Observation of unrepairable lesions in DNA by using 3-MeV proton microbeams produced by glass capillaries

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Microbeams allow extremely efficient alteration of or damage to a small region in the target with minimum beam intensity. To date, X-ray, UV, and visible light laser have been used as microbeams. However, to create double strand breaks (DSBs) in DNA, which are difficult to repair, multiple photons are required and have to be focused on the DNA. Because of the finite size of the focal spot, the beam intensity needs to be high. This intense beam creates multiple DSBs. In contrast, when ion beams are used, a single ion can create a DSB via ballistic electrons and radicals generated along the ion's trajectory. This is an advantage of ion-beam irradiation. We developed a method involving ion-microbeam irradiation to single cells in culture using tapered glass capillaries with outlet diameters of ~1 μm.[1,2] The capillary is known as glass pipet for microinjection or a glass electrode for real time measurements of the voltage potential within the neurons. The number of ions irradiated to single cells is controlled by a pulsed beam. However, some DSBs can be repaired quickly even if ions hit the DNA. Here, we measured the fluorescence brightness corresponding to unrepaired DSBs after irradiation for different numbers of ions input per cell to confirm that a small number of protons can cause unrepairable damage.

The beam used for irradiation was composed of 3-MeV protons that were generated by a RIKEN Pelletron accelerator and transported to the cell irradiation port, which uses an inverted microscope (OLYMPUS IX-71). A glass capillary optics system with a thin plastic end-window (diameter, 2 μm) was mounted at the beam port at 45° so that a petri dish filled with solution can be used. The capillary tip can go close to the cells which is advantageous for suppressing multiple scattering of ions before they reach the target. To adjust the number of protons, which is proportional to the dose, the time window of the beam pulse, which included a maximum of 10 ions, was set at 1 - 5 μs, wherein the number of ions in a pulse follows Poisson distribution. We irradiated 50-1,000 ions per cell to reduce statistical error.

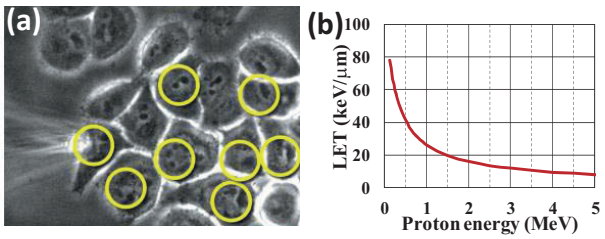

Fig. 1. (a) HeLa cells (yellow circles) and the tip of the glass capillary, (b) LET in water vs. proton energy.

Figure 1(a) is a microscopic view of HeLa cells during irradiation. Cell nuclei marked by yellow circles were selected. The capillary tip, which was above the cells, is at the lower left in Fig. 1(a). It took ~20 min to irradiate 60 cells in one dish. After the irradiation, cells grown in glass-bottom dishes 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).

We obtained clear fluorescence distributions only in the irradiated nuclei. The brightness of the foci (determined in terms of Alexa488 fluorescence) in each irradiated nucleus was proportional to the number of *unrepaired* lesions in DNA at the time cell fixation was initiated. The brightness data were displayed in a histogram by using the software, *Image-J*.[3] After subtracting the low background level of foci seen for the non-irradiated cells, histogram integration was compared according to the microbeam intensities [Table 1]. The brightness increased with but was not proportional to microbeam intensity, perhaps because of saturated damage due to the large number of ions.

The linear energy transfer (LET) of 3-MeV proton was ~10 keV/μm [Fig. 1(b)]. When heavier ion pieces are selected, higher LETs will be obtained. Moreover, the ion stopping positions can be localized at a certain depth inside a cell because of their short ranges. In this case, extremely high energy deposition corresponding to the Bragg peak is available for destroying a single cell or a small region of tissue.

Table 1. Integrated brightness of the foci.

Microbeam intensity (ions/cell)	Brightness of Alexa488 (arbitrary unit)
50	733
200	875
1,000	940

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3) *Image-J* 1.47v, downloaded from http://rsbweb.nih.gov/ij/