Microbeam Studies of Radiation-Induced Bystander Cell Killing Using Synchrotron Radiation

Radiation-induced bystander response is defined as a response in cells which have not been directly targeted by radiation, but which are in the neighborhood of cells which have been directly exposed. Bystander cell killing effects in normal human diploid fibroblasts were assessed by irradiating an exact number of cell nuclei in confluent cultures with synchrotron X-ray microbeam. It was found that the bystander cell killing showed a parabolic relationship to the irradiating dose. In addition, nitric oxide (NO) was shown to be a strong candidate for the initiator/mediator of the bystander response which was induced by X-ray microbeam irradiation.

Initial damage produced in cellular DNA in response to radiation has been thought to induce biological effects such as cell death, mutation and transformation. Recently, however, non-DNA-targeted effects, which are not a direct consequence of the initial lesions produced in cellular DNA, have been found and are defining a new paradigm [1]. Bystander response is the most characteristic of non-targeted effects and is generally defined as cellular responses which have not been directly induced by radiation, but are induced in the neighborhood of cells which have been directly irradiated [1]. Thus the bystander response may have important biological consequences under low dose irradiation conditions where non-targeted or non-irradiated cells are affected in the irradiated population. However, to date, there have been no reports on well described bystander responses observed after exposure to X-rays or γ-rays.

In the present study, bystander cell killing effects in normal human diploid fibroblast WI-38 cells were assessed by irradiating an exact number of cell nuclei in confluent cultures with a 5.35 keV monochromatic synchrotron X-ray microbeam delivered in a 5 μm x 5 μm square [2]. About 7 x 10^5 cells formed a confluent culture on the microbeam irradiation dishes. First we determined the surviving fraction of bystander cells as a function of the number of irradiated cells in the culture. Targeted cells were irradiated with 0.93 Gy from a microbeam. All of the cells on the culture dishes, including the targeted cells, were harvested 24 h after irradiation, and the surviving fraction was determined with a colony formation assay. The surviving fractions were found to decrease significantly if 5 or more cell nuclei were irradiated. Next, five cell nuclei in the center of a dish were irradiated with different doses of microbeam, and the dose responses of surviving

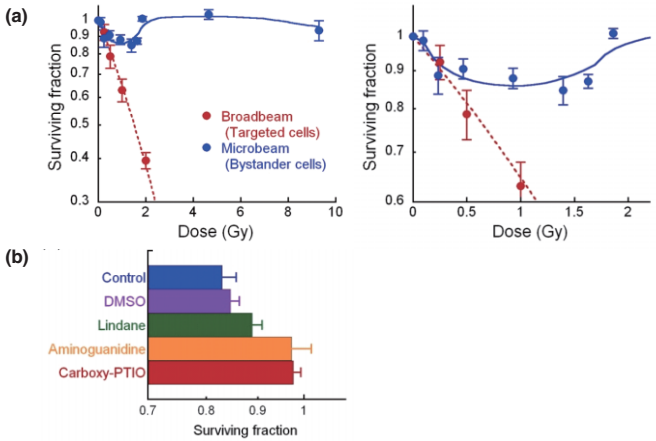

Figure 1
Bystander cell killing effects. (a) In the microbeam study, 5 cells in the center of a dish were irradiated with microbeam. Broadbeam irradiation was performed using conventional X-rays. The left panel shows the overall surviving fraction for cells exposed to doses up to 10 Gy, while the right panel is its magnified view under 1.9 Gy. (b) Effect of inhibitors or scavengers on cell survival. DMSO (0.1%), lindane (50 μM), aminoguanidine (20 μM) or carboxy-PTIO (20 μM) were added to the culture medium 2 h before irradiation. Five cells in the center of a dish were irradiated with 0.93 Gy of microbeam. Error bars represent the standard errors of the mean (SEM) which were obtained from three to five independent experiments.

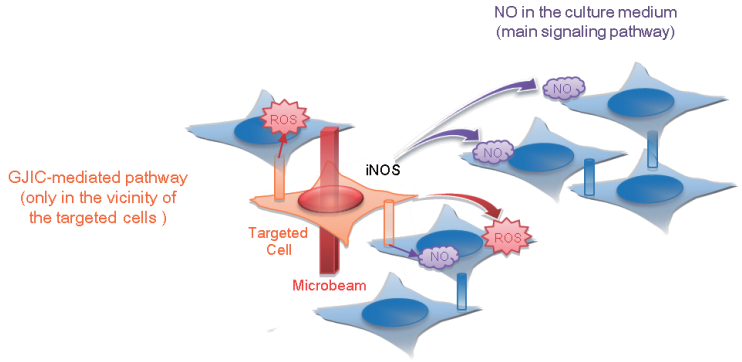

Figure 2
Schematic model of the bystander cell signaling pathway.

fractions were determined [Fig. 1(a)]. The surviving fraction decreased at doses above 0.09 Gy and fell to 0.85 at 1.4 Gy. However, at 1.9 and 4.9 Gy, the measured surviving fractions recovered approximately to the control. In the case of confluent cultures irradiated with conventional broadbeam X-rays, the dose resulting in a 37% cell survival rate, at which one lethal lesion per cell is induced on average, was about 2.0 Gy [Fig. 1(b)]. The observed parabolic relationship between the irradiation dose and survival suggests that the induction of bystander cell killing may require physiological activity in the targeted cells. In other words, the bystander signals may have been released from the living cells which did not have the lethal lesions.

Concerning the mechanisms underlying these bystander responses, at least two signaling pathways are known to be functional: one is through a direct physical connection between cells such as gap-junction intercellular communication (GJIC), and the other is through the culture medium (Fig. 2) [1]. To determine which cell signaling pathways might be involved in the bystander response, the effects of scavengers of reactive oxygen species (ROS) and nitric oxide (NO), and inhibitors of GJIS and inducible NO synthase (iNOS) were examined [Fig. 1(b)]. Cells were pretreated with the drugs, and 5 cell nuclei were irradiated with 0.93 Gy of microbeam radiation. Dimethyl sulfoxide (DMSO), which is a scavenger of ROS, did not prevent bystander cell killing. Lindane, which is an inhibitor of GJIC, partially suppressed bystander cell killing, suggesting that the working distance of GJIC is restricted in the vicinity of the targeted cells. In contrast, Aminoguanidine, which is an inhibitor of iNOS, and carboxy-PTIO, which is a scavenger of NO, effectively suppressed bystander cell killing. In addition, we recently reported that the bystander cell killing effect in Chinese hamster V79 cells irradiated with synchrotron X-ray microbeams was also effectively inhibited by carboxy-PTIO [3]. These results suggest that bystander response induced by X-rays is mainly mediated through the culture medium rather than GJIC, and NO is a prime candidate for the initiator/mediator of the bystander response.

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