Report on “Radiation Disaster Recovery Studies”

Course: Radiation Disaster Medicine
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○Regarding “Radiation Disaster Recovery Studies”

Nominal coefficients of radiation risks were formulated by the International Commission of the Radiological Protection (ICRP). These standards were established uniformly to the public. However, numerous scientific publications have been postulated that individual difference of radiosensitivity exists in human population [1,2]. A line of studies in the field of radiation biology suggests that the individual difference is probably attributed to single nucleotide polymorphisms on DNA repair genes.

Radiation sciences may take an advantage to investigate effects of mutations in genes that are encoding proteins involved in DNA damage response and repair [3]. For example, functionality of the genes encoding molecular sensors and protein machines participating in reparation of damaged DNA. Dampened DNA repair system causes chromosomal aberrations and cell death to induce acute and/or chronic radiation syndromes. Hence, analysis of size effects of gene mutations is required for investigation of individual radiosensitivity.

While studying at Radiation Disaster Medicine course of Phoenix Leader Education Program, Hiroshima Initiative, I was engaged in the research project focused on development of strategy for quantitative assessment of individual radiosensitivity. Screening of individual-specific gene alterations with established methodology can be used as reliable genetic biomarker to predict radiation response of given person and precisely calculate its consequences for health.

○Title of Doctoral Thesis

Evaluation of *ATM* heterozygous mutations underlying individual differences in radiosensitivity using genome editing in human cultured cells. *Scientific reports* 2017, in press.
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○Summary of Doctoral Thesis

Introduction: Ataxia-telangiectasia is a rare autosomal-recessive disorder characterized by hyper-radiosensitivity, cancer predisposition, immunodeficiency and neurodegeneration. A-T is caused by germline mutations in the ataxia-telangiectasia mutated (*ATM*) gene encoding ATM kinase, which modulates DNA damage response. A-T heterozygous carriers exist at a rate of approximately 1% in human populations and are clinically asymptomatic. Previous epidemiological studies demonstrated that A-T carriers has several-fold increased risk of cancer in comparison with normal individuals. It is therefore important to quantify the precise effect of *ATM* heterozygous mutations on

radiosensitivity in the primary cells.

Purpose: Main purpose of my study was to clarify size effect of *ATM* gene variants on individual differences in radiosensitivity using various biological assays. Since the radiosensitivity of human primary cells might be affected by confounding factors such as age, gender, smoking and the diverse genetic backgrounds within human populations, it was necessary to generate a system for evaluating genetic factors underlying individual differences in radiosensitivity in a human cultured cell line with a uniform genetic background. Since in my doctoral study the term “radiosensitivity” was attributed to biological end-points reflecting number of DNA double-strand breaks that were unrepaired/misrepaired even after an operation of DNA repair systems.

Methodology: In my doctoral study, I have established semi-automatic cytokinesis-blocked micronucleus assay for evaluation of radiosensitivity of human adherent cell lines using Metafer system. Since evaluation of size effects of mutations on DNA repair genes using CBMN assay might be affected by genetic heterogeneity and confounding factors (such as age, gender, smoking, life style, etc.) existing in human population, I generated human cell lines with uniform genetic background (not affected by confounding factors) using CRISPR/Obligare genome-editing strategy. Generated model cell lines were used for evaluation of impact of homozygous and heterozygous mutations on *ATM* gene on individual radiosensitivity. Results were compared with chromosomal aberration assay. The coefficients representing DNA repair capacity were extracted from dose-response calibration curves using Cabas software.

Results: Cytokinesis-blocked micronucleus assay and chromosome aberration assay showed that the radiosensitivity of *ATM*+/− cell clones was significantly higher than that of *ATM*+/+ cells, suggesting that *ATM* gene variants are indeed involved in determining individual radiosensitivity. Importantly, the differences in radiosensitivity among the same genotype clones were small, unlike the individual differences in fibroblasts derived from A-T-affected family members. Targeted introduction of heterozygous mutation in *ATM* gene had an effect of increasing cellular radiosensitivity by 2.6-fold, and suggested that they were indeed a genetic factor underlying individual differences in radiosensitivity within human populations.

Conclusions: In my research, I have established an experimental flow combined with a semiautomatic CBMN assay and genome editing technology in a human cultured cell line as a unique system for evaluating genetic factors underlying individual radiosensitivity. Obtained results suggests that the genome editing technique could be implemented into radiation biology in order to investigate size effects of mutations on DNA repair genes on individual radiosensitivity. In addition, this methodology can be applied for estimation of radiation-induced cancer risk in human populations.

○Other theses published in academic research journals

1. Miyamoto T, Hosoba K, Ochiai H, Royba E, Izumi H, Sakuma T, Yamamoto T, Dynlacht BD, Matsuura S: The microtubule-depolymerizing activity of a mitotic Kinesin protein KIF2A drives primary cilia disassembly coupled with cell proliferation. *Cell Reports* **10**, 664-673, 2015.
2. Matsuura S, Royba E, Akutsu SN, Yanagihara H, Ochiai H, Kudo Y, Tashiro H, Miyamoto T. Analysis of individual differences in radiosensitivity using genome editing. *Ann ICRP* **45**, 290-296, 2016

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1. Foray N, Colin C, Bourguignon M. 100 Years of Individual Radiosensitivity: How We Have Forgotten the Evidence. *Radiology* **264**, 627-631, 2012
2. Foray N, Bourguignon M, Hamada N. Individual response to ionizing radiation. *Mutat Res Mutat Res* **770**, 369-386, 2016
3. Herskind C, Talbot CJ, Kerns SL *et al.* Radiogenomics: A systems biology approach to understanding genetic risk factors for radiotherapy toxicity? *Cancer Lett* **382**, 95-109, 2016