

Ferritin overexpression as a tool for detection of live cells transplanted into infarcted heart

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Introduction

Transplantation of embryonic stem cell derivatives is a promising therapeutic approach for heart failure. Current methods of studying stem cell engraftment rely heavily on postmortem histological sampling. This precludes longitudinal monitoring and hinders studies of biodistribution, survival and proliferation. Noninvasive MR imaging of grafted cells is possible through cell labeling using iron oxide nanoparticles [1-2]. Recent breakthroughs permit direct imaging of gene expression through over-expression of non-toxic proteins, such as ferritin, responsible for *in vivo* uptake of MRI-detectable probes [3-4]. The potential of using MR reporter genes to study fate of stem cells engrafted into infarcted myocardium has not been explored. The purpose of this work was to compare efficacy of MRI detection of live cells engrafted to the infarcted heart after preliminary *in vitro* cell labeling by iron oxide particles or by genetic manipulations leading to ferritin overexpression.

Methods

Mouse skeletal myoblasts (C2C12 cells) were labeled *in vitro* by overnight co-cultivation with Bangs iron oxide particles (Bangs Laboratories, Fishers, IN) without use of transfection agents [2]. In parallel, other C2C12 cells were engineered to overexpress ferritin. Murine ferritin heavy-chain cDNA with an HA (influenza hemagglutinin) epitope tag was donated by Dr. Neeman and Dr. Cohen at the Weizmann Institute, Israel [3]. C2C12 cells were transduced with pcDNA3-HA ferritin plasmid vector using a FuGENE6 reagent. Neomycin was added to the cell culture media at 1.2 mg/mL to select for stably transduced cells. We assessed viability of labeled as well as transduced cells, their proliferation and differentiation into multinucleated myotubes. Expression of ferritin was monitored by Western blot analysis (using monoclonal mouse HA-antibody). Prussian Blue staining was used to confirm iron accumulation in cytoplasm. Iron oxide labeled cells as well as transduced C2C12 cells overexpressing ferritin were transplanted into the infarcted left ventricle wall of synergic C3H mice. Infarction in the mouse heart was induced by permanent ligation of the left coronary artery. 150,000 cells were directly injected into the infarcted left ventricle wall of the mouse heart in 7 μ l of serum/antibiotics-free medium per animal. Along with live cell transplantation, two groups of mice were injected with dead C2C12 cells (cells were killed by repeated freeze-thaw cycles) previously labeled with Bangs particles or genetically modified for ferritin overexpression. All mouse hearts were imaged non-invasively on an Achieva 3T Philips scanner three weeks after the surgery using a dedicated solenoid mouse coil (Philips Research Laboratories, Hamburg, Germany). The imaging protocol included ECG-gated bright-blood 2D cine gradient echo (TR/TE=15/9.3ms; flip angle 15°; slice thickness 0.8 mm, resolution 195x160 μ m) and black-blood 2D iMSDE-prepared (improved motion sensitized driven equilibrium) turbo field echo pulse sequences (TR/TE=16/9.8ms; flip angle 15°; slice thickness 0.8 mm, resolution 195x160 μ m) [5]. The MRI data were validated against established histological and immunohistochemical methods (Picrosirius red histochemical staining to define the infarct zone, immunostaining for embryonic skeletal myosin, Prussian Blue iron staining).

Results

***In vitro* studies.** Labeling of C2C12 cells by Bangs particles as well as overexpression of ferritin did not effect cell viability, proliferation and differentiation. Prussian Blue staining indicated significant accumulation of iron in transgenic C2C12 cells overexpressing ferritin, as well as in wild type cells labeled with iron oxide particles. No blue cells were observed in the non-labeled wild type C2C12 cells. Expression of ferritin in transgenic cells was confirmed by Western blot analysis. Cell phantom studies showed highly significant changes in T1 and T2 relaxation time in cells overexpressing ferritin as well as in iron oxide labeled cells in comparison with wild type control.

***In vivo* studies.** Presence of cellular grafts in mouse heart was detected by MRI as dark areas caused by signal void effect of iron oxide particles as well as by ferritin overexpression led to increased accumulation of iron in those cells (Fig. 1). Improved artifact- and blood-suppression was achieved by implementation of a 2D iMSDE-prepared black blood turbo field echo pulse sequence to mouse heart imaging. Cellular grafts showed strong MRI signal decrease 3 weeks after transplantation of live C2C12 cells labeled with Bangs particles, live transgenic cells overexpressing ferritin and after intra-myocardial injection of dead particle-labeled cells; however no signal from ferritin was detected in hearts injected with dead transgenic cells. This shows that MRI signal from iron oxide particles does not represent live transplanted cells; and vice versa, that overexpression of MRI gene reporter ferritin can distinguish between live and dead transplanted cells. Histological staining for mouse embryonic myosin confirmed the presence of skeletal muscle grafts in the left ventricle of the mouse heart (Fig. 2). Prussian Blue staining indicated accumulation of iron in the hearts of mice that received live or dead C2C12 labeled by iron oxide particles as well as in the hearts transplanted with live transgenic cells overexpressing ferritin (Fig. 3).

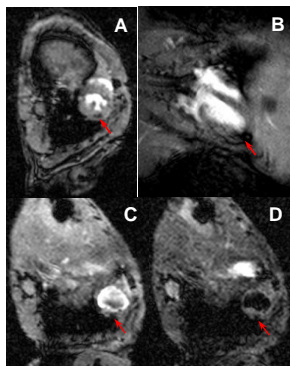


Figure 1. *In vivo* MRI of the mouse heart after transplantation of C2C12 cells. Stable signal void is revealed at the same area of the left ventricle (arrow) indicating presence of cellular graft.

A: live C2C12 labeled with Bangs particles. **B:** dead C2C12 labeled with Bangs particles. **C and D:** live transgenic C2C12 expressing ferritin. **C:** 2D bright blood turbo gradient echo cine pulse sequence. **D:** 2D iMSDE-prepared black blood turbo field echo pulse sequence.

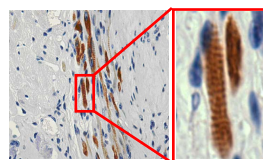


Figure 2. Embryonic myosin staining confirms presence of skeletal muscle graft in the left ventricle of mouse heart. Graft cells are well differentiated and contain sarcomeres.

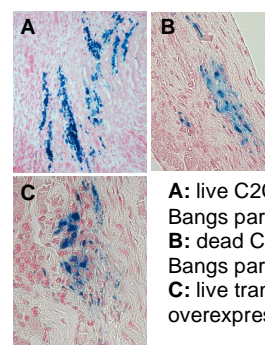


Figure 3. Prussian Blue staining indicates accumulation of iron in the mouse heart.

A: live C2C12 labeled with Bangs particles. **B:** dead C2C12 labeled with Bangs particles. **C:** live transgenic C2C12 overexpressing ferritin.

Conclusion

These data suggest that using iron oxide particles for cell imaging cannot distinguish between live and dead transplanted cells; therefore iron oxide particles cannot be used for MRI assessment of cell viability and for longitudinal monitoring of graft size. However, using MRI reporter gene ferritin instead can be a valuable tool for noninvasive detection of live cells transplanted into the heart. To our knowledge, this is the first use of MR imaging for the detection of gene expression in cardiac grafts. A combined genetic and non-invasive imaging approach may be useful to study the biology of cells transplanted into infarcted hearts.

References

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