USING FERRITIN AS A TRANSGENIC MRI REPORTER FOR MONITORING OF EMBRYONIC STEM CELL GRAFT IN VIVO

H. Mao[1,2], J. Liu[2,3], E. C. Cheng[2], R. C. Long[4], S. H. Yang[2], L. Wang[4], P. H. Cheng[2], J. J. Yang[2], and A. W. Chan[2]

[1]Department of Radiology, Emory Unversity School of Medicine, Atlanta, Georgia, United States, [2]Yerkes National Primate Research Center, Emory University School of Medicine, Atlanta, GA, United States, [3]Neuroscience program, Emory University School of Medicine, Atlanta, GA, United States, [4]Department of Radiology, Emory University School of Medicine, Atlanta, GA, United States

Introduction

Embryonic stem (ES) cells have shown great potentials in regenerative medicine and cell based therapeutics. The ability to visualize cell transplant *in vivo* over extended period of time is critical to the development of successful stem cell therapy. Tracking cell transplant with MRI can be done with pre-label cells with magnetic nanoparticles ex vivo. However, pre-graft labeling with exogenous contrast agents constitutes only a short term monitoring solution as MRI contrast may diminish overtime as magnetic nanoparticles diluted with cell division and degraded through cell metabolism. A molecular MRI reporter system based on endogenous gene expression for MRI contrast offers significant advantages in longitudinal cell graft monitoring. Using MRI reporter gene to genetically engineer ES cells to enable long term *in vivo* tracking with reporter genes can greatly facilitate stem cell research and its translation into clinical applications. Here we report our results on introducing a metalloprotein based MRI reporter gene, ferrotin, into the mouse ES (mES) cell and successful monitoring of the transgenic mES cell graft in mice.

Materials and Methods

Construct Design A lentiviral vector FU-IRES-GW derived from FUGW with internal ribosomal entry site inserted before EGFP sequence is used in the current study. Full length human Ferritin Heavy Chain (hFTH) cDNA was PCR modified to remove Iron Response Element (IRE) and create HA-tag at the N-terminus of hFTH before subcloned into the multiple cloning site of FU-IRES-GW. The resultant pLVUbi-HA FTH-IRES-EGFP vector constitutively express FTH under the ubiquitin promoter and co-express downstream EGFP through IRES.

Establishment of Clonal Transgenic mES Cell Lines Mouse embryonic stem cells (AB2.2 line) were cultured in mES maintenance medium. Human FTH carrying lentivirus was generated by co-transfecting pLVUbi-HA FTH-IRES-EGFP with packaging plasmid pΔ8.9 and envelope vector pVSV-G into 293FT packaging cells. Culture medium was collected 48hrs post transfection. mES cells were passaged as single cells and seeded at low densities onto inactivated MFF. Positive colonies were selected manually based on GFP expression. Each positive colony was passaged as single cells onto individual 35mm culture plates with MFF and the process was repeated if necessary till clonal transgenic lines were established. The resulted transgenic mES cell line was named HFG-mES.

Transplantation of HFG-mES in SCID mice Teratoma formation of FTH transgenic mES cells were confirmed by two subcutaneous (SC) inoculation of $1x10^6$ HFG-mES cells into one SCID mouse. At day 30 post inoculation, tumors were removed, paraffin embedded, sectioned at 8 μm and stained with hematoxylin-eosin to visualize tissue morphology characteristic of different germ layers.

In vivo Magnetic Resonance Imaging CD-1 nude mice (n=4) were chosen for *in vivo* MRI study. Following a within subject design, $3x10^6$ WT mES and HFG-mES cells were inoculated subcutaneously onto opposite flanks of each mouse to facilitate direct comparison. First MRI scans were performed on day 14 post transplant when tumor diameters reached 7-10mm. Repeat scans were performed on day 21. Mice were scanned using a 4.7-Tesla MRI scanner under anesthesia using 2% isoflurane through out the MRI experiments. High resolution MRI were performed in full extent of tumors with T_2 weighted fast spin echo sequence with TR of 5000ms multiple effective TE of 20, 40, 60, 80 ms, matrix 256 x 256. Typically, an FOV 40mm x 70mm, slice thickness 0.5mm, gap 0.15mm were used. Multi-echo images of slices cutting through the center of tumors across samples were used for calculating T_2 maps using the program ImageJ.

Iron Concentration, Histology & Immunohistochemistry Animals were sacrificed upon completion of MRI to confirm transgene expression in cell transplants (1 at D14 and 3 at D21 post inoculation). Tumor tissues were sectioned at 20 μm followed by staining with monoclonal HA antibody for HA tagged FTH expression as well as confirmation of GFP expression. Fe content of WT and FTH transgenic tumor samples were determined using ICP-OES analysis.

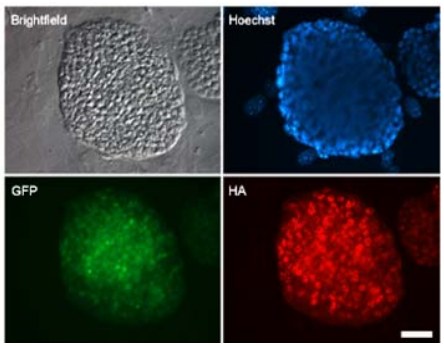

Fig 1. The homogenous expression of transgene was confirmed by expression of GFP and immunostaing using antibody specifically recognized the HA tag.

Results and Discussions

Transgenic mES cell lines carrying human ferritin heavy chain were established using lentiviral vector pLVUbi-HA-FTH-IRES-EGFP (pLVU-HFG) which constitutively expresses under ubiquitin promoter hemagglutinin (HA) tagged human ferritin heavy chain (HA FTH) and enhanced green fluorescent protein (EGFP). The resultant transgenic HFG-mES cells have maintained healthy morphology and stable homogenous transgene expression confirmed with ICC (**Fig. 1**) and western blot using the antibody against HA. HFG-mES cells have proliferation rate closely matches that of the wide type (WT) mES cell controls, with doubling time estimated at 12.25 hrs (transgenic) and 12.15hrs (WT) respectively. FTH transgenic mES cell line maintained its pluripotency as expression of early stem cell markers in HFG-mES cells were confirmed by immunostaining using antibody specific for Oct 4 and SSEA-1. FTH as a MRI reporter in mES cells were validated in vivo using a mouse model with WT mES and HFG-mES cells graft (n=4). Measurements of MRI signal decay at multiple TE points showed significantly decreased T_2 relaxation time in transgenic mES line overexpressing ferritin. Repeated scans in one week with the same animal revealed continued decrease in T_2 relaxation time observed in transplants of HFG-mES origin (Fig. 2). FTH induced change of MRI contrast is estimated at an increase in transverse relaxation rate ($R_2=1/T_2$) between 15%-28% (**Fig. 2**). Post-mortem analysis showed that transgenic mES neoplasia can be distinguished under fluorescence microscope. Homogenous HA tagged FTH transgene expression was confirmed with immunstaining of tissue sections. An average 80% increase of the iron content was found in transgenic ES cell graft (Fe 226.9 ppm) compared to that of WT sample (Fe 126.1 ppm). Our results suggested that MRI reporter based cell tracking method may allow for non-invasively localize and follow the implanted graft, assess the risk of neoplasia growth and study *in vivo* gene expression linked to that of reporter genes.

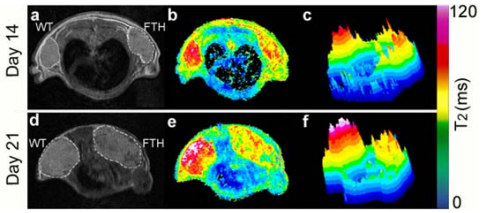

Fig 2. T_2 weighted fast spin echo images showing the same pair of tumors from ES grafts (left: WT, right: FTH transgenic), at day 14 (a) and day 21 (d). Corresponding color coded T_2 maps (b & e) showed significant reduction of T_2 relaxation time in the tumor overexpressing FTH transgene. Surface plot of T_2 values (c & f) suggests the greater T_2 difference between WT and transgenic tumors.