Quantitative cellular tracking MRI of human mesenchymal stem cells home to the metastatic breast cancer in the rat brain

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Introduction

To validate the efficacy of stem cell transplantation, tracking of implanted or infused stem cells by MRI has been perform by visually localizing and counting of hypointense voxels on T_2* sensitive images. However, the threshold of lesion detection will vary according to the pulse sequences used (1). Micron sized iron oxide particles (MPIO) have been used to increase conspicuity of MRI cellular tracking (2). In this study, the ability to track human mesenchymal stem cells (hMSC) labeled with either MPIO (2), Ferumoxides-Protamine Sulfate (FE-Pro) (3), $MnFe_2O_4$ nanocrystal (MnMEIO) (4) was evaluated in a metastatic breast cancer model.

Methods

After intracardiac injection of 10^6 MDA-MB-231BR luciferase positive human breast cancer cells in nude rats, metastasic tumor development was monitored with MRI and bioluminescence imaging(BLI) . Two weeks later, rats were infused (IV) with 10^6 human mesenchymal stem cells (hMSCs) labeled for 12 hours with: FEPro, $MnFe_2O_4$, or MPIO particles (n=8 rats per group). Serial 3T MRI (TSE and Multigradient echo T_2* weighted images) and BLI scanning were performed day 3, weeks 1-2 after MDA-MB-231BR injection, and day 1-3 , week 1-2 after infusion of hMSCs. The MR pulse sequences were as follows: T_2 weighted TSE, TR/TE = 3200/60 ms, TSE factor 12, NAV 8, FOV 50 mm, slice thickness 0.5 mm, matrix 224×256, resolution 100×100 μm; T_2* weighted multi-shot EPI, TR/TE = 4560/28 ms, 15 echos, flip angle 30°, NAV 2, FOV 50 mm, slice thickness 0.5 mm, matrix 176×256, resolution 200×200 μm. Post-processed T_2* map histograms of the brain were used to quantitate the hMSCs homing to the tumors. All rats were euthanized at week 2 after IV infusion of hMSCs and were examined by Prussian blue stain and immunohistochemistry for STRO-1 MSC marker. MRI T_2* map histogram analyzed to investigate the difference of each group.

Results

Prussian blue staining and Relaxometry analysis of the MSCs labeled with 3 labeling agents showed variable amounts of uptake of the FePro (7.1pg Fe/cell), MPIO (57pg Fe/cell) and MnMEIO (35.2 MnFe/cell). There was no difference in viability and proliferation capacity of hMSCs after labeling with 3 agents (Fig. 1a,b). Prussian blue staining of the lesion showed multiple blue colored cells surrounding the tumor mass that correlated with immnunohistochemical stained g anti-human STRO-1 antibody positive cells for human MSC. Histogram of the number of voxels from T2* maps rfor each group of animals demonstrated a shifting to lower T_2* value at day 2 and 3 following IV injection of magnetically labeled hMSCs. Quartile (Q) analysis of the histogram revealed an increased number of voxels in Q2 (T2*=25-50ms) following injection of labeled cells and no significant difference was observed among for animals receiving FePro, MPIO and MnMEIO labeled cells.

Discussion

Quantitative tracking of superparmagnetically labeled hMSCs homed to diffuse metastatic brain tumor was performed using MRI T_2* map histogram analysis and confirmed by histology. The amount of hMSCs that homed to the metastatic brain tumor was expressed as histogram shifting to lower T2* values at each time point following injection of labeled hMSCs. Quartile analysis revealed that there was no difference when animals were injected with FEPro, MnFe2O4, or MPIO particles labeled hMSC. The results demonstrate the use of T2* maps to track intravenously injected magnetically labeled homing to sites of metastatic breast cancer in the brain. Differences in the induced T2* susceptibility by FEPro, MnFe2O4, or MPIO particles due to size and chemical composition that is observed in vitro following cell labeling is not apparent when tracking cells in vivo on cellular MRI at 3T following IV injection of labeled cells. Quantitative T2* mapping and analysis could be used to quantitatively track genetically engineered stem cells in the treatment of metastatic human breast cancer.

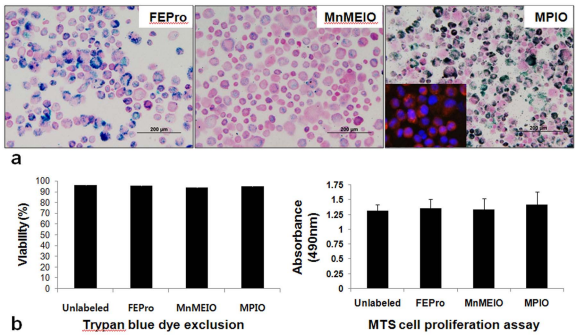

Fig. 1. a. Prussian blue staining of the MSC. Cells were labeled with 3 labeling agents. Inset in right image shows red fluorescence from the MPIO particle. b. Viability test. No difference in viability and proliferation capacity of hMSCs after labeling with 3 different superparamagnetic stem cell labeling agents were observed.

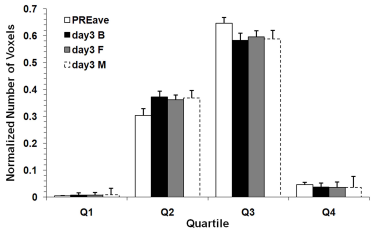

Fig. 3. Averaged normalized histogram of the whole brain ROI at day 3 after IV injection of 10^6 hMSCs. At two weeks after IC tumor cell injection, the PREave: averaged baseline scan B: MPIO, F:FEPro, M: MnFe2O4 were similar despite the presence of labeled cells in metastatic brain tumors.

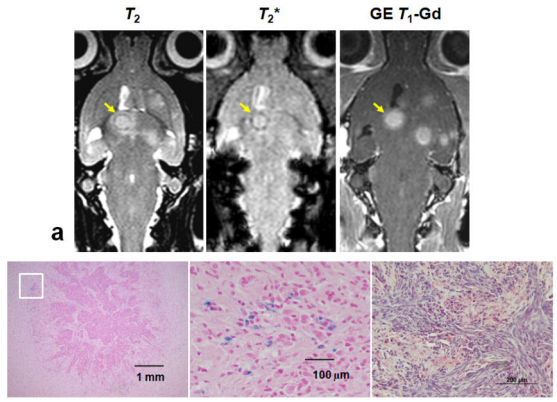

Fig. 2. Representative MRI and histopathology which were taken at 2 weeks after injection of 10^6 FEPro labeled hMSCs. **a.** MR images show multiple cerebral hyper-signal intensity metastatic tumor lesions. Yellow arrow indicates one of the representative lesion that is surrounded by hypointense rim. **b.** Left. Prussian blue staining of the lesion indicated by arrow at **a** shows multiple blue colored cells surrounding the tumor mass. **Middle.** Magnified view of box in the left figure. **Right.** Immnunohistochemical staining using anti-human STRO-1 antibody to detect human MSC shows integration of cells (Red color) into metastatic tumor.

References.

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