

The Lowest Radiation Dose Having Molecular Changes in the Living Body

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Abstract

We herein attempted to identify the lowest radiation dose causing molecular changes in the living body. We investigated the effects of radiation in human cells, animals, and humans. DNA double-strand breaks (DSBs) formed in cells at γ - or X-ray irradiation doses between 1 mGy and 0.5 Gy; however, the extent of DSB formation differed depending on the cell species. The formation of micronuclei (MNs) and nucleoplasmic bridges (NPBs) was noted at radiation doses between 0.1 and 0.2 Gy. Stress-responsive genes were upregulated by lower radiation doses than those that induced DNA DSBs or MN and NPBs. These γ - or X-ray radiation doses ranged between approximately 10 and 50 mGy. In animals, chromosomal aberrations were detected between 50 mGy and 0.1 Gy of low linear energy transfer radiation, 0.1 Gy of metal ion beams, and 9 mGy of fast neutrons. In humans, DNA damage has been observed in children who underwent computed tomography scans with an estimated blood radiation dose as low as 0.15 mGy shortly after examination. The frequencies of chromosomal translocations were lower in residents of high background areas than in those of control areas. In humans, systemic adaptive responses may have been prominently expressed at these radiation doses.

Keywords

lowest radiation dose, molecular changes, DNA damage, chromosomal aberrations, genomic instability

Introduction

Many researchers have reported the harmful effects of high-dose radiation exposure. Although it is considered important to clarify the dose limit at which the effects of radiation on health become undetectable for its regulation, few studies have investigated the harmful effects of low-dose radiation exposure. Since the human population is not typically exposed to high doses of radiation, the effects of very low doses such as environmental radiation on the living body need to be examined in more detail.

The International Commission on Radiological Protection recommended that the linear no-threshold (LNT) hypothesis be applied to doses lower than 200 mSv, which has been defined as a low radiation level by the United Nations Scientific Committee on the Effects of Atomic Radiation.¹ Regarding cancer development, they stated that even very low doses of radiation need to be considered as being harmful in order to achieve radiation protection. However, the effects of ultra-low doses on the living body were investigated and the findings obtained did not fit an LNT model. Furthermore, systemic adaptive responses were observed in animals exposed to low-dose irradiation at the same level that causes molecular changes in cells. Although individual cells are injured, adaptive responses may systemically appear.

Ionizing radiation interacts with atoms and molecules in cells and causes damage such as DNA damage, which increases the risk of cancer. Single-strand breaks (SSBs), double-strand breaks (DSBs),²⁻⁷ DNA base alterations, and DNA–DNA or DNA–protein cross-links are induced by radiation.⁸⁻¹² These molecular changes cause genomic instability, which is generally detected by examining DSBs, chromosomal aberrations, the frequencies of micronuclei (MNs) and nucleoplasmic bridges (NPBs), and/or the upregulation of stress-responsive genes. The abovementioned indices have been extensively examined in cells irradiated at higher doses; however, the

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Table 1. Effects of Low-Dose Irradiation on Human Cells.

| Indices of Genome Instability | Cells | Irradiation Protocol | The lowest Radiation Dose Studied That Cause some Effect | Reference |
|---|--|--|---|-----------|
| Foci of γ -H2AX | MRC-5 | 1.2, 5, 20, and 200 mGy X-ray single exposure | 1.2 mGy | 17 |
| | AG01522 | 0.5 Gy of γ -rays, protons, carbon ions, and α particles | 0.5 Gy | 18 |
| | HFL III | Carbon ions and γ -rays at 1 mGy once | 1 mGy of carbon ions | 23 |
| MN and NPBs | GMI5036, GMI5510, GMI5268, and GMI5526 | 50 mGy, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, or 4 Gy of ^{60}Co γ -rays | 0.2 Gy | 30 |
| MN, NBUDs nucleocytoplasmic bridges, polynucleate cells, and chromosomal aberrations | Peripheral blood lymphocyte | 0.1, 0.25, 0.5, 1, and 2 Gy of X-rays | 0.1 Gy | 31 |
| Chromosome aberrations | HMEC | 1, 2, 4, or 6 Gy of X-rays. 0.5, 1, 1.5, 2, or 3 Gy of iron ion beams | 0.5 Gy of 1 GeV/nucleon iron ions | 36 |
| Foci of γ -H2AX, stress-responsive genes or protein | HUVECs | 0.125, 0.25, and 0.5 Gy single dose of X-rays or fractionated dose of 2×0.125 Gy and 2×0.25 Gy | 0.5 Gy for DNA DSBs, 0.125 Gy for surface protein levels, and 2×0.125 Gy for ICAM-1 mRNA | 38 |
| Cyclin D1 expression | Human ATM- and NBS1-deficient cell lines | 10 mGy or 0.5 Gy of X-ray per fraction and total doses delivered over 31 days were 0.46 or 2.3 Gy, respectively | 10 mGy per fraction, total radiation dose was 0.46 Gy | 39 |
| Pattern of gene expression (modulated genes) | CD ⁺ T lymphocytes | Ex vivo irradiation of ^{60}Co γ -rays. Total exposure doses were 5, 10, 25, 50 mGy, 0.1, and 0.5 Gy | 5 mGy | 50 |
| Stress-responsive genes | ML-1 | 20 mGy to 0.5 Gy of γ -rays | 20 mGy | 51 |
| | Keratinocytes | 10 mGy or 2 Gy of γ -rays | 10 mGy | 54 |
| | Normal human fibroblasts | 20 mGy and 4 Gy of X-rays | 20 mGy | 55 |
| | Lymphocytes | 50 mGy and 0.5 Gy of ^{60}Co γ -rays | 50 mGy | 56 |
| | Lymphocytes | Irradiation doses were 20 mGy, 0.1, 0.5, 1, 2, and 4 Gy of ex vivo irradiation of ^{137}Cs γ -rays | 20 mGy | 57 |
| Raman spectrometry | Lymphocytes from peripheral blood | Irradiation doses were 50 mGy and 0.5 Gy ex vivo irradiation of ^{60}Co γ -rays | 50 mGy | 58 |
| Stress-responsive genes | hESCs | Irradiation with 0.4, 2, or 4 Gy of γ -radiation using ^{137}Cs irradiator | 0.4 Gy | 59 |

Abbreviations: HFL III, human lung fibroblast III; MN, micronucleus; NPBs, nucleoplasmic bridges; NBUDs, nuclear buds; HMEC, human mammary epithelial cell; HUVECs, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule-1; ATM, ataxia telangiectasia, mutated; hESCs, human embryonic stem cells.

effects of a lower irradiation dose currently remain unclear. Therefore, the lowest dose of radiation that induces these phenomena has not yet been established. Even ultra-low doses of irradiation may induce these changes in cells. We intend to clarify the dose limit of radiation that induces changes in cells, which may be harmful and/or beneficial for the whole body.

We herein reviewed previous studies that focused on the relationship between low-level irradiation and genomic instability in experimental cell lines, animals, and humans living in a high-level natural radiation area (HLNRA). Our purpose is to estimate the lowest dose that induces detrimental changes in these groups, even those that are not entirely dependent on the direct effects of radiation.

Effects of Low-Dose Ionizing Radiation on Cells

DNA DSBs by Low-Dose Irradiation

DNA DSBs are the main cytotoxic lesions caused by ionizing radiation and are potent inducers of mutations and cell death.¹³ The DSBs are induced linearly with radiation doses^{14,15} and are always followed by the phosphorylation of the histone, H2AX, which is termed γ -H2AX. γ -H2AX has been widely used as a tool to measure the induction of DNA damage because there is always a constant number or percentage of γ -H2AX per DSB (Table 1).¹⁶

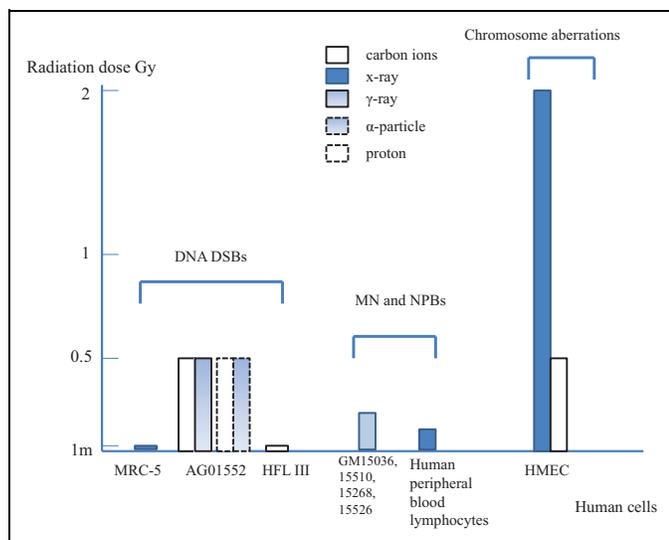


Figure 1. The estimated smallest dose causing DNA DSBs, MN, NPBs, and chromosomal aberrations reported by several researchers. The doses are the smallest that were tried by each report. The cells examined were MRC-5,¹⁷ AG01552,¹⁸ HFL III,²³ GMI5036, GMI5510, GMI5268, GMI5526,³⁰ peripheral blood lymphocytes,³¹ and HMEC.³⁶ DSBs indicates double-strand breaks; MN, micronucleus; NPBs, nucleoplasmic bridges; HMEC, human mammary epithelial cell.

DNA damage responses induced by acute single radiation at a high dose have been extensively examined; however, the effects of acute or long-term exposure to radiation at low doses have also recently been investigated by several research groups.

Rothkamm and Löbrich examined DSBs in cultures of the nondividing primary human fibroblast cell line, MRC-5, in the G1 phase of the cell cycle after a very low dose of X-ray irradiation.¹⁷ They detected γ -H2AX foci using immunofluorescence in order to establish the lowest irradiation dose and background level of damage. Measurements of the background level of DSBs revealed that confluent MRC-5 cells had approximately 0.05 DSBs per cell. This group also assessed γ -H2AX foci in MRC-5 cells at an irradiation dose range between 1.2 mGy and 2 Gy and found a linear relationship between the number of foci induced per cell 3 minutes after irradiation and irradiation doses. They also showed that the number of foci did not change with a repair time up to 24 hours. The initial number of foci linearly depended on the dose but not after a 24-hour repair incubation. An examination of remaining foci 24 hours after irradiation at doses of 1.2, 5, 20, and 200 mGy revealed the same level of approximately 0.1 foci per cell, which was significantly different from the background level. Therefore, they indicated that DSBs after X-ray exposure at 1.2 mGy remained unrepaired. From their findings, although the tested doses were not the lowest, a single exposure to X-rays at a dose of 1.2 mGy may be one of the lowest doses that induces persistent foci per cell and therefore exhibit some molecular changes in MRC-5 cells (Figure 1).

The assumption of higher radiation linear energy transfer (LET) causing more complex DNA damage has generally been accepted. Antonelli et al examined the kinetics of radiation-induced γ -H2AX foci after exposure to γ -rays, protons, carbon ions, and α particles using primary human foreskin fibroblasts (the AG01522 cell line).¹⁸ The number of foci after exposure to 0.5 Gy of each radiation type differed. γ -Radiation caused the largest number of foci among the types of radiation tested. The maximum focus numbers following γ -ray, proton, carbon ion, and α particle radiation were seen 30 minutes after irradiation, and the values were 12.64 (0.25), 10.11 (0.40), 8.84 (0.56), and 4.80 (0.35), respectively. The numbers gradually decreased, and at 24 hours postirradiation, the α particle induced focus about 2DSBs; however, the other had less than 1. Control cells also showed γ -H2AX foci and the average number of foci per cell was 0.48 (0.04), which was markedly lower than that of irradiated cells. These differences in the number of foci may be due to the indirect effects of low-LET radiation, such as γ -ray radiation. The indirect effects of low-LET radiation under aerobic conditions have been reported to account for 50% to 85% of radiation damage in cells.¹⁹⁻²¹ However, high-LET radiation induced unique DNA damage by direct effects, and this damage was less likely to be properly repaired²²; therefore, the persistence of γ -H2AX foci was also dependent on LET. The higher the LET, the longer foci persisted in the cell nucleus. High-LET radiation, such as α particles, induced γ -H2AX foci of different sizes and morphologies from γ -ray-induced foci. Although there are differences among the radiation types and effects of reactive oxygen species produced by free radicals, 0.5 Gy was one of the indices of low-dose radiation. Figure 1 shows the lowest dose that induced maximum number of foci in MRC-5 cells based on the result of 30 minutes postirradiation. However, the injured cells were recovered at 24 hours postirradiation.

Okada et al investigated the long-term biological effects of low-dose ionizing radiation, which was carbon ions and γ -rays, at 1 mGy once at a low dose rate (1 mGy/6-8 hours).²³ They used normal human lung fibroblast III (HFL III) cells, irradiated them with carbon ions, and found that the growth of irradiated cells started to markedly slow down earlier than that of nonirradiated cells and reached senescence. They highlighted the correlation between cellular end points and the duration of persistence of γ -H2AX foci. They showed that the senescence process itself seemed to produce DNA DSBs, as the number of foci increased in all samples at cell passage 26 by irradiation. The yield of the average number of foci increased further, particularly in carbon ion-irradiated cells. However, in the fully senescent (passage 30) cells, the number of foci was significantly reduced. They found that a single exposure to γ -rays at a similar dose and dose rate did not shorten the life span of cells, and it slightly extended it. A total of 1 mGy of carbon ions appears to be the lowest dose that examined as small as possible by them. In order to clarify the smallest dose, further study must be done (Figure 1).

On the other hand, the induction of γ -H2AX is highly influenced by the dose rate.²⁴⁻²⁶ Irradiation of high dose by high

dose rate (1.8 Gy/min) causes many γ -H2AX foci per cells, approximately 35 foci per cells by 5 Gy. The number of foci per nucleus increased in proportion to the total dose. However, irradiation by low dose rate (0.3 mGy/min) is not influenced by total dose. Therefore, we consider that there is very few dose-rate effect in the reports described earlier because the irradiations were performed at a low dose rate.

Detection of MN and NPBs

The MN, NPBs, and nuclear buds (NBUDs) are biomarkers of genotoxic events and chromosomal instability.²⁷ These genome damage events may be measured using the cytokinesis-block micronucleus cytome (CBMN cyst) assay, which is regarded as a reliable and precise method to establish genotoxicity.^{28,29}

Joshi et al investigated cells exposed to ^{60}Co γ -rays in the G2 phase by comparing the slopes of the dose responses for MN and NPBs in the low-dose region (<50 mGy) to those at higher doses (>60 mGy).³⁰ In order to achieve this, they used 4 normal human lymphoblastoid cell lines: GM15036, GM15510, GM15268, and GM15526, and cells were acutely exposed to ^{60}Co γ -radiation. Each cell line was individually exposed to 0, 50 mGy, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, or 4 Gy. They evaluated cytogenetic damage as MN and NPBs in G1 and G2 cells irradiated with 0 to 4 Gy using the CBMN assay. The findings obtained suggested the existence of a nonlinear response in the G2 phase for doses <1 Gy. The hyperradiosensitivity (HRS) effect, which increases cell death, was observed at an irradiation dose of <0.2 Gy as indicated by multifold increases in low-dose slopes in G2- versus G1-irradiated cells. They also indicated that the steepest slopes were obtained from between 0 and 50 mGy. However, they stated that the data used to assess low-dose slopes were limited; therefore, the ratio of low-dose and high-dose slopes may have been higher and, correspondingly, more significant. Nevertheless, they demonstrated HRS responses in G2-irradiated cells for MN and NPBs at doses up to 0.2 Gy in 4 normal human lymphoblastoid cell lines. Although Joshi et al reported the result from only a small part of the cell cycle (G1 and G2), they showed HRS effect in G2-irradiated cells for MN and NPBs at doses up to 0.2 Gy in these human lymphoblastoid cell lines (Figure 1).

Peripheral blood lymphocytes were used by Tewari et al as an in vitro model instead of cell lines to assess the genomic instability induced by an exposure of low-dose radiation.³¹ They collected blood from 35-year-old healthy females, and samples were divided into 6 groups according to radiation doses: 0, 0.1, 0.25, 0.5, 1, and 2 Gy. X-ray irradiation of blood samples was conducted using linear accelerators. After irradiation, blood samples were cultured and subjected to the CBMN assay. The very low formation of MN was observed in the control group. However, MN numbers significantly increased at an irradiation dose of 0.1 Gy. As other markers of genomic instability, the number of NBUDs, nucleocytoplasmic bridges, and polynucleated cells were examined and found to increase

in lymphocytes at exposure doses between 0.1 and 0.5 Gy but decrease at doses between 1 and 2 Gy. Radiation-induced changes were observed at the lowest dose of 0.1 Gy. The types of aberrations at 0.1 and 0.25 Gy were reported to be similar. They found that dicentric fragments were the most abundant at 0.5 Gy but then exponentially decreased. No measurement below 0.1 Gy was reported. Therefore, the lowest dose that induced NBUDs or NPBs is not clear. However, the MN assay revealed that distinct MNs along with NBUDs and NPBs were present at doses as low as 0.1 Gy (Figure 1).

Detection of Chromosomal Aberrations

The frequency of chromosomal aberrations has been one of the most reliable markers of DNA damage.^{32,33} Chromosomal aberrations have been detected by whole chromosome painting-fluorescence in situ hybridization (WCP-FISH), which is a powerful technique for chromosome-type structural changes.^{34,35}

Sudo et al attempted to compare the genomic instability induced by sparsely ionizing X-rays to that by densely ionizing iron ions.³⁶ However, the radiation doses in that study were relatively high because they did not establish the radiation dose limit causing genomic instability; they investigated differences between the effects of X-rays and iron ion beams. Finite life span human mammary epithelial cells (HMECs) were exposed to a graded X-ray dose of 0, 1, 2, 4, or 6 Gy and a graded dose of 0, 0.5, 1, 1.5, 2, or 3 Gy of iron ion beams. Cytotoxicity was evaluated by examining the survival of HMEC exposed to X-rays and iron ion beams in noncycling conditions seeded for colony formation at 48 hours postirradiation. Iron ions were found to be more cytotoxic to HMEC than X-rays. Chromosomal aberrations were detected by WCP-FISH. These findings revealed a severe karyotypic instability in colonies exposed to the lowest dose of 0.5 Gy of iron ions. Regarding X-ray exposure, severe karyotypic instability was only noted in colonies that survived exposure to 2 Gy or more. They concluded that doses as low as 2 Gy of X-rays and 0.5 Gy of iron ions may induce severe karyotypic instability (Figure 1). They also indicated that exposure to a low dose of 0.5 Gy of 1 GeV/nucleon iron ions resulted in an average of 2.4 traversals per cell nucleus, suggesting that a single iron ion track is sufficient to elicit a persistent state of genomic instability in finite life span HMEC.

Roch-Lefèvre et al recently compared the induction of chromosomal damage in mouse lymphocytes after acute γ -irradiation with that in humans.³⁷ They revealed that the ratio of the yield of chromosomal breakpoints in mice versus humans was approximately 2 at all γ -doses delivered (0.1, 0.2, or 0.5 Gy) using an FISH analysis. They suggested that this was partly due to the smaller size of the mouse genome from that of the human genome. In either case, after 0.1 Gy of acute γ -irradiation, the yield of chromosomal breakpoints was significantly higher than the mean control yield.

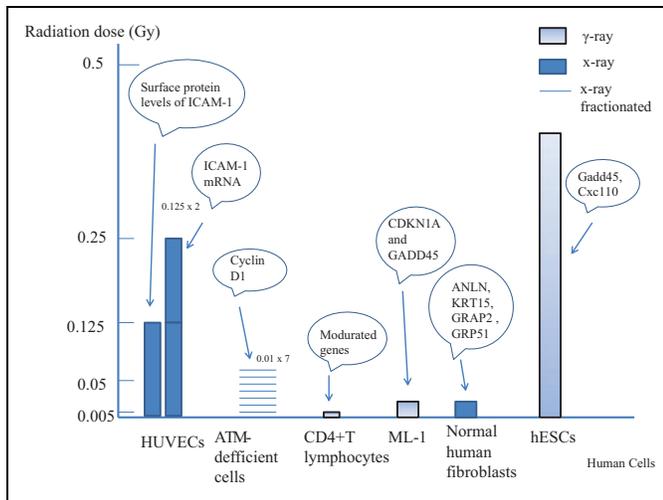


Figure 2. The estimated smallest dose causing the upregulation of stress-response genes or proteins that reported by several researchers. The doses are the smallest that were tried by each report. The cells examined were HUVECs,³⁸ ATM-deficient cells,³⁹ CD4+ T lymphocytes,⁵⁰ ML-1,⁵¹ normal human fibroblast cells,⁵⁵ and hESCs.⁵⁹ HUVECs indicates human umbilical vein endothelial cells; ATM, ataxia telangiectasia, mutated; hESCs, human embryonic stem cells.

Upregulation of Stress Responsive Genes or Proteins

The effects of single and fractionated low-dose irradiation were investigated using human umbilical vein endothelial cells (HUVECs) by Cervelli et al.³⁸ Cells were irradiated with X-rays at single doses of 0.125, 0.25, and 0.5 Gy or fractionated doses of 2×0.125 and 2×0.25 Gy. They examined the surface protein and messenger RNA (mRNA) levels of intercellular adhesion molecule 1 (ICAM-1). They demonstrated that the surface exposure of ICAM-1 was upregulated after 0.125 and 0.25 Gy, and this increase was potentiated further by fractionated doses rather than single doses. Irradiation with single doses was also found to induce the surface exposure of ICAM-1 but did not significantly increase its mRNA level. These findings suggested that low-dose irradiation affects the posttranscriptional regulation of baseline ICAM-1 mRNA, leading to an increase in ICAM-1 protein exposure. The DNA DSBs visualized with γ -H2AX foci also increased in a dose-dependent manner, whereas the number of foci per nuclei only significantly differed from that of the control after 0.5 Gy. Furthermore, the kinetics of γ -H2AX foci were not affected by fractionated doses. They attempted to clarify the relationship between atherosclerosis and low-dose X-ray irradiation and indicated that fractionated low doses accelerated chronic vascular inflammation by increasing ICAM-1 mRNA levels. These findings indicated that the dose limit inducing DNA DSBs in HUVECs was 0.5 Gy. Regarding the upregulation of surface protein levels, 0.125 Gy was the lowest dose studied. Doses lower than 0.125 Gy were not examined, so we do not know whether the protein levels were upregulated or not. Concerning mRNA, the upregulation of ICAM-1 mRNA was noted at a fractionated dose of 2×0.125 Gy; therefore, the total dose was 0.25 Gy (Figure 2).

Low-dose, long-term fractionated radiation (FR) was examined by Shimura et al.³⁹ Their index of DNA damage responses was cyclin D1, a regulatory subunit of cyclin-dependent kinases (CDKs), which controls cell cycle progression from the G1 phase to the S phase. The overexpression of cyclin D1 has been shown to prevent DNA repair.⁴⁰ Shimura et al investigated whether abnormal nuclear cyclin D1 accumulation occurred following low-dose long-term FR and induced defects in DNA replication and the resulting DNA DSBs.³⁹ They used human ataxia telangiectasia, mutated (ATM)-deficient and the product of the gene underlying Nijmegen breakage syndrome (NBS1)-deficient cell lines and the corresponding cell lines expressing ATM and NBS1. The ATM is a protein product of the gene mutated in the human genetic disorder, AT, which is characterized by high radiosensitivity and neurodegeneration.⁴¹⁻⁴⁴ On the other hand, NBS is a chromosomal instability disorder that differs from AT and is characterized by immunodeficiency, microcephaly, growth retardation, increased sensitivity to ionizing radiation, and a high frequency of malignancies.^{45,46} *NBS1* is the gene mutated in NBS, and the NBS1 protein includes meiotic recombination 11 (MRE11) and RAD50, which are involved in DNA repair and cell cycle regulation in response to DNA. NBS1 amplifies ATM activation by accumulation of the MRN (MRE11-RAD50-NBS1) complex at damaged sites.⁴⁷⁻⁴⁹ In Shimura's study, low-dose X-ray FR was conducted at 10 mGy or 0.5 Gy per fraction, and the total doses delivered over 31 days were 0.46 and 2.3 Gy, respectively. The nuclear accumulation of cyclin D1 was induced within 7 days after ATM-deficient cells, but cyclin D1 appeared later (from days 21 to 31) in cell lines expressing ATM. After 21 days of FR, NBS1- and ATM-deficient cells showed a decrease in the percentage of nuclear cyclin D1-positive cells and an increase in that of apoptotic cells. The ATM is associated with protection against cell death induced by the nuclear accumulation of cyclin D1 in irradiated cells. The ATM may play an important role in preventing the abnormal nuclear accumulation of cyclin D1 at early time points after low-dose FR. Based on these findings, 10 mGy per fraction of X-ray irradiation was sufficient to cause the accumulation of cyclin D1; however, it is not clear whether even lower doses per fraction could lead to accumulation of cyclin D1 (Figure 2).

Nosel et al reported that they used blood samples exposed to 6 low doses between 5 mGy and 0.5 Gy.⁵⁰ In contrast to Tewari et al,³¹ Nosel et al investigated whether the pattern of gene expression showed dose dependence. Venous peripheral blood samples from 5 healthy male donors were submitted to examinations. Ex vivo irradiation was performed with a ⁶⁰Co source at a low-dose rate, and total exposure doses were 5, 10, 25, 50 mGy, 0.1, and 0.5 Gy. After cell sorting and a flow cytometry analysis, total RNA was extracted from CD4⁺ T lymphocytes. RNA was then amplified and submitted to microarray hybridization. Gene expression modifications in response to low-dose radiation ranges have been investigated in CD4⁺ T lymphocytes. The number of modulated genes did not appear to be markedly affected by the dose delivered, even at the lowest

dose of 5 mGy. At an irradiation dose of 0.5 Gy, 864 genes were selected as being upregulated, while 577 were downregulated, at least at one of the postirradiation times tested. In that study, the number of modulated genes decreased significantly at 5 mGy when postirradiation times increased. The activation of gene regulation appeared to start at the lowest tested dose of 5 mGy and remained constant regardless of the dose delivered. Their analysis confirmed the involvement of signaling pathways partly related transcription factor p53 response from 25 mGy; 5 mGy was the lowest dose that tried (Figure 2). Even lower doses should be examined. Previous studies reported gene modifications in various cells following low-dose irradiation. In 1999, Amundson et al reported that several stress-responsive genes were induced in a human myeloid tumor cell line (ML-1) by γ -irradiation at doses <0.5 Gy.⁵¹ They observed the upregulation of CDKN1A, which is involved in the inhibition of cellular proliferation in response to DNA damage,⁵² and GADD45, which acts as a sensor of environmental and physiological stress, interacts with and/or modulates the activities of proteins involved in, for example, cell survival, the maintenance of genomic stability, and DNA repair.⁵³ However, the induction of genes produces little toxicity, and surviving cells contribute significantly to the stress responses observed. Regarding gene expression, the γ -irradiation doses tested, ranging between 20 mGy and 0.5 Gy from a ^{137}Cs source, were the lowest doses that induce specific gene expression effects in the ML-1 cell line (Figure 2).

The effects of single acute doses of 10 mGy or 2 Gy of γ -rays in normal human keratinocytes were tested by Franco et al.⁵⁴ They showed that 140 low-dose-specific genes were modulated after 48 hours of irradiation using a microarray technique. Their findings demonstrated that irradiation at a dose as low as 10 mGy was sufficient to induce specific transcriptional responses in human keratinocytes. Normal human fibroblast cells were irradiated with 20 mGy of X-rays, and a complementary DNA microarray analysis was conducted.⁵⁵ The findings obtained showed that several genes such as the cytoskeleton components ANLN and KRT15 and the cell-cell signaling genes *GRAP2* and *GPR51* responded to low-dose, but not high-dose (4 Gy) radiation (Figure 2).

Irradiation was delivered at doses as low as 50 mGy of ^{60}Co γ -rays or 20 mGy or 0.1 Gy of ^{137}Cs γ -rays to blood samples from donors, and the modulation of gene expression in lymphocytes was examined.^{56,57} The expression of 5 genes was induced 24 hours after irradiation using ^{60}Co γ -rays and the number of downregulated genes was 10-fold greater in CD4⁺ cells than in other cell types 3 hours after exposure.⁵⁶ The expression of 144 genes was found to be significantly changed after the irradiation of blood lymphocytes.⁵⁷

Regarding other analytical methods, Maguire et al used Raman spectroscopy to detect radiation-induced damage responses in lymphocytes isolated from peripheral blood γ irradiated at doses of 50 mGy and 0.5 Gy from a ^{60}Co source.⁵⁸ Lymphocytes from blood in a cohort of volunteers were cultured ex vivo and irradiated. They concluded that Raman spectroscopy demonstrated its capacity for detecting changes in the

spectral profiles of irradiation at doses as low as 50 mGy. Raman spectroscopy measures increases in RNA levels, transcription, and gene expression after irradiation. In contrast to γ -H2AX detection, which only represents the phosphorylation of H2AX, changes in spectral profiles also contain signatures of damage and cellular responses, not only from DSBs but also from SSBs and other lesion types. Their study demonstrated the capacity of the detection limit of Raman spectrometry, and changes were more clearly detected in 50 mGy-irradiated samples than in the sham-irradiated control (0 Gy). However, they did not attempt doses <50 mGy in this study; lower doses may also cause structural changes in lymphocytes. Further study is needed.

Regarding the limits of radiation exposure for pregnant women, Wilson et al analyzed genome-wide transcriptional responses to ionizing radiation in human embryonic stem cells (hESCs).⁵⁹ Undifferentiated hESCs were irradiated with 0.4, 2, or 4 Gy of γ -radiation using a ^{137}Cs irradiator. They demonstrated that low-dose irradiation upregulated the known stress-responsive genes *Gadd45* and *Cxcl10*⁶⁰ using microarrays to analyze global gene expression. They also found that apoptosis and cell death were slightly more prominent at 2 and 4 Gy than at 0.4 Gy and under control conditions. In order to confirm that surviving hESCs were pluripotent, cells were injected into immune-compromised mice and the formation of teratomas was monitored. Teratoma formation from hESCs was observed at all irradiation doses, providing definitive proof of pluripotency. They also analyzed the progression of gene and pathway changes that occur in hESCs at radiation doses between 0 and 0.4 Gy. An irradiation dose of 0.4 Gy was found to affect cellular functions, such as cell death, cancer, and signaling pathways including p53. They concluded that irradiated hESCs underwent significant cell death and apoptosis after irradiation, whereas the expression of pluripotency genes was unaffected, and these cells still formed teratomas; 0.4 Gy was the lowest dose that studied. We do not know whether lower doses induce changes in hESCs. However, this dose seems to be taken as an approximate indicator (Figure 2).

Effects of Low-Dose Ionizing Radiation on Animals

Mice have been utilized by many researchers to examine whole-body irradiation effects. BALB/c and C57BL/6 mice are the most commonly used mouse strains in radiation research. The BALB/c strain is reported to be radiation sensitive, whereas the C57BL/6 strain is radiation resistant.^{61,62} Newman et al recently investigated whether radiation sensitivity influences the modulation of DNA methylation, which plays a role in maintaining genomic stability, following high-dose radiation exposure, namely, 1 Gy of X-rays. They concluded that radiation exposure elicited time-dependent changes in the methylation of repeat elements that were influenced by the genetic background, gender, and type of repeat element.⁶³ Another study examined the effects of 7 Gy of total-body γ -irradiation from a ^{137}Cs source on mitochondrial DNA.⁶⁴ The findings obtained from the 4 strains investigated BALB/c, C57BL/6,

Table 2. Effects of Low-Dose Whole-Body Irradiation on Animals.

| Indices of the Detection of Abnormalities | Animals | Irradiation Protocol | Samples | The Lowest Radiation Dose Studied That Cause Some Effect | Reference |
|---|-------------------------------|---|---|---|-----------|
| External malformations, skeletal abnormalities of fetuses, fertility, and embryonic death | BALB/c, CF1 female mice | 50 mGy, 0.1, 0.2, 0.5, or 1 Gy X-rays 7 hours after presumed fertilization | Fetuses and embryos | 0.5 Gy single exposure in CF1 mice | 66 |
| The inversion frequency in spleen cells | pKZ1 mice | Single whole-body X-radiation doses from 1 μ Gy to 2 Gy. | Spleen cells | 5 μ Gy | 67 |
| Chromosome aberrations by FISH assay | Male BALB/c, C57BL/6J mice | 50 mGy, 0.1, or 1.0 Gy of ^{137}Cs γ -rays (at dose rate of 0.75 Gy/min) using γ cell | Bone marrow cells | 0.1 Gy | 71 |
| Chromosome aberrations, cytogenetic assays, FISH assay | SCID/J mouse | Whole-body total doses of, 50 mGy, 0.1, or 1.0 Gy of ^{137}Cs γ -rays (dose rate 0.75 Gy/min) | Bone marrow cells | 50 mGy at 4 hours postirradiation, 0.1 Gy at 1 and 6 months postirradiation | 72 |
| MN and NPBs | Female C57BL/6 or BALB/c mice | A single dose of ^{60}Co γ -irradiation (dose were 20 mGy, 0.1, 0.5, 1, or 2 Gy) | Spleen cells | 0.1 Gy | 76 |
| Bilallelic <i>PU.1/Sfpil</i> alterations by FISH analysis | Male CBA/Caj mice | 0.1, 0.2, 0.4, or 1.0 Gy of 1 GeV/nucleon ^{56}Fe ion radiation | Spleen cells | 0.4 Gy of ^{56}Fe ions | 79 |
| Chromosome aberrations DNA methylation patterns | CBA/Caj mice | Whole-body exposure to total-body doses of 0.1, 0.25, or 0.5 Gy of 300 MeV/nucleon ^{28}Si ions | Hematopoietic stem/progenitor cells (HSPCs) | 0.1 Gy of ^{28}Si ions | 83 |
| Chronic inflammation and altered level of DNA hydroxyl-methylation | CBA/Caj mice | Whole-body exposure to total-body doses of 0.1, 0.25, or 0.5 Gy of 1 GeV/nucleon ^{48}Ti ions | Somatic and germinal tissues | 0.1 Gy of ^{48}Ti ions | 84 |
| Chromatin and DNA by FTIR and comet assay | Female Wister rats | Whole-body exposure of 9 mGy of neutron at a dose rate of 0.2 mGy/h from a 185 GBq $^{241}\text{Am-Be}$ neutron source capsule. | Peripheral blood mononuclear cells (PBMC) | 9 mGy of fast neutron | 85 |

Abbreviations: MN, micronucleus; NPBs, nucleoplasmic bridges; FISH, fluorescence in situ hybridization; FTIR, Fourier transform infrared.

and F1 hybrid (BALB/c female \times C57BL/6 male and BALB/c male \times C57BL/6 female), indicating that calcium-induced mitochondrial swelling was strain dependent and mitochondrial permeability transition pores opened sooner in radiosensitive strains (Table 2).

At low radiation doses, radiosensitive BALB/c and radioreistant C57BL/6 mice exhibit differences in their Th1/Th2 lymphocyte and M1/M2 macrophage phenotypes, radiosensitivity, and incidence of postirradiation tumors. Nowosielska et al investigated the effects of repeated low-level exposure to X-rays at 10, 20 mGy, and 0.1 Gy daily and found significantly reduced numbers of neoplastic colonies in the lung following an intravenous injection of syngeneic tumor cells. Ten daily doses of low-level irradiation with X-rays induced similar antitumor reactions in both strains.⁶⁵ These phenomena suggested that low-level repeated irradiation induces adaptive responses.

Fertility and Embryonic Death, External Malformations, and Skeletal Abnormalities in Fetuses

In 1995, Jacquet et al reported embryonic death, dwarfism, and fetal malformations following the delivery of irradiation to

embryos at the zygote stage.⁶⁶ Female mice of the BALB/c and CF1 strains were mated and irradiated with 50 mGy, 0.1, 0.2, 0.5, or 1 Gy of X-rays at a dose rate of 900 mGy/min. Only 1 Gy was delivered to the CF1 strains. They found that embryonic mortality in pregnant animals predominantly occurred during the preimplantation stages in BALB/c mice in the irradiation groups. In contrast, irradiated CF1 females mainly exhibited greater early postimplantation loss. More than 60% of the litter died at a dose of 1 Gy. Mortality in the later stages did not increase. They also noted malformed fetuses and, among the irradiated groups, found that abnormalities included exencephaly and altered numbers of fingers (hypodactyly and polydactyly) in CF1 mice. The proportion of malformed fetuses was low but slightly increased with elevations in the dose of radiation delivered. Irradiation had no effect on the frequency of abnormal fetuses in BALB/c mice; however, depending on the criterion, 0.5 or 1 Gy significantly increased the frequency of abnormalities in fetuses of the CF1 strains. On the other hand, the findings obtained indicated that irradiation did not increase the frequency of skeletal abnormalities. Therefore, an irradiation dose of 0.5 Gy may have detrimental effects on fetuses of the CF1 strains (Figure 3).

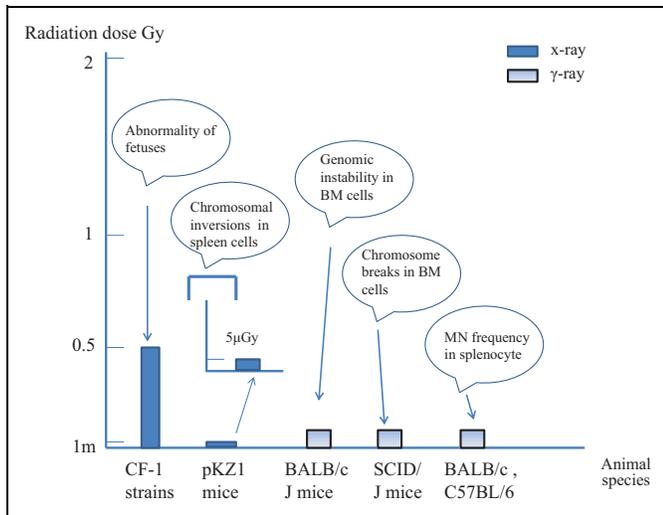


Figure 3. The estimated smallest dose causing abnormalities in fetuses, chromosomal inversions, genomic instability, chromosomal breaks, and MN in mice reported by several researchers. The doses are the smallest that were tried by each report. MN indicates micronucleus.

Detection of Chromosomal Aberrations

Hooker et al demonstrated that the number of chromosomal inversions in spleen cells after single acute low-dose X-ray irradiation was not consistent with the LNT model.⁶⁷ They used a pKZ1 transgenic mouse model, which is extremely sensitive, to detect chromosomal inversions in the spleen after exposure to low doses of DNA-damaging agents. The pKZ1 mouse recombination mutagenesis assay enables the study of the mutational effects of ultra-low doses of low LET radiation in a whole animal model.⁶⁸ After pKZ1 mice were exposed to a single dose of X-ray irradiation ranging between 1 μ Gy and 2 Gy, Hooker et al examined the frequency of inversions in spleen cells from mice that were killed 3 days postirradiation. An increasing number of inversions were observed when the dose was increased from 0.1 to 2 Gy. However, at doses between 10 and 1 mGy, a reduction to less than the endogenous inversion frequency was observed. On the other hand, lower doses from 10 to 5 μ Gy increased the frequency of inversions, and this returned to endogenous levels at a dose of 1 μ Gy (Figure 4). These findings indicated that the number of chromosomal inversions in spleen cells after a single acute low dose of X-ray irradiation is not consistent with the LNT model. They suggested overestimations at doses between 10 and 1 mGy and underestimations at ultra-low doses between 10 and 5 μ Gy. They indicated that ultra-low doses between 5 and 10 μ Gy induced DNA damage and may have lead to an increase in recombination activity, resulting in detectable increases in the frequency of inversions. At low doses between 1 and 10 mGy, induced DNA damage may have lead to a direct decrease in recombination activity or an indirect decrease resulting from the induction of protective mechanisms and, thus, a decrease in the stimulus for recombination activity. Sykes et al reported

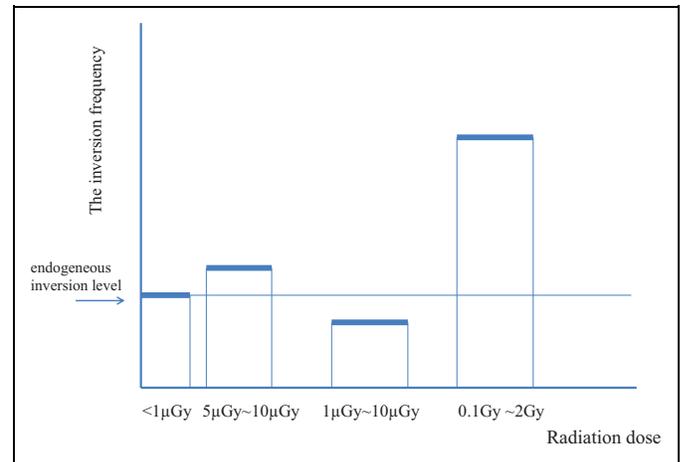


Figure 4. A conceptual diagram of the relationship between the chromosomal inversion frequency in the spleen of pKZ1 mice and radiation doses. The arrow shows the endogenous inversion level. Radiation doses between 1 and 10 μ Gy revealed a lower inversion level than those of other radiation doses. This figure was made from the findings of Hooker et al.⁶⁷

similar findings using spleen sections from irradiated pKZ1 mice. They also detected an increase in the frequency of inversions at very low doses (<10 μ Gy), whereas decreases to less than the endogenous frequency were noted between 100 μ Gy and 0.1 Gy.⁶⁹ Temporal responses to X-ray irradiation exposure in the spleen of the pKZ1 mouse were previously reported by Ormsby et al.⁷⁰ They employed a wide radiation dose range. PKZ1 mice were irradiated with a single whole-body X-ray dose of 10 μ Gy, 1 mGy, or 1 Gy, and spleen sections were analyzed for inversions 7 hours, 1 day, or 7 days after exposure. On day 1, an increase in the frequency of inversions was observed in response to 10 μ Gy. On the other hand, a decrease in the frequency of inversions to less than the sham-treated frequency was noted for 1 mGy. They found that the inversion frequency for both doses returned to that of the sham-treated frequency by day 7. Based on these findings, 5 μ Gy of acute X-ray irradiation may be the lowest dose causing chromosomal inversions in pKZ1 mice (Figure 3).

In order to evaluate the *in vivo* induction of genomic instability expressed as late-occurring chromosomal aberrations, Rithidech et al attempted to examine their incidence in bone marrow (BM) cells from BALB/cJ and C57BL/6J mouse strains.⁷¹ Whole-body irradiation was performed using ¹³⁷Cs γ -rays (0, 50 mGy, 0.1, and 1.0 Gy). They observed no increases in the frequencies of abnormal cells or any type of chromatid aberration in 50 mGy-exposed mice at 1 or 4 hours postirradiation in both strains of mice. However, exposure to 0.1 or 1.0 Gy of ¹³⁷Cs γ -rays resulted in significant damage to the BM cells of mice. At 1 and 6 months postirradiation, they observed significant decreases in the frequencies of abnormal cells and chromosomal damage in BM cells from both strains related to that in BM cells collected at early time points. On the other hand, slightly persistent elevations were observed in all types of chromosomal damage in cells collected from only BALB/cJ

mice at 6 months postirradiation with 0.1 Gy of γ -rays; therefore, exposure to 0.1 Gy was considered to be the lowest dose causing detrimental effects on the chromosomes of radiosensitive BALB/cJ mice (Figure 3). However, this phenomenon was not found in C57BL/6J mice, suggesting that differences in responses to radiation reflect differences in the DNA repair capacity of these 2 strains. They concluded that 50 mGy of ^{137}Cs γ -rays was incapable of inducing significant in vivo genomic instability in the BM cells of the 2 mouse strains.

Rithidech et al subsequently provided evidence for the lack of genomic instability in the BM cells of severely combined immunodeficiency (SCID/J) mice exposed to whole-body γ -radiation at a dose of 50 mGy.⁷² The differentiation of T and B lymphocytes was impaired in SCID mice,^{73,74} and they had extremely low levels of DNA-PKcs activity.⁷⁵ At an early time point postirradiation (1 hour), no significant differences were noted between the frequencies of chromatid exchanges in the BM cells of SCID/J mice after irradiation at 50 mGy or 0.1 Gy by γ -rays and those detected in the BM cells of controls. However, they indicated that a significant increase in the frequency of iso-chromatid breaks occurred in the BM cells of mice exposed to 50 mGy of γ -rays only. At 4 hours postirradiation, all types of aberrations, except for chromatid exchanges, were significantly more abundant than those found in the controls at irradiation doses of 50 mGy and 0.1 Gy. At later time points (1 and 6 months postirradiation), a reduction was noted in the frequencies of chromosomal breaks in the BM cells of SCID/J mice exposed to 50 mGy or 0.1 Gy of γ -rays. They concluded that there were no evidence for the in vivo induction of genomic instability by low-dose radiation, in spite of using SCID/J mice, because their findings indicated no increase in the frequency of late-occurring chromosomal damage in 50 mGy-exposed SCID/J mice at 6 months postirradiation. Based on these findings, 0.1 Gy of γ -rays seems to be approximate indicator (Figure 3). Even lower doses may also show the effect on BM cells of SCID/J mice.

Detection of MN and NPBs

Bannister et al examined MN induction in the spleen cells of C57BL/6 and BALB/c mice exposed to a single dose of ^{60}Co γ -irradiation (20 mGy, 0.1, 0.5, and 1.2 Gy) using the CBMN assay.⁷⁶ Regarding the C57BL/6 mouse, sampling 24 or 28 hours after irradiation revealed that the frequency of MN did not change after the 20 mGy dose but was 1.8-, 2.1-, 4.5, and 13-fold higher than that in control animals after exposure to 0.1, 0.5, 1, and 2 Gy, respectively. The frequency of MN in BALB/c mice was examined at irradiation doses of 20 mGy and 2 Gy only. A dose of 20 mGy also had no effect on the frequency of MN in the splenocytes of BALB/c mice. However, BALB/c mice showed increased radiosensitivity for the induction of MN at a high irradiation dose of 2 Gy, with the frequency of MN being approximately 1.9-fold greater than that in C57BL/6 mice. They concluded that the in vivo radiation dose of 20 mGy had a negligible effect on MN frequencies in the splenocytes of either mouse strain. The dose limit

affecting the frequency of MN may be 0.1 Gy in the above-mentioned mouse strains (Figure 3).

Effects of High-LET Radiation

The effects of high-LET radiation on animals have been reported by several researchers. The aims of their studies were to clarify the health risks of exposure to heavy ions and improve radiation protection guidance for astronauts and patients receiving heavy-ion radiation therapy. Some of these studies attempted to use ^{56}Fe , ^{48}Ti , or ^{28}Si ion exposure in CBA/CaJ mice, which is a leukemogenesis-sensitive CBA mouse strain. These studies attempted to elucidate whether the risk of leukemia was increased using this mouse model, which is known to be sensitive to the development of radiation-induced myeloid leukemia (ML).^{77,78} Steffen et al reported that high-LET ^{56}Fe ion radiation exposure may result in murine acute ML (AML) with biallelic *PU.1/Sfpil* (a hematopoietic transcription factor gene) alterations using an FISH analysis,⁷⁹ because it was previously demonstrated that radiation-induced AML in mice correlated with the deletion of *PU.1/Sfpil* on chromosome 2.⁸⁰⁻⁸² Doses of 0.1, 0.2, 0.4, or 1.0 Gy of 1 GeV/nucleon ^{56}Fe ion radiation were used by Steffen et al, and they found that mutant frequencies significantly increased at a low dose of 0.4 Gy. Regarding the molecular characterization of radiation-induced AML, the effects of 1.0 Gy of γ -irradiation appeared to have the same effects as 1.0 Gy of ^{56}Fe ions based on mutant frequencies. They indicated that the relative biological effectiveness of iron ion leukemogenesis was approximately 1. Based on their findings, 0.1 Gy of iron ions had no effect on the deletion of *PU.1*; therefore, 0.4 Gy of ^{56}Fe ions was the lowest dose that caused the *PU.1* deletion and point mutations (Figure 5).

Rithidech et al reported chromosomal aberrations and DNA methylation in the hematopoietic stem/progenitor cells (HSPCs) of CBA/CaJ mice exposed to ^{28}Si ions.⁸³ They subjected these mice to whole-body irradiation at various doses using 300 MeV/nucleon ^{28}Si ions: 0.1, 0.25, or 0.5 Gy. At 6 months postirradiation, they examined the frequencies of late-occurring chromosomal aberrations in the myeloid lineage of HSPC-derived clones using genome-wide multicolor FISH. A dose-dependent increase in the frequencies of chromosomal aberrations was detected, and thus, genomic instability was induced after the exposure to ^{28}Si ions in all irradiation groups tested. Therefore, genomic instability in HSPC-derived myeloid colonies of CBA/CaJ mice was observed from a dose of 0.1 Gy of 300 MeV/nucleon ^{28}Si ions.

Rithidech et al focused on the induction of chronic inflammation and altered levels of DNA hydroxymethylation in the somatic and germinal tissues of CBA/CaJ mice exposed to ^{48}Ti ions.⁸⁴ Mice were whole-body exposed to total body doses of 0.1, 0.25, or 0.5 Gy of 1 GeV/nucleon ^{48}Ti ions. They examined the level of activated nuclear factor- κ B (NF- κ B), tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), IL-6, 5-methylcytosine (5mC), and 5-hydroxymethylcytosine (5hmC). They found that activated NF- κ B, TNF- α , IL-1 β ,

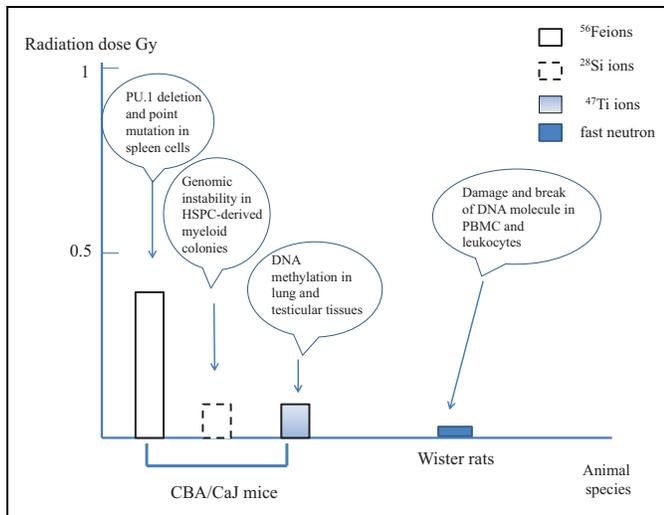


Figure 5. The estimated smallest dose causing molecular changes by high-LET radiation. Spleen cells, HSPCs, and lung and testicular tissues of CBA/CaJ mice were used for the detection of chromosomal deletions, chromosome aberrations, and DNA methylation, respectively. Wistar rat PBMCs and leukocytes were used for the detection of DNA breaks and chromatin fragmentation. The doses are the smallest that were tried by each report. LET indicates linear energy transfer; HSPCs, hematopoietic stem/progenitor cells; PBMCs, peripheral blood mononuclear cells.

IL-6, and 5mC levels dose dependently increased. In contrast, significant dose-dependent decreases were noted in 5hmC levels in the lungs of exposed mice. Moreover, a dose-dependent reduction occurred in global 5hmC levels in testicular tissues collected at 6 months postirradiation. 1 GeV/nucleon ^{48}Ti ions may induce chronic inflammation and the persistence of altered DNA methylation in the lung and testicular tissues of CBA/CaJ mice. They indicated that ^{48}Ti ion exposure induces disturbances in cytokine production, reflecting chronic inflammation and impairments in the immune system. Although significant increases in global 5mC levels were only observed following exposure to 0.5 Gy, dose-dependent decreases in 5hmC levels were noted in the lung tissues of exposed mice from 0.1 Gy; therefore, altered DNA methylation occurred following the exposure to 0.1 Gy. Based on these findings, 0.1 Gy of 1 GeV/nucleon ^{48}Ti ions may be the lowest dose affecting the immune system and DNA of CBA/CaJ mice.

The effects of very low-dose fast neutrons on the chromatin and DNA of rat peripheral blood mononuclear cells (PBMCs) and leukocytes were reported by Nafee et al.⁸⁵ Female Wistar rats were irradiated at a dose rate of 0.2 mGy/h to a total dose neutron dose of 9 mGy from a 185 GBq $^{241}\text{Am-Be}$ neutron source capsule. Using Fourier transform infrared and single-cell gel electrophoresis (comet assay), they examined PBMC spectra and detected DNA strand breaks. They indicated that fast neutrons at a very low dose of 9 mGy induced changes in the DNA of PBMCs at a submolecular level. They demonstrated that fast neutrons may cause SSBs, DSBs, and the chromatin fragmentation of the DNA of PBMCs as well as

low-level damage in the DNA of leukocytes using the comet assay. This study revealed that a fast neutron dose of 9 mGy may damage and break DNA molecules.

Systemic Adaptive Responses

Previous studies reported systemic adaptive responses in animals exposed to low-dose irradiation at the same level that induces some detrimental effects. For example, low-dose irradiation (20 mGy and 0.1 Gy, ^{60}Co γ -rays) enhanced the rate of DNA DSB repair after a challenge irradiation (2 Gy, ^{60}Co γ -rays),⁸⁶ 10 repeated doses of low-level exposure to X-rays at 10, 20 mGy, and 0.1 Gy upregulated antitumor cytotoxic function,⁶⁵ and decreased apoptosis was noted in the splenocyte subpopulations studied most prominently in natural killer cells and dendritic cells after γ -radiation (10, 50 mGy, 0.1, and 0.5 Gy).⁸⁷ Moreover, a continuous very low dose of γ -radiation (100 mGy/yr) did not have any adverse effects on the life span or incidence of lymphoma in SJL mice.⁸⁸ Seed et al reported accommodative responses to a chronic, low, daily dose of γ -irradiation on the blood-forming system of canines (beagles).⁸⁹ Canines were chronically exposed to γ -rays with a ^{60}Co γ -irradiator, with increasing doses between 3 and 128 mGy/d. They demonstrated weak but significant suppression of blood leukocyte and platelet levels at 3 mGy/d, the rate of suppression increased by approximately 8-fold, and the time to accommodate declined from 2000 to approximately 150 days. The duration of the initial suppressive phase increased as the irradiation rate increased from 3 to 75 mGy/d. In addition, blood leukocyte levels decreased negatively with radiation dose rates from 3 to 75 mGy/d. They concluded that the highly radiosensitive hematopoietic system adapts and becomes radioresistant under protracted very low-dose rate γ -ray irradiation. Based on their findings, 3 mGy/d of chronic irradiation was the lowest dose causing blood responses. On the other hand, Cuttler et al showed that the optimum life span of beagles increased at 50 mGy/yr of chronic γ -irradiation,⁹⁰ corresponding to 0.137 mGy/d. Therefore, this dose rate appears to be the hormesis level. The threshold for harm (decreased life span) was 700 mGy/yr for 50% mortality in dogs, corresponding to 1.9 mGy/d, and 1100 mGy/yr for short-lived dogs, corresponding to 3 mGy/d. Combined with the findings of Seed et al,⁸⁹ doses between 1.9 mGy/d (700 mGy/yr) and 3 mGy/d (1100 mGy/yr) may be the lowest doses causing detrimental effects in beagles.

Effects of Low-Dose Ionizing Radiation in Humans

DNA DSBs by Low-Dose Irradiation

Computed tomography examination for medical reasons. The formation of γ -H2AX foci in peripheral blood lymphocytes from young children who underwent computed tomography (CT) examinations for medical reasons was investigated by Halm et al.⁹¹ Three children aged between 21 months and 3 years

undergoing CT examinations were irradiated at effective doses that ranged between 1.57 and 2.86 mSv, corresponding to blood doses of 0.22 to 1.22 mSv, respectively. Although γ -H2AX focus values were similar among patients before CT, they increased in all 3 patients 1 hour after the CT scans. Their pilot study revealed that a very low dose of ionizing radiation could induce somatic DNA damage 1 hour after the CT scan. This pilot study was limited because the samples from pediatric patients could not be repeatedly obtained. And therefore, the DNA DSBs measurement was performed shortly after the CT scan. However, there are reports that the foci loss seemed to be dose dependent and was noted up to 24 hours at which time the background level was reached.⁹² This decrease in foci may be the defensive response.

An investigation on DNA damage induced by CT X-rays in pediatric patients was conducted by Vandevoorde et al.⁹³ Blood T lymphocytes from 51 patients with an average age of 3.8 years were examined in order to establish whether DNA damage was induced by scoring γ -H2AX foci after patients had undergone a CT examination of the chest or abdomen. They observed an increase in DNA DSBs in every patient, except for 1 chest CT patient exposed to a very low dose of 0.14 mGy. The average postexposure level of γ -H2AX foci was 0.72 foci per cell, while the preexposure level was 0.56 foci per cell. Blood doses were in the range of 0.14 to 8.85 mGy by the calculation of the full Monte Carlo simulation from the pediatric CT protocol typically adopted at every radiology department. This study showed that most CT examinations induce DNA DSBs in the T lymphocytes of pediatric patients exposed to even low doses (blood doses in the range of 0.15-8.85 mGy) shortly after the examination. There are no data from the longer time after the examination. DNA DSBs in the lymphocytes may be decreased by self-defensive response.

High-level natural radiation area. Several studies have been conducted on DNA DSBs from the residents of HLNRA. Jain et al reported the lack of an increase in DNA DSBs in PBMCs from the residents of these areas.⁹⁴ They examined the residents of HLNRA in Kerala, located on the south west coast of India. The background radiation level in this area varies between <0.1 and 45.0 mGy/yr. Therefore, they compared the spontaneous level of DNA DSBs in the PBMCs of the residents of HLNRA (the mean annual dose received was 8.28 [4.96] mGy/yr) to those in a normal natural-level radiation area (NLNRA; the mean annual dose received was 1.28 [0.086] mGy/yr). They found that the spontaneous frequencies of DSBs in terms of γ -H2AX foci among NLNRA and HLNRA individuals were 0.095 (0.009) and 0.084 (0.004) per cell, respectively. Moreover, they further classified the residents of HLNRA as a low-dose group (LDG; 1.51-5.0 mGy/yr, mean dose: 2.63 [0.76] mGy/yr) and high-dose group (HDG; >5.0 mGy/yr, mean dose: 11.04 [3.57] mGy/yr). The spontaneous frequencies of γ -H2AX foci per cell in LDG and HDG were 0.096 (0.008) and 0.078 (0.004), respectively. These findings suggested that this low frequency of γ -H2AX foci was due to the weaker induction or better repair of DSBs in individuals in the HDG of HLNRA.

Table 3. Accumulated Doses and the Frequencies of Chromosome Translocation in Residents Who are Elderly Individuals or Children in a High Background Radiation Area in the South of China.^a

| Age Ranges in Residents (years old, mean [SD]) | Accumulated Dose (mSv) (mean [SD]) | Mean Frequencies of Chromosome Translocations |
|---|------------------------------------|---|
| Elderly persons (53.2-89.5, 61.6 [9.9]) | 132.3-261.3 (172.3 [36.0]) | 11.4 [3.6] |
| Control of elderly person (55.3-70.5, 60.4 [4.6]) | 32.5-49.1 (39.6 [4.3]) | 12.0 [3.8] |
| Children (10.8-13.5, 12.5 [0.9]) | 25.9-41.4 (34.2 [5.4]) | 3.8 [1.1] |
| Control of children (10.3-13.8, 12.3 [1.3]) | 5.6-11.1 (8.9 [2.2]) | 3.2 [2.0] |

Abbreviation: SD, standard deviation.

^aRef. 96.

The elimination of damaged cells or better antioxidant defense mechanisms was more prominent in the HDG than in the LDG and NLNRA. They concluded that 5.0 mGy/yr (mean dose) may be the threshold dose for DSB induction with chronic low-dose radiation exposure in vivo.

Chromosomal Aberrations

High background radiation area. High background radiation areas (HBRAs) exist in South China, and the level of radiation in these areas is 3- to 5-fold higher than that in a control area. The reasons for the high background are the soil and building materials containing Th-232 and U-238 decay products.

Zhang et al examined radiation-induced stable chromosomal aberrations (translocations) in the lymphocytes of the residents of HBRA.⁹⁵ Their statistical analysis revealed no significant differences in the frequencies of translocations in children and the elderly individuals between the HBRA and control groups (Table 3). The worldwide average annual exposure to natural radiation sources appears to be approximately 2.4mSv/year while that of HBRA ranges between 7.2 and 12 mSv/year. Based on their findings, the frequencies of translocations in lymphocytes in HBRA were similar to those in the control area; however, it currently remains unclear whether the dose range was the limit having detrimental effects on lymphocytes.

Zhang et al attempted to clarify dose limits from the frequencies of translocations in the lymphocytes of individuals living under normal conditions.⁹⁶ Table 4 shows the mean frequencies of translocations in lymphocytes (standard deviation [SD]) in 1000 cells. As shown in Table 4, the mean value and variations were the smallest in children, while those in the elderly individuals in a remote village were slightly lower than those in a large city (Beijing). They concluded that the SD of the calculated dose was the dose level below which the effects of radiation became undetectable due to background variations,

Table 4. Chromosome Analysis and the Calculated Doses of People Living in Beijing and a Remote Village.^a

| People Living in Different Area (Average Years) | Mean Frequencies of Translocation in Lymphocytes (SD) in 1000 Cells | Calculated Doses (SD) for Chronic Irradiation (in Case of Acute Irradiation), mSv |
|---|---|---|
| Nonsmokers in Beijing (61.2) | 9.6 (5.0) | 384 ± 200 (248 [153]) |
| Older people in remote village (64.4) | 8.4 (3.1) | 336 ± 124 (225 [104]) |
| Children in remote village (12.3) | 3.2 (2.0) | 128 ± 80 (104 [72]) |

Abbreviation: SD, standard deviation.

^aRef 97.

even if assuming that all translocations had been induced by radiation. The frequencies of translocations under these conditions showed the same variations in the effects of all types of mutagenic factors. An epidemiological study may not be able to show significant increases in malignant diseases. Hayata et al reported that HBRA may contribute to an elevated induction rate of translocations; however, there were no significant effects from other mutagenic factors such as chemicals and/or metabolic factors.⁹⁷

Therefore, the induction of stable-type chromosomal aberrations may not be significantly induced by background radiation, even in high background areas. The lowest dose of radiation that causes chromosomal aberrations was higher than natural and high background levels (approximately 2.4-4 mSv/yr).

Radiation Hormesis in Humans

As for low-LET radiation in the range of 10 mGy to 0.5 Gy total absorbed dose, stimulatory effects could occur to living body.⁹⁸ The effect of low-dose radiation could contribute to antioxidant potential,⁹⁹ reduced cancer incidence,^{100,101} modulated a variety of immune responses,¹⁰² and so on. Radiation hormesis is interpreted to be adaptation to higher radiation exposures dependent on metabolic protection from the stresses in the environment. For example, cancer mortality survey in Misasa spa area (Japan) which has a high radon background, and the relative risk among the inhabitants of Misasa was significantly lower than in the control area for deaths from cancer.^{103,104} However, these epidemiological studies were not completely controlled because the individual exposure level was not measured, and major confounding factors such as smoking and diet could not be controlled. There are few reports of useful information about humans.

As shown in Table 3, the accumulated doses of residents in a high background area in the South of China are almost the same level that induced stimulatory effects described earlier. The epidemiological studies performed in this area detected no significant increase in either cancer morbidity or

mortality.¹⁰⁵ As shown in Table 4, the calculated doses for chronic irradiation are also the same level of the abovementioned doses that cause the stimulatory effects. Also, about this report, epidemiological studies performed in this area could not detect increase in cancer attributable to the high levels of natural radiation.

Therefore, it may be difficult to demonstrate the relationship between the changes in levels of biomarkers, such as translocations in lymphocyte, and cancer morbidity or mortality or otherwise, radiation hormesis.

Discussion

As discussed earlier, 200 mSv is defined as a low radiation level¹; therefore, many researchers have attempted to elucidate the effects of radiation at this dose level. Although the cell species, cell cycles, and radiation types tested differed, the foci of γ -H2AX, one of the experimental indices of DSBs, appeared from approximately 1 mGy to 0.5 Gy (Table 1, and Figure 1). Considering the radiation weighting factor, these radiation levels are similar to 1 mSv to 0.5 Sv with low-LET radiation such as γ - or X-rays. Carbon ions appear to produce DSBs at lower radiation doses (Table 1; Figure 1). On the other hand, Autonelli et al showed that low-LET radiation was prone to induce DSBs because the number of γ -H2AX foci was the highest among the radiation types tested, namely, γ -rays, protons, carbon ions, and α particles.¹⁸ However, foci persisted longer with high-LET radiation than with low-LET radiation. The characteristics of foci induced by high-LET and low-LET radiation may differ.¹⁸

As shown in Figure 1, 1 mGy of carbon ions caused foci of DSBs in HFL III cells, and this may be the lowest dose. Okada et al attempted to examine γ -ray irradiation and found that a single exposure to γ -rays at 1 mGy did not result in a shortened cell life span; a slight extension was noted. Low-LET radiation may produce DSBs in cells; however, this phenomenon did not lead to senescence.²³ These findings suggest the differences in the characteristics induced by high-LET and low-LET radiation.

Both MN and NPBs were detected at a γ -ray irradiation dose of 0.2 Gy in normal human lymphoblastoid cell lines and at an X-ray irradiation dose of 0.1 Gy in human lymphocytes (Figure 1; Table 1). Based on these findings, genotoxic events and chromosomal instability may be induced by 0.1 to 0.2 Gy of low-LET irradiation. Regarding chromosomal aberrations, X-ray irradiation caused severe karyotypic instability at an exposure dose of 2 Gy. This phenomenon appears to occur at a high dose of radiation in the case of low-LET radiation. In contrast, a single exposure to 0.5 Gy of iron ions was sufficient to elicit a persistent state of genomic instability; therefore, a difference may exist between low- and high-LET irradiation.

Some stress-responsive genes or proteins appeared to be affected by a slightly lower dose than that causing DNA DSBs, MN, or chromosomal aberrations (Figure 2). We found several studies in the literature that examined stress-responsive genes or proteins (Table 1). The cell surface protein levels of ICAM-1

were upregulated by an X-ray irradiation dose of 0.125 Gy, and elevated ICAM-1 mRNA levels were noted at a fractionated dose of 2×0.125 Gy as shown in Figure 2. Cyclin D1 accumulation was induced in ATM-deficient cells by 7 days of irradiation at 10 mGy per fraction. Several genes such as *ALIN*, *KRT15*, *GRAP2*, and *GPR51* in human fibroblast cells responded at an X-ray irradiation dose of approximately 10 mGy. Higher doses appear to be required for the upregulation of *CDKN1A*, *Gadd45*, or *Cxc110*. Although the species of cells examined were different, the lowest dose of low-LET radiation that caused the upregulation of genes ranged between 10 mGy and 0.5 Gy (Figure 2).

The effects of low-dose ionizing radiation on animals were also studied by several researchers. Mice were often selected and their spleen or BM cells were examined in order to establish whether chromosomal inversions or breaks were induced by low-dose irradiation (Figure 3).

The occurrence of abnormalities in fetuses was noted at higher doses (0.5 Gy), while biomarkers of genotoxic events, such as chromosomal inversions, breaks, or a high frequency of MN, were observed from very low doses (5 μ Gy-0.1 Gy) of low-LET radiation.

Figure 5 shows the effects of high-LET radiation on mice or rats. Ions or fast neutrons caused chromosomal aberrations or DNA damages in the radiation dose range of 0.9 mGy to 0.4 Gy. Fast neutrons may damage and break DNA molecules at very small doses (0.9 mGy). This neutron source was reported to be $^{241}\text{Am-Be}$ and the average energy was approximately 4.5 MeV.⁸¹ Therefore, considering the radiation weighing factor, approximately 9 mGy of γ - or X-rays have similar effects on the living body.¹⁰⁶

In animals, systemic adaptive responses are induced by low-dose radiation at the same dose level of radiation that causes changes in levels of biomarkers in the living body. Negative effects on the living body may resolve by adapting to good conditions. Homeostasis may be maintained at the individual level. However, although systemic adaptive responses appear, cells were damaged by DNA DSBs, apoptosis, and a defective immune system. A previous study demonstrated the occurrence of DNA DSBs in children subjected to CT examinations for medical reasons.^{91,93} Halm et al indicated that blood doses ranging between 0.22 and 1.22 mSv may induce somatic DNA damage after the blood samples were collected 1 hour post-CT examination.⁹¹ Moreover, Vandevoorde et al showed that a small blood dose, 0.15 mGy caused γ -H2AX foci 5 minutes after the CT examination. Extremely low-dose radiation as low as 0.15 mGy by the blood dose to children may inevitably affect DNA immediately after the CT examination.⁹³ The data from the longer time after CT examination could not be shown in this study. However, they noted that the γ -H2AX foci yield was only 70% of that from samples taken 30 minutes post-CT examination. Löbrich et al indicated the foci loss seemed to be noted up to 24 hours, at which time, background level was reached.⁹² The foci loss was thought to be the self-defensive effects.

However, the residents of HLNRA present with less DNA damage than those in lower level NRA.⁹⁴ The elimination of damaged cells and better antioxidant defense mechanisms in the residents of HLNRA may function well and represent adaptive responses.

Accumulated doses and the frequencies of chromosomal translocations in the residents of HLNRA are summarized in Table 3 from the study by Zhang et al.⁹⁶

The mean frequencies of chromosomal translocations were lower in the elderly individuals and children with high accumulated doses; therefore, some adaptive responses may have occurred in the living body. We tried to identify the lowest dose that causes some molecular changes in the living body. However, this study has limitation because we do not know the examined radiation dose of each report is the lowest. Moreover, we do not estimate whether each molecular changes cause the detrimental effect on living body or not and whether these changes may cause good effects on living body (hormesis) or not. In future, the relationship between clinical responses and the molecular changes should be examined.

Conclusions

For conclusions with respect to the lowest dose that causes molecular changes in the living body, the present study has limitations, but the smallest radiation dose causing the changes in the levels of biomarkers appears to be between approximately 0.1 and 0.5 Gy. This dose may overlap with the induction of some adaptive responses.

Declaration of Conflicting Interests

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