

# Establishment of patient-derived models of renal cell carcinoma to study metabolism and develop relevant clinical biomarkers

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**Target audience:** Investigators of tumor metabolism and therapy response.

**Purpose:** Renal cell carcinomas (RCCs) are a heterogeneous group of tumors with a wide range of aggressiveness. There is a current lack of noninvasive biomarkers that can confidently predict the behavior of RCCs to guide treatment selection and to monitor treatment response. Development of clinically relevant biomarkers of RCC aggressiveness and response to novel therapeutics requires robust models that recapitulate the human situation. The purpose of this study is to establish both an *ex vivo* and an *in vivo* model of RCCs using patient-derived tissue slices for metabolism studies in conjunction with hyperpolarized (HP) <sup>13</sup>C MR.

**Methods:** *Tissue slice cultures (TSCs):* RCC tissues were obtained from surgical specimens, sliced into 300 μm thick disk (optimal thickness to allow maximal oxygen and nutrient diffusion), and maintained in culture on a rotating plate<sup>1</sup>. Tissue viability over time was assessed by H&E, Ki67 (proliferation), and Caspase-3 (apoptosis) staining, and by Live/dead assays (Invitrogen). *Ex vivo model of TSCs in a bioreactor:* 4 tissue slices were perfused in a custom-designed cartridge construct<sup>2</sup>. NMR data were acquired on a narrow-bore 14.1T Varian INOVA (150MHz <sup>13</sup>C) equipped with a 5mm broadband probe. <sup>31</sup>P spectra were obtained to monitor tissue viability (via quantification of βNTP) during the bioreactor experiment. HP <sup>13</sup>C MR was acquired dynamically (10° pulses, 3 s interval for 300 s) following injection of 1mL of 4mM of [1-<sup>13</sup>C] pyruvate to assess pyruvate metabolism in the RCC tissue slices. *Animal model:* The RCC tissue slices were also implanted under the renal capsule of immuno-compromised (RAG2) knockout female mice of 5-7 weeks as previously described<sup>1</sup>. After the tumor grafts reached about 5mm in diameter, mice were imaged at 14T MR (Agilent microimaging system). Multi-parametric proton imaging (T<sub>2</sub> weighted, DWI and DCE), and dynamic HP <sup>13</sup>C pyruvate MR were performed. For the HP pyruvate MR, a 3D echo-planar based GRASE<sup>3</sup> sequence was used to obtain dynamic lactate images, post injection of 80mM of HP [1-<sup>13</sup>C]pyruvate.

**Results and Discussion:** Fig.1 shows good viability of TSC (n=3), as assessed by Live/Dead assay over 48 hours. Fig. 2A shows representative <sup>31</sup>P spectra of RCC TSCs in the bioreactor at 5- and 48-hours with similar βNTP levels, again indicating maintenance of viability over 48 hrs. The observed <sup>31</sup>P metabolites are typical of those seen in renal cancers clinically, with dominant phosphor-ester peaks<sup>4</sup>. Following injection of HP [1-<sup>13</sup>C] pyruvate into the bioreactor, a dynamic flux to lactate of 0.05 nmols/s/mg tissue was observed in the RCC tissue slices (Fig. 2B). The pyruvate-to-lactate flux in the tissue slices was 24% lower than flux observed in immortalized human renal proximal tubule cells (HK2)<sup>5</sup>. This suggests that the patient-derived RCC tissue slice model more closely mimics the human situation when compared to models derived from immortalized cells, since even the normal immortalized renal tubule cells (where most human RCCs originate from) have unnaturally elevated lactate levels, likely secondary to abnormally high proliferation rates of cells in culture. Fig. 3 shows multi-parametric imaging of a patient-derived clear cell RCC tumor graft under the renal capsule. Fig. 3B shows the ADC map with the mean tumor ADC of 1.23e<sup>-3</sup> mm<sup>2</sup>/sec, which is similar to what has been reported in renal cancers clinically<sup>6</sup>. Fig 3C shows gadolinium enhancement of the tumor, and confirms tumor engraftment and perfusion. Fig. 3D shows the lactate metabolic map overlaid on the T<sub>1</sub> weighted axial image demonstrating elevated flux to lactate in the tumor similar to the bioreactor.

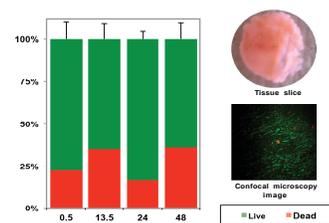


Fig. 1: Tissue viability in time measured by Live/Dead assay, with inset shows the tissue slice and the corresponding microscopic

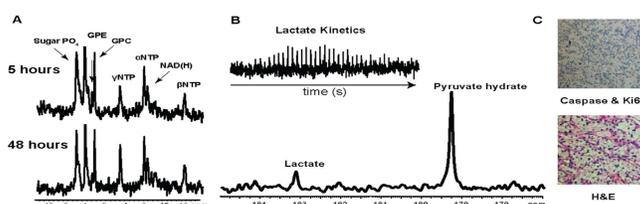


Fig. 2: A) <sup>31</sup>P spectra from 4 living renal cancer TSCs demonstrating the preservation of bioenergetics over 48 hours. B) HP Lactate Kinetics after injection of HP pyruvate. C) Caspase and Ki67 combined (above) and H&E (below) staining.

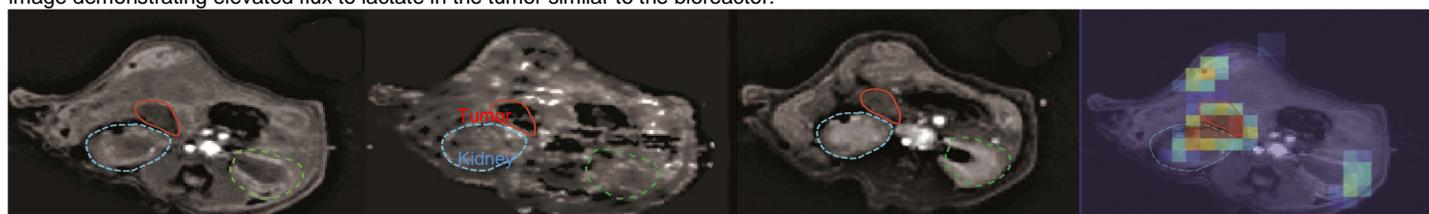


Fig.3: Multi-parametric imaging of a patient-derived RCC tumor graft (clear cell RCC) under the renal capsule of a RAG2 mouse A) T1 weighted proton image B) ADC map C) Contrast enhanced image D) Lactate metabolic map (obtained 35Sec post injection) overlaid on the T1 weighted image clearly showing increased lactate over the tumor region. ROIs are drawn over the tumor (solid red line), tumor bearing kidney (dashed blue line) and the contralateral kidney (dashed green line).

**Conclusion:** In this preliminary study, we have shown the feasibility of establishing both an *ex vivo* and an *in vivo* model of RCC using patient-derived tumor tissues, and used it to investigate a HP biomarker of RCC. Such models facilitate metabolic assessment of renal tumors of a range of histology, tumor grades and stages, which is not possible with currently available pre-clinical models of renal tumors. The combination of patient-derived tumor tissue slices and the MR-compatible bioreactor provides a unique *ex vivo* model that permits investigation of tumor metabolism in a physiologic and controlled setting. This *ex vivo* model facilitates testing of novel therapies and identification of clinically translatable biomarkers in a more efficient manner. The *in vivo* model allows additional interrogation of the contribution of tumor perfusion and microenvironment to tumor phenotype. The juxtaposition of the *ex vivo* and *in vivo* models may further enhance our understanding of renal cancer metabolism and development of clinically relevant biomarkers of tumor aggressiveness and therapy response.

**References:** – 1. H. Zhao et al, *Am J Pathol*, 2010, 177, 229–239. 2. K. R. Keshari et al, *Magn Reson Med*, 2010, 63, 322–329. 3. K. Oshio and D. A. Feinberg, *Magn Reson Med*, 2005. 4. B. Ross et al, *Kidney Int*, 1986, 29, 131–141. 5. K. R. Keshari et al, *Cancer research* in press. 6. G. Erbay et al, *Acta Radiologica*, 2012, 53, 359–365. **Acknowledgements:** Sukumar Subramaniam, Robert Bok, Romelyn DeLos Santos, Justin DeLos Santos and Rosalie Nolley. **Grants:** NIH P41EB013598 (JK), R01CA166766 (DMW), K99EB014328 (KRK), Department of Defense Peer Reviewed Cancer Research Concept Award (ZJW), Radiological Society of North America Scholar grant (ZJW).