Characterising the time-dependent changes in ^{31}P-MRS detectable metabolites resulting from MAPK signalling inhibition in HCT116 human colon cancer cells

M. Beloueche-Babari[1], L. E. Jackson[1], N. M. Al-Saffar[1], P. Workman[2], M. O. Leach[1]

[1]Cancer Research UK Clinical Magnetic Resonance Research Group, Institute of Cancer Research and Royal Marsden NHS Foundation Trust, Sutton, Surrey, United Kingdom, [2]Cancer Research UK Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, Surrey, United Kingdom

Introduction: The Ras-Raf-MEK-ERK (also known as the MAPK) signalling pathway is deregulated in many human cancers and inhibitors of this pathway, such as the MEK inhibitor CI-1040 and the Raf inhibitor BAY 43-9006, are now under clinical evaluation. Using MRS to detect metabolic markers of MAPK signalling down-regulation in cancer cells could, in principle, enable non-invasive monitoring of response to this novel therapy.

We have previously shown that treatment with the prototype MEK inhibitor U0126 in human breast cancer cells correlates with a drop in phosphocholine (PC) levels (1). In the present study we focus on a model where CI-1040 and BAY 43-9006 show promising in vivo anti-tumour activity namely human colon cancer cells (2,3). Our in vitro investigation assessed a) whether MAPK signalling inhibition with U0126 could be associated with MR detectable metabolic biomarkers and b) the timing at which any such markers would be visible to help guide response monitoring in clinical trials.

Methods: HCT116 human colon carcinoma cells were treated with 30 μM U0126 or 0.1% (v/v) DMSO for 6h, 16h and 24h. Inhibition of MAPK signalling was assessed using Western blotting for phosphorylated (i.e activated) ERK and bands representing P-ERK were further quantified by densitometry using Image Quant version 5. Control and U0126-treated cells were extracted using a dual phase extraction method and the aqueous fractions analysed by ^{31}P MRS using a 500MHz Bruker spectrometer, a 2s repletion time and a 30° flip angle. Metabolite levels were corrected for saturation and cell number.

Results & Discussion: Inhibition of MAPK signalling with U0126 in HCT116 cells was associated with a time-dependent reduction in cell number per flask to 84±3% relative to control (p=0.003) by 24h post-treatment and which was indicative of decreased cell proliferation. In addition, Western blot analysis showed that U0126 treatment induced a substantial drop in ERK phosphorylation that was detectable from 6h and sustained following 24h of exposure to U0126 (Figure 1A). P-ERK levels dropped to 3±4%, 7±8% and 1±1% relative to control at 6h, 16h and 24h post U0126 treatment respectively (p≤0.004) (Figure 1C).

We next assessed whether the effect on P-ERK levels was associated with changes in the levels of ^{31}P-containing metabolites of treated cells. ^{31}P MR analysis of the water soluble fraction of cell extracts showed that PC levels were not significantly altered at 6h (equal to 86±16% relative to control; n=3, p=0.22), but decreased to 80±8% (n=3, p=0.01) and 61±4% (n=4, p=0.0004) at 16h and 24h post-treatment respectively (Figure 1B, C). A drop in nucleotide tri-phosphate (NTP) levels to 78±12% (p=0.005) relative to control was also detectable at 24h but not earlier. Consequently the PC/NTP ratio also decreased to 78±11% (p=0.04) relative to control following 24h of continuous exposure to U0126.

In conclusion, our results have shown that, in addition to a drop in NTP and PC/NTP, inhibition of MAPK signalling in HCT116 human colon cancer cells was associated with a time-dependent drop in PC levels that is temporally preceded by the drop in P-ERK levels. This suggests that the effects of MAPK signalling inhibition on phospholipid metabolism could occur downstream of P-ERK. Our findings further reinforce the role of PC as a surrogate marker for monitoring inhibition of the MAPK pathway using MRS, and highlight the importance of defining the appropriate time point for response assessment during clinical trials.

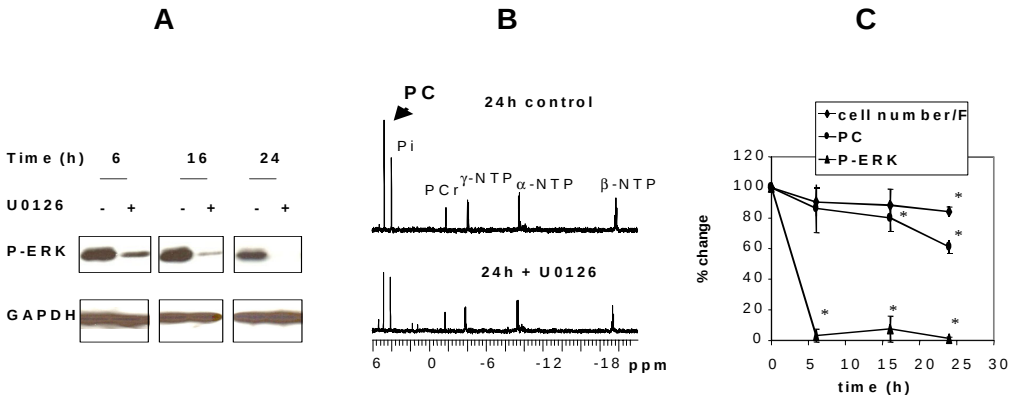

Figure 1: The effect of MAPK signalling inhibition with U0126 in HCT116 cells on P-ERK levels (A,C), cell number per flask and cellular PC content (B,C).

References: 1) M. Beloueche-Babari et al (2003) Proc. ESMRMB Rotterdam, The Netherlands, abstract no 131. 2) J. S. Sebolt-Leopold et al (1999) Nature Med., 3) J. F. Lyons (2001) Endocr.-Related Cancer.

Acknowledgements: This work was funded by Cancer Research UK (Grant number C1060/A808).