Short Communication

Differences in heavy-ion-induced DNA double-strand breaks in a mouse DNA repair-deficient mutant cell line (SL3-147) before and after chromatin proteolysis

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DNA double-strand breaks induced by X- or neon beam-irradiation in a DNA double-strand break-repair-deficient mutant cell line (SL3-147) were examined. The increase in the number of DNA double-strand breaks was dose depend after irradiation with X-rays and neon beams and was enhanced by chromatin-proteolysis treatment before irradiation. These results suggest that the induction of DNA double-strand breaks by ionizing radiation, including heavy-ions, is influenced by the chromatin structure.

INTRODUCTION

The chief biological effects of heavy-ion radiation are thought to be mediated by DNA damage. The extent of cell death after charged-particle irradiation may depend on unrepairable DNA double-strand breaks3).

Recently, pulsed-field gel electrophoretic analysis has been used to study radiation-induced DNA double strand breaks in several mammalian species2)–6). The number of DNA double-strand breaks induced by ionizing radiation is greater in proteolysed than in intact chromatin7).
Some types of radiation-sensitive mutants of mammalian cells are reported to be DNA repair-deficient. Many mutant cell lines that are deficient in DNA repair have been established\(^{8-12}\).

The ionizing radiation-sensitive mouse mutant cell line SL3-147 was established from the mouse LTA cell line after MNNG treatment (Sato, unpublished). The DNA double-strand break repair deficiency of this cell line was shown by pulsed-field gel electrophoretic analysis after X-ray irradiation\(^3\).

The nature of DNA breaks induced by an ion beam with a high LET may differ from those induced by X-rays because the ratio of non-reparable to total breaks is higher for high LET ion beams. We elsewhere report the biological effects of neon-beam irradiation on SL3-147 cells and the parental LTA cells\(^\text{13}\). Differences in the inactivation cross sections of SL3-147 and LTA cells decrease with increasing LET, but the inactivation cross section of a neon-beam on SL3-147 cells is larger than that on LTA cells even in high LET regions (LET \(~ 300\) keV/\(\mu\)m). The reason for the larger inactivation cross section on SL3-147, however, is not clear. This result suggests that the dsb repair system works correctly for dsb induced by a neon beam with high LET in normal cells even though the working efficiency decreases. Another possible reason is a difference in chromatin structure between the SL3-147 and parental LTA cells.

We analyzed the DNA double-strand breaks induced by X-irradiation and by neon beam irradiation in SL3-147 cells using pulsed-field gel electrophoresis. Induction of DNA double-strand breaks was detected after exposure to X-rays and to neon beams of low and high LET. We also obtained findings on the relationship of the chromatin structure and the X-ray or neon beam induced DNA double-strand breaks.

**MATERIALS AND METHODS**

**Cell culture**

Mouse cell line SL3-147 was cultured in α-MEM medium (Gibco) supplemented with 10% fetal bovine serum and under 5% CO\(_2\) at 37°C. SL3-147 cells deficient in DNA double-strand break repair were established from mouse LTA cells after N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) treatment (Sato, unpublished and Warters et al.\(^3\)).

**X-irradiation**

Cells growing exponentially in a culture dish (\(r = 30\) mm, Falcon) after 2 days of culture or agarose plugs containing naked DNA (see later) were exposed to X-radiation from a Shimazu model Shinai X-ray Irradiator at 200 kVp, 20 mA, with 0.5 mm aluminum and 0.5 mm copper filtration.

**Neon-beam irradiation**

SL3-147 cells grown in monolayer in a 25 cm\(^2\) culture flask (Falcon) for 2 days or agarose plugs containing naked DNA (see later) were exposed to the neon-beam from a cyclotron (RIKEN, Wako-shi, Japan) at the initial energy of 135 MeV/u. The apparatus for irradiation
and heavy-ion dosimetry have been described elsewhere\textsuperscript{14,15}. In brief, a combination of wobbler magnets and a gold scatterer was used to obtain a uniform irradiation field of 5 cm radius at the sample position. For irradiation with heavy ions an auto sample changer was used. The irradiation dose was monitored with a parallel plate ionization chamber placed next to the vacuum window. The energy of the heavy-ion at the sample was changed by inserting PMMA sheets of a range shifter in the beam course. The bragg curve was measured by changing the thickness of the PMMA sheets, a small ionization chamber being placed at the sample position. The dose distribution obtained was calibrated by measuring the particle fluence at zero absorber thickness\textsuperscript{14,15}. The dose for the agarose plug containing the naked DNA was taken to be the dose at the center of the agarose cylinder \((r=1 \text{ mm})\). After irradiation, cells recovered from the monolayer culture using trypsin were the sample used for pulsed-field gel electrophoresis.

\textbf{Pulsed-field gel electrophoresis}

After irradiation with X-rays or neon beams, cells were collected using trypsin treatment then washed with PBS. The samples for pulsed-field gel electrophoresis were prepared according to the modified method of Warters et al. (1993)\textsuperscript{8}. Cells were resuspended in Lysis buffer (LB) (100 mM EDTA, 20 mM NaCl, 10 mM Tris-HCl, pH 8.0) and mixed with an equal volume of LB containing low melting point agarose (Takara Tokyo, 1\% in LB) previously incubated at 50°C. This cell suspension was placed in a tygon tube to form an agarose cylinder \((r=1 \text{ mm})\) at 4°C. The cylinders were removed from the tygon tubes and treated for 24 hr with 1\% N-lauroyl sarcosine (Sigma) and 1 mg/ml proteinase K (Wako Pure Chemicals, Osaka) in LB at 50°C to make the naked DNA. The agarose cylinders then were cut into small plugs containing about 1 \(\times 10^5\) cells then applied to an 0.8\% agarose gel (BRL) and electrophoresed for 68 hr at 60 V and 15°C in a Hexafield horizontal gel electrophoresis apparatus (BRL) with the electrical field alternating every 30 min. After electrophoresis, the gel was stained with ethidium bromide and photographed.

\textbf{Agarose plug containing naked DNA}

For irradiation of the naked DNA, cells cultured for 2 days were collected, embedded in agarose plugs, and treated with proteinase K as described above. The agarose cylinders \((r=1 \text{ mm})\) containing the naked DNA were irradiated with neon beams (The scheduled LET=60 keV/\(\mu\)m or 200 keV/mm). After irradiation, the cylinders were cut into small plugs, each containing about 1 \(\times 10^5\) cells, that were analyzed by pulsed-field gel electrophoresis.

\textbf{RESULTS AND DISCUSSIONS}

The amount of DNA double-strand breaks with X-irradiation was estimated by pulsed-field gel electrophoresis of SL3-147 cells (Fig. 1). SL3-147 cells are a good material for this type of study because DNA double-strand breaks are not repaired during the experimental procedure. The increase in the number of double-strand breaks was dose dependent. After X-irradiation, 5.7 Mbp DNA bands were present. These bands were scarcely observed in the X-irradiated
Fig. 1. DNA double-strand breaks after X-ray irradiation of intact or naked DNA. M, DNA marker (Schizosaccharomyces Pombe); lane 1, unirradiated SL3-147 cells (control); lane 2, SL3-147 cells irradiated with 30 Gy of X-rays; lane 3, SL3-147 cells irradiated with 80 Gy of X-rays; lane 4, unirradiated naked DNA (control); lane 5, naked DNA irradiated with 30 Gy of X-rays; lane 6, naked DNA irradiated with 80 Gy of X-rays; M, DNA marker (Saccharomyces Cerevisiae).

In contrast, the number of double-strand breaks found for irradiated naked DNA was greater than that found for intact cell irradiation. The 5.7 Mbp bands seen in the 80 Gy exposure of the intact cells were absent.

To estimate the biological effects of heavy ion radiation, SL3-147 cells were irradiated with neon beams from the cyclotron at RIKEN (Wako-shi, Japan). In a previous report\textsuperscript{13}, the inactivation cross section of SL3-147 cells irradiated with neon beams was larger than that of LTA cells at high LET regions (∼300 keV/μm). The cause of this larger inactivation cross section is not clear.

We analyzed the DNA double-strand breaks caused by neon beam irradiation using pulsed field gel electrophoresis (Fig. 2). The number of DNA bands eluted at the lower LET irradiation (scheduled LET=60 keV/μm; measured LET=62.8 keV/μm) and at high LET irradiation (scheduled LET=200 keV/μm, measured LET=205.5 keV/μm) increased with the dose (Fig. 2a,b). The 5.7 Mbp DNA bands were similar to those in X-irradiation, whereas no bands were present in the neon beam-irradiated LTA cells (data not shown). Possibly there are susceptible sites on each 5.7 Mbp of SL3-147 DNA. Chromosome analysis showed several double minutes in the SL3-147 cells (data not shown), and double minutes are reported to be closely related to highly repetitive genes\textsuperscript{10,17} that have much to do with drug resistance. Taking into consideration the results of Nevaldine et al.\textsuperscript{10} this band, may be the double-strand broken double minute's DNA. The fact that no or few double minutes were recognized in the LTA cells supports this hypothesis.
Proteolyzed chromatin samples embedded in agarose plugs (r=1 mm) were irradiated (Fig. 2c). The LET at the center of the agarose plug was 62.9 keV/μm for the low LET irradiation. The LET of the agarose plugs of the low LET neon beam irradiation ranged from 61.8 to 64.0 keV/μm, whereas for the high LET irradiation the LET at the center of the agarose plug was 209.3 keV/μm and the range was 162.6–401.3 keV/μm.

Both the low and high LET neon beam irradiations showed remarkable dose responses for the DNA double-strand breaks on naked DNA irradiation. The number of DNA double-strand breaks induced by neon beam irradiation after proteinase K treatment was greater than that for nontreated cells. An obvious decrease in the 5.7 Mbp DNA band concurrent with the increase in the relatively higher mobility bands (−1 Mbp) was seen. Preliminary quantification using the NIHImage supported this result (data not shown). This result is a strong indication that deproteinized chromatin shows hypersensitivity to heavy ion radiation as seen in X-ray irradiation.

Story et al (1993)\(^2\) reported that prior protease treatment enhanced the susceptibility of DNA double-strand-break induction to ionizing radiation. Removal of histones before γ-irradiation also increased the yield of DNA double-strand-breaks\(^1\). These findings were confirmed by our study. We also found a similar enhancement in heavy-ion radiation exposure. Destruction of the chromatin structure accounts for the hypersensitivity to heavy-ion radiation.
DNA DSB INDUCTION WITH HEAVY-ION

after proteolysis treatment.

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REFERENCES

M. MURAKAMI ET AL.


