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***In Vivo* Metabolic Evaluation of Breast Tumor Mouse Xenografts for Predicting Aggressiveness Using the Hyperpolarized ¹³C-NMR Technique**

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Abstract

In vivo imaging/spectroscopic biomarkers for solid tumor aggressiveness are needed in the clinic to facilitate cancer diagnosis and treatment strategies. In mouse models of human melanoma and breast cancer we were able to detect the metabolic differences among tumors of different metastatic potential and between normal and cancer tissues by optical imaging of the mitochondrial redox state of snap-frozen tissue samples. Such metabolic differences indicate that tumors of different aggressiveness have different metabolic homeostasis, which supports that kinetic parameters such as rate constant(s) can also serve as bio-markers for cancer aggressiveness and treatment response. Here we present our preliminary study on the mouse xenografts of the aggressive and indolent human breast cancer cell lines using the hyperpolarized ¹³C-NMR (HP-NMR) technique. By recording the time courses of ¹³C-pyruvate tracer and its metabolite signals *in vivo*, particularly the ¹³C-lactate signal, the apparent rate constants of both the forward and reverse reactions catalyzed by lactate dehydrogenase (LDH) were extracted via the ratiometric modeling of the two-site exchange reaction that we developed. Data from four breast tumors (MCF-7, MDA-MB-468, and MDA-MB-231 medium and large) with different aggressiveness are included. We demonstrate the feasibility to quantify the apparent rate constants of LDH reactions in breast tumor xenografts.

1 Introduction

Cancer mortality is mainly the result of tumor metastasis. *In vivo* imaging of solid tumor aggressiveness or metastatic potential is needed in the clinic to facilitate cancer treatment strategies. Cancer is considered as “a paradigm of genetically-defined metabolic abnormalities” [1]. Several studies have shown tumor abnormalities in glucose metabolism, Krebs cycle, and the mitochondrial electron transport chain [2–5]. Our previous *ex vivo* studies on animal models demonstrated that the quantitative mitochondrial redox state imaging biomarkers differentiated solid tumor aggressiveness, and distinguished normal from malignant tissues and may therefore be potentially useful for clinical diagnosis and treatment of cancer [6–8]. All these results show that the difference in aggressiveness of tumors can be manifested in their intracellular metabolic state including the mitochondrial redox status. Since the *in vitro* cell cultures or perfused organ models hardly represent the

living tissue conditions [9], it is desirable to quantitatively interrogate *in vivo* metabolism in living tissue by non-invasive techniques.

The HP-NMR technique can produce ^{13}C -NMR signals ~10,000 times stronger than the regular NMR technique and thus has been employed to probe cell metabolism *in vivo* [10]. A rapidly growing number of HP-NMR experiments have been carried out to investigate the *in vivo* enzymatic reactions for various purposes, such as tumor grading [11], redox status imaging [12], and monitoring therapeutic effects of anti-cancer drugs [13]. Usually these studies are achieved by monitoring the time courses of the injected hyperpolarized ^{13}C -enriched substrate such as ^{13}C -pyruvate and its metabolites such as ^{13}C -lactate produced by lactate dehydrogenase (LDH) catalyzed reaction, followed by extracting the kinetic parameters, such as apparent reaction rate constants. Higher lactate levels in tumors than in normal tissue have been shown by many metabolic studies on prostate cancer patients [14–16] and have been correlated with prostate tumor grades [11]. Increased aerobic glycolysis in cancer - the Warburg effect - has been associated with the observed lactate elevation [17].

Our long-term goal is to investigate whether the *in vivo* metabolism can provide biomarkers for cancer aggressiveness. We hypothesize that tumor aggressiveness may be predicted based on the difference in LDH reaction rate constants. In the present study, we employed the HP-NMR technique on several breast tumors with different aggressiveness to interrogate the LDH catalyzed reactions using ^{13}C -NMR signals of pyruvate and lactate.

2 Materials and Methods

Breast cancer lines MCF-7, MDA-MB-468, and MDA-MB-231 with increasing order of metastatic potential were cultured and inoculated subcutaneously into athymic nude mice to grow xenografts [8, 18, 19]. During the experiment, tumor-bearing mice were sedated and maintained with 1.5% isoflurane. A 1.4-cm home-made dual-tuned surface coil was placed over the tumor. The mouse was placed in a 9.4-T wide-bore, vertical bore magnet equipped with a Varian console. Body temperature was monitored via a fluoroptic rectal probe (Luxtron) and maintained in the range of 34–37 °C with heated air. A hyperpolarized [1- ^{13}C]-pyruvic acid sample was prepared by the dynamic nuclear polarization (DNP) method using a HyperSense polarizer (Oxford Instruments) [20]. The final concentration of the hyperpolarized tracer was ~75 mM in neutral isotonic solution. This sample (~10 μL /g mouse body weight) was then injected into mice via tail vein catheter over a period of ~10 sec. Single-pulse ^{13}C -NMR spectra were collected with a 9–15° nominal flip angle every 1 or 2 seconds for about 2 minutes.

A customized MATLAB® program was used for data analysis. Line broadening of 20 Hz was applied before Fourier transform of the FID signals. The spectral baseline was removed by fitting it as a fourth order polynomial function. A coarse fitting was performed first by summing up the spectra of all time points followed by fitting the sum to Lorentzian functions to obtain an estimate of the peak position and width of the major ^{13}C -labeled pyruvate and lactate. Their peak areas at each time point t were then obtained by fitting the individual NMR spectrum at time t to Lorentzian functions. The time courses of the pyruvate signal $P(t)$ and the converted lactate signal $L(t)$ as well as their ratio $R_{lp}(t)$ ($=L(t)/P(t)$) were obtained. SNR threshold was set at 2.5. Using the ratiometric analysis method for ^{13}C exchange (see Appendix) [21], we modeled the $R_{lp}(t)$ with customized MATLAB® (MathWorks, 2010b) programs on the basis of the following equation:

$$R_{lp}(r) = \left\{ r [1 + R_{lp}(t_0)] + [R_{lp}(t_0) - r] e^{-s(t-t_0)} \right\} / \left\{ 1 + R_{lp}(t_0) + [r - R_{lp}(t_0)] e^{-s(t-t_0)} \right\}$$

where $r = k^+/k^-$ and $s = k^+ + k^-$, and k^+ is the apparent rate constant for the forward reaction and k^- the rate constant for the backward reaction catalyzed by LDH, i.e.,

3 Results and Discussions

The top row of Figure 1 shows the time courses of the signals of ^{13}C -pyruvate and its metabolites (e.g. ^{13}C -lactate) in three breast cancer mouse xenografts with different aggressiveness. The signals of ^{13}C -pyruvate peaked at ~4, 15, and 12 sec for MCF-7, MDA-MB-468 and MDA-MB-231, respectively, after the injection of the hyperpolarized tracers ($t = 0$ s). The ^{13}C -lactate signals peaked later and surpassed the ^{13}C -pyruvate signals demonstrating the significant conversion of ^{13}C -pyruvate to ^{13}C -lactate by LDH *in vivo*. The bottom row of Figure 1 shows the time courses of the ^{13}C -lactate/ ^{13}C -pyruvate ratios and the ratiometric fits. Apparently we can see that the indolent MCF-7 tumor had the fastest pyruvate to lactate conversion. The quantitative results of rate constants are summarized in Table 1. From the calculated rate constant ratios we can see that the indolent MCF-7 tumor had the largest k^+/k^- and the most aggressive MDA-MB-231 (large) had the smallest one.

We have demonstrated the feasibility of obtaining both the apparent forward and backward LDH reaction rate constants in breast tumor mouse xenografts using the ratiometric fitting. Usually the rate constant modeling is based on the time courses of the metabolite signals, which are sensitive to various instrument/protocol parameters. The modeling of the time courses of the metabolite ratios can cancel out some confounding factors such as variations in the degree of spin polarization and the flip angles of radiofrequency pulse, etc.

In further studies with more tumor samples we hope to investigate the possible tumor size-dependence of the kinetic parameters as obtained in this study, and whether these parameters can be used for the *in vivo* detection of cancer aggressiveness.

4 Conclusions

In this report we presented our preliminary results of using the HP-NMR technique to probe the LDH catalyzed reaction in three types of breast cancer xenografts with different aggressiveness. Ratiometric modeling was demonstrated to quantify the apparent reaction rate constants. Further experiments are being carried out to investigate the metabolic flux differences among tumors with different aggressiveness.

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5 Appendix

The MR signals of pyruvate, $P(t)$, and lactate, $L(t)$, can be described by the two-site exchange model using the following differential equations:

$$\frac{dP}{dt} = -\rho P - k^+ P + k^- L \quad (\text{A1a})$$

$$\frac{dL}{dt} = -\rho L + k^+ P - k^- L \quad (\text{A1b})$$

where we have assumed the relaxation rates ρ for L and P are the same [21]. The solution of Eq. A1 is

$$P = P(0) [k^- e^{-\rho t} + k^+ e^{-(\rho + k^- + k^+)t}] / (k^- + k^+) + L(0) [k^- e^{-\rho t} - k^- e^{-(\rho + k^- + k^+)t}] / (k^- + k^+) \quad (\text{A2a})$$

$$L = P(0) [k^+ e^{-\rho t} - k^+ e^{-(\rho + k^- + k^+)t}] / (k^- + k^+) + L(0) [k^+ e^{-\rho t} + k^- e^{-(\rho + k^- + k^+)t}] / (k^- + k^+) \quad (\text{A2b})$$

where $L(0)$ and $P(0)$ are the signal intensities at $t=0$. Let us divide Eq. A2b by Eq. A2a, and we have

$$L(t)/P(t) = \left\{ r [1 + L(0)/P(0)] + [L(0)/P(0) - r] e^{-st} \right\} / \left\{ 1 + L(0)/P(0) + [r - L(0)/P(0)] e^{-st} \right\} \quad (\text{A3})$$

where $r = k^+/k^-$, $s = k^+ + k^-$. If we also generalize the initial time point to $t = t_0$, Eq. A3 becomes

$$R_{lp}(t) = L(t)/P(t) = \left\{ r [1 + R_{lp}(t_0)] + [R_{lp}(t_0) - r] e^{-s(t-t_0)} \right\} / \left\{ 1 + R_{lp}(t_0) + [r - R_{lp}(t_0)] e^{-s(t-t_0)} \right\} \quad (\text{A4})$$

Fig. 1.

Upper row: Time courses of the metabolites in mouse xenografts of MCF-7 (*left*), MDA-MB-468 (*middle*), and MDA-MB-231 (*large*) (*right*); Bottom row: the time courses and their fits of the corresponding tumor's lactate/pyruvate ratios.

Table 1

Apparent rate constants of individual tumors with different aggressiveness.

Tumor	k^+	k^-	k^+/k^-	size (mm ³)
MCF-7	0.068 ± 0.028	0.011 ± 0.007	6.4 ± 2.3	228
MDA-MB-468	0.040 ± 0.010	0.010 ± 0.004	3.9 ± 0.8	397
MDA-MB-231(large)	0.047 ± 0.007	0.013 ± 0.003	3.5 ± 0.4	1380
MDA-MB-231(medium)	0.065 ± 0.004	0.014 ± 0.001	4.6 ± 0.3	157