Cell size matters in gamma-H2AX assay for low-dose alpha particle effect assessment

Ui-seob Lee* and Eun-hee Kim
Department of Nuclear Engineering, Seoul National University
1 Gwanak-ro, Gwanak-gu, Seoul, 151-744, Republic of Korea
**colorpink1@snu.ac.kr, Corresponding Author: eunhee@snu.ac.kr*

1. Introduction

Gamma-H2AX assay is an immuno-fluorescence experiment that enables detecting the location and number of DNA double strand breaks (DSBs) in cells. Assuming that a single gamma-H2AX focus corresponds to a single DSB, one can convey the severity of cellular damage by *the number of gamma-H2AX foci per cell* (FPC) [1]. Under uniform radiation beam intensity, cells would respond with similar numbers of gamma-H2AX if they are similar in cross section. If cells are not represented by a common size, however, a larger cell has a greater chance of radiation exposure and has a better chance of counting a greater number of foci. In other words, the cell size distribution would be reflected in the FPC distribution.

In the conventional gamma-H2AX assay, the mean FPC value solely indicates the level of cellular damage under a certain radiation exposure. The purpose of this study is to investigate the FPC distribution in connection with the cell size distribution. The high-LET alpha beam was employed for radiation exposure so that a single track of radiation leaves a meaningful amount of energy in the cell.

2. Methods

2.1 Cell culture and Irradiation

Normal human lung epithelial cells (Nuli-1, CRL-4011, ATCC) were seeded on a collagen-coated flask, cultured in airway epithelial cell basal medium (PCS-300-030, ATCC, VA, USA) containing bronchial epithelial cell growth kit (PCS-300-040, ATCC), and incubated at 37 ℃ under humidified 5% CO2 environment.

Cell irradiation was conducted in the alpha particle irradiation chamber of the Radiation Bio-engineering Laboratory at Seoul National University. The alpha dose rate was 0.064 Gy/min.

2.2 Gamma-H2AX assay

After irradiation, cells were washed with Dulbecco's Phosphate-Buffered Saline (DPBS, 17-512F, Lonza) and fixed in 4 % paraformaldehyde (163-20145, Wako). After fixation, samples were permeabilized with 1 % Triton X-100 (T9500-010, GenDEPOT) solution for 15 min. Those cells were then blocked with 10 % Bovine Serum Albumin (BSA) solution. Anti-gamma-H2AX phosphor S139 antibody (Ab2893, Abcam. primary antibody) in 1 % BSA was added to the samples. One hour later, goat anti-rabbit IgG H&L (Ab6717, Abcam. secondary antibody) in 1 % BSA was put into samples. Cells were stained with DAPI in fluoroshield mounting medium.

The gamma-H2AX foci images were taken by using a fluorescence microscopy (BX53F, Olympus). The countings of gamma-H2AX foci per cell were analyzed by the image analyzing program 'CellProfiler' (Broad Institute's Imaging Platform). The FPC values at several dose levels were recorded after subtracting the background from each of the FPC readings.

2.3 Estimation of nucleus sizes and alpha particle tracks per cell

In this study, we distinguished cells of different sizes by the size of their nuclei considering that our sample cells were observed to roughly go along with their nuclei in size distribution.

The sizes of individual sample cells were figured out from the image data of cells by using the CellProfiler program. This program can count the number of nuclei within a specified size range when the boundary values are given in pixel unit. Sample cells were categorized by their nucleus diameters in an interval of 10 pixels. With the human normal lung epithelial cells, the nuclei took ~22% of the cell pixels in average. The average nucleus size of our sample cells was about 14 μm in diameter, which corresponds to around 54 pixels in image data.

From the 371.1 kBq ^{241}Am source, 371,100 alpha emissions were expected per second. Considering the distance between the source and the cell dish, the average number of alpha particles entering a single cell was about 0.011 per every second on the condition that the particle emission was isotropic.

3. Results

3.1 Conventional gamma-H2AX foci counting

The FPC values observed after alpha particle exposures are shown in Fig. 1 in comparison with the expected values on the condition of uniform-sized sample cells. The observed FPC values, increasing with dose, overlapped with the expected ones at low dose region. At over 0.2 Gy, however, the observed value did

not increase with dose anymore, turning from slightly higher to lower than the expected.

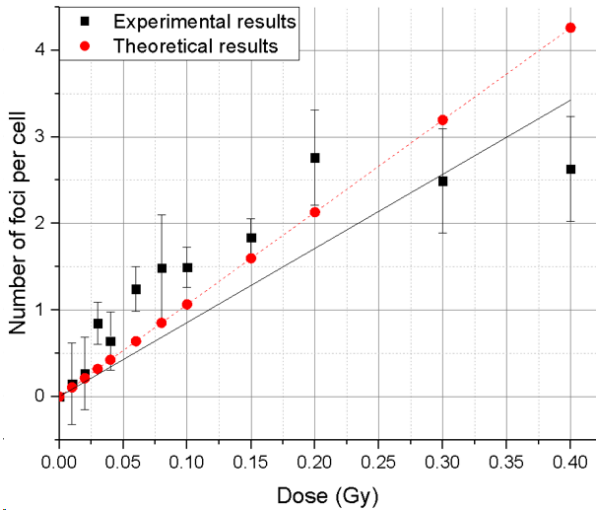

Fig. 1. Mean numbers of gamma-H2AX foci per cell observed with normal human lung epithelial cells after alpha particles exposures at different doses (square) in comparison with those expected from cells of uniform size (circle). Error bars show the standard errors.

3.2 Gamma-H2AX foci per cell vs. cell size

The nucleus-size distributions from sample cells are given in Fig. 2. The size difference among sample cells was not ignorable. The size distributions of sample cells at 0.15 Gy and 0.2 Gy were comparable to each other.

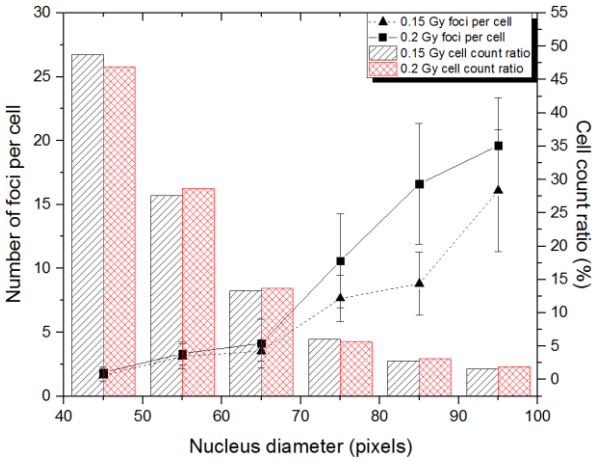

Fig. 2. Mean numbers of gamma-H2AX foci per cell (line graph) observed with normal human lung epithelial cells after alpha particle exposures at 0.15 Gy (triangle) and 0.2 Gy (square) along with the nucleus size distributions of the sample cells (bars). The size of a single pixel is 0.2567 μm x 0.2567 μm.

Fig. 2 also presents the mean FPC values varying with the nucleus size after alpha particle exposures at 0.15 and 0.2 Gy. The FPC values were averaged among sample cells of the same size. The mean FPC value was slightly greater at 0.2 Gy than at 0.15 Gy for all sizes of cells. As presumed, the mean FPC value increased with the nucleus size approximately in proportion of the nucleus cross section. The mean FPC from the whole sample cells without consideration of nucleus size was 1.9 for the cells at 0.15 Gy and 2.8 for those at 0.2 Gy.

4. Conclusion

Gamma-H2AX is a powerful tool for investigating the cellular response at low-dose exposure. If the gamma-H2AX assay is performed with cells of the same size, "the average number of foci per cell" may accord with the overall response of sample cells to radiation exposure. With cells of non-uniform size, however, one should be cautious in taking the value as an index of the severity in cellular effect of radiation exposure. According to our experiments, a portion of sample cells carried DSBs of more than 5 times greater number than the mean FPC value and might play a critical role in radio-response. Also, the saturation in the FPC counting at doses over 0.2 Gy guides one to take a look not only at the number of foci but also at the sizes of individual foci.

REFERENCES

[1] SV. Costes, A. Boissière, S. Ravani, R. Romano, B. Parvin and MH. Barcellos-Hoff. Imaging features that discriminate between foci induced by high- and low-LET radiation in human fibroblasts. *Radiat Res*. 165; 505-15 2006