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Effects of fission neutrons on human thyroid tissues maintained in SCID mice

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ABSTRACT

Morphology and function (secretion of thyroid hormone) of human thyroid tissues from Graves' disease patients are well maintained in C57BL/6J-*scid* mice. Serum level of thyroid hormone was reduced by fission neutrons from the nuclear reactor UTR-KINKI, and changes in thyroid hormone by fission neutrons were bigger than those by low LET radiations, X-rays and ^{137}Cs γ-rays, suggesting high relative biological effectiveness (RBE; 6.5) of fission neutrons. Microarray analyses revealed that about 3% of genes showed more than 4-fold change in gene expression in the unexposed thyroid tissues against surgically resected thyroid tissues from the same patient, probably due to the difficult oxygen and nutrient supply shortly after transplantation. Dose-dependent changes in gene expression against unexposed concurrent controls were observed with increasing doses of fission neutrons (0.2–0.6 Gy) and ^{137}Cs γ-rays (1.0–3.0 Gy) and showed high RBE (4.2). Furthermore, there were some specific genes which showed more than 4-fold change in gene expression in all the thyroid tissues exposed to higher doses of radiation, especially neutrons (0.4 and 0.6 Gy), but none at lower doses (0.2 Gy of neutrons and 1.0 and 2.0 Gy of γ-rays). These genes related to degeneration, regeneration, apoptosis, and transcription, respond specifically and very sensitively to neutron injury in human thyroid tissues. This is the first experimental report that fission neutrons can induce some morphological and functional disorders in human tissues, showing high RBE against γ-ray exposure. These results are useful to evaluate the risks of fission neutrons and cosmic rays to humans.

1. Introduction

Radiation induces various types of damage in human and animals. Among various radiation sources, neutrons are several times more effective than X- and γ-radiation in inducing neoplastic cell transformation, mutation *in vitro*, germ-cell mutation *in vivo*, chromosomal aberrations *in vivo* and *in vitro* and cancer in experimental animals [1]. In spite of the evidence in experimental animals, there is a scarcity of evidence in humans; epidemiological study of A-bomb survivors on the difference between Hiroshima and Nagasaki and a few accidents at nuclear sites [1]. In humans, exposure to neutrons can occur from the nuclear fission reactions usually associated with the production of nuclear energy and from cosmic radiation (in the flying body) in the natural environment [2–4]. Consequently, it is of utmost importance to study the direct effects of fission neutrons on human organs and tissues for investigating the precise risk.

In the improved severe combined immunodeficient (super-SCID) mice, normal human organs and tissues are well maintained in morphology and function for a long period (~3 years) by the consecutive transplantation of these tissues [5–10]. For example, no substantial histological changes were observed in the human thyroid tissues maintained in SCID mice for 18 months, and rapid and high uptake of radioiodine into the transplanted human thyroid tissue was observed [10]. Furthermore, transplanted human thyroid tissues secreted thyroid hormone (T3), and T3 secretion was stimulated by the injection of human thyroid stimulating hormone (TSH)

Abbreviations: SCID, severe combined immunodeficiency; SPF, specific pathogen free; SSCP, single strand conformational polymorphism; RBE, relative biological effectiveness.

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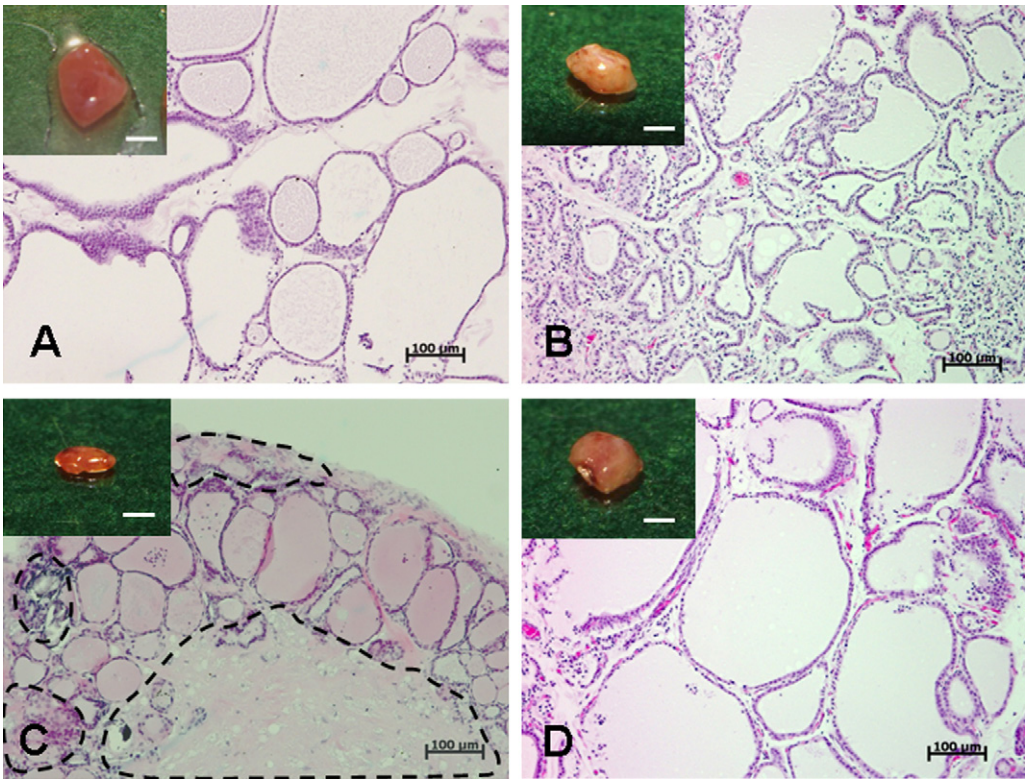

Fig. 1. Macroscopic and microscopic views of transplanted human thyroid tissues with or without radiation exposures. (A) Surgically resected human thyroid tissue from Graves' disease patient (20 years, female). (B) Human thyroid tissues from a Graves' disease patient (20 years, female) exposed to neutrons (0.2 Gy × 4 times at 1 week interval) and exposed tissue was removed 5 weeks after transplantation, (C and D) transplanted thyroid tissues exposed to neutrons (0.2 Gy × 6 times) and removed 7 months after transplantation (C) and concurrent unexposed controls (D), respectively. Histologically, follicles became small, some disappeared and were replaced to connective tissues (C) (marked by broken line). Scale bars in gross features: 3 mm. Microscopic views; haematoxylin and eosin staining. Scale bars: 100 μm.

[10]. Expression analysis by microarray indicated that gene expression was also well maintained in the transplanted human thyroid tissues [11]. The expression of only 3% of genes showed more than 4-fold change in gene expression during the first week after transplantation of surgically resected original tissues. However, further changes were not observed 2–4 weeks after transplantation, but instead recovered slightly [11].

The thyroid gland is one of the most important endocrine organs for the development and growth, and one of the most sensitive organs to radiation. Radiation exposure, therefore, causes general disorder in human beings [12]. In fact, consecutive irradiation with X-rays or ^{137}Cs γ-rays for approximately 2 years resulted in the disappearance of follicles and significant decrease of thyroid hormone secretion [11]. Mutations in *p53* and *c-kit* genes were induced significantly by high dose and high dose rate of X-rays and γ-rays in human thyroid tissues from old head and neck cancer patients and a Graves' disease patient, while mutations were not detected by low dose rate exposure [11]. Furthermore, lower doses (1–3 Gy) of ^{137}Cs γ-rays can induce changes in gene expression in the transplanted human thyroid tissues.

In the present study, human thyroid tissues from Graves' disease patients were transplanted into the improved SCID mice, and exposed to fission neutrons or ^{137}Cs γ-rays to examine the induced changes in morphology, function, cancer-related genes, and changes in gene expression in the transplanted human thyroid tissues to confirm the direct link between radiation sources.

2. Materials and methods

2.1. Human thyroid tissues

Thyroid tissues resected from two Graves' disease patients (20 and 23 years, females) were used for heterotransplantation to the SCID mice. Goiter was resected because of cosmetic problem, and blood level of thyroid hormone of the patients before the surgical resection was within normal range. Only the human tissues free of mycoplasma, human hepatitis B and C antigens/antibodies, adult T cell leukemia, human immunodeficiency virus, and Wassermann reaction were accepted into the SPF room of the barrier section of the Institute of Experimental Animal Sciences, Osaka University. Use of human tissues were permitted by the ethics committees of Osaka University, Graduate School of Medicine, Kuma Hospital and National Institute of Biomedical Innovation, and all experiments were performed following the guidelines of the Ministry of Education, Science and Culture and the Ministry of Health and Labor for the use of human tissues.

2.2. SCID mice

C57BL/6J-*scid*/*scid* mice ($N_{12}F_{22-24}$) were used for the experiment. C.B17-*scid*/+ male and female mice were provided by Dr. M.J. Bosma [13], Institute of Cancer Research, Philadelphia, in 1986, and then C.B17-*scid*/*scid* mice were maintained by selective sister–brother inbreeding of C.B17-*scid*/*scid* homozygote showing undetectable serum IgG and IgM (<1 μg/ml) by T. Nomura to diminish the leaky and leukemic mice [5,6,14]. C.B17*scid*/*scid* male (N_1F_3) was mated with C57BL/6J female (F_{153}) (provided by E.S. Russell, Jackson Laboratory at F_{129} in 1976 and inbred by sister–brother mating for further generations). Progeny was crossed and *scid* homozygote mouse was repeatedly back-crossed to C57BL/6J mouse to make congenic strain of C57BL/6J-*scid*/*scid* ($N_{12}F_{20}$) by T. Nomura [15]. Mice were maintained in the complete barrier condition, lit from 4:00 to 18:00, at 23 ± 1 °C and 50–70% humidity with autoclaved mouse diet CRF-1 (Charles River Japan, Kanagawa, Japan) and acidified, chlorinated, and filtrated (by MILLIPORE) water. Serum IgG and IgM were examined at 4–6 weeks after birth by enzyme-linked immunosorbent assay [5,6], and 2 months old C57BL/6J-*scid*/*scid* mice showing undetectable serum IgG and IgM (<1 μg/ml) were used for the heterotransplantation of human thyroid tissues. Animal experiments were carried out in the barrier section of the Institute of Experimental Animal Sciences following the Osaka University Guidelines for Animal Experimentation.

2.3. Maintenance of human thyroid tissues in SCID mice

Procedures for the heterotransplantation of human organs and tissues into the SCID mice were reported previously [5–11]. Briefly, resected human thyroid gland was cut into 5–6 mm cubic masses in a 0.9% NaCl solution contain-