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Irradiation of Fucci-expressing HeLa cells using a tapered glass capillary microbeam

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ABSTRACT

Fluorescent, ubiquitination-based cell cycle indicator-expressing HeLa cells have been irradiated with a microbeam set-up at RIKEN for examining the effect to cell cycle after bombarding with protons of 1 MeV incident energy. Here, single cells were exposed in their nucleus with different doses (100, 1000, 10,000 and 100,000 protons). Subsequently, the cells were monitored using time-lapse microscopy after irradiation. In this paper, we present the preliminary results for such irradiation for 8 cells. The irradiated cells with up to 10,000 protons showed the change in fluorescence from red to green. This indicates that the transformation from G_1 to S/G_2 in cell cycle seemed not to be affected by the irradiation up to 10,000 protons. However, the irradiated cell with 100,000 protons died in red fluorescence at ~4 h after the irradiation.

1. Introduction

Ion microbeams are an attractive technology to the field of radiobiology because of its capability to selectively expose a single cell or even sub-cellular organelles with defined numbers of bombarded ions or doses [1–6]. Commonly, magnetic and electrostatic systems are used for focusing microbeams. However, a simplified and compact technique has also been demonstrated to focus the ion beams into a micrometer scale using a tapered glass capillary [7]. This method was then extended for cell irradiation in liquid solution, by adding a few micrometer thin window at the end of the tip [8]. After the successful test above, we built a new beamline for dedicating this methodology at RIKEN, where three bending magnets scheme was adopted to deliver ions from above at an angle of 45 degrees into a capillary holder, placed above the target stage [6]. With this set-up, cells can be kept in a normal cell culture dish during the irradiation process, without requiring special cell targeted holders as used in other microbeam systems [9–14].

Recently, fluorescent, ubiquitination-based cell cycle indicator (Fucci)-expressing HeLa cells were developed by Sakaue-Sawano et al. [15] for labeling their nuclei with two different fluorescent colors depending on the phases of the cell cycle. In this cell line, the mKO2 protein was fused in Cdt1, which expresses red fluorescence in G_1 phase. Additionally, the mAG protein was fused in Geminin, which expresses green fluorescence in S, G_2 and M phases. By this, the effect to cell cycle can directly be observed through the fluorescent color changes. This enables real time, continuous and non-destructive measurements on living cells in contrast to flow cytometric technique [16].

In this work, the RIKEN microbeam was used to irradiate the Fucci-expressing HeLa cells for investigating the influence to cell cycle induced by energetic protons. This cell line was obtained from the RIKEN BioResource Center (BRC). Here, current results of this study are shown.

2. Experimental details

The cells were irradiated using the microbeam facility at RIKEN as previously described in detail elsewhere [6]. Shortly, a proton beam with an initial energy of 1 MeV was collimated to a diameter below 10 μm by using a tapered glass capillary. Fig. 1 shows an optical micrograph of one of the tapered capillaries used in this experiment. The typical inner diameter was ~6 μm, with an approximately 4 μm thick polystylene at the exit. Before irradiating the targeted cells, a silicon surface barrier detector (SSBD) was used to measure a proton count rate. The dose was set by the count rate and the exposure time for 1 s irradiation. In principle, the uncertainty of the number of irradiated protons can be determined by $\sqrt{N}$, where N is the number of protons. However,

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the fluctuation can be varied by factor of 2 due to the stability of the system.

Fig. 2 illustrates the Fucci-expressing HeLa cells, which show different colors corresponding to different phases. In early G_1 phase, no fluorescence is emitted from the nucleus about 1–2 h as seen in a pair of daughter cells (cell Nos. 1 and 2) after a Fucci-expressing HeLa cell division. When the cell is in G_1 phase, the nucleus expresses red color (see cell No. 3). During the transition from G_1 to S phase, the ratio of the red protein with respect to the green protein reduces. This causes a color change of the nucleus, first to orange, and then to yellow, as can be seen in cells Nos. 4 and 5, respectively. Subsequently, the red emission disappears and only the green fluorescence is visible in S, G_2 and M phases. As seen in cell No. 6, it is in S/G_2 phase. Additionally, the cell morphology changes to a round shape when it is in M phase, see cell No. 7. More detail of the Fucci-expressing HeLa cells can be found elsewhere [15].

The Fucci-expressing HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) with supplementing by 10% FBS (fetal bovine serum), at 37 °C in a 5% CO_2 humidified environment. Normally, cells were prepared by seeding in 35 mm glass-bottom cell dishes, about 2 days before irradiation. One day before irradiation, the cells were transferred and kept in an incubator (9100E, Wakenyaku) next to the beamline. During the irradiation, the cell dish was kept in a top stage incubator (TOKAI Hit), for keeping the same environment as described above, mounted on the top of an inverted microscope (IX71, Olympus) using with a 60× objective lens, at the end of beamline. The lid of the top stage incubator has a hole (~2.5 cm × ~3 cm) for inserting the capillary into the incubator. This microscope is configured for phase contrast and equipped with filter systems for the mAG amd mKO2 fluorescent proteins.

We bombarded a single cell in a colony with different number of protons (100, 1000, 10,000 and 100,000). Cells were irradiated when they expressed in red or orange color. After finishing the irradiation, the cells were then placed back to the incubator. Finally, they were monitored using an incubator fluorescence microscope (LCV110, Olympus) for time lapse imaging with a 20× objective lens. The positions of the irradiated cells were labeled by a microgrid from the glass cell dish. In the same cell dish, colonies without any irradiated cells were monitored as a control. Differential interference contrast (DIC) [17], as well as fluorescences in red and green, were sequentially acquired by using a control program (MetaMorph). The images were taken every 10 min, except for 5 min in the case of irradiation with 100,000 protons. It usually takes a few hours before starting to acquire the first image, primarily because several cells in the same dish but from different

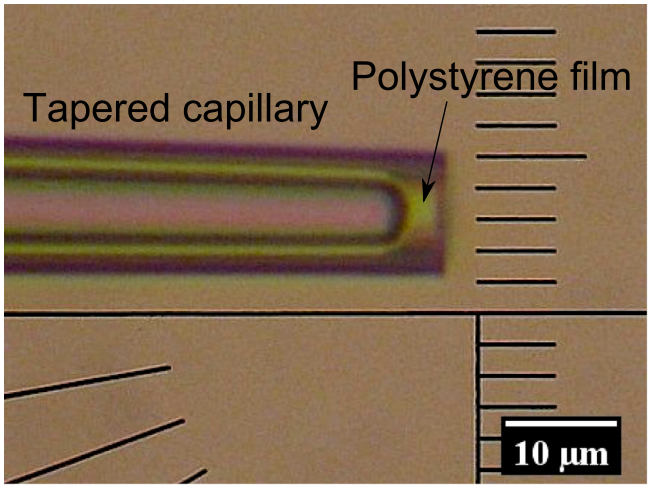

Fig. 1. Optical micrograph taken by a microforge (MF-900, NARISHIGE) of one of the tapered glass capillaries that were used for this experiment. A polystyrene film of an average thickness of ~4 µm was used to cover the tip of the capillary, with a typical inner diameter of ~6 µm. The distance of the scale bars is 2.8 µm/div.

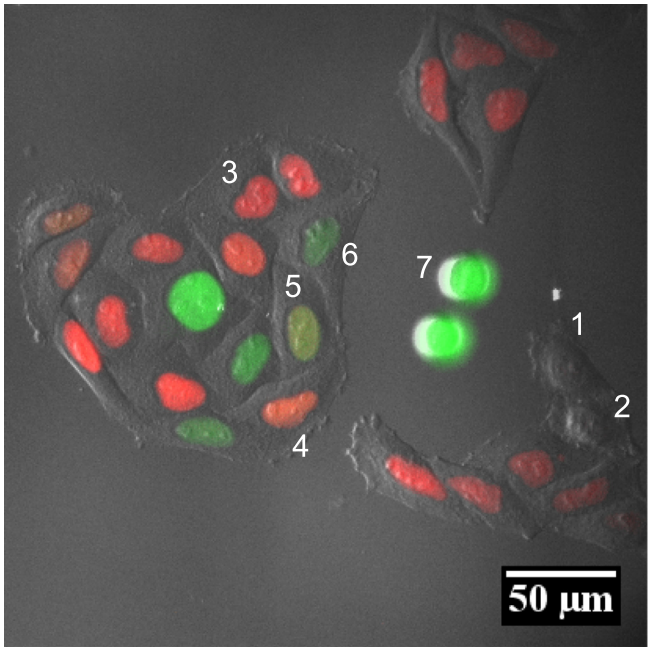

Fig. 2. Example of Fucci-expressing HeLa cells showing different fluorescent colors in the nuclei when the cells are in early G_1 phase, G_1 phase, the transition from G_1 to S phase, S/G_2 phase, as well as M phase, as marked with the Nos. 1 and 2, 3, 4 and 5, 6, as well as 7, respectively. The image was obtained by merging the DIC, as well as both red and green fluorescent channels, sequentially taken by the LCV110 microscope. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

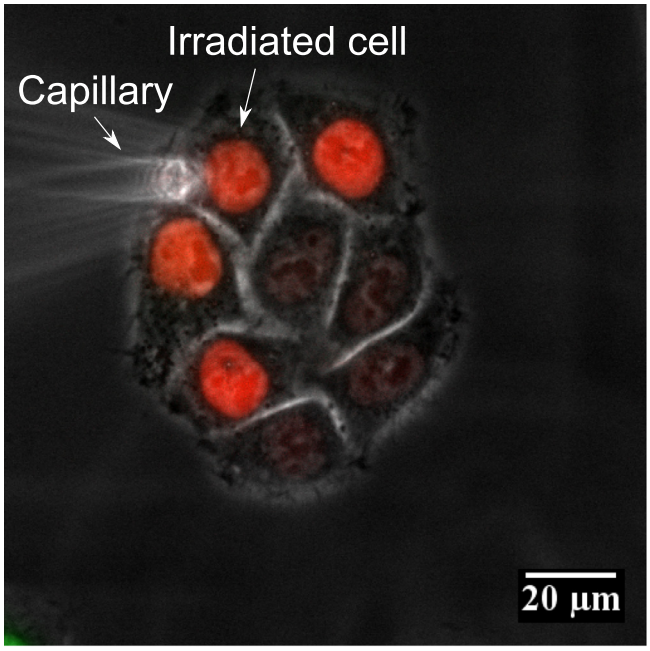

Fig. 3. The capillary (top left) placed ~14 µm above the targeted cell, before irradiation. The image was obtained by merging a phase contrast and red as well as green fluorescent images, taken by the IX71 microscope using 60× objective lens. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)