Hyperpolarized ^{13}C MR Spectroscopic Imaging of Disease State in a Switchable MYC-Oncogene Model of Liver Cancer

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Introduction: Development of hyperpolarized technology utilizing dynamic nuclear polarization has enabled the monitoring of ^{13}C metabolites *in vivo* at very high SNR [1]. In this work, hyperpolarized ^{13}C 3D-MRSI was used to measure liver metabolism in mice after expression of the MYC proto-oncogene was switched on and then off in the liver. Mice in various disease stages were studied, and significant differences in hyperpolarized lactate and alanine levels were detected ($P < 0.01$). In addition, biochemical assays showed increased LDH expression and activity in the MYC-driven tumors.

Methods: Tet-o-MYC/LAP-tTA double-transgenic mice in which the human MYC proto-oncogene is overexpressed only in the liver and can be switched off with doxycycline administration were used [2]. All studies were performed on a GE 3T scanner with a custom $^{1}H/^{13}C$ mouse coil. ^{13}C 3D-MRSI data (TE/TR = 140ms/215ms, 0.034 cm^3 voxel size, 16 second acquisition time) were acquired with a double spin-echo compressed sensing pulse sequence [3] after injection of 0.35 mL of 80mM hyperpolarized $^{13}C_1$-pyruvate. Lactate area/total carbon (Lac/tCar) and alanine area/total carbon (Ala/tCar) ratios were derived from the spectral arrays. LDH activity assays were performed on a subset of the mice, and Lac/tCar was correlated with LDH Vmax. LDH-A expression assays (microarray analysis) were also performed on different cohorts of MYC and control mice.

Results: Figure 1a shows a representative case of disease progression. Elevated Ala/tCar was observed before a tumor was apparent on anatomic images, and dramatically elevated Lac/tCar was observed afterward. Figure 1b shows a representative case of disease regression after MYC expression was switched off with doxycycline. Reductions in both tumor size and Lac/tCar were observed. Figure 2 shows Lac/tCar and Ala/tCar data from all mice studied. For Lac/tCar, statistically significant differences were detected among no disease (no MYC or MYC on < 30 days), late disease (tumor detectable on anatomic images), and regressed disease groups ($P < 0.01$). For Ala/tCar, the early disease group (MYC on > 30 days and no tumor on anatomic images) was significantly different from the other groups ($P < 0.01$). These results paralleled the examples in Figure 1. Figure 3a shows a comparison of LDH-A expression between control and MYC mice. As expected, LDH expression was significantly ($P < 0.002$) elevated in the MYC group. Figure 3b shows LDH activity assay data collected from mice that were sacrificed after hyperpolarized experiments. A strong correlation ($r = 0.82$, $P < 0.02$, one outlier excluded) was found between Vmax of LDH activity and Lac/tCar. Note that Figure 3b contains data from both healthy and late disease animals.

Discussion: The inducible transgenic animal model allowed for direct analysis of *de novo* tumor formation driven by a defined oncogenic event. Metabolic changes following MYC activation/deactivation were monitored by using hyperpolarized [1-^{13}C]pyruvate to probe the LDH pathway, a direct transcriptional target of MYC. Significant changes in hyperpolarized lactate and alanine levels were detected with oncogene expression and inhibition. This study demonstrated the potential of hyperpolarized ^{13}C to monitor cancer progression and gene therapy in liver and other oncogene-driven cancers.

References: [1] Ardenkjaer-Larsen et al. PNAS (2003) 100:10158 [2] Shachaf et al. Nature (2004) 431:1112 [3] Hu et al. 3D Compressed Sensing for Highly Accelerated Hyperpolarized ^{13}C MRSI with *In Vivo* Applications to Transgenic Mouse Models of Cancer. MRM (2009) In Press.

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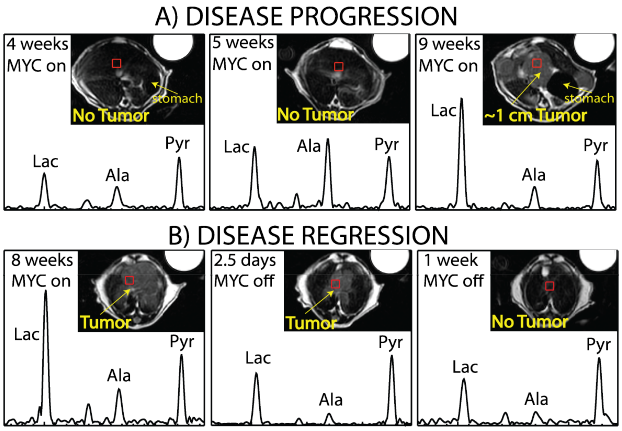

Figure 1: A) Representative example of disease progression after switching expression of MYC on. Elevated hyperpolarized alanine was detected before gross anatomic changes were observed. Subsequently, dramatically elevated hyperpolarized lactate was detected. B) Representative example of disease regression after switching expression of MYC off. A dramatic reduction in hyperpolarized lactate was detected.

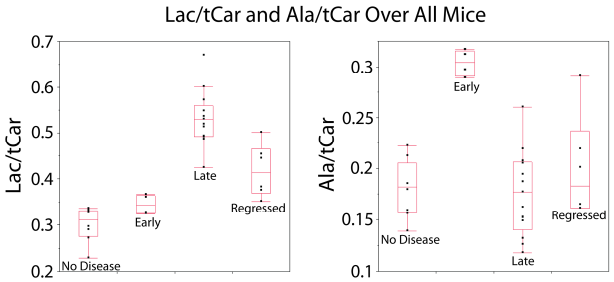

Figure 2: Hyperpolarized Lac/tCar and Ala/tCar over all disease stages in all mice examined with hyperpolarized ^{13}C 3D-MRSI. The plots show the minimum, maximum, and upper quartiles. As shown in the left-hand plot, Lac/tCar rose dramatically as disease progressed and fell during regression, paralleling the data shown in Figure 1. Multiple comparisons tests showed statistically significant differences ($P < 0.01$) between groups A and C, A and D, B and C, and C and D (A = no disease, B = early, C = late, D = regressed). The right-hand plot shows elevated Ala/tCar in the early stage disease group ($P < 0.01$). Ala/tCar values were similar among the other groups.

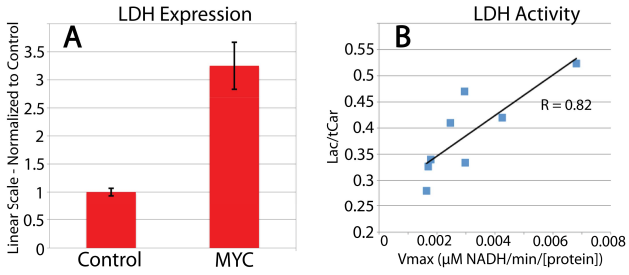

Figure 3: LDH expression and activity assays. A) Microarray analysis was used to compare LDH-A expression between Tet-o-MYC/LAP-tTA and Tet-transactivator control mice (n = 4 for each group). Higher expression was found for the MYC group ($P < 0.002$, means and standard errors shown). B) Regression analysis of Lac/tCar and LDH activity (Vmax). For mice that were dissected close to their last hyperpolarized exam, LDH activity assays were performed on normal and tumor liver tissues and compared with their Lac/tCar values averaged over regions of interest identified from anatomical imaging. A strong correlation ($r = 0.82$, $P < 0.02$) was observed.