3.0T MRI of Auto-transplantation of Bone Marrow-Derived Stem-Progenitor Cells: Toward Cell-Based Repair of Injured Arteries

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Introduction: Injured arteries and ruptured atherosclerotic lesions can recruit circulating bone marrow (BM)-derived stem-progenitor cells (SPCs) that are capable of differentiating into proliferating smooth muscle cells (SMCs) and endothelial cells (ECs) [1]. The proliferated SMCs and ECs may contribute to strengthening the fibrous caps of plaques and maintaining the integrity of the endothelial layer to prevent additional damage to the arterial wall. Allogenic transplantation of BM-SPCs from donors to recipients can cause immune rejection [2]. Autologous cell transplantation is a strategy to solve this problem. The aim of this study was to validate the feasibility of using clinical 3.0T MRI to monitor the migration of auto-transplanted BM-SPCs to the injured arteries of near-human-sized animals for potential cell-based repair of injured arteries.

Materials & Methods: Ten domestic pigs were divided into (i) Group 1 (n=4) with auto-transplantation of BM cells labeled by a T2 MR contrast agent (Feridex) and/or a fluorescent marker (PKH26); (ii) Group 2 (n=3) with auto-transplantation of BM cells labeled by Feridex only; and (iii) Group 3 (n=3) with auto-transplantation of non-labeled BM cells or without cell transplantation. For in vitro confirmation, approximately 12-18 ml BM cells were endogenously extracted from the iliac crests of each pig, and then labeled with Feridex (25μg/ml) and/or PKH26. Viability and proliferation function of labeled cells were assessed by Trypan blue exclusion with cell counting and MTS, respectively. The efficacies of Feridex and/or PKH26 labeling were determined by cell counting with Prussian blue staining and fluorescent microscopy, respectively.

For in vivo validation, under ultrasound guidance or through a surgical cutting down, a Fogarty balloon or a cutting balloon was advanced to injure the iliofemoral artery. Labeled or non-labeled BM-SPCs were autologously transplanted back to each of the pigs. Approximately 3 weeks post-cell transplantation, 3.0T MRI, with a surface coil or a 0.032-inch intravascular MR image-guidewire, was performed to detect Feridex-created signals of the transplanted SPCs that migrated to the injured arterial segments. MRI with improved Motion Sensitized Driven Equilibrium (iMSDE) sequence was acquired for (i) T2-imaging at 4500-5000ms TR & 40ms TE; and (ii) T1-imaging at 800ms TR & 8.6ms TE. The targeted and control artery segments were subsequently harvested, cryosectioned, and then stained with (a) H&E to grade the injured arteries; (b) Prussian blue to detect Feridex-positive cells; (c) immunofluorescent stain to detect dextran, the capsules of Feridex particles; and (d) fluorescent microscopy to detect PKH26-positive cells.

Results: In vitro experiments showed the viability of Feridex- and/or PKH26-labeled BM cells at 95-98%. Regarding to the proliferation function, there was no significant difference among different cell groups of Feridex-labeled cells (0.359±0.047), PKH26-labeled cells (0.333±0.032), Feridex/PKH26dual-labeled cells (0.340±0.029), and non-labeled (control) cells (0.355±0.050)(Fig.1A, ANOVA, p>0.05). Cell labeling efficacy was achieved at approximately 90% for Feridex and 100% for PKH26 (Fig. 1). In vivo validation study demonstrated Feridex-created MR signal voids along the injured iliofemoral artery segments on T2-MRI, while such findings were not visualized in control arteries. Histology confirmed the MR findings, demonstrating: (i) Feridex-positive cells as blue spots with Prussian blue staining and green fluorescent spots with dextran immunofluorescent staining, and (ii) PKH26-positive cells as emitted red fluorescent dots under fluorescent microscope through the injured arterial walls. These findings were not seen in the control group of arteries (Fig. 2).

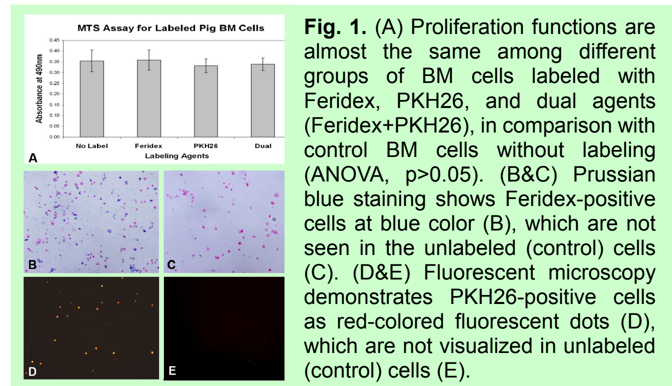

Fig. 1. (A) Proliferation functions are almost the same among different groups of BM cells labeled with Feridex, PKH26, and dual agents (Feridex+PKH26), in comparison with control BM cells without labeling (ANOVA, p>0.05). (B&C) Prussian blue staining shows Feridex-positive cells at blue color (B), which are not seen in the unlabeled (control) cells (C). (D&E) Fluorescent microscopy demonstrates PKH26-positive cells as red-colored fluorescent dots (D), which are not visualized in unlabeled (control) cells (E).

Conclusions: This study initially demonstrates the capability of using clinical 3.0T MRI to monitor auto-transplantation of BM-SPCs migrated to the injured arteries of human sized animals, which may lead to a new technology — *MRI of cell-based repair of injured arteries*.

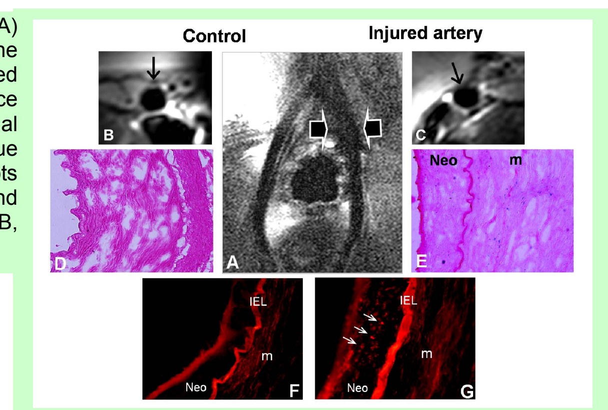

Fig. 2. MRI-histology correlation for the auto-transplantation of BM cells in pigs. (A) Near-coronal-view of 3.0T, T2-MRI shows hypo-signal (between arrows) along the iliofemoral artery due to the migration of Feridex-labeled BM cells to the injured iliofemoral artery. (C) Axial-view of an intravascular 3.0T MRI shows the disappearance of the anterior wall of the left iliofemoral artery (arrow) due to Feridex-induced hypo-signal in the injured artery. This is confirmed by histology, showing Feridex-positive cells as blue spots with Prussian blue staining (E) and PKH26-positive cells as red fluorescence dots (arrows on G, from another pig) under fluorescent microscopy. These MRI and histological findings are not seen in the right uninjured (control) iliofemoral arteries (A, B, D&F). Neo= neointima hyperplasia; IEL = internal elastic lamina; m = media.

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References: [1] Werner N, et al. Ann Med. 2007;39:82. [2] Qiu B, et al. JMRI.2007;26:339.