Real-Time In-vivo Metabolic Characterization in Spontaneous Hepatocellular Carcinoma Using a Novel Transgenic Mouse Model

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Introduction - Cellular metabolism in tumours is significantly changed in order to adapt to environmental influences like rapid cell proliferation and hypoxia. Some of the metabolic changes can be followed by observing the fate of Carbon-13 (13C) labels using magnetic resonance spectroscopy (MRS). The low detection sensitivity of 13C MRS restricted its use in vivo. Recently, a revolutionary technique has emerged, enabling unprecedented visualization of pyruvate metabolism in vivo in live animals, and empowering the investigation of enzyme fluxes under normal and pathological conditions. In this study, we developed the hyperpolarized 13C technology to measure in vivo real-time metabolic fluxes in hepatocellular carcinoma (liver cancer), using a clinically relevant mouse model. Our work brings crucial insights into the comprehension of HCC metabolism and proves a useful tool to follow tumour development and progression in pre-clinical mouse models.

Materials and Methods - Hepatocellular Carcinoma Mouse Model (HCC): We used a combination of Sleeping Beauty transposon system and HBV transgene to mimic in mice the human hepatitis B-induced HCC(1-2). 20 male mice of age 13 – 23 months were scanned in a preclinical 9.4 T MRI system. Three distinct tumour development stages were identified. **Pyruvate Polarization and Dissolution:** Approximately 40mg of [1-13C] pyruvic acid, doped with 15mM trityl radical and 0.6mM of 3-Gd, was hyperpolarized in a polarizer. The sample was subsequently dissolved in a pressurized and heated alkaline solution, containing 100mg/liter EDTA, to yield a solution of 80mM hyperpolarized sodium [1-13C] pyruvate with 30% polarization at physiological temperature and pH [3]. Intravenous infusion of 300uL was followed by a 100uL saline flush. **Slice-selective 1D Spectroscopy:** 120 spectra of the metabolites were collected immediately upon infusion of hyperpolarized pyruvate (TR=1s, flip angle=20°, axial slice thickness(THK)=6mm, sweep width(SW)=40ppm, spectra points(SP)=2048). MR spectra were analyzed with AMARES algorithm in the jMRUI package. Flux quantification was implemented according to [4]. C**hemical Shift Imaging:** A 2D gradient-echo CSI pulse sequence was implemented in a 9.4 T MRI scanner. Imaging begins about 16 seconds after infusion of hyperpolarized pyruvate to ensure phase encoding starts only when all metabolites began to form. They were then axially imaged over a time-course of 2mins with TR=120ms, TE=0.6ms, FA=5°, SW=80ppm, SP=256, FOV=25.6x25.6mm^2, matrix=16x16, THK=5mm. Post-processing with Matlab picked up the peak of each metabolite in the spectra, filled up its k-space, zero-filled to 32 x 32, followed by 2D Fourier Transform. A reference proton image was acquired with Matrix=128x128, TR=100ms, TE=1.28ms and THK=1mm. **Glutaminase (GLS) activity assay:** 15 mice were sacrificed and their livers were excised and categorized into normal and carcinomas. Spectrophotometric determination of GLS activity was subsequently implemented using a suitable ammonia assay kit [5].

Results & Discussion - Different HCC development stages can be clearly distinguished from one another with respect to the number and size of tumours; as seen in Figure 1a. The lower pyruvate dehydrogenase (PDH) flux in advanced-stage carcinomas suggests a significant reduction in carbon entry into the Krebs cycle via decarboxylation (Fig. 1b). The obvious enhancement in glutaminase activity advocates the anaplerotic role of glutamine in hepatocellular carcinoma. Figure 1c illustrates the intense metabolic reduction of pyruvate to lactate (via lactate dehydrogenase LDH) as well as transamination to alanine (via alanine amino-transferase ALT) within the tumour.

Conclusion – We have successfully optimized the use of hyperpolarized 13C biomolecules to measure metabolic fluxes in hepatocellular carcinomas in-vivo and in real time. Metabolic imaging offers both a probe into tumour energetics and also a spatial aspect of its functional activity. In addition, the potential to detect aberration in biochemical fluxes using this technique might prove invaluable in longitudinal tumourigenesis studies.

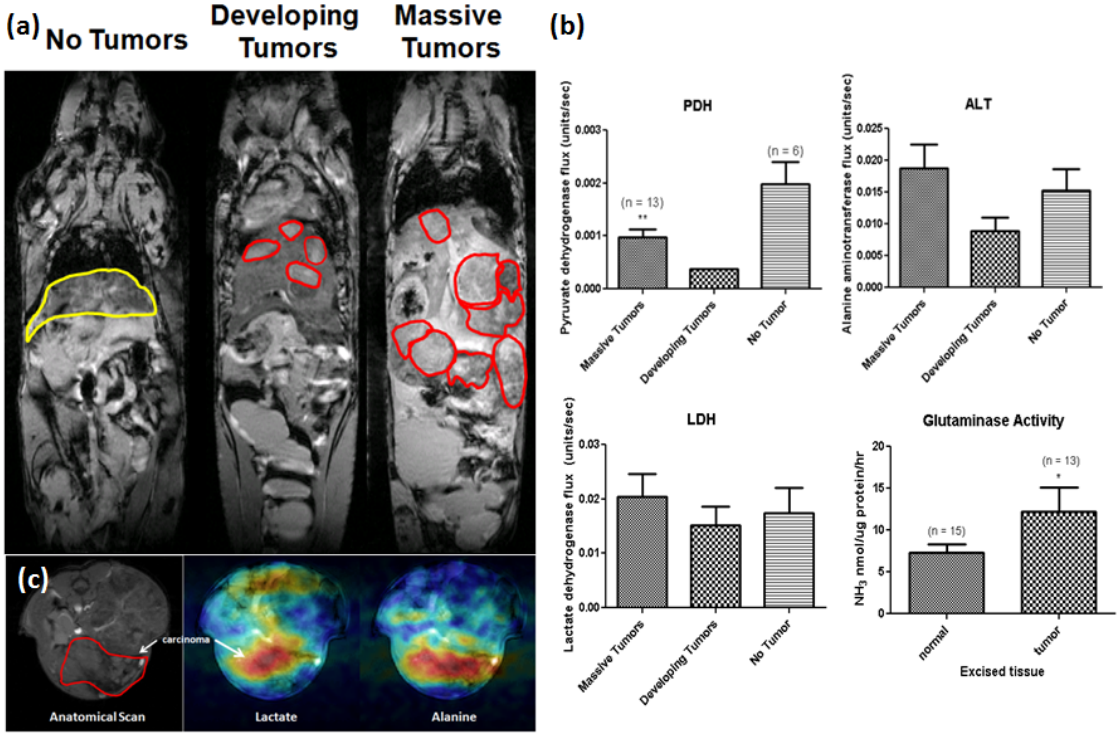

Figure 1: (a) T2-weighted MRI illustrating HCC progression in the liver. (b) Metabolic flux at each live cancer stage. Glutaminase assay compares the anaplerotic activity between healthy and tumour tissues.

References – [1] Cancer Res. 69(20), 8150 (2009); [2] Cell. 59(6):1145 (1989); [3] Cardiovas. Res. 86(1):82 (2010); [4] J. Mag. Res. 202: 85 (2010);