MEK1/2 signalling inhibition in human melanoma cells leads to reduced lactate production via inhibition of glucose uptake and lactate dehydrogenase activity

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Background: RAS-BRAF-MEK1/2-ERK1/2 signalling is deregulated in several cancer subtypes and represents an important focus for advancing mechanism-based cancer therapy, with inhibitors of BRAF and MEK1/2 currently in clinical development. We have previously reported that MEK1/2 targeted therapeutics alter the glycolytic capacity of cancer cells as shown by reduced levels of lactate production[1]. We now analyse the time-course of the response and investigate the mechanism behind this effect by assessing glucose uptake and lactate dehydrogenase (LDH) activity.

Methods: WM266.4 melanoma cells (V600E *BRAF* mutant) were treated with 1 μM of the MEK1/2 inhibitor CI-1040 and lactate production in the growth media was monitored at 30 min, 2 h, 6 h, 16 h, 24 h, and 48 h using ^{1}H MRS as previously described[1]. To evaluate the effect on glucose uptake, cells were treated with vehicle or CI-1040 for 24 h and 10 μM of fluorescent glucose analogue 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) was added at the last 2 h of treatment. Median fluorescence intensities of 2-NBDG uptake were obtained by measuring 20 000 control and treated cells on a BD FACSAria™ Flow Cytometer. LDH activity was measured with a dynamic nuclear polarisation (DNP) assay. 1-^{13}C Pyruvic acid containing trityl radical was polarised in a HyperSense DNP polariser and then dissolved in a neutralised solution of lactate and EDTA which was added to a suspension of 10^{7} cells followed by serial ^{13}C MRS acquisitions. Rates were derived from non-linear least squares fitting of the bi-exponential time dependence of the hyperpolarized lactate build-up and corrected for cell number. Statistical analysis was performed using a Student t-test with $p < 0.05$ considered to be significant.

Results: Time-course analysis indicated that lactate levels were unchanged at 30 min, 2 h and 6 h but decreased significantly at 16 h (79±3%), 24 h (76±4%) and 48 h (80±6%) as compared to controls (n=3, $p \leq 0.006$) as shown in Figure 1A. CI-1040 treatment led to a reduction in 2-NBDG uptake to 88±2% relative to the control (n=3, p=0.003). LDH activity, as measured by lactate generation with the DNP assay, in control and treated cells was 0.83±0.26 and 0.48±0.08 nmol/s/ 10^{-6} cells respectively (n=5, p=0.02) i.e. reduced by ~40% compared to the control as shown in Figure 1B.

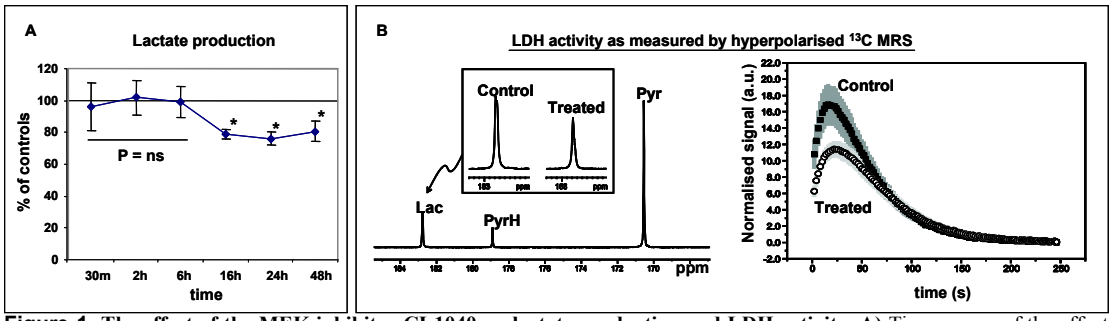

Figure 1. The effect of the MEK inhibitor CI-1040 on lactate production and LDH activity. A) Time-course of the effect of MEK1/2 inhibition on lactate levels in the growth media. **B)** Representative data from control and treated cells showing ^{13}C lactate production from hyperpolarised ^{13}C pyruvate. * denotes $p \leq 0.006$ and ns $p > 0.5$.

Conclusions: Our findings demonstrate that MEK1/2 signalling inhibition leads to decreased lactate production through modulation of both glucose uptake and LDH activity. These results show lactate as a potential non-invasive MRS biomarker of response to MEK1/2 targeted therapeutics in human cancer cells. Further studies are required to establish the molecular processes linking MEK1/2 inhibition to decreased lactate production.

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References: 1) M. Falck Miniotis, P. Workman, M. O. Leach and M. Beloueche-Babari (2009). ^{1}H MRS Reveals Altered Lactate Levels in Cancer Cells Subjected to MEK1/2 Signalling Inhibition. *17th ISMRM Scientific Meeting and Exhibition, Honolulu, USA.* Abstract # 2315.