^{13}C MRS and DNA microarray analysis demonstrate increased phosphatidylcholine breakdown following inhibition of the PI3K signalling pathway in HCT116 colon cancer cells

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Introduction

The phosphoinositide 3-kinase (PI3K) pathway plays an important role as a regulator of mammalian cell proliferation and survival pathways that drive tumour progression. Thus, small molecule therapeutics that block PI3K signalling may kill cancer cells by blocking many aspects of the tumour cell phenotype (1). Some anti-cancer treatments may cause tumour stasis rather than regression (2), hence methods that measure response at the level of the molecular pathway rather than depending on volume change are required. Such methods will help to confirm the mode of action at an early stage in development, and ultimately, provide a tool to assess activity in early stage clinical trials. The aims of this work are: first, to develop MRS as a non-invasive tool for the detection of markers for PI3K pathway inhibition. Second, to investigate mechanisms underlying the detected changes. Using MRS we have monitored response to the PI3K inhibitor LY294002. In line with our previously reported results (3;4), we have detected an accumulation in phosphodiesters (PDEs). PDEs are intermediates in phospholipid metabolism and may provide a specific surrogate marker for response to treatment. ^{13}C MRS and DNA microarrays were used to investigate the mechanism underlying the accumulation of PDEs.

Methods

The human colon adenocarcinoma cell line HCT116 was treated with the PI3K inhibitor LY294002 (200 μM) for 24 h. Adherent cells (1-2x10^8) were extracted using a dual extraction method. A ^{13}C MR wash out experiment was performed by pre-incubating cells with medium in which choline was replaced with 99.0% [1,2-$^{13}C_2$]-choline for up to 68 h. Cells were then treated as above in the presence of medium containing normal choline. At the required time point, changes in the levels of ^{13}C labelled metabolites formed out of the labelled precursors were analysed by ^{13}C MRS. MR spectra were acquired at room temperature on a 500 MHz Bruker spectrometer using a 30° flip angle, a 1 s relaxation delay for ^{31}P and a 2 s relaxation delay for ^{13}C with broad band decoupling during acquisition. Metabolite content was determined by integration and normalised relative to internal standards and cell number. Results represent (average ± SD, n ≥ 3). Western blotting for P-PKB was used to confirm the molecular response to LY294002 and DNA microarrays were used to establish the expression profile of the LY294002. Our DNA expression arrays are based on cDNAs from the Unigene set of ESTs and slides were scanned using an Axon Genepix 4000 scanner.

Results & Discussion

Treatment of the HCT116 colon adenocarcinoma cell line with LY294002 has resulted in a drop in cell number via inhibition of PI3K signalling pathway as confirmed by western blotting. ^{31}P MR spectra of treated cells showed a significant accumulation of the PDEs, glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE) relative to controls (6.7 ± 1.8 vs. 1.5 ± 0.2 fmol / cell, $P < 0.05$). A significant drop in phosphocholine (3.8 ± 1.7 vs. 8.6 ± 1.1 fmol / cell, $P < 0.03$) was also detected (Figure 1). This supports results from our group using MRS to monitor response to 17AAG treatment which inhibits both Ras and PI3K pathways and suggests that the increase in GPC is specific to inhibition of the PI3K pathway (3;4). Because the increase in PDEs following tumour treatment has not been detected before, it may provide a specific surrogate marker for response to treatment. Furthermore, the average PDEs/NTP ratio, a parameter independent of cell number, also increased significantly (0.6 ± 0.02 vs. 0.2 ± 0.04 , $P < 0.001$), indicating that this ratio could serve as an *in vivo* MRS marker of PI3K inhibition.

PDEs are intermediate metabolites in phospholipid metabolism and their accumulation could reflect an increase in phospholipid breakdown via the activation of phospholipase A_2 (PLA_2) or an inhibition at the level of PDEs phosphodiesterases. A ^{13}C wash out experiment using [1,2-$^{13}C_2$]-choline was performed in order to investigate the mechanism underlying GPC accumulation. ^{13}C MR spectra from treated HCT116 but not control showed an accumulation of ^{13}C labelled GPC (Figure 2). DNA expression array analysis showed down-regulation of annexin I, an enzyme that is known to inhibit PLA_2 (5). Taken together, our data demonstrate that the accumulated GPC may be coming from enhancement of phosphatidylcholine breakdown.

In summary, we have shown that MRS could be used as a non-invasive technique to monitor response to inhbition of PI3K signalling pathway. This could, in the long term, translate into the clinic to aid early detection of response to novel PI3K-targeted therapeutic drugs.

Acknowledgements

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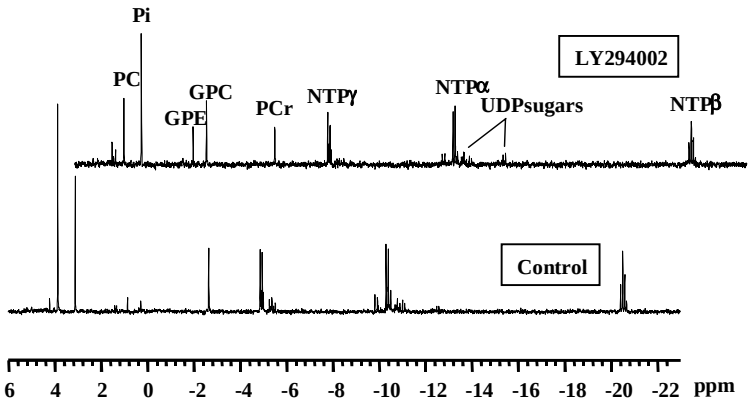

Figure 1 ^{1}H decoupled ^{31}P MR spectra of the water soluble fraction of HCT116 cell extracts. Spectra are the result of 10 K scans plotted with line broadening of 1Hz

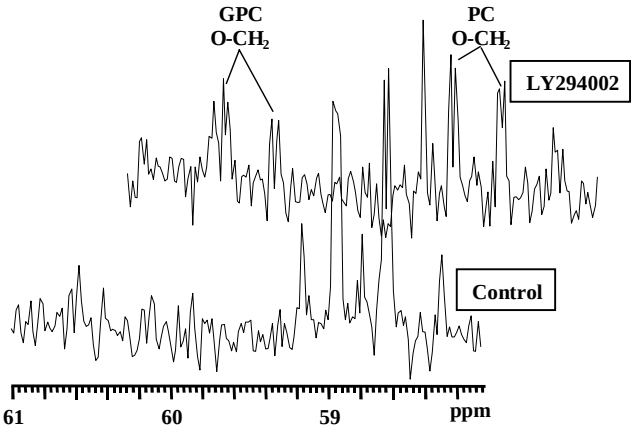

Figure 2 An expanded region of the ^{1}H decoupled ^{13}C MR spectra of the water soluble fraction of HCT116 cell extracts from the ^{13}C wash out experiment. Spectra are the result of 30K scans plotted with line broadening of 1 Hz