CO6-1 Localization Estimation of Abasic Sites in DNA Irradiated with Ionizing Radiation under a Cell-Mimetic Condition

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INTRODUCTION:

DNA lesions induced by ionizing radiation and chemicals can cause mutation and carcinogenesis. In particular, "clustered damage" site, that is a DNA region with multiple lesions within one or two helical turns, is believed to hardly be repaired. This damage is considered to be induced, *e.g.*, around high-LET ionizing radiation tracks. However, detail of the damage is not known. We have already developed a method for estimating degree of localization of abasic sites (APs) in DNA using Förster resonance energy transfer (FRET). The FRET efficiency (*E*) was calculated using the donor fluorescence intensities before/after enzymatic digestion of the labeled AP-DNA [1]. We have attempted to apply the method to $^{4}He^{2+}$- and ^{60}Co γ-irradiated DNA. In the first trial, we used the $^{4}He^{2+}$ (9 MeV, LET: ~70 keV/μm) beam which is near the Bragg peak. The beam, however, could not penetrate the layer of DNA solution (~1 mm) in a sample folder, and moreover, the DNA solution was not buffered so that surroundings of the DNA was far from that in a cell [2]. Thus, we here show a new result using the faster He beam (50 MeV) as a high-LET radiation source and a cell-mimetic DNA solution as a sample for irradiation.

EXPERIMENTS:

●*Sample preparation and He beam irradiation*

The plasmid DNA digested by Sma I was used (linear form). The DNA was dissolved to be 0.5 g/L in 0.2 M Tris-HCl buffer (pH 7.5) which is a cell-mimetic condition in relation to radical scavenging capacity. One hundred microliters of the DNA solution was transferred to an irradiation folder [2], and was irradiated with the $^{4}He^{2+}$ beam (50 MeV, LET: ~20 keV/μm; TIARA, Japan Atomic Energy Agency) and ^{60}Co γ-rays (LET: ~0.2 keV/μm; Kyoto University Research Reactor Institute: KURRI) were also used as a standard radiation source.

●*Preparation of fluorophore-labeled irradiated DNA and the FRET observation*

The irradiated DNA (10 μL in water) and 10 μL of 100 mM Tris-HCl (pH7.5) were mixed in a microtube. Two microliters of a mixture containing AF350 (donor fluorescent probe) and AF488 (acceptor one) with a given molar ratio was added to the DNA solution and was incubated for 24 h at 35°C. The fluorophore-labeled DNA was purified by precipitation by ethanol. Twenty microliters of water was added to the residue. The fluorescence intensities were measured both at 449 nm (ex. 347 nm for AF350) and at 520 nm (ex. 460 nm for AF488). After the measurement, the enzyme cocktail containing DNase I and phosphodiesterase I was added to the solution, followed by being incubated for 3 h at 37°C. *E* values were calculated from the donor intensity before/after the digestion.

RESULTS AND DISCUSSION:

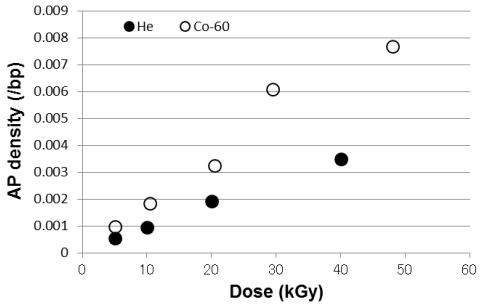

Fig. 1. Relationship between absorbed dose and AP density for He ion beam (●) and ^{60}Co γ-rays (○).

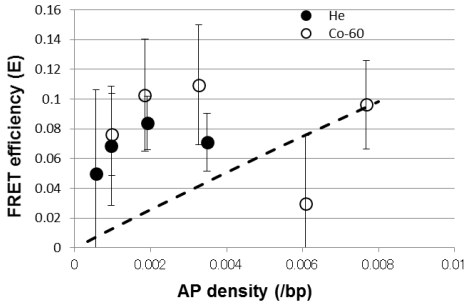

Fig. 2. Relationship between AP density and FRET efficiency for He ion beam (●) and ^{60}Co γ-rays (○). Dashed line indicates a theoretical curve for randomly-distributed APs in DNA.

The AP yield (AP density/Gy) of He was clearly lower than that of ^{60}Co γ-rays (Fig. 1). The finding is consistent with previous results [*e.g.*, 3]. The lower damage yield shown in a higher-LET radiation is due to the higher frequency of radical recombination reaction between OH radicals around the particle track. In Fig.2, there was no difference in FRET efficiency (*E*) between the two radiation sources. This tendency is similar to the result using dry DNA as sample for irradiation [4]. We consider that most diffusible radicals should be scavenged by Tris to find difference in the FRET results among radiations. The DNA concentration set here (0.5 g/L) might be too high to find the difference.

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採択課題番号 25001　放射線誘発ＤＮＡ損傷スペクトルの線質依存性に関する研究　共同通常
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CO6-2 The Mechanism of Radiation Induced Bystander Effect

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INTRODUCTION: The effects of ionizing radiation have been examined in biological model. One of the impacts recently is that some signals are interacted between targeted cells and non-targeted cells. This effect, called as "bystander effect" has impact on risk estimation in lower dose range, because non-targeted cells may be leading to cancer. Some factors from irradiated cells are thought to be effective in non-targeted cells. In this study, culture medium with human tumor cells in a culture flask was gamma- ray irradiated, and then the medium was transferred to other culture flasks in which non-irradiated human tumor cells were plated. It is expected that irradiated cells as donor cells release some soluble bystander factors into the medium, so that the non-irradiated cells as recipient cells may be affected by the factors. The result showed that bystander factors from irradiated cells modulate the function of mitochondria in non-irradiated cells. This response is thought to be a trigger of following responses such as the induction of reactive oxygen species (ROS), and gene mutation [1]. In the present study, we examined the role of superoxide (O_2^-) in mitochondria in bystander responses.

EXPERIMENTS: Human glioma cell line, U251 was used in this study. Cells were cultured in DMEM medium supplemented with 10%FBS. Cells were maintained at 37^0C in a humidified atmosphere with 5% CO_2. Cells were seeded onto T25 flask one day prior to irradiation. Immediately after irradiation, medium was changed and cells were irradiated with 4 Gy of gamma-rays. Cells were incubated for 4 days following irradiation. After the culture medium was centrifuged at 1,200 rpm for 5 min, supernatant was transferred to unirradiated cultured cells on T25. Twenty four hours after transfer, number of cells were counted to know the effect of cell growth by bystander effect. Also, in order to know the sensitivity to H_2O_2, cells were treated with 50 μM H_2O_2 during colony formation. Reactive oxygen species were analyzed by the MitosoxRed dye, which is a marker of superoxide radical (O_2^-) in mitochondria.

RESULTS: In U251 cells, growth ratio in treated with conditioned medium from 4 Gy-irradiated cells was significantly higher compare to that in control (non-irradiated) conditioned medium (Fig. 1). This result suggests that the secreted factors from 4Gy-irradiated cells are effective on the stimulation of cell growth. We hypothesized reactive oxygen species (ROS) should be involved in this response. Reactive oxygen species (ROS) were examined by MitosoxRed analysis, however no increased levels of ROS, which is thought to be superoxide radical (O_2^-) in mitochondria, were detected in this condition. Therefore we examined the sensitivity of bystander cells after the treatment of H_2O_2, because antioxidant status should be reflect in this sensitivity. The result showed significant higher sensitivity to H_2O_2 in the treated cells with conditioned medium from 4 Gy-irradiated cells compare to the cells treated with control medium (Fig. 2). This result suggests that reduced activity of antioxidants in the cells treated with conditioned medium from 4Gy-irradiated cells. Therefore, it is thought that consumption of antioxidants was occurred in bystander cells.

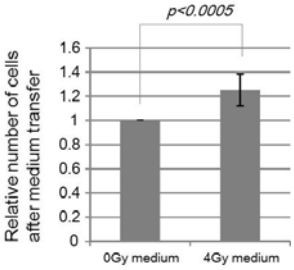

Fig.1 Relative ratio of cell growth in cells treated with conditioned medium from irradiated cells. Conditioned mediums were collected 4 days after irradiation. Twenty-four hour after treatment, numbers of cells were counted. Results were indicated by the average of seven independent studies.

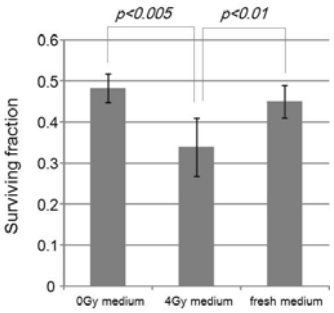

Fig.2 Surviving fractions after H_2O_2 treatment in cells treated with conditioned medium from irradiated cells. Conditioned mediums were collected 4 days after irradiation, then transferred to non-irradiated cells. Twenty-four hour after each treatment (both conditioned mediums from 0 and 4 Gy irradiated cells, and fresh medium), cells were seeded into 100 mm dish for colony formation assay. Cells were treated with 50 μM H_2O_2 during colony formation. Results were indicated by the average of three independent studies.

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採択課題番号 25003　　放射線誘発バイスタンダー効果の機構解明　　共同通常

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CO6-3 Specificity of DNA Damage Induced by Neutron Radiation

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INTRODUCTION: Ionizing radiation leads to fatal result on living organisms. There have been many studies about the mechanism with using gamma-rays and X-rays. Hitherto we have focused on role of DNA damage in the mechanism with gamma-rays [1] and heavy ion beams [2, 3]. On the other hand, we have less information about neutron radiation. In this study, we analyzed the DNA damage induced by neutron radiation from atomic reactor for elucidation of the mechanisms of the biological effect with neutron radiation. Our result might lead to contribute the radiation protection for atomic industry.

EXPERIMENTS: We used a DNA molecule and a cultured cell as the irradiated samples for the analysis of DNA damage. The purified pUC19 DNA molecules as the target DNA were dissolved in 10 mM Tris-HCl (pH7.5) to 300 μg mL^{-1}. Each 100 μL of the DNA solution was in a polypropylene microtube, and was irradiated by the neutron radiation from the deuterium water apparatus of the Kyoto University Research Reactor (KUR). The apparatus provided mixed thermal and epithermal neutron radiations. For cell irradiation, Chinese hamster ovary AA8 cells were exponentially grown, and the cells were recovered into polypropylene microtube as pellet with trypsinization. The cell pellets were irradiated by the neutron radiation like the DNA as mentioned above. The dose rate of the neutron radiation was around 2 Gy h^{-1}, which were measured by radioactivation of gold. The neutron radiations included "contaminated" gamma-rays with various energies. Contribution of the gamma-rays for the dose rate was estimated by thermoluminescent dosimeter. Both irradiated samples were quickly frozen by dry ice until analysis. The irradiated DNA was analyzed by the procedure reported previously for yields of strand break and oxidative base lesions [2]. The cell analysis is in process, and the result will be reported later.

RESULTS: In the irradiated DNA, double strand break (DSB) generated in a dose related fashion (Fig. 1a). We used two specific DNA glycosylases to detect oxidative base lesions induced by neutron radiation. Endonuclease III and Fpg from *Escherichia coli* can expose pyrimidine and purine oxidative bases, respectively [1]. After treatments of these enzymes, the electrophoresis analysis revealed the yields of clustered DNA damages consisting of oxidative pyrimidine and purine base lesions (Fig. 1b and 1c). The yields of oxidative pyrimidine and purine clusters were similar level. These base lesions also showed dose-association.

The yields of all these DNA damages including DSB and both oxidative base clusters were around 10–20 times higher than those with gamma-rays [2]. The present result conforms to the relative biological effect of neutron radiation reported previously.

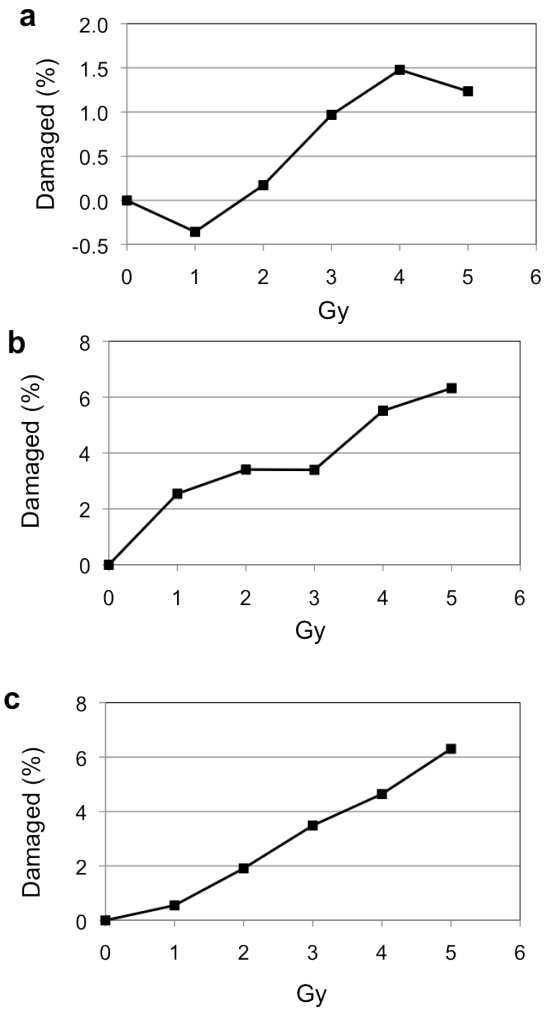

Fig. 1. The yields of DNA damage in pUC19 DNA irradiated by the neutron radiation. a) DSB, b) oxidative pyrimidine cluster, c) oxidative purine cluster.

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採択課題番号 25064　　中性子線によって生じるＤＮＡ損傷の特異性解析　　共同通常
（佐賀大・総合分析セ）寺東宏明、（京大・原子炉）齊藤毅、木梨友子、櫻井良憲、鈴木実

CO6-4 Multi-Element Neutron Activation Analysis of Canadian Food Samples by Short Irradiation

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INTRODUCTION: It is important to obtain multi element levels in food samples for nutritional purposes. For the analysis of multi elements in food samples, atomic absorption spectrometry (AAS) and induced coupled plasma spectrometry (ICP-AES) are widely used after pre-treatment of acid extraction or acid digestion. Though complete acid digestion is needed for obtaining total levels of elements in food samples, it is not easy by the presence of high concentrations of lipids, polysaccharides, etc preventing acid digestion. Short irradiation neutron activation analysis using Ge detector with Compton suppression system (CSS) is strong tool for analyzing multi elements in food samples, especially with high levels of NaCl. In this paper, multi-elements were analyzed in typical Canadian food samples by short neutron irradiation and gamma measurement by Ge detector with CSS.

EXPERIMENTS: (1) Samples: The samples were supplied from Health and Welfare Canada in 1992 as freeze-dried powders. In the series of samples, totally 95 kinds of samples were selected and in them milk products, meat products, eggs, fish products, soups, cereals, vegetables, fruits, sweets, fast foods, frozen dinner were included. (2) SRMs: For confirming the accuracy of data, the following standard reference materials were used; NIST SRM 1577b Bovine Liver, NIST SRM 1515 Apple Leaves, NIST SRM 1566b Oyster Tissue, NIST SRM 1573a Tomato Leaves, NIST SRM 1570a Trace Elements in Spinach Leaves, and BCR CRM 414 Plankton. (3) NAA: 0.3-0.5 g of samples, SRMs, and CRM were weighed and doubly sealed in a plastic sheet. Samples, SRMs, and CRM were irradiated for 1 minute in TcPn site. After 1.5 minutes decay, gamma spectrum was measured for 5 minutes by Ge detector withCSS. Samples containing extremely high NaCl were irradiated for 20 seconds for reducing dead time. After appropriate decay time, the second gamma counting was done for 2000 sec. Elemental levels were obtained by comparing sensitivity of each radionuclides between samples and SRMs.

RESULTS: Regarding the first gamma counting, Se-77m (161.9 keV, $T_{1/2}$ = 17.45 s), Ti-51 (320.1, 5.76 m), Rb-86m (556.3, 1.02 m), Br-80 (616.3, 17.68 m), Mg-27 (1014.4, 9.46 m), Cu-64 (1039.2, 5.10 m), Na-24 (1368.6, 14.96 h), V-52 (1434.1, 3.75 m), K-42 (1524.6, 12.36 h), Cl-38 (1642.7, 37.24 m), Mn-56 (1810.7, 2.58 h), and Ca-49 (3084.5, 8.72 m) were detected. In those of peaks, Mg-27, Na-24, K-42, and Cl-38 were seen in most of the samples. Results obtained for Ca, K, and Cl in SRMs are shown in Table 1, and good agreements with certified values were obtained. Regarding trace elements, Ti was detected in BCR CRM 414 Plankton, Rb in LAMB sample, V in NIST SRM 1566b Oyster Tissue, Cu in ORGAN MEATS_LIVER & KIDNEY sample, SEEDS_SHELLED sample, VEGETABLES_PEAS sample, NIST SRM 1566b Oyster Tissue, and NIST SRM 1577b Bovine Liver. By further calculations, information on several more trace elements in Canadian Food Samples will be obtained.

Table 1 Levels of major elements in SRMs. (n=3)

	SRM	This work	Certified value
Ca	NIST 1570a	1.49± 0.13 %	1.520 ± 0.066 %
Ca	NIST 1573a	4.08 ± 0.93 %	5.05 ± 0.09 %
K	NIST 1573a	2.64 ± 0.33 %	2.70 ± 0.05 %
K	NIST 1515	1.73 ± 0.42 %	1.61 ± 0.02 %
Cl	NIST 1570a	6560 ± 510 ppm	(6600) ppm
Cl	NIST 1573a	703 ± 300 ppm	579 ± 23 ppm

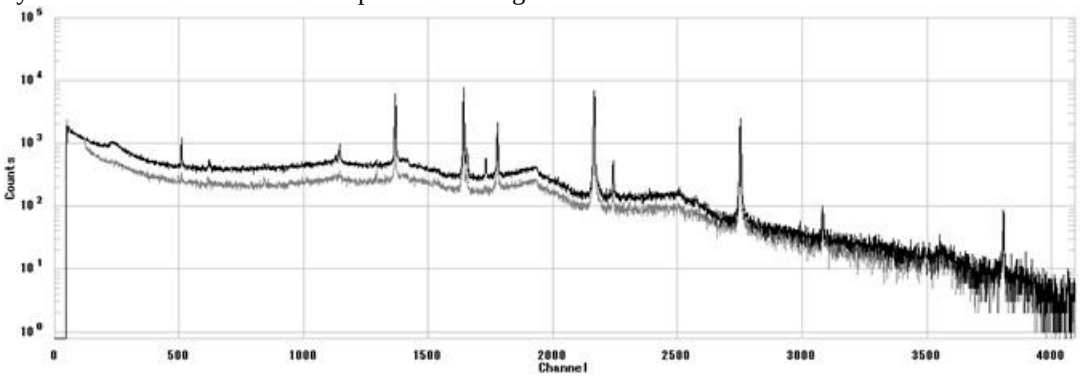

Fig.1 Gamma spectra of COTTAGE CHEESE sample. 1 min irradiation, 1.5 min decay, 5 min gamma counting with CSS (gray) and without CSS (black).

CO6-5 The Role of Human Oxidation Resistance 1 (OXR1) in Cellular Response to Radiation

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INTRODUCTION: OXR1 (oxidation resistance 1) is a gene highly conserved in eukaryotes. It is suggested that OXR1 prevents cellular oxidative damage. Previous studies showed that human OXR1 suppressed spontaneous mutation in *E. coli mutH nth* [1]. OXR1 is induced by oxidative stresses such as H_2O_2 and is localized to mitochondria [2]. However, the function of OXR1 remains to be elucidated. Reactive oxygen species (ROS) act as a mediator of ionizing radiation-induced cellular damage. To clarify the protective functions of OXR1 against oxidative damage, we studied the effects of OXR1 on radiation-generated damage. In this study, we used high LET heavy-ion beams, which are known of stronger cell killing effect than X-rays [3]. Our studies showed that OXR1 proteins in HeLa cells were induced by carbon-ion beam, and that OXR1- depleted HeLa cells were highly sensitive to heavy-ion beams. We are now under constructing OXR1-knockout DT40 cells.

EXPERIMENTS:

Protein level

HeLa cells were irradiated with 2.5Gy carbon ion beam at high LET. OXR1 protein levels was analyzed by western blotting.

Cell survival assay

HeLa cells stably expressing shRNA targeting OXR1 or luciferase were constructed. The cells were seeded in T25 Flask 10-16 hours before irradiation. Cells were irradiated with carbon-ion (0, 2, 4 or 6Gy, 290MeV/nucleon, 87.0keV/um) and iron-ion (500MeV/nucleon, 64.83 mmH_2O) beams generated by the Heavy Ion Mediacl Accelerator in Chiba (HIMAC). The colonies were stained with crystal violet after 10 – 14 days of incubation, and the colonies containing more than 50 cells were counted

Targeted disruption of OXR1 in DT40 cells

We prepared chicken OXR1 disrubtion constructs using puromycin, blasticidin or histidinol resistance gene. The chicken B lymphoma cells DT40 are cultured and transfected with the three gene disruption constructs.

RESULTS: The chicken OXR1 targeting constructs, OXR1-pur, OXR1-bsr and OXR1-his were generated. DT40 cells are now transfected with these constructs.

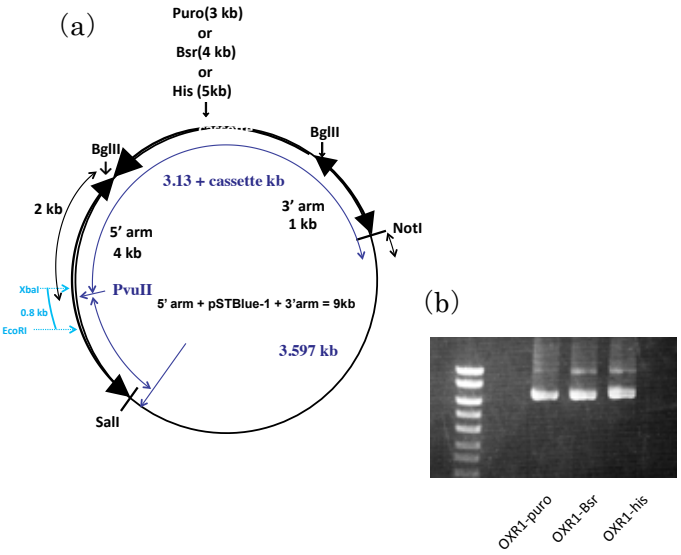

Fig. 1. (a) Schematic representation of the OXR1 disruption constructs. (b) Electrophoresis of three constructs.

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採択課題番号 25084　　酸化ストレス誘導性タンパク質 OXR1 の機能解析　　共同通常
（京大院・理）秋山　秋梅、松井　亜子、吉川　幸宏、山崎　晃
（京大・原子炉）田野　恵三、齊藤　毅、藤井　紀子

CO6-6 Establishment of Innovative Combination Therapy with Boron Neutron Capture Therapy and Immune Therapy

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INTRODUCTION: Boron neutron capture therapy (BNCT) is an attractive therapy for local tumor control in the treatment of brain tumor, melanoma, and so on [1,2]. However, to achieve successful tumor regression, some important issues are remained: the tumor-specific accumulation of highly concentrated boron, the real-time quantification of boron concentration in local tumor tissues, and the prevention of re-growth and metastasis of residual tumor cells after BNCT. In this study, we have designed and synthesized new boron compounds based on the structure of sulfoquinovosylacylpropanediol (SQAP), for BNCT and B-MRI. Aiming at systemic antitumor effect, we try to establish the combination treatments with BNCT and immunotherapy [3,4].

Synthesis of boron compound based on SQAP

It has been reported that SQAP, which consists of 5-sulfonylglucose and a long-chain carboxylic acid parts, functions as a potent radiosensitizer and is accumulated in tumor tissues [5]. Besides, we previously report on the in-cell ^{11}B NMR probes for the detection of d-block metal ions in cells [6].

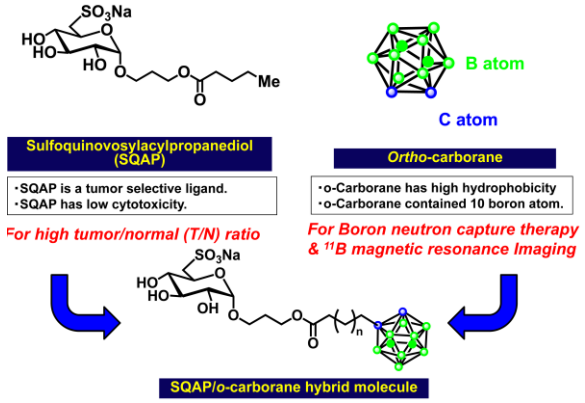

Fig. 1. Design of SQAP-carborane hybrids

These facts prompted us to design and synthesize the SQAP derivatives that have a hydrophobic boron cluster for the BNCT and the MRI measurement of its distribution in patients' body (Fig 1).

Immune-therapy combined with BNCT

To establish the combination therapy, we examined the immunological response after BNCT using mouse model, C57BL/6 mice, which were subcutaneously administrated with 250 mg/kg BPA, and then treated with thermal neutron irradiation (1 MW, 50 min in the heavy water facility of KUR). To assess the immune response, mice were immunized, and the immunological response of splenocytes and lymphocytes was examined by IFN-γ production at 10 days after immunization. Although transient lymphopenia was observed after neutron irradiation, the number of immune cells was restored at 11 days after treatment. Assessing IFN-γ production, the antigen specific immune response was comparable in neutron irradiated mice, compared with control mice (Fig. 2), suggesting that antigen-specific immune response could be induced even after neutron irradiation, indicating that it is possible to induce tumor-specific immunity after BNCT.

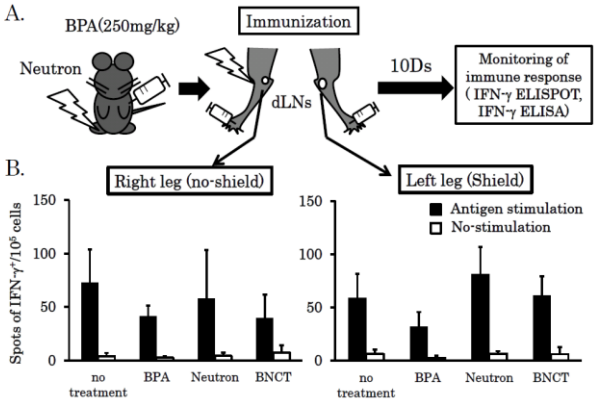

Fig. 2. Antigen-specific immune response could be iduced after neutron-beam irradiation. A; Schema of experiment. B; Immunological responses (IFN-γ production of T cells) in draining lymph node (dLNs).

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採択課題番号 25104　ホウ素中性子捕捉療法(BNCT)と免疫療法の併用に係る基礎研究　　共同通常
（東理大・薬学部）青木　伸、久松洋介、有安晋也、田中智博、西浦由紀子、月本光俊、森　夏樹、西野圭佑、棚町圭佑、北原大輔、（東理大・生命研）安部　良、鈴木利宙、小川修平、若松　英、堀江和峰、大塚旬子（京大・原子炉）櫻井良憲、田中浩基、小野公二