Project 1 Analyzing Tumor Microenvironment and Exploiting its Characteristics in Search of Optimizing Cancer Therapy Including Neutron Capture Therapy (R2P1)

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I-1. PROJECT RESEARCHES

Project 1

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

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

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

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

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

RESEARCH SUBJECTS:

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

ARS-1 (R2P1-1)*: 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 (R2P1-2): Development of Hypoxic Micro-environment-Oriented ^{10}B-Carriers. (H. Nagasawa, *et al.*)

ARS-3 (R2P1-3)*: Search and Functional Analysis of Novel Genes that Activate HIF-1, and Development into Local Tumor Control. (H. Harada, *et al.*)

ARS-4 (R2P1-4)*: Radiochemical Analysis of Cell Lethality Mechanism in Neutron Capture Reaction. (R. Hirayama, *et al.*)

ARS-5 (R2P1-5)*: Development of Neutron Capture Therapy Using Cell-Membrane Fluidity Recognition Type Novel Boron Hybrid Liposome. (S. Kasaoka, *et al.*)

ARS-6 (R2P1-6): Drug Delivery System Aimed at Adaptation to Neutron Capture Therapy for Melanoma. (T. Nagasaki, *et al.*)

ARS-7 (R2P1-7)*: Molecular Design, Synthesis and Functional Evaluation of Hypoxic Cytotoxin Including Boron. (Y. Uto, *et al.*)

ARS-8 (R2P1-8)*: Bystander Effect on Malignant Trait of Tumor Cells by Irradiation. (H. Yasui, *et al.*)

ARS-9 (R2P1-9): Analysis of the Response of Malignant Tumor to BNCT. (M. Masutani, *et al.*)

ARS-10 (R2P1-10)*: Cell Survival Test by Neutron Capture Reaction Using Boron Compound and Inhibitory Effect on Tumor Growth. (K. Nakai, *et al.*)

ARS-11 (R2P1-11)*: Multilateral Approach Toward Realization of Next Generation Boron Neutron Capture Therapy. (Y. Matsumoto, *et al.*)

ARS-12 (R2P1-12): Analysis of Radiosensitization Effect through Targeting Intratumoral Environmental. (Y. Sanada, *et al.*)

(*There were no allocated time for experiments using reactor facilities during their operation periods of FY 2020, partially due to the impact of measures to prevent the spread of new coronavirus infection.)

PR1-1 **Significance of combination with both continuous administration of hypoxic cytotoxin, tirapazamine and mild temperature hyperthermia in BNCT in terms of local tumor control and lung metastatic potential**

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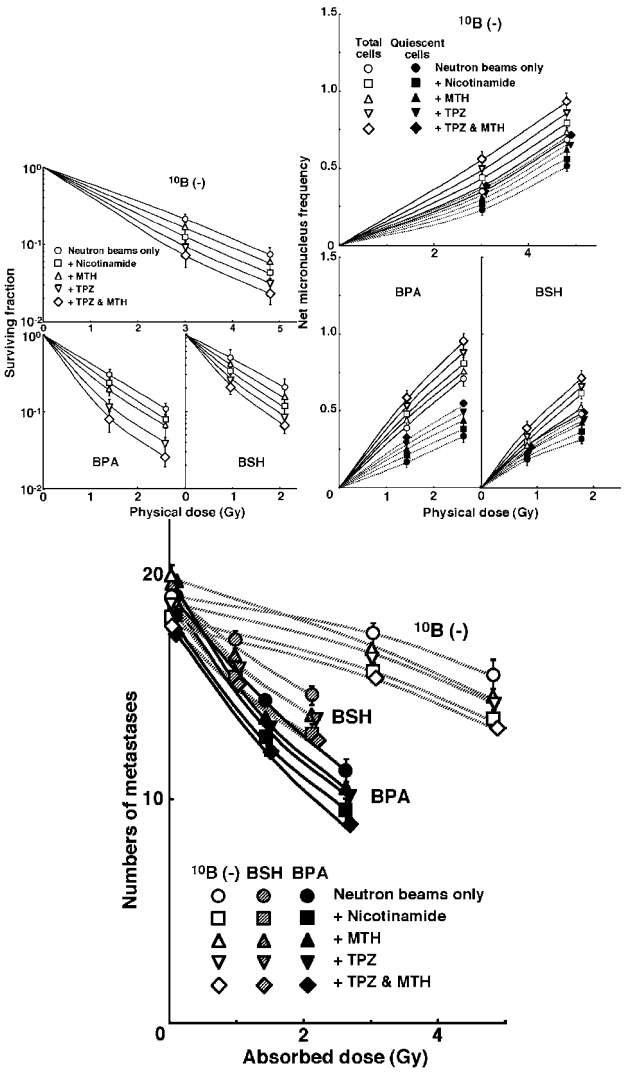
INTRODUCTION: To evaluate the usefulness of combined treatment with both continuous administration of a hypoxic cytotoxin, tirapazamine (TPZ) and mild temperature hyperthermia (MTH) in boron neutron capture thrrapy (BNCT) in terms of local tumor response and lung metastatic potential, referring to the response of intratumor quiescent (Q) cells.

MATERIALS AND METHODS: B16-BL6 melanoma tumor-bearing C57BL/6 mice were continuously given 5-bromo-2'-deoxyuridine (BrdU) to label all proliferating (P) cells. The tumors received reactor thermal neutron beam irradiation following the administration of a ^{10}B-carrier (L-para-boronophenylalanine-^{10}B (BPA) or sodium mercaptoundecahydrododecaborate-^{10}B (BSH)) after single intraperitoneal injection of an acute hypoxia-releasing agent (nicotinamide), mild temperature hyperthermia (MTH, 40 °C for 60 min), 24h continuous subcutaneous infusion of TPZ or combined treatment with both TPZ and MTH. Immediately after irradiation, cells from some tumors were isolated and incubated with a cytokinesis blocker. The responses of the Q and total (= P + Q) tumor 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: BPA-BNCT increased the sensitivity of the total tumor cell population more than BSH-BNCT. However, the sensitivity of Q cells treated with BPA was lower than that of BSH-treated Q cells. With or without a ^{10}B–carrier, combination with continuously administered TPZ with or without MTH enhanced the sensitivity of the both total and Q cells, especially Q cells. Even without irradiation, nicotinamide treatment decreased the number of lung metastases. With irradiation, BPA-BNCT, especially in combination with combined treatment with both TPZ and MTH as well as nicotinamide treatment, showed the potential to reduce the number more than BSH-BNCT.

CONCLUSION: BSH-BNCT combined with TPZ with or without MTH improved local tumor control, while BPA-BNCT in combination with both TPZ and MTH as well as nicotinamide is thought to reduce the number of lung metastases. It was 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 [1,2].



REFERENCES:
[1] Masunaga S. *et al., Int J Radiat Biol* **95**(2019) 1708–1717.
[2] Masunaga S. *et al., J Radiat Res* **61**(2020) 876–885.

PR1-2 Development of Amino Acid Derivatives Containing ^{10}B-Clusters for BNCT

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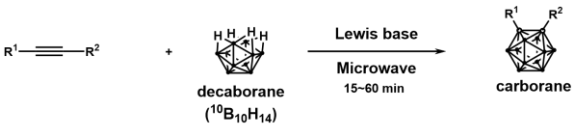
INTRODUCTION: Malignant tumor cells exhibit metabolic alterations that have been recognized as one of the hallmarks of cancer. Proliferating cancer cells increase their uptake of glutamine for energy metabolism, leading to glutamine addiction. Glutamine is transported into cells through ASCT2, and the imported glutamine can be used or exchanged through the L-type amino acid transporter (LAT1 or SLC7A5) for hydrophobic or aromatic amino acids such as isoleucine, valine, methionine, tryptophan, and phenylalanine. It is known that these transporters are overexpressed in a variety of tumor cells. Focusing on this characteristic of malignant tumor "glutamine addiction," we have designed, synthesized, and evaluated boron cluster-containing amino acid derivatives to develop boron carriers that can efficiently accumulate ^{10}B atoms in tumors via amino acid transporters that are highly expressed in tumors.

EXPERIMENTS AND RESULTS: Since carborane ($C_2B_{10}H_{12}$) is hydrophobic and has three-dimensional aromaticity, we designed and synthesized various amino acid derivatives possessing carborane as a hydrophobic pharmacophore (Fig. 1). They were efficiently obtained from the corresponding amino acid alkynes and decaborane in a short step using our microwave-assisted reaction [1]. The obtained compounds and L-boronophenylalanine



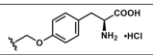
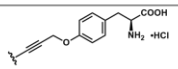
	R_1	R_2
BC-1	H	
BC-2	H	
BC-3	Ph	
BC-4	H	

Fig. 1. Structures of new boron carriers synthesized from corresponding alkynes and decaborane by micro-wave assisted reaction.

(BPA) were administered to T98G cells at 10 μg ^{10}B/mL and treated for 20 hours, after which the collected cells were dissolved in nitric acid to be measured the boron concentration by ICP-AES. As a result, intracellular boron uptake was highest in BC-2, with a very high value of about 185 ng ^{10}B/10^6 cells, which is more than 10 times higher than BPA (Fig. 2).

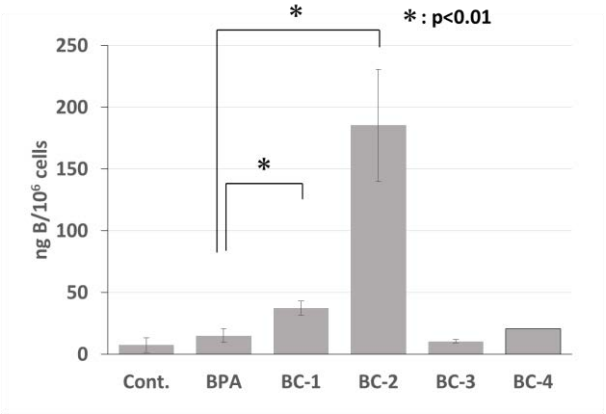


Fig. 2. Uptake of boron carriers in T98G cells.

Next, to evaluate neutron sensitizing ability of the compounds, T98G cells were treated with 10 μg ^{10}B/mL boron carriers for 20 h. Then the cells were washed with PBS, suspended in serum containing medium and aliquoted into Teflon tubes for irradiation. Cells were irradiated using the neutron beam at the Heavy Water Facility of the Kyoto University Research Reactor (KUR) operated at 1 MW power output. The survival rates of the irradiated cells were determined using conventional colony assays. The D_{10} of BNCT was calculated from survival curve shown in Fig 3.

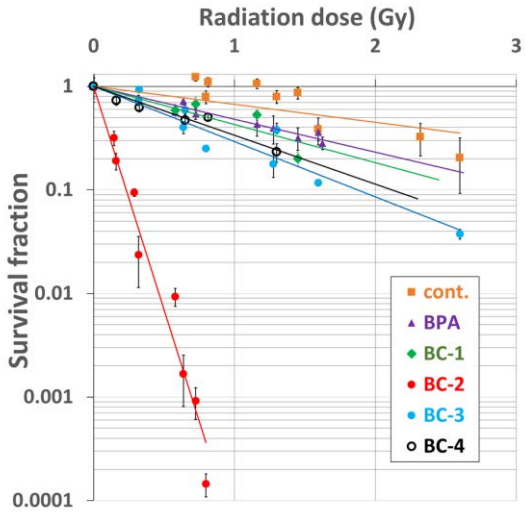


Fig. 3. Survival curves after irradiation on T98G with mew boron carries.

BC-2 showed the highest enhancement of the cell killing effect of thermal neutrons on T98G cells, with a D_{10} of 0.23 Gy and an enhancement ratio of 24.8. These results suggest that BC-2 is a promising candidate for a new boron carrier. We will investigate the mechanism of cellular uptake and the biodistribution of BC-2.

REFERENCES:

[1] S. Kimura *et al.,* Bioorg. Med. Chem., **19**(2011), 1721-1728.

PR1-3 Proteolysis of a Histone Acetyl Reader Protein Induces Chemoresistance of Cancer Cells under Hypoxia by Inhibiting Cell Cycle Progression in S Phase

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INTRODUCTION: Cancer cells acquire resistance to conventional chemotherapy in hypoxic regions of solid tumors [1,2], which is suggested to be at least partly due to reduction of their proliferative activity. However, mo-lecular mechanisms behind it have not been fully eluci-dated.

EXPERIMENTS: We performed both basic studies using cultured cells and tumor-bearing mice and clinical immunohistochemical analyses and found the importance of active proteolysis of a histone acetylation reader protein in the chemoresistance of cancer cells under hypoxic conditions.

RESULTS: We identified the histone acetylation reader protein as a target of proteasomal degradation under hypoxic conditions. Moreover, we found that inactivation of an O_2/Fe^{2+}/α-ketoglutarate-dependent dioxygenase triggered the degradation of the histone acetylation reader protein by the proteasome system upon hypoxia. The proteolysis was observed in hypoxia-inducible factor 1β (HIF-1β) knockout cells as well as their parental cells, suggesting HIFs'-independency. Consistently, the expression levels of the histone acetylation reader protein were markedly lower in perinecrotic hypoxic regions in both xenografted and clinical tumor tissues. The proteolysis of the histone acetylation reader protein was accompanied by a decrease in the amount of acetylated histone H4 and inhibited cell cycle progression from the early to late S phase under hypoxia. The S phase retardation caused cancer cell chemoresistance, which was blocked by the overexpression of the histone acetylation reader protein.

CONCLUSIONS: Degradation of the histone acetylation reader protein induces chemoresistance of cancer cells under hypoxia through heterochromatinization and the subsequent S phase retardation; therefore, inhibition of the proteolysis is expected to be a strategy to overcome chemoresistance of hypoxic tumor cells.

REFERENCES:

[1] S. Kizaka-Kondoh *et al.*, Tumor hypoxia: a target for selective cancer therapy. Cancer Sci, **94** (2003) 1021-1028.

[2] S. Kizaka-Kondoh *et al*. The HIF-1-active microenvironment: an environmental target for cancer therapy. Adv Drug Deliv Rev, **61** (2009) 623-632.

[3] H. Harada. Hypoxia-inducible factor 1-mediated characteristic features of cancer cells for tumor radio-resistance. J Radiat Res. **57** (2016) 99-105.

[4] S. Koyasu, M. Kobayashi, Y. Goto, M. Hiraoka, H. Harada. Regulatory mechanisms of hypoxia-inducible factor 1 activity: Two decades of knowledge. Cancer Sci. 109 (2018) 560-571.

[5] A. Nagao, M. Kobayashi, S. Koyasu, C.C.T. Chow, H. Harada. HIF-1-dependent reprogramming of glucose metabolic pathway of cancer cells and its therapeutic significance. Int J Mol Sci. 20 (2019) 238.

PR1-4 Cancer-Targeting Hyaluronic Acid/Carboranyl Pyrene Complex for Boron Neutron Capture Therapy

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INTRODUCTION: With minimal invasiveness and spatiotemporal therapeutic effects, boron neutron capture therapy (BNCT) is one of the most robust candidates for the treatment of cancer. In this therapy, destruction of cancer cells is achieved by the energy of particle beam generated by nuclear reaction between boron (^{10}B) and thermal neutrons, that is boron neutron capture reaction [1]. Moreover, the effective range of the energy is corresponding to the size of single cells, suggesting BNCT can eliminate cancer cells without affecting healthy cells if boron agents can be delivered to cancer cells with high specificity. For these points of views, success of BNCT is highly dependent on the selective and efficient delivery of boron agents to tumor cells. Currently, two types of boron agents, L-boronophenylalanine (L-BPA) and sodium boronocaptate (BSH) are clinically available. However, several issues such as water-solubility, tumor selectivity, tumor accumulation, and retention capacity in bloodstream are remaining in delivery with these two boron agents. Therefore, development of platforms for boron delivery has been desired to exploit therapeutic efficacy of BNCT. In this study, we developed the complex of pyrene substituted carborane (CBP-H) with sodium hyaluronate (HA) as cancer cell targeted boron agents for BNCT (Fig. 1). HA enabled to solubilize hydrophobic carboranes and strong fluorescence properties derived from aggregation induced emission (AIE) are powerful means to visualize subcellular distribution of boron agents [2]. The excellent deliverability of the HA/CBP-H complex through CD44 [3] that is overexpressed on cancer cells enabled to boost the efficacy of BNCT.

RESULTS and DISCUSSION: The complex of CBP-H with HA was prepared by high-speed vibration milling method as previously reported [4]. The formation of the CBP-H/HA complex was confirmed by measuring UV-Vis absorption spectra and fluorescence spectra. Current systems enabled to dissolved hydrophobic carborane derivatives at most 730 μM (boron concentration, 7300 ppm). Moreover, the UV-Vis absorption spectra of CBP-H got broadened after complexation with HA, suggesting the CBP-H encapsulated with HA forms self-aggregate. In addition, the complex exhibited strong AIE properties that is powerful means to visualize the subcellular distribution of CBP-H. Hydrodynamic diameter of the complex

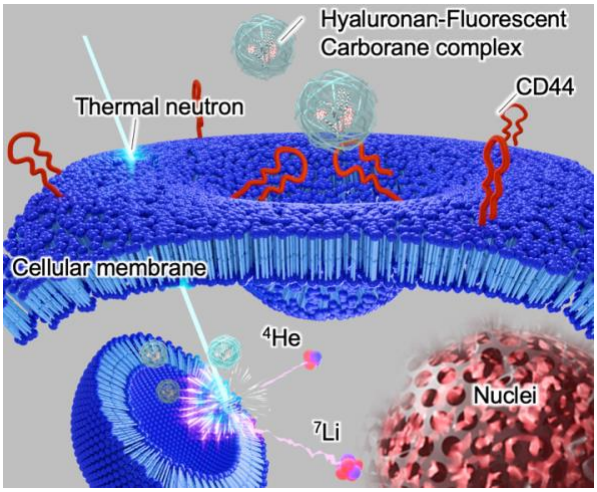

Fig. 1 Schematic illustration of BNCT using the complex of fluorescent carborane with hyaluronic acid.

obtained was estimated to be 200 nm with relatively narrow dispersity (PDI, 0.12). The size of complexes corresponds to passive tumor targeting, enhanced permeable and retention effect (EPR effect).

We evaluated the efficacy of BNCT in affecting cell viability of HA/CBP-H complex against murine colon cancer cell lines (colon26). After 24 h co-incubation with HA/CBP-H, colon26 cells were treated with thermal neutron irradiation with a fluence (6.3×10^{11} n·cm^{-2}). No apparent cytotoxicity was found in the absence of thermal neutron irradiation, suggesting current system is appropriate as a boron agent for BNCT. On the other hand, thermal neutron irradiation induced cytotoxicity against colon 26 treated with HA/CBP-H complex. Moreover, the IC_{50} value of HA/CBP-H complex is 0.074 ppm, which is slightly lower than that of clinically available boron agent, L-BPA/fructose complex (0.080 ppm). The competitive inhibition assay using free HA revealed cellular uptake mechanism of current system is dominantly relying on CD44-mediated endocytosis, as we expected. Furthermore, we successfully visualized the delivered CBP-H from their strong fluorescent properties and large part of CBP-H was distributed in lysosomes.

In conclusion, we developed cancer-targeting fluorescent carborane/hyaluronic acid complex as a boron agent for BNCT. Current system enabled to visualize the subcellular distribution of boron agents and exhibited excellent therapeutic efficacy of BNCT. These results suggest that our system has a promising potential as a boron agent for BNCT.

REFERENCES:

[1] J. A. Coderre, G. M. Morris, *Radiat. Res*. **7**, 1511. (1999).
[2] J. Ochi, K. Tanaka, Y. Chujo, *Angew. Chem. Int. Ed.*, **59**, 9841. (2020).
[3] M. Zöller, *Nat. Rev. Cancer*, **11**, 254. (2011).
[4] R. Kawasaki, K. Yamana, R. Shimada, K. Sugikawa, A. Ikeda, *ACS Omega*, **6**, 3209. (2021).

PR1-5 An evaluation of stratified mouse model and the response of tumor cells to BNCT

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INTRODUCTION: Boron neutron capture therapy (BNCT) is based on nuclear reactions and the reaction occurs between thermal neutron and boron-10. Generated alpha particle and lithium nuclei in a short length causes cancer cell killing. For the clinical trials, boron compounds such as ^{10}B-boronophenylalanine (BPA) have been used. Accelerator-based BNCT systems have been developed recently. Therefore, accurate assessment of the extent of cell and DNA damages by boron neutron capture reaction (BNCR) is important to improve BNCT. However, it is difficult to measure or calculate the irradiated dose. This is because that the neutron beam is attenuated in objects and delivered dose by BNCT is affected by various factors including boron uptake and neutron dose. We previously observed extensive DNA damage such as γ-H2AX foci, and an increased poly(ADP-ribose) level in the rat lymphosarcoma model[1]. We also performed comprehensive analysis of proteome for human squamous carcinoma SAS cells after BNCR treatment[2]. These results suggested that the changes in the particular protein levels may be involved in the early response of BNCT. In this study, we tried to evaluate the deliverd doses to beam-depth direction in mice stratified in three layers. We also investigated the dynamics of genes and proteins after BNCR or neutron beam irradiation in comparison with the γ-ray irradiation.

EXPERIMENTS: We used human squamous cell line SAS, C57BL/6J male mice of 5 weeks old. For BNCR experiment, ^{10}B-BPA fructose complex (BPA) was used as a boron compound, and neutron irradiations at KUR reactor was operated at 1 MW. Three mice were positioned in three layers (stratified model) or in a single layer against irradiation port. The local irradiations to the mouse hind legs were also performed at the same time. Local irradiations to mouse legs were operated using ^{6}LiF containing thermal neutron shield. BPA were administrated to all mice approximately 30 min before irradiation and added to vials of cell suspension at least 60 min before the irradiation. We used gold foil activation analysis for the measurement of thermal neutron fluences and thermoluminescence dosimeter (TLD) for the measurement of the γ-ray doses including secondary γ-ray. Total physical dose calculation was carried out using the flux-to-dose conversion factor by the sum of the absorbed doses resulting from ^{1}H(n, γ)^{2}D, ^{14}N(n, p)^{14}C, and ^{10}B(n, α)^{7}Li reactions, as previously described. To analyze the acute cellular responses, cell culture supernatants were filtrated and analyzed 6 and 24 hrs after irradiation. RNA and proteins were also isolated and RNA expression levels were examined using real-time PCR and protein levels were analyzed by ELISA.

RESULTS:

Table 1. Irradiated doses at stratified positions (three layers) of mice (cart, irradiation room)).

Irradiation time [min]	Position	Thermal neutron fluence [/cm^2]	Thermal neutron [Gy]	Epi-thermal neutron [Gy]	Fast neutron [Gy]	Gamma-ray [Gy]	Physical dose [Gy]
60	Closest	3.5E+12	0.47	0.050	0.35	0.21	1.1
	Center	1.7E+12	0.22	0.024	0.17	0.27	0.68
	Farthest	8.7E+11	0.12	0.013	0.09	0.23	0.44

Table 2. Delivered doses for local irradiation of mouse legs (cart, irradiation room).

Irradiation time [min]	Thermal neutron fluence [/cm^2]	Thermal neutron dose [Gy]	Epi-thermal neutron dose [Gy]	Fast neutron dose [Gy]	Gamma-ray dose [Gy]	Physical dose [Gy]
60	4.8E+12	0.63	0.068	0.47	0.7	1.9
60	5.2E+12	0.70	0.075	0.52	0.67	2.0

Table 3. Irradiated doses of mice (single layer, E-4 rail port).

	Irradiation time [min]	Sample	Thermal neutron [/cm^2]	Thermal neutron [Gy]	Epi-thermal neutron [Gy]	Fast neutron [Gy]	Gamma-ray [Gy]	Total dose [Gy]
1	50	Top	4.6E+12	0.62	0.07	0.47	1.0	2.1
		Center	4.7E+12	0.63	0.07	0.47	1.0	2.2
		Bottom	4.7E+12	0.63	0.07	0.47	1.0	2.2
2	50	Top	4.6E+12	0.61	0.07	0.46	1.3	2.4
		Center	4.9E+12	0.66	0.07	0.49	1.3	2.5
		Bottom	4.8E+12	0.65	0.07	0.48	1.3	2.5

The measurement of thermal neutron fluence and doses for stratified mice model (three layers) and local irradiation to legs were performed twice, respectively, and the average data are shown in Tables 1 and 2. For the stratified positions, the physical doses between the two mice, closest and farthest from the irradiation port, showed approximately 2-fold difference. Depending on the distance from the irradiation port, delivered thermal neutron doses decreased thereby allowing the evaluation of biological effects on beam-depth direction. Irradiation data of 3 mice set in a single layer to rail port showed similar doses at the irradiated surface of mice (Table 3).

We also observed that *HMGB1* mRNA level in culture supernatant increased depending on the increase in doses 24 hrs after neutron beam irradiation with BPA. The results suggest a potential role of HMGB1 as a biomarker for evaluation of BNCT response.

REFERENCES:

[1] M. Masutani et al., Appl. Rad. Iso., 104 (2014) 104-108.

[2] A. Sato et al., Appl. Rad. Iso., 106 (2015) 213-219.

PR1-6 Attempts to sensitize tumor cells by exploiting the tumor microenvironment

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INTRODUCTION: Hypoxia and glucose deprivation have been suggested to play important roles in resistance to radiation [1]. Attempts to sensitize tumor cells by exploiting the tumor microenvironment have been studied. A major mediator of the cellular hypoxic response, hypoxia inducible factor 1 (HIF-1), is a potential target for cancer therapy, because it transcriptionally regulates a number of genes, including those involved in glucose metabolism, angiogenesis and resistance to chemotherapy and radiation therapy [2]. We previously reported that the disruption of Hif-1α enhanced the sensitivity of murine squamous cell carcinoma (SCC VII) cells to gamma-ray [3]. We have investigated whether the disruption of Hif-1α affects the sensitivity of SCC VII cells to the boron neutron capture reaction (BNCR). Previous studies reported that HIF-1 is likely involved in DNA damage and DNA repair. We have found that Hif-1α-deficient SCC VII cells exhibit higher amount of DNA damage than SCC-VII cells. The intracellular ^{10}B levels in Hif-1α-deficient cells was higher than SCC-VII cells. In the present study, we investigated SLC7A5 expression profiles in SCC VII cells. Unfortunately, antibodies against mouse SLC7A5 protein in SCC VII cells were not available. Therefore, we established SCC VII cells expressing SLC7A5-6xHis proteins.

EXPERIMENTS: In order to establish SCC VII cells expressing SLC7A5-6xHis proteins, a targeting vector was generated. SCC VII cells were transfected with this targeting vector and treated with G418 for selection. A CreER expression vector was used for recombination between loxP sites. G418-senstitive clone (SCC VII -S7A5-H) was isolated and used for analysis of SLC7A5 expression profiles.
SCC VII-S7A5-H and SCC VII-S7A5-H-Hif-1α-deficient cells were grown under hypoxic conditions. SLC7A5 expression profiles were analyzed by Western blotting.

RESULTS: We examined whether SLC7A5 protein levels were affected under hypoxic conditions and the disruption of HIF-1α. SLC7A5-6×His protein levels decreased after 4 h and then increased near to the base level. HIF-1α protein levels continued to be elevated. If Hif-1α gene was disrupted, SLC7A5-6×His protein levels were not significantly decreased under hypoxia.

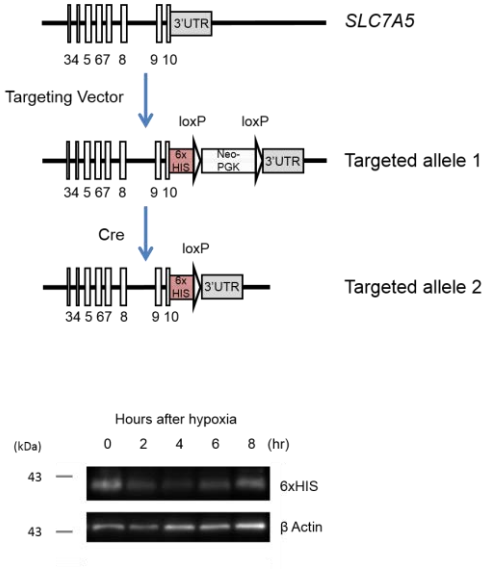

Fig. 1. (Top) Generation of SCC VII cells expressing SLC7A5-6xHis proteins. (Bottom) Effects of hypoxia on the expression of SLC7A5-6xHis protein levels in SCC VII. β Actin was used as a loading control.

REFERENCES:

[1] S. Masunaga et al., Int. J. Rad. Biol. 92 (2016) 187–194.
[2] Z. Luo et al., Neuropharmacology. 89 (2015) 168–174.
[3] Y. Sanada et al., Int. J. Rad. Biol. 94 (2018) 88-96.