Number of Fe Ion Traversals Through a Cell Nucleus for Mammalian Cell Inactivation Near the Bragg Peak

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**H2AX spots/DNA double strand break/Ion traversal/Cell inactivation/Fe ion.**

HeLa and CHO-K1 cells were irradiated with Fe ions (1.14 MeV/nucleon) near the Bragg peak to determine how many ion traversals through a cell nucleus are necessary to induce cell inactivation. The ion traversals through a cell nucleus were visualized by immunostaining the phosphorylated histone H2AX (γ-H2AX), as an indicator of DNA double strand breaks (DSBs), to confirm that DSBs are actually induced along every Fe ion traversal through the nucleus. The survival curves after irradiation with Fe ions decreased exponentially with the ion fluence without a shoulder. The inactivation cross sections calculated from the slope of the survival curves and the standard errors were 96.9 ± 1.8 and 57.9 ± 5.4 µm² for HeLa and CHO-K1 cells, respectively, corresponding to 0.442 and 0.456 of the mean value of each cell nucleus area. Taking the distribution of the cell nucleus area into consideration with an equation proposed by Goodhead et al. (1980), which calculates the average number of lesions per single ion track through the average area of a sensitive organelle (mainly nucleus), these two ratios were converted to 0.705 and 0.659 for HeLa and CHO-K1 cells, respectively. These ratios were less than one, suggesting that the average numbers of lethal hits per cell produced by a single ion traversal were less than one. We thus considered two possible explanations for ion traversals of more than one, necessary for cell inactivation.

**INTRODUCTION**

Survival curves of mammalian cells irradiated with few MeV α-particles usually show a single-hit kinetics, which appear as an exponential decrease along with an increase in the fluence or dose. According to a naïve hit theory the single-hit kinetics shows that a single α particle traversing a sensitive site (target) somewhere in a cell is sufficient for cell inactivation. Barendsen et al. reported that the inactivation cross section of human T-1 cells after irradiation with 5.2 MeV α-particles was not much smaller than the cross section of the cell nucleus,1 which is considered to be a main target.2 They also reported that the probability of cell inactivation per 3.4 MeV α-particle traversing the nucleus was 0.86.3 Similar results were reported: a single traversal of a 3.26 MeV α-particle inactivates pre-B cells4,5 and radiosensitive CHO-K1 cells (irs2).6

In contrast to the above reports, there are many reports concluding that multiple traversals of α-particles were needed for cell inactivation as follows: for C3H10T1/2 cells 10 to 20 of 5.6-MeVα-particles7 and 6 of 3.2-MeV α-particles,8 for synchronized V79 cells 11.6 and 4.3 of 4.6-MeV3He ions in late-S and G1/S cells, respectively,9 for T-1 cell 4-5 of 3.5-MeV α-particles,10 for five different cell lines (CHO-10B, HS-23, C3H10T1/2, V79 and AG1522B) 2-6 of 4.3-MeV α-particles,11 for radio-sensitive CHO-K1 cells (xrs5) 1.7 of 3.26-MeV α-particles, and for radio-sensitive irs1 and irs3 cells 2.2–3.2 of 3.26-MeV α-particles.6

Microbeam techniques are suitable for determining the exact number of α-particle traversals necessary for cell inactivation: 3.7 traversals for human-hamster hybrid (A1) cells,12 and 4.3 for C3H10T1/2 (estimated from the survival data by Miller et al.13) Using “Biostack” method (superimposing two microscopic images of cells and etch pits produced in a plastic track detector) the inactivation of an individual V79 cell was studied, and it was concluded that 2.1 of 4.3-MeV α-particles were required for inactivation.14 Several authors reported that multiple traversals of ions

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heavier than the α-particle were needed for cell inactivation. Deering et al. reported that the survival curves of HeLa cells showed single-hit kinetics after the irradiation of C and O ions of 8.7 and 8.2 MeV/nucleon, respectively, and the inactivation cross section was about 55 μm², which was about the half of the area of the cell nucleus. Wulf et al. measured inactivation cross sections for four strains of rodent cells using 14 ion species from He to U, mainly below 20 MeV/nucleon, and reported that the extrapolation of the inactivation cross sections of the V79 cells to the zero energy, representing the area of the sensitive target in the over kill approach, occurred at an inactivation cross section of about 40 μm², which was only half of the cell nucleus area.

Recently, Tsuruoka et al. reported on the LET dependence, up to 400 keV/μm for Fe, of the inactivation cross sections of normal human skin fibroblast cells (NB1RGC) using energetic C, Ne, Si, and Fe ions (130–500 MeV/nucleon) and Lucite absorbers at the Heavy Ion Medical Accelerator in Chiba (HIMAC). The maximum cross section was 122.6 μm², which approached approximately 70% of the geometrical cross section of the cell nucleus (172 μm²). It is interesting to investigate whether the inactivation cross section increases to the geometrical cross section of the cell nucleus at higher LETs. However, the combination of energetic heavy ions, such as 500-MeV/nucleon Fe, and the absorbers has a serious drawback in that the number of lighter secondary ions, such as H and He ions, increases after passing through the absorber. As a result, the inactivation cross sections could not be determined directly from the fluence giving 37% survival, but were calculated indirectly using an equation, cross section (μm²) = 0.16 × LET (keV/μm)/D₂₁(Gy), where the LET and the dose were the average values for a mixed beam containing the original ions and lighter secondary ions. Therefore, the cross sections obtained as above must be regarded as being superficial.

Thus, a correct measurement for the cross section near the Bragg peak requires irradiation systems of heavy ions without lighter secondary ions near the Bragg peak for biological studies. Since such irradiation systems of ions are still rare, we have developed a new irradiation system (described in reference) of low energy ions (1 ≤ Z ≤ 54) near the Bragg peak at energies of up to 6 MeV/nucleon at the Medium Energy Beam (MEXP) course at HIMAC, the National Institute of Radiological Sciences (NIRS).

Around the Bragg peak, LET shows the maximum value and energies of the secondary electrons are relatively low, indicating that radiation energy concentrates most densely around the track center. Thus biological effects around the Bragg peak are interesting, since we expect most ion-specific effects. In this work, HeLa and CHO-K1 cells, with different cell nucleus sizes, were irradiated with Fe ions of 1.14 MeV/nucleon using the new irradiation system, to clarify whether a pure single Fe ion is enough for cell inactivation. Since for the broad beam irradiation used in this work the number of ions passing through a cell nucleus inevitably varied according to the Poisson distribution, this distribution was taken into account for data analysis using an equation proposed by Goodhead et al., the distribution of cell nucleus areas was also taken into account. We discussed two possible explanations for ion traversals, more than one being necessary for cell inactivation. We showed, furthermore, that a single Fe ion traversal through a cell nucleus induced a cluster of DNA double strand breaks (DSBs) along the ion track using immunostaining of the phosphorilated histone, H2AX (γ-H2AX).

**MATERIALS AND METHODS**

**Cell Strains and Culture Media**

A human cervix carcinoma cell line, HeLa, and a Chinese hamster ovary cell line, CHO-K1 purchased from the Japanese Collection of Research Resources Cell Bank (JCRB), were used in this study. These two cell lines were suitable for this study; the cells attaches firmly to the surface of a plastic dish, which must be set vertically during irradiation (see later), and the nucleus area of HeLa cell being 1.7-times larger than that of CHO-K1.

The HeLa and CHO-K1 cells were cultured at 37°C in 95% air, 5% CO₂ in Dulbecco’s modified Eagles’s medium nutrient mixture F-12 HAM (cat# D8062, SIGMA), and α-MEM (cat#1257-063, GIBCO), respectively, supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin).

**Irradiation**

The irradiation system, described elsewhere, at MEXP-HIMAC, NIRS, was used, and is briefly described below. Fe ions of 6 MeV/nucleon were led out into the air through a metal foil window with 20-mm diameter and 6-μm thickness. An apparent Bragg curve in the air was measured by a flat type ionization chamber (IC); a typical measured Bragg curve is shown in Fig. 1. The relative values of the outputs of the IC to those of the secondary electron monitor (SEM) are plotted against the irradiation positions of which a datum point is defined as a point giving half of the maximum (the Bragg peak), as indicated in Fig. 1. The cell samples were irradiated at a position of -14 mm (Fig. 1), at which the LET in water and the energy calculated by the SRIM-2003 code were 4.67 MeV/μm and 1.14 MeV/nucleon, respectively.

The absolute ion fluence was determined by the solid track detector (CR-39 (Fukuvi Chemical Industry Co., Ltd)). CR-39s were irradiated at the irradiation position of biological samples, etched in 7N NaOH at 70°C for 3 minutes, rinsed with water, and dried in air. The surface of the CR-39 was scanned by an atomic force microscope (Nanoscope 4, Digital Instruments), the number of etch pits was...
scored, and then the fluence was calculated in units of ions/\(\mu m^2\). The linear relationship between the fluence measured with the CR-39 as above and the integrated SEM output was confirmed in the range of the fluences of \(1.7 \times 10^{-3} \sim 4.0 \times 10^1\) ions/\(\mu m^2\). For every experiment the fluence was measured with the CR-39 at 5 different integrated values of the SEM output in the fluence range of 50–300 ions/100 \(\mu m^2\) at the center of the ion beam. The ion fluence irradiated to the cell samples was calculated using this linear relationship between the fluence and the integrated SEM output.\(^{22}\)

**Colony formation assay**

A cell suspension of 0.1 ml containing \(6.0 \times 10^4\) cells for HeLa or \(3.0 \times 10^4\) cells for CHO-K1 was spread over an area of 10–12 mm diameter at the center of a 35-mm plastic dish (cat #353001, Falcon), incubated for 24 hours; and the cells then reached a subconfluent state. The dishes were sealed with 5-\(\mu m\) Mylar film to prevent the cells from drying and contamination during ion irradiation (Fig. 2). The samples were irradiated at the –14 mm position of the Bragg curve through Mylar film.

Irradiated and control cells were harvested using trypsin solution, and plated in a 60 mm plastic dish after appropriate dilution to obtain approximately 100 surviving cells. After incubation for 13 days for HeLa and 9 days for CHO-K1, cells were fixed with a 10% formalin solution in PBS, and stained with 1% methylene blue solution. The number of colonies that contained more than 50 cells was counted as the survivors.

**Measurement of cell nucleus area**

The samples were prepared as exactly the same way as those for the survival curves, as mention above. After being fixed with a fixative solution (modified Streck Tissue Fixa-
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Table 1. Summary of the results of the cross sections for the inactivation of HeLa and CHO-K1 cells by Fe ions near the Bragg peak.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Experiment series number</th>
<th>σ: cross-section (µm²)</th>
<th>σ/A&lt;sup&gt;1)&lt;/sup&gt;</th>
<th>l&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;2)&lt;/sup&gt;</th>
<th>l&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;eff&lt;/sup&gt;&lt;sup&gt;3)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa</td>
<td>1</td>
<td>100.1</td>
<td>0.457</td>
<td>0.611</td>
<td>0.819</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>97.0</td>
<td>0.443</td>
<td>0.585</td>
<td>0.652</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>99.1</td>
<td>0.456</td>
<td>0.609</td>
<td>0.703</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>97.0</td>
<td>0.443</td>
<td>0.585</td>
<td>0.672</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>90.2</td>
<td>0.412</td>
<td>0.531</td>
<td>0.677</td>
</tr>
<tr>
<td>Average±SE</td>
<td>96.8±1.8</td>
<td>0.442±0.008</td>
<td>0.584±0.014</td>
<td>0.705±0.030</td>
<td></td>
</tr>
<tr>
<td>CHO-K1</td>
<td>1</td>
<td>58.0</td>
<td>0.457</td>
<td>0.611</td>
<td>0.646</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>56.3</td>
<td>0.443</td>
<td>0.585</td>
<td>0.619</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>44.9</td>
<td>0.354</td>
<td>0.437</td>
<td>0.455</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>48.1</td>
<td>0.379</td>
<td>0.476</td>
<td>0.501</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>79.0</td>
<td>0.622</td>
<td>0.973</td>
<td>1.038</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>61.1</td>
<td>0.481</td>
<td>0.656</td>
<td>0.696</td>
</tr>
<tr>
<td>Average±SE</td>
<td>57.9±5.4</td>
<td>0.456±0.039</td>
<td>0.623±0.078</td>
<td>0.659±0.084</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1)</sup> “A” is the average nucleus area given in Table 2.
<sup>2)</sup> The l<sub>1</sub> is number of lethal lesion per single Fe ion track that was calculated by the equation, l<sub>1</sub> = -ln(1- σ/A), which was derived from the equations of the single hit survival curve, S = exp(-σF), and the equation., S = exp[-[1 – exp(-l<sub>1</sub>)]AF], proposed by Goodhead et al.<sup>26</sup>.
<sup>3)</sup> l<sub>1</sub><sup>eff</sup>, see the discussion section.

SE: Standard error.

Table 2. Average nucleus areas of HeLa and CHO-K1 cells.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Number of cells</th>
<th>Nucleus area in µm²</th>
<th>± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa</td>
<td>293</td>
<td>219</td>
<td>3.5</td>
</tr>
<tr>
<td>CHO-K1</td>
<td>216</td>
<td>127</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Visualization of ion traversal by an Immunofluorescence assay

A cell suspension of 1 ml containing 6.0 × 10⁴ HeLa cells was centrifuged (S.T.F., Streck Inc.) supplemented with 50 mM EDTA-2Na) and stained with 0.1 µg/ml propidium iodide (PI), the areas of the nuclei were measured as follows: (1) images of cells were taken by a confocal laser scanning system (LSM 510, Carl Zeiss) equipped with an Axiovert 200M microscope, with 40 × lens (C-Apochromat 40X/1.2W), with a 4–6 × digital zoom, which correspond to 37.5–56.3 nm/pixels by Multi Track mode, (2) the PI fluorescent area was surrounded by a trace of the mouse on the display using the software LSM ver. 3.0, (3) the software automatically returned the value of the surrounded area. The accuracy of the area was estimated to be within 2% from the accuracy of the length, ±1.4%, which was obtained from a measurement of using 100-µm objective micrometer.

Fig. 4. Distribution of nucleus areas of HeLa and CHO-K1 cells. The averages and standard errors were 219 ± 3.5 µm² for 293 HeLa cells and 127 ± 1.2 µm² for 216 CHO-K1 cells. The arrows in the figure show the average. Each bar in the figure represents the fraction of the nuclear area in the 10-µm² range.

Table 2. Average nucleus areas of HeLa and CHO-K1 cells.
or 4.0\times 10^4 CHO-K1 cells was plated on a two-well chamber slide (Lab Tek, Nalge Nunc), and incubated for 24 hours. Just before irradiation, the culture medium was removed; the slides were sealed with 5-µm Mylar, and then irradiated with ions under the same conditions as the survival experiments. Within no longer than 10 minutes after irradiation, the cells were washed with PBS three times, and fixed with the fixative solution for 15 minutes at room temperature. The cells were permeabilized with a solution of 100 mM Tris pH 7.5, 50 mM EDTA and 1% Triton-X-100 for 20 minutes at 56°C, blocked for 60 minutes in 10% FBS in PBS at 37°C, and incubated with anti-γ-H2AX antibody (cat# 4411-PC-020, Trevigen), diluted to 100 µg/ml in PBS supplemented with 0.5% Tween 20 and 1% BSA for 1 hour at 37°C. The cells were then incubated with secondary anti-rabbit Alexa 488 antibodies (Molecular Probes) diluted to 30 µg/ml in PBS.

Fig. 5. γ-H2AX spots formed in the nuclei of HeLa (B) and CHO-K1 (D) cells after irradiation with Fe ions at a fluence of 4.0 \times 10^{-2} and 5.0 \times 10^{-2} ions/µm², respectively. Well-defined fluorescent spots of γ-H2AX were recognized in the nuclei. Non-irradiated control HeLa cells (A) and CHO-K1 cells (C). Bar size: 10 µm.

Fig. 6. Top (A) and cross-sectional views (B, C) of a single irradiated HeLa cell after γ-H2AX immunostaining. Three dimensional images were constructed from 20 sliced images of an irradiated cell with 0.55-µm intervals. Panel A is a top view image at the twelfth slice, 6.05 µm from the top, which is the left edge of panels B and C. Panels B and C are cross-sectional images along the longitudinal lines, B and C in panel A, respectively. Cross-sectional views of the fluorescent stripes are shown in the panel B and C, corresponds to those fluorescent spots, the positions of which indicated by the arrows in the panel A. Bar size, 5 µm.
including 0.5% Tween 20 and 1% BSA for 1 hour at 37°C. The cells were rinsed with PBS three times after each treatment, mentioned above. The cells were counter stained with 0.1 µg/ml PI, and mounted in an antifade solution containing 2.3% diazabicyclo {2,2,2} octane, 90% glycerol, 10 mM Tris-HCl (pH 7.5).

The γ-H2AX immunostained cells were observed with the confocal microscope, mentioned above. The cross-sectional view images of a single cell were reconstructed from 20 sliced images taken with an interval of 0.55 µm over 10.45 µm using with the software installed in the LSM system.

**RESULTS**

**Cell inactivation cross section and number of lethal hits**

Figure 3 shows typical survival curves of HeLa and CHO-K1 cells after Fe ion irradiation. Since the survival curves showed an exponential decreased with an increase of the fluence, the survival fractions (S) were fitted with the equation, S = exp (-σF), where σ is the inactivation cross section, and F is the ion fluence; the inactivation cross sections were 100.1 and 58.0 µm² for HeLa and CHO-K1 cells, respectively. The cross sections for independent experiments, five for HeLa and six for CHO-K1, are summarized in Table 1; the average values with standard errors were 96.6 ± 1.8 and 57.9 ± 5.4 µm² for HeLa and CHO-K1 cells, respectively.

To compare the inactivation cross sections with the areas of the cell nuclei, 293 nuclei for HeLa and 216 nuclei for CHO-K1 were measured (Fig. 4); the averages were 219 and 127 µm² for HeLa and CHO-K1, respectively (Table 2). The ratio between the average inactivation cross sections and the average nucleus areas were 0.442 ± 0.014 for the HeLa cells and 0.456 ± 0.039 for the CHO-K1 cells, the reciprocal values of which, 2.3 and 2.2, indicate the average numbers of Fe ion traversals through a cell nucleus necessary to induce cell inactivation. The result that more than one Fe ions was needed to induce cell inactivation, though the survival curves showed single-hit kinetics, is discussed below.

**Distribution of γ-H2AX spots in the cell nucleus**

Representative confocal microscope images of HeLa and CHO-K1 cells after irradiation with Fe ions and immunostaining with anti-γ-H2AX antibody are shown in Fig. 5. In the cells irradiated with the Fe ions (panels B and D), fluorescent spots were seen in the nucleus very brightly and locally, in contrast to the control cells (panels A and C), in which no such spot was seen. These images indicate that an ion traversal through a cell nucleus produces a cluster of DSBs locally along its track, and can be seen as a spot of the fluorescence after immunostaining with an anti-γ-H2AX antibody. Since the DSB cluster must be a columnar shape along the ion track, the fluorescent spots may be seen differently from different point of views; if seeing from the side, rod-like shape must be seen.

To prove the above prediction, we constructed a three dimensional image of an irradiated cell from 20 sliced images. Figure 6 displays 3 views of the same HeLa cell; the pan-

![Fig. 7. Correlation between the expected number of ion traversals through nucleus and the observed number of γ-H2AX spots in HeLa (A) and CHO-K1 cells (B). The average number of ion traversals, n, was calculated by the equation n = AF, where A is the cross-sectional area of the individual cell nucleus, and F the ion fluence. Linear fits to the data plots are shown by the solid lines, of which slopes and vertical intercepts were 0.92 and 2.4 for HeLa cells and 0.96 and 0.90 for CHO-K1 cells. The plot A contains 13 non-irradiated (including 4 cells without spot) and 51 irradiated HeLa cells, and plot B contains 22 non-irradiated (including 14 cells without spot) and 50 irradiated CHO-K1 cells. For control cells, the average number of foci observed was plotted; the average values with standard errors were 2.38 ± 0.53 for HeLa, and 0.455 ± 0.143 for CHO-K1. The dotted line represents the width of the Poisson distribution around the expected value, next, given by c + n ± √n, which c is the average number of foci in the control cells.](http://jrr.jstage.jst.go.jp)
el A shows a top-view image at the twelfth slice, 6.05 μm from the top, and panels B and C are cross-sectional images along the longitudinal lines, denoted by the letters B and C on panel A, respectively. Figure 6B and C clearly shows rod-like shapes of green fluorescence, which are interpreted as a cross-sectional view of the γ-H2AX column corresponding to the column of DSBs.

**Correlation between number of Fe ion traversals and fluorescent spots**

In Fig. 7, the numbers of the fluorescent spots counted per nucleus are plotted to the expected numbers of ion traversals per nucleus, which was calculated as the product of the ion fluence and the area of the cell nucleus. For HeLa cells, 38 irradiated cells and 13 control cells (4 without spots), and 50 irradiated cells and 22 control cells (14 without spots) for CHO-K1 cells were plotted. The slopes of the regression line were 0.92 ± 0.09 for HeLa and 0.96 ± 0.05 for CHO-K1. The fact that the slopes were practically unity, together with the images of Fig. 6, suggests that (1) a column of DSBs are formed along each ion track, (2) H2AXs in and near the column are phosphorylated, and thus the column of γ-H2AX is formed. (3) Immunostaining with fluorescent anti-γ-H2AX antibody visualizes the column, and (4) a top view of the cell shows a circular fluorescent spot.

**DISCUSSION**

After HeLa and CHO-K1 cells were irradiated with Fe ions of 1.14 MeV/nucleon and 4.67 MeV/μm, we obtained survival curves, as well as confocal microscope images of the DSB clusters after the immunostaining of γ-H2AX, which is frequently used as a DSB indicator.27–29 The results are summarized as follows: (1) The cell inactivation cross sections were 0.442 and 0.456 of the cell nucleus areas of the HeLa and CHO-K1 cells, respectively (Table 1). (2) Taking the Poisson distribution of a number of ion traversals per cell nucleus and the distribution of the cell nucleus area into consideration, the average numbers of lesions per single ion track through a cell nucleus were 0.705 and 0.659 for HeLa and CHO-K1 cells, respectively (Table 1). (3) Confocal microscope images of cell nuclei indicated that DSBs were induced along with the ion track (Fig. 6), and each Fe ion traversing the cell nucleus induced a DSB cluster along the track (Fig. 7).

Here, we estimate the number of DSBs induced by a single ion traversal as basic information. The cross section (σcell) of DSB induction for a cell nucleus is assumed to be the product, σcell = σbr × b × cind, where σbr is the cross section for dry pBR322 plasmid DNA (4.3 kb), 7.96 × 10^{-15} m^2, after irradiation by the Fe ions with the same energy and the same LET as that for the present irradiation experiments (unpublished result), b is the ratio of the number of base pair between pBR322 DNA and the cell, and cind is the correction factor for the indirect action. The numbers of base pairs in HeLa and CHO-K1 cells were assumed 6 × 10^9 for simplicity, although the HeLa cell is not diploid, and the distribution in the cell cycle should be considered. If cind is assumed to be 2, σcell is calculated as 2.22 × 10^{-8} m^2 (=7.96 × 10^{-15} m^2 × 1.4 × 10^6 × 2). A single ion traversal through a cell nucleus with a area of A is equivalent to a fluence of 1/A, thus 4.57 × 10^8 and 7.87 × 10^9 m^{-2} for HeLa and CHO-K1 using the nucleus area shown in Table 1, respectively. The number of DSBs induced by a single Fe ion traversal through HeLa and CHO-K1 cells is estimated to be 101 (= 2.22 × 10^{-8} × 4.57 × 10^8) and 175 (= 2.22 × 10^{-8} × 7.87 × 10^9), respectively. In addition to this large number of DSBs, the quality of DSBs induced by heavy ions is known as clustered DNA damage, or a locally multiply damaged site (LMDS), which must be difficult to repair.32,33 These two facts may lead to an assumption that a single Fe ion traversal is sufficient for cell inactivation, although our results showed that a single Fe ion traversal did not seem to be enough to induce a lethal hit.

For the broad beam condition, the number of ions traversing individual nucleus follows a Poisson distribution. Goodhead et al.20 explicitly considered the influence of the Poisson distribution, and obtained an equation, S = exp(- AF[1-exp(-l_1)]), where S is the survival fraction, A the total projected area of the cell nucleus, F the fluence of ions (tracks), and l_1 the average number of lethal lesions per cell produced by a single track through the nucleus. Since the survival curve is expressed by a single-hit kinetics, S = exp(-σF), AF[1-exp(-l_1)] equals σF, and thus l_1 = -ln(1-σ/A) is obtained. The l_1 values calculated for each experiment are listed in Table 1; the average values were 0.584 (0.570–0.598) and 0.623 (0.545–0.701) for HeLa and CHO-K1, respectively. Since l_1 = 1 corresponds to σ/A = 0.63 and our results of σ/A were 0.442 and 0.456, indicating the difficulty to determine the precise value of l_1 using data obtained from broad beam experiments. To determine the l_1 precisely near l_1 = 1, experiments in which precise number of Fe ions traversing individual nucleus can be determined, such as a microbeam technique, are highly desirable.

The l_1s in Table 1 were calculated using the average value of the nucleus area, but they are widely distributed, especially for HeLa cells (see Fig. 4). Thus, we calculated the survival values while taking into account the distribution for the experiment series 1 of HeLa cells (Fig. 3), on the assumption that the l_1 is constant for every nucleus area (the value listed in Table 1), and that the survival values, S, are given by

\[ S = \sum p_i \exp(-AF(1-\exp(-l_{i1}))) \]

where \( p_i \) is the probability of the ith nucleus area, \( A_i \), in μm^2 units shown in Fig. 4, and F is the ion fluence per μm^2 at the same value as that for the obtained experimental data. The crosses in Fig. 8 represent the calculated values, and deviate remarkably from the experimental data (open circles). Under the above assumption, cells with larger nuclei are more sensitive than...
cells with smaller nuclei, and thus the survival curve at low fluences was dominated by sensitive cells with larger nuclei; consequently, the net survival curve was concave upwards as can be seen in Fig. 8. We, therefore, defined \( l_{\text{eff}} \) as the \( l_1 \) which gives the same cross section as the experimental values for the set of survival values (rhombuses in Fig. 8) calculated by the above equation; the value of the \( l_{\text{eff}} \) was 0.819 instead of the \( l_1 \) of 0.611 for HeLa cells. As seen in Fig. 8, the calculated survival values (rhombuses) could not be distinguished from the experimental survival values, which inevitably contained errors. The \( l_{\text{eff}} \) obtained as above for each experiment is listed in Table 1: the average value and the differences between the \( l_{\text{eff}} \) and \( l_1 \) were 0.705 and 0.121 for HeLa cells, and 0.659 and 0.036 for CHO-K1 cells. As expected the differences between the \( l_{\text{eff}} \) and \( l_1 \) was much greater for HeLa cells with a wider distribution of the nucleus area than for CHO-K1 cells. We concluded that, from the present experiments using the broad ion beam, these \( l_{\text{eff}} \) values were the best estimates for the average number of lethal lesions per cell produced by a single ion track through the cell nucleus.

The \( l_{\text{eff}} \) values obtained in the present results, 0.705 and 0.659 (Table 1), are apparently less than one; even a DSB cluster was induced in a columnar distribution along the ion traversal, as visualized with \( \gamma \)-H2AX immunostaining. Deerling et al.\(^{15}\) and Wulf et al.\(^{16}\) reported that the maximum of the cell inactivation cross sections for heavy ion irradiation were approximately half of the nucleus size, indicating that a heavy ion traversal through a cell nucleus does not inevitably lead to cell inactivation. Recently, Funayama et al.\(^{17}\) reported that more than 10% of the CHO-K1 cells irradiated with a single Ar ion of 11.5 MeV/nucleon divided more than 3 times.\(^{18}\) This result supports the idea that some cells could survive even after heavy ion traversing through their nuclei. The result that the \( l_{\text{eff}} \) values are apparently less than one may be explained by the following two explanations. The first possibility is that sensitivity of the cell nucleus is not uniform; in other words, the size of the sensitive area is approximately 0.7 of the nucleus area. This sensitive distribution or the “critical site” in the cell nucleus was also mentioned by Barendsen et al.\(^3\) and Raju et al.\(^11\) for alpha particles irradiation.

The second possibility is that DNA damage is necessary to induce cell death, but DNA damage alone is not sufficient to cause cell inactivation, and an unidentified second radiation target (non-DNA) must be irradiated simultaneously. This model was proposed by Schneiderman et al.\(^{36}\) after they observed that the survival of CHO-irs20 cells labeled with 5-[\(^{125}\)I]-iodo-2’-deoxyuridine (\(^{125}\)dU) remained at approximately 30%, even after 1265 decays/cells of \(^{125}\)I.

Further investigations are necessary to determine which explanations are more appropriate.

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