International Workshop

*on*

The Biological Effects of Low Dose Radiation

# PROGRAM & ABSTRACTS

21 October 2013

Sanjo Conference Hall

The University of Tokyo

Institute for Environmental Sciences

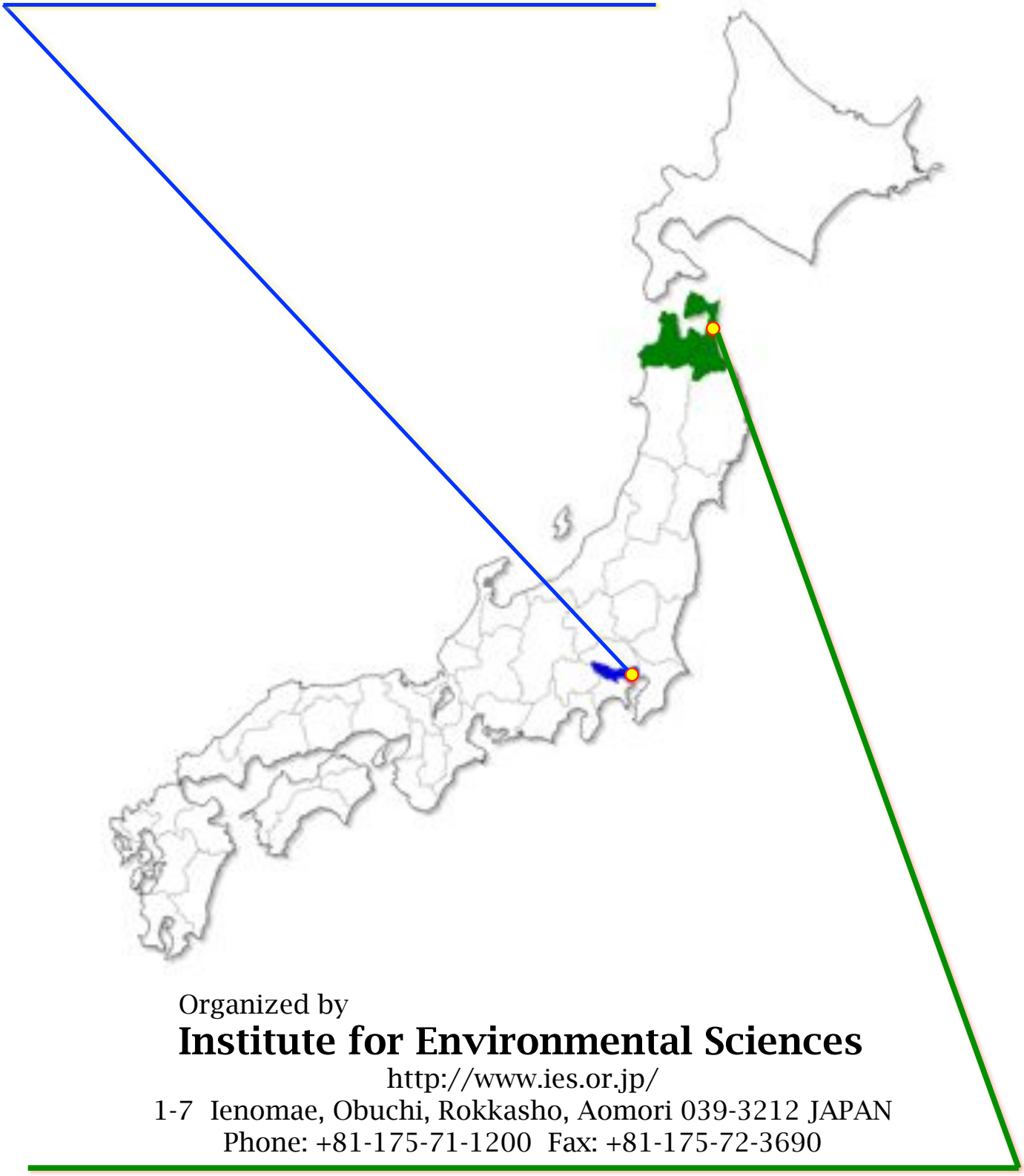
# International Workshop on the Biological Effects of Low Dose Radiation

21 October 2013

Venue:

**Sanjo Conference Hall, The University of Tokyo**

7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8654 JAPAN



Organized by

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1-7 Ienomae, Obuchi, Rokkasho, Aomori 039-3212 JAPAN

Phone: +81-175-71-1200 Fax: +81-175-72-3690

# WELCOME

Dear Colleagues,

On behalf of the organizing committee, I welcome you to the "IES International Workshop on the Biological Effects of Low Dose Radiation."

Although the estimation of the effects of low dose and low dose rate radiation is important for human welfare, it has been hampered by inevitable low signal to noise ratios. New technologies in various fields of biological sciences however, enable us to overcome this difficulty.

In this workshop, recent prominent progress in our knowledge on the effects of low dose and low dose rate radiation will be presented. I hope that the future research on this subject will be promoted by penetrating opinion in lively discussion.

Yours sincerely,
Jun-ichiro Komura
Institute for Environmental Sciences


Memo

21 October, 2013, Monday, Tokyo

**IES International Workshop on the Biological Effects of Low Dose Radiation**
**Sanjo Conference Hall, The University of Tokyo**

**9:00-9:30 Registration**

**9:30-9:35 Opening**

**9:35-12:30 Session I Presentations by Invited Speakers**

(Chair: Dr. Hiroshi MITANI, The University of Tokyo)

**I-1** Dr. Mary Helen BARCELLOS-HOFF (New York University)
Radiation Carcinogenesis: Microenvironment Matters

**I-2** Dr. Yoshiya SHIMADA (National Institute for Radiological Sciences)
Age Dependence of Radiation Carcinogenesis in Animal Models

**(Coffee Break, 10:40-10:55)**

**I-3** Dr. Christophe BADIE (Public Health England)
A Mouse Model of Radiation-Induced Leukemia, Past, Present and Future Work

**I-4** Dr. Michael M. WEIL (Colorado State University)
Low Dose Rate Exposure Enhances Interindividual Differences in DNA DSB Repair

**I-5** Dr. Keiji SUZUKI (Nagasaki University)
DNA Damage Accumulation in Tissues/Tissue Stem Cells Exposed to Low-Dose-Rate Radiation

**12:30-13:30 Lunch**

**13:30-16:25 Session II Presentations by IES speakers**

(Chair: Dr. Toshiyasu IWASAKI, Central Research Institute of Electric Power Industry)

**II-1** Dr. Tetsuya ONO
Outline of the IES Research Projects on Biological Effects of Low-Dose-Rate Radiation

**II-2** Dr. Ignacia Braga TANAKA
Transgenerational effects in Mice Exposed to Continuous Low-Dose-Rate Gamma-rays, Pathological Study

**II-3** Dr. Keiji OGURA
Transgenerational effects in Mice Exposed to Continuous Low-Dose-Rate Gamma-rays, Genome Mutation Study

**II-4** Dr. Daisaku TAKAI
Response of B6C3$F_1$ Mice Continuously Irradiated with Low-Dose-Rate Gamma-rays to Transplanted Tumor Cells

**(Coffee break, 14:55-15:10)**

(Chair: Dr. Keiji SUZUKI, Nagasaki University)

**II-5** Dr. Shingo NAKAMURA
Relationship between Radiation-Induced Menopause and Body Weight Gain in Mice Continuously Irradiated with Low-Dose-Rate Gamma-rays

**II-6** Dr. Tokuhisa HIROUCHI
Mutations and Alterations of Expressions in Genes Associated with DNA Repair System in Leukemia Induced by Continuous Exposure to Gamma-rays at Low-Dose-Rates

**II-7** Dr. Atsushi KOHDA
Chromosomal Translocation Rates in Splenocytes of Mice Chronically Exposed to Low-Dose-Rate Gamma-rays

**16:25-16:30 Closing**


Memo


Memo

**I-1**

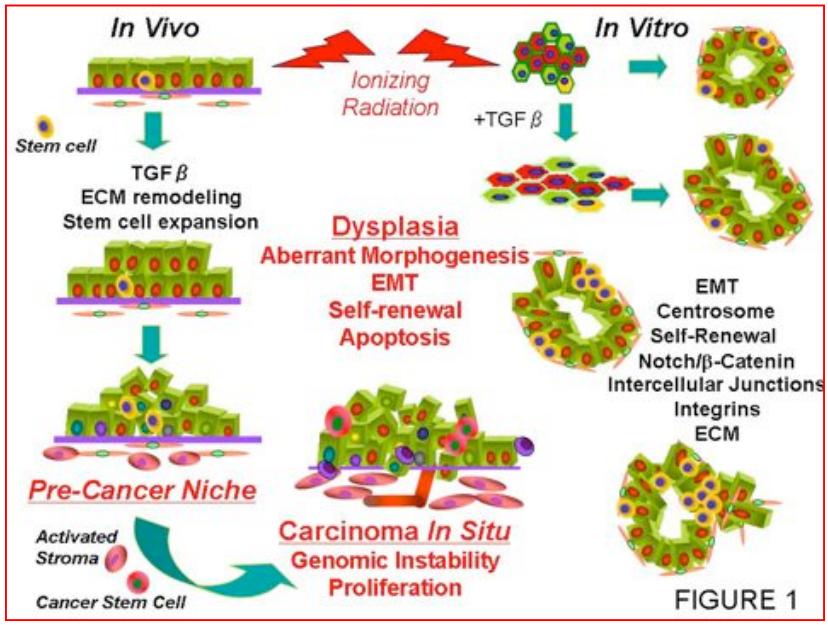
# Radiation Carcinogenesis: Microenvironment Matters

Mary Helen BARCELLOS-HOFF
Departments of Radiation Oncology and Cell Biology, New York University School of Medicine, New York, NY 10016 USA.

Ionizing radiation can alter genomic sequence as a result of damage due to targeted effects from the interaction of energy and DNA, and the response to damage can alter phenotype and multicellular interactions, referred to herein as non-targeted effects (NTE). We have previously demonstrated that high and low LET radiation affects epithelial phenotype, e.g. epithelial to mesenchymal transition (EMT), stromal remodeling and genomic instability (GIN), in human cells and mouse tissues, and have recently shown radiation affects stem/progenitor self-renewal *in vivo* (reviewed in (Barcellos-Hoff and Nguyen, 2009; Nguyen et al., 2010). We have demonstrated the relevance of NTE to radiation carcinogenesis using a mouse model to show that radiation effects on the microenvironment significantly alter the frequency and course of mammary cancer (Nguyen et al., 2011a). Our hypothesis is that radiation induced signaling disrupt tissue-level interactions that suppress malignant progression of genomically unstable epithelial cells.

Tissue microenvironments can either suppress or promote tumorigenesis. Our published and preliminary studies suggest that radiation exposure has very early and persistent effects on the tissue microenvironment prior to the development of cancer. We have identified one critical signal, TGFβ, in prior studies. Our published low LET studies have identified three event modified by radiation in irradiated mammary gland: Stem cell self-renewal, macrophage activation, and persistent stromal activation, all of which occur well before cancer develops (Nguyen et al., 2011b). Recent analysis of expression profiles from HZE irradiated tissues confirms that high LET irradiation also affects key macrophage and fibroblast pathways.

Our model is shown in Figure 1. We propose that 1) a *pre-cancer niche* is established between activated stromal cells (fibroblasts and/or macrophages) and epithelial cells undergoing neoplastic transformation; and 2) that such pre-cancer niches mediate the efficiency of mammary cancer development (Barcellos-Hoff et al., 2013). Our data lead shows that radiation actually primes the target epithelium by increasing the frequency of stem cells by inducing Notch and TGFβ stimulated symmetric division (Tang et al., 2013). This primes the host by recruitment of macrophages and activation of fibroblasts to form pre-cancer niches, which in turn malignant progression. This component of radiation carcinogenesis is particularly important to preventing cancer risk in susceptible populations such as those treated with radiation for childhood cancers.




**References**

Barcellos-Hoff, M. H., Lyden, D., and Wang, T. C. (2013). The evolution of the cancer niche during multistage carcinogenesis. Nat Rev Cancer 13, 511-518.Barcellos-Hoff, M. H., and Nguyen, D. H. (2009). Radiation carcinogenesis in context: How do irradiated tissues become tumors? Health Physics 97, 446-457.

Nguyen, D. H., Bochaca, I. I., and Barcellos-Hoff, M. H. (2010). The biological impact of radiation exposure on breast cancer development. In Breast Cancer and the Environment, J. Russo, ed. (New York: Springer ).

Nguyen, D. H., Oketch-Rabah, H. A., Illa-Bochaca, I., Geyer, F. C., Reis-Filho, J. S., Mao, J. H., Ravani, S. A., Zavadil, J., Borowsky, A. D., Jerry, D. J., et al. (2011a). Radiation Acts on the Microenvironment to Affect Breast Carcinogenesis by Distinct Mechanisms that Decrease Cancer Latency and Affect Tumor Type. Cancer Cell 19, 640-651.

Nguyen, N. H., Oketch, H. A., Geyer, F. C., Reis-Filho, J. S., Mao, J.-H., Ravani, S. A., Zavadil, J., Borowsky, A. D., Jerry, D. J., Dunphy, K. A., et al. (2011b). Radiation Acts on the Microenvironment to Affect Breast Carcinogenesis by Distinct Mechanisms that Decrease Breast Cancer Latency and Affect Tumor Type. Cancer cell In press.

Tang, J., Fernandez-Garcia, I., Vijayakumar, S., Martinez-Ruiz, H., Illa-Bochaca, I., Nguyen, D. H., Mao, J.-M., and Barcellos-Hoff, M. H. (2013). Irradiation of juvenile, but not adult, mammary gland increases stem cell self-renewal and estrogen receptor negative tumors. Stem Cells 10.1002/stem.1533.

**I-2**

# Age Dependence of Radiation Carcinogenesis in Animal Models

Yoshiya Shimada[1], Mayumi Nishimura[1], Kentarou Ariyoshi[1], Shusuke Tani[1], Yuka Ishida[2], Chizuru Tsuruoka[1], Tatsuhiko Imaoka[1], Yutaka Yamada[1], Shino Takeda[1], Yoshiko Amasaki[1], Yi Shang[1], Tomoko Sawai[1], Shinobu Hirano[1], Ken-ichi Iwata[1], Ayaka Hosoki[1], Takamitsu Morioka[1] and Shizuko Kakinuma[1]

[1]Radiobiology for Children's Health Research Program,
[2]Department of Technical Support and Development,
National Institute of Radiological Sciences

With the advance of radiological techniques in medicine, radiation dose of child patients has been rapidly increased in these two decades. Since children are highly susceptible to radiation-induction of cancer, the cancer risk of radiation has been a great concern. Therefore, it is important to access the cancer risk after childhood exposure accurately.

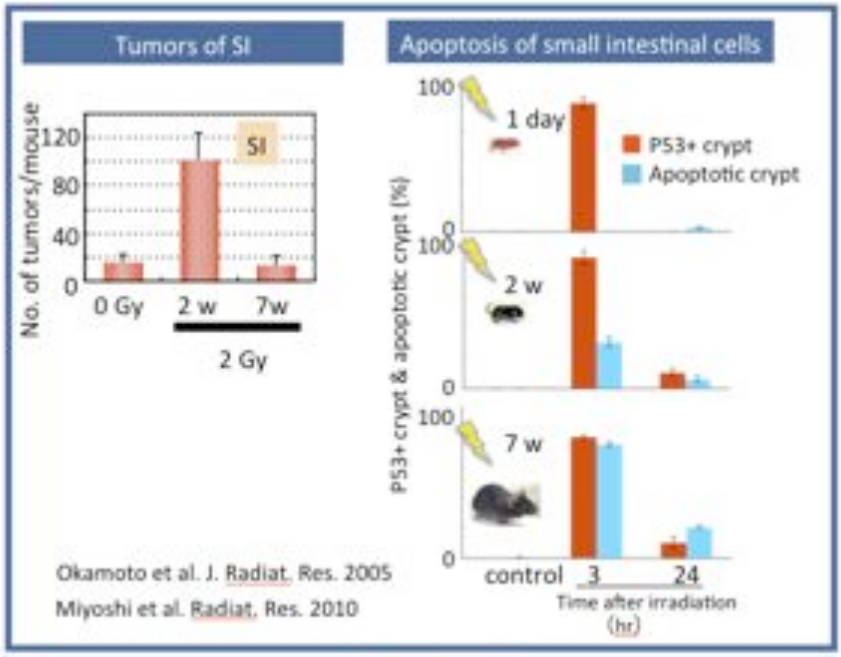
Recently we have started the project to elucidate the age dependence of radiogenic cancer risk and the underlying mechanism. We here introduce the scheme of our project, and show recent results on the effect of early-life radiation exposure on lifespan shortening and cancer induction in mouse and rat models.

**Life span shortening**: Life shortening was investigated in both genders of B6C3F1 (C57BL/6 x C3H) mice exposed to gamma rays, carbon ions (290-MeV/u; 13 keV/um) and neutrons (1-2 MeV). Mice were irradiated in utero or at 1, 3, 7 and 15 weeks after birth. Linear dose-response curves were obtained. For mice exposed to gamma rays at 7 weeks of age, the slope was steeper for female than male, indicating that female is more susceptible than male. Irradiation at 1 week of age further increased the slope and no gender difference was observed. In utero exposures had negligible effect for both genders. The experiments on carbon ions and neutrons are now in progress.

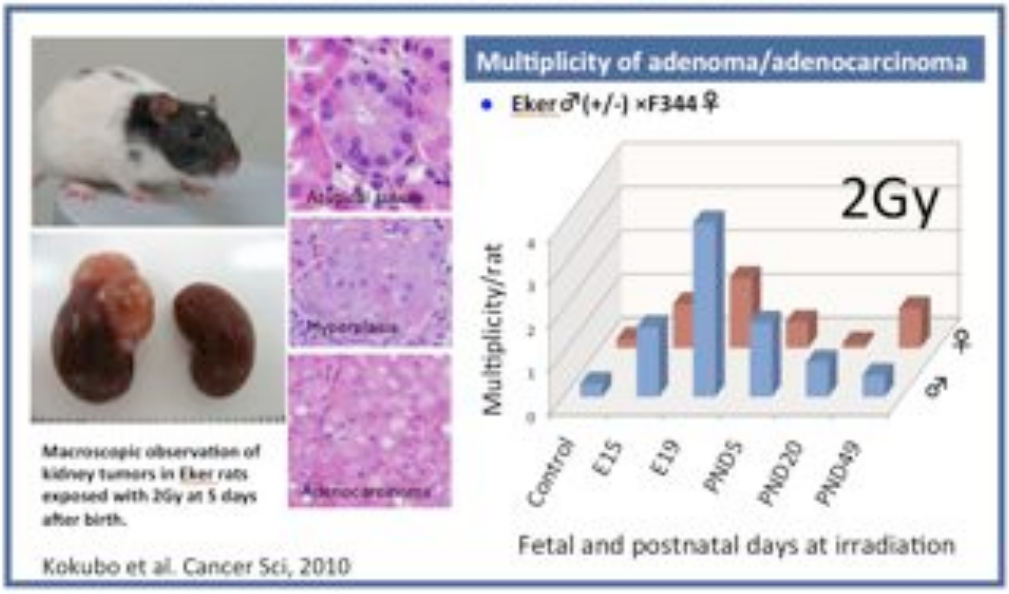
**Cancer induction**: It was reported that there is a susceptible age-window for radiation carcinogenesis in a tissue dependent (Figures). We further extended the tissue types with additional tumor models and demonstrated the susceptible age-window for each organ. These include brain, kidney, intestine, lung and mammary glands.

**Biological mechanisms of age dependence**: High incidence of tumors after irradiation at juvenile is ascribed to extensive cell division during growth, which results in the rapid fixation of DNA damage and gives chance for mutant cells to clonally expand. We introduce the unique response of immature/progenitor cells and tissue microenvironment to ionizing radiation, which are dependent on the age-at-exposure.

## Unique characteristics of radiation-induced apoptosis in infant intestinal cells




## Induction of kidney tumor in Eker rats



**I-3**

# A Mouse Model of Radiation-Induced Leukemia, Past, Present and Future Work

Christophe BADIE

Cancer Genetics and Cytogenetics group, Biological Effects Department
Centre for Radiation, Chemical & Environmental Hazards Public Health England

Animal models of human cancers are extremely valuable for a better understanding of the molecular mechanisms of tumour initiation and development, in particular for cancers induced by environmental agents such as ionizing radiation (IR). Radiation-induced acute myeloid leukaemia (rAML) is one IR induced tumour for which mouse models are available. The CBA/H mouse model of rAML has been studied for decades to bring to light the molecular mechanisms associated with multistage carcinogenesis. A specific interstitial deletion of mouse chromosome 2 found in a high proportion of rAML is recognised as the initiating event; this partial hemizygous deletion is a common feature in CBA/H and several other susceptible strains. The deletion is an early event detectable 24 hours after exposure in bone marrow cells. The deletion leads to the loss of Sfpi, a gene essential for haematopoietic development. Its product, the transcription factor PU.1 acts as a tumour suppressor in this model. Although the deletion can be detected by cytogenetic techniques, precise characterization of the haematopoietic cells carrying the deletion and the study of their fate in vivo couldn't so far be achieved. It is assumed that leukaemia originates in an early progenitor cell or haematopoietic stem cell but it is unknown whether the original chromosome damage occurs at a similar frequency in committed progenitors and stem cells. In a recent study we monitored the frequency of chromosome 2 deletions in immature bone marrow cells (Lin−) and haematopoietic stem cells/multipotent progenitor cells (LSK) by several techniques, using a reporter gene model. We showed that partial chromosome 2 deletions are present in the LSK subpopulation (Table 1). After transplantation, these cells didn't show an advantage for growth and in vivo repopulation, at least at early stages following occurrence.

Using the same genetically engineered CBA/H mouse model expressing the GFP fluorescent molecule under the control of the Sfpi1 promoter, we demonstrated that GFP expression did not interfere with X-ray induced leukaemia incidence and that GFP fluorescence in live leukaemic cells is a surrogate marker of radiation-induced chromosome 2 deletions (Fig.1). This is the first experimental evidence for the detection of this leukaemia initiating event in live leukemic cells.

New mouse models are currently being developed for investigating individual pre-leukaemic cell fate within its natural microenvironment in order to dissect the steps in tumour initiation, promotion and malignant progression. This could lead to new insights into the nature of the molecular mechanisms and identification of the cell of origin in radiation-induced leukaemia.

This report is commissioned by the National Institute for Health Research. Financial support was also provided by the European Union FP7 DoReMi network of excellence.

**Table 1**

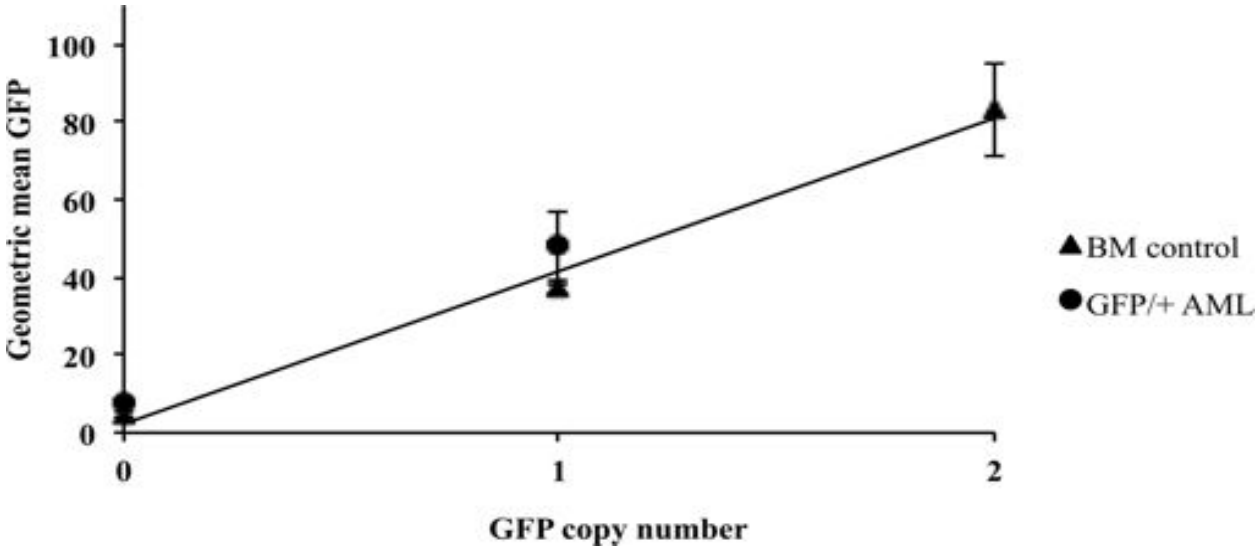
Chromosome 2 interstitial deletions in LSK cells sham exposed (control) or exposed to a 3 Gy X-rays dose (irradiated) detected by BAC-FISH.

| | Metaphase scored | Retained | Deleted | PU.1 loss (%) |
|---|---|---|---|---|
| LSK control | 67 | 66 | 1 | 1.5 |
| LSK irradiated (*in vivo*) | 100 | 94 | 6 | 6** |
| LSK irradiated (*in vitro*) | 32 | 30 | 2 | 6.2*** |

** $p = 0.0064$.
*** $p = 0.0068$ for differences between irradiated and control populations.

Olme CH et al. Mutat Res. 2013 Aug 30;756(1-2):119-26

**Figure 1**




Correlation between geometric mean of GFP expression and GFP copy number in unirradiated mouse bone marrow cells and rAML
Olme CH et al. Leuk Res. 2013 Oct;37(10): 13

**I-4**

# Low Dose Rate Exposure Enhances Interindividual Differences in DNA DSB Repair

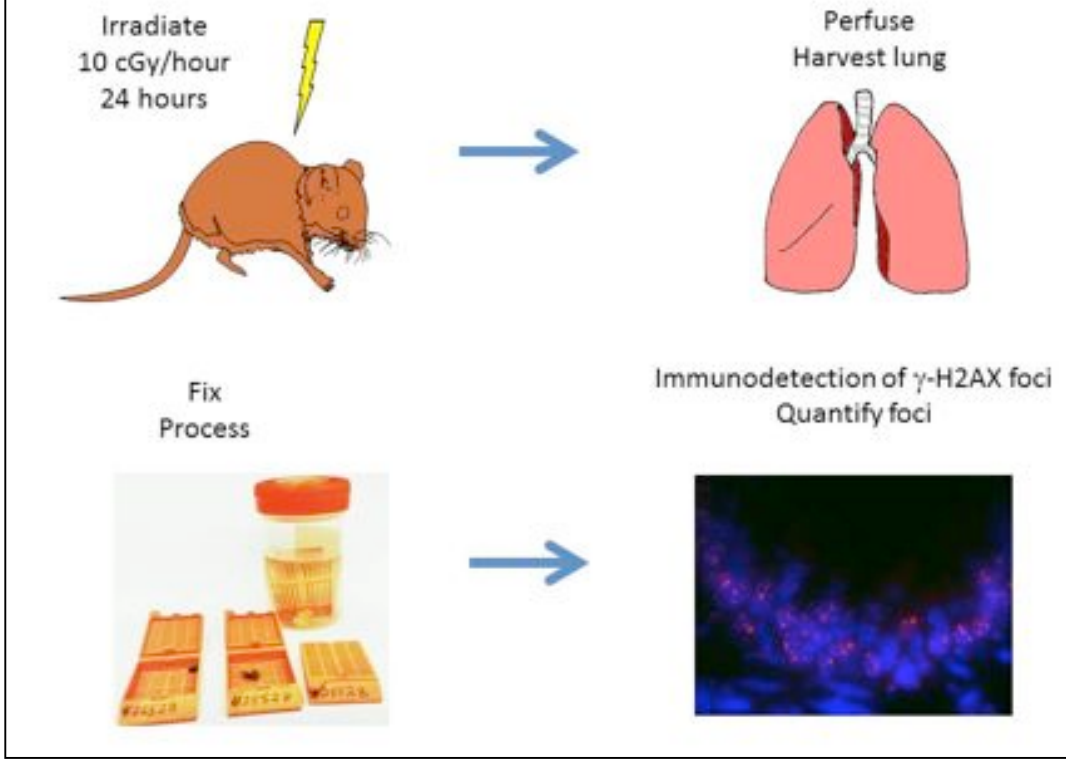
Michael M. WEIL, Takamitsu KATO, Peter DEMANT, Donasian OCHOLA, and Joel S. BEDFORD

Department of Environmental and Radiological Health Sciences, Colorado State University

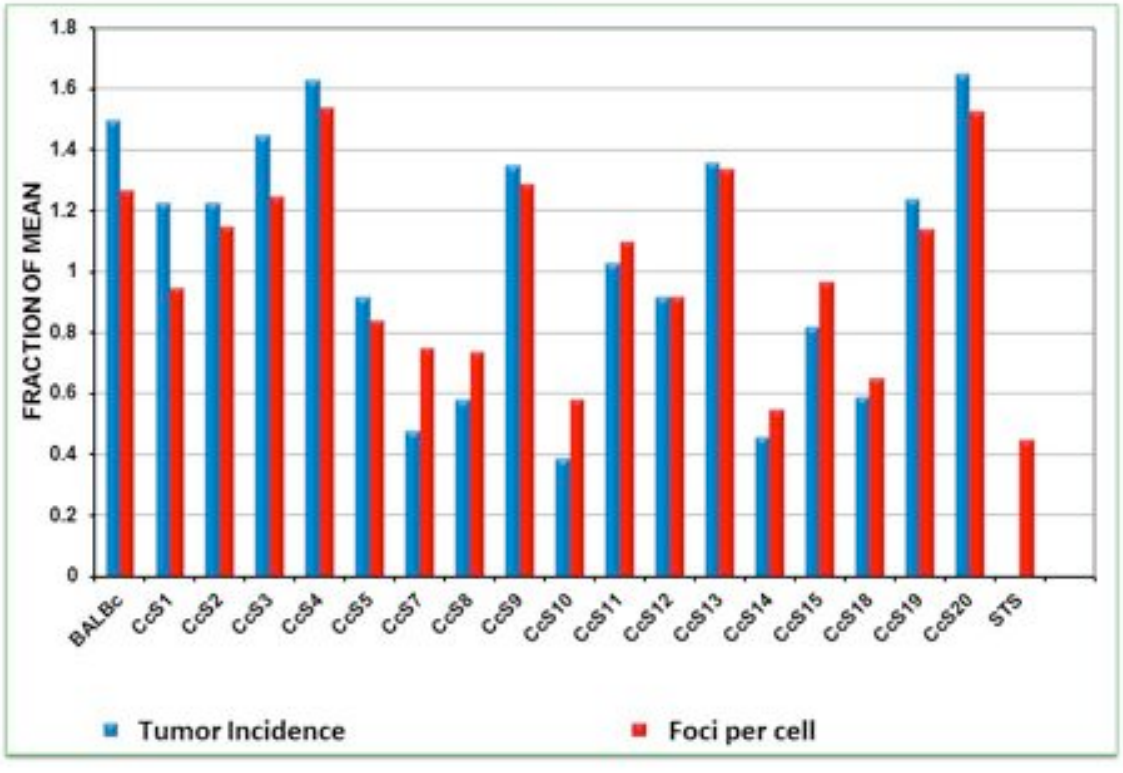
Individuals differ in their capacity to repair radiation-induced DNA double strand breaks (DSB) efficiently. Low dose rate radiation exposure followed by immediate quantification of repair foci in exposed cells provides a much more sensitive assay for DNA DSB repair efficiency than time-delayed measurements after acute high dose rate exposures. We have used this technique to assess the repair proficiency of fibroblasts derived from retinoblastoma patients, ataxia-telangiectasia (A-T) patients, their unaffected family members and clinically normal, control individuals. The assay can identify members of A-T families who carry a single mutated copy of the *ATM* gene. Fibroblasts from these individuals, while not nearly as deficient in DNA DSB repair as fibroblasts from A-T patients with two defective *ATM* alleles, are nevertheless less adept at repair than fibroblasts from most normal controls. While the number of families studied is small, it also appears that the parents of retinoblastoma patients with *de novo RB1* mutations are less repair proficient than most normal controls. This observation, along with the finding of interindividual differences in DNA DSB repair in clinically unremarkable individuals, may contribute to our understanding of the events underlying the transmission of *de novo* germline mutations.

We are currently using fibroblasts from recombinant inbred mouse strains to examine the role of specific genetic polymorphisms in DNA DSB repair proficiency. Also using these mouse strains we are examining the possible connection between the genetically based differences in relative susceptibilities that have been observed for radiogenic cancers in the recombinant congenic mouse strains and the corresponding sensitivities as measured in the low dose rate based DNA DSB repair assay. For this, we are using the molecular focus assay directly in cells of relevant tissues in low dose rate irradiated mice. This will further allow a direct comparison of possible tissue dependent heterogeneity in cellular sensitivity using this assay.

# METHODS




## Comparison of Lung Tumor Incidence and Foci per Cell



**I-5**

# DNA damage accumulation in tissues/tissue stem cells exposed to low-dose-rate radiation

Keiji SUZUKI

Department of Radiation Medical Sciences, Atomic Bomb Disease Institute, Nagasaki University.

Absorption of radiation energy into DNA gives rise to DNA damage, among which DNA double strand breaks (DSBs) are the most lethal and carcinogenic ones. The spatiotemporal dynamics of DSBs is well examined after high-dose and high-dose-rate exposure. However, the information is still limited on the effects of low-dose and low-dose-rate exposure, particularly *in vivo*. Therefore, we aim to investigate accumulation of DSBs in various tissues and organs obtained from mice exposed to γ-rays at different dose-rates.

B6C3F1 mice were irradiated with $^{137}$Cs γ-rays at low-dose-rate (LDR) of 400 mGy/22 h/day (0.3 mGy/min) for 10 days (Figure 1). For high-dose-rate (HDR) exposure, mice received 4 Gy at 850 mGy/min. After completion of radiation exposure, various tissues and organs, including lung, were isolated. Formalin-fixed samples were embedded in paraffin, and then, tissue slices were obtained. Antigen retrieval was performed at 95°C for 40 min. DSBs and proliferating cells were quantified by immunofluorescence using anti-53BP1 and anti-Ki-67 antibodies, respectively. The images were captured and analyzed by FW4000 software.

DSBs were detectable in the epithelial cells of the bronchioles obtained from unirradiated control mice (approx. 0.04 foci/cell), however, HDR exposure significantly increased the number of DSBs in all bronchiolar epithelial cells, whose yield was more than 15 foci/cell (Figure 2). While DNA repair pathway efficiently removed DSBs, approximately 8 foci/cell were still remained in 50% of cells even 3 days after irradiation. LDR exposure also induced DSBs but to a much lesser extent, and approximately 1 focus/cell was observed in about 60% of cells. After 3 days, the DSB levels were dropped down to 0.11 foci/cell, and foci-positive cells were about 10%. Intriguingly, the frequency of Ki-67 was significantly increased in the bronchioles 3 days after HDR exposure.

These results demonstrated that the induction and repair of DSBs were detectable *in vivo*. HDR exposure efficiently induced DSBs, whereas not the same effect was observed by LDR exposure, even if the total dose was the same. Moreover, a half of exposed cells still harbored at least one focus 3 days after HDR exposure, which was in contrast to LDR exposed cells that repaired DSBs in approximately 90% of them. Thus, the effect of HDR exposure is far greater than that of LDR exposure, indicating that multiplicity and complexity of DSBs could be different between HDR and LDR exposures. Since excess tissue proliferative response was observed only after HDR exposure, this should take into account to estimate cancer risk from radiation exposure.

This work has been performed in collaboration with IES.

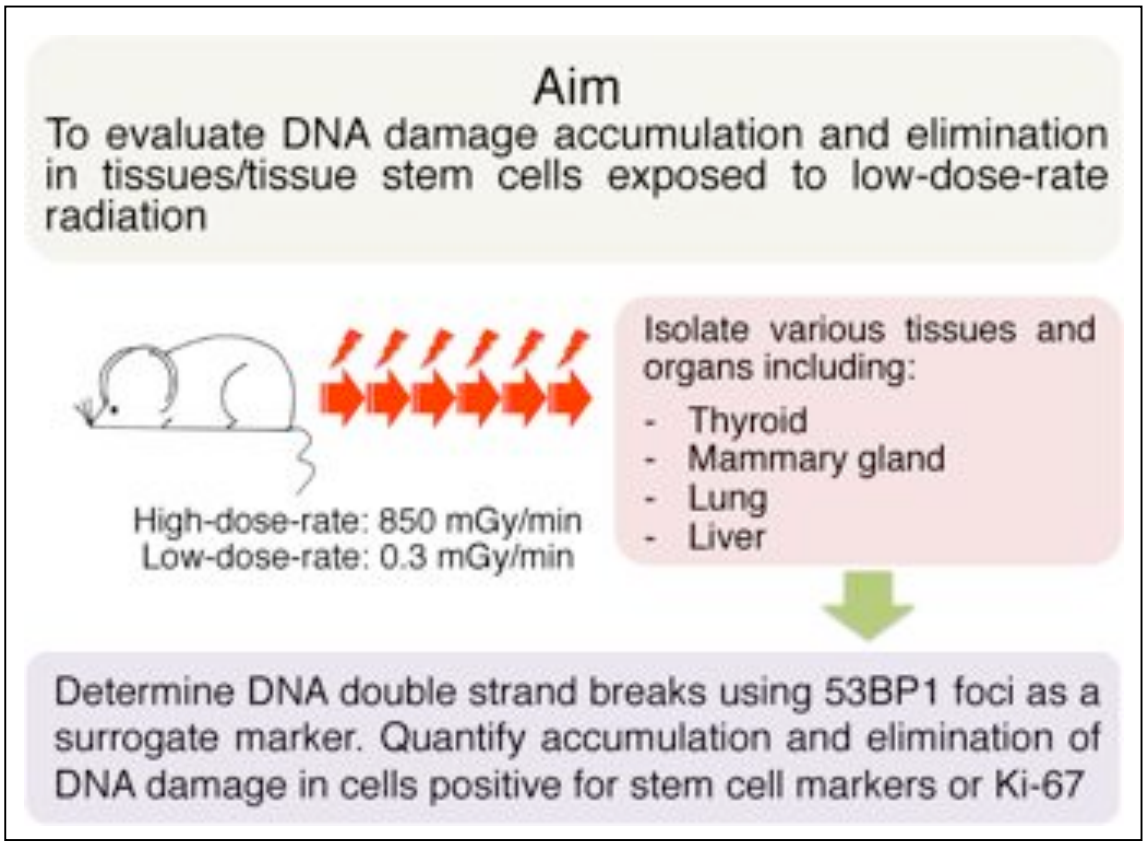



Figure 1

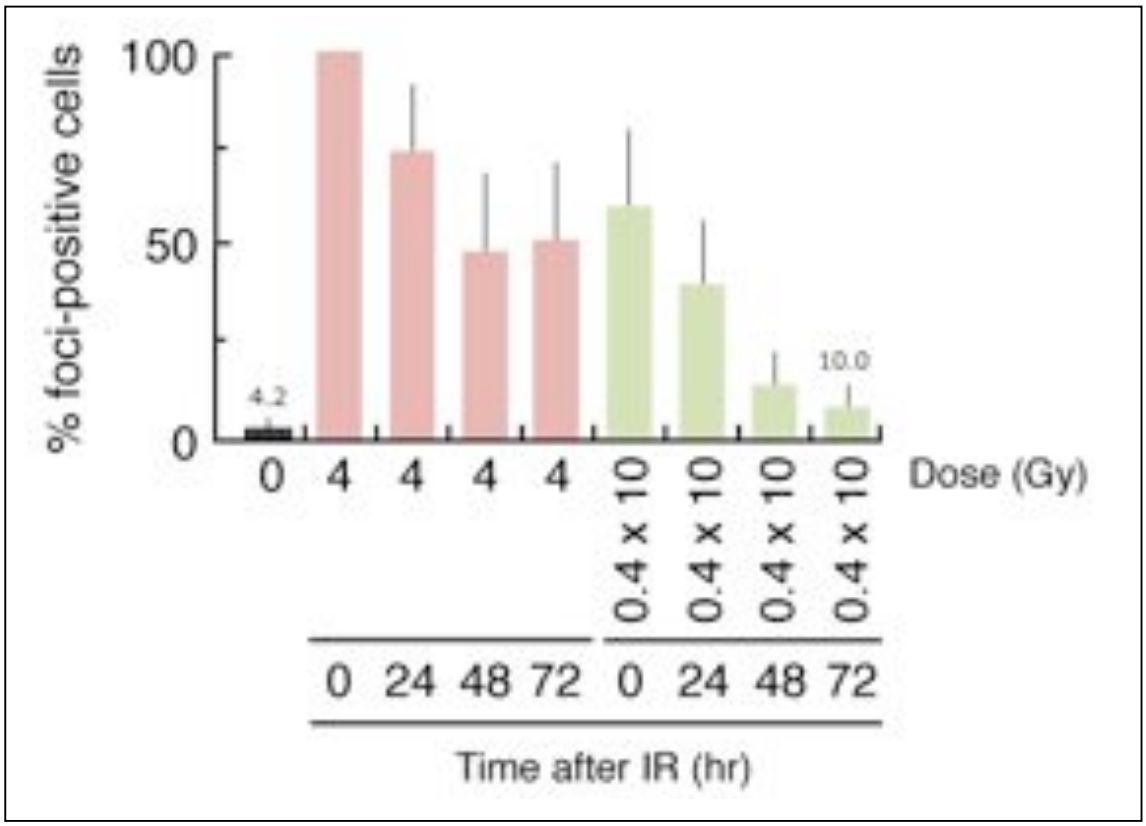



Figure 2


Memo


Memo

**II-1**

# Outline of the IES Research Projects on Biological Effects of Low-Dose-Rate Radiation

Tetsuya ONO
Institute for Environmental Sciences

The biological effects of low dose radiation are not understood well and the risk estimation is based on a hypothesis called a linear non-threshold model. It was not a big issue when the low dose exposure was limited mostly to a specific group of people like radiation workers, medical practitioners, scientists, etc. However, the two nuclear power plant accidents in Chernobyl and Fukushima have pulled out people's strong attention to the risk of low dose exposure. Elucidation of the biological responses to the low dose radiation is now an urgent theme for radiation biology.

The low dose of radiation can be exposed in either acute or chronic ways. IES founded in 1990 has chosen a long-term low-dose-rate chronic irradiation as a target to challenge. The dose-rate was set at two levels, 400 mGy/400 day and 20 mGy/400 day. These are comparable to the exposure in space station and the legal dose limit for radiation workers, respectively. As a positive control, the effects of 8,000 mGy/400 day were also examined in parallel. Mice were used as experimental animals. They were irradiated with gamma-rays from $^{137}$Cs for 22 hours every day and it was continued for 400 days under SPF (specific pathogen-free) conditions. The remaining 2 hours (10:00-12:00) were not used for irradiation but spent for health check of the mice, supply of food and water, etc. The biological indices studied so far were life span, cause of death, cancer incidence, pathological change, body weight, chromosome abnormality, somatic mutation frequency, alteration in gene expression, immunological competence against cancer cells, and trans-generational effects examined on life span, cancer incidence and genomic change. All of these indices revealed some influence if the total dose of radiation was 8,000 mGy. One exception was cancer incidence in the offspring of irradiated males. Slight but significant effects were observed for limited indices in 400 mGy-irradiated mice; life span in females, chromosomal abnormality and gene expression. The total dose of 20 mGy, on the other hand, revealed little detectable effects (Table 1).

Now, we are planning to extend our studies to the following three questions:

(1) Dose-rate dependency for trans-generational effects on life shortening and cancer induction.
(2) Effects of low-dose-rate irradiation on embryo and fetus.
(3) Molecular and cellular mechanisms of low-dose-rate irradiation.

Our goal is to clarify the biological effects of low-dose-rate irradiation in experimental animals. It will provide important information for understanding low dose radiation effects in humans.

This study was performed under contract with the Aomori Prefectural Government, Japan.

## Table 1. Summary of the effects observed in IES

| Index \ Total dose | 20 mGy | 400 mGy | 8,000 mGy | Ref. |
|---|---|---|---|---|
| Life shortening | – | +/– | + | 1 |
| Cancer induction | – | – | + | 2 |
| Trans-generational effect | – | – | + | 3 |
| Chromosome aberration | (–) | + | + | 4 |
| Gene mutation | – | – | + | 5, 6 |
| mRNA level | + | + | + | 7, 8 |
| Protein level | – | + | + | 9 |

– ; no effect
+/– ; shortening in female, but no effect in male
+ ; effective
(–) ; preliminary result


1. Radiat Res 160: 367 (2003)
2. Ibid 167: 417 (2007)
3. Unpublished results
4. Radiat Res 171: 290 (2009)
5. Data Sci J 8: 36-41 (2009)
6. Radiat Res 173: 138 (2010)
7. J Radiat Res 50: 241 (2009)
8. Radiat Res 174: 611 (2010)
9. J Radiat Res 49: 661 (2008)

**II-2**

# Transgenerational Effects in Mice Exposed to Continuous Low-Dose-Rate Gamma-Rays – Pathological Study –

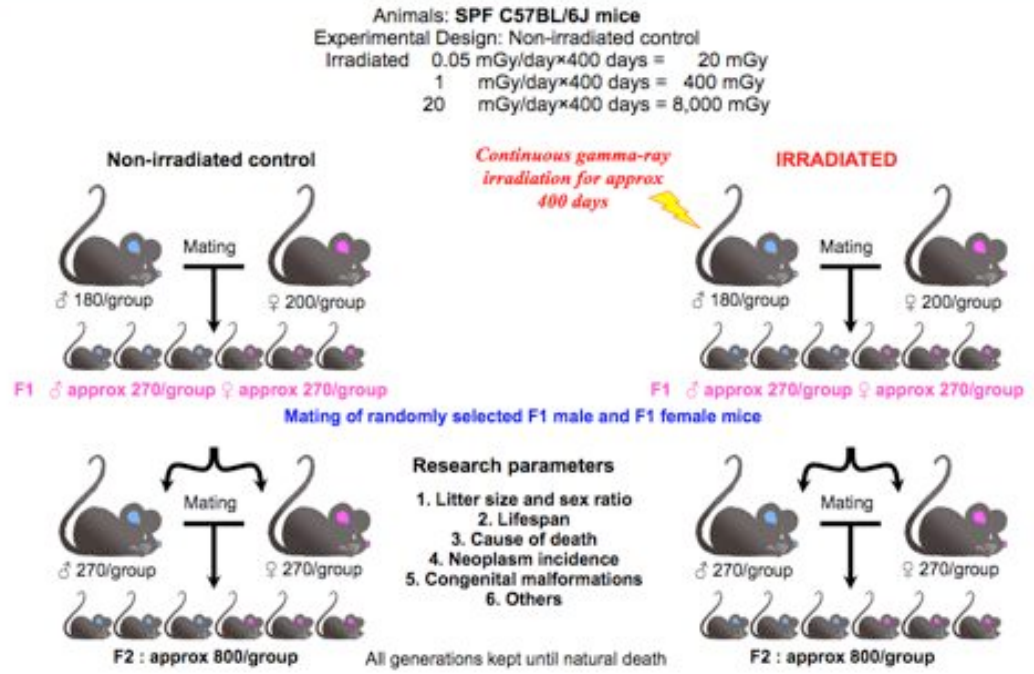
Ignacia TANAKA and Satoshi TANAKA

Department of Radiobiology, Institute for Environmental Sciences.

To study the effects of continuous low-dose-rate gamma-ray irradiation on the progeny of mice, males (sires) were irradiated for 400 days with $^{137}Cs$ gamma-rays at low-dose-rates of 20 mGy/22 h/day, 1 mGy/22 h/day, and 0.05 mGy/22 h/day with accumulated doses equivalent to 8000 mGy, 400 mGy, and 20 mGy, respectively. Immediately after completion of irradiation, the male mice were bred with non-irradiated females to produce $F_1$ mice. Randomly selected $F_1$ males and females were bred to produce $F_2$ mice. All mice, except the dams of $F_1$ mice, were subjected to pathological examination upon natural death. Lifespan, cancer incidence and number of offspring were used as parameters to evaluate the biological effects of low-dose-rate irradiation (Fig 1). There were no significant differences in the pregnancy rate and weaning rate in the parent generation. There were, however, significant decreases in the mean litter size ($p$=0.029), as well as the mean number of weaned pups ($p$=0.023) per female bred to males exposed to 20 mGy/22 h/day compared to the non-irradiated controls (Table 1). Partial results show that significant decreases in the lifespan of male parent mice (F0, $p$=0.001) exposed to 20 mGy/22 h/day and their male progenies ($F_1$, $p$=0.006) were observed. No significant differences were found in the cause of death and cancer incidence in $F_1$ and $F_2$ progeny mice.

This study was performed under contract with the Aomori Prefectural Government, Japan.

## Transgenerational Effects in Mice Exposed to Continuous Low-Dose-Rate Gamma-Rays – Pathological Study –




## Breeding results of F0

| | Mean birth rate (%) | Mean litter size | Mean weaning rate (%) | Mean weaning size |
|---|---|---|---|---|
| Non-irradated | 65 | 5.4±2.1 | 75.3±33.3 | 4.4±2.4 |
| 0.05 mGy/22h/day | 59.8 | 5.1±1.9 | 76.5±36.2 | 4.2±2.4 |
| 1 mGy/22h/day | 58.4 | 5.2±1.8 | 79.7±31.6 | 4.3±2.1 |
| 20 mGy/22h/day | 59.8 | 4.9±1.8* | 69.8±33.3 | 3.7±2.4* |

*; $p < 0.05$

**II-3**

# Transgenerational effects in Mice Exposed to Continuous Low-Dose-Rate Gamma-Rays, Genome Mutation Study

Keiji OGURA[1], Hidekazu NEGISHI[2], Chihiro HARADA[2], Katuyoshi FUJIKAWA[1], Satoshi TANAKA[1], Ignacia BRAGA-TANAKA III[1], Junichiro KOMURA[1]

[1]Department of Radiobiology, Institute for Environmental Sciences, [2]Japan Animal Care

In the past, trans-generation effects of radiation have been examined using phenotypic markers like coat color. Recent advances in technology enabled us to examine a whole genome at molecular level. Here we adopted one of these techniques called comparative genome hybridization (CGH) and studied trans-generational effects on DNA copy number aberrations (CNAs). C57BL/6J mice were exposed to low-dose-rate (LDR) gamma rays (20 mGy/22 h/day; 909 μGy/h) for 400 days (total dose: 8000 mGy) from 8 weeks of age. We analyzed, so far, a total of 333 genomes from mice (111 progenies from 20 pairs of parents in 20 mGy/22 h/day irradiated group, and 140 progenies from 21 pairs of parents in non-irradiated group) using oligo-microarray CGH (Agilent Technologies). The results indicate that the progeny from the 20 mGy/22 h/day irradiated group had significantly higher frequencies of genomic aberrations compared to non-irradiated mice (21.6% vs 11.4%, Table 1). Next we asked if the F1 mice harboring CNAs could have shorter life spans than the F1 that did not have aberration (Table 2). Although some trends were suggested, the statistical analysis reveled no significant difference between these two groups. The difference in life shortening between mice with or without DNA aberrations after exposure to LDR radiation was not significant due to the small number of samples. In addition, we will discuss the possibility of genomic instability in somatic tissues being induced in the 20 mGy/22 h/day irradiated group mice.

This study was performed under contract with the Aomori Prefectural Government, Japan.

Table 1. Results of the genomic aberrations analysis using the oligo-microarray CGH

| | No. of analyzed $F_1$ mice | No. of mice with aberrations | No. of loci with aberrations |
|---|---|---|---|
| 20 mGy/ 22h/day irradiated group | 111 | 24 (21.6 %) | 175 (Ave. 1.58 /generation) |
| Non irradiated group | 140 | 16 (11.4 %) | 21 (Ave. 0.15 /generation) |
| | | P =0.03 | P<0.01 |

Table 2. Relationship between the presence of aberrations and life span in $F_1$ mice

| | | Aberrations | Life span | Cause of death: Category | Cause of death: Major | |
|---|---|---|---|---|---|---|
| 20 mGy/ 22h/day irradiated group (111 mice) | ♂ | Yes 13 (20.6%) | 794.8 ±170.7 | Neoplasms 8 (66.6%)<br>Inflammation 1 (8.3%)<br>Others 3 (25.0%) | Lymphoma, Malignant | 38% |
| | | No 50 (79.3%) | 843.7 ±231.2 | Neoplasms 23 (48.9%)<br>Inflammation 13 (27.7%)<br>Others 11 (23.4%) | Lymphoma, Malignant | 14% |
| | ♀ | Yes 11 (22.9%) | 706.1 ±182.7 | Neoplasms 9 (81.8%)<br>Inflammation 1 (9.1%)<br>Others 1 (9.1%) | Lymphoma, Malignant | 45% |
| | | No 37 (77.1%) | 799.8 ±154.3 | Neoplasms 29 (78.4%)<br>Inflammation 4 (10.8%)<br>Others 4 (10.8%) | Lymphoma, Malignant | 43% |
| non irradiated group (140 mice) | ♂ | Yes 7 (10.4%) | 787.9 ±340.5 | Neoplasms 3 (42.9%)<br>Inflammation 2 (28.6%)<br>Others 2 (28.6%) | Lymphoma, Malignant | 14% |
| | | No 60 (89.6%) | 905.5 ±172.2 | Neoplasms 34 (58.6%)<br>Inflammation 14 (24.1%)<br>Others 10 (17.2%) | Lymphoma, Malignant | 22% |
| | ♀ | Yes 9 (12.3%) | 699.0 ±167.0 | Neoplasms 7 (77.8%)<br>Inflammation 0 (0.0%)<br>Others 2 (22.2%) | Lymphoma, Malignant | 67% |
| | | No 64 (87.7%) | 804.5 ±158.5 | Neoplasms 43 (69.4%)<br>Inflammation 12 (19.4%)<br>Others 7 (11.2%) | Lymphoma, Malignant | 41% |

## II-4

# Response of B6C3F$_1$ Mice Continuously Irradiated with Low-Dose-Rate Gamma-Rays to Transplanted Tumor Cells

Daisaku TAKAI

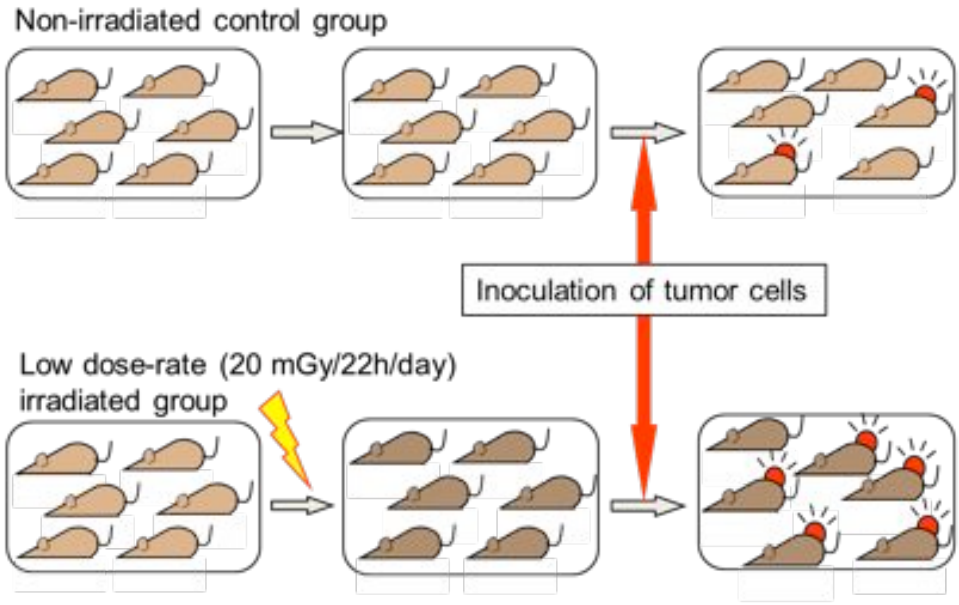
Department of Radiobiology, Institute for Environmental Sciences

Previously, Tanaka et al. reported significant life-shortening due to early neoplastic death in specific-pathogen-free B6C3F$_1$ mice irradiated continuously with low dose-rate (20 mGy/22 h/day) gamma-rays to a total dose of 8000 mGy (high dose). To understand the mechanisms responsible for the early neoplastic death, we transplanted syngeneic ovarian granulosa tumor cells, OV3121, into age-matched irradiated and non-irradiated control mice and found enhanced transplantability of the tumor cells in irradiated mice. Then we examined the expression of chemokine receptors relevant to tumor immunity in recipient mice and found that compared with non-irradiated control mice, expressions of some chemokine receptors were reduced in irradiated mice. Moreover, mice with reduced expression of chemokine (C-C motif) receptor type 5 (Ccr5) showed enhanced tumor transplantability. We hypothesized that immune cells with Ccr5 migrated to the transplantation site in recipient mice and contributed to the eradication of tumor cells. Based on the assumption that the migration of the immune cells to the transplantation site would be induced by a chemokine ligand from the transplanted tumor cells, we have examined whether the transplantability of OV3121 cells in non-irradiated B6C3F$_1$ mice is enhanced if the expression of a chemokine (C-C motif) ligand 8 (Ccl8), which is known to bind Ccr5, is knocked down in the inoculated cells. The result showed that the tumor transplantability of OV3121 (Ccl8$^-$) was enhanced compared with OV3121 (Ccl8$^+$).

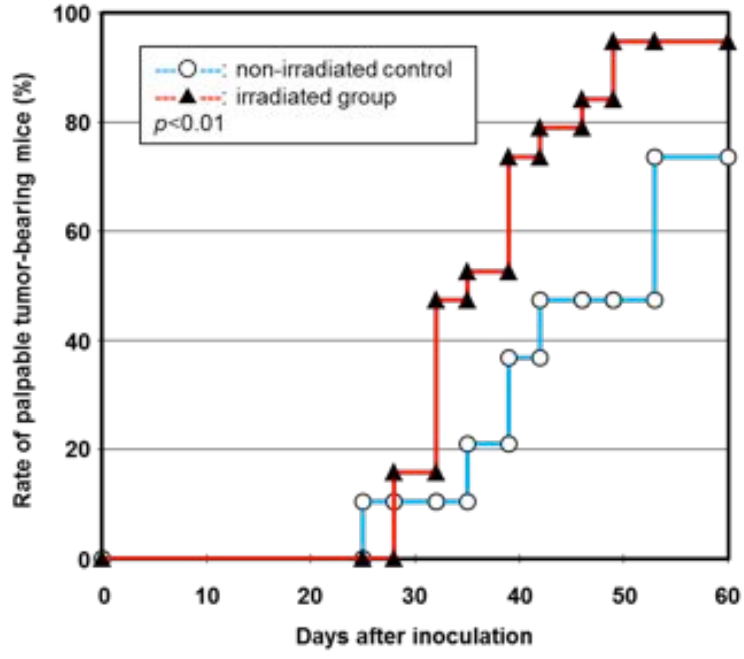
These suggest that low dose-rate gamma-irradiation disturbs the balance of chemokine network and reduces anti-tumor immunity. The understanding of the detailed role of chemokine network in tumor transplantability in irradiated mice requires further investigation.

This study was performed under contract with the Aomori Prefectural Government, Japan.

# Tumor cell transplantation assay




# Tumor cell transplantability



## II-5

# Relationship between Radiation-Induced Menopause and Body Weight Gain in Mice Continuously Irradiated with Low-Dose-Rate Gamma-Rays

Shingo NAKAMURA, Satoshi TANAKA, Ignacia Braga-Tanaka III

Department of Radiobiology, Institute for Environmental Sciences.

We previously reported a significant increase in body weights of B6C3F1 female mice continuously exposed to low dose-rate (20 mGy/22 h/day) γ-rays as compared to that of age-matched non-irradiated control mice (*Radiat Res.* 2010, **173**, 333-341). To clarify the underlying mechanisms of body weight gain observed after exposure to continuous low dose-rate irradiation, we examined adipose tissue weights, liver and serum lipid contents, and factors related to lipid metabolism (adipocytokines), as well as ovarian dysfunction in female B6C3F1 mice continuously irradiated with γ-rays at 20 mGy/22 h/day from 9 to 44 weeks of age. Significant body weight gains, related to tissue adiposity, were observed from 30 to 44 weeks of age (accumulated dose = 3 to 5 Gy) in the irradiated mice (Fig. 1). Histopathological analyses of ovaries and vaginal smears from irradiated mice showed gradual depletion of viable oocytes as irregular estrus cycles become increasingly frequent leading menopause and that these were observed to occur within the same period the increased body weight gain (Fig.2 & Fig. 3). These results suggested a possibility that the radiation-induced premature menopause could trigger body weight gain due to adiposity in B6C3F1 female mice continuously irradiated with low dose-rate γ-rays at 20 mGy/22 h/day.

This study was performed under contract with Aomori Prefectural Government, Japan.

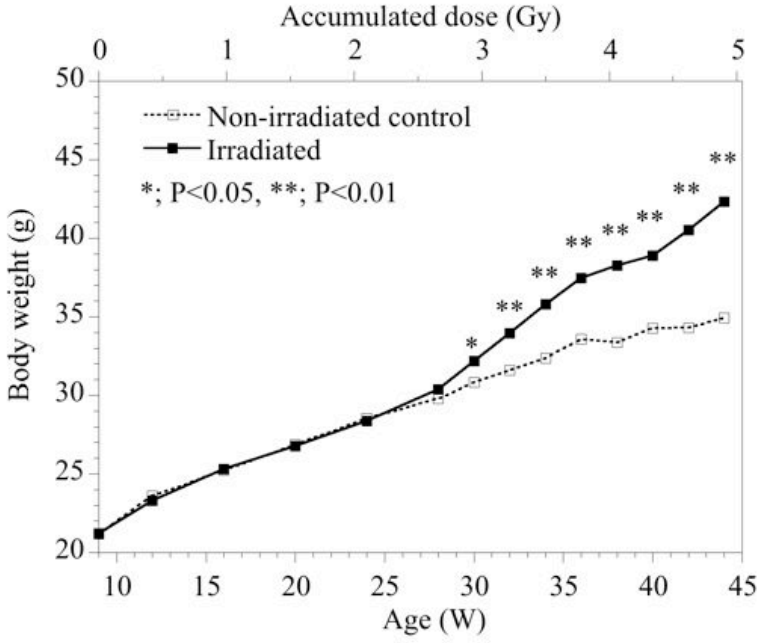



Fig. 1

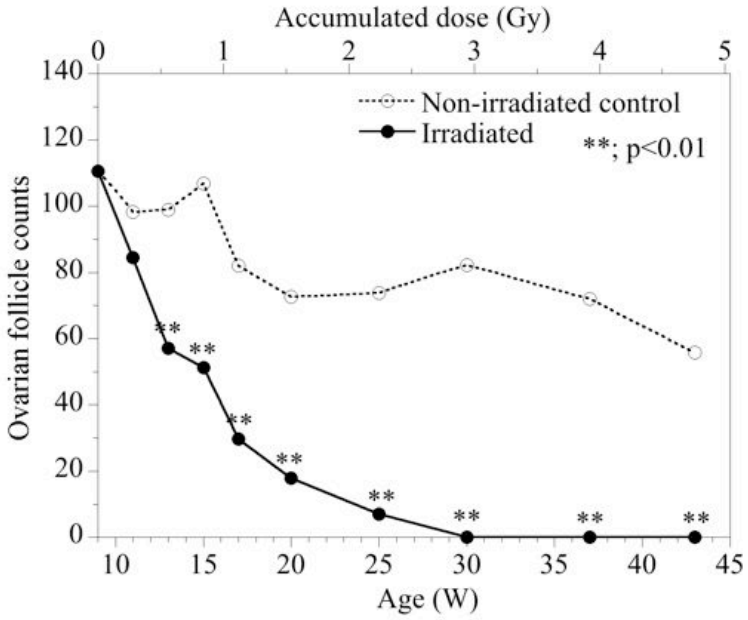

Accumulated dose (Gy)
0
1
2
3
4
5
140
120
100
80
60
40
20
0
Ovarian follicle counts
Non-irradiated control
Irradiated
**; p<0.01
**
10
15
20
25
30
35
40
45
Age (W)

Fig. 2

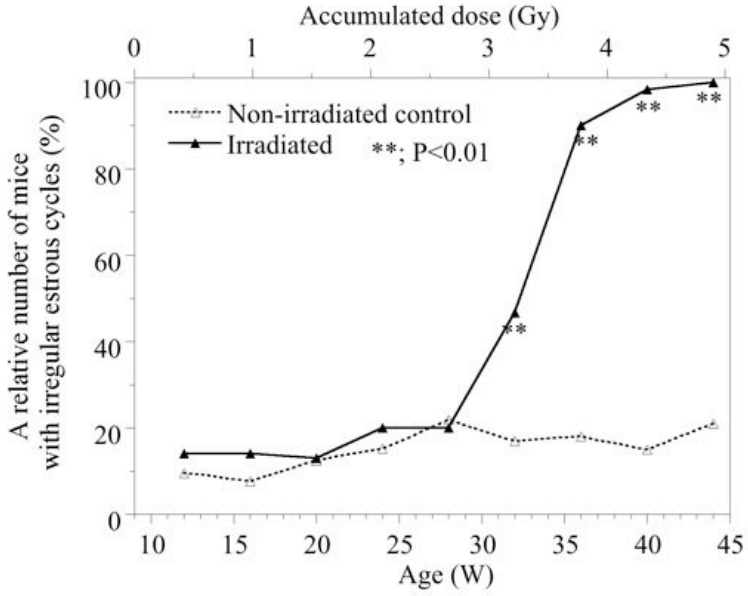

Accumulated dose (Gy)
0
1
2
3
4
5
100
80
60
40
20
0
A relative number of mice
with irregular estrous cycles (%)
Non-irradiated control
Irradiated
**; P<0.01
**
10
15
20
25
30
35
40
45
Age (W)

Fig. 3

## II-6

# Mutations and Alterations of Expressions in Genes Associated with DNA Repair System in Leukemia Induced by Continuous Exposure to Gamma-Rays at Low-Dose-Rates

Tokuhisa HIROUCHI

Department of Radiobiology, Institute for Environmental Sciences.

It is important to understand why mice exposed to high-dose γ-rays at a low-dose rate (LDR) have a significantly higher leukemia incidence than non-irradiated mice, although LDR γ-ray irradiation induces very few DNA double strand breaks. This study focused on characterizing the leukemic stem cells of LDR radiation-induced leukemias by comparing them with those of high-dose-rate (HDR) or middle-dose-rate (MDR) γ-ray-induced leukemias. The LDR, MDR and HDR γ-ray-induced leukemias were obtained from mice that were exposed to 8,000 mGy at 20 mGy/22h/day, 4,000 mGy at 400 mGy/22h/day and 3,000 mGy at 890 mGy/min, respectively. Leukemic stem cells in leukemias induced by irradiation at the three dose rates were identified by transplanting 100 cells of different hematopoietic differentiation stages to syngeneic mice. The stages were hematopoietic stem cell, multipotent progenitor, common myeloid progenitor, common lymphoid progenitor, macrophage, erythrocyte, and B/NK cell. The leukemic stem cells from HDR and MDR γ-ray-induced leukemias had hemizygous deletions of chromosome 2 at around *PU.1* allele and showed identical CD antigen profiles. Microarray analysis of the mRNA revealed similar gene expression profiles to those of normal common myeloid progenitor (Table 1). In contrast, leukemic stem cells from LDR and some of the MDR γ-ray-induced leukemias with intact *PU.1* allele did not reveal similar profiles of CD-antigen and mRNA gene expression profiles to those of normal common myeloid progenitor, but frequently showed profiles resembling those of common lymphoid progenitor cells, granulocytes or monocytes. Thus, the origin of leukemic stem cells of LDR γ-ray-induced leukemias seems to be different from that induced by HDR γ-ray. Annotation analysis of gene expression profiles showed that down-regulation of p53 in the LDR and MDR leukemic stem cells influenced on several DNA damage response-associated biological pathway in database of Ingenuity Systems Co. such as, “Role of BRCA1 in DNA damage response”. However, no alterations of DNA damage responses were found in HDR leukemic stem cells. These suggest a possibility that DNA damage repair systems contribute to leukemogenesis in different ways when dose rate is changed.

This study was performed under contract with the Aomori Prefectural Government, Japan.

Table 1. CD antigen profiles and prediction of origin of the stem cells of radiation-induced acute myeloid leukemias (rAML) on the basis of gene expression profiles.

| AML sample | CD antigen profile[a] | Population with highest similarity with rAML stem cell[b] | Confidence Measure[c] |
|---|---|---|---|
| LSK- | 0.9 Gy/min-2 | Common myeloid progenitor | 0.63 |
| | 0.9 Gy/min-3 | Common myeloid progenitor | 0.94 |
| | 0.9 Gy/min-9 | Multipotent progenitor | 0.95 |
| LSK+ | 0 mGy/day-2 | Multipotent progenitor | 0.95 |
| | 0.9 Gy/min-10 | Multipotent progenitor | 0.83 |
| CMP | 0.9 Gy/min-1 | Common myeloid progenitor | 0.97 |
| | 0.9 Gy/min-2 | Common myeloid progenitor | 0.89 |
| | 0.9 Gy/min-3 | Common myeloid progenitor | 0.98 |
| | 0.9 Gy/min-6 | Common myeloid progenitor | 0.97 |
| | 0.9 Gy/min-9 | Common myeloid progenitor | 0.93 |
| | 0.9 Gy/min-10 | Common myeloid progenitor | 0.91 |
| CLP | 0 mGy/day-7 | Common lymphoid progenitor | 0.66 |
| | 20 mGy/day-1 | Multipotent progenitor | 0.65 |
| | 20 mGy/day-3 | Multipotent progenitor | 0.95 |
| Gr-1$^{pos}$ | 400 mGy/day-4 | Common lymphoid progenitor | 0.91 |
| | 0.9 Gy/min-9 | Common myeloid progenitor | 0.95 |
| | 0.9 Gy/min-10 | Common myeloid progenitor | 0.93 |
| CD45R/B220$^{pos}$ | 20 mGy/day-1 | Common myeloid progenitor | 0.83 |
| | 20 mGy/day-3 | Common lymphoid progenitor | 0.97 |
| | 400 mGy/day-3 | Common lymphoid progenitor | 0.88 |
| | 0.9 Gy/min-10 | Hematopoietic stem cell | 0.55 |

**II-7**

# Chromosomal Translocation Rates in Splenocytes of Mice Chronically Exposed to Low-Dose-Rate Gamma-Rays

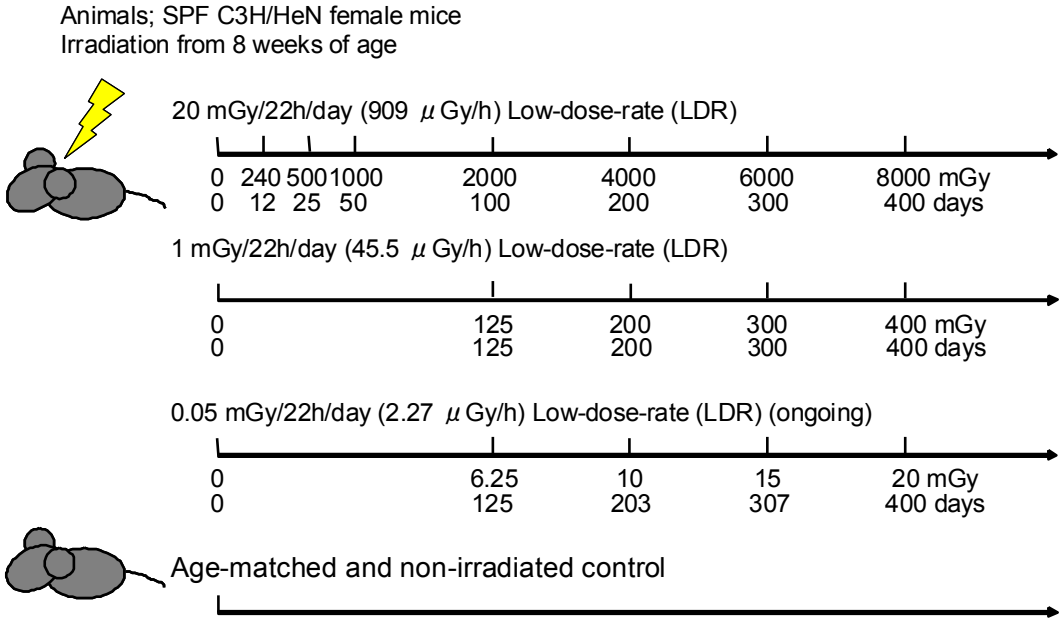
Atsushi KOHDA, Jun-ichiro KOMURA, Kimio TANAKA

Department of Radiobiology, Institute for Environmental Sciences.

The rates of chromosomal translocations were estimated in splenic lymphocytes of specific pathogen-free (SPF) female C3H mice exposed to low-dose-rate (LDR; 20 mGy/22h/day, 1 mGy/22h/day and 0.05 mGy/22h/day) $^{137}$Cs-γ-rays continuously from 8 weeks of age to a maximum of about 400 days (Fig 1) and compared with those after irradiation at a high dose rate (HDR; 890 mGy/min) or a medium dose rate (MDR; 400 mGy/day). Splenic lymphocytes from irradiated and non-irradiated control mice were cultured for 46 h in the presence of LPS, Con A, and 2-ME to obtain metaphase spreads, and translocations were identified under a fluorescent microscope using the multiplex-fluorescence *in situ* hybridization (M-FISH) method. Translocations increased linearly with the total dose of LDR (20 mGy/22h/day and 1 mGy/22h/day) as well as MDR (400 mGy/day) γ-rays. This was in contrast with a linear-quadratic increase induced by HDR (890 mGy/min) γ-rays (Fig 2). So far, significant increases in translocations have not been detected in mice irradiated at the lowest dose rate (0.05 mGy/22h/day). The dose rate effect was quite obvious when comparing HDR (890 mGy/min) and MDR (400 mGy/day), but negligible between 400mGy/day and 1 mGy/22h/day. The dose and dose-rate effectiveness factor (DDREF) was calculated to be about 2 from the rates of translocation for HDR and LDR at a total dose of 100 mGy. These results will be useful for estimating the risk of LDR radiation.

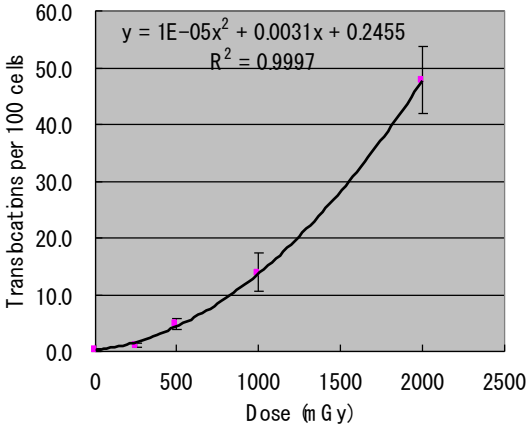
This study was performed under contract with the Aomori Prefectural Government, Japan.

## Irradiation procedures

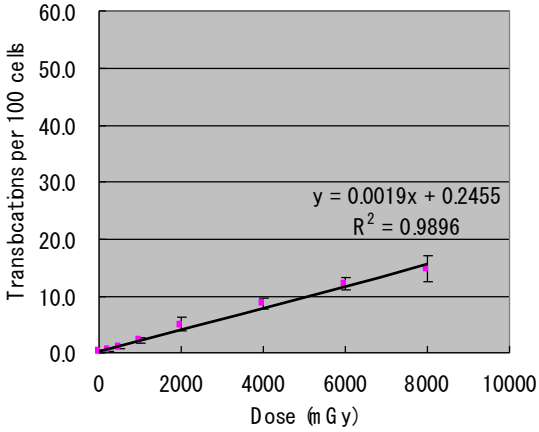



# Chromosomal translocation rates in mouse splenic lymphocytes

**HDR (890 mGy/ min)**




**LDR(20 mGy/22h/day)**



Secretariat:
**Institute for Environmental Sciences**
http://www.ies.or.jp/
2-121 Hacchazawa, Takahoko, Rokkasho, Aomori 039-3213 JAPAN
Phone: +81-175-71-1966 Fax: +81-175-71-1982