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Time-lapse study on cell cycle modifications of irradiated and non-irradiated bystander HeLa-Fucci cells with X-ray microbeam

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1 Introduction

It has been known that living cells exposed to ionizing radiation postpone their cell division (mitosis) to obtain time to repair DNA damage induced by irradiation in a cell nucleus. There are several checkpoint mechanisms in mammalian cells to control the cell cycle, and irradiated cells shows cell cycle arrest in a specific cell cycle stage. Recently a fluorescent ubiquitination-based cell cycle indicator (Fucci) technique has been practically utilized to visualize cell cycle as live cell images [1]. The cell cycle of the Fucci-cells can be easily identified with their fluorescent colors. Using this advantage, we can select cells in a specific cell stage, G1 or S/G2, as targets of X-ray microbeam exposure. We reported that HeLa-Fucci cells showing red or green color for G1 or S/G2 stage, showed cell cycle arrest in G2/M stage irrespective of their cell cycle when irradiated [2]. The G2/M arrest seemed to continue for over 24 hours.

On the other hand, we noticed some technical difficulties in a long microscopic observation such as condensation of culture dish surface and defocusing originated from a membrane used as the dish-bottom materials. The time-lapse images should be taken manually every a few minutes or hours for a couple of days, so that the observers took a great deal of strain. Further, we sometimes lost the targeted cells under microscopic field by cellular motility during interval of taking images.

In order to overcome these issues, we developed new instruments to hook up an X-ray microbeam irradiator of BL27B. Using this new system we observed cell cycle dynamics of microbeam irradiated and non-irradiated bystander cells in a micro-colony of HeLa-Fucci cells.

2 Experiment

In order to observe the cells exposed to X-ray microbeam, we used an off-line microscope (KEYENCE, BZ-9000). This microscope equips with a CO_2 incubator on its stage and enables us to fully automatically take cell images at every few minutes or hours for a couple of days. We also prepared a polyethylene film with a micro-pattern as shown in Fig. 1. This pattern indicated a certain type of positional coordinates to calibrate the origins for both the X-ray microbeam irradiator and the off-line microscope. Before irradiation experiments, we observed this film by the two microscopy systems, and determined the origins for them based on the pattern in the viewing fields.

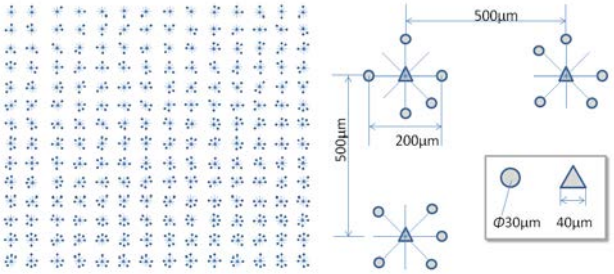

Fig. 1: The Micro-pattern to calibrate the origins for both the X-ray microbeam irradiator and the off-line microscope.

HeLa-Fucci cells obtained from RIKEN (BRC2812) were cultured on a commercial 35 mm ϕ dish a couple of days before irradiation. X-ray microbeam (5.32 keV) obtained at BL-27B was exposed to cells from bottom of the culture dish. Details of the irradiation setup were described elsewhere [3]. The transmittance of the X-rays through the dish bottom was 20 %.

One of cells in a colony containing more than a dozen cells was exposed to the microbeam with an absorbed dose calculated to be 5 Gy. After exposure, the dish was removed from the stage of the irradiator and set in the incubator of the off-line microscope. Time-lapse observation was performed using the software to control the off-line microscope. The images were automatically taken every 20 minutes for 48 hours by switching the filter systems for the UV-light to observe both red and green fluorescence of the Fucci-cell nuclei.

3 Results and Discussion

We successfully obtained time-lapse images of the irradiated cell samples. Figure 2 shows a typical set of the image data of the colony in which one cell were exposed to X-ray microbeam. At the irradiation, the cell nucleus color was red, indicating that the cell cycle was G1 stage when irradiated. After irradiation the cell cycle progressed to S/G2 stage and underwent cell division during 24 to 36 hours after irradiation. However the daughter cells seemed to be often killed by presumably apoptosis or catastrophic cell death mechanism until 48 hours after irradiation. Further some bystander cells also underwent apoptosis as shown in Fig. 2 because presumably of bystander effect caused by signal transduction from the irradiated cell to non-irradiated bystander cells. Further data are undergoing analysis.

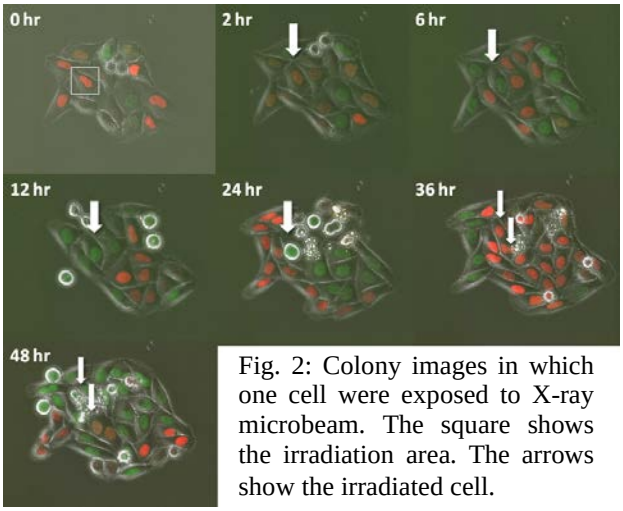

Fig. 2: Colony images in which one cell were exposed to X-ray microbeam. The square shows the irradiation area. The arrows show the irradiated cell.

In conclusion we have established a time-lapse observation system for living cells exposed to X-ray microbeam. This system will be enormously valuable to study dynamics of functional changes in cells by irradiation.

References

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