

# Is higher lactate generation rate an indicator of tumor metastatic risk?-A pilot study using hyperpolarized $^{13}\text{C}$ -NMR

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## Target audience

Cancer researchers, particularly in the field of metabolism, and clinical practitioners would benefit from this study.

## Purpose

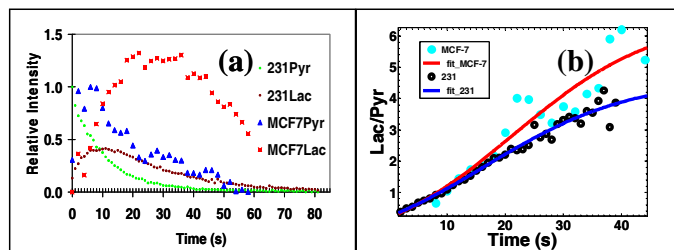
Tumor metastatic risk determination remains one of the greatest clinical challenges due to the lack of reliable biomarkers of metastatic potential. Increased glycolysis resulting in higher lactate production under conditions of sufficient oxygen supply (the Warburg effect) has been demonstrated in tumor tissues in numerous studies including some that employed hyperpolarized  $^{13}\text{C}$ -NMR. A positive correlation between the hyperpolarized lactate level and prostate tumor histological grades was demonstrated in transgenic mouse models<sup>1</sup>. High grade metastatic tumors exhibited more rapid growth than low grade non-metastatic tumors. It is, however, not clear if the difference in levels of hyperpolarized lactate reflects differences in tumor growth rate or tumor metastatic potential or both? To answer this question, we examined two breast tumor mouse models<sup>2</sup>, the less metastatic but faster growing (MCF-7) and the more metastatic but slower growing (MDA-MB-231) tumor and quantitatively compared their metabolism using the hyperpolarized  $^{13}\text{C}$ -NMR technique.

## Methods

Human breast cancer cells (MCF-7 or MDA-MB-231, 10 million/site) were subcutaneously inoculated into athymic nude mice to produce tumor xenografts<sup>2</sup>. All NMR experiments were performed with a 1.4-cm  $^1\text{H}/^{13}\text{C}$  dual-tuned home-made surface coil in a 9.4-T Varian vertical bore NMR spectrometer. Mice were anesthetized by administering oxygen doped with 1% isoflurane while the body temperature was maintained in the range of 31-37 °C with heated air. Tail vein injection of 250  $\mu\text{L}$  75mM hyperpolarized (DNP method by GE HyperSense)  $^{13}\text{C}$ -1-pyruvate ( $\sim 10 \mu\text{L/g}$  mouse body weight) was completed in  $\sim 10$  sec. Single-pulse or slice-selective (for localizing smaller tumors)  $^{13}\text{C}$ -NMR spectra were collected over a period of 2 minutes with a  $9\sim 15^\circ$  nominal flip angle every 1 or 2 seconds. Ratiometric analysis<sup>3</sup> of the time course of the lactate to pyruvate ratio was performed using a customized Matlab<sup>®</sup> program to determine the forward ( $k^+$ ) and reverse ( $k^-$ ) rate constants of the lactate dehydrogenase-catalyzed reaction. The SNR threshold = 2.5 for pyruvate signals.

## Results and Discussion

Fig. 1(a) shows typical time courses of both hyperpolarized pyruvate and lactate signals in MCF-7 and MDA-MB-231 tumors. Fig. 1(b) displays typical time courses of the hyperpolarized lactate to pyruvate ratio and the quality of curve fitting using the ratiometric analysis of the same tumors shown in Fig. 1(a). As shown in Table 1, the MCF-7 tumors have a significantly larger apparent forward rate constant  $k^+$  than the MDA-MB-231 tumors ( $p=0.002$ , unpaired t test). There is no significant difference in the reverse rate constant  $k^-$  or in the ( $k^+/k^-$ ) ratio between the two groups ( $p>0.05$ ), presumably related to the larger error in  $k^-$ . We did not detect a significant dependence of the rate constants on tumor volume. Our preliminary data indicate that the lactate conversion from  $^{13}\text{C}$ -1-pyruvate is positively associated with tumor growth rate, consistent with the reported data<sup>4</sup> indicating that the lactate level correlates with the hepatoma growth rate, whereas the tumors with higher metastatic risk but slower growth rate exhibited lower lactate generation rate. The result suggests that lactate generation rate is not necessarily associated with breast tumor metastatic risk.



**Table 1:** Quantification of the apparent rate constants ( $\text{s}^{-1}$ ) (mean  $\pm$  SD, N=4 for each line)

Tumor	$k^+$	$k^-$	$k^+/k^-$	size( $\text{mm}^3$ )
MDA-MB-231	$0.054 \pm .009$	$0.021 \pm .009$	$2.98 \pm 1.4$	$762 \pm 744$
MCF-7	$0.086 \pm .005$	$0.019 \pm .007$	$4.88 \pm 1.3$	$256 \pm 96$
p	0.002	0.740	0.097	0.267

## Conclusions

The preliminary results showed that the less metastatic but faster growing MCF-7 breast tumors have higher lactate generation rate than the more metastatic but slower growing MDA-MB-231 tumors, contradicting the assumption that lactate generation rate is positively associated with tumor metastatic potential.

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## References:

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