Metabolic Profiling of Primary and Recurrent Mammary Gland Tumors in an Inducible Her2/neu Breast Cancer Mouse Model Using ^{1}H 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 the female population worldwide [1]. Among the women afflicted with this disease, breast cancer recurrence represents the principal cause of death from this disease [2]. While recurrence represents a vastly important clinical problem, little is known about the cellular and molecular mechanisms underlying its development. To dissect those mechanisms, our lab has developed an inducible transgenic mouse model that accurately reproduces key features of the natural history of human breast cancer progression: primary tumor development, tumor dormancy and recurrence [2,3]. Dysregulated metabolism has been previously shown to be a key feature in tumorigenesis [4]. The goal of this study was to investigate the role of ^{1}H MRS metabolic profiling as a potential breast cancer progression marker. Specifically, we evaluate the metabolic changes in primary and recurrent mammary gland tumors using proton spectroscopy and we correlate those changes with the expression levels of the enzymes involved.

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. 9 primary and 9 recurrent mammary gland tumors were dissected from a cohort of 18 mice. Samples were immediately frozen in liquid nitrogen and perchloric acid extraction was performed as previously described by Lehnhardt et al. [5]. NMR spectroscopy was performed at 400MHz on a Bruker Avance DMX 400 wide-bore spectrometer. Fully relaxed proton spectra were acquired with a 5 mm inverse probe using the following conditions: PW 45°, TR 8s, water saturation during the relaxation delay, 6775 Hz SW, TD 64k and 64 scans. An external standard made of trimethylsilylpropionic acid (TSP) was introduced in the NMR tube and used as a chemical shift reference and as a quantitation standard. Statistical significance was determined using a student's t-test. Gene expression levels of metabolic enzymes of interest were obtained from microarray studies previously conducted in our lab.

Results and Discussion: We find that primary and recurrent tumors do differ with respect to their ^{1}H MRS metabolic profiles (Table 1). Recurrent tumors exhibit higher lactate levels (P=0.009), lower succinate levels (P=0.009), lower phosphocholine (PC) levels (p=0.013) and higher glycine levels (P=0.001) than primary tumors. Some of our findings are in agreement with prior studies conducted on primary and recurrent brain tumors [5]. Our results can largely be attributed to differences in the levels of enzymes involved in the production or consumption of these metabolites (Fig. 1). Succinate is converted by succinate dehydrogenase (SDH) into fumarate in the TCA cycle. Higher levels of *sdhb* expression in recurrence accounts for lower succinate being present. Phosphocholine is produced from choline by choline kinase (CHK). Lower *chka* expression can explain the lower phosphocholine levels in recurrence. Glycine is metabolized for inclusion into nucleotides by glycine decarboxylase (GLDC). Markedly lower *gldc* levels in recurrence can account for the accumulation of glycine observed in recurrent tumors. Lactate is produced from pyruvate by lactate dehydrogenase (LDH). Higher levels of lactate in recurrence cannot be explained by the *ldha* expression profiles. We speculate that pathways other than aerobic glycolysis might be involved in lactate production in recurrent tumors. Studies are currently underway to confirm this hypothesis.

Conclusion: In conclusion, the preliminary results presented here indicate that ^{1}H MRS metabolic profiling has the potential to allow for both major improvements in our understanding of the molecular pathways involved in breast cancer recurrence and to 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)Moody et al., Cancer Cell (2002). (4)DeBerardinis et al., Cell Metab (2008), (5) Lehnhard et al., NMR Biomed. (2005).

Table 1: Metabolite Concentrations in Primary and Recurrent Mammary Gland Tumors by ^{1}H MRS.

Metabolite	[Primary] (µmol/g ww)	[Recurrent] (µmol/g ww)	P-value
Lactate	8.48 ± 2.44	11.06 ± 1.62	0.009
Alanine	2.04 ± 0.48	2.05 ± 0.33	0.475
Acetate	1.17 ± 0.89	0.78 ± 0.43	0.130
Glutamate	3.61 ± 0.53	4.92 ± 1.41	0.103
Succinate	0.58 ± 0.12	0.46 ± 0.06	0.009
Creatine	1.80 ± 0.55	1.78 ± 0.75	0.476
Choline	0.36 ± 0.11	0.30 ± 0.06	0.094
PC	0.95 ± 0.43	0.55 ± 0.18	0.013
GPC	1.50 ± 0.48	1.22 ± 0.29	0.079
Taurine	10.95 ± 1.86	16.62 ± 9.66	0.059
Glycine	1.27 ± 0.31	2.08 ± 0.59	0.001
Inositol	10.87 ± 9.18	5.76 ± 1.82	0.068
NADH	3.62 ± 0.93	3.56 ± 1.37	0.457
Formate	1.35 ± 1.33	0.72 ± 0.31	0.09

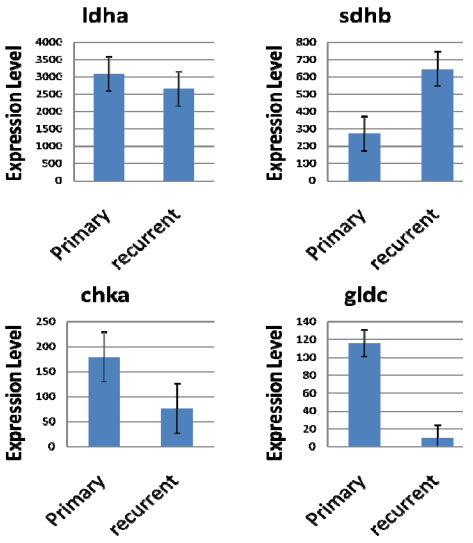

Figure 1: Enzyme expression levels in primary and recurrent mammary gland tumors. Expression levels reflect the average signal in mircroarray studies for 6 samples in each category. Only enzymes responsible for the production or consumption of lactate, succinate, PC and glycine are shown here.