I. Project Research

Project 3

PR 3 Preclinical study for development of new drug for NCT

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In this research project, twenty two research projects were included. In this summary, five research projects (P3-5, P3-6, P3-9, P3-10) could not be reported due to unexpected or uncontrolled events. Details of each project is referred to the following contents. Details of each project is referred to the following contents.

P3-1: The many novel boron compounds from China, US, Sweden and Turkey have been investigated in 2019. All of the China boron compound are extremely low toxic and highly water soluble. Some are excellent and promising B-com for BNCT.

P3-2: In this project, peptide DDS system with A6K peptide was focused. The new boron delivery system with A6K peptide and BSH show new direction of boron agent in next generation of BNCT.

P3-3: Boron-containing nanoparticle (B-NP) which is promising for which is promising nanodevice for BNCT was functionalized with polyglycerol. The resulting B-NP-PG was dispersed in a phosphate buffer saline (PBS) at very high concentration and the resulting dispersion is very stable for more than one month.

P3-4: The results in this project show that the chicken egg chorioallantoic membrane (CAM) model can be used as a reliable model to examine the efficacy of the BNCT therapy. The CAM tumor can be dramatically decreased by the exposure to a neutron beam. The result suggest that the CAM model is particularly suited for the BNCT experiments.

P3-7: In this project, the peptide series IF7-B series (IF7-BSH and IF7-BPA) newly developed as a boron drug was investigated using tumor-bearing mice. Comparing the changes in tumor size in unirradiated tumor models with IF7-BPA and IF7-BSH respectively, it seems that IF7-BPA suppresses tumor growth relatively.

P3-8: In this project, newly developed *in vitro* model of tumor tissue for BNCT demonstrated the pharmacokinetics of BPA and the efficacy of neutron irradiation by direct observation of α -ray/recoiled Li particle tracks that are corresponding to the distribution of BxPC3 cells.

P3-11: In this project, the novel boron compound (BADB) was tested. The combination group of simultaneous use of both BADB (CED) and BPA (i.v.) gave the most significant prolongation of survival (38 (36-39) days).

P3-12: In this research, importance of controlled intracellular locations of boron compounds was shown by the cell-penetrating peptides (CPP) conjugation for achieving their sophisticated BNCT biological activity. This experimental techniques and findings will contribute to development for BNCT methodology.

P3-13: In this project, a novel boron compound containing carbon nanostructure like a horn was investigated. The carbon nanohorns showed better tumor suppression effect on colony formation test in vitro compared to radiation only group.

P3-14: In this project, a phenylboronic acid (PBA)-installed polymeric nanoparticle was tested. In vivo evaluation on a melanoma-bearing mouse model could reveal that the PBA-NP possesses a highly potent antitumor efficacy, which could be only provoked by neutron irradiations.

P3-16: In this project, poly(vinyl alcohol) (PVA) -BPA was tested. PVA-BPA revealed the enhanced inhibition of the tumor growth probably because of the higher intratumoral BPA concentration than sorbitol-BPA during the thermal neutron irradiation.

P3-17: In this project, maleimide-functionalized *closo*-dodecaborate (MID)- transferrin (TF) conjugates was investigated regarding to their in vivo selective boron delivery to tumor in colon 26 tumor bearing mice.

P3-18: A novel boron agent OKD-001 was ef- fective for BNCT against orthotopic xenograft glioblas- toma model. As shown in Fig. 1, OKD-001 plus neutron irradiation significantly prolong the overall survival of the mice in a dose-dependent manner.

P3-19: Pegylated BSH (BAMP) significantly suppressed the tumor growth as compared to other control groups without remarkable side effect (e.g. weight loss).

P3-20: In this study, oligodeoxynucleotides (ODNs) bearing hydrophobic and fluorescent BODIPY unit at uridine base (^{B}U) was investigated.as a new boron compound.

P3-21: In this study, the effectiveness of BPA-BNCT for 5-FU resistance OSCC is evaluated.

P3-22: Arg-Gly-Aso(RGD) binding Gd-DTPA-incorporated calcium phosphate nanoparticles (Meo) was investigated.as a new boron compound.

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PR3-1 Screening of boron compound for BNCT International collaboration studies

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For the purpose of boron sciences, we have continuously screening the many novel boron compounds mainly for BNCT collaborating with China, US, Sweden, and Turkey in 2019. Global paucity of neutron sources is the main reason for international demands of collaboration. We investigated/ screened many compounds mainly for BNCT for Cancer. We report a brief summary of 2019 year.

Materials and Methods.
We already uploaded our standardized experimental maneuvers on the following URL, <https://1458ab30-7501-42df-8c2e-ff59d20cecb7.filesusr.com/ugd/ddd07a_cbe194d92fd14397a5db1690d68a185c.pdf >.
The minimum requirement for our screening protocol of Boron compound samples (B-com) for BNCT is water (and/or DMSO)-soluble 30mg for first look screening of experiments 1, 2 and 3. Whole serial steps are as follow.

1. Solubility in a physiological condition
2. Cell toxicity; IC_{50}
3. Cellular BNCT (in-vitro BNCT)
4. Bio-distribution study (neutron induced boron autoradiography)
5. Animal BNCT (in-vivo BNCT)
6. Pre-clinical study

Results and Discussion.
The figure 1 shows a summary of in-vitro BNCT effect of China B-coms. All of them are extreamy low toxic and highly water soluble. Some are excellent and promising B-com for BNCT and following bio-distribution study of them are investigating on the step 4.
The figure 2 is a schematic drawing of tumoricidal effect of carborane-conjugated anti cancer drug, MMP inhibitor of USA. The idea of such conjugation to evaluate synergetic effect is interesting and exciting. Our data on tumoricidal effect of carborane conjugated MMP inhibitor shows MMP inhibitor is fur beyond BNCT effect within acceptable dosage.

Acknowledgment: This study has been financially supported by National Research Fund No. 17Ki8536 2017-19 from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

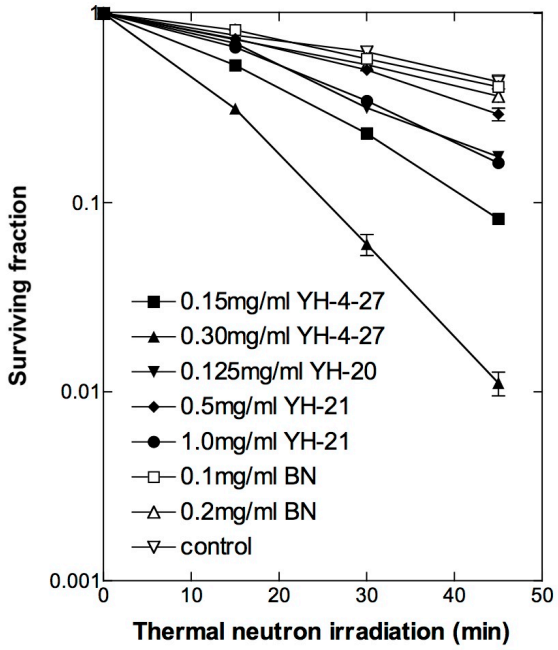

Fig.1. A summary of in-vitro BNCT effect of China B-coms.

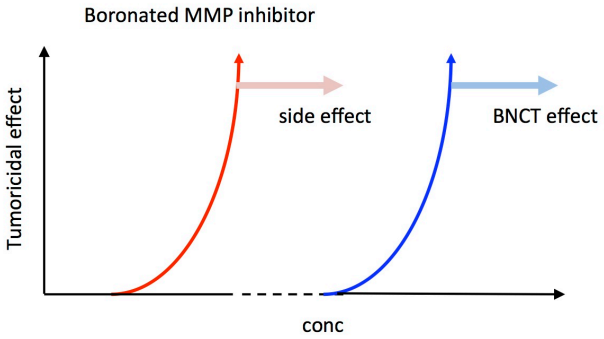

Fig.2. A schematic drawing of tumoricidal effect of carborane-conjugated anti cancer drug, MMP inhibitor of USA.

PR3-2 The self-assembling A6K peptide nanotube as BSH delivery system

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INTRODUCTION: In present advanced cancer therapy with surgery, chemotherapy, and radiation therapy, Glioblastoma multiforme (GBM) is the most treatment-resistant malignant primary brain tumor, with a median survival of approximately 1.5 year. Therefore, the development of novel anti-GBM treatment with the combination of drug and radiotherapy is promising method of treatment to GBM after surgery, especially for elderly GBM patients. BNCT is one of the most promising GBM treatment based on the neutron capture and fission reactions occurred by ^{10}B atoms capture with low-energy neutrons on the irradiation of brain tumor tissues with thermal or epithermal neutron beams. The BNCT reaction consists of high energy transfer alpha particle (^{4}He) and ^{7}Li nuclei with particle range of approximately within the size cell diameter (<10μm). Recently, the neutron source changed to the accelerator neutron source that could be installed in hospital from reactor neutron source. One of the most difficult problem in BNCT developing has been reported the new boron drug applied to several malignant tumor except BPA. The BPA is the leading BNCT boron drug that started from amino acid analogue fused boron against malignant melanoma. Next, many researcher reported that the system L-amino acid transporter-1 (LAT-1) expressed in many malignant tumors. The BSH was used in primary malignant glioma BNCT with the combined usage of BPA. In that BNCT project, the combination use of BPA and BSH group showed significantly longer survival outcome compared to that of single BPA BNCT.

EXPERIMENTS: All of protocol was administrated in committee of KURNS. Before 24hr neutron irradiation, 200μM A6K/2mM BSH (final concentration) or 100μM A6K/1mM BSH (final concentration) was administrated to U87 deltaEGFR cell line. Just before neutron irradia-tion, all the cell sample were collected in collecting tube and got 1MW neutron irradiation (thermal neutron flux 1.4X109 neutron/cm2/sec) for 5min (thermal neutron fluence 4.4X1011 neutron/cm2, γ-ray 0.7X10-1 Gy), 15min (thermal neutron fluence 1.1X1012 neutron/cm2, γ-ray 1.7X10-1 Gy) or 30 min (thermal neutron fluence 2.8X1012 neutron/cm2, γ-ray 3.1X10-1 Gy). After irradiation, all glioma cells were re-cultured in 96 well (9×103 cells/well) and checked cell proliferation with Cell Proliferation Reagent WST-1 (Roche, Basel) by microplate reader (Vient XS, DS Pharma Promo Co., Ltd) for 48hr. And colony formation assay was done after 2 weeks culture with U87 delta EGFR in 60 mm culture dish (n=4) and all culture cells were stained with 0.5% Crystal Violet (CV) in 20% methanol. The colony of CV staining sample was counted automatically with aCOLyte 3 automatical colony counter machine, (Synbiosis, A Division of Synoptics Ltd) and all data was performed statistical analysis.

RESULTS: As shown in Fig. 1,The boron neutron reaction in vitro with low or high dose A6K/BSH complex to U87 delta EGFR cells in KURRI: (A) The colony formation assay of U87 delta EGFR with CV staining after 2 weeks of different neutron irradiation time (blue line: control group, orange line: low dose 100μM A6K/ 1mM BSH and gray line: high dose of 200μM A6K/ 2mM BSH, each n=4).

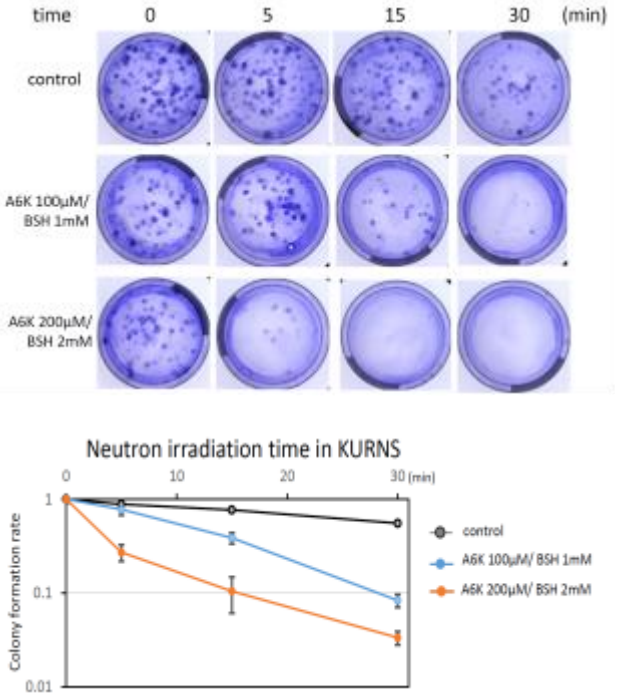

Fig. 1. The boron neutron reaction in vitro with low or high dose A6K/BSH complex to U87 delta EGFR cells in KURRI: the colony formation assay of U87 delta EGFR (blue line: control group, orange line: low dose 100μM A6K/ 1mM BSH and gray line: high dose of 200μM A6K/ 2mM BSH, each n=4) .

CONCLUSION:

The boron DDS agent with polymeric macromolecule DDS such as liposomes,or several boron compounds were very interesting and expansible tools in preclinical BNCT experiments. In this time, we focused peptide DDS system with A6K peptide. In this time, we tried to show the new boron delivery system with A6K peptide and BSH, and this would show new direction of boron agent in next generation of BNCT.

PR3-3 Chemical Functionalization of Boron-Containing Nanoparticle and its Application to Boron Neutron Capture Therapy

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Boron neutron capture therapy (BNCT) is one of the promising cancer therapy with minimized side ef-fect, because ^{10}B atoms located in the cancer tissue generate alpha particles locally upon neutron irradia-tion. Herein, we will report that boron-containing na-noparticle (B-NP) is functionalized with polyglyc-erol (B-NP-PG) and the resulting B-NP-PG exhibits good dispersibility in a physiological environment.

B-NP was functionalized with PG through ring-opening polymerization of glycidol, as shown in Scheme 1, according to the method previously reported by us [1-4]. The resulting B-NP-PG was fully characterized by nuclear magnetic resonance (NMR) spectroscopy, Fourier transfer infrared (FTIR) spec-troscopy and thermogravimetric analysis (TGA). B-NP-PG is dispersed in a phosphate buffer saline (PBS) at very high concentration and the resulting dispersion is very stable for more than one month.

We are planning to carry out pharmacokinetic experiments to see the biodistribution; especially if B-NP-PG is trapped in the lever and/or spleen or not. If we can confirm tumor accumulation of B-NP-PG in enough amonunt, BNCT experiments should be carried out. We hope that the tumor decreases in size and disappears finally after neutron irradiation.

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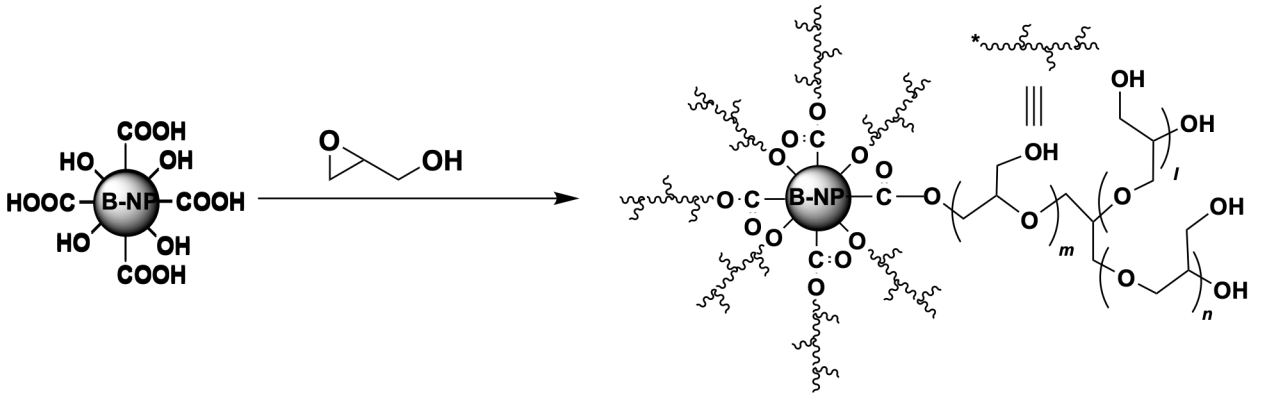
Scheme 1. Chemical Functionalization of Boron-Containing Nanoparticle (B-NP) with Polyglycerol.



PR3-4 Examining efficacy of tumor models for BNCT

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INTRODUCTION:
Boron Neutron Capture Therapy (BNCT) provides a promising approach for cancer treatment. While BPA and BSH have been used as a boron reagent for BNCT, we have seen development of novel boron agents in the past years. These include various nano-formulated boron agents. To evaluate the efficacy of these new agents, it is necessary to use tumor models. In the past, tissue culture cells and mouse models have been used. More recently, tumor spheroids and the CAM (chorioallantoic membrane) assay using fertilized chicken eggs have been developed [1]. We have evaluated the use of these models for BNCT.

EXPERIMENTS:
(Exp.1)
Tumor spheroids were prepared by growing GFP labeled ovarian cancer cells on a spheroid forming plate for fourteen days. The spheroids were incubated with boron-10 agents which resulted in the distribution of the agents throughout the spheroids. The spheroids were exposed to neutron beam at the nuclear reactor for one hour and changes to the spheroids were examined.

(Exp.2) A similar experiment was carried out with ovarian tumor formed on the CAM membrane of fertilized chicken eggs. This was prepared by transplanting human ovarian cancer cells onto the CAM membrane. After confirming tumor formation, boron-10 agents were injected intravenously and the eggs were exposed to neutron at the nuclear reactor for 1 hour. Effect on tumor growth was examined by observing tumor size as well as by examining tumor weight three days after the exposure.

RESULTS:
Exp.1:
Tumor spheroids were formed from human ovarian cancer cells labeled with GFP. They had uniform shape and size (0.2 x 0.2 mm). Incubation with boron agents such as BPA-loaded nanoparticle (Rhodamine-B labeled) resulted in uniform distribution of the agent throughout the spheroids as revealed by the analysis of confocal microscopy. These spheroids were then irradiated with a neutron beam for one hour at the nuclear reactor. Two days after the exposure, the spheroids were examined. The results showed that little changes have occurred by the neutron exposure.

Exp.2:
Three days after transplanting human ovarian cancer cells onto the CAM membrane, tumor was formed. Boron re agents such as BPA or BPA-loaded nanoparticles were injected intravenously into the chicken eggs. The amount of boron loaded onto the nanoparticle that was used in this experiment was examined by ICP-OES. This analysis revealed the presence of 5.47 ppm of boron in a 10 ml solution containing 2 mg of nanoparticles, which amounts to the weight of boron constituting 2.5% of the nanoparticle. The eggs were exposed to neutron beams for one hour at the nuclear reactor. Two or three days after the exposure, tumor size and weight were examined. The results showed that the tumor size was significantly decreased by the exposure.

CONCLUSION AND FUTURE PROSPECTS:
Our results show that the chicken egg CAM model can be used as a reliable model to examine the efficacy of the BNCT therapy. We have reproducibly demonstrated that the CAM tumor can be dramatically decreased by the exposure to a neutron beam. The tumor weight after the exposure was less than 10 mg while that of the non-exposure control was around 50 mg. This type of dramatic effect was not observed with the use of tumor spheroids. These results suggest that the CAM model is particularly suited for the BNCT experiments.

In the future, we plan to use the patient-derived chicken egg tumor model (PDcE model). We have established the PDcE model by transplanting tumor fragments from the ovarian cancer patients as well as by transplanting biopsy samples from esophageal cancer patients. This will enable testing of the BNCT efficacy with an individual patient tumor.

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PR3-5 Development of new boron drug for next-generation A-BNCT treatment

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INTRODUCTION: Aomori Prefecture is one of the shortest-lived prefectures in Japan, and the return of short-lived prefectures is a long-cherished desire. Among them, the death rate due to cancer is the highest in both men and women nationwide, so it is an urgent task to overcome cancer death as soon as possible. Therefore, in this research, the aim is to introduce the first BNCT technology in Aomori Prefecture[1-2] and to develop original technology mainly for (1) A (advanced Aomori) -BNCT and (2) regenerative treatment using artificial tissue after BNCT[3-4]. In this report, we mainly report on (1) item mainly on the development of high-performance BNCT device and animal experiments using newly developed boron drug using this device.

EXPERIMENTS: For animal experiments, the QSC accelerator and irradiation facility installed by Aomori Prefecture in 2018 were used as A-BNCT devices. The peptide series IF7-B series (IF7-BSH and IF7-BPA) newly developed as a boron drug was administered to tumor model nude mice (about 80 mice) at a maximum dose of 70 mg / kg by TVI and IP. The irradiation dose was 1.2×10^{12} n / cm^2 and the maximum irradiation time was up to 1 hour.

RESULTS: Fig. 1 shows the tumor volume change of each tumor model injected with IF7-BPA and IF7-BSH after irradiation with A-BNCT device (indicated by Hot in the figure), respectively, and unirradiated tumor model (indicated by Cold in the figure).

According to this result, although the variation in tumor size increases with the passage of irradiation time, in the case of IF7-BPA, the tumor size rapidly increases 5 days after irradiation in the non-irradiated tumor model (Cold), whereas the irradiation(Hot), it can be seen that the growth rate in the tumor model is suppressed to some extent.

On the other hand, in the case of IF7-BSH, the tumor size rapidly increases 6 days after irradiation in the unirradiated tumor model (Cold), whereas the growth rate in the irradi-ated tumor model (Hot) is suppressed to some extent and it was after 10 days.

Comparing the changes in tumor size in unirradiated tumor models with IF7-BPA and IF7-BSH respectively, it seems that IF7-BPA suppresses tumor growth relatively.

On the other hand, when comparing the suppressive effect of tumor growth rate with IF7-BPA and IF7-BSH, almost the same suppressive effect is observed, but the time when the suppressive effect of IF7-BSH appears is slightly later than that of IF7-BPA.

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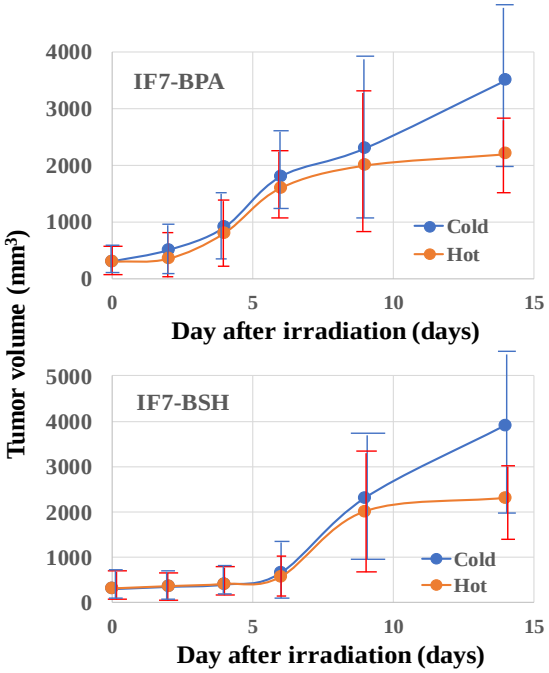

Fig. 1. Change in tumor volume of tumor seeding model after IF7-BPA and IF7-BSH dope and irradiation. In the figure, Cold shows the data of the tumor seeding model that was not irradiated after the boron drug administration, and Hot shows the data of the tumor model that was irradiated after the drug administration.

PR3-6 Research and development on next-generation A-BNCT treatment

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INTRODUCTION: Aomori Prefecture is one of the shortest-lived prefectures in Japan, and the return of short-lived prefectures is a long-cherished desire. Among them, the death rate due to cancer is the highest in both men and women nationwide, so it is an urgent task to overcome cancer death as soon as possible. Therefore, in this research, the aim is to introduce the first BNCT technology in Aomori Prefecture[1-2] and to develop original technology mainly for (1) A (advanced Aomori) -BNCT and (2) regenerative treatment using artificial tissue after BNCT[3-4]. In this report, we mainly report on (2) item mainly on the development of high-performance BNCT device and animal experiments using newly developed boron drug using this device.

EXPERIMENTS:

2.1 Cells, reagents and instruments: Normal human dermal-fibroblast (NHDFs) and red fluorescent protein (RFP)-labeled human pancreatic cancer line BxPC3were used in the experiment. Dulbecco's modified Eable7s medium (DMEM) containing 10% fetal bovine serum (FBS) was used to proliferate cells prior to construction of the tumor tissue model. The cells were cultivated at 37℃, 5% carbon dioxide. Bovine plasma-derived fibronectin (FN) and porcine skin gelatin(G) were applied in this process. Cell cultivation were conducted on transwell inserts with porous polyester bottom(pore size: 0.4 μ m) for 12-well culture plate(12mm diameter, 112mm2 area, cat. No.3401). 2.2 Preparation of *in vitro* human three-dimensional tumor tissue model: Human three-dimensional tumor was pre-pared by cell accumulation method. First, connective tis-sue-like structures were fabricated by three-dimensional lamination of NHDFs. As shown in Fig.1, NHDFs are cul-tured under the conditions mentioned above, then ECM-nano film (about 10nm thick) was formed on each cell sur-face by coating cells with fibronectin and gelatin dissolved in Tris-HCL buffer. The cells are seeded on the transwell inserts at a density of 27.2×105 cells/insert (8 layers) and cultured under the conditions of 5% carbon dioxide at 37℃ for 12 to 24 hours. We regarded this connective tis-sue-like structure as an artificial human normal tissue model, termed as NHDF3D. Next, RFP-labeled BxPC3 cells which have been cultured and proliferated were col-lected by trypsin treatment, washed, and uniformly seeded on the upper surface of NHDF3D at a density of 300 cells/mm^2, then further cultured for 24 hours under the above culture conditions.

2.3 BPA immersion treatment and fixation: The BPA solution (3%w/v) was diluted to a concentration of 40ppm with DMEM containing 10% FBS. After removing the culture solution of NHDF3D or NHDF3D/BxPC3, 750 μ L of BPA treatment solution was added, and incubated for 2 hours (BPA exposure) under condition of 5% carbon dioxide at 37℃(Fig. 1). After that, the BPA treatment solution was removed and the tissues were washed 3 times with 0.01 M phosphate-buffered saline (PBS, PH 7.3). Subse-quently, the tissues were fixed by 4% paraformaldehyde/0.1M phosphate buffer (PH 7.3) for 30 minutes at room temperature shading the light. After the fixation, the cellular nucleus was stained by 4',6-diamidino-2-phenyl-indole(DAPI).

2.4 Neutron irradiation experiment: The above-mentioned NHDF3D or NHDF3D/BxPC3 in the transwell inserts were cut out with a knife together with the polyester base, mounted on the solid track detector CR-39 with close con-tact, and used as a sample for track image acquisition(Fig.1(d)9. Irradiation experiments using these samples were conducted at the heavy water neutron irradiation facility of Kyoto university reactor(KUR), and irradiation was performed for 30minitues under an irradiation flux of 1.4×109 n/cm2/s(Fig.1). After the neutron irradiation, the above sample was etched (6N NaOH, 70℃×2 hours) to visualize the α-ray/recoiled Li particle tracks generated on the CR-39 surface.

RESULTS: From these results, our *in vitro* model of tumor tissue for BNCT demonstrated the pharmacokinetics of BPA and the efficacy of neutron irradiation by direct observation of α-ray/recoiled Li particle tracks that are corresponding to the distribution of BxPC3 cells. Moreover, the evaluated number of α-ray/recoiled Li particle tracks per single BxPC3 cell or NHDF provide the compa-rable value with T/N ratio of BPA in the previous stud-ies[2-3].

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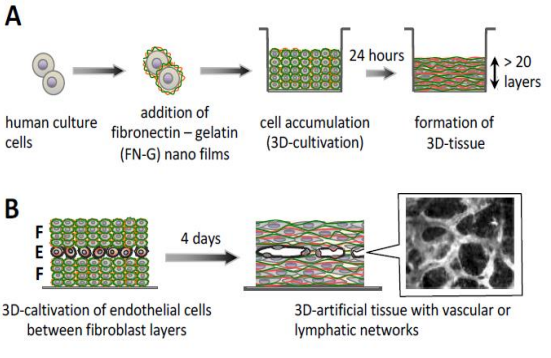

Fig. 1. Procedure for detection of BNCT reaction by using in vitro three-dimensional artificial human tumor tissue model.

PR3-7 Evaluation of boron neutron capture therapy (BNCT) using brain tumor bearing rats or mice model

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Introduction

Boron neutron capture therapy (BNCT) has been performed as an adjuvant therapy of malignant glioma. We treated patients with malignant glioma by surgical removal followed by BNCT, and recently reported with good results.[1]

Many new drugs have been proposed to date, but clinical experience is limited to BPA (boronophenylalanine) and BSH (borocaptate sodium).[2] The development of effective boron compounds is a major theme. We have been conducting the basic study on how the novel boron compound (BADB) that combines the advantages of using amino acid demand (BPA) and containing a large amount of ^{10}B per molecule (BSH) for BNCT will affect rat brain tumor model.

Materials and Methods

We used two boron compounds: BPA and BADB. For in vivo BNCT study, the therapeutic effect was evaluated in terms of the survival time for all rats divided into six groups: untreated controls, neutron irradiation controls, non-neutron irradiation with BADB (CED) controls, BNCT with BPA (i.v.), BNCT with BADB (CED), and BNCT with combination of BPA (i.v.) and BADB (CED). The rats were irradiated at a reactor power of 1 MW for 1 hour.

Results

The survival data following BNCT are summarized in Table. 1. Median survival time (MST) in all neutron irradiation groups were significantly longer than that in the untreated control group ($p < 0.005$ by Log-rank test, respectively).

The combination group of simultaneous use of both BADB (CED) and BPA (i.v.) gave the most significant prolongation of survival (38 (36-39) days). BADB (CED) and BPA (i.v.) combined group had a significant survival prolongation compared with the single-agent group. (vs. BPA (i.v.): 34 (33-36) days, $p < 0.05$ by Log-rank test, vs. BADB (CED): 31 (29-34) days, $p < 0.05$ by Log-rank test). In addition, the combined group showed the highest percent increase in life span value (ILS) among all treated groups (43.4%).

	Survival Time		
Group	n	Median	Range(95% CI)
control	6	26.5	25-28
Irradiated	5	28	27-29
BADB control	5	28	27-29
i.v. BPA	6	34	33-36
BADB (CED)	8	31	29-34
BADB (CED)+ i.v. BPA	7	38	36-39

Table 1. Survival times of F98 glioma-bearing rats after neutron irradiation

Ongoing study

We developed another novel boron drug that human serum albumin conjugated maleimide-functionalized closo-dodecaborate (MID-HSA)[3] as ^{10}B carrier for BNCT. The MID-HSA utilizes the accumulation in the tumor through the well-established Enhanced Permeability and Retention (EPR) effect[4] of solid tumors. We evaluated the biodistribution of these following BPA, BSH, and MID-HAS. In biodistribution study, boron uptake in tumor boron concentration was confirmed by intravenous administration of MID-HSA. Blood concentration was high after i.v. administration. In vivo further therapeutic experiments of MID-HSA using intravenous and CED as a boron delivery agent are ongoing and the results will be reported soon.

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PR3-8 Intracellular target delivery of cell-penetrating peptide-conjugated dodecaborate for boron neutron capture therapy (BNCT)

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INTRODUCTION: In BNCT, internalization of ^{10}B-boron atom by cancer cells leads to the induction of cell death by generation of alpha particles and recoiling ^{7}Li nuclei with high linear energy transfer and short range when irradiated with neutrons [1]. Effective therapeutic benefits on intractable cancer, such as brain tumor and head and neck cancer have been attained through current BNCT technology. However, insufficient accumulation and cellular uptake efficacy of second-generation boron compounds such as thiododecaborate (BSH) under clinical research have been pointed out. In this research, we aim to develop BNCT technology using cell-penetrating peptides (CPPs) [2] for enhanced cellular uptake of boron compounds and their controlled locations inside cells.

EXPERIMENTS: Chemical synthesis of all peptides were conducted via Fmoc solid-phase peptide synthesis. Dehydration condensation of carboxyl dodecaborate derivative [$B_{12}H_{11}S(CH_2)_2COOH$] to the N-terminal of CPPs was conducted for their conjugation. Cellular uptake of fluorescently labeled dodecaborate-conjugated CPPs were assessed using a confocal laser microscopy. Regarding BNCT assay, after cellular treatment with each dodecaborate-conjugated CPP (30 min, 37°C), the cells were irradiated with neutrons, and cell-killing effects were detected by e.g. cellular colony assay.

RESULTS: When cell death is induced by organelle damage, the cell death efficacies and pathways are possibly affected by the damaged organelles (e.g. plasma membrane, mitochondria, or nucleus) by BNCT. We designed and synthesized organelle-targeted peptide-conjugated boron clusters to increase their cellular uptake and to control the intracellular locations for induction of sophisticated cancer cell-killing activity (including efficacies and mechanisms) under BNCT. For example, boron compounds conjugated with mitochondria target CPP, *RLA* (amino acid sequence: $_{D}$[RLARLAR]$_2$) [3], showed significantly enhanced cellular uptake efficacy and mitochondrial accumulation of the boron compounds (Figure 1). Once the accumulation of the boron compounds in mitochondria (30 min treatment), the compounds were highly retained even after 24 hrs incubation. In BNCT experiments, we found that *RLA* peptide-conjugated dodecaborate showed higher effects of cancer cell-killing activity than that of other CPPs (endosomes and cytosolic release) when irradiated with neutrons *in vitro* BNCT assay [4].

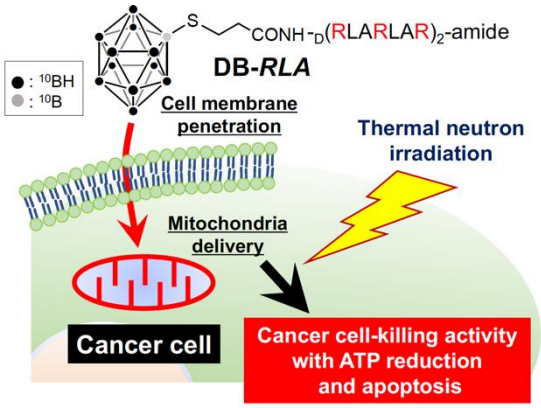

Figure 1. Schematic representation of the intracellular targeted delivery of boron compounds. Boron compounds (dodecaborates, DB) are conjugated to *RLA* peptides for mitochondria delivery. The controlled intracellular delivery by the conjugated peptide affects the efficacy of the cancer cell-killing activity and the cell death types.

CONCLUSION: In this research, we showed importance of controlled intracellular locations of boron compounds by the CPP conjugation for achieving their sophisticated BNCT biological activity. Our experimental techniques and findings will contribute to development for BNCT methodology.

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PR3-9 **A novel carbon nanohorns for BNCT**

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INTRODUCTION: For Boron Neutron Capture Therapy (BNCT), less toxic and tumor-selective accumulation by ideal drug delivery systems are mandatory. Many types of newly boron compound such as liposome, porphyrin, and others were developed, none of novel boron compound has been used in BNCT trials other than p-boronophenylalanine (BPA), sodium borocaptate (BSH). Morever, only BPA proceeded to phase II trial in the field of BNCT.

We developed a novel boron compound containing carbon nanostructure like a horn provided by national institute of advanced industrial science and technology. This compound contains borons not only inside horn structure but also the outer surface of this horn. For selective accumulation in tumor cells, the BN-CNH was coated with phospholipid polyethylene glycol having folate, BN-CNH/PLPEG-FA [1] called carbon nanohorns. This carbon nanohorns conjugated with FA can selectively accumulate in tumor tissues. Also, it can be observed under light microscope to check whether it is in the tumor tissues and tumor cells [1] .

EXPERIMENTS: CT26, mice colon tumor cells, and GL261, mice glioma cells were used for colony formation test. The $1*10^6$ cells were plated on wells of 6 well plate 24 hours before experiment. The drug, carbon nanohorns and BPA were added to each well respectively 24 hours before irradiation compared to control, radiation only group. The cells were trypsinized and counted. The irradiation was performed with thermal neutrons with a flux of 1.6×10^{12} neutrons/cm2 over 15min at the Kyoto University Research Reactor (KUR). The 200 cells and 600 cells per dish were plated respectively. Plating efficiencies were checked after counting colonies on day 14. This time carbon nanohorns only contains natural boron.

RESULTS: The carbon nanohorns showed better tumor suppression effect on colony formation test in vitro compared to radiation only group. BPA showed the best tumor supression effect (Figures 1&2). However, our carbon nanohorn consists of natural borons, not contains ^{10}B. therefore, if we use carbon nanohorns consisting of ^{10}B, we can expect better results the same as BPA or more.

Figure 1

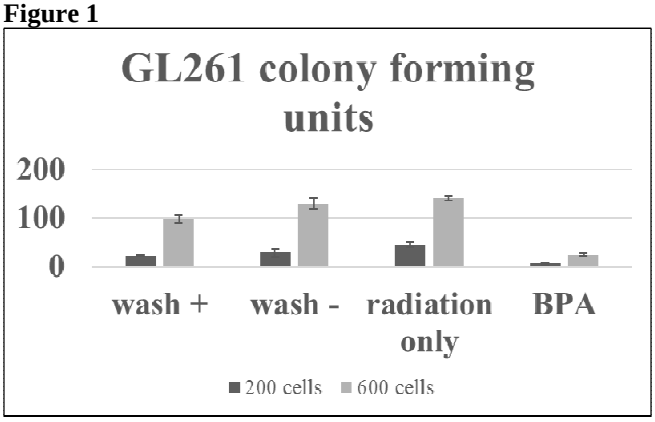
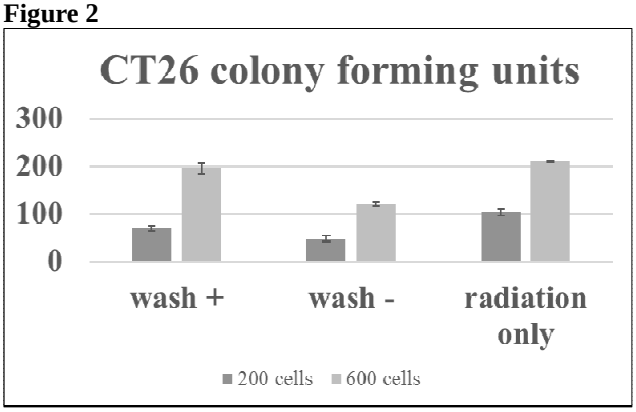


Figure 2



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PR3-10 Development of a multifunctional nanoparticle towards next-generation boron neutron capture therapy

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INTRODUCTION

Boron neutron capture therapy (BNCT) utilizes nuclear fission in a few μm-range to achieve target-specific tumoricidal effects. The location of the boron-10 determines where the nuclear fission takes place, thus the biodistribution of the boron-10 should be regulated to afford damage in the tumors, but not to provoke adverse effects elsewhere. To this end, we designed a phenylboronic acid (PBA)-installed polymeric nanoparticle (Fig. 1), of which tethered PBA moieties provide neutron capture capacity as well as strong affinity to sialic acids. Because hypersialyation of the cancer cells is generally accompanied by metastatic invasiveness, the PBA might facilitate accumulation and retention of the nanoparticle, especially in metastatic tumors. Furthermore, in contrast to conventional BNCT agents, polymeric nanoparticles are not prone to an acute renal clearance, due to a supramolecular structure comprised of synthetic polymers. Thus, our design is supposed to have a prolonged systemic circulation, which may result in even higher tumor accumulation.

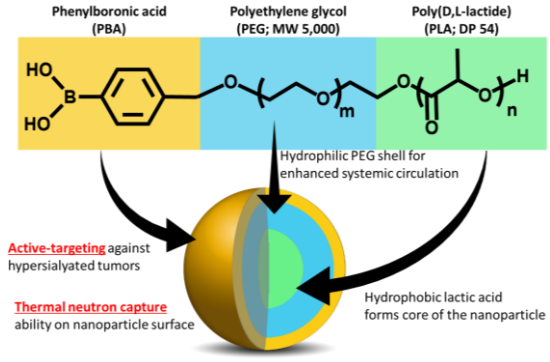

Fig. 1. Schematic design of the PBA-installed nanoparticle.

EXPERIMENTS

Sequential anionic polymerization of ethylene oxide and D,L-lactide was carried out on a PBA pinacol ester bearing alkoxide initiator, resulted in an amphiphilic copolymer composed of pinacol-protected PBA, poly(ethylene glycol) (PEG), and poly(lactic acid) (PLA). Subsequently, the PBA-installed polymeric nanoparticle (PBA-NP) was self-assembled by dissolving aforementioned polymer in an organic solvent, followed by dialysis against distilled and ion exchanged (DI) water. The PBA-NP was then evaluated with a series of in vitro analyses, including surface plasmon resonance (SPR) technique, confocal laser scanning microscopy (CLSM) observations, and cytotoxicity assays. In vivo feasibility of the PBA-NP was validated on a B16 melanoma-bearing C57BL6/j mouse model, by subcutaneous injection of either the PBA-NP or boronophenylalanine-fructose complex (BPA-f), subsequently irradiated with non-invasive neutrons. Furthermore, microdistribution of the boron-10 could be indirectly monitored by embedding tumor sections on a CR-39 solid state nuclear track detector, followed by thermal neutron irradiations, then observed with phase contrast microscope. Pharmacokinetics of the nanoparticles could be validated by applying an inductively coupled plasma mass spectrometry (ICP-MS) on homogenized tissue samples.

RESULTS

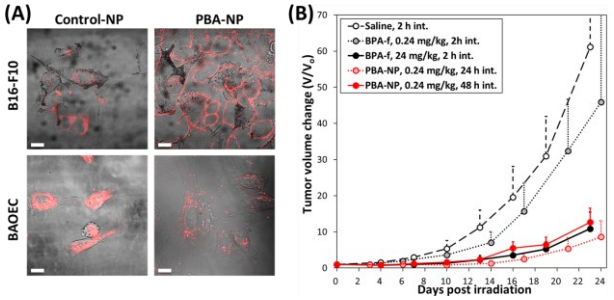

Fig. 2. (A) CLSM observation of B16-F10 cancer cell line and BAOEC primary cells treated with 5 min with rhodamine-labeled nanoparticles, scale bars represent 20 μm. (B) Antitumor efficacy of PBA-NP on BNCT, compared with BPA-fructose (BPA-f), n = 6.

As confirmed by ^{1}H NMR spectra, although the PBA moiety on the synthetic polymer was initially protected with pinacol, it could be readily deprotected while the nanoparticle was being purified, presumably because of hydrolysis-driven deprotection on dialysis. Thus, no further procedure was required to allow functional PBA moieties to be exposed, and to obtain the PBA-NP. The hydrodynamic diameter of the PBA-NP was around 75 nm, which was stably sustained under a physiological condition in the presence of serum, for at least 24 h. With an SPR analysis, the PBA-NP demonstrated unusually strong and selective binding to a sialic acid-immobilized surface, ensured a targeting effect. This was consistent with the CLSM observation (Fig. 2A), where brief incubation of the PBA-NP with hypersialyated cancer cell lines clearly showed selective localization onto the cell membranes. Notably, in vivo evaluation on a melanoma-bearing mouse model could reveal that the PBA-NP possesses a highly potent antitumor efficacy, which could be only provoked by neutron irradiations. It is important to state although the PBA-NP was administered at a 100-folds lower effective dose (0.24 mg ^{10}B/kg) than that of the BPA-f (24 mg ^{10}B/kg), their antitumor efficacies on neutron irradiations were comparable to each other (Fig. 2B). No distinct side effect was observed in the PBA-NP injected mice, as there was no apparent body weight change in both irradiated and non-irradiated control groups. Moreover, assured with a series of cytotoxicity assays, none of the normal primary cells and cancer cell lines manifested palpable response to the PBA-NP. In conclusion, here we propose a highly efficient BNCT agent which affords an active targeting capability.

PR3-11 Design, Synthesis, and Evaluation of Glucose and Macrocyclic Polyamine-type Boron Carriers for BNCT

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INTRODUCTION: Boron neutron capture therapy (BNCT) is one of powerful therapies for local tumor control in the treatment of brain tumor, melanoma, and so on [1]. To date, only two boron-containing drugs, L-4-boronophenylalanine (BPA) and BSH (sodium mercaptoundecahydrododecaborate, $Na_2B_{12}H_{11}SH$) (Fig. 1) have been approved as clinically test compounds, and development of better BNCT agents is highly required.

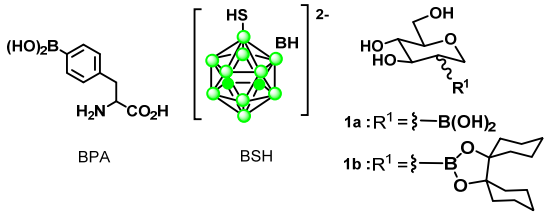

Fig. 1. Chemical structures of BSH, BSH, and **1.**

An enhanced uptake of D-glucose and glucose transporter expression are common in cancer cells. In this context, we previously reported on the design and synthesis, of 2-boryl-1,2-dideoxy-D-glucose derivatives **1** (Fig. 1), although its intracellular uptake is not so high [2]. In this paper, we report on new D-glucose-based drugs **2a,b** and **3** having boryl parts at the C-2 and C-1 positions and **4a-d** having BSH unit at the C6 position of D-glucose and cationic sulfonium unit to neutralize the dianion of BSH part (Fig. 2).

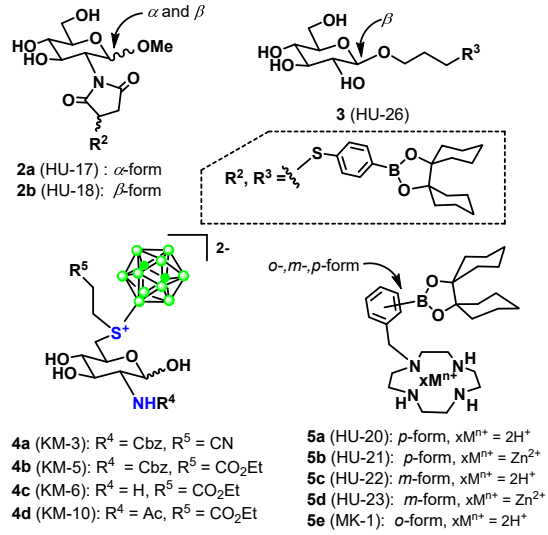

Fig. 2. Structures of boron-containing agents based on D-glucose and macrocyclic polyamine scaffolds.

EXPERIMENTS and RESULTS: The synthesis of **2a,b** and **3** was carried out from 2-deoxy-2-amino-D-glucose and D-glucose, respectively. The synthesis of **4a-e** was conducted from the 1,4-addition of BSH with acrylonitrile and ethyl acrylate, followed by the alkylation reaction with 6-TsO derivatives of the *N*-protected D-glucosamines (the details will be reported elsewhere). The synthesis of **5a-e** was achieved according to our previous paper [3]. Cytotoxicity and cellular uptake activity of the synthesized compounds in cancer cells were evaluated by MTT assay and ICP-MS (inductivity coupled plasma-mass spectrometer).

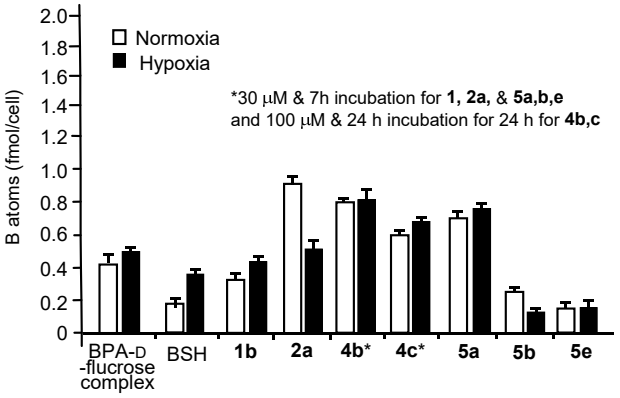

Fig. 3. The results of intracellular uptake evaluatoin of BPA-D-fructose complex, BSH, **1**, **2a,b**, **4b,c**, and **5a,b,e** in HeLa S3 cells, as determined on ICP-MS after the incubation with 30 μM (for **1**, **2a,b**, and **5a,b,e**) and 100 μM (for **4b,c**) of the drugs under normoxic and hypoxic conditions at 37 ºC for 7−24 hr. Data represent the mean ±SD of at least three replicates.

The intracellular uptake values of the aforementioned compounds are presented in Fig. 3, which implies that the intracellular uptake of **2a**, **4b**, **4c**, and **5a** is somewhat better than BPA and BSH. The uptake of **5b**, a zinc(II) complex of **5a** is lower than that of **5a**. The comparison of **5a**, **5c** (data not shown) and **5e** suggests the potion of boryl unit on the benzyl groups of these compounds is important for the uptake efficiency [4]. The evaluation of BNCT effect of these molecules and the improvement of the design of these B-carriers and the attempts at ^{11}B MRI of these agents are now in progress.

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[4] H. Ueda *et al.,* Unpublished result.

PR3-12 Boron Neutron Capture Therapy using Polymer-Based Boron Delivery Systems

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INTRODUCTION: In boron neutron capture therapy (BNCT), *p*-boronophenylalanine (BPA) has been the most powerful drug in clinical studies because it can accumulate selectively within malignant tumors by targeting LAT1 amino acid transporters overexpressed on many tumor cells. However, due to the antiport mechanism of LAT1, the intratumoral BPA concentration gradually decreases during thermal neutron irradiation in some cases [1], thereby compromising the ultimate therapeutic potential. Thus, it has been expected that the prolonged BPA retention in the tumor may enhance the BNCT effect.

In this regard, we recently found that simple mixing of biocompatible poly(vinyl alcohol) (PVA) and BPA results in the formation of PVA-BPA complexes via the formation of boronate esters. The PVA-BPA complex could be internalized into the cells through LAT1-mediated endocytosis and localized in endo-/lysosomes while conventional BPA was localized in cytosol. The PVA-BPA complex in the endo-/lysosomes eventually slowed the unfavorable efflux from the tumor cells. Even in *in vivo* study, the PVA-BPA exhibited the enhanced tumor accumulation and prolonged retention, thereby accomplishing the strong BNCT effect [2].

In these previous studies, we used PVA that was synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization of vinyl acetate and subsequent hydrolysis. Here, to examine whether this concept can be applied to commercially available PVA, we used GMP-grade PVA (PE-05JPS provided by JAPAN VAM & POVAL CO., LTD.) for the preparation of PVA-BPA and investigated its BNCT effect.

EXPERIMENTS: BALB/c mice bearing subcutaneous CT26 tumors were used in this study. PVA-BPA or sorbitol-BPA complex was intravenously injected to the mouse (10 mg BPA/mouse), and the thermal and epithermal neutrons were irradiated to the tumor using KUR 3 h after the injection. Size of the tumor was measured using a caliper, and tumor volume (V) was calculated using the following equation:

$$V = 1/2 \times a \times b^2$$

where a and b denote major and minor axes of a tumor, respectively.

RESULTS: As shown in Fig. 1, both sorbitol-BPA and PVA-BPA exhibited strong BNCT effect and significantly inhibited the tumor growth. While sorbitol-BPA-treated tumors showed slight regrowth of the tumor, PVA-BPA revealed the enhanced inhibition of the tumor growth probably because of the higher intratumoral BPA concentration than sorbitol-BPA during the thermal neutron irradiation. These results strongly indicate that the concept of PVA-BPA can be applied even to commercially available PVA, which is important for smooth clinical translation of PVA-BPA.

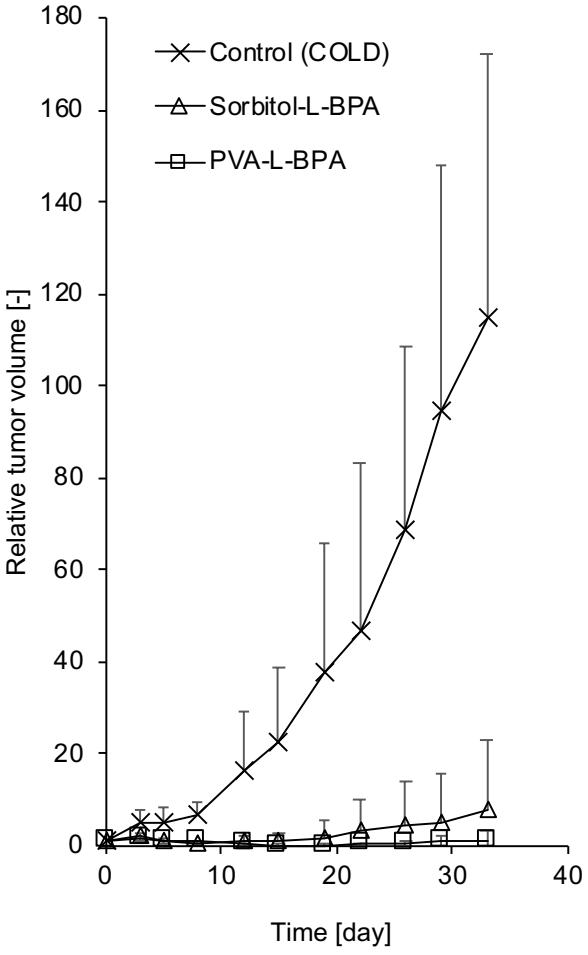

Fig. 1. BNCT effect of sorbitol BPA and PVA-BPA.

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PR3-13 Development of Carrier Protein-based Boron Delivery System

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INTRODUCTION: Boron Neutron Capture Therapy (BNCT) of tumor has been focused as one of the minimally invasive cancer therapies. The successful treatment of BNCT is highly dependent on the sufficient and selective accumulation of ^{10}B atoms to tumor.

We have studied carrier protein-based boron delivery system. Recently, we developed maleimide-functionalized *closo*-dodecaborate (MID) [1] aiming to conjugate to serum albumin, as known to accumulate in malignant and inflamed tissues due to the combination of leaky capillaries with the absence or defect of the lymphatic drainage system, at Cys34 which has only a free SH group among 33 cysteines. Interestingly, MID was found to conjugate not only to a free SH of cysteine residue but also to lysine residues in albumin.[2] MID was also conjugated to transferrin (TF), which does not have any free cysteine residues. TF is an iron carrier protein and accumulated into cells via TF receptor-mediated endocytosis. It is known that TF re-ceptor is overexpressed on the surface of many tumor cells, thus TF has been used as a ligand for active targeting in drug delivery system. Herein we report the preparation of MID-TF conjugates and their in vivo selective boron de-livery to tumor in colon 26 tumor bearing mice.[3].

EXPERIMENTS: MID and isothiocyanate-functionalized *closo*-dodecaborate (ISD) were synthesized from the commercially available *closo*-dodecaborate triethylammonium form, $(Et_3NH)_2[B_{12}H_{12}]$ and ^{10}B-enriched *closo*-dodecaborate sodium form, $Na_2[^{10}B_{12}H_{12}]$ according to our previously reported procedures.[1] TF was treated with MID (1.0 mM) or ISD (1.0 mM) in PBS (50 μL) at room temperature for 1 h. The mixture was subjected to SDS-polyacrylamide gel electrophoresis (PAGE). Immunoblotted with anti-$B_{12}H_{11}SH$ (BSH) antibody was ten carried out. After further incubation with horseradish peroxidase (HRP)-conjugated secondary antibody, MID-conjugated TF was visualized with a Molecular Imager ChemiDoc XRS System. The quantification of MID-conjugated TF was determined by image analysis program, Image J. Total proteins (MID-conjugated and unreacted TF) were visualized by coomassie brilliant blue (CBB) staining. Conjugation of MID or ISD to bovine serum albumin (BSA) was also carried out for comparison.

RESULTS: Figure 1(a) shows the evaluation of the conjugation of MID and ISD to TF. MID and ISD were conjugated to BSA under physiological pH conditions although the conjugation efficacy of ISD was lower than that of MID against BSA (lane 2 *vs.* 3). Interestingly, ISD similarly conjugated to both BSA and TF even though TF has no free SH cysteine residue (lane 5 and 6). In our previous report, we investigated that MID binds to the lysine residues located in the drug binding sites I and II in albumin. Based on the observation, efficient conjugation of MID compared to ISD was probably caused by their different binding modes in the case of BSA.

We next examined the uptake of MID-TF conjugates by colon 26 cells which overexpress TF receptors on the cell surface. The colon 26 cells were incubated with MID-TF conjugates at 10, 30, and 300 ppm boron concentrations for 3 h and the boron accumulation was measured by inductively coupled plasma (ICP). As shown in Figure 1(b), the concentration-dependent uptake of MID-TF conjugates by colon 26 cells was observed. The uptake was not drastically arrested by addition of TF, indicating that the uptake of MID-TF conjugates is considered to be not only through the TF receptor mediated mechanism but also through other pathways.

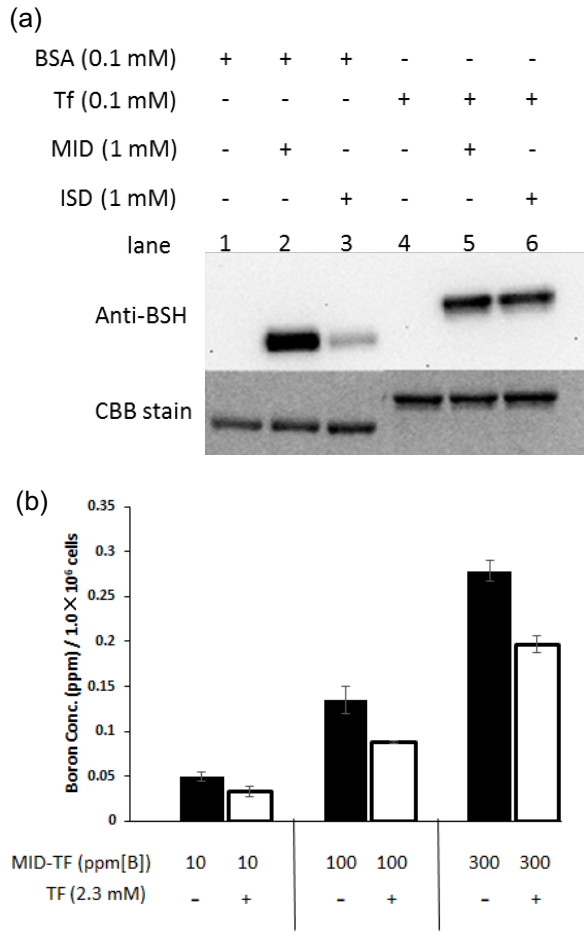

Fig. 1. (a) Western blot analysis of MID conjugation to BSA and TF. (b) Concentration-dependent uptake of MID-TF conjugates by colon 26 cells (black bars).

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PR3-14 Development of Novel Boron Agents for BNCT against Malignant Brain Tumors

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INTRODUCTION: Boron neutron capture therapy (BNCT), a well-controlled intracellular atomic reaction of boron ^{10}B, is a cutting-edge cancer therapy. This *nanobomb* technology utilizes a combination of ^{10}B delivery and neutron irradiation, and consequent ^{10}B nuclear decay in cell, thus requires high selectivity of ^{10}B delivery into cancer cells for safety and efficacy. Currently, primary boron agent for BNCT against several kinds of cancers is BPA (4-borono-L-phenylalanine). BPA is a ^{10}B-added derivative of tyrosine/phenylalanine, and is imported through amino acid transporters such as LAT1[(1)]. Although LAT1 expression levels are upregulated in almost all cases of head and neck cancers or melanomas, there are many cases with low expression levels of LAT1 in the other kind of malignant tumors including glioblastoma. In these cases, the other types of boron agents are needed. Our goal of this study is to develop new boron agent for BNCT against malignant brain tumors, to cover the patients whose LAT1 expression levels are not high.

EXPERIMENTS: NOD-SCID mice were transplanted with 100,000 cells of patient-derived glioblastoma stem-like cells MGG8[(2)] 15 days before BNCT. On 14th day after transplantation, the mice were injected with 0, 10, 20, 40 mg/kg of OKD-001 (our novel boron agent). On the next day, these mice were irradiated with neutron for 1 hour at KUR. After BNCT, the mice were checked twice a week to draw the overall survival curve. Animal experiment was approved by the animal experiment ethical committees of Okayama (OKU-2019315) and Kyoto (#17) Universities.

RESULTS: Our novel boron agent OKD-001 was effective for BNCT against orthotopic xenograft glioblastoma model. As shown in Fig. 1, OKD-001 plus neutron irradiation significantly prolong the overall survival of the mice in a dose-dependent manner.

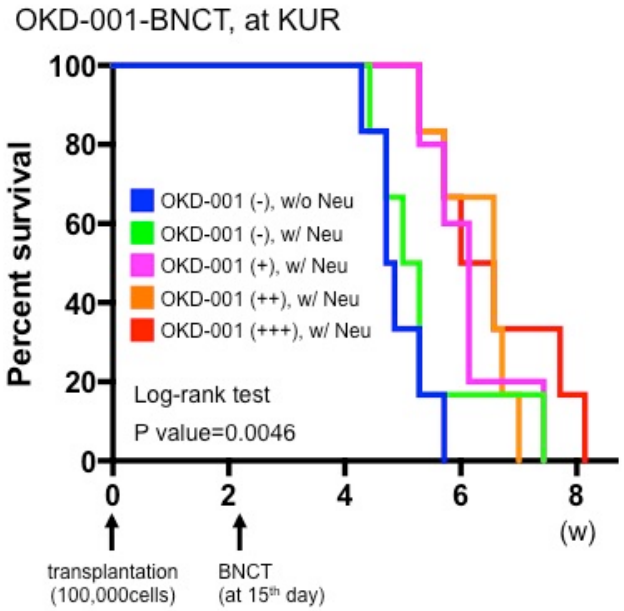

Fig. 1. Orthotopic xenograft glioblastoma model mice are treated with OKD-001-BNCT. OKD-001-BNCT significantly improves the overall survival of the mice in a dose-dependent manner.

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2) M.L. Suva *et al.,* "Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells." ***Cell*** 157 (2014) 580-594.

PR3-15 Experiment on the Anti-tumor Effect of Pegylated BSH by Thermal Neutron Irradiation

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INTRODUCTION: Nowadays, novel boron compounds are frequently demanded to achieve a good anti-tumor effect. Because, Boron Neutron Capture Therapy (BNCT) using *p*-boronophenylalanine (BPA) for carcinoma such as malignant glioma has a limited therapeutic effect, and there are many recurrence cases.

Therefore we synthesized novel boron compound named BAMP [1]. BAMP combined polyethylene glycol (PEG) and mercaptoundecahydrododecaborate (BSH) by covalent bond. Consequently, pegylated BSH (BAMP) leads to improvement of blood retention time and accumulation to tumor.

This paper presents the experimental results obtained on anti-tumor effect *in vivo* using BAMP.

EXPERIMENTS: The tumor-bearing mice were prepared by grafting 5 x 10^6 of mouse colon carcinoma cells (CT26) to the right thigh of female BALB/cA mice (4 weeks old, weighing 16-20 g) to have a tumor diameter of 6-8 mm. These mice were purchased at the age of 3 weeks from CLEA Japan Inc. (Tokyo, Japan) and tamed in Institute for Integrated Radiation and Nuclear Science, Kyoto University.

About 11 days after, 200μL of BAMP and BPA (as control) were administrated by tail vein injection before irradiation. The dosage was 10mg^{10}B/kg and 24mg^{10}B/kg. At the interval BAMP was 36 hours and BPA was 2hours, the irradiation was performed with thermal neutrons with a flux of 1.4-5.9 x 10^{12} neutrons/cm^2 over 1 hour. The tumor size was measured over time after the irradiation until Day 24 and calculated using the general formula [2].

Also, a significant difference in tumor size on the last measurement day of each group was calculated by independent t-test. The value of the significant difference and the number of asterisks are as follows.
(*: $p<0.05$, ** : $p<0.01$, *** : $p<0.005$, **** : $p<0.001$, ns: No significant difference)

RESULTS: As shown in Fig. 1, Fig. 2 and Fig. 3, BAMP significantly suppressed the tumor growth as compared to other control groups without remarkable side effect (e.g. weight loss).

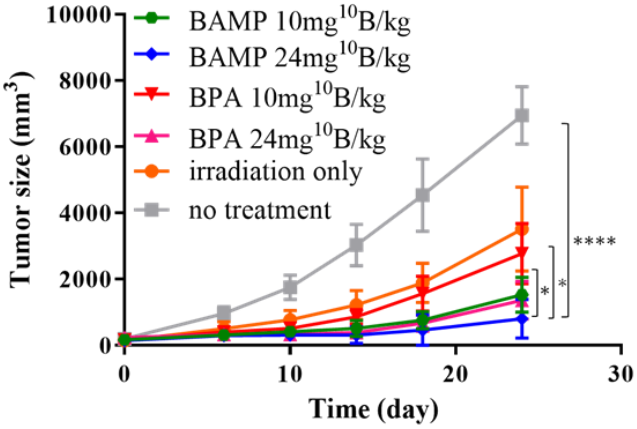

Fig.1) Anti-tumor effect of BNCT by BAMP. (BAMP 24mg^{10}B/kg vs. BAMP 10mg^{10}B/kg : *, vs. BPA 10mg^{10}B/kg : *, vs. BPA 24mg^{10}B/kg : ns, vs. irradiation only : *, vs. no treatment : ****)

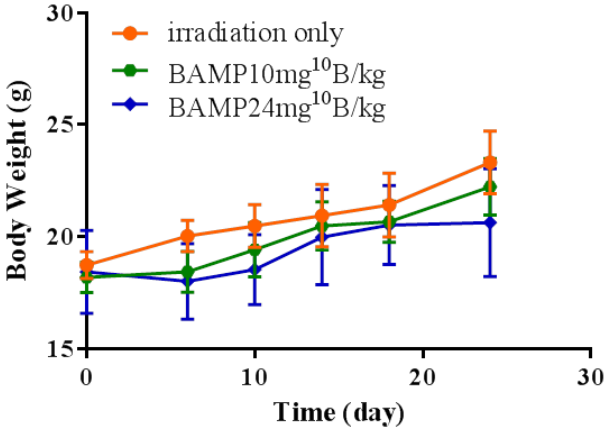

Fig.2) Body weight of mice after irradiation with the injection of BAMP.

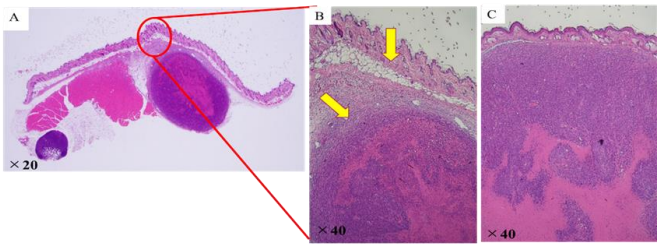

Fig.3) HE stained image of tumor including skin. (A) Image in the BAMP administration group with neutron irradiation after 24 days. (B) Enlarged image of A (x 40). (C) Image in neutron irradiation group without administration after 24 days.

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PR3-16 Preparation and Characterization of BODIPY-Tethered Oligonucleotides for BNCT

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INTRODUCTION: Amphiphilic oligonucleotides with hydrophobic substituents have been widely used as functional materials, and a variety of them have important functions in biological control. For example, modifications of cell membranes, fusion of nanostructures such as liposomes, and gene manipulations have all been produced by these amphiphilic and functional oligonucleotides.

One of the most useful properties of these amphiphilic oligonucleotides is their aggregate formation that showed efficient cellular uptake and high level of biological stability within cells. In our recent studies, we demonstrated the successful delivery of the aggregate consisting of oligonucleotides into the cells to regulate the gene expression.[1]

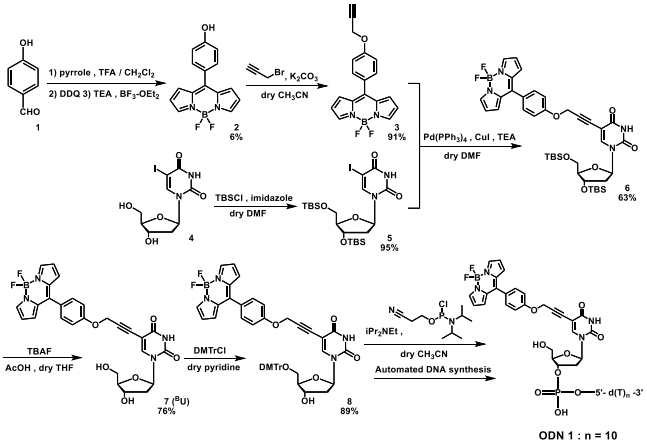
In this study, we prepared oligodeoxynucleotides (ODNs) bearing hydrophobic and fluorescent BODIPY unit at uridine base (^{B}U). We expected that the ODNs bearing BODIPY unit would form aggregate to be taken into the cells and express their cytotoxic effect upon irradiation of thermal neutron. Herein, we synthesize the ODNs and characterize their properties.

EXPERIMENTS:

Cellular experiments using ODNs bearing BODIPY unit. ODN 1 (30 μM) were administered to the A549 cells and then the cells were incubated for 23 h. After incubation, the cells were irradiated (neutron, 1 MW) for 45 min at KUR. After incubation, WST 8 was added to the cells, and the cell viability assay was performed using Microplate Reader.

RESULTS: The synthesis of ODNs bearing ^{B}U is outlined in Scheme 1. Phenol group was introduced into BODIPY unit to give 2, which was alkylated by propargyl bromide under basic conditions. The resulting BODIPY derivative 3 and iododeoxyuridine 5 were coupled by Sonogashira reaction, and then the silyl groups were removed by treatment with TBAF. The uridine derivative 7 (^{B}U) was tritylated and incorporated into DNA via phosphoramidite, using a DNA synthesizer. We prepared 10 mer oligodeoxynucleotides bearing one ^{B}U (ODN 1) at the 5'-end. The structures of ODNs was confirmed by MALDI-TOF mass spectrometry.

We then conducted cellular experiments with ODN 1 using a human cell line of lung adenocarcinoma, A549. A549 cells were incubated with the aggregates consisting of ODN 1 at a concentration that was sufficient to form aggregates, and the fluorescence emission of BODIPY units from the cells was imaged by means of confocal microscopy. We observed robust emissions from all cells, indicating that the aggregates of ODNs were smoothly transported into cells.



Scheme 1. Synthesis of ODN 1.

Based on the above reaction characteristics, an attempt was also made to demonstrate the radiolytic onset of drug potency using A549 cells. We investigated the radiation-dependent cytotoxic effect of ODN 1 aggregates. After administration of ODN 1 aggregates to A549 cells and incubation for 23 h to allow penetration into the cells, the cells were exposed to the thermal neutrons for 45 min. As shown in Figure 1, negligible cytotoxic effect in the presence of 30 μM ODN 1, was observed. These results strongly indicate that the concentration of boron atoms in the cells are not enough to emerge their cytotoxic effects. Thus, the improvement of the drug delivery method is still challenging.

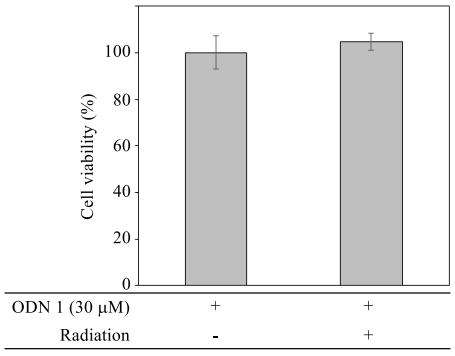


Figure 1. Cytotoxic effect of ODN 1 (30 μM) toward A549 cells upon thermal neutron irradiation (1 MW, 45 min).

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PR3-17 *In vivo* evaluation of BNCT for 5-FU resistant oral squamous cell carcinoma

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INTRODUCTION: The incidence of oral squamous cell carcinoma (OSCC) is increasing gradually with aging society in Japan. In consideration of the preservation of organ function, as well as appearance, Boron Neutron Capture Therapy (BNCT) for head and neck cancer is one of the effective treatments instead of surgical procedures, radiotherapy, chemotherapy and combined therapy. OSCC is the most common malignant neoplasm of the oral cavity and has been treated with 5-florouracil (5-FU) as an anticancer drug. However, the acquisition of resistance to 5-FU is a major problem for successful cancer treatment. In this study, the effectiveness of BNCT for 5-FU resistance OSCC is evaluated.

EXPERIMENTS: 5-FU resistant oral squamous cell carcinoma cell line [2] were subcutaneously injected into the left hind legs of 6-week-old female Balb/c nude mice (Clea Japan Inc., Japan). Using L-boronophenylalanine (BPA, Katchem, Czech), fructose-BPA complex (200 mg/kg) was injected to the tumor bearing mouse before 40 minutes' irradiation. After neutron irradiation, the body weight and the diameter of tumor were measured. The tumor size was calculated according to the following formula.

Tumor volume [mm^3] = (Long diameter [mm]) x (Short diameter [mm])^2 /2

RESULTS and DISCUSSION: As shown in Fig. 1, BPA group showed significantly decrease in tumor size compared to non-boron group at 2 weeks after BNCT for 5-FU resistant SCC bearing mice. The mortality of 5-FU resistant SCC bearing mice is 80 % in non-boron group and 0 % in BPA group.

These results indicate that BNCT is effective to 5-FU resistant OSCC. Moreover, it is important to validate the BNCT for cisplatin resistant OSCC [3]. In near future, the multidisciplinary approach including BNCT is proposed for refractory OSCC.

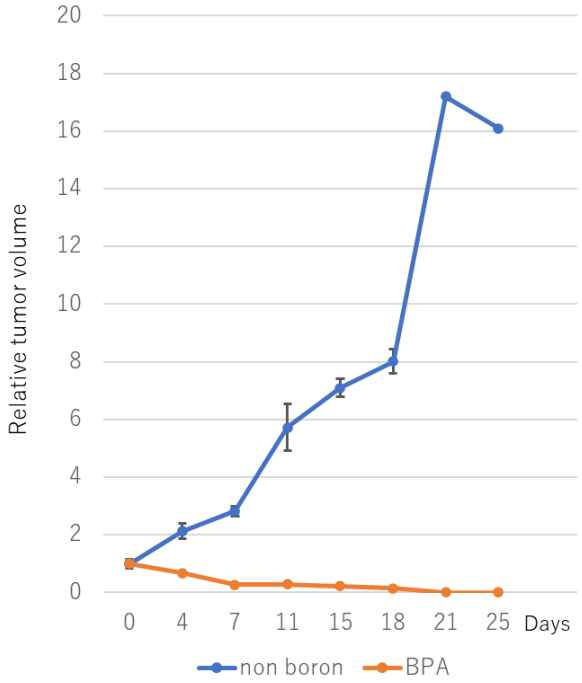

Fig. 1. Tumor growth ratio after thermal neutron irradiation with or without BPA (each group n=5) .

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PR3-18 Anti-tumor evuluation of Gadolinium Neutron Capture Therapy through comparison of tumor size using Gd-DTPA-incorporated calcium phosphate nanoparticles

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INTRODUCTION:

In our previous report, the anti-tumor effect of Gd-DTPA-incorporated calcium phosphate nanoparticles (Meo) was improved, and it had the possibility to be applied to the intensive cancer treatment in the future [1,2,3].

However, although the Gd-DTPA-incorporated calcium phosphate nanoparticle can bind tumor tissues through the EPR pathway, considering to improve the tumor targeting ability and drug accumulation in tumor, we introduced Arg-Gly-Aso(RGD), which is a peptides that can target many kinds of cancer cells through the integrin.

In this work, we checked the antitumor effect of this material again and focused on the change of the tumor size. We took the photos at the 30st day after irradiation to compare the antitumor effect of each samples.

EXPERIMENTS:

Tumor-bearing mouse models were developed with colon 26 tumor cells and after synthesis of Meo and RGD binding Meo, they were injected into mice, respectively.

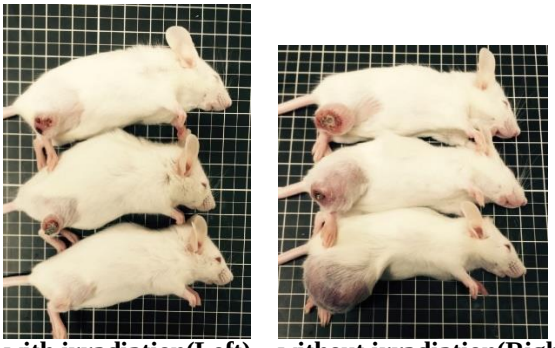
At the 24h after administration, these mice received thermal neutron irradiation at Nuclear Reactor Facility of Kyoto Univ Institute for Integrated Radiation & Nuclear Science with average neutron fluence of 2.0×10^{12} n/cm^2. Moreover, considering the influence of neutron, the same samples were also injected into mice but without irradiation.

After almost one-month observation, the situation of all mice was recorded and the conclusion about antitumor effect was showed below according to the tumor size comparison photos.

RESULTS:

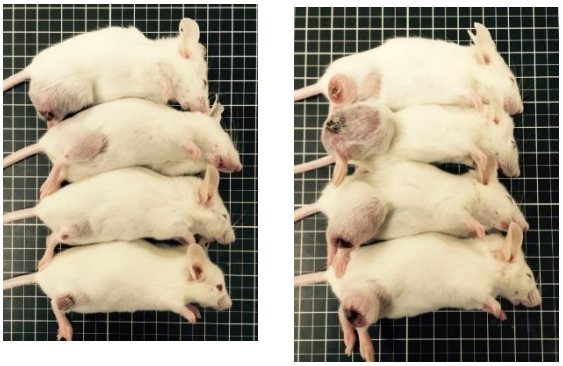
In this experiment, tumor growth was suppressed in the groups of RGD sequence binding Meo nanomicelle and Meo nanomicelle compared with the non-irradiated groups with the injection of same DDS. In this time,the tumour decresence by RGD nanomicelle and Meo nanomicelle was almost same effect by NCT.

We will check the expression of integrin receptors in Colon 26 cancer cell line, and evaluate the uptake of Gd atoms in the cancer cells by endocytosis. We also evaluate the possibility of RGD sequence binding Meo nanomicelle for clinical applications of NCT.



with irradiation(Left) without irradiation(Right)
RGD group

with irradiation(Left) without irradiation(Right)
Meo group



with irradiation(Left) without irradiation(Right)
Control group

Figure 1. Tumor growth suppression by GdNCT using RGD-Meo / Meo nanomicelle.

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