Targeted MR Imaging of CD44-positive Breast Cancer Stem-Like Cell Phenotype

D. Artemov[1], W. Zhu[1], Y. Kato[1], M-F. Penet[1], F. Vesuna[1], Z. Bhujwalla[1], and V. Raman[1]
[1]Radiology, Johns Hopkins University, Baltimore, MD, United States

Introduction: Recent studies have identified stem-like populations in tumors that contribute significantly to the propagation and dissemination of pathogenic metastatic cells. These cells were initially identified in acute myelogenous leukemia (1), and have subsequently been detected in several tumors including breast cancer. These highly tumorigenic cells, referred here as breast cancer stem-like cells (BCSLC) are characterized by their ability to form tumors with low inoculums (2), resistance to chemo and radiation-therapy (3), and expression of the breast cancer resistance proteins (MDR1, BCRP/ABCG2) (4, 5). These cells are also involved in breast cancer invasion and metastasis (6). One of the phenotypic characters of BSCLCs is that they exhibit high CD44 expression along with low CD24 levels on the cell membrane (7). In this study, we have developed gadolinium- and SPIO-based targeted contrast agents for specific MR imaging of CD44 expressing BCSLC and tested them for *in vitro* imaging of isolated breast cancer cells. Significant MR contrast was observed in MDA-MB-231 human CD44-positive breast cancer cells.

Materials and Methods: Human breast cancer cell lines MCF-7 and MDA-MB-231 were used in the studies. Cells were characterized for expression of CD44 and CD24 membrane markers using FACS analysis with fluorescently-labeled anti-CD24 and anti-CD44 monoclonal antibodies obtained from AbCam and BD Biosciences. Fluorescent microscopy was performed with cells grown in transwell chambers, labeled with fluorescent mAbs at 4^0C, fixed and mounted on the slides. For MR imaging we used anti-Human CD44 monoclonal antibody (Cambridge, MA, clone A3D8). The antibody was biotinylated with NHS-LC-biotin reagent from Pierce according to the manufacture's protocol. The biotinylation ratio was estimated as 5-7 biotins per antibody by HABA assay (Pierce, Rockford, lL). AvidinGdDTPA (8) and Streptavidin-SPIO (Miltenyi Biotech, Auburn, CA) were used as T1 and T2 targeted contrast agents respectively. All MR imaging experiments were performed with MDA-MB-231 and MCF-7 cells incubated with the biotinylated-mAbs (20µg/ml for 30 min at 4^0C) washed and probed with avidin-GdDTPA (5mg/ml for 30min at 4^0C) and streptavidin-SPIO microbeads according to Myltenyi's protocol. Control samples were treated with contrast agents only without the antibody prelabeling step. MR imaging of cell pellets was performed on a Bruker 9.4T horizontal bore scanner using T1 and T2 weighted fast spin echo MRI sequences. Quantitative T1 maps were reconstructed from T1 weighted images acquired with recovery delays in the range of 250ms to 8s.

Results: FACS data presented in Fig. 1A demonstrate that MCF-7 and MDA-MB-231 have significantly different expression patterns of CD44 and CD24 antigens (6) and more than 90% of MDA-MB-231 cells are CD44 positive. Fluorescent microscopy of MDA-MB-231 cells with fluorescent anti-CD44 mAb confirmed cell surface localization of the antigen: Fig. 1B. Superparamagnetic nanoparticles (SPIO) produced strong negative T2 contrast in the images (Fig.1C) and can be used as highly sensitive platform for in vivo MR imaging of CD44 receptors in the tumor. On the other hand, the positive T1 contrast agent, avidin-GdDTPA, provided a significantly lower contrast enhancement of about 8% due to the lower intrinsic relaxivity in comparison to SPIO nanoparticles (Fig. 1D). However a significant advantage of this type of CA is a relatively low molecular weight of ~100kDa that can provide more efficient delivery and extravasation of the agent in solid tumors. No significant MR signal enhancement was detected in MCF-7 cells for both labeling protocols.

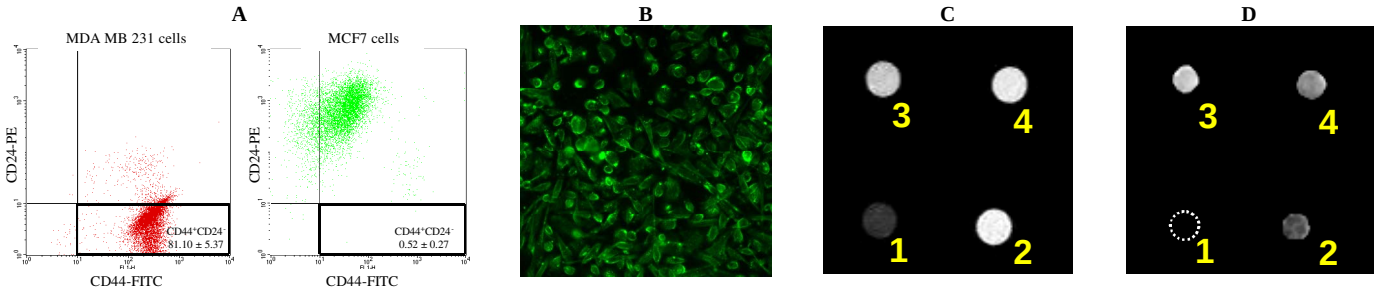

Figure 1. **A.** FACS analysis of CD44 and CD24 expression in MCF-7 and MDA-MB-231 breast cancer cells. **B.** Fluorescent microscopy of MDA-MB-231 cells with anti-CD44 mAb. **C, D**: MR images of MDA-MB-231 cell pellets labeled with CD44 specific contrast agents acquired on a 9.4T MR scanner. **C**. T2 weighted image acquired with TE/TR = 5/1000ms, **D**. Quantitative T1 map reconstructed from T1-weighted images. Cell pellets labeled with: 1-biotin-mAb & Streptavidin-SPIO; 2- biotin-mAb & Albumin-GdDTPA; 3- Streptavidin-SPIO only; 4- Avidin-GdDTPA only. T1 relaxation time of the specimens are $T_1(3) = 2488 \pm 92$ ms, $T_1(4) = 2272 \pm 87$ ms, and $T_1(2) = 2097 \pm 52$ ms respectively.

Discussion: As cancer stem-like cells presumably are resistant to treatment and can repopulate a tumor even after the bulk of the tumor is eradicated by therapy, they present an important novel target for anticancer therapy. Noninvasive imaging technology that can accurately determine tumor areas with high density of cancer stem-like cells can provide invaluable information to direct the treatment and to access its efficiency. Due to the high spatial resolution of MRI, one can utilize its imaging capabilities to distinguish the variant CD44 levels in these BCSLCs. In this study, we have developed an MR imaging platform targeted to CD44 receptors that are markers of breast cancer stem-like cells. Both positive gadolinium-based and negative SPIO-based contrast agents provide specific MR enhancement in cells expressing high levels of the CD44 receptor. Contrast agents will be further validated in preclinical models of human breast cancer.

References: [1] Lapidot, T., Grunberger, T., Vormoor, J., et al. Blood, *88:* 2655-2664, 1996. [2] Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., et al. Proc Natl Acad Sci US A, *100:* 3983-3988, 2003. [3] Phillips, T. M., McBride, W. H., and Pajonk, F. J Natl Cancer Inst, *98:* 1777-1785, 2006. [4] Vander Borght, S., Libbrecht, L., Katoonizadeh, A. et al. J Histochem Cytochem, *54:* 1051-1059, 2006. [5] Donnenberg, V. S. and Donnenberg, A. D. J Clin Pharmacol, *45:* 872-877, 2005. [6] Sheridan, C., Kishimoto, H., Fuchs, R. K., et al. Breast Cancer Res, *8:* R59, 2006. [7] Fillmore, C. and Kuperwasser, C. Breast Cancer Res, *9:* 303, 2007. [8] Artemov, D., Mori, N., Ravi, R., et al. Cancer Res, *63:* 2723-2727, 2003.

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