I. ANNUAL SUMMARY OF EXPERIMENTAL RESEARCH ACTIVITIES

I-1. PROJECT RESEARCHES

PR1 Analyzing Tumor Microenvironment and Exploiting its Characteristics for Controlling Malignant Solid Tumors and Distant Metastatic Potential

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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 or bevacizumab may be promising for reducing numbers of lung metastases. Consequently, BPA-BNCT in combination with nicotinamide and/or bevacizumab treatment 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 (26P1-1): 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 (26P1-2): Development of Hypoxic Microenvironment-Oriented ^{10}B-Carriers. (H. Nagasawa, *et al.*)

ARS-3 (26P1-3): Clarification of Mechanism of Radio-Resistance in Cancer Using Optical Imaging at Tissue Level. (H. Harada, *et al.*)

ARS-4 (26P1-4)*: Analysis of Radiation-Induced Cell-Killing Effect in Neutron Capture Reaction. (R. Hirayama, *et al.*)

ARS-5 (26P1-5): Transdermal Drug Delivery System using Hyaluronan-Conjugated Liposomes as ^{10}B-Carrier in Boron Neutron Capture Therapy for Melanoma (S. Kasaoka, *et al.*)

ARS-6 (26P1-6): Evaluation of Inclusion Complex of Carborane Modified Kojic Acid and Cyclodextrin as ^{10}B-Carrier in Boron Neutron Capture Therapy. (T. Nagasaki, *et al.*)

ARS-7 (26P1-7)*: Molecular Design and Synthesis and Functional Evaluation of Anticancer and Molecular Targeting Agents. (Y. Uto, *et al.*)

ARS-8 (26P1-8)*: Analyzing Biological Effect of BNCT from the Viewpoint of the Changes in Oxygenation Level. (H. Yasui, *et al.*)

ARS-9 (26P1-9): Analyses on the Responsiveness of Malignant Tumors to BNCT. (M. Masutani, *et al.*)

ARS-10 (26P1-10): Assay for Tumor Cell Survival and Tumor Growth Delay through Neutron Capture Reaction according to the Changes in Intracellular Concentrations within Solid Tumors of Newly- Developed ^{10}B-Carriers. (K. Nakai, *et al.*)

ARS-11 (26P1-11)*: Antitumor and Metastasis-Reppresing Effect of BNCT on Human Breast and Pancreatic Cancer Cell Lines. (Y. Matsumoto, *et al.*)

(* Due to the irregular and short operation period of our reactor in 2014, the data could not be shown here.)

採択課題番号 26P1　　局所腫瘍制御と転移抑制を同時に目指す　　プロジェクト
治療法開発のためのがん微小環境解析とその応用

（京大・原子炉）増永慎一郎

PR1-1 Significance of Fractionated Thalidomide Administration Combined with γ-Ray Irradiation from the Viewpoint of Local Tumor Response and Lung Metastasis

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INTRODUCTION: Thalidomide has been reported to induce tumor blood vessel normalization in a mouse model [8, 9]. Today, thalidomide is being mainly applied as a treatment of certain cancers (multiple myeloma) and of a complication of leprosy. Here, using a readily metastasizing murine melanoma cell line, we tried to analyze the significance of combined treatment with thalidomide in radiotherapy with γ-rays in combination with an acute hypoxia-releasing agent nicotinamide or mild temperature hyperthermia (MTH), already shown to have the potential to release tumor cells from diffusion-limited chronic hypoxia [1, 2], in terms of local tumor response and lung metastatic potential. Concerning the local tumor response, the effect not only on the total (= proliferating (P) + quiescent (Q)) tumor cell population but also on the Q cell population was evaluated using our original method for selectively detecting the response of Q cells in solid tumors.

Materials and Methods: B16-BL6 melanoma tumor-bearing C57BL/6 mice were continuously given 5-bromo-2'-deoxyuridine (BrdU) to label all P cells. The tumor-bearing mice then received γ-ray irradiation after thalidomide treatment through a single or two consecutive daily intraperitoneal administrations up to a total dose of 400 mg/kg in combination with nicotinamide or mild MTH. Immediately after the irradiation, cells from some tumors were isolated and incubated with a cytokinesis blocker. The responses of the Q and total (= P + Q) cell populations were assessed based on the frequency of micronuclei using immunofluorescence staining for BrdU. In other tumor-bearing mice, 17 days after irradiation, macroscopic lung metastases were enumerated.

Results: Thalidomide raised the sensitivity of the total cell population more remarkably than Q cells in both single and daily administrations. Daily fractionated administration of thalidomide elevated the sensitivity of both the total and Q cell populations, but especially the total cell population, compared with single administration. Daily administration, especially combined with MTH, decreased the number of lung metastases.

Discussion: Thalidomide was originally introduced as a non-barbiturate hypnotic, but withdrawn from the market due to teratogenic effects. However, it has been reintroduced and used for a number of immunological and inflammatory disorders due to its immunosuppressive and anti-angiogenic activity. It inhibits release of tumor necrosis factor from monocytes, and modulates other cytokine action. Thalidomide is racemic, and contains both left and right handed isomers in equal amounts: one enantiomer is effective against morning sickness, and the other is teratogenic. The enantiomers are converted to each other at physiological conditions (pH = 7.0) *in vivo*. That is, if a human is given D-thalidomide ((+)- or R-) or L-thalidomide ((-)- or S-), both isomers can be found in the serum. Hence, administering only one enantiomer will not prevent the teratogenic effect in humans. It is employed for the acute treatment of the cutaneous manifestations of moderate to severe erythema nodosum leprosum (ENL). Available data from *in vitro* studies and preliminary clinical trials suggest that its immunologic effects can vary substantially under different conditions, but may be related to suppression of excessive tumor necrosis factor production and down-modulation of selected cell surface adhesion molecules involved in leukocyte migration [3].

As a cancer treatment, thalidomide was shown to inhibit basic fibroblast growth factor (bFGF) as well as VEGF, two promoters of angiogenesis. It was reported to measure the modifications in the tumor environment early after an angiogenic treatment of thalidomide, with a special focus on possible normalization of the tumor vasculature that could be beneficial for radiotherapy [4]. Incidentally, angiogenesis also is critical during limb development of the foetus. Thalidomide directly inhibits angiogenesis induced by bFGF or VEGF *in vivo*. In 2009, it was confirmed that loss of newly formed blood vessels is the primary cause of thalidomide teratogenesis, and that developing limbs are particularly susceptible because of their relatively immature, highly angiogenic vessel network. Thus inhibition of angiogenesis is now thought to be a main mechanism of its teratogenicity [3,5].

Conclusion: It was elucidated that control of the chronic hypoxia-rich Q cell population in primary solid tumors has the potential to impact the control of local tumors as a whole, while control of the acute hypoxia-rich total tumor cell population has the potential to impact the control of lung metastases. Namely, in conventional radiotherapy, daily fractionated administration of thalidomide combined with MTH is thought to have a great potential to control both local solid tumors and lung metastases from the local tumors [6].

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[3] M. E. Franks, *et al.*, Lancet **363** (2004) 1802-1811.
[4] R. Ansiaux, *et al.*,Clin Cancer Res **11** (2005) 743-750.
[5] C. Therapontos, *et al.*, Proc Natl Acad Sci U S A **106** (2009) 8573-8578.
[6] S. Masunaga *et al.*, World J Oncol **5** (2014) 155-165.

PR1-2 Development of Nucler-targeting Boron Carriers Based on the Chemistry of Direct Cell-penetrating Pepducins

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INTRODUCTION: For the success of boron neutron capture therapy (BNCT), it is essential to selectively deliver sufficient amount of 10B atoms to tumor cells. To achieve intracellular molecular targeting of drug delivery, we focused on the direct cell-penetrating lipopeptides, pepducins which were developed as allosteric modulators of GPCRs[1]. By the study of fluorescence resonance energy transfer (FRET)-based imaging of pepducins, we demonstrated that they penetrate directly into cells by trans-membrane lipid translocation [2]. Accordingly, we envisaged that the pepducin moiety may be exploitable for intracellular deliver of membrane impermeable molecules such as anionic boron claster, sodium borocaptate (BSH).

MOLECULAR DESIGN: As shown in Fig. 1, we designed new hybrid molecules comprising pepducin (Pep) as a vehicle, and boron cluster (BS) attached with fluorescent dyes such as 7-hydroxycoumarin (Cou) and Hoechst 33342 dye (Hoe) as a cargo; Pep-SS-BS-Cou and Pep-SS-BS-Hoe. The cargo is supposed to be released into cytosol after reductive cleavage of the disulfide bond by intracellular glutathione when it is carried into the cytosol.

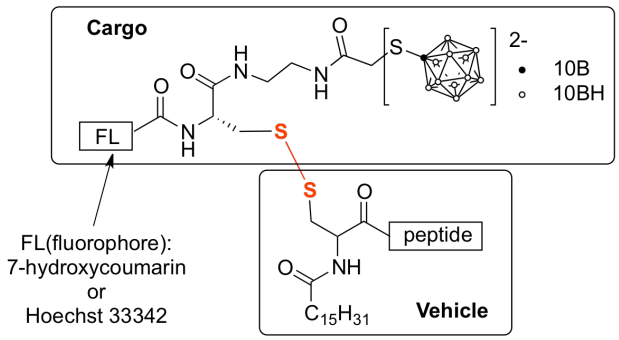

Fig. 1 Design of boron carriers

RESULTS AND DISCUSSION: The lipidated peptides were prepared by solid-phase synthesis and then combined with BSH and the corresponding fluorophore through an appropriate liker to afford the boron carriers (Fig. 2 and 3). We performed live cell imaging experiments using MCF-7 cells with the probes and confocal microscopy. By the treatment with Pep-SS-BS-Cou, the fluorescence signal was localized on cell-membrane for more than 30 min, which suggested that the boron carrier was likely to be anchored on the plasma membrane but could not be translocated into the cytosol. To evaluate the structure activity relationship of the peptide sequence for controlling cellular distribution, we synthesized pepducins containing various peptides to conjugate with the cargo moiety.

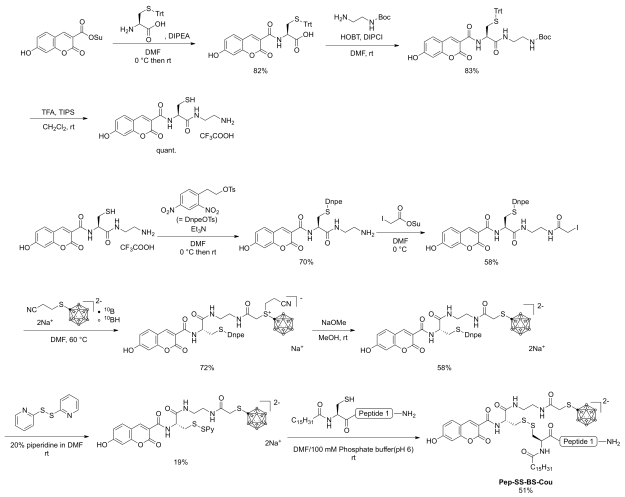

Fig. 2 Synthesis of Pep-SS-BS-Cou

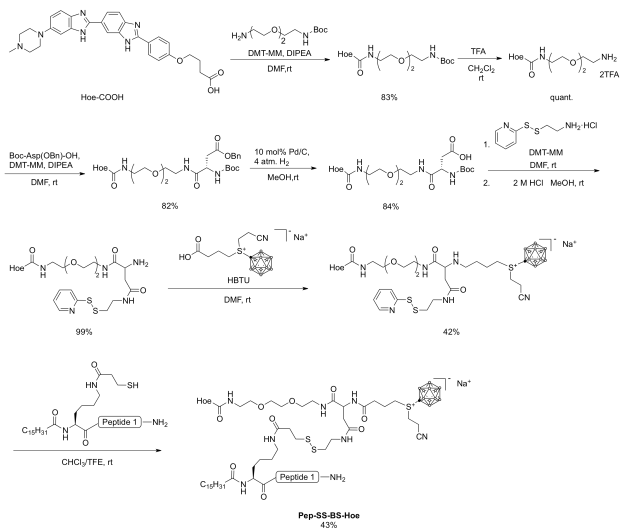

Fig. 3 Synthesis of Pep-SS-BS-Hoe

We also synthesized Pep-SS-BSs by connecting of various pepducins to the boron cluster without any fluorophores. To quantify their intracellular boron concentration, ICP-MS analyses of nuclear and cytoplasmic extracts will be conducted.

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[1] L. Covic *et al.*, Proc. Natl. Acad. Sci. USA., **99** (2002) 643–648.
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採択課題番号 26P1-2　低酸素微小環境を標的とするボロンキャリアの開発　　プロジェクト
（岐阜薬大・薬化学）磯野　蒼、奥田健介、平山　祐、永澤秀子（京大・原子炉）増永慎一郎

PR1-3 Genetic Screening of Upstream Activators of Hypoxia-inducible Factor 1 for the Devrlopment of Novel Anti-cancer Strategies

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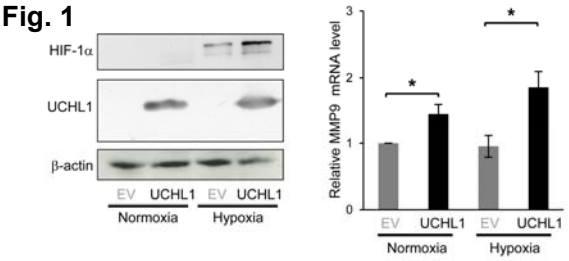
INTRODUCTION: Accumulating evidence has shown that hypoxia-inducible factor 1 (HIF-1) plays critical roles in distant tumor metastases at multiple steps [1] and eventually causes death among cancer patients. Clinical studies have demonstrated consistent data that HIF-1 could be used as an adverse prognostic factor for not only local tumor recurrence but also distant tumor metastasis in cancer patients. These findings justify targeting HIF-1 for cancer therapies.

HIF-1, a heterodimeric transcription factor composed of an α-subunit (HIF-1α) and a β-subunit (HIF-1β), is known to become activate mainly through the stabilization of HIF-1α protein. Under normoxic conditions, prolyl-4-hydroxylases (PHDs) hydroxylate proline residues, P402 and P564, in the oxygen-dependent degradation domain (ODD domain) of HIF-1α in an oxygen-dependent manner. The hydroxylations trigger polyubiquitilation and subsequent proteasomal degradation of HIF-1α. On the contrary, HIF-1α becomes active under hypoxic conditions because of the inactivation of the hydroxylases, and then, interacts with its binding partner, HIF-1β. Resultant heterodimer, HIF-1, binds to its cognate enhancer sequence, the hypoxia-responsive element (HRE), and induces transcriptions of various genes related to the escape from hypoxia (invasion and metastasis of cancer cells) as well as the improvement of oxygen-availability (angiogenesis) and adaptation of cellular metabolism to hypoxia (metabolic reprogramming).

In order to explore novel genes which are responsible for the HIF-1-mediated tumor metastasis, we recently established a new genetic screening method [2] and found that overexpression of ubiquitin C-terminal hydrolase L1 (UCHL1) is responsible for the activation of HIF-1. In the present study, we analyzed both the molecular mechanisms underlying the UCHL1-mediated activation of HIF-1 and the involvement of UCHL1-HIF-1 axis in distant tumor metastases.

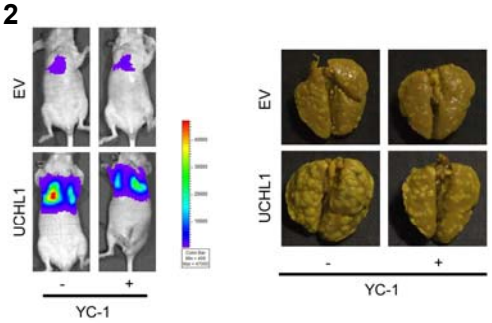
EXPERIMENTS & RESULTS: We established stable transfectants of HeLa cells with the UCHL1 expression vector (HeLa/UCHL1) or its empty vector (HeLa/EV) [3]. We found that aberrant expression of UCH-L1 in cancer cells abrogated von Hippel-Lindau-mediated ubiquitination of HIF-1α, leading to the stabilization of HIF-1α and subsequent activation of HIF-1 (Fig. 1) [3].

Fig. 1



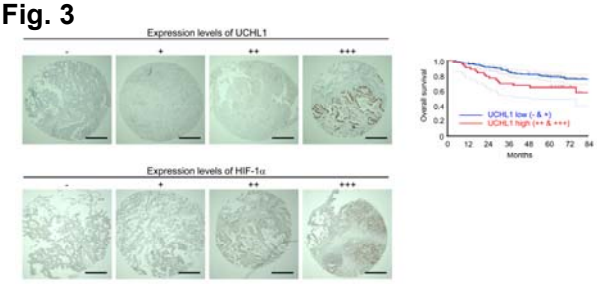
A murine model of pulmonary metastasis demonstrated that the forced expression of UCH-L1 facilitated tumor metastases in a HIF-1-dependent manner (Fig. 2) [3].

Fig. 2



On the other hand, silencing of the aberrantly expressed UCH-L1 suppressed the metastatic tumor formation by inactivating HIF-1[3].
Moreover, we revealed that UCH-L1 expression levels were associated with poor prognosis of patients with breast and lung cancers (Fig. 3) [3].

Fig. 3



DISCUSSIONS: Together, these results demonstrate that UCH-L1 functions in distant tumor metastasis as a deubiquitinating enzyme for HIF-1α, and thus, justify exploiting it as a prognostic marker and therapeutic target for individualization of cancer therapy.

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[2] Zeng L et al. *Oncogene*. in press.
[3] Goto Y et al. *Nature Communications*. **6**:6153. 2015.

採択課題番号26 P1-3　低酸素誘導性因子1（HIF-1）を活性化する新規遺伝子の探索と　プロジェクト機能解析、および局所腫瘍制御への展開

（京大・院医）原田浩（京大・原子炉）増永慎一郎

PR1-4 Quantitative Detection of Metastasized B16BL6 Cells in Lung by Using TaqMan RT-PCR Assay

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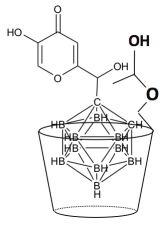
INTRODUCTION: Although melanoma boron neutron capture therapy (BNCT) with L-^{10}B-p-boronophenyl alanine has been treated for melanoma patient, the enhancement of ^{10}B accumulation in melanoma cells would lead to improved efficacy of malignant melanoma BNCT. Recently, we have evaluated the inclusion complex of kojic acid-appended carborane (CKA) with hydroxypropyl-ß-cyclodextrin (HP-β-CD) as a novel boron agent for BNCT toward melanoma. CKA/HP-β-CD complex showed melanoma cells selectivity, unique nuclear localization, and high tumor-suppression effect on BNCT toward melanoma-bearing mice. Furthermore, CKA/HP-β-CD complex was found to be the potent inhibitor of hypoxia-induced HIF-1α and to have a strong effect on tumor metastasis [1]. Herein, in order to evaluate quantitatively metastasis-suppression, TaqMan RT-PCR assay of melanoma gene GP100 was carried out toward melanoma B16BL6 cells metastasis from thigh subcutaneous to lung in melanoma-bearing mice [2].



Structure of CKA/HP-β-CD

EXPERIMENTS: B16BL6 cells (2.5×10^{12} cells) were implanted into thigh subcutaneous. Mice were euthanized and lung and spleen were isolated and snap frozen in liquid nitrogen before being stored at -80ºC. Total RNA was extracted using NucleoSpinRNA Kit (Takara). Total RNA concentration and purity were measured using NanoDrop 2000c Spectrometer (Thermo Scientific). Subsequently, cDNA synthesis was performed by using PrimeScript™ RT reagent Kit (Perfect Real Time) (Takara). Quantitative real time polymerase chain reaction (Q-PCR) was performed according to the manufacturer's manual using FastStart Universal Probe Master (Roche) and Q-PCR was run in a STRAGENE Mx3000P using the following program: 95ºC/10 min+40×(95ºC/15 s+60ºC/1 min+72ºC/1 min). Primers and probe were used: GP100 forward: 5' AGC ACC TGG AAC CAC ATC TA 3', GP100 revers: 59 CCA GAG GGC GTT TGT GTA GT 3', GP100 probe: 5'Hex-CAC TAC AAA AGT TGT GGG TAC TAC ACC TG-BHQ-1-3'.

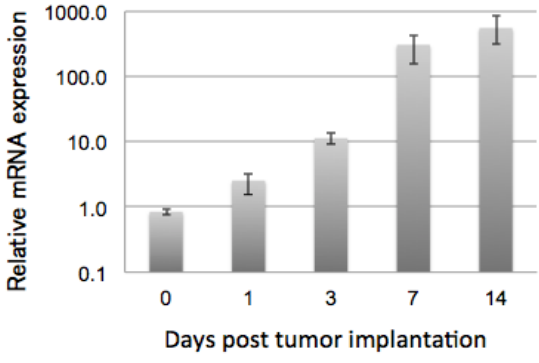


Fig.1 Relative mRNA expression of melanoma gene GP100 in lungs.

RESULTS AND DISCUSSION: GP100 is highly expressed in melanoma B16BL6 cells, but not to any substantial degree in normal tissues. GP100 expression exceeded background levels when at least 5×10^3 B16BL6 cells are contained in metastasized tissue. In order to quntify the increase of metastasized B16BL6 cells, lungs were collected 1, 3, 7, or 14 days post tumor implantation. On day 7 increase of GP100 expression reached a level of about 100 times the lowest point (Fig. 1).

TaqMan RT-PCR Assay toward GP100 is potent to estimate quantitatively the metastasis of melanoma cells to lung. The quantitative evaluation of metastasis-suppression effect of CKA/HP-β-CD complex is proceeding in our laboratory.

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[1] K. Shimizu *et al*., Bioorg. Med. Chem. Lett., **20** (2010) 1453-1456.
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採択課題番号 26P1-6　メラノーマ中性子捕捉療法への適応を目指した薬物送達システムに関する研究　プロジェクト

（阪市大院・工）長﨑　健、川口純貴、河崎　陸、櫻本昌士（阪府大・21 研究機構）切畑光統、服部能英（京大・原子炉）増永慎一郎、櫻井良憲

PR1-5

Response of Tumor Cells to BNCT

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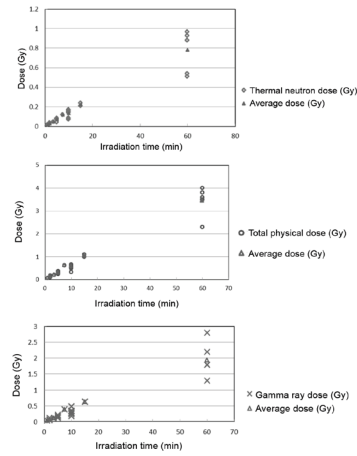
INTRODUCTION: Tumors are consisted of heterogenous populations of cells, including hypoxic cells, cancer stem cells and differentiating cells. These populations affect the consequence of the boron neutron capture therapy (BNCT) [1]. Boron neutron capture reaction (BNCR) causes extensive DNA double strand breaks and micronuclei formation has been used to estimate the level of DNA damage induced by BNCR [2]. In rat xenograft model, BNCR caused the up-regulation of γH2AX and HMGB1, then induction of poly(ADP-ribose) [3]. The cell death process and the factors affecting tumor cell killing, side effects and effectiveness of BNCT are not fully understood. To elucidate the cell death process after BNCR, we have taken approaches of comprehensive analysis of the tumor cells by using transcriptome and proteome analysis.

EXPERIMENTS: Human oral squamous cancer SAS cells [1] was used for the comprehensive analysis with transcriptome and proteome analysis. ^{10}B-boronophenylalanine (^{10}B-BPA)-fructose solution was prepared as described previously [4]. SAS cell was suspended and incubated 2 h with or without 25 ppm of ^{10}B-BPA as previously reported [3]. After neutron-beam irradiation operated at 1 MW, cells were inoculated for colony formation, RNA and protein preparation. To analyze the early response of cancer cells, RNA and protein were prepared 6 and 24 hrs after irradiation at the supposal doses of 4 Gy-eq and 24 Gy-eq conditions.

For mouse xenograft model, male BALB/c-nu/nu mice were used and SAS cells were injected subcutaneously into left legs of the mice. ^{10}B-BPA at the dose of 250 mg/kg bodyweight was intraperitoneally injected 1 h before irradiation operated at 1 MW. Tumors, tissues, and blood were sampled for the analysis of early responses after irradiation. The animal studies were performed in accordance with the relevant laws and institutional guidelines of KUR and NCC. ^{10}B concentration was measured by prompt-gamma ray analysis (PGA). Thermal neutron fluence was measured with gold foils activation analysis. Gamma-ray dose was measured with thermaluminescence dosimeter. These physical radiation doses were measured with the kind help of Drs. Yoshinori Sakurai and Hiroki Tanaka of KUR.

RESULTS:

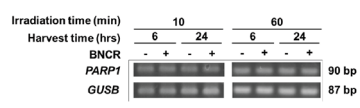
Fig. 1. The physical dose measurement.



The measured results of total physical dose, neutron and gamma ray dose were shown in Fig. 1 and confirmed that the dose estimation was in the expected range. Twenty-four hrs after 24 Gy-eq irradiation, SAS cells showed the cleavage of PARP1, caspase-9 and caspase-3, suggesting that apoptosis started to occur. Meanwhile, the low levels of necrotic cleavage pattern of PARP1 were also observed, implying that necrotic cell death is also involved after BNCR. The isolated RNA 6 and 24 hrs after irradiation was evaluated by electrophoresis and RT-PCR. As shown in Fig. 2, the expression levels of *PARP1* and *GUSB* were not different among the irradiation conditions, confirming the quality of RNA. In the microarray analysis, we found that the expression of the genes related to cell death, transcription, and inflammatory and immune responses was augmented after BNCR and the further evaluation is ongoing.

By proteome analysis, proteins involved in the vesicle regulation, mRNA processing, transcription were observed with changes after BNCR and being studied further. The observation suggests that dynamic changes in cellular responses could be induced after BNCR reaction at an early phase. SAS xenograft model in nude mice was also set up, and the boron concentration in the blood and tissues was measured. Using the experimental system, the genes and proteins associated with BNCR response will be further elucidated.

Fig. 2. Gene expression analysis of *PARP1* and *GUSB* by RT-PCR.



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採択課題番号 26P1-9　　ホウ素中性子捕捉療法に対する腫瘍応答機構の分析と奏効バイオマーカーの探索　　プロジェクト

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PR1-6 Additive effect of Gadolinium to cell survival analysis for neutron capture therapy

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INTRODUCTION: In neutron capture therapy; the therapeutic effect of a boron compound is based on alpha particle production in a $^{10}B(n, \alpha)\ ^{7}Li$ reaction, while a gadolinium compound effect is based on producing gamma rays derived from a $^{157}Gd(n, \gamma)$ reaction[1,2]. Gadolinium containing agents has been used for contrast enhance of MRI scan, and are commercially available as pharmaceutical products.

If gadolinium is administered simultaneously with boron and irradiated with neutrons, it might become a source of additional local gamma rays, which correspond with recent BNCT clinical trial protocol for glioblastoma. We analyzed the additive effect of gadolinium on boron based neutron capture reaction.

Materials and Methods:

Boron and Gadolinium compounds

p-boronophenylalanine (BPA) was obtained from Interpharma Praha (Praha, Czech Republic). Gd-DTPA (Magnevist) was purchased from Shering (Berlin, Germany). BPA and fructose ware dissolved and prepared as previously described. Briefly, BPA was converted to BPA-fructose complex by mixing BPA and fructose in NaOH. The pH was adjusted to 7.4 with HCl. Dissolution solvent for Gd-DTPA was cell culture medium.

Cell lines; C6 rat glioma cell lines and V79 Chinese hamster cell lines were maintained in Eagle's Minimum Essential Medium (MEM; Sigma-Aldrich), supplemented with 10% fetal bovine serum.

Colony Formation Assay ;V79 cells and C6 cells after exposition with the compounds were irradiated with neutron mixed beam for 90 min at the Kyoto University Research Reactor (KUR). We use the combination of BPA (0, 10, 20, 40 Bu $\mu\mu$g/mL) and Gd-DTPA (0, 0.5, 5, 10 Gdμg/mL) with 10^4 of cells of each line. The number of cells was sufficient to produce 20-100 colonies at each boron concentration. After 7days of incubation, the plates were stained with 0.25 % methylene blue in 90 % ethanol. Colonies of more than 50 cells were counted.

RESULTS: As shown in Fig. 1, Radiation-related damage significantly increased with the increase of gadolinium concentration. When 5 ppm of Gd-DTPA was added to 40 ppm of BPA in C6 cells, the additive effect was obtaine. In radiosensitive V79 cells the survival fraction dramatically decreased after irradiation and was lower than 10% at boron concentration of 40ppm.

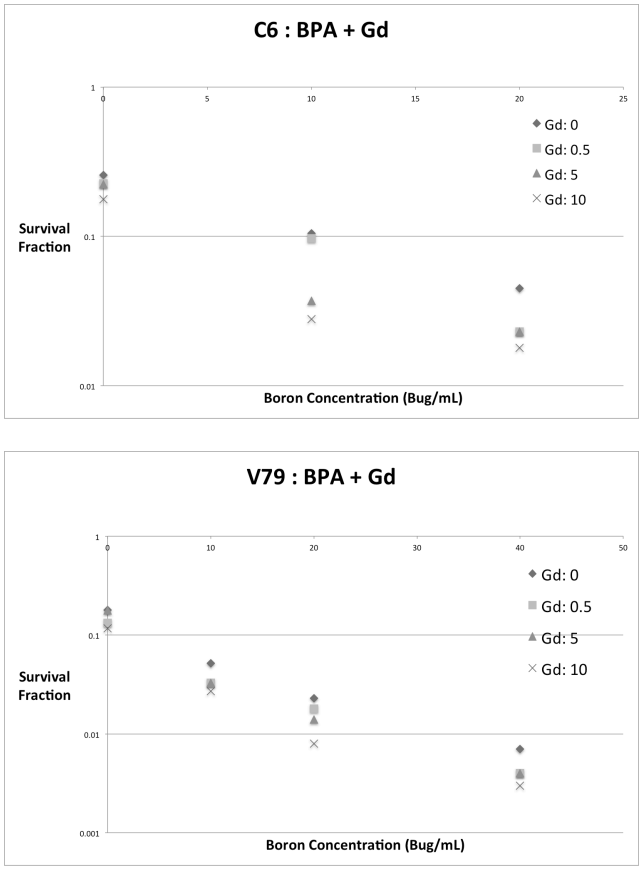

Fig. 1. Influence of Gd on BNCT in C6 (upper), V79 (lower) cell lines. The symbols ◆ show the control (BPA only); ■ show the result of additional 0.5 ppm of Gd; ▲ show the result of additional 5 ppm of Gd and × show the result of additional 10 ppm of Gd.

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採択課題番号26P1-10 新規ホウ素化合物等を用いた中性子捕捉反応による細胞生存試験および腫瘍増殖抑制効果　　プロジェクト　（筑波大・医）中井　啓、吉田文代、山本陽平、高　振宇、栗田　正（国際医療大・薬）白川　真（京大・原子炉）田中浩基、櫻井良憲、増永慎一郎