Study on the Low Dose Hyper-Radiosensitivity through γ-H2AX Foci Analysis

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1. Introduction

Hyper radiosensitivity (HRS) is a phenomenon in which cells respond with greater sensitivity to lower doses of radiation exposure. [1] An early study suggested that the repair mechanism of DNA damage is not related to the HRS phenomenon. [2] Recently, the DNA repair process has been discussed as a decisive factor in the HRS phenomenon. [3] Also, in a study based on clonogenic assay, dose rate was suggested as an additional effector for HRS phenomenon. [4] The underlying mechanism HRS phenomena has not yet been fully explained. This study was conducted to investigate the effect of dose rate on HRS phenomenon by observing HRS in terms of γ -H2AX foci formation due to DNA double strand break (DSB).

2. Materials and Methods

2.1 Cell lines and cell culture

Preliminary experiments were performed with rat gliosarcoma cells [9L/lacZ, Catalog No. CRL-2200, ATCC, Manassas, VA, USA]. Cells were cultured in the mixture of 90% Dulbecco's modified eagle medium-and 10% fetal bovine serum. Cells in flasks were incubated at 37 ℃ with humidified 10% CO2. The culture medium was changed at least three times a week.

2.2 X-ray irradiation

X-ray irradiation was conducted at the SNU-HARDX facility, which consisted of the YXLON 450-D08 beam tube. [5] The beam tube operated at 150 kVp with beam currents of 1.25, 5, or 15 mA. X-ray exposures were made at varying dose rates of 0.125, 0.5, and 1.5 Gy/min. X-rays were delivered at doses up to 0.5 Gy at intervals of 0.1 Gy.

2.3 γ-H2AX assay

DNA DSBs induce phosphorylation of nearby H2AX histones. The phosphorylated H2AX histones are detected as individual γ-H2AX focal images. In this than the fitting level study, γ-H2AX foci were counted at 1 and 24 h post-irradiation. 1 h post-irradiation was practically the fastest time for foci counting whereas 24 h post-irradiation was chosen to observe the decrease in the number of γ-H2AX foci as a result of DNA damage repair.

After 1 or 24 h incubation following irradiation, cells on slides were fixed with 4% paraformaldehyde for 5 minutes, permeabilized in phosphate-buffered saline with 1% Triton X-100 for 10 minutes and blocked for 40 minutes in PBST (phosphate-buffered saline with 0.05% Tween 20) containing 10% bovine serum albumin. Next, cells were stained with primary antibody against γ-H2AX (anti-γ-H2AX phosphor S139) for 1 hour. Then cells were stained with goat anti-rabbit IgG H&L secondary antibody for 1 h in dark room and counterstained with 4'6-diamidino-2-phenylindole. All processes were performed at room temperature.

γ -H2AX foci were observed using a fluorescence microscope [Catalog No. BX53F, Olympus, Tokyo, Japan] and analyzed with "Cellprofiler" software. At least three independent experiments were performed to obtain one data value, and at least 800 cells were analyzed in each experiment.

3. Results

The excess numbers of γ-H2AX foci were recorded at 1 h (Fig 1) and 24 h (Fig 2) after irradiation. Data points were linearly fitted complying with the LNT model.

3.1 γ -H2AX foci formation

The excess number of γ-H2AX foci observed 1 h post-irradiation suggests the extent of DNA DSB production in cells. At 1 hour after irradiation, more excess γ-H2AX foci were counted at higher doses and higher dose rates for the same dose. The excess number of γ-H2AX foci increased with the dose at a greater slope when dose was delivered at higher dose rate. When X-rays were delivered at the highest dose rate of 1.5 Gy/min, γ-H2AX foci were observed more than the linear fitting level at 0.1 and 0.2 Gy. At lower dose rates of exposure, the number of excess γ-H2AX foci at 0.2 Gy dropped below the fitting level.

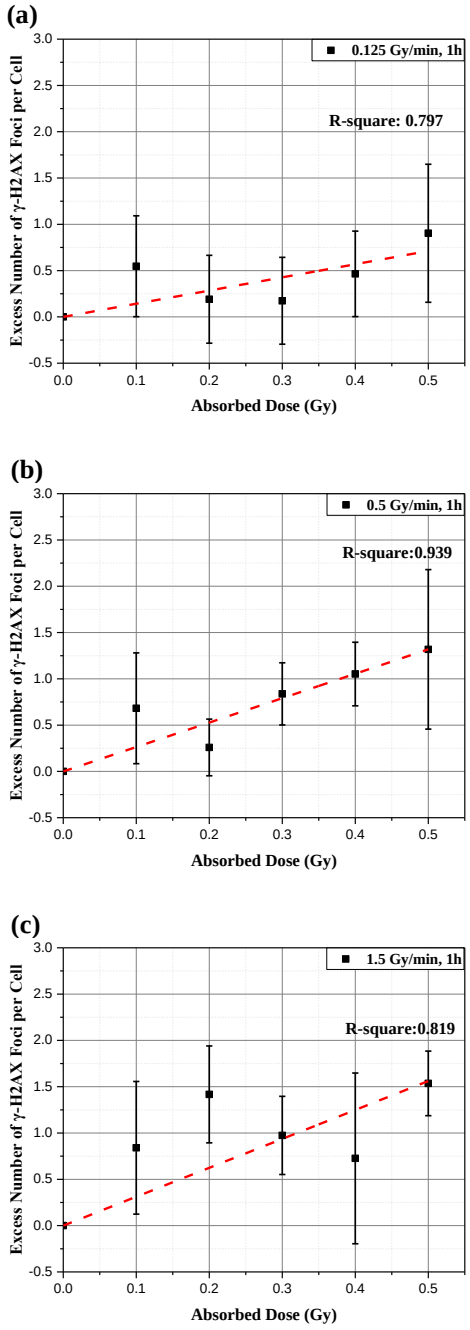

Figure 1. Excess numbers of γ-H2AX foci recorded 1 h post-irradiation in rat gliosarcoma cells at dose rates of (a) 0.125, (b) 0.5, (c) 1.5 Gy/min.

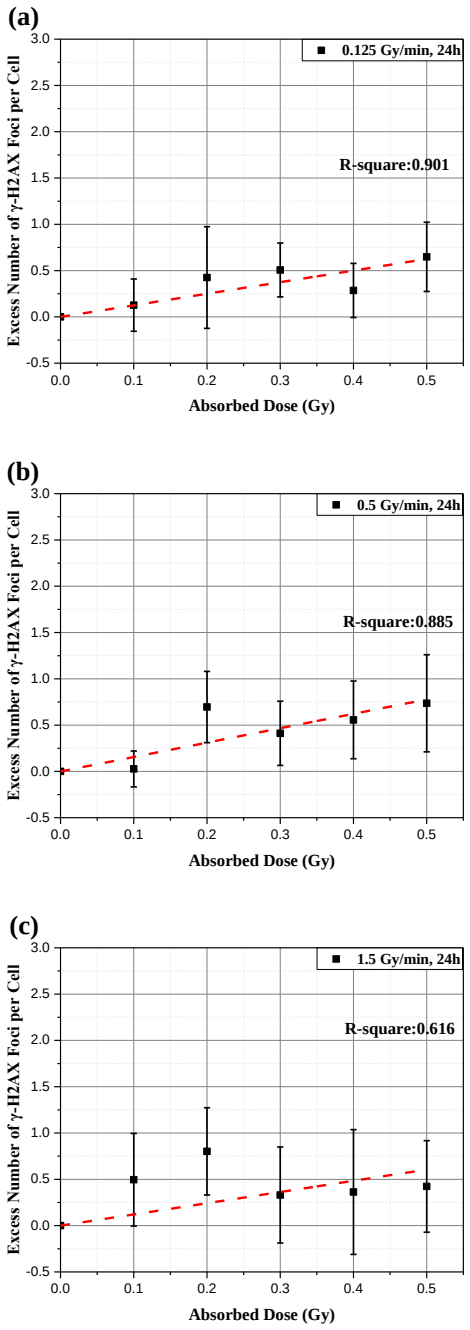

Figure 2. Excess numbers of γ-H2AX foci-recorded 24 h post-irradiation in rat gliosarcoma cells at dose rates of (a) 0.125, (b) 0.5, (c) 1.5 Gy/min.

3.2 DSB repair quantification

The γ-H2AX foci observed 24 h post-irradiation suggests a residue of DSBs recorded 1 h post-irradiation. Reduction in foci count would result from DSB repair. The following quantity can be defined as DSB repair rate over time from 1 to 24 h post-irradiation:

$$R_{D,DR} = 1 - \frac{FPC_{24h}}{FPC_{1h}}$$

$R_{D,DR}$ denotes the DSB repair rate after dose D is delivered at dose rate DR. FPC_t is the number of excess foci per cell at time t elapsed after irradiation. Figure 3 summarizes DSB repair rates over time 1 to 24 h post-irradiation in rat gliosarcoma cells.

The negative repair rates in Fig 3 are invalid and can be interpreted to indicate negligible repair rates. At 0.1, 0.4, and 0.5 Gy of exposure, cells showed positive repair rates that γ-H2AX foci produced 1 h post-irradiation decreased over time to 24 h post-irradiation. In the cells exposed to 0.3 Gy, repair rates were positive at 0.5 and 1.5 Gy/min, but became nullified at lower dose rate of 0.125 Gy/min. At 0.2 Gy of exposure, repair rate was nullified at 0.5 Gy/min as well as at 0.125 Gy/min.

4. Conclusion

Rat gliosarcoma cells responded to 0.2 and 0.3 Gy of X-ray exposure by expressing a limited number of γ-H2AX foci 1h post-irradiation. Considering that phosphorrrylation of H2AX followed by dephospho-rylation of γ-H2AX is the process of DSB repair, [6] expression of a limited number of γ-H2AX foci soon after irradiation may imply less chance of DSB repair resulting in more residue of DNA damage.

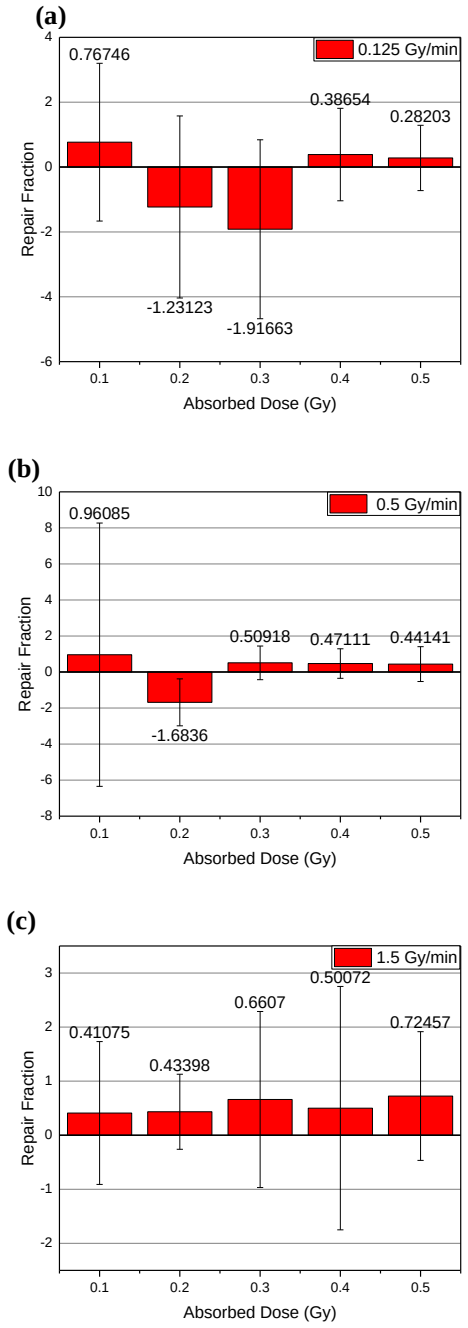

Figure 3. DSB repair rates over time 1 to 24 h post-irradiation in rat gliosarcoma cells exposed to 0.1 to 0.5 Gy at dose rates of (a) 0.125, (b) 0.5, and (c) 1.5 Gy/min.

REFERENCES

[1] B. Marples and M. C. Joiner, The response of chinese hamster V79 cells to low radiation doses: Evidence of enhanced sensitivity of the whole cell population, Radiat. Res., Vol. 133, No. 1, pp. 41-51, 1993.

[2] Wykes. S., Piasentin. E., Joiner. M., Wilson. G., and Marples. B., Low-dose hyper-radiosensitivity is not caused by a failure to recognize DNA double-strand breaks. Radiation Research, 165(5), 516-524, 2006

[3] Martin. L. M., Marples. B., Lynch. T. H., Hollywood. D., and Marignol. L., Exposure to low dose ionising radiation: Molecular and clinical consequences. Cancer Letters, 349(1), 98-106, 2014
[4] Thomas. C., Martin. J., Devic. C., Bräuer-Krisch. E., Diserbo. M., Thariat. J., and Foray, N., Impact of dose-rate on the low-dose hyper-radiosensitivity and induced radioresistance (HRS/IRR) response. International Journal of Radiation Biology, 89(10), 813-822, 2013
[5] Lee. K.-M., Kim. S.-R., and Kim. E.-H., Characterization of dose delivery in a hard X-ray irradiation facility. Journal of nuclear science and technology, 49(6), 655-661, 2012
[6] Rothkamm. K., Krüger. I., Thompson. L. H., & Löbrich. M., Pathways of DNA Double-Strand Break Repair during the Mammalian Cell Cycle. Molecular and Cellular Biology, 23(16), 5706-5715, 2003