Treatment with the MEK inhibitor U0126 induces a decrease in hyperpolarized pyruvate to lactate conversion in breast but not in prostate cancer cells

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Introduction. The clinical availability of an increasing number of new targeted therapies and treatment options requires timely and effective methods to evaluate individual response in order to improve the outcome by personalizing treatment. However, the evaluation of response to targeted therapies using traditional imaging methods is complicated by the fact that this response is frequently associated with inhibition of growth or tumor stasis and therefore only detectable after prolonged treatment. The development of novel methods to evaluate early response is therefore of key importance. Recently, $^{13}$C magnetic resonance spectroscopy (MRS)-detectable hyperpolarized pyruvate to lactate conversion was validated in cancer models as a noninvasive imaging method for the detection of tumors and treatment response, and is currently in phase I clinical trials. Importantly, responses to radiation, chemotherapy and targeted PI3K signaling inhibitors have all been associated with a drop in hyperpolarized lactate production, albeit via different mechanisms. The goal of this study was to assess the effect of treatment with another therapeutic approach entering the clinic, namely inhibition of the MAPK pathway. To this end we studied the effect of the MEK inhibitor U0126.

Methods. Prostate (PC3) and breast (MCF-7) cancer cells were treated for 48 hours with the MEK inhibitor U0126 (50 and 25 μM, respectively). To monitor treatment-induced modulation of hyperpolarized pyruvate to lactate conversion, live cells grown on beads were investigated in a bioreactor system and $^{13}$C MRS was performed to compare treatment-induced changes in intracellular lactate levels, cells were imaged immediately after 3 s using 5 s pulses. To determine treatment-induced changes in intracellular lactate levels, cells were extracted using the dual phase approach to enhance our understanding of the metabolic sequelae of treatment, and illustrate the potential use of this new methodology for monitoring response to MEK inhibitors.

Results. U0126 treatment lead to MAPK signal inhibition and inhibition in cell proliferation in both cell lines (data not shown). Figure 1A illustrates the temporal evolution of hyperpolarized lactate production in control and U0126-treated cells. The maximum hyperpolarized lactate levels in treated MCF-7 cells dropped to 62±26% (p=0.05) compared to control cells. In contrast, in PC3 cells treatment with U0126 induced an increase to 182±23% (p=0.02) of control. To mechanistically explain our findings, and in particular the unexpected increase in hyperpolarized lactate observed in PC3 cells in response to administration of U0126, we investigated treatment-induced changes in the different factors known to affect pyruvate to lactate conversion, namely NAD(H) concentration, total intracellular lactate levels, LDH expression and activity, and expression of MCT1. In both PC3 and MCF-7 cells, LDH activity and NAD(H) levels remained unchanged. Total intracellular lactate levels increased in both cell lines following treatment with U0126, to 134±16% (p=0.002) of control in PC3 and to 206±42% (p=0.001) in MCF-7 cells (Fig. 1B). We also observed an increase in LDH expression (Fig. 1C) and activity to 136±18% (p=0.037), and to 245±38% (p=0.02) of control in PC3 and MCF-7 cells, respectively (Fig. 1D).

Discussion and Conclusions. The elevation of LDH expression and activity as well as the elevation of total intracellular lactate could all contribute to the formation of higher levels of hyperpolarized lactate. Indeed, in PC3 cells we observed a treatment-induced increase in the hyperpolarized pyruvate to lactate conversion. In contrast and in spite of an even greater elevation of LDH expression and activity as well as total intracellular lactate levels in MCF-7 cells, we observed a drop in the pyruvate to lactate conversion in these cells. This was associated with a clear drop in cellular MCT1 levels. Thus, the reduction in hyperpolarized pyruvinate delivery into the cytosol is likely the explanation for the drop in hyperpolarized lactate production observed following U0126 treatment in MCF-7, but not in PC3 cells. Our results highlight the complexity of the interactions between signaling pathways and metabolic pathways, and illustrate the need for a good mechanistic understanding of links between cell signaling and metabolism. Moreover, our findings demonstrate the value of the hyperpolarized $^{13}$C approach to enhance our understanding of the metabolic sequelae of treatment, and illustrate the potential use of this new methodology for monitoring response to MEK inhibitors.

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