

Noninvasive Assessment of Renal Tumor Aggressiveness using Hyperpolarized ^{13}C MR

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INTRODUCTION: The widespread use of cross-sectional imaging has led to the incidental discovery of many renal tumors (1). However, triage of therapies is currently difficult due to our inability to reliably differentiate benign from malignant, or indolent from aggressive renal tumors noninvasively by conventional imaging techniques. Hyperpolarized (HP) ^{13}C MR is a new metabolic imaging technique that highlights the increased glycolysis in many aggressive and metastatic cancers. This technique has already been successfully applied to the evaluation of prostate cancer presence and aggressiveness in animal models (2,3) and a clinical trial of hyperpolarized ^{13}C MR in patients with prostate cancer is currently underway. HP ^{13}C MR may similarly provide an excellent opportunity to characterize renal tumor aggressiveness *in vivo*. In this study, we aim to test the hypothesis that HP ^{13}C MR can differentiate among normal renal tubule epithelial cells, indolent renal cell carcinoma (RCC), and aggressive/metastatic RCC in cell culture.

METHODS: *Cell lines:* HK2 (normal human renal tubule epithelial cell line), UMRC6 (localized non-metastatic human RCC cell line), and UOK262 (highly aggressive and metastatic human RCC cell line) (4), with doubling times 85, 45, and 24 hours respectively, were grown in DMEM medium.

Assessment of steady-state ^1H and ^{13}C metabolite concentrations - HR-MAS NMR Cell Labeling Studies:

Cells were incubated with medium containing 4.65mM [3- ^{13}C]pyruvate and 1mM unlabelled glucose at 37°C for 120 min, followed by washing and trypsinization. Cell pellets (n=3, per cell line) and cell media were analyzed in custom designed 35µL ZrO₂ rotors containing 3.0µL D₂O + 0.75% TSP. HR-MAS data were acquired at 11.7T, 1°C, and 2,250 Hz spin rate using a Varian INOVA spectrometer, equipped with a 4mm gHX nanoprobe. Quantitative 1D ^1H spectra were acquired with 40,000 points, 20,000 Hz spectral width, and 256 transients (at = 2s, T_R = 6s). ^1H data of the cells were quantified using HR-QUEST, a custom version of QUEST adapted for HR-MAS [5] with an electronic reference (ERETIC) for absolute quantification [6]. Concentrations of ^{13}C metabolites were quantified using a 2D ^1H - ^{13}C HSQC experiment with GARP ^{13}C decoupling (at=0.82s, nt=8, ni=128, T_R =3.8s), as previously described [7], and statistically compared using a students t-test (p< 0.05). *Assessment of Metabolic Fluxes - HP ^{13}C Bioreactor Studies:* Cells were encapsulated as described previously (8). The encapsulated cells were then maintained in a custom designed 10mm NMR-compatible bioreactor system, with a continuous flow of 37°C DMEM media oxygenated with 95% Air/5% CO₂. 1mL of 4mM HP [1- ^{13}C] pyruvate (HypersenseTM, Oxford Instrument) was injected into the bioreactor. ^{13}C NMR spectra were acquired at 3sec intervals using 10° pulses for 300sec on a 11.7T Varian INOVA (Varian Instruments) equipped with a 10mm direct detect broadband probe. Both prior to and after injection, cell viability was assessed by measuring β -NTP peak areas from ^{31}P spectra (90° pulse nt=1024, at=1sec, 1 hour). The relative integrals of pyruvate and lactate were plotted as a function of time to determine maximum flux to lactate for each of the three cell lines.

RESULTS AND DISCUSSION: *HR-MAS Labeling Studies:* Fig. 1a summarizes the mean \pm sdev concentrations (N=3) of renal cell metabolites obtained from ^1H HR-MAS spectra of the 3 cell lines after incubation with 4mM [3- ^{13}C]pyruvate. Notably, steady state lactate concentrations increased significantly with aggressiveness of the cell lines. Other significant metabolite concentration differences included elevated glutamate in the UMRC6 RCC cells relative to the HK2 cells consistent with an increase in the tricarboxylic acid (TCA) cycle metabolism in cancer, and decreased glutamate and lower TCA cycle flux in UOK262 cells consistent with the known mutation in fumarate hydratase. Fig. 1b summarizes the mean (N=3) \pm sdev concentrations of ^{13}C labeled renal cell metabolites obtained from 3 cell lines after incubation with 4mM [3- ^{13}C]pyruvate. Notably, the fractional enrichment (FE) of lactate significantly increased from 42 \pm 7% for HK2 cells to 57 \pm 4% and 61 \pm 17% for UMRC6 and UOK262 cells, respectively. FE patterns are also consistent with increased TCA flux in UMRC6 RCC cells and reduced TCA flux in UOK262 cells. *HP ^{13}C Bioreactor Studies:* Figure 2a shows a representative ^{31}P spectrum of viable UMRC6 cells while perfused in the bioreactor. Plots of β -NTP versus time of the cell lines in the NMR compatible bioreactor demonstrated viable metabolism, with cell growth rates consistent with known doubling times. Figure 2b is a representative HP ^{13}C spectra post-injection of 4mM hyperpolarized [1- ^{13}C]pyruvate in UMRC6 cells, demonstrating flux to lactate at high signal to noise. Fig 2c shows the time-course of HP [1- ^{13}C]lactate production after injection of HP [1- ^{13}C]pyruvate. Notably, the HP [1- ^{13}C]lactate was higher in the highly aggressive, metastatic UOK262 cells compared with the UMRC6 cells and benign HK2 tubule epithelial cells, with the maximum HP [1- ^{13}C]lactate to [1- ^{13}C]pyruvate ratio increasing from 0.034 (HK2) to 0.063 (UMRC6) and 0.184 (UOK262).

CONCLUSIONS: We showed that hyperpolarized ^{13}C MR has the potential to noninvasively characterize renal tumor aggressiveness. Specifically, HP [1- ^{13}C]lactate may be a useful biomarker for discriminating benign from malignant, and indolent from aggressive renal tumors. Additionally, steady state ^{13}C labeling studies suggest that HP probes of the TCA cycle could provide further characterization of renal tumors. Although the present study is preclinical, hyperpolarized ^{13}C MR can be readily applied in patients with renal tumors as it allows fast imaging with breath-hold acquisition (which is not possible with conventional ^1H spectroscopy). The technique offers an unprecedented opportunity for *in vivo* metabolic imaging of renal tumors, which may significantly improve the management of patients by rationally guiding treatment.

REFERENCES: [1] Silverman SG et al. Radiology 2006; 240(1): 6-22. [2] Albers MJ et al. Cancer Res 2008; 68(20): 8607-15 [3] Day S et al. Nat Med 2007; 13(11): 1382-7. [4] Yang Y et al. Cancer Genetics and Cytogenetics 2010;196:45-55, Grossman HB et al. Journal of Surgical Oncology 1985;28:237-244, [5] Ratiney H et al. NMR in Biomed 2010 [6] Albers MJ et al. Magn Res Med 2009; 61(3): 525-32. [7] Levin YS et al. Magn Res Med 2009; 62(5): 1091-8. [8] Keshari KR et al. Magn Res. Med. 2010; 63(2): 322-9. NIH R01 EB007588, and R21 GM075941-01.

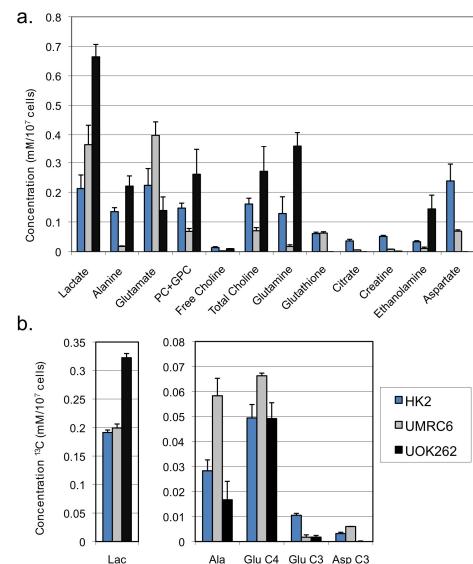


Figure 1. (a) ^1H derived steady-state and (b) ^{13}C metabolite concentrations post-incubation with 4mM [3- ^{13}C]pyruvate for HK2, UMRC6 and UOK262 cells.

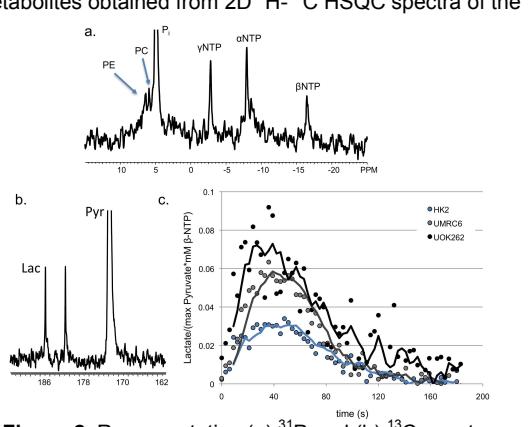


Figure 2. Representative (a) ^{31}P and (b) ^{13}C spectra post-injection of 4mM hyperpolarized [1- ^{13}C]pyruvate in UMRC6 cells. (c) Normalized Lac/max Pyr dynamics for the 3 cell lines.