Characterization of Glycolytic Activity and Perfusion in a Renal Cell Carcinoma Model During Sunitinib Treatment and Resistance with Hyperpolarized ^{13}C MRI

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Purpose: Renal cell carcinomas (RCC) demonstrate a high rate of glycolysis, associated with high expression of the glucose transporter GLUT1, which is in turn regulated by hypoxia-induced factors (HIF)[1]. Here we use hyperpolarized ^{13}C pyruvate (h^{13}C-pyruvate) to assess for *in vivo* changes in glycolytic metabolism in an RCC xenograft mouse model treated with sunitinib, and to correlate these findings with tumor perfusion as measured by ^{13}C-tert-butanol (h^{13}C-tert-butanol), and with GLUT1 and CD34 expression using quantitative immunohistochemistry.

Methods: Four mice were implanted with A498 VHL-deficient RCC. Two were treated with sunitinib, and two controls were administered phosphate-buffered saline (PBS). One sunitinib-treated mice was imaged 7 days after treatment initiation, the other 32-days post-treatment, at resistance. Control mice were imaged pre-PBS and 6-7 days following PBS. MRI was performed at 9.4 T horizontal-bore animal system (Bruker Biospec) using methods approved by our Institutional Animal Care and Use Committee. h^{13}C-pyruvate and h^{13}C-tert-butanol were polarized by dynamic nuclear polarization (DNP) as described previously[2]. T2-weighed proton images were acquired for anatomical localization with rapid acquisition with refocused echoes (RARE) sequence (TR/TE 3000/80 ms, echotrain of 8, 5.5 cm FOV, 2 mm slice, 270^2 µm resolution). h^{13}C-pyruvate imaging was performed with echo-planar spectroscopic imaging (EPSI, 16 x 16 matrix, 10 mm slice thickness, 512 spectral points, 64 frames), following injection of 200 µL of 100 mM ^{13}C-pyruvate via the tail vein. h^{13}C-tert-butanol imaging was performed using a balanced steady-state free precession (bSSFP) sequence (60° flip angle, TR/TE 4/2 ms, 5.5 cm FOV, 3 mm slice, 1^2 mm resolution, 512 ms/frame, 100 frames) following injection of 200 µL of 230 mM ^{13}C-tert-butanol via the tail vein. Tumors were harvested after final images for immunohistological analysis with CD34, GLUT1, and Hoechst immunofluorescent (IF) labeling on 4 µm slices across the center of the tumor, matching slice orientation to the MR images. Confocal scanning microscopy was performed across the entire slice. IF and MR analysis were performed with dedicated software (Volocity, ImageJ, Mathematica).

Results: Control tumors treated with PBS show high uptake of h^{13}C-pyruvate and conversion into ^{13}C-lactate (Top row, left figure). Sunitnib-treated tumor at 7d demonstrated markedly decreased perfusion correlating directly to decreased C13-pyruvate uptake, which was partially restored at resistance (Bottom row, left figure). Lower lactate:pyruvate ratios are suggested during resistance in comparison to pre-treatment (0.26 ± 0.04 vs 0.85 ± 0.005). High GLUT1 expression was sustained during growth, treatment, and at resistance, while CD-34 expression was reduced during sunitinib response (right figure) and restored at resistance.

Conclusion: Glycolytic metabolism is altered during RCC response to sunitinib and subsequent resistance, suggested by lower relative lactate levels, though overall glycolytic activity is promoted as demonstrated by persistent GLUT1 expression. C13-pyruvate uptake correlates with perfusion as measured by both h^{13}C-tert-butanol and CD34 expression.

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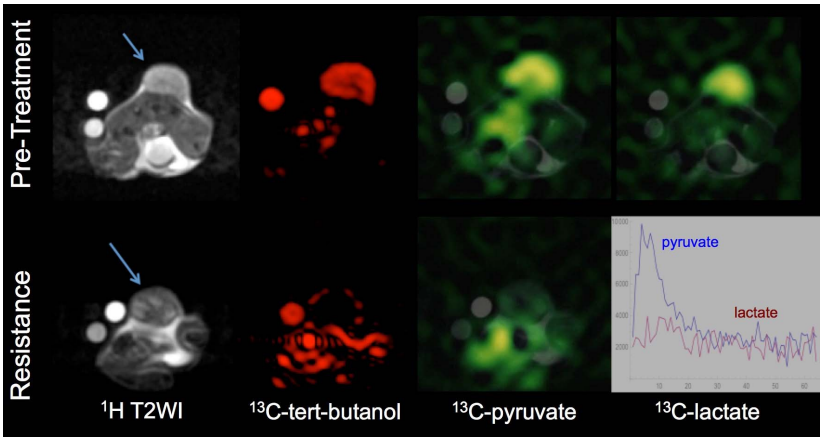
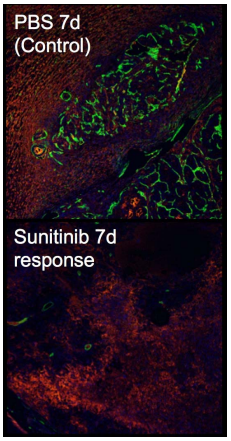
Left figure: ^{1}H and ^{13}C images of tumors at pre-treatment (top row) and at resistance (bottom row). ^{13}C-lactate signal is low during resistance due to reduced perfusion and difficult to visualize on imaging; amplitude plots integrated over the entire tumor for ^{13}C-lactate and ^{13}C-pyruvate are shown instead. **Right figure**: Control tumor (top) and treatment tumor during sunitnib response (bottom) demonstrating sustained high levels of GLUT1 expression (red), while CD34 expression is suppressed during treatment response (green).





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2. Grant AK et al. Perfusion imaging with a freely diffusible hyperpolarized contrast agent. Mag Reson Med. 2011 66:746-755.