Ion track observation in cell nucleus irradiated by 3 MeV He ion microbeams produced with glass capillaries

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Microbeam irradiation of cultured single cells using tapered glass capillaries with outlet diameters of the order of 1 μm has been performed employing RIKEN Pelletron accelerator. Apparatus with a 3-μm outlet diameter is reported in elsewhere[1]. Microbeam is a unique scheme [2] that can actively select an irradiation volume of ~μm^3. When a microbeam of He ions of a few MeV hits a target, e.g., a biological cell, the ions will stop at the depth corresponding to their range (10~20 μm). Any other parts downstream of the stopping volume will not be damaged. This is one of advantages of employing beams of a few MeV. The glass capillaries with thin end-windows play an important role in delivering the ions directly to the cells in solution.

At RIKEN, we have developed a unique technology for mutation breeding using high-energy heavy-ion beams from RIKEN Ring Cyclotron. Such high linear energy transfer (LET) ions produce clustered DNA damage that cannot be repaired by the cell itself, leading to cell death, or can only be repaired incompletely by the cell, which induces mutations. In order to investigate both the lethality and the effectiveness of mutation induction, the position selectivity of the microbeam will be needed because hitting of different parts may cause unwanted effect. Here we demonstrate the DNA damages along ion tracks in a cell nucleus using a microbeam with relatively high LET.

A 3-MeV He ion microbeam was used for the irradiation of the nucleus of human cells (HeLa cells) because it is known that there is a similarity in the radiation response between plant and animal cells. The ions were generated by the Pelletron and transported to the cell irradiation port[1]. A 5-cm-long glass capillary optics with a 4-μm-thick end-window whose diameter was 3 μm and with an inlet diameter of 0.8 mm was installed at the beam port with an angle of 45° with respect to the horizontal plane so that widely-used petri dish filled with liquid solution can be used. The LET of a 3-MeV He ion is 150 keV/μm in water. The range of the ions is only 12 μm in water after the end-window. In order to adjust the dose of the irradiation to each cell, a beam chopper consisting of an electrostatic beam deflector was employed so that a few to ten ions were included in a short pulse of 0.8 μs. The pulse was repeated 100-1000 times according to the number of the required ions to the target.

It took approximately 12 min to irradiate about 40 cells in one dish, and totally 168 cells in 4 dishes were irradiated. After the irradiation, the Double Strand Breaks (DSBs) at DNAs were fluorescent-labeled as follows. The time for the repair process based on an enzymatic reaction was 20 min after the irradiation, and then the cells were washed three times with ice-cold phosphate-buffered saline (PBS) and fixed with 4% formaldehyde in PBS at 4°C for 20 min. Then, the cells were permeabilized with 0.5% Nonidet P-40 in PBS at 4°C for 5 min, and phosphorylated histone H2AX was detected by using rabbit antibody (Millipore) and an Alexa488-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories).

Figures 1(a) and (b) are the cross-sectional and bottom views of the irradiated cells, respectively, reconstructed from the photos taken by changing the focus position along the z-axis (vertical axis). Cell nuclei are identified as bean-shaped blue regions with a width of ~15 μm and have fluorescence from a stain DAPI binding to A-T rich regions in DNA. The outlines of the cells are not seen. The DSBs are detected as bright points with green color from Alexa488. Even without irradiation, DSBs can take place as an activity of a living cell. However, the concentration of DSB bright points along a line is an evidence of artificial lesion. The ion track with the angle of 45° is clearly seen as a fluorescent line in Fig. 1(a). We succeeded in observation of a visible track for MeV ions inside a cell nucleus. It is confirmed that MeV-ion irradiation made DSB lesions in the DNA, which may cause gene defects. Further experiments considering other conditions, e.g., LET, control of cell cycle, and so on are needed as well as the number of ions necessary to form a track.

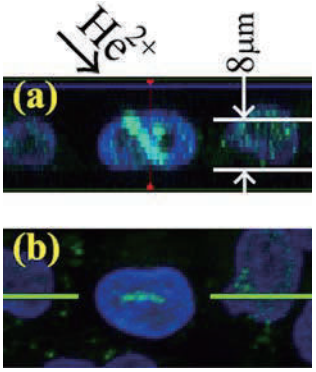

Fig. 1. Fluorescent lines corresponding to the ion track in a human cell nucleus. (a) Cross-sectional view of the irradiated cells at the horizontal line in the bottom view. (b) Bottom view of the cell.

References

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2) Y. Iwai et al.: Appl. Phys. Lett. **92**, 023509 (2008).

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