Lactate Detection in Inducible and Orthotopic Breast Cancer Models

S. Magnitsky[1], G. Belka[2], C. Sterner[2], L. A. Chodosh[2], and J. D. Glickson[1]
[1]Radiology, University of Pennsylvania, Philadelphia, PA, United States, [2]Cancer Biology, University of Pennsylvania, Philadelphia

Introduction. The steady state lactate (Lac) level reflects a balance of lactate production and lactate washout by the tumor vasculature. A selective quantum coherence filter technique was successfully implemented to measure Lac levels in xengofraft models of lymphoma, breast and prostate cancers [1-3]. However, xenograft models only partially mimic normal tumor biology and cannot be used for investigation of several important properties of cancer. To overcome this problem, orthotopic and inducible tumor models have been utilized. Inducible models are considered the closest to naturally occurring human cancers. We studied MTB/TAN mice, which conditionally expressed the human breast cancer oncogene *neu*, in response to doxycycline treatment as well as an orthotopic model of a cultured cell line with the same mutation [4]. I*n vivo* detection of Lac in MTB/TAN mice was difficult and not reproducible, while orthotopic and xenograft models yielded consistent results. The objective of this study was to determine why the detection of Lac was difficult in the inducible tumor model of breast cancer.

Methods. Starting at the age of 6 weeks, the MTB/TAN mice were administered doxycycline 0.1 mg/mL in their drinking water. The administration of doxycycline induced transgene expression in a mammary-specific manner. Mice were monitored twice a week for tumor formation. Palpable tumors were found approximately 15 weeks after starting induction. MR experiments were initiated on a mouse when the tumor size was approximately 8-10 mm in diameter.

All MRS experiments were performed with a Unity INOVA console interfaced to a 9.4 T magnet with 25 G/cm max gradient insert. Animals were anesthetized by continuous inhalation of 1–1.5% isoflurane in oxygen. Tumors were positioned in a home-built loop-gap resonator, which provided a uniform field. The core body temperature of the animal was maintained at 36±1 ^{0}C. A selective multiple quantum coherence (SelMQC) pulse sequence [5, 6] was used to detect Lac and filter out overlapping lipid signals.

Results. Fig 1A depicts a typical Lac spectrum acquired from orthotopic (top) and intact (bottom) tumors with the SelMQC sequence. The peak on the left (i.e., low field) originates from the residual water signal, whereas the peak on the right (high field) is the CH_3 resonance of lactic acid. Much stronger Lac signals were detected in the orthotopic tumor compared to the signal of Lac in the intact model. However perchloric acid extracts obtained from the same snap-frozen tissues revealed similar lactate concentrations (Fig 1B). To determine the reason why the *in vivo* Lac signal was low in the MTB/TAN mice, we conducted histological studies. Very different tumor architecture was detected in these two models (Fig 1C), and a much higher amount of fat (red) tissue was detected in the intact model, while the orthotopic tumor exhibit very low fat content (Fig 1D).

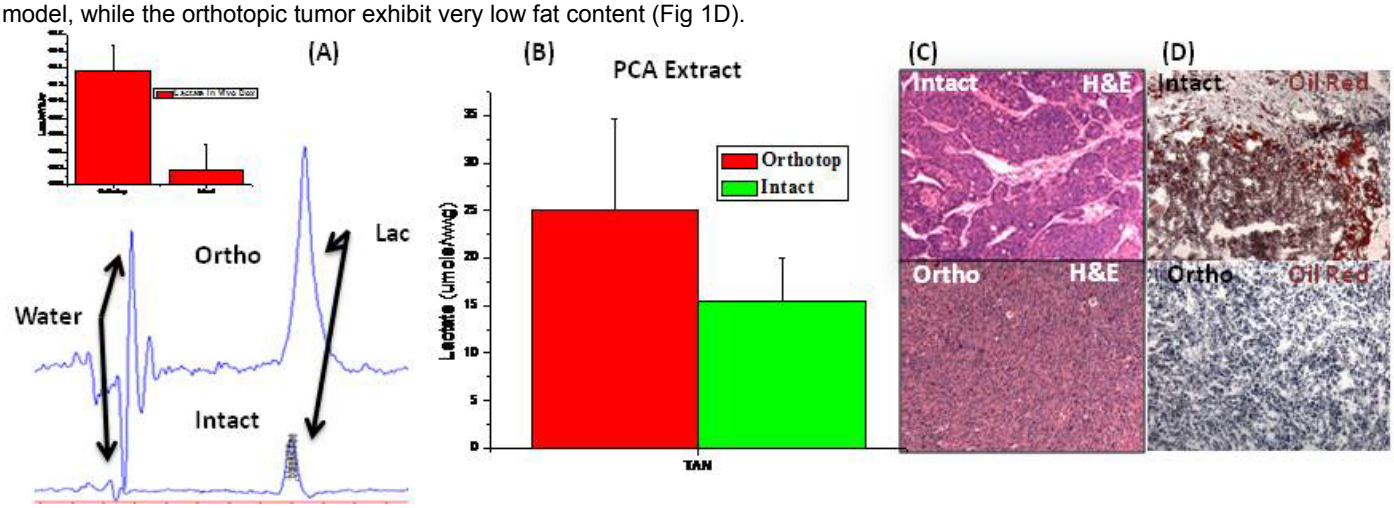

*Fig.1 (A) – In vivo Lac spectra of Orthotopic (top) and Intact (bottom) tumors. (**Lac level in the inducible model was lower and not reproducible, while Orthotopic model revealed consistent data**). (B) Concentration of Lac in Orthotopic and Intact tumors (**the same high concentration of Lac was found in both models ex vivo**). (C) – H&E staining of Intact (top) and Orthotopic (bottom) tumor tissues (**Different tumor structure**), (D) – Oil Red (fat staining) of Intact (top) and Orthotopic (bottom) tumor tissue (**High amount of fat (red) was detected in the inducible tumor**).*

Discussion. Promising preclinical data demonstrating the utility of Lac levels as markers of therapy response have been reported [7]. The SelMQC pulse sequence has been suggested for clinical use as a robust method for *in vivo* detection of Lac by NMR. However this technique was not able to reliably detect Lac signal in MTB/TAN mice. We attributed the low Lac signal in MTB/TAN mice to higher amounts of fat tissue in MTB/TAN mice compared to those in the orthotopic model. Our histological findings also indicate significant differences between the inducible and the orthotopic models. The inducible breast cancer model better reflects biological aspects of human disease and provides additional details Lac detection, which was not available with xenograft and orthotopic models. Our results suggest that the SelMQC method may have distinct limitations in tissues with relatively high levels of mobile lipids. This may limit the utility of this method to breast and other tumors that exhibit high lipid content.

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