

In vivo detection of β -gal in PC3 Prostate Xenograft by ^{19}F NMR

Li Liu, Vikram Kodibagkar, Jianxin Yu and Ralph P. Mason

Cancer Imaging Program, Department of Radiology, The University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, Texas 75390-9058, USA

Introduction

Gene therapy shows promise for treating prostate cancer and has been successfully exploited in several clinical trials. A major challenge remains establishing a method of verifying transgene activity *in situ*. The lacZ gene encoding β -galactosidase has historically been the most popular reporter gene for molecular biology. We have designed a non-invasive ^{19}F NMR approach to reveal LacZ gene expression by assessing β -galactosidase (β -gal) activity *in vivo*. 2-Fluoro-4-nitrophenol- β -D-galactopyranoside (OFPNPG) is a ^{19}F NMR active molecule, which is highly responsive to the action of β -gal. Upon cleavage by β -gal, the single ^{19}F NMR peak of OFPNPG decays accompanied by appearance of a new signal 4-6 ppm up field corresponding to the aglycon OFPNP. We now demonstrate utility of this reporter molecule using ^{19}F NMR spectroscopy and Chemical Shift Imaging (CSI) in PC3 prostate cancer cells and xenografts in mice. Most significantly, we demonstrate this approach for differentiating wild type and LacZ xenografts in mice.

Methods

Stably transfected PC3 cell line: *E. coli* lacZ gene was inserted into high expression phCMV giving a recombinant vector phCMV/lacZ, which was used to transfect PC3 cells using GenePORTER2. The highest β -gal expressing colony PC3/lacZ was selected using G418.

X-gal and S-gal staining: standard procedures were used with 1 mg/ml X-gal, 1 mM MgCl_2 , and 5mM $\text{K}_4\text{Fe}(\text{CN})_6$ or 25 mg/ml S-gal (Sigma) and 125 mg/ml Ferric Ammonium Citrate (FAC) for 2 hours at 37°C in PBS.

NMR/MRI: ^{19}F NMR measurements were performed with a Varian Unity INOVA (188.2 MHz). A solution of 78 mg/ml OFPNPG in 1:1 aqueous DMSO, with 10 mg/ml sodium trifluoroacetate (NaTFA) as chemical shift reference, was injected intra-tumorally. MRI parameters were: field of view = 30X30 mm, spectral window = 70 ppm, slice thickness = 10 mm, matrix = 16x16, TR/TE= 1000/12 ms. NMR data were reconstructed and analysed with homebuilt programs written using the MATLAB programming language.

Histology, western blot and β -gal assay: Tumors were excised after imaging. Thin sections were collected on gelatin-coated glass slides and 8 μm sections stained with X-gal and Eosin (Sigma) for β -gal activity. The protein was extracted for western blot and β -gal assay.

Results

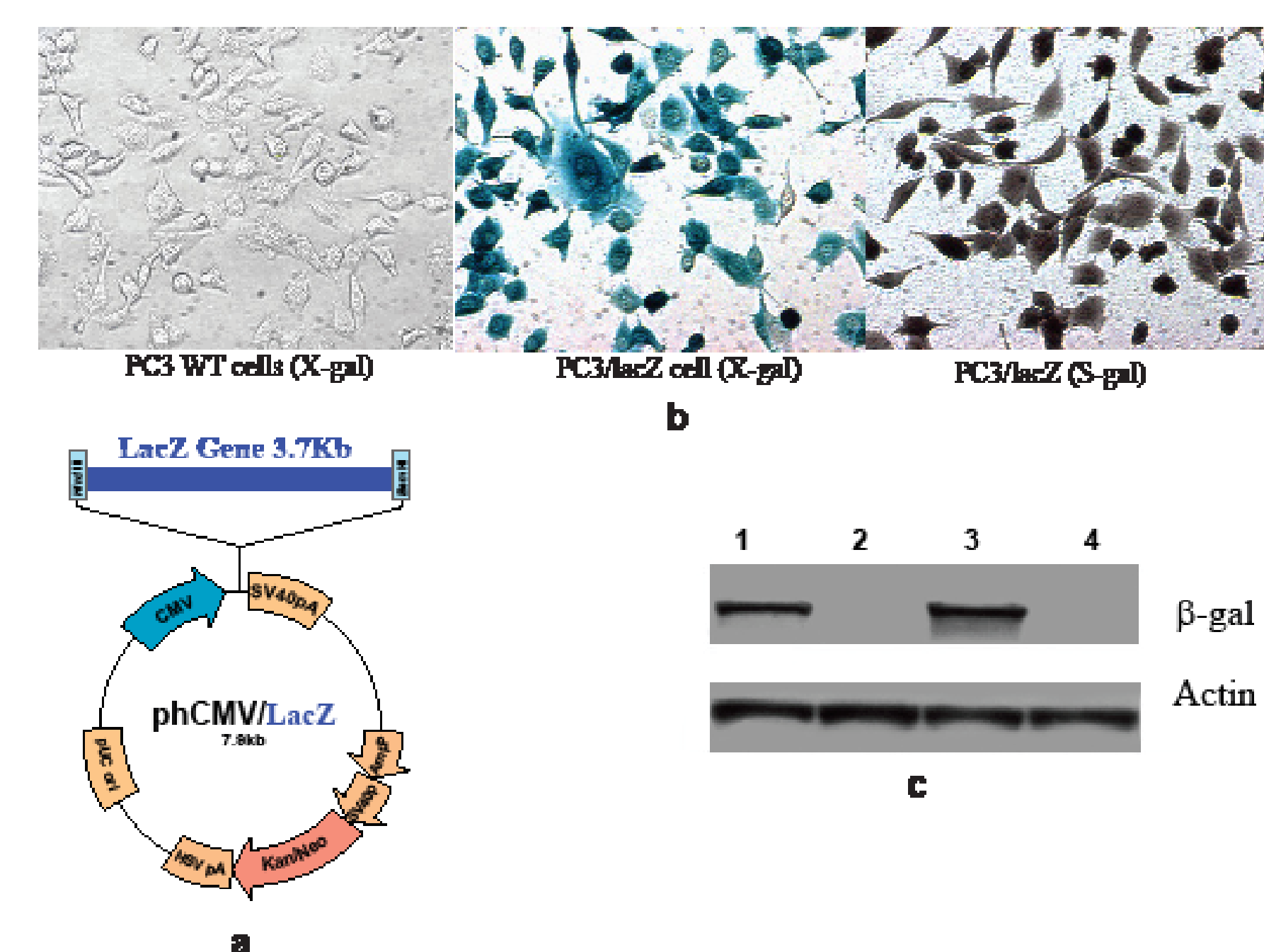


Figure 1. Generation of PC3 cells stably expressing of β -gal. a) Map of recombinant lacZ vector. b) PC3 and PC3/lacZ cells were stained using X-gal and S-gal: over 90% of PC3/lacZ cells were stained blue or black respectively, while the PC3 wild type cells did not stain. c) Western blot: lanes 1, 3 PC3/lacZ cell extraction; lanes 2, 4. PC3 WT cells extraction.

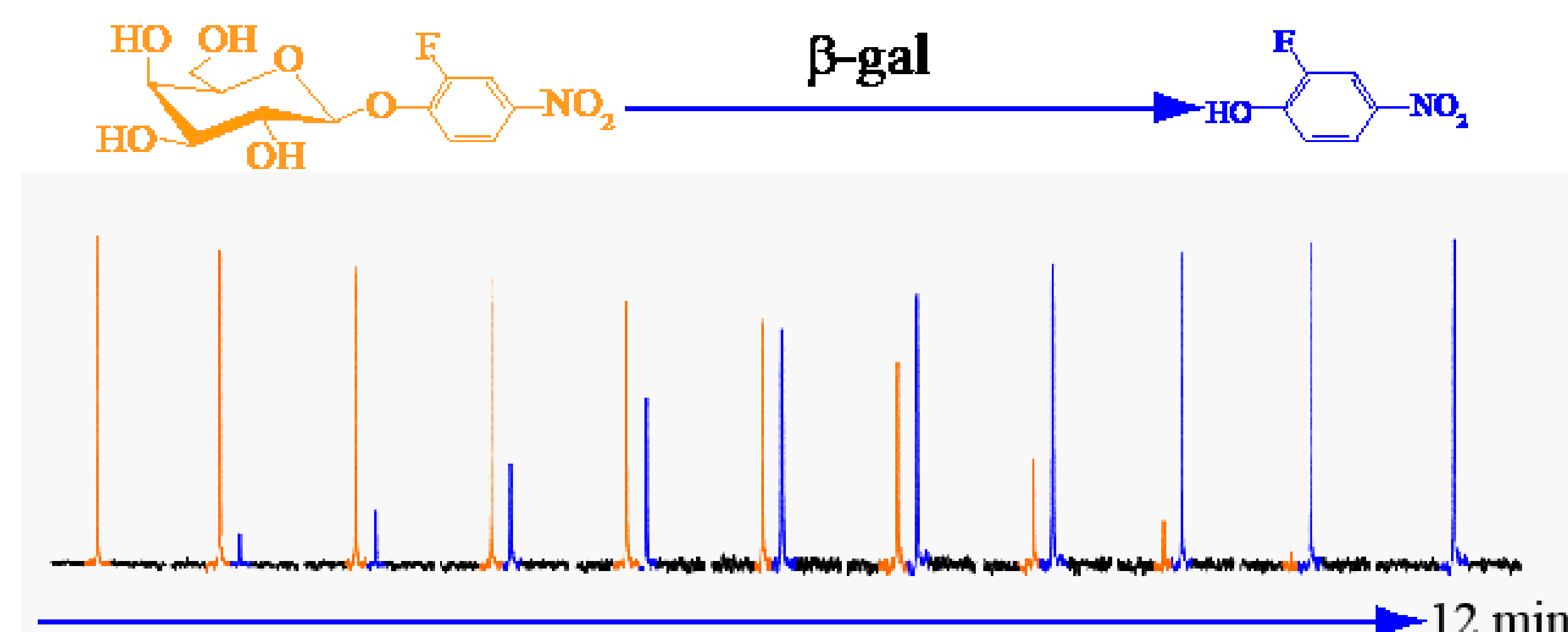


Figure 2. Conversion of OFPNPG to OFPNP by stably transfected PC3/lacZ-LL1 cells (clone 1) Addition of OFPNPG to PC3/lacZ prostate cancer cells (5×10^5) in PBS at 37°C was rapidly followed by generation of a new signal 4-6 ppm upfield corresponding to the product aglycon OFPNP. Spectra acquired in 60 seconds each over 12 min.

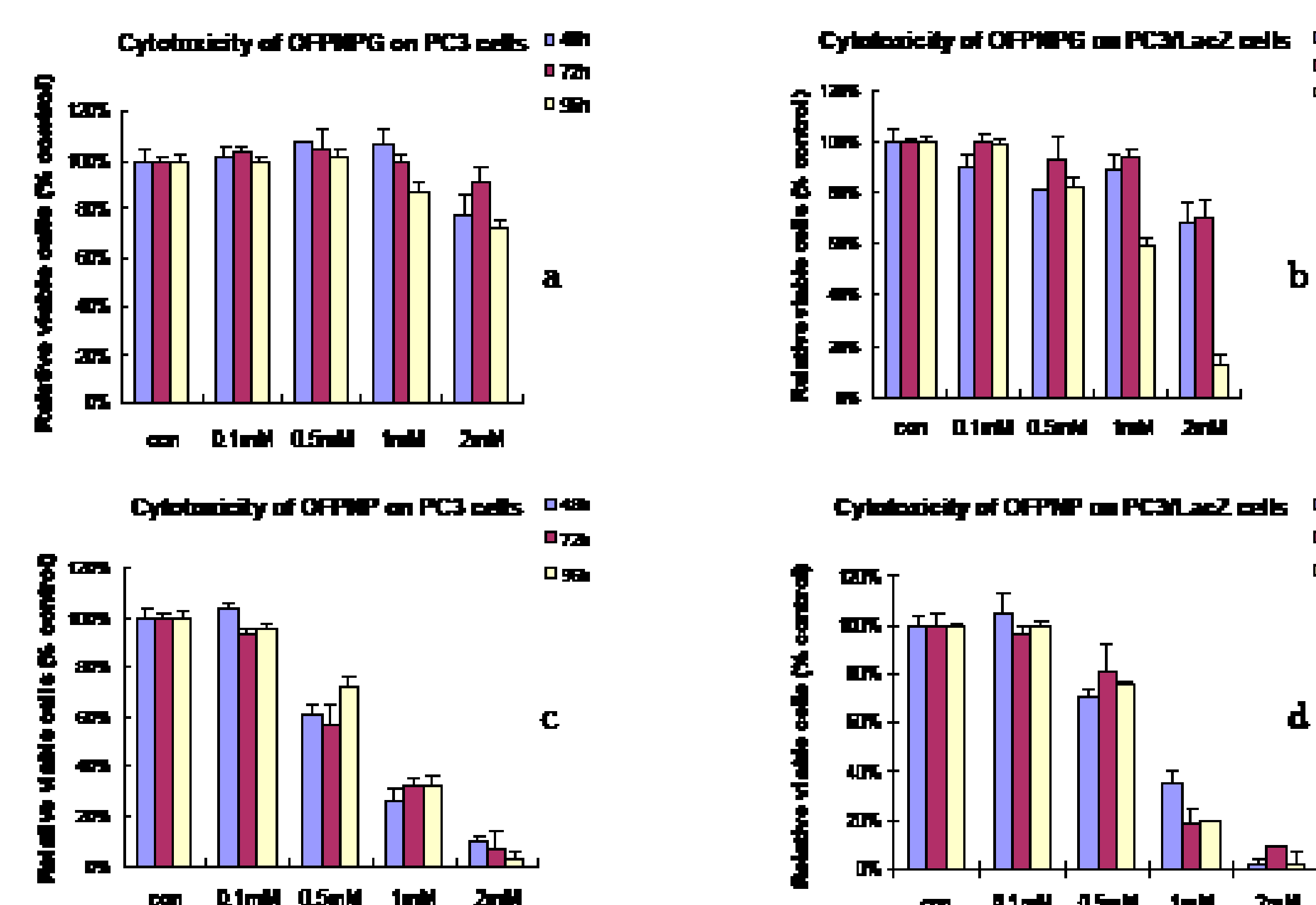


Figure 3. Toxicity of gene reporter molecules. When OFPNPG was added to PC3 wild type cells, there was minimal toxicity. PC3/lacZ cells showed some toxicity, particularly with longer incubation times. (c and d) Addition of the aglycon OFPNP to PC3-WT or PC3/lacZ cells showed considerable toxicity. This indicates that toxicity arises from the product aglycon. This could be the basis for enzyme activated chemotherapy. In terms of reporter molecules, it suggests that less toxic aglycons are needed. Cell viability was assessed using the crystal violet method.

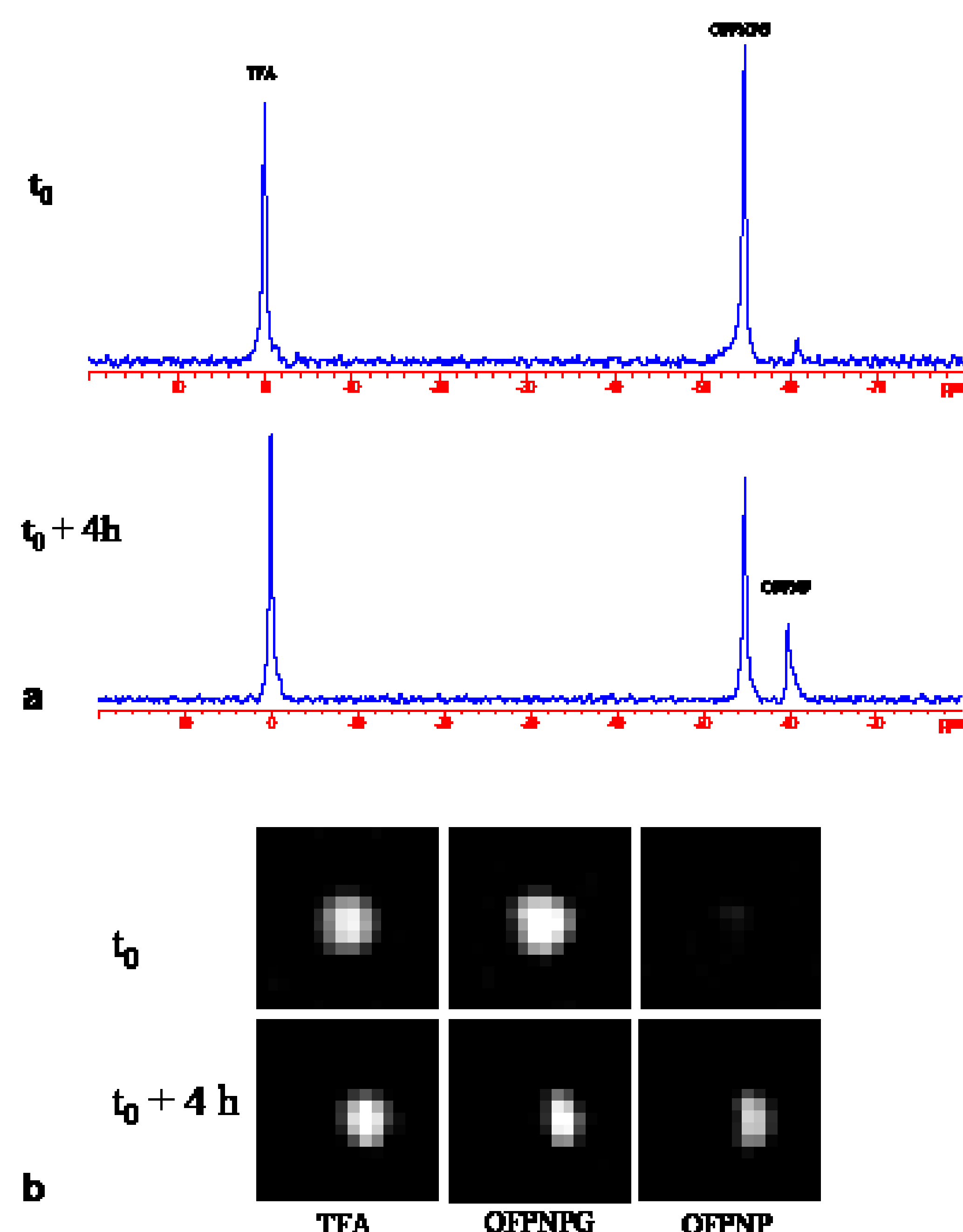


Figure 4. Detection of β -gal activity by CSI in PC3/LacZ cells *in vitro*. a) Spectra b) CSI

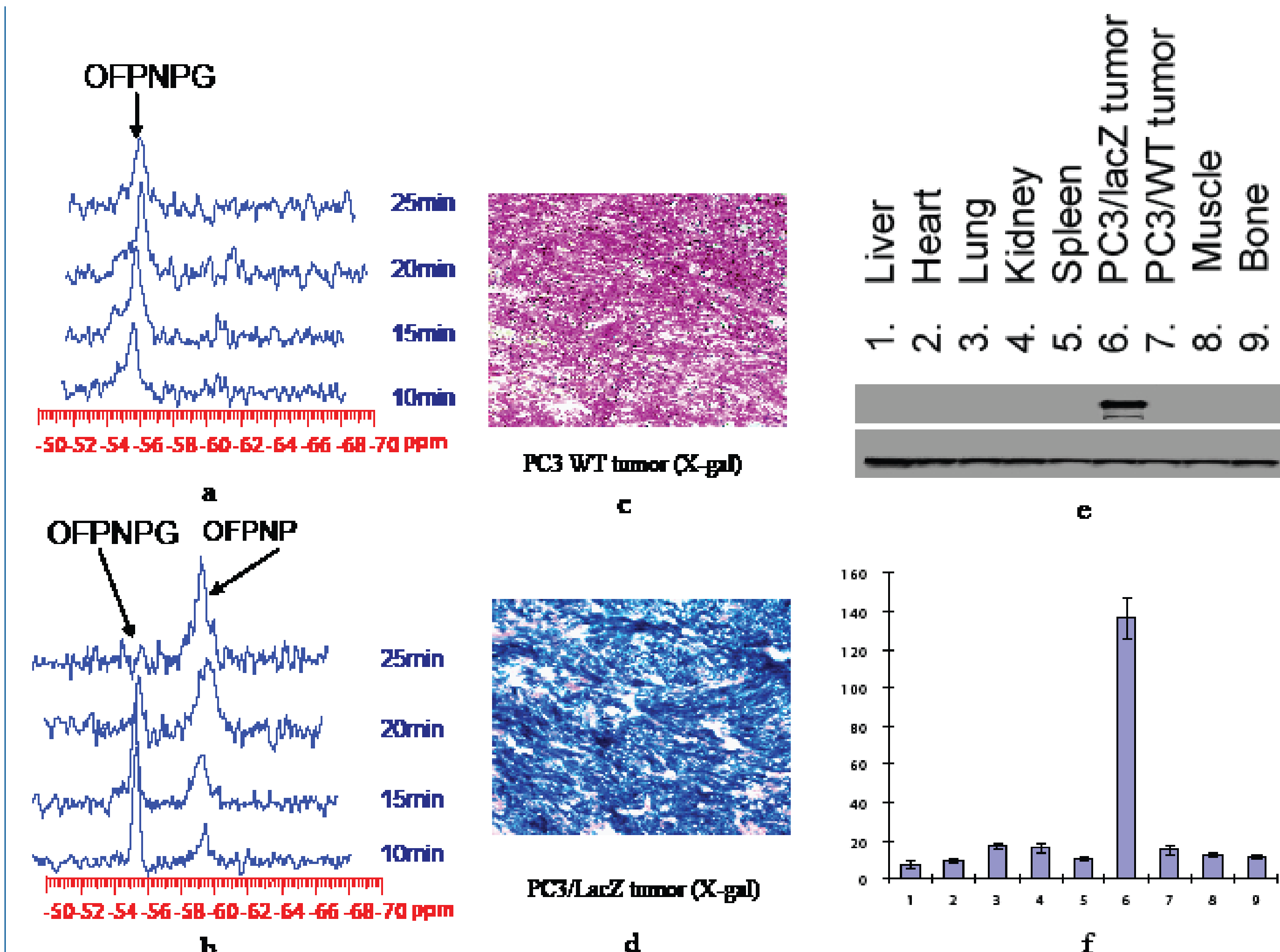


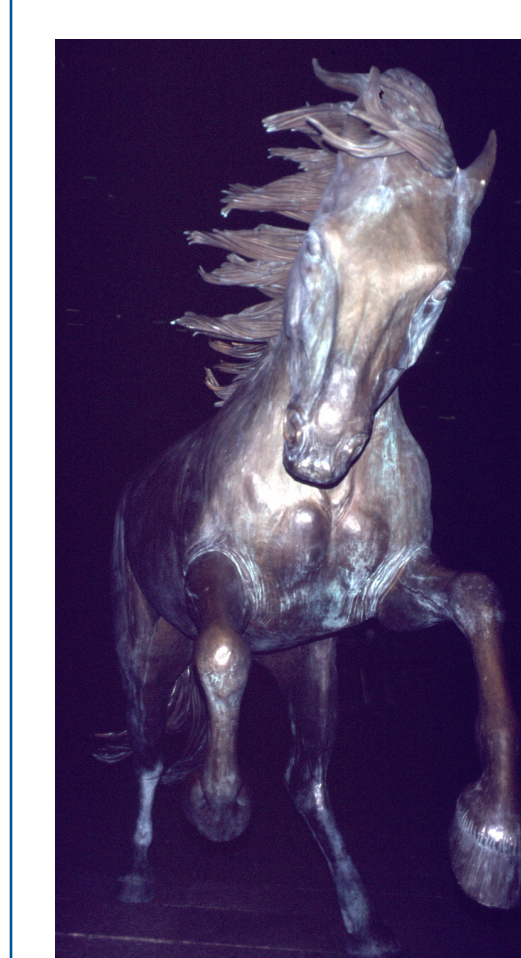
Figure 5. *In vivo* detection of β -gal expression. Time-course ^{19}F NMR spectra of (a) PC3 wild type tumor (1.3 cm x 1 cm x 0.6 cm) and (b) PC3/lacZ tumor (1.4 cm x 1.5 cm x 0.8 cm) demonstrating ability to detect β -gal activity *in vivo*. (c) X-gal staining of PC3 WT and (d) X-gal staining of PC3/lacZ tumor. (e) Western blot of tissues (f) β -gal assay of tissues. 1. Liver; 2. Heart; 3. Lung; 4. Kidney; 5. Spleen; 6. PC3/lacZ tumor; 7. PC3/WT tumor; 8. Muscle; 9. Bone.

Conclusions

- OFPNPG is a promising lacZ gene reporter molecule for ^{19}F NMR spectroscopy *in vitro* and *in vivo*, though less toxic analogs will be preferable (Yu & Mason, *J. Med. Chem.* 49, 1991-1999 (2006)).
- The chemical shift difference is sufficient to allow ^{19}F NMR Chemical Shift Imaging (CSI) to detect β -gal activity.
- For the first, we demonstrate detection of β -gal *in vivo* in PC3 human prostate xenograft using ^{19}F NMR
- This approach directly reveals β -gal activity, which could be used in tandem with therapeutic genes to monitor therapy. It could both accelerate the transfer of gene therapy to patients in the clinic and provide a new tool for evaluation of gene therapy.

Acknowledgements

This work was supported by DOD Prostate Cancer Initiative post doctoral fellowship 17-03-1-0101 (LL) NMR experiments were conducted in the Advanced Imaging Research Center at UT Southwestern and supported by NIH P41-RR02584. We also recognize valuable advice and access to facilities provided by Dr. Steve L. Brown (Henry Ford Hospital System, Detroit, MI) and J.T. Hsieh (Department of Urology, UTSW)



UT SOUTHWESTERN MEDICAL CENTER

Abstract ID Number 372
Contact: Li.Liu@utsouthwestern.edu
SMI 2006