**Objective/Significance**

In the near future, we are obliged to travel or live in the cosmic space, and the invention of new technology using the cosmic space is much expected. Therefore, it is necessary to evaluate the effects of cosmic radiations and circumstances on humans, and to protect from radiation-induced cancers and chronic diseases. However, there have been no proper methods to evaluate such effects on humans, except for post-mortem or post-hazard epidemiological studies, animal studies, and research works with cultivated cells. As there exist some serious problems in animal studies, such as species and strain differences, it is necessary to establish a novel technique using human organs and tissues. In this ground-based study, the thyroid gland and the bone marrow, which are very sensitive, even fatal sometimes, to radiation and also are the organs playing an important role in human growth, were transplanted and maintained in the severe combined immunodeficient mice (SCID mice), and then exposed to radiations for evaluating the effects of cosmic radiations and circumstances on human tissues, suggesting the significance of the effects of cosmic radiations on the health of space pilots, which is the matter of discussion in the National Radiation Protection Committee.

**Methods**

By the improvement of SCID mouse, which is deficient of immunological rejection to human tissues, we have succeeded to maintain morphology and function of normal human tissues for more than 2 years. C57BL/6J-scid and C.B17-scid mice transplanted with human thyroid tissues (Graves disease) and bone marrow cells were exposed to $^{137}$Cs γ-rays or fission neutron, and their macro- and micro-scopic structural, functional, and genetic changes and abnormal expression in human organs and tissues were examined by the advanced morphological and molecular techniques.

**Results**

1) **Improvement of SCID mice:**

SCID mice were improved by the selective inbreeding of scid/scid homozygous male and female which showed undetectable level of IgG and IgM (<1μg/ml) for more than 20 generations, producing non-leukemic and non-leaky SCID mice which were completely deficient of T cell and B cell function. C57BL/6J-scid/scid (N11F20) and C.B17-scid/scid (F51) were produced and used for study.

2) **Effects on microstructure and function of the thyroid gland:**

Human thyroid tissues from patients of Graves disease were cut into 5-6 mm cubic masses and transplanted subcutaneously into the right and left back of the SCID mice. When SCID mice died or became moribund, the thyroid tissues were removed and transplanted into another SCID mice, and maintained over the mouse generation. Using Gammacell 40 Exactor and Computed $^{137}$Cs Exposure
Instrument, SCID mice with human thyroid tissues were exposed to 1 Gy of γ-rays weekly at a rate of 1.07 Gy/min or 0.23 mGy/min. The thyroid tissues transplanted into SCID mice were biopsied and microscopic changes were examined. Peripheral blood samples were taken from the SCID mice and human thyroid hormone (T₃) levels were measured by radio-immunoassay.

High dose rate exposure to 8 Gy of ¹³⁷Cs γ-rays caused strong atrophy to the transplanted thyroid tissues, and the disappearance of the follicle was highly observed, while low dose rate exposure caused little changes. Concerning the secretion of thyroid hormone (T₃) from the transplanted thyroid tissues, though less amounts of T₃ were measured in the SCID mice exposed to 8 Gy at high dose rate (303 ± 69 pg/ml, mean ± SE, n=10) compared to those of the un-exposed tissues (446 ± 19, n=10), such difference was not observed in the SCID mice with thyroid tissues exposed to low dose rate γ-rays (440 ± 15, n=5). This result indicates that there exist strong dose rate effects (reduced morphological and functional damage by the low dose rate exposure) in human thyroid tissues.

3. Detection of genetic changes:

Mutational changes in p53, k-ras, c-kit, β-catenin, and RET genes were examined by PCR- ‘Cold SSCP’ analysis following by direct sequencing (ABI PRISM 3100 Genetic Analyzer). Though no mutational changes of above genes were detected in the human thyroid tissues exposed to 8 Gy, three p53 mutations were detected in 2 of 7 thyroid tissues exposed to 8-16 Gy at a high dose rate. Of three mutations found in this study, two were silent mutations at codon 148 and 152, and one was deletion mutation at codon 180 (GAG>G: GA-AG deletion). No mutations were observed in the un-exposed tissues nor in the tissues exposed to 8-16 Gy at a low dose rate.

4. Detection of abnormality in gene expression:

We are now analyzing the abnormality in gene expression for 8500 human genes using GeneChip (Agilent Technologies Co. Ltd). Human thyroid tissues transplanted to SCID mice were exposed to 2 Gy of ¹³⁷Cs γ-rays at a dose rate of 1.07 Gy/min or 0.46 mGy/min. On day 7 and day 14, the transplanted thyroid tissues were taken from the SCID mice and gene expression status was analyzed by GeneChip. In the γ-ray exposed thyroid tissues at a high dose rate, changes in gene expression were observed in 6 genes on day 7 and in 25 genes on day 14 (increase and decrease more than 4 times) compared to the unexposed thyroid tissues. In the γ-ray exposed thyroid tissues at a low dose rate, changes in gene expression were observed in only 4 genes on day 7 and in 7 genes on day 14 compared to the unexposed tissues. The dose rate effect was also observed in gene mutations and abnormality in gene expression. Human thyroid tissues transplanted to SCID mice were exposed to fission neutron (0.2 Gy of neutron and 0.2 Gy of γ-rays) by UTR-KINKI at Kinki University. On day 7 after neutron exposure, changes in gene expression were observed in 215 genes in comparison to those of unexposed controls.

5. Bone marrow transplantation:

Human bone marrow cells were injected into SCID mice intravenously. All of the SCID mice exposed to 4 Gy of ¹³⁷Cs γ-rays died of intestinal death irrespective of the transplantation of human bone marrow. SCID mice exposed to 2 Gy without human bone marrow transplantation became moribund at 4 weeks after γ-ray exposure, while SCID mice exposed to 2 Gy and transplanted with human bone marrow survived more than 4 months. The remaining human bone marrow cells in the mouse leukocytes were confirmed by the human cell specific fluorescent staining.

Potential for space experiment

Microscopic changes, decrease of thyroid hormone secretion, gene mutations and changes in gene expression were induced in transplanted human thyroid tissues by ¹³⁷Cs γ-ray exposure at a high dose rate. However, no changes were induced by low dose rate exposure (1/5000). The strong effects of fission neutron were observed on the transplanted human thyroid tissues, indicating the necessity to evaluate the effects of cosmic radiations for the protection of space pilots and passengers.

9. Publication List

No publication in English.

10. URL

To be planned.