BL-27B/2021G539

Radio-adaptive response induced by the targeted cytoplasmic irradiation in normal human fibroblasts with X-ray microbeams via bystander effects by gap-junction mediated cell-to-cell communication

Masao SUZUKI [1,*] and Noriko USAMI [2]
[1]Institute for Quantum Medical Science, QST, 4-9-1 Anagawa, Chiba, 263-8555, Japan
[2] Photon Factory, KEK, 1-1 Oho, Tsukuba, 305-0801, Japan

1 Introduction

Radio-biological effects for low-dose or low-fluence irradiations should be one of the major concerns in the fields of radiology or health physics. It is very useful for understanding fundamental radio-biological responses, such as bystander effects, genomic instability and radio-adaptive response, to use a microbeam-irradiation system and it enables us to irradiate with a targeted cell nucleus or cytoplasm. The study of such biological cellular responses for low linear energy transfer (LET) radiation can surely provide the critical information for inducing secondary carcinogenesis after tumor radio-therapy and for evaluating radiation risk such as the accident of Fukushima Daiichi Nuclear Power Plants caused by the Great East Japan Earthquake at 2011.

There are many studies available to examine cellular responses such as bystander effect using microbeam radiations. They, however, are carried out using high-LET-particle radiations and only limited studies are available to use low-LET electromagnetic radiations, such as X or gamma rays. The X-ray microbeams produced by PF at BL-27 are unique in the world for the radio-biological-study use.

We reported so far that the cellular bystander effect, such as chromosomal damage, in normal human fibroblasts via gap-junction mediated cell-to-cell communication was not induced in cells immediately after random irradiations with both cell nucleus and cytoplasm of the X-ray microbeams [1]. On the other hand, the bystander cellular lethal effect was induced in normal human fibroblasts when cells were irradiated with targeted cell nucleus alone [2]. And also, the higher frequency of gene mutation at the hypoxanthine-guanine phosphoribosyl transferase (*HPRT*) locus was induced in the progeny of the following 20-cell generations from the X-ray-microbeam irradiated cells than that in the progeny of non-irradiated control cells and it was reduced to the non-irradiated control level when treating with a specific inhibitor of gap-junction mediated cell-to-cell communication [3, 4].

We also have been studying the radiation-quality dependent bystander cellular effects, such as cellular lethal effect and mutagenic effect induced by high-LET heavy-ion microbeams at the Takasaki Ion Accelerator for Advanced Radiation Application, Takasaki Advanced Radiation Research Institute, National Institutes for Quantum Science and Technology. Together with the data of X-ray microbeams in PF, so far we understand the cellular responses as follows:

(1) In the case of both irradiations with cell nucleus and cytoplasm at the same time, the bystander cellular effects were observed in the cells irradiated with medium-LET carbon-ion microbeams, but not higher-LET neon- or argon-ion microbeams and lower-LET X-ray microbeams.
(2) Secondary radiations with low-LET components were calculated to produce from higher-LET heavy-ion tracks in proportion to LET values by the Monte Carlo simulation. They irradiated the cells located in the neighborhood of the heavy-ion tracks.
(3) The bystander cellular effects were induced by the targeted cell-nucleus irradiations even if low-LET X-ray microbeams were used.
(4) The cell-killing effect for low-LET X rays were higher in the cells with the targeted cell-nucleus irradiations than those in the cells of both irradiations with cell nucleus and cytoplasm at the same time.

Thus we can set up a hypothesis from the above scientific evidence as follows:

“When the cytoplasm of cells is irradiated with low-LET X rays, unknown cellular response(s) is induced in the cell and in consequence the cell becomes protective to radiation damage (Radio-adaptive response).”

We have been studying for some years ahead to make clear the radio-adaptive response induced in each cell by intracellular response, targeted cytoplasm of 100% cells on the microbeam-irradiation dish were irradiated with X-ray microbeam (10R=0.092Gy) beforehand and then targeted cell nuclei of 100% cells were irradiated with X-ray microbeam (0.092Gy). The cell survival in the cells irradiated with the targeted cell nuclei when the cells

were irradiated the cytoplasm beforehand was increased at 98%, suggesting the intracellular radio-adaptive response. [5-9]. This year we reported the preliminary result to make clear whether the radio-adaptive response was induced in intercellular response or not.

2 Experiment

Normal human skin fibroblasts distributed by the RIKEN BioResource Center Cell Bank (Cell No.: RCB0222, Cell name : NB1RGB,) were used in this study. The method of the microbeam irradiation was described in the user report of 2020. Briefly, approximately 1,000 exponentially growing cells were inoculated into the center of each microbeam dish, which was stretching a 2.5μm-thick Mylar film over the bottom of the hole for X-ray window, one day before microbeam irradiations. Each cell nucleus stained by Hoechst 33342 was captured by the computerized-cell-irradiation system. Targeted cytoplasmic irradiations with the monochromatic X-ray microbeams (5.35keV) to NB1RGB cells were carried out using the cell-irradiation system [5]. We made the microbeam covering the areas of 30μm x 30μm in which the center of the microbeams the gold-made mask that was 22 μm in diameter and 20 μm in height on a thin SiN film was set in order to shield the nucleus. When cell nucleus was irradiated, we used the X-ray microbeams collimating the beam size of 10μm x 10μm. The irradiation doses were selected to be 10R (0.092Gy). In order to examine the radio-adaptive response induced in intercellular response, targeted cytoplasm randomly selected 10% of all cells in the microbeam-irradiation dish were irradiated with X rays (0.092Gy) beforehand and then targeted cell nuclei of 100% cells were irradiated with X rays (0.092Gy) at the 180min interval within which the cells were kept in a CO_2 incubator at 37°C after the first cytoplasmic irradiation. A specific inhibitor of gap-junctions (40 μM of gamma-hexachlorocyclohexane) was added to the culture medium 1hr before the first cytoplasmic irradiation to the second cell nucleus irradiation in order to examine the effects of bystander responses via gap-junction mediated cell-to-cell communication. Cellular lethal effect was detected with a colony-forming assay as a reproductive cell death. Immediately after irradiation, cells were trypsinized and a defined number of cells plated onto 100mm plastic dish to make 60-70 colonies per dish. The colonies fixed and stained with 20% methanol and 0.2% crystal violet for 16-day incubation. The colonies consisting of more than 50 cells were scored as a survivor.

3 Results and Discussion

The result was shown in Fig.1 regarding the cellular lethal effect for randomly selected 10% of targeted cytoplasmic irradiation (0.092Gy) ---> (180min interval) ---> 100% of targeted nuclear irradiation (0.092Gy) with the gap-junction inhibitor. The cell survival irradiated with all cell nuclei alone (0.092Gy) was 79% and no cellular lethal effect was observed in the cytoplasmic irradiation alone (0.092Gy). They were rising to 95-98% survivals when either 10% or 100% of cytoplasm was irradiated beforehand. The data suggest that intercellular responses must play an important role of reducing lethal effect. The next we have been examining the effect of intercellular responses focusing on gap-junction mediated cell-to-cell communication.

When treating with the gap-junction inhibitor, it was 73% survival with the same level as the cell nucleus irradiation alone with the gap-junction inhibitor, showing NO radio-adaptive response. There is evidence that observed radio-adaptive response is induced in the cells by intercellular responses via gap-junction mediated cell-to-cell communication.

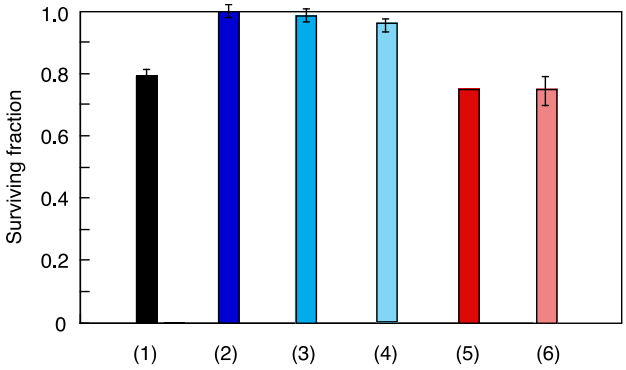

Fig.1: Cellular lethal effect of normal human fibroblasts irradiated with either targeted nuclear or cytoplasmic irradiation of monochromatic X-ray microbeams. (1) 100% of targeted nuclear irradiation (10R=0.092Gy) alone, (2) 100% of targeted cytoplasmic irradiation (0.092Gy) alone, (3) 100% of targeted cytoplasmic irradiation (0.092Gy) ---> (180min interval) ---> 100% of targeted nuclear irradiation (0.092Gy), (4) Randomly selected 10% of targeted cytoplasmic irradiation (0.092Gy) ---> (180min interval) ---> 100% of targeted nuclear irradiation (0.092Gy), (5) 100% of targeted nuclear irradiation (10R=0.092Gy) alone with the gap-junction inhibitor, (6) Randomly selected 10% of targeted cytoplasmic irradiation (0.092Gy) ---> (180min interval) ---> 100% of targeted nuclear irradiation (0.092Gy) with gap-junction inhibitor. The data showed the average and the standard error of 8 independent experiments for (1), (2) and (3), 6 independent experiments for (4). The data of (6) showed the average and the standard deviation of 4 independent experiments.

References

[1] N. Autsavapromporn *et al., Radiat. Res*. **180**, 367 (2013).

[2] M. Suzuki *et al*., *Photon Factory Activity Report* 2010, #28 Part B (2011).
[3] M. Suzuki *et al*., *Photon Factory Activity Report* 2014, #32, 54 (2015).
[4] N. Autsavapromporn *et al*., *Int. J. Radiat. Biol*. **91**, 62 (2015).
[5] M. Suzuki and N.Usami, P*hoton Factory Activity Report* 2015, #33 (2016).
[6] M. Suzuki and N.Usami, *Photon Factory Activity Report* 2016, #34 (2017).
[7] M. Suzuki and N.Usami, *Photon Factory Activity Report* 2017, #35 (2018).
[8] M. Suzuki and N.Usami, *Photon Factory Activity Report* 2018, #36 (2019).
[9] M. Suzuki and N.Usami, *Photon Factory Activity Report* 2020, #38 (2021).

* suzuki.masao@qst.go.jp