Differential expression of the DREAM MuvB core complex component Lin37 in cancers of the breast.

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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 the MuvB core complex and DREAM component Lin37 when comparing primary tumors of the breast to the tissue of origin, the normal breast. Expression of Lin37 in primary tumors of the breast was significantly higher than in normal breast tissue, and patients with low expression of Lin37 in primary tumors had greater relapse-free survival. Lin37 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, Lin37, MuvB core complex, DREAM complex, 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 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. The DREAM MuvB core complex component Lin37 emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE109169² and GSE124648³ for this differential gene expression analysis of female breast cancer. 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 and; analysis was performed using platform GPL5175. The tissues from this first dataset are paired tissues (25 tumors matching 25 breast tissues from 25 patients). GSE124648 was generated using Affymetrix Human Genome U133A Array technology with $n=10$ normal breast tissue and $n=126$ tumors of the breast and; analysis was performed using platform GPL96. 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 Lin37 expression was significantly between primary breast tumors and breast tissue using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455). For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of Lin37 mRNA expression levels with relapse-free survival (disease-free survival) in $n=3951$ breast cancer patients.

Results

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

Lin37 is differentially expressed in primary tumors of the breast.

Comparison of 25 normal breast tissues to 25 tumors of the breast² revealed that the DREAM MuvB core complex component Lin37 was among the genes most differentially expressed in tumors of the breast in female breast cancer (Table 1). When sorting each of the genes expressed in tumors of the breast based on significance of difference as compared to normal breast tissue, Lin37 ranked 198 out of 19076 total transcripts, equating to 99.0% differential expression (Table 1). Differential expression of Lin37 in female breast cancer was statistically significant (Table 1; $p=3.67E-13$).

Analysis of a second microarray dataset³, here in 126 tumors of the breast as compared to 10 normal breast tissues, again revealed significant differential expression of Lin37 in tumors

of the breast in female breast cancer (Table 2). When sorting each of the genes expressed in tumors of the breast based on significance of difference as compared to normal breast tissue, Lin37 ranked 7872 out of 22283 total transcripts, equating to 64.7% differential expression (Table 2). Differential expression of Lin37 in female breast cancer was statistically significant (Table 2; $p=1.65E-02$).

Lin37 is expressed at significantly higher levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for Lin37 from the breast and from breast tumors to understand the magnitude and direction of Lin37 expression change. In both datasets, Lin37 was expressed at higher levels in tumors of the breast as compared to normal breast tissue (Figure 1). Increased expression of Lin37 in primary breast tumors was statistically significant (Figure 1: $p<0.000$). We calculated a mean fold change of 1.08 ± 0.03 when comparing primary tumors of the breast to normal breast tissues (Table 1).

Lin37 expression associates with survival outcomes in female breast cancer.

We performed Kaplan-Meier survival analysis in an attempt to correlate Lin37 mRNA expression levels with patient survival, in 3951 breast cancer patients for relapse-free survival. We found a statistically significant correlation between Lin37 expression and relapse-free survival (also known as disease-free survival) in patients with breast cancer (Figure 2; log rank p -value: $8.6e-10$ for relapse-free survival, hazard ratio: 1.42 (1.27-1.58) (Fig. 2)). Higher Lin37 mRNA levels were a negative prognostic indicator. Median RFS was 228.85 months for patients whose tumors expressed low levels of Lin37 while median RFS was 191.21

1 months for patients whose tumors expressed high levels of Lin37 (Table 3).

2 Thus, through comparative transcriptome analysis of primary tumors of the breast and
3 normal breast tissue, we found that differential expression and up-regulation of Lin37 was
4 among the most significant transcriptional features in primary tumors from patients with breast
5 cancer. Lin37 expression in primary tumors of the breast demonstrated a strong and significant
6 correlation with relapse-free survival in patients with breast cancer, with lower expression of
7 Lin37 correlating with improved survival.
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9 10 **Discussion**

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12 Invasive breast cancer is medical problem with a 27% 5-year survival rate for women
13 whose disease has spread to distant sites. To facilitate understanding of the basic
14 transcriptional differences between primary tumors of the breast and the tissues from which
15 these tumors originate, normal breast tissues, we performed comparative transcriptome
16 analysis using two independently published microarray datasets^{2,3} and identified significant
17 differential expression of Lin37 in breast cancer.
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19 The multivulva class B, or MuvB core complex is composed of five components: Lin9,
20 Lin37, Lin52, Lin54 and RBBP4/Lin53. The MuvB core complex, in conjunction with p130,
21 E2F4, and DP1 forms the DREAM complex⁸. The DREAM complex functions in control of G0 /
22 cell cycle quiescence as a general repressor of both early and late genes in the G0 gene
23 expression program⁸. In this context, DREAM can occupy the promoter of more than 800 genes
24 during G0⁸. Another function of the MuvB core complex involves association with B-Myb and
25 FoxM1. Once cell cycle progression into the S-phase has been initiated, the MuvB complex
26 dissociates from DREAM, associates with B-Myb, and occupies late gene promoters⁹. In G2,
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1 FoxM1 is recruited to the promoter of late genes, for which B-Myb and FoxM1 are required, and
2 FoxM1 remains associated with MuvB core complex until B-Myb loses its association with
3 FoxM1 and the MuvB core complex late in S-phase following proteasomal degradation
4 dependent on phosphorylation⁹. Thus, the MuvB core complex, of which Lin37 is a component,
5 has functions both in quiescence and during cell cycle progression^{8,9}.

7 However, a subsequent study has demonstrated that Lin37 is an essential component of
8 the DREAM complex, and not simply a MuvB core complex component. Despite the ability of
9 p130/p107 to occupy the promoter of genes in G0, DREAM loses its ability to repress G0 gene
10 expression in the absence of Lin37. Since cells lacking both the retinoblastoma protein (Rb)
11 and Lin37 are unable to exit cell cycle, together these data demonstrate that Rb and Lin37
12 function together to execute quiescence¹⁰.

14 We could not identify literature describing a role for Lin37, the MuvB core complex,
15 DREAM, or any components of these cellular machinery in human breast cancer. However,
16 mutations in the nuclear localization signal of p130 (Rb2) have been identified in cell lines and
17 primary tumors from patients with Burkitt's Lymphoma¹¹. Differential expression of p130 based
18 on histological grade has been found in some lung cancers¹², and a study screening 17 small-
19 cell lung cancer cell lines found that the GLC2 cell line expressed no p130 protein¹³. Loss of
20 p130 in the GLC2 small-cell lung cancer cell line was found to be caused by a point mutation in
21 the splice acceptor site in the second intron of genomic DNA, resulting in generation of a stop
22 codon due to loss of the second exon in the aberrant p130 RNA¹³.

25 We identified significant differential expression of the MuvB core complex component
26 Lin37 in primary tumors from patients with breast cancer. Lin37 expression was significantly
27 correlated with relapse-free survival in patients with breast cancer. The transcriptional induction
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1 of Lin37 in tumors of the breast suggests that the DREAM complex and/or the MuvB core
2 complex independent of p130, E2F4 and DP1 may be of importance to breast cancer
3 pathogenesis, and future studies can determine by which mechanisms these molecules might
4 function to contribute to breast cancer initiation, maintenance or progression. We report here,
5 as among the most significant transcriptional features of tumors the breast, the up-regulation of
6 the multivulva class B core complex component Lin37 and its association with breast cancer
7 patient survival.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
198	3830789	3.67E-13	9.69	19.780213	1.08 ± 0.03	LIN37	lin-37 DREAM MuvB core complex component

Table 1: Lin37 is differentially expressed in 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, fold change of Lin37 in primary breast tumors as compared to normal breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
7872	213526_s_at	1.65E-02	2.428012	-3.95302	LIN37	lin-37 DREAM MuvB core complex component

Table 2: Lin37 is differentially expressed in 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, fold change of Lin37 in primary breast tumors as compared to normal breast, gene and gene name are listed in this chart.

Figure 1: Lin37 is expressed at significantly higher levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of Lin37 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 Lin37 expression between normal breast tissue and primary tumors of the breast, a *p*-value, listed above.

Figure 2: Lin37 expression correlates with relapse-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of relapse-free survival (RFS) for $n=3951$ total patients stratified into two groups, based on low or high expression of Lin37 in patient primary tumors. The log rank p -value denoting statistical significance of difference in relapse-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 Lin37 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.

Low expression cohort (months)	High expression cohort (months)
228.85	191.21

Table 3: Median relapse-free survival is greater in breast cancer patients with low primary tumor expression of Lin37.

The relapse-free survival of $n=3951$ breast cancer patients based on stratification into low or high tumor expression of Lin37 is listed in this chart.