Imaging of Elevated Branched Chain Amino Acid Metabolism in Tumors with Hyperpolarized ^{13}C Ketoisocaproate

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Introduction: Powerful analytical tools are vital for characterizing the complex molecular changes underlying oncogenesis and cancer treatment, in particular if information is to be collected *in vivo* by non-invasive approaches. Hyperpolarized ^{13}C magnetic resonance (MR) spectroscopy has in many cases the potential to deliver the sensitivity and detailed spectral information to report on the chemical fate of tracer molecules in different tissues. In a preclinical study we here show that α-ketoisocaproic acid (KIC) can be used to assess molecular signatures of tumors using hyperpolarized MR spectroscopy. KIC is metabolized to leucine by the enzyme branched-chain aminotransferase (BCAT)(Fig. 1), which is a putative marker for metastasis and a target of the proto-oncogene *c-myc*.

Methods: The *in vivo* MR experiments were performed on a 2.35T Bruker Biospec Avance II system. EL4 tumor bearing c57BL/6 mice were anaesthetized and ECG, breathing rate and temperature was monitored (SA instruments). ^{13}C spectra were acquired with a 20-mm surface coil. Hyperpolarized [1-^{13}C]KIC (20 mM) were injected *i.v.* (175 µl / 6 s) and a double slice ^{13}C- CSI was acquired. [1-^{13}C]KIC was polarized to 32±3% in the liquid state using a setup previously decribed[1].

Results and discussion: Upon [1-^{13}C]KIC injection into an EL4 mouse, ample ^{13}C signal is detectable in mouse tissue within few seconds *in vivo*, both for [1-^{13}C]KIC (δ^{13}C=172.6 ppm) and its transamination product [1-^{13}C]leucine (δ^{13}C=176.8 ppm). No other reaction product than [1-^{13}C]leucine gets detectable. Murine lymphoma [1-^{13}C]KIC and [1-^{13}C]leucine signals were imaged in 5 mm slices through the tumor 20-32 seconds after intravenous injection of hyperpolarized [1-^{13}C]KIC (n=5). Resultant ^{13}C chemical shift images were overlaid with anatomical ^{1}H images (Fig. 2) to yield functional images of [1-^{13}C]KIC distribution and [1-^{13}C]leucine synthesis. Images obtained in this way show that [1-^{13}C]KIC has been distributed to the tumor within the first 20 seconds after injection. After injection of 4 µmol [1-^{13}C]-KIC, the signal to noise ratio (SNR) of [1-^{13}C]-leucine in the EL4 lymphoma model is determined to be 13.3±6.3 (mean ± SD). Most notably, [1-^{13}C]leucine signal is detected within the tumor at a high contrast of 6.9±1.0 relative to the highest [1-^{13}C]leucine signal in surrounding tissues. For reference [1-^{13}C]pyruvate was used in the same tumors revealing SNR of 34.2±11.1 and a contrast of 5.6±2.5.

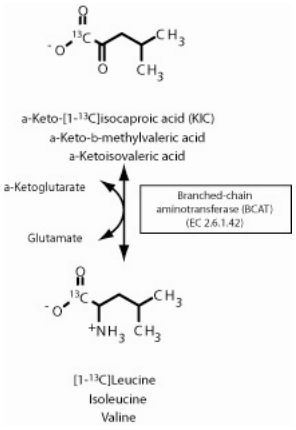

Figure 1. Reaction scheme for the metabolic production of leucine from α-ketoisocaproic acid. The reaction is catalyzed by BCAT, which catalyzes the transamination of leucine, isoleucine and valine to the respective α-ketoacids.

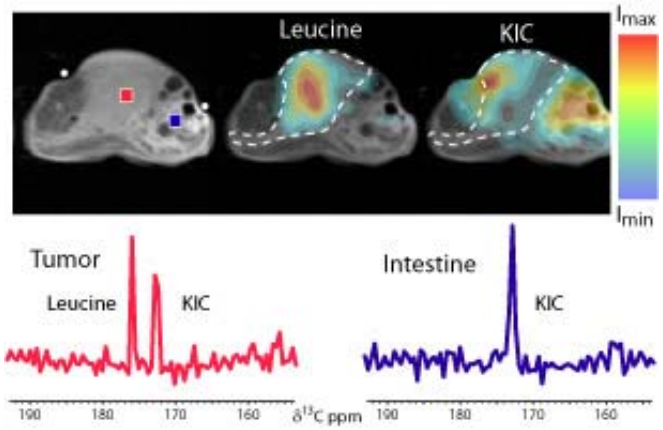

Figure 2. Imaging of BCAT activity *in vivo*. The anatomical ^{1}H image of an EL4 mouse is shown on the top left. Chemical shift images of [1-^{13}C]leucine and [1-^{13}C]KIC after injection of hyperpolarized [1-^{13}C]KIC are overlaid onto the anatomical image. 1D ^{13}C spectra for volume elements of the tumor and intestine (blue and red dots) demonstrate the difference in [1-^{13}C]leucine signal between tumor and surrounding tissue.

Conclusion: We describe a pre-clinical study showing that hyperpolarized KIC is suitable for the profiling of tumors at the single gene level. To this end, hyperpolarized KIC is introduced as a novel imaging modality for tumors with high BCAT activity.

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References: [1] Ardenkjaer-Larsen et al., PNAS 100:10158-63, 2003.