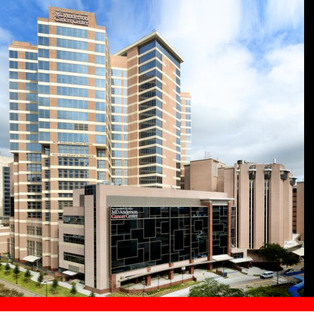

Quantification of DNA Repair Rad51 Foci Using Fluorescence Intensity

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Background

Radiation can induce DNA double-strand breaks. Rad51 is a well-established biomarker of DNA homologous recombination repair (HRR) for DNA damage assessment. The current widely-accepted quantification method requires manual counting, which is time-consuming.

Herein, we investigate the potential of using the nucleus fluorescence intensity (FI) to reflect Rad51 foci changes after radiation. This method is referred to as fluorescence measurement in the following sections.

Methods

Radiation & Immunostaining

Head and neck cancer cells (SqCC/Y1) were exposed to a single dose of 4-Gy X-ray or proton radiation.

At 2 hours, 5 hours, and 24 hours post irradiation, immunocytochemical analysis were used to assess Rad51 foci formation. Secondary antibody was conjugated to Cy3 to visualize immunoreactivity.

DNA was stained with 4',6-diamidino-2-phenylindole (DAPI). Immunoreactions were visualized with a Leica Microsystem.

Fluorescence measurement & Manual counting

The FI was measured using ImageJ and the workflow is presented in **Fig. 1**. In ImageJ, the integrated density, which is the product of Area and Mean Gray Value, was used to represent the FI. The FI of regions of interest (ROI) was calculated by subtracting background readings using the below formula:

$$FI = Integrated\ Density - (Area\ of\ ROI) \times (mean\ background\ fluorescence\ readinngs)$$

To take the nucleus' variation in size into account, we divided the FI by pixels to get the average fluorescence intensity per area (AFIA). The AFIA of treatment groups were compared to the control with a ratio to reflect the changes in Rad51 FI after radiation. The number of Rad51 foci in the nucleus was obtained through manual counting. The results derived from the two methods were compared.

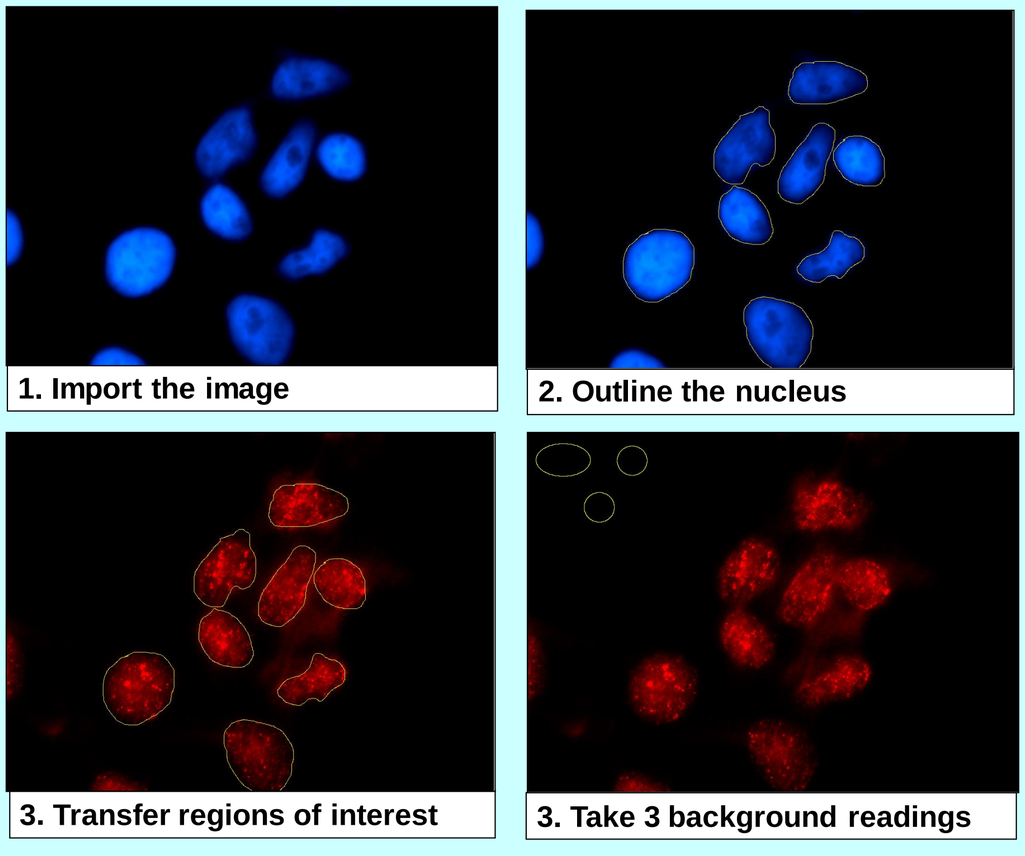

Fig. 1 Four steps to measure the nucleus fluorescence in ImageJ. Freehand tool was used to draw outlines for the nucleus and Control+ Shift+ E command was used to transfer the regions. The area and the integrated density of the ROI and the mean integrated density of the background were collected for calculation of the FI.

Results

For the SqCC/Y1 cell line at 2 hours post radiation, compared with the control group, manual counting indicated a 10% increase of Rad51 foci in cells treated with X-ray versus a 20% increase of foci in cell treated with proton; whereas, the increase of Rad51 foci was 19% after X-ray versus 39% after proton in average fluorescence intensity of nucleus (**Fig. 2 & Fig. 3**).

Increased Rad51 foci were observed at both 5 hours (57% in the X-ray group; 122% in the proton group) and at 24 hours (60% in the X-ray group; 123% in the proton group) in manual counting. Nevertheless, less to no increase of average fluorescence intensity of nucleus was observed after radiation at both 5 hours and 24 hours.

There is no statistical significance between the increase detected by manual counting and fluorescence measurement for Rad51 at 2 hours post radiation (**Fig. 3**). However, the changes detected by the two methods are significantly different when they measure the increase at 5h and 24h post radiation.

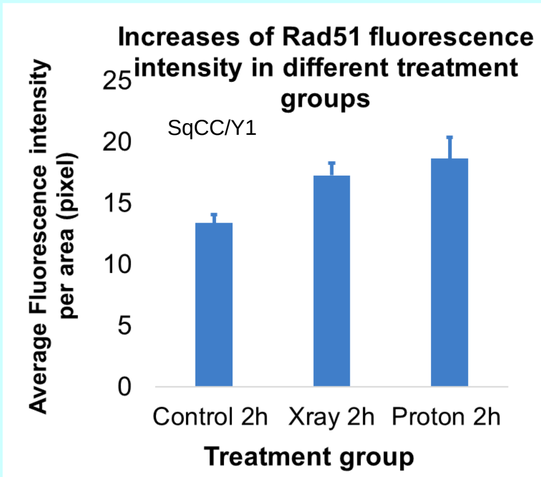

Fig. 2 AFIA of nucleus at 2 hours post radiation in different treatment groups. Both X-ray group and Proton group showed an increase in the AFIA. A t-test suggests that there is no statistical significance between the two groups (P-value = 0.51).

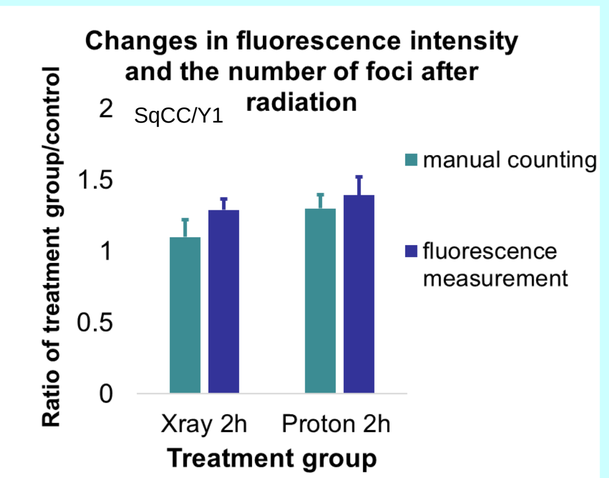

Fig. 3 Changes in AFIA and the number of foci at 2 hours post radiation. There is no statistical significance between the manual counting and the AFIA detected radiation induced Rad51 changes (P-value = 0.09 and 0.21 respectively).

Conclusions

- The average fluorescence intensity of nucleus may be able to reflect the radiation-induced increase of Rad51 foci at an early time point (2 hours after radiation) in the SqCC/Y1 cell lines, but not for late time points.
- This method needs to be tested in more foci, such as γH2AX and 53bp1, and in more cell lines for its ability to reflect the extent of radiation-induced DNA damage.

Acknowledgement

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