**Background:** To meet the energy demands of the tumour micro-environment, cancer cells exhibit an increased rate of aerobic glycolysis also known as the Warburg effect which results in intracellular lactate accumulation. RAS-B-RAF-MEK-ERK signalling is often deregulated in cancer and represents a significant target for mechanism-based drugs with inhibitors of B-RAF and MEK currently in clinical development. Our aim was to investigate whether inhibition of this signalling pathway in human cancer cells could lead to magnetic resonance spectroscopy (MRS) detectable changes in glycolysis that may serve as biomarkers of target suppression. In addition, and as part of the V600E B-RAF mutation has been shown to sensitize cells to MEK inhibition, we assessed whether any changes in glycolysis would be dependent on B-RAF mutational status.

**Methods:** We used the specific MEK1/2 inhibitors CI-1040 and PD325901 on four human cancer cell lines with various tissue origin and B-RAF mutation status: HCT116 (colorectal, WT B-RAF), MDA-MB-231 (breast, intermediate activity G463V B-RAF), SKMEL-28 and WM266.4 (melanoma, highly active V600E B-RAF). The effect of drug treatment on cell proliferation was assessed using the SRB assay and Glc values derived. For MRS analyses, equipotent concentrations of MEK1/2 inhibitors that achieved target modulation in all cell lines were used. For CI-1040, cells were treated with 20 µM for 16 h and 24 h (HCT116) or with 4 µM for 24 h (MDA-MB-231) or with 1 µM for 24 h (SKMEL-28 and WM266.4). These concentrations were equivalent to 10 x Glc values. For experiments with PD325901, cells were treated with 5 x Glc i.e. 10 nM for WM266.4 cells and 15 nM for SKMEL-28 cells for 24 h. Post-treatment, 10^5 cells were extracted with a dual phase method and ^1H MRS spectra of the water soluble metabolites acquired on an 11.7 T Bruker Avance spectrometer. In addition, cell culture media from these treatments were analysed. Integrals were referenced to an internal standard (TSP, 3-trimethylsilyl-[2,2,3,3]-2H4-propionic acid) and corrected for cell numbers. Levels of P-ERK1/2 and downstream targets cyclin D1 and pRB, were evaluated with Western blotting to confirm successful MEK1/2 signalling inhibition.

**Results:** In agreement with previous reports (Solit et al., Nature. 2006), cell lines with the V600E B-RAF mutation showed the highest sensitivity to MEK1/2 inhibition compared to intermediate activity or WT B-RAF harbouring cells (Figure 1A). Exposure to CI-1040 and PD325901 led to a marked decrease in ERK1/2 phosphorylation as well as levels of cyclin D1 and pRB as shown in Figure 1B. Figure 1C&D shows SKMEL-28 lactate levels post-treatment. MRS evaluation of intracellular lactate levels post-treatment with CI-1040 shows that lactate levels decreased significantly in all cell lines and using both MEK1/2 inhibitors. Lactate levels decreased to 44 ± 9% at 16 h (n = 3, p = 0.001) and 57 ± 13% at 24 h in HCT116 cells (n = 4, p = 0.001), 78 ± 13% in MDA-MB-231 cells (n = 4, p = 0.03), 46 ± 2% in WM266.4 cells (n = 3, p = 6 x 10^-5) and 44 ± 19% in SKMEL-28 cells (n = 3, p = 0.01) following CI-1040 treatment. Evaluation of extracellular lactate levels showed that these increased significantly in HCT116 cells 24 h post-treatment to 135 ± 8% (n = 5, p = 2 x 10^-6), were unchanged in MDA-MB-231 cells (101 ± 10%, n = 6, p = 0.9) and decreased in SKMEL-28 cells (to 72 ± 3%, p = 0.0001) and WM266.4 cells (to 76%, n = 1). Treatment with PD325901 also showed a reduction in lactate levels in WM266.4 and SKMEL-28 cells (to 66 ± 31, n = 2 and 59 ± 19, n = 2). Extracellular lactate levels also decreased in WM266-4 (to 82 ± 7%, n = 4, p = 0.002) and SKMEL-28 cells (to 73 ± 3, n = 2) post-treatment with PD325901. These results are similar to the effects of CI-1040 in these two cell lines.

**Conclusion:** Our findings demonstrate that MEK1/2 signalling inhibition with CI-1040 or PD325901 leads to decreased intracellular lactate levels in human melanoma, colorectal and breast carcinoma cell lines regardless of their B-RAF mutation status. The effects on extracellular levels of lactate were less uniform suggesting potential mechanistic variations in the changes observed. Our results suggest lactate as a potential non-invasive MRS biomarker of response to MEK1/2 targeted therapeutics in human cancer cells. Further studies are required to elucidate the mechanisms underlying the observed effects and to validate these changes in vivo.

**Cellular and metabolic effects of MEK1/2 inhibition in human melanoma cells**

**Figure 1.** A. Growth inhibition of CI-1040 as shown by SRB. B. Representative Western blots of MEK1/2 target protein expression 24 h post-treatment with CI-1040 and PD325901 in SKMEL-28 cells. C. Representative ^1H MRS spectra of SKMEL-28 cells, zoomed in on the lactate peak at 1.33 ppm. D. MRS evaluation of SKMEL-28 intracellular and extracellular lactate levels after treatment with CI-1040 or PD325901 (24 h treatments). The data are presented as percent change as compared to controls. Values are corrected relative to internal reference (TSP) and cell numbers. Error bars indicate standard deviation.

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