Novel MRI Reporter Gene for Determination of Cell Viability

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Background

MRI is emerging as a main diagnostic modality in preclinical investigations of cell therapy for myocardial restoration. Dual ability to track iron-oxide labeled cells and characterize the myocardial tissue has enabled longitudinal monitoring of therapeutic efficacy. However, one limitation in iron-oxide based MR cellular imaging is the inability to determine cell viability. An MRI reporter gene may provide molecular marker of cell viability.

Objective

A novel MRI reporter gene driven by constitutively expressed CMV/ubiquitin promoter has been targeted to specific recombinant fusion gene designed to express antigenic epitopes on the surface of mouse embryonic stem cells (mESC). Employing commercially available SPIO-tagged monoclonal antibodies (SPIO-MAb) specific to these surface epitopes, viable mESC transfected with a reporter gene will generate MRI signal from the SPIO-MAb. *In vitro* proof-of-concept was performed to demonstrate generation of MR signal from the molecular marker of mESC viability.

Methods

MRI reporter gene was constructed employing a CMV/ubiquitin hybrid promoter (Invitrogen, Carlsbad, CA) to provide a robust expression of novel recombinant fusion protein that contains hemagglutinin (HA) and hCD4 surface epitopes and IRES-green fluorescent protein (GFP) moiety as shown in Figure 1. The fusion protein is targeted to the cell membrane by the hCD4 receptor transmembrane domain. The surface HA and hCD4 epitopes provide a target for MR signal amplification scheme employing commercially available SPIO-MAb specific to HA and hCD4 antigens. A streptavidin conjugated SPIO imaging agent, MACS Streptavidin Microbeads, have been bound to biotinylated monoclonal antibodies specific to HA and hCD4 antigens (Miltenyi Biotec, Auburn, CA). Expression of the synthetic fusion protein containing the surface epitopes will provide a specific molecular marker of viability. Plasmid DNA containing the transcript has been cloned using a mammalian expression vector pDisplay (Invitrogen, Carlsbad, CA) with neomycin resistance gene for homologous recombination in mESC. Using the clinical scale system of Magnetic Cell Sorting (MACS) Microbeads (Miltenyi Biotec, Auburn, CA), mESC expressing HA and hCD4 surface epitopes were selected.

Results

CMV/Ubq driven MRI reporter gene was constructed and amplified to transfect the undifferentiated mESC. Fluorescent activated cell sorting (FACS) demonstrated strong expression of GFP^+ confirming successful expression of reporter gene as shown in Figure 2. Transfection efficiency of almost 50% was achieved. Selection of mESC transfected by MRI reporter gene was performed using MACS Microbeads specific to HA and hCD4 surface antigens. This process generated approximately 4 x 10^5 HA^+ and 2.5 x 10^5 $hCD4^+$ mESC. *In vitro* MR images of the 2 magnetically sorted cell populations are shown in Figure 3. Both HA^+ and $hCD4^+$ cells (left) demonstrate significant dephasing signal using GRE sequence in comparison to 3 x 10^6 HA^- and $hCD4^-$ cells (right). Measurement of MRI signal demonstrated a highly significant dephasing signal in the MACS Microbead labeled (HA^+ and $hCD4^+$) cells versus the non-labeled cells (p<0.001).

Conclusion

MRI signal for molecular marker of cellular viability has been demonstrated. This method may enable both *in vivo* molecular and cellular MRI of stem cells in addition to the precise tissue characterization of the surrounding myocardium. *In vivo* molecular and cellular MRI detection of viable mESC in a mouse myocardium is currently underway.

Figure 1. MRI reporter gene.

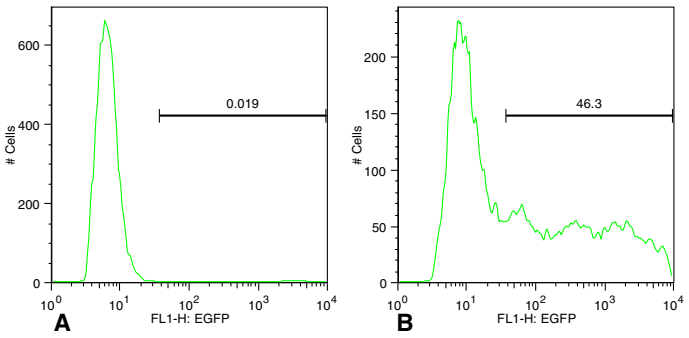

Figure 2. (A) non-transfected mESC without evidence of GFP activity and **(B)** transfected mESC demonstrating high GFP activity of 50%.

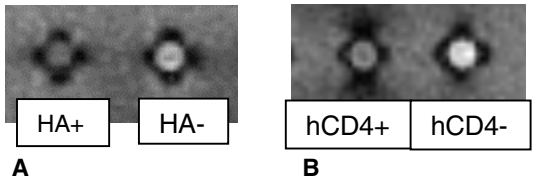

Figure 3. (A) *In vitro* MRI of HA^+ MACS Microbead labeled cells on the left and non-labeled cells on the right. Negative contrast from the signal dephasing generated from SPIO-MAb on the HA^+ cell surface can be detected visually and **(B)** $hCD4^+$ MACS Microbead labeled cells on the left and non-labeled on the right demonstrating similar signal dephasing.