SPIO-labeled cells as an ffective vehicle for tracking GFP gene marker with MRI

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Introduction: Recent advances in non-invasive cellular and molecular imaging have provided new research tools for monitoring the expression of different genes and the activities of various signal transduction pathways. Cell and gene imaging has primarily been accomplished through fluorescence microscopy methods. More recently, it has become possible to depict biological processes *in vivo* using MRI complementing the work at the *in vitro* cellular and molecular level. The ability to non-invasively map gene expression *in vitro* or *in vivo* has tremendous implications for biomedical research and is crucial in gene medicine(1). We have developed a new generic method for *in vitro* MRI of iron-loaded cells with GFP expression capabilities, which may permit *in vivo* MRI of gene expression of transplanted cells in the near future. These GFP-expressing cells were labeled with superparamagnetic iron-oxide (SPIO) MR contrast agents. Due to the compatibility of SPIO nanoparticles used for MRI cell tracking and GFP-expressing gene markers used for fluorescent imaging monitoring, we hypothesized that SPIO can be used to effectively label GFP-expressing cells with no effects on cell viability, proliferation, or GFP expression. The iron-loaded GFP-expressing cells can be visualized using MRI to track these cells indirectly. This technique may be expanded to a clinical gene transfer research setting and be used in deep organs and tissues to monitor targeted genes and cells for longevity, efficacy, and safety.

Methods: The GFP-expressing R3230Ac cell line (kindly provided by the Dewhirst group, Duke University, USA) was incubated for 24 hours using SPIO (Feridex®, Advanced Magnetics, USA) at a concentration of 20 µg Fe/mL. Labeled cells were then visualized with microscopy and MRI successively. Cell samples were prepared for iron content analysis and cell function evaluation. Pellets of labeled cells (0.5, 1, and 2 million per pellet) were imaged using a 3.0T scanner (Trio, Siemens, Erlangen, Germany) with a 3D T2*-weighted GRC sequence, T_R/T_E = 1000/22.4 ms, voxel size = 0.5 x 0.5 x 0.5mm^3.

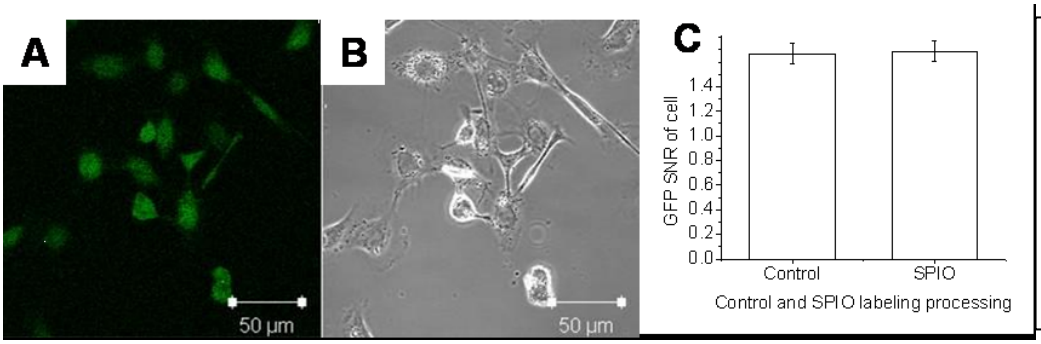

Fig.1Confocal microscopic slice of labeled GFP-cells with PB staining: GFP expression of labeled cells on panel A, and microscope slice of the same view verifying the uptake of SPIOs (dark spots in cytoplasm) on panel B. no difference in GFP expression between labeled and unlabeled cells panel C.

Results: SPIO was used to effectively label GFP-expressing cells with no effects on cell function and GFP expression (Fig.1). Iron-loaded GFP-cells were successfully imaged with both fluorescent microscopy (Fig.1) and T2*-weighted MRI (Fig. 2). Prussian blue staining revealed intracellular iron accumulation on histological slices, with a 100% labeling efficiency. Average iron content per cell was 4.75 ± 0.11 pg Fe/cell ($p<0.05$ versus control).

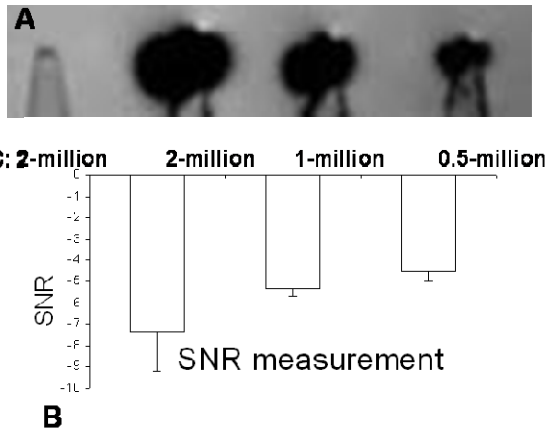

Fig.2. Panel A: a representative T2* weighted MR image of GFP-cell pellets: Labeled 2, 1, and 0.5 million labeled cells. All labeled cell pellets showed a marked susceptibility effect compared with 2 million unlabeled cells as control group (C: 2-million). Measurement of signal intensity of samples on images on panel B.

Discussion/Conclusion: A commercially-available FDA-approved MR contrast agent was used to effectively label GFP-expressing cells with no effects on cell viability, proliferation, or GFP expression. Clinical experience in use of MR contrast agents should allow translation of this MRI-based method from the experimental setting *in vitro* to *in vivo* or clinical trials to indirectly monitor gene markers. SPIO-labeling of cells with specific gene expression holds promise for monitoring the temporal and spatial migration of gene markers and is likely to enhance the understanding of gene-based therapeutic strategies.

References: [1] Chudakov DM et. al. TRENDS in Biotechnology 2005; 23(12): 605-613. [2] Li CY et. al. J Natl Cancer Inst 2000; 92(2): 143-147.