Improved Identification of Ferritin-Tagged Grafts in Mouse Heart at Higher Magnetic Field Strength

Anna V Naumova[1,2], Vasily L Yarnykh[1,2], Niranjan Balu[1,2], Hans Reinecke[2,3], Charles E Murry[2,3], and Chun Yuan[1,2]

[1]*Radiology, University of Washington, Seattle, WA, United States,* [2]*Center for Cardiovascular Biology, University of Washington, Seattle, WA, United States,* [3]*Pathology, University of Washington, Seattle, WA, United States*

Introduction

Stem cell transplantation has potential to repair injured tissues such as myocardial infarctions. Non-invasive detection of transplanted cells in the damaged organ and longitudinal follow-up of cell fate and graft size is important for the evaluation of cell therapy. Genetically-induced cell labels, such as overexpression of the natural iron storage protein ferritin, have significant advantages over standard particles-based approaches ensuring longitudinal tracking of live dividing cells without dilution of the label. Previous studies described applications of ferritin as MRI gene-reporter [1-5], but effects of magnetic field strength on relaxation properties of ferritin-tagged grafts in the infarcted heart have not been described. Aims of this study: 1) to quantify MRI signal properties of wild type and transgenic cells overexpressing ferritin at the clinical 3T and research 14T scanners; 2) to compare signal intensity changes in ferritin-tagged grafts in the infarcted mouse heart at two different magnetic field strengths.

Methods

In vitro studies. Ferritin was overexpressed in mouse skeletal myoblasts (C2C12 cells) using pcDNA3 plasmid vector and FuGENE6 transduction reagent. Effects of gene overexpression to cell viability, proliferation and differentiation were assessed using standard cell biology techniques. T2 relaxation time was measured in cell pellets of wild type (WT) and ferritin-transduced C2C12 using spin-echo multi-echo sequences at the 3T Achieva Philips clinical scanner and 14T Bruker research spectrometer. Using 3T scanner we scanned with 32 equally spaced echoes (TE from 10ms to 320ms) and TR of 5000 ms. With the 14T scanner 16 equally spaced echoes (TE from 10ms to 160ms) and TR of 2500 ms were used.

In vivo studies. Stably transduced cells overexpressing ferritin were transplanted into the infarcted mouse heart (n=10). Cardiac MRI was performed *in vivo* one month after cell transplantation using the 3T and 14T scanners. Imaging protocol included ECG-gated T2* weighted cine gradient echo (GRE) multislice sequences. GRE parameters for the 3T scanner included TR/TE = 14/9 ms; slice thickness 1mm; flip angle 15°; field of view 50x50 mm, matrix 200x198; 6 signal averages, image resolution 250x250 μm. Imaging parameters on the 14T scanner for flow-compensated GRE: flip angle 15°; TR/TE = 8.44/2 ms; slice thickness 1 mm; acquisition matrix 256x256; field of view 25x25 mm, 2 signal averages, image resolution 98x98 μm. Graft-to-myocardium signal intensity ratio (SIR) was calculated from the regions of interest centered in the middle of the graft and in the non-infarcted area of myocardium.

Results

In vitro results. Ferritin overexpression did not alter cell viability, proliferation or differentiation, but significantly changed MRI contrast. T2 relaxation time was shorter for cells overexpressing ferritin in comparison with unlabeled WT cells: T2=161.21 ms for WT cells and 72.45 ms for transgenic cells measured with the 3T scanner (55% change); T2=35 ms for WT and 13 ms for ferritin-tagged cells at the 14T scanner (63% difference in T2).

In vivo results. Unlabeled WT C2C12 cells transplanted to the mouse heart did not alter MRI signal intensity, SIR=1, and therefore were indistinguishable from host tissue (figure 1A). Signal intensity of transgenic grafts overexpressing ferritin decreased by 30% at the 3T magnetic field strength (SIR=0.7) and by 50% at the 14T (SIR=0.5). The difference in SIR between ferritin-tagged and unlabeled grafts was statistically significant at both field strengths ($p<0.0001$). Difference in SIR measured at 3T and 14T magnetic field strength was also statistically significant ($p<0.05$).

Conclusion

Ferritin-tagged cells are better visualized at the higher magnetic field strengths due to increased T2 and T2* relaxivity of ferritin-tagged cells. Thus, use of higher field strengths could potentially improve MRI graft identification and assessment of graft size in stem cells overexpressing ferritin.

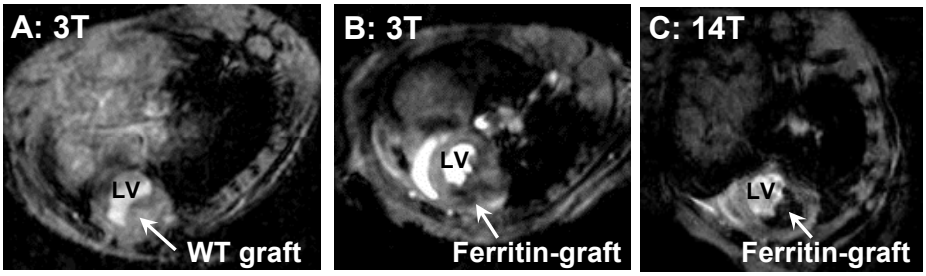

Figure 1. MRI identification of the wild-type (**A**) and transgenic ferritin-tagged C2C12 graft (**B,C**) in a mouse heart *in vivo.*

A,B: T2* GRE at the 3T.
C: T2* GRE at the 14T.
B,C: same animal imaged at the 3T and 14T scanners.
LV: left ventricle.

References

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