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

M. Falck Miniotis1, T. R. Eykyn1, P. Workman1, M. O. Leach1, and M. Beloueche-Babari1
1CRUK and EPSRC Cancer Imaging Centre, The Institute of Cancer Research & The Royal Marsden Hospital, Sutton, Surrey, United Kingdom, 2CRUK Centre for Cancer Therapeutics, The Institute of Cancer Research & The Royal Marsden Hospital, Sutton, Surrey, United Kingdom

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 production1. 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 1H MRS as previously described1. 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. 1-13C 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 13C 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 ≤ 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.

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.

Acknowledgements: This work was funded by Marie Curie Action: Early Stage Training (grant MEST-CT-2005-020718) and CRUK (grants C309/A8274, C1060/A5117 and C1060/A6916). In addition, we also acknowledge the support received from the CRUK and EPSRC Cancer Imaging Centre in association with the MRC and Department of Health (England) grant C1060/A10334 and NHS funding to the NIHR Biomedical Research Centre.

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