Assessment of Glutamine Metabolism in Mammary Tumor Recurrence Using ^{13}C MRS

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Introduction: Breast cancer is the most commonly diagnosed malignancy in women and the leading cause of cancer-related death in this population worldwide [1]. Tumor recurrence represents the principal cause of death from breast cancer [2]. Despite being a critical clinical problem, little is known about the cellular and molecular mechanisms underlying tumor recurrence. Our laboratory has developed an inducible transgenic mouse model that accurately reproduces key features of the natural history of human breast cancer progression including metastasis, tumor dormancy and recurrence [2,3]. Dysregulated metabolism has been previously shown to be a key feature in tumorigenesis [4]. Particularly, increased glutaminoloysis is considered to be a major component of the metabolic profile of cancer cells [5]. To date, no association has been established between changes in glutamine metabolism and breast cancer recurrence. The goal of this study was to investigate the role of ^{13}C-glutamine as a potential breast cancer progression marker using MRS. In particular, we assess changes in glutamine metabolism between primary and recurrent tumors and identify their underlying molecular determinants.

Materials and Methods: MMTV-rtTa;TetO-HER2/neu doxycycline-inducible bitransgenic mice in which the *HER2/neu* proto-oncogene can be conditionally overexpressed in the mammary gland were used. A total of 8 mice, 4 bearing primary tumors and 4 bearing recurrent mammary tumors, were infused through a tail vein catheter with an 8 mM solution of [3-13C]-glutamine over a period of 45 minutes. At the end of the infusion period, tumors were dissected and flash frozen in liquid nitrogen. Perchloric acid extraction was performed as described by Lehnhardt et al. [6]. NMR spectroscopy was performed at 9.4T on a Bruker Avance III 400 wide-bore spectrometer. Carbon spectra were acquired overnight with a 5 mm BBO probe using the following conditions: PW 45 degrees, TR 1.4s, 24kHz SW, 64K data points and 35,000 to 40,000 scans. Spectral analysis was conducted using the Acorn NMR NUTS software. Statistical significance was determined using a student's t-test. Gene expression levels of metabolic enzymes of interest were assessed using qRT-PCR.

Results and Discussion: We find that primary and recurrent tumors display substantial differences in glutamine metabolism (Fig. 1). Recurrent tumors exhibit higher conversion of glutamine to glutamate than primary tumors. In particular, the integral ratio of labeled [3-13C]-glutamate (Glu-3) to [3-13C]-glutamine (Gln-3) is higher in recurrent tumors when compared to primary tumors. The Glu-3/Gln-3 ratio computed across all the tumors included in this study display statistically significant differences (Fig. 2). Assessment of the potential underlying molecular determinant of these changes reveals differences in glutaminase expression levels as tumors progress to recurrence. Glutaminase catalyzes the rate-limiting step in glutaminolysis by converting glutamine to glutamate. Quantification by qRT-PCR of glutaminase expression levels reveals higher levels of glutaminase in recurrent tumors compared to primary tumors (Fig.3). Experiments are currently underway to quantify the difference in the activity of the glutaminase enzyme in vitro in both primary and recurrent tumors. It has been previously shown that glutaminase is required for tumor growth [7]. The results of this study lead us to speculate that the observed changes in glutamine metabolism and the underlying differences in glutaminase expression might, in addition to its known role in primary tumor development, be contributing to cancer progression and recurrence.

Conclusion: Our preliminary results indicate that ^{13}C MRS of glutamine metabolism has the potential to allow for both major improvements in our understanding of the molecular pathways involved in breast cancer recurrence and to provide a clinical marker of breast cancer progression. Ultimately, this has the potential to enable improvements in the prediction, prevention and treatment of breast cancer recurrence.

References: (1) Parkin et al., CA Cancer J Clin (2005). (2) Moody et al., Cancer Cell (2005), (3) Moody et al., Cancer Cell (2002). (4) DeBerardinis et al., Cell Metab (2008), (5) Wise et al, PNAS, 2009, (6) Lehnhard et al., NMR Biomed. (2005), (7) Lobo et al., Biochem J, 2000.

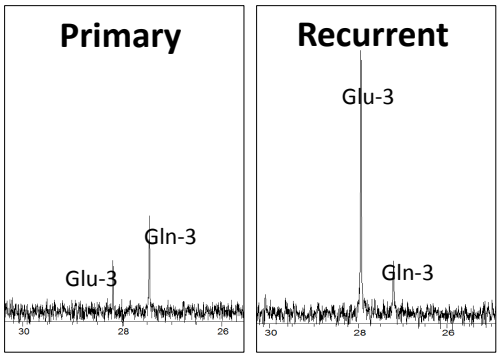

Fig.1: Sample ^{13}C spectra from a primary and a recurrent tumor following infusion of [3-13C]-glutamine. Glutamate production from glutamine is higher in recurrent tumors compared to primary tumors.

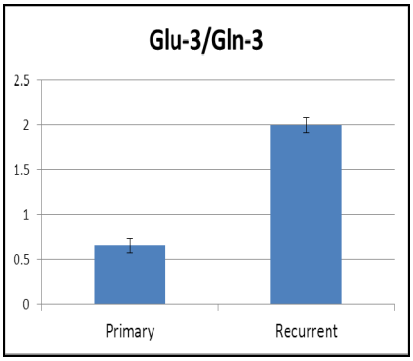

Fig.2: Quantification of the ratio of [3-13C]-glutamate (Glu-3) to [3-13C]-glutamine (Gln-3) following computation of the peak integrals. Ratio is higher in recurrent tumors ($p<0.001$).

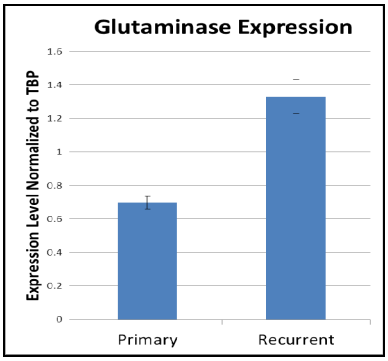

Fig.3: Gene expression levels of glutaminase in primary and recurrent tumors by qRT-PCR. Recurrent tumors express higher levels of glutamines than primary tumors ($p<0.001$).