Evaluation of radiotherapy using Manganese-enhanced MRI (MEMRI)

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INTRODUCTION

Radiotherapy using high-energy X-rays or ionized-particles has been applied to treat malignant tumor with the intention of killing or inactivating cells while preserving normal tissue integrity [1]. The intracellular contrast agent $MnCl_2$ can be used to assess cell viability in heart ischemia [2]. In addition, Aoki et al. reported that Mn^{2+} can enter non-phagocytotic/non-depolarizing cells [3]. The aim of our research is to investigate Mn uptake in X-ray irradiated tumor cells both in-vitro and in-vivo. We demonstrate that manganese-enhanced MRI (MEMRI) can detect radiation-induced alterations to cells in a colon-26 cell line and to tissue in a tumor-baring mouse model.

MATERIALS AND METHODS

Animal model and X-ray irradiation: **In vitro study:** Colon-26 cells were exposed to a single X-ray irradiation at a dose of 20 Gy one day before MRI measurement. The X-ray irradiation conditions were 200kVp, 20mA, 0.5 mm Cu + 0.5 mm Al filter, 500mm distance from focus to object and 1.3 Gy/minute dose rate. **In vivo study:** Male Balb/c nude mice (n = 8, 20.5 ± 0.4 g) were divided into two groups (MRI experiment: n=3, Tumor growth: n=8). Colon-26 cancer cells (1×10^7 cells) were subcutaneously transplanted in both upper hind legs of the mice under 2% isoflurane anesthesia. The tumors were allowed to grow for 7 days before MRI measurements. One side of the tumor was exposed to X-rays with the same parameters as for the in vitro study.

In vitro MEMRI: The Colon-26 cells with or without X-ray irradiation were incubated in a medium containing $MnCl_2$ (0.1mM) for 30 min at 37 °C under 5% CO_2. After incubation, the medium was carefully removed by washing with phosphate-buffered saline (PBS). The cells were harvested, transferred to a ninety-six well PCR tube, and pelleted by gravity. The MRI acquisitions were performed with a 7.0-T MRI (Magnet: Kobelco and Jastec, Japan; Console: Bruker Biospin, Germany). A 30 mm inner-diameter mouse volume coil (Rapid Biomedical, Germany) was used to measure the cell samples. T_1-weighted MR images (T_1WIs) were acquired with a conventional spin echo (SE) sequence (TR = 400 ms, echo time, TE = 9.57 ms, matrix size = 256 × 256, field of view (FOV) = 51.2 × 51.2 mm^2, slice thickness = 1.0 mm, fat suppression on, and number of acquisitions (NA) = 8). A RARE sequence with inversion-recovery was used to estimate T_1 (TR = 10,000 ms, TE = 10 ms, inversion time = 51, 100, 200, 400, 800, 1600, 3200, 6400 ms, matrix size = 128 × 128, FOV = 51.2 × 51.2 mm^2, RARE factor = 4). Quantitative T_1 maps were calculated with a non-linear least-squares fitting to inversion-recovery MRI data. Regions-of-interest (ROI) were defined in the precipitated cell region. All calculations and analysis were performed using the MRVision image analysis software (Version 1.5.8, MRVision Co.).

Flow cytometry: Cell viability was measured using Guava Viacount reagent. The Colon-26 cells with or without X-ray irradiation were set in six-well plates. $MnCl_2$ (50 mM) was added to the medium. After thirty minutes of incubation, cells were washed with PBS and trypsinized. A 20µl aliquot of the cell suspension was then incubated with 180 µl of Viacount reagent for 5 min at room temperature. Cells were then analyzed on a Guava PCA machine using the Viacount Acquisition Module (Guava Technologies, USA).

In vivo MEMRI Animal models: T_1WI acquisitions were performed under 1.5-2.0 % isoflurane anesthesia in the following order: 1) pre-administration (control), 2) Gd-enhanced, and 3) Mn-enhanced experiments. After Gd-DTPA administration (gadopentetate dimeglumine, 50 mM, 150 µmol/kg, Bayer Japan, Japan) and before the MEMRI scans, tumor vasculature was evaluated with T_1WI acquisitions repeated 2 times every 16 minutes. After starting Mn^{2+} infusion (50 mM, osmotic pressure-controlled, 380 µmol/kg, 4.0 ml/hour) through the tail vein, T_1WI acquisitions were repeated 4 times every 32 minutes. 2D single-slice transversal T_1WI was obtained using a SE sequence with the following parameters: TR = 400 ms, TE = 9.57 ms, matrix size = 256 × 256, FOV = 40.0 × 40.0 mm^2, Slice thickness = 1.0 mm, fat suppression on, and NA = 4.

Data analysis: The "manganese uptake ratio (MUR)" was defined with the following method; 1) a ROI (ROI_Out) was outlined by hand on the T_1WI, 2) the Gd-enhanced area (ROI_Gd) was defined by the parts of ROI_Out over a threshold (+2 s.d. of the mean signal intensity over ROI_Out in the control image), 3) the Mn-enhanced area (ROI_Mn) was defined as ROI_Out - ROI_Gd, 4) ROI_Muscle was selected from muscle near the tumor, and 5) MUR was calculated with MUR = ROI_Mn signal intensity / ROI_Muscle signal intensity.

RESULTS and DISCUSSION

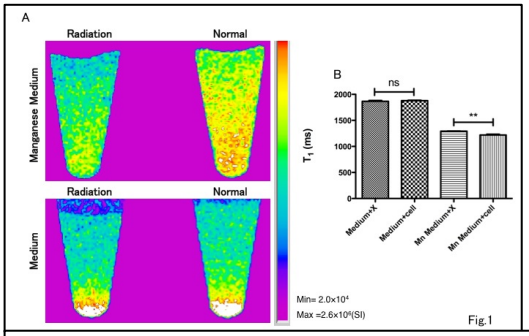

Fig.1

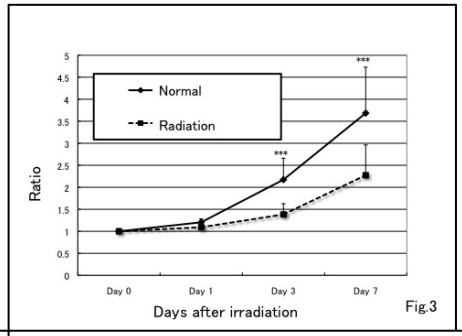

Fig.3

Fig. 1A shows T_1WIs of the colon-26 cell pellets with (left column) or without (right column) X-ray irradiation incubated with (upper row) or without (bottom row) 0.1 mM $MnCl_2$. We found that signal from normal colon-26 cells was enhanced by $MnCl_2$ in comparison with x-ray-exposed colon-26 cells (upper row: normal).

Fig. 1B presents T_1 for the cells. T_1 for the radiation-exposed colon-26 cells with $MnCl_2$ incubation was higher than for the normal cells. **Fig. 2** shows the results of flow-cytometry analysis. ~~A~~poptotic cells were detected only in the radiation-exposed samples (right graph: blue arrow).

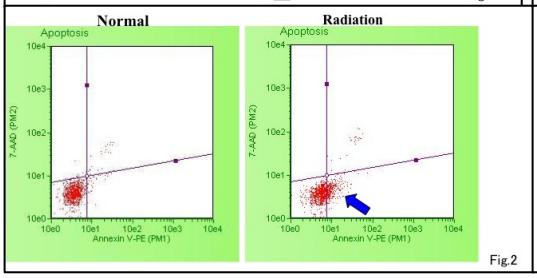

Fig.2

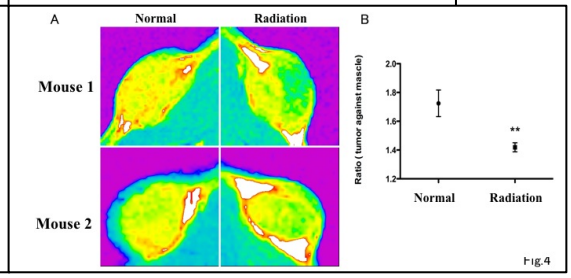

Fig.4

Fig. 3 shows the change in tumor volume with and without radiation treatment. Tumor growth was significantly lower for the radiation-exposed group after 3 and 7 days ($P < 0.001$). **Fig. 4A** shows T_1WIs of a tumor site with (right column) or without (left column) x-ray exposure. The signal intensity of the tumor decreased in radiation-exposed tumor one day after irradiation. **Fig. 4B** shows MUR in the tumors with or without x-ray irradiation. The MUR decreased significantly in radiation-exposed tumor 24 hours after irradiation.

CONCLUSION

We demonstrated that MEMRI signal enhancement was reduced after x-ray irradiation for both in-vitro and in-vivo models. MEMRI may be used to evaluate the cellular viability of tumor after radiotherapy.

REFERENCE [1] Wim Ceelen. Int. J. Radiation Oncology Biol. Phys; 2006 [2] Tom C.- C. Hu. MRM; 2005 [3] Ichio Aoki. NMR in biomedicine; 2006.

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