**Metabolic Microenvironments in Normal Breast and Breast Cancer**

Metabolomic Analysis: NIH Eastern Regional Comprehensive Metabolomics Resource Core (RTI RCMRC)

PI, RTI RCMRC P&F Study: Melissa Troester, PhD., MPH., University of North Carolina at Chapel Hill (UNC-CH)

IRB Number: Study # 09-1436: Gene expression profiles of histologically normal breast, LCCC 0913.

**Abstract:** This describes a collaboration with Melissa Troester at the University of North Carolina at Chapel Hill, (U01 ES019472-03S2 trial funded by NCI, RO1 CA138255-05, Troester and Perou, PhD. MPIs). The purpose of this study was to understand the genomic and metabolomic changes in the breast tissue microenvironment at various stages of cancer development and progression (i.e. normal breast, DCIS, benign disease and invasive cancer). It was previously reported that integrated analyses of metabolite and gene expression data from breast tumors could identify phenotypically distinct groups of tumors.1 The stroma (normal breast tissue) has also been shown to play an important role in dictating the metabolic phenotype. Since intrinsic breast cancer subtypes are very distinct from each other in terms of clinical features as well as their genomic profiling, these subtypes may also have distinct metabolic microenvironment signatures. To understand the way in which metabolic microenvironments evolve with breast cancer, this study analyzed normal breast tissue adjacent to benign and malignant lesions at various stages of cancer development and studied samples adjacent to invasive cancers of distinct molecular breast cancer subtypes.

Breast tissue samples were collected from patients in the UNC hospital system under The Normal Breast Study (IRB #09-1436). Normal breast tissue collected from patients undergoing reduction mammoplasty, or with DCIS and invasive breast cancers were analyzed in this study. Tissue samples 2-4 cm away from the tumor were collected and stored at -80°C after surgery. Samples were cut on dry ice to yield samples for metabolomics analyses comprising 50-120 mg tissue. The tissue was placed in a tissue homogenization tube with ceramic beads and stored at -80°C prior to shipment. The samples were shipped on dry ice to the RTI RCMRC and stored at -80°C until processing. The goal of this metabolomics pilot and feasibility study was to apply broad spectrum metabolomics methods (GC-MS and NMR) to determine whether characteristic metabolic changes occur in the surrounding normal tissue during breast cancer progression and if any identified signatures can be correlated with disease subtype.

Of the 120 study samples shipped, 2 were excluded prior to sample preparation due to insufficient biomass. Prior to all data acquisitions, the samples were thawed on ice in a 4°C room and homogenized and extracted using a high-throughput method to generate 0.1 mg/µL homogenates. Phenotypic pool samples were created by combining equal volume aliquots from each sample in each phenotype based on breast cancer subtype, invasive status or non-malignancy (Reduction mammoplasty). Equal volumes of the phenotypic pool samples were also combined to create a pooled QC sample for the entire study (Total Pool 1). A second QC pool sample was created using unequal volumes of the phenotypic pool samples (Total Pool 2) and used only for conditioning the GC inlet and column prior to sample analysis during the GC-MS acquisition of data.

For NMR data, a volume corresponding to 50 mg of tissue/experimental samples was then dried on a speed-vacuum concentrator, the lipids and proteins were extracted, and re-dried. The extracted samples were resuspended in D2O with the addition of Chenomx ISTD (+ Imidazole) and transferred to NMR tubes for acquisition on a 700 MHz spectrometer for 139 total samples.

For GC-MS data, a proportion of NMR retrieved samples corresponding to 25 mg of tissue/experimental sample, were further prepared in an approach similar to the methods published by Dr. Oliver Fiehn, Director, RCMRC UC Davis.2 Briefly, samples were chemically derivatized after a D2O exchange and analyzed by GC-TOF-MS (Agilent 7890 gas chromatogram and Leco Pegasus 4D time of flight mass spectrometer). The run order was re-randomized and samples were analyzed in four batches over 4 separate days. A new Total Pool 1 sample was analyzed between every 6 sample injections in each batch. The Total Pool 2 sample was injected multiple times at the beginning of each batch to condition the column and the system. Metabolomics analysis by GC-MS was performed on a total of 174 sample injections.

The data required for the GC-MS metabolomics analysis can be found in the accompanying files:

Procedures: 1. MT Breast Tissue Procedures.docx

Flowchart: 1a. MT Breast Tissue GCMS flowchart.pdf

1b. GCMS Preparation of fatty acid methyl esters mixture.pdf

Study Design Table: 2. MT Breast Tissue Study Design Table.xls

Metadata: 3. MT Breast Tissue Metadata and Analytical Metadata.xlsm

Processed Data: 4. MT Breast Tissue Phenotypic and Processed Data.xlsx

Raw Data: 5. MT Breast Tissue Raw Data.zip

Notes:

Full sample preparation and analysis procedures are available in the accompanying document entitled **1. MT Breast Tissue Procedures** for GC-MS-specific methods. A flowchart describing the full GC-MS sample preparation is also available at **1a. MT Breast Tissue GCMS flowchart.pdf**. The preparation of the fatty acid methyl esters (FAME) mixture is located in accompanying file **1b. GCMS Preparation of fatty acid methyl esters mixture.pdf**.

Descriptions of abbreviations for factors are available in the Variable Dictionary in the accompanying files no. **2. MT Breast Tissue Study Design Table.xls** for GC-MS.

The spreadsheets in the GC-MS processed data file **4. MT Breast Tissue Phenotypic and Processed Data** contain a BinBase Processed Data tab. This tab shows the peak height value (not normalized) output from BinBase. Sample ID and factors can be found in the top 5 rows in the spreadsheet.

For GC-MS, the raw data in netCDF format is available in the accompanying file **5. MT Breast Tissue Raw Data.zip**. A table linking datafile names to Sample IDs is present in accompanying file **2. MT Breast Tissue GCMS Study Design Table.xlsx**.

**References:**

1. Brauer HA, Makowski L, Hoadley KA, Casbas-Hernandez P, Lang LJ, Roman-Perez E, D’Arcy M, Freemerman AJ, Perou CM, Troester MA: Impact of tumor microenvironment and epithelial phenotypes on metabolism in breast cancer. Clin Cancer Res 2012;19:571-585.

2. J Budczies, C Denkert, B Muller, S Brockmoller, F Klauschen, B Gyorffy, M Dietel, C Richter-Ehrenstein, U Marten, R Salek, J Griffin, M Hilvo, M Oresic, G Wohlgemuth, O Fiehn: Remodeling of central metabolism in invasive breast cancer compared to normal breast tissue – a GC-TOFMS based metabolomics study. BMC Genomics 2012; 13:334.