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Proteomic analysis of cellular response induced by boron neutron capture reaction in human squamous cell carcinoma SAS cells

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HIGHLIGHTS

- BNCR in human squamous carcinoma cells caused typical apoptotic features.
- BNCR induced fragments of LRMP, in human squamous carcinoma and rat tumor model.
- The fragmentation of LRMP could be involved in cellular response to BNCR.

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ABSTRACT

To understand the mechanism of cell death induced by boron neutron capture reaction (BNCR), we performed proteome analyses of human squamous tumor SAS cells after BNCR. Cells were irradiated with thermal neutron beam at KUR after incubation under boronophenylalanine (BPA)(+) and BPA(−) conditions. BNCR mainly induced typical apoptosis in SAS cells 24 h post-irradiation. Proteomic analysis in SAS cells suggested that proteins functioning in endoplasmic reticulum, DNA repair, and RNA processing showed dynamic changes at early phase after BNCR and could be involved in the regulation of cellular response to BNCR. We found that the BNCR induces fragments of endoplasmic reticulum-localized lymphoid-restricted protein (LRMP). The fragmentation of LRMP was also observed in the rat tumor graft model 20 hours after BNCT treatment carried out at the National Nuclear Center of the Republic of Kazakhstan. These data suggest that dynamic changes of LRMP could be involved during cellular response to BNCR.

1. Introduction

Boron neutron capture therapy (BNCT) is based on the preferential uptake of ^{10}B containing compounds, such as ^{10}B-boronophenylalanine (BPA) into tumor cells, coupled with subsequent irradiation with thermal neutrons to yield high linear

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energy-transfer (LET) alpha-particles and ^{7}Li nuclei, resulting in the killing of tumor cells (Barth et al., 2005; Wittig et al., 2000). Thus, tumor cells that incorporated ^{10}B are destined to die in this process of therapy.

For supporting development of clinical application of BNCT, identification of good early and late stage markers for evaluating the tumor cellular responses to BNCT are important (Imahori et al., 1998a, 1998b; Kato et al., 2009). Micronuclei formation has been used to estimate the amount of DNA damage induced by boron neutron capture reaction (BNCR) (Dagrosa et al., 2011; Kinashi et al., 2007; Ono et al., 1996). As indicated for various types of radiation, γH2AX level/foci and 53BP1 foci formation, two DNA double strand break markers, are shown to be useful as biomarkers for DNA damage in BNCT (Kinashi et al., 2011). We have been studying the comprehensive analysis of mechanisms of tumor cell death induced by BNCT. Previously, we reported the histological and biochemical features of DNA damage after BNCR in a rat tumor model (Masutani et al., 2014). In this model, BNCR caused the up-regulation of γH2AX and HMGB1, then induction of poly(ADP-ribose). These changes might be useful as biomarkers for monitoring the DNA and cellular damage induced by BNCR. Here, we investigated the molecular mechanisms involved in the BNCR of human squamous cell carcinoma SAS cells by using comprehensive proteomic analysis.

2. Materials and methods

2.1. Reagents, and cell culture

A human oral squamous carcinoma cell line, SAS was cultured as described previously (Masunaga et al., 2002). Briefly, SAS cells were maintained in Dulbecco's Modified Eagle's Medium (Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum containing penicillin–streptomycin. The cells were cultured in a humidified atmosphere with 5% CO_2 at 37 °C. The cells were passaged every 3 days in numbers appropriate to ensure logarithmic growth.

2.2. Boron neutron capture reaction

^{10}B-Boronophenylalanine (^{10}B-BPA)-fructose solution was prepared as described previously (Masutani et al., 2014; Yoshino et al., 1989). Before neutron beam irradiation, SAS cells were suspended using trypsin and dispensed aliquots of cells into 2 ml tube, and 2 h incubation with or without 25 ppm of ^{10}B-BPA as previously reported (Kinashi et al., 2011). This dose was chosen based on previous reports, where the maximum blood concentration in animal models reaches around 10–30 ppm after subcutaneous administration of 250–750 mg/kg bodyweight ^{10}B-BPA (Masunaga et al., 2002). This dose range is reported to generate satisfactory levels of BNCR using cancer cell lines (Diaz, 2003; Kinashi et al., 2011; Morris et al., 2002).

Cell samples in 2 ml polypropylene vials were irradiated with thermal neutron beam (neutron flux: 1×10^{9} cm^{-2} s^{-1}, fluence: 6×10^{11} cm^{-2}) at the Heavy Water Neutron Irradiation Facility of Kyoto University Reactor (KUR) operated at 1 MW. The prompt gamma ray analysis (PGA) using a thermal neutron guide tube installed at KUR was used to measure ^{10}B concentration. Thermal neutron fluence was measured with gold foil activation analysis and the γ-ray dose including secondary γ-ray was measured with thermoluminescence dosimeter (TLD) as previously described (Kinashi et al., 2011). The TLD used was beryllium oxide (BeO) enclosed in a quartz glass capsule (Panasonic Corporation, Osaka, Japan) (Sakurai and Kobayashi, 2000). Total absorbed dose was calculated 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 (Kobayashi et al., 2000). For colony formation assay, the total dose of control (−BPA) cells was 64 cGy after 10 min irradiation and the dose of BPA-treated cells was 184 cGy. As dose components, thermal neutron was 8.7 cGy, epithermal neutron was 0.92 cGy, fast neutron was 6.2 cGy, gamma-ray dose was 48 cGy and ^{10}B dose rate was 4.8 cGy/ppm. After irradiation, 0.6 ml of cells was immediately transferred into 3 ml of culture medium in a 60 mm dish. For immunostaining, western blot and proteome analysis, 60 min irradiation was carried out to give the total dose of 17.5 Gy for BPA-treated cells and the total dose of 4.0 Gy for control (−BPA). As dose components, thermal neutron dose was 97 cGy, epithermal neutron dose was 10 cGy, fast neutron dose was 72 cGy, gamma-ray dose was 2.2 Gy, and 1 ppm of ^{10}B dose was 54 cGy. After incubation for 6 or 24 h, conditioned medium and cells were separately harvested, and samples were kept at −80 °C.

The experiments of rat BNCT model were carried out at the National Nuclear Center of the Republic of Kazakhstan (Masutani et al., 2014). In the rat tumor model, Sarcoma-45 cells were injected subcutaneously into left groin of two Wistar rats as previously reported (Potapnev et al., 2004). Two weeks after subcutaneous injection, tumors reached diameter of 3–4 cm and were forty-five min after injection of 330 mg/kg bodyweight ^{10}B-BPA (+BPA) or saline (−BPA) from tail vein. The neutron beam irradiation was carried out (neutron flux: 1×10^{9} nvt/s, fluence: 6×10^{11} nvt) with the presence of background γ-irradiation (33 Gy/10 min) as described previously (Masutani et al., 2014). The BPA dosage of 330 mg/kg bodyweight was chosen based on the previous reports that achieved effective BNCT (Diaz, 2003; Kinashi et al., 2011; Morris et al., 2002). The neutron flux, fluence data was provided by the facility as estimated values and during the experiments, the measurement of neutron flux, and fluence was not performed.

The animal studies were performed according to the Minister of Health of the Republic of Kazakhstan dated November 19, 2009, No.745 “On approval of preclinical (non-clinical) studies of biologically active substances”, and the national standard of the republic of Kazakhstan for Good Laboratory Practice. ST RK 1613-2006).

2.3. γ-ray irradiation

Cells were γ-irradiated using a Cs-137 Gammacell® 40 Exactor at the National Cancer Center Research Institute in Japan at a dose rate of 1 Gy/min.

2.4. Protein identification

The protein identification, namely, two dimensional gel electrophoresis, image analysis, in gel tryptic digestion, matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/MS) and high performance liquid chromatography (HPLC)-chip-MS analyses were performed as described previously (Sato et al., 2010). Briefly, the tumor cell protein samples (40 μg/gel) were separated by two dimensional gel electrophoresis. The gel images were analyzed using Quantity One software (Bio-Rad) and the protein spots to be identified were excised from the gels. The protein was digested with trypsin and identified using Autoflex-III (Bruker Daltonics) and HPLC-Chip-MS (Agilent Technologies). To search for human proteins, that matched to the peptide fragments, the NCBInr database and the Swiss-Prot database were used.