Treatment with the MEK inhibitor U0126 induces increased lactate production in prostate and breast cancer cell lines

A. Lodi[1], S. M. Woods[1], R. M. Danforth[1], and S. M. Ronen[1]
[1]University of California San Francisco, San Francisco, California, United States

Introduction. Mitogen-activated protein kinase (MAPK) pathways control fundamental cellular processes including proliferation, differentiation and apoptosis and are often deregulated in cancer. Among the MAPK pathways, the extracellular signal-regulated kinase (ERK) is of particular relevance as it is deregulated in approximately one-third of all human cancer and targeted inhibitors are being developed as cancer therapeutics. U0126 is a highly selective inhibitor of the ERK-activating MEK1/2. In this study we investigated the metabolic consequences of U0126 treatment in human prostate and breast cancer cell lines using ^{1}H and ^{13}C magnetic resonance spectroscopy (MRS) as well as gene expression data.

Methods. Prostate (PC3 and LNCaP) and breast (MCF7) human cancer cells were treated for 48 hrs with U0126. Cells were extracted using a dual phase extraction and the dried polar extracts were redissolved in 90% H_2O/10% D_2O phosphate buffer. ^{1}H MR data were acquired at 600 MHz using a 90° pulse and 4 s relaxation delay and the water signal was suppressed using excitation sculpting. Repeated univariate statistical testing with false discovery rate correction was performed on each point of the ^{1}H MR spectra to determine significant signal changes. The rate of glucose uptake and lactate production were determined from dynamic studies in live cells grown on beads by using ^{13}C MRS to acquire sequential 2 hour proton-decoupled ^{13}C spectra (60° pulse and 6 s relaxation delay) over 15-22 h while the cells were perfused with medium containing 1-^{13}C glucose as described (1). Protein and gene expression levels were measured using Western blotting and microarray analysis as described (1).

Results and Discussion. The effect of U0126 was confirmed by western blot analysis demonstrating a drop in p-ERK (Fig. 1). ^{1}H MRS spectra were recorded for all cell lines. The univariate statistical analysis (Fig. 2a) performed on the data of the prostate cells revealed that the 48 hour drug treatment induced several significant metabolic changes, including depletion of intracellular aspartate, succinate and phosphocholine. In addition, surprisingly, in all three cell lines a substantial increase in intracellular lactate levels was observed following treatment, with lactate up 134±16% (p=0.002), 149±20% (p=0.001) and 213±43% (n=2) of control in PC3, LNCaP and MCF7 cells, respectively. Dynamic studies in live MCF7 cells were in line with the extract data and showed that the rate of lactate production increased following treatment 169±43% from 260±98 fmol/cell/hour to 439±114 fmol/cell/hour (p=0.027; Fig 2b and c). The rate of glucose uptake increased 140±40% but did not reach significance (p=0.18). Dynamic studies are underway for prostate cells with initial findings in line with the MCF7 data. Consistent with the metabolic observations, overexpression of several genes associated with glycolysis, including 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), pyruvate dehydrogenase kinase isozyme 1 (PDK1), hexokinase 2 (HK2) and glucose transporters (GLUT1) were observed in PC3 cells pointing to an increase in glycolytic enzymes induced by the U0126 treatment. In an effort to explain this observation we assessed the expression of other signaling proteins and found that, although U0126 inhibits its target kinase, cross-talk between the MAPK and the PI3K pathways triggers increased Akt phosphorylation (Fig 1). The activation of Akt following MAPK inhibition has been previously observed (2,3). Moreover, Akt activation has been previously observed to modulate glycolysis (4). We therefore hypothesize that the observed increases in lactate production and its intracellular accumulation following inhibition of MAPK kinase signaling with U0126 result from activation of the PI3K/Akt pathway induced by cross-talk between the two pathways.

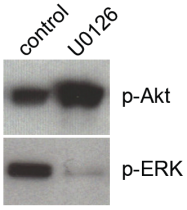


Fig. 1. Western blots probing for p-Akt and p-ERK in untreated and U0126 treated LNCaP cells.

Conclusion. Deregulation of the MAPK pathway is often involved in oncogenesis and tumor growth, and therefore inhibitors are being investigated as targeted cancer therapeutics. Our results highlight the value of MRS in identifying previously unidentified metabolic consequences of treatment that could serve to inform on drug action.

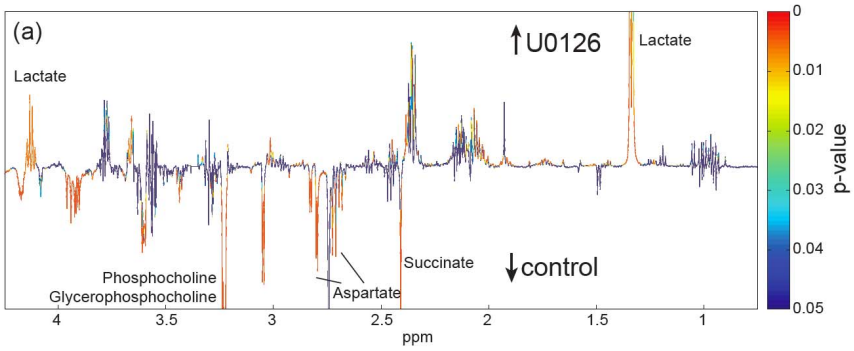
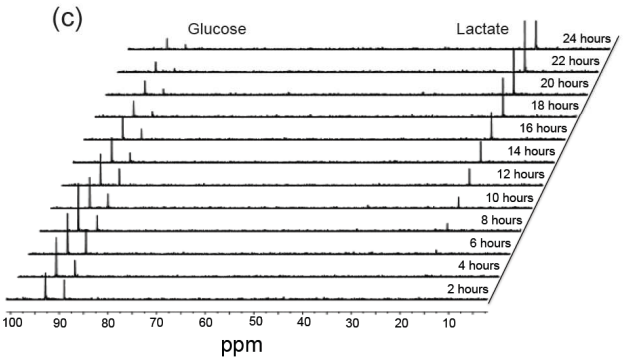


Fig. 2. (a) Average ^{1}H difference spectrum for PC3 cells treated with U0126 minus control. Significantly different points are color-coded according to the p-values color-bar scale. (b) Lactate production over a 24-hours period in untreated and U0126-treated MCF7 live cells continuously perfused with medium containing 1-^{13}C glucose as obtained from ^{13}C MR spectra (c) acquired at 2-hours intervals).



References:
(1) Brandes AH et al., Breast Cancer Res, 2010;12(5):R84.
(2) Halilovic E et al., Cancer Res, 2010;70(17):6804-6814.
(3) Wee S et al., Cancer Res, 2009;69(10):4286-4293.
(4) Elstrom RL et al., Cancer Res, 2004;64(11):3892-3899.

Acknowledgements: This work was supported by NIH grant RO1 CA130819.