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 CO_2 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/cfC_3H mouse (5). Orthotopic 4T1 tumors form metastatic macroscopic 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 mm^3) and large (~300mm^3) 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 mm^3) 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 metastastatic potential, and that SelMQC may provide a noninvasive approach for monitoring tumor progression and metastatic potential noninvasively.

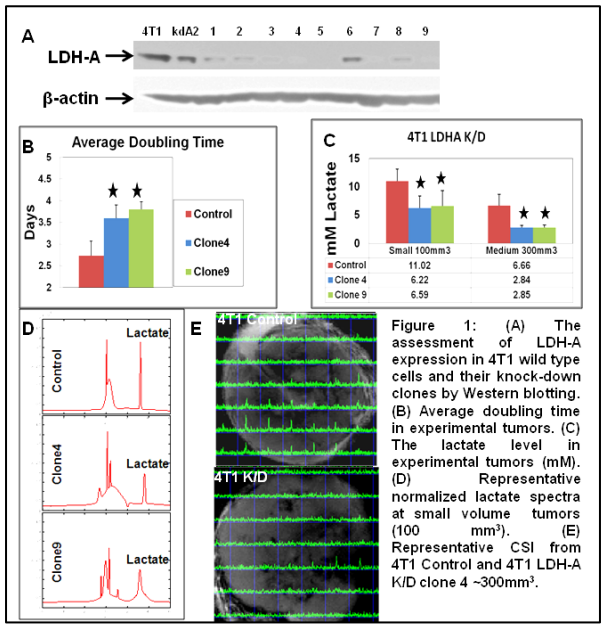

Figure 1: (A) The assessment of LDH-A expression in 4T1 wild type cells and their knock-down clones by Western blotting. (B) Average doubling time in experimental tumors. (C) The lactate level in experimental tumors (mM). (D) Representative normalized lactate spectra at small volume tumors (100 mm^3). (E) Representative CSI from 4T1 Control and 4T1 LDH-A K/D clone 4 ~300mm^3.

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