MRS Observed Effects of Phorbol Myristate Acetate on Lipid Metabolism in DU145 Prostate Tumor Cells

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Introduction

Tumors have been shown to have a disregulation in choline lipid metabolism. It has been demonstrated that upregulations in choline kinase, the enzyme responsible for the phosphorylation of choline to phosphocholine (PC), may explain the abnormally high levels of PC in a panel of tumors, ranging from breast to prostate tumor cell lines [1, 2]. However, upon drug treatment, tumor cells often exhibit a switch in the [GPC]/[PC] ratio compared to controls [3]. It remains unclear if this is caused by choline uptake, phosphorylation, or the actions of phospholipases. Additionally, often ignored are the peripheral enzymes that contribute to choline lipid metabolite formation, such as protein kinase C (PKC). PKC has been shown to mediate the apoptotic effect of phorbol esters (e.g., PMA) [4] and inhibit proliferation via phosphorylation of EGFR at threonine 654 [5] in prostate cancer cells. Phorbol myristate acetate (PMA) is a known activator of PKC and mimetic analogue of diacylglycerol, the cleavage product of PLC. PMA has also been shown to cause a redistribution of PLC to the plasma membrane in fibroblasts [6]. In this work, we have investigated the effect of PKC activation by PMA on lipid metabolism in DU145 prostate tumor cells. Perfused DU145 cells were treated with PMA and analyzed with ^{31}P MRS to measure levels of PC. Cultured DU145 cells were also treated with PMA, as well as the phosphatidylcholine-specific phospholipase C (PC-PLC) inhibitor D609, and extracts were measured by high-resolution ^{1}H MRS to examine levels of choline, PC, and GPC. The data shows that PMA affects lipid metabolism and has antiproliferative effects on DU145 prostate tumor cells.

Methods

Cell Culture: DU145 human prostate adenocarcinoma cells were cultured in MEM (10% FBS in 5% CO_2 95% O_2 at 37°C). *NMR Spectroscopy:* Biosilon microcarriers (1.8 grams) were inoculated with 5.0 x 10^6 cells/ml and cultured for 48 h. The microcarriers were transferred to a 10 mm MR tube and perfused with medium (1.8 ml/min) equilibrated with 5% CO_2 in O_2. Cells were treated with 100 nM PMA in the presence or absence of 50 µM D609. MR spectra were acquired on a Varian 9.4 T INOVA spectrometer equipped with a 10 mm Doty multinuclear probe. ^{31}P MR spectra were acquired (TR = 2 s; data size, 2K; flip angle, 45°; spectral width 5 kHz; 2500 scans) hourly for 16 h and integrated resonance intensities compared to baseline. *High-resolution ^{1}H MRS:* DU145 cells were treated under corresponding conditions and extracted with perchloric acid. Spectra were obtained with a pulse-acquire sequence with a 45° flip angle, TR = 8.8 s, SW =6775 Hz, NP = 65536, and NT = 128 on a Bruker Avance DMX400 spectrometer. A capillary containing sodium 3-(trimethylsilyl)-[2,2,3,3,-2H4]-1-propionate (TSP) was inserted as an external reference into the 5 mm NMR tube. Spectra were the curve fitted using MestReC. *Cell cycle:* Cell cycle was analyzed by flow cytometry using by staining double-stranded DNA with propidium iodide.

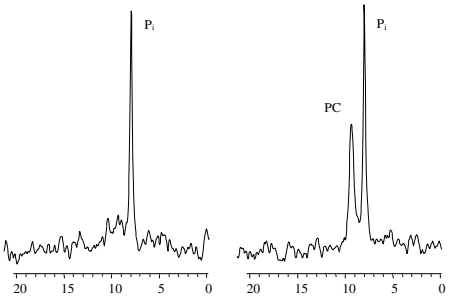

Figure 1. PC formation induced by PMA in perfused prostate tumor cells. *Left* shows control ^{31}P spectra of DU145 cells. Hour 16 of DU145 cells grown in the presence of 100 nM PMA, *right.*

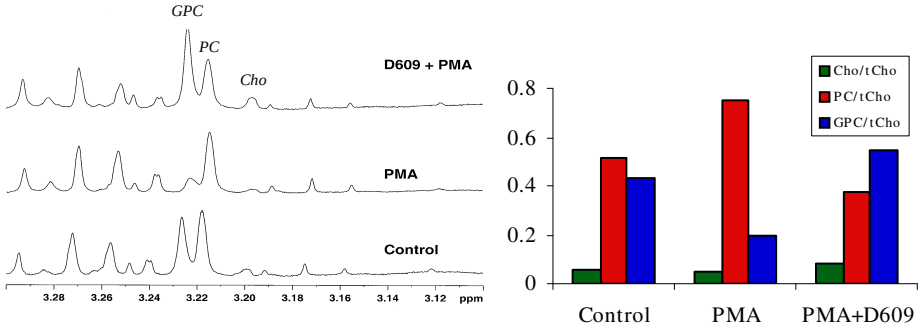

Figure 2. PC formation induced by PMA in prostate tumor cells. Cells were treated with PMA ± D609 for 18 hours. *A.* High-resolution ^{1}H spectra of DU145 cell extracts. *B.* Quantified peak integrations from A as a ratio of total choline. Concentrations of drugs: 100 nM PMA, 50 µM D609.

Results and Discussion

To obtain insight on the mechanisms of choline metabolism peripheral to the phosphatidylcholine cycling pathway, this study used PMA as a DAG mimetic to investigate the association between PKC and PC. PMA stimulates PKC activity by acting as a DAG analog. Here, we show that PMA is also able to affect the phosphatidylcholine pathway directly, enhancing MR-visible PC in perfused DU145 cells (Figure 1). Additionally, high-resolution extracts show that D609, a specific PLC inhibitor, is able to attenuate the effects of PMA on PC production and reverse the [GPC]/[PC] ratio changes induced by PMA (Figure 2). This is suggestive that a positive feedback system through DAG exists, as shown by an increase in PC, and implicates the actions of PC-PLC in PC production. PMA may be antiproliferative via PKC activation. Figure 3 demonstrates a significant increase in percentage of cells in G1 phase and a decrease in percentage of cells in S phase when treated with PMA. This suggests that cells treated with PMA are in G1 arrest. The co-administration of D609 with PMA did not inhibit the effects of PMA on the cell cycle, indicating that PKC-induced PLC activity is not involved in PMA's antiproliferative effects.

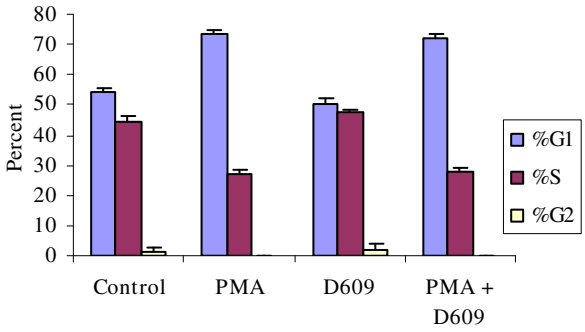

Figure 3. Cell cycle analysis of prostate tumor cells performed by flow cytometry. Concentrations of drugs: 100 nM PMA, 50 µM D609.

References

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