Differential expression of RAP2A/RAP2B 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 RAP2A and RAP2B, members of the RAS oncogene family, encoded by RAP2A/RAP2B, in cancer of the vulva. RAP2A/RAP2B may be of pertinence to understanding transformation and disease progression in vulvar cancer (3).

Keywords: vulvar cancer, vulvar carcinoma, RAP2A/RAP2B, RAP2A and RAP2B, members of the RAS oncogene family.

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 GSE5563 (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. GSE5563 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=10$ normal vulvar tissues and $n=9$ VIN (vulvar intraepithelial neoplasia) tissues; analysis was performed using platform GPL570. 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 RAP2A/RAP2B expression was significantly different between normal vulvar tissue and vulvar carcinoma 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.

RAP2A/RAP2B is differentially expressed in vulvar cancer.

We identified a probe that recognized RAP2A and RAP2B, members of the RAS oncogene family, encoded by RAP2A/RAP2B, corresponding to a differentially expressed gene, at the transcriptome level, 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, RAP2A/RAP2B ranked 87 out of 22277 total transcripts, equating to 99.6% differential expression (Chart 1) (1). Differential expression of RAP2A/RAP2B was statistically significant (Chart 1; $p=9.16E-06$).

We validated our findings by performing a similar analysis using a second published microarray dataset (2), but here measuring gene expression in vulvar intraepithelial neoplasia, a dysplastic cell state from which vulvar carcinomas are said to originate from (6). When sorting each transcript whose expression was measured across the transcriptome based on significance of change between the normal vulva and vulvar cancer tissues, RAP2A/RAP2B ranked 5908 out of 54662 total transcripts (Chart 2) (2), equating to 89.2% differential expression. Differential expression of RAP2A/RAP2B was again statistically significant (Chart 2; $p=1.40E-02$).

RAP2A/RAP2B expression is higher in cancer tissue as compared to the vulva.

We obtained exact mRNA expression data from the first microarray dataset we mined to directly

1 compare expression levels between control and cancer tissues. RAP2A/RAP2B was expressed at
2 significantly higher levels in cancer of the vulva as compared to normal vulva. RAP2A/RAP2B was
3 expressed at 6.55 ± 0.17 arbitrary units (A.U.) in normal vulva, but at 7.77 ± 0.41 A.U. in vulvar cancer
4 tissues (Figure 1). We calculated a mean fold change of 1.19 in RAP2A/RAP2B expression when
comparing vulvar carcinoma to vulva.

5 **Discussion**

6 We documented here that RAP2A/RAP2B is differentially expressed at the transcriptome level in
7 cancer and dysplasia of the human vulva (1, 2). RAP2A/RAP2B is a candidate gene of interest in
8 understanding the transcriptional biology of vulvar cancer.
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Rank: 87
Probe ID: 214487_s_at
p-value: 9.16E-06
t: -6.891995
B: 3.87202
Gene: RAP2A/RAP2B
Gene name: RAP2A and RAP2B, members of the RAS oncogene family

Chart 1: Differential expression of RAP2A/RAP2B 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: 5908
Probe ID: 214487_s_at
p-value: 1.40E-02
t: 2.7001712
B: -3.279027
Gene: RAP2A/RAP2B
Gene name: RAP2A and RAP2B, members of the RAS oncogene family

Chart 2: Differential expression of RAP2A/RAP2B in VIN tissues.

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: RAP2A/RAP2B is expressed at significantly higher levels in vulvar cancer as compared to normal vulva.

The mRNA expression level of RAP2A/RAP2B 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 RAP2A/RAP2B expression between the two groups, a *p*-value, listed above.