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Membrane lipid peroxidation in lung cancer cells after monoenergetic X-ray irradiation at the K-shell photoabsorption peak of phosphorus

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1 Introduction

The mean energy deposition (MED) in a small volume by photoninduced K-shell absorption followed by Auger cascades in an appropriate atom may be sufficient to cause ionizing radiation damage due to moderate radiation clusters. According to calculations by Humm (1984), the MED after phosphorus K-absorption in a volume with a 2 nm diameter is about 150 eV.

Phosphorus atoms are important constituents of DNA and of the membrane lipid bilayer. In our previous work, we showed that phosphorus K-absorption could efficiently induce lethality of mammalian cells and inactivation of yeast cells, *Escherichia coli* and bacteriophages. Only a few studies have investigated the membrane damage induced by phosphorus K-absorption. Indo et al. demonstrated that the formation of intracellular membrane lipid peroxidation products, such as 4-hydroxy-2-nonenal (HNE)-modified proteins, by X-rays (120kVp) correlated with the production of mitochondrial reactive oxygen species

The purpose of this study is to experimentally verify whether membrane lipid peroxidation is enhanced in cells by Auger cascades after K-shell photoionization of phosphorus.

2 Experiment

One day before irradiation, lung cancer cells were plated on glass bottom dishes in DMEM supplemented with 10% fetal bovine serum.

Monoenergetic X-ray irradiation was performed using a Si crystal monochromater at BL-27A. Three energies were chosen for irradiation: 2.147, 2.153 (phosphorus K-shell resonance absorption peak, P-K peak), and 2.160 keV. To assess the production of membrane lipid peroxide, cells were exposed to 0.516 C/kg for each energy radiation.

After irradiation, the cells were incubated at 37 °C for 2 hours and subsequently immunofluorescently labeled with anti-4-HNE mouse monoclonal antibody and Alexa Fluor 488 goat anti-mouse IgG(H+L) conjugate. Fluorescence images were obtained using a confocal laser scanning unit, inverted microscope (20x objective lens), and color chilled 3CCD camera. The intensity of the laser beam was set at a constant level to allow quantitative comparison of the fluorescence for the different samples. IPLab Spectrum software was used to obtain the values of the average fluorescence intensity per cell.

3 Results and Discussion

The formation of intracellular HNE-modified proteins, observed in green, represents lipid peroxidation in A549 cells (Fig. 1). All three tested irradiation energies led to a significant increase in immunofluorescent intensity as compared to that for the control. The fluorescent intensity measured after irradiation at 2.153 keV was higher than that for 2.146 and 2.160 keV irradiation ($P < 0.01$). Reproducibility of these results was confirmed through three experiments. The increment (21%) in relative fluorescent intensity after 2.153 keV radiation was about 2 times as high as that after radiation at the other two energies. Therefore, these results indicate that 2.153 keV (P-K peak) radiation induced the peroxidation of membrane phospholipids more efficiently than P-K off-peak radiation. We suggest that the energy deposition in the vicinity of phosphorus in the Auger cascade produced efficient reactive oxygen species (e.g., superoxide, hydrogen peroxide) in mitochondria.

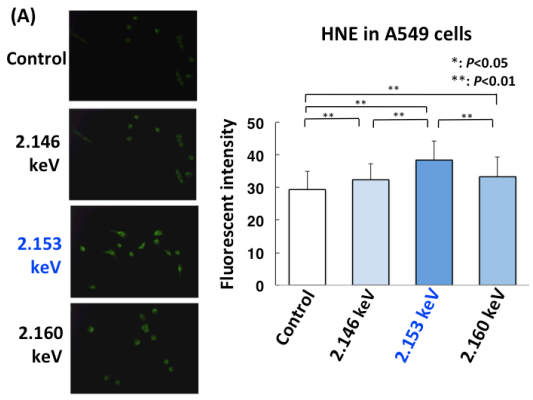

Fig. 1: Intracellular HNE generation in A549 cells following irradiation. Representative images of immunofluorescence staining (green) and the quantification of the fluorescence intensity are shown for irradiated and non-irradiated control cells. Bar represents the mean ± s.d. (n>100);**: P <0.01; Scheffe's F test.

References

[1] J. L. Humm, *KFA Report*, 100 (1984).
[2] H. Maezawa *et al.*, *Acta Oncologica*, **35**, 889 (1996).
[3] H. P. Indo *et al.*, *Free Radical research*, **46**, 1029, (2012).

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