VIII-Ⅱ-1. Project Research

Project 8

PR8-1 The Effect of Boron Neutron Capture Therapy Targeting Tumor Endohtelial Cells to Clinically Relevant Radioresistant Cells

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INTRODUCTION: Radiotherapy is one of the major therapeutic modalities for eradicating malignant tumors. However, the existence of radioresistant cells remains one of the most critical obstacles in radiotherapy and radiochemotherapy. Standard radiotherapy for tumor treatment consists of approximately 2 Gy once a day, 5 days a week, over a period of 5-8 weeks. To understand the characteristics of radioresistant cells and to develop more effective radiotherapy, we established a novel radioresistant cell line, SAS-R, from SAS derived from human oral squamous cell carcinoma. SAS-R cells continue to proliferate under daily exposure to 2 Gy of X-rays for more than 30 days. We call those "clinically relevant radioresistant" (CRR) cells. Our preliminary experiments showed that tumor tissues of CRR cells transplanted into nude mice were richer in tumor blood vessels compared with their radiosensitive parental cell lines, suggesting that the growth of SAS-R cells is dependent on blood vessels. Therefore, we think that targeting tumor endothelial cells in radioresistant tumors could be a promising strategy to overcome radioresistant tumor. In this study, we performed boron neutron capture (BNC) method targeting tumor endothelial cells using polyethylene glycol (PEG)-^{10}B-liposomes (PEG-10B).

EXPERIMENTS: Three days before experiments, $1x10^6$ cells of SAS and SAS-R were injected subcutaneously into hind legs of male Balb/c nude mice (4 weeks old). The day of the exposure experiment tumor diameter was approximately 3-4 mm. For irradiation of tumor endothelial cells by α-particles, PEG-^{10}B were administered. The compound was suspended in physiological saline at a concentration of 2500 ppm and was injected via the tail vein. Three hours after the administration, mice were exposed to neutron radiation at the Research Reactor Institute, Kyoto University (RRIKU). We determined the exposure period at the calculation dose of 28 Gy-Eq at an organ level. Tumor size was determined by caliper measurements daily. Blood vessels in tumor were immunohistochemically stained for CD34.

RESULTS: We first examined the tumor size of SAS and SAS-R daily after exposure. Within 20 days after exposure to α-particles, the tumor size of SAS and SAS-R was not significantly different (data not shown).

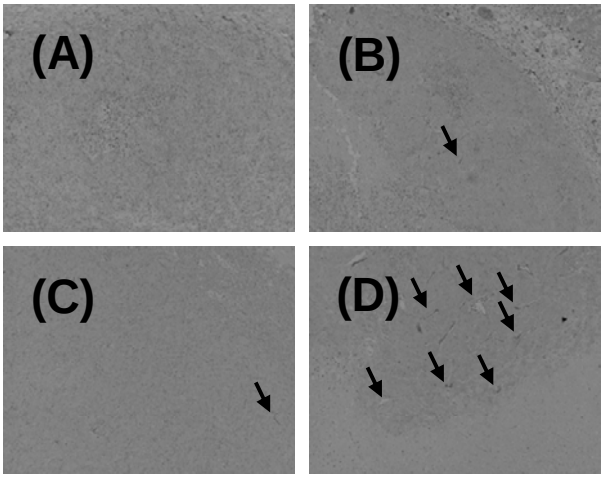

Fig.1. Histological analysis of tumor end othelial cells14 days after exposure to 28 Gy of a-particles. Blood vessels were visualized by CD34 staining. (A) SAS tumor without a- particles. (B) SAS tumor without a- particles. (D) SAS-R tumor after exposure to a-particles. Arrow; blood vessels.

Mice were sacrificed on days 14 after the exposure. Histological examination showed that the center of the tumor was entirely necrotic both in SAS and SAS-R tumors. As was our preliminary study, CD34 positive blood vessels were more abundant in SAS-R tumors than in SAS-tumors (Fig. 1). Surprisingly, 14 days after BNC the density of CD34 positive blood vessels increased in SAS-R tumor exposed to α-particles compared to control SAS-R tumor but did not in SAS tumor irrespective of α-particles exposure.

DISCUSSION: In this research we tried to target tumor endothelial cells of radioresistant SAS-R tumors, because our preliminary experiment showed that the density of blood vessels in SAS-R tumors was higher than that in SAS tumors. We selectively exposed endothelial cells to α-particles, we used BNC with PEG-^{10}B. However, the tumor volume of SAS and SAS-R was not significantly different after exposure to 28 Gy of α-particles for the examination period. We think that the necrotic part contributes to the ostensible volume of tumors. Therefore, we need exclude necrotic part for the assessment of experimental radiotherapy. Moreover, to our surprise blood vessel density in SAS-R tumors increased by BNC therapy. This tendency was not observed in SAS tumors. Therefore, further studies are needed to confirm how blood vessel density contributes to tumor radiotherapy.

採択課題番号 22P8-3　　腫瘍内の血管は腫瘍の放射線耐性にどのように関わっているのか　　プロジェクト

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（京大・原子炉）櫻井良憲、小野公二、鈴木 実、劉 勇

PR8-2 **The Role of Vascular Damage to Radiation Lung Injurry**
- An Analysis by BNCR -

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INTRODUCTION: The vascular damage is considered to be cause of radiation injury of the lung. However, this is an indirect proof mainly based on histopathology. General radiation like as X-ray or gamma-ray is homogeneously delivered to all kinds of cell consisting tissue. Therefore, it not apparent which kind of cell plays a leading role of radiation injury in general. New idea is necessary to overcome this point. We made a new clever idea to solve this difficulty. It is a use of boron neutron capture reaction (BNCR). This reaction emits He nucleus (alpha-particle) and Li nucleus with extremely short range not exceeding one cell diameter.

MATERIALS AND METHODS : Sodium borocaptate (BSH-2Na) involving 12 B-10 atoms in a molecule is encapsulated into liposome. If the diameter of liposome is large enough, it stays in blood vessel and does not leak into the tissue. When B-10 in the liposome receives neutron, two particles mentioned above are emitted and hit endothelium of blood vessel selectively. We prepared 400nm diameter liposome containing BSH at the level of 10,000ppm B-10. They were injected into C3H/He female mice of 7 week old via tail vein. Mice were irradiated 7.5 Gy of gamma-ray 24 hour before liposome injection and neutron irradiation. The liposome emulsion just before injection contained B-10 at 5,000ppm. So, 0.1, 0.2 and 0.3 ml of it were injected 1 hour before neutron ir radiation. Neutron irradiation time was 30 min. As a control, mice of gamma-ray irradiation alone were prepared. They received pre-dose of 7.5 Gy and following test dose of 3, 6 and 9 Gy of gamma-ray. Three and 6 months later, each 3-4 mice were killed and the lungs were examined macro- and microscopically.

RESULTS: Only 1 mouse died by 6 months after treatment. The activity of all mice seemed not to be impaired. Figures show lung epecimens excised from mice 6 months after the neutron or gamma-ray treatment.

Fig. 1. 7.5Gy(γ)+Liposome+Neutron.

Fig. 2. 7.5Gy(γ)+γ-ray.

There exists no apparent macroscopically difference among them. At present, microscopic examination is in progress.

採択課題番号 22P8-4　放射線肺障害に及ぼす血管損傷の役割の BNCR を用いた解明　プロジェクト（京大・原子炉）小野公二、劉 勇、鈴木 実（京大院・医）澁谷景子（東北大・加齢研）福本 学（学習院・理）中村浩之