Detection of Glutaminase Activity *In Vivo* in a MYC Mouse Model of Liver Cancer Using Hyperpolarized [5-^{13}C]Glutamine

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Introduction: Development of hyperpolarized technology utilizing dynamic nuclear polarization has enabled the measurement of ^{13}C metabolism *in vivo* at very high SNR [1]. In this work, hyperpolarized [5-^{13}C]glutamine was used to probe glutaminase activity *in vivo* in mice with MYC driven liver cancer and in normal mice. A significant increase in glutamate to glutamine ratio (P = 0.0021) was detected in liver tumor versus normal tissue. This is consistent with previous *in vitro* experiments in which glutamate production was detected in human hepatocellular carcinoma cells [2].

Methods: Tet-o-MYC/LAP-tTA double-transgenic mice in which the human MYC proto-oncogene is overexpressed only in the liver [3] and normal FVB strain mice were used. All studies were performed on a GE 3T scanner with a custom ^{1}H/^{13}C mouse coil. ^{13}C slab-localized spectroscopic data (10 degree flip, TE = 35ms, 32 averages, 3 seconds between averages, 40mm slab thickness) were acquired with a double spin-echo pulse sequence [4] after injection of 0.35mL of 25-30mM hyperpolarized [5-^{13}C]glutamine (3-5% polarization). For the MYC mice, the 40mm slab covered tumor tissue, and for the normal mice, it covered abdominal tissue that spanned the liver and kidneys. Spectra were apodized with a 5 Hz filter, and the area under the peaks around the main [5-^{13}C]glutamine peak were quantified (Figures 1 and 2). The left peak is the sum of [5-^{13}C]glutamate and ^{13}C pyroglutamic acid, which overlap at the 3T field strength. The right peak is unenriched [1-^{13}C]glutamine. The [5-^{13}C]glutamate to [1-^{13}C]glutamine ratio was calculated and statistically compared (the contribution of pyroglutamic acid was accounted for and subtracted off by measuring the ^{13}C pyroglutamic acid / [1-^{13}C]glutamine ratio in a separate non-*in vivo* syringe experiment).

Results: Figure 1 shows a representative spectrum from a 40mm tumor slab. As demonstrated by Figure 1, the left peak, consisting of [5-^{13}C]glutamate and some pyroglutamic acid, is comparable in size to the [1-^{13}C]glutamine peak. Conversely, in Figure 2, which shows a spectrum from a normal tissue slab, the left peak to right peak ratio is noticeably lower. Figure 3 summarizes the results from all animals studied (n = 3 for tumor, n = 3 for normal). Figure 3 shows a significant difference (P = 0.0021) in glutamate/glutamine ratio (contribution from pyroglutamic acid subtracted off) between the two groups studied (standard error bars shown).

Discussion: In this study, *in vivo* detection of glutaminase activity with hyperpolarized [5-^{13}C]glutamine was shown for the first time. A significant increase in glutaminase mediated glutamate production was detected in MYC transgenic mice. This is consistent with prior *in vitro* studies [2] and the concept of "glutamine addiction," in which MYC regulates glutamine metabolism and promotes increased glutamine consumption as a mechanism for cell growth [5].

References: [1] Ardenkjaer-Larsen et al. PNAS (2003) 100:10158 [2] Gallagher et al. MRM (2008) 60:253. [3] Shachaf et al. Nature (2004) 431:1112 [4] Cunningham et al. JMR (2007) 187:357. [5] Wise et al. PNAS (2008) 105:18782.

Acknowledgments: Funding from NIH EB007588 & CA137298.

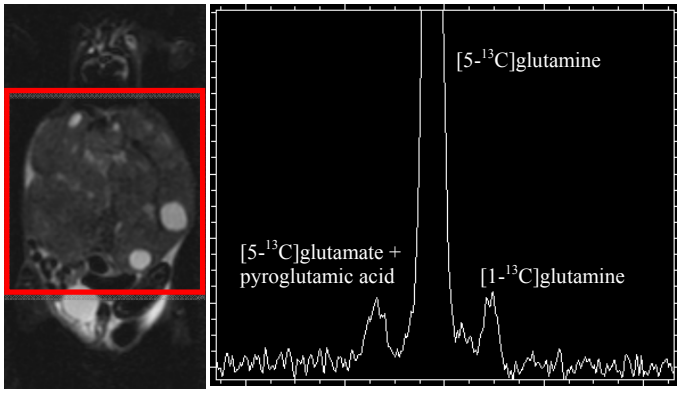

Figure 1: Representative spectrum from a tumor slab (location shown on the left in red). Note that the left peak is a combination of [5-^{13}C]glutamine and ^{13}C pyroglutamic acid, which overlap at the 3T field strength. The middle peak is the injected enriched [5-^{13}C]glutamine. The right peak is glutamine in the unenriched 1-position. In tumor mice, the ratio of the left peak to right peak is close to one.

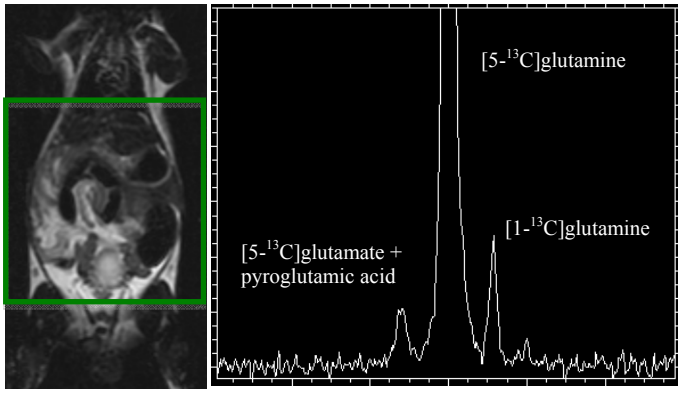

Figure 2: In contrast to Figure 1, a typical spectrum from a normal tissue slab (location shown on the left in green) is characterized by a much lower left peak to right peak ratio. This indicates decreased flux through glutaminase in normal tissue.

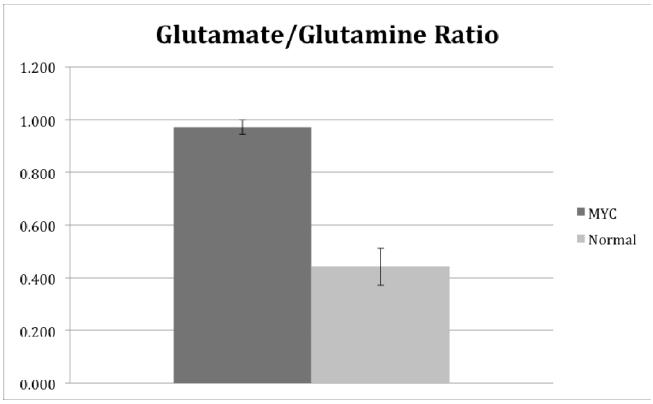

Figure 3: A summary of all the animal data (n = 3 for tumor, n = 3 for normal tissue). The contribution from pyroglutamic acid was subtracted off, and the [5-^{13}C]glutamate/[1-^{13}C]glutamine ratio was computed. There was a significant difference (P = 0.0021) between MYC and normal groups (t-test used, standard error bars shown).