Differential expression of lipase E, hormone-sensitive type in cancers of the breast.

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Breast cancer affects women at relatively high frequency[1]. We mined published microarray datasets[2,3] to determine in an unbiased fashion and at the systems level genes most differentially expressed in the primary tumors of patients with breast cancer. We report here significant differential expression of the gene encoding lipase E, hormone-sensitive type, LIPE, when comparing primary tumors of the breast to the tissue of origin, the normal breast. LIPE mRNA was present at significantly lower quantities in tumors of the breast as compared to normal breast tissue. Analysis of human survival data revealed that expression of LIPE in primary tumors of the breast was correlated with recurrence-free survival in patients with HER2+ subtype cancer, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by PAM50 molecular subtype. LIPE may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, LIPE, lipase E, hormone-sensitive type, systems biology of breast cancer, targeted therapeutics in breast cancer.

Invasive breast cancer is diagnosed in over a quarter of a million women in the United States each year[1] and in 2018, breast cancer was the leading cause of cancer death in women worldwide[4]. While patients with localized breast cancer are provided a 99% 5-year survival rate, patients with regional breast cancer, cancer that has spread to lymph nodes or nearby structures, are provided an 86% 5-year survival rate[5,6]. Patients with metastasis to distant sites, like the brain, are provided a 27% 5-year survival rate[5,6]. Understanding how primary tumors are most transcriptionally different from the tissue from which they originate, the breast, can facilitate development of novel diagnostic and therapeutics to promote early detection and enhanced treatment, and contribute to efforts to prevent progression to metastatic stages. We mined published microarray data[2,3] to understand at the transcriptome level and in an unbiased fashion genes most differentially expressed in primary tumors of the breast as compared to normal breast tissue. Lipase E, hormone-sensitive type emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE42568[2] and GSE109169[3] for this global differential gene expression analysis of female breast cancer. GSE42568 was generated using Affymetrix Human Genome U133 Plus 2.0 array technology with *n*=17 normal breast tissue biopsies and *n*=104 primary breast tumor biopsies from patients with breast cancer; analysis was performed using platform GPL570; the majority of patients whose tumors were analyzed were age 50 or older. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array technology with *n*=25 normal breast tissue and *n*=25 tumors of the breast; analysis was performed using platform GPL5175. The tissues whose expression was profiled in this dataset are paired tissues (25 tumors matching 25 breast tissues from 25 patients). The Benjamini and Hochberg method of *p*-value adjustment was used for ranking of differential expression but raw *p*-values were used to assess statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether LIPE expression was significantly different between primary breast tumors and normal breast tissue using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool[7] for correlation of LIPE mRNA expression levels with recurrence-free survival (RFS) in *n*=953 patients with basal-like subtype cancer, *n*=1809 patients with luminal A subtype cancer, *n*=1353 patients with luminal B subtype cancer, *n*=695 patients with HER2+ cancer, and *n*=119 patients with normal-like subtype cancer, in the lower quartile.

Results

We performed discovery of genes associated with breast cancer in females by mining two independently published microarray datasets[2,3].

LIPE is differentially expressed in primary tumors of the breast.

Studying the global gene expression profiles of 104 breast cancers from patients aged 31 to 89 revealed that the gene encoding lipase E, hormone-sensitive type, LIPE, was among the genes most differentially expressed in tumors of the breast in human breast cancer (Chart 1). When sorting each of the genes expressed in tumors of the breast based on significance of difference as compared to normal breast tissue, LIPE ranked 113 out of 54675 total transcripts, equating to 99.8% differential expression (Chart 1). Differential expression of LIPE in female breast cancer was statistically significant (Chart 1; *p*=4.03E-30).

Analysis of a second microarray dataset[3], here studying global gene expression patterns in the tumors of 25 patients with early-onset breast cancer, again revealed significant differential expression of LIPE in human breast cancer (Chart 2). When sorting each of the genes expressed in the tumors of patients

with breast cancer based on significance of difference as compared to normal breast tissue, LIPE ranked 1351 out of 19076 total transcripts, equating to 92.9% differential expression (Chart 2). Differential expression of LIPE in the tumors of patients with breast cancer was statistically significant (Chart 2; p=4.55E-08). These data suggested that differential expression of LIPE was not an artifact of a single microarray dataset, nor was it strictly associated with early-onset breast cancer, rather a general feature of cancers of the breast.

LIPE is expressed at significantly lower levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for LIPE from the breast and from breast tumors to understand the magnitude and direction of LIPE expression change. LIPE was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of LIPE in primary breast tumors was statistically significant (Figure 1: p<0.0001). LIPE was expressed at 10.48 ± 2.38 arbitrary units (AU) in normal breast tissue, while it was expressed at 5.89 ± 0.80 AU in tumors of the breast. We calculated a mean fold change of 0.56 in LIPE mRNA levels when comparing primary tumors of the breast to normal breast tissues.

LIPE expression correlates with survival outcomes in HER2+ subtype human breast cancer.

We performed Kaplan-Meier survival analysis to study relationships between tumor LIPE mRNA expression levels and survival outcomes in patients with breast cancer. We observed a correlation between LIPE expression and recurrence-free survival (RFS) in patients with HER2+ subtype breast cancer which trended towards statistical significance, in the lower quartile (Figure 2; log rank p-value: 0.09 for recurrence-free survival, hazard ratio: 0.8 (0.61-1.04) (Fig. 2)). LIPE mRNA levels were a positive prognostic indicator in HER2+ subtype breast cancer patients. Median RFS was 122.64 months for HER2+ patients with low tumor expression of LIPE while median RFS was 171.43 months for HER2+ patients with high tumor expression of LIPE (Chart 3). LIPE primary tumor expression was not correlated with recurrence-free survival in basal-like breast cancer (Figure 2; log rank p-value: 0.64 for RFS, hazard ratio: 1.06 (0.83-1.35) (Fig. 2)), luminal A subtype breast cancer (Figure 2; log rank p-value: 0.1 for RFS, hazard ratio: 1.23 (0.96-1.58) (Fig. 2)), in luminal B breast cancer (Figure 2; log rank p-value: 0.78 for RFS, hazard ratio: 1.03 (0.84-1.26) (Fig. 2)) or in patients with normal-like breast cancer (Figure 2; log rank p-value: 0.11 for RFS, hazard ratio: 0.57 (0.28-1.14) (Fig. 2)).

Thus, through comparative transcriptome analysis of primary tumors of the breast and normal breast tissue, we found that differential expression and down-regulation of LIPE was among the most significant transcriptional features in primary tumors from patients with breast cancer. LIPE expression in primary tumors of the breast was correlated with recurrence-free survival in patients with HER2+ subtype disease, with mRNA levels of LIPE a positive prognostic indicator for HER2+ breast cancer patients.

Discussion

Invasive breast cancer is a medical problem with a 27% 5-year survival rate for women whose disease has spread to distant sites[5,6]. To facilitate understanding of the basic transcriptional differences between primary tumors of the breast and the tissues from which these tumors originate, normal breast tissues, we performed comparative transcriptome analysis using two independently published microarray datasets[2,3], providing evidence here that differential expression of lipase E, hormone-sensitive type, encoded by LIPE, is a defining transcriptional feature of human breast cancer: in patients diagnosed after age 50, and in early onset-breast cancer. LIPE was expressed at significantly lower levels in primary tumors from patients with breast cancer as compared to normal breast tissue. Importantly, in patients with HER2+ subtype breast cancers, expression of LIPE was correlated with recurrence-free survival. Lipase

E, hormone-sensitive type and the molecular processes to which it pertains to may be relevant to the initiation or progression of human breast cancer.

References

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Rank: 113
Probe ID: 208186_s_at
p-value: 4.03E-30
t: 1.54E+01
B: 57.9597258
Gene: LIPE
Gene name: lipase E, hormone-sensitive type

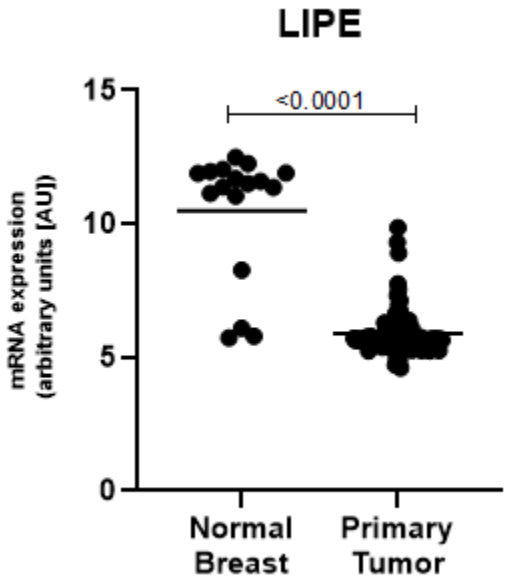
Chart 1: LIPE is differentially expressed in the primary tumors of patients with breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Rank: 1351
Probe ID: 3863606
p-value: 4.55E-08
t: 6.42
B: 8.222965
Gene: LIPE
Gene name: lipase E, hormone-sensitive type

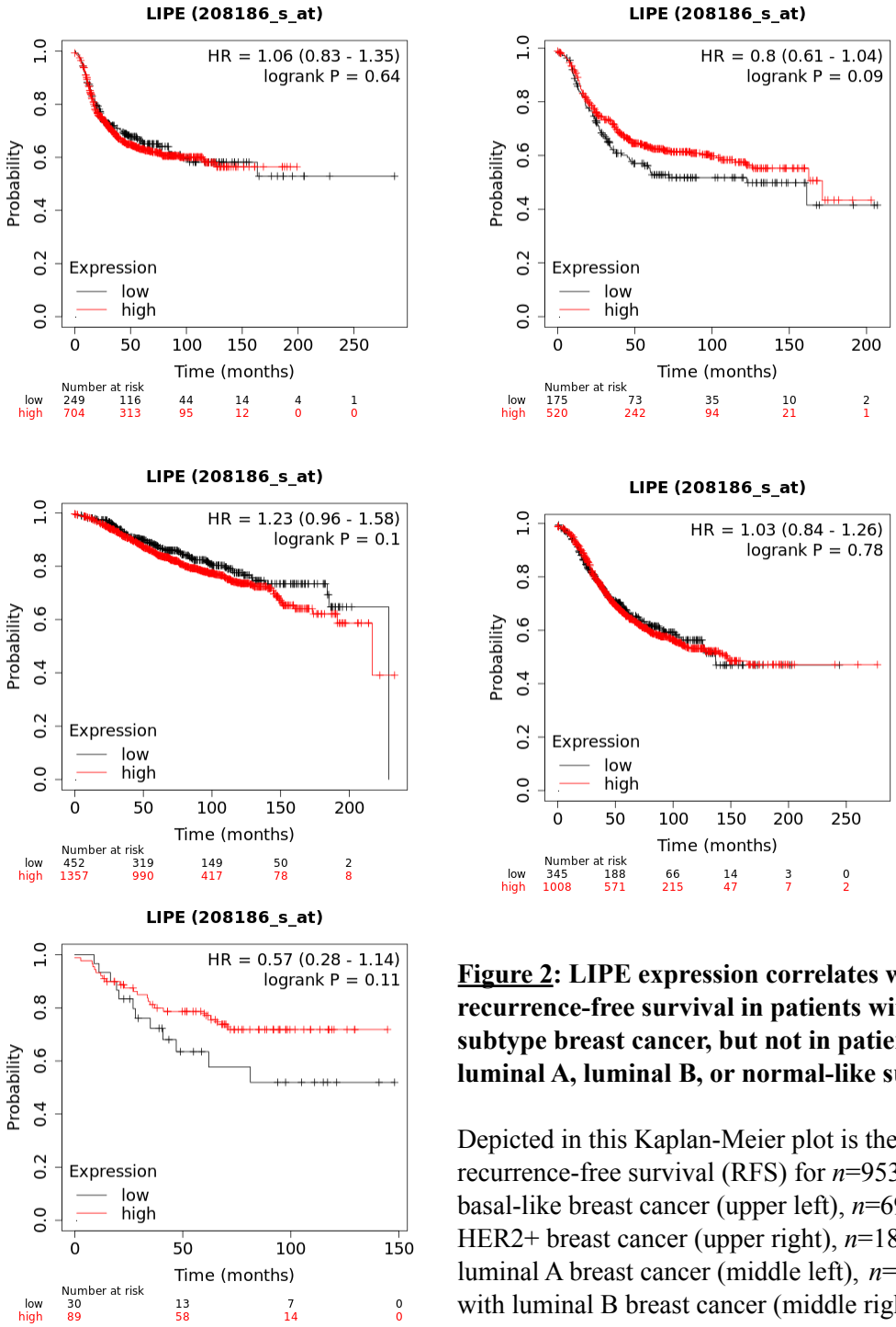
Chart 2: LIPE is differentially expressed in the primary tumors of patients with early-onset human breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.



<u>Figure 1:</u> LIPE is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of LIPE in normal breast tissue (left) and in primary tumors of the breast (right) is graphically depicted with the result of a statistical test evaluating significance of difference in LIPE expression between normal breast tissue and primary tumors of the breast, a *p*-value, listed above.



<u>Figure 2</u>: LIPE expression correlates with recurrence-free survival in patients with HER2+ subtype breast cancer, but not in patients with basal, luminal A, luminal B, or normal-like subtype cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival (RFS) for *n*=953 patients with basal-like breast cancer (upper left), *n*=695 patients with HER2+ breast cancer (upper right), *n*=1809 patients with luminal A breast cancer (middle left), *n*=1353 patients with luminal B breast cancer (middle right), and *n*=119 patients with normal-like breast cancer (below) stratified into two groups, based on low or high expression of LIPE in patient primary tumors, in the lower quartile. The log rank p-value denoting statistical significance of difference in recurrence-free survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on LIPE expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

HER2+ patients

Low LIPE expression: 122.64 months
High LIPE expression: 171.43 months

Chart 3: In HER2+ subtype breast cancer, median recurrence-free survival is superior in patients with high primary tumor expression of LIPE.

The recurrence-free survival of *n*=1353 HER2+ subtype breast cancer patients based on stratification into low or high tumor expression of LIPE, in the lower quartile, is listed in this chart.