METABOLIC PROFILING OF EXPERIMENTAL TUMORS USING HYPERPOLARIZED [1-^{13}C] PYRUVATE: EVALUATING CELL TYPE SPECIFICITY AND EFFECTS OF TUMOR ENVIRONMENT.

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INTRODUCTION: Hyperpolarized ^{13}C magnetic resonance spectroscopy and imaging is a rapidly expanding field with many applications, in particular in the field of cancer. Polarization of a number of enzyme substrates enabled the studies of key steps in glycolysis and TCA cycling [1]. [1-^{13}C]-pyruvate has been extensively used in this context. The most commonly used experimental tumor models are (i) mice implanted with tumors and (ii) genetically manipulated mice that develop spontaneous lesions [2]. In this study we compared the lactate dehydrogenase (LDH) activity by measuring the conversion of [1-^{13}C]-pyruvate to [1-^{13}C]-lactate by (i) using same type of tumor cells (4T1 mammary carcinoma cell line derived from BALB/c mammary tumor) growing at different implantation sites (subcutaneous versus orthotopic) and using mice of different genetic background (immune compromised versus immune competent) thereby assessing effects of the tumor environment on the enzymatic conversion rate, and (ii) by using different tumor cell lines (4T1, C51 colon carcinoma, GL261 glioma) in order to assess differences in the metabolic status amongst the tumor cell lines.

MATERIAL and METHODS: *Animals*: 9 Balb/c nude (forming 3 groups), 4 Balb/c and 2 C57BL/6 female mice of 6 - 8 weeks of age were used. The mice were anesthetized using isoflurane (2 - 2.5%) and oxygen - air mixture (1/4), with the body temperature maintained at 36.5±0.5°C and respiration monitored. All animal experiments were performed in strict adherence to the Swiss Law for Animal Protection. *Tumor:* The animals were injected 6 days before the first experiment with 100ul or 50ul of a suspension containing 10^5 4T1 breast cancer cells either into the right thigh flank (in Balb/c) or fourth left mammary fat pad (inguinal group) (in Balb/c or Balb/c nude) respectively. Alternatively, 10^5 C51 colon carcinoma cells were injected in the right thigh flank of Balb/c nude, while the mouse glioma GL261 cell line was intracerebrally injected in a population of 2×10^4. *Sample production:* Samples of [1-^{13}C] pyruvic acid containing 15 mM trityl radical and 1.5mM Gd(DOTA) were hyperpolarized at 1.4K using a homemade DNP hyperpolarizer [3]. The sample was rapidly dissolved with Tris/NaOH/EDTA buffer solution to a concentration of 90mM and pH 7.5, and a bolus of 0.25ml was injected intravenously. *MRS experiment:* All in vivo MRS measurements were performed on a Bruker BioSpec 94/30 (Bruker BioSpin MRI, Ettlingen, Germany) horizontal bore MR system using a combination of volume resonant coil, for proton imaging and a custom built 16mm diameter surface coil for ^{13}C measurements. A FLASH sequence was used for anatomical reference images, which were used to accurately position the slices for the spectroscopy experiments and also to calculate the tumor volume. The MRS experiments were carried out using a slice selective spectroscopy sequence with the following parameters: 1 slice with thickness 7.5mm, echo/repetition time TE/TR = 0.5712ms/3000ms, Gaussian shape excitation pulse with 0.1ms duration and 15 degree angle, band width = 2740 Hz, number of sampling points = 4096. *Analysis of MRS data:* All spectroscopy data were processed using AMARES fitting of jMRUI (Version 4, http://www.mrui.uab.es/mrui/). The post-processing was done using MATLAB (R2012a, Mathworks) and Origin (OriginPro 8.6.0, OriginLab). For calculation of rate constant of the enzymatic reaction the method proposed by Harris et al [4] was used. Peak assignments were based on published data [5].

RESULTS: Fig.1 shows a representative spectrum and time profile of pyruvate and lactate intensity for an orthotopic 4T1 tumor in Balb/c mice. The signal intensity was used to calculate the rate constant, k_{LDH}, for LDH catalysed enzymatic reaction. The LDH forward rate constant (averaged over the tumor volume) for 4T1 tumors implanted either orthotopic in Balb/c or nude Balb/c mice or subcutaneous in nude Balb/c mice was found not to depend on the site of implantation and the mouse strain used. Also we did not observe any change in the conversion rate in relation to the tumor volume (Fig. 2). Based on the observation that the rate constant was found constant under these different conditions (implantation site, mouse strain, tumor volume), group statistics were calculated irrespective of tumor size for the different tumor types studied. We found significant differences in the LDH rate constant when comparing subcutaneous C51 and 4T1 tumors as well as orthotopic 4T1 tumors and gliomas (Fig.3).

DISCUSSION: This study showed that the pyruvate to lactate conversion depends on the tumor type as we found significant differences in the LDH rate constant for three distinct tumor types. On the other hand, the influence of the tumor environment on these metabolic processes appears to be minimal, as we did not detect any difference in the LDH activity when implanting 4T1 tumor cells at different implantation sites or when using different host mouse strains. This indicates that the pyruvate processing through the LDH reaction reflects an intrinsic property of the tumor cell line, which at least for 4T1 tumors is not altered during tumor growth. Important next steps would be to consider tumor heterogeneity, as the current analysis is based on data averaged over the entire tumor. Furthermore, the differences found among different tumor types have to be validated by comparing the in-vivo MRS finding with immunochemical analysis of LDH activity in these in vivo evaluated tumors.

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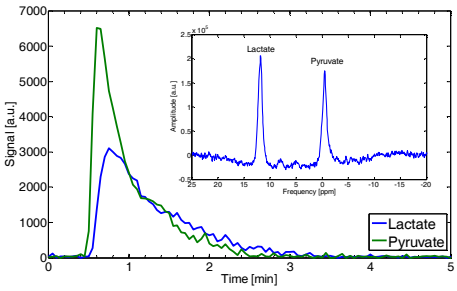

Fig. 1: Time profile of the pyruvate and lactate intensity, as calculated using AMARES. Insert figure show a representative spectrum 30sec after pyruvate injection.

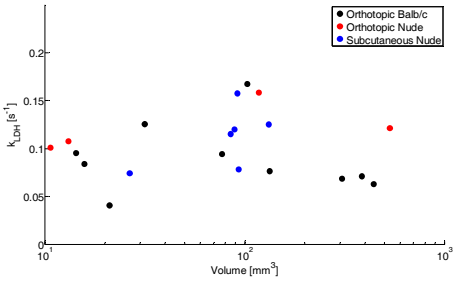

Fig. 2: Values for the enzymatic conversion through the LDH reaction for 4T1 mammary carcinoma as a function of the tumor volume for different implantation sites and for animals of different genetic background (horizontal axis in logarithmic scale).

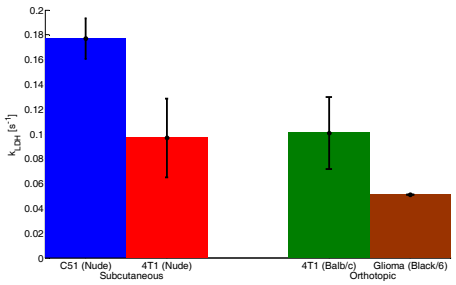

Fig 3: Mean values ± standard deviation of the mean k_{LDH} values for different tumor types (blue: C51, red: 4T1 subcutaneous inoculated in nude mice, green: 4T1 and brown: glioma orthotpically inoculated in Balb/c mice and Black/6 mice)