MR molecular imaging of HER-2 in a murine tumor xenograft by SPIO labeling of anti-HER-2 Affibody.

M. Kinoshita[1], Y. Yoshioka[2], Y. Okita[1], N. Hashimoto[1], and T. Yoshimine[1]

[1]Department of Neurosurgery, Osaka University Medical School, Suita, Osaka, Japan, [2]High Performance Bioimaging Research Facility, Osaka University, Graduate School of Frontier Biosciences, Suita, Osaka, Japan

Abstract

In vivo molecular imaging is a rapidly growing research area both for basic and clinical science. Non-invasive imaging of *in vivo* conditions in a molecular level will help understand the biological characteristics of normal and diseased tissues without performing surgical invasive procedures. Among various imaging modalities, magnetic resonance imaging (MRI) has gained interest as a molecular imaging modality for its high special resolution. In this research, we have demonstrated that the combined use of HER-2 targeting Affibody, a small 7kDa molecule that behaves similarly to antibodies, and superparamagnetic iron oxide (SPIO) can non-invasively image HER-2 expressing cells or tissues both *in vitro* and *in vivo* by MRI. This preliminary study demonstrates that Affibody-SPIO is a feasible target specific contrast agent for *in vivo* MR-molecular imaging.

Methods

In vitro HER-2 imaging by MRI using anti-HER-2 Affibody: The concept of HER-2 detection in MRI is illustrated in **Fig. 1A**. HER-2 will be recognized by Biotinated-anti-ErbB2 (HER-2) Affibody, followed by SPIO labeling using biotin-streptavidin linkage. The SPIO tagged HER-2/Affibody complex will be detected using MRI. After both C6 and SKOV-3 cells were harvest, they were suspended in 1ml phosphate buffer saline (PBS) at a final concentration of $1x10^6$cells/ml. Biotinated-anti-ErbB2 (HER-2) Affibody was reacted to the cells at a final concentration of 10mg/ml for 30 minutes at 4 °C. The samples were subsequently washed with PBS and then suspended in 100ml PBS with 10ml of Streptavidin SPIO MicroBeads for 15 minutes at 4 °C. The estimated Fe concentration during SPIO labeling was 0.4mmol/Fe/ml. Finally the cells were washed with PBS and collected at the bottom of a 1.5ml tube in a pellet for MR imaging **(Fig. 1B)**. The samples were imaged in an 11.7 tesla Bruker vertical bore MRI scanner (Bruker BioSpin, Ettlingen, Germany). The images were aquired by using a fast spin echo sequence with TE = 27.7msec and TR = 4000msec.

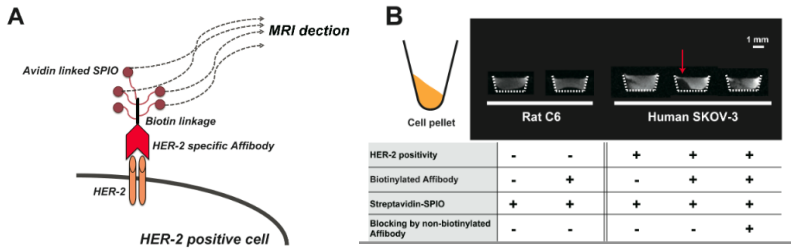

Fig. 1. ***A*****,** A schematic drawing of the concept of Affibody-SPIO based MR molecular imaging. HER-2 will be labeled with SPIO by HER-2 specific Affibody using biotin-streptavidin linkage. ***B*****,** *In vitro* experiment showing the feasibility of HER-2 imaging by Affibody-SPIO MR molecular imaging. Cell pellet can be identified on MR images using HER-2 targeting Affibody and SPIO only when the cells are expressing HER-2 (red arrow), which can be blocked by non-Biotinylated Affibody.

SKOV-3 murine tumor xenograft model: $1x10^7$ SKOV-3 cells suspended in PBS were injected subcutaneously into the left flank of 5- to 7-week-old female nude mouse (BALB/c nu/nu). It took approximately 4 weeks for the injected cells to form a tumor with a diameter of 1 to 1.5cm.

In vivo HER-2 MR imaging using anti-HER-2 Affibody: For *in vivo* HER-2 MR imaging, the above-mentioned animal model was first intravenously injected with 28.5mg Biotinated-anti-ErbB2 (HER-2) Affibody via orbital venous plexus. Pre-contrast MR images were acquired 2 to 3 hours post Affibody injection, followed by 100ml (0.4mmol/Fe) Streptavidin SPIO MicroBeads injection again via orbital venous plexus. Subsequently, post-contrast MR images were acquired 3 to 4 times **(Fig. 2B)**. Both T2-wighted images and gradient echo images were obtained around the tumor for obtaining anatomical information with the distribution of SPIO accumulation. Regions of interest (ROIs) were placed on both contrast enhanced area and the paravertebral muscle (normal tissue). The tumor to normal tissue ratio (T/Nr) of the absolute MR intensities on gradient-echo images were calculated **(Fig. 2C)**. For those animals without Affibody injection, a randomly selected tumor tissue was placed as the target ROI.

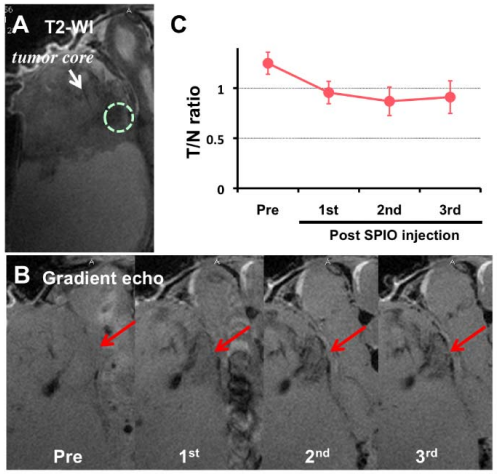

Fig. 2 ***A*****,** T2-weighted images were obtained for achieving anatomical information. Mice were inoculated with SKOV-3 HER-2 positive cells at their right flank. RF coil was position at the center of the tumor. ***B*****,** Gradient echo images were scanned before (left) and after (right 3 images) SPIO injection, which was done 4 hours after Affbody administration. Repeated image acquisition was performed up to 4 times after SPIO injection. SPIO accumulation can be appreciated (red arrows). ***C*****,** Tumor to normal tissue ratio (T/Nr) was calculated at the target ROI indicated as green circle and reference ROI set at the paravertebral muscle. Decrease in T/Nr is observed.

Results and Discussion

MRI has a great advantage over other modalities for its high special resolution and its freedom of choosing different image sequences for different purposes, such as diffusion images for cerebral infarction and diffusion tensor imaging for tracking white matter fibers in the brain. In this study, we have pursued the possibility of using MRI for imaging cell surface receptors using a recently developed molecule named "Affibody". Affibody is a molecule that behaves similarly to antibodies with a much smaller molecular weight of 7kDa. We fist show in **Fig. 1B** that HER-2 molecular imaging is possible with the combination of MRI, Affibody and an MRI contrast agent superparamagnetic iron oxide (SPIO). HER-2 targeting Affibody is tagged by SPIO by biotin-streptavidin linkage **(Fig. 1A)**. It is clearly shown in an *in vitro* model that HER-2 positive cell pellets were detectable on MRI with the combination of HER-2 targeting Affibody and SPIO. We next evaluated this technique in an *in vivo* model. HER-2 targeting biotinated Affibody and streptavidin SPIO were injected into a HER-2 positive SKOV-3 xenograft mouse, and we clearly show in **Fig. 2B** that the tumor is marked with SPIO when imaged by a gradient-echo sequence. The solid component of the tumor showed a heterogenous low intensity signal indicating that the SPIOs have accumulated at the lesion. This was also supported by the fact that histological examination revealed iron accumulation within the tumor, especially around the tumor vasculature **(Fig. 3)**. In previous reports, antibodies were used for similar purposes. There are several advantages of using Affibody compared to antibodies. One is its small size. This has the potential for the agent to penetrate into tissues supplied by vascular networks comprised of tighter endothelial cell junctions, such as in the brain. Another advantage is its faster clearance from the system compared to antibodies. Pharmacokinetic study showed that Affibody has a faster clearance compared to antibodies, a feature that could be considered beneficial for diagnostic imaging agents, as consecutive multiple imaging using different Affibodies targeting different molecules could be possible. Considering these features and our presented data, we propose that Affibody based MR molecular imaging could be an appealing alternative for PET for molecular imaging *in vivo.*

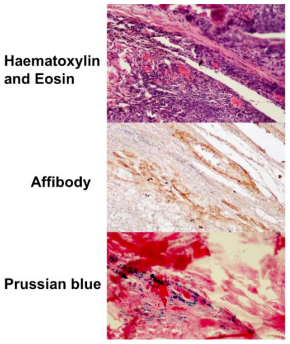

Fig. 3 Histological assessment of the Affibody-SPIO labed *in vivo* tumor. Affibody localization is mainly detected around the tumor microvesseles. SPIO accumulation is also detected corresponding to the Affibody localization.