In vivo molecular MRI of mouse embryonic stem cell viability

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Background
MRI has become one of the predominant modalities to monitor the efficacy of cell therapy to restore the injured myocardium. MRI enables robust tracking of *ex vivo* labeled cells and precise evaluation of myocardial function. However, MRI-guided assessment of the fundamental biology regarding cell viability remains a challenge. The transplanted stem cells ***at the very least must survive*** to restore the injured myocardium. Development of *in vivo* molecular MRI to image both the viability of the transplanted mESC and the myocardium will provide a critical link to evaluate cell therapy efficacy.

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 (FeMAb) specific to these surface epitopes, viable mESC transfected with a reporter gene will generate MRI signal from the FeMAb. *In vivo* proof-of-concept was performed in mouse to demonstrate generation of MR signal from the molecular marker of mESC viability.

Methods
MRI reporter gene is constructed employing pDisplay vector driven by CMV/ubiquitin hybrid promoter (Invitrogen, Carlsbad, CA) to provide a robust expression of novel recombinant fusion protein that contains hemagglutinin (HA) and c-myc surface epitopes and firefly luciferase gene (luc) (Fig 1). The luc gene is inserted to enable bioluminescence imaging (BLI) to validate mESC viability (Fig 2A). The fusion protein is targeted to the cell membrane by PDGFR-TM transmembrane domain. The surface HA and c-myc epitopes will provide a target for MR signal amplification scheme employing commercially available FeMAb specific to HA and cmyc antigens (Miltenyi Biotec, Auburn, CA). Expression of the synthetic fusion protein containing the surface epitopes provides the basis for a MRI-based reporter gene (RG) of cell viability. Stable mESC-RG line has been established with neomycin resistance gene for homologous recombination in mESC. $3x10^6$ each of mESC-RG and mESC (negative control) treated with 50ul of FeMAb-HA and FeMAb-cmyc are washed 3 times to remove excess FeMAb. These cells are transplanted directly into syngeneic mouse hind limb and the mice undergo GRE imaging (TE 10ms, TR 500 ms, FA 30°, FOV 12, NEX 1, 256x256) using 3-inch receive coil.

Results
Insertion of luc at Sac II/Sal I restriction sites is confirmed with restriction endonuclease (Fig 2B). Functional expression of mESC-RG is confirmed by BLI signal (Fig 2C) and HA-luc-cmyc on cell surface by red immunofluorescence (Fig 2D). $3x10^6$ mESC-RG and mESC (negative control) treated with FeMAb specific to HA and cmyc are injected into right and left murine hindlimbs, respectively. *In vivo* MRI of the murine hindlimbs in both axial and coronal views demonstrates strong dephasing signal generated from Fe-MAb bound to HA and cmyc antigens in the right hindlimb (Fig 2E). In contrast, the left hindlimb, which received mESC (negative control), demonstrates minimal dephasing signal (Fig 2E). Measurement of MRI signal demonstrates significant dephasing signal in the right hindlimb from the Fe-MAb bound to HA and cmyc surface antigens of mESC-RG whereas mESC in left hindlimb demonstrates minimal signal.

Conclusion
In vivo molecular MRI signal of mESC viability has been demonstrated. This method will enable dual *in vivo* molecular MRI of stem cell viability and physiologic tissue characterization of the surrounding tissue.

Fig 1. MRI reporter gene.

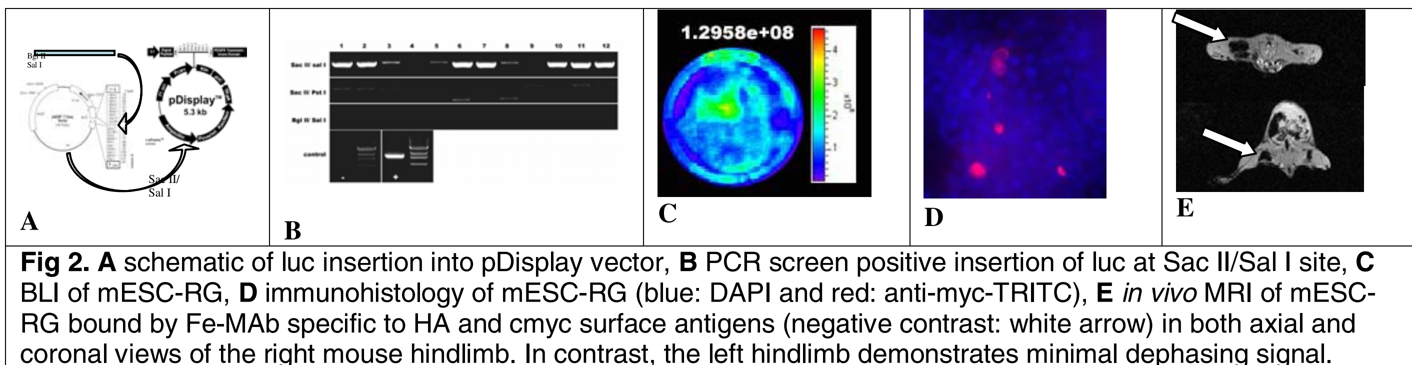

Fig 2. A schematic of luc insertion into pDisplay vector, **B** PCR screen positive insertion of luc at Sac II/Sal I site, **C** BLI of mESC-RG, **D** immunohistology of mESC-RG (blue: DAPI and red: anti-myc-TRITC), **E** *in vivo* MRI of mESC-RG bound by Fe-MAb specific to HA and cmyc surface antigens (negative contrast: white arrow) in both axial and coronal views of the right mouse hindlimb. In contrast, the left hindlimb demonstrates minimal dephasing signal.