Differential expression of thyrotropin-releasing hormone-degrading enzyme in cancers of the breast.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY 11730

Breast cancer affects women at relatively high frequency¹. 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 thyrotropin-releasing hormone-degrading enzyme, TRHDE, when comparing primary tumors of the breast to the tissue of origin, the normal breast. TRHDE 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 TRHDE in primary tumors of the breast was correlated with recurrence-free survival in patients with luminal A subtype cancer, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by PAM50 molecular subtype. TRHDE may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, TRHDE, thyrotropin-releasing hormone-degrading enzyme, 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 year¹ and in 2018, breast cancer was the leading cause of cancer death in women worldwide⁴. 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. Thyrotropin-releasing hormone-degrading enzyme emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE42568² and GSE109169³ 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 TRHDE 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 tool⁷ for correlation of TRHDE 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 A subtype cancer, $n=695$ patients with HER2+ cancer, and $n=119$ patients with normal-like subtype cancer.

Results

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

TRHDE 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 thyrotropin-releasing hormone-degrading enzyme, TRHDE, 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, TRHDE ranked 83 out of 54675 total transcripts, equating to 99.8% differential expression (Chart 1). Differential expression of TRHDE in female breast cancer was statistically significant (Chart 1; $p=5.69E-32$).

Analysis of a second microarray dataset³, here studying global gene expression patterns in the tumors of 25 patients with early-onset breast cancer, again revealed significant differential expression of

TRHDE 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, TRHDE ranked 3124 out of 19076 total transcripts, equating to 83.6% differential expression (Chart 2). Differential expression of TRHDE in the tumors of patients with breast cancer was statistically significant (Chart 2; $p=1.62E-05$). These data suggested that differential expression of TRHDE 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.

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

We obtained exact mRNA expression levels for TRHDE from the breast and from breast tumors to understand the magnitude and direction of TRHDE expression change. TRHDE was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of TRHDE in primary breast tumors was statistically significant (Figure 1; $p<0.0001$). TRHDE was expressed at 5.14 ± 1.64 arbitrary units (AU) in normal breast tissue, while it was expressed at 2.40 ± 0.26 AU in tumors of the breast. We calculated a mean fold change of 0.47 in TRHDE mRNA levels when comparing primary tumors of the breast to normal breast tissues.

TRHDE expression correlates with survival outcomes in luminal A subtype human breast cancer.

We performed Kaplan-Meier survival analysis to study relationships between tumor TRHDE mRNA expression levels and survival outcomes in patients with breast cancer. We observed a correlation between TRHDE expression and recurrence-free survival (RFS) in patients with luminal A subtype breast cancer which approached statistical significance (Figure 2; log rank p -value: 0.069 for recurrence-free survival, hazard ratio: 0.83 (0.67-1.02) (Fig. 2)). TRHDE mRNA levels were a positive prognostic indicator in luminal A subtype breast cancer patients. Median RFS was 108.6 months for luminal A patients with low tumor expression of TRHDE while median RFS was 130.72 months for luminal A patients with high tumor expression of TRHDE (Chart 3). TRHDE primary tumor expression was not correlated with recurrence-free survival in basal-like breast cancer (Figure 2; log rank p -value: 0.31 for RFS, hazard ratio: 0.9 (0.72-1.11) (Fig. 2)), luminal B subtype breast cancer (Figure 2; log rank p -value: 0.24 for RFS, hazard ratio: 0.9 (0.76-1.07) (Fig. 2)), in HER2+ breast cancer (Figure 2; log rank p -value: 0.67 for RFS, hazard ratio: 0.95 (0.75-1.21) (Fig. 2)) or in patients with normal-like breast cancer (Figure 2; log rank p -value: 0.46 for RFS, hazard ratio: 1.29 (0.66-2.54) (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 TRHDE was among the most significant transcriptional features in primary tumors from patients with breast cancer. TRHDE expression in primary tumors of the breast was correlated with recurrence-free survival in patients with luminal A subtype disease, with mRNA levels of TRHDE a positive prognostic indicator for luminal A 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 thyrotropin-releasing hormone-degrading enzyme, encoded by TRHDE, is a defining transcriptional feature of human breast cancer: in patients diagnosed after age 50, and in early onset-breast cancer. TRHDE was expressed at

1 significantly lower levels in primary tumors from patients with breast cancer as compared to normal breast
2 tissue. Importantly, in patients with luminal A subtype breast cancers, expression of TRHDE was
3 correlated with recurrence-free survival. Thyrotropin-releasing hormone-degrading enzyme and the
4 molecular processes to which it pertains to may be relevant to the initiation or progression of human breast
5 cancer.
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Rank: 83
Probe ID: 219937_at
p-value: 5.69E-32
t: 1.62E+01
B: 62.1682925
Gene: TRHDE
Gene name: thyrotropin-releasing hormone-degrading enzyme

Chart 1: TRHDE 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: 3124
Probe ID: 3422458
p-value: 1.62E-05
t: 4.76
B: 2.482466
Gene: TRHDE
Gene name: thyrotropin-releasing hormone-degrading enzyme

Chart 2: TRHDE 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.

Figure 1: TRHDE is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of TRHDE 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 TRHDE expression between normal breast tissue and primary tumors of the breast, a *p*-value, listed above.

Figure 2: TRHDE expression correlates with recurrence-free survival in patients with luminal A subtype breast cancer, but not in patients with basal, luminal B, HER2+, 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 TRHDE in patient primary tumors. 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 TRHDE 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.

Luminal A patients

Low TRHDE expression: 108.6 months

High TRHDE expression: 130.72 months

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

The recurrence-free survival of $n=1809$ luminal A subtype breast cancer patients based on stratification into low or high tumor expression of TRHDE is listed in this chart.