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-MR spectroscopic imaging (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 1H/13C mouse coil. $^{13}$C 3D-MR spectroscopic imaging data (TE/TR = 140 ms/215 ms, 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 80 mM hyperpolarized $^{13}$C-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 progression 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 $^{13}$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.


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