MRI of Bone Marrow Cell-Mediated Interleukin-10 Gene Therapy of Atherosclerosis

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Objectives: Recent studies have demonstrated that atherosclerosis can recruit circulating bone marrow cells (BMC)(1), and the migration of BMCs to atherosclerotic lesions can be monitored, in vivo, by molecular magnetic resonance imaging (MRI)(2). The aim of this study was to evaluate the feasibility of using MRI to monitor interleukin-10 (IL10) gene-transfected BMCs migrated to atherosclerosis for preventing progression of plaques.

Methods: For in vitro confirmation, BMCs were extracted from donor mice and then transduced by IL10 cDNA-lentivirus. The IL10-BMCs were labeled with a T2-MR contrast agent (Feridex). Success of simultaneous IL-10 gene transduction and Feridex-labeling of BMCs was confirmed by cytologic staining for IL10-gene expression and intracellular iron particle localization. For in vivo validation, atherosclerotic ApoE$^{-/-}$ recipient mice were intravenously transplanted with IL10/Feridex-BMCs (Group I, n=5) or Feridex-BMCs (Group II, n=5), while a group of five atherosclerotic ApoE$^{-/-}$ mice was not transplanted with BMCs to serve as a control (Group III). Approximately four weeks later, the migration of IL10/Feridex-BMCs and Feridex-BMCs to aortic atherosclerotic lesions of ApoE$^{-/-}$ mice was monitored, in vivo, with 3.0T MR imaging using a Philips mouse coil. All aortic tissues were then harvested for subsequent histological correlation and confirmation. To evaluate the therapeutic effect of BMC-mediated IL10 gene therapy in preventing the progression of atherosclerotic plaques, we measured quantitatively the normalized wall index (NWI) of ascending aorta of recipient ApoE$^{-/-}$ mice using a formula of dividing the aortic wall area by the total aortic area at cross-sectional views of digitized microscopic images. Subsequently, we statistically compared the mean NWIs among the three mouse groups with different treatments (one-way ANOVA).

Results: Of in vitro experiments, the success of simultaneous lentivirus-IL10/Feridex transduction/labeling of BMCs were confirmed by cytologic staining. Of in vivo experiments, molecular T2-MRI of the animal group I and group II presented signal voids of the aortic walls due to Feridex-created artifacts from the migrated IL10- and/or Feridex-BMCs in atherosclerotic lesions, which were confirmed by histological staining as Feridex- and/or IL10-positive cells. These findings were not seen in the control group III (Figure 1). Histologic quantitative measurements showed that the mean NWI of group I was significantly lower than those of group II and group III (P<.05), while there was no significant difference on the mean NWIs between the study group II and group III (Figure 2).

Conclusion: This study demonstrates that it is possible to use in vivo MRI to track IL10/Feridex-BMCs recruited to atherosclerotic lesions, where IL10 genes potentially function to prevent the progression of atherosclerotic lesions. This technique may open a new avenue for treatment of atherosclerotic cardiovascular diseases using MR-integrated, BMC-mediated IL-10 gene therapy.

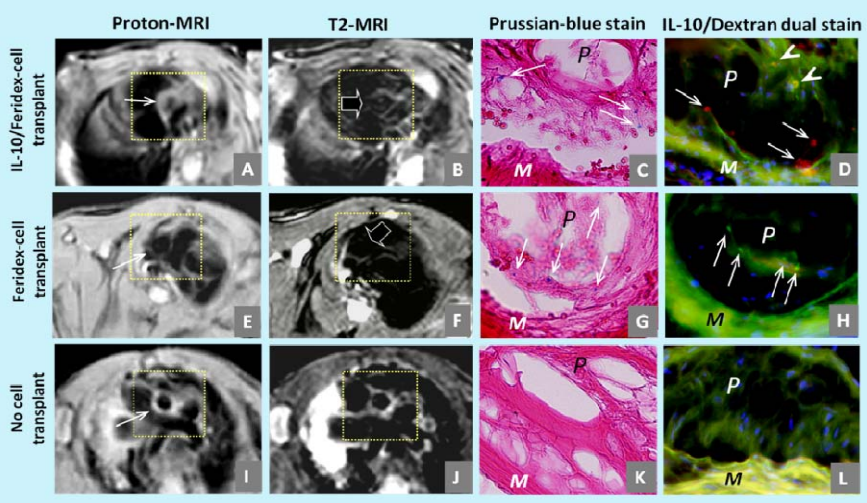

Figure 1. Representative MRI-histologic correlation of atherosclerotic ascending aorta (insets) of ApoE mice with IL10/Feridex-BMC transplantation (A-D), Feridex-BMC transplantation (E-H) and with no cell transplantation as a control (I-L). (A, E&I) Proton-weighted MRI shows thickening of the aortic walls due to formation of atherosclerosis lesions (arrows on A, E&I). (B, F&J) T2-weighted MRI shows MR signal void (arrows on B&F) at the aortic walls with IL10 and/or Feridex-BMC transplantation, which is not seen in the control aorta (J). (C, G&K) Prussian-blue staining detects Feridex-positive BMCs migrated to the atherosclerotic aortic wall (arrows in C&G), which are not visualized in the control aortic wall (K). (D, H&L) Immunofluorescent dual staining confirms simultaneous IL-10 gene expression (as red-colored spots, arrows on D), IL-10 overlapped with dextran shells of Feridex particles (as orange-colored dots, arrowheads on D) and dextran shells of Feridex particles (as green-colored dots, arrows on H), which are not seen in the control aortic wall (L), 400X. P=atherosclerotic plaque; M=medial. Blue color indicates nuclei.

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Reference

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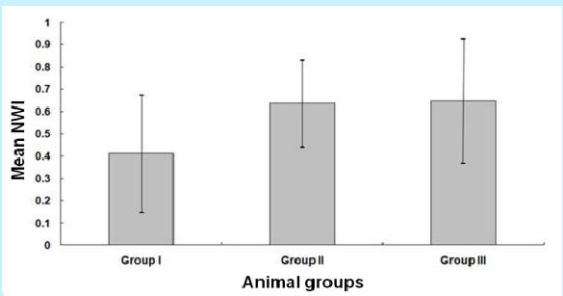

Figure 2. Comparison of normalized wall index (NWI) of ascending aortas of ApoE$^{-/-}$ mice among group I with IL10/Feridex-BMC transplantation, group II with Feridex-BMC transplantation, and group III with no cell transplantation. The mean NWI of group I is significantly lower than those of group II and group III (P<.05), while there is no significant difference between group II and group III.