Report on "Radiation Disaster Recovery Studies

Course: Radiation Disaster Medicine

Name: Silvia Natsuko Akutsu

- **Regarding "Radiation Disaster Recovery Studies"**

  The "Phoenix Leader Education Program (Hiroshima Initiative) for Renaissance from Radiation Disaster" was created as a "Program for Leader Graduate Schools" by the Minister of Education, Culture, Sports, Science and Technology of Japan (MEXT) with an interdisciplinary education to build and cultivate experts in Radiation Disaster Recovery Studies in a global system.[1]

  Japan had the misfortune to come up against nuclear disaster and incidents; Hiroshima and Nagasaki atomic bombs (1945), Tokaimura nuclear accident (1999) and Fukushima Daiichi Nuclear Power Plant (2011). However, those incidents made possible the concentration of expertise in medical, environmental and social radiation fields. In the Phoenix Program, I belong to the "Radiation Disaster Medical course", where I had opportunity to learn with an interdisciplinary broad view of how to recover from radiation disaster. Mainly, the focus is having the ability to record the lessons learned documented from previous incidents to avoid and manage future radiation disasters. [1]

  Phoenix Program has national collaborations, such as Fukushima University, Fukushima Medical University, Tohoku University, Nagasaki University, Radiation Effects Research Foundation and the National Institute of Radiological Sciences. In addition, some international collaborations, including International Atomic Energy Agency (IAEA), REAC/TS and Universities. Those links made possible a visiting of expertise to Hiroshima University and opportunities for me to go to those facilities or institutions for fieldwork, internship, symposium, conference and training courses. Then, the exchange of scientific knowledge, valuable experiences and fruitful discussions enable to develop me as a person, researcher and future leader for radiation recovery process. [1]

  During the four years of the doctoral program, I have several lectures in Radiation Disaster Recovery, special subjects, common course work, fieldwork and internship that brought me a broad knowledge. In addition, I could focus in my research theme to understand the risks and genetic damage of radiation to the human body.

  The studies of Atomic Bomb Casualty Commission (1950)[2] reported that the Japanese atomic bomb survivors who were exposed to the ionizing radiation (IR) in vitro had affected their brain development. The mental retardation and small head size (microcephaly) were shown in those individuals. There were also an increase of perinatal loss, miscarriage, anencephaly and death during infancy due to radiation exposure. Microcephaly was found in those who were exposed to IR in utero at 8 to 15 weeks of gestation[3]. The International Commission on Radiological Protection (ICRP)-60 recommends that pregnant women with occupational radiation exposure should keep dose below 1mGy due to a high sensitivity of fetus to IR[4]. The embryonic brain is very vulnerable to IR. There were several studies in mouse fetus exposure to IR that showed

reduction of the brain size formation in a dose-dependent manner, due to a radiation induces apoptosis of neural progenitors and development of supernumerary centrosomes causing mitotic catastrophe[5].

In my doctoral study, I attempted to identify the microcephaly causing mutations in Japanese patients by whole exome sequencing analysis in order to elucidate the pathological mechanism of microcephaly.

References

1. http://phoenixprogramlp.hiroshima-u.ac.jp/en/
2. http://www.rerf.jp/radefx/genetics_e/birthdef.html
3. Committee on the Biological Effects of Ionizing Radiation (BEIR V), Health Effects of Exposure to Low Levels of Ionizing Radiation: BEIR V. **National Research Council ISBN**: 0-309-58970-3, 436 pages, 6x9, (1990).
4. ICRP, 1991. 1990 Recommendations of the International Commission on Radiological Protection. ICRP Publication 60. **Ann. ICRP 21** (1-3).
5. Shimada M., Matsuzaki F., Kato A. *et al*. Induction of Excess Centrosomes in Neural Progenitor Cells during the Development of Radiation- Induced Microcephaly. **PLoS One**. 2016.

- **Title of Doctoral Thesis:**

"PLK1-mediated phosphorylation of WDR62/MCPH2 ensures proper mitotic spindle orientation" (PLK1 による WDR62 のリン酸化を介した細胞分裂軸制御機構).

- **Summary of Doctoral Thesis**

Introduction

The primary microcephaly (MCPH) is an autosomal recessive neurodevelopmental disorder characterized by reduction of occipitofrontal head circumference at birth. The MCPH gene products localize to centrosome organelles, which regulates the microtubule (MT) organization during cell division and chromosome segregation. There are about 13 genes described in primary microcephaly development and implicated in spindle pole orientation control. However, the molecular mechanism remains unclear. In this study, I identified compound heterozygous (missense and non-sense) mutations of *WDR62/MCPH2* gene in two siblings with microcephaly in Japanese family by whole-exome sequencing. Previous studies demonstrated that WDR62 localizes to mitotic centrosomes to assemble the MT composed of spindles, and that Polo-like kinase 1 (PLK1) is a player in stabilizing spindle orientation through astral MT formation. Here, I demonstrated that PLK1 phosphorylates WDR62 at S897 to develop astral MT, which stabilize the spindle orientation parallel to the substratum, resulting in symmetrical cell division.

Methods and Results

To better understand the role of WDR62 protein, we introduced CRISPR/Cas9 expression vector and ssODN with WDR62 c.731 C>T variant (missense mutation), silent mutation mediated BamHI restriction enzyme site into HCT116 cell lines to generate WDR62 *in vitro* model cells. The mRNA and protein levels of WDR62 were significantly reduced in those cells. The immunostaining analysis with anti-pericentrin and alpha-tubulin antibodies of WDR62 mutant cells detected a bipolar spindle mis-orientated by different confocal z-section planes and a significant reduction of astral microtubule formation. The same phenomena were observed in WDR62$^{S244L/S244L}$ HEK293T cells generated by the same procedure. These results indicated that WDR62 regulates the correct spindle orientation by promoting astral MT assembly.

The immunoprecipitation analysis demonstrated that PLK1 physically interacts with WDR62 in a Polo-box domain dependent manner. According to the consensus phosphorylation motifs of PLK1, I constructed the same AcGFP tagged alanine-substituted mutants of WDR62 (T575A, S897A, S987A and S1038A), in order to identify the essential residue on WDR62 for PLK1 dependent spindle orientation. The introduction of S897A mutant did not rescue the proper spindle orientation in WDR62 null cells. To confirm whether this residue is indeed the site of PLK1 phosphorylation, I generated anti-phospho WDR62(S897) polyclonal antibody and performed an in vitro kinase assay. The results confirmed that PLK1 phosphorylate WDR62 at Ser897.

Moreover, exogenous WDR62 S897E enable to maintain the spindle orientation and astral MT development in the WDR62$^{+/+}$ cells treated by PLK1 inhibition.

Conclusion

I demonstrated that the whole exome analysis of Japanese MCPH family is a useful tool to identify novel mutations of the WDR62/MCPH gene. The use of genome editing technology to generate human disease model cell lines is important to understand the biological mechanism. The PLK1 phosphorylates WDR62 at S897 to assemble astral MT and ensure the proper spindle orientation.

- **Other theses published in academic research journals**

1. Tatsuo Miyamoto, Silvia Natsuko Akutsu, Shinya Matsuura. Update summary of genome editing technology in human cultured cells linked to human genetics studies. **Journal of Human Genetics.** 2017, in press.

2. Ekaterina Royba, Tatsuo Miyamoto, Silvia Natsuko Akutsu, Kosuke Hosoba, Hiroshi Tauchi, Yoshiki Kudo, Satoshi Tashiro, Takashi Yamamoto, Shinya Matsuura. Evaluation of *ATM* heterozygous mutations underlying individual differences in radiosensitivity using genome editing in human cultured cells. **Scientific Reports.** 20;7(1):5996, 2017.

3. Shinya Matsuura, Ekaterina Royba, Silvia Natsuko Akutsu, Hiromi Yanagihara, Hiroshi Ochiai, Kudo Y, Satoshi Tashiro, Tatsuo Miyamoto. Analysis of individual differences in radiosensitivity using genome editing. **Annals of the ICRP.** 45: 290-296, 2016.