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学位論文要旨

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論文題名

The accelerator-based boron neutron capture reaction evaluation system for head and neck cancer (頭頚部がんに対する加速器ベースのホウ素中性子捕捉反応評価システムに関する研究)

論文内容の要旨（2000字程度）

Introduction

Boron neutron capture therapy (BNCT) is a binary therapy based on Boron-10 (10B) containing in tumor cells and thermal neutron irradiation. When 10B react with thermal neutrons, high-linear energy transfer (LET) alpha (α)-particles (4He) and recoiling lithium-7 (7Li) nuclei are emitted to destroy tumor cells. Since α-particles have very short pathlengths (5–9 μm), their destructive effects are limited to boron- containing cells. In theory, α-particles can selectively destroy tumor cells and spare adjacent normal cells, subsequently BNCT improves the quality of life (QOL). Clinically, BNCT has focused primarily on high grade gliomas, recurrent tumors of the head and neck cancers, and a much smaller number of osteosarcomas. Although additional data are needed to support expanding the indications for BNCT, there are few reports because of the limited number of neutron sources. Colony formation assays (CFAs) were initially described for studying the effects of irradiation on cells, and they have played an essential role in radiobiology. To date, CFAs also have often been used to evaluate the effects of boron neutron capture reaction (BNCR). The high-density survival (HDS) assay can be applied to cells with extremely low cloning efficiencies by facilitating cell-to-cell interactions. Similarly as CFA, the HDS assay involves the incubation of cells for prolonged periods after X-ray irradiation and therefore provides an integrated measure of all cytotoxic responses, encompassing early and late cell death. The purpose of this study was to evaluate BNCR using CFAs and HDS assays for head and neck cancer, which has low sensitivity to conventional radiotherapy. It has been reported BNCT improves clinical outcomes in squamous cell carcinoma. Although there are few reports for osteosarcoma in clinical practice, we also decided to evaluate BNCR using a human osteosarcoma cell line.

Cell lines and cell culture

Human oral squamous cell carcinoma (SAS) and human osteosarcoma (MG-63) cell lines were cultured in Dulbecco's modified essential medium (DMEM; Ham's F12 medium [1:1 mixture] supplemented with 2 mM L-glutamine and 5% fetal bovine serum [FBS]) supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin, and 100 μg/mL streptomycin in a humidified atmosphere of 5% CO2 and 95% air at 37 ◦C. A total of 2 ×10^6 cells were seeded in each T25 flask, and cells were harvested via trypsinization (0.5% trypsin). Exponentially growing cells were seeded at 5 ×105/25-cm2 flask and incubated for 48 h. Subsequently, cells were treated with 10B for 24 h, and the medium containing boron was discarded. Cells were harvested using 0.5% trypsin in phosphate-buffered saline (PBS) containing 0.53 mM ethylenediaminetetraacetic acid (EDTA, 4 min incubation at 37 ◦C) and suspended in 5 mL of PBS. Using a 5-mL pipette, the cells were pipetted up and down several times, forcing them through the tip of the pipette to break up the clumps. After centrifugation for 5 min at 300×g, cells were counted using a cell counter.

Method

Experimental Groups: Setting up for control, ^{10}B low (0.2 mg/ml), ^{10}B high group (0.4 mg/ml), which the human oral squamous cell carcinoma (SAS) & Human osteosarcoma cell (MG-63). We used Borono-phenylalanine (BPA) as ^{10}B.

^{10}B uptake: Control, ^{10}B groups with drug for 24 hours, cleaned by PBS and heating after treated with HNO_3, after analyzed by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), we got the data of boron absorption in SAS and MG-63.

X-ray irradiation: All groups treated with 24 hours, different groups were set under X-ray irradiation for 0, 1, 2, 4 and 6 Gy.

Neutron irradiation: All groups treated with 24 hours, different groups were set under Neutron irradiation for 0, 1, 2, 4 and 6×10^{11} n/cm^2.

Colony formation assay: After radiation, the cells were cultured for 300 cells each 6 well dish for different radiation conditions and groups. After 14 days of culture, the colony were stained and counted.

High Density Survival (HDS) assay: After radiation, the cells were cultured for different radiation conditions and groups. After 14 days of culture, the total number of surviving cells were counted.

Results

^{10}B uptake: Compared with the control group levels, boron was noticeably accumulated in SAS and MG-63 cells

SAS & MG cell survival after X-ray irradiation: Compared with control, the survival rate of the cell lines was similar in the B high group. Compared CFA to HDS assay, the survival rate trend of the cell lines was the same.

SAS & MG-63 cell survival after Neutron irradiation: Compared with control, the survival rate of the cell lines has significant difference in the B high group in neutron irradiation. Compared CFA to HDS assay, the survival rate trend of the cell lines was the same

Discussion

To apply BNCT, it is important to ensure that boron accumulates in the tumor region but not in healthy normal tissues surrounding the tumors. The CFA is the gold standard for evaluating early and late responses after BNCR in vitro; however, it is difficult for human normal cells to form colonies. Opposed to CFAs, which are generally used to evaluate radiation therapy, the HDS assay was verified for BNCR in human cell lines using an accelerator-based neutron source. The CFA and HDS assay produced extremely similar data, and the HDS assay is thus useful for evaluating the effects of BNCR. The CFA is limited to cells with the ability to form colonies. However, the HDS assay can be applied to non-adherent cells before and after irradiation. In addition, the HDS assay is easy to perform via simple cell counting without special devices or materials, similarly as CFA. The evaluation of SAS cells indicated the BNCR is moreeffective than a neutron source alone or X-ray irradiation, as reported previously. Boron uptake by human osteosarcoma cells was examined in this study as previously performed for rat osteosarcoma cell lines. The evaluation of MG-63 cells indicated that BNCR is also effective compared with the effects of X-ray irradiation. BNCT for HNSCC is one of the best treatments for elderly patients.

Conclusion

The utility of BNCR using an accelerator-based neutron source was verified in human tumor cell lines. Our results imply that the HDS assay has similar utility as the CFA for evaluating BNCR. In the future, our BNCR evaluation system will require further validation in normal human cells.