Chapter 5: DNA damage and biological responses induced by Boron Neutron Capture Therapy (BNCT)

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Abstract

Boron Neutron Capture Therapy (BNCT) is a tumor cell selective high LET (Linear energy transfer) particle beam therapy. The patient is administrated a boron (^{10}B) compound via intravenous injection or infusion, and when ^{10}B is sufficiently accumulated in the tumor, neutron beams containing epithermal neutrons as the main component are irradiated. Epithermal neutrons lose energy in the body and become thermal neutrons. The captured ^{10}B undergoes a (n, α) reaction with thermal neutrons, and the resulting α particles and ^{7}Li nuclei have short ranges of 9-10 μm and 4-5 μm, respectively, and do not reach the surrounding cells in normal tissues. Therefore, these high LET - heavy charged particles can selectively kill cancer cells. The cell-killing effect of these heavy charged particles is thought to be triggered by DNA damage. It is known that DNA

damage caused by heavy charged particles is more serious and difficult to repair than DNA damage caused by Low LET radiation such as X-rays and γ-rays. This review focuses on DNA damage, e.g., DNA strand breaks and DNA damage repair caused by BNCT and describes the resulting biological response.

Introduction

Mechanism of BNCT

Boron neutron capture therapy (BNCT) is a tumor selective particle radiation therapy using high- linear energy transfer (LET) radiations produced by nuclear capture and fission reactions. BNCT consists of the binary approach. First, the stable isotope boron-10 (^{10}B)-containing drug is administered to the patient to obtain a sufficient tumor ^{10}B concentration. For successful treatment, ~20~35 μg/g of ^{10}B must be selectively delivered to the tumor cells. Second, the patient is irradiated with enough neutrons (~10^{12} n/cm^2) mainly consisting of epithermal neutrons from an external radiation source [1]. The epithermal neutrons are moderated to thermal neutrons in the body. The resulting ^{10}B(n,α)^{7}Li capture reaction with the thermal neutrons produces alpha particle and lithium-7 nucleus. The ranges of these high LET particles are short to 9–10 and 4-5 μm, respectively, so tumor cells are selectively killed while sparing adjacent normal tissues as shown in Fig. 1. Unlike other particle beam therapies such as proton beam and carbon beam, which use the Bragg peak to physically concentrate the dose on the cancer tissue, BNCT is a particle beam therapy that uses the biological feature of selective boron drug accumulation in the tumor to deliver the dose. Therefore, there are high expectations for BNCT against infiltrative or disseminated tumors into normal tissues such as malignant gliomas which are difficult to control with other particle therapy methods.

Neutron Beam Component

Neutron beams are mixed beams which contain fast neutrons, thermal neutrons, low-LET gamma-rays. Therefore, except the dose from capture reactions in ^{10}B: [$^{10}B(n,\alpha)^{7}Li$], the background dose consists of a) low LET gamma rays resulting from the capture of thermal neutrons by hydrogen atoms in tissue: [$^{1}H(n,\gamma)^{2}H$] but also present as incident gammas in the beam, b) high-LET protons, either recoiling hydrogen nuclei due to collisions with fast neutrons: [$^{1}H(n,n')^{1}H$] or ejected due to capture of low-energy neutrons in nitrogen: [$^{14}N(n,p)^{14}C$], and c) High-LET heavier charged particles ^{14}C, released as products of capture reactions in ^{14}N [$^{14}N(n,p)^{14}C$] [2]. The background dose is much lower compared with the dose from capture reactions in ^{10}B: [$^{10}B(n,\alpha)^{7}Li$] [2]. However, as long as normal tissue receives the background dose, BNCT protocols should ideally be maximize the ratio between the tumor selective boron dose and the non-selective background dose [1,2].

^{10}Boron compounds in clinical use

Ideal boron compounds would a) be able to maintain the enough boron concentration in tumor tissues during neutron irradiation, b) have systemic low or non-toxicity and be cleared rapidly from normal tissues and blood after neutron irradiation, and c) not accumulate in normal tissues and guarantee the high ratio of 'concentration in tumor tissue / concentration in normal tissue'. Various low and high molecular weight boron compounds have been developed and their

efficacy has been shown *in vitro* and in experimental animal tumor models, however only two boron compounds have been mainly used in clinical BNCT studies [1]. One is sodium mercapto-undecahydro-*closo*-dodecaborate ($Na_2B_{12}H_{11}SH$), commonly known as sodium borocaptate or BSH and the other is, a boron-containing amino acid (L)-4-dihydroxy-borylphenylalanine, known as boronophenylalanine or BPA (Fig. 2). BSH was used clinically for malignant glioma by Hatanaka and Nakagawa in Japan for the first time and showed significant antitumor effects [3,4]. Successively, phase I/II clinical trial for malignant glioma was performed in Europe [5]. Although BSH is not selectively taken up by tumor cells, it is considered to diffusely accumulate in brain tumor stroma where the blood-brain barrier is disrupted. On the other hand, BPA is selectively taken up by tumor cell taken up via L-Type Amino Acid Transporter 1 (LAT1), and LAT1 expression has been reported to be higher in various cancer types than in cells of normal tissue [6]. BPA was clinically introduced for melanoma BNCT by Mishima et al [7] and was demonstrated to be taken up by other histologic type of tumor, rat brain tumor, 9L gliosarcoma by Coderre et al [8]. Increased water solubility as BPA-fructose complex (BPA–F) [9] promoted its clinical use for malignant glioma in the United States [10,11], Finland [12], Sweden [13], and Japan [14] and it became clear that BPA was more therapeutically effective than BSH. After that, BPA was evaluated successfully in BNCT for head and neck cancers [15, 16] and then became the central Boron compound in BNCT.

Transition of neutron irradiation field and neutron source

Since the first BNCT of gliomas at BNL in 1951, BNCT has used thermal neutrons from nuclear reactors in United States or Japan. In 1994, a modification of the reactor irradiation field at BNL made it possible to use epithermal neutrons [17]. Since the late 1990s, BNCT using epithermal neutrons has become possible in nuclear reactors and enabled BNCT clinical studies in Europe [18] and Japan [19] of not only brain tumor but also head and neck cancers [15] or other organ cancer such as mesotheliomas in lung [20], sarcomas in limbs [21,22]. Basic and clinical research was also conducted in Argentina, China, Taiwan, and Korea using epithermal neutrons from reactors [23]. In parallel with the successful BNCT clinical studies in nuclear reactors, the development of accelerator neutron sources has progressed around the world. In 2008, the Cyclotron Irradiation System for BNCT (C-BENS) was installed at KURNS, Japan in collaborative work with Sumitomo Heavy Industries, Ltd [24]. Clinical trials using this system for malignant glioma and recurrent head and neck cancer were conducted for the first time in the world, from October 2012 to April 2018, with favorable results [25, 26]. In 2020, this accelerator system was approved as a medical device. At the same time, BPA was also approved as borofalan (^{10}B), a boron drug for BNCT in 2020. In Japan, the National Cancer Center is currently conducting clinical trials for malignant melanoma and angiosarcoma using accelerator BNCT system with a solid lithium target-linac-vertical beam [27]. In Finland, Korea, and China, clinical

trials with accelerator based BNCT are under preparation [28].

In order to develop BNCT into a safer and more effective therapy, which will be in increasing demand in the future with the availability of accelerator-based therapies, it is very important to know the biological effects of BNCT resulting from DNA damage, and following DNA repair, checkpoint, and cell death.

DNA damage and repair mechanism induced by BNCT

Radiation damages various parts of the cell, including the cell membrane, cytoplasm, and nucleus, but DNA damage is thought to be the most important cause of mitotic death. Low and high linear energy transfer ionizing radiations induces a plethora of DNA damage, and the proportion of the locally clustered damage increases with LET of the radiation [29]. Isolated DNA lesions (mainly induced by low LET radiation), including DSBs, SSB, and damaged bases located at a distance from other damage, are generally repaired efficiently. High-LET radiation induces complex DNA damage, a unique class of DNA lesions that includes two or more individual lesions within one or two helical turns of the DNA. Complex DNA lesions are more difficult to repair than isolated lesions and in some instances are irreparable [30].

The cell-killing effect of BNCT is supposed to result from DNA damage caused by high linear energy transfer (LET) radiation, i.e. alpha particles and Li recoil nuclei produced by the

capture reaction of a thermal neutron by a ^{10}B atom, [$^{10}B(n,\alpha)^{7}Li$]. The DNA damages induced by BNCT were investigated using plasmid DNA and boric acid [31,32]. These studies indicated that SSBs and DSBs were produced and increased as the absorbed dose increases in the BNCT mixed field. The DSB/SSB ratio for the boric acid consisting of pure ^{10}B isotopes with 0.3 M was 3–4 times greater than that for boric acids with low concentration, 0.03M[32]. High-LET alpha and ^{7}Li particles were considered to be produced in larger amounts by the boron neutron capture reaction [^{10}B (n, a) ^{7}Li] and consequently the DSB/SSB ratio was increased for the boric acids with high concentrations [32].

One of the earliest responses to an ionizing radiation-induced DSB is the phosphorylation of a histone, H2AX, at serine 139, yielding a focal product (gamma-H2AX) that can be detected by a fluorescent antibody. Other proteins, such as TP53BP1, then colocalize at these sites of DSBs and so become candidate signatures for processing and repair of this damage. These nuclear foci are strikingly apparent after their induction by ionizing radiation and can be counted [33]. Comparing with gamma -ray, the numbers of gamma-H2AX, or TP53BP1 produced by BNCT were not different at 30 min after irradiation, while they became higher after 2 - 24 hours after irradiation [34,35]. And, the analysis of focus size showed that foci are larger in cells treated by BNCT 30 or 120 min after irradiation as compared to those in the gamma-ray or neutron only groups [34, 35]. These results indicated boron neutron capture reaction [^{10}B (n, a) ^{7}Li]

induced complex DSBs [34, 35].

In mouse intracranial glioblastoma model, the gamma H2AX foci in brain tumor disappeared in saline treated mouse, i.e., in the absence of ^{10}B, 24 hours after neutron irradiation. However, in the tumor of 500 mg/kg BPA-treated mouse, i.e., in the presence of ^{10}B, the gamma H2AX foci remained (4 foci/cell), indicating that unrepairable DSBs were induced by [$^{10}B(n,\alpha)^{7}Li$] [36] (Fig. 3(a), (b)). In this model, the gamma H2AX foci produced by the component of contaminated gamma-ray dose were investigated and found that they disappeared 24 hours after irradiation (Fig. 4) in the tumor, indicating that only the gamma H2AX foci produced by [$^{10}B(n,\alpha)^{7}Li$] remained in the tumor after BPA-BNCT [36]. From these results, the [$^{10}B(n,\alpha)^{7}Li$] can induce severe and unrepairable DSBs, suggesting BNCT has a remarkable effect on radiation-resistant glioblastoma. Similar results were reported by rat lymphosarcoma xenograft model. In the study, 20 hours after BPA-BNCT, the positivity of staining with gamma H2AX and Poly(ADP-ribose) which is another immediate marker for both single strand break (SSB) and DSB, synthesized by poly(ADP-ribose) polymerase (PARP) in nucleus, was higher than that of saline-treated group (without ^{10}B) [37]

DNA repair mechanisms induced after BNCT were investigated by *in vitro* studies. The two major DSB repair pathways (homologous recombination repair: HRR and non-homologous end-joining: NHEJ) have been analyzed. HRR uses the sister chromatid in the late S and G2/M

phase in cell cycle, therefore completes accurate repair. Error-prone NHEJ can function in all the time of cell cycle, including G0/G1 phase where most of somatic cells belong [33]. NHEJ repair protein deficient cell, Ku80 [34] and DNA ligase IV [38], showed higher sensitivity to the [$^{10}B(n,\alpha)^{7}Li$], compared to the wild or parental cells, and Ku80 and DNA ligase IV were supposed to contribute to the repair of DSBs caused by BNCT. Depending on cancer cell lines, the proportion of contributory DSB repair pathways induced by BPA-BNCT may be different. In the human undifferentiated thyroid follicular cancer cell line, representing the Rad51 and Rad54, HRR was predominantly activated, while in the human melanoma cell line, both HRR and Ku70, on behalf of NHEJ were activated [35]. In the hepatocellular carcinoma, boric acid (BA)-mediated BNCT induced G2/M arrest, and HRR was activated [39]. More detailed studies are necessary regarding DSB repair pathway involved in BNCT.

Biological effects of BNCT

Mild DNA damage normally leads to the induction of cell-cycle arrest, whereas severe and irreparable injury shifts the cellular response towards induction of the senescence or cell death programs, such as apoptosis, mitotic catastrophe (MC), autophagy and necrosis [40]. BNCT causes severe and irreparable DNA damage as stated before, and is expected to induce these responses.

Apoptosis

P53, as one of the main targets of ATM/ATR, is a universal sensor of genotoxic stress and has a key role in cellular responses to DNA damage, determining the fate of cells towards either survival, which involves cell-cycle delay accompanied by repair of DNA damage, or cell death [40]. *P53* mutation occurs frequently in tumor cells and that makes tumor cells more resistant to radiation therapy [41]. *P53 mt* squamous cell carcinoma (SAS), and glioblastoma cells were more resistant to BNCT, comparing with *p53 wild* tumor cells [41,42]. Regarding apoptosis, in *p53 wild* SAS cells, G1 arrest and apoptosis occurred after 6 hours, while in *p53mt* SAS cells, G1 arrest did not occur, only G2/M arrest occurred after 12 hours, and apoptosis occurred after 48 hours [42]. Thus, BNCT causes both p53 dependent apoptosis and p53 independent apoptosis. Similarly, both p53 wild and mt glioblastoma cells were induced apoptosis by BNCT [43].

Glioma stem cells, which are also known to resistant to X-ray and chemotherapy, take up BPA [44] and BNCT induced apoptosis via mitochondrial pathway [45].

Mitotic catastrophe

To maintain genome integrity, cells respond to DNA damage by either a delay in cell-cycle progression, allowing time for proper DNA repair, or by the elimination of cells that are irreparably injured. When checkpoints are compromised, cells can enter mitosis prematurely before the completion of DNA repair and initiate MC [40]. MC is not an ultimate manifestation

of cell death but rather a process leading to apoptosis or necrosis [46]. MC occurs either during or shortly after a dysregulated/failed mitosis and can be accompanied by morphological alterations, including micronucleation (which often results from chromosomes and/or chromosome fragments that have not been distributed evenly between the daughter nuclei) and multinucleation (the presence of two or more nuclei of similar or heterogeneous sizes, derived from a deficient separation during cytokinesis) [40]. Micronuclei were observed after BNCT with BPA in melanoma [47], and with BPA or BSH in EL4 lymphoma, SCC VII squamous cell carcinoma, FM3A mammary carcinoma, and EMT6/KU sarcoma, followed by apoptosis [48]. Chromosomal aberrations, which also lead to MC, increased in human lymphocytes after BSH or BPA-BNCT in a dose dependent manner [49].

Although BPA-BNCT suppressed the growth of both SAS/*neo* and SAS/*mp53* tumors to undetectable levels in vivo, half of SAS/*mp53* tumors showed regrowth and histological examination of BNCT-treated tumors revealed chromosomal condensation, micronucleation, nuclear segmentation and intra- and intercellular vacuolation [50]. Notably, multinucleated giant cells appeared in SAS/*mp53* tumors early after BNCT, suggesting mitotic catastrophe. In SAS/*mp53* tumors treated with BNCT, a rapid decrease in phosphorylated cell division cycle 2 (cdc2) and a high level of cyclin B1, required for premature mitosis, were observed [50].

Necrosis

Necrosis is an acute form of cell death usually initiated following energy loss. Until recently, necrosis was considered to be an unregulated form of cell death; however, accumulating experimental data demonstrate that, except under extreme conditions, necrosis may be a genetically regulated process. Poly(ADPribosyl)ation was shown to have a central role in modulating the cellular response to severe genotoxic stress and induction of cell death through necrotic mechanisms [40]. After DNA damage, the activation of poly(ADP)-ribose polymerase (PARP) regulates the translocation of HMGB1, the immunostimulatory histone binding protein, from the nucleus to the cytosol [51]. BPA-BNCT increased Poly(ADPribosyl) foci in xenograft tumor tissue slightly at 6 hours, and remarkably at 20 hours post irradiation, and HMGB1 expression in the tumor tissue 6 hours after irradiation [37]. In another study, at 3 days post-BPA-based BNCT irradiation in a SAS xenograft mouse model, plasma HMGB1 levels were higher than those in the non-irradiation control, and HMGB1 was detected in both nuclei and cytoplasm in tumor cells [52]. Additionally, increased plasma HMGB1 levels post-BNCT irradiation were detected even when tumors decreased in size. Therefore, BNCT induced necrosis with HMGB1 release, and HMGB1could be a biomarker for BNCT for evaluating the therapeutic response during BNCT [52].

Abscopal Effect

Abscopal effect indicates ‘remission of metastases outside the field of irradiation’ and

has occurred very rarely before the immune-checkpoint inhibitors in combination radiation therapy were introduced [53]. Abscopal effect must be mediated by an antitumor immune response. The main effectors of tumor cell killing are cytotoxic T lymphocytes, which rely on neoantigen presentation by dendritic cells [53]. Neoantigens are abundantly produced by DNA damaging agents, radiation or chemotherapy. Exposure of radiation -induced micronuclear DNA to cytoplasm is the potent trigger of type I interferon signaling and contribute to the onset of abscopal effect [53].

BNCT was firstly shown by Trivillin et al., to cause abscopal effects using colon cancer xenografts [54] and this abscopal effect was enhanced by immune-therapy agent Bacillus Calmette-Guerin (BCG) [55]. The mechanism is not fully elucidated, but BNCT-induced micronuclear DNA would trigger the abscopal effects by BNCT. Misrepaired DNA damage after BNCT might be the source of tumor neoantigens and contribute to the abscopal effects by BNCT.

Closing Remarks

BNCT can induces apoptosis, mitotic catastrophe, and necrosis due to irreparable DNA damage in a tumor cell-specific manner by [$^{10}B(n,\alpha)^{7}Li$]. These powerful biological effects should lead to the excellent results of BNCT even against the most miserable tumor, glioblastoma, or refractory head and neck tumors. Recently, we have obtained the accelerator-based BNCT and more cancer

patients will have chance to choose BNCT. In addition, further research will develop more tumor-selective boron compounds, sophisticated drug delivery or better combined therapy (for example, immuno-modulating agents) with BNCT. Clinical and basic research on BNCT will become more and more necessary and noteworthy in the future.

Figure legends

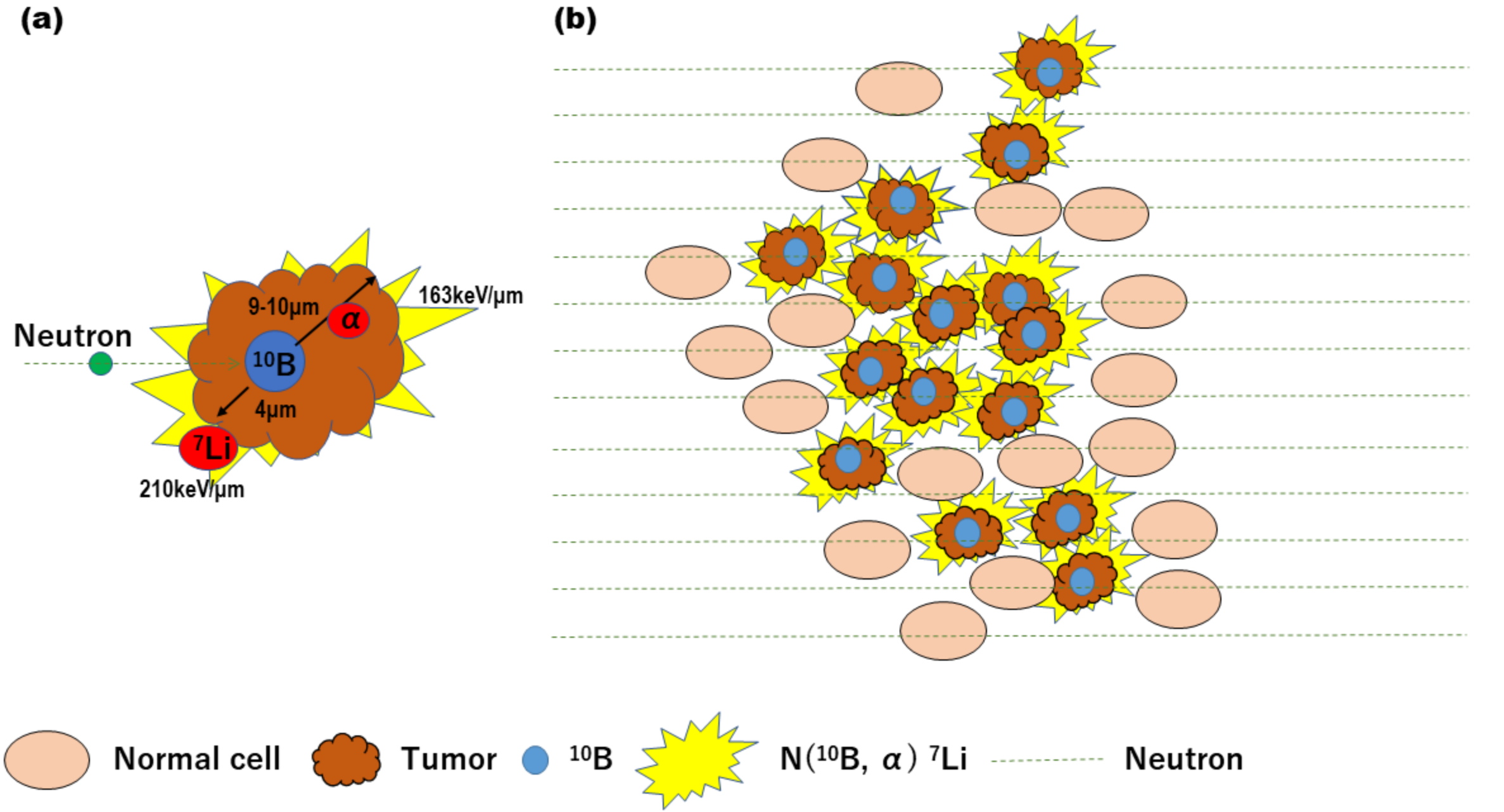
Fig. 1. Schema of mechanistic action of BNCT (a) $^{10}B(n, \alpha)^{7}$ Li capture reaction occurring in a tumor cell taken up by ^{10}B. When ^{10}B is irradiated by low energy thermal neutron, the resulting $^{10}B(n, \alpha)^{7}$ Li capture reaction produces high linear energy transfer (LET) α paricles (^{4}He) and recoiling lithium-7 (^{7} Li) atoms with short path lengths 9-10, and 4 μ m within the diameter of a single cell, respectively (b) The $^{10}B(n, \alpha)^{7}$ Li capture reactions mainly occur in the tumor cells which include ones invading into normal tissues. Thus, BNCT can selectively kill the tumor cells and spare the surrounding normal cells.

Fig. 2. L-4-dihydroxy-borylphenylalanine, BPA (boronophenylalanine) and undecahydro-mercapto-*closo*-dodecaborate, BSH (sodium borocaptate)

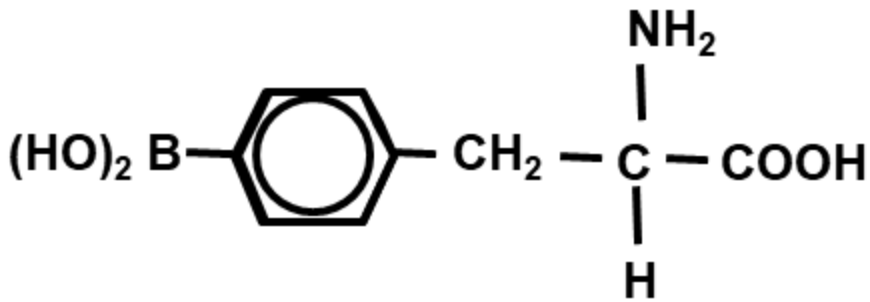
Fig. 3. γH2AX foci in brain tumor model after neutron irradiation. (a) Representative images of nuclear γH2AX foci of brain tumor cells. Upper image shows saline-treated mouse, and lower

shows BPA treated mouse. DAPI = staining of nuclear DNA. (b) The number of γH2AX foci 24 h after irradiation. Bars represent the standard errors. Reproduced with permission. Copyright 2016, Elsevier [37]

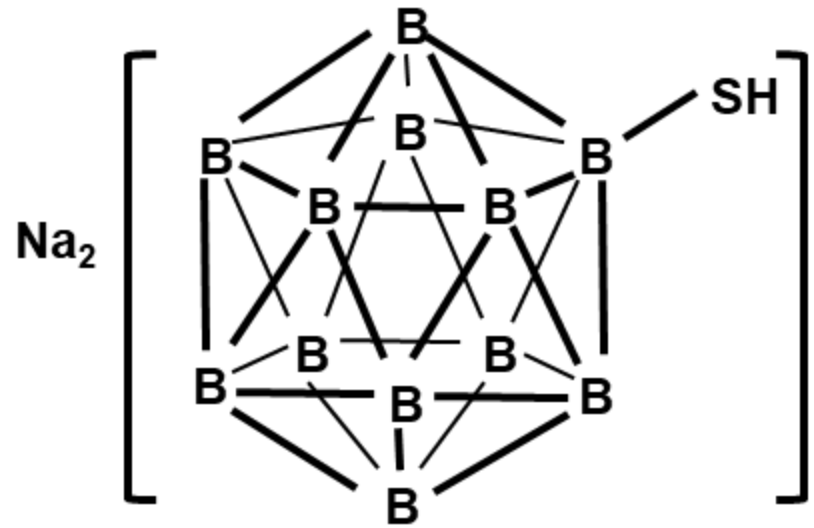
Fig. 4. γH2AX foci in normal brain and brain tumor model after γ-irradiation. The number of γH2AX foci 24 h after irradiation at the times indicated post-irradiation. Reproduced with permission. Copyright 2016, Elsevier [37]


(a)
Neutron
9-10μm
α
163keV/μm
10B
4μm
7Li
210keV/μm
(b)
Normal cell
Tumor
10B
N(10B, α) 7Li
Neutron

Boronophenylalanine (BPA)

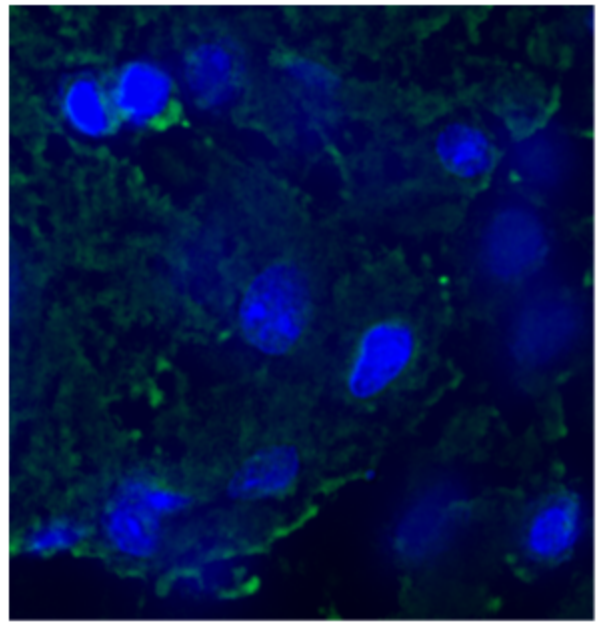


Sodium borocaptate (BSH)

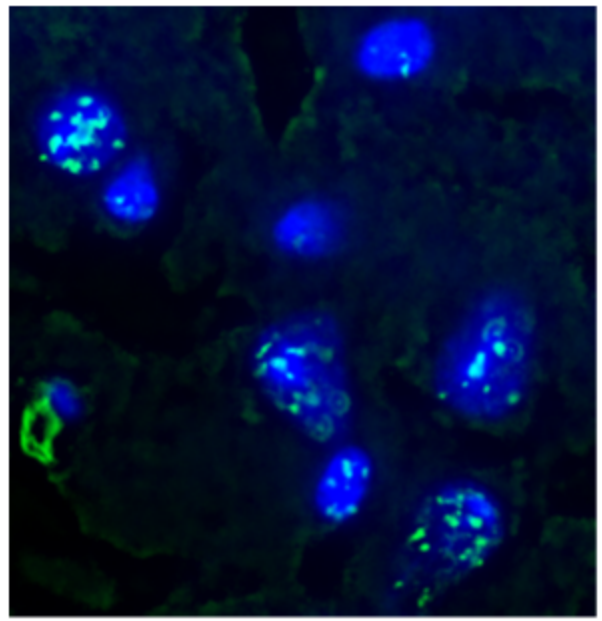


(a)

Saline

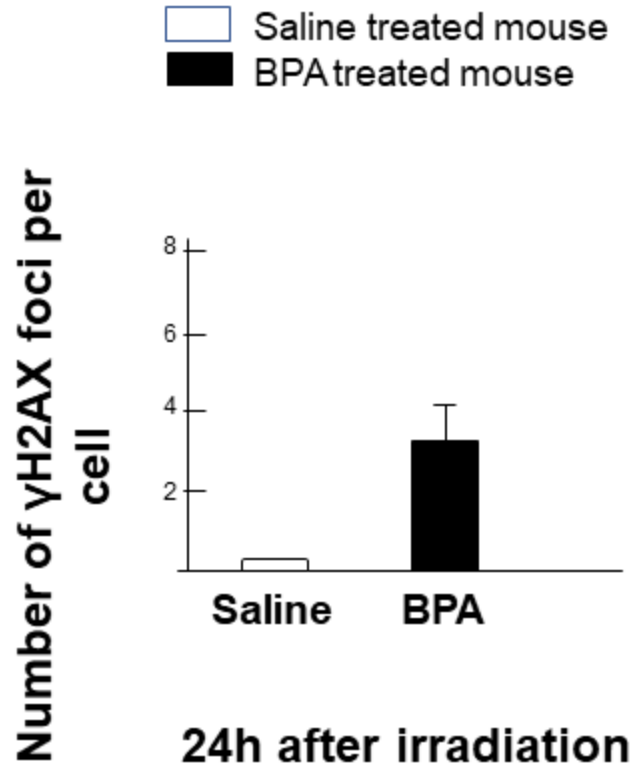


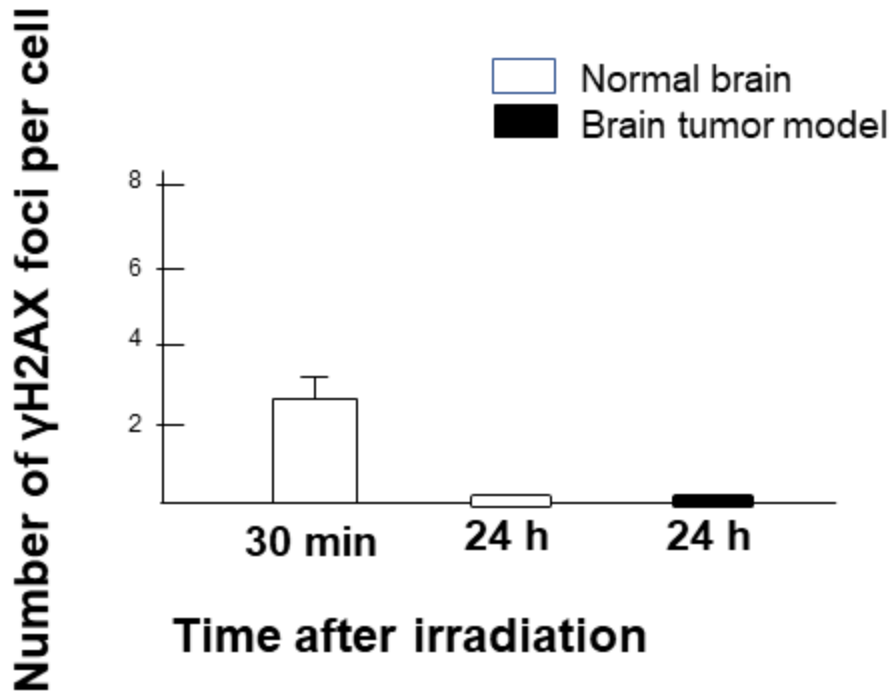
BPA



24hrs after irradiation

(b)




Normal brain
Brain tumor model
Number of γH2AX foci per cell
8
6
4
2
30 min
24 h
24 h
Time after irradiation

Reference

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