A New Approach to Investigate Cell-Cycle Modification Using Synchrotron X-Ray Microbeam

Fluorescent ubiquitination-based cell-cycle indicator (FUCCI) HeLa cells were exposed to a synchrotron X-ray microbeam. The cells were irradiated at the G1 (red color) and S/G2 (green color) phases selectively in the same microscopic field. Live-cell images of the cells were obtained 24 h after irradiation. The G1 irradiated cells indicated the progress of the cell cycle, while the S/G2 irradiated cells arrested the cell-cycle. These results show that the new method combining FUCCI cells, X-ray microbeam and live-cell imaging is a useful technique to elucidate selectively the effects of irradiation on the cell cycle.

It is well known that the cell cycles of eukaryotic cells delay or arrest after exposure to ionizing radiation because the irradiated cells need time to repair DNA damage, such as base lesions and strand breaks. The delay or arrest of the cell cycle is controlled by cell-cycle checkpoints, which are one of the mechanisms that determine the fate of the cell. To investigate the cell-cycle arrest, flow cytometry methods have been typically applied using the cells detached from a culture dish. The results of such methods can be used for statistical trend analyses of cell populations, but the data are not enough to track in detail the fate of irradiated single cells.

Live-cell imaging techniques enable us to observe the fate of cells in real time. This technique makes it possible to track a single cell under microscopic observation. Recently, the fluorescent ubiquitination-based cell-cycle indicator (FUCCI) has been developed to visualize the cell cycle as live-cell images (Fig. 1a, b) [1]. The functional proteins in the nucleus existing in a specific phase fuse with fluorescence proteins. The color of the nucleus of FUCCI cells changes with each phase of the cell cycle because of the differences in the expressing functional proteins. Thus, the cell cycle can be distinguished easily by observing the color of the nucleus.

To investigate the cell-cycle modification caused by ionizing radiation using FUCCI cells, this study used an X-ray microbeam, which is a powerful tool to sharpshoot a single cell on a culture dish. The method that combines the FUCCI technique and the X-ray microbeam can selectively irradiate the cell-cycle phase on a dish without chemical treatment of cell-cycle synchronization.

In this study, we introduced a new experimental system for observing the effects of radiation on eukaryotic cells depending on the cell cycle (Fig. 1c). The system consists of three constituent parts, FUCCI cells, X-ray microbeam and live-cell imaging technique. FUCCI HeLa cells were irradiated with a monochromatic X-ray microbeam having a fixed energy of 5.35 keV. The absorbed dose corresponds to 5 Gy. The cells in G1 or S/G2 phase were selectively irradiated, then live-cell images were obtained for 24 h after irradiation. Histone H2AX phosphorylation (γ-H2AX) was also detected by the fluorescence immunostaining method to confirm the DNA damage caused by X-ray microbeam irradiation.

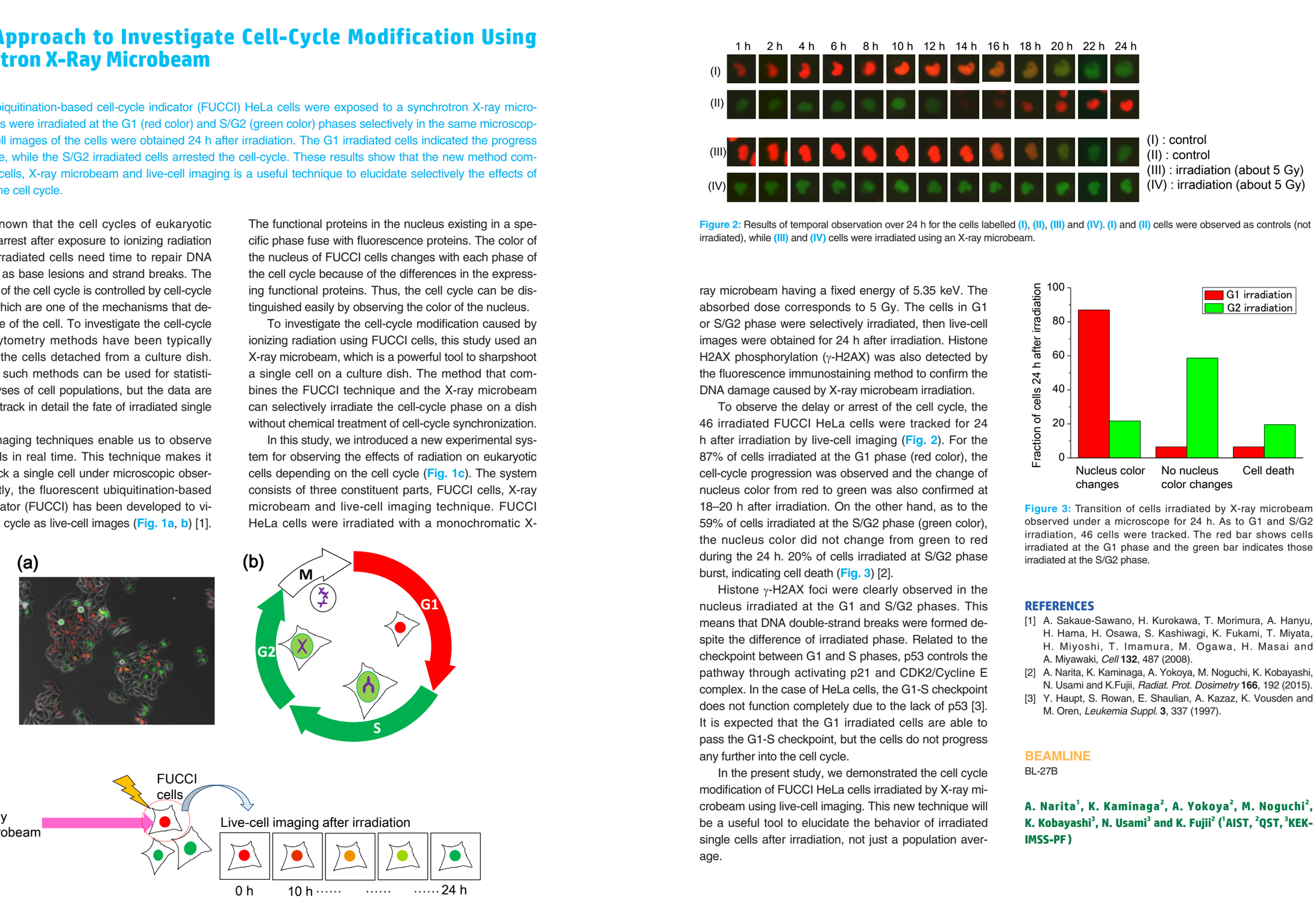

Figure 1: (a) Image of FUCCI HeLa cells, **(b)** schematic diagram of cell cycle of FUCCI and **(c)** concept of new experimental system using FUCCI cells, X-ray microbeam and live-cell imaging.

Figure 2: Results of temporal observation over 24 h for the cells labelled **(I)**, **(II)**, **(III)** and **(IV)**. **(I)** and **(II)** cells were observed as controls (not irradiated), while **(III)** and **(IV)** cells were irradiated using an X-ray microbeam.

To observe the delay or arrest of the cell cycle, the 46 irradiated FUCCI HeLa cells were tracked for 24 h after irradiation by live-cell imaging (Fig. 2). For the 87% of cells irradiated at the G1 phase (red color), the cell-cycle progression was observed and the change of nucleus color from red to green was also confirmed at 18–20 h after irradiation. On the other hand, as to the 59% of cells irradiated at the S/G2 phase (green color), the nucleus color did not change from green to red during the 24 h. 20% of cells irradiated at S/G2 phase burst, indicating cell death (Fig. 3) [2].

Histone γ-H2AX foci were clearly observed in the nucleus irradiated at the G1 and S/G2 phases. This means that DNA double-strand breaks were formed despite the difference of irradiated phase. Related to the checkpoint between G1 and S phases, p53 controls the pathway through activating p21 and CDK2/Cycline E complex. In the case of HeLa cells, the G1-S checkpoint does not function completely due to the lack of p53 [3]. It is expected that the G1 irradiated cells are able to pass the G1-S checkpoint, but the cells do not progress any further into the cell cycle.

In the present study, we demonstrated the cell cycle modification of FUCCI HeLa cells irradiated by X-ray microbeam using live-cell imaging. This new technique will be a useful tool to elucidate the behavior of irradiated single cells after irradiation, not just a population average.

Figure 3: Transition of cells irradiated by X-ray microbeam observed under a microscope for 24 h. As to G1 and S/G2 irradiation, 46 cells were tracked. The red bar shows cells irradiated at the G1 phase and the green bar indicates those irradiated at the S/G2 phase.

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