In vivo MRI Signal Features of Transgenic Grafts Overexpressing Ferritin in the Murine Myocardial Infarction Model

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Introduction

Cell labeling by iron oxide particles is a well-established technique for MRI cell tracking [1, 2]. A newer approach for non-invasive imaging of transplanted cells is genetic modification of those cells for stimulation of endogenous production of MRI-detectable proteins, such as iron-binding protein ferritin [3, 4]. It was shown that ferritin overexpression increased cellular iron content and transverse relaxation rate of transduced cells both, *in vitro* and *in vivo* in C6 glioma tumors [3] and mouse brain [4]. Ferritin overexpression was detected *in utero* in transgenic mice [5]. Use of MRI gene reporter ferritin would be benificial for non-invasive imaging of therapeutic cells engrafted to the infarcted heart. Aims of this study were to quantify MRI signal intensity changes in transgenic grafts overexpressing ferritin in the infarcted mouse heart and to identify an optimal MRI sequences for visualization of ferritin-labeled cardiac grafts.

Methods

Ferritin overexpression: Mouse ferritin heavy-chain was overexpressed in mouse skeletal myoblasts (C2C12 cells) using pcDNA3 plasmid vector and FuGENE6 reagent. Stably transduced cells were selected by neomycin (1.2 mg/mL in cell culture media). Expression of ferritin was monitored by Western blot analysis with rabbit monoclonal antibody to ferritin (Abcam Inc, Cambridge, MA). Prussian Blue staining was used to confirm iron accumulation in transduced cells overexpressing ferritin. *Cell transplantation:* Unlabeled and ferritin-tagged C2C12 were transplanted in hearts of syngeneic C3H mice. Infarction in the mouse heart was induced by permanent ligation of the left coronary artery. 500,000 cells per mouse were injected to the border of infarction in 7µl of serum/antibiotics-free medium. *MRI protocol:* Animals were imaged on a 3T Philips Achieva scanner one month after the surgery using a solenoid mouse coil (Philips Research Laboratories, Hamburg, Germany). The imaging protocol included ECG-gated proton-density weighted black-blood turbo spin echo (PD TSE) multislice sequence (TR/TE = 480/9.9 ms; slice thickness 1mm; matrix 248x245; flip angle 90°; FOV 50mm; 3 signal averages); bright-blood T2* cine gradient echo (GRE) multislice sequence (TR 15.4 ms; TE 9.4ms: slice thickness 1mm; flip angle 15°; matrix 200x198; 6 signal averages), and black-blood iMSDE (improved motion sensitized driven equilibrium [6]) pulse sequences (TR/TE=16/8ms; flip angle 13°; slice thickness 1 mm, resolution 168x165 µm). *Image analysis:* Graft-to-myocardium signal intensity ratio (SIR) was calculated from *in vivo* MRI using regions of interest centered in the middle of the graft and in the non-infarcted area of myocardium (mid-septum). MRI data were validated by established histological and immunohistochemical methods: Picrosirius Red staining was used to define the infarct zone; immunostaining for embryonic skeletal myosin for graft identification; Prussian Blue staining for detection of iron deposition in cardiac tissue.

Results

One month after infarction all hearts exibited contractile dysfunction and left ventricle remodeling detectable by MRI *in vivo*. Unlabeled wild-type C2C12 cells as well as transgenic cells overexpressing ferritin formed large skeletal muscle grafts in the hearts of syngeneic C3H mice one month after transplantation. Presence of grafts was confirmed by immunostaining for embryonic myosin. Unlabeled C2C12 grafts did not cause signal intensity changes in mouse myocardium in PD TSE and T2*-weighted GRE MR images (figure 1a-b): SIR in wild-type C2C12 grafts was about 1. Skeletal muscle C2C12 grafts exhibited MRI signal enhancement when black-blood iMSDE sequence was used: average SIR 1.46 (figure 1c).

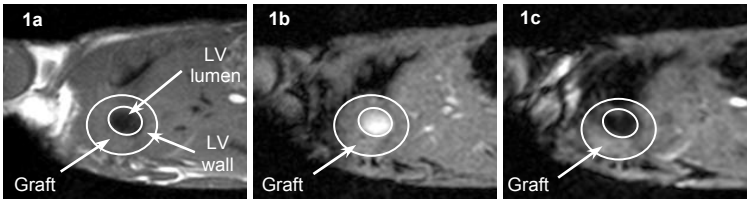

Figure 1. Unlabeled wild-type C2C12 grafts in the mouse heart. **1a**, PD TSE black-blood sequence (SIR=1.04); **1b**, T2* GRE bright-blood (SIR=1.07); **1c**. iMSDE black-blood (SIR=1.26). **LV**: left ventricle.

Transgenic C2C12 grafts overexpressing ferritin decreased MRI signal intensity more than 30% in T2*-weighted GRE (mean SIR=0.67) and by 20% in iMSDE sequences (mean SIR=0.82). SIR differences between ferritin-tagged and unlabeled grafts were statistically significant ($p<0.05$) in T2*-weighted GRE (figure 2 and table 1). PD TSE black blood sequence allowed clear deliniation of the left ventricle borders in mouse heart, but was not sensitive to moderate effect of ferritin overexpression into MRI signal change (SIR=1).

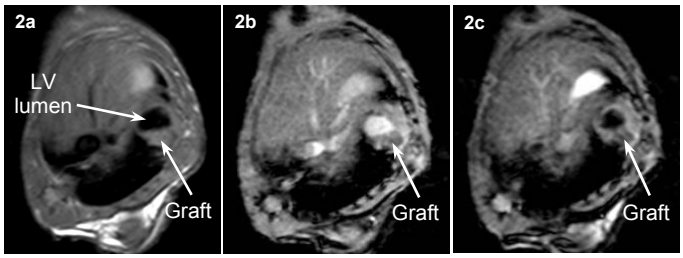

Figure 2. Transgenic C2C12 grafts overexpressing ferritin in the mouse heart. **2a**, PD TSE black-blood sequence (SIR=1.02); **2b**, T2* GRE bright-blood (SIR=0.7); **2c**. iMSDE black-blood (SIR=0.7).

Table 1. Graft-to-myocardium signal intensity ratio (SIR) in transgenic C2C12 overexpressing ferritin and unlabeled wild-type grafts.

	PD TSE	T2* GRE	iMSDE
Unlabeled graft (n=3)	1.0 ± 0.04	0.98 ± 0.12 **# $**	1.46 ± 0.26 **$**
Ferritin-tagged graft (n=6)	1.0 ± 0.07	0.67 ± 0.07 **#**	0.82 ± 0.06

All values shown as mean ± standard deviation.
#, statistically significant difference between ferritin and control; $p < 0.05$ (independent t-test, unequal variances).
$, statistically significant difference between different sequences at the same animal group; $p < 0.05$ (paired t-test).

Conclusion

Transgenic C2C12 grafts overexpressing ferritin were successully imaged *in vivo* in the infarcted mouse heart. Ferritin-tagged grafts moderately decreased MRI signal intensity; T2*-weighted GRE sequence was most sensitive for imaging of transgenic grafts capturing about 30% change in MRI signal intensity. Unlabeled wild-type C2C12 cells transplanted to the mouse heart did not change MRI signal intensity in T2*-weighted images. This study showed possibility of non-invasive visualization and quantification of cardiac grafts overexpressing ferritin. The important advantage of this approach is that the gene reporter divides with each round of cell division retaining a desired MRI contrast over the entire volume of growing grafts.

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