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The acceleration of boron neutron capture therapy using multi-linked mercaptoundecahydrododecaborate (BSH) fused cell-penetrating peptide

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ABSTRACT

New anti-cancer therapy with boron neutron capture therapy (BNCT) is based on the nuclear reaction of boron-10 with neutron irradiation. The median survival of BNCT patients with glioblastoma was almost twice as long as those receiving standard therapy in a Japanese BNCT clinical trial. In this clinical trial, two boron compounds, BPA (boronophenylalanine) and BSH (sodium borocaptate), were used for BNCT. BPA is taken up into cells through amino acid transporters that are expressed highly in almost all malignant cells, but BSH cannot pass through the cell membrane and remains outside the cell. We simulated the energy transfer against the nucleus at different locations of boron from outside the cell to the nuclear region with neutron irradiation and concluded that there was a marked difference between inside and outside the cell in boron localization. To overcome this disadvantage of BSH in BNCT, we used a cell-penetrating peptide system for transduction of BSH. CPP (cell-membrane penetrating peptide) is very common peptide domains that transduce many physiologically active substances into cells *in vitro* and *in vivo*. BSH-fused CPPs can penetrate the cell membrane and localize inside a cell. To increase the boron ratio in one BSH-peptide molecule, 8BSH fused to 11R with a dendritic lysine structure was synthesized and administrated to malignant glioma cells and a brain tumor mouse model. 8BSH-11R localized at the cell nucleus and showed a very high boron value in ICP results. With neutron irradiation, the 8BSH-11R administrated group showed a significant cancer killing effect compared to the 100 times higher concentration of BSH-administrated group. We concluded that BSH-fused CPPs were one of the most improved and potential boron compounds in the next-stage BNCT trial and 8BSH-11R may be applied in the clinical setting.

1. Introduction

For decades, malignant glioma, especially glioblastoma (GBM) was not curable, but in 2004, a randomized phase III trial by the European Organization for the Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada Clinical Trials Group (NCIC) reported improved median and 2-year survival of patients with glioblastoma treated with concomitant and adjuvant temozolomide (TMZ) and radiotherapy [1]. The median survival was 14.6 months with radiotherapy plus TMZ and 12.1 months with radiotherapy alone. Furthermore, recently, the Japanese boron neutron capture therapy (BNCT) group against malignant brain tumor reported that the median survival of newly-diagnosed glioblastoma patients with BNCT was 23.5 months [2]. This BNCT outcome is excellent and hopeful for malignant glioma patients.

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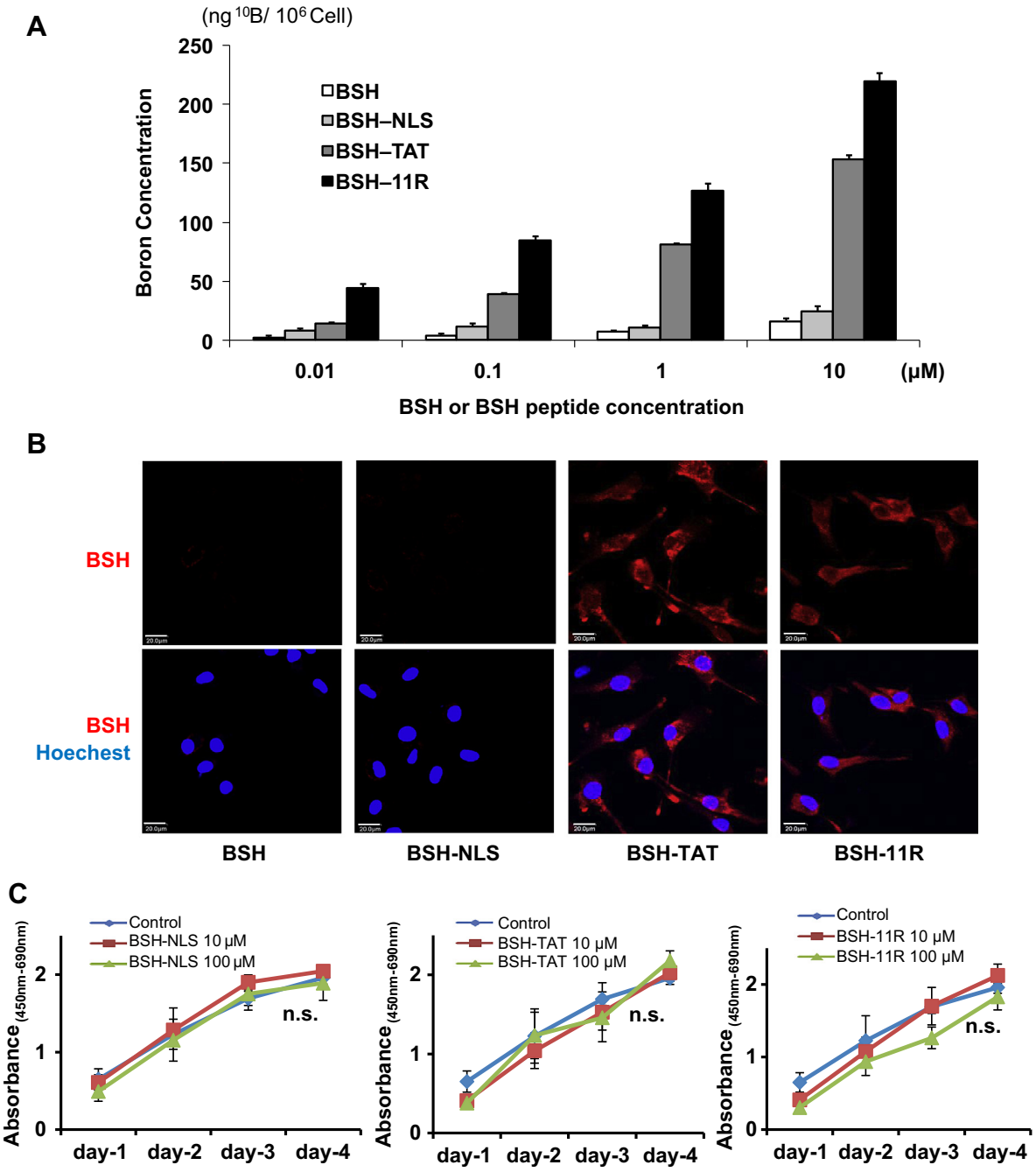

Fig. 1. Administration of BSH-peptide into U87 delta EGFR cell and evaluation of intra-cellular function 1-A: Boron concentration of U87 delta EGFR cells administrated different kinds of BSH and BSH-peptide at 0.01, 0.1, 1 and 10 μM for 12 h. 1-B: Immunocytochemistry showing BSH (red) and nucleus (blue) with identified BSH antbody and Hoechst 33342 by confocal microscopy. Scale bar = 20 μm 1-C: Three graphs showing cell proliferation after administration of each BSH or BSH-peptide at 10 or 100 μM for 4 days by the measurement of absorbance (450 nm-690 nm) with WST-1 reagent. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

BNCT is based on the nuclear capture and fission reactions that occur when boron-10, a non-radioactive isotope and a constituent of natural elemental boron, is irradiated with low energy (<0.025 eV) thermal neutrons (*n*th) to produce high energy (2.3 MeV) alpha (α) particles and recoiling lithium-7 nuclei ($^{10}B + nth \rightarrow [^{11}B] \rightarrow {}^4He\,(\alpha) + {}^7Li + 2.38$ MeV) [3,4]. BNCT has been used to treat patients with high-grade primary brain tumors and a much smaller number of patients with other types of brain tumors [2,5,6]. For BNCT to be successful, there must be high uptake of ^{10}B by the tumor and low levels in the normal brain, and sufficient fluence of thermal neutrons must be delivered to the tumor. In their BNCT, two low molecular weight ^{10}B-containing drugs, boronophenylalanine (BPA) and/or sodium borocaptate (BSH), were used as capture agents [7]. Two boron compounds have been used clinically: sodium undecahydro-mercaptocloso-dodecaborate ($Na_2B_{12}H_{11}SH$, also referred to as sodium borocaptate or 'BSH') in Japan and Europe and 4-dihydroxyborylphenylalanine (boronophenylalanine or 'BPA') in the United States [4,8]. BPA can enter and accumulate in malignant cells, but BSH shows leakage from the tumor area and cannot transduce into cells [4]. The nuclear reaction caused by BNCT outside cells is mildly effective against malignant cells, but a precise simulation dependent on the intracellular