PR1 Analyzing Tumor Microenvironment and Exploiting its Characteristics in Search of Optimizing Cancer Therapy Including Neutron Capture Therapy

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BACKGROUNDS AND PURPOSES: Human solid tumors contain moderately large fractions of quiescent (Q) tumor cells that are out of the cell cycle and stop cell division, but are viable compared with established experimental animal tumor cell lines. The presence of Q cells is probably due, in part, to hypoxia and the depletion of nutrition in the tumor core, which is another consequence of poor vascular supply. As a result, Q cells are viable and clonogenic, but stop cell division. In general, radiation and many DNA-damaging chemotherapeutic agents kill proliferating (P) tumor cells more efficiently than Q tumor cells, resulting in many clonogenic Q cells remaining following radiotherapy or chemotherapy. Therefore, it is harder to control Q tumor cells than to control P tumor cells, and many post-radiotherapy recurrent tumors result partly from the regrowth of Q tumor cells that could not be killed by radiotherapy. Similarly, sufficient doses of drugs cannot be distributed into Q tumor cells mainly due to heterogeneous and poor vascularity within solid tumors. Thus, one of the major causes of post-chemotherapy recurrent tumors is an insufficient dose distribution into the Q cell fractions.

With regard to boron neutron capture therapy (BNCT), with ^{10}B-compounds, boronophenylalanine-^{10}B (BPA) increased the sensitivity of the total cells to a greater extent than mercaptoundecahydrododecaborate-^{10}B (BSH). However, the sensitivity of Q cells treated with BPA was lower than that in BSH-treated Q cells. The difference in the sensitivity between the total and Q cells was greater with ^{10}B-compounds, especially with BPA. These findings concerning the difference in sensitivity, including other recovery and reoxygenation following neutron irradiation after ^{10}B-compound administration were mainly based on the fact that it is difficult to deliver a therapeutic amount of ^{10}B from ^{10}B-carriers throughout the target tumors, especially into intratumor hypoxic cells with low uptake capacities.

Hypoxia is suggested to enhance metastasis by increasing genetic instability. Acute, but not chronic, hypoxia was reported to increase the number of macroscopic metastases in mouse lungs. We recently reported the significance of the injection of an acute hypoxia-releasing agent, nicotinamide, into tumor- bearing mice as a combined treatment with γ-ray irradiation in terms of repressing lung metastasis. As the delivered total dose increased with irradiation, the number of macroscopic lung metastases decreased reflecting the decrease in the number of clonogenically viable tumor cells in the primary tumor. The metastasis-repressing effect achieved through a reduction in the number of clonogenic tumor cells by irradiation is much greater than that achieved by releasing tumor cells from acute hypoxia. On the other hand, more ^{10}B from BPA than from BSH could be distributed into the acute hypoxia-rich total tumor cell population, resulting in a greater decrease in the number of highly clonogenic P tumor cells with BPA-BNCT than with BSH-BNCT and with neutron beam irradiation only. BPA-BNCT rather than BSH-BNCT has some potential to decrease the number of lung metastases, and an acute hypoxia- releasing treatment such as the administration of nicotinamide, bevacizumab, wortmannin or thalidomide may be promising for reducing numbers of lung metastases. Consequently, BPA-BNCT in combination with the treatment using these agents may show a little more potential to reduce the number of metastases. Now, it has been elucidated that control of the chronic hypoxia-rich Q cell population in the primary solid tumor has the potential to impact the control of local tumors as a whole, and that control of the acute hypoxia-rich total tumor cell population in the primary solid tumor has the potential to impact the control of lung metastases.

The aim of this research project is focused on clarifying and analyzing the characteristics of intratumor microenvironment including hypoxia within malignant solid tumors and optimizing cancer therapeutic modalities, especially radiotherapy including BNCT in the use of newly-developed ^{10}B-carriers based on the revealed findings on intratumor microenvironmental characteristics.

RESEARCH SUBJECTS:

The collaborators and allotted research subjects (ARS) were organized as follows;

ARS-1 (29P1-1): Optimization of Radiation Therapy Including BNCT in terms of the Effect on a Specific Cell Fraction within a Solid Tumor and the Suppressing Effect of Distant Metastasis. (S. Masunaga,*et al.*)

ARS-2 (29P1-2): Development of Hypoxic Microenvironment-Oriented ^{10}B-Carriers. (H. Nagasawa, *et al.*)

ARS-3 (29P1-3)*: Search and Functional Analysis of Novel Genes that Activate HIF-1, and Development into Local Tumor Control. (H. Harada, *et al.*)

ARS-4 (29P1-4)*: Radiochemical Analysis of Cell Lethality Mechanism in Neutron Capture Reaction. (R. Hirayama, *et al.*)

ARS-5 (29P1-5): Development of Neutron Capture Therapy Using Cell-Membrane Fluidity Recognition Type Novel Boron Hybrid Liposome. (S. Kasaoka, *et al.*)

ARS-6 (29P1-6)*: Drug Delivery System Aimed at Adaptation to Neutron Capture Therapy for Melanoma. (T. Nagasaki, *et al.*)

ARS-7 (29P1-7)*: Molecular Design, Synthesis and Functional Evaluation of Hypoxic Cytotoxin Including Boron. (Y. Uto, *et al.*)

ARS-8 (29P1-8)*: Bystander Effect on Malignant Trait of Tumor Cells by Irradiation. (H. Yasui, *et al.*)

ARS-9 (29P1-9)*: Analysis of the Response of Malignant Tumor to BNCT. (M. Masutani, *et al.*)

ARS-10 (29P1-10): Cell Survival Test by Neutron Capture Reaction Using Boron Compound and Inhibitory Effect on Tumor Growth. (K. Nakai, *et al.*)

ARS-11 (29P1-11)*: Multilateral Approach Toward Realization of Next Generation Boron Neutron Capture Therapy. (Y. Matsumoto, *et al.*)

ARS-12 (29P1-12): Analysis of Radiosensitization Effect through Targeting Intratumoral Environmental. (Y. Sanada, *et al.*)

(*There was not assignment time for experiment using reactor facilities during its operation period of FY 2017.**)**

PR1-1 Estimation of Therapeutic Efficacy of BCNT Based on the Intra- and Intercellular Heterogeneity in ^{10}B Distribution

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INTRODUCTION: In the current treatment planning of boron neutron capture therapy (BNCT), the absorbed doses deposited by ^{10}B(n,α)^{7}Li, ^{14}N(n,p)^{14}C, and ^{1}H(n,n)p reactions as well as photons are separately calculated, which are generally referred to as boron, nitrogen, hydrogen, and photon components, respectively. The absorbed doses for each component are weighted by their relative biological effectiveness (RBE) or compound biological effectiveness (CBE) [1] in the treatment planning for estimating the doses equivalent to conventional photon therapy. Note that the concept of CBE, the weighting factor on the boron component, was introduced to express the difference of biological effectiveness between the types of ^{10}B compounds because the therapeutic efficacy depends on the intra- and intercellular heterogeneity in ^{10}B distribution besides RBE. However, the sum of the absorbed dose weighted by fixed RBE or CBE (hereafter, RBE-weighted dose) of each component may not be an adequate index for representing its biological impact, since RBE and CBE vary with the absorbed dose, and the synergistic effect exists in the radiation fields composed by different types of radiation. Thus, the concept of the photon-isoeffective dose that represents the photon dose giving the same biological effect was recently proposed for the treatment planning of BNCT [2]. We therefore developed a model for estimating the RBE-weighted and photon-isoeffective doses of BNCT considering the intra- and intercellular heterogeneity in ^{10}B distribution.

MATERIALS AND METHODS: Our developed model is based on the stochastic microdosimetric kinetic (SMK) model [3], which can estimate the cellular surviving fraction (SF), not from the profiles of radiation imparting energy such as LET, but from the probability densities of the absorbed doses in cell nucleus and its intra-nuclear domains. Thus, the SMK model considers the synergistic effect intrinsically. For extending SMK model to be applicable to BNCT, we calculated the probability densities for each dose component of BNCT using the Particle and Heavy Ion Transport code System, PHITS [4]. Then, the probability densities for actual BNCT radiation fields inside patients are determined by summing up the calculated data for each dose component weighted by its absorbed dose. In this summation, the intra- and intercellular heterogeneity in ^{10}B distribution are also considered. The SF of tumor cells in patients can be evaluated from the calculated probability densities using the SMK model. Four parameters that express cellular characteristics must be evaluated in the SMK model. In this study, their numerical values were determined by the least-square (LSq) fitting of the SF of tumor cells, which we previously determined *in vivo*/*in vitro* experiments of mice exposed to reactor neutron beam with concomitant BPA or BSH treatment at various concentrations [5].

RESULTS AND DISCUSSION: Figure 1 shows the experimental and calculated therapeutic efficacy of BNCT in comparison to X-ray therapy as a function of the absorbed dose in tumor. The photon-isoeffective dose can be calculated by the absorbed doses weighted by this relative therapeutic efficacy. The data for three drug conditions, administration of BPA and BSH with 17 ppm, and without ^{10}B compound, are shown in the graph. It is evident from the graph that the relative therapeutic efficacies for the BPA administration are higher than the corresponding data for the BSH case, and they decrease with increase of the absorbed dose in tumor. Our model can satisfactorily reproduce these tendencies, though it slightly overestimates the therapeutic efficacies for the BPA administration. This overestimation is probably due to the ignorance of the inter-cellular heterogeneity in ^{10}B distribution in this calculation. More detailed discussions can be found in our recently published paper [6].

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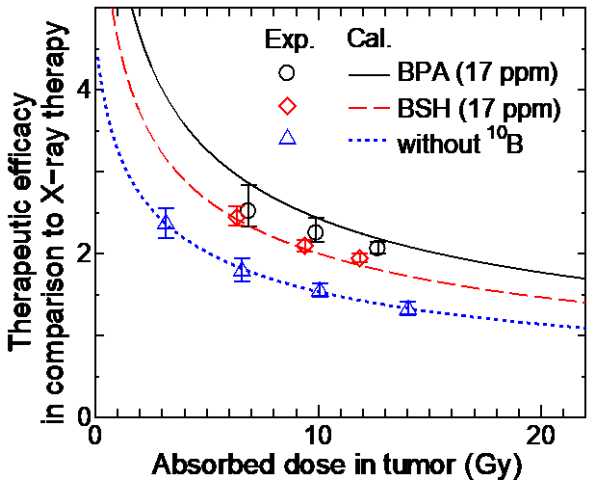

Fig. 1. Experimental and calculated therapeutic efficacy of BNCT in comparison to X-ray therapy.

PR1-2 Design, Synthesis and Biological Evaluation of Pepducin-BSH Conjugates for BNCT

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INTRODUCTION: Selective delivery of sufficient quantity of ^{10}B to tumor cells is essential for the success of boron neutron capture therapy (BNCT). The clinically used boron carrier, sodium mercaptoundecahydro-closo-dodecaborate (BSH: $Na_2B_{12}H_{11}SH$) is impermeable to plasma membrane due to its highly hydrophilic and anionic property. We found that pepducins, which are artificial lipidated peptides developed as G protein-coupled receptor (GPCR) modulators, enable fluorescein, an anionic molecule, to penetrate membrane directly. From this study, we envisaged that the anionic boron cluster can be delivered into cytosol by using the pepducin as a delivery unit. So, we designed and synthesized pepducin-BSH conjugates and performed structural optimization to improve cellular uptake. (Fig.1)

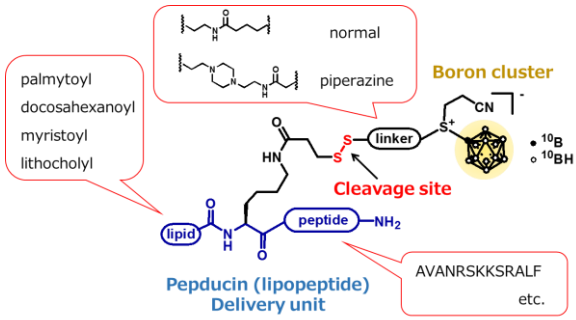
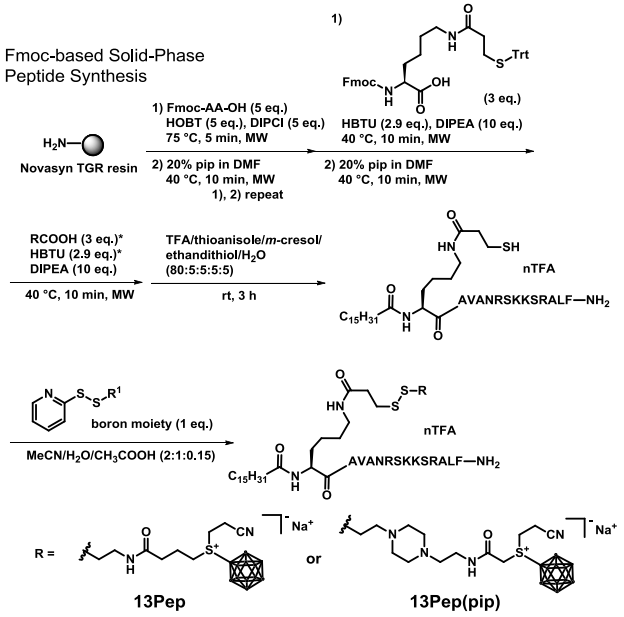


Fig. 1 Structural optimization of pepducin-BSH conjugates.

In the present study, we investigated the biological effects on BNCT of the selected pepducin-BSH conjugates using T98G cells.

EXPERIMENTS: 13Pep and 13Pep(pip) were synthesized based on solid-phase synthesis.(Scheme 1) T98G, cells were treated with the Peps (10 or 20 μM) at 37 °C for various times, then, washed with PBS three times, and dissolved in 200 μL HNO3 for 1 h. The boron concentrations of these extracts were measured by inductively coupled plasma-atomic emission spectrometry. To evaluate neutron sensitizing ability of the compounds, T98G cells were treated with 20 μM boron carriers for 24 h. Then the cells were washed with PBS, suspended in serum containing medium and aliquoted into Teflon tubes for irradiation. Cells were irradiated using the neutron beam at the Heavy Water Facility of the Kyoto University Research Reactor (KUR) operated at 1 MW power output. The survival rates of the irradiated cells were determined using conventional colony assays.

RESULTS: Pep13 and Pep13(pip) were showed highly cellular uptake into T98G cells. Pepducin carrier was clearly useful for membrane penetration of BSH. (Fig. 2)



Scheme 1 Synthesis of pepducin-BSH condugates and structures of 13Pep and 13Pep(pip).

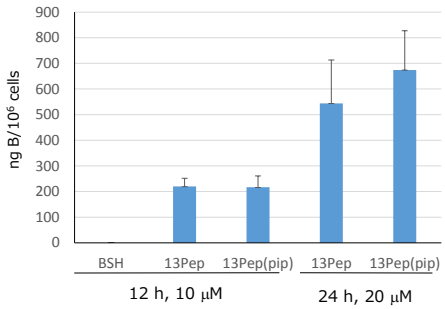


Fig. 2 Intracellular uptake of 13Pep and 13Pep(pip).

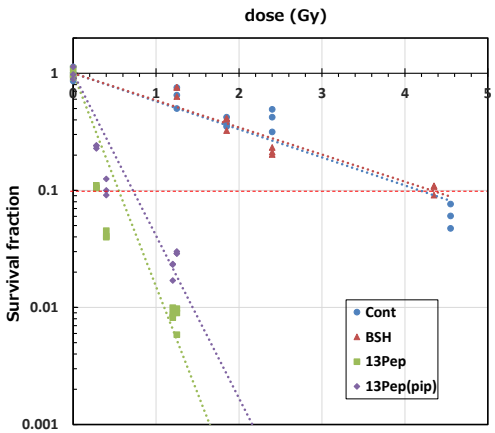


Fig. 3 Survival fraction of T98G cells treated with 13Pep and 13Pep(pip) and irradiated by mixed-neutron beam for BNCT.

The D_{10} of BNCT was calculated from survival curve shown in Fig. 3. Each D_{10} was 0.54 Gy for 13Pep, 0.72 Gy for 13Pep(pip) and 4.32 Gy for BSH. Form these results, the novel boron carriers, 13Pep and 13Pep(pip) were promising candidates for BNCT. We are now investigating bio distribution and *in vivo* activity.

PR1-3 HIF-1 Maintains a Functional Relationship between Pancreatic Cancer Cells and Stromal Fibroblasts by Upregulating Expression and Secretion of Sonic Hedgehog

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INTRODUCTION: Pancreatic cancer is a deadly disease because it is highly resistant to conventional therapies. Characteristic features of pancreatic cancer strongly associated with the poor prognoses of patients and therapeutic resistance are the existence of both hypoxic regions and stroma-rich microenvironments.

Accumulating evidence has suggested that a factor associated with the poor prognosis as well as malignant progression of pancreatic cancers is a hypoxia-inducible transcription factor, hypoxia-inducible factor 1 (HIF-1). Once the regulatory subunit of HIF-1, HIF-1α, becomes stabilized and activated under hypoxic conditions, it, in combination with its binding partner, HIF-1β, induces the expression of hundreds of genes responsible for malignant cancer progression. Although the positive correlations between HIF-1α expression levels as well as the volume of hypoxic regions and both the poor prognosis of pancreatic cancer patients and decreased anti-tumor effects of HIF-1α-targeting drugs in pancreatic tumors have been repeatedly reported, key molecular mechanisms behind them are still unclear.

Another characteristic feature of pancreatic cancers is the stroma-rich microenvironment, which has been reported to result from the activation of the Sonic hedgehog signaling pathway, aberrant proliferation of fibroblasts, and overproduction of extracellular matrix (ECM). Specifically, the mature form of Sonic hedgehog protein (SHH) is secreted from pancreatic cancer cells after removal of the signal peptide and autocatalytic processing. The secreted SHH protein then cancels the negative regulation of smoothened (SMO) by patched (PTCH) through the direct binding of SHH to PTCH on the surface of fibroblasts, leading to the activation of a transcription factor, Gli-1, in fibroblasts. Because Gli-1 has an activity to upregulate cellular proliferation, differentiation, and survival by inducing the expressions of target genes, such as cyclin D1, c-myc, bcl2, and snail, the paracrine signaling is thought to be important in the formation of the stroma-rich microenvironment of pancreatic cancers. Thus, marked efforts have been devoted to clarify the characteristic features of each hypoxic condition and the stroma-rich microenvironment in pancreatic cancers; however, whether and how HIF-1 and the Sonic hedgehog signaling pathway influence each other and eventually create the pancreatic cancer-distinctive microenvironments have yet to be fully elucidated.

In the present study, we investigated the functional and mechanistic linkage between HIF-1 and Sonic hedgehog signaling to better understand whether and how the stroma-rich microenvironment arises in pancreatic cancers [1]. We revealed that pancreatic cancer cells secrete more SHH under hypoxic conditions by increasing the efficiency of secretion as well as expression of SHH in a HIF-1-dependent manner, and promote the growth of fibroblast cells by stimulating the hedgehog signaling pathway in a paracrine manner.

EXPERIMENTS and RESULTS: Performing Western blotting using antibody against SHH protein, we found that pancreatic cancer cells secreted more Sonic hedgehog protein (SHH) under hypoxia by upregulating its expression and efficiency of secretion in a HIF-1-dependent manner (Fig. 1). Recombinant SHH, which was confirmed to activate the hedgehog signaling pathway, accelerated the growth of fibroblasts in a dose-dependent manner (Fig. 1). The SHH protein secreted from pancreatic cancer cells under hypoxic conditions promoted the growth of fibroblasts by stimulating their Sonic hedgehog signaling pathway. The SHH-mediated growth acceleration was significantly suppressed by a SMO inhibitor, TAK-441. These results suggest that the increased secretion of SHH by HIF-1 is potentially responsible for the formation of detrimental and stroma-rich microenvironments in pancreatic cancers, therefore providing a rational basis to target it in cancer therapy (Fig. 1).

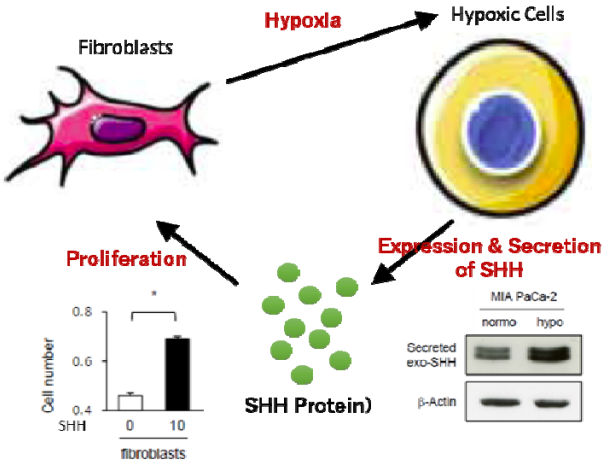

Fig. 1 Positive feedback loop among the upregulation of both expression and secretion of SHH, accelerated proliferation of fibroblasts, and development of hypoxic regions in malignant pancreatic tumor tissues.

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PR1-4

Selective Accumulation of Boron-conjugated Liposomes Composed of Dimyristoylphosphatidylcholine to B16F10 Murine Melanoma Cells in Relation to Fluidity of Cell Membranes.

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INTRODUCTION: There are many reports that membranes in cancer cells are relatively more fluid compared to healthy cells. Higher membrane fluidity in cancer cells closely relates to their invasive potential, proliferation, and metastatic ability [1]. Liposomes composed of dimyristoylphosphatidylcholine (DMPC) and polyoxyethylenedodecylether were found to inhibit the growth of human promyelocytic leukemia (HL-60) cells without using any drugs [2]. In this study, we have developed a novel boron delivery system using the membrane-fluidity sensitive boron liposomes (MFSBLs) composed of DMPC and borocaptate (BSH)-conjugated chemical compounds for boron neutron capture therapy (Fig. 1).

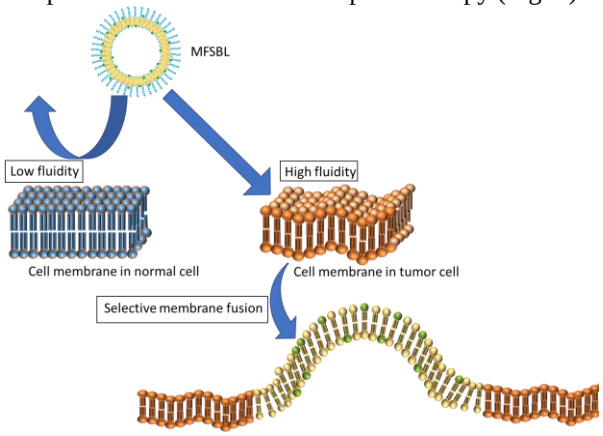

Fig. 1. Selective membrane fusion of boron-conjugated liposomes composed of DMPC to B16F10 murine melanoma cells in relation to fluidity of cell membranes.

EXPERIMENTS: Octadecylamine and 1,2-dimyristoyl-sn-glycero-3-phosphorylethanolamine were conjugated with BSH using the optimal hetero-crosslinking agents for boron compounds. MFSBLs composed of DMPC, polyoxyethylenedodecylether and boron compounds at mole ratios of 8:0.9:1.1 were prepared by sonication method in 5% glucose solution at 45°C with 300 W , followed by filtration with a 0.45 μm filter. The diameter of MFSBLs was measured with a light scattering spectrometer. The boron concentration was measured by inductively coupled plasma atomic emission spectrometry. B16F10 murine melanoma cells were pre-incubated with 2.5-10 ppm of ^{10}B at 37°C for 24 hours before neutron irradiation. The cells were rinsed twice in PBS and suspended in fresh medium. After neutron irradiation the cells were plated into plastic Petri dishes 60 mm in diameter at 200 cells per dish. They were incubated for an additional 7 days to allow colony formation.

RESULTS: MFSBLs had a mean diameter of 59.6 nm and a zeta potential of -11.3 mV. High encapsulation efficiency value from 55% to 89% of ^{10}B in MFSBLs were obtained. MFSBLs had high stability (95-99%) in the retention of ^{10}B during storage at 4°C for 4 weeks. All borocaptate-loaded formulations had low cytotoxic effects in human fibroblast cells. MFSBLs were efficiently fused to melanoma cells, but were inefficiently fused to human fibroblast cells. Thus, it is essential to elevate the ^{10}B concentration in melanoma cells, while maintain low levels of ^{10}B in normal fibroblast cells. The tumor/normal ratio (T/N ratio) was 3.0. As shown in Fig. 2, MFSBLs showed higher suppression of growth of melanoma cells than BSH solution. This result suggested novel MFSBLs composed of DMPC is useful for ^{10}B carrier on BNCT for melanoma.

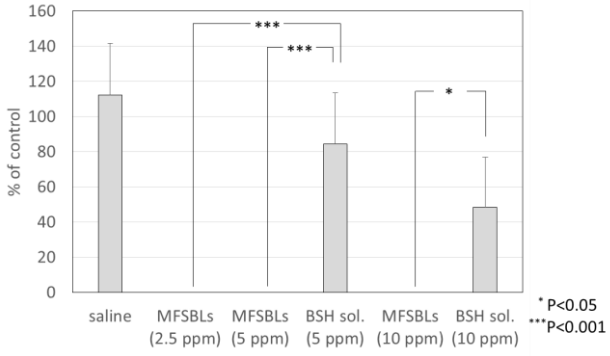

Fig. 2. Suppression of the colony formation of B16F10 cells after in vitro BNCT.

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PR1-5 The Response of Cancer Cells after BNCR

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INTRODUCTION: Boron neutron capture therapy (BNCT) is based on nuclear reactions between thermal neutron and boron-10 in the cancer cells. The reaction causes alpha particle and lithium nuclei short length with high energy. Boron compounds such as ^{10}B-boronophenylalanine (BPA) are introduced into cancer cells and neutron beam using nuclear reactor or accelerator-based BNCT system are irradiated. Boron neutron capture reaction (BNCR) efficiently introduces DNA damages[1)], however, tumor cell killing is affected by various factors including the uptake of boron compounds and thermal neutron fluence[2)]. Therefore, it is difficult in BNCT to calculate the irradiated dose on tumor and normal tissues. We previously observed extensive DNA damage responses including those for DNA double strand breaks after BNCR by the observation of remaining gamma-H2AX and poly(ADP-ribose) in the rat lymphosarcoma model of BNCT[3)]. We also performed comprehensive analysis of mRNA expression and proteome using human squamous carcinoma SAS cells after BNCR[4)]. From the comprehensive analysis, expressions of particular mRNAs were increased after BNCR. These gene products may be involved in early response of BNCT. We focused on factors present in culture supernatant including *CSF2* gene product, granulocyte-macrophage colony stimulating factor (GM-CSF), which was increased after BNCR, and metabolites and investigated the functions and dynamics after BNCR or neutron beam irradiation in comparison with the gamma-ray irradiation.

EXPERIMENTS: The experiments with neutron-beam irradiation with KUR Nuclear Reactor was planned but not carried out during FY2017. Neutron-beam irradiations were carried out in the previous experiments at 1 MW in the KUR facility. Human oral squamous cancer SAS cells and melanoma A375 cells were irradiated after 2 hrs incubation with or without ^{10}B-BPA at 25 ppm. Gamma-ray irradiations were operated at National Cancer Center Research Institute (Tokyo) and Nagasaki University with the ^{137}Cs source. The cellular responses including factors and metabolites present in culture supernatants were filtrated and analyzed 6 and 24 hrs after irradiation of therapeutic dose of BNCT and gamma-ray. Cell survival was analyzed by colony formation assay. The siRNA was transfected with Lipofectamine™ 3000 Reagent (Thermo Fisher Scientific). The knockdown efficiency of siRNA for *CSF2* was evaluated in the cancer cell lines by measurement of the mRNA levels. The *GUSB* gene was used as a control. GM-CSF levels in culture supernatant were measured using ELISA.

RESULTS: The relative biological effectiveness (RBE) of BNCR for A375 cells was around 2-3 like in the case with SAS and HSG cells. A375 cells show a relatively high basal level of *CSF2* mRNA expression. The siRNA of *CSF2* caused a decrease in *CSF2* mRNA level to around 20% at 10 nM in A375 cells. A decrease of gene product GM-CSF in the culture supernatant was also observed after the siRNA treatment by ELISA analysis. The siRNA treatment of *CSF2* limitedly affected the cell survival after gamma-ray irradiation.
The results suggest that CSF2 may be involved in cancer cell survival after various kinds of radiation as an autocrine factor. This possibility and other biological significances of CSF2 will be investigated after BNCR in the KUR facility.

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PR1-6 Radiobiological Effect of Extracellular Boron Distribution and Neutron Irradiation

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INTRODUCTION: Boron Neutron Capture Therapy (BNCT) is a particle radiation therapy for malignant diseases. The clinical trial of BNCT for malignant brain tumor and head and neck cancers is ongoing. However, Boron distribution of extra-cellular fluid or interstitial tumor tissue during the neutron irradiation and radiobiological effect of neutron irradiation is still unclear. In the previous studies, we have focus on intra-cellular boron concentration and tumor tissue boron concentrations. The goal of this study is, to clarify a role of extra-cellular boron neutron reaction in BNCT.

EXPERIMENTS: U251 human glioma cell lines were cultured in D-MEM supplemented with 10% fetal bovine serum and maintained at 37℃ in a humidified atmosphere with 5% CO_2. After trypsinized and counted, cells were suspended in culture medium. To mimicking tumor stroma condition, we made four groups (Fig.1).
First, from 6hr before to end of neutron irradiation, cells were continuously exposed to Boronophenylalanine (BPA 6hr). The second, BPA exposure was from 6hr before irradiation to just before irradiation, neutron irradiation was done after changing culture medium to that of BPA free (BPA 6hr wash). add BPA and Boric Acid (BA) just before neutron irradiation on single cell suspensions (BPA 0hr, Boric Acid). These cells were irradiated at the KUR irradiation system, with boron concentration of 10 and 40 μg/mL ^{10}B. The cells were assayed for colony formation to determine survival fraction.

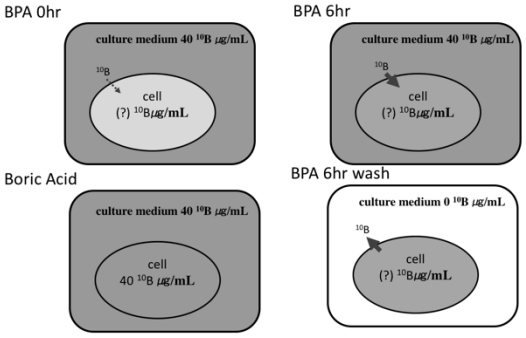

Fig. 1. Schema of the extracellular boron circumstances. Each group has exposed 10 and 40μg/mL ^{10}B. 0hr BPA, 6hr BPA,6hr BPA wash, Boric Acid.

RESULTS: As shown in Fig. 2, boron effected the survival fraction of U251, but it is not simple concentration-dependent manner. Neutron irradiation time resulted the dose-dependent manner. The reproducibility of these experiments did not assess. 10μg/mL ^{10}B of Boric Acid effected intensely. It may reflect the intra-cellular 10μg/mL ^{10}B play the role of cell killing effect. Comparing the "6hr BPA wash" and "6hr BPA", wash group almost constantly low killing effect, it suggested that extra cellular boron contribute to cell killing.

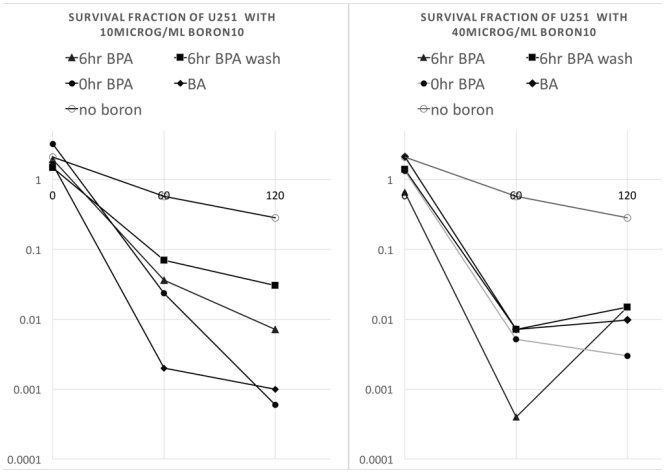

Fig. 2. Survival fraction of U251 cells. Each group has exposed 10 and 40μg/mL ^{10}B. BPA were added in culture medium, exposure time was 6hr (▲6hr BPA) and just before neutron irradiation (●0hr BPA), 6hr exposure BPA and culture medium was changed before irradiation (■6hr BPA wash), ^{10}B enriched Boric Acid was added to culture medium, before irradiation(♦BA). White circles indicated survival fraction of cells without boron.

Previous reports [1,2] indicated 6hr or overnight incubation with BPA or sodium borocaptate[3], intracellular concentration of boron was higher than boron concentration in cell culture medium. Chandra *et al.* reported subcellular boron uptake and retension from BPA study[4], from SIMS observations.comparing these previous report, this experiment is insufficiently verified, and it is still in progress, further study is required.

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Multilateral Approach toward Realization of Next Generation Boron Neutron Capture Therapy

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INTRODUCTION: Currently, the development of next-generation boron neutron capture therapy using accelerator that can be installed together with hospitals at multiple facilities is underway. Despite its clinical usefulness, BNCT utilizes neutrons from conventional nuclear reactors, and it is difficult to disseminate it due to complications of handling and regulatory matters when handling it as a medical device, and adaptive diseases are also caused by brain tumors and other diseases Head and neck cancer etc. are limited. Since accelerator BNCT can be safely operated compared to nuclear reactor BNCT and the high neutron flux can treat cancer of the trunk which was not targeted by BNCT in the past. However, there are very few environments in which basic biological experiments can be conducted by BNCT, and it is difficult to say that accumulation of experimental data is sufficient. We will develop an accelerator neutron source for clinical medicine as a priority issue of the Tsukuba International Strategy Comprehensive Special Zone and some biological experiments were started using accelerator BNCT at May of 2017, and we confirm the biological effect of reactor BNCT in parallel. The purpose of this research project is to accumulate knowledge as the foundation of the development of BNCT from the viewpoint of the possibility of expansion of adaptive disease, the treatment effect by new boron compound, and the change of treatment effect accompanying the change of irradiation condition.

EXPERIMENTS: In FY2007, we refuse that the accelerator BNCT device in Tokai village is not a state where biological experiments can be steadily performed, but it is only one experimental result on January 30, 2018 in KUR. The cytotoxic effects were examined using two novel boron drugs that are going to collaborate with external companies (ND201-BSH). ND201-BSH is a novel boron drug in which BSH is encapsulated in folate - modified cyclodextrin (ND201) and active accumulation on cancer cells with highly expressed folate is added to BSH. BPA and BSH were also used as reference boron compounds.

Cells were seeded in T75 cell culture flasks and treated with each compound. The concentration of ^{10}B in medium were fixed to 20 ppm for BPA and BSH and 2 ppm for ND201-BSH, respectively. After 1h, medium including ^{10}B were washed out and cells were washed with PBS (-), soaked with trypsin-EDTA solution and harvested with new fresh medium. Adequate number of cells were put into the irradiation cryotube (Japan Genetics Co, Ltd.) and irradiated with thermal neutron produced by KUR. After irradiation, cells were analyzed with classical colony formation assay technique. Cells were harvested from cryotube and the cell number was counted with blood cell counting board. Then, cells were diluted to adequate concentration and reseeded into 6 cm cell culture dishes. After 14 days, colonies were fixed with 100% ethanol and transported from KUR to University of Tsukuba. Then, colonies were stained with 1% methylene blue solution and counted. Kaleida graph software was used for the drawing and analysis of the survival curves, and cells showed a linear survival curve without shoulder after BNCT, therefore LQ model with β value set to 0 was used to fit the experimental data.

RESULTS: As shown in Fig. 1, colon-26 showed linear survival curves after each BNCT. The survival curves after thermal neutron alone and BSH treatment group showed straight with a gradual slope. On the other hand, the BPA and ND201-BSH groups showed steep survival curves. Surprisingly, ND201-BSH shows a significant cell killing effect at only 1 ppm, which is ^{10}B concentration of 1/10 of BPA. From these results, it was suggested that the use of ND201-BSH in the case of cancers in which the folate receptor was highly expressed also proved to be more useful with BNCT with a smaller amount of ^{10}B.

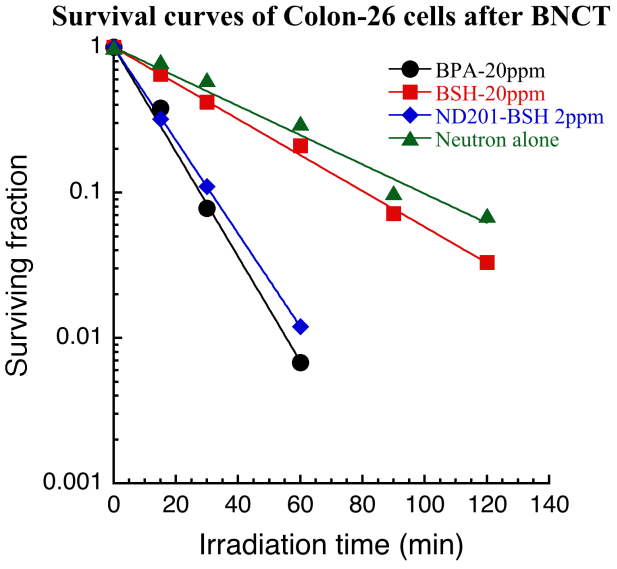

Fig. 1. Survival curves of Colon-26 cells after BNCT with BPA, BSH and ND201-BSH.

PR1-8 Attempts to Sensitize Tumor Cells by Exploiting the Tumor Microenvironment

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INTRODUCTION: Hypoxia and glucose deprivation have been suggested to play important roles in resistance to radiation [1]. Attempts to sensitize tumor cells by exploiting the tumor microenvironment have been studied. A major mediator of the cellular hypoxic response, hypoxia inducible factor 1 (HIF-1), is a potential target for cancer therapy, because it transcriptionally regulates a number of genes, including those involved in glucose metabolism, angiogenesis and resistance to chemotherapy and radiation therapy [2]. Moreover, many cytotoxic agents that selectively kill tumor cells under low glucose conditions, including metformin, were reported [3]. In the present study, we investigated whether the disruption of Hif-1α affects the sensitivity of murine squamous cell carcinoma (SCC VII) cells to metformin and if metformin functions as a radiosensitizer using SCC VII cells [4].

EXPERIMENTS: Hif-1α-deficient SCCVII cells were established through the CRISPR/Cas9 system. In vitro, cell death was evaluated using image-based cytometer; propidium iodide (PI)-positive cells were identified as dead cells. In vivo, tumor-bearing mice were intraperitoneally administered metformin and then subjected 2 h later to acute whole-body γ-ray irradiation. A clonogenic cell survival assay and micronucleus (MN) assay were performed after tumors were disaggregated by stirring in PBS containing 0.05% trypsin.

RESULTS: The disruption of Hif-1α enhanced the cytotoxicity of metformin against SCC VII cells under glucose-free and/or hypoxia-mimetic conditions in vitro. SCC VII Hif-1α-deficient cells from tumor-bearing mice exhibited lower cell survival than SCC VII cells, suggesting that the disruption of Hif-1a strongly influenced viability in the tumor microenvironment; however, additional decreases were not observed in the survival of SCC VII Hif-1α-deficient cells after the in vivo administration of metformin. While no radiosensitivity was found in SCC VII tumors after the in vivo treatment with metformin, a significant enhancement in radiosensitivity was noted in Hif-1α-deficient SCC VII tumors (Fig. 1). Metformin increased the micronucleus frequency in SCC VII Hif-1α-deficient SCC VII cells, which may reflect a reduced DSB repair capacity because micronucleus are a consequence of unrepaired DSBs. Although metformin itself was shown to reduce the stabilization of HIF-1α, our results suggest that the additional downregulation of HIF-1α is effective for sensitizing tumor cells.

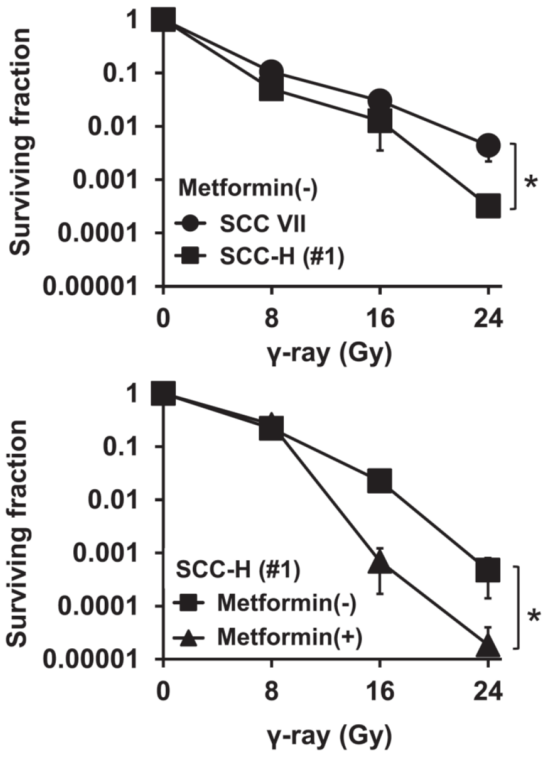

Fig. 1. Cell survival curves for the total cell population from SCC VII and SCC VII Hif-1α-deficient (SCC-H) tumors irradiated with γ-rays on day 14 after the tumor cell inoculation

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