MR Tracking of Hematopoietic Progenitor Cells in Animal Models of Atherosclerosis

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Abstract:

Hematopoietic progenitor cells have been known to play an important role in neointima formation in atherosclerotic lesions. Several studies have demonstrated the presence of bone marrow (BM)-derived smooth muscle cells (SMC) at the site of plaque formation. Traditionally, the confirmation of migration/differentiation of BM-derived SMCs in the neointimal hyperplasia relies on staining the tissues obtained from either biopsy or autopsy. We attempted to develop a method, using high resolution MR imaging to track the migration and differentiation of hematopoietic progenitor cells in mouse models of plaque, i.e., models with injury resembling post-angioplasty restenosis and hyperlipidemia-induced atherosclerosis.

Materials and Methods:

The study was carried out in two phases: (1) confirming the migration of the progenitor cells to the site of neointima formation; and (2) monitoring the BM-derived progenitor cells using MR imaging. In the first phase, BM cells from transgenic mice expressing green fluorescent protein (GFP) were transplanted intravenously into sublethally irradiated balb/c nude mice (n=4). After successful BM transplantation, the intima of unilateral iliac-femoral arteries was injured mechanically using either a wire-mediated endovascular denudation approach or a cuff-constriction approach. In the second phase, BM cells from transgenic mice expressing LacZ (β-Galactosidase) were labeled with the superparamagnetic MR T2 contrast agent, Feridex, and then transplanted intravenously into lethally irradiated ApoE$^{-/-}$ mice (n=4). These recipient animals were then fed an atherogenic diet (15% butter and 2 % cholesterol) for 10-12 weeks to induce hyperlipidemic plaques in their blood vessels.

Of the first phase group, the recipient animals were sacrificed after 5-6 weeks of the mechanical injury, and both the control and the targeted artery segments were harvested for fluorescence microscopy detection of GFP^{+} BM-derived cells at the site of the plaques. In addition, the histology slides were also stained for α-smooth muscle actin (SMA, a marker for SMCs). In the second phase group, the aortas of the recipient animals were excised and perfused in 4 % paraformaldehyde. Then, high-resolution T2-weighted images of the sample were acquired on an 11.7T magnet (Bruker) using a 3-D fast spin-echo (FSE) sequence (RARE-3DBIO) with image parameters of 1-s repitition time (TR); 32.15-ms echo time (TE); 256×512×256 image matrix; 9×24×9 mm3 field of view (FOV); 208×384×208 image resolution; and number of excitations, 3 (NEX). For histological correlation, the aorta tissues were also stained for Feridex, α-SMA, and LacZ.

Results:

Fluorescence microscopy revealed stronger green signal from the targeted arteries compared to the uninjured control arteries of the nude mice (n=3) transplanted with GFP expressing-BM cells. This points to the presence of circulating BM-derived cells at the injured intima of the target vessels. Ex-vivo MR imaging demonstrated multiple MR signal voids on the aortic wall (Fig. 1) of the ApoE$^{-/-}$ mice (n=2), associated with positive staining of Feridex (Fig. 2.) in the atherosclerotic aortic tissues.

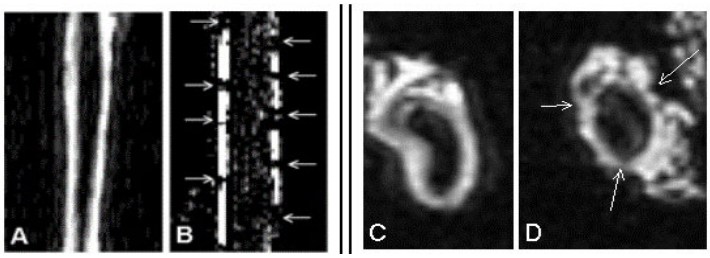

Fig. 1. Ex-vivo high resolution T2-weighted MR imaging of excised aorta of the control mice without BM transplantation (A&C) and with Feridex-labeled BM transplantation (LacZ-ApoE$^{-/-}$)(B&D). Coronal sections of the control model showed a smooth vessel wall with no hypoenhancement (A), whereas multiple signal voids (arrows) are visualized in the vessel wall of animals transplanted with Feridex-labeled BM Cells (B). The hypointentense spots represent migration of the magnetically labeled cells into the vessel wall. Axial MR imaging demonstrates similar findings: the control model with a smooth vessel wall (C) and the plaque model (D) with signal voids (arrows) in the vessel wall.

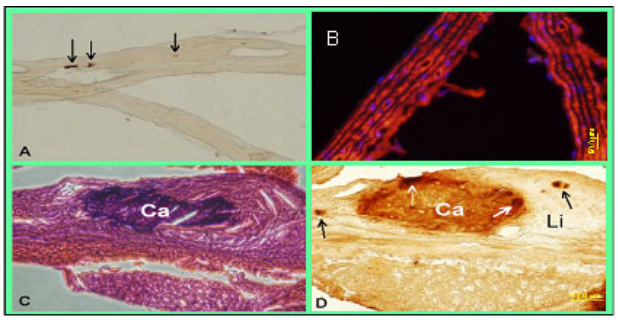

Fig. 2. Immunohistochemical staining of the aorta of ApoE$^{-/-}$ mice showing (A) positive staining for Feridex, (B) α-SMA in the vessel wall, (C) plaque deposits with calcification (Ca) within the atherosclerotic lesion (H&E staining), and (D) Prussian blue staining showing Feridex-labeled cells in the plaque region. (Li=Lipid core)

Conclusion:

These preliminary results provide evidence to support the potential of using MR imaging to track the migration of magnetically-labeled hematopoietic progenitor cells into atherosclerotic lesions. This could be used to investigate the role of BM progenitor cells in the pathogenesis of atherosclerosis, and target these cells for therapeutic interventions.

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