VALIDATION OF FACS-BASED ANALYSIS OF yH2AX FOCI FORMATION AND DECAY IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES AND U2OS CELLS.

Magdalena Płódowska[1], Aneta Węgierek-Ciuk[1], Anna Lankoff[2,1], Paweł Wołowiec[3], Andrzej Wójcik[4,1], *Halina Lisowska[1]**

1. Department of Medical Biology, Institute of Biology, Jan Kochanowski University, Kielce, Poland
2. Institute of Nuclear Chemistry and Technology, Warsaw, Poland
3. Department of Medical Physics, Holy Cross Cancer Center, Kielce, Poland
4. Centre for Radiation Protection Research, MBW Department, Stockholm University, Sweden

INTRODUCTION

The formation and decay of γH2AX foci is measured to assess the response of cells to ionizing radiation. γH2AX foci occur at sites flanking DNA double-stranded breaks (DSBs) and reflect the number of DSBs in a cell. Foci can be measured microscopically or with the help of flow cytometry (FACS). The aim of the study was to investigate a flow cytometry-based method optimized to measure γH2AX in peripheral blood lymphocytes (PBL) and U2OS cells. Manual counting on images was compared with a method measuring foci intensity by flow cytometry.

MATERIALS AND METHODS

Experiments were carried out with human osteosarcoma U2OS cells and PBL. Human venous blood was collected three times from three healthy donors. Peripheral blood lymphocytes were isolated by LymphoPrep. To measure the kinetics of γH2AX foci formation by flow cytometry in U2OS and PBL, cells were washed with PBS and fixed in Cytofix Fixation Buffer for 10 minutes. Then cells were again washed with PBS and incubated in cold methanol for next 5 minutes. After centrifugation, cells were washed in Perm/Wash and next with PBS. After that cells were incubated 1h with γH2AX antibody (Alexa Fluor 647 Mouse anti-H2AX; pS139) in Stain Buffer. Following a final wash, fluorescent cells were analyzed with a FACS (Becton, Dickinson and Co., San Jose, CA, USA). 20,000 cells per point were analyzed for γH2AX intensity.

AIM

The aim of the study was to compare the kinetics of foci formation and decay in U2OS cells and peripheral blood lymphocytes. Foci in U2OS cells were analysed by FACS after 2 Gy of gamma radiation at 0, 15, 30, 60, 120, 180 min and 24 h of repair time. In PBL foci were analysed manually after 2 Gy and 60, 120, 180 min and 24 h of repair time.

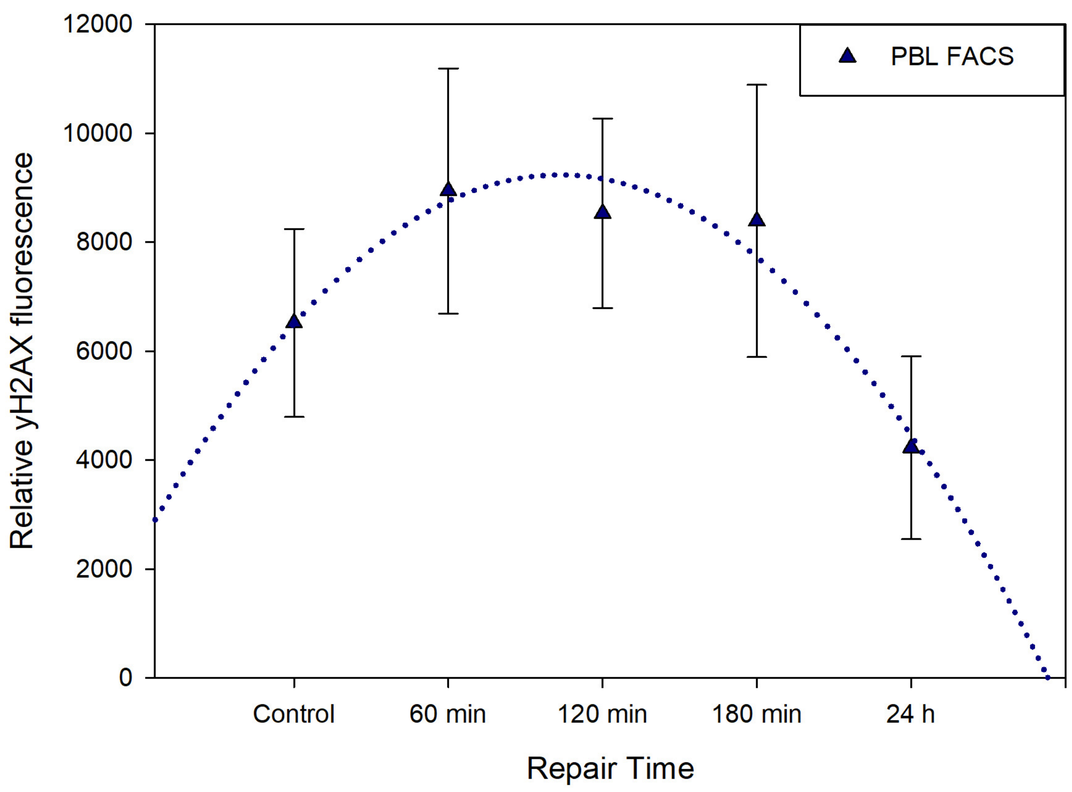

Figure 2

Kinetics of γH2AX foci formation and decay in in human peripheral blood lymphocytes exposed to 2 Gy of gamma radiation and allowed to repair at 37°C. Time scale is not proportional. The relative γH2AX fluorescence is expressed as mean from three donors and three independent experiments per donor. Error bars represent standard deviations from 12 experiments.

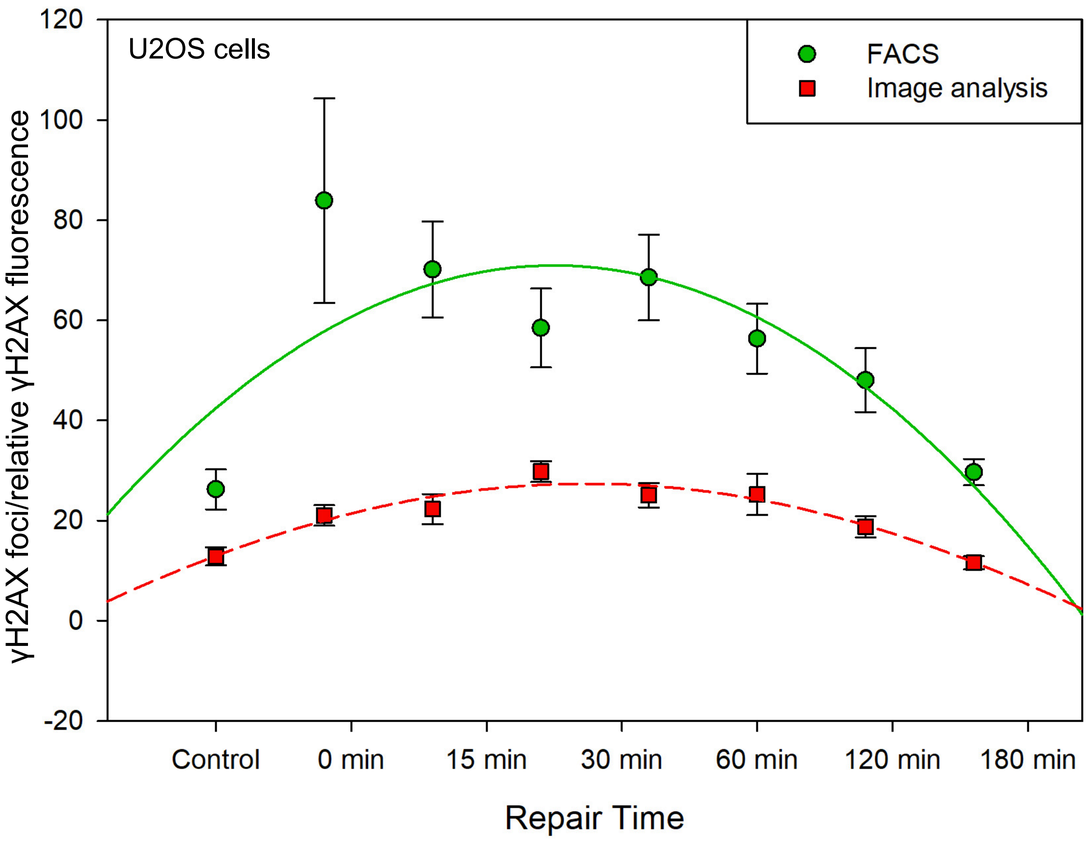

Figure 1

Kinetics of γH2AX foci formation and decay in U2OS cells exposed to 2 Gy of gamma radiation and allowed to repair at 37°C. Time scale is not proportional. Image analysis results are from manually counting foci per cell on images from confocal microscope. FACS analysis: a relative γH2AX fluorescence from flow cytometry. For clarity reasons, results from FACS analysis were divided by 10. Error bars represent standard deviations from 3 experiments.

RESULTS AND CONCLUSION

The results demonstrate that the kinetics of foci formation and decay in U2OS cells and PBL counted manually are more expressive and dynamic than results obtained by FACS. Fluorescence intensities measured by FACS are more spread-out than foci frequencies measured microscopically, resulting in smaller signal differences between selected repair times than in the manual method. This is probably due to the differences in the nature of measured signals: cell fluorescence intensity vs focus frequency. The advantage of FACS analysis is that the measured signal level is less dependent on the precision of sampling time post irradiation. This is an asset when the γH2AX focus assay is used to measure differences in individual response to radiation.

REFERENCES

1. Marková et al.l International Journal of Radiation Biology, 2007
2. Lisowska et al. International Journal of Radiation Biology, 2013
3. Plodowska et al. Scientific Reports, 2022