A gene reporter system for detection of cellular LacZ expression by magnetic resonance imaging

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INTRODUCTION:

The purpose of this study was to evaluate the use of a histochemical substrate, commonly used for the in vitro detection of marker gene expression, for the in vivo detection of β-gal expression in bone marrow cells (BMCs). Contrast agents have been extensively used for MR cell tracking studies but in many cases their use has been limited to following either the initial delivery of cells or to track slowly symmetrically, dividing cells(1). It may not always be possible to avoid rapid cell turnover or loss in many therapeutically relevant cases. For example following myeloablation and bone marrow transplantation, a small subset of BMCs called hematopoietic stem cells (HSCs) will respond to replenish the depleted bone marrow and restore homeostasis by rapid cell division. In order to track HSCs and their progeny *in vivo*, a genetic approach would be a valuable tool to follow this dynamic cell population. In this study we determine whether the widely established gene marker, β-gal, could be detected by MRI following exposure to S-gal and ferric ammonium citrate (FAC) *in vitro* and *in vivo*. The effects of adding S-gal to β-gal expressing BMCs, were determined by monitoring cell viability and changes in T_2 and T_2* in cell phantoms followed by *in vivo* transplantation studies of labeled BMCs between 4.7-17.6T.

METHODS:

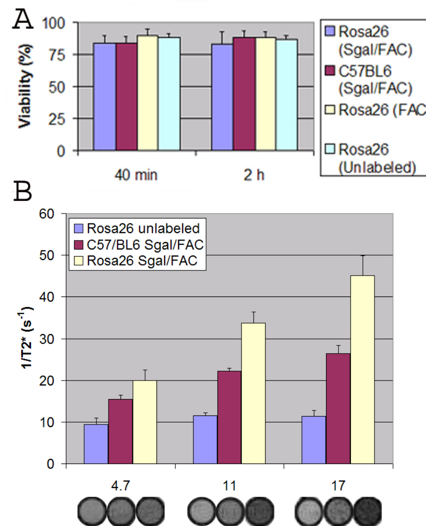
BMCs were harvested from C57BL/6 and B6ROSA26 mice by flushing bone marrow from femurs and tibias. Cells were re-suspended with 1 mg/ml S-Gal (Sigma) and 0.5 mg/ml FAC (Sigma) in 1ml PBS, 0.5 mg/ml FAC in 1 ml PBS or 1 ml PBS in microcentrifuge tubes and incubated at 37°C for 2 hours. BMCs were then washed twice with PBS to remove free S-Gal and FAC and filtered through FACS tubes with cell strainer caps to remove large aggregations of S-Gal and cells. Finally, BMCs were counted, assayed for viability by Trypan blue exclusion test (n=5), and re-suspended at a concentration of 8×10^7 cells/ml in 100 µl PBS. Cell phantoms were made by adding 100 µl 2% Agarose to the labeled BMCs and injecting the solution into 100 µl capillary tubes to keep cells stationary during imaging (n=5). Phantoms with a final cell concentration of 4×10^7cells/ml were placed in a water filled FACS tube to minimize susceptibility artifacts at the edges of the samples. Phantoms were kept on ice and imaged with T_2* weighted FLASH gradient echo (GE) scan sequences within an hour after being produced at 4.7, 11.1 and 17.6 T magnetic field strength magnets using Paravision® software (PV3.02;Bruker Medical). TR was kept constant at 500 ms with varying TEs of 4, 8, 12, 16, 20, 30, 40, 60, 80 and 100 ms, FOV:2.8×2.8 cm, Matrix size:256×256, 2 signal averages, Spectral width = 60 kHz and 1 mm slice thickness. ImageJ software (NIH) with the MR analysis calculator plug-in was used to quantify T_2* between field strengths of 4.7-17.6 T. Data are presented as the mean±SD of measurements. Subsequently, 0.5×10^6 S-Gal/FAC labeled B6ROSA26 and C57BL6 bone marrow cells in 40 µl PBS were injected into C57BL6 tibialis anterior (TA) muscles (left and right legs respectively) (n=3). The animals were anesthetized using a mix of 2% Isofluorane in oxygen. The hind limbs were placed inside a 1cm solenoid coil or loop-gap coil and imaged with a 3D GE scan sequence at 4.7 and 11.1 T magnetic field strengths with; TR = 100 ms, TE = 5 ms, Spectral width = 100 kHz, 30º pulse angle, FOV = 1.45×1.20×2.40 cm and matrix size 384×192×64. Images were acquired with Paravision® - and analyzed with OsiriX software.

RESULTS:

Trypan blue exclusion test indicate that labeling BMCs with S-gal/FAC has minimal effect on cell viability (Figure 1A). BMC phantom data shows a reduction in T_2* relaxation due to the specific reaction between S-gal and β-galactosidase and that this T_2* effect is markedly increased with increasing magnetic field strengths (Figure 1B). The *in vivo* detection of S-gal/FAC labeled B6ROSA26 BMC (green) was dramatically increased compared to labeled C57BL6 BMCs (purple), as indicated by the much larger T_2*-effect generated by the β-gal expressing BMCs in the left TA (Figure 1c). Furthermore, in vivo T_2*-contrast from the labeled cells was also enhanced with increasing magnetic fields (4.7T vs. 11.1T).

CONCLUSIONS:

S-gal reacts with β-galactosidase to produce a dark iron-rich precipitate that can be detected simultaneously by MRI and histology. We found that S-gal labeling reduced T_2* relaxation time for β-gal expressing BMCs more than control cells and the change in T_2* increased with increasing magnetic field strengths. The dramatic decrease in T_2* relaxation time translated into increased sensitivity and detection capabilities of transplanted BMCs *in vivo* at 11.1T compared to 4.7T.



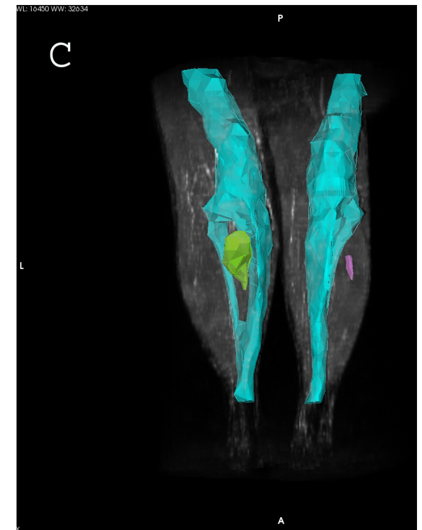


Figure 1: A) Viability assay by Trypan blue exclusion test at 40 minutes and 2 hours of labeling. **B)** 1/T_2* values from 4.7, 11 and 17T. Insets depict phantom images from the various field strengths with TE=30ms. **C)** 3D rendering image (OsiriX) of implanted S-gal/FAC labeled B6ROSA26-(left) and C57BL6 (right) BMCs acquired at 11T.

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