VIII-II-1. Project Research

Project 3

PR3 Biological Effects of Low Dose Radiation

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OBJECTIVES AND RESEARCH SUBJECTS:

It has been believed that the first target of radiation carcinogenesis is DNA. However, this hypothesis is not proved as a main route for carcinogenesis of low dose radiation yet. After analyzing our results of research of malignant cell transformation with low dose radiation during the past 30 years, we came to strongly believe that a radiation cancer-causing primary target is not DNA itself. Recently, several reports including our reports suggested that non-target effects, such as bystander effect and delayed effect, modify cell transformation frequency. From these results, we speculate that non-genomic damage plays an important role in an initial process of cellular malignant transformation.

Therefore, the aim of this project is focused on elucidation of non-genetic factors related to carcinogenesis.

The collaborators and allotted research subjects (S) are as follows;

S-1: Mechanism of Non-targeted Effects Radiation (M. Watanabe, K. Watanabe, H. Nawata, H. Nishiura,T. Okada, K. Tano, G. Kashino)

S-2: Low Dose Radiation-induced Telomeric Instability and Carcinogenesis (S. Kodama, M. Takebe, K. Shiraishi, M. Watanabe)

S-3: Effect of Vitamin C on Radiation Induced Thymic Lymphomagenesis and *Ikaros* Mutation (S. Kakinuma, Y. Amasaki, N. Kowatari, K. Yamauchi, M. Nishimura, T. Imaoka, K. Ariyoshi, G. Kashino, M. Watanabe, and Y. Shimada)

S-4: Long-Lived Radicals for Radiation Carcinogenesis (J. Kumagai, K. Mioki, G. Kashino, M. Watanabe)

S-5: Aneuploidy Induced by Low Dose Radiation and Carcinogenesis (K. Yamamoto)

S-6: Expression Cancer-related Genes in Response to Low-dose Radiation (K. Suzuki, M. Watanabe)

S-7: Radiation-quality Dependent Cellular Response of Gene Mutation in Normal Human Fibroblasts(M. Suzuki, C. Tsuruoka, M. Watanabe)

MAIN RESULTS OF THIS PROJECT:

As a result, we found that the intracellular oxidation degree, such as reactive oxidative radicals and long lived radicals, was elevated by high density culture and radiation exposure both in mammalian cells (S-1 and 4). Specially, long-lived radicals (LLRs) play an important role of genetical effects of radiation. These radicals attack several proteins, such as telomere related protein and centrosome, and destroy their structure (S-1, 2 and 5). Telomere destabilization induces telomere fusion and makes dicentric chromosome (S-2) and reason of chromosome instability. In fact, dicentric chromosome is dominant aberration induced by low dose radiation (S-1 and 2). Radiation induced radicals also attacked centrosome (S-1). Centrosome destabi- lization induces nondisjunction and raises the frequency of aneuploid (S-1 and 5). In early process of cell transformation, structural aberration of chromosome does not occur, but aneuploid is seen in high frequency (S-1). Aneuploid is also induced by deficiency in cell cycle checkpoint at G_2-M (S-5). By treatment of vitamin C (VC) prevent induction of LLRs, aneuploid, and thymic lymphomas in B6C3F1 mice (S-3 and 4). Interestingly, the thymic lymphomas in VC treated mice lacked point mutation of *Ikaros*, suggesting a suppression of point mutation by VC (S-3 and 4).

Low dose radiation activated repair capacity of DNA damage in irradiated cells (A-7). Because radiation-induced genomic instability is induced in some fraction of the progeny of a single survived cell, not a single gene mutation but some epigenetic changes may be involved in the initiation of radiation-induced genomic instability. Oxidative stress and altered chromatin structure have been proposed as the mechanisms of perpetuation of radiation-induced genomic instability (S-1 and 6).

These results suggest a possibility that a main target of radiation carcinogenesis is not DNA, but is centrosome, which are the proteins to constitute chromosomal homeostasis maintenance mechanism. If our results are right, “Mutation Theory of Carcinogenesis” is to be wrong. I will suggest a new hypothesis about radiation carcinogenesis, which was named as "protein target theory" by this report.

課題番採択号21P3 低線量放射線の生物影響に関する研究 プロジェクト

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PR3-1 Mechanism of Non-Targeted Effects Radiation

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INTRODUCTION: It has been believed that the first target of radiation carcinogenesis is DNA. However, this hypothesis is not proved for carcinogenesis of low dose radiation directly yet. However, this hypothesis is not proved as route for carcinogenesis of low dose radiation yet. We analyzed our results of research of malignant cell transformation by low dose radiation during the past 30 years and came to strongly believe that a radiation cancer-causing primary target was not DNA itself. One evidence which supports our thought is that transformation frequency in Syrian hamster embryo fibroblast (SHE) cells irradiated with low dose of X-rays is 500-1,000 times higher than that of somatic mutation [1-3]. This contradicts "the multistage mutation theory" which carcinogenesis produces in accumulation of 3-5 independent mutations. Transformation frequency should be smaller than independent mutation frequency theoretically.

Recently, several reports including our reports suggested that non-target effects, such as bystander effect and delayed effect, modify cell transformation frequency [2,4]. From these results, we speculate that non-genetic damage plays an important role in an initial process of cellular malignant transformation. However, it is the other important finding that this process is strictly inhibited in a human cell *in vitro* [5]. Therefore, we were searching for an intracellular target related to carcinogenesis in mouse embryo fibroblast (ME), SHE and human embryo fibroblast (HE) cells.

MATERIALS AND METHODS: We cultured normal human cells and rodent cells (HE and SHE) under atmospheric (20%) and physiological (hypoxic; 2% and 0.5%) oxygen conditions, and measured cell growth, levels of intracellular oxidative stress, and quantity and function of mitochondria of each.

RESULTS: As a result, we found that the intracellular oxidation degree, such as reactive oxidative radicals and long lived radicals, was elevated by high density culture and radiation exposure in ME, SHE cells and HE cells [6,7]. These radicals attack several proteins, such as telomere related protein and centrosome, and destroy their structure [8,9]. Telomere destabilization induces telomere fusion and makes dicentric chromosome. In fact, dicentric chromosome is dominant aberration induced by low dose radiation [10]. Centrosome destabilization induces non-disjunction and raises the frequency of aneuploid. In early process of cell transformation, structural aberration of chromosome does not occur, but aneuploid is seen in high frequency.

DISCUSSION: These results suggest a possibility that a main target of radiation carcinogenesis is not DNA, but is centromere and centrosome, which are the proteins to constitute chromosomal homeostasis maintenance mechanism. If our results are right, “mutation theory of carcinogenesis” is to be wrong. I will suggest a new hypothesis about radiation carcinogenesis, which was named as "Protein Target Theory".

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採択課題番号 21P3-1　放射線による非標的影響発現メカニズムに関する研究　　プロジェクト
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PR3-2 Low Dose Radiation-Induced Telomeric Instability and Carcinogenesis

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INTRODUCTION: Chromosomal instability is a hallmark of most cancer cells. Multiple mechanisms are proposed to explain the induction of chromosomal instability in carcinogenesis. Recently, it is widely accepted that telomeres play a vital role in preventing chromosome fusion. Thus, loss of telomere function is a mechanism for chromosome instability in cancer. For example, telomere loss results in sister chromatid fusion and breakage-fusion-bridge cycles, leading to extensive chromosome rearrangements including gene amplification, large deletions, and non-reciprocal translocations [1]. All of those chromosome rearrangements have been associated with cancer development. It is well known that ionizing radiation initiates cancer development with extensive chromosomal instability. However, induction of telomeric instability by radiation remains obscure. In the last KURRI Progress Report 2008, we reported that chromosomal instability is transmitted via the irradiated chromosome to the clonal progeny of unirradiated cells by means of chromosome transfer. To know the involvement of telomeric instability in the induction of chromosomal instability by ionizing radiation, we investigated intrachromosomal rearrangement in the irradiated human chromosome by examining subtelomeric FISH in microcell hybrids, where an irradiated human chromosome 8 is transferred into mouse recipient cells.

MATERIALS AND METHODS: *Cells and Cell Culture*: Mouse A9 cells containing a single copy of human chromosome 8 were used as chromosome donors. Mouse m5S cells established from embryonic skin fibroblasts were used as recipient cells.
Chromosome Transfer: The method for microcell-mediated chromosome transfer was described previously [2].
X-irradiation: The donor cells were irradiated with 4 Gy of X-rays using a soft X-ray generator (OM-B205; OH-Mic, Tokyo) operating at 70 kVp and 5 mA with a 0.5 mm Al filter at a dose rate of 0.596 Gy/min.
Chromosome Samples: Metaphases were harvested by the treatment with 60 ng/ml Colcemid for 30 min, treated with hypotonic KCl (0.075 M) solution for 25 min, and fixed in fixative (methanol:acetic acid, 3:1). The mitotic cell suspensions were dropped onto a slide glass and dried for 24 h at room temperature.
Fluorescence In Situ Hybridization: The stability of the human chromosomes 8 was investigated by fluorescence *in situ* hybridization (FISH) as described previously [2]. The stability of subtelomeric regions of short and long arms of chromosome 8 was examined by subtelomere FISH as instructed by the supplier.

RESULTS AND DISCUSSION: *Stability of a Transferred Human Chromosome*: We finally examined the stability of the transferred chromosome in twelve microcell hybrids including unirradiated human chromosome 8 and nineteen microcell hybrids including irradiated human chromosome 8. No structural abnormalities were observed in ten out of twelve microcell hybrids transferred with the unirradiated human chromosome 8. Although the rearranged human chromosome 8 was seen in two microcell hybrids, their fractions were low (6%), suggesting that an unirradiated human chromosome was structurally stable in mouse m5S cells. In contrast to this, three out of nineteen microcell hybrids transferred with the irradiated human chromosome 8 exhibited more than three different types of aberrations and those cells with the aberrations consisted of more than 88% of total cell populations. This indicates that the irradiated chromosome is more unstable than the unirradiated chromosome, suggesting that radiation-induced lesions that trigger subsequent rearrangements involving the irradiated chromosome remain at long times after exposure. We investigated intrachromosomal rearrangements of chromosome 8 by subtelomere FISH in eleven microcell hybrids transferred with the irradiated human chromosome 8. The result indicated that 74% or more cells showed the intrachromosomal rearrangements in three out of eleven microcell hybrids. However, we could not find a clear correlation between intrachromosomal rearrangements and induction of chromosomal instability, suggesting that intrachromosomal rearrangements may not be prerequisite to proceed to chromosomal instability.
Conclusions: This study confirms the previous our conclusion that chromosomal instability can be transmitted via the irradiated chromosome to the clonal progeny of unirradiated cells. This suggests that the irradiated chromosome itself enhances the potential for genomic rearrangement, and this promotes genomic instability possibly involved in radiation carcinogenesis. However, in the present study, we could not find evidence to show that telomeric instability is involved in the induction of chromosomal instability induced by ionizing radiation. Further study is needed to clarify a mechanism for radiation-induced chromosomal instability.

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採択課題番号 21P3-2　　低線量放射線によるテロメア不安定化と発がん　　プロジェクト
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PR3-3 Effect of Ascrobic Acid on Radiation Induced Rat Mammary Carcinogenesis

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INTRODUCTION:

Ascorbic acid (AsA) exerts anti-carcinogenic effects through anti-oxidant action in various systems. Under in vitro conditions, excess amount of AsA decreases carcinogen-induced gene mutation, chromosomal aberration, and cell transformation [1]. In rodents, it reduces tumor development after exposures to UV radiation, nitroso-compounds and benzo(a)pyrene [2]. In humans, AsA has been reported to decrease chromosomal damage in peripheral lymphocytes of coal-tar workers [3]. Epidemiological studies show a negative association of stomach cancer and larynx cancer with AsA intake [4]. A case-control study in New York has shown that ASA intake per day of 80 mg, compared to 107 mg, yielded 10-fold increase in the risk of cervical dysplasia [5]. However, it also has a tumor promoting effect [1].

The DNA damage of radiation is caused by both direct action and indirect action. Indirect action is mediated not only by short-lived but also by long-lived radicals. The generation of radiation-induced long-life radicals in cells is reported to be suppressed by addition of AsA [6]. Furthermore, post-treatment of AsA on irradiated cells reduces the frequency of mutations and morphological transformations, suggesting long-lived radicals may be involved in these biological phenomena [7]. The reduction of point mutation by the post-treatment of AsA was also found following neutron irradiation [8] and high-LET carbon-ion irradiation [9]. However, the data on the role of ASA and long-lived radicals in radiation carcinogenesis *in vivo* are insufficient.

Rat mammary cancer can be reproducibly induced by exposure to ionizing radiation or chemical carcinogens. This animal model is considered useful in the search for the mechanism of the development of human breast cancer. OSD rat is a mutant Wistar rat with a lack of L-glono-gamma-lactone oxidase, which catalizes the terminal step of ascorbic acid biosynthesis as a result of single base mutation in the gene of this enzyme [10] (Kawai et al. 1992). This model has been providing the robust evidences on the physiological role of AsA.

AsA is anti-oxidative when physiological concentrations are available in the plasma [11]. However, most patients with malignant disease have minimal store of AsA. For instance, patients with breast tumors had low leukocyte AsA [12]. Clinically, it is a concern that AsA deficiency at irradiation influences the radiation effects. Although a number of studies have been done on the effects of large dose of exogenous ASA administration, little information is available on the deficiency of AsA on the carcinogenic action of ionizing radiation.
In this study, we aimed to clarify the effect of reduced level of AsA at irradiation on radiation carcinogenesis, using the OSD rats.

EXPERIMENTS:

Female OSD rats were purchased from Clea (Kanagawa). All rats were allowed free access to drinking water containing 0.1 % AsA (~50mg/rat/day) and chow diet, which did not contain AsA. At eight weeks of age, they were administered to gamma-rays at a dose of 2 Gy with or without AsA deficiency. We set the following three experimental groups, (i) irradiation alone, (ii) treatment with AsA deficiency for 10 days, and (iii) irradiation at the midst day of AsA deficiency. It is reported that AsA deficiency for 14 days reduced renal and hepatic concentrations of ASA to one twentieth and one-tenth, respectively [13]. All animals were followed the development of mammary tumors until they got moribund.

RESULTS:

Mammary tumor, most of which are fibro-adenomas, developed after 6 months after irradiation. Latent period of mammary tumor induction was not significantly different among the groups, suggesting AsA at the time of irradiation has little effect for tumor induction at least in this experimental protocol.

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採択課題番号 21P3-3　　低線量放射線の生体影響に関する研究　　プロジェクト
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PR3-4 Long-Lived Radicals on Radiation Carciogenesis

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INTRODUCTION: Ionizing radiation-induced bystander effects have been recognized that non-irradiated cells received some bystander factors from irradiated cells via gap junctions and/or culture medium express biological responses similar to radiation biological effects such as mutation, transformation, and so on [1-3]. Bystander effects must be one of the important steps for radiation carcinogenesis. Mechanisms of induction of bystander effects are quite interesting and should be elucidated. It is almost certain that NO molecules play important crucial roles in induction of bystander effects [4]. In this study we have designed radiation bystander experiments to induce point mutation by medium transfer method. Culture medium with Chinese hamster ovary cells in a culture flask was X- or γ-ray irradiated, and then the medium without irradiated cells was transferred to other culture flasks in which non-irradiated CHO cells were plated. It is expected that irradiated cells as donor cells release some soluble bystander factors into the medium, so that the non-irradiated cells as recipient cells may be affected by the soluble bystander factors. Because of lifetime of nitric oxide, the soluble bystander factors in the medium are not nitric oxides but other unknown secret factors. In this system, we have succeeded in detecting bystander responses as elevations of both point mutation frequency and concentration of long-lived radicals in CHO cells. The radicals in the recipient cells were measured by electron spin resonance spectroscopy without any spin-trapping agents. We will show that both endpoints of bystander effect by medium transfer are in good correlation by using an ascorbate as an inhibitor of the medium transfer bystander effect. The long-lived radicals are likely to be related to inducing point mutations to the recipient cells.

EXPERIMENTS: Initially 7 x 10^6 of normal Chinese Hamster Ovary (CHO) cells for donor of soluble bystander factor were introduced to a T175 flask with 35 ml of culture medium (D-MEM) supplemented with 10% fetal calf serum and have incubated for 24 h at 37 °C. The medium in the flask was exchanged to 100 mL of new culture medium, and the flask was γ-ray irradiated for 0.2 or 1 Gy at Co60 γ-ray irradiation facility in Nagoya University. After irradiation the flask was placed in the incubator for 24 h. We denote the medium in the flask at 24 h after irradiation as "bystander medium". Other five flasks initially having 3.5 x 10^6 of normal CHO cells for recipient cells (bystander cells) were incubated for 48 h with the culture medium. After the incubation, the medium in each flask was exchanged to the 18 mL of the bystander medium. The flasks were stored in the incubator for 24 h, then recipient cells were corrected and moved into a Suprasil quartz tube for ESR measurement. The ESR tube with the cells was sealed and frozen in liquid nitrogen. ESR spectra of recipient cells were recorded by JEOL JES-RE1X EPR spectrometer in Nagoya University.

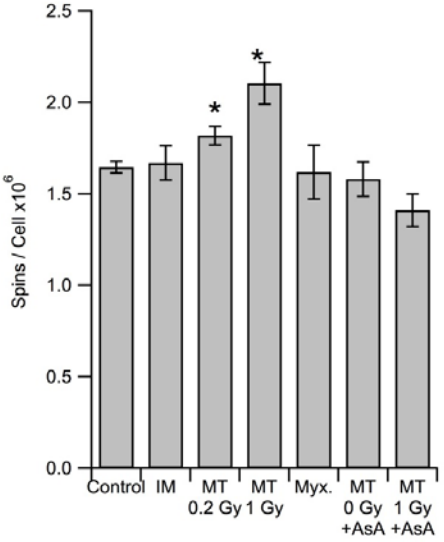

Fig. 1. Levels of long-lived radicals in a CHO cell after medium transfer (MT) of bystander medium. *: $p < 0.05$.

RESULTS: Increase in the levels of long-lived radicals as 10 and 27% in recipient CHO cells were detected by ESR when the bystander medium was exposed (Fig. 1 MT 1 Gy). Exposing irradiated medium (IM) without donor cells to recipient cells did not increase the level, so that unknown secret bystander factors were released from the irradiated donor cells. Treatment of a myxothiazol (Myx), as an inhibitor of electron transport in mitochondria [5], in 0.5 μM did not affect to the increase in the levels of the radicals. It indicates that induction of the soluble bystander factors are related to dysfunctional mitochondria. Ascorbate (AsA) addition to the transferred bystander medium in 1 mM decreased the level as for control. Point mutation frequency in HPRT locus increased by the medium transfer with significant difference and was reduced by the ascorbate addition. These results indicate that long-lived radicals are responsible for the induction of point mutation.

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採択課題番号 21P3-4　　放射線発がんにおける長寿命ラジカルの関与　　プロジェクト
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PR3-5 Aneuploidy Induced by Low Dose Radiation and Carcinogenesis

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INTRODUCTION: It has been predicted that missegregation during cell divisions could result to chromosome instability (CIN), also known as aneuploidy, leading cancer. Human cancer cells frequently have abnormal chromosome complements suggesting that aneuploidy may cause cancer. On the other hand, the concept that cancer develops through the accumulation of gene mutations of protooncogenes and/or tumor-suppressor genes is widely accepted. Thus, whether aneuploidy is a contributing factor, or merely a consequence of tumorigenesused is has long been debated.

To understand whether aneuploidy has a causal role in tumor formation, we made a usage of *Saccharomyces cerevisiae* and HCT116 human cell. We investigated the effect of chemicals that were tested negative in Ames system and positive in tumor formation or positive in Ames system and negative in tumor formation. In general, the presence of Ames system negative carcinogen suggests the absence of gene mutation for tumorigenesis. We observed that Ames test negative carcinogens can cause G_2/M arrest and aneuploidy, on the other hand, Ames test positive non-carcinogens can cause gene mutation and recombination but not aneuploidy. Thus, we argue that aneuploidy is a contributing factor for carcinogenesis.

EXPERIMENTS: We constructed genetic system to measure aneuploidy in yeast [1]. Cell cycle progression was measured by FACS analysis. Budding or spindle morphology was observed by microscopic analysis. Western blotting was performed to understand the signals for aneuploidy formation. The human colon cancer cell line HCT116 was used to measure aneuploidy [2]. Immunofluorescence microscopy was used to measure the amount of DNA damage, and the activation of signal proteins. Cell cycle progression was measured by FACScan analysis. Western blotting was performed to understand cleavage, and phosphorylation of signal proteins.

RESULTS: As a representative of Ames test negative carcinogens, we used phenyl hydroquinone (PHQ), which is a hepatic derivative of fungicide *o*-phenyl phenol. Using yeast system [3], we demonstrate that PHQ can arrest the cell cycle at the G_2/M transition as a result of the stabilization of Swe1 (Wee1 homolog), probably leading to inactivation of the Cdc28 (Cdk1/Cdc2 homolog). Furthermore, Hog1 (p38 MAPK homolog) was robustly phosphorylated by PHQ, which can stabilize Swe1. On the other hand, Chk1 and Rad53 were not phosphorylated by PHQ, indicating that Mec1/Tel1 DNA damage checkpoint was not functional. Mutation of *swe1* and *hog1* abolished the PHQ-induced arrest at the G_2/M transition and became resistant to PHQ lethality and aneuploidy formation. These data suggest that PHQ-induced G_2/M transition checkpoint which is activated by the Hog1-Swe1 pathway plays a role in the formation of aneuploidy.

To further delineate the mechanism of action of PHQ, we examined its effect on human cells [2]. Treatment of the colon cancer cell line HCT116 with 0-150 mM PHQ caused a concentration-dependent inhibition of growth, accumulation of cells having G_2/M DNA content, and increase in the mitotic index, suggesting delay at metaphase to anaphase transition. Moreover, a dose-dependent increase in apoptotic cells was observed. Finally, a high frequency of aneuploid cells was found. No increase in γ-H2AX foci was observed. The results show that PHQ does induce mitotic arrest, apoptosis and aneuploidy in the absence of DNA damage. We next demonstrated, using histone H3 antibody, that PHQ caused G_2/M delay. G_2/M delay, but not metaphase/anaphase delay, is directly correlated with aneuploidy, since p53-null cell lines did not show G_2/M delay and not aneuploidy. Thus, even in the absence of DNA damage, PHQ activates p53 phosphorylation, causes G_2/M delay which is probably the signal for centrosome amplification, leading to aneuploidy.

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採択課題番号21P3-5　　低線量放射線による染色体異数化と発がん　　プロジェクト
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PR3-6 Expression Cancer -Related Genes in Response to Low-Dose Radiation

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GACKGROUND AND AIM: Accumulating evidence suggests that ionizing radiation can cause various delayed effects in cells that have not directly absorb radiation energy. These effects, observed in non-irradiated cells, are now collectively described as non-targeted effects, which include radiation-induced genomic instability. Genomic instability is manifested in the progeny of surviving cells and is measured as the expression of various delayed effects such as delayed reproductive death or lethal mutation, delayed chromosomal instability, and delayed mutagenesis. Since radiation-induced genomic instability leads to the accumulation of gene mutations and chromosomal rearrangements, it is thought to play a pivotal role in radiation-induced carcinogenesis.

Recent advances in stem cell biology suggest the possible involvement of tissue stem cells in the development of cancer. Stem cells are able to proliferate both asymmetrically and symmetrically, and until they are stimulated to divide, some stem populations undergo quiescence in contact with a stem cell niche. Such quiescence in niche has been hypothesized to account for why cancer stem cells are refractory to chemotherapy and radiotherapy. In contrast to the proliferating tissue stem cells, whose surviving progenies manifest radiation-induced genomic instability during the successive cell divisions, survived quiescent stem cells remain residing in the radiation-exposed tissues until they face circumstances that trigger their proliferation and increase the risk of manifesting genomic instability. Since genetic changes leading to carcinoma are thought to accumulate in non-hematopoietic stem cells, and most of these cells remain in a quiescent state for the better part of their life span, it is highly relevant to examine the persistence of radiation-induced genomic instability in cells maintained in a quiescent state. As reported recently, sustained excess relative risk of solid cancers demonstrated in Atomic bomb survivors has suggested that radiation-exposed tissue stem cells residing in a niche may undergo proliferation after a long period of quiescence. Thus, our present study was designed to determine whether or not irradiated cells that have remained in a quiescent state for long time are indeed capable of inducing delayed phenotypes after they are forced to divide. Current studies are also indispensable for the better understanding of the late effects of radiation, because non-cancerous late effects are also known to be stemmed from delayed dysfunction in stem cells of various adult tissues.

RESULTS AND DISCUSSIONS: In the present study, normal human diploid cells were maintained in a confluent (quiescent) state for up to 24 months after irradiation. We found that those cells stimulated to divide after the confluence showed the delayed induction of DNA double strand breaks, as well as various delayed phenotypes, including delayed reproductive death and delayed chromosome instability, thereby indicating the persistence of radiation-induced genomic instability. Interestingly, no significant increase in ROS levels were detected in long-term cultured cells, which implicated ROS-independent mechanism(s) capable of contributing to the succession and perpetuation of the initial insults of the genome caused by ionizing radiation. Furthermore, the results indicated the involvement of DNA repair pathways, particularly in manifestation of radiation-induced genomic instability. These involvement is further pronounced if the cells were exposed to low-dose radiation, as low levels of DNA double strand breaks tend to be ignored by DNA repair pathway, especially by non-homologous end-joining. Thus, a set of genes is tightly regulated the process of radiation-induced carcinogenesis by differential expression of the gene transcripts. Those involved in DNA repair and oxidative stress are constitutively expressed under physiological condition, and their expression may not be affected by low-dose radiation. Further studies will uncover the biological significance of transcription of such genes.

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[2] K. Suzuki *et al.*, Mutat Res., **671** (2010) 33-39.

PR3-7 **Radiation-Quality Dependent Cellular Response of Gene Mutation in Normal Human Fibroblasts**

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INTRODUCTION: A central paradigm in radiation biology has been that only cells "hit" by a track of radiation would be affected to induce radiobiological consequences, and cells "not hit" should not be. This paradigm is the basis for the current system for risk estimation from radiation and the risk of radiation-induced cancer after high and moderate doses are relatively well known, based on the data from detailed epidemiological studies of the Japanese atomic bomb survivors in Hiroshima and Nagasaki[1)]. However, it recently has been challenged by so called non-targeted effects, such as genomic instability, adaptive response and bystander effect, and such radiation-induced non-targeted effects may have important implications for risk evaluation of low dose / low dose rate radiations.
In this study, we have examined radiation quality dependent cellular response in gene mutation induced by low dose rate radiations.

EXPERIMENTS: Normal human skin fibroblasts distributed by the RIKEN BioResource Center Cell Bank (NB1RGB : Cell No. RCB0222) in Tsukuba, Japan were used in this study. Mutation induction was measured as 6-thioguanine resistant clones at the *hprt* locus. The cells were pretreated with low-dose-rate irradiation (~1mGy/7-8h) of ^{137}Cs gamma rays, ^{241}Am-Be neutrons, helium (LET=2.3keV/μm), carbon (LET=13.3keV/μm) and iron ions (LET=200keV/μm) before following irradiation with an X-ray challenge dose (1.5Gy). Radiation-quality dependent cellular response was evaluated by changing X-ray-challenging dose induced mutation frequency, comparing to non-pretreatment of low-dose-rate radiations. Heavy-ion beams, such as helium, carbon and iron, were generated at the Heavy Ion Medical Accelerator in Chiba (HIMAC) in Japan.

RESULTS: As shown in Fig. 1, X-ray-induced mutation frequency in cells pretreated with 1mGy of ^{137}Cs gamma rays was reduced to around 70% in unpretreated control cells, which were irradiated with X-ray challenging dose alone. In cells pretreated with 1mGy of neutrons derived from ^{241}Am-Be source, it was reduced to around 15%, comparing to the control cells. In the case of the pretreatment of heavy ions, X-ray-induced mutation was around 1.9 times higher in helium-ion pretreated and 4.0 times higher in carbon-ion pretreated cells than in unpretreated control cells. However, the mutation frequency in cells pretreated with iron ions was the same level as either unpretreated control cells. Furthermore, reduced mutation pretreated with ^{137}Cs gamma rays and neutrons and enhanced mutation pretreated with helium and carbon ions were returned to the control level, when using a specific inhibitor of gap-junction mediated cell-cell communication (40μM lindane).
There is evidence that cellular responses induced by low-dose-rate irradiations with different radiation types are radiation-quality dependent and gap-junction mediated cell-cell communication plays an important role of inducing cellular responses, such as genomic instability and radioadaptive response.

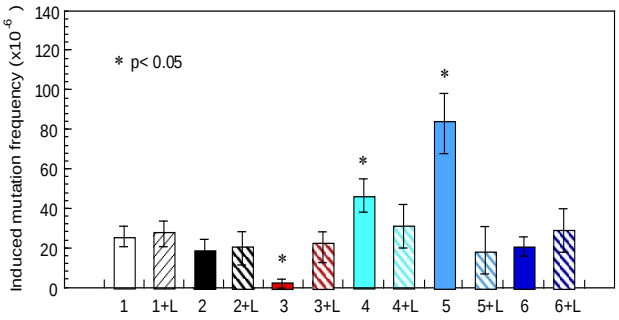

Fig. 1. Cellular response in mutation induced by low-dose-rate irradiation. 1; 200 kV X rays 1.5Gy alone, 2; gamma-ray pretreatment (1mGy/8h) → X rays 1.5Gy, 3; neutron pretreatment (1mGy/8h) → X rays 1.5Gy, 4; helium-ion pretreatment (1mGy/6.8-7.4h)→X rays 1.5Gy, 5; carbon-ion pretreatment (1mGy/7.1-7.5h) → X rays 1.5Gy, 6; iron ion pretreatment (1mGy/7.1-7.3h) → X rays 1.5Gy. +L ; with specific inhibiter of gap-junction mediated cell-cell communication.

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採択課題番号 21P3-7　　低線量放射線による遺伝子突然変異に対する細胞応答の放射線線質依存性　　プロジェクト

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