Differential expression of LTBP4 in cancer of the vulva.

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In these brief notes we document work using published microarray data (1, 2) to pioneer integrative transcriptome analysis comparing vulvar carcinoma to its tissue of origin, the vulva. We report the differential expression of latent transforming growth factor beta binding protein 4, encoded by LTBP4, in cancer of the vulva. LTBP4 may be of pertinence to understanding transformation and disease progression in vulvar cancer (3).

Keywords: vulvar cancer, vulvar carcinoma, LTBP4, latent transforming growth factor beta binding protein 4.

Gynecologic cancers include cervical cancer, ovarian cancer, endometrial (uterine) cancer, and vulvar cancer (4, 3). Vulvar cancer is thought to be the least prevalent of these, with 6190 cases reported in the United States in 2018 (5). We integrated separate published microarray datasets to understand, at the systems level, the transcriptional landscape of vulvar carcinoma.

Methods

We utilized microarray datasets GSE63678 (1) and GSE38228 (2) for this integrated, transcriptome-level differential gene expression analysis of human vulvar cancers in conjunction with GEO2R. GSE63678 was generated using Affymetrix Human Genome U133A 2.0 Array technology with $n=7$ normal vulvar tissues and $n=6$ vulvar carcinoma tissues; analysis was performed using platform GPL571. GSE38228 was generated using Illumina HumanHT-12 V4.0 expression beadchip technology with $n=5$ normal vulvar tissues and $n=13$ vulvar SCC (squamous cell carcinoma) tissues; analysis was performed using platform GPL10558. 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 LTBP4 expression was significantly different between normal vulva and vulvar cancer using a two-tailed t -test.

Results

We mined published microarray data (1, 2) to understand the most significant transcriptional changes between the normal vulva and cancer of the vulva.

LTBP4 is differentially expressed in vulvar cancer.

We found that latent transforming growth factor beta binding protein 4, encoded by LTBP4, was among the most differentially expressed genes, transcriptome-wide, in cancer of the vulva (1). When sorting each transcript whose expression was measured across the transcriptome based on significance of change between the normal vulva and vulvar cancer tissues, LTBP4 ranked 132 out of 22277 total transcripts, equating to 99.4% differential expression (Chart 1) (1). Differential expression of LTBP4 was statistically significant (Chart 1; $p=2.09E-05$).

We validated our findings by performing this same analysis using a second published microarray dataset (2). When sorting each transcript whose expression was measured across the transcriptome based on significance of change between the normal vulva and vulvar cancer tissues, LTBP4 ranked 641 out of 47323 total transcripts, equating to 98.6% differential expression (Chart 2) (2). Differential expression of LTBP4 was again statistically significant (Chart 2; $p=9.43E-06$).

LTBP4 expression is lower in cancer tissue as compared to the vulva.

We obtained exact mRNA expression data from the first microarray dataset we mined to directly compare expression levels between control and cancer tissues. LTBP4 was expressed at significantly lower levels in cancer of the vulva as compared to normal vulva. LTBP4 was expressed at 10.37 ± 0.61 arbitrary units (A.U.) in normal vulva, but at 7.80 ± 0.95 A.U. in vulvar cancer tissues (Figure 1). We

1 calculated a mean fold change of 0.75 in LTBP4 expression when comparing vulvar carcinoma to vulva.
2

3 **Discussion**

4 We documented here that LTBP4 is differentially expressed at the transcriptome level in cancer of
5 the human vulva (1, 2). LTBP4 is a candidate gene of interest in understanding the transcriptional biology
6 of vulvar cancer.
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Rank: 132
Probe ID: 204442_x_at
p-value: 2.09E-05
t: 6.371548
B: 3.08541
Gene: LTBP4
Gene name: latent transforming growth factor beta binding protein 4

Chart 1: Differential expression of LTBP4 in vulvar 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: 641
Probe ID: ILMN_1665219
p-value: 9.43E-06
t: 6.0487075
B: 3.58798
Gene: LTBP4
Gene name: latent transforming growth factor beta binding protein 4

Chart 2: Differential expression of LTBP4 in vulvar 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: LTBP4 is expressed at significantly lower levels in vulvar cancer as compared to normal vulva.

The mRNA expression level of LTBP4 in the normal vulva (left) and in vulvar carcinoma (right) is graphically depicted with the result of a statistical test evaluating significance of difference in LTBP4 expression between the two groups, a *p*-value, listed above.