Differential expression of RRAGD in cancer of the vulva.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

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 Ras-related GTP-binding D, encoded by RRAGD, in cancer of the vulva. RRAGD may be of pertinence to understanding transformation and disease progression in vulvar cancer (3).

Keywords: vulvar cancer, vulvar carcinoma, RRAGD, Ras-related GTP-binding D.

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 RRAGD 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.

RRAGD is differentially expressed in vulvar cancer.

We found that Ras-related GTP-binding D, encoded by RRAGD, 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, RRAGD ranked 117 out of 22277 total transcripts, equating to 99.5% differential expression (Chart 1) (1). Differential expression of RRAGD was statistically significant (Chart 1; $p=1.62E-05$).

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, RRAGD ranked 354 out of 47323 total transcripts, equating to 99.3% differential expression (Chart 2) (2). Differential expression of RRAGD was again statistically significant (Chart 2; $p=1.01E-06$).

RRAGD 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. RRAGD was expressed at significantly lower levels in cancer of the vulva as compared to normal vulva. RRAGD was expressed at 7.78 ± 0.71 arbitrary units (A.U.) in normal vulva, but at 5.73 ± 0.43 A.U. in vulvar cancer tissues (Figure 1). We

1 calculated a mean fold change of 0.74 in RRAGD expression when comparing vulvar carcinoma to vulva.
2

3 **Discussion**

4 We documented here that RRAGD is differentially expressed at the transcriptome level in cancer of
5 the human vulva (1, 2). RRAGD is a candidate gene of interest in understanding the transcriptional
6 biology of vulvar cancer.
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Rank: 117
Probe ID: 221523_s_at
p-value: 1.62E-05
t: 6.530305
B: 3.32978
Gene: RRAGD
Gene name: Ras-related GTP-binding D

Chart 1: Differential expression of RRAGD 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: 354
Probe ID: ILMN_1699772
p-value: 1.01E-06
t: 7.1690247
B: 5.79886
Gene: RRAGD
Gene name: Ras-related GTP-binding D

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

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