Quantitation of gamma-H2AX expression for the assessment of radiation quality

Ui-seob Lee and Eun-Hee Kim*
Nuclear Engineering Dept., Seoul National Univ., 1 Gwanak-ro, Gwanak-gu, Seoul, 151-744, Korea
**Corresponding Author: eunhee@snu.ac.kr*

1. Introduction

Radiations can be differentiated according to the amount of energy they deliver to the medium per unit length of track (linear energy transfer or LET). Different LET values imply different extents of DNA damages caused by exposure to different radiations. Attempts have been made to quantitate DNA damages from different points of view, counting chromosome aberrations or measuring DNA double strand breakages [1-3]. The gamma-H2AX assay enables one to identify the complexity of DNA damages by the size of gamma-H2AX foci [3, 4].

In this study, we investigated the gamma-H2AX expression, in terms of quantity and quality of gamma-H2AX foci, as an indicator of radiation impact and discussed the grounds with the data from computer simulations.

2. Methods

2.1 Cell culture and irradiation

Normal human lung epithelial cells (Nuli-1, CRL-4011, ATCC, VA, USA) were cultured in airway epithelial cell basal medium (PCS-300-030, ATCC) containing bronchial epithelial cell growth kit (PCS-300-040, ATCC). Cells were incubated at 37 °C under humidified 5 % CO_2 environment.

Cells were irradiated with X-rays and alpha particles in the HardX-SNU facility [5] operating with YXLON 450-D08 beam tube at 150 kVp and in the ALPHACELL-SNU [6] irradiator with a 371.1 kBq Am-241 disc source, respectively. Dose rates were quite comparable at 0.064 Gy/min and 0.079 Gy/min for X-ray and alpha particle exposures, respectively.

2.2 Gamma-H2AX assay

One hour after irradiation, cells were fixed with 4% paraformaldehyde (163-20145, Wako) for 15 minutes. Fixed cells were permeabilized by 1 % Triton X-100 (T9500-010, GenDEPOT) solution for 15 minutes. 10 % bovine serum albumin (BSA) solution was added to get only the desired gamma-H2AX signal. Cells were treated with anti-gamma-H2AX phosphor S139 antibody (Ab2893, Abcam. primary antibody) in 1 % BSA and then with goat anti-rabbit IgG H&L (Ab6717, Abcam. secondary antibody) in 1 % BSA for one hour each. Cells were then stained with 4' 6-diamidino-2-phenylindole (DAPI) in fluoroshield mounting medium.

The foci images were taken by using a fluorescence microscope (BX53F, Olympus). We used 'CellProfiler' (Broad Institute's Imaging Platform) to count the number of gamma-H2AX foci per cell (FPC). Foci were categorized according to their sizes. The number of FPC were normalized to that of control cells.

2.3 PHITS simulation

The PHITS (Particle and Heavy Ion Transport code System, Version 3.02) was employed to assess the cellular dose distribution. Geometries of the HardX-SNU and the ALPHACELL-SNU systems were simplified. The cell geometry was set as a square lattice of 10 μm. Using the T-heat (dose for photons) and T-deposit (dose for charged particles) tallies, the size of energy deposition at each site of cells was estimated.

3. Results

3.1 Number of gamma-H2AX foci per cell

Fig. 1 shows the average number of gamma-H2AX foci in individual cells induced by X-rays and alpha particles. At each dose level ranging up to 0.5 Gy, greater numbers of gamma-H2AX foci were observed in the cells exposed to X-rays than in those to alpha particles, which did not correspond to the idea that alpha particles of higher LET would cause more DNA double strand breakages than X-rays of lower LET.

3.2 Size distribution of gamma-H2AX foci

Fig. 2 presents the probability distribution of gamma-H2AX foci size in the cells exposed to X-rays at 0.15 or 0.2 Gy in comparison with that in the cells exposed to alpha particles at 0.0625 or 0.125 Gy, respectively. The distributions by alpha particle exposures were shifted toward the greater foci as compared to those by X-rays. The center of distribution transition was the cell size category of 1.3 ~ 1.9 μm^2.

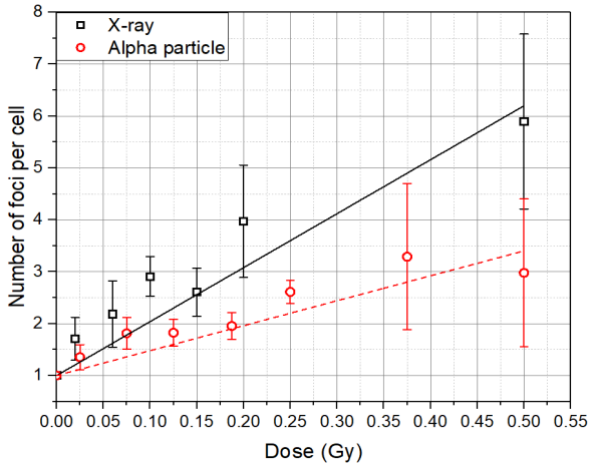

Fig. 1. Numbers of gamma-H2AX foci per cell in normal human lung epithelial cells induced by X-ray and alpha particle exposures. The error bars represent the standard errors.

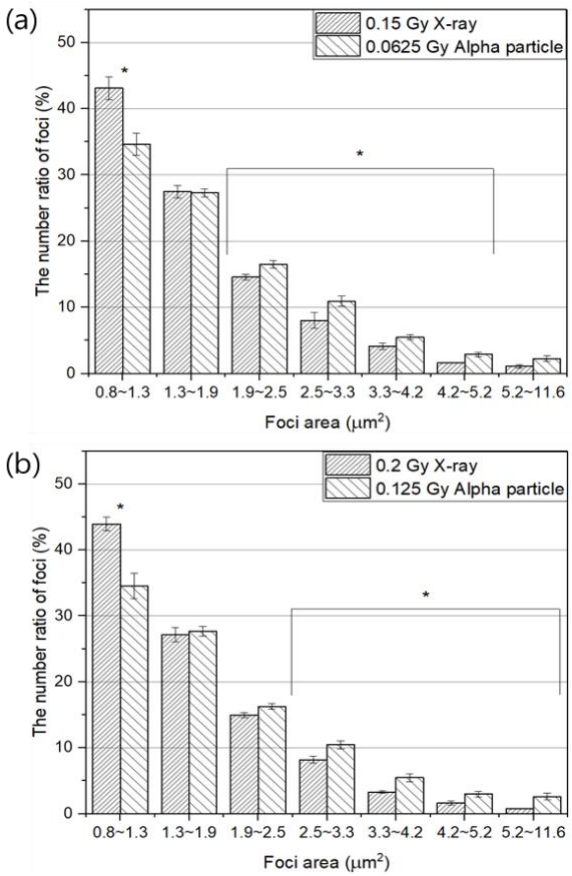

Fig. 2. Distributions of gamma-H2AX foci size in cells exposed to (a) 0.15 Gy of X-rays and 0.0625 Gy of alpha particles and to (b) 0.2 Gy of X-rays and 0.125 Gy of alpha particles. The error bars represent the standard errors. The asterisks indicate the significant differences between data for two groups (p-value < 0.05).

3.3 Energy deposition in cellular targets

Fig. 3 depicts the spatial distribution of energy deposition by 0.1 mGy of X-ray and alpha particle exposures of cells in square dishes. From exposure to alpha particles, only 0.055% of cells were affected whereas approximately 4% of cells were affected by X-ray exposure. On the other hand, the energy deposited in each cell is much lower from X-ray exposure as compared to alpha particle exposure.

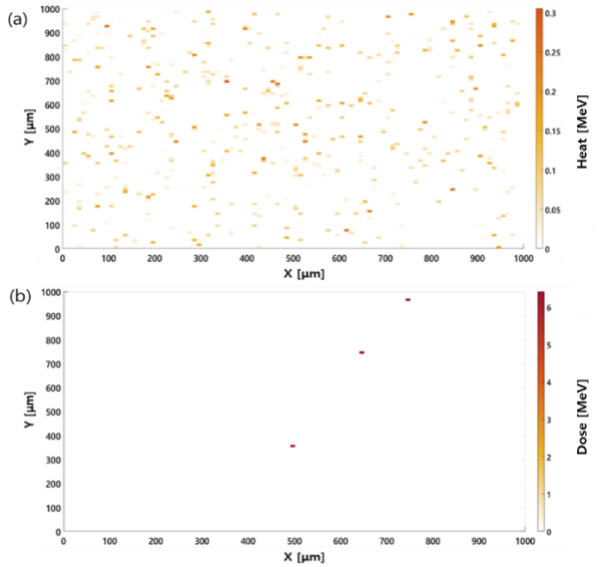

Fig. 3. Energy depositions in each mesh (10 μm × 10 μm) of cell dish by (a) X-ray and (b) alpha particle exposures both at 0.1 mGy.

4. Conclusion

Regarding the induction of DNA double strand breakages, the number of gamma-H2AX foci per cell did not properly indicate the radiation quality whereas the size distribution of gamma-H2AX foci would better inform the quality of radiation.

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