Phosphocholine depletion is a non-invasive MRS biomarker for PI3K inhibition in childhood high-grade glioma

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Introduction

Children with high grade-glioma have a poor outcome with a 5-year survival rate of less than 20% (1). There is increasing evidence to suggest that the phosphoinositide 3-kinase (PI3K) pathway is activated in pediatric glioma (2). Novel inhibitors of the PI3K pathway have been developed at our institution and it is proposed to carry out early clinical studies of these inhibitors. In order to determine the optimum dose and schedule of these PI3K inhibitors, biomarkers are required. Obtaining serial biopsies in pediatric glioma presents both practical and ethical difficulties. Non–invasive biomarkers would thus be of particular value in assessing inhibition of PI3K in this group. The aims of this work are: First, to establish biomarker changes using ^{1}H and ^{31}P MRS in pediatric glioma cell lines treated with PI3K inhibitors. Second, to correlate the observed metabolic changes with the underlying biology of pathway inhibition. Using MRS we have monitored response to PI103, a novel class 1A PI3K inhibitor (3), using the pediatric high-grade glioma cell line SF188, the most sensitive CNS line yet tested. Our results show that inhibition of PI3K as evidenced by depletion of Akt phosphorylation results in a decrease in PC levels detected by ^{1}H and ^{31}P MRS. This indicates that MRS could be used to non-invasively monitor response to PI3K inhibition in children with high-grade glioma.

Materials and Methods

The PTEN wild type cell line SF188 is very sensitive to PI103 with IC_{50} of 0.2 μM. Cells were treated with PI103 at a concentration equivalent to $5xIC_{50}$ for 24h. PI3K pathway inhibition was verified using Western blotting for P-Akt (Ser^{473}) and P-S6r. Cell cycle distribution was determined by FACS analysis. Adherent control and PI103-treated cells ($5x10^{7}$) were extracted using the DPE method and the aqueous fractions were analyzed by ^{1}H and ^{31}P MRS at room temperature on a 500 MHz Bruker spectrometer using a 30° flip angle and a 1s relaxation delay. Metabolite levels were corrected for saturation and cell number. Results represent (average ± SD, $n \geq 3$).

Results & Discussion

The number of PI103-treated SF188 cells was significantly reduced to 61±5% ($P<0.01$) relative to controls, consistent with decreased proliferation. PI103 caused a decrease in P-Akt as well as P-S6r, providing molecular evidence for PI3K inhibition. Cell cycle distribution by flow cytometry was performed to further characterize the effects of PI103 on SF188 cells. A 24h exposure to PI103 caused an increase in G1 cellular population (51±10% to 80±5%, P=0.03) as well as a decrease in S phase (37±7% to 13±6%, P=0.03) and G2 (13±3% to 6±1%, P=0.05). ^{31}P MRS showed that PI103 treatment for 24h led to a drop in PC levels relative to controls (66±4%, $P<0.01$) (Fig. 1). The change in PC levels was also confirmed using ^{1}H MRS. This and our previous reports (4, 5) show that the reduction in PC is robust, reproducible and consistent across a wide range of tumor cell lines regardless of their molecular phenotype and is indeed related to PI3K signaling blockade and hence could potentially be used as an early marker for response to PI3K inhibition in treated tumors.

Conclusions

Using MRS, we have detected a decrease in PC levels following inhibition of PI3K pathway by the novel inhibitor PI103 in the human pediatric high-grade glioma cell line SF188. Monitoring the pharmacodynamic effects of PI3K inhibitors by MRS may provide a non-invasive pharmacodynamic biomarker(s) for PI3K inhibition and potentially of tumor response in children and adolescents with malignant glioma.

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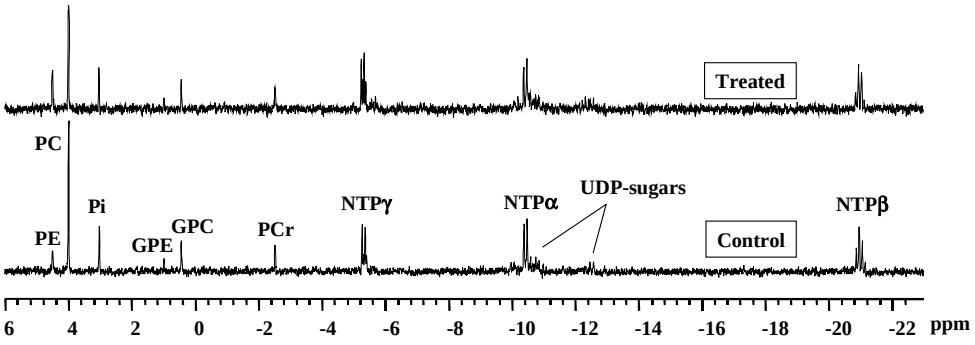

Fig. 1 (A) Representative ^{31}P MR spectra of control and PI103 treated SF188 cell extracts showing the drop in PC levels.