Dual Transfer of Gene and MR Contrast Agent into Stem-Progenitor Cells:Toward in vivo MR imaging of stem cell-mediated gene therapy

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PURPOSE: Stem-progenitor cells can migrate to specific lesions, such as atherosclerosis and cancers (1). Hematopoietic stem-progenitor cells circulate in the blood system, flow through the entire body, and thus home to wherever atherosclerotic and cancerous lesions exist. Transfer of therapeutic genes into hematopoietic stem-progenitor cells prior to their transplantation to the body may enable to explore a stem cell-mediated target-specific delivery of therapeutic genes. Recent efforts to use magnetic resonance (MR) imaging to serially track and quantify cell transplantation have focused on labeling the cells with MR imaging-detectable contrast agents, such as superparamagnetic iron oxide agents (2). These two concepts, that (a) hematopoietic stem-progenitor cells can be pre-transferred with genes and pre-labeled with MR contrast agents and (b) the transferred and labeled hematopoietic stem-progenitor cells can circulate through the body and thus carry genes and contrast agents to the targets, motivated us to further develop a target-specific molecular MR imaging technique, to monitor stem-progenitor cell-mediated gene therapy. As the first step, the current study focused on validation of the feasibility to co-transfer a marker gene and an MR contrast agent into stem cells and bone marrow (BM) cells.

METHODS: Since T2 MR contrast agents, such as a superparamagnetic iron oxide agent, can create too strong signal void on T2 MR imaging to visualize the details of the target and its background, we attempted to solve this problem by labeling cells with a T1 MR contrast agent, MGd (Pharmacyclics Inc, CA). This agent, a gadolinium texaphyrin analog, is primarily designed as an intracellularly localized anti-cancer agent (3). In addition, MGd itself can produce red-colored fluorescence when stimulated by blue light, and thereby, it can be used as an excellent optical bio-tissue marker that can be easily tracked under fluorescence microscopy. Thus, the characteristics of MGd (i.e., MR and fluorescent microscopy detectable) provided us the possibility to correlate MR imaging with histology confirmation.

Using a lipofectamine approach, GFP plasmid (2 μg/mL) and MGd (50 μg/mL) were simultaneously transferred into (a) six groups of C17.2 mouse neural stem cells; and (b) six groups of mouse hematopoietic BM cells, with approximately 6 x 10^5 per group for 36 hours. Additional a group of C17.2 stem cells and three groups of BM cells were not transferred to serve as controls. The transferred and non-transferred cells were harvested by trypsin and the nuclei were stained with DAPI. Then, we examined cells under fluorescent microscopy to confirm successful GFP expression and MGd labeling. For quantitative comparison between the transferred and non-transferred cells, the efficiencies of GFP gene expression and MGd labeling in both C17.2 and BM cell groups were subsequently measured using flow cytometry.

In addition, we performed MR imaging to confirm the capability of using MRI to detect MGd-labeled cells. Approximately 10^6 (0.2-mL) transferred and non-transferred C17.2 cells were stored within 1-mL plastic tubes, which were then centrifuged to obtain cell pellets at the bottom of the tubes. The cell pellets were imaged in a 1.5T MR scanner. The MR parameters included spin echo (SE) sequence, 500/11-ms TR/TE, 256x256 matrix, 16x16-cm FOV, NEX 4, and 5-mm thickness. We also suspended the same amount of BM cells within 500-μL 4% w/w gelatin, and performed the MR imaging in an 9.4T (Bruker Biospin, Billerica, MA) MR scanner. The parameters of 9.4T MR imaging were SE, 500/11-ms TR/TE, 256x256 matrix, 16x16-cm FOV, NEX 4, and 5-mm thickness. MR images were reconstructed and then analyzed using the scanner consoles. We set the same-sized regions of interests (ROI) onto the bottom of each tube, and then compare the MR signal intensities of cell pellets between the transferred and non-transferred cell groups.

RESULTS: Fluorescent microscopy detected simultaneous GFP expression (green fluorescence emission) and MGd signals (red fluorescence emission) from the transferred cells, while only autofluorescence was visualized in non-transferred cells (Figure 1). Flow cytometry showed that (i) for the C17.2 cell group, 46.73±25.27% of cells generated positive GFPs and 95.95±6.32% of cells generated positive MGds; and (ii) for the BM cell group, positive GFP cells were detected at 55.84±12.4% and positive MGd cells were detected at 57.03±11.4%. For both C17.2 and BM cells, MR imaging demonstrated greater signal intensity in transferred cells, compared to non-transferred cells (Figures 2&3).

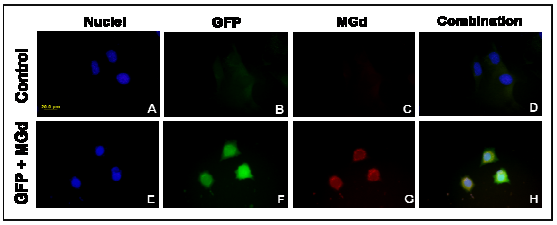

Fig. 1. Fluorescent microscopy imaging of non-transferred cells (upper row) and the cells transferred simultaneously by GFP gene and MGd (lower row). Images are obtained at blue channel for nuclei with DAPI stain (A&E), green channel for GFP (B&F), red channel for MGd (C&G), and the combination of three colored images (D&H). Green light due to GFP expression and red-colored spots (due to MGd labeling) surrounding the nuclei are detected in GFP/MGd-transferred cells, while only auto-fluorescence from cells themselves is visualized in non-transferred cells.

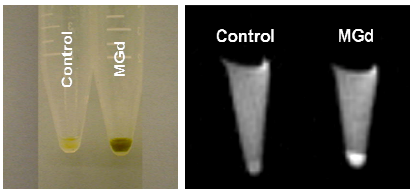

Fig. 2. (Left) A photo taken immediately after centrifuging cells at the bottoms of tubes, showing the success of C17.2 cells labeling by MGd as dark-brown color with MGd-trasnferred cells. (Right) Representative 1.5T MR imaging of cell pellets at bottoms of tubes, demonstrating clearly brighter signals in transferred cells compared to non-transferred cells.

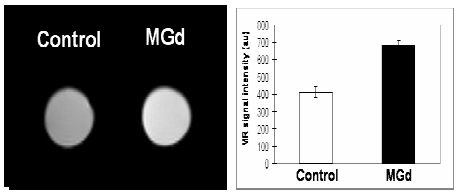

Fig. 3. (Left) Representative 9.4T MR imaging (cross-sectional view) of BM cells in 4% gelatin, showing brighter MR signal in transferred cells than in non-transferred cells. (Right) Comparison of average MR signal intensities between non-transferred and transferred BM cells, demonstrating greater average signal intensity in MGd-transferred cells. au= arbitrary unit.

Conclusion: This study demonstrates initially the success of co-transferring a marker gene and a T1 MR contrast agent into the stem cells and hematopoietic bone marrow cells. In vivo studies on MR tracking of gene/MGd-transferred BM cells that migrated to atherosclerosis of animal models are underway to further validate the feasibility of this new technique. These studies should establish the groundwork to develop target-specific molecular MR imaging of stem-progenitor cell-mediated gene therapy of atherosclerotic cardiovascular diseases.

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