Dual ^{1}H and ^{19}F MR LacZ Gene Reporter Molecule

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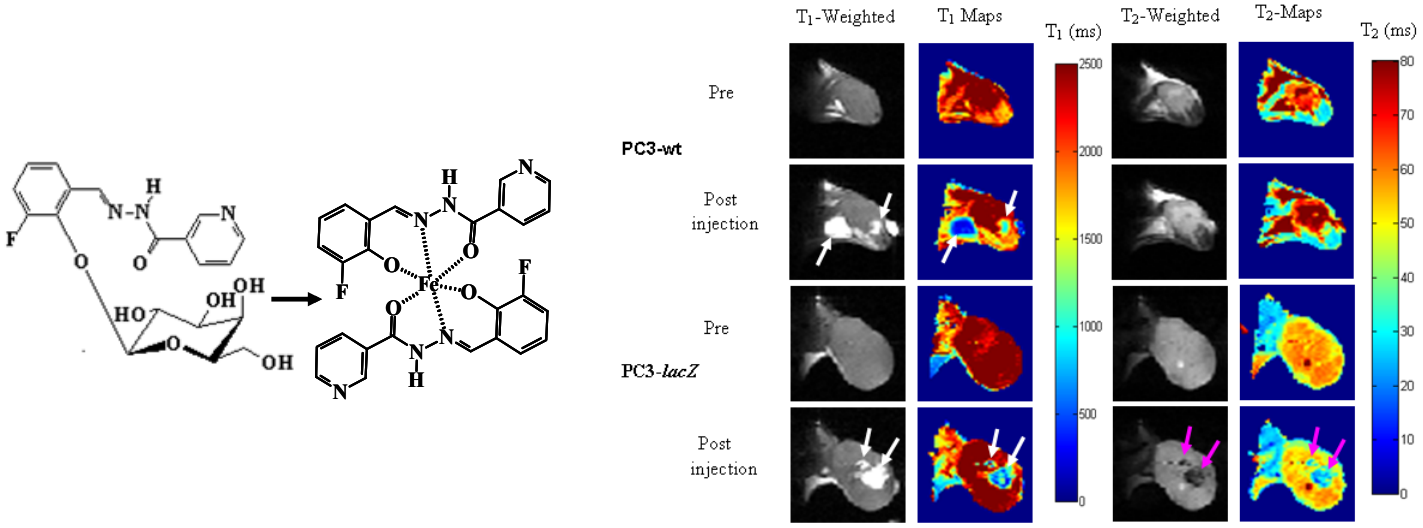
INTRODUCTION: The *lacZ* gene encoding enzyme beta-galactosidase (β-gal) is widely used as a reporter gene in diverse biological investigations including gene therapy. However, assessing β-gal activity *in vivo* remains challenging. Many colorimetric substrates have been developed over the years to assess β-gal activity and more recently *in vivo* imaging has been demonstrated based on fluorescence, bioluminescence, PET, SPECT, optoacoustics, and NMR. Notably, Meade *et al.* pioneered proton MRI contrast agents [1], and Mason *et al*. demonstrated ^{19}F-labeled molecules as NMR active substrates [2]. Proton MRI alone can suffer from difficulty in identifying induced contrast in heterogeneous tissues, while ^{19}F NMR often lacks SNR for effective imaging and is restricted to spectroscopy. A major strength of ^{19}F NMR is the ability to observe substrate and product simultaneously based on chemical shift. A novel combined dual ^{19}F/^{1}H MR *lacZ* gene detection approach based on (2-[(β-*D*-galactopyranosyl)oxy]-3-fluorobenzaldehyde nicotinoyl hydrazone; GFBNH- figure below) was demonstrated *in vitro,* whereby ^{19}F chemical shift reveals substrate and product and ^{1}H MRI shows contrast from product [3]. We now show *in vivo* applications of GFBNH as a *lacZ* gene reporter molecule in mice.

METHODS: PC3 cells (wild type and stably transfected to express *lacZ*) were implanted subcutaneously in thighs of SCID mice (n=3). A solution of GFBNH (50 µL 50 mM, DMSO/PBS 1:1 V/V') was injected directly into the tumor (~0.8 cm in diameter), followed by ferric ammonium citrate (FAC) (50 µL 50 mM, PBS). T_1 and T_2 weighted ^{1}H MR images were obtained before GFBNH injection and after FAC injection using a 2 cm diameter home built volume coil (tunable from 188.2 MHz for ^{19}F to 200.1 MHz for ^{1}H). ^{19}F MR spectra were obtained after GFBNH injection and after FAC injection using the same coil. The same procedure was followed for both wild type and *lacZ* tumors. The animal temperature was maintained throughout the experiment at 37 ^{o}C by a warm pad with circulating water. Control experiments were also performed in the muscles of live and dead wild type and ROSA26 mice.

RESULTS AND DISCUSSION: A significant drop in T_1 values was observed after injection of GFBNH and FAC, attributed to the FAC, in both wild type and *lacZ* tumors verifying injection. The corresponding T_2 maps showed a significant drop in T_2 only in the *lacZ*-transfected tumors with no change in wild type tumors, indicating the formation of superparamagnetic precipitate in *lacZ*-transfected PC3 tumor. ^{19}F spectroscopy showed the presence of GFBNH only in the wild type tumors, whereas no substrate or product signal was observed in *lacZ*-transfected tumors. The absence of GFBNH and its product in ^{19}F NMR spectroscopy indicates the rapid conversion of the substrate and precipitation of product. This was confirmed in tissues post sacrifice where signal decline was observed in the absence of any possible perfusion.



Left**:** Molecular structure of substrate and predicted iron-chelating product. Right: T_1 and T_2 maps indicate signal intensity drop at injection site. First column presents the T_1-weighted images and T_1 maps. Signal drop, indicated by small arrows, can be noticed in both wild type and *lacZ* tumors. T_2-weighted and T_2 maps in the second column indicate a signal drop in *lacZ* tumor, but no change in wild type tumor.

CONCLUSION: The strong negative contrast in the T_2 maps in *lacZ* tumors indicates that GFBNH is a promising ^{1}H MR gene-reporter molecule. T_1 contrast and ^{19}F signal each reveal the presence of substrate providing evidence for lack of β-gal activity. Importantly, GFBNH had appeared to be a poor substrate in cell culture, but contrast was observed almost instantaneously *in vivo*.

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REFERENCES 1) *Nature Biotechnol*.2000;18:321; 2) *NMR Biomed.* 2008;21:704; 3) *The World Molecular Imaging Conference*, *#676*, Nice, France, September 10-13, 2008.