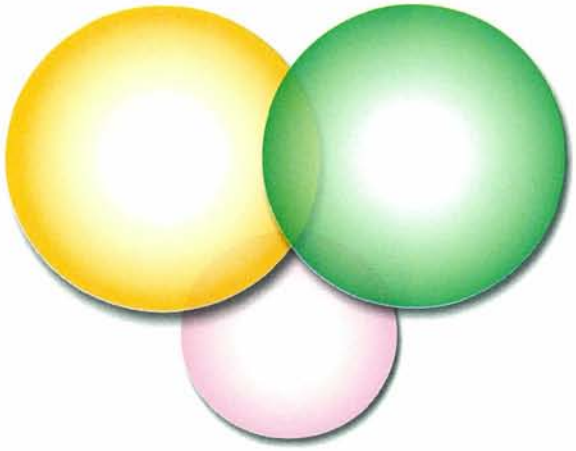
NIRS-M-211
ISBN 978-4-938987-51-0

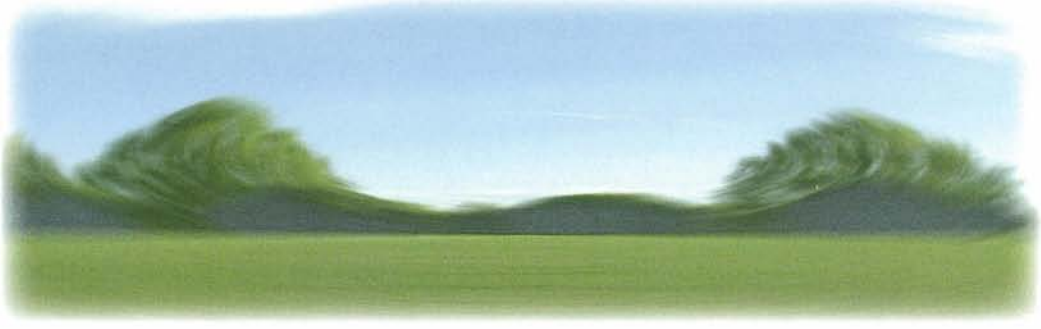
Proceedings of

NIRS International Symposium on the Effects of Low Dose Radiation



February 13-14 , 2008

National Institute of Radiological Sciences
Chiba, Japan



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NIRS International Symposium on the Effects of Low Dose Radiation

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Organized by
National Institute of Radiological Sciences

Co-sponsored by:
Health Research Foundation

In co-operation with
Japan Radioisotope Association
The Japan Radiation Research Society
Japan Health Physics Society

Preface

In January 2006 the National Institute of Radiological Sciences was designated as an IAEA Collaborating Center on the Effect of Low Dose Radiation. The projects under this program include (1) to understand biological effects of ionizing radiation specifically observed at low doses, (2) to investigate the effect of radiation on the health of people living in high background radiation area, (3) to understand the age dependency of low dose radiation with emphasis on its effects on embryos/children, and (4) to disseminate the information on the low dose effects in the training course operated under an IAEA program for Regional Collaboration Agreement.

We chose the first two topics for this year in the present Symposium; other topics will be covered in another opportunity we are planning next year. In addition to present the research outcomes obtained in the NIRS, we invited distinguished scientists in this field as guest speakers from China, India, Korea, United Kingdom and United States.

The Symposium itself was not the goal of our activity; It was intended that it would becomes a trigger to stimulate the research in this field, and, furthermore to promote the collaboration among the participants. In this regard, I believe the Symposium was a great success in that it stimulated not only the information exchange within the each area, but also the interaction between the two to end up, hopefully, with the integration of epidemiology and life sciences.

On behalf of the staff organizing this Symposium, I sincerely express profound appreciation to all speakers and participants for their presentation and exciting discussion.

February 2008

Kazuo Sakai
Chair Organizer of the Symposium
Director
Research Center for Radiation Protection
National Institute of Radiological Sciences

Contents

Subject 2: Epidemiology in High Background Radiation Areas

NIRS Programme on Low Dose Radiation as IAEA CC

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1. Outline

The year 2006 was the first year for the National Institute of Radiological Sciences (NIRS) as an IAEA Collaborating Center on Biological Effects of Low Dose Radiation; NIRS was designated as one of the IAEA Collaborating Centers on January 18, 2006. The ceremony for the designation was held on February 8 with the presence of Professor Pedro Andreo, Director, Division of Human Health, Department of Nuclear Sciences and Applications, IAEA.

In April 2006, NIRS initiated the second 5-year plan. The aim of the plan is to pursue comprehensive research and development on radiation and human health. The missions include the research on the effects of radiation on human bodies, medical countermeasures against radiation, diagnosis and treatment of diseases using radiation and radioisotopes, dissemination of research, and promotion of their uses. Among the missions of Research Center for Radiation Protection, both an understanding of the biological effects of low dose radiation and training of the skills of researchers and radiation workers have been the most important aspects.

2. Work Plan and Progress Report

The plan comprises three programs: (i) biological measurements, data analysis, and mechanisms including carcinogenesis using rodents, age-related effects and epigenetic effects, (ii) biological indicators of low-level radiation in terrestrial environments, and (iii) IAEA/RCA training course.

2.1 Biological measurements, data analysis, and mechanisms

2.1.1 Carcinogenesis: Experimental Studies

The use of interventional and fluoroscopic imaging for children has increased. For children, scattered radiation dose during radiotherapy may be significant because of their smaller body size than adults. Use of proton and heavy ion beams for children has also increased. The scattering foil to produce a field of useful size of pencil-proton beams produces neutrons. These situations raise another concern:

the effectiveness of the high LET radiations for carcinogenesis in children. Therefore, since 2006, we started animal experiments to determine the radiosensitivity for cancer induction during fetus and childhood stages together with measurements of the RBE of heavy ions and neutrons for children. We focused on both hematopoietic and solid tumors: myeloid leukemia, thymic lymphoma, and mammary, kidney, uterine, liver and lung tumors.

We irradiated B6C3F1 mice (both male and female) and female SD and Wistar rats *in utero* on gestation day 3, 13 and 17, as well as infant (1 and 3 weeks after birth), young (7-8 weeks) and mature (15 weeks) adulthood stages at doses of 0.2 to 2 Gy, and have been keeping observations of their health condition. At the moribund stage, mice were autopsied for pathological examination. Autopsy will be completed by 2009.

The results obtained were as follows: (i) SD rats and $Min^{Apc/+}$ mice at pre-pubertal age (<3 weeks after birth) were more resistant to gamma-rays at 2 Gy in induction of mammary carcinomas than those at mature age (7 weeks) (Imaoka et al. 2006). This may be ascribed to the high susceptibility of immature oocytes to radiation, which causes ovarian malfunction to secrete estrogens. (ii) $Min^{Apc/+}$ mice were susceptible at pre-pubertal age to radiation induction of intestinal tumors, while $Mlh1^{-/-}$ mice were susceptible at adult age (Okamoto and Yonekawa 2005, Tokairin et al. 2006). (iii) RBE value for 290MeV/u carbon ions in induction mammary carcinomas in adult rats was 2 at typical therapeutic dose per fraction, while it was 10 at lower doses (Imaoka et al., 2007). These data indicate that susceptible age window for radiation carcinogenesis is dependent on both target tissues and genetic background.

2.1.2 Age-Related Effects: A Literature Survey

To estimate the risk of radiation for children, it should be useful to collect and re-evaluate the epidemiological data on the diagnostic and therapeutic exposures, and the observed effects. Dose estimation for each radiological diagnosis and therapy is important. These epidemiologic cohorts include children exposed for benign disease such as tinea capitis and hemangiomas, malignant childhood cancer and in Chernobyl accident. Since excellent review has been performed by UNSCEAR 2000 and IARC, we reviewed the literatures published within these couple of years (2003-2007). So far, we reviewed papers on thyroid and brain tumors after irradiation for tinea capitis, mortality after radiotherapy for skin hemangioma, thyroid and skin tumors after therapeutic irradiation registered in Childhood Cancer Survey Study (CCSS), and thyroid tumors after Chernobyl and brain. The result of Chernobyl is summarized in Table 1.

Table 1. Cancer risks among children from studies of the effects of Chernobyl accident

Cancer site	Population, Author	No. of cancer cases	Age at exposure	Mean or median of dose (Gy)	ERR/Gy (95% CI)
Thyroid	Belarus and Russia, Cardis et al. 2005	276	0-14	0.365 (Belarus), 0.040 (Russia)	5.5 (2.2-8.8) from linear ERR model
	Belarus and Ukraine, Jacob et al. 2006	512 (Ukraine), 577 (Belarus)	0-18	0.180 (Belarus), 0.079 (Ukraine), 0.118 (Total)	18.9 (11.1-26.7)*
	Russia, Ivanov et al. 2006	199	0-17	0.08	Female: 45.3 (5.2-9953), 10.1 (-0.1-84.7), 1.0 (-5.3-15.0), exposed at 0-4, 5-9, 10-14 years, respectively. Male: 68.6 (10.0-452), exposed at 0-9 years. based on internal control
	Ukraine, Tronko et al. 2006	45	0-17	2.00 (cases), 0.78 (controls)	5.25 (1.70-27.5)
	Russia, Kopecky et al. 2006	66	0-19	0.0435 (cases), 0.016 (controls)	48.7 (4.8-1151)
	Ukraine, Likhtarov et al. 2006	232	1-18	0.353	8.0 (4.6-15)

* Linear coefficient of LQ model

2.1.3 Epigenetic effects (bystander responses and genomic instability)

A central paradigm in the field of radiation biology has been that only a cell "hit" by a track of radiation would elicit radiobiological effects, while a cell not hit should not be. This paradigm is the basis for the current system for risk estimation of radiation. However, recently, this paradigm has been challenged by so called non-targeted effects, including genomic instability and bystander effects.

Radiation-induced genomic instability can be observed in cells at delayed times after irradiation and manifested in the progeny of exposed cells multiple generations after the initial insult. Furthermore, an effect of irradiated cells is communicated to neighboring non-irradiated cells to elicit radiobiological effects ("bystander effects"). In laboratory studies *in vitro*, radiation-induced instability has been measured using many kinds of biological endpoints, such as mini-/microsatellite instabilities, gene amplifications, chromosomal alterations, micronuclei formation, mutation induction and decreased plating efficiency. Such radiation-induced instability may have important implications for risk

evaluation of low dose/low dose rate radiation.

We have recently investigated cellular responses of normal human fibroblasts after exposure to low fluences of different types of radiation followed by challenging dose of X-rays. The cells were pre-treated with low-fluence irradiation (~1mGy/7-8h) of ^{137}Cs gamma rays, ^{241}Am-Be neutrons, helium ions and carbon ions before an X-ray challenge dose (1.5Gy). Preirradiation of several types of radiation had profound effect on X-ray-induced mutation induction at *hprt* locus depending upon radiation quality. There was no effect of gamma-ray-preirradiation on mutation induction, while pre-treatment by high-LET ions such as carbon and helium ions increased mutation frequency. On the contrary, mutation frequency was reduced in neutron pre-treated cells. These results indicate that genome stability is affected by the low fluence of the high LET radiation, but not by the low LET radiation.

2.2 Biological indicators of low-level radiation in terrestrial environments

The Talesh Mahalleh area in Ramsar has the highest terrestrial radiation in Iran, mainly due to hot springs with high concentrations of radium-226 and its decay products, which flow into the surrounding areas. From 2004, NIRS has prepared the cooperative research with the Atomic Energy Organization of Iran, Tarbiat Modarres University (Iran) and Health Research Foundation (Japan) with the aim to determine the relationship of dose with dicentrics and ring chromosomes in lymphocytes of the residents in the high background radiation area (HBRA) of Ramsar.

Until 2006, two Iranian researchers have learned the cytogenetic techniques necessary for making good preparations, which were developed in NIRS. They were trained how to culture lymphocytes separated from human peripheral blood, to make chromosome preparations, to analyze chromosome aberrations under the microscope and to capture images with high resolution. NIRS also provided the instrument such as a metaphase spreader. In October 2006, NIRS invited an Iranian researcher to NIRS to make chromosome preparations for 15 elderly housekeeping women living in Talesh Mahalleh and 10 matched elderly women in the nearby control area (CA) of Katalom.

Physical dose measurement has been also carried out in some high natural background areas, including Iran and China.

2. 3 IAEA/RCA training course

The IAEA/RCA training course is one of the training and educational systems in NIRS. Aiming to promote the researches and medical technologies in the developing countries, IAEA and NIRS have been organizing the IAEA/RCA training courses for the radiation workers in these countries. RCA means the Regional Co-operative Agreement, to which 17 countries (Australia, Bangladesh, China,

India, Indonesia, Korea, Japan, Malaysia, Mongolia, Myanmar, New Zealand, Pakistan, Philippines, Singapore, Sri Lanka, Thailand, and Vietnam) have joined.

In May, eighteen trainees from 12 countries participated in the course on Radiation Biology for Radiation Oncology, which provided the participants the basic and modern knowledge about Radiation Biology. All participants considered that the course had been useful. This is because that few Radiation Biology classes have been opened in any University of their countries. They were also interested in the technologies for genome analyses. It is urgent for them to learn the genome studies to consider and understand the mechanisms of radiation-related carcinogenesis.

3. Acknowledgements

We highly appreciate Professor Jolyon Hendry, IAEA retiree, and Dr. Hideo Tatsuzaki, NIRS, for the effort to designate NIRS as IAEA Collaborating Center.

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Effects of Low Doses of Radiation

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Abstract

The Department of Nuclear Sciences and Applications at the International Atomic Energy Agency (IAEA) has a scheme of Collaborating Centres. A Collaborating Centre is an institution designated to assist the IAEA in implementing its programme through research and development and training in any nuclear technology. NIRS was appointed in 2006 as the Collaborating Centre for Biological Effects of Low Dose Radiation.

In addition to this collaboration, this writer initiated a review article on High Natural Background Radiation (HNBR). This involved authors in various organizations including NIRS, and some of the material in that review is summarized here. Natural radiation is the major source of human exposure, and the largest component of arises from the inhalation of the ^{222}Rn and its daughter products. An understanding of the long-term health risk of chronic public exposure is important for providing a rational basis for regulation of radiation applications in today's society. Only a few opportunities exist to quantify the health risks of such chronic exposures.

The possibility that studies of health risks among populations exposed in HNBR areas could increase our knowledge about health risks from chronic low-level exposure has been long considered, but never fully realized. A variety of investigations have taken place in some notable HNBR areas, e.g. Ramsar, Iran; Kerala, India; Yangjiang, China and Guarapari, Brazil over the past twenty years. Those include dosimetric studies, epidemiologic investigations and studies of chromosome abnormalities. Most studies from those locations, however, have not had sufficient sample size or been designed with the necessary methodologic strategies to detect small effects. Moreover, certain specific weaknesses were common including lack of cancer registries to confirm cases as well as accurate dosimetry on an individual basis. Other studies were designed specifically to estimate the lung cancer risk associated with indoor radon exposure. More than 20 studies have been conducted, especially in Europe, North America and China. These studies, thanks to a collaborative approach and a specific effort toward standardization and data quality, succeed in providing direct evidence of the risk associated with long term protracted radon exposure among the general population.

Studies in areas of HNBR continue today, generally with improved methodologies and with

peer-review in place, thereby increasing the chance that they can yield definitive conclusions. Such studies, however, have limitations that are difficult or expensive to overcome. At present, informative studies been conducted only for radon and the risk of lung cancer, that provide convincing association between long-term protracted radiation exposures in the general population and disease incidence. The success of those studies is due to the fact that, for radon, tissue doses are quite elevated and large-scale collaborative studies have been conducted, with careful individual reconstruction of exposures and collection of information on potential confounding factors. While it is difficult today to evaluate the existence and possible magnitude of a health risk associated with residence in HNBR areas, careful studies conducted on a large scale, with individual assessment of exposure and a common protocol for those studies conducted in different countries may, in the future, provide direct information on the effects of low dose protracted exposures on other organs and tissues. Steps that have been taken in China and India, including the establishment of cohort and case-control studies, provide a model framework for studies of low dose risks from high background radiation and could be used as a model in other areas of the world. The difficulties outlined regarding health issues also apply to biological measurements, and potentially interesting future studies including molecular techniques need to take the above considerations into account.

Overview: Adaptive Responses

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The radiation adaptive responses have been typically demonstrated as an acquired resistance induced by a low dose of radiation, referred as a conditioning, adapting, or priming dose, against a large dose (challenge dose) given after some interval time. The endpoint in the first report on this subject [1] was chromosome aberration in human lymphocytes *in vitro*. Since then, the responses have been shown in various types of cultured cells; the endpoints were micronuclei[2], cell survival[3] and malignant transformation[4, 5]. Studies in cultured cells have revealed some characteristics of the adaptive response (e.g. ref 6): (i) It takes some time; typically 4-6 hr of incubation after the priming irradiation is needed for the development of the resistance; (ii) the acquired resistance is transient to decay during prolonged incubation after the priming; (iii) there is an optimal dose "windows" for the priming dose for the induction of the resistance, typically in the range of tens of mGy, and the doses less or more than this level tend to be less effective. Another important feature of the adaptive response is that dependent on genetic background as is revealed by the dependency on individual donors of lymphocytes[7].

The induction of radioresistance by a small dose of radiation was observed at whole body level[8]. Half a Gy of conditioning dose given 2 weeks before the lethal dose increased the survival of ICR mice significantly. In this case, too, the induction of the resistance was highly dependent on the dose of the conditioning and the period of the interval also the resistance was not observed in another strain of mice, indicating a dependency on the genetic background [9]. The endpoints at whole body level have been malformation[10], and radiation-induced[11,12] or carcinogen-induced [13] tumorigenesis.

Studies on the mechanism underlying the adaptive responses have suggested the involvement of stimulation or enhancement of some protective functions, including antioxidative capacity, DNA repair, apoptosis, and immune functions, suggesting that the adaptive response could be considered essentially as an enhancement of the protective functions by low level radiation (for review see ref. 14).

As the adaptive response is not limited to cellular level, but also observed at whole body level, and it would modify the incidence of cancer, which is one of the major concerns in the current radiological protection, at lease in some cases, it should potentially have a great impact on the protection system. Currently, the system of radiological protection is based on the Linear-No-Threshold model, which claims that the increment of the risk of stochastic effects from ionizing radiation is proportional to the dose, no

matter how low the dose is. The adaptive response certainly works in a protective way against radiation damage, suggesting lower risk at low dose range than currently predicted based on the LNT model. Although there have been intensive discussion on this issue, the adaptive response has not been fully appreciated in this sense (see refs. 15 and 16 for discussion).

In the context of radiation therapy, the adaptive response might be an advantage, if the lower dose inevitably given to the normal tissue surrounding the target tumor should increase its resistance; the timing of the fractionated irradiation may be determined taking the timing of the development and decay of the adaptive response into the account. Currently, biological effects of ionizing radiation are used mostly for radiotherapy of malignant tumors, in which the damaging effect of radiation of large doses is utilized to kill tumor cells. If the enhancement of protective functions by low dose radiation can be used to treat/prevent certain types of diseases, it would be a novel application of ionizing radiation. Such possibility has been discussed, using conventional irradiation[14, 17] and using technique of nuclear medicine[18]. In fact, for certain types of tumors, successful "low dose therapy" has been reported.[19]

Since its discovery, the adaptive response has been an interesting and an intriguing topic in radiation biology. Now it has been becoming a challenging issue in both radiological protection and medical use of ionizing radiation.

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Systemic Effects of Low-Dose and Low-Dose-Rate Irradiation in Mice

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1. Introduction

The harmful effects of high-dose (HD) radiation on human beings and living organs have been well documented through studies on the survivors of the atomic bomb attacks on Japan and the explosion of the Chernobyl Nuclear Power Plant. There are some reports that low-dose (LD) radiation can contribute to life span elongation[1)], strengthen the immune system[2)], and inhibit the occurrence of diseases such as diabetes and rheumatoid disorders. However, because those studies used HD or high-dose-rate (HDR) radiation, they did not deliver sufficient explanation of the effects that LD (less than 200mGy) or low-dose-rate (LDR) (less than 6mGy/hr), which UNSCEAR (2000) suggested as the proper level, may have on human beings[3)]. For these reasons, experiments on the effects of radiation on cells and experimental animals are still being carried out by a number of radiation relevant organizations and institutes such as the Department of Energy and the National Committee of Radiation Protection in the U.S., and the National Institute of Radiological Science, the Institute of Environmental Sciences and the Central Research Institute of Electric Power Industry in Japan. Their studies have shown parts of the mechanisms of adoptive response[4)], apoptosis, the bystander effect, genome instability[5)] and the repair mechanism[6)]. If additional results of such studies are accumulated, they will reveal biological phenomena caused by radiation on the molecular cytology level or an individual organism. In the "NIRS International Symposium on the Effects of Low Dose Radiation," I report the frequency of micronuclei in erythrocytes of peripheral blood as an index to evaluate its proper response after irradiating the LD and LDR radiation. I also report relative effects of the LD and LDR radiation to the HDR radiation on chromosomal aberration in bone marrow cells, apoptosis of splenocytes and sperm abnormality in the epididymis.

2. Materials and Methods

In this study, I used IRC mice (female, 6-7 weeks old) of SLC (Japan) after adapting them for a week. Feed and water were sterilized with an autoclave, and the cages and straw were irradiated. A γ-irradiator (Cs-137, 0.8Gy/min) was used for the HDR irradiation. For the low-dose-rate irradiation, I used a Low

Dose Irradiation Facility (Cs-137, 185GBq,) which was equipped in the Radiation Health Research Institute of the Korea Hydro & Nuclear Power Corporation. In the first experiment, I observed how well the mice were able to protect themselves from physical damage after irradiating with HD radiation the mice that had been previously irradiated with the LD radiation. I divided the mice into three groups, because prior irradiation dose plays an important role in inducing a proper response. The HDR radiation was administered to the first group of mice as much as 0, 0.01, 0.05, 0.1Gy, and their physical responses were observed. The second group was irradiated with the HDR (0, 0.01, 0.1Gy) once a day for 10 days until the final dose reached 0, 0.1, 1Gy. The third group was exposed to an environment where the mice were irradiated at the dose-rate of 0, 12, 98mGy/day, and their final dose was adjusted to about 0, 0.1, 1Gy. The term for physical adaptation of the mice that had already been irradiated with the LD of radiation plays an important role in the experiment to observe the adaptation response to radiation. In this study, I set it to 6 hours after reviewing existing reports. The mice were irradiated with as much as 2Gy after the adaptation period of 6 hours with the prior low-dose irradiation. I irradiated with 2Gy of the challenge dose because it is well documented that 2Gy of radiation can cause more micronuclei in the erythrocytes of peripheral blood than any other dose of radiation. Blood was collected from the tail vein 24 hours after the high-dose radiation. The micronuclei in erythrocytes were stained with Acridine-orange dye. In the second experiment, I observed the physical damage of each individual organism of the two irradiated groups of mice. One group was irradiated with the dose-rate of 0.7mGy/hr for 11 days until their accumulated final dose reached 0.2Gy. The other group was irradiated with the dose-rate of 4mGy/hr for 20 days until their accumulated final dose reached 2Gy. I used as the indices the frequency of micronuclei in the red blood-cells of peripheral blood, chromosomal aberration in bone marrow cells, apoptosis of splenocytes, and sperm abnormality in the epididymis to evaluate the physical damage of each individual organism.

3. Results and Discussion

The results showed that the frequency of micronuclei in the mice that were irradiated with the HD radiation increased in proportion to the dose ($P<0.0001$). On the contrary, the mice irradiated with the HD radiation 6 hours after the prior irradiation with a LD 0.01Gy showed the lowest frequency of micronuclei ($P<0.001$). The mice that were irradiated with the HD radiation 6 hours after the prior irradiation with 0.05 and 0.1Gy showed a higher frequency of micronuclei than those irradiated with the prior radiation of 0.01Gy. This result indicates that the LD prior radiation contributed to the higher capability to inhibit physical damage from the HD radiation. When the frequency of micronuclei in polychromatic erythrocytes of the mice was observed after they were irradiated with 0.01 and 0.1Gy

once a day for 10 days, it showed an increase as the accumulated dose surpassed 0.1Gy ($P<0.0001$). However, the mice, which were irradiated with the HD radiation after they were previously irradiated with 0.01 and 0.1Gy once a day for 10 days to ensure that the accumulated final dose reached 0.1Gy, showed a lower frequency of micronuclei than mice under other conditions ($P<0.0001$). For the last experiment on the adaptation response to radiation, the third group was raised in an environment in which radiation was introduced at the dose-rate of 0, 12, 98mGy/day, and the final dose was adjusted to 0.1 and 1Gy, respectively. The mice were irradiated with the HD radiation; however, no difference was found in the frequencies of micronuclei. On the other hand, they showed a lower frequency than the prior-irradiated mice ($P<0.05$). In addition, when I administered the HD radiation to mice that had been constantly irradiated in the LDR environment, they showed a lower frequency of micronuclei than those irradiated only with the HD. Furthermore, the mice that were irradiated with the accumulated dose of 0.1 and 1Gy, respectively, did not show any difference in their frequencies of micronuclei, even though their irradiated doses were ten times different. It appeared that the mice that were raised in the LDR environment had proteins relating to the recovery of damaged DNA that were continuously being activated so that the proteins could more effectively respond to the damage caused by the HD radiation. I compared the physical damage of the individual organisms caused by the LDR radiation with the damage caused by the HDR (0.8mGy/min). The results showed that when mice were irradiated once with the high-dose-rate, the micronuclei in the erythrocytes and chromosomal aberration increased in proportion to the amount of the dose ($P<0.0001$). However, there was no difference in physical damage among the LDR irradiated mice even though their final doses were quite different ($P<0.001$). In addition, when splenocytes were stained with Annexin V to compare the rates of apoptosis, only mice irradiated with 4mGy/hr for 20 days until the accumulated dose reached 2Gy, showed a higher apoptosis rate than others ($P<0.03$). This indicates that the mice exposed in the LDR environment suffered not only less damage to the chromosome in the bone marrow cells and splenocytes but, also, recovered their damaged cells faster than the mice irradiated with the HD. Finally, male ICR mice were exposed to the second experiment conditions until the dose reached the final dose. On the 8th day after the radiation was completed, their caudal epididymides were collected, chopped, minced and stained with Eosin. When the sperm abnormalities in caudal epididymides were compared, the abnormality of the mice that were irradiated with the high-dose-rate increased in proportion to the amount of the irradiated dose ($P<0.01$). However, the sperm abnormality in the mice irradiated with the LDR was lower than that of mice irradiated with the HDR or non-irradiated ($P<0.0001$). In particular, the sperm abnormality in the mice that were irradiated with the HDR until the final dose reached 2Gy was much lower than the sperm abnormality in the mice that were raised in the LDR environment ($P<0.0005$). On the other hand, when

the whole sperm abnormality in the epididymis was compared with the sperm abnormality in the control group, the whole sperm abnormality increased in proportion to the dose until 1Gy, but decreased in the dose higher than 1Gy. When the sperm abnormality in the mice that were irradiated with the high-dose-rate was compared to the sperm abnormality in the mice that were exposed to the LDR (0.7mGy/hr) environment, their relative difference was about 0.6. This indicates that LDR radiation can contribute to the removal and recovery of damaged reproductive cells in the testis through the apoptosis mechanism[7].

4. Conclusion

In terms of the mice irradiated with the HD, the damage of bone marrow cells, splenocytes and reproductive cells increased in proportion to the dosage amount. However, the damage in the cells of the mice that were irradiated with the LDR was lower than that in the cells of the mice that were irradiated with the HDR. In particular, there were no harmful effects caused by the LD (less than 200mGy) and LDR (less than 6mGy/hr) radiation that UNSCEAR (2000) suggested as the proper level.

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Reduction in Malformations by Low Dose Pre-Irradiation: The Early Beginning of Adaptive Response Story in Fetal Mice

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1. Introduction

Radiation-induced adaptive response is defined as the phenomenon of priming low-dose-induced resistance to subsequent irradiation at higher doses. Research on adaptive response, which provides important scientific basis for radiation risk estimates and offer significant insight into the defense mechanisms regarding radiation protection, is of great concern for both public health and academic research[1]. In the *in vitro* examinations, diminished detrimental effects such as chromosome aberrations, mutations, transformation and cell death are documented[1, 2]. In the *in vivo* investigations, adaptive response is recorded mainly as a rescuing effect resulting in an improved survival[3, 4]. In a series of studies using an *in utero* model with fetal mice, we demonstrated the existence of adaptive response and characterized the experimental conditions for its successful induction[5-7].

2. Materials and Methods

Animals: Mice of ICR, C57BL, BALB/c and C3H strains were either prepared in our institute (NIRS) or purchased from both SLC, Inc. (Japan) and ANMED BIOSAFE, Inc. (U. S. A.) via IBL, Co. Ltd. (Japan).

Irradiations: The pregnant mice were exposed to whole-body irradiations at room temperature with an X-ray machine (Pantak-320S, Shimadzu, Japan) operated at 200 kVp using a filter of 0.5 mm aluminum plus 0.5 mm copper. The dose rates were measured using an exposure rate meter (AE-1321M, Applied Engineering Inc.). Priming irradiations were of different combinations of dose and dose rate. Challenging irradiation was delivered 24 h later after the priming dose. The challenging doses to the fetuses *in utero* and lethal doses to the animal *in vivo* were delivered at a dose rate of 1.8 Gy/min.

Endpoints: Radiation induces high incidences of abnormalities and neonatal death in late organogenesis in mice[8]. Gross malformations in limbs and prenatal deaths were used as endpoints, and a priming-radiation-induced reduction of a subsequent challenging-radiation-induced prenatal deaths and digital defects was applied to the judgment of adaptive response.

All experimental protocols involving mice were reviewed and approved by the Animal Care and

Use Committee of NIRS and were performed in strict accordance with the NIRS Guidelines for the Care and Use of Laboratory Animals.

3. Results

On the timing of irradiations, priming doses, and mouse stains

A variety of physical and biological conditions with respect to both irradiation and irradiated subjects are crucial to the induction of adaptive response. An adaptive response *in utero* was first demonstrated by irradiating the animals with a priming dose of 0.3 Gy at 0.34 Gy/min on E11 before a changing the dose of 5 Gy at 1.8 Gy/min on E12 in ICR strain fetal mice[5], manifesting as a significant reduction of challenging irradiation-induced prenatal death and digital defects (Fig.1). This set of efficient conditions for the successful induction of adaptive response was obtained out of 30 combinations of both the irradiation timing from E9 to E12 with one day of interval and the priming dose ranging from 0.05 to 0.5 Gy. Subsequent examinations were then performed in C57BL, BALB/c and C3H strain animals. In C57BL fetal mice, both 0.05 Gy and 0.3 Gy delivered at 0.34 Gy/min were efficient for a successful induction[6]. However, neither of these two doses was efficient in BALB/c and C3H strain animals. Results indicate that induction of adaptive response *in utero* is related to the timing of irradiations, priming doses, and mouse stains.

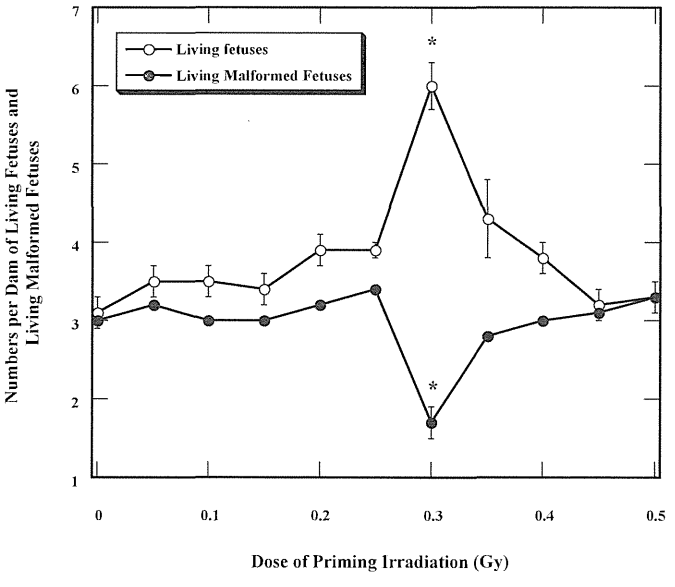

Fig.1. Reduction of challenging irradiation-induced prenatal death and digital defects by priming irradiation in ICR fetal mice. Statistical significance ($P<0.05$) compared to the group receiving only the challenging dose of 5 Gy was indicated with a star symbol.

On the p53 gene status and radiation-induced apoptosis

The developing limb provides a good model to correlate the initial pathological changes in early limb buds with final digital defects, which are due to an excess induction of p53 dependent apoptosis by the high dose of irradiation[9, 10]. To study the cellular response under adaptive response *in utero*, investigation in C57BL fetal mice with different p53 gene status was conducted for the correlation between both the p53 gene status and the radiation-induced apoptosis with the induction of digital defects. With a priming dose at either 0.05 Gy or 0.3 Gy at 0.34 Gy/min, adaptive response was observed in p53 wild type but not in heterozygous type fetal mice[6], suggesting both alleles of p53 gene are essential for a successful induction. In p53 wild type fetal mice, both of the two efficient priming doses brought a significant decrease in the incidence of apoptosis in predigital regions in living fetal mice (Fig.2). Results indicate that induction of adaptive response *in utero* is dependent on the p53 gene status and related to the suppression of challenging-irradiation-induced apoptosis[12]. Suppression of apoptosis suggests an anti-apoptotic pathway involved in the adaptive response.

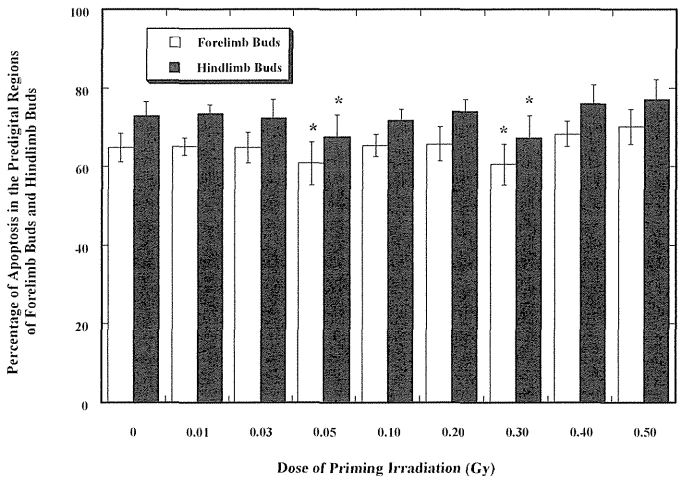

Fig.2. Induction of apoptosis in the predigital regions of forelimb buds and hindlimb buds 6 h after in utero challenging irradiation at 3 Gy on E12 in C57BL fetal mice with wild type p53 gene status. A priming dose of 0 to 0.5 Gy on E11 was delivered prior to the challenging dose. Statistical significance ($P<0.05$) compared to the group receiving only the challenging dose was indicated with a star symbol.

On the dose rate of priming irradiation

Low dose rate and low dose are so closely related issues in the field of adaptive response research. To verify any possible dose-rate effect on induction of adaptive response, the efficient priming dose of 0.3 Gy was administered to ICR fetal mice on E11 in a range from 0.06 to 5.0 Gy/min, followed by a challenging dose of 3.5 Gy at 1.8 Gy/min on E12[7]. Unexpectedly, successful induction of adaptive response was observed within two dose-rate ranges, from 0.18 to 0.98 Gy/min and from 3.5 to 4.6 Gy/min (Fig.3). At any dose rate outside of these two ranges, no adaptive response was observed.

Results indicate that induction of adaptive response *in utero* is related to the dose rate of priming irradiation. These findings further suggest the existence of multiple pathways of signal transduction activated by different conditioning doses and dose rates, which may involve multiple cell populations with various susceptibilities to radiation-induced apoptosis.

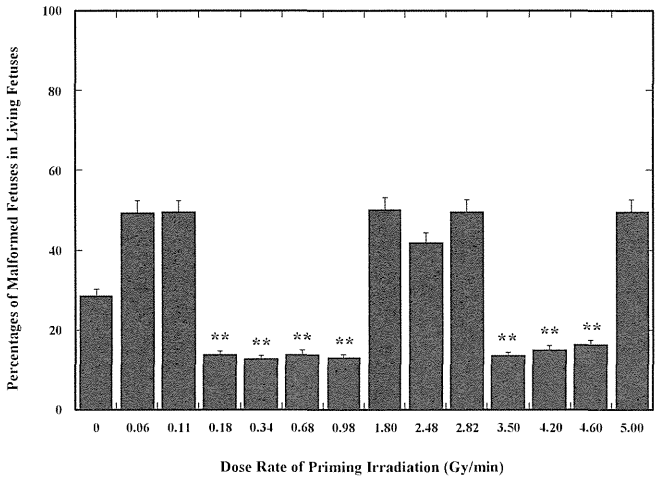

Fig.3. Two efficient dose-rate ranges for 0.3 Gy of priming irradiation to adapt fetal mice against digital defects in ICR fetal mice. Statistical significance ($P<0.01$) compared to the group receiving only the challenging dose was indicated with two star symbols.

4. Discussion

Radiation-induced adaptive response is a simple phenomenon involved in complicated mechanisms. In a pile of documented investigations, radiation-induced adaptive response is predominately studied *in vitro*. However, the margin of limited applicable endpoints and the simplicity of cell line due to its one tissue origin make any comprehensive study hardly possible. It is of the most practical significance that all the conclusions drawn from the *in vitro* studies should be finally verified and confirmed in the *in vivo* models. In this regard, research on the suppression of death at the whole body level, which is based on the rescue of all the critical organs and tissues, is of great importance. The molecular mechanisms underlying the radiation effects such as gene expression are tissue specific, thus characterization of the efficient conditions for successful induction of adaptive response *in utero* and *in vivo* would play a critical role in elucidating possible universal mechanisms involved in adaptive response. It is worthy of mentioning that *in utero* and *in vivo* study is of more and more concern in the field of adaptive response research[13-15].

While it should be also noticed that judgment for existence of adaptive response largely depends on the endpoints and a comprehensive study should also take the late consequences of adaptive response into account. In a series of studies on adaptive response in fetal mice different consequences were

observed depending on the endpoints. In fact, it was just the early beginning of our adaptive response research story that demonstrated the existence of adaptive response in fetal mice by showing the efficient priming doses were capable of preventing prenatal death and malformations from the challenging irradiation. Further studies on the molecular mechanisms under this phenomenon are in progress. On the other hand, to look into the late consequences of adaptive response, the follow-up study is being performed on physiological maturation, behavior, carcinogenesis and life span in the postnatal survivals.

In the mean time, as one of the vanguards of paradigm shift in radiation biology, adaptive response should be studied in a closed relation to other radiation-induced effects at low doses and low dose rates such as hormesis, bystander responses and genomic instability[16, 17].

5. Conclusion

Adaptive response *in utero* is a simple phenomenon but with complicated mechanisms and late consequences. The findings in a series of our studies indicate that radiation-induced adaptive response existed in fetal mice, which manifested as reduction in malformations and prenatal fetal deaths. This adaptive response was due to a complex interplay between dose, dose rate and animal factors such as the strain, developmental stage, and p53 gene status. These results further confirm that radiation could be a double-edged sword in late organogenesis: radiation at high doses induces detrimental effects while radiation at low doses could trigger a protective mechanism to mitigate those detrimental effects.

6. Acknowledgement

Deep gratitude is expressed to Drs. Shiro Aizawa, Osami Yukawa, Harumi Ohyama, and late Takeshi Yamada, for their guidance and warm encouragement throughout this study. This work was supported in part by the Budget for New Nuclear Crossover Research from the Ministry of Education, Culture, Sports, Sciences and Technology, Japan.

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Effects of Smoking on Chromosomes Compared with Those of Radiation in a High-Background Radiation Area and of Environmental Mutagenic Factors in a Large City

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1. Introduction

Smoking is the most influential factor among the environmental mutagens to increase cancer incidence. In order to know how environmental mutagens affect the induction of translocations caused by smoking we analyzed the translocations in the lymphocytes of smokers and nonsmokers in a large city, Beijing, and compared them with those reported by us in a high background radiation area (HBRA) and in its control area (CA), remote villages, in the south of China. This paper is the review of our reported studies [1,2,3,4] performed under China-Japan collaborative study on HBRA in China conducted by Prof. Tsutomu Sugahara, Japan, and Prof. Luxin Wei, China.

2. Materials and methods

2.1 Subjects and individual dose measurement

Subjects analyzed consist of 15 non-smokers and 10 smokers in HBRA, 16 non-smokers and 7 smokers in CA, and 20 non-smokers and 10 smokers who lived in Beijing more than 40 years. They had no history of occupational exposure to chemicals and medical radiation except for routine chest X-ray examination during their lifetimes. Subjects in HBRA and CA are farmers. Chemicals such as insecticide were rare-used in those areas, since the places locate in the remote area. Before blood collecting, eligible participants read and signed an Informed Consent statement. Ethical approval was obtained from Ethics Committee of National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention. The radiation dose exposed to the environmental natural radiation of each individual was measured by an electric pocket dosimeter (Aloka PDM-101) for 24 hours and/or NaI scintillation survey meter (Aloka TCS-166) for 2 months in their homes prior to blood collection. The accumulated dose of each individual by the time of blood collection was estimated from the measured dose.

2.2 Blood collection, cell culture and chromosome painting

Blood was taken at HBRA, control area and Beijing and cultures were set up within 8 hours after taking blood. Chromosome preparation was made according to a high yield chromosome preparation method for low dose radiation study [5,6]. Chromosome painting was performed using Biotin-labeled whole chromosome painting probes specific to chromosomes 1, 2 and 4 (Cambio, UK), representing 22.71% and 22.34% of the human genome in male and in female, respectively. The frequencies of translocations per 1000 cells were converted to genome-equivalent frequencies (F_G) by the formula [7] as follows: $F_G=F_p/2.05f_p(1-f_p)$, where F_p is the frequency of translocations detected by painting and f_p is the fraction of the genome painted.

2.3 Statistical analysis

Mann-Whitney U test was used to compare the frequencies of translocations between groups.

3. Results

Data obtained in non-smokers and smokers in HBRA, CA and Beijing are summarized in Table 1. Their average age was about 60 year-old. Average accumulated dose (air karma) in HBRA was about 3 times higher than those in CA and in Beijing. Mean genome equivalent frequencies of translocation per 1000 cells of non-smokers and smokers in HBRA, CA and Beijing were 11.7±4.7 and 11.1±3.6, 8.4±3.1 and 13.4±3.4, and 9.6±5.0 and 10.6±3.1, respectively.

Table 1. Summary of examinations in 6 sub-groups in HBRA, CA and Beijing

	HBRA		CA		Beijing	
	Nsmokers	Smokers	Nsmokers	Smokers	Nsmokers	Smokers
No. of cases	15	10	16	7	20	10
Age	63.9±5.2	57.4±4.2	64.4±5.3	61.2±5.2	61.2±3.0	58.5±3.5
Total cells analyzed	65490	50654	67510	20743	50329	32972
Average cells analyzed	4366	5065	4219	2963	3871	3297
Dose	167.6±17.9	158.3±20.1	48.2±10.1	40.1±4.4	54.9±5.4	50.1±3.4
F_G/1000 cells						
Mean±SD	11.7±4.7	11.1±3.6	8.4±3.1	13.4±3.4	9.6±5.0	10.6±3.1
Range	4.7-23.6	7.1-18.4	4.2-13.5	9.3-17.8	3.5-23.8	3.7-14.2
Median	10.5	10.25	7.9	14.1	8.75	10.6

Result of statistical analysis is shown in table 2. Significant difference was found between CA smokers and CA non-smokers as well as between CA smokers and Beijing non-smokers. But no other

possible comparisons between groups showed significant difference.

Table 2. Comparison of the frequency of translocations among 6 sub-groups in HBRA, CA and Beijing showing p values analyzed with Mann-Whitney U test

Group		HBRA		CA		Beijing	
		Nsmokers	Smokers	Nsmokers	Smokers	Nsmokers	Smokers
		15	10	16	7	20	10
HBRA	Nsmokers						
	Smokers	0.846					
CA	Nsmokers	0.075	0.108				
	Smokers	0.259	0.241	0.009			
Beijing	Nsmokers	0.142	0.194	0.787	0.031		
	Smokers	0.739	0.850	0.120	0.157	0.262	

4. Discussion

There is discrepancy in the results of the studies on the effects of smoking to the chromosomal translocations in the peripheral lymphocytes. Whitehouse et al. (2005)[8] did not find increase of the frequency of translocations, while Sigurdson et al. (2008)[9] showed that smoking increase the frequency.

In the present study, we detected increase of the frequency in CA but not in HBRA nor in Beijing. The effect of smoking seems to be suppressed by the environmental mutagens including the elevated level of natural radiation in HBRA and ever-existed continuous air pollution in Beijing.

Ikeda et al. (2007)[10] reported that genotoxic effects in the lung of gpt delta transgenic mice decreased when low-dose-rate radiation was given together with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), the most carcinogenic tobacco-specific nitrosamine. They suggested that NNK induced adaptive response that eliminated the cells bearing radiation induced double strand breaks in DNA.

Analysis with more subjects in those three areas may be needed to confirm the suppressive effect of environmental mutagens to the induction of chromosomal aberrations caused by smoking. Further study is now in progress by us.

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Combined Effects of X-rays and *N*-ethyl-*N*-nitrosourea on Thymic Lymphoma Induction

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Abstract

Since we are living in the environment with numerous natural and man-made radiation and chemicals, cancer development in human is considered as a result of interaction with these factors. While high dose of ionizing radiation is well known to contribute to tumor induction, carcinogenic effect of low dose (< 0.2 Gy) is still controversial. To understand the effect of low dose radiation on carcinogenesis, we examined mode and mechanism of combined effect of low dose radiation and chemical carcinogen comparing to those of high dose. For induction of thymic lymphoma (TL), which is a good animal model for human T-cell acute lymphoblastic leukemia, female B6C3F1 mice at 4-weeks of age were exposed weekly to whole body X-irradiation at 0.2 to 1.0 Gy for consecutive 4 weeks, and then given *N*-ethyl-*N*-nitrosourea (ENU) in drinking water at 50 to 200 ppm for 4 weeks. Dose-response curve for induction of TL by fractionated X-irradiation or ENU administration had threshold dose, therefore the dose range including threshold dose were used for the combined exposure. The mode of combined effect of high dose X-rays followed by ENU was synergistic, and that of low or threshold dose of X-rays was unexpectedly antagonistic. We previously reported that X-ray-induced TL was characteristic of a high frequency of loss of heterozygosity (LOH) in the centromeric region of chromosome 11, in which we mapped *Ikaros,* a master gene of lymphopoiesis, but not in ENU-induced or spontaneously developing TL[1]. In the X-ray-induced TL, a variety of *Ikaros* alteration including transcriptional silencing, unusual splicing, point mutation and small insertion, most of which were associated with LOH, were observed[2]. On the other hand, in ENU-induced TLs Ikaros was altered by only point mutations without LOH[3]. Thus, the mutation of *Ikaros* was distinguished between X-ray- and ENU-induced TL. In TL after combined treatment, *Ikaros* alteration was mainly point mutation without LOH. The analysis of mutation in thymus of B6C3F1 *gpt*-delta mice demonstrated that combined exposure of 0.2 Gy X-rays with ENU dramatically decreased mutant frequency, especially G:C to A:T and A:T to T:A mutations, compared to ENU treatment alone[4]. In contrast, 1.0 Gy X-rays combined with ENU enhanced mutant frequency, indicating a good correlation with carcinogenic

response. In conclusion, the mode of combined effect of X-rays and ENU on TL induction and mutant frequency was dependent upon the dose of X-rays.

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Low Dose Irradiation and Antioxidants Protect against High Dose Radiation Induced Lymphoma in Mice

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Abstract

Ionizing radiation-induced tumor induction in thymus and its suppression by pre-exposure to low dose irradiation has been investigated in Swiss albino mice in our laboratory. These studies showed that a single dose of whole body gamma irradiation (3 Gy) induced thymic lymphoma (TL) after 3-4 months followed by shortening of the life span of tumor bearing animals. These findings have been extended to detailed investigations on the mechanisms of radiation-induced occurrence of tumor and its modification by antioxidants and low dose exposures prior to tumor causing radiation dose. The induced tumor has been found to exhibit sensitivity to therapeutic doses of gamma radiation and concentration dependent anti-tumor drug, doxorubicin. Moreover, transplanted tumor growth was found significantly reduced by exposure to fractionated doses of radiation. Studies have further confirmed that pre-exposure of animals to low doses of radiation significantly suppressed the growth of the transplanted tumor. In addition, tumor cells exposed to 1cGy of radiation and transplanted to mice showed 30 % reduction in the incidence of tumor. The development of TL was found associated with the enlargement of spleen and induction of anaemia. Recent results have shown that whole body exposure of animals to sub-lethal doses (1-5 Gy) resulted in dose-dependent increase in reactive oxygen species (ROS) level in thymocytes from irradiated animals. I*n vitro* studies on thymocytes of irradiated mice showed increased percent apoptosis, as measured by annexin V fluorescence method, which was inhibited by antioxidants such as vit E and curcumin. More recent results have shown that radiation induced tumor induction was dependent on the age of animal at the time of irradiation. The younger age irradiation showed greater sensitivity to tumor induction. In addition, radiation mediated tumor induction was found gender dependent. This brief review presents a highlight of involvement of γ radiation generated ROS in cell/membrane oxidative damage and the role of cellular apoptosis in the mechanism of radiation-induced lymphoma tumor in mice.

1. Introduction

Ionizing radiation interaction with biological systems involves generation of free radicals and subsequent damage to biomolecules like proteins, lipids and nucleic acids (DNA/RNA). These damage processes to living organisms may be manifested in resultant mutation, cellular transformation and cell death (1-3). The major risk posed by radiation exposure is cancer induction, which has been investigated after radiation exposure using different experimental models. Many laboratories around the world are actively investigating to understand the mechanism of radiation action on cells/tissues and its modification by various treatment modalities (4-7). The phenomenon of apoptosis is believed to be associated with manifestation of radiation-induced carcinogenesis and many other related diseases (8). Radiation induced cellular oxidative damage is initiated by the generation of intracellular reactive oxygen species (ROS) modulating the cellular redox status. Modification of various cellular damage related effects like cancer induction (9) by priming dose of low radiation have been investigated. However, the molecular mechanism of the cellular response by low dose irradiation and its mechanism in modification of induced thymic lymphoma (TL) in animals exposed to acute dose of Whole Body Irradiation (WBI) remains to be elucidated. We have investigated role of ROS and apoptosis in the mechanism of radiation-induced TL incidence. In addition, inhibition of TL incidence by treatment with antioxidants and pre-exposure of low dose was investigated in order to understand the molecular steps involved in the mechanism of radiation-mediated thymic lymphoma induction in laboratory mice.

2. Materials and Methods

Animal experiments were performed adhering to the Institutional Animal Ethics Committee (IAEC) guidelines. Swiss mice were irradiated by Co-60 γ-rays (dose rate: 0.5 Gy/min.) using Junior Theratron (MDS, Canada). Animals were dissected after overdose of ether and the obtained thymus or thymic lymphoma tissue was rinsed with PBS followed by preparation of cell suspension in PBS at room temperature as mentioned in Ref. (10). The lysates were prepared from cell pellets using mammalian cell lysis kit (Sigma, St. Louis, MO, USA) followed by measurement of protein estimation by Lowry Method (11) . The protein samples were fractionated on 10 % SDS-PAGE followed by staining with Comossie blue (12).

3. Results and discussion

Previous studies from our laboratory have shown the induction of TL in Swiss mice 120 days after 3 Gy dose of Whole Body Irradiation (9, 13). Physiological alterations in these mice and changes in protein profile in control and tumor tissue were further investigated. Alteration in body weight gain in

male and female mice animals was studies after WBI either at the age of 3-4 or 6-8 weeks (Table 1). Compared to sham irradiated controls, animals exposed to WBI showed significant decrease in gain of the body weight measured after 120 days of exposure (Table 1). It was interesting to observe that in females the decrease in gain in body weight was more prominent than in male mice. The decrease in body weight has been known to indicate physiological alterations and sickness under different pathological conditions. In present investigation, the decrease in body weight may be associated with the incidence of tumor. As observed in our previous study, an increase in TL incidence was observed with the increased post-irradiation time, which was 47, 80 and 93 % after 90, 120 and 150 days of WBI, respectively in female mice. Animals were found to show higher incidence of TL incidence when animals were exposed to WBI at younger age than adults. The incidence of TL is found to be associated with sex of animals and age at the time of irradiation (13). In addition, it was interesting to observe that the TL incidence was significantly higher in females than in males exposed to WBI at same age. Results showed typical thymic lymphoma incidence in mice exposed to 3 Gy of WBI followed by 120 days (Fig. 1). The thymic lymphoma is very prominent and depending on the size of tumor, it covers partial / whole thoracic region including heart. Animals bearing thymic lymphoma showed many physiological alterations including hematological parameters and markers of oxidative stress (data not shown). In addition, effect of antioxidants on radiation-induced TL was investigated in mice orally fed with different antioxidants after WBI (3 Gy). Thymic lymphoma incidence in irradiated animals without feeding of antioxidant was ~90 % (13). However, the incidence of TL was prevented significantly when animals were fed with antioxidants for some days prior to irradiation. Feeding with ascorbic acid (1000 mg/ kg body weight), eugenol (25 mg/ kg body weight) showed inhibition (20 %) in TL incidence; whereas prevention in TL incidence was 55 % in animals fed with curcumin (1 % mixed in feed). It is generally considered that radiation generated reactive oxygen species reacted and caused alterations in biomolecules leading to cellular malfunction. Natural plant products of dietary and medicinal values have shown the potential to prevent the oxidative damage and consequent apoptosis by ionizing radiation (4, 6, 7, 14). It has been proposed that prevention in TL incidence WBI mice involved protection of bone marrow in addition to thymus (data not shown). Studies on mechanisms of radiation induced tumorigenesis have indicated that cellular transformation and associated molecular changes lead to appearance of tumor phenotype. The phenomenon may involve alterations in protein and gene expression, which might contribute directly or indirectly in the mechanism of radiation induced incidence of TL. Figure 2 shows the pattern in protein profile by SDS-PAGE in control and thymic lymphoma tissues. It was interesting to observe that TL samples showed significant difference in alterations in protein profile than the respective control. It appears that in TL many proteins are

downregulated and a few new proteins are formed as inferred from appearance of new bands on the gel. It seems possible that alterations in some of the proteins in TL tissue may be the cause of tumor transformation by radiation. It may be mentioned that further identification and characterization of these proteins using Western blotting and proteomics is under investigation in our laboratory.

Table 1. Alterations in body weight in mice exposed to 3 Gy and respective sham irradiated controls.

Age at the time of irradiation (Weeks)	Treatment	Body Weight at Gain (%) in Females	Body Weight at Gain (%) in Males
3-4	Sham irradiated	239	185
	Irradiated	158	156
6-8	Sham Irradiated	46	45
	Irradiated	28	41

Mice of either 3-4 or 6-8 weeks in age were exposed to whole body irradiation by ^{60}Co γ-rays in specially designed Perspex box using Junior Theratron Radiation Source. Body weight of these animals was measured before radiation exposure and after 120 days of irradiation. The gain in body weight was calculated as follows: ((body weight after irradiation-body weight before irradiation)/body weight before irradiation) x100).

It has been reported that low radiation doses can protect/modify the radiation response in a variety of cell systems (12, 15-19). We have also investigated modification in incidence of TL after pre-exposure of animals with low doses. Our results suggest that animals pre-exposed to low doses prevented the incidence of tumor induced by subsequent challenge dose (9). These observations were further correlated with the role of intracellular ROS and cellular apoptosis in mechanism of radiation induced TL(20). Our results showed that treatment of animals with increasing fractions of low doses resulted in increased level of ROS as well as apoptosis. However, the generation of ROS after irradiation by 3 Gy was significantly inhibited, when animals were pre-treated with priming doses of low radiation. Results suggest that pre-exposure of animals with low dose resulted in significant prevention in oxidative stress and induction of apoptosis. The induction of apoptosis has been known to contribute significantly in the manifestation and pathogenesis of radiation-induced cellular transformation. Results of our experiments have shown significant increase in ROS as well as apoptosis in thymocytes of irradiated mice depending on applied radiation dose and post-irradiation time. WBI with 3 and 5 Gy yielded 2 and 6.5 times increase in ROS generation in thymocytes. Moreover, an increase (3 to 4.5 times) in apoptosis was observed after 3-5 Gy WBI. Our results show good correlation between the generation of intracellular ROS and apoptosis in WBI mice. Based on observation made in many laboratories in human cells

exposed to 'prime' low dose ionizing radiation it has been postulated that a priming low dose enhance inducible excision base repair response, which might be underlying in the mechanism of low dose adaptive response. Involvement of ROS and apoptosis has been elucidated in the mechanism of induction and pathogenesis of cancer (8). Moreover, our results explain the mechanism of modification of radiation-induced TL incidence in mice mediated by dietary antioxidants and low dose radiation possibly through neutralization of radiation oxidative stress induced species.

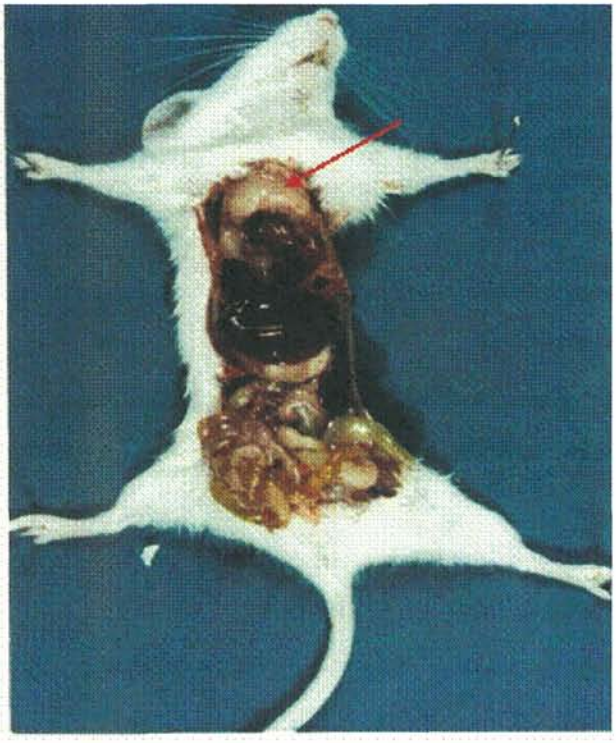

Figure 1. Incidence of thymic lymphoma in female mice after WBI. Animals were irradiated to WBI followed by measurement of thymic lymphoma after 120 days. The red arrow showed lymphoma, which has spread over the thoracic region.

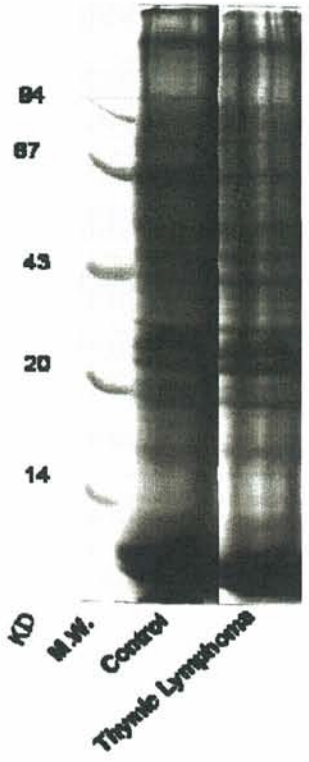

Figure 2. Protein profile in control and thymic lymphoma tissue. The M.W.: Molecular weight markers. The lysate prepared from these samples were fractioned on 10 % SDS-PAGE followed by staining with Comossie blue.

4. Summary and Conclusions

Induced thymic lymphoma in irradiated mice provide convenient and sensitive *in vivo* model to investigate the mechanism for tumor induction by radiation and its modification by dietary antioxidants and priming low doses, which may provide substantial information about radiation damage and associated risk of cancer induction. Our studies using *in vitro* and *in vivo* experiments suggest involvement of cellular oxidative stress and magnitude of apoptosis in thymocytes obtained from WBI mice in the induction of TL induction by γ radiation. It has been shown that radiation induced generation

of ROS and apoptosis in thymocytes of whole body irradiated mice were significantly elevated. However, it is significant to note that they were found significantly reduced when animals were pre-exposed to low doses of radiation or fed with antioxidants. These results imply low dose radiation and antioxidants may prevent high dose radiation induced tumor induction which may be applicable to protection of human against cancer inducing doses of radiation exposure.

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Change of Protein Arginine Methylation during Radio-Adaptive Response and Role in Cell Proliferation

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Abstract

Exposure of cells to low doses of radiation has been well known to elicit adaptive responses, but the underlying regulatory mechanisms are still poorly understood. Arginine methylation is a post-translational modification that results in the formation of asymmetric and symmetric dimethylarginines, and involved in a growing number of known cellular processes, including transcriptional regulation, cell signaling, RNA processing and DNA repair. The present study evaluated the epigenetic change involving arginine methylation of proteins in radioadaptive response. A normal human liver cell-line, Chang-liver cells, was irradiated with 0.02, 0.2, 2 or 4 Gy (60) Co-gamma-ray. Adaptive response by exposure to 0.2 Gy prior to 4 Gy irradiation was observed by comparing arginine methylation status with cells that received a single 4 Gy dose. In a 27-kDa protein, asymmetric dimethyl-arginine was found to increase at 24 h after low dose radiation (0.2 Gy). In a 73-kDa protein, asymmetric dimethyl-arginine increased and symmetric dimethyl-arginine decreased at 24 h after high dose radiation (4 Gy). The aforementioned proteins were identified by mass spectrometry to be a hypothetical protein (27 kDa) and mortalin (73 kDa; a member of the heat shock 70 protein family which is known to be correlated with the radioadaptive response, control of cell proliferation and can also act as a chaperone). Intriguingly, the increase of asymmetric dimethyl-arginine in mortalin decreased and the decrease of symmetric dimethyl-arginine in the 73-kDa protein increased as compared to the arginine methylation status by a single high dose (4 Gy) irradiation, when cells were preirradiated with 0.2 Gy before a 4 Gy irradiation at a 4 h interval. Thus, the present study showed that radiation induced a change in arginine methylation status of some proteins, and also was involved in radioadaptive response eliciting radioresistance. Change of arginine methylation profile during rat liver regeneration was observed to investigate the role for arginine methylation in proliferation, which provides the information on some important proteins that seems to be involved in the regulation of cell

prolifereration. Previously, we showed that the protein arginine methyltransferase (PRMT) activity underwent time-dependent changes in the cytosol of the rat hepatocytes upon partial hepatectomy, indicating that the PRMT activity was directly correlated with the degree of proliferation. The present study particularly investigated the change of arginine methylation profile of the in vivo substrates during liver regeneration, using anti-asymmetric or anti-symmetric dimethylarginine antibodies. Asymmetric or symmetric dimethylarginine formation in proteins generally appeared at 1-3 days following hepatectomy, and disappeared thereafter. The nature of the proteins that showed the most remarkable changes at the early period post hepatectomy were identified employing 2-D electrophoresis and mass spectroscopy. Identified were 17 asymmetric arginine dimethylated proteins and 14 symmetric arginine dimethylated proteins. Some proteins had both asymmetric and symmetric dimethylarginine on the same protein. Many of them were found to be enzymes involved in the regulation of oxidative stress, including catalase, carbonic anhydrase 3, peroxiredoxin6 and argininase-1 that contain asymmetric dimethylarginine, and glutathione S-transferase, catalase and hsp-70 that contain symmetric dimethylarginine. Collectively, the present study demonstrated relevance of arginine methylation to radioadaptive response and regulation of cellular proliferation. The biological mechanism of the phenomenon needs to be clarified.

Keywords: low dose radiation, arginine methylation, radio-adaptive response, cell proliferation

Genes Activated by Low Dose Radiation

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1. Introduction

The major risk caused by radiation is cancer. The cancer incidence has been estimated based on the epidemiological studies on atomic bomb survivors in Hiroshima and Nagasaki, which are the cases of high dose-rate exposure over intermediate dose ranges. The cancer risk due to low-dose or low-dose-rate radiation has not been well established, because sufficient epidemiological data have not been available (Fig.1). It is generally considered that the animal study is an alternative approach to this issue if it is combined with the study on mechanism of radiation effects to extrapolate the animal data to humans.

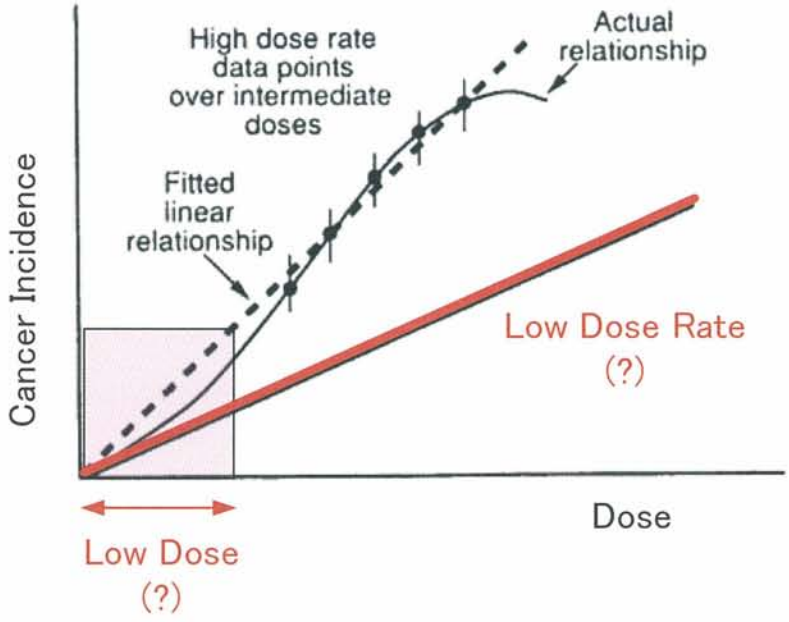

Figure 1 Risk of Radiation-induces cancers

A large-scale study of the biological effect of continuous low dose-rate irradiation has been performed at Institute for Environmental Sciences (IES) in Japan. Assessment of low-dose-rate radiation effect is a difficult issue, because a long-term experiment using a lot of animals in specific pathogen free (SPF) conditions is required. Continuous irradiation of 4,000 SPF mice for 400-days was carried out at IES, and the result was published by Tanaka[1] in 2003 (Fig.2). He reported that the life

spans of mice irradiated at the dose-rate of 16,000 nGy/min were significantly shortened, but not at the dose-rate of 40 nGy/min. A significant life span-shortening in female mice irradiated at 800 nGy/min was observed. In order to investigate the molecular mechanisms for the life span-shortening caused by low dose-rate irradiation, we examined gene expression profiles at the time of termination of continuous irradiation.

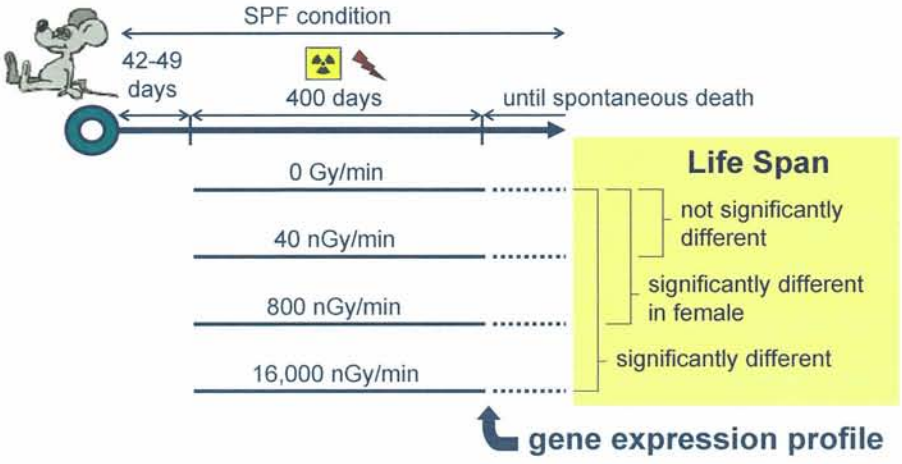

Figure 2 Experiment of long-term low-dose-rate irradiation at IES

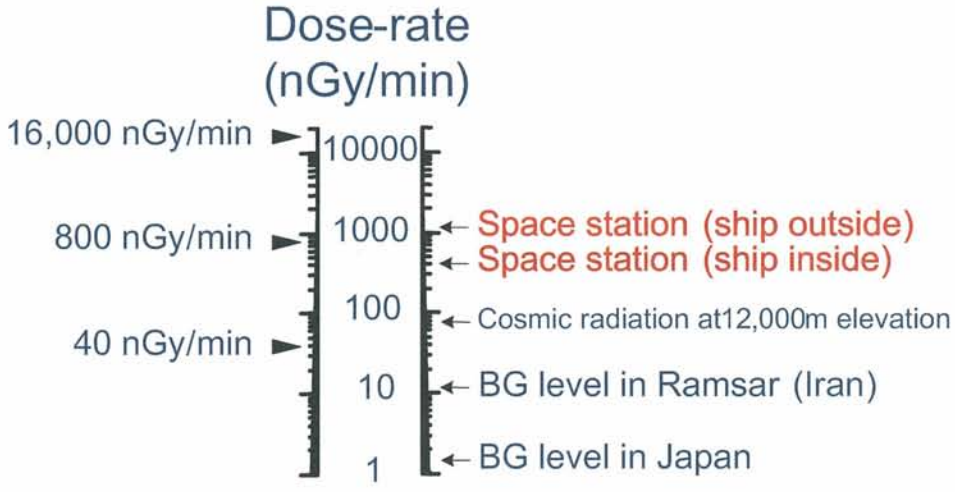

Figure 3 Dose-rate range utilized in the experiment at IES

Figure 3 shows the dose-rate range used in the life span study at IES in comparison with various natural radiation levels. The lowest dose-rate level is 20 times higher than the natural radiation level in Japan, and close to the cosmic radiation at 12,000 elevation and natural radiation level in Ramsar in Iran, which is known as one of the high background radiation areas. The middle level is comparable to the

dose-rate that the astronauts working inside or outside the space station are exposed to. The highest level is 20 times higher than this level. The pathological analysis of the mice used in the life span study at IES was completed recently, and it was reported by Tanaka[2] that the observed life spans-shortening was due to early death from a variety of neoplasms and not from increased incidence of specific neoplasms. This result suggests that effects of the low-dose-rate radiation may be somewhat ubiquitous over various organs.

2. Materials and Methods

Our experiment was designed similarly to that of the life span study at IES (Fig.4). We used the C57BL/6J male mice of 7-8 weeks of age at the onset of irradiation. Irradiation was carried out with cesium-137 γ rays for 485 days in the same facility as that used in the life span study. The dose-rates, a little bit smaller than those used in the life span study were used. In sampling, three 3 mice per dose rate group were killed immediately after irradiation, and kidneys were isolated and stored in a freezer of -70°C in RNAlater. Gene expression was analyzed by use of the cDNA microarray of Illumina, Sentrix Mouse-6, which contains 46k transcripts. This array is constructed by the very small beads coated with oligonucleotide probes. And the beads are densely arrayed on the surface of silicon substrates. Because of the high density of the beads, the signal from one gene is taken 30 times on average across the array, and this redundancy causes increased accuracy of the measurement.

- Continuous irradiation of mice
  - mouse: C57BL/6J, male, 7-8 weeks of age (at the onset of irradiation)
  - Irradiation: ^{137}Cs γ-rays, 485 days (22h/d)
  - Dose rate: 32 nGy/min, 650 nGy/min, 13,000 nGy/min
- Sampling
  - 3 mice/group were killed immediately after irradiation.
  - kidneys were isolated and stored at -70C in RNAlater
- cDNA microarray
  - Illumina, Sentrix Mouse-6 (46k transcripts)

Figure 4 Design of the experiment

3. Results and Discussion

Quality control of microarray data

When the distribution of the normalized signal intensity from 12 arrays, which contain 3 replicates in each of 4 dose-rate conditions were checked, a sample from the mouse irradiaited at 32 nGy/min showed an anomalous distribution. And this sample was simply removed from the further analysis.

When the scatter plot for signal intensities from 2 of 3 unirradiated control mice was checked, a lot of genes showed a large variation in signal intensity caused by both interindividual differences and the statistical errors of the data with small signal intensity. We removed the genes with large variation within the array as well as among individuals from the analysis. As a result, 4,061 genes were remained out of 46k genes. The variation of the signal intensity of the remained genes were reduced with the relative ratio mostly less than 1.3.

Largely modulated genes in kidneys

We here defined the largely modulated genes as the genes whose expression level was changed more than 1.6-fold after irradiation. As a total, 50 of largely modulated genes were identified. By examining the physiological roles of the largely modulated genes, it was found that four genes whose expression was up-regulated after irradiation at 650 and 13,000 nGy/min were involved in mitochondrial oxidative phosphorylation pathway. These four genes, *Uqcrb*, *Ndufb9*, *Atp5k* and *Ndufv2*, were up-regulated with increase in the dose-rate. As these genes play positive roles in mitochondrial respiration, it is expected that the activity of mitochondrial oxidative phosphorylation was elevated after irradiation at these dose-rates. The dose rate-denpendent increase in expression of the *Ndufb9* gene could be confirmed by real-time PCR.

Significanty modulated genes in kidneys

Next, we examined the genes whose expression levels were significantly modulated by low-dose-rate irradiation. 621 genes were extracted by a Welch's ANOVA with p-value cutoff of 0.05 (Multiple Testing Correction was not utilized). For these significantly modulated genes, clustering analysis was performed. On the basis of expression pattern, the genes were classified into 16 clusters, and we could observe that the genes belonging to the biggest cluster show increased expression depending on dose-rates. There is a database called "Gene Ontology". The Gene Ontology database consists of gene groups categorized according to their function. We compared the gene clusters with Gene Ontology category. And it was found that the biggest cluster is significantly similar to the Gene Ontology category of cytoplasm, mitochondria and energy pathways. This result further supports the idea that the activity of mitochondrial respiration was elevated after low-dose-rate irradiation.

Genes modulated in testes

Next I move on to a little bit different topics, hereditary effects of low-dose-rate radiation. Actually this subject is now ongoing at IES. Therefore it is too early to carry out a microarray study for the purpose of elucidating the mechanism for the hereditary effects of low-dose-rate radiation. However we considered that we may obtain something suggestive from the analysis of gene expression profiles. And

here we have set the question; Are DNAs in reproductive organs and non-reproductive organs maintained in a same way? In order to address this question, we performed another microarray analysis using the testes from the same mice as those used in the kidney study. After quality control of the data, approximately 8,000 genes remained after removal of unreliable data. And we could extract 110 of largely modulated (fold-induction>1.6) genes.

When largely modulated genes were compared between kidneys and testes, we found only 2 genes commonly modulated. For the *Hspa8* gene, the direction of modulation was opposite, that is, down-regulation in kidneys but up-regulation in testes. We concluded that the gene modulation after low-dose-rate irradiation is not so similar between kidneys and testes.

When clustering analysis was performed, we found that the genes whose expression was increased with dose-rates were significantly similar to the Gene Ontology category of "response to temperature" and "response to heat". In contrast, the genes whose expression was decreased with dose-rates were significantly similar to the Gene Ontology category of "DNA repair", "response to DNA damage", "DNA replication" and "mitotic cell cycles". Here we can draw an image that cells in testes responded to low-dose-rate radiation as if they were preparing for emergency by shutting down the general metabolisms as well as DNA repair activity.

4. Conclusion

In conclusion, mitochondrial oxidative phosphorylation was suggested to be elevated after irradiation at 650 nGy/min and 13,000 nGy/min. And mice irradiated with low dose-rate radiation in this range may undergo oxidative stresses caused by elevated mitochondrial respiratory activity. This oxidative stress may be one of the causes for life spans-shortening. In addition, expression of multiple genes, including mitochondria-related genes, was found to be modulated after 32 nGy/min. Our study suggested the importance of mitochondrial respiratory activity in life-span shortening after low dose-rate irradiation. This idea must be further tested by various experimental systems including those with genetically modified animals.

Alteration of gene expression profile after low-dose-rate irradiation was different between kidneys and testes. And cells in testes responded to low dose rate radiation in a similar way to that of heat shock responses, which is a type of biological responses for emergency. It should be emphasized that the genes related to DNA repair were repressed. It seems as if the cells in testes damaged by low dose rate radiation would not be actively repaired but eliminated from the organs. It may be suggested that, in testes, mutation frequency due to low-dose-rate radiation would be low because miss-repair of damaged DNA is infrequent.

5. Acknowledgment

This study was performed as a part of Crossover Research Project which is supervised by Dr. Ono of Tohoku University, Japan. The collaboration of Tsuneya Matsumoto, Yoichi Oghiso, Kimio Tanaka, Kazuaki Ichinohe, Shingo Nakamura, Satoshi Tanaka of Institute for Environmental Sciences is much appreciated. This study was supported in part by the Budget for Nuclear Research of the Ministry of Education, Culture, Sports, Science and Technology of Japan, based on screening by, and in consultation with, the Atomic Energy Commission of Japan.

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Radiation-Adaptive-Response-Based Cancer Risk Modeling

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1. Introduction

Ionizing radiation spans the universe and there are two basic forms: electromagnetic and particulate. Electromagnetic radiation is comprised of uncharged photons that interact with electrons in matter causing ionizations if photon energy is sufficiently high. Examples of ionizing electromagnetic radiation are X-rays and gamma rays. Examples of particulate ionizing radiation are alpha and beta particles emitted by radioisotopes and protons from the sun. Neutrons do not directly cause ionizations but can cause them indirectly through secondary charged particles such as protons.

Natural background ionizing radiation we encounter on earth comes from the sun (solar radiation), outer space (cosmic rays), and terrestrial sources (e.g., radionuclides in our environment, homes, and bodies). Radionuclides in our bodies include ^{40}K and ^{14}C. We are also exposed to low doses of radiation from some diagnostic medical procedures (e.g., computed tomography [CT scans] and chest X-rays).

Cancer risk assessment for humans exposed to ionizing radiation is currently based on the linear-no-threshold (LNT) model[1]. With the LNT model, any radiation dose, no matter how small, supposedly causes some cancers among a very large irradiated population. Doubling the dose supposedly doubles the number of cancer cases. The BEIR VII Report[1] has implicated diagnostic X-rays (e.g., chest X-rays, mammograms, CT scans) and nuclear medicine diagnostic procedures as causing harm through inducing excess cancers, based on use of the LNT model. Brenner and Hall more recently arrived at similar conclusions for CT scans.[2]

This paper focuses on modeling lung cancer induction by combined exposure to low doses of alpha + gamma radiation. Evidence is provided for low doses and dose rates of gamma rays preventing sporadic and hereditary lung cancers and also for preventing high linear-energy-transfer (LET) alpha-radiation-induced lung cancer. Such findings as well as others discussed below invalidate the LNT model so far as its application to low-LET radiation or combined exposure to low- and high-LET radiations. The possibilities for using low-dose, low-LET radiation in cancer prevention and for cancer therapy are also discussed.

There is abundant evidence for reduced cancer and some other health risks after low doses and dose rates of low-LET radiation.[3-16] Low doses or low dose rates of low-LET radiation have been

demonstrated to do each of the following: (1) protect against spontaneous genomic damage;[3] (2) protect against spontaneous and high-radiation-dose-induced mutations;[4] (3) protect against spontaneous neoplastic transformation;[5-7] (4) protect against high-dose chemical-[8] and alpha-radiation-induced cancers[9]; (5) enhance immune system defense against cancer;[10-11] (6) suppresses metastasis of existing cancer;[12] (7) extend tumor latency period;[13] (8) protect against diseases other than cancer;[14-15] and (9) protect against heritable mutations and fetal malformation.[16]

The indicated beneficial effects of low-LET radiation do not support the LNT risk model. Low doses and dose rates of low-LET radiation activate a system of cooperative, protective processes in the body. The protective processes are transient and include (1) presumably p53-related high-fidelity DNA repair/apoptosis, (2) a novel auxiliary **p**rotective **a**poptosis **m**ediated (PAM) process that selectively eliminates cells with genomic instability[6,7], and (3) induced immunity against cancer cells[10]. The scavenging of reactive oxygen species and other toxins also contribute to protection.[3]

The PAM process has been demonstrated to involve reactive oxygen and nitrogen chemical species[6,7], specific cytokines (e.g., transforming growth factor β)[6,7], and can occur independently of the *p53* gene[17]. The PAM process and stimulated immunity, which are activated by low doses and dose rates of low-LET radiation, appear to be inhibited by moderate and high doses as well as by high radiation dose rates. The PAM process appears to be inefficiently activated by high-LET alpha radiation.[17] For exposure to neutrons, the gamma-ray component to the dose appears to activate the PAM process, thereby protecting from deleterious neutron-induced stochastic effects.[18] The level of protection appears to increase as the gamma-ray contribution to the dose increases, which is a function of neutron energy.[18]

2. Materials and Methods

A previously published adaptive-response-based relative risk (*RR*) model[17] is used here and is schematically represented in Figure 1 as it applies to the population average *RR*. Because the dose-response curve has a hormetic shape (i.e., J shape), the model has been called the **h**ormetic **r**elative **r**isk (HRR) model. Absorbed radiation doses considered range from absolute zero, 0, to above the current natural background exposure level, *b*. With the indicated model, low doses and dose rates of radiation are considered to stimulate the system of protective processes already discussed. However, the dose for stimulation of protection differs for different individuals and therefore is stochastic. The threshold dose may also differ for different tissues of the body.

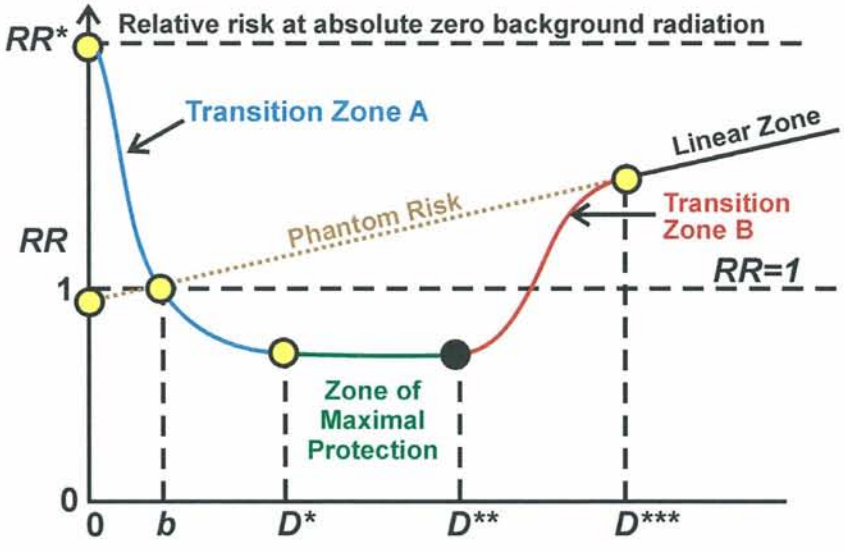

Figure 1. HRR model.[17] See text for detailed explanation.

The indicated stimulation of protection causes *RR* to decrease progressively as radiation dose increases since more thresholds are progressively exceeded. The cancer *RR* decreases over this dose zone which is called Transition Zone A (dose range 0 to D^* in Figure 1). The total radiation dose D is made up of a low-LET component D_L and high-LET component D_H. When the total radiation dose D decreases below the natural background radiation level of b, *RR* is expected to increase as the low-LET dose component D_L decreases due to a progressive loss of adapted protection. The loss occurs as D_L falls below the individual-specific threshold for activating the protective processes that contributed to adapted protection.[17]

Above the total dose D^*, *RR* is roughly constant at $RR = 1 - PROFAC$ (protection factor) over the Zone of Maximal Protection that is relatively wide after low-rate exposure and narrow after high-rate exposure. At higher doses, protection is lost (PAM process and immune system stimulation) as doses exceed inhibitory stochastic thresholds causing a progressive rise in the dose-response curve (Transition Zone B: doses in the Rang D^{**} to D^{***}). Just above a dose D^{***} where protection is inhibited in each irradiated person, the curve then enters the Linear Zone that has been investigated in many epidemiological studies and inappropriately used to justify an LNT extrapolation of cancer risk down to dose b. For the Linear Zone, immunity and the PAM process are considered to be maximally suppressed. Total-body radiation doses in this zone and higher may therefore promote metastasis of existing cancer. For Transition Zone A, changes in *RR* are determined by changes in D_L.[17] For the Zone of Maximal Protection, *RR* changes very little. For Transition Zone B, *RR* can depend both on D_L and D_H. For the Linear Zone *RR* can also depend on both D_L and D_H.[17] The value for RR^* in Figure 1 cannot exceed $1/B$,

where B is the baseline cancer incidence when the natural background radiation dose is b.

3. Results

The cancer RR equation for the HRR model depends on the radiation exposure scenario. The solution provided below applies for combined exposures of the lung to low doses and dose rates of alpha + gamma radiation for $D \geq b$, which is the focus of this paper. At and above natural background radiation exposure, the population average cancer RR is characterized by the following equation:

$RR = 1$, for background radiation exposure.

$RR = (1 - PROFAC)[1 + f(B)K_{\alpha}D_{\alpha}]$, for doses > background.

Here, $f(B)$ represents the quotient $(1 - B)/B$, where B is the fixed baseline cancer frequency (incidence of mortality depending on the endpoint modeled) of interest and is not a free parameter in our current applications. K_{α} is a slope parameter (actually a pseudo parameter) and is associated with the high-LET alpha radiation dose. The corresponding term for gamma rays appears negligible for low doses and dose rates and is not included.[17] The $PROFAC$ in the above equation accounts for radiation activated natural protection (ANP) influences and relates only to the gamma-ray component to the dose. It takes on a value of zero when only alpha radiation is involved.

For cancer mortality considerations, the $PROFAC$ represents the expected proportion of deaths that were avoided among persons with ANP. For cancer incidence considerations, the $PROFAC$ represents the expected proportion of cancer cases that were prevented among persons with ANP. The $PROFAC$ differs for different cancer types and can differ for different exposure scenarios, being larger for low rate extended exposure than for brief exposure at a high rate.[17] Groups with different radiation susceptibilities can have different $PROFAC$ values (indicated by $PROFAC_j$ for the jth group). Thus, $PROFAC$ is a population average of $PROFAC_j$. Similarly, K_{α} is a population average over different susceptibility groups j. The group-specific value is indicated by $K_{\alpha,j}$.

Bayesian inference methods implemented via Markov chain Monte Carlo have been used to obtain estimates of population distributions of $K_{\alpha,j}$ and $PROFAC_j$ and associated averages (K_{α} and $PROFAC$) for lung cancer induction in adult humans by combined alpha + gamma irradiation.[17] Results obtained are based on applying the HRR model to lung cancer data for Mayak plutonium facility workers[19] in Russia who were exposed over years and at low rates to alpha + gamma radiation. The alpha irradiation was associated with inhaled ^{239}Pu. Gamma-ray exposure related to a radionuclide-contaminated

workplace and was presumed protective for the low doses and dose rates considered. Alpha radiation doses for the different dose groups used span wide dose ranges (0 to 12, 12.1 to 50, 50.1 to 200, 201 to 800, and 801 to 3200 mGy, respectively) and were assumed uniformly distributed over the indicated groups. Cancer cases for a given level of exposure were assumed to follow a Poisson distribution.[17] Model simulated (predicted) and observed group averaged *RR* values are presented in Figure 2 and are almost identical. Baseline incidences *B* (averages used) differed for each dose group.[19]

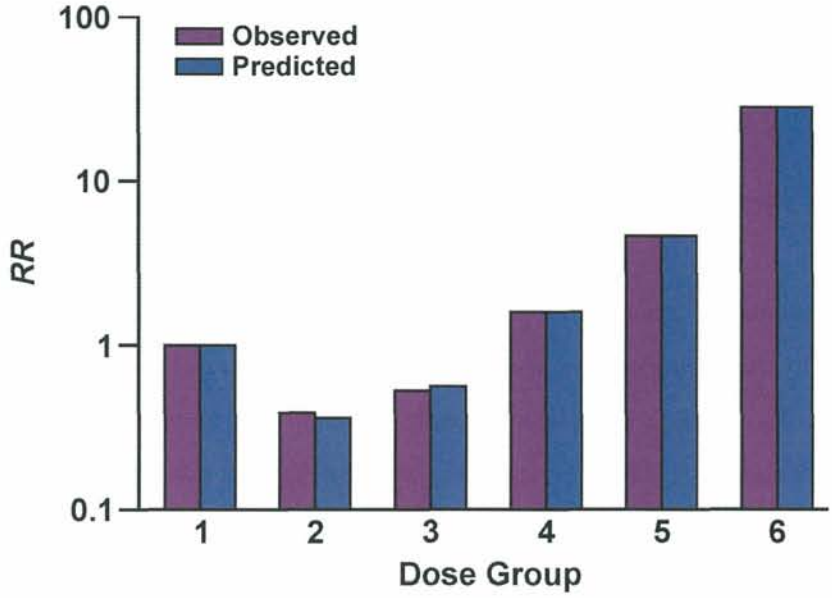

Figure 2. Model simulated (predicted) and observed group average lung cancer *RR* for Mayak workers exposed to alpha and gamma radiations at low rates over many years based on Scott and Di Pamla[17]. Alpha radiation doses by group: Group 1 (unexposed), Group 2 (0 – 12 mGy), Group 3 (12.1 – 50 mGy), Group 4 (51 – 200 mGy), Group 5 (201 – 800 mGy), Group 6 (801 – 3200 mGy).[19] For Group 1, $RR = 1$.

The population distribution obtained for $K_{\alpha j}$ was highly nonuniform. The population average K_α (Bayesian posterior distribution mean) was 1.2×10^{-4} mGy^{-1} with 5%, 50%, and 95% percentile values of 3.7×10^{-5}, 8.6×10^{-5}, and 3.3×10^{-4} mGy^{-1}, respectively. The indicated results can be used to predict lung cancer risk in adult humans when chronically exposed to alpha radiation only. A population average of 0.86 was obtained for *PROFAC*. The 5%, 50% and 95% percentile values for *PROFAC* were 0.73, 0.87, and 0.96, respectively. The indicated results for *PROFAC* can be used to predict lung cancer risk in adult humans when exposed at low rates over an extended period to low doses of gamma radiation. For such gamma-ray exposures the population average *RR* would equal 1 - *PROFAC*.

4. Discussion

The population average for humans ($K_\alpha = 1.2 \times 10^{-4}$ mGy^{-1}) is quite similar to that obtained for dogs and rats exposed to alpha radiation via inhaled plutonium (^{239}Pu or ^{238}Pu) aerosols[20], suggesting that for

mammals and for lung cancer K_α may be evolutionarily conserved. Widely varying estimates of *PROFAC* for different human populations[17] and for lung cancer suggest that this parameter may be evolutionarily diverse for mammals and may be influenced by age, gene-environment interactions and epigenetic processes.

The fact that low doses and dose rates of low-LET radiation can prevent sporadic and hereditary lung cancers as well as those associated with alpha irradiation suggests that low-dose-radiation ANP could be used to prevent future cancers in high risk groups (e.g., heavy long-term smokers). The fact that low doses of low-LET radiation stimulate immunity against cancer suggests that such doses could be used in curing existing cancer (i.e., low-dose cancer therapy). With respect to cancer prevention, factors such as age and genetic characteristics of the irradiated individual would need to be taken into consideration. There is some evidence that the efficiency of radiation ANP against breast cancer increases as age increases above about 50 years.[17] Similar age-related influences may also apply for lung and other cancers. The increase efficiency is thought to relate in part to an increased efficiency of protective intracellular and intercellular signaling related to the PAM process. The body burden of cells with genomic instability increases with increasing age. The unstable cells participate in the signaling process that leads to their destruction (autocrine self destruction)[6,7], with the signaling intensity between unstable and normal cells thought to increase as the local concentration of unstable cells increases.[17] Regarding low-dose cancer therapy for lung and other cancers, one would have to take into consideration that ANP is age-dependent and transient, and the onsets and durations of the protective components (PAM process, stimulated immunity against cancer) have not been resolved. The fact that low-rate exposure to gamma rays over an extended period enhances the level of ANP suggests that the protective processes can be repeatedly reactivated. This points to the use of multiple small doses of low-LET radiation in cancer therapy. However, cancer cells are known to resist undergoing apoptosis, implicating a resistance to the PAM process. Thus, combined therapy involving multiple low doses of low-LET radiation in combination with multiple low doses of an agent that sensitize cancer cells to undergoing apoptosis might effectively destroy cancer cells. Adding multiple low doses of an antiangiogenic agent targeted to tumors might be optimal for curing cancer. Common low-LET radiation sources used in medical diagnostics could be used in this form of combined, low-dose therapy. Further, low-dose and/or low-dose-rate radioimmunotherapy could be employed in combination with applications of apoptosis-sensitizing and antiangiogenic agents, for curing cancer while minimizing side effects. Low-dose radiation therapy has already been reported to be successful for treating non-Hodgkin's lymphoma, ovarian, colon, and hematologic cancer.[17]

5. Conclusions

- Systems radiation biology does not support the LNT risk model so far as it's application to cancer risk assessment when low-LET radiation is involved. A new low-dose caner risk assessment paradigm is therefore needed.
- Low doses and dose rates of low-LET radiation can prevent cancer occurrence, including alpha-radiation-induced lung cancer and chemically-induced skin cancer.
- Low doses of diagnostic X-rays could be used to prevent future lung cancer among heavy smokers. Optimal scheduling of doses requires new research.
- Multiple low doses of diagnostic X-rays or continuous low-rate exposure to low-LET radiation in combination with multiple low doses of apoptosis sensitizing and antiangiogenic agents could be used in curing existing cancer.

6. Acknowledgements

This research was supported by Lovelace Respiratory Research Institute. I am grateful to Vicki Fisher for editorial assistance.

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Implication of the Adaptive Response - A Summary of the General Discussion

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In the session of "Adaptive Response at the Whole Body Level", the adaptive responses observed in pre-irradiated mice were described. The endpoints included micronuclei, chromosome aberrations apoptosis and sperm abnormality (Kim), digital malformation (Wang), thymic lymphoma induced by a chemical carcinogen (Kakinuma) or by gamma rays (Mishra). In the same session an intriguing observation in an epidemiological study on high natural background area was reported (Zhang); the increase in chromosomal translocation observed among smokers in the control area seemed to be cancelled in the high natural background area, suggesting possible adaptive response against smoking by the increased level of background radiation.

All of these presented results suggested that the adaptive responses can be considered as an increase in some protective functions against detrimental effects of ionizing radiation of high doses or some types of chemicals. Although intensive attempts have been made through a genome wide approach (Nenoi) and an approach at protein modification level (Park), the mechanism underlying the adaptive response has not been fully elucidated.

The implication of the adaptive response in radiological protection was discussed. A model for risks from low level radiation with the adaptive response taken into account was presented based on experimental data (Scott). The model predicts, in certain low dose area, even lower risk than control due to the suppression of "background" carcinogenesis by the enhanced protective functions.

Implication of the adaptive response in medical area was also discussed. The enhancement of antioxidative capacity, one of the suggested mechanisms for the adaptive responses (Mishra), makes it promising to apply the adaptive response in prevention/treatment of so-called "reactive oxygen related diseases", if one can identify and choose appropriate cases, and the justification is achieved by taking the balance between potential benefit and risk, based on the information on mechanisms underlying the biological effects of low level radiation.

As a conclusion, the needs of research in the following area were proposed.

(1) To elucidate the mechanism underlying the adaptive response.

(2) To bridge the epidemiological human data with experimental animal data.

(3) To establish biomarkers, which reflect the dose exposed to the individual and bioindicators, which reflect responses going on in the exposed individual and predict the outcome of the exposure.

(4) To identify cases the adaptive response is applicable.

With the information obtained from these researches, the effects of low level radiation would be better understood, and better use of ionizing radiation could be developed.

Overview of Epidemiology in High Background Radiation Areas

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Abstract

Radiation is a natural part of the environment. The air we breathe, the food we eat and the homes we live in contain small amounts of radioactivity. Because of the interest in the health effects from low-levels of radiation it is not surprising that studies over the years have been conducted on populations residing in areas of high background radiation.

Early studies have been ecological, that is, geographical correlation studies contrasting population rates of cancer with estimated levels of gamma radiation or radon (and radon decay products). Subsequent studies have been case-control investigations where measurements of gamma radiation or radon have been made in the homes of children or adults who developed specific cancers such as leukemia or lung cancers.

Finally, there have been relatively large-scale investigations of populations residing in areas of high background radiation in China, India and most recently Iran. These studies have involved detailed exposure measurements and dose reconstructions, chromosomal cytogenetic analyses and even physical screenings for specific disease such as of the thyroid. Cancer and congenital malformations have been a focus.

The advantages of the high background radiation studies include relatively large populations, careful environmental dosimetry programs, comparison populations, low migration rates and genetic homogeneity. There are limitations, however. The weaknesses include the uncertainties in dose estimates actually received by individuals, the somewhat low and narrow range of cumulative doses, the possibility that important demographic and lifestyle factors might differ between the high background and the comparison populations, and the somewhat low statistical power to accept or reject estimates of risk from higher dose studies. For example, typical individuals in the China high background area (6.4 mSv y^{-1}) might by age 50 years receive cumulative doses about 200 mSv greater than persons living in the lower-dose control areas. Based on recent estimates of lung cancer risk from the study of atomic bomb survivors (ERR = 0.81 Sv^{-1}) (Preston 2007), acute doses of about 200 mSv might be expected to result in a relative risk (RR) of lung cancer of the order of 1.20. Relative risks of this magnitude are difficult to detect epidemiologically.

The high background radiation studies, however, should receive greater attention in light of recent reports from studies of radiation workers and of populations living in contaminated environments. The 15-country international worker study (Cardis 2007) and the Techa River study (Krestinina 2007) report significant risks following chronic radiation exposures --- remarkably at lower cumulative doses than in most areas of high background radiation. The average cumulative dose in the 15-country worker study was only 19.4 mSv and only about 1 percent received > 200 mSv. The all cancer (ERR = 0.97 Sv^{-1}) and lung cancer (ERR = 1.86 Sv^{-1}) risks were higher than estimates from the study of Japanese atomic bomb survivors, and possibly confounded by smoking. The mean cumulative dose in the Techa River study was 40 mSv, more than half from ingested radionuclides, and only about 3 percent of the population received doses > 200 mSv. The risk estimate for solid cancers (ERR = 1.0 Sv^{-1}) was twice as high as the recent estimate from the atomic bomb survivor study (ERR = 0.47 Sv^{-1}).

In comparison, the ERR Sv^{-1} for total cancers in China was -0.11 (95% CI -0.67 to 0.69) (Wei and Sugahara 2000). Continued and perhaps combined studies of high background areas might be sufficiently powerful to accept or reject the possibility of high risks from chronic exposures to low-dose radiation experienced throughout life.

Japan-China Collaborative Epidemiological Study on Health Effects of High Background Radiation Area

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Abstract

The epidemiological study on health effects in the residents in high background radiation area in Yangjiang, China was initiated in 1972 by the Chinese group and its first report was published in 1980 in Science. The report attracted the interest of Japanese radiobiologists whose personal contacts with the Chinese scientists had been done since 1980. But no collaborative study has been done until 1991 when we started the discussion on the possibility of collaborative study with revised protocol.

Main epidemiological studies on radiation effects have been the study on A-bomb survivors in Hiroshima and Nagasaki. They were exposed to acute dose of radiation. But usually expected exposure to radiation may be chronic or low-dose continuous irradiation. The residents in high background radiation areas have been exposed to such radiation for a long time. Thus the study on these people would be very important for risk assessment in practical situations. But practically it is very difficult to detect any effects of radiation at such low doses and low dose rates. This is the reason why we spend more than one year for discussion and feasibility study.

After the feasibility study in 1991, the long-term collaborative study between Japanese and Chinese scientists has been initiated in 1992 and continued until now. The study has been reviewed almost every 3 years by international experts. Thus the protocol of the study has been revised and expanded from cancer mortality to non-cancer mortality. The related laboratory work also expanded from unstable chromosome aberration to stable aberration and some laboratory tests in peripheral blood.

Every possible efforts have been carried out to obtain personal cumulative dose for all residents as far as possible with personal history and personal record on daily activities. We now have a data on mortality in nearly 2 million person-years but still have difficulty to demonstrate a significant result on cancer mortality.

Our personal comments on our work are that non-cancer mortality is more important than that of cancer in this area and the importance of laboratory investigation should be recognized depending on the development of molecular biology.

1. Introduction

In 1980 a paper published in Science gave a strong impression to Japanese radiobiologists. The paper was as follows: High-Background Radiation Research Group: Health survey in high background radiation areas in China[1]. The Japan Radiation Research Society invited Dr.Wei Luxin, the chief investigator of the group to give a special lecture on the report at its annual meeting in Nagasaki. Personal contact and exchange of scientists had been continued between the Japanese and Chinese scientists since then.

The final report of this work was published in 1996 edited by Wei Luxin entitled " High Background Radiation Research in Yangjiang, China". The book[2] includes the observation of the area from 1972 to 1986. Sugahara engaged in IAEA project on the improvement of cancer therapy by the combination of conventional radiation and chemical or physical means during 1980s. He had personal contacts with the Chinese scientists in this respect but did not directly related to the project. But a big change happened in 1990.

2. The initial phase

In March, 1990, Dr.Tao, one of the chief investigators of the Chinese group, who stayed in Kinki University as a visiting scientist visited Sugahara in Kyoto and asked him for the collaboration on their HBRA study. The reason of the appeal was that the Chinese government did not support the research after 1986. But they would like to continue their research because they thought that the research was very important for the evaluation of low dose rate effects of radiation. I agreed with their opinion but the Health Research Foundation had no extra budget for this project. Sugahara, chairman of the foundation at that time, started his action to raise money.

In September, we published the Japanese translation of the Chinese report on this topics[3] and distributed it to the Nuclear Safety Commission and Power companies. In October, when Sugahara visited Beijing, he had discussion with the Chinese group on the scale of the budget they expected. Through these processes the Foundation decided to start the joint project with Chinese group. In January, 1991, the foundation organized a workshop to discuss the practical protocol of the research at the international level asking as many related scientists as possible in Japan to join.

Major changes of the research protocol from the original one are shown in Fig.1.

- Geographical comparison→a cohort study
- C vs H →dose response in 4 dose levels
- Area dose →personal dose
- Cancer diagnosis →revised confirmation system
- Cytogenetic study: old technique →newly developed technique with mutual comparison between China and Japan
- Set up new laboratories in China
- Introduction of a reviewing system

Fig.1 Major Changes in the research protocol proposed in the 1991 workshop

From 1987 to 1998 the report on person-years of 1,993,016 including 1,202 cancer death and 11,242 non-cancer death were collected for analysis. At the same time a cross-sectional survey of confounding factors on food intake were carried out and found no significant difference among four levels of radiation. Cumulative dose of each resident was calculated using data on occupancy factors for age and sex surveyed in advance. The summarized report was published in 2000 as a supplement of the Journal of Radiation Research edited by L.-X.Wei and T.Sugahara[4].

3. Changes in research protocol thereafter

Case control studies on site specific cancers such as leukemia, nasopharyngeal cancer, cancers in children and lung cancer were introduced since 1998. With regard to cytogenetic study the analysis on stable type aberrations (translocation) were introduced since 2001 including possible effects of smoking and air pollution in mind Since 2003, the analyses on non-cancer such as tuberculosis have been introduced with laboratory studies.

Concerning population studies, it is estimated that about 382,282 person-years would be accumulated by extending the follow-up for another 4 years since 1998. During this period 3,189 deceased persons were ascertained, 9.6% of them were reported died of various cancers, 7.7% from suspected cancers,36.9% died of non-cancer diseases, 35.3% with unknown causes and 8.7% die from external causes.

Unfortunately, however, these data have not yet published. Urgent publication of these studies in English has been waited. But the reviewing and recommendation on research protocol have been continued by the consultants:

In 1995: W.J.Schull, S.Wolf, D.L.Lloyd, E.Tazima. I.Shigemastu,T.Numakunai,and S.Okada.

In 1998: W.J.Schull, W.Burkart, S.Wolf, M.Sohrabi, P.C.Kesavan S.Okada, Y.Aoki, Y.Sasaki, T.Numakunai. and M.S.Sasaki.

In 2001: W.K.Sinclair, C.R.Muirhead, C.Streffer, I.Shigemastu,and J.Mastubara.

In 2004: E.Cardis, W.Burkart, T.K.Hei, Y.Sasaki,and O.Niwa.

In 2006: Four institutions enganged in HBRA study received the China Medical Award.

4. Psychology of the residents

The psychology of the residents at the high background area in China was studied by questionnaire to the scientists engaged in the study as follows:

1) Q: When and how did the residents recognized the area they lived to be at high natural radiation level? A: Since 1972s, by our study and explanation to the residents when we started the survey.
2) Q: Did the residents know in advance that the area is different from other areas? A: No. they did not know.
3) Q: When the residents knew the fact, were they uneasy in their mind? A: No, I do not think so. But when they were educated well and their family member or friends suffered from cancer, they would have some uneasiness.
4) Q: What is the most concern of the residents now? A: In my opinion, they do not care of it so much.in Yangxi, there is a rumor that their blood has some problems such as anemia.
5) Q: How do you summarize the psychology of the residents based on theabove finding? A: Because they have been exposed to such radiation since their birth and no real effects have been found so far, they do not have any serious feeling about HBRA.

Our comments: We should have more detailed study on the psychology of the residents.

5. High background areas in the world so far observed

In the world there are four well-known high background areas, e.i., Yangjiang, China, Garapari, Brazil, Kerala, India and Ramsar, Iran. But the main sources of radiation are different in different places. In Yangjiang, China, the main radiation source is brick for houses. there are mostly farmers and living in similar brick houses. So personal accumulated doses may be easily estimated. But recent changes in economical situation in China may affect the dose for young people there.

In Garapari, the city became a resort and most soil have been covered by asphalt or concrete. So only sea shores remain at high radioactive level. Visitors enjoy the sand bath there.

In Kerala, India, radioactive sea sand covers the ground. Out-door radiation dose is always higher than

that on indoor. Indoor radiation doses are largely dependent on flour materials depending on the economical situation of the house residents.

In Ramsar, Iran, Ra-hot spring is the source of radiation. Ra-contaminated ground and house wall materials are the main source of high radiation level. Thus only in limited houses the radiation levels are exceptionally high.

Our project on the health effects of high background radiation has been expanded to India and Iran supported by the Radiation Safety Research Center, the Central Research Institute of Electric Power Industry (CRIEP) as a part of its research program since 2003. The Center has cellular as well as animal studies on low dose radiation effects besides the present one. The health Research Foundation greatly acknowledges the financial support of the Center.

6. Concluding remarks on the study in China

In developed countries, the risk of cancer has been a main concern of radiation protection. But in high background area in China, cancer is not the main cause of death. Non-cancer death should be analyzed more carefully including laboratory studies. The population there are rather homogeneous and stable but the influence of recent economic development in China can not be avoided.

To expand the dose range and man-year for more accurate risk assessment, meta-analysis with other regions such as India would be expected.

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Thoron(^{220}Rn) Impact in the Radon-and-Lung-Cancer Epidemiological Study

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Abstract

Since radon is internationally noted as the second cause of lung cancer, many countries are about to solve the problem worldwide. In addition, a new evidence of lung cancer risk has been recently found out with a low level below 200 Bq m^{-3}. Thus the action level will have to be set lower than before. Importance of radon exposure has been further recognized and accurate radon concentrations will be required. Recently thoron has also been recognized from the viewpoint of accurate radon measurements. The present paper describes specification of the NIRS thoron chambers, passive measurement technique of radon and thoron and thoron interference on radon measurements from both experimental studies and field experiences on epidemiological study area.

1. Introduction

Over the past 30 years, radon (^{222}Rn) has been recognized to be one of the most important contributors to natural radiation sources[1)]. Subsequently recent studies have revealed that there is a clearly positive relationship between indoor radon concentration and lung cancer risk even at the low exposure level below 200 Bq m^{-3} [2),3)]. Thus the WHO launched the International Radon Project in January, 2005. In this project, radon is regarded as the global burden of disease and the second leading cause of lung cancer followed by tobacco smoking. Many countries are about to solve the problem worldwide. In general, residential radon is regulated by the action level with 200-600 Bq m^{-3} of radon concentration based on the ICRP recommendation[4)]. On the other hand, the WHO is planning to recommend a new guideline of radon exposure. The action level will be revised with a lower level (100-400 Bq m^{-3}) than before. From such international circumstances, importance of radon issues has been recognized again. If the new guideline is set up, an indoor radon survey will be definitely initiated. Although radon concentrations are to be measured in this survey, measurement data have to be sufficiently assured from the viewpoint of their reliability.

For radon measurements, there are many radon measuring devices: alpha track detectors, charcoal

canisters, electrets and so on. In particular, alpha track detectors and electrets are suitable for large-scale and long-term surveys so as to obtain annual radon concentrations. Those detectors are also often used in some epidemiological studies. They are generally calibrated in a well-controlled environment such as a radon chamber. They give us radon signals only because there is radon only in the chamber. However, thoron is also everywhere together with radon. In fact, thoron has not been well studied in the past studies over a long time because it was often considered that thoron was much less than radon. There are also some difficulties in measurement and calibration. In addition, there have been no epidemiological data on thoron exposure so far. Tokonami (2005) has pointed out that some of alpha track detectors are sensitive to thoron[5]. This finding implies that radon readings will be overestimated and consequently may lead to incorrect estimates of lung cancer risk.

The present paper describes specification of the NIRS thoron chambers, passive measurement technique of radon and thoron, and thoron interference on radon measurements from both experimental studies and field experiences on epidemiological study area.

2. Specification of NIRS thoron chamber

Figure 1 illustrates an overview of the NIRS thoron chamber. The chamber consists of four main components: exposure system, calibration system, monitoring system and humidity control system. The exposure chamber is a 150 liter stainless steel cylindrical vessel and a fan is amounted in the chamber so as to obtain homogeneous distribution of thoron concentration. The thoron gas is supplied through a column filled with many layered lantern mantles with a pump. The thoron concentration is continuously monitored with a RAD7 (electrostatic collection radon monitor). For quality control of thoron concentration, thoron concentrations are accordingly measured with a single scintillation cell technique and grab sampling[6]. Temperature and relative humidity are also continuously monitored. The quality assurance of thoron concentrations are explained as follows: the thoron concentration is determined with the aforementioned single scintillation cell method. Firstly, counting efficiencies for radon and its progeny are calculated by Monte Carlo simulation. Secondly, they are compared with experimental results for their verification. Thus radon concentrations are traceable here. This verified Monte Carlo simulation can be applied to counting efficiencies for thoron and its progeny. Consequently, thoron concentrations can be determined with their counting efficiencies. **Figure 2** exemplifies time variation of thoron, temperature and relative humidity in the thoron chamber.

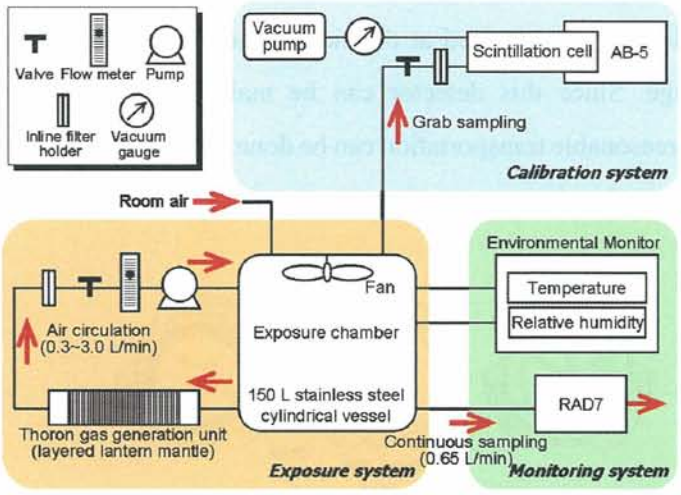

Figure 1 Overview of the NIRS thoron chamber.

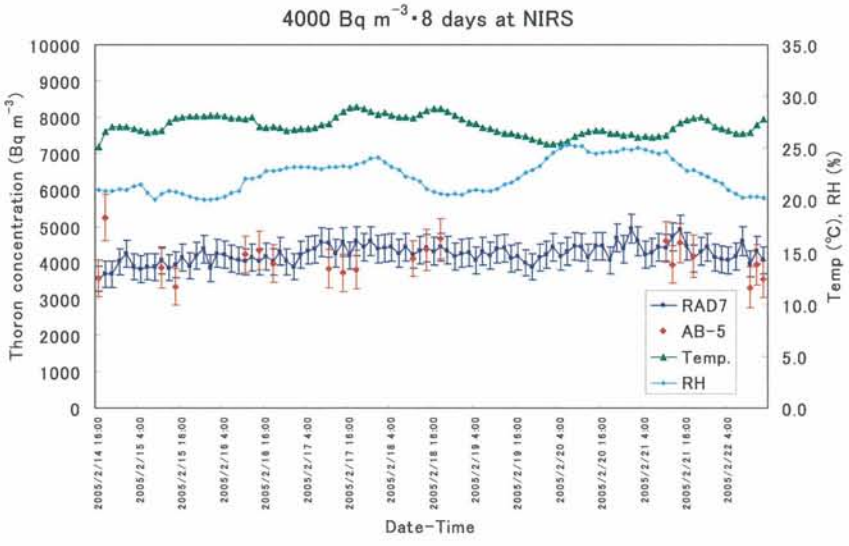

Figure 2 Time variation of thoron, temperature and relative humidity in the thoron chamber.

3. Passive radon-thoron discriminative detector (RADUET)

Figure 3 illustrates an overview of passive radon-thoron discriminative detector[7]. The detector consists of two different diffusion chambers. Each chamber is made of an electroconductive plastic and is cylindrical with an inner volume of about 30 cm^3. The A CR-39 is used as the detecting material and placed at the bottom of the chamber with sticky clays. Radon in air can penetrate into the chamber through an invisible air gap between its lid and bottom through diffusion. Since this air gap functions as the high diffusion barrier, thoron can scarcely go into the chamber with a small pathway due to its very

short half life (55.4 s), compared with that of radon (3.82 d). In order to detect thoron more effectively, six holes of 6 mm in diameter are opened at the side of the other chamber and are covered with an electroconductive sponge. Since this detector can be mailed into a postbox anywhere with this dimension, an easy and reasonable transportation can be done. The conversion factors are determined by the NIRS radon and thoron chambers.

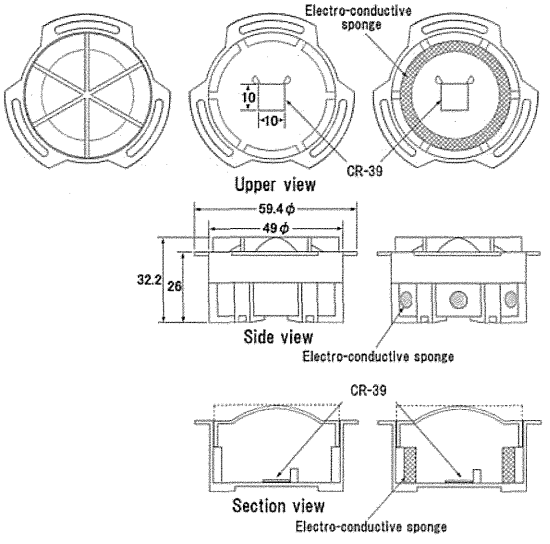

Figure 3 Overview of passive radon-thoron discriminative detector.

4. Thoron interference on radon measurements

Table 1 summarizes relative sensitivities of typical alpha track-etch detectors. Some of them were used in the nationwide surveys and epidemiological surveys. In fact, we have some experience on thoron interference on radon measurements in the first nationwide survey in Japan with KfK monitors, conducted from 1985 to1991[8]. In field experiences on some epidemiological study area, the thoron interference was obviously found. The indoor radon study on the residential radon and lung cancer risk was conducted in Gansu Province, China, 1994-1998[9]. This result shows some evidence that residential radon may cause lung cancer. Many dwellings are located at Chinese loess plateau and most people live in caves. In the epidemiological study area, radon measurements were made with Radtrak. **Table 2** summarizes comparison of the survey result with other studies. The previous study gave us high radon concentrations only, but our new survey gave low radon but high thoron concentrations in the same area[10]. Therefore, thoron interference on radon measurements may result in incorrect risk estimates in several epidemiological studies on residential radon. Although former studies might give us low risk estimates, future studies may give us high risk. A new case control study is now going on in Gansu

Province in cooperation with Chinese scientists.

Table 3 Relative sensitivities of typical alpha track-etch detectors.

Measuring device	*Sensitivity*		*Remarks*
	Radon	*Thoron*	
RADUET with low air exchange rate	1	0.02	Tokonami, et al.(2005)
RADUET with high air exchange rate	1	0.90	
RADOPOT with low air exchange rate	1	0.05	Zhuo et al. (2002)
RADOPOT with high air exchange rate	1	0.59	Tokonami et al. (2003)
KfK monitor	1	0.78	Tokonami et al. (2001)
Radtrak	1	0.68	Tokonami et al. (2001)
NRPB/SSI	1	0.05	Tokonami (2005)
Radon-thoron discriminative dosimeterc with low air exchange rate (Japan)	1	0.08	Tokonami et al. (2001)
Radon-thoron discriminative dosimeter with high air exchange rate (Japan)	1	0.50	

Table 4 Comparison of the survey result with other studies.

Items	*NCI, 2002*	*NIRS, 2005*	*Wiegand et al., 2000*	*NIRS, 2004*
Study area Province	Pingliang, Qingyang Gansu	Qingyang Gansu	Yan'an Shaanxi	Yan'an Shaanxi
Radon (Bq m^{-3})	223	87	92a	76
Thoron (Bq m^{-3})	No data	289	215a	255
EETC (Bq m^{-3})	No data	2.6	21.5b (F of 0.1 used)	2.2
Excess odds ratio (Lung cancer risk)	0.19 at 100 Bq m^{-3} (95%CI:0.05,0.47)	On-going	No data	No data

5. Conclusion

The present study describes thoron interference on radon measurements and its related topics. In particular, the following three components are briefly mentioned: specification of the NIRS thoron chamber, passive radon-thoron discriminative detector (RADUET), and thoron interference on radon measurements from both experimental studies and field experiences in some epidemiological area. Thoron problem still remains unsolved.

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Chromosome Study on the Residents in High Natural Background Radiation Area and Control Area in the South of China and in Beijing

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1. Introduction

Chromosome aberration frequencies in the peripheral blood lymphocytes have increasingly been used for the study of the effect of various occupational and environmental mutagens. In the south of China there is a high background radiation area (HBRA) where the level of natural radiation is 3 to 5 times higher than control areas (CA). The level of natural radiation at HBRA is high due to radionuclides such as Thorium 232 and Uranium 238 decay products in the soil and in the building materials of houses. The elevated dose rate level of natural radiation in HBRA is about equal to that of routine chest X-ray examination of about once a week. To determine the effect of low-dose radiation on human health, we analyzed the chromosomes of peripheral lymphocytes of the residents in the HBRA and compared the results with those obtained from the residents in a control area in Guangdong Province and in Beijing.

In the present paper we review our cytogenetic studies performed in the above 3 places [1-5] and discuss the effect of low dose radiation on human health. All the results presented here were obtained by the China-Japan collaborative study on the HBRA conducted by Prof. Tsutomu Sugahara, Japan, and Prof. Luxin Wei, China.

2. Materials and methods

2.1 Subjects

HBRA and CA are nearby hamlets and the genetic and cultural backgrounds of the residents in both areas are very similar. For the determination of unstable chromosome aberrations (dicentric and ring), 22 members from 8 families in HBRA and 17 members from 5 families in control were studied. Each family consists of 3 generations. For stable chromosome aberrations (translocation), 27 aged persons

and 6 children in HBRA, 25 aged persons and 8 children in control and 30 aged persons in Beijing were studied.

2.2 Measuring individual dose

In our study, each individual dose was measured with electric pocket dosimeter (Aloka PDM-10) for 24 hours and/or thermoluminescence dosimeter (National UD-200S) for 2 months. The level of natural radiation (gamma rays) in HBRA was 3 to 5 times higher than that in CA and Beijing. Accumulated dose were calculated by multiplying the measured dose rate with the age of each individual at the time of blood sampling.

2.3 Cytogenetic preparation

About 3ml of blood was taken from each subject in HBRA, CA and Beijing. Lymphocyte cultures were set up within 8 hours after taking blood. Colcemid was supplemented in cultures from the beginning to the end for 48 hours. Chromosome preparations were made according to the high yield chromosome preparation method [6]. Air-dry slides were stained with Giemsa or with fluorescent dyes using whole chromosome painting probes for Nos. 1, 2, and 4.

2.4 Observation

Analyses were done using microscopes equipped with an automated stage. All the results of analyses were reviewed by more than 2 examiners. Unstable types of chromosome aberrations (dicentrics, Dic and rings, R) were examined in 22 members of 8 families in HBRA and 17 members of 5 families in CA. From each family 3 generations participated in this study. Dic with or without fragments are pooled in the present results. Twenty-seven elders and 6 children in HBRA, 25 elders and 8 children in CA and 30 elders in Beijing were examined with respect to stable type aberrations (translocations, Tr). The frequencies of Tr per 1000 cells were scaled to genome-equivalent frequencies (F_G) by the formula reported by Lucas et al. [7] as follows: $F_G=F_p/2.05fp(1- fp)$, where F_p is the frequency of Tr detected by painting and fp is the fraction of the genome painted.

2.5 Statistical analysis

The frequencies of chromosome aberrations in the HRBA, CA and Beijing were compared by using the Mann-Whitney's U test. A variance test of the homogeneity of the Poisson distribution was used to test for homogeneity.

3. Results

3.1 Unstable aberrations

Regarding unstable aberrations (Dic and R), 101,395cell in total and on average 2600 cells per subject were analyzed. Frequencies of Dic+R in relation to accumulated dose are shown in Fig. 1, which indicates positive dose response of the frequencies. As shown in Fig. 2, the frequencies seem to increase with age in both groups. The rate of increase is about 3 times higher in HBRA than that in CA. Difference of the frequencies between HBRA and CA groups becomes statistically significant after middle age. Effect of high level of natural radiation to the chromosome aberrations is detected.

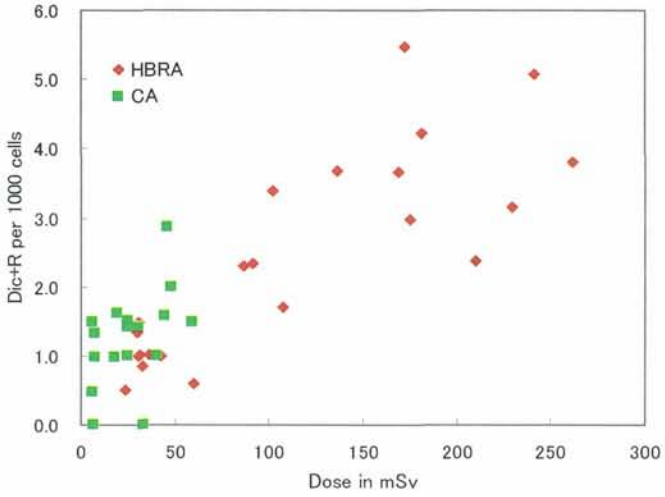

Fig. 1. Frequency of dicentrics and ring chromosomes in relation to the accumulated dose of subjects.
Symbols in red: HBRA. Symbols in green: CA

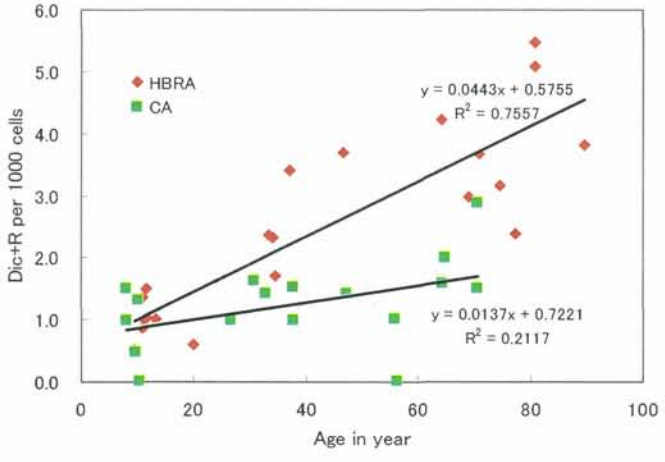

Fig. 2. Frequency of dicentrics and ring chromosomes in relation to the age of subjects.
Symbols in red and solid regression line: HBRA.
Symbols in green and broken regression line: CA.

3.2 Stable aberrations

In the case of stable aberrations (Tr) 448,582 cells in total and 4,313 cells per subject on average were analyzed. As shown in Fig. 3, the frequencies of Tr are much higher than those of Dic+R both in HBRA and in CA. Statistically there is no difference in the frequencies of Tr between HBRA and CA, but the frequencies in children are significantly lower than those in elders in both groups. Individual variation is small in children while that in elders is large. The frequencies in elders among HBRA, CA and Beijing are compared (Fig. 4). The frequency in Beijing is similar to those in rural areas. Results of the homogeneity test are summarized in Table 1. Statistically significant poor homogeneity is found in elders in CA and Beijing. The individual variation in Beijing is the largest among three elderly groups.

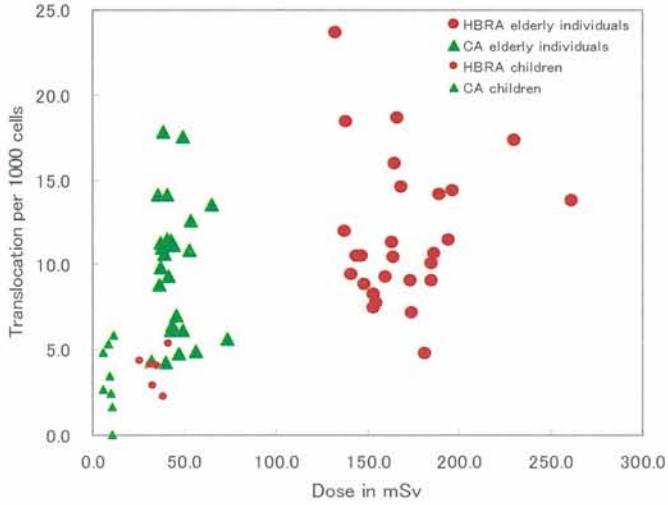

Fig. 3. Frequency of translocations in relation to the accumulated dose of subjects.
Large (elders) and small (children) symbols in red: HBRA.
Large (elders) and small (children) symbols in green: CA.

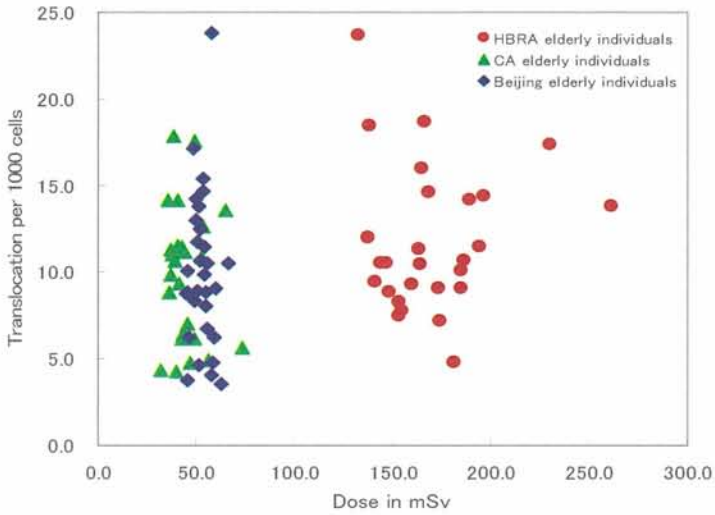

Fig. 4. Comparison of the frequencies of translocations in elders among HBRA, CA and Beijing.
Symbols in red: HBRA. Symbols in green: CA. Symbols in blue: Beijing.

Table 1. Results of the homogeneity test in 5 sub-groups.

Area	HBRA		CA		Beijing
Sub-group	elders	children	elders	children	elders
No. of cases	27	6	25	8	30
Age	62.8±8.1	12.5±0.9	62.9±5.5	12.3±1.3	60.3±3.4
Total cells analyzed	120470	45535	93770	56198	111977
Average cells analyzed	4462	7589	3751	7025	3733
Dose	170.0±28.7	34.2±5.4	45.1±9.5	8.9±2.2	53.3±5.3
F_G/1000 cells Mean±SD Range Median	 11.8±4.2 4.7-23.6 10.5	 3.8±1.1 2.2-5.3 4.05	 9.8±3.9 4.2-17.8 10.6	 3.2±2.0 0-5.8 3	 10.0±4.4 3.5-23.8 9.4
χ^2 df P value	38.88 26 >0.05	1.6 5 >0.9	37.2 24 <0.05	8.8 7 >0.25	56.1 29 <0.005

4. Discussion

Dicentrics and Ring chromosomes are unstable type aberrations, while translocations are stable type aberrations. Those unstable type aberrations are very specific and very sensitive indicators of radiation exposure, while translocations reflect the effects of all kind of mutagenic factors [8]. Increase of the unstable type aberrations was observed in HBRA where the dose rate was lower than 5 mGy per year. This dose rate is the condition that less than one track of radiation passes through the cell in two months. Therefore, the chromosome aberrations detected in HBRA must be induced by a single track of radiation. There seems to be no threshold dose for the induction of chromosome aberrations.

Dicentrics and translocations are induced by radiation in about equal frequency [9], but the effect of radiation to translocations in HBRA was not detected. The frequencies of translocations varies widely among subjects and are much higher than those of dicentrics and rings. Translocations induced by the radiation at the dose rate 3 to 5 times higher than that of the normal level of natural radiation seems to be within the range of individual variation of total chromosome aberrations which are caused by all kinds of mutagenic factors, such as chemicals, radiation, and metabolic factors in the normal living conditions.

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Environmental Dosimetry and Individual Dosimetry

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1. Introduction

Some epidemiological and cytogenetic studies on inhabitants living in high levels of natural radiation areas (HLNRAs) have been performed to examine the health effects of exposure to low dose radiation. As a part of international collaborative studies in this field, we have carried out radiological surveys in some HLNRAs in Iran and China[1-7]. The present report deals with environmental dosimetry and individual dosimetry applied for estimation of individual external doses in these surveys.

2. Materials and Methods

2.1 Environmental dosimetry

Measurement of indoor and outdoor radiation dose rates was made at one meter above the ground and at the earth's surface using an Aloka TCS-166 NaI(Tl) scintillation survey meter. Indoor measurement was made also with Luxel optically stimulated luminescence dosimeters (OSLDs).

2.2 Individual dosimetry

Direct method

Individual external doses of inhabitants in HLNRAs were determined with Aloka PDM-111 electronic personal dosimeters (EPDs) and OSLDs worn by subjects.

Indirect method

Individual external doses were also estimated from ambient radiation dose rates determined by environmental dosimetry, and occupancy factors which were fractions of time spent in a certain place.

3. Results and Discussion

3.1 Environmental dosimetry

A radiological survey was carried out at locations around several hot springs, and inside about 20 dwellings of inhabitants living in HLNRAs in Ramsar, in 1999, 2000 and 2005[1-3].

Some spots with high level of natural radiation were scattered around springs. At a spot near a spring, outdoor radiation dose rates were found to reach levels of 32 μGy/h at 1m height, and 97 μGy/h on the surface of the ground[3]. These results are consistent with those observed by Sohrabi et al.[8-9]. Although hot water from some springs is used for spas, most springs are located far away from dwellings. Particular attention should be given to internal exposure from ingestion of food crops gathered from farms situated around springs, since the spring water flowing into a stream is used for irrigation together with river water[10].

Among all the houses where we surveyed, radiation dose rates inside Taleshi's house were very high, and the highest values detected were 22 μGy/h at 1m height and 110 μGy/h on one wall of a bedroom[3]. This arose as a result of the use of travertine - the Ra-226 content of which was very high - as a building material in the wall[2]. This maximum value for the radiation dose rate on the wall's surface is nearly equal to the value 105 μGy/h reported by Sohrabi et al.[9]. Twenty-three OSLDs were placed at various points in Taleshi's house for one month, where we measured indoor dose rates with a survey meter. Figure 1 shows the relationship of observed dose rate values between OSLDs and a survey meter. From this figure, it is clear that the dose rate values obtained with the two kinds of devices gave a positive correlation.

Outdoor dose rates varied largely and irregularly even in a narrow area. This variation was significant in the vicinity of hot springs. There was also a large difference within indoor dose rates among rooms, because of the non-uniform distribution of travertine contained in building materials. For this reason, individual dosimetry as well as environmental dosimetry was necessary for studying health effects of low dose radiation on inhabitants living in HLNRAs.

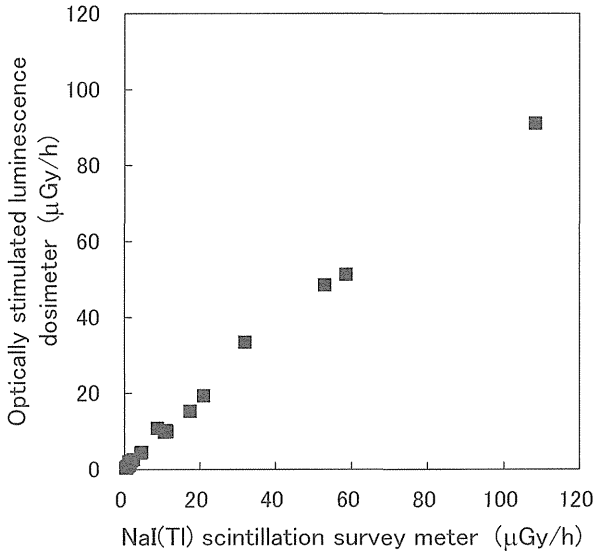

Figure 1 Relationship of observed dose rate values between with OSLDs and a survey meter.

3.2 Individual dosimetry

3.2.1 Direct and indirect methods

We carried out surveys of annual doses that inhabitants received from natural radiation sources in HLNRAs in Yangjiang of Guangdong Province, China[4-7]. Geographical averages for field radiation doses could not be used because of a large difference in radiation dose among houses. Although personal measurement, as well as those indoor and outdoor, appeared to be required, the number of dosimetric subjects was too large to determine directly each dose of all inhabitants with personal dosimeters. For the above reason, an indirect method was applied for estimating individual doses from ambient radiation dose rates obtained by environmental dosimetry and occupancy factors[5-7]. To confirm the validity of this estimation method, we determined actual doses with personal dosimeters for appropriately selected families and compared them with those estimated. The result proved that an adequate correlation existed between the dose values obtained through our estimation and personal measurement. This fact enabled us to estimate individual doses from ambient radiation dose rates and occupancy factors.

In a cytogenetic study conducted in Ramsar, Iran, both direct and indirect methods were applied for determination of individual doses of all subjects[11]. Each of 15 inhabitants in HLNRAs and 10 inhabitants in a control area carried an EPD for one day in April and December 2005. Their individual doses were also estimated from ambient radiation dose rates determined with a NaI(Tl) survey meter and occupancy factors. Figure 2 reveals the correlation of observed dose rate values between April and December 2005. It is indicated from this figure that results of personal measurement carried out twice were essentially similar. Figure 3 shows the relationship of dose rate values between direct and indirect methods. This result indicates a definite correlation between the dose rate values obtained through personal measurement and estimation.

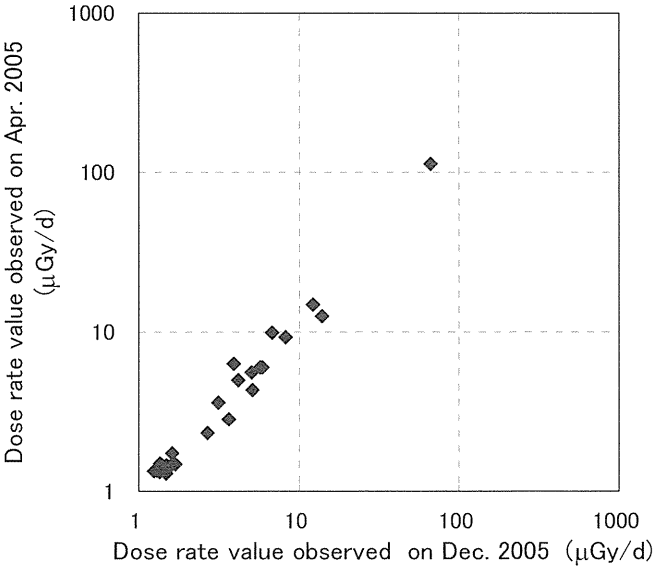

Figure 2 Correlation of observed individual dose rate values between April and December 2005.

3.2.2 Long-term and short-term measurements

In the above cytogenetic study, each of the dosimetri c subjects carried also an OSLD for about one

month in September 2005. Figure 4 reveals the relation of observed dose rate values between one-day measurement with an EPD and one-month measurement with an OSLD. Figures 3 and 4 suggest that a few values obtained by one-month measurement deviates widely from those estimated by the indirect method as well as those obtained by one-day measurement. This deviation would be ascribed to the fact that these OSLDs were left behind somewhere in houses, since it was too troublesome for a few inhabitants to always wear dosimeters for a month. As a result, observed dose values depended heavily on the place where dosimeters had been left, because of the non-uniform distribution of Ra-226 contained in materials of their houses built in the HLNRAs.

For this reason, one-day measurement with an EPD sometimes produced more reliable results than one-month measurement with an OSLD.

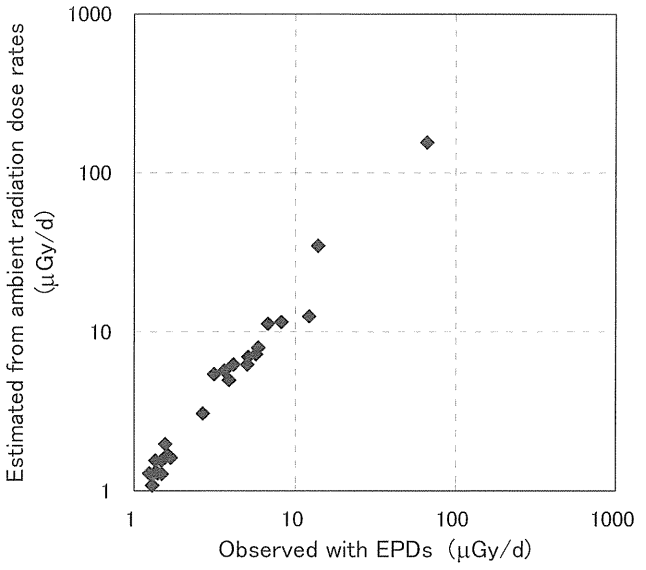

Figure 3 Relationship of dose rate values between direct and indirect methods.

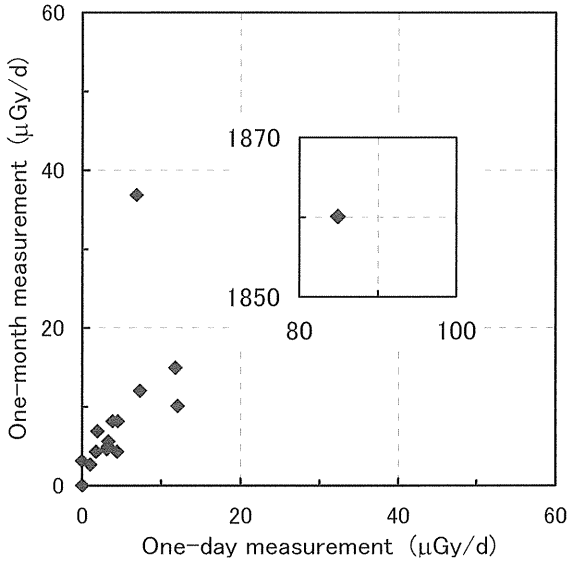

Figure 4 Relation of observed dose rate values between one-day measurement and one-month measurement.

4. Conclusions

The following have been pointed out from the results of radiological surveys in HLNRAs.

(1) In addition to environmental dosimetry, individual dosimetry is essential for studying health effects of low dose radiation, since there is a large difference within indoor dose rates among rooms, as well as among houses built in HLNRAs.

(2) Although individual doses of all inhabitants in HLNRAs should be directly determined with personal dosimeters, in cases where it is difficult, it would be reasonable that direct and indirect methods are applied properly according to purposes of study and some conditions such as the

number of inhabitants and level of individual doses.

(3) While it is desirable that the period of measurement with a personal dosimeter is long enough to estimate individual annual doses, extension of measurement period increases difficulties of always wearing a dosimeter. As a result, short-term measurement with a sensitive dosimeter would frequently produce more reliable results than long-term measurement, if short-term measurement is repeated every season and the results are confirmed by comparison with those estimated from ambient radiation dose rates. In both cases, it is invariably necessary to confirm whether each dosimeter was not left behind somewhere in a house during the measurement period and whether each dosimetric subject took usual patterns of movements, in order to exclude incorrect individual dose values.

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Non-cancer Disease Mortality among Inhabitants in the High Background Radiation Area of Yangjiang, China (1979-1998)

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1. Introduction

Much is still unknown about the health effects of low-level radiation on human health, particularly on the risk of non-cancer diseases. A review by RERF scientists 1) has concluded that the association of atomic-bomb radiation and cardiovascular disease (CVD) is almost certain since the relationship was shown by incidence and prevalence studies of various endpoints of atherosclerosis. In addition to the studies of atomic bomb survivors, the association of CVD with radiation exposure was observed in several other studies, including the follow-up study of patients treated for Hodgkin lymphoma, ankylosing spondilitis, peptic ulcer and scoliosis as well as nuclear workers2-4) .

The inhabitants in the high background radiation area (HBRA) of Yangjiang, Guangdong Province, China receive the external radiation doses of 1.33 mSv in excess every year, mainly from exposure to terrestrial gamma ray, when compared to the residents in the neighboring control area (CA) [5)]. Our most recent analysis of cancer mortality among residents in the HBRA and the CA showed no evident difference between the two areas [6)] . It should be of note that the crude cancer mortality rate in the study population was 60/100, 000 person-years, which is much lower than cancer mortality in developed countries. On the other hand, non cancer deaths accounted for 90% of all deaths [7)]. In this report, we present the results of mortality analysis for non-cancer diseases during the period 1979-1998.

2. Materials and Methods

2.1 Study subjects and mortality follow-up

The HBRA consists of 384 hamlets in the HBRA, and 142 in the CA. A dynamic population had been followed up since 1979. New born babies and immigrants moved into the study areas were not

included in the study cohort since the cohort was fixed in 1987. The methods of mortality follow-up survey and data linkage used in the risk analysis in detail were described elsewhere [7-8]. Briefly, trained local census takers in each hamlet surveyed the subjects to collect information on deaths and migrations among inhabitants in each hamlet, and recorded in the demographic survey sheet. The task group on mortality follow-up survey, then, visited the studied areas and reviewed the survey sheets. In order to ascertain the cause of death, they visited the related hospitals, and reviewed medical records of the deceased subjects and extracted relevant information. When necessary, the task group revisited the local village doctors and next of kin including the family members to collect further information on cause of death. The underlying cause of death thus ascertained was coded according to the 9th revision of the International Classification of Diseases (ICD-9) [9].

2.2 Dosimetry

The method used for estimating the lifetime cumulative dose of each individual was described elsewhere [10,11]. Briefly, the annual external exposure dose of an individual was calculated by multiplying his/her hamlet's environmental dose (indoor and outdoor doses) with a sex- and age-specific occupancy factor for each age group. Then cumulative lifetime dose was calculated by taking the sum of the annual exposure dose of each age.

2.3 Statistical methods

Risk analysis was based on the tabulated person-years data, cross-classified by the variables having the following categories: sex, attained age (0, 1, 2, 3, 4… 89, ≥90 years old), and the follow-up period (1979-1981, 1982-1986, 1987-1990, 1991-1995 and 1996-1998). Relative risk (RR) was calculated to compare the mortality in the HBRA and the CA using the following model: $R=\alpha e^{\beta\chi}$, where α represents the stratum background mortality rates specific for sex, attained age group and follow-up period categories; and χ is an indicator variable of living in the HBRA ($\chi=1$ for HBRA inhabitants, and $\chi=0$ for CA). The estimated β is the log relative risk for the comparison between the HBRA and the CA. All the p-values presented are two-sided. RRs and their 95% confidence interval (CI) were obtained from Poisson regression analysis using the AMFIT in Epicure [12].

3. Results

The follow-up of 125,079 subjects accumulated 1,992,940 person-years at risk, and ascertained 12,444 deaths during the period between 1979 and 1998. The deaths from cancer, non-cancer diseases and external causes numbered 1,202, 10,038 and 1,204, respectively. Among 10,038 non-cancer disease

deaths, only 99 cases were diagnosed at provincial or prefecture hospitals; and 1,058 in county hospitals (data not shown).

The sex- and age-adjusted RR comparing the all cancer mortality in the HBRA and the CA was 1.00 (95% CI= 0.89 to 1.14) as shown in **Table 1**, indicating the absence of difference in cancer mortality. Considering the quality of non-cancer diagnoses, we grouped the cause of death on the basis of categorization used in ICD-9, usually did no attempt on specific disease. The external causes of death (injuries and poisoning) was slightly less common in the HBRA (RR=0.96, 95% CI=0.84-1.08) but the difference was not statistically significant. The RR of non-cancer disease mortality was 1.06 (95% CI=1.01-1.10), indicating a statistically significant increase (p=0.015) in the HBRA, and the excess was virtually limited to those aged under 50 years and the latter half of the observation period (1987-1998) (data not shown).

A statistically significant increase in the HBRA was observed in diseases of the digestive system (RR=1.30; 95% CI, 1.11-1.53), and endocrine, nutritional and metabolic diseases and immunological disorders (RR=1.99; 95% CI=1.25-3.31). The excess of digestive system diseases was mainly due to chronic liver diseases, including liver cirrhosis (RR=1.50; 95% CI= 1.17-1.95). The excess deaths of chronic liver diseases in the HBRA might have been at least partially due to misdiagnoses between chronic liver diseases (RR=1.50) and liver cancer (RR=0.89, 95% CI=0.70-1.13). Indeed, if those two diseases were lumped together, the RR was 1.15 (95% CI=0.97-1.37), which was not a statistically significant increase. The increase of endocrine, nutritional and metabolic diseases and immunological disorders was mainly attributable to diabetes mellitus (RR=2.19, 95%CI=0.98-5.81). The risk of diseases of the circulatory system did not increase in the HBRA compared with the CA (RR=1.02, 95% CI: 0.96-1.09). No more detailed analysis was conducted for CVD risk, because of the poor quality of this diagnosis in the present study.

Although the mortality of infectious and parasitic diseases did not show evident excess, when separated in to subcategories, significant excess was observed in viral hepatitis (RR=4.79, 95% CI, 1.73-19.83), and intestinal infection (RR=1.98, 95% CI, 1.41-2.78). On the other hand, the mortality of tuberculosis, the most common chronic infection in the study area, was lower in the HBRA than in the CA (RR=0.63, 95% CI=0.54-0.74), even when tuberculosis was combined with lung cancer, which might have been the case of misdiagnosis, the RR was 0.67 (95% CI=0.58-0.78). The decrease of tuberculosis mortality in the HBRA was observed both among men and women, and its magnitude increased with attained age, which is closely related to cumulative lifetime radiation dose (**Table 2**). Tuberculosis mortality decreased with lifetime cumulative radiation dose (P<0.001) (**Table 3**).

Table 1 Relative risks for mortality of major non-cancer causes of death

Cause of death	No. of cases		RR (95% CI)	P-value
	HBRA	CA		
All deaths	8905	3539	1.04 (1.00-1.08)	0.047
All cancers	855	347	1.00 (0.89-1.14)	0.950
Injury and poisoning (external causes)	859	345	0.96 (0.84-1.08)	0.482
All noncancer deaths	8050	3192	1.04 (1.00-1.09)	0.039
All non-cancer diseases	7191	2847	1.06 (1.01-1.10)	0.015
Diseases of the circulatory system	3765	1561	1.02 (0.96-1.09)	0.445
Diseases of the respiratory system	899	363	1.03 (0.92-1.17)	0.595
Infectious and parasitic diseases	850	378	0.93 (0.82-1.05)	0.220
All tuberculosis	380	261	0.63 (0.54-0.74)	<0.001
Pulmonary tuberculosis	365	247	0.64 (0.55-0.75)	<0.001
Viral infection	118	23	1.94 (1.26- 3.11)	0.002
Diseases of the digestive system	620	199	1.30 (1.11-1.53)	0.001
Chronic liver disease incl. cirrhosis	269	75	1.50 (1.17-1.95)	0.001
Other than chronic liver disease	351	124	1.18 (0.96-1.45).	0.116
Diseases of the genitourinary system	235	81	1.20 (0.94-1.56)	0.147
Mental disorders	123	62	0.81 (0.60-1.10)	0.176
Endocrine, nutritional & metabolic diseases and immunologic disorders	97	20	1.99 (1.25-3.31)	0.003
Diabetes mellitus	32	6	2.19 (0.98-5.81)	0.056
Symptoms, signs and ill-defined conditions	112	37	1.17 (0.81-1.71)	0.417

4. Discussion

The most concern in interpretation of the present results is the comparability between the HBRA and its control area. In order to compare the distribution of factors potentially confounding the relationship between radiation exposure and cancer or noncancer risk, our study group conducted a series of studies on confounding factors including diet and nutrition, drinking water, pesticide residue and Aflatoxin B1 in rice, medical exposures, tobacco smoking, alcohol consumption and others. Those studies showed that the distributions of those potential confounding factors did not differ much in the two areas [13]. However, the information on those factors could not be used as covariates in our statistical analysis since only a small number of subjects (less than 1000 subjects in each survey) were selected from among the cohort and interviewed.

Table 2. Modification effects by selected factors for all tuberculosis

Factors	Tuberculosis deaths		RR	95% CI
	HBRA	Control area		
All	380	261	0.63	0.54-0.74
Period				
1979-86	195	135	0.63	0.50-0.78
1987-98	185	126	0.63	0.50-0.79
				P-value=0.975
Sex				
Female	128	75	0.75	0.56-0.995
Male	252	186	0.58	0.48-0.71
				P-value=0.154
Age				
0-39	42	14	1.22	0.66-2.24
40-49	35	19	0.68	0.39-1.19
50-59	72	41	0.72	0.49-1.06
60-69	122	79	0.72	0.54-0.95
70+	109	108	0.44	0.34-0.58
				P for trend<0.001

Table 3. Relative risk by cumulative dose (external terrestrial gamma only)

Cumulative dose, mSv	Pulmonary tuberculosis death	PYR	RR (95%CI)
0	254	1407355	1
50-	102	377558	0.77 (0.58-1.01)
100-	212	177614	0.62 (0.51-0.75)
150+	44	30412.5	0.44 (0.31-0.62)

LR statistic =41.92, P=<0.001

The present study showed a significant excess of non-cancer disease deaths in the HBRA. The excess was, however, limited to those aged under 50 and was observed only in the latter half of the observation period (1987-1998), suggesting that the excess mortality may be due to changes in lifestyles in younger generations in recent years. The elevated mortality of diabetes mellitus supports the notion. Regarding the elevated mortality of viral infection in the HBRA, it may be because hygienic status in the HBRA is worse than in the CA, suggesting the lower socioeconomic status (SES) in the HBRA.

Another noteworthy finding in the present study was a decreased mortality of tuberculosis in the HBRA when compared to the control area. The decrease was more evident in older residents. In addition, tuberculosis mortality decreased with the increase of cumulative lifetime radiation dose from terrestrial gamma-ray exposure. The diagnosis of tuberculosis, made in local anti-tuberculosis institutions or town-level above hospitals, is reliable. Even the SES is lower in the HBRA, it is unlikely that this lower SES results in the lower tuberculosis mortality. Also, it is unlikely that the deficit of deaths from pulmonary tuberculosis, the majority of tuberculosis in the HBRA, is due to misdiagnosis since the

diagnosis of pulmonary tuberculosis is not difficult to make. Supporting the notion, the mortality of lung cancer, a possible differential diagnosis, was not different in the HBRA and the CA [6,7].

The relationship between tuberculosis and radiation exposure was also examined in the follow-up studies on patients treated with radiotherapy [14] and nuclear workers [15-16]. A study of radiation workers at Sellafield plant in the UK[15] found an evident decrease of tuberculosis mortality among nuclear workers when compared to non-nuclear workers. In other studies, tuberculosis mortality among radiation workers was similar to that in the corresponding control group.

We compared the rate of newly diagnosed tuberculosis cases among the residents using the official records kept at municipal offices (data not shown). The frequency of registered tuberculosis cases in the HBRA did not differ much from those in the control area. It is unlikely for chemotherapy regimen to be different in the HBRA and CA, since the local patients were mainly treated by local anti-tuberculosis institutions or town-level above hospitals using province-wide standard chemotherapy for pulmonary tuberculosis. In other words, the difference of tuberculosis risk between the HBRA and the CA was evident only in mortality but not in the occurrence of newly registered cases. Those findings suggest that the decreased tuberculosis mortality in relation to radiation exposure may be mainly due to its better prognosis, rather than its decreased incidence.

The better prognosis of tuberculosis maybe relate to enhancement of the immune functions even adaptive response. There is a large body of evidence demonstrating that low-dose radiation exposures can activate the immune functions. However, the observed effects in animal are highly dependent on the range of dose and dose-rate and upon the animal and strain of animal studied; human data are inconsistent [17]. Few convincing data have been published concerning the impact on the immune system of people living in high level natural radiation areas. The interaction of T cells and infected macrophages is central to protective immunity against M. tuberculosis infection, several cytokines such as IL-2, TNF-a, and IFN-g are known to play central roles in regulation of the immune response to M. tuberculosis. We compared the sIL-2r levels between 9 cases of newly diagnosed pulmonary tuberculosis and 14 such cases in the control area, and found a marginally statistical significant difference ($p=0.067$), however no significant difference of sIL-2r was found between the 59 healthy subjects from the HBRA and its counterparts in the control area ($p=0.557$).

In conclusion, the present study showed a significant excess of non-cancer disease in the HBRA, but limited to those aged under 50 years and in the latter half of the follow-up period, suggesting that the excess mortality may be due to changes in lifestyles in younger generations in recent years. A noteworthy finding is the decreased mortality of tuberculosis with lifetime cumulative dose, which is difficult to attribute to confounding factors. We think the decreased tuberculosis mortality may be due to

an improved prognosis of tuberculosis, possibly caused by long-term exposure to low-dose radiation. Further studies seem warranted to confirm our findings and elucidate the underlying mechanism.

5. Acknowledgement

The work was supported by grants from Ministry of Health, National Science and Technology Commission, National Nuclear Safety Agency, the People's Republic of China during 1972-1990, the Health Research Foundation, Japan (Chairman: Dr Kanji Torizuka, Professor Emeritus, Kyoto University) and the Low-level Radiation Research Center of Central Research Institute of Electric Power Industry (Director: Dr Ishida Kenji).

The authors wish to express their gratitude to He WH, Lin ZX, Liang MY, Feng RL, Zhang SZ, , Liu YS, Li J and Lei SW, and other colleagues in the investigated areas for their contributions to the work involving the data collection. The authors also wish to express their sincere gratitude to Nakai S and Kato H for their contributions to this study.

S Akiba was supported by Grants-in-Aid for Scientific Research (12218231) and International Scientific Research, Special Cancer Research (09042007), Ministry of Education, Science, Sports and Culture, Japan.

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Excess Cancer Risk among Inhabitants in the High Background Radiation Area of Yangjiang, China (1979-1998)

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1. Introduction

The mortality among the inhabitants in the high background radiation area (HBRA) of Yangjiang, Guangdong Province, China was first reported internationally in 1980 [1]. Most of the inhabitants lived in the study areas for six or more generations. External radiation dose from the natural sources, including thorium, was estimated to be 2.10 mSv/a in the HBRA and 0.77 mSv/a in the control area (CA) [2,3]. Regarding internal radiation exposure, it was estimated that internal radiation doses in the HBRA and the CA were 4.27 mSv/a and 1.65 mSv/a, respectively [4]. The epidemiological studies had failed to show any excess risk of cancer [5,6].

The major purpose of the present study is to evaluate the effects of continuous low dose-rate exposure to low-LET ionizing radiation on excess cancer risk. In this report, we present the results of cancer mortality analysis during the period 1979-1998, adding 3 years to the previous reports [5,6].

2. Materials and Methods

2.1 Study subjects and mortality follow-up

The mortality data for the period 1979-1986 were collected by follow-up survey of dynamic populations, consisting of around 80 000 inhabitants in the HBRA and as many in the CA. The mortality data for 1987-1998 were obtained from follow-up survey of a fixed cohort consisting of 106,517 individuals (78614 in the HBRA and 27903 in the CA) alive as of the January 1, 1987. The study subjects lived in 227 hamlets in Dong-anling region, 157 hamlets in Tongyou region, and 142 hamlets in the CA. The methods of mortality follow-up survey were described in detail elsewhere [5,7]. Underlying cause of death was determined and coded based on ICD-9.

2.2 Dosimetry

The annual dose received by an individual was estimated based on hamlet-specific environmental dose and age- and sex-specific occupancy factors. The cumulative lifetime dose of each individual was calculated by taking the sum of the annual dose of each age. The methods used in dose estimation were described in details elsewhere [6,8].

The radon and thoron (EEC_{Tn}) exposure level in the HBRA was estimated to be 48 Bq/m^3 and 6.51 Bq/m^3, respectively (data not shown). Therefore, radon/thoron exposure is unlikely to affect our risk estimate substantially. To our knowledge, there is no good evidence that internal exposure causes excess cancer risk except for thyroid carcinoma after Chernobyl accident and some medical therapy using radionuclides including ^{131}I. In the present study, we decided to ignore the radiation dose related to internal exposure.

2.3 Statistical methods

Risk analysis was based on the tabulated person-years data, cross-classified by the variables having the following categories: sex, attained age (0, 1, 2, 3, 4... 89, ≥90 years old), and the follow-up period (1979-1981, 1982-1986, 1987-1990, 1991-1995 and 1996-1998). Relative risk (RR) was calculated to compare the mortality in the HBRA and the CA using the following model: $R=\alpha e^{\beta\chi}$, where α represents the stratum background mortality rates specific for sex, attained age group and follow-up period categories; and χ is an indicator variable of living in the HBRA ($\chi=1$ for HBRA inhabitants, and $\chi=0$ for CA). The estimated β is the log relative risk for the comparison between the HBRA and the CA. All the p values presented are two-sided.

Based on the estimated lifetime individual doses of external radiation from natural sources, the excess relative risk (ERR) per sievert dose (Sv) was estimated using the following model: $r = r_0 \times[1+ERR(dose)]$, where r is the mortality rate for given age, sex, and calendar period; r_0 is the background or baseline of the mortality rate specific for the period of follow-up (calendar period), sex, and attained age; and *dose* is the cumulative dose in sievert. RRs and ERRs, and their 95% confidence interval (CI) were obtained from Poisson regression analysis using the AMFIT in Epicure.

3. Results

The follow-up of 125,079 subjects accumulated 1,992,940 person-years at risk, and ascertained 12,444 deaths including 1,202 cancer deaths during the period 1979 through 1998. Among 819 and 334 solid cancer deaths in the HBRA and the CA, respectively, 24.3% and 24.9% of those causes of death were ascertained on the basis of pathological evidence, and 83.8% and 88.9% were based on pathology, radiology or ultrasonography. For site-specific cancer, the proportion of deaths with pathological

information was highest in nasopharyngeal cancer deaths (54.2% in the HBRA and 47.0% in the CA), and lowest in the liver cancer deaths (only 1.8% in the HBRA and 2.0% in the CA). For leukemia, hematological diagnosis could not be obtained for only 2 out of 49 cases of deaths, one case each in the HBRA and in the CA. The cancer mortality reaches its peak at ages 75-79 and goes down in older age group. On the other hand, non-cancer death mortality jumps up at the age group of 85+ (figure not shown).

The RRs were not substantially modified by sex, age or calendar period. As shown in **Table 1**, all cancer mortality in the HBRA was almost the same as in the CA, the sex and age-adjusted RR comparing the all cancer mortality in the HBRA and the CA was 1.00 (95% CI= 0.89-1.14). When cancer deaths were limited to those with pathological (or hematological) diagnosis, the RR changed only slightly (RR=0.99; 95% CI, 0.78-1.25).

Table 1 Relative risks of all-cancer deaths and its modification by sex, age or calendar period

Variable		No of cancer deaths		RR (95% CI)
		HBRA	CA	
All		855	347	1.00(0.89-1.14)
Period	1979-86	317	129	1.04(0.84-1.27)
	1987-98	538	218	0.98(0.84-1.15)
Sex	Female	284	122	0.96(0.77-1.19)
	Male	571	225	1.03(0.88-1.20)
Age	0-39	165	59	1.04(0.77-1.41)
	40-49	144	65	0.85(0.63-1.14)
	50-59	199	76	1.01(0.77-1.32)
	60-69	215	82	1.17(0.90-1.51)
	70+	132	65	0.92(0.68-1.23)

In site-specific cancer-mortality analysis (**Table 2**), only cancer of the esophagus showed a statistically significant excess in the HBRA (RR=2.61; 95% CI=1.11-7.66). The excess was observed among both men and women. The mortality of all types of leukemia for all ages in the HBRA was slightly higher than that in the CA, but the difference was not statistically significant (RR=1.03, 95% CI=0.56-2.02). There were 4 cases of chronic lymphocytic leukemia (CLL) deaths, 3 in the HBRA and 1 in the CA. The RR comparing the mortality of leukemia except CLL in the HBRA and the CA was 1.31 (95% CI= 0.67-2.81).

Table 2 Relative risks (95% CI) for major cancer sites

Site of cancer	No of cancer deaths		RR (95% CI)
	CA[a]	HBRA	
All cancers	347	855	1.00 (0.89-1.14)
Leukemia	13	36	1.03 (0.56-2.02)
Solid cancers	334	819	1.00 (0.88-1.14)
Nasopharynx	66	153	0.94 (0.71-1.26)
Esophagus	5	31	2.61(1.11-7.66)
Stomach	37	81	0.90 (0.61-1.34)
Colon	7	12	0.70 (0.28-1.89)
Rectum	4	13	1.40 (0.49-4.97)
Liver	100	218	0.89 (0.70-1.13)
Pancreas	4	17	1.69 (0.62-5.87)
Lungs	38	81	0.89 (0.70-1.13)
Bone	5	12	0.99 (0.36-3.11)
Skin	7	28	1.74 (0.80-4.33)
Female breast	8	12	0.65 (0.27-1.66)
Cervix uteri	1	9	4.01 (0.75-742)
Brian and CNS	7	24	1.32 (0.60-3.33)
Thyroid	2	5	1.09 (0.23-7.60)
Lymphoma	6	23	1.48 (0.64-4.01)

[a] RR=1 in control area

The results of dose-response analysis are presented in **Table 3**. Neither all cancer nor solid cancer risk indicates any increasing trend with radiation dose. Cumulative lifetime dose was calculated using only external exposure since there is no good evidence to suggest internal exposure to increase cancer risk except for cancers of the thyroid and lung. It is unlikely that thyroid cancer, which is usually non-fatal, affects our cancer mortality risk substantially.

Table 3 Relative risks (95% confidence interval) by dose group (external doses only, mSv)

Site of cancer	0-49	50-99		100-149		150+		P-value for trend test
	N	N	RR (95%CI)	N	RR (95%CI)	N	RR (95%CI)	
All cancers	420	357	1.16 (0.98-1.38)	365	0.97 (0.82-1.14)	60	0.90 (0.66-1.22)	0.900
Leukemia	35	10	3.08 (0.96-9.89)	3	0.61 (0.13-2.86)	1	1.50 (0.12-18.47)	0.681
Solid cancers	385	347	1.14 (0.96-1.35)	362	0.97 (0.83-1.14)	59	0.89 (0.65-1.21)	0.950
Nasopharynx	73	94	1.14 (0.81-1.61)	48	0.80 (0.53-1.19)	4	0.43 (0.14-1.30)	0.319
Esophagus	5	6	5.43 (1.19-24.86)	20	2.94 (1.00^2-8.64)	5	2.54 (0.64-10.10)	0.095
Stomach	40	28	0.85 (0.49-1.49)	42	0.85 (0.53-1.35)	8	1.07 (0.45-2.51)	0.646
Colon	6	6	1.06 (0.29-3.85)	7	0.75 (0.25-2.78)	0	-	0.312
Rectum	3	5	1.19 (0.24-5.78)	8	2.62 (0.59-11.65)	1	0.57 (0.04-8.85)	0.467
Liver	112	109	0.99^7 (0.73-1.35)	89	0.80 (0.59-1.09)	8	0.55 (0.25-1.19)	0.115
Pancreas	8	3	0.56 (0.12-2.53)	9	2.34 (0.58-9.49)	1	2.83 (0.22-35.78)	0.320
Lungs	36	30	1.23 (0.69-2.18)	45	0.85 (0.54-1.35)	8	1.13 (0.48-2.67)	0.905
Female breast	8	4	0.67 (0.16-2.83)	7	0.74 (0.26-2.13)	1	0.30 (0.03-2.71)	0.227
Cervix uterus	2	1	2.96 (0.17-52.69)	6	3.83 (0.46-31.78)	1	4.24 (0.23-77.31)	0.158
Others	87	58	1.61 (1.02-2.53)	75	1.35 (0.91-1.99)	22	1.55 (0.87-2.77)	0.050

Note: Relative risk (RR) is 1 for the group of 0-49 mSv.

Table 4. Excess relative risks (1/Sv) of all cancer, solid cancer

Cancer site	ERR (95% CI)/Sv for all age	ERR (95% CI)/Sv for those aged 50-79 years
All cancer	-0.40 (-1.81, 1.48)	0.19 (-1.58,2.53)
Solid cancer	-0.52 (-1.89, 1.32)	0.20 (-1.47, 2.57)
Solid cancer excluding lung cancer	-0.60 (-2.05, 1.38)	0.12 (-1.66, 2.71)
Solid cancer excluding nasopharynx, cervical, and liver cancer	1.17 (-1.10, 4.64)	1.70 (-0.89, 6.01)

Table 4 presents excess relative risk per Sv for solid cancer. It was -0.52 (95% CI=-1.89, 1.32), assuming a latent period of 10 years and ignoring internal exposure. When study subjects were limited to those aged 50-79 years, the ERR /Sv was 0.20 (95% CI= (-1.47, 2.57).

4. Discussion

The studies of cancer risk among nuclear workers and the HBRA residents provide important information on cancer risk related to low dose-rate exposure to low-LET ionizing radiation. In the present study, the ERR/Sv for all solid carcinomas was estimated to be -0.52 when we assumed a latent period of 10 years, and ignored internal exposure. We also calculated an ERR/Sv for those aged 50-79 years. The reasons why we placed such an age restriction were i) non-cancer mortality analysis suggested a lifestyle change among those aged less than 50 years and the possibility that lifestyles may be related to radiation dose in recent years [7]; and ii) deaths among those aged 80 or older may not be as accurate for younger age groups. After the age restriction, the estimate became 0.20, which is less than half of ERR/Sv estimate of 0.47 reported from atomic bomb-survivors [9]. It is of note that the recent pooled analysis of nuclear workers in 15 countries [10] showed the ERR/Sv of 0.97, which is much larger than the estimate obtained from atomic-bomb survivor data. However, the wide confidence interval of the risk estimate obtained from the present study precludes any definitive conclusion regarding this problem.

Yangjiang HBRA consists of two separate regions, Tongyou, the western part of Yangjiang area, and Dong-anling, the eastern part of Yangjiang area. Dong-anling is 50 km west to Wudianmeihua region of the control area in Enping, and Tongyou is 60 km west to Dong-anling. The average annual dose received by the inhabitants from the natural sources of external exposures in Dong-anling was a little lower than that in Tongyou: 2.05 (1.33-2.68) mSv vs 2.27 (1.25-3.08) mSv [2,3]. It is suspected that the socioeconomic status of Tongyou is lower than that of Dong-anling; and therefore, Dong-anling is more similar to the CA in terms of socioeconomic status. However, cancer and non-cancer mortality did not show any evident differences between the two regions, and the distribution of causes of deaths did not differ markedly in the two regions, either (data not shown).

A noteworthy finding in the present study is an excess of esophageal cancer deaths in the HBRA. The RR of esophagus cancer mortality was 3.22 (95% CI= 1.16-8.96) in Tonyou, 2.29 (95% CI=0.84-6.23) in Donganling, and 2.61 (96% CI=1.11-7.66) for the entire HBRA when compared to the CA. The trend test of dose-response is not statistically significant (p=0.095). The risk of such magnitude is difficult to be entirely explained by radiation exposure in view of recent risk estimates obtained from the study of A-bomb survivors [9]. Interestingly, a recent study of Japanese radiation workers found a strong relationship between esophageal cancer risk and radiation exposure received at nuclear facilities [11]. Another Asian study reporting the excess of esophageal cancer is the study of medical x-ray workers in China [12]. The both authors suggested that confounding effects of smoking and drinking might explain the excess. However, our findings are unlikely to be explained by the synergistic effect of smoking and drinking since the distributions of smoking and drinking do not seem to be markedly different in the HBRA and in the CA according to our previous surveys [13,14]. Furthermore, the HBRA did not show any excess mortality of lung cancer, which is strongly related to smoking. It should also be pointed out that the excess deaths of esophageal cancer among women couldn't be attributed to smoking and drinking because most of women in the study areas did not smoke or drink alcohol. Further studies are necessary to explain the observed excess deaths of esophageal cancer in the Yangjiang HBRA.

Terrestrial gamma irradiation is a virtually unavoidable source of radiation for any bios living on the earth. We believe that the epidemiological data obtained from HBRA give important information on the health effects of continuous exposure to low-dose and low-dose-rate radiation. When compared with other important populations such as nuclear workers, HBRA studies have some advantages. The study population of HBRA includes both men and women of all ages while nuclear worker studies are usually dominated by working-age men. In addition, HBRA residents in China have much less chances of medical radiation exposure when compared to nuclear workers, where lifetime dose from diagnostic x-ray exposure may exceed lifetime occupational radiation exposure in some developed countries. The migration of HBRA residents in China has been much less common than in other countries and, therefore, the subjects lost to follow-up are low. In the case of nuclear workers, it is sometimes difficult to follow contract workers and retirees. The major disadvantage of HBRA studies is its difficulty in estimating lifetime radiation dose. However, such difficulties are not specific to the studying the effects of terrestrial gamma irradiation but also common to examining lifetime radon exposure. Unfortunately and fortunately, the indoor and outdoor dose in Yangjiang HBRA is not the same high as HBRAs in other countries but homogeneously distributed within a hamlet, this will warrantee the estimation of lifetime dose based on hamlet-specific environmental dose and occupancy factors. Another possible disadvantage of the Yangjiang study is the high incidence of virus-related cancers in this area. The

leading cancers in this area are nasopharyngeal carcinomas and liver cancer, which are strongly related to EBV and HBV, respectively, in southern China. The excess relative risk changed substantially when excluding those virus-related cancer from the solid carcinomas (0.20 vs 1.70 for those aged 50-79 years).

In conclusion, the cancer mortality did not show any evident difference between the HBRA and the CA. Compared with the estimate reported by the follow-up study of atomic-bomb survivors, our ERR estimate per Sv of solid cancer was much lower for all ages and less than half for those aged 50-79 years, but the wide confidence intervals of our estimate preclude any definitive conclusion. Further follow-up of the cohort seems warranted to obtain more precise estimates.

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Epidemiological Studies in High-Background Radiation Areas Its potential contribution to evaluating risk of low-level radiation

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1. Introduction

The health effect of low-level ionizing radiation is yet unclear. As pointed out by Upton in his review (**Upton, 1989**), low-level ionizing radiation seems to have different biological effects from what high-level radiation has. Its evaluation requires epidemiological studies of scale-large cohorts (ICRP 99, 2005) such of atomic bomb survivors and nuclear workers. Epidemiological studies in high-background radiation (HBR) areas are also expected to make a significant contribution toward this end. Among several HBR areas in the world, Yangjiang, Guangdong Province in China, Karunagappally in Kerala State of India, Manawalakurichi and Koodankulam in Tamil Nadu of India, and Ramsar in Iran are important areas where epidemiological studies are possible, because of their relatively high background radiation levels and large population sizes.

2. Yangjiang study

In the 1970s, Chinese researchers led by Dr Wei Luxin of Laboratory of Industrial Hygiene in Beijing conducted epidemiological surveys in Yangjiang, china, and their early results were published in the Science journal in 1980 (**HBR research group, 1980**). In the 1980s, Dr Wang Zuoyuang of Laboratory of Industrial Hygiene and Dr John Boice of the US NCI conducted a collaborative work in Yangjiang to examine the prevalence of thyroid nodularity that seems to be the most sensitive endpoint to radiation (**Wang et al., 1990**). They found an increase of chromosome aberrations in lymphocytes from HBR area residents. However, the prevalence of thyroid nodularity was not increased among them.

Since the 1990s, Health Research Foundation (HRF, President: Dr Torizuka; former President: Dr T Sugahara) started a collaborative work with Dr Wei's research group. In this China-Japan collaborative work, the lifetime dose of each resident was estimated on the basis of average indoor and outdoor doses of each hamlet, and occupancy factors specific for sex and age. The cytology study, examining blood drawn from the residents, showed a positive correlation between natural radiation levels and the frequency of unstable-type chromosome aberration in lymphocytes (**Jiang et al., 2000**). On the other

hand, the mortality study, after following more than 100,000 subjects for 16 years, did not show a significant excess risk of all cancer or leukemia (**Tao et al., 2000**).

3. Karunagappally study

The coastal belt of Karunagappally in Kerala, India is known for HBR from thorium-containing monazite sand (**Mistry et al. 1965**). In 1959, a WHO Expert Committee first made this observation and pointed out a potential for epidemiological studies to examine the effect of chronic exposure of low-level radiation on human health in this area with a high-density of population (**WHO 1959**). In the 1960s, the Medical Research Council, UK conducted studies on rats caught from this area but noted no significant genetic effects (**Gruneberg 1964**).

In the 1990s with the help of Bhabha Atomic Research Centre in Mumbai, Dr. MK Nair and his colleagues in Regional Cancer Center (RCC), Trivandrum started the baseline survey of Karunagappally residents to establish a cohort, which involves as large as 400,000 subjects. During the survey, advice was sought from Radiation Effects Research Foundation (RERF) and its then director Dr. Jack Schull. A regional cancer registry was also established in 1990. Those surveys are described in a brief paper in Radiation Research published in 1999 (**Nair et al. 1999**) and in more detail in the technical report published in 2004 (**Nair et al. 2004**). In the late 1990s, the HRF also started to a collaborate work with the RCC scientists. Currently, the database of cancer incidence, radiation dosimetry, and covariates for statistical analysis are being developed.

4. HBR studies in the other areas

The proceedings of 6th High Level Natural Radon and Radiation Area present epidemiological studies other than Yangjiang or Karunagappally (**Sugahara et al. eds, 2004**). In Ramsar, Iran, several groups are studying the effect of high-level natural radiation on the health of residents. Dr Mosavi-Jarrahi of Shaheed Beheshti University of Medical Sciences and his colleagues examined the cancer incidence in 2003 and cancer mortality during the period 2001-2003, and calculated Standardized Incidence Ratio and Standardized Mortality Ratio, taking the entire nation as the reference population. Cancer incidence and mortality rates among women showed slight increases in the HBR area when compared with the control area. On the other hand, no such an increase was observed among men. In Brazil, three major HBRA areas are known: Poços de Caldas, Araxá and Guarapari. Dr Lene Veiga of Institute of Radioprotection and Dosimetry evaluated cancer mortality in the HBRAs of Brazil, using the vital statistics data for the period 1991-2000. SMRs of all neoplasm for Poços de Caldas, Guarapari and Araxa were 120, 109 and 100, respectively. Internal comparison to examine dose-response relationship

was not conducted. Given the weakness of methodology, these findings do not allow for convincing conclusions at this moment. In Brazil and Iran, no further epidemiological studies are planned.

5. Dosimetry

Estimation of cancer risk in relation to HBR requires the follow up of a large study population for many years (**ICRP 99, 2004**). However, it is practically impossible to measure lifetime cumulative exposure to HBR in any epidemiological stydies. Therefore, epidemiological studies in Yangjiang and Karunagappally estimated lifetime cumulative dose in various approaches.

5.1 Indirect estimation on the basis of indoor and outdoor doses and occupancy factors.

Yangjiang study in China estimated cumulative lifetime effective dose for each individual on the basis of average indoor and outdoor doses in each hamlet, and sex- and age-specific house occupancy factors. A similar approach is taken in Karunagappally study in India. In both studies, attempts are/were made to estimate organ dose for each individual using conversion factor presented in ICRP74 (**1996**). There are, however some unscertainties regarding the conversion factor when it comes to the magnitude of its dependence on age at exposure, geometries of exposure, energy spectra, which are dependent on radiation sources. It is desirable to establish an internationally standardized protocol in order to make it possible to compare study results obtained from different countries.

5.2 Direct individual measurement

Individual dosimetry gives the current radiation dose, rather than the cumulative dose. In Yangjiang, China, TLD was carried by volunteers for about 3 months to directly measure individual radiation dose. A similar survey was conducted in Karunagappally using optically stimulated luminescent dosimeters (OSLD) worn by residents. However, this kind of approach does not give lifetime dose. Since the cumulative lifetime dose is necessary for radiation-induced cancer risk estimation, this approach can be used only for evaluating the validity of radiation dose estimated by other approaches.

5.3 ESR using teeth

A retrospective measurement of cumulative dose in the past 10-20 years is possible if teeth are taken and are subjected to ESR dosimetry.

5.4 Radon and thoron measurements

Residential radon exposure is suspected to cause lung cancer (**Darby et al, 2005, Krewski et al,**

2005). Although water and foods in HBR areas are known to contain radioactive nuclei, whether internal exposure through water and diet causes cancer or not is not well-understood. Radon exposure is accompanied by thoron exposure in some areas, particularly in Asian countries. However, radiation dose inflicted on lung by thoron exposure may be smaller than what is once understood since a recent study in China has revealed the equilibrium factor of thoron is much lower than 0.1, which is the value given by UNSCEAR report (**Tokonamin et al., 2004**). A study by Tokonami et al. in Karunagappally and Manavalakurichy, another HBR area near Kerala state showed that the radon concentration, thoron concentration and Equilibrium Equivalent Thoron Concentration ranged from 2-70, 6-690 and 0.1-1.6 Bq m^{-3}, respectively (**Tokonami et al. 2002**). Unless lung cancer is the health effect of interest, it is not necessary to conduct residential radon-thoron measurements. It may also be of interest to examine the prevalence of abnormal sputum cytology with respect to internal exposure of the lung to radon and thoron and their progenies. Indeed, a study among uranium miners suggested the use of sputum cytology to examine the association of lung cancer risk with radon exposure (**Michaylov MA et al., 1995**).

5.5 Biological dosimetry

Lymphocyte chromosome aberration, particularly unstable type chromosome aberration, is a radiation-sensitive parameter. The frequency of unstable type chromosome aberration increased proportionally with natural background radiation level in Yangjiang, China (**Jiang et al. 2000**). DNA point mutation can also be used as a biological marker. Glycopholin A and HPRT point mutation were used in several epidemiological studies, including Tibetian cosmic ray study (**Jensen et al. 1997**). However, biological dosimetry has some drawbacks in epidemiological studies: i) it does not give lifetime cumulative dose; ii) it is difficult to conduct biological dosimetry for a large number of subjects necessary for cancer risk estimation; and iii) estimated dose is not reliable and/or sensitive enough to be used in an epidemiological study, particularly in low dose ranges. It should be noted that stable-type chromosome aberration may reflect radiation exposure for a relatively long period but is also affected by environmental factors other than ionizing radiation (**Hayata et al. 2004**).

6. Health effects and health-related parameters of interest

The parameters used to assess the health risk are cancer incidence, cancer and non-cancer mortality, and various physiological functions.

6.1 Cancer incidence

In order to estimate cancer incidence in the study area, it is necessary to establish a high-quality

cancer registry system. When it is difficult to estimate cancer incidence, cancer mortality is used. In Karunagapapally, its regional cancer registry enables us to estimate cancer incidence. The registry reports have been presented in "Cancer Incidence in Five Continents" vol. VII (**Nair et al. 1997**), vol. VIII (**Nair et al. 2002**) and vol IX (**Jayalekshmi and Rajan 2007**), indicating the high quality of the registry's cancer incidence data. On the other hand, Yangjiang study in China uses only mortality data. Note here, however, most of malignancies, including brease cancer, are fatal since it is difficult for local farmers, which make up the majority of the HBR area residents, to undergo expensive cancer treatment.

6.2 Non-cancer risk

Collecting information on date of death is necessary to calculate person-years of follow-up even if cancer registry enables us to ascertain all the cancer cases occurring among the population of interest. If cause of death is collected as well, the association of non-cancer mortality and radiation exposure can also be examined. Recent radiation epidemiology studies suggested the possibility of the association between non-cancer mortality and radiation exposure among atomic-bomb survivors in Hiroshima and Nagasaki (**Preston et al., 2003**), and radiation workers in the US (**Howe et al., 2004**). However, it is difficult to obtain accurate information on non-cancer diseases through death certificate.

6.3 Prevalence of benign tumors and precancerous lesions

A number of non-malignant disorders are suspected to be related to ionizing irradiation. Among them are thyroid nodularity, thyroid function, parathyroid function, endometriosis, viral hepatitis, and so on. It is particularly of interest to examine the frequency of thyroid nodularity focusing on middle-aged women in the HBR and control areas. Such a study was already conducted in Yangjiang (**Wang et al. 1990**). A thyroid nudularity study was also conducted in Karungappally (**Kouchipillai, 1976**). Neither study found any excess prevalence of thyroid nudularity. A few epidemiological studies suggested that external radiation exposure increases hyperparathyroidism in the dose ranges as low as 500 mSv or even in lower dose ranges (Fujiwara et al., 1992; Schneider et al., 1995; Holmberg et al., 2002). It is of interest to examine the prevalence of hyperparathyroidism in HBR areas. Studies of atomic-bomb survivors showed that serum calcium levels and PTH (parathyroid hormone) were also increased with radiation exposure. However, the increase of serum PTH, which was 6.8% per Gy, may not be larger enough to be detectable in the HBR areas.

6.4 Pathological features of cancer

It is of interest to examine the expression of various oncogenes and suppressor genes, including

those involved in p53 and Rb pathways, at mRNA and protein levels in the HBRA and the control area, using immune-histochemical staining of paraffin embedded blocks and frozen materials, and PCR of DNA/RNA specimens extracted from those materials. The expression of oncogenic virus, including human papillomavirus, is also of interest in Karunagappally, where oropharyngeal cancer is common. It is desirable to establish a tissue bank system to facilitate the use of those specimens and to store those specimens in a safe and secured condition.

6.5 Malformation and genetic disorders

Studies of residents were also conducted, and found no unequivocal association of HBR exposure with abortion, genetic disorders, Down syndrome (**Kouchupillai et al. 1964**), congenital malformation (Jaikrishan et al. 1999), or chromosomal aberrations among neonates (**Cherian et al. 1999**). Recently, however, Forster et al. reported that the natural radioactivity in the study areas was associated with mitochondrial DNA mutations in the residents (2002). It is of interest to examine patients with genetic disorders and their family members in order to evaluate their DNA repair capacity, telomere length and its functions. It is also of interest to follow the patients of genetic disorders (Down's syndrome, Ataxia Telangiectasia, AT heterozygotes, childhood cancer, malformation, etc) in HBR and the control areas to compare the risk of leukemia and solid cancer development.

7. Cell biology and molecular biology, and Immunological responses

7.1 Chromosome aberrations

Chromosome aberrations, both stable and unstable types, are radiation-sensitive parameters. The frequency of unstable-type chromosome aberration increases proportionally to HBR levels. Aging causes telomere deletion and an increase in dicentric and other chromosomal aberrations. Interaction between aging and radiation is also an interesting topic. It is also of interest to quantify the cytochalasin-induced cytokinesis blocking micronucleus, and to detect X chromosome loss in females employing FISH (Fluorescence In situ Hybridization) technique with a probe specific for a centromere of chromosome X. If subjects with chromosomal aberrations are followed to examine their cancer risk, that will make an invaluable scientific contribution.

7.2 Adaptive response among individuals exposed to different doses and dose rates.

Iranian researchers have reported that adaptive response is induced in the residents of the HBR area (**Mohammadi et al. 2006**). However, much remains to be learned about the adaptive response whereby exposure to very low doses of radiation results in less damage being induced by subsequent exposure to

high radiation doses. Uncertainties still exist in many aspects of adaptation and its underlying mechanisms. What is certain, however, is that the phenomenon is real, and that a vigorous worldwide efforts are now under way to understand the basic mechanisms involved. Those efforts are stimulated both by a desire to understand the basic cell biology behind the adaptive response and a desire to see if, indeed, this phenomenon affects the estimation of the risks of low-level radiation exposure.

7.3 Immunological and other studies.

It is well known that radiation affects immunological systems (**UNSCEAR2000**). It is of interest to conduct immunologoical function tests and flow cytometry measurements of T, B, and NK cells in peripheral blood lymphocytes. Studies on cytokines IL2, I L6, p53 are also of interest.

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Detectable Low Dose Limit in Health Effect Based on the Background Frequency of Chromosome Aberrations

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1. Introduction

In order to know the effect of low dose radiation on health, it is important to know the detectable dose limit by an epidemiological study. In this paper I will first describe biological significance of chromosome aberrations in the peripheral lymphocytes and then report the results of calculation of radiation doses based on the observed frequencies of translocations in the healthy individuals. Finally detectable low dose limit in health effect predicted by the study on the background frequency of chromosome aberrations is discussed.

2. Biological significance of chromosome aberrations in the peripheral lymphocytes

Bonassi et al. (2004)[1] reviewed the studies showing positive relation between risk of cancer and the frequency of chromosome aberrations in the peripheral lymphocytes. In those studies subjects whose chromosomes were analyzed were classified into three groups according to the frequencies of chromosome aberrations detected in the peripheral lymphocytes: low frequency group, medium frequency group, and high frequency group.

Table 1. Studies showing the risk of cancer associated with the frequency of chromosome aberrations in lymphocytes.

Study	Subjects	Person -year	Follow-up period	Cancer cases (death)	SIR (95% CI)	SMR (95% CI)	Hazard Ratio (95% CI)
Nordic Study Group on the Health Risk of Chromosome Damage, 1990	1,787	7,547	1970-1985	26	0.90 (0.33-1.98)[1] 0.92 (0.34-2.04)[2] 1.80 (0.98-3.01)[3]		
Hagmar et al., 1994	1,979	17,666	1970-1988	66	0.90 (0.50-1.40)[1] 0.70 (0.30-1.20)[2] 2.10 (1.50-2.80)[3]		
Bonassi et al., 1995	1,455	16,190	1969-1994	44		0.83 (0.36-1.63)[1] 1.79 (1.02-2.90)[2] 1.82 (1.11-2.81)[3]	
Hagmar et al., 1998	3,541	43,827	1970-1995	91(64)	0.78 (0.50-1.18)[1] 0.81 (0.52-1.25)[2] 1.53 (1.13-2.05)[3]	0.83 (0.46-1.37)[1] 1.16 (0.71-1.80)[2] 2.01 (1.35-2.89)[3]	
Smerhovsky et al., 2001	3,973	37,775	1975-1999	144			1.6 (1.01-2.37)[3]

SIR: Standardized Incidence Ratio; CI: Confidence Interval; SMR: Standardized Motality Ratio. ;[1]Low frequency of CA; [2]Medium frequency of CA; [3]High frequency of CA (after Bonassi et al., Cytogenet. Genome Res. 104, 376-82, 2004)

As shown in Table 1, the follow up studies revealed that the rate of cancer incidence or mortality were

associated with the frequency of chromosome aberrations. Large scaled studies reported by Rossner et al. (2005)[2] and Boffetta et al. (2007)[3] confirmed the results of those studies. The association was found in chromosome type aberrations but not in chromatid type aberrations.

There are increasing evidences showing the higher frequency of chromosome type aberrations (translocations) in the peripheral lymphocytes of the individuals exposed to the environmental clastogens such as the gases in traffic air pollutions[4] as well as smoking[5,6] when such frequency was compared with that of the less exposed to them.

Chromosome aberrations in the peripheral lymphocytes are a very sensitive indicator of radiation exposure. Lloyd et al. (1988)[7] observed the increase of dicentrics at the dose of 20 mSv. According to Lloyd and Edwards (1983)[8] the induction rate of dicentrics by gamma irradiation at minimum low dose rate is about 2-2.5 in 10,000 cells per cSv. In case of acute exposure the frequency follows the dose response reported by Sasaki et al. (2001)[9]: $Y = 2.31 \times 10^{-5} D + 6.33 \times 10^{-8} D^2$, where Y is the frequency of dicentrics per 1,000 cells, and D is the dose in mSv. Since radiation induces dicentrics and translocations in about equal frequency[10], the dose response of dicentrics and translocations should be about the same.

3. Calculation of radiation doses based on the standard deviations of the observed frequencies of translocations in the healthy individuals

The healthy individuals analyzed in the present study consist of 20 elders (61.2 year-old on the average) in a large city, Beijing, and 16 elders (64.4 year-old on the average) and 8 children (12.3 year-old on the average) in the control group in the remote village, who were reported by C. Wang et al. and W. Zhang in this proceedings (Fig. 1).

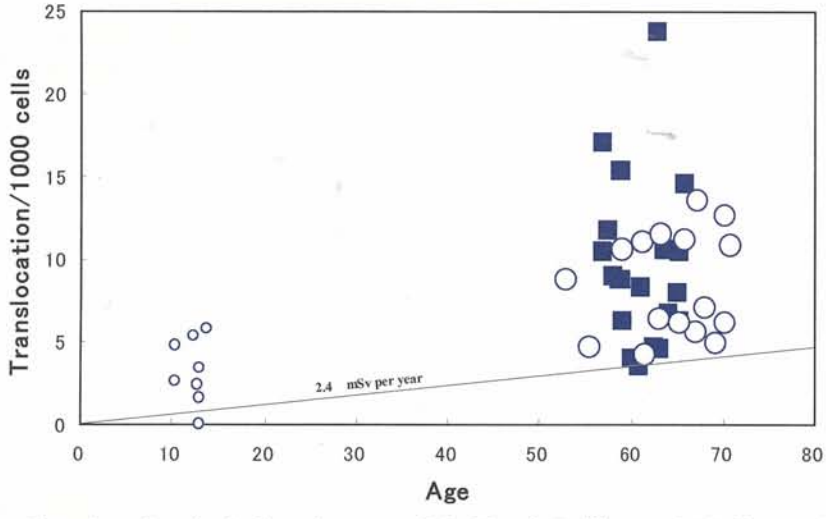

Fig. 1. Frequencies of translocations in the lymphocytes of 20 elders in Beijing, and 16 elders and 8 children in the remote village. The solid line indicates level of the translocations induced by 2.4 mSv per year (world average radiation level from natural source). The translocations above this line are induced by mutagenic factors other than natural radiation.

They were all non-smokers who had no history of the exposure to radiation except for the natural background radiation or routine medical diagnostic procedures such as chest X-ray examinations. As shown in Fig. 1, a large variation among subjects in each group was observed. Since radiation doses can be calculated by the dose response relation of chromosome aberrations, the frequencies of translocations detected in the healthy individuals were converted to radiation doses assuming that all those translocations had been induced by radiation. In case of the calculation of radiation dose by chronic low dose exposure, induction rate of 2.5 translocations in 10,000 cells per cSv mentioned above was used. In case of acute exposure, the dose response formula reported by M. S. Sasaki et al. was applied. Calculated doses converted from the standard deviations should be the detectable low dose limit of health effects of radiation originated from the DNA rearrangements, because it is not possible to detect significant difference of incidences between cohort and control when these differences are within the standard deviations of the causative factors.

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Future Directions of Epidemiological Study in the High Background Areas

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Abstract

In the current epidemiological study of the High Background Radiation Areas(HBRA), the main criticized point is that the study has no statistical power of low dose risk. Based on the A-bomb survivor study, at least 10,000 persons should be followed up to detect the cancer risk due to 100 mGy if no dose-rate effects are considered. The excess accumulated dose more than 100 mGy can be attained until age of 60 in HBRA. Thus, a deliberate design may produce statistically significant result of cancer risk if no dose-rate effects exist. What is the meaning of the non-significant result of the current study? Quantitative analysis should be carried out to evaluate the current attained status how high is cancer risk from low dose rate. On the other hand, it is interesting to focus on the biological results that no increase of stable chromosome aberrations was observed while unstable aberrations increased. Further investigation of life-style related factors other than radiation would be an important information that enable us to analyze cancer-related factors. Another aspect should be emphasized regarding the epidemiological study of HBRA. Negative results necessarily means no excess risk due to radiation exposure. In the light of risk communication with the public, however, we should focus on the result of the study when we understand how high is the health risk from continuous exposure at low-dose rate, since current radiation protection use the risk estimates based on the A-bomb survivor study. The essence of radiation protection is sophisticated risk management to reduce radiation exposure to rather lower risk compared with other risk from life-style related factors.

High Background Radiation Exposures and Biomarkers of Response

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Abstract

There have been a number of epidemiological studies over some 30 years on the potential adverse health effects resulting from exposure to high background radiation in specific geographic areas of the world. In general, there has been no consistently demonstrated increase in cancer risks associated with these exposures. There have been isolated reports of increases in congenital abnormalities (particularly Down syndrome). However, the consensus appears to be that there is no clear increase in congenital malformations in newborns as a result of high radiation background exposures. Similarly, from a collection of cytogenetic monitoring studies conducted in high background radiation areas, it appears that there is no clearly established increase in chromosome aberrations (either stable or unstable) in exposed versus control populations. However, it should be noted that such cytogenetic monitoring studies are being conducted at predicted levels of effect that are quite close to background ranges and thus are subject to variation as a result of confounding effects (e.g. smoking, diet, age distributions, and other unknown exposures). Thus, reliable data for predictive purposes can only be generated if the exposed and control populations are quite large (say, more than 30 individuals in each), that these populations are well-characterized as regards potential confounders, and that reciprocal translocations (measured by FISH) are the endpoint of study. Even under these conditions, it is essential that a statistical power analysis be conducted prior to initiating a study for establishing the likelihood of detecting a significant difference between exposed and control groups. The study design would need to be adjusted to meet the needs of the statistically derived conditions for detection. The question of whether or not increases in chromosomal aberrations can be attributed to high background radiation is an important one, especially as it is generally accepted that at the population level, increases in chromosome aberrations can predict increases in cancer.

February 14, 2008

Proceedings of
NIRS International Symposium
on the Effects of Low Dose Radiation

Issued on March 25, 2008

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Printed by Sakura Printing Co. Ltd., Japan