Differential expression of KLHL3 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 kelch-like family member 3, encoded by KLHL3, in cancer of the vulva. KLHL3 may be of pertinence to understanding transformation and disease progression in vulvar cancer (3).

Keywords: vulvar cancer, vulvar carcinoma, KLHL3, kelch-like family member 3.

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 KLHL3 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.

KLHL3 is differentially expressed in vulvar cancer.

We found that kelch-like family member 3, encoded by KLHL3, 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, KLHL3 ranked 125 out of 22277 total transcripts, equating to 99.4% differential expression (Chart 1) (1). Differential expression of KLHL3 was statistically significant (Chart 1; $p=1.79\text{E-}05$).

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, KLHL3 ranked 1203 out of 47323 total transcripts, equating to 97.5% differential expression (Chart 2) (2). Differential expression of KLHL3 was again statistically significant (Chart 2; $p=8.08\text{E-}05$).

KLHL3 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. KLHL3 was expressed at significantly lower levels in cancer of the vulva as compared to normal vulva. KLHL3 was expressed at 7.89 ± 0.38 arbitrary units (A.U.) in normal vulva, but at 6.66 ± 0.27 A.U. in vulvar cancer tissues (Figure 1). We

1 calculated a mean fold change of 0.84 in KLHL3 expression when comparing vulvar carcinoma to vulva.
2

3 **Discussion**

4 We documented here that KLHL3 is differentially expressed at the transcriptome level in cancer of
5 the human vulva (1, 2). KLHL3 is a candidate gene of interest in understanding the transcriptional
6 biology of vulvar cancer.
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References

1. Pappa, K.I., Polyzos, A., Jacob-Hirsch, J., Amariglio, N., Vlachos, G.D., Loutradis, D. and Anagnou, N.P., 2015. Profiling of discrete gynecological cancers reveals novel transcriptional modules and common features shared by other cancer types and embryonic stem cells. *PLoS One*, 10(11), p.e0142229.
2. Micci, F., Panagopoulos, I., Haugom, L., Dahlback, H.S.S., Pretorius, M.E., Davidson, B., Abeler, V.M., Tropé, C.G., Danielsen, H.E. and Heim, S., 2013. Genomic aberration patterns and expression profiles of squamous cell carcinomas of the vulva. *Genes, Chromosomes and Cancer*, 52(6), pp.551-563.
3. Canavan, T.P. and Cohen, D., 2002. Vulvar cancer. *American family physician*, 66(7), p.1269.
4. Grimes, D.A. and Economy, K.E., 1995. Primary prevention of gynecologic cancers. *American journal of obstetrics and gynecology*, 172(1), pp.227-235.
5. Weinberg, D. and Gomez-Martinez, R.A., 2019. Vulvar cancer. *Obstetrics and Gynecology Clinics*, 46(1), pp.125-135.

Rank: 125
Probe ID: 221221_s_at
p-value: 1.79E-05
t: 6.467292
B: 3.23326
Gene: KLHL3
Gene name: kelch-like family member 3

Chart 1: Differential expression of KLHL3 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: 1203
Probe ID: ILMN_1770641
p-value: 8.08E-05
t: 5.0393513
B: 1.4589
Gene: KLHL3
Gene name: kelch-like family member 3

Chart 2: Differential expression of KLHL3 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: KLHL3 is expressed at significantly lower levels in vulvar cancer as compared to normal vulva.

The mRNA expression level of KLHL3 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 KLHL3 expression between the two groups, a *p*-value, listed above.