Functionalized mesoporous silica nanoparticles for innovative boron-neutron capture therapy of resistant cancers

‡Guillaume Vares[1], ‡Vincent Jallet[1], Yoshitaka Matsumoto[2], †Cedric Rentier[3], Kentaro Takayama[3], Toshio Sasaki[1], Yoshio Hayashi[3], Hiroaki Kumada[2], §Hirotaka Sugawara[1]*

[1]Okinawa Institute of Science and Technology (OIST), Onna, Okinawa 904-0495, Japan.

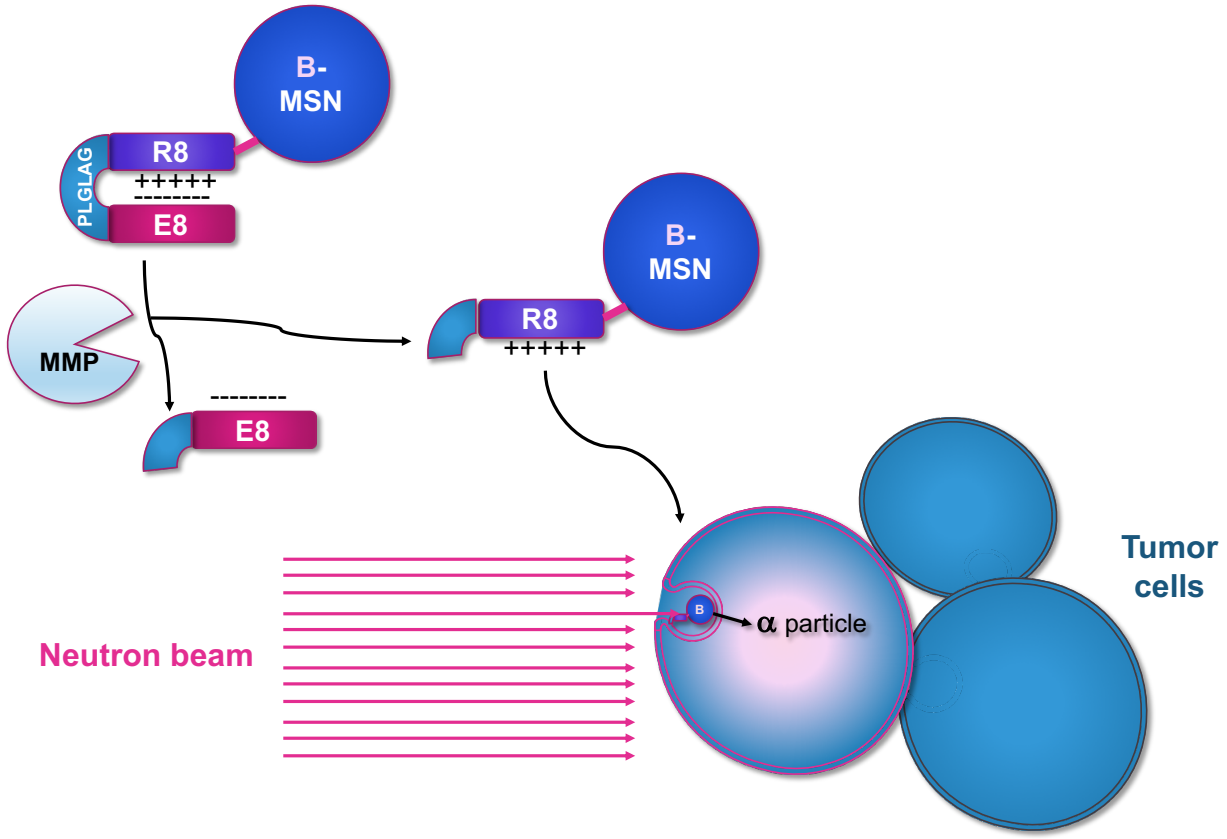
[2]University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan.

[3]Department of Medicinal Chemistry, Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo 192-0392

‡These authors contributed equally

*Correspondance: guillaume.vares@oist.jp

Table of Contents Graphic



Abstract

Treatment resistance, relapse and metastasis remain critical issues in some challenging cancers, such as chondrosarcomas. Boron-neutron Capture Therapy (BNCT) is a targeted radiation therapy modality that relies on the ability of boron atoms to capture low energy neutrons, yielding high linear energy transfer alpha particles. We have developed an innovative boron-delivery system for BNCT, composed of multifunctional fluorescent mesoporous silica nanoparticles (B-MSNs), grafted with an activatable cell penetrating peptide (ACPP) for improved penetration in tumors and with Gadolinium for magnetic resonance imaging (MRI) *in vivo*. Chondrosarcoma cells were exposed *in vitro* to an epithermal neutron beam after B-MSNs administration. BNCT beam

exposure successfully induced DNA damage and cell death, including in radio-resistant ALDH+ cancer stem cells (CSCs), suggesting that BNCT using this system might be a suitable treatment modality for chondrosarcoma or other hard-to-treat cancers.

Keywords

Mesoporous silica nanoparticles; Boron-neutron capture therapy; Chondrosarcoma; Radiation therapy; Cancer stem cells

Results and discussion

Remarkable progress has been made in the understanding and treatment of human cancer, resulting in a greatly improved survival rate for many patients. However, such achievements remain incomplete or out of reach for some hard-to-treat cancers, such as pancreatic cancer, glioblastoma or bone tumors. Chondrosarcomas are cartilaginous tumors which represent the second most common primary bone tumor in adults[1]. Chondrosarcomas are notoriously resistant to conventional radiation therapy and to chemotherapy, and complete surgical resection remains to this day the primary treatment. A number of patients experience relapse, metastasis or present unresectable disease with poor clinical outcome[2].

Tumors are heterogeneous and are comprised of cells with various morphological and molecular features, including a subset of tumor-initiating dedifferentiated cells with self-renewing abilities. These cancer stem cells (CSCs) are capable of reconstituting tumor heterogeneity, and a large amount of evidence strongly suggests that they may contribute to treatment resistance, relapse and metastasis[3]. CSCs have been identified in a number of tumors, including chondrosarcomas[4]. Several features of CSCs have been reported to explain their intrinsic radioresistance: lower levels of basal and radiation-induced reactive oxygen species (ROS), improved DNA damage repair activation and apoptosis inhibition or relative quiescent state[5]. New approaches are thus highly expected to address treatment-resistant tumors, which may include targeting CSCs.

In addition to conventional X-ray therapy, new radiation therapy modalities have emerged which might finally contribute overcoming treatment resistance, such as boron neutron capture therapy (BNCT) or carbon-ion particle therapy[6]. BNCT is an innovative experimental treatment modality that relies on the ability of ^{10}B to capture thermal neutrons, resulting in the release of high-linear

energy transfer (LET) α (^{4}He) particles and lithium (^{7}Li) nuclei, with a path length shorter than 10 μm[7]. Therefore, it is crucial to maximize the concentration of boron-enriched compounds in tumor tissues while minimizing levels in surrounding normal tissues. Furthermore, intracellular boron delivery should be achieved, due to the short path length of α (^{4}He) particles. Because BNCT releases high-LET radiation, it should provide improved relative biological effectiveness (RBE) and a lower oxygen enhancement ratio (OER), compared to conventional X-ray therapy. BNCT clinical trials have been performed on patients suffering from head and neck, brain, lung and liver cancers[7], with some encouraging results in terms of overall survival, recurrence and metastasis. For those reasons, BNCT might also be an effective strategy for the treatment of radioresistant tumors, such as clear cell sarcoma (CSS)[8], osteosarcoma[9] or chondrosarcoma.

Even though the first BNCT trials have been performed more than half a century ago, BNCT has not yet become an established treatment modality, due to two main limiting factors[10]. First, only two boron-delivery drugs are routinely used in BNCT clinical trial studies: sodium mercaptoundecahydrododecaborate ($Na_2{}^{10}B_{12}H_{11}SH$; $Na_2{}^{10}BSH$) and L-*p*-boronophenylalanine (L-^{10}BPA). Reported tumor-to-normal tissue (T/N) ratio for BSH does not always reach 1. New advances are necessary to improve T/N ratio with low toxicity. Second, the sole neutron sources traditionally available for BNCT were nuclear reactors. Recent advances in nanotechnologies for drug delivery and the development of new accelerator-based neutron sources promise to overcome those limitations. Here, we report a new theranostic multi-functional boron-delivery system based on mesoporous silica nanoparticles (B-MSNs). We tested this system using an accelerator-based neutron beam for BNCT.

Nanoparticles (NPs) have recently emerged as a promising therapeutic tool for a variety of medical applications. NPs allow the encapsulation of therapeutic compounds with higher

protection against metabolic degradation and the ability to control and target drug release preferentially in tumor tissues[11]. The accumulation of NPs in tumor tissues has been attributed to the poor alignment of neovascularization and lymphatic drainage in those areas, so called enhanced permeability (EPR) effect[12]. In particular, much attention has been devoted to the design of mesoporous silica nanoparticles (MSNs), which present a number of advantageous features: tunable size (usually 50 to 200 nm diameter), easy surface functionalization, large mesopore volume for efficient drug loading, *in vitro* and *in vivo* tolerance[13,14]. They might therefore serve as ideal boron-delivery agents for BNCT.

To this end, we have developed multifunctional MSNs, which can serve as boron-delivery carrier and can be monitored for BNCT dosimetry (Figure 1a, Scheme S1). The diameter of inorganic core, as determined by transmission electronic microscopy (TEM), was around 100 nm (Figures 1b, S3). In order to improve biocompatibility, a layer of polyethylene glycol (PEG, 5kDa 95w%, 10 kDa 5 w%) was grafted onto the surface of nanoparticles by simple peptide bond (Figure 2a, Table S2). PEG also allowed steric stabilization of the nanoparticles, which did not form significant aggregates in culture media[15]. The hydrodynamic radius of B-MSNs, measured by dynamic light scattering (DLS), was around 200 nm (Figure 2b). The nanoparticles didn't show any significant toxicity *in vitro* (Figure S4) or *in vivo* (Figure S5) and exhibited good stability and dispersion properties, as confirmed by analysis of zeta potential values (consistently lower than -25 mV).

a **b**

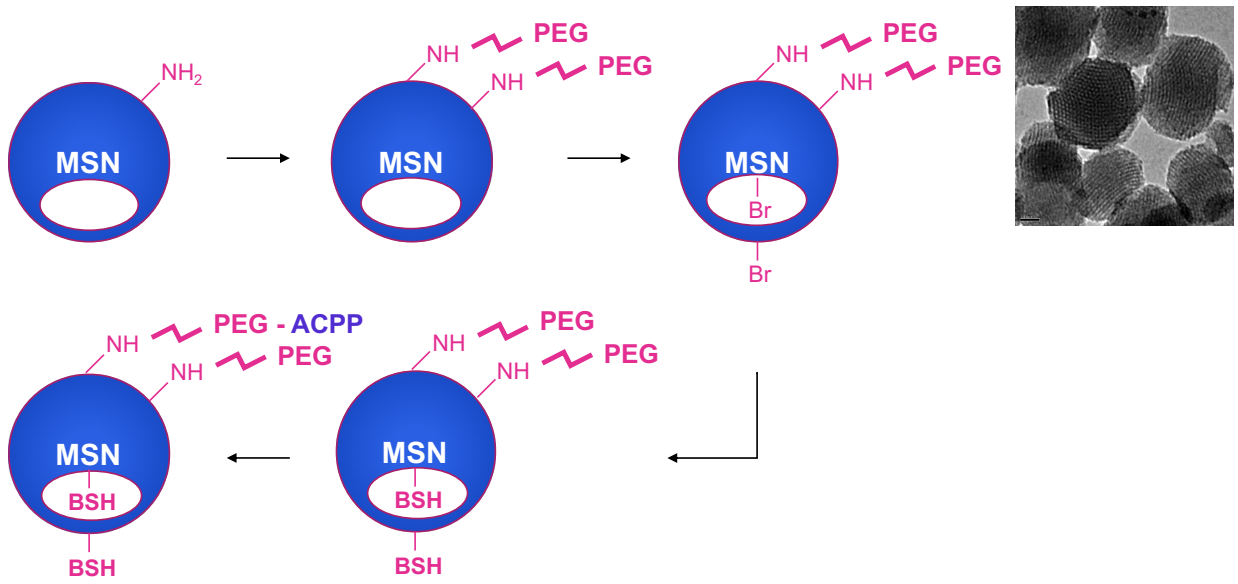


Figure 1. Synthesis of Boron-delivery mesoporous silica nanoparticles (B-MSNs). (a) Mesoporous silica MCM-41 fluorescent nanoparticles were PEGylated, then BSH (B10-enriched) was incorporated. Finally, an activatable cell penetrating peptide (ACPP) was grafted. Synthesis steps are detailed in Supplementary Information. (b) Visualization of B-MSNs by transmission electronic microscopy (TEM).

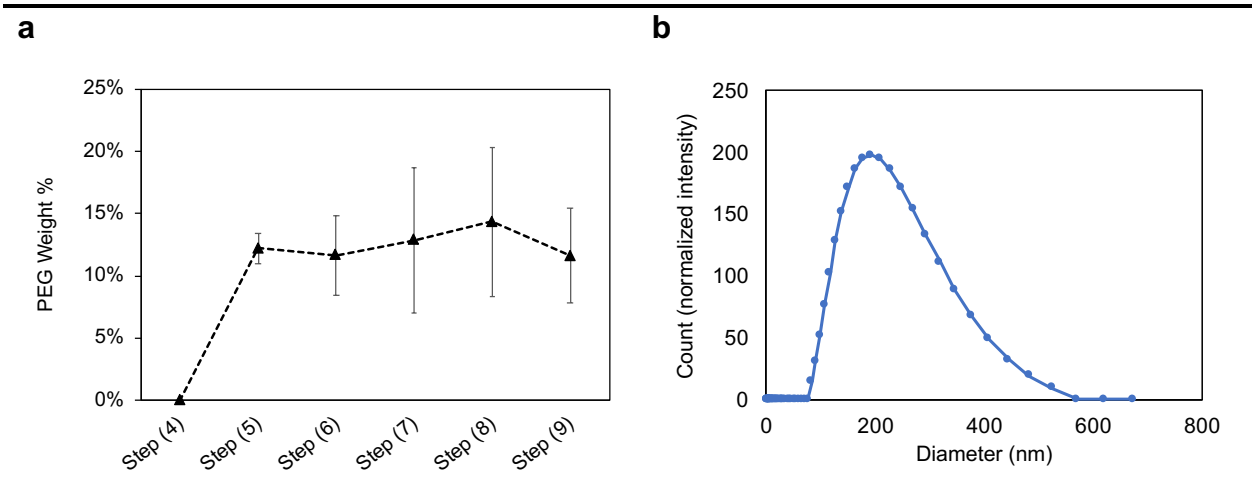


Figure 2. Characterization of Boron-delivery mesoporous silica nanoparticles (B-MSNs). (a) Quantification of PEG grafted on MSNs at various synthesis steps (see Scheme S1). (b) Measurement of the hydrodynamic radius of B-MSNs by dynamic light scattering (DLS).

Sufficient amounts of ^{10}B (about 20 μg/g weight or about 10^9 atoms/cell) need to be delivered to tumor cells for the success of BNCT[16]. Furthermore, because the track of α particles generated by boron-neutron capture is 10 μm at most, it is necessary that a sufficient proportion of ^{10}B penetrate inside cells for optimal efficiency. Large amounts of boron might be loaded into nanoparticle mesopores as o-carborane[17], however there is a risk of carborane leakage and unpredictable boron distribution. Here, we propose to attach ^{10}B-enriched BSH inside mesopores, using an aminosilane coupling agent. Inductively coupled plasma mass spectrometry (ICP-MS) measurements confirmed the successful accumulation of ^{10}B on B-MSNs, which contain 1.27% mass fraction of boron (95% ^{10}B), representing around 5 x 10^{17} atoms of ^{10}B per mg nanoparticles (Table S1). Subsequent steps of nanoparticle synthesis did not lead to release of BSH. This suggests that if *in vivo* B-MSN delivery to tumors could be optimized, the amount of boron reaching tumor cells might be sufficient for BNCT treatment.

In order to efficiently enter cells by endocytosis, nanoparticles are commonly surface-modified with cell penetrating peptides (CPPs). Surface functionalization of the nanoparticles with an activatable cell penetrating peptide (ACPP) allows for efficient tumor targeting. Our ACPP consists of three regions (Figure 3a): a polyanionic autoinhibitory domain (octaglutamic acid E_8), a PLGLAG linker region (sensitive to proteases) and a cell-penetrating polycationic domain (octaarginine R_8)[18,19]. In addition, an Acp (aminohexanoic acid) moiety is grafted on the C-terminal portion of the peptide to serve as a spacer between the polycationic domain and the Cys residue for a better efficiency in the thiol-maleimide coupling strategy used. In the intact ACPP, the

polyanionic peptide domain prevents uptake of the polycationic domain. Matrix metalloproteinases (MMP) 2 and 9 (generally overexpressed in tumors[20]) cleave the PLGLAG linker, releasing the cell-penetrating R_8 portion grafted on the nanoparticle. Indeed, chondrosarcoma cells expressed higher levels of MMP-2 than normal chondrocytes (Figure 3b), leading to enhanced relative cellular uptake, compared to nanoparticles grafted with polyethylene glycol (PEG) (Figures 3c and 3d).

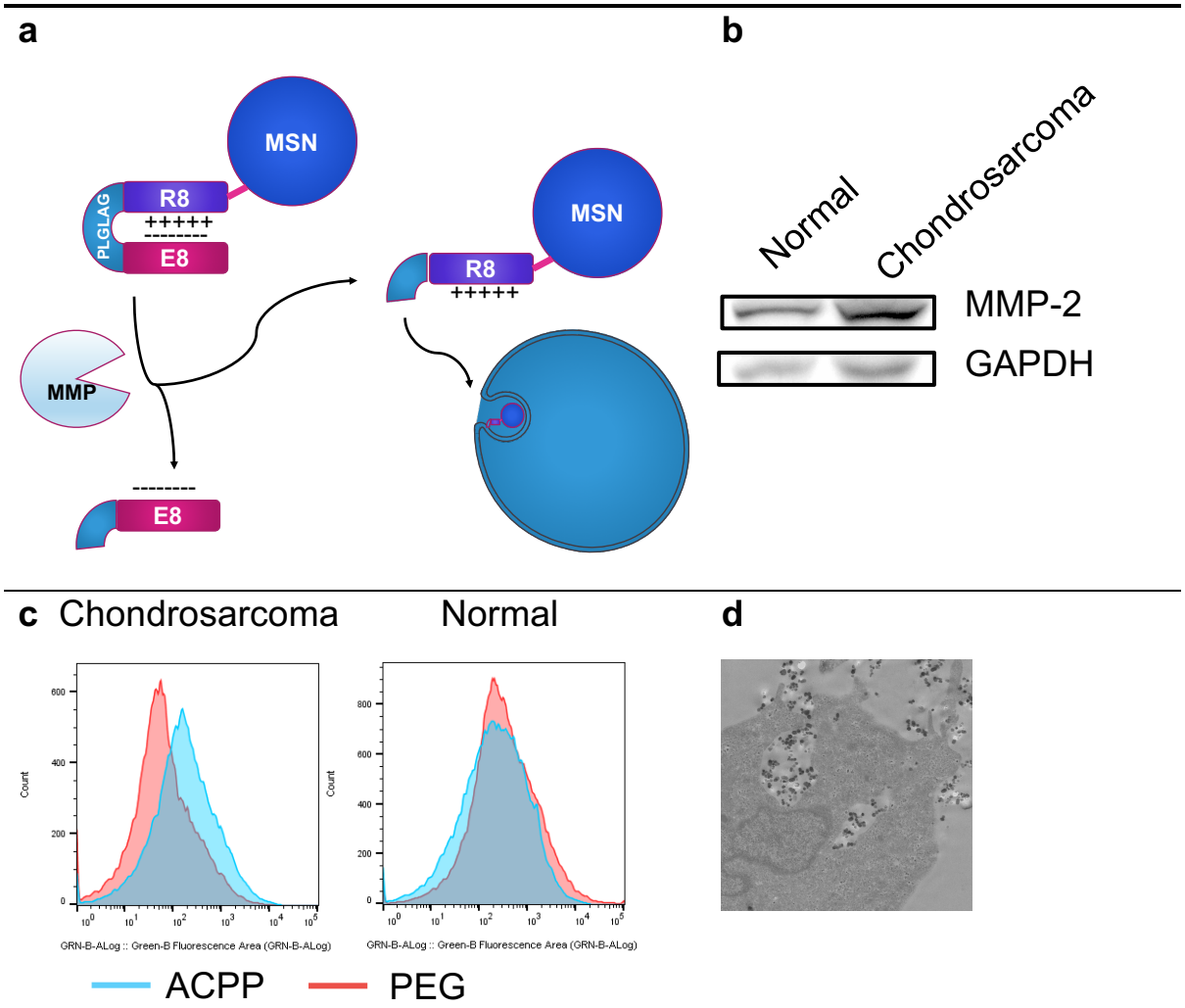


Figure 3: Cellular uptake of functionalized boron-delivery nanoparticles (B-MSNs). (a) Matrix metalloproteinase (MMP)-mediated cleavage of the linker PLGLAG region releases the polycationic octaarginine (R8) region of the activatable cell penetrating peptide (ACPP). (b)

Chondrosarcoma cells express more MMP-2 protein than normal chondrocytes. (c) B-MSN cellular uptake is improved in chondrosarcoma cells, as measured by flow cytometry three hours after MSN administration. (d) B-MSNs functionalized with ACPP penetrate cells by endocytosis, as observed by transmission electronic microscopy (TEM).

Unlike other radiation therapy modalities (X-rays or charged particle beams), calibration and dosimetry for BNCT relies on many parameters, including neutron beam properties and boron uptake in tumors. In this context, it is crucial to properly monitor the biodistribution of boron-delivery compounds, if possible in a non-invasive way. Although our B-MSNs include Fluorescein isothiocyanate (FITC), allowing fluorescent tracking for *in vitro* and small animal studies, the depth limitation of optical imaging methods seriously hampers their clinical utility. We have therefore also developed MSNs grafted with Gadolinium for *in vivo* visualization using magnetic resonance imaging (MRI)[21,22]. These nanoparticles were injected into the tail vein of nude mice bearing xenograft chondrosarcoma tumors. Longitudinal (T_1) and transverse (T_2) relaxation times were measured for 24h in the tumor (Figures 4, S6). Due to its paramagnetic properties, gadolinium shortens T_1 and T_2 when it accumulates. While T_1 values did not change significantly after injection, we observed a clear decrease in T_2 values, reflecting nanoparticles tumor uptake. Relative lack of T_1-weighted contrast is expected at high magnetic fields (11.7 T), as reported previously[23]. Accordingly, increased loading of gadolinium on nanoparticles led to changes in T_2, but not T_1 values (Figure S6). Better overall T_1-weighted contrast may be expected at lower fields in MRI systems for routine clinical use.

a **b**

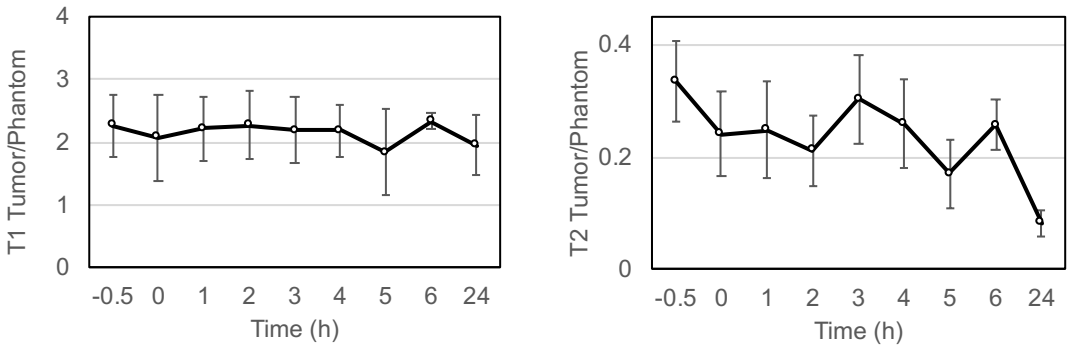


Figure 4. Time-course of T_1 and T_2 values in xenograft chondrosarcoma tumors before and after intravenous nanoparticle injection. (a) Average T1 values in the tumor, normalized to phantom. (b) Average T2 values in the tumor, normalized to phantom.

In order to verify the efficiency of our boron-delivery system *in vitro*, CH-2879 chondrosarcoma cells[24] and an ALDH+ radioresistant CSC subpopulation[25] (Figure S8) were exposed to an epithermal neutron beam[26] (Table S3). Although apoptosis induction after 24h was limited (Figure 5b), BNCT beam exposure resulted in significant DNA damage levels and lower clonogenic survival (Figure 5ad). Comparison of survival curves may allow for a rough estimation of RBEs for dosimetry. Doses resulting in 10% survival (D_{10}) were 5.86 Gy for X-rays and 0.42 mA.h (about 6.7×10^{11} n/cm^2) for neutron beam. Interestingly, while CSCs were more resistant to conventional X-ray therapy than the general CH-2879 cell population (Figure 5c), no significant difference was observed in cells exposed to neutron beam (Figure 5d), suggesting that high-LET radiation exposures such as BNCT might be more efficient at targeting CSCs than other treatment modalities[27].

0 mA.h 0.5 mA.h

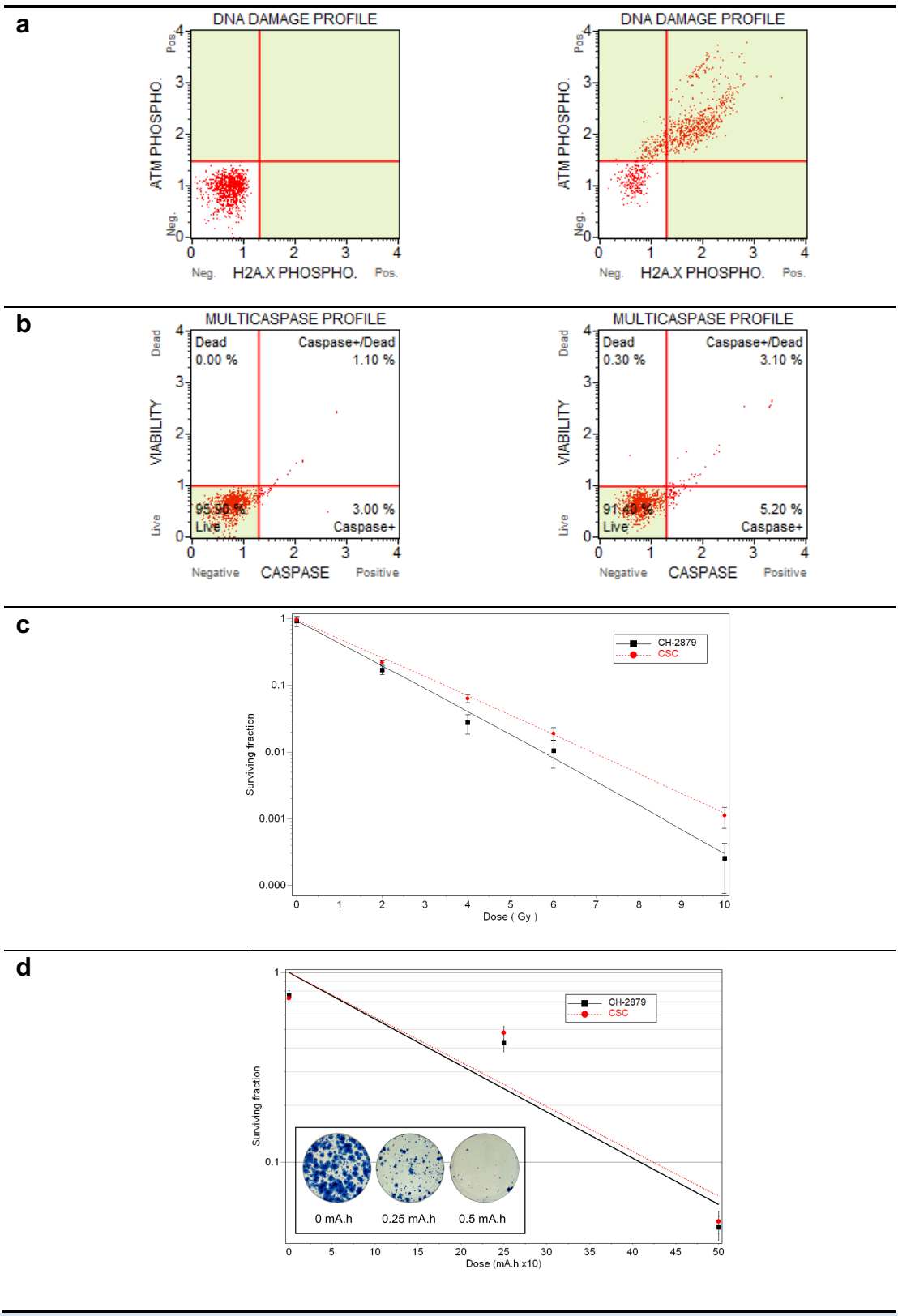


Figure 5. Boron-neutron capture therapy (BNCT) of chondrosarcoma cells with boron-delivery nanoparticles. (a) BNCT induces DNA damage. ATM and H2AX phosphorylation

levels were measured in non-irradiated and irradiated CH-2879 cells, respectively. (b) BNCT induces limited apoptosis 24h after irradiation. (c-d) CSCs are comparatively resistant to X-rays (c), but not to BNCT (d), compared with non-CSC CH-2879 cells, as observed after performing clonogenic assays. Neutron beam exposures were performed on cells after administration of mesoporous silica nanoparticles (MSNs) functionalized with activatable cell-penetrating peptide (ACPP).

In summary, we suggest that multi-functional B-MSNs may be a suitable delivery system for BNCT of resistant cancers. Other boron-delivery strategies have included the encapsulation of boron-curcumin in poly(lactic-co-glycolic acid) (PLGA) nanoparticles[28], the use of boron cluster-containing polyion complex (PIC) nanoparticles[29] or a boron-rich MAC-TAC liposomal system[30]. Our B-MSNs exhibit several advantageous features when compared with other organic and inorganic drug delivery systems: easily tunable particle and pore size, high flexibility for further functionalization, suitability for theranostic approaches (such as non-invasive imaging for biodistribution measurements and BNCT dosimetry).

Overcoming treatment resistance might require an effective targeting of radioresistant CSCs. Therapeutic strategies against CSCs have included inhibition of WNT and NOTCH pathways, ablation using antibody-drug conjugates (ADCs) or epigenetic therapy, each with potential drawbacks or limitations[31]. High-LET radiation treatment, in combination with other targeted therapies (such as chemotherapeutic agent cisplatin or PARP inhibitor talazoparib), has shown favourable results in bypassing tumor and CSC radioresistance[6,27,32]. Using our boron-delivery system, BNCT might be capable of efficiently targeting radioresistant CSCs in hard-to-treat tumors, such as chondrosarcoma. The ability of nanoparticle-based systems to target specific or diffuse tumor sites, as observed in malignant mesothelioma[33], is also of particular interest for BNCT. Recently, a number of proton accelerator-based neutron sources have been

commissioned for research and clinical use[34], opening new perspectives for the potential development of BNCT as a viable new cancer therapy modality.

Supporting Information.

Supporting information available.

Corresponding Author

* guillaume.vares@oist.jp

Present Addresses

†Biotage Japan, Koto, Tokyo 136-0071

§High Energy Accelerator Research Organization (KEK), Tsukuba, Ibaraki 305-0801

Author Contributions

GV and VJ designed the experiments. VJ designed and synthesized the nanoparticles. GV performed biological experiments. YM and GV performed neutron beam experiments. CR and KT synthesized ACPP. YT, VJ and GV performed TEM experiments. VJ and GV performed MRI experiments. All authors interpreted the results and contributed to writing the manuscript. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

Funding Sources

This study was funded by Okinawa Institute of Science and Technology (OIST) and by the R&D Cluster Research Program (Okinawa Prefecture / OIST).

Acknowledgements

The authors thank Keigo Hikishima (Okinawa Institute of Science and Technology Graduate University, OIST) for performing MRI measurements, Yoshiteru Iinuma (OIST) for performing ICP-MS measurements and the iBNCT support team (University of Tsukuba) for neutron experiments. The authors also thank Ichio Aoki (National Institutes for Quantum and Radiological Science and Technology) and Sergey Taskaev (Budker Institute of Nuclear Physics) for scientific discussions.

Abbreviations

ACPP: Activatable cell penetrating peptide. ADC: antibody-drug conjugate. B-MSN: Boron-delivery mesoporous silica nanoparticle. BNCT: Boron neutron capture therapy. BPA: Boronophenylalanine. BSH: Mercaptoundecahydrododecaborate. CSC: Cancer stem cell. CSS: Clear cell sarcoma. DLS: Dynamic light scattering. EPR: Enhanced permeability effect. FITC: Fluorescein isothiocyanate. ICP-MS: Inductively coupled plasma mass spectrometry. LET: Linear energy transfer. MMP: Matrix metalloproteinase. MRI: Magnetic resonance imaging. NP: Nanoparticle. OER: oxygen enhancement ratio. PEG: Polyethylene glycol. PIC: Polyion complex. PLGA: poly(lactic-co-glycolic acid). RBE: Relative biological effectiveness. TEM: Transmission electronic microscopy.

References

(1) Mery, B.; Espenel, S.; Guy, J.-B.; Rancoule, C.; Vallard, A.; Aloy, M.-T.; Rodriguez-Lafrasse, C.; Magné, N. Biological Aspects of Chondrosarcoma: Leaps and Hurdles. *Critical Reviews in Oncology/Hematology* **2018**, *126*, 32–36.
(2) Gelderblom, H.; Hogendoorn, P. C. W.; Dijkstra, S. D.; Rijswijk, C. S. van; Krol, A. D.; Taminiau, A. H. M.; Bovée, J. V. M. G. The Clinical Approach Towards Chondrosarcoma. *The Oncologist* **2008**, *13* (3), 320–329.
(3) Singh, A.; Settleman, J. EMT, Cancer Stem Cells and Drug Resistance: An Emerging Axis of Evil in the War on Cancer. *Oncogene* **2010**, *29* (34), 4741–4751.

(4) David, E.; Blanchard, F.; Heymann, M. F.; De Pinieux, G.; Gouin, F.; Rédini, F.; Heymann, D. The Bone Niche of Chondrosarcoma: A Sanctuary for Drug Resistance, Tumour Growth and also a Source of New Therapeutic Targets https://www.hindawi.com/journals/sarcoma/2011/932451/ (accessed May 30, 2018).
(5) Pajonk, F.; Vlashi, E.; McBride, W. H. Radiation Resistance of Cancer Stem Cells: The 4 R's of Radiobiology Revisited. *STEM CELLS* **2010**, *28* (4), 639–648.
(6) Sai, S.; Suzuki, M.; Kim, E. H.; Hayashi, M.; Vares, G.; Yamamoto, N.; Miyamoto, T. Effects of Carbon Ion Beam Alone or in Combination with Cisplatin on Malignant Mesothelioma Cells in Vitro. *Oncotarget* **2017**, *9* (19), 14849–14861.
(7) Nedunchezhian, K.; Aswath, N.; Thiruppathy, M.; Thirugnanamurthy, S. Boron Neutron Capture Therapy - A Literature Review. *J Clin Diagn Res* **2016**, *10* (12), ZE01–ZE04.
(8) Andoh, T.; Fujimoto, T.; Suzuki, M.; Sudo, T.; Sakurai, Y.; Tanaka, H.; Fujita, I.; Fukase, N.; Moritake, H.; Sugimoto, T.; et al. Boron Neutron Capture Therapy (BNCT) as a New Approach for Clear Cell Sarcoma (CCS) Treatment: Trial Using a Lung Metastasis Model of CCS. *Appl Radiat Isot* **2015**, *106*, 195–201.
(9) Futamura, G.; Kawabata, S.; Siba, H.; Kuroiwa, T.; Suzuki, M.; Kondo, N.; Ono, K.; Sakurai, Y.; Tanaka, M.; Todo, T.; et al. A Case of Radiation-Induced Osteosarcoma Treated Effectively by Boron Neutron Capture Therapy. *Radiation Oncology* **2014**, *9*, 237.
(10) Moss, R. L. Critical Review, with an Optimistic Outlook, on Boron Neutron Capture Therapy (BNCT). *Appl Radiat Isot* **2014**, *88*, 2–11.
(11) Brigger, I.; Dubernet, C.; Couvreur, P. Nanoparticles in Cancer Therapy and Diagnosis. *Advanced Drug Delivery Reviews* **2002**, *54* (5), 631–651.
(12) Blanco, E.; Shen, H.; Ferrari, M. Principles of Nanoparticle Design for Overcoming Biological Barriers to Drug Delivery. *Nature Biotechnology* **2015**, *33* (9), 941–951.
(13) Hudson, S. P.; Padera, R. F.; Langer, R.; Kohane, D. S. The Biocompatibility of Mesoporous Silicates. *Biomaterials* **2008**, *29* (30), 4045–4055.
(14) Lu, J.; Liong, M.; Li, Z.; Zink, J. I.; Tamanoi, F. Biocompatibility, Biodistribution, and Drug-Delivery Efficiency of Mesoporous Silica Nanoparticles for Cancer Therapy in Animals. *Small 6* (16), 1794–1805.
(15) Graf, C.; Gao, Q.; Schütz, I.; Noufele, C. N.; Ruan, W.; Posselt, U.; Korotianskiy, E.; Nordmeyer, D.; Rancan, F.; Hadam, S.; et al. Surface Functionalization of Silica Nanoparticles Supports Colloidal Stability in Physiological Media and Facilitates Internalization in Cells. *Langmuir* **2012**, *28* (20), 7598–7613.
(16) Barth, R. F.; H Vicente, Mg.; Harling, O. K.; Kiger, W.; Riley, K. J.; Binns, P. J.; Wagner, F. M.; Suzuki, M.; Aihara, T.; Kato, I.; et al. Current Status of Boron Neutron Capture Therapy of High Grade Gliomas and Recurrent Head and Neck Cancer. *Radiation Oncology* **2012**, *7*, 146.
(17) Lai, C.-H.; Lai, N.-C.; Chuang, Y.-J.; Chou, F.-I.; Yang, C.-M.; Lin, C.-C. Trivalent Galactosyl-Functionalized Mesoporous Silica Nanoparticles as a Target-Specific Delivery System for Boron Neutron Capture Therapy. *Nanoscale* **2013**, *5* (19), 9412–9418.
(18) Mei, L.; Zhang, Q.; Yang, Y.; He, Q.; Gao, H. Angiopep-2 and Activatable Cell Penetrating Peptide Dual Modified Nanoparticles for Enhanced Tumor Targeting and Penetrating. *International Journal of Pharmaceutics* **2014**, *474* (1–2), 95–102.
(19) Jiang, T.; Olson, E. S.; Nguyen, Q. T.; Roy, M.; Jennings, P. A.; Tsien, R. Y. Tumor Imaging by Means of Proteolytic Activation of Cell-Penetrating Peptides. *PNAS* **2004**, *101* (51), 17867–17872.

(20) Egeblad, M.; Werb, Z. New Functions for the Matrix Metalloproteinases in Cancer Progression. *Nat. Rev. Cancer* **2002**, *2* (3), 161–174.
(21) Di Corato, R.; Gazeau, F.; Le Visage, C.; Fayol, D.; Levitz, P.; Lux, F.; Letourneur, D.; Luciani, N.; Tillement, O.; Wilhelm, C. High-Resolution Cellular MRI: Gadolinium and Iron Oxide Nanoparticles for in-Depth Dual-Cell Imaging of Engineered Tissue Constructs. *ACS Nano* **2013**, *7* (9), 7500–7512.
(22) Marangoni, V. S.; Neumann, O.; Henderson, L.; Kaffes, C. C.; Zhang, H.; Zhang, R.; Bishnoi, S.; Ayala-Orozco, C.; Zucolotto, V.; Bankson, J. A.; et al. Enhancing T1 Magnetic Resonance Imaging Contrast with Internalized Gadolinium(III) in a Multilayer Nanoparticle. *PNAS* **2017**, *114* (27), 6960–6965.
(23) Hagberg, G. E.; Scheffler, K. Effect of R1 and R2 Relaxivity of Gadolinium-Based Contrast Agents on the T1-Weighted MR Signal at Increasing Magnetic Field Strengths. *Contrast Media & Molecular Imaging 8* (6), 456–465.
(24) Gil-Benso, R.; Lopez-Gines, C.; López-Guerrero, J. A.; Carda, C.; Callaghan, R. C.; Navarro, S.; Ferrer, J.; Pellín, A.; Llombart-Bosch, A. Establishment and Characterization of a Continuous Human Chondrosarcoma Cell Line, *Ch-2879*: Comparative Histologic and Genetic Studies with Its Tumor of Origin. *Laboratory Investigation* **2003**, *83* (6), 877–887.
(25) Xu, X.; Chai, S.; Wang, P.; Zhang, C.; Yang, Y.; Yang, Y.; Wang, K. Aldehyde Dehydrogenases and Cancer Stem Cells. *Cancer Letters* **2015**, *369* (1), 50–57.
(26) Kumada, H.; Naito, F.; Hasegawa, K.; Kobayashi, H.; Kurihara, T.; Takada, K.; Onishi, T.; Sakurai, H.; Matsumura, A.; Sakae, T. Development of LINAC-Based Neutron Source for Boron Neutron Capture Therapy in University of Tsukuba. *Plasma and Fusion Research* **2018**, *13*, 2406006–2406006.
(27) Sai, S.; Vares, G.; Kim, E. H.; Karasawa, K.; Wang, B.; Nenoi, M.; Horimoto, Y.; Hayashi, M. Carbon Ion Beam Combined with Cisplatin Effectively Disrupts Triple Negative Breast Cancer Stem-like Cells in Vitro. *Molecular cancer* **2015**, *14* (1), 166.
(28) Alberti, D.; Protti, N.; Franck, M.; Stefania, R.; Bortolussi, S.; Altieri, S.; Deagostino, A.; Aime, S.; Geninatti Crich, S. Theranostic Nanoparticles Loaded with Imaging Probes and Rubrocurcumin for Combined Cancer Therapy by Folate Receptor Targeting. *ChemMedChem 12* (7), 502–509.
(29) Gao, Z.; Horiguchi, Y.; Nakai, K.; Matsumura, A.; Suzuki, M.; Ono, K.; Nagasaki, Y. Use of Boron Cluster-Containing Redox Nanoparticles with ROS Scavenging Ability in Boron Neutron Capture Therapy to Achieve High Therapeutic Efficiency and Low Adverse Effects. *Biomaterials* **2016**, *104*, 201–212.
(30) Heber, E. M.; Hawthorne, M. F.; Kueffer, P. J.; Garabalino, M. A.; Thorp, S. I.; Pozzi, E. C. C.; Hughes, A. M.; Maitz, C. A.; Jalisatgi, S. S.; Nigg, D. W.; et al. Therapeutic Efficacy of Boron Neutron Capture Therapy Mediated by Boron-Rich Liposomes for Oral Cancer in the Hamster Cheek Pouch Model. *Proceedings of the National Academy of Sciences* **2014**, *111* (45), 16077–16081.
(31) Batlle, E.; Clevers, H. Cancer Stem Cells Revisited. *Nature Medicine* **2017**, *23* (10), 1124–1134.
(32) Lesueur, P.; Chevalier, F.; El-Habr, E. A.; Junier, M.-P.; Chneiweiss, H.; Castera, L.; Müller, E.; Stefan, D.; Saintigny, Y. Radiosensitization Effect of Talazoparib, a Parp Inhibitor, on Glioblastoma Stem Cells Exposed to Low and High Linear Energy Transfer Radiation. *Scientific Reports* **2018**, *8* (1), 3664.

(33) Alberti, D.; Deagostino, A.; Toppino, A.; Protti, N.; Bortolussi, S.; Altieri, S.; Aime, S.; Geninatti Crich, S. An Innovative Therapeutic Approach for Malignant Mesothelioma Treatment Based on the Use of Gd/Boron Multimodal Probes for MRI Guided BNCT. *Journal of Controlled Release* **2018**, *280*, 31–38.
(34) Kreiner, A. J.; Bergueiro, J.; Cartelli, D.; Baldo, M.; Castell, W.; Asoia, J. G.; Padulo, J.; Suárez Sandín, J. C.; Igarzabal, M.; Erhardt, J.; et al. Present Status of Accelerator-Based BNCT. *Reports of Practical Oncology & Radiotherapy* **2016**, *21* (2), 95–101.