A Generic Gene Reporter for MR Microscopy

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Introduction:

Recent advances in both MR Microscopy (MRM) allow for imaging on the cellular level similar to light microscopy. Likewise there have been recent advances in the use of MR stains to enhance cellular contrast in MRM but there have been few MRM based reporters of gene expression similar to those used in fluorescent microscopy, such as targeted fluorescent protein expression. Here we report on the use of a generic gene reporter, commonly used for the histological analysis of gene expression using light microscopy but is also able to generate MR contrast based on iron accumulation.

Experimental:

Two general experimental paradigms were explored using the expression of β-galactosidase (LacZ) as the genetic marker. The first investigation assesses cytoplasmic expression of LacZ by muscle stem cells *in vitro*. The second study investigates nuclear LacZ (nLacZ) expression in a transgenic mouse model. In both investigations, ferric ammonium citrate (FAC) was combined with S-Gal™ as substrates for LacZ to produce an iron containing black deposit, visible both optically and by MR[1]. In addition, we have previously shown[1] that this MR stain can work *in vivo* and that image contrast increases with increasing magnetic field leading to an ideal MR gene marker for MRM. All experiments were performed at 14T using either a 5 mm volume coil (Bruker) for the whole muscles (cytoplasmic LacZ in regenerated muscle fibers (Fig 1) or with a micro surface-coils of 200 or 500 µm (Bruker Biospin) in diameter for single muscle fibers expressing nLacZ (Fig 2). All muscle samples were imaged after the incubation in FAC/S-Gal™ as previously described[1]. Whole muscles were imaged in PBS and individual muscle cells were isolated then embedded in low-melting point agarose after serial PBS washes.

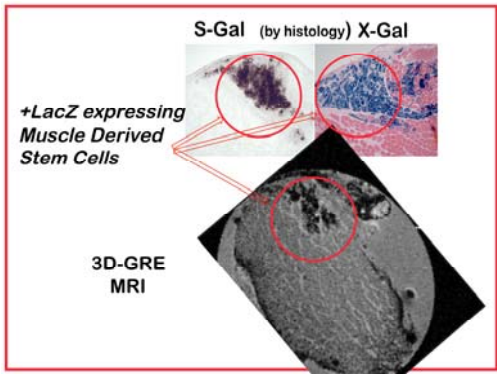

Fig 1: Microscopic Imaging of cytoplasmic LacZ expression

Results and Discussion:

Displayed in Fig 1 are histological images of cytoplasmic LacZ expression from S-Gal (black) or X-Gal (Blue) staining along with the corresponding axial 3D gradient echo MRI slices taken. The ability of MRM to image individual muscle cells and nLacZ expressing nuclei was obtained with a 500 µm and 200 µm diameter surface coils capable of image resolutions of 8 µm and 6 µm isotropic, respectively (Fig 2 bottom). Optical and MRI contrast generated by S-GAL/FAC in the presences of LacZ appeared dark both by MRI and light microscopy inside regenerated muscle cells Fig 1 and within individual myonuclei (Fig 2). In the absence of LacZ, both optical (Fig 1 and 2 top) and MRI image contrast is homogenous throughout the muscle cells (Fig 1-bottom, Fig 2 bottom -right).

Conclusions:

MRM using microcoils at 14T demonstrated that not only can single muscle cells be directly visualized with MRI, but with the use of a genetic reporter, so can individual myonuclei and cytoplasmic gene expression can be detected. This genetically targeted MRI contrast methodology could have widespread applications, including the tracking of stem cells as well as the verification of specific cellular structures and proteins using MRM in a similar fashion to fluorescent microscopy.

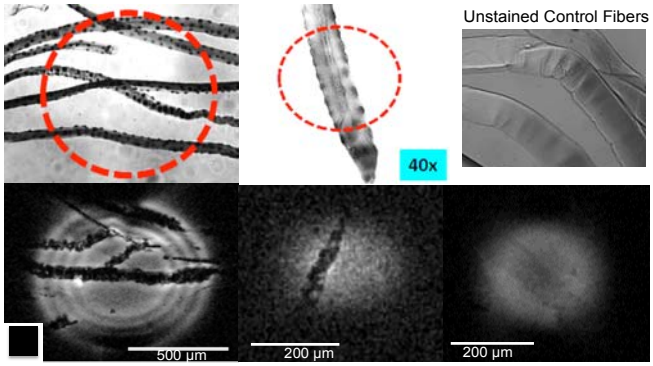

Fig 2: Microscopic Imaging of nuclear LacZ expression
Top Row: Light microscopic images (top row) and MRI (bottom row) of Sgal labeled myonuclei imaged with 500 µm (left) and 200µm coils (middle and right) and control unstained fibers(right). Red circle indicates approximate coil size

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References:

[1] Bengtsson NE et al. MRM. 63(3): 745, 2010.
[2] Stuelsatz P et al, Dev Biol. Jan 1;397(1):31,2015.[3]Walter et al, MRM, 51(2):273,2004.