–2016, Tsukuba, pp 113–118
 49. Nakajima H, Yamaguchi Y, Yoshimura Y et al (2015) Fukushima simulation experiment: assessing the effects of chronic low-dose internal ¹³⁷Cs radiation exposure on litter size, sex ratio, and biokinetics in mice. *J Radiat Res* 56:i29–i35. Special Issue-Fukushima
 50. Rogakou EP, Pilch DR, Orr A et al (1998) DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem* 273:5858–5868
 51. Rogakou EP, Boon C, Redon C et al (1999) Megabase chromatin domains involved in DNA double-strand breaks *in vitro*. *J Cell Biol* 146:905–915
 52. Nakajima H, Tsuboi R, Nomura T (2002) Biodosimetry by detecting H2AX foci in peripheral WBC and SCID lymphoma cell line after ¹³⁷Cs gamma-rays irradiation (abstract). *J Radiat Res* 43:445
 53. Rothkamm K, Löbrich M (2003) Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. *Proc Natl Acad Sci U S A* 100:5057–5062
 54. Nakajima H, Tsuboi R, Nomura T (2003) Biodosimetry by detecting H2AX foci in human peripheral lymphocytes and mouse organ tissues after ¹³⁷Cs γ -ray irradiation (abstract). *J Radiat Res* 44:406
 55. Hall EJ, Giaccia AJ (2019) Radiobiology for the radiologist, 8th edn. Wolters Kluwer, USA, Philadelphia, p p10. (9781496335418)
 56. Egashira A, Yamauchi K, Yoshiyama K et al (2002) Mutational specificity of mice defective in the MTH1 and/or the MSH2 genes. *DNA Repair* 1(11):881–893
 57. Ohno M, Sakumi K, Fukumura R et al (2014) 8-oxoguanine causes spontaneous de novo germline mutations in mice. *Sci Rep* 4:4689
 58. Tsuzuki T, Piao J, Isoda T et al (2011) Oxidative stress-induced tumorigenesis in the small intestine of various types of DNA repair-deficient mice. *Health Phys* 100(3):293–294
 59. Piao J, Nakatsu Y, Ohno M et al (2014) Mismatch repair deficient mice show susceptibility to oxidative stress-induced intestinal carcinogenesis. *Int J Biol Sci* 10(1):73–79
 60. Drost JB, Lee WR (1995) Biological basis of germline mutation: comparisons of spontaneous germline mutation rates among drosophila, mouse, and human. *Environ Mol Mutagen* 25(Suppl 26):48–64
 61. Roach JC, Glusman G, Smit AFA et al (2010) Analysis of genetic inheritance in a family quartet by whole-genome sequencing. *Science* 328:636–639
 62. Conrad DF, Keebler JE, DePristo MA et al (2011) Variation in genome-wide mutation rates within and between human families. *Nat Genet* 43:712–714
 63. Campbell CD, Chong JX, Malig M et al (2012) Estimating the human mutation rate using autozygosity in a founder population. *Nat Genet* 44:1277–1281
 64. Kong A, Frigge ML, Masson G et al (2012) Rate of de novo mutations and the importance of father's age to disease risk. *Nature* 488:471–475
 65. Michaelson JJ, Shi Y, Gujral M et al (2012) Whole-genome sequencing in autism identifies hot spots for de novo germline mutation. *Cell* 151:1431–1442

66. Segurel L, Wyman MJ, Przeworski M (2014) Determinants of mutation rate variation in the human germline. *Annu Rev Genomics Hum Genet* 15:47–70
67. Besenbacher S, Liu S, Izarzugaza JM et al (2015) Novel variation and de novo mutation rates in population-wide de novo assembled Danish trios. *Nat Commun* 6:5969
68. Rahbari R, Wuster A, Lindsay SJ et al (2016) Timing, rates and spectra of human germline mutation. *Nat Genet* 48:126–133
69. Wong WS, Solomon BD, Bodian DL et al (2016) New observations on maternal age effect on germline de novo mutations. *Nat Commun* 7:10486
70. Jónsson H, Sulem P, Kehr B et al (2017) Parental influence on human germline de novo mutations in 1,548 trios from Iceland. *Nature* 549:519
71. Uchimura A, Higuchi M, Minakuchi Y et al (2015) Germline mutation rates and the long-term phenotypic effects of mutation accumulation in wild-type laboratory mice and mutator mice. *Genome Res* 25:1125–1134

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