Development of γ-H2AX Assay for Radiation Biodosimetry : A Model Study in Cell Culture

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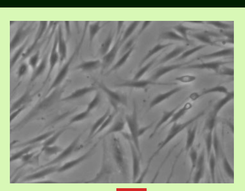
INTRODUCTION

Double-strand breaks (DSBs) of DNAs caused by ionizing radiation can pose detrimental damages on organisms which include genetic instability and cell death. In the case of either accidental or therapeutic exposures to radiation, it is of extreme importance to be able to quickly assess the potential health risks in patients for proper medical treatments. As part of the biodosimetry project initiated by the Office of Atoms for Peace in Thailand that started in 2013, we developed a biodosimetric measure based on the quantification of phosphorylated variant histone H2AX (γ-H2AX) formed at DSB sites. The presence of γ-H2AX marked one of the early key events in the repair process of DNA following the damage. In the first phase of this project, we established a linear dose response relationship based on a 2-dimensional cell culture model.

EXPERIMENTAL SETUP



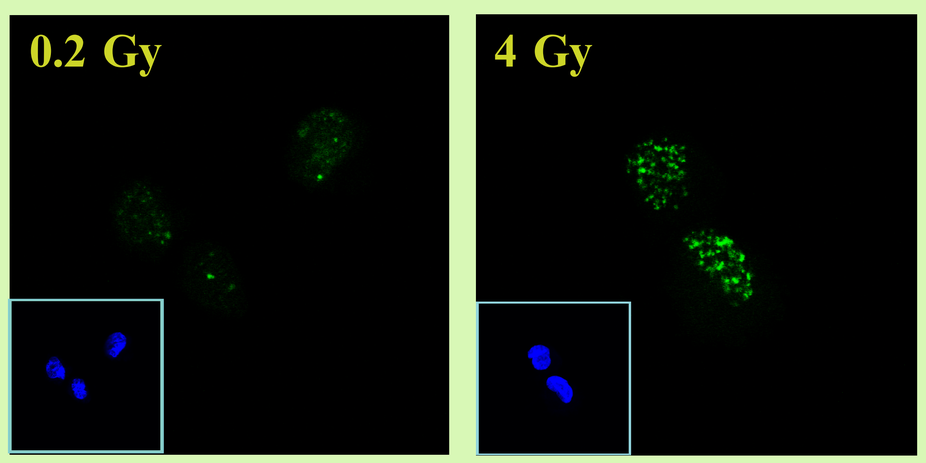
Cell culture model: human dermal fibroblasts (HDFBs) passages 8-12

Gamma irradiation
doses : 0, 0.2, 1, 2 and 4 Gy
dose rate : 0.21 Gy/min

Data collection and analysis

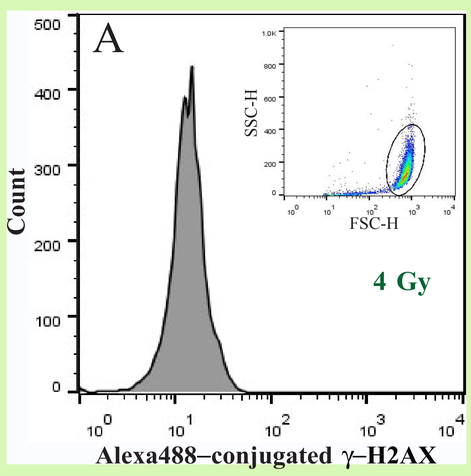
Detection: immunofluoresence labeling of γ–H2AX, cell viability and DNA fragmentation

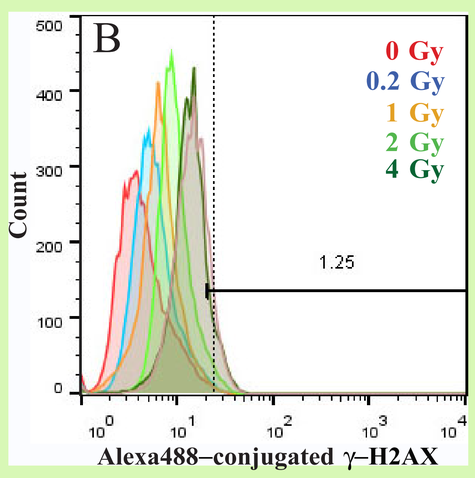
VISUALIZATION OF γ-H2AX

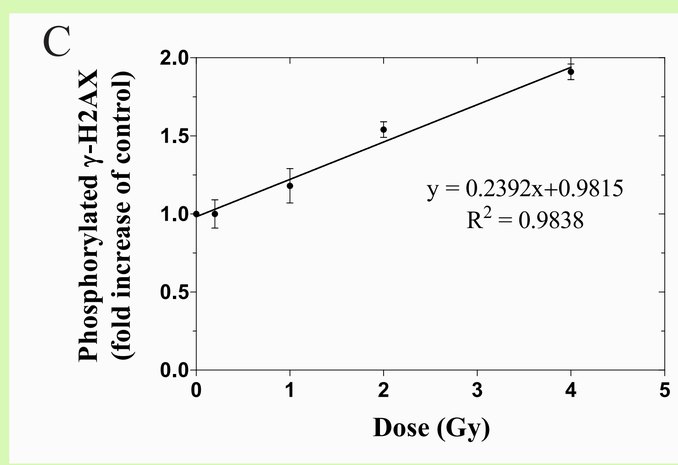


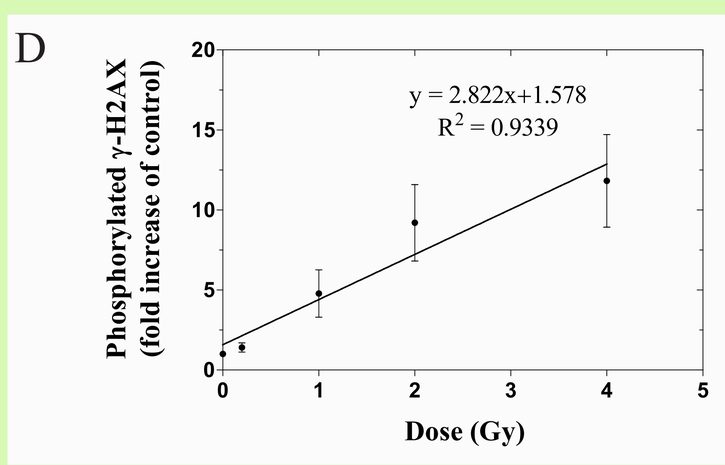
Bright foci (green) mark the presence of γ–H2AX inside the cell nuclei (blue, insets). Images are taken with a 60x objective lens.

ANALYSIS OF γ-H2AX : A DOSE RESPONSE RELATIONSHIP



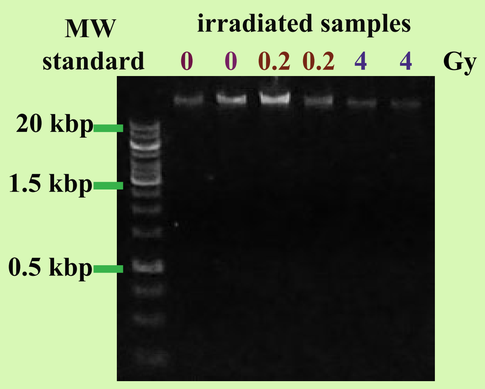




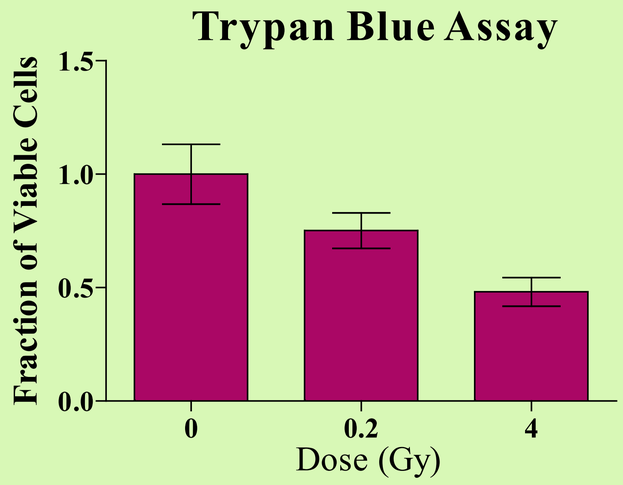


Analysis of γ-H2AX foci by (A)-(C) flow cytometry and (D) confocal imaging. A linear dose response relationship can be established based on (C) flow cytometric as well as (D) image analysis of foci intensity collected from the same sample set. Data are represented as mean ± SD (n = 5) normalized to control at 0 Gy.

CELL VIABILITY



0.6% agarose gel



At day 4 post irradiation, the number of viable cells declines with the increase in irradiation doses although DNA fragmentation was not observed on agarose gel.

CONCLUSION

In this present work, the dose response to gamma irradiation was successfully established for doses in the range of 0-4 Gy using cell culture as a model study. Analysis of γ-H2AX foci showed a linear increase with irradiation dose, accompanied by the decrease in fraction of viable cells.

ACKNOWLEDGEMENT

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