Assessment of the Serine Synthesis Pathway during Breast Cancer Progression using ^{13}C-MRS

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Introduction: Breast cancer is the most commonly diagnosed malignancy in women and is the second leading cause of cancer-related death in females in the U.S. [1]. Among women with breast cancer, tumor recurrence represents the principal cause of mortality [2]. Nevertheless, little is known about the molecular mechanisms underlying how breast cancer cells survive therapy and ultimately recur. In particular, while dysregulated metabolism has long been recognized as a key feature of cancer development, the metabolic changes accompanying cancer recurrence are largely unexplored [3]. To address this gap, our laboratory has developed a series of inducible bitransgenic mouse models that accurately recapitulate human breast cancer progression, including primary tumor development, minimal residual disease, tumor dormancy and recurrence [2.4]. Increased serine biosynthesis has been recently found to be a key feature of mammary tumorigenesis [5]. To date, no association has been established between this metabolic pathway and breast cancer progression. In this study, we investigate the differences in the serine biosynthetic pathway between primary and recurrent mammary tumors and assess their role as a potential prognostic marker.

Materials and Methods: MMTV-rtTa;TetO-NeuNT doxycycline-inducible bitransgenic mice in which the HER2/*neu* proto-oncogene is overexpressed specifically in the mammary glands were used. A total of 6 mice, 3 bearing primary tumors and 3 bearing recurrent mammary tumors, were infused through a tail vein catheter with a 300 mM solution of D-[1,2-^{13}C]-glucose over a period of 45 minutes. At the end of the infusion period, tumors were dissected and were immediately frozen in liquid nitrogen. Perchloric acid extraction was performed as previously 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, SW 24 kHz, 64K data points and 35,000 to 40,000 scans. Spectral analysis was conducted using the Acorn NMR NUTS software. Gene expression levels of metabolic enzymes of interest were assessed using qRT-PCR. Human association analysis was conducted using publically available microarray data.

Results and Discussion: ^{13}C-MRS following D-[1,2-^{13}C]-glucose infusion revealed increased serine biosynthesis pathway activity in recurrent tumors compared to primary tumors. The glycolytic intermediate, 3-phosphoglycerate, can be converted into 3-phophohydroxypuruvate contributing glycolytic carbon to the serine biosynthesis pathway where glycine is produced. Our results suggest increased diversion of glucose-derived carbon into serine synthesis during tumor progression and show higher labeled glycine production from glucose in recurrent tumors relative to primary tumors (Fig.1). These results were confirmed by mass spectrometry which also revealed increased ^{13}C enrichment of glycine in recurrent tumors. The rate-limiting step in the diversion of glucose carbon into the serine pathway is catalyzed by phosphoglycerate dehydrogenase (Phgdh) [7]. Consistent with the observed increase in glycine signal, recurrent tumors exhibited increased expression levels of *Phgdh* compared to primary tumors (Fig. 2). Human association analysis revealed an association between increased *PHGDH* expression and reduced recurrence-free survival in human breast cancer patients, further highlighting the translational potential of our findings (Fig.3). Combined, our results suggest a potential role of serine biosynthesis in breast cancer progression.

Conclusion: In conclusion, the preliminary results presented here indicate that ^{13}C-MRS of glucose metabolism can be used to assess the activity of the serine biosynthesis pathway and can provide for a clinical marker of breast cancer progression. This can eventually allow for much-needed 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)DeBerardinis et al., Cell Metab (2008), (4)Moody et al., Cancer Cell (2002). (5) Possemato et al, Nature, 2011, (6) Lehnhard et al., NMR Biomed. (2005), (7) Locasale et al. Nature Genetics, 2011.

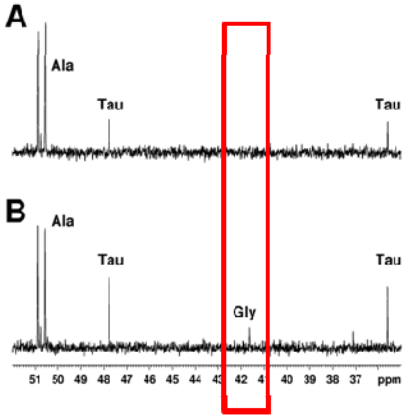

Fig.1: Sample ^{13}C spectra from a primary (A) and a recurrent (B) tumor following infusion of D-[1,2-^{13}C]-glucose. Glycine production from glucose is higher in recurrent tumors compared to primary tumors.

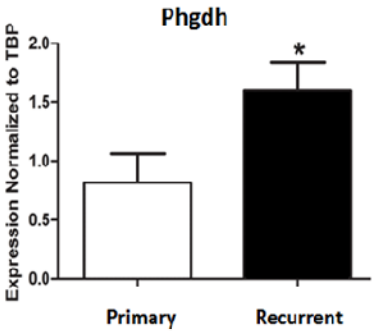

Fig.2: Gene expression levels of phosphoglycerate dehydrogenase (Phgdh) in primary and recurrent tumors by qRT-PCR. Recurrent tumors express higher levels of Phgdh than primary tumors ($p<0.001$).

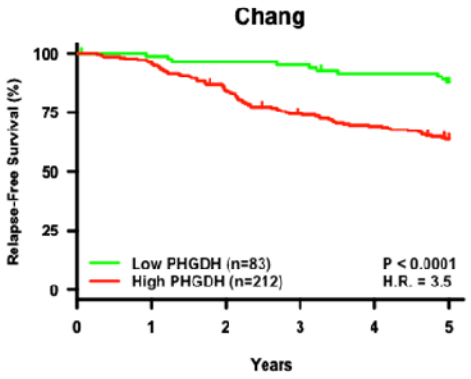

Fig.3: Human association analysis assessing the relation between *PHGDH* levels and cancer relapse. Higher *PHGDH* levels are associated with decreased relapse-free survival in breast cancer patients.