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Dose Responses of Bystander Cell Death are Modified by Sites of Energy Deposition within Cells Irradiated with a Synchrotron X-Ray Microbeam

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Introduction

The bystander response is a phenomenon whereby biological responses occur in cells that are located nearby cells that have been traversed by charged particles, but which themselves have not been traversed. Therefore, microbeam irradiation system, which enables the observation of cellular responses of individual nonirradiated and irradiated cells, has become a powerful tool to elucidate the mechanisms underlying the bystander responses. Using a synchrotron X-ray microbeam irradiation system with a changeable beam size developed at the Photon Factory, KEK [1, 2], we recently demonstrated that cell death is more prevalent in cells irradiated with low doses of radiation when only nuclei are irradiated than when whole cells are irradiated [3]. In this study we examined the relationship between bystander cell death and intracellular energy-deposited sites.

Material and Methods

2×10^3 Chinese hamster lung V79 cells were plated on specially designed Mylar-based dishes. We irradiated 5 cells located in the center of the scanned area (6 mm × 6 mm) of the dish in two ways: (1) by aiming at nuclei with 10 μm × 10 μm X-ray beams, and (2), by aiming at whole-cells with 50 μm × 50 μm X-ray beams. Surviving fractions of bystander cells in the scanned area were measured by using a single-cell clonogenic assay [3]. In some cases, to clarify the contribution of nitric oxide (NO) in bystander signalling, cells were incubated with medium containing carboxy-PTIO, a specific scavenger of NO, instead of normal fresh medium.

Results and Discussion

As shown in Fig. 1, in the nucleus-irradiated case, when nuclei were irradiated with a nuclear-averaged dose of approximately 1 Gy, the surviving fraction of bystander cells decreased to 90%, but at higher doses, the surviving fraction was stable at 96%. In contrast, in the whole-cell-irradiated case, the surviving fraction of bystander cells decreased monotonically to 92% and remained stable with higher doses of radiation. Dose-dependent parabolic enhancement in bystander cell death was observed only with low doses in the nucleus-irradiated case [4]. Moreover, addition of carboxy-PTIO suppresses bystander cell death under both irradiation conditions [4]. These results suggest that NO acts as a mediator in the induction of 2 types of bystander cell death, namely, the parabolic type and the monotonic type. Our findings clearly demonstrate that the induction of bystander cell death depend on the sites of energy deposition in irradiated cells.

In cases where both irradiated [3] and bystander cells [4] die after low doses of radiation, cell death is enhanced in the absence of energy deposition to the cytoplasm. Energy deposition in the cytoplasm may play an important role in cell protection at low doses of radiation. We propose that radiation-sensing mechanisms exist in the cytoplasm that may induce some kind of intracellular signaling event that controls a cell's protection mechanism, such as the generation of repair-related factors, and that the collaboration of intercellular signaling mechanisms may induce bystander responses.

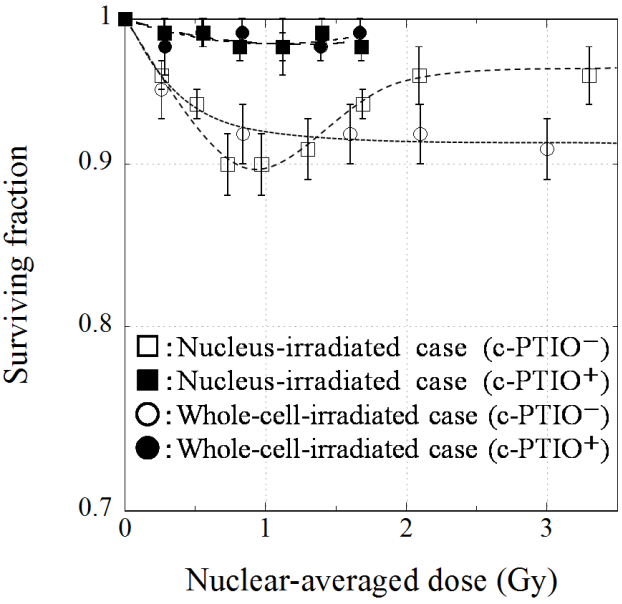

Fig. 1 The surviving fraction of bystander V79 cells surrounding the V79 cells irradiated with different sizes of 5.35 keV X-ray microbeams [4].

References

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