

Imaging analysis of DNA repair proteins using synchrotron X-ray microbeam

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Introduction

DNA double-strand breaks (DSBs) are the most lethal radiation-induced DNA damage and are subject to misrepair. In higher vertebrate cells, there are at least two major DSB repair pathways, namely non-homologous end-joining (NHEJ) and homologous recombination (HR) [1,2]. It has been identified many DSB repair proteins, which are engaged in NHEJ and/or HR.

Recently, imaging analysis of foci formation of DSB repair proteins is one of the available methods to elucidate the mechanisms of DSB repair.

However, it is difficult to observe the initial response of DSB repair proteins on the site of DSBs induced by X-rays, because conventional X-ray machines cannot perform target irradiation. In this study, we irradiated the targeted area of cell nuclei using synchrotron X-ray microbeam system[3] and observed the localization and phosphorylation of DNA repair and cell cycle checkpoint proteins by immunofluorescence.

Materials and Methods

Cells were seeded onto the original sample dishes with a polypropylene film coated with fibronectin sterilized by UV. Just before irradiation, cells were stained with Hoechst33258 to visualize each cell nucleus. Cells were irradiated with 5.35 keV monochromatic X-ray microbeam. Beam size was 5 $\mu\text{m} \times 5 \mu\text{m}$ square and the range of secondary electrons is 0.8 μm . After irradiation, cells were fixed with cold methanol and rinsed with cold acetone. Following blocking, cells were incubated with indicated primary antibodies overnight at 4°C. The dishes were incubated with Alexa-488-conjugated anti-rabbit IgG with or without Alexa-546-conjugated anti-mouse IgG (Molecular Probes) and were analyzed using an off-line laser scanning microscope.

Results and Discussions

We first identified the induction of DSBs in the irradiated site using both γ -H2AX specific antibody and phosphorylated DNA-PKcs at Thr2609 specific antibody (Fig. 1). The localization of γ -H2AX and phosphorylated DNA-PKcs was observed in the HeLa cell nuclei irradiated with microbeam. In addition, Localization of NBS1, 53BP1 and phosphorylated ATM at Ser1981 on the site of target irradiation could be observed predictably (data not shown). Next we observed the localization of cell cycle checkpoint proteins Chk1, Chk2 and SMC1.

Phosphorylated Chk2 and SMC1 are co-localized in the irradiated part of cell nuclei (Fig. 2). On the other hand, phosphorylated Chk1 observed entire irradiated cell nuclei. These results suggest that the response of DNA repair and cell cycle checkpoint proteins to DNA damage induced in part of cell nucleus by synchrotron X-ray microbeam varies in the each process of DNA repair and/or genome surveillance.

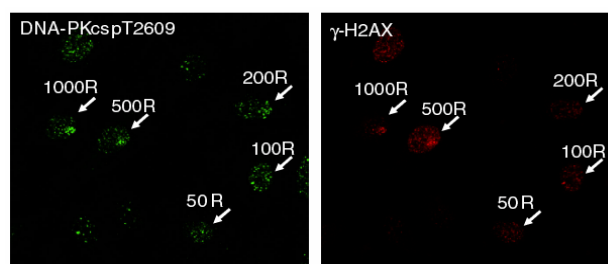


Figure 1 Localization of γ -H2AX and DNA-PKcs pT2609 on the site of target irradiation with microbeam.

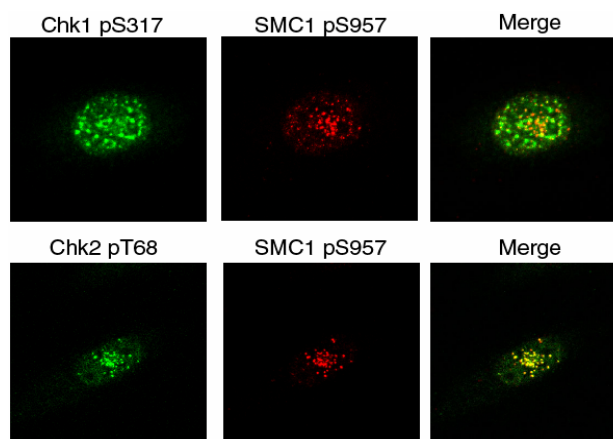


Figure 2 Localization of phosphorylated cell cycle checkpoint proteins Chk1, Chk2 and SMC1 in the microbeam-irradiated cell nuclei.

References

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