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Visualization of cell cycle modification by synchrotron X-ray-microbeam exposure in single HeLa cells using fluorescent ubiquitination-based cell cycle indicator

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1 Introduction

X-ray exposure to a living cell at a specific cell cycle stage has been expected as a key technique to elucidate the dynamics of biological responses to radiation damage. Recent progress in a life science has put a fluorescent ubiquitination-based cell cycle indicator (FUCCI) technique into practical use in visualizing cell cycle as live cell images [1]. In this study, we applied this new biological technique to expose single cells using synchrotron X-ray microbeam. After selectively exposure to human cancer (HeLa) FUCCI cells at G1 or S/G2 stages, we performed a time lapse observation for 24 hours.

2 Experiment

FUCCI-HeLa cells express monomeric Kusabira-Orange 2 (mKO2) and Azami-Green 1 (mAG1) fused to the ubiquitination domains of the hCdt1 and mGem proteins, respectively. The mKO2-hCdt1 labels the nuclei of G1 phase cells red and mAG1-mGem labels the nuclei of S/G2 phase cells green. The cells were cultured on specially designed Mylar-based dishes with grown in the medium at 37 °C in a 5% CO_2 humidified atmosphere during 24 hours. Irradiation to the cells were performed microbeam apparatus at BL27B. The X-ray energy was 5.32 keV, and the beam size was fixed to 20 μm × 20 μm square. The exposure was about 500 C/kg, roughly corresponds to an absorbed dose calculated to be 5 Gy. After irradiation, the dish was immediately removed from the beam line, and set on the stage of off-line microscope equipped with a chamber to keep the dish at 5% CO_2, 37 °C during the time lapse observation for 24 hours. We obtained cell images by the CCD camera every 2 hours.

3 Results and Discussion

Figure 1(a) shows a typical image of FUCCI-HeLa cells before irradiation. For irradiation, we selected two cells indicated as C and D for a G1 and S/G2 stage cell, respectively. Two cells indicated by A and B were non irradiated cells as controls. Figure 1(b) shows time-lapse fluorescent images of the irradiated and non-irradiated cells for 24 hours. The nucleus of the non-irradiated cells gradually changed. When the G1 cell (C in Figure 1) was irradiated, the cell showed a progression of the cell cycle, and the nucleus color changed from red to green at roughly 18-20 h after irradiation (Figure 1(b)). There was no noticeable difference between the G1-irradiated and the control cell (A). On the other hand, the nucleus color of the D cell irradiated at the S/G2 phase kept green color during the observation.

Quantitative analysis of the fluorescence intensities of the cells revealed that the two colors of the control cell became reversed every 14-18 hours. The cells irradiated at the G1 phase also showed a reverse at 20 hours after irradiation. On the other hand, the green color for cells irradiated at the S/G2 phase showed a much higher intensity than the red color. The cells did not show any increases of red. Although the intensity of green reached a minimum around 20 hours after irradiation, no color reversal was observed.

In summary, we demonstrated that cell cycle modulation of a single cell can be tracked by the live cell imaging of FUCCI cells. The time lapse images show that the cell cycle of HeLa cell exposed to X-rays is arrested at the G2 phase before mitosis. However, when the cells are irradiated at the G1 phase, the cell cycle progresses and the cells might replicate their genomic DNA which may contain radiation damage.

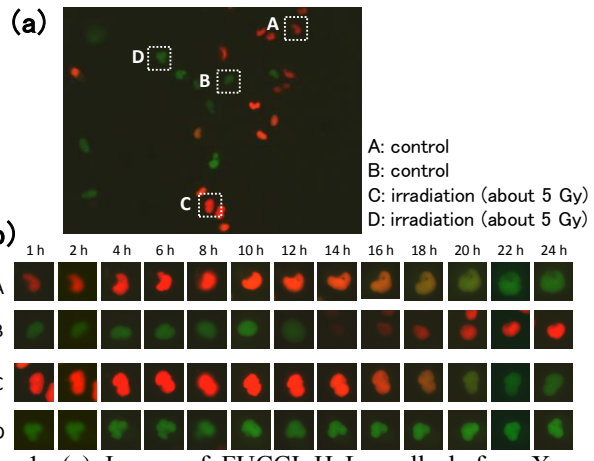

Fig. 1: (a) Image of FUCCI HeLa cells before X-ray microbeam irradiation and (b) results of temporal observation over 24 h for cells of A, B, C and D. Cells of A and B were controls, while cells of C and D were irradiated by X-ray microbeam.

References

[1] A. S. Sawano, *et al,*. Cell, **132,** 487 (2008).

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