# Characterization of localized and metastatic renal cell carcinoma metabolism using hyperpolarized and thermal $^{13}$C MR

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**Introduction:** A hallmark of cancer cell metabolism, first postulated by Warburg[1], is elevated glycolysis, even in the presence of oxygen. Elevated glycolysis leads to an increased flux of pyruvate to lactate, which can lead to cancer invasiveness and metastasis[2]. The flux of pyruvate to lactate, catalyzed by the enzyme lactate dehydrogenase enzyme (LDH), is a function of the enzyme activity and cofactors, and monocarboxylate transporters for pyruvate (MCT1) and lactate (MCT4). In order to understand the various factors that contribute to the observed HP pyruvate/lactate kinetics, we performed thermal labeling with [3-$^{13}$C] pyruvate in human renal cell carcinomas (RCC) cell lines established from localized RCCs (UMRC6) and metastatic RCC (UOK262), and compared the thermal labeling data to the HP MR data and the expression of LDH enzyme and MCT transporters.

**Methods:** UMRC6 and UOK262 cells (n=3 each) were grown to 50% confluency in DMEM medium (supplemented with 10% FBS and Penicillin/Streptomycin). The cells were incubated in medium containing 15mM [3-$^{13}$C] pyruvate and 1g/L glucose for 24 hours. Following incubation, the medium was collected and the cells were extracted with methanol and chloroform and were lyophilized[3]. The extracts were reconstituted in 600µl $D_2O$ containing 1.27mM TSP as a concentration reference. The extracts and medium were measured on Bruker Avance II 800 MHz spectrometer fitted with a triple resonance cryo probe. 1D $^1$H spectra were obtained with presaturation with TD=24K points, SW = 12KHz, TR=3s and 256 repetitions. Direct Carbon detection was also used to measure the carbon labeled metabolites with proton decoupling using a 30° pulse, TR=3s, NS=2048. Spectra were quantified using the deconvolution algorithm provided with TOPSPIN. HP [1-$^{13}$C] pyruvate experiments were also conducted on theses cells encapsulated in alginate beads in the 10mm bioreactor as previously described[3]. Bioreactor HP experiments were conducted in a narrow-bore 11.7T Varian INOVA (125MHz $^{13}$C, Varian Instruments). HP $^{13}$C signals were acquired at intervals of 3s using 10° pulses. Relative mRNA expression of LDH and MCTs was obtained by qRT-PCR.

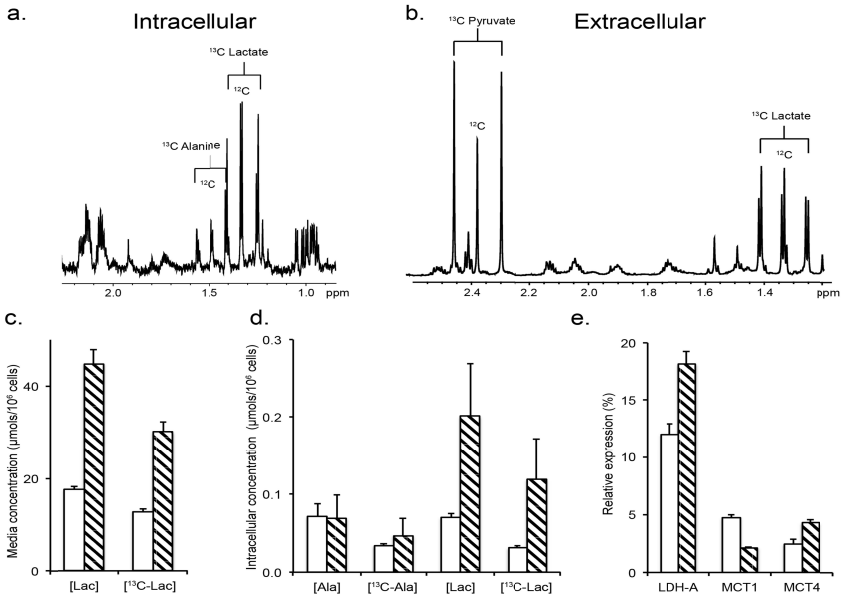


**Figure 1**. Representative 1D $^1$H spectra of (a) intracellular and (b) extracellular labeling of lactate and alanine after incubation for 24 hours with [3-$^{13}$C] pyruvate in UOK262 cells. Lactate concentrations in the media (c), intracellular metabolite concentration and relative mRNA expression of LDH-A and MCTs to betaactin (e), in UMRC6 (white) and UOK262 (striped) cells.

**Results and Discussion:** **Fig 1a** is a representative proton spectrum of UOK262 extracts, demonstrating clearly, peaks of $^{13}$C satellites of intracellular alanine and lactate. **Fig** 1b demonstrates [3-$^{13}$C] satellite peaks of pyruvate and lactate in the media. The high resolution proton spectra enable direct measurements of the labeled pools of lactate, alanine and pyruvate. **Fig 1c** shows that the medium of UOK262 cells had a 2.5 fold higher lactate concentration than that of the UMRC6 cells. However, the fractional enrichment of lactate in the media of UMRC6 cells is higher than that of the UOK262 cells ($p<0.01$). **Fig 1d** shows the intracellular metabolite concentrations in the two cell lines. The intracellular concentrations of alanine in both cell lines are similar. Although the $^{13}$C labeled alanine is elevated in the UOK262 cells by 26%, it is not significantly different from that in the UMRC6 cells. In contrast, the total intracellular pool of lactate as well as the $^{13}$C labeled lactate (derived from [3-$^{13}$C] pyruvate) is higher in the metastatic UOK262 cells ($p=0.03$ and 0.05 respectively). It is clear from **Fig 1c and 1d** that 99% of the $^{13}$C labeled lactate was transported out of the cell by 24 hours in both RCC cell lines. **Fig 1e** shows the relative mRNA expression of LDH-A, MCT1 (influx of pyruvate), and MCT4 (efflux of lactate) in the two RCC cell lines. UOK 262 cells had 50% higher expression of LDH enzyme ($p<0.02$), and 75% higher expression of MCT4 ($p<0.03$) than UMRC6 cells. The combination of the higher LDH and MCT4 expression in the UOK 262 cells likely contributed to the increased extracellular lactate observed in the UOK262 cells. Even though UMRC6 cells demonstrate 2 fold higher MCT1 expression, this does not result in an observable increase in intracellular $^{13}$C labeled lactate. This suggests that the production of intracellular $^{13}$C labeled lactate may be rate-limited by the LDH enzyme in these two RCC cells. The dynamic flux of pyruvate to lactate in these two RCC lines was then measured after the injection of HP [1-$^{13}$C] pyruvate in an MR-compatible bioreactor. The flux of HP pyruvate to lactate was similar in the UMRC6 cells and UOK262 cells (4.8±0.6 and 5.1±0.5 nmols/s/$10^7$ cells, respectively) within the timeframe of the bioreactor experiment. The measured HP pyruvate-to-lactate flux is likely influenced by a combination of factors including LDH, MCT1 and MCT4 expression and/or activity in these RCC cells. Future studies, such as measurement of time-to-maximum HP lactate and HP lactate total area under the curve, may further elucidate which of the above factors has the most influence on the observed HP pyruvate/lactate kinetics. The steady-state thermal labeling study revealed additional interesting isotopomer pattern in the glutamate pool of the two RCC cell lines. [5]The 4-$^{12}$C glutamate pool in the UOK262 cells (0.166±0.034 µmols/$10^6$ cells) is three fold higher ($p<0.02$) than that of UMRC6 cells (0.051±0.002 µmols/$10^6$ cells). Similarly, the 4-$^{13}$C labeled glutamate was significantly increased (three fold, $p=0.029$) in the UOK262 cells than the UMRC6 cells. These observations are consistent with the known mutation in the enzyme fumarate hydratase in the UOK262 cells[5].

**Conclusion:** Our thermal labeling studies suggest that the biotransporters of pyruvate and lactate (MCT1 and 4), LDH, and other key TCA cycle enzyme are important determinants of RCC aggressiveness and metastatic potential. The observed HP pyruvate/lactate dynamics in the RCC cells is likely influenced by a combination of factors including MCT and LDH expression. Understanding the metabolic underpinning of the observed HP MR data in these cells is the first step towards clinical translation of this technology for renal cancer evaluation.

**References**: [1] O. Warburg, *Science* **1956**, *123*, 309. [2] R. A. Gatenby, et al., *Cancer research* **2006**, *66*, 5216. [3] K. R. Keshari, et al., *Magn Reson Med* **2010**, *63*, 322. [4] P. Sonveaux, et al., *J Clin Invest* **2008**, *118*, 3930. [5] Y. Yang, et al., in *Cancer Genetics and Cytogenetics, Vol. 196*, **2010**, 45.

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