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Laboratory of Radiation Biology

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This laboratory was established as the first laboratory for Radiation Biology in a veterinary medical school in Japan in June 1969. Since this laboratory is not only responsible for radiation biology research but also for education on the safe use of radioisotopes (RI) for researchers and students in this faculty, it is equipped with a liquid scintillation counter (Packard 2100TR), auto well gamma system (Aloka ARC-300), Geiger Müller (GM) counter (Aloka GM2503B), bioimaging analyzer (Fuji BAS-1000) and X-ray generator (Toshiba KXC-18). Furthermore, every year there is an RI safety and emergency drill.

The teaching staff is composed of a professor, an associate professor and an instructor, and three graduate students and four undergraduate students are enrolled. Our research interests are mainly focused on mechanisms of radiation-induced or oxidative-stress-induced damage, repair and protection in biological systems, application of the magnetic resonance imaging (MRI) technique in animal experiments and development of RI techniques in molecular biology. The main topics in our current research include the following five fields.

1. Sensitive detection of oxygen radical-induced DNA damage by a method combining spin-trapping and electron spin-resonance spectroscopy (ESR)

Since lethal injuries to the living cell by ionizing radiation and oxidative stress are mainly due to oxygen-radical-induced DNA damage, the reaction mechanism of OH radicals, which are the most highly reactive species among oxygen radicals, with DNA and related components is being investigated. For this purpose, a highly sensitive techniques for detection of free-radical induced DNA lesions consisting of spin-trapping and ESR was developed and various free radicals that induce strand breaks have been identified[1].

2. Mechanisms for generation of reactive oxygens from stimulated neutrophils.

Bovine leukocyte adhesion deficiency (BLAD) is a genetic deficiency of $\beta 2$ integrin as one component of CR3 on the surface of the neutrophil membrane and such neutrophils show low bactericidal activity. By the spin-trapping and ESR methods, it was found that stimulation of BLAD neutrophils with serum-opsonized zymozan bring about lower generation of superoxides than in normal calves, whereas stimulation of BLAD neutrophils with phorbol 12-myristate 13 acetate (PMA) results in the normal generation of superoxides. These results suggest that the activation of NADPH oxidase is greatly affected by the presence of CR3[2].

3. MRI of tissue damage in diseased laboratory animals.

Recently, we introduced MRI with 7.05T magnet (Siemens SIS300/183) which we share with the Faculty of Engineering of Hokkaido University (Fig. 1) and the physiological and pathological changes in the aged rat brain[3] and the LEC rat liver were clearly observed. In application of MRI to mice, three-dimensional MR images of thoracic and abdominal organs were constructed by superimposing multislice MRI on a computer with volume ray-tracing software. This technique makes it possible to clearly distinguish individual organs with respect to position, size and shape. Furthermore, pathological changes such as the foci in the liver and lung tumors produced by exposure to urethane and transplantation of tumor cells were also identified in anesthetized mice. These results suggest that this new 3-D technique for small animals can be widely applied to observe not only normal tissues but also pathological changes in various organs to judge the degree of progress of malignancy.

4. Responses of mammalian cells to ionizing radiation.

In radiation therapy for malignant tumors, low radiosensitivity due to their hypoxic condition is a serious problem. Therefore, the development of radiation-sensitizing agents and studies

to clarify their sensitizing mechanisms are necessary. Thus, a project to test the effects of various synthesized-nitroimidazol derivatives on radiation-induced cell death under hypoxic conditions was planned in this laboratory. For irradiation under hypoxia, a special gastight chamber was designed and viability of X-irradiated cells (V79, L5178Y and molt-4, etc.) under hypoxic conditions was assessed by clonogenic assay and dye exclusion assay. Furthermore, agarose gel electrophoresis for the apoptosis-induced DNA ladder, pulse-field gel electrophoresis for radiation-induced genomic DNA damage and the western blotting technique for expression of the apoptosis-related proteins p53, Waf1, BCL-2, etc., were used to examine cell damage and the repair process. Using these techniques, experiments aimed at examination of the sensitizing mechanisms in tumor cells by various nitroimidazol derivatives are in progress.

5. Sequence and functional analysis of Marek's disease virus oncogenes.

Radiation emitted from radioisotopes is also useful for life science research, especially for molecular biotechnology. A method combining RI and molecular biotechnology was applied to analyze the gene expression of the Marek's disease virus (MDV) which is an oncogenic herpesvirus of the chicken. Using sensitive and quantitative reverse-transcriptase-polymerase chain reaction combined with RI-probing hybridization and bioimage analysis, it was shown that the MDV gene, which is a homolog of ICP4 of herpes simplex virus type1, is immediately expressed after the infection and transactivates itself in *in vitro* infected cells[4]. These data suggest that the ICP4 homolog can be used as a primary probe for MDV infection.

The significant data obtained from these research projects have been presented to the public in several journals and at meetings organized by the following scientific societies: The Japanese Radiation Research Society; The International Society of Radiation Research; The Japanese Society of Veterinary Science; The Japanese Society of Magnetic Resonance in Medicine; The International Society for Free Radical Research and The Japanese Cancer Association.

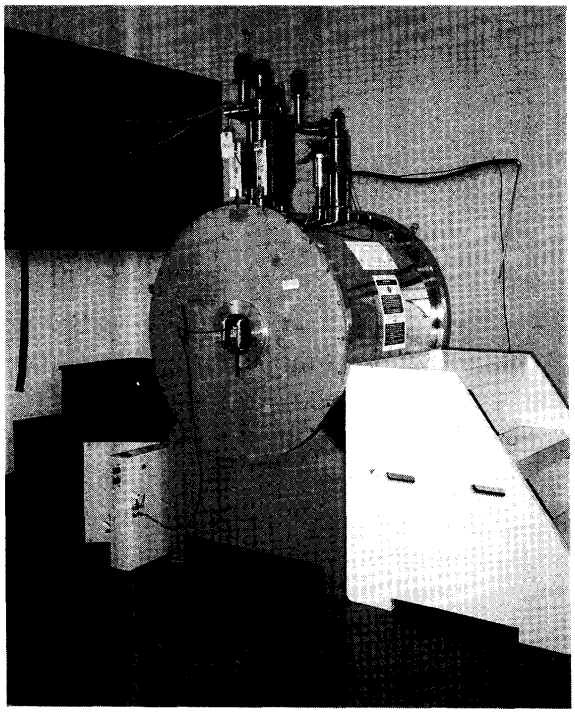

Fig. 1. 7.05T MRI (Siemens SIS300/183)

References

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