Detection of *LacZ* Gene Expression In PC3 Prostate Xenograft By ^{19}F NMR

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Introduction

Gene therapy shows promise for treating prostate cancer and is being exploited in several clinical trials. A major hurdle is establishing a method of verifying transgene activity *in situ*. β-galactosidase (β-gal) was historically the most popular reporter gene for molecular biology. We are designing non-invasive ^{19}F NMR approaches to reveal β-gal activity *in vivo*. 2-Fluoro-4-nitrophenol-β-D-galactopyranoside (OFPNPG) belongs to a novel class of NMR active molecules, which are highly responsive to the action of β-gal. OFPNPG has a single ^{19}F peak at -55 ppm relative to aqueous sodium trifluoroacetate (NaTFA). Upon cleavage by β-gal, the pH sensitive aglycone OFPNP is observed at a chemical shift of -59-61 ppm [1]. The change in the chemical shift allows assessment of β-gal activity with magnetic resonance chemical shift spectroscopy and imaging.

Methods

PC3/LacZ tumor cells were generated by recombinant plasmid phCMV/lacZ transfection and a high expressing clone selected by X-gal and S-gal staining and Western blot (Fig. 1). $2X10^6$ PC3 wild type and lacZ stably transfected cells were implanted in the flank of nude mice and allowed to grow to about $1cm^3$ during 1 month. Following an intra-tumoral injection of 50 µl OFPNPG (78mg/ml, water:DMSO::1:1 mixture with sodium trifluoroacetate (TFA) as a chemical shift reference standard), the tumors were placed in a volume coil and ^{19}F NMR spectra were obtained on a 4.7T Varian scanner (188.2 MHz). Over a period of 30 min conversion of OFPNPG to product OFPNP was revealed by development of a new upfield signal unequivocally demonstrating β-gal activity. Each spectrum took 5 min with 128 averages. After the NMR experiment, the mice were sacrificed for histology, Western blot and β–gal assay.

Conclusions

These results provide our first observations that the chemical shift response is sufficient to observe β-gal activity by ^{19}F NMR in PC3 human prostate tumor xenografts in mice. This approach directly reveals β-gal activity, which could be used in tandem with therapeutic genes to monitor therapy. As gene therapy becomes a reality, the ability to detect transgene expression non-invasively will become increasingly important for treatment planning and optimization. The ^{19}F NMR approach is feasible in vivo in a prostate tumor growing in a mouse. Meanwhile, we are also developing new generations of ^{19}F NMR reporter designed to minimize toxicity. This study provides further evidence of the utility of OFPNPG as a gene-reporter molecule to develop gene therapy in animals. More importantly, it could both accelerate the transfer of gene therapy to patients in the clinic and provide a new tool for evaluation of gene therapy.

Results

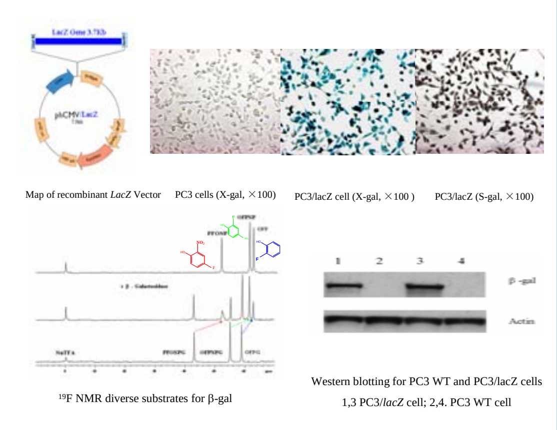

Fig 1. Screen for stable expression of β-gal PC3 cell lines

The phCMV/*lacZ* plasmid map is top left. When PC3 and PC3/LacZ cells were stained by X-gal and S-gal, more than 90% PC3/*lacZ* cells were stained blue or black, while the PC3 wild type cells were not colored. This result shows *lacZ* gene expression in PC3 cells after passage for 30 generations. To develop enhanced reporter molecules, diverse substrates were synthesized and the activity of β-gal is shown for 3 representatives. OFPNPG has a single ^{19}F peak with chemical shift of -55ppm. It is cleaved by β-gal to OFPNP, which has a pH sensitive chemical shift of -59-61ppm.

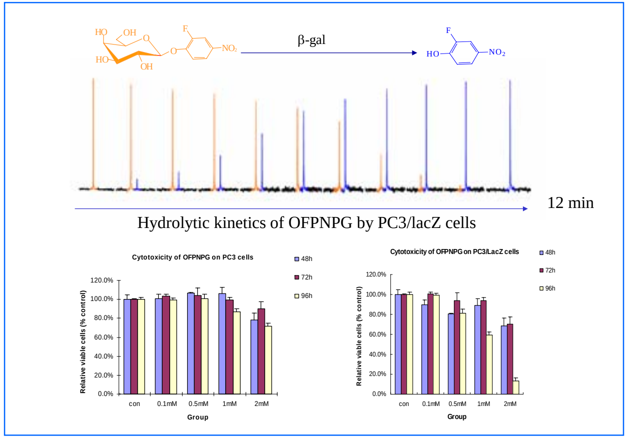

Fig 2. *In vitro* detection of β-gal expression

Top: Hydrolytic kinetics of OFPNPG by PC3/LacZ prostate cancer cells (5×10^5) in PBS buffer at 37 °C (orange lines--signals of OFPNPG; blue lines-signals of product aglycone OFPNP). Spectra acquired in 60s over 12min.
Bottom: PC3 WT and PC3/lacZ cell viability for OFPNPG detected by crystal violet methods showed less toxicity in PC3 WT cells than in PC3/lacZ cells.

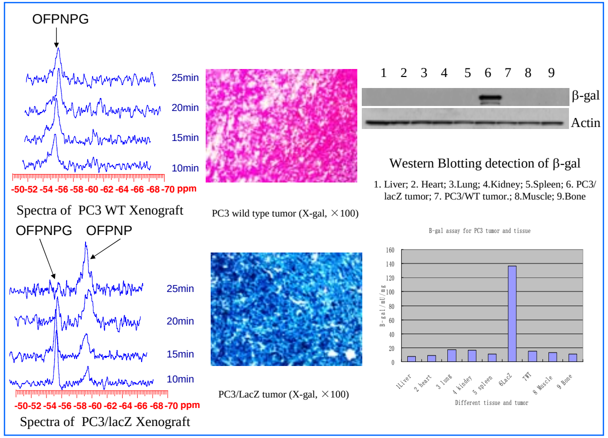

Fig 3. *In vivo* detection of β-gal expression

Over a period of 30min conversion of OFPNPG to product OFPNP was observed by ^{19}F NMR unequivocally demonstrating β-gal activity. Left: Shows spectra of wild type tumor (1.3cm x 1cm x 0.6cm) and PC3/lacZ tumor (1.4cm x 1.5cm x 0.8cm). Tumor and tissues were also examined by Western blots and β-gal assay. High β-gal activity was found in the tumor, with minimal activity in normal tissues. The definition of β-gal unit is that one unit will hydrolyze 1.0µmole of o-nitrophenyl β-D-galactoside to o-nitrophenol and D galactose per min.

Reference

1. Yu JX, Otten P, Ma Z, Cui W, Liu L, Mason RP. Bioconj. Chem. 2004;151334-41

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