In vivo MR imaging of human glioblastoma cell derived tumor by using anti MT1-MMP antibody conjugated ultrasmall superparamagnetic iron oxides (USPIOs)

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Introduction

Glioblastoma is the most malignant brain tumor in humans. Glioma cells are highly proliferative and are very invasive within the CNS. The median survival of patients with malignant glioma is less than 1 year [1]. MT1-MMP is highly expressed in different cancers, and overexpression promotes migration, invasion and metastasis of cancer cells in vitro as well as in vivo [2]. In this study, we aimed to obtain the *in vivo* MR imaging of human glioblastoma cell U87-MG derived tumor by using anti MT1-MMP antibody conjugated ultrasmall superparamagnetic iron oxides (MT1-MMP-USPIOs).

Material and Methods

Identification of MT1-MMP in Cells ; Immunofluorescence staining were tested for staining of MT1-MMP.

Nude mouse tumor model ;Xenotransplanted nude mice of the BALB/c Slc-nu strain (Japan SLC., Inc.) were used as tumor model (male, 20-25 g body weight). After anesthesia, the human colon cancer cell line HT-29 cells (1×10^6 cells, s.c.) and the human glioblastoma cell line U87-MG cells (2×10^6 cells, s.c.) were transplanted in the left and right lower flanks of the mouse respectively. 4 weeks after transplantation, nude mice with 5~7 mm tumor diameter were used in the experiment. After experiment, tumor masses were removed for Prussian blue staining.

Conjugation of MT1-MMP-USPIOs ;Monoclonal anti-human MT1-MMP antibody was dialyzed using biotin-labeling buffer. N-hydroxysuccinimide ester biotin (10 µl of 10 mg/ml in DMSO) was added to antibody, and then incubated 1hr 30min at room temperature. Biotinylated anti-MT1-MMP antibodies (25 µg/50 µl) were covalently coupled to lyophilized USPIO (1 ml of lyophilized USPIO agent powder/30 µl of PBS) particles labeled with a streptavidin. The USPIO agent consist of an iron crystal (~10 nm diameter, 55-59 % iron oxide, w/w) coated with polysaccharide resulting in a ~50 nm diameter particle.

MRI ;The MR examination were performed with 3 Tesla (T) MR unit with 8 channel phased array brain coil. MRI was performed using susceptibility weighted imaging (SWI) technique (TR = 50 ms, TE = 11 ms, number of excitations [nex] = 4, matrix = 384×160, FOV = 100 mm). After anesthesia, the animals were examined 0 hr, 1 hr, 3 hr, 5 hr, 7 hr, 9 hr, 15 hr, 18 hr, and 24 hr after intravenous administration of the MT1-MMP-USPIOs into the tail vein.

Results and Discussion

The result of immunofluorescence shows that MT1-MMP was strongly stained in U87-MG cells but not in HT-29 cells [Fig. 1]. Since the intracellular region of cells may be the trans-Golgi network, our data seem to suggest that MT1-MMP is activated or maturated prior to presentation of the plasma membrane. After 7 hr intravenous injection of MT1-MMP-USPIOs, the dark signal was visualized at the margin of established U87-MG derived tumor mass. On the other hand, no detectable MR signal change was shown within the established HT-29 derived tumor mass [Fig. 2]. These results therefore suggest that a combination of the USPIO properties with the specificity of MT1-MMP-mediated endocytosis allows in vivo detection of glioblastoma cells by MR imaging. Our study used MT1-MMP as molecular marker for active targeting of glioblastoma cells. Prussian blue staining in matched coronal sections revealed iron staining with blue colors at the margin within the U87-MG derived tumor mass. However, HT-29 cells did not show any Prussian blue-positive cells due to low level of MT1-MMP expression as well as much lower internalization of the particles [Fig. 3].

In this study, we demonstrated that MRI can monitor grafted human glioblastoma cell derived tumor mass by using conjugation of biotinylated anti MT1-MMP antibody and streptavidinylated USPIOs (MT1-MMP-USPIOs) system. The MR molecular imaging of glioblastoma (U87-MG) cells is therefore feasible by using a targeted superparamagnetic iron oxide nanoparticle and MT1-MMP is a useful molecular marker of tumor.

References

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[2] Y. Itoh, M. Seiki. TRENDS in Biochemical Sciences 2004; 29(6):285-289.

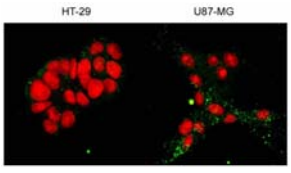

Fig 1. Spatial expression patterns of MT1-MMP in U87-MG cells

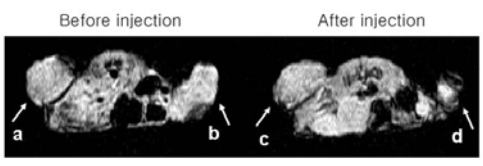

Fig. 2. Axial MR images of a nude mouse with subcutaneous HT-29 (a and c) and U87-MG (b and d) tumor before and after 7 hr administration of MT1-MMP-USPIOs.

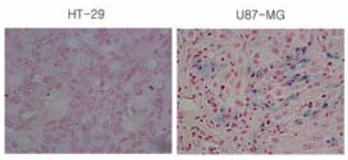

Fig. 3. Photomicrographs of Prussian blue staining of HT-29 (left) and U87-MG (right) cells related tumors (×200).