^{19}F CSI of gene-reporter molecule OFPNPG

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Introduction:

The *lacZ* gene, encoding the enzyme β-galactosidase (β-gal) was historically the most attractive reporter gene for molecular biology. Many chromogenic or fluorogenic substrates are well established, but they are generally limited to histology or *in vitro* assays. 2-Fluoro-4-nitrophenol-β-D-galactopyranoside (OFPNPG) belongs to a novel class of NMR active molecules (fluorophenyl-β-D-galactopyranosides), which are highly responsive to the action of β-gal. OFPNPG has a single ^{19}F peak with chemical shift of 55 ppm. It is cleaved by β-gal to OFPNP, which has a pH sensitive chemical shift of 59-61 ppm. The large change in the chemical shift allows us to image β-gal activity with magnetic resonance chemical shift imaging (CSI).

Methods:

A standard 2D spin-echo CSI sequence was used at 4.7 T for the imaging studies. Sodium trifluoroacetate (Na-TFA) was used as a standard for quantifying dynamic changes on a voxel by voxel basis. The imaging parameters were FOV = 30X30 mm, spectral window = 65 ppm, slice thickness: 10 mm, matrix=16X16, TR/TE= 1000/12 ms, na = 1. Total imaging time was 4.5 min. The data were analyzed using homebuilt software written in the MATLAB programming language. Tumor cells transfected with lacZ to stably express β-gal were examined in culture or implanted in mice to grow as solid tumors.

Results and Discussion:

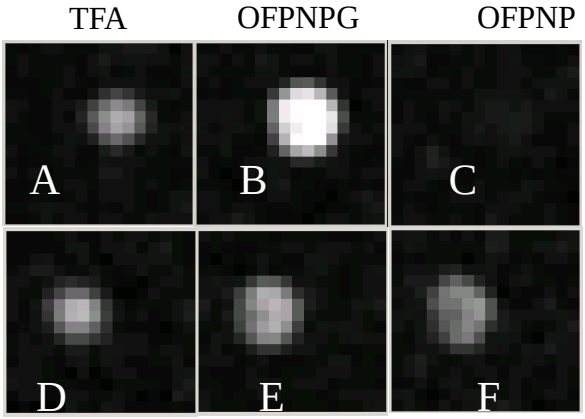

Fig1: *^{19}F CSI and spectroscopy of β-gal activity of 10^8 9L-lacZ cells* ***(A-F).*** *TFA, OFPNPG and OFPNP images at t=0 min (**A**, **B** and **C**, respectively) and t=70 min (**D**, **E** and **F**, respectively).*

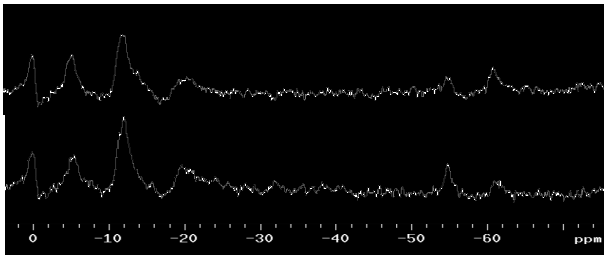

Fig 2: *^{19}F NMR spectroscopy of PC3-lacZ tumor following i.p injection of 0.5 ml OFPNPG (76 mg/ml), lower spectrum after 10 min and upper spectrum after 55 min). The peaks are: TFA (0 ppm), isoflurane (5 and 12 ppm), OFPNPG (55 ppm) and OFPNP (61 ppm).*

At room temperature, conversion of OFPNPG to OFPNP was observed *in vitro* by 9L-lacZ and PC3-lacZ cells. Evidence for β-gal expression was also found for PC3 tumor in mouse. These results provide further evidence for the utility of this class of substrate to generate *reporter products for magnetic resonance (RPMs).* OFPNPG is a promising lacZ gene reporter molecule using ^{19}F MR spectroscopy and CSI.

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