Dual Transfer of Gene and MR Contrast Agent into Stem-Progenitor Cells for in vivo MR Imaging of Stem Cell-mediated Gene Therapy

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PURPOSE: Atherosclerotic cardiovascular disease is a unique illness that produces diffuse and multiple atherosclerotic lesions in nearly all arteries of the body. Hematopoietic stem-progenitor cells circulate in the blood system, flow through the entire body, and thus home to wherever atherosclerotic plaques exist [1,2]. 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 [3]. 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. This study focused on validation of the feasibility to co-transfer a reporter gene and an MR contrast agent into bone marrow (BM) cells in vitro and in vivo.

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, motexafin gadolinium (MGd, Pharmacyclics Inc, CA). This agent, a gadolinium texaphyrin analog, is primarily designed as an intracellularly localized anti-cancer agent [4]. 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 [(5)]. Thus, the characteristics of MGd (i.e., MR and fluorescent microscopy detectable) provided us the possibility to correlate MR imaging with histology confirmation.

For in vitro study, we used a lipofectamine approach to simultaneously transfer green fluorescent protein (GFP) plasmid (2 μg/mL) and MGd (50 μg/mL) into six groups of mouse hematopoietic BM cells, with approximately 6 x 10^5 per group, for 36 hours. Additional 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 gene expression and MGd labeling. For quantitative comparison between the transferred and non-transferred cells, the efficiencies of GFP gene expression and MGd labeling were subsequently measured using flow cytometry. In addition, we performed MR imaging to confirm, in vitro, the capability of using MRI to detect MGd-labeled cells. Approximately 10^6 (0.2-mL) transferred and non-transferred BM cells were suspended within 500-μL 4% w/w gelatin, and imaged using 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) to compare MR signal intensities of cell pellets between the transferred and non-transferred cell groups.

For in vivo study, we first collected BM cells from LacZ transgenic mice and then transferred and/or labeled the LacZ BM cells by GFP and MGd. Ten atherogenic ApoE$^{-/-}$ mice were divided into three study groups, including: (a) Group I without MGd- and/or GFP-transferred BM transplantation (n=4); (b) Group II with GFP-transferred BM transplantation (n=3); and (c) Group III with MGd- and GFP-transferred BM transplantation (n=3). Migrated GFP/MGd-LacZ-BM cells to atherosclerotic aortic walls were monitored, *in vivo,* using a 9.4T MR scanner and correlated with histopathological findings.

RESULTS: Fluorescent microscopy could detect 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. Flow cytometry showed GFP-positive cells at 55.84±12.4% and MGd-positive cells at 57.03±11.4%. In vitro MR imaging demonstrated greater signal intensity in transferred cells compared to non-transferred cells. In vivo MR imaging revealed the partial enhancement of the aortic walls in the GFP/MGd-BM-transferred mice (Group III), and no such enhancement in two control groups (Group I and II). These MR findings were correlated with histological confirmation (Figure 1).

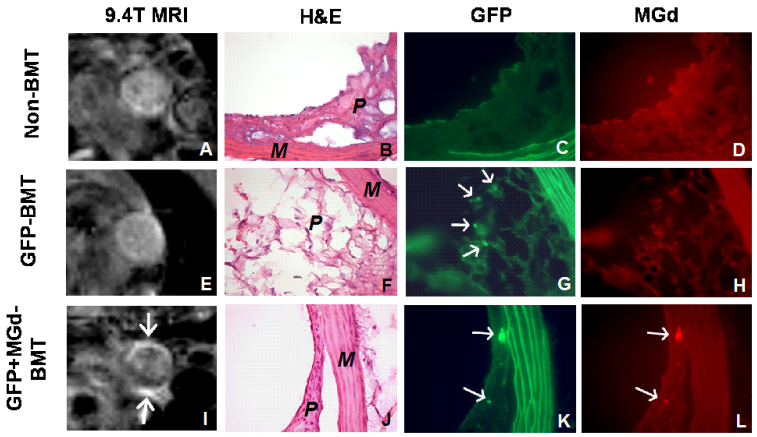

Figure 1. Representative in vivo 9.4T MRI of atherosclerotic mouse aorta with transplant of GFP/MGd-BM cells, which are correlated with histology confirmation. MR image (I) demonstrates MGd-enhanced partial ascending aortic wall (arrows) due to migrated GFP/MGd-BM cells, which is confirmed by both GFP expression (green fluorescent emission, arrows on K) and MGd-positive cells (red fluorescent emission, arrows on L). The MGd enhancement of the aortic wall is not visualized on the two control groups with either no BM transplat (BMT, A) or GFP-BMT (E). On G with GFP-BMT only, GFP-positive cells are detected (arrows on G), while GFP- or MGd-positive cells are not seen on control histological slides (C, D, & H). P=plaque and M=media of aortic wall. In vivo MRI parameters: spin Echo, TR/TE=500/9.8, NEX=2, THK=0.5mm, FOV=3x3cm, Matrix=256x256. Histology, 40X.

Conclusion: This study demonstrates the initial success of co-transferring a marker gene and a T1 MR contrast agent into the hematopoietic stem-progenitor cells. In vivo studies have initially validated the feasibility of using MR to monitor GFP/MGd-transferred BM cells that migrated to atherosclerosis of animal models. 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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