MRI Tracking of Transplanted Cell Viability and Function Using a Multimodal Quadruple Fusion Gene Reporter

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Target audience: Basic and translational science researchers who are interested in non-invasive methods for assessment of transplanted cells survival, growth and therapeutic benefits.

Purpose: Transplantation of therapeutic cells is a potent approach to regenerate damaged tissues. The unmet needs in the cell transplantation studies are the following: 1) non-invasive detection of transplanted cell survival and longitudinal follow up of graft size; 2) evaluation of graft viability and proliferation; 3) assessment of structural and functional integration of graft with host tissue; 4) discrimination of immediate versus long term benefits of cell transplantation. Currently there is no single imaging modality that can evaluate all these endpoints. We propose an integrative approach to monitor transplanted cells non-invasively by combining MRI based structural and functional evaluation with other imaging modalities such as bioluminescence imaging, fluorescence and positron emission tomography (PET); for this we engineered and tested a novel genetically-based sensing system that makes graft detectable by multiple imaging modalities.

Methods: We have created a unique quadruple-fusion reporter gene construct for simultaneous expression of the following proteins in human stem cells: 1) ferritin, a natural iron storage protein detectable by MRI. Overexpression of endogenous ferritin enables high resolution longitudinal imaging of graft size without administration of contrast agents. 2) Herpes simplex virus thymidine kinase type 1 (HSV1-tk) is detectable by PET with 18FHBG and used for assessment of grafted cell proliferation and expansion. HSV1-tk transduced cells are sensitive to death induction by ganciclovir; the targeted induction of graft death can distinguish permanent versus transient effects of cell therapy. 3) Luciferase is an oxidative enzyme detectable by bioluminescence imaging and provides a highly sensitive method for assessment of graft cell survival. 4) GCaMP3 is a genetically encoded high-affinity calcium sensor, composed of a green fluorescence protein (GFP)-calmodulin fusion protein which is activated upon binding of calcium to calmodulin; this approach can be used to evaluate electro-mechanical coupling between graft and host heart tissue. A multifunctional gene construct was knocked into the AAVS1 locus using zinc-finger nuclease technology, creating a human embryonic (RUES2) stem cell line simultaneously expressing transgenes.

Results: Quadruple gene reporter construct did not affect RUES2 cell viability, proliferation and differentiation into the functional cardiomyocytes (fig. 1A). Transgenic cells demonstrated strong fluorescence (fig. 1B) and bioluminescence properties: 663,132 au vs. 24 au in WT control. Prussian Blue staining was used to detect increased accumulation of iron in overexpressed ferritin. Quantification of iron load was assessed by in vitro MRI: T2 relaxation time was measured in cell pellets using the 3T Achieva Philips clinical scanner and spin-echo multi-echo sequences with 32 equally spaced echoes (TE from 10 ms to 320 ms) and TR of 5000 ms. Q-PCR was used for mRNAs quantification. GCaMP3 green fluorescence was detected in beating cardiomyocytes by fluorescence microscopy. Luminescence was used to detect emission of light after luciferin administration to transgenic cells. Cytotoxicity test to ganciclovir was used to assess functionality of HSV1-tk. Western blot was quantified to protein expression.

Discussion: Transgenic RUES2 stem cell line carrying a novel quadruple-fusion gene reporter construct demonstrated strong expression of all transgenes. In vitro tests confirmed expression and functionality of each protein: Prussian Blue staining and MRI (ferritin), bioluminescence (luciferase), ganciclovir sensitivity test (HSV1-tk) and fluorescence (GCaMP3). Transgenic construct did not affect stem cell viability, proliferation and cardiac differentiation.

Conclusion: We have created a unique human embryonic stem cell line simultaneously expressing ferritin, luciferase, GCaMP3, and HSV1-tk that can be detected by different imaging modalities. Transgenic cells expressing quadruple gene reporter demonstrated T2 shortening effect in MRI, strong bioluminescence and fluorescence properties as well as ganciclovir sensitivity. Each reporter has unique properties that can be used to answer specific important biological questions related to stem cell transplantation. This integrative approach enables longitudinal non-invasive monitoring of the transplanted cells. Future in vivo multimodality imaging studies using a novel reporter will have an important scientific and practical value for studies in stem cell therapy.