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Preliminary study of X-ray sensitizer for cancer-specific therapy

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Introduction

To develop a cancer-specific radiotherapy, we have proposed a novel method that introduces a heavy atom, such as Nb or Ba, into cancer cells and induces cell death with active oxygen species produced by Auger process after X-ray irradiation. We previously prepared the Nb-containing cationic liposome and transfected L-929 cells with the liposomes, however, the liposomes aggregated on the cell surface and no lethal effect was shown by X-ray irradiation [1]. Here we report that the new liposomes could introduce Nb atoms into the cells, electroporation could also introduce Nb atoms into the cells, and active oxygen species were produced by X-ray irradiation on Nb aqueous solution.

Experiments and Results

Preparation of Nb-containing liposomes

$Nb(OEt)_5$ was diluted with water in the presence of diisopropylethylamine and the aqueous solution of desired Nb concentration was obtained. Cationic liposomes composed of DOTAP:DOPC:DOPE=1:3:1 [2] aggregated on the cell surface. Therefore we prepared new cationic liposomes composed of DOTAP:DOPE=1:1 [3]. When we transfected HeLa cells with the old liposomes including pGFP [4], the cells showed no green fluorescence, however, about 30% of the cells transfected with the new liposomes including pGFP showed fluorescence of GFP. The results show that the newly formed liposomes can indeed introduce included molecules into the cells. Therefore, Nb-containing liposomes having the new phospholipid composition were prepared and transfected to HeLa cells. Incorporated Nb atoms measured by MIP-MS (Hitachi, P-6000) were about 8×10^7 atoms/cell at 1×10^{-3} M Nb solution and about 5×10^8 atoms/cell at 1×10^{-2} M Nb solution, respectively.

Introduction of Nb atoms by electroporation

HeLa cells were suspended in PBS containing 1×10^{-5} M or 1×10^{-4} M Nb atoms. The cell suspension was charged with 960 μF and 300 V electric pulse using GenePulser (BIO-RAD). Viability of the cells after electroporation was more than 95% in any conditions, and incorporated Nb atoms were about 5×10^8 atoms/cell at 1×10^{-5} M Nb solution and about 3×10^9 atoms/cell at 1×10^{-4} M Nb solution, respectively. Electroporation may introduce a larger number of Nb atoms into the cells than the method using the cationic liposomes.

X-ray irradiation of the cancer cells containing Nb atoms

Our previous work revealed that the aqueous solution of Nb compounds showed sharp K-edge near 19 keV in the X-ray absorption spectrum. After including Nb atoms in HeLa cells with the cationic liposomes or electroporation, the cells were irradiated by 2 Gy of monochromatic X-ray beam from BL-27B with the energy just above or below K-edge of Nb atoms. However, no lethal effect was caused by X-ray irradiation.

Active oxygen species in the cells induced by X-ray irradiation

The Nb PBS solution (1×10^{-3} M or 1×10^{-2} M) was irradiated using X-ray fluorescence analyzer (Horiba, MESA-500W). Concentration of active oxygen species in the irradiated solutions was measured as the method reported previously [1]. Only the 1×10^{-2} M Nb solution showed significant increase of active oxygen species after the X-ray irradiation. Our previous study using H_2O_2 showed that the similar concentaration of active oxygen species could induce significant cell death [1]. These results mean that Nb atoms as high as 1×10^{-2} M in the cell may produce active oxygen species and may promote cell death by X-ray irradiation.

Conclusion

We selected Nb atom as an X-ray sensitizer, and up to 10^9 Nb atoms were incorporated to the cells with newly prepared cationic liposomes or electroporation. However, there was no significant increase of cell death after X-ray irradiation at a dose of 2 Gy. We could detect active oxygen species only at 1×10^{-2} M of Nb solution by X-ray irradiation. These results show that Nb atom is not so efficient to produce active oxygen species enough to promote cell death by X-ray irradiation. Heavier atom, such as Ba, which has higher efficiency both to absorb X-ray and to produce active oxygen species, may be needed for development of a useful cancer-specific therapy.

References

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