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学位論文の題目	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)
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学位論文内容の要旨

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 $\times 10^6$ 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.

論文審査結果の要旨

ホウ素中性子捕捉療法 (BNCT）は，癌細胞がホウ素を捕捉する性質を利用し，ホウ素 (^{10}B) と熱中性子との核反応で生じるα粒子を用いて癌細胞のみを破壊する放射線治療で，古くからその概念はあるものの，普及しなかった。近年，腫瘍集積率の高い ^{10}B-para-boronophenylalanine (BPA) が開発され，^{18}F-BPA を使用した陽電子放出断層撮影システム（^{18}F-BPA PET）や中性子発生器機の開発により注目を浴びるようになり，本邦でも BNCT センターが設立されつつある。しかしながら BNCT は臨床先行で普及しようとしており，その基盤研究に必要なホウ素中性子捕捉反応（BNCR）を評価するシステムが求められている。本研究は，頭頸部癌での in vitro BNCR 評価システムの確立を目的に，加速器ベースの中性子源（千葉放医研）を使用して，放射線療法の評価に使用されてきた Colony Formation Assay (CFA) ならびにより簡便な High Density Survival (HDS) Assay でヒト口腔扁平上皮癌細胞株（SAS）を用いて検証した。また BNCT の骨肉腫の有用性に関する報告がないため，ヒト骨肉腫細胞株（MG-63）についても検証を行った。

研究結果は以下の内容である。

1) SAS，MG-63 細胞共に，ホウ素の取り込みが BPA の濃度依存的に上昇することが Coupled Plasma-Mass Spectrometer で確認された。
2) SAS，MG-63 細胞共に，BPA 添加群では非添加群と比較して中性子源単独または X 線照射と比較し BNCR が有意に高く，BPA の濃度依存性を認めた。
3) CFA および HDS assay は非常に類似したデータを得たため，より簡便な HDS assay は BNCR の効果を評価するのに有効であった。

臨床ではホウ素の集積は ^{18}F-BPA PET で効果評価が行われているが，本研究で示した BNCR 評価システムは，癌細胞のみならずコロニーを形成しない正常細胞での応用も可能であり，新規ホウ素製剤やホウ素捕捉促進薬の創薬開発におけるスクリーニングアッセイへの応用，細胞レベルでのホウ素取り込みの差異を応用したホウ素集積の制御分子の探索や分子機構の解析への展開が期待され評価できる。本論文は Applied Radiation and Isotopes, 2020 Nov;165:109271（2020 年 7 月 6 日）に掲載されており，国際的にも評価されている。

よって，審査委員会は本論文に博士（歯学）の学位論文としての価値を認める。