Differential expression of NRN1 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 neuritin 1, encoded by NRN1, in cancer of the vulva. NRN1 may be of pertinence to understanding transformation and disease progression in vulvar cancer (3).

Keywords: vulvar cancer, vulvar carcinoma, NRN1, neuritin 1.

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 NRN1 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.

NRN1 is differentially expressed in vulvar cancer.

We found that neuritin 1, encoded by NRN1, 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, NRN1 ranked 88 out of 22277 total transcripts, equating to 99.6% differential expression (Chart 1) (1). Differential expression of NRN1 was statistically significant (Chart 1; $p=9.33E-06$).

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, NRN1 ranked 521 out of 54662 total transcripts (Chart 2) (2), equating to 99.0% differential expression. Differential expression of NRN1 was again statistically significant (Chart 2; $p=2.43E-05$).

NRN1 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 compare expression levels between control and cancer tissues. NRN1 was expressed at significantly higher levels in cancer of the vulva as compared to normal vulva. NRN1 was expressed at 7.75 ± 0.48

arbitrary units (A.U.) in normal vulva, but at 5.71 ± 0.65 A.U. in vulvar cancer tissues (Figure 1). We calculated a mean fold change of 0.74 in NRN1 expression when comparing vulvar carcinoma to vulva.

Discussion

We documented here that NRN1 is differentially expressed at the transcriptome level in cancer and dysplasia of the human vulva (1, 2). NRN1 is a candidate gene of interest in understanding the transcriptional biology of vulvar cancer.

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. Santegoets, L.A., Baars, R.V., Terlou, A., Heijmans-Antonissen, C., Swagemakers, S.M., van der Spek, P.J., Ewing, P.C., Beurden, M.V., van der Meijden, W.I., Helmerhorst, T.J. and Blok, L.J., 2012. Different DNA damage and cell cycle checkpoint control in low- and high-risk human papillomavirus infections of the vulva. *International journal of cancer*, 130(12), pp.2874-2885.
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.
6. Del Pino, M., Rodriguez-Carunchio, L. and Ordi, J., 2013. Pathways of vulvar intraepithelial neoplasia and squamous cell carcinoma. *Histopathology*, 62(1), pp.161-175.

Rank: 88
Probe ID: 218625_at
p-value: 9.33E-06
t: 6.879615
B: 3.85379
Gene: NRN1
Gene name: neuritin 1

Chart 1: Differential expression of NRN1 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: 521
Probe ID: 218625_at
p-value: 2.43E-05
t: 5.4970331
B: 2.761564
Gene: NRN1
Gene name: neuritin 1

Chart 2: Differential expression of NRN1 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: NRN1 is expressed at significantly higher levels in vulvar cancer as compared to normal vulva.

The mRNA expression level of NRN1 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 NRN1 expression between the two groups, a *p*-value, listed above.