Measurement of Lactate Concentrations in the Breast Mammary Tumors Using Selective Multiple Quantum Coherence Editing Sequence at 4.7T

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Introduction: Detection and quantification of metabolic markers using magnetic resonance spectroscopy (MRS) is a non-invasive powerful tool for early cancer diagnosis and treatment monitoring (1-2). Recent studies have shown that a high level of lactate concentration [Lac] is associated with tumor aggressiveness in breast cancer mouse models and prostate cancer rat models (3-4). Methods were previously reported for lactate detection in the presence of lipids using a multiple quantum editing sequence, SS-SelMQC (5). The purpose of this study is to measure [Lac] in breast mammary tumors with contrasting growth rates, tumor aggressiveness, and metastatic risk. In this work, we implemented the SS-SelMQC using higher order binomial pulses (SS1-SelMQC) for lactate detection as well as T1- and T2- versions of SS1-SelMQC for relaxation measurements. Absolute [Lac] were calculated by considering the T1 and T2 relaxation correction factors.

Materials and Methods: Animal studies were conducted in compliance with protocols approved by our institutional IACUC committee. 4 to 6 weeks old athymic mice were randomly classified into four groups for four different cell lines viz., MCF7 and BT474, MDA231 and MDA435. Two days before implantation, estrogen pellet was inserted in MCF7 and BT474 group mice. These models were selected because they have contrasting levels of IHC-based molecular biomarkers such as ER, PR, HER2, and metastatic potential, which are the controlling parameters for the clinical outcome. $5 * 10^6$ cells were inoculated on the mammary fat pad and the tumor growth was monitored every week. The tumor volume was calculated by measuring the length (l) breadth (b) and height (h) of the tumor using the formula π*(l*b*h)/6. ***MRI and MRS:*** Scans were performed on a 4.7T Bruker scanner. The mice were anesthetized using a mixture of isoflurane and oxygen (10% O_2) and was placed inside a 2 turn home built 11 mm ($\leq$ 300 $mm^{3)}$ and 15 mm (> 300 mm^3) diameter tuned coil. The animal body temperature was maintained at 37 °C. The FWHM is less than 50 Hz. Non-localized lactate signal from whole tumors was obtained using a SS-SelMQC sequence (5) with the higher order binomial bp1, bp2 and bp3 for spectral selection (SS1-SelMQC); bp1= $[(\pi/16)_{-x} - \Delta_1 - (3\pi/16)_x - \Delta_1 - (3\pi/16)_{-x}) - \Delta_1 - (\pi/16)_x]$, bp2=$[(\pi/16)_x - \Delta_1 - (3\pi/16)_x - \Delta_1 - (3\pi/16)_x) - \Delta_1 - (\pi/16)_x]$, and bp3= $[(\pi/8)_{-x} - \Delta_2 - (3\pi/8)_x - \Delta_2 - (3\pi/8)_{-x} - \Delta_2 - (\pi/8)_x]$. Each pulse width in bp1 and bp2 is 200 us, and in bp3 is 400us sinc pulses with their phases set to 0(x) or 180(-x). 16 averages used. Other MRS parameters are similar to SS-SelMQC (5). Relaxation constants were measured by modifying the SS1-SelMQC sequence similar to Muruganandham et al. (6). Lactate spectra were obtained with 16 transients for T_1 by varying the inversion time (TI) and 32 transients for T_2 measurements. For T2 scans, TR of 10s was used. Relaxation measurements were done using 3-4 mice per group. For concentration measurements 6-8 mice per group were used. ***Data Analysis:*** The T_1 was calculated using $S= S_0 (1\text{-}2 \exp^{(-TI/T_1)}) - S_0$ Where S is lactate signal integral at variable inversion delay, TI is inversion delay. T_2 was calculated using $S= S_0 \exp^{(-TR/TE)}$, where TR is recycle delay and TE is echo time. For calculating [Lac], time domain spin echo 1D data were processed using Matlab in-house software to calculate the power spectrum. The area under the lactate peak was calculated with a peak fit to a Gaussian function. [Lac] was computed using a 15mM external lactate phantom as a reference. Statistical analysis was performed using SPSS. Lac concentrations along with the T1 and T2 estimates for each tumor models were compared using Wilcoxon test. The level of significance was set at $P < 0.05$.

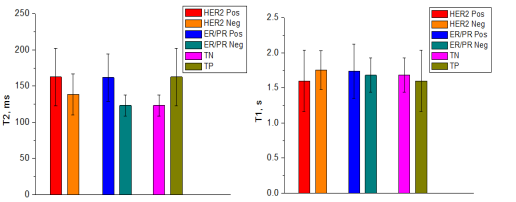

Figure 1. Comparison of relaxation constants, T1 (A) and T2 (B) values in breast mammary tumors with different growth rates and protein expression levels

Results: There was significant difference in the growth behavior among the four models ($p < 0.0001$). MCF7, BT474, MDA231 and MDA435 tumors were also classified into HER2 Pos/HER2 Neg, ER/PR Pos/ ER/PR Neg, and Triple negative (TN)/Triple positive (TP) groups. As shown in the figure 1, significant differences in T2 were observed between ER/PR Pos versus ER/PR Neg groups ($p = 0.02$), TN versus TP ($p = 0.0667$), and HER2 Pos versus HER2 Neg ($p = 0.2398$) (Figure 1). No significant differences were observed in T1 values between any two groups ($P = 0.31$). The [Lac] in tumors were measured at different tumor volumes. In all four tumor types, the [Lac] was found to be higher at tumor volume (100-200mm^3), and as tumor volume increases, [Lac] tend to decrease (Figure 2). Additionally, no significant differences in [Lac] were found among different models.

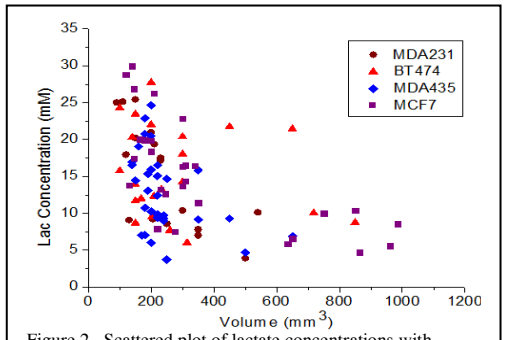

Figure 2. Scattered plot of lactate concentrations with increasing tumor volumes

Conclusion: Higher [Lac] were observed in all tumors at small tumor volumes and these levels were significantly reduced at higher tumor volumes. Further studies, investigating the lactate spatial distribution within these tumors based on tumor heterogeneity need to be explored.

References: 1) Glunde K et al, NMR Biomed 24, 673 (2011). 2) Luo Y et al., Magn Reson Med. 41, 676 (1999). 3) Serganova I et al., 17, 6250 (2011). 4) Yaligar J et al., NMR in Biomed 25, 113 (2011). 5) Thakur SB et al., Magn Reson Med. 62, 591 (2009). 6) Muruganandham M et al., 52, 902 (2004).

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