Magnetically labeled Human Breast Cancer Cell In Vivo MR Imaging to assess tumor metastasis; Experimental Animal Pilot Study

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Introduction

Magnetically labeled-cells imaging has been a crucial corner of *in vivo* molecular MR imaging. Recent studies have focused on *in vivo* imaging of the implanted stem cell cells. Trafficking of magnetically labeled tumor model has not been addressed because growing and division rate of the tumor cell is too fast to contain transfected iron crystals in the cytoplasm for a long time.

The purpose of this study is to evaluate whether implanted intracellular superparamagnetic iron oxide (SPIO) nanoparticles-labeled tumor cells in mouse can be used as a model for cancer metastasis.

Materials and Methods

Cell line: Rapidly growing and adherent human breast cancer cells (MDA-MB231) were allowed to grow until 90% confluence of the surface area of the culture flask before co-culture with Feridex-PLL complex. Size and number of the cells were measured on hemacytomer (Brihgt-Line; Hausser Scientific, Horsham, PA). Mean size of the cell was 15μm.

Preparation of Feridex-PLL complex: Dextran coated superparamagnetic iron oxide (Feridex IV; Berlex Laboratories, Inc., Wayne, NJ) was used for labeling agent and Poly-$_L$-lysine (PLL; Sigma, St. Louis, MO) was used for transfection agent. Feridex at a concentration of 25 μg/mL was put into a conical tube containing 16mL serum free media and then PLL was added to the solution at a concentration of 0.5 μg/mL. The solution was slowly mixed in a rotator for 60 minutes at 4°C. After 60 minutes, old media was discarded and replaced with Feridex-PLL complex solution, and then incubated for 18 hours.

Validation of labeling and implantation: Cells were washed and centrifuged three times by phosphate buffered saline for removing excess Feridex-PLL complex. Cellular viability of labeled cells was evaluated using trypan blue dye exclusion test. Labeled cellular histology was evaluated after transferred to cytospin slides. Cells were fixed 95% alcohol, washed, incubated for 20 minutes 2% potassium ferrocyanide (Perl's reagent for Prussian blue staining) in 3.7% hydrochloric acid, washed again and counterstained with nuclear fast red. Labeling efficiency was determined by manual counting of Prussian blue stained and unstained cells using microscope. Labeled cells were injected subcutaneously at the both proximal thigh at a concentration of $2.7x10^5$/200 μL to the six-week-old BALB/C-nude mouse. Procedures were approved by the Animal Care and Use Committees of our institute.

***In vitro* cellular MR imaging:** For high resolution cellular imaging, cells were incubated with Feridex-PLL complex in a concentration of 25:1.5 μg/mL. Imaging was done using 14.1 Tesla system based on a Bruker DMX 600. The imaging protocol consisted of gradient echo sequences with TR/TE = 1000/15 ms, matrix size = 64 x64, slice thickness = 27 μm, in-plane resolution 23 x 23 μm, acquisition bandwidth =1553Hz, averaging = 768, and an experimental imaging time of 13 hours 39 minutes 12 seconds.

***In vivo* MR imaging of SPIO labeled tumor and *ex vivo* correlation:** After the 25 days implantation, *in vivo* MR imaging was done using 4.7 Tesla research magnet (Varian Medical Systems, Inc., Palo Alto, CA). The imaging protocol consisted of spin echo sequences with TR/TE = 200/10.6 and 2000/70 ms, matrix size = 128 x 128, slice thckness = 1 mm, FOV = 25mm, averaging = 1. Gradient echo sequences with TR/TE = 10/3.6, flip angle = 30°, averaging = 1. For histologic correlation, mouse was sacrificed and specimen fixed in formalin. Specimen embedded in paraffin block and sectioned for Prussian blue, activated DAB-enhanced Prussian blue and H-E staining.

Results and Discussion

Cell viability after labeling was 98.3% and labeling efficiency was 99.8%. *In vitro* cellular MR imaging of the single tumor cells show dark signal voids in a capillary tube fixed with 4% gelatin (Fig.1.) *In vivo* MR imaging of SPIO labeled tumor mass showed significant dark signal intensities in T1-, T2, and T2* weighted images. With DAB enhance Prussian blue staining of the pathologic specimen proved intracytoplamic SPIO containing vacuoles (Fig.2.). Concentration of Feridex-PLL complex as 25:0.5 was slightly low for complete transfection of incubated cells. However clumping of excess complex which not transfected intracellularly was minimized yet sufficient intracytoplasmic endocytosis of iron oxide for MR imaging was achieved. Pathologic specimen confirmed that tumor cells contained comparable amount of irons in the cytoplasmic vacuoles over 3 weeks.

Conclusion

SPIO labeled rapidly growing tumor cell could be traced until at least 25 days after the implantation in mouse using in vivo molecular MR imaging. It is believed that MR imaging for implantation model of magnetic labeled tumor cells could provide new insight for understanding tumor growth and spread such as metastasis.

References

1. Frank et al. Radiology. 2003 Aug;228(2):480-7
2. Lewin et al. Nat biotechnol 2000 Apr;18(4):410-4

Figures and Legends

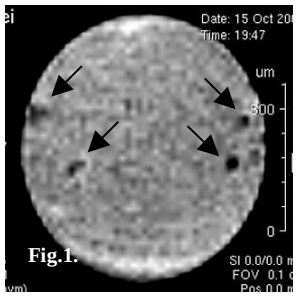

Fig.1. . Single cell imaging of magnetically labeled MDA- MB231 with voxel size of 23 x 23 x 27 μm (arrows).

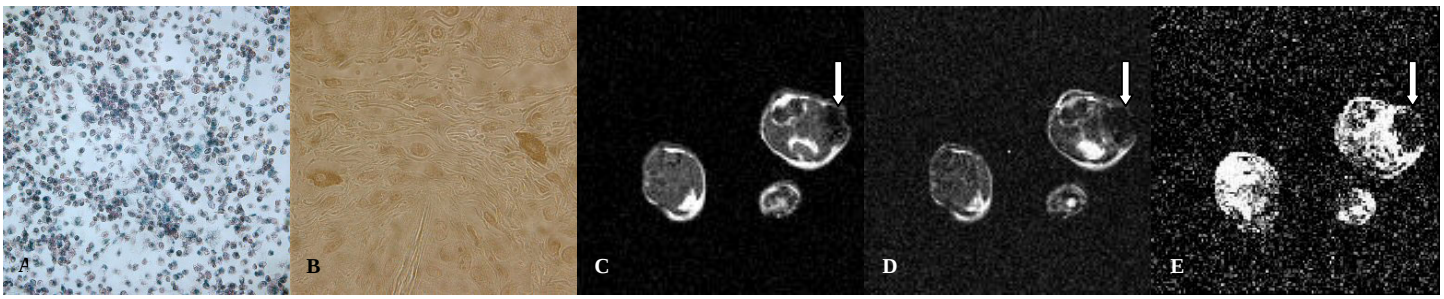

Fig.2. A. Photomicrograph shows Prussian blue staining of intracellular labeled SPIO in MDA-MB231(x 200). **B.** Pathologic photomicrograph of tumor mass stained by activated DAB-enhanced Prussian blue shows intracytoplasmic vacuoles containing SPIO, stained dark brown (x 1000). **C.D.E.** T1-, T2 -, and T2* weighted axial images show profound low signal intensity at the proximal thigh of the mouse, resulting from blooming artifact due to intracellular labeled SPIO(arrows).