Ferritin Overexpression for Molecular Imaging of Transplanted Cells

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Introduction

Application of MR reporter genes is a new strategy in molecular imaging based on over-expression of non-toxic proteins responsible for *in vivo* uptake of MRI-detectable probes [1-2]. Gene-based production of contrast agents for MR cell tracking has many advantages over the standard approach using exogenous administration of superparamagnetic particles, where the imaging signal does not reflect cell viability and quantity [3]. Ferritin has been proposed as an endogenous MRI reporter for noninvasive imaging of gene expression in C6 glioma tumors [1] and for *in vivo* studies in the mouse brain [2]. The potential of using MR reporter genes to study fate of stem cells engrafted into infarcted myocardium has not been explored. We aim to develop genetically-based technique for molecular imaging of the MRI gene reporter ferritin to enable noninvasive assessment of cell survival and biodistribution after transplantation into infarcted rodent heart.

Methods

We obtained the murine ferritin heavy-chain cDNA with an HA (influenza hemagglutinin) epitope tag as a gift from Dr. Neeman and Dr. Cohen at the Weizmann Institute, Israel [1]. Mouse skeletal myoblasts (C2C12 cells) were used as model for ferritin overexpression by pcDNA3 plasmid vector. C2C12 cells were transduced with plasmid DNA using FuGENE6 reagent. Neomycin was added to the cell culture media at 1.2 mg/mL to select for stably transduced cells. Expression of ferritin was monitored by Western blot analysis (using monoclonal mouse HA-antibody). Prussian Blue staining was used to confirm iron accumulation in transduced cells. Because standard DMEM media contains only very low amounts of iron (as ferric nitrate, ~0.2 μM of iron), we facilitated iron loading by supplementing the medium with ferric citrate in different concentrations (from 1 μM to 1mM). Effect of ferritin overexpression into viability, proliferation and differentiation of transgenic cells was compared with wild type control. T1 and T2 relaxation measurements were obtained from a single slice aligned through the center of the alive cell pellets in epiendorff tubes using a 3T Achieva Philips scanner. Control measurements were performed with non-transduced cells using the same media. For T1 measurements, an inversion-recovery sequence was used with 12 inversion times (TI) in a range 50-4000 ms, TE=9ms and TR=6000 ms. T2 measurements were performed using a multiple spin echo pulse sequence with 32 equally spaced echoes (10 ms echo spacing) and TR=5000 ms.

Results

Prussian Blue staining indicated significant accumulation of iron in transgenic C2C12 cells overexpressing ferritin (Fig.1), whereas no blue cells were observed in the non-transduced control. Expression of ferritin was confirmed by Western blot analysis (Fig. 2). Ferritin overexpression did not affect C2C12 viability, proliferation and differentiation into multinucleated myotubes (Fig. 3). To test the capability of MRI to detect the ferritin gene expression, we measured relaxation times in suspensions of transgenic C2C12 cells, cultured with and without iron supplementation. Overexpression of ferritin in C2C12 cells was detectable by MRI *in vitro* yielding significant decrease in T2 and T1 relaxation times (table 1).

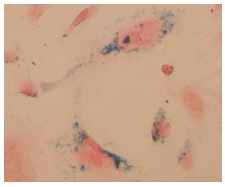

Figure 1. Prussian Blue staining indicating iron accumulation in C2C12 cells transduced by by pcDNA3-HAFerr plasmids.

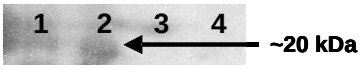

Figure 2. Western Blot analysis with mouse monoclonal HA-antibodies indicating ferritin expression in sense clones (1 and 2) of C2C12 cells transduced by pcDNA3-HAFerr plasmids, but not in antisense clones (3 and 4).

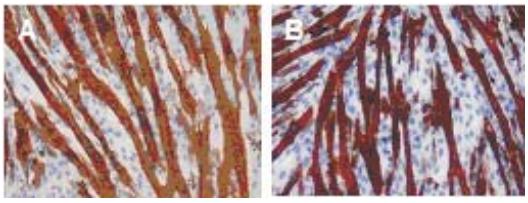

Figure 3. Skeletal myosin heavy chain immunostaining indicating comparable differentiation pattern into multinucleated myotubes in wild type (A) and in transgenic C2C12 cells overexpressing ferritin (B).

Table 1. *In vitro* MR relaxation times of live WT C2C12 and cells transduced by pcDNA3-HAFerr.

	WT C2C12 control, no Fe citrate	WT C2C12 + Fe citrate	Transfected C2C12, no Fe citrate	Transfected C2C12 + Fe citrate
T2, ms	629 ± 4	500 ± 4	467 ± 3	259 ± 4
T1, ms	2858 ± 39	2588 ± 15	2705 ± 24	1985 ± 25

Conclusion

Thus, in our preliminary studies we successfully constructed pcDNA3-HAFerr transduction vector, encoding ferritin. Mouse skeletal myoblasts transduced by the vector increased intracellular iron stores, confirmed by Prussian blue staining and yielding a robust detectable effect on T2 and T1 relaxation times *in vitro*. Our pilot studies are encouraging for further *in vivo* studies of transplanted cells in a beating heart.

References

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