Quantification of lactate concentrations in orthotopic breast tumors with different growth rates

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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). Lactate (Lac) a metabolic product of glycolysis plays a vital role in energy metabolism. Recent studies have shown that high level of lactate concentration [Lac] is associated with breast cancer and prostate cancer (3-4). The predominant use of glycolysis for energy demand due to poor tissue oxygenation has been observed in cancerous tissues. We used the modified Spectral-Selective Pulses in Selective MQ coherences (SS-SelMQC) method (5) to detect Lac by effectively suppressing water and fat with signal enhancement. Lac signal enhancement was achieved by using short binomial frequency selective pulses, which reduces signal loss in the evolution period due to the effects of scalar coupling and molecular diffusion. In the present work, we measured Lac with improved lipid and water suppression using 1331 binomial composite pulses (SS1-SelMQC). This sequence was used to detect and quantify Lac levels in orthotopic breast tumors with different growth rates. MCF-7(n=6) and BT-474 (n=6) breast tumors were used to represent fast growing tumors compared with slow growing MDA-MB-231(n=6) and MDA-MB-435 (n=6) tumors. We discreminated the molecular prognostic markers with respect to [Lac] viz., ER(Estrogen) PR(Progesterone) +/-, Her2(Human Epidermal growth factor Receptor 2)+/- and TN(Triple)+/-.

Materials and Methods: Animal studies were conducted in compliance with protocols approved by MSKCC's IACUC committee. 4 to 6 weeks old Athymic nu/nu nude mice were used for the study. Animals were randomly classified into four groups for four different cell lines viz.**,** MCF-7 and BT-474, MDA-MB-231 and MDA-MB-435. Two days before estrogen pellet was inserted in MCF-7 and BT-474 group mice. 5 * 10^6 cells in each mouse for all four groups were inoculated on the mammary fat pad. Tumor growth was started after one week of cell inoculation and its growth was monitored every week **(Fig.1A).** Tumors were categorized into three groups: small (<**150** mm^3), medium (**200-350** mm^3), and large (>**450**mm^3). 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. All MR imaging and spectroscopy experiments were performed on a 4.7 Tesla Bruker Biospin spectrometer (40 cm horizontal bore). The mice were anesthetized using a mixture of isoflurane and oxygen (10% O_2) and placed in the animal holder. The tumor was placed inside a 2 turn home built 15 mm diameter tuned coil. A rectal fiberoptic probe was used to monitor the mouse body temperature. Warm air was blown to maintain the animal body temperature at 37 °C. The magnet was shimmed to a half height line width of less than 50 Hz for the ^{1}H water signal. Spectra were obtained for localized (5mm thick) to detect Lac signal from the tumor. The SS1-SelMQC, spectral editing technique to measure [Lac] by efficiently suppressing lipid (1.3 ppm) and water (4.7 ppm) signal in a single shot was used to measure Lac in tumor. One-dimensional slice spectra in the sagittal plane were acquired with TR = 3 sec, echo time of 72 ms, number of excitations 16, 1024 data points and spectral width of 2510 Hz. The One-dimensional spectra were processed by a 1D Fourier transform, similar to our previous report (5). The magnitude mode of the spectra were fitted in in-house written matlab (Matworks 7.6.0) program and normalized to the slice volume. Quantification of Lac was performed by the phantom replacement technique using a 15 mM Lac (4). Statistical analysis was performed using SPSS software. Non-Parametric Mann-Whitney U test(2 sample) were used to differentiate molecular prognostic markers. Correlation coefficient b/n slice [Lac] with tumor volume was performed by Pearson two tailed test.

Results and Discussion: The [Lac] in MCF-7, BT-474 (Fig 1B) MDA-MB-231and MDA-MB-435 (Fig 1C) tumors were measured using MRSI with respect to tumor volume. In all four tumor types, the [Lac] was found to be higher in small tumor volume (100-150mm^3), as tumor reaches 400mm^3, [Lac] level tend to decrease, at higher tumor volume (> 400mm^3) [Lac] level declined (Fig 1D&E). [Lac] found to be significantly higher (p=0.00 for all three types) in ER PR +, Her2+ and TN+ compared to ER PR -, Her2- and TN- markers respectively (Fig 1F).

Conclusion: Our results, inferred the decrease in Lac level as tumor volume increases. At smaller tumor volumes, in fast growing tumors (low metastatic potential), [Lac] was higher, whereas in slow growing tumors (high metastatic potential) has lower [Lac]. In all these tumors [Lac] is negatively correlated with tumor volume. Significant correlation was found in MDA-MB-231 (r=-0.84), MCF-7 (r=-0.76) and moderate correlation was found in BT-474 (r=-0.51) and in MDA-MB-435 (r=-0.69). [Lac] is significantly differentiate the molecular prognostic marker.

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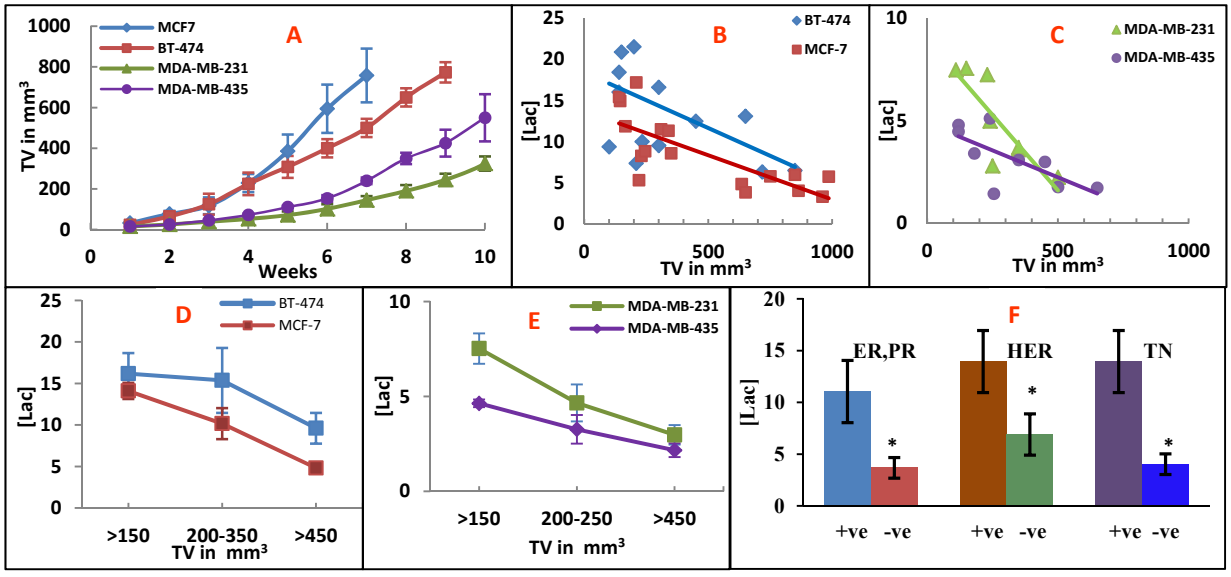

Fig 1: Tumor volume (TV) growth curve (A) , [Lac] level with respect to TV in fast [B] and slow growing [C] tumors, mean [Lac] level in small , medium and bigger TV in slow [D] and fast [E] growing tumors and [Lac] level with respect to protein prognostic factor with statistical analysis p=0.00 * (F).