

## Killing and migration on human cancer cells induced by bystander effect after X-ray microbeam irradiation on cell nucleus

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### Introduction

Technique of microbeam irradiation targeted on nuclei of cells is a useful tool to understand the mechanisms of biological effects of ionizing radiation at low-dose and -dose rate, and at exposure in a limited part of organ.

It has been reported that bystander cell killing and mutation induction were induced in normal fibroblasts by proton or He-ion microbeams. Study of bystander effects with X-rays is however restrictive. Tomita *et al.* have recently reported the bystander cell killing in human fibroblasts after irradiation of synchrotron microbeams [1]. Aim of present study was to investigate if the enhancement of killing and migration in unexposed human cancer cells were present after irradiation with X-ray microbeams on defined number of cells.

### Materials and Methods

#### Cell culture and preparation of irradiation sample:

Human astrocytoma U251MG(KO) cells were grown in DMEM supplemented with 10% fetal bovine serum and antibiotic. Cells ( $2 \times 10^5$ ) were placed onto polypropylene film (5 micrometer thick) of dishes and cultured in a complete medium for 12~16 hours at 37 C.

Nuclei of cells were stained with 1 micromolar Hoechst 33258 for 1 hour at 37 C.

#### X-ray microbeam irradiation:

Monochromatic X-ray microbeams of 5.35 keV (beam size of 10x10 micrometer square) was generated with the cell irradiation system, which was composed of Si double crystal monochromator and a metal slit system, at BL27B, PF, KEK [2, 3]. After set the dish on a fluorescence microscope on beam line, image and position on x-y moving-stage of nuclei of cells on dish were taken using the image detection and memory system. Defined number of nuclei was irradiated at a given dose.

#### Immunofluorescence:

For detection of DNA double strand breaks (dsb) in nuclei exposed to X-rays, cells were treated with anti-mouse or -rabbit gamma-H2AX antibody and Cy2 conjugated secondary antibody. Images were obtained with a laser scanning confocal fluorescence microscope.

#### Colony formation assay for cell survival:

After irradiation cells were incubated in the complete medium for 24 hours, washed with PBS (-) and suspended in medium with trypsinization. For colony formation assay appropriate number of cells were cultured in medium for 2 weeks at 37C, fixed and then

stained with methylene blue. Surviving fraction of cells was calculated based on the colony forming ability.

#### Migration assay:

Migration of cells through 8 micron pores was assessed using insertwell culture chamber precoated with fibronectin. Cells cultured for 24 hours after irradiation were suspended in medium, seeded in the upper chamber and cultured for 20 hours. The number of cells that had migrated to the lower surface of the chamber was counted under a light microscope.

### Results and Discussion

The localization of gamma-H2AX foci within the target cell nucleus is shown in Fig.1.

When 40-cells on a dish were irradiated at 0.129 C/kg, survival of cells in dish decreased to about 87% as compared with that of sham irradiation (control). Cell survivals after 4-cells irradiation at 0.129 C/kg were not significantly changed from the control. In the presence of carboxy-PTIO (nitric oxide (NO) scavenger, 10 micro molar), reduction of cell survival after 40-cells irradiation was inhibited.

After irradiation on 40-cells at 0.129 C/kg, migration of cells, which were able to accept the bystander effect, tended to reduce as compared with that of control. However, there was a large variation of the migration rates at every experiment.

Present results suggested that bystander cell killing were induced by irradiation of X-ray microbeams on defined number of human cancer cells and might depend on NO molecule. More studies need to elucidate the mechanism of migration on bystander cells.

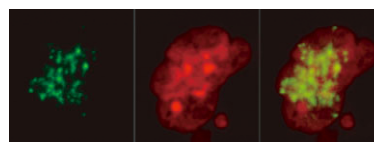


Fig.1. Immunofluorescence images of target U251MG(KO) cells exposed to 0.0774 C/kg of microbeams: panel left: gamma-H2AX focus; center: nucleus; right: merge.

### References

- [1] M.Tomita et al., Radiat Res., 173, 380 (2010)
- [2] N.Usami et al., Radiat.Prot.Dosimetry, 122, 307 (2006).
- [3] M.Maeda et. al., J.Radiat.Res., 49, 171 (2008).

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