**Multimodality Imaging of Phospholipase Activity in Prostate Cancer Cells treated with Differentiating Agents**

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**Introduction:** Numerous MRS studies have shown that chemotherapy causes increased levels in glycerophosphocholine (GPC) in cells and tumors, often suggested to arise through phospholipase A2 (PLA2) activation[1-4]. Treatment of the prostate cancer cell line DU145 with phenylbutyrate (PB), a differentiating agent in clinical trials for treatment of hematological and solid tissue malignancies, results in apoptosis in conjunction with a number of alterations in lipid metabolism including an increase in GPC and total choline in $^{31}$P and $^{1}$H NMR spectra respectively[5]. Since one of the main pathways for GPC production is through phosphatidylcholine catabolism, we hypothesized that PLA2 activity could account for the observed spectroscopic changes occurring during apoptosis. Phospholipases catalyze phospholipid breakdown and perform a number of critical regulatory functions within cells and tissues[6-7]. Phospholipase A2 (PLA2) catalyzes the hydrolysis of the *sn*-2 position of phospholipid, and levels of this enzyme are increased during inflammation, hyperproliferation and apoptosis. To verify the information obtained by MRS, we used reporter probes that release fluorescent moieties upon phospholipase cleavage. In this study, we monitored the effects of PB with $^{31}$P and diffusion-weighted (DW) $^{1}$H NMR, and measured phospholipase activity with PED-6 and B7701. PED-6 is a self-quenching fluorescent phospholipid analog incorporating a BODIPY label at the *sn*-2 position of the phospholipid glycerol backbone, with a *p*-dinitroanilino quencher attached to a modified ethanolamine headgroup. The molecule has low intrinsic fluorescence, but cleavage of the dye-labeled chain destroys the intramolecular quenching, leading to increased fluorescence[8-10]. B7701 is also a self-quenching fluorescent phospholipid analog, however this molecule incorporates a BODIPY label at the *sn*-1 and *sn*-2 position of the phospholipid glycerol backbone with a choline moiety at the headgroup. As with PED-6, B7701 has low intrinsic fluorescence, but cleavage of either dye-labeled chains (through phospholipase A1 or A2 mediated release) eliminates intramolecular quenching, again leading to increased fluorescence[11].

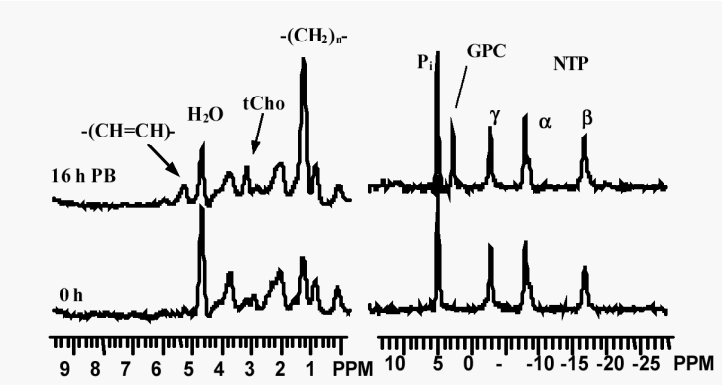


Figure 1: Diffusion-weighted $^{1}$H (*left)* and single pulse $^{31}$P (*right)* NMR spectra of perfused DU145 prostate carcinoma cells. The bottom traces show control cells, the top traces show cells treated with PB (10 mM, 16 h). Note increases in mobile fatty acid resonances at 1.3 and 5.3 ppm and tCho at 3.2 ppm in $^{1}$H spectra and in GPC at 0.5 ppm in $^{31}$P spectra.

**Results and Discussion:** DU145 prostate cells were treated with the differentiating agent phenylbutyrate to induce MR-visible increases in MR visible lipid, total choline and GPC (Figure 1). *In situ* activation of PLA2 was probed using the phospholipase-activated self-quenching fluorophores PED-6 and B7701. The significant time-dependent increase in mobile lipids, total choline and GPC measured by $^{1}$H and $^{31}$P MRS in perfused cells was accompanied by increased cytosolic fluorescence from BODIPY released from the *sn*-2 position of PED-6. In contrast, increased nuclear and perinuclear fluorescence from B7701 was observed in both PB-treated and control DU145 cells. The combination of these modalities provides evidence that MR-detectable metabolic changes can be attributed to PLA2 activation, and gives insight as to the identity of the PLA2 being activated. These data suggest the existence of two separate pools of phospholipase activity in DU145 cells: one that is constitutive and primarily nuclear in location, and one that is inducible by PB, leads to GPC production and occurs in the cytoplasm. These results provide evidence that the metabolic changes detected by NMR, increased mobile lipids, total choline, and GPC, can be attributed to cytoplasmic phospholipase activation that precede or accompany apoptosis. Multiple isoforms of PLA2 exist, including calcium-dependent and calcium-independent forms, and these have both been shown to be upregulated in different forms of apoptosis. Thus this approach shows promise for deciphering the often contradictory changes in choline levels observed with MR spectroscopy. Since PLA2 has been implicated in a wide range of illnesses, this study has significant ramifications for *in-vivo* imaging of disease processes and treatment regimens.

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