VIII-Ⅱ-1. Project Research

Project 4

PR4-1 **The Effect of Boron Neutron Capturetreatment Targeting Tumor Endohtelial Cells of Clinically Relevant Radioresistant Tumors**

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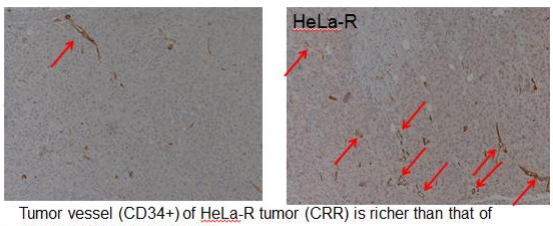
INTRODUCTION: Radiotherapy is one of the major therapeutic modalities for eradicating malignant tumors. However, the existence of radioresistant cells remains one of the most critical obstacles in radiotherapy. To understand the characteristics of radioresistant cells and to develop more effective radiotherapy, we have established clinically relevant radioresistant (CRR) cell lines. The density of blood vessels in tumor tissues of CRR cells transplanted into nude mice was higher compared with their radiosensitive parental cell lines. Therefore, we performed boron neutron capture treatment (BNCT) targeting tumor endothelial cells using PEG-10B.

EXPERIMENTS: Three days before experiments, $1x10^6$ cells of HeLa and HeLa-R were injected subcutaneously into hind legs of male Balb/c nude mice (4 weeks old). Tumor size was determined by caliper measurements. The day of BNCT treatment tumor diameter was approximately 3-4 mm and was measured every 2 days. For irradiation of tumor endothelial cells by α-particles, PEG-^{10}B was administered. The compound was suspended in physiological saline at a concentration of 2,500 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). The calculated dose of α-particles was 8 Gy-Eq. Endothelial cells of blood vessels in tumor tissues were immunohistochemically stained for CD34 and CD31. Pericytes were detected by immunohistochemistry for Type IV collagen and functional blood vessels was determined by tomatolectin injected from the tail vain. We counted the number of vessels in 10 high power field (x400) and calculated the average (n=3).

Figure 1



Tumor vessel (CD34+) of HeLa-R tumor (CRR) is richer than that of parental HeLa tumor (arrow).

Figure 3

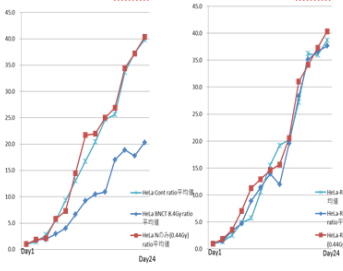


Figure 4

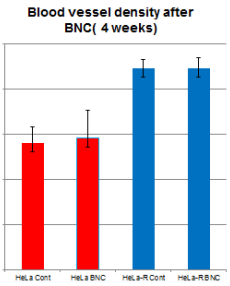


Fig.3 Tumor size of HeLa was significantly decreased in Day 24.
Fig.4 Blood vessel density in CRR tumors was higher than in parental cell tumors after BNC using PEG-10B (10 fields of view).

Figure 4 red: TypeIV Collagen blue: CD31 green: tomatolectin **Figure 5**

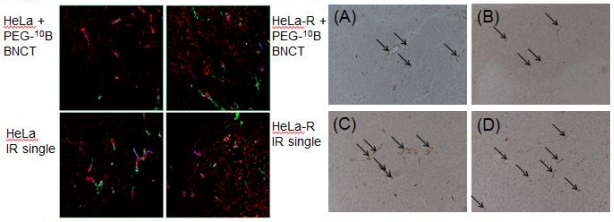


Fig.5 Histological analysis of tumor endothelial cells 7 days after BNCT (8 GyE of α-particles). The intensity of blood vessels of CRR tumors are richer than that of parental cell tumors. And after BNCT using PEG-^{10}B, the number of blood vessels of CRR tumors was recovered more rapidly than that of parental cells.
Fig.6 Histological analysis of tumor endothelial cells 30 days after BNCT. Blood vessels were visualized by CD34 staining. (A) HeLa tumor without BNCT. (B) HeLa tumor after BNCT. (C) HeLa-R tumor without BNCT. (D) HeLa-R tumor after BNCT. Arrow; blood vessels.

RESULTS: Within 24 days after BNCT, the size of HeLa-R tumors was not significantly different from HeLa-R tumors without BNCT. But the size of HeLa tumors was significantly smaller compared with HeLa tumors without BNCT. Mice were sacrificed on day 30 after BNCT. Histological examination showed that the tumor blood vessel density of HeLa-R tumors were larger compared with their radiosensitive HeLa tumors. CD34 positive blood vessels were also more abundant in SAS-R tumors than in SAS-tumors (Data not shown). After BNCT using PEG-10B, the density of CD31 positive blood vessels of HeLa-R tumors recovered more rapidly than HeLa tumors on 7 days. However, 4 weeks after BNCT, the density of CD34 positive blood vessels was almost the same between tumor with BNCT and without BNCT in both HeLa-R tumors and HeLa tumors.

DISCUSSION: Our preliminary experiments showed that the density of blood vessels in SAS-R tumors was higher than that in SAS tumors. In order to determine the significance of blood vessel concentration in radiorsistance, we tried to target tumor endothelial cells by selective exposure of endothelial cells to α-particles, using BNC with PEG-^{10}B.

Seven days after BNC the density of CD31 positive blood vessels were destroyed in HeLa tumor exposed to α-particles compared to control HeLa tumor but did not in HeLa-R tumor irrespective of α-particles exposure. Moreover, to our surprise, 4 weeks after BNCT the desity of CD34 positive blood vessels were almost same irrspective of α-particles exposure in both HeLa-R tumor and HeLa. Therefore, further studies are needed to confirm how blood vessel density contributes to tumor radiotherapy.

採択課題番号 23P4-3 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
（東北大・加齢研）桑原義和、福本 基、福本 学（京大・原子炉）桜井良憲、鈴木 実、小野公二