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Judgement on "Hit or Non-hit" of CHO cells Exposed to Accelerated Heavy-ions (Fe- or Ar-ions) using Division Delay and CR-39 Plastics as an Indicator

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Abstract The cell killing effect of ionizing radiation depends on the degree of linear energy transfer (LET). The relative biological effectiveness (RBE) reaches a maximum at LET of around 100-200 keV/μm and decreases at higher levels. The ion clusters produced by high-LET radiation are not uniformly distributed. The incidence of non-hit cell events is higher in high LET irradiation than in the cases of low-LET irradiation. This fact could explain the decrease in the cell killing effect at higher levels of LET irradiation. Since the cell killing effect may be related to the nuclear traversal of heavy-ions, it is necessary to establish methods to distinguish the hit cells from the non-hit cells, especially in case with high LET irradiation. Using time-lapse photography, we first examined the hit events by observing the division delay in the cells caused by high-LET irradiation. In addition, we explored the use of CR-39 plastics to detect the exact position of heavy-ion traversal on the surface of a flask where cells were growing. When Chinese hamster ovary (CHO-K1) cells were exposed to 4 Gy of accelerated Fe-ions (2000 keV/μm) or Ar (1640 keV/μm)-ions, the surviving fraction decreased to about 30% in both cases of irradiation. Eighty percent of the irradiated cells, suffered a division delay in contrast to the remaining 20% of the cells which showed a normal division time (12-13 hrs). The later 20% of the cells is considered to be a population of cells which were not actually traversed by heavy-ions. The difference between the higher values of the surviving fraction (approximately 30%) and the non-hit cell population (20%) indicates that some hit cells can grow even after being hit by heavy-ions. The fraction of recovered cells determined by the time-lapse photography method was 10%, and this value closely correlated with the difference between the surviving fraction and the non-hit cells. We used the Poisson distribution of the hit-events by heavy-ions among the cell population in order to calculate the fraction of cells receiving at least a single-hit in the cell nucleus (130 μm^2 in average size). From this calculation we determined that 80% of the cells had a single hit to their nuclei by a heavy-ion which induced such early cellular responses as division delay. Our finding in the experiments using CR-39 plastics as a detector for hit-sites further supported the idea that the hit lethality of a cell is related to heavy-ion traversal through its nucleus. This study idicates the possible usefulness of both the division delay and CR-39 plastic methods for evaluating the biological effects of heavy-ions, especially when these two methods are combined.

Key words: Heavy-ion, Division delay, CR-39 as a hit indicator.

Introduction

The cosmic environment is considered to be a complicated mixture of high-energy photons and radiation particles. Its spectrum consists of atomic nuclei which are highly energetic and densely ionized (HEZ)[17]. Highly energetic heavy-ions such as iron ions are known to contribute significantly to the estimated total radaition exposure during manned space missions[5]. For an accurate assessment of potential health risks to humans during such missions, it is necessary to understand the biological significance of such highly energeric particles.

According to the classical hit-theory on the biological effects of radiation, cell killing is an inevitable consequence of the accumulation of the number of hit-events in intracellular targets[8]. The cell killing effect of ionizing radiation is known to depend on its linear energy transfer (LET)[1)19)]. The biological effectiveness for a lethal effect in most of living cells has been shown to reach a maximum in the LET region at around 100-200 keV/μm. An over-killing effect hypothesis has been proposed to explain the reduced efficiency of cell killing when the LET increases to even higher levels[2)3)]. In contrast to the sparse and homogeneous distribution of energy deposition due to low-LET radiation, the accelerated high-LET charged particles produce dense ionization along their tracks. Therefore, the possibility that such high-LET radiation provides no ionization within the cell target is greater than at a lower level of LET radiation for the same absorbed dose.

The exposure of mammalian cells to ionizing radiation can result in a rapid arrest of cell division. Such division delay is reported to be dependent on the cell-cycle[13)20)], but the cells exposed to ionizing radiation might also be delayed to some extent throughout their cell-cycle. In this study we analyzed the cell division delay in relation to cell-hit by heavy-ions. The cell hit was also physically determined using the plastic ion track detector, CR-39, which is usually used for monitoring surface contamination by alpha-particles[6)9)]. We herein present a new method for evaluating the biological effects caused by heavy-ion traversal.

Materials and Methods

Cell culture

Chinese hamster ovary cell line (CHO-K1) provided by the RIKEN Cell Bank, Japan, was used in this study. The cells were cultured as a monolayer at 37°C in humidified 5% CO_2 atmosphere with Ham's F12 medium supplemented with 10% fetal calf serum. The generation time of the CHO cells was 12 hours.

Irradiation

Iron (Fe)- and Argon (Ar)-ion were accelerated by the RIKEN (The Institute of Physical and Chemical Research, Japan) Ring Cyclotron (RRC) and the LET at the target cell site was adjusted to 780, 1200, 2000 keV/μm for Fe-ions and 1640 keV/μm for Ar-ions. X-ray irradia-

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tion was carried out with a Shimazu Pantak X-rays generator (200 kVp, 20 mA ; Filter 0.3 mm copper plus 1.0 mm aluminum) at a dose rate of 1 Gy/min in air for comparison as a low LET irradiation. For the exposure of cells to heavy-ions, the beam-ions were injected from the bottom side of the cell culture flask. On the other hand, X-ray irradiation was done from the upper side of the flask. The details of the beam transport systems and dosimetry have all been reported previously[10)11)]. The dose and LET distribution were measured with a parallel-plate ionization chamber and a proportional counter field with air and tissue-equivalent gas, respectively, and by changing the thickness of the water column and polymethyl methacrylate sheets. The dose-averaged LET was controlled by inserting a range shifter with the appropriate thickness.

Colony formation assay

The surviving fraction of the cells was determined by a standard colony formation assay. Briefly, the exponentially growing CHO cells were dispersed with trypsin and then were plated on a plastic flask (Falkon T25). Five cell culture flasks were prepred for each dose of irradiation. 200 cells were seeded in each flask for 0, 2 or 4 Gy irradiation and 2,000 cells for 6, 8 or 10 Gy irradiation, respectively. The cells were incubated at 37°C for 5 hours before irradiation. Following irradiation, the cells were incubated under normal growth conditions for 10 days before fixation. The cells were stained with giemsa solution and the colonies containing ~50 or more cells were scored as surviving colonies. The surviving fraction was calculated as the ratio of plating efficiency of irradiated cells to non-irradiated cells. The same measurments were done three times, two times, and one time after exposure to X-rays, Fe-ion and Ar-ion, respectively.

Time lapse observation

Changes in the doubling time of the individual cells, the division delay, was monitored by time-lapse photography during this period for a total of six days ; three days each before and after irradiation with 4 Gy of Fe-ion or Ar-ion. The cells were allowed to divide from one to three times before being exposed to monoenergetic beams of heavy-ions. For comparison purposes, the same experiment with X-ray irradiation was carried out as a low LET control. Exponentially growing asynchronous cells in plastic flasks were placed in a plastic box with an atmosphere of 5% CO_2, 95% air. Photographs were taken every 15 min using Minicopy film (Fuji Photo Film Co., Ltd.) with a 35-mm motor-driven camera (Nikon) affixed to a phase-contrast microscope. The results of the time-lapse photographical observations were recorded as the pedigree of the individual cells after irradiation. In such a pedigree, each horizontal line represents the life of a cell, relative to the time in hours. When a cell divides, the line branches off into two horizontal lines. The arrow in the pedigree indicates the time point when each cell was exposed to heavy-ions. The scale number (length of the line) on the left side of the arrow point corresponds to the time interval between cell division and irradiation, while the scale number (length of the line) on the right-hand side represents the time interval from irradiation to the first post-irradiation division. Therefore, the sum of these two numbers represents the time of a single cell-cycle for the irradiated cell.

CR-39 plastic as an indicator

In this study we used CR-39 plastic (solid state nuclear track detector : Harzlas/TNF-1) for detecting the exact position of heavy-ion traversals. When accelerated Fe- and Ar-ions interact with CR-39 they were registered as

latent tracks[24)25)]. To amplify the latent tracks, we treated the CR-39 plastic in very mild conditions with PEW (KOH : Ethanol : Water, respectively 15% : 65% : 20%) as an etching solution, for 10 min at 37°C (recommended by Dr. H. Yasuda : NIRS, Chiba, Japan). The CR-39 plastic attached to the bottom surface of a culture flask. After the exposure to heavy-ions the latent tracks were amplified in CR-39 while it was still attached to the flask. To determine the particle fluence at 1, 2 and 4 Gy, the mean track numbers were counted under phase contrast microscopy (magnification : 20 ×) with twelve different areas of 100 μm × 100 μm on the CR-39 plastic after heavy-ions exposure.

Results

Dose-survival relationship in irradiated CHO cells

We determined the colony-forming ability as an indicator of cell survival. The dose-response curve for heavy particle exposure appears to be linear over this dose range (Fig. 1), whereas the survival curve of the X-ray irradiated cells showed a typical initial shoulder followed by an exponential part with higher doses. The surviving fraction of the cells exposed to 4 Gy of Fe-ion (2000 keV/μm) and 4 Gy Ar-ion (1640 keV/μm) was 30% and 28%, respectively. As shown in Fig. 1, the slope of the survival curve for Fe-ion becomes more gentle as the LET increases and the cell killing effect at LET 2000 keV/μm in dose-ranges higher than 4 Gy becomes less than that after X-rays. A similar LET-dependent decrease in the cell killing effect is usually observed LET regions with an RBE peak higher than 100 to 200 keV/μm.

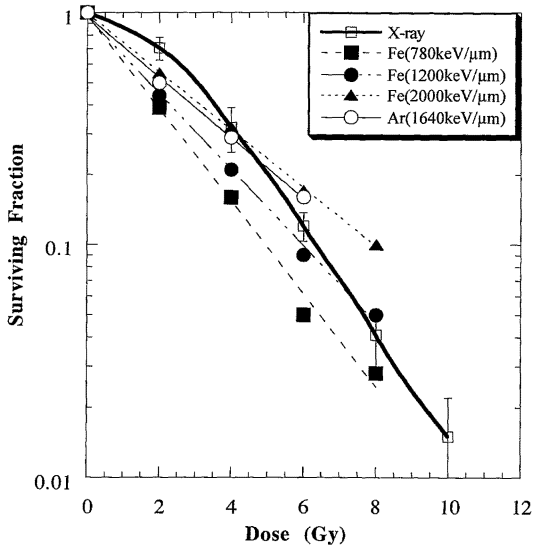

Fig. 1 Survival curves of the CHO cells exposed to X-ray (200 kVp, 20 mA, 1 Gy/min), Fe-ion (LET : 780, 1200, 2000 keV/μm) and Ar-ion (LET : 1640 keV/μm).
The X-ray irradiated cell survival curve has an initial shoulder which was followed by an exponential part ; Fe- and Ar-ions showed exponential curve. The error bars present the standard error.

Assessment of "hit or non-hit" cells by time-lapse observations

In this study, we estimated the fraction of non-hit cells based on the idea that division delay should be the result of a "hit" event by heavy ions. Using time-lapse photography, we determined whether division delay did or did not occur (hit or non-hit) for individual cells during an observation period of six days in total ; three days each before and after 4 Gy exposure of Fe- (2000 keV/μm) or Ar- (1640 keV/μm) ions. The fate of each cell after irradiation was recorded as the pedigree for the individual cells. Based on the microscopic analyses of the 142 cells that were observed three times by time-lapse observations, the cells were classified into three categories. Exam-

ples of time-lapse photographs are shown in Fig. 2 (for further detailes see the following section).

(1) Hit-cells

An example of the pedigree for hit cells, classified as a type 1 cells, is shown in Fig. 3. The cell-cycle time of this type 1 cell was prolonged to 30.5 h in post-irradiation generation, compared to 12 h in prior-irradiation. Furthermore, this cell divided into two daughter cells after irradiation. One of the resulting sister-cells stopped dividing and transformed into a giant cell during the first post-irradiation generation, while the other one continued to divide and showed apoptotic morphological changes in the 3rd post-irradiation generation. Moreover, another pair of daughter cells following the 3rd incomplete division leading to sister-cell fusion and binucleate-cell formation at the 4th post-irradiation generation. In these cells, 98 out of 142, suffered from division delay and later died. We therefore determined them to be hit-cells.

(2) Non-hit cells

Regarding the 20% of the cells which survived (29 out of 142), we did not see any division delay and the cells continued to divide with a normal generation time (12 h-13 h), even after irradiation (Table 1). These cells appeared to not be hit by heavy-ions. An example of the pedigree for such non-hit cells is shown in Fig. 4. Judging from the pedigree of this cell type, which

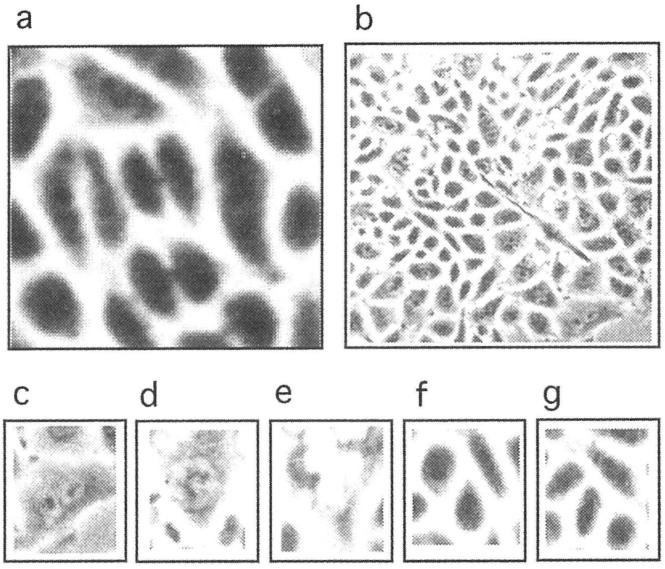

Fig. 2 Photographs of the CHO cells after exposure to 4 Gy of Fe-ion (LET 2000 keV/μm).
a : unirradiated cells (control) ; b : the same cells as shown in panel a, 40 h after exposure. The magnified image of the cells in panel b are shown in the following panels, c to g. Panels : c to e (hit-cells) ; c : binucleaed cells ; d : giant cells undergoing interphase cell death ; e : morphological apoptotic cells ; f : representative photo of non-hit cells ; g : representative photo of hit-survival cells.

Table 1 Correlation between cell survival and the fraction of non-hit cells in the CHO cells after exposure to 4 Gy Fe-ion (LET : 2000 keV/μm), Ar-ion (LET : 1640 keV/μm)

Ions	Surviving Fraction (%)	Fraction of non-hit cells (%)	
		Observed value	Calculated value
Fe	30%	(12/64)[a] (9/44) (8/34) 20%[c]	19% ($e^{-1.66}$)
Ar	28%	(14/80)[b] (8/42) 18%[c]	18% ($e^{-1.69}$)

[a,b] The ratios in parentheses indicate the fraction of non-hit cells per total observed cells, [c] The average fraction value of the non-hit cells

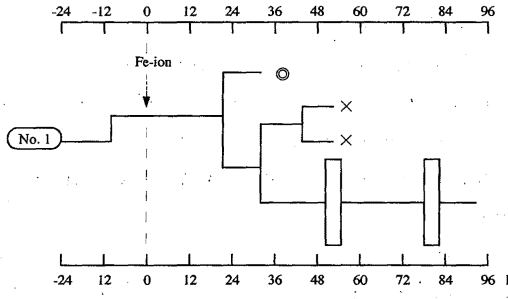

Fig. 3 The pedigree of the hit-cells after exposure to 4 Gy Fe-ion (LET 2000 kev/μm). The No. 1 cell was irradiated at the position indicated by the arrow. The pedigree shows an example of type 1 cell with giant cell (◎) in the first post irradiation generation, two cells with apoptotic morphological changes (×) in the 3rd post-irradiation generation, the inferior branch (□) refers to cells that divide but their daughter cells fused two times, respectively, and such cells are known as multinucleated cells.

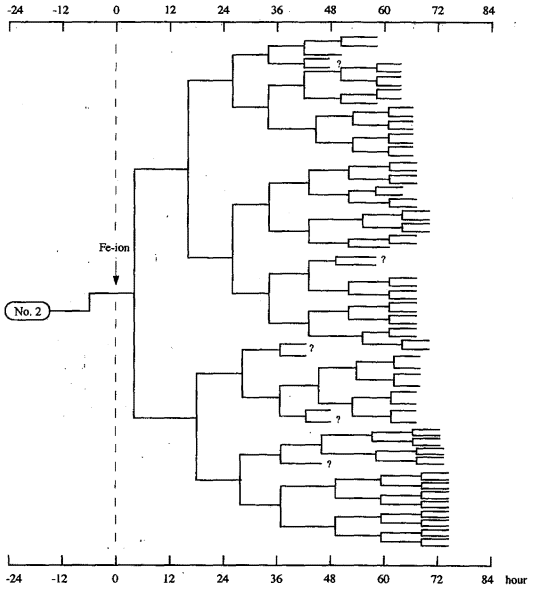

Fig. 4 The pedigree of non-hit cells which survived after exposure to 4 Gy Fe-ion (LET 2000 keV/μm).
The No. 2 cell was irradiated at the point indicated by the arrow. A question mark (?) indicates the moment when the cell disappeared from the visual field and therefore the fate of the cell could no longer be followed up.

was designated type 2 cells, there is no difference between the cell generation times before (12.5 h) and after (7 h+5.5 h) irradiation. These cells countinued to divide with a generation time of 11 h-13 h up to the seventh generation observed after irradiation. We did not find any such type 2 cells, non-hit cells, when we examined the time-lapse photographs for the 115 cells exposed to 4 Gy X-rays (survival fraction 30%).

(3) Hit-survival cells

The fraction of non-hit cells (20%) was slightly lower than the surviving fraction (30%). This fact suggests that there is a fraction of cells that retained their reproductive integrity even after being hit by Fe-ions. An example of the pedigrees for this type of cell, designated type 3, is shown in Fig. 5. Since such cells suffered from division delay during the irradiated generation (8 h+14 h), we determined this type 3 cell to be a hit-cell. However, as shown in this case, lethal sectoring was often observed among the type 3 cells. The one sister cell (see the cell lineage A in Fig. 5) which arose in the first generation of post-irradiation continued to divide with a prolonged generation time (>12 h-13 h). In contrast, the other sister cell (see the cell lineage B in Fig. 5) continued to grow with a generation time closely similar to that of the unirradiated cells (11 h-13 h). No morphological changes was observed with this sister cell lineage B of type 3. We thus classified type 3 cells to be hit-survival cells. The percentage of type 3 cells among the irradiated cell population with Fe-ions was 10%, or 15 out of 142, which apparently matched with the difference in the cell population between the surviving fraction (30%) and non-hit cells (20%). Essentially the same results were obtained with the pedigrees of 122 cells observed after exposure to Ar-ion (data not shown).

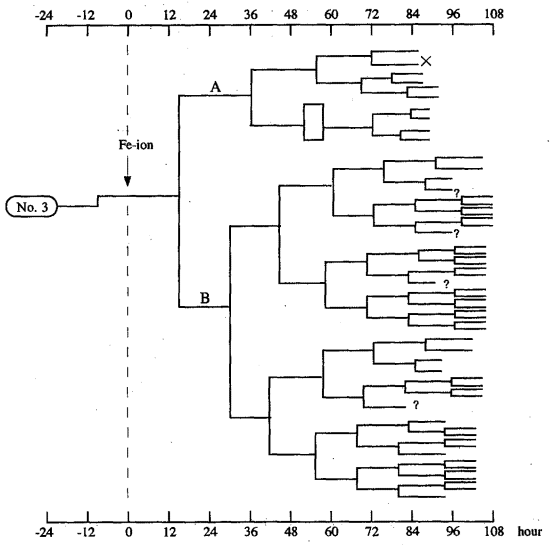

Fig. 5 The pedigree of the hit-survived cell after exposure to 4 Gy of Fe-ion (LET 2000 keV/μm).
The No. 3 cell was irradiated at the point indicated by the arrow. The upper branch (A) was classified as a lethal sector because cell death (×) and fusion (□) took place. However, in the B branch normal colony formation was observed. A question mark (?) indicates the moment when the cell disappeared from the visual field and therefore the fate of the cell could no longer be followed up.

Calculation assays for fraction of non-hit cells

Statistical aspects are especially important when considering the dose absorbed in a small region of an irradiated target, such as in the cell nuclei. Radiation dose for heavy-ions was calculated by converting the absorbed dose to the fluence, while assuming that the target size is equivalent to cell nucleus. Based on the Poisson nature of the hitting process, the probability of non-hit to the cell nuclei was thus calculated according to the following equations[12)7)]:

$$F\ [n/\mu m^2] = K.\ D\ [Gy]/LET\ [keV/\mu m] \quad (a)$$

$$S = e^{-F.\sigma} \quad (b)$$

(a) Where F is the number of traversed heavy-ions per unit area, LET is the linear energy transfer, K is the conversion factor=6.242 (1 Gy=6.242 keV/μm^3), D is the dose of Fe- or Ar-ions (Gy).

(b) S is the fraction of non-hit cells, F is the calculated fluence in (a), σ is the cell nucleus area=130 μm^2 [16)].

The calculated value of the cells whose tar-

gets are not traversed by the accelerated heavy-ions was 19%. This value is quite similar to that for the fraction of non-hit cells determined by time-lapse observations. The correlation between the cell survival and the fraction of non-hit cells is summarized in Table 1.

Assessment of hit using CR-39 plastics

In this study we used CR-39 plastics to detect the cellular hit-sites (nucleus or cytoplasm) through which the heavy-ions traversed. First, the CR-39 plastics were exposed to 1, 2 and 4 Gy, Fe-ion (2000 keV/μm), (Fig. 6). The average number of heavy-ion traversals per 100 μm $\times$ 100 μm (10^4 μm^2) was 40, 77, 149 for 1, 2 and 4 Gy, respectively (Fig. 7). The pit-size was almost the same for all defferent doses examined (ϕ : $\sim$2 μm). For detecting the position of heavy-ion traveral, the CR-39 plastic was placed on the surface of the bottom side of a culture flask, when the cells were exposed to 4 Gy accelerated Fe-ion. After the irradiation, the CR-39 plastic was treated with an etching solution in order to allow the amplification of the latent tracks. Then two photographs were taken using a phase-contrast microscope within the same area with one focused on the cells and the other focused on the pits detected on the surface of the CR-39 plastic. These two microscopic photographs were next superimposed on each other using of computer graphics. An example of such a superimposed photograph is shown in Fig. 8. The cell nucleus becomes visible at the center of cell (dark area) in the green field of the fluorescence microscope. Heavy-ion tracks are depicted by yellow-orange circles. The hit or non-hit cells were judged by heavy-ion traversal through their nucleus. We did not see any pits, which were considered to show the track of traversal, in 20% of the cell nuclei after irradiation. This ratio correlated with the non-hit fraction which was observed by time-lapse photography. The results of the experiment detecting the hit-sites after Ar-ion (1640 keV/μm) irradiation were essentially the same as after Fe-ion irradiation. Accordingly, we concluded that the detection of hit sites based on the resulted pits on CR-39 plastic is a useful method for determing the hit cells by heavy-ions, especially when combined with division delay observations after irradiation.

Discussion

Heavy-charged particles can produce ion-clusters along the track and kill cells more efficiently than low LET radiation such as X- or γ-rays[26]. The biological significance of

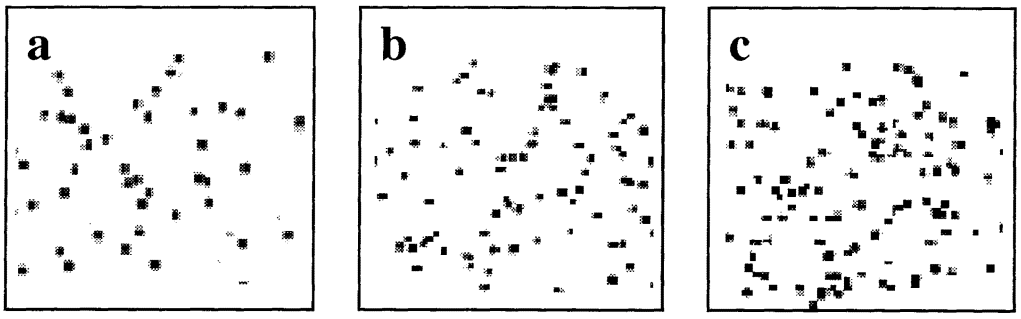

Fig. 6 Photographs of CR-39 plastics exposed to Fe-ion (LET 2000 keV/μm) after etching. a : 1 Gy ; b : 2 Gy ; c : 4 Gy

iron-ion is of particular interest due to its high LET and its relative abundance compared to the other heavy nuclei in space radiation environment[5]. The division delay is an early cellular response which is observed in almost all-irradiated cells[14] in which the proliferation of cell cycle is temporarily arrested at G_1 and G_2. According to previous reports, the division delay especially during G_2 arrest in the cells exposed to the accelerated heavy particles is substantial[15)18]. In this study, we observed the division delay during the cell cycle for individual cells after the exposure to either accelerated iron- or argon-ions and applied it as an indicator of the cell hit by heavy-ions. Eighty percent of the irradiated cell population, suffered a delay in division at the irradiated generation while the remaining of 20% of the cells showed a normal division time (12~13 h). A similar figure was obtained from the theoretical calculations based on the following target theory (Table 1). The "nucleus" can be considered a sensitive target of the cells. This enables the calculation of the ratio of surviving cells by applying the Poisson distribution to determine the number of hits in its sensitive target through which the heavy-ion traversed. We speculated that the higher value of the surviving fraction (30%) compared to the non-hit cell fraction (20%) could be attributed to the presence of

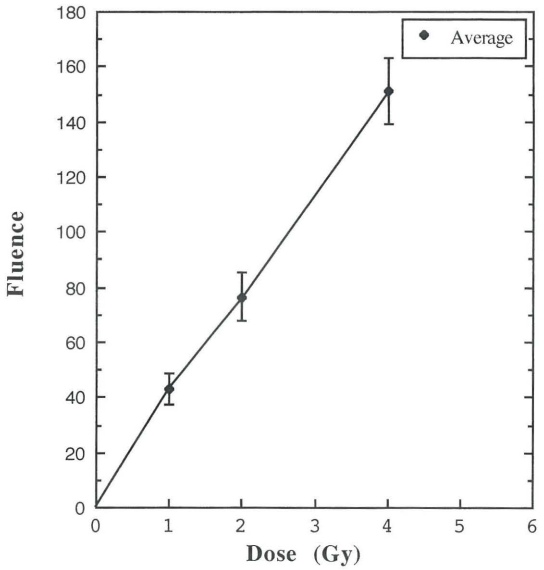

Fig. 7 The relationship between the fluence (n/10^4 μm^2) and dose (Gy) detected with the CR-39 plastics exposed to Fe-ion.
Error bars presents the standard error.

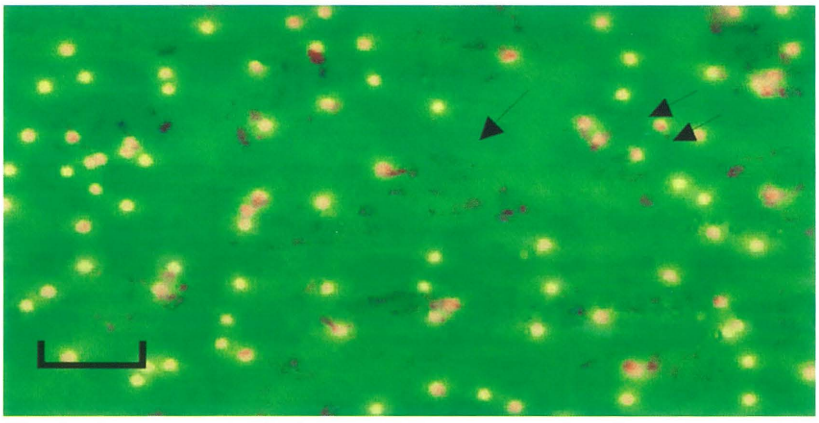

Fig. 8 A superimposed photograph showing CHO cells and the CR-39 detector with the tracks of traversed 4 Gy of Fe-ion.
The single arrow show a non-hit cell, while the double arrows show a hit-cell. Scale bar=20 μm.

surviving cells which had been hit by heavy ions. The physical hit event can be divided into two subfractions consisting of a lethal and unlethal damage but with lethal sectoring. Based on the pedigree of the cells we could determine whether a cell had died by a lethal hit (Fig. 3). If a cell did not sustain a lethal hit, it could survive (Fig. 5). The percentage of hit-survival cells among the irradiated cell population was 10%, which was coincidentaly the same value as the difference between the surviving fraction and the non-hit cells. Accordingly, hit lethality can be considered to be related to its traversed locality. In addition, a single hit of either Fe or of Ar-ion to the cell nucleus is considered to be a lethal hit event.

The cell survival curve is predicted to be exponential in a single-hit lethal case. If more than one hit is required to the observed effect, a threshold by definition, exists for the biological response. Although, the dose-response curves in Fe- or Ar-ions do not exhibit a discontinuous threshold response rather bend smoothly away from the origin. Judging from the random distribution of hits per cell in the irradiated population, some cells may receive one or more hits in their nuclei, while others are not hit. In multi-hit lethal cases, the survival curve has smoothly bending shoulders with a zero initial slope. Assuming that heavy charged particles deposit almost their total energy when traversing through a cell nucleus[22], multi hit case at higher LET can be expected to produce excess biological damage to inactivate the cell (the "over killing effect").

We showed that cell survival can be measured as a function of the particle traversals through the cell nucleus or cytoplasm (Fig. 8). The biological response to heavy-ions differs from sparsely ionizing radiation, depending on the physical nature of the hit of heavy charged particles. The merits of using CR-39 plastic as a detector of hit cell events[24)25)] include its portability, low cost and high sensitivity to heavy-ion traversal. The potential application of heavy-ion therapy for clinical use especially in cancer therapy, is therefore expected. This expectation is based on the biological characteristics of heavy charged particles and their precise dose-localization[4)21)23)]. Therefore, the use of iron and argon-ions are recommended for cancer therapy. In conclusion, these results showed that the evaluation of cell death by a single-hit traversal of heavy-ions through the cell nucleus can be determined based on the delay in cell division. The difference between the surviving fraction (30%) and non-hit cells (20%) was 10%, which means that some cells could survive even after exposure to high-LET radiation. This fraction corresponds to the population with an unlethal hit (hit-survival cell). In order to evaluate the biological effects of heavy ions, more difinitive studies have to be done on the direct relationship between the hit site in the cells and the resulting division delay after heavy-ions exposure. The combined use of the time-lapse observations and CR-39 plastics for determining the cell killing effect should therefore be further studied in the future.

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(和文抄録)

分裂遅延とCR-39プラスチックを指標とした重イオン（鉄，アルゴンイオン）を照射されたCHO細胞におけるヒットの判定

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加速された粒子線の細胞致死作用はLET（線エネルギー付与）に依存し，コロニー形成能の喪失を指標とした増殖死の誘発効果は，LETが100-200 keV/μmで最大（RBE値，約3）に達するが，LETがこれを越えると効果はかえって低下することが知られている．LETが非常に高い粒子線では，細胞に当たると細胞死に必要な量以上のエネルギー付与をするので，単位線量当たりの細胞致死効果で観察すると効果が低下すると考えられている．重イオンの致死効果を評価する第一歩として，重イオンでヒットされた細胞を正しく判定する方法を確立することが必要であると考え，ヒットと非ヒットを各々の細胞の分裂遅延の有無で判定し，さらにCR-39プラスチック上のピット像を細胞の像と対応させることにより，重イオンの通過点すなわちヒット部位の判定を行った．

鉄イオン4 Gy（コロニー生存率が30%に相当）を照射した個々のチャイニーズハムスター由来CHO細胞について，微速度写真法による観察で調べた結果，20%の細胞が分裂遅延を示さなかったが，この値は，細胞の核の断面積を平均130 μm^2としてポアソン分布に従って計算して求めた場合の，鉄イオンが核にヒットしなかった細胞の割合に等しかった．次に，単層培養した細胞面上の重粒子線の通過点を正しく判定する目的で，培養フラスコの底にCR-39プラスチック板を貼付して同様な実験を行った．その結果，細胞核に対応する部分にイオン通過により形成されるピット（穴）が認められない細胞（すなわちヒットされなかった細胞）の割合が20%であり，微速度写真撮影法による観察で分裂遅延を示さなかった細胞の割合と一致した．同様な実験をアルゴンイオンによる細胞ヒットについても検討し，上記2通りの方法によって重イオンによる細胞ヒットを判定できることが明らかとなった．なお，コロニー形成法による生存率との間で認められる10%の違いは，細胞集団の中に重イオンが核にヒットしたものの致死的損傷とならなかったものがあることを示している．