

## Cytochemical Detection of Active Oxygen Species Produced by Monochromatic X-rays

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### Introduction

We have been studied about the cytochemical detection of the nucleic DNA damages of the irradiated cells by monochromatic X-rays as reported in our previous reports.<sup>[1-8]</sup> The DNA damages were enhanced by bromodeoxyuridine (BrdU) at 0.918A.<sup>[2-3]</sup> It was also reported that OH radicals increased in the presence of the BrdU as the intermediate at the energy level (0.918A) of Br-atom absorption by ESR spin-trapping method.<sup>[9]</sup>

However, it will be not done to observe the cytochemical changes by the active oxygen species produced in the eelles irradiated by monochromatic X-rays using the fluorescent probes without the fixation of the cells.

### Manuscript preparation

*Human leukaemia cultivated (HL-525) cells:*

The HMF cells were treated with and/or without 100 µg/ml BrdU in the RPMI1640 medium for 6hr before the harvesting.

*Chemicals:*

HL-525 cells ware washed with new medium RPMI 1640 and were stained by the fluorescent probes for active oxygen species [2',7'-dichlofluorescence in diacetate succinidyl ester Oxy BURST Green, Molecular Probes, Inc.] for H<sub>2</sub>O<sub>2</sub> and luminol for OH radical] before the irradiations of the monochromatic X-rays.

*Radiation of monochromatic X-rayss:s*

The sample cells in the special vessel (2x1.3x0.1cm<sup>3</sup>) were irradiated by the monochromatic X-ray (0.918A, 2, 5, 10 Gy) with and/or without of BrdU.

*Cytochemical observation of the fluorescence:s*

The irradiated samples were observed by a fluorescent microscope (AH-2 type, Olympus) after the irradiation. Furthermore, the irradiated cells were stained by Hoechst 33342 (bisbenzimidide) for the nucleus and 8-Amilino-1-naphthlene sulfonic acid (ANS) for the membrane after 12 hr incubation and were observed the fluorescence imaging.

### Results and Discussion

The green fluorescence of the Oxy BURST Green reacted specifically with H<sub>2</sub>O<sub>2</sub> in the HL-525 cells was observed in the damaged cells irradiated by the monochromatic X-ray at 0.918A, especially, the intensity of the fluorescence enhanced in the case of BrdU presented. This enhancement was reasonable comparing from the data of ESR signal intensity (Increasing) of DMPO-OH radical adduct. In the results, the nucleus DNA of HL-525 cell was damaged by the OH radicals produced by the radio-sensitization of BrdU and the monochromatic X-ray of 0.918A because the nucleus DNA damages increased by the adding of BrdU in the cells. The presence of H<sub>2</sub>O<sub>2</sub> in the cells will be caused by the chain-reaction from the OH radicals.

Furthermore, the membrane damages also were observed by the ANS fluorescent staining, which induced to the apoptotic cell death detected by the Hoechst 33342 staining. This cell death also will be induced by the OH radicals.

### References

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