Detection of beta-galactosidase activity in a human tumor xenograft by 1H MRI in vivo using S-gal

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Introduction

Gene-based therapy has been stimulated by remarkable progress in understanding molecular biology. The development of non-invasive reporter techniques based on appropriate molecules and imaging modalities may help to assay gene expression. LacZ, which produces beta-galactosidase, has been the primary choice of reporter gene to verify effective transfection in biochemistry for many years, and due to its broad spectrum of activity, many reporter molecules are available for biological and histological analysis. Last year, it was shown that the "black stain", S-Gal™ had potential as a proton MRI reporter in *E. coli* (Cui *et al*, *#1712*, ISMRM Kyoto, 2004). Upon cleavage by beta-galactosidase in the presence of ferric ions (Fe^{3+}), the aglycone chelates iron to produce an intense black stain, which is not only visible, but also paramagnetic. We now report the first results *in vivo* demonstrating this novel approach to detecting gene activity in LacZ transfected MCF-7 tumor cells and MCF-7/LacZ xenograft tumor.

Materials and Methods

Sodium - S-gal (3,4-cyclohexenoesculetin-beta-D-galactopyranoside) sodium and ferric ammonium citrate (FAC) were obtained from Sigma. MR images were obtained using a Varian Unity INOVA 4.7 T 40 cm horizontal MR system. As an in vivo tests to show cell expression, a mixture of 10^6 human breast tumor MCF7 or MCF7/LacZ cells in PBS with S-gal Na (300 µg/ml) and FAC (500 µg/ml) was put into 10 mm NMR tubes as an interlayer between agarose. For *in vivo* experiments MCF-7/LacZ tumor cells were implanted in the thigh of SCID mice and allowed to grow to about 0.7 cm^3. Then 50 mg/kg S-gal-Na and 25 mg/kg FAC in saline were injected intraperitoneally into the anesthetized mouse and tumor observed using a volume coil at 4.7 T over a period of 2 h.

Results

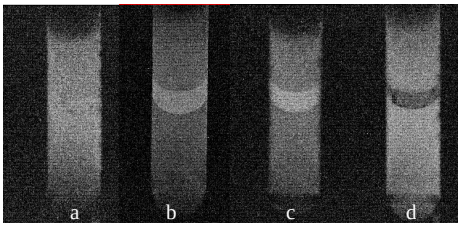

Fig1 a) MCF-7WT cells with S-gal Na and FAC; b) MCF-7-LacZ cells with FAC; c) MCF-7-LacZ cells with S-gal; d) MCF-7-LacZ cells with S-gal and FAC; (TR/TE=1000/30ms, in plane resolution 80x60 µm, thickness= 2 mm).

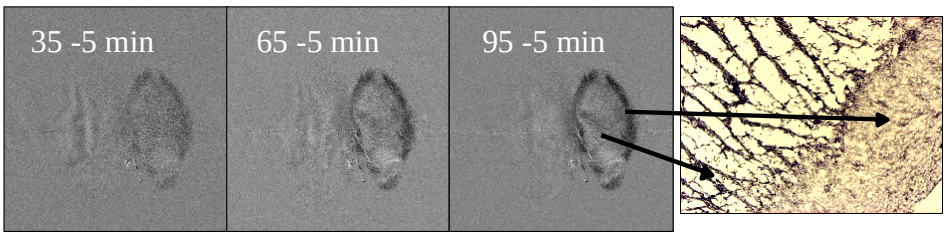

Fig 2. Subtraction images over time from T2* weighted data obtained using gradient echo MRI sequence (TR=500 ms, TE=8 ms, FOV= 256x256) of MCF-7/LacZ tumor. Contrast is primarily in tumor periphery. Post mortem histology showed intense stain in the tumor periphery with central tumor necrosis.

The images demonstrate that S-Gal is sensitive to beta-galactosidase, generating a paramagnetic black precipitate in the presence of FAC revealed as intense T2* contrast. The contrast may be detected in LacZ transfected MCF-7 breast tumor cells and the corresponding xenograft tumor. The progressive contrast occurred as signal loss as 16.7%, 22.5% and 29% in the tumor periphery.

Conclusion

S-Gal™ is commercially available and readily enters cells. Action of beta-gal rapidly generates an intense black precipitate, which induces strong T2* relaxation and intense MRI contrast in beta-gal expressing tumor cells and tumor. We believe this holds great promise as a novel proton MRI approach for imaging gene activity and detect gene function *in vivo*.

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