Assessment of lactate in LDH-A silenced 4T1 tumors with selective multiple-quantum coherence transfer
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Introduction: The assessment of the metastatic potential of a tumor could alter treatment during the early stages of therapy. Tumor lactate levels has been considered as a prognostic marker of aggressiveness tumors (1). Pyruvate is largely derived from both glucose and glutamine metabolism, and is converted to lactate by the lactate dehydrogenase (LDH) complex and/or enters the TCA cycle for conversion to CO2 and ATP. The conversion of pyruvate to lactate is catalyzed by LDH. LDH is a tetrameric enzyme, containing two major subunits (A and B) coded by two different genes (LDH-A and LDH-B), resulting in five isozymes (2). All five isozymes can catalyze the forward and backward conversion of pyruvate and lactate. LDH-A (LDH-5, M-LDH, or A4) kinetically favors the conversion of pyruvate to lactate, while LDH-B (LDH-1, H-LDH, or B4) predominantly converts lactate to pyruvate, which will be further oxidized through the TCA cycle (3). Recently, a link between tumor lactate levels (monitored by MRSI and LDH-A expression) and tumor phenotype has been demonstrated (4). The current study employs transfecting cells with LDH-A shRNA to down regulate the level of LDH-A expression. We show in this abstract that a decrease in the level of LDH-A leads to less lactate production (measured by MRSI) and slower growth rates (in orthotopic 4T1 breast tumors). Studies evaluating metastatic burden are ongoing.

Materials and methods: The metastatic breast cancer line model used in this study, 4T1, was derived from a spontaneous breast tumor that developed in the a BALB/cfC3H mouse (5). Orthotopic 4T1 tumors form metastatic macrscopic nodules in lung and other organs (5). 4T1 cells were transfected with Sure Silencing™ shRNA plasmids that were designed to specifically knock down the expression of the mouse LDH-A gene. Two clones with highest knock down level of LDH-A were selected, based on Western blot assessments under normoxia (21% oxygen) and hypoxia (1% oxygen). 4T1 cells were also transfected with shRNA plasmid bearing a scrambled shRNA as a control. Cells were injected into mammary fat pad of athymic nu/nu female mice (5 mice for each group). The lactate signal was acquired using a selective multiple-quantum coherence transfer (SelMQC) editing sequence in combination with chemical shift imaging (CSI) (6). The tumors were scanned at small (~100 mm3) and large (~300mm3) volumes. The spectra were then quantitated by means of the phantom replacement technique to estimate the in vivo lactate concentration. Results: The assessment of LDH-A expression in 4T1 control cells (4T1, kdA2) and the knock-down clones (columns 1-9) by Western blotting are shown (Fig. 1A). Clones 4T1, 4 and 9 were used for further in vivo experiments. We found that the in vivo growth rate of the LDH-A knock-down clones was significantly slower the control; the average doubling time of the tumors increased 1.5 fold (p<0.01) (Fig. 1B). Moreover, the LDH-A down regulated tumors also produce less lactate on average, at both small and large tumor volumes (Fig. 1C). Representative whole tumor MRS lactate spectra from a control and two LDH-A knock-down tumors is shown (Fig 1D). Representative CSI spectra from a large tumor (300 mm3) 4T1 control and clone #4 are also shown (Fig.1E). Visual inspection indicates that the LDH-A down-regulated tumors do not generate lung metastases at early stages. Further experiments are in progress to verify whether less lactate production by tumors is correlated with a lower metastatic potential. Discussion: Transfecting wild type 4T1 mouse breast cancer cells with LDH-A shRNA significantly reduces LDH-A expression and lactate production, when compared to 4T1 cells transfected with scrambled shRNA (control). This difference can be detected and measured in vivo by MRSI. High lactate levels in control 4T1 tumors were associated with more rapid growth, in comparison to the LDH-A knock-down clones. Preliminary results also suggest that tumor lactate levels, even at an early stage of tumor development, is associated with a greater propensity to develop metastases. We suggest that LDH-A expression and tumor lactate levels may be potential markers of high metastatistc potential, and that SelMQC may provide a noninvasive approach for monitoring tumor progression and metastatic potential noninvasively. References: (1) Walenta S, Mueller-Klieser WF. Lactate: mirror and motor of tumor malignancy. Semin Radiat Oncol. 2004;14:267-74.(2) Everse J, Kaplan NO. Lactate dehydrogenases: structure and function. Adv Enzymol Relat Areas Mol Biol. 1973;37:61-133.(3) Stambaugh R, Post D. Substrate and product inhibition of rabbit muscle lactic dehydrogenase heart (H4) and muscle (M4) isozymes. J Biol Chem. 1966;241:1462-7. (4) Serganova I, Rizwan A, Ni X, Thakur SB, Vider J, Russell J, et al. Metabolic Imaging: A Link between Lactate Dehydrogenase A, Lactate, and Tumor Phenotype. Clin Cancer Res. 2011;17:6250-61. (5) Aslakson CJ, Miller FR. Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. Cancer Res. 1992;52:1399-405. (6) He Q, Shungu DC, van Zijl PC, Bhujwalla ZM, Glickson JD. Single-scan in vivo lactate editing with complete lipid and water suppression by selective multiple-quantum-coherence transfer (Sel-MQC) with application to tumors. J Magn Reson B. 1995;106:203-11.