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Preparation and Preliminary Study of X-ray Sensitizer for Cancer-Specific Therapy

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Introduction

In the cancer therapy, there are surgical treatments, chemotherapy, radiotherapy, etc., and improvement in cancer selectivity has been made by combination of the therapy. Photodynamic therapy using porphyrin derivative as a photosensitizer, which is activated by laser beam and makes radicals, has been reported[1]. Though specificity to cancer cells is high because of double targeting, it is restricted to the superficial tumor which the laser beam reaches. Then, we investigated a novel therapy in which a heavy atom was used as an X-ray sensitizer and the included heavy atoms in cancer cells were irradiated by monochromatic X-ray with energy equivalent to K-edge of the heavy atom.

Experiments and Results

Selection of safe heavy atom

We selected the heavy metal that was safe to organism and that had large difference in X-ray absorption efficiency from the atoms including in organism. The X-ray absorption efficiency is proportional to power 5 of the atomic number. For example, Ba has fifty times higher absorption efficiency than that of Fe which is included in hemoglobin at high concentration. In this experiment we selected Nb having K-edge near 19 keV within the energy range covered by BL-27B.

Preparation of Nb-containing liposomes

$Nb(OEt)_5$ was diluted with water in the presence of amine and the aqueous solution of desired Nb concentration was obtained. The liposome containing Nb aqueous solution was made from phospholipids in which the composition was DOTAP:DOPC:DOPE = 1: 3: 1 [2].

Nb concentration in cancer cells

Nb-containing liposomes were incubated with mouse tumor cell line, L929, for 4 h and incubated another 20 h after the medium exchange. Then, cell number was counted and the cells were washed thoroughly with 0.02% EDTA/PBS. Concentrated HNO_3 was added to solve the cells homogeneously and the Nb concetration in the obtained solution was measured by MIP-MS (Hitachi, P-6000). Intracellular Nb atom was increased proportionally to Nb concentration in the liposome.

Concentration of active oxygen species in the cells

The preliminary study was carried out using H_2O_2 as a radical source in order to establish the method of determining concentration of intracellular radicals, such as active oxygen species, which was produced by Auger process after X-ray irradiation. Dichlorodihydro-fluorescein diacetate and various concentration of H_2O_2 were simultaneously added to the L929 cells, and fluorescence (Ex: 485 nm, Em: 530 nm) was measured by fluorescence plate reader. The significant fluorescence was observed over 400 μM of H_2O_2, and it was proportional to the lethality of the cell observed by PI.

X-ray absorption spectrum of Nb aqueous solution

X-ray absorption spectrum of Nb aqueous solution was measured using monochromatic X-ray obtained by BL-27B. Nb_2O_5 powder was also used as control. Both spectra showed obvious K-edge near 19 keV which is equal to the reported value.

X-ray irradiation of the cancer cells containing Nb atoms

After including Nb atoms in L929 cells using Nb-containing liposome, the cells were irradiated by 2 Gy of monochromatic X-ray from BL-27B with the energy above or below K-edge of Nb. However there was no lethal effect caused by X-ray irradiation.

Conclusion

We selected Nb atom as an X-ray sensitizer, and included Nb atom into L929 cells using Nb-containing lipospmes. Even at maximum those, 8.6×10^9 atoms/cell, there was no increase of lethality by X-ray irradiation. This phenomenon might be caused by the fact that Nb atom was not really included into the cell but Nb-containing liposomes aggregated on the cell surface. Therefore it need to improve the conditions for including Nb atoms into the cell.

References

[1] J. G. Levy, TIBTEC., 13, 14 (1995).
[2] K. Yagi et al., BBRC., 196, 1042 (1993).

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