PEG3 is differentially expressed in high-grade serous ovarian cancers.

Shahan Mamoor¹

¹shahanmamoor@gmail.com

East Islip, NY 11730

Ovarian cancer is the most lethal gynecologic cancer (1-3). We sought to identify genes associated with high-grade serous ovarian cancer (HGSC) by comparing global gene expression profiles of normal ovary with that of primary tumors from women diagnosed with HGSC using published microarray data (4, 5). We identified paternally expressed gene 3 (PEG3) (6) as among the genes whose expression was most different in HGSC ovarian tumors. PEG3 expression was significantly lower in ovarian tumors relative to normal ovary. In one dataset, an anti-sense transcript produced at the PEG3 locus was among those most differentially expressed between HGSC tumors and benign ovarian tissue. These data indicate that significant changes in expression at the PEG3 imprinted locus could be a feature of high-grade serous ovarian cancers.

Keywords: ovarian cancer, high-grade serous ovarian cancer, HGSC, targeted therapeutics in ovarian cancer, systems biology of ovarian cancer, PEG3.

1 The five-year survival rate for women diagnosed with high-grade serous ovarian cancer
2 is between 30-40% and has not changed significantly in decades (2, 3). The development of
3 novel, targeted therapeutics to treat HGSC can be facilitated by an enhanced understanding of
4 the transcriptional behavior of ovarian tumors relative to that of the normal ovary. We mined
5 published microarray data (4, 5) to compare global gene expression profiles between HGSC
6 ovarian tumors and that of normal ovarian tissue. We identified the gene encoding PEG3 as
7 among the most differentially expressed in HGSC tumors of the ovary. PEG3 may be a gene of
8 interest when prioritizing the study of target genes and pathways for the development of novel
9 therapeutic interventions in high-grade serous ovarian cancers.
10

11 12 **Methods**

13 We used microarray data from datasets GSE146556 (4) and GSE124766 (5) for this
14 differential gene expression analysis of high-grade serous carcinomas. The Benjamini and
15 Hochberg method of p-value adjustment was used for ranking of differential expression but raw
16 p-values were used for assessment of statistical significance of global differential expression.
17 Log-transformation of data was auto-detected, and the NCBI generated category of platform
18 annotation was used. GSE146556 (4) was generated using Affymetrix Human Gene 1.0 ST
19 Array with n=3 for normal ovarian tissue and n=40 for tumors from patients with high-grade
20 serous ovarian cancer. GSE124766 (5) was generated using Agilent-014850 Whole Human
21 Genome Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors
22 from patients with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels
23 only for the top 250 most differentially expressed genes.
24

25 A statistical test was performed to evaluate whether PEG3 expression was significantly
26 different when comparing normal ovarian tissue from control subjects and primary tumors from
27 women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's correction. Only
28

p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined published microarray data (4, 5) to identify differentially expressed genes in high-grade serous ovarian cancer (HGSC), the type of ovarian cancer responsible for 70-80% of deaths resulting from the most lethal gynecologic malignancy.

PEG3 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified PEG3 as among the genes whose expression was quantitatively most different when comparing primary HGSC tumors to normal ovarian tissue (Table 1) (5). When sorting all of the transcripts measured by microarray based on change in expression between HGSC tumors and the normal ovary, PEG3 ranked 84 out of 41093 total transcripts (Table 1). Differential expression of PEG3 in HGSC tumors was statistically significant (Table 1; $p=1.52E-06$).

We analyzed a second microarray dataset (4) generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of PEG3 could be observed in a separate group of patients. We again found that a transcript corresponding to the PEG3 gene was differentially expressed in primary HGSC tumors (Table 2). In this case, the transcript corresponded to overlap between the PEG3 and ZIM2 genes, which share 5' exons (7). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, the ZIM2/PEG3 ranked out 57 of 29088 total transcripts (Table 2). Differential expression of ZIM2/PEG3 in HGSC tumors was statistically significant (Table 2; $p=6.51E-10$).

An anti-sense transcript is differentially expressed in HGSC tumors.

We also identified an anti-sense non-coding RNA produced at the PEG3 locus as among the genes most differentially expressed when comparing HGSC tumors to normal ovary (Table 3) (5). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, PEG3-AS1 ranked 146 out of 41093 total transcripts (Table 2). Differential expression of PEG3-AS1 in HGSC tumors was statistically significant (Table 2; $p=6.5E-06$).

PEG3 mRNA and PEG3-AS1 ncRNA are expressed at significantly lower levels in HGSC when compared to the normal ovary.

We then obtained exact mRNA expression levels for the differentially expressed PEG3 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. PEG3 was expressed at significantly lower levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 1; $p=0.0184$). We calculated a mean fold change of 0.7223 ± 0.0351 in PEG3 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

The ZIM2/PEG3 transcript that was found to be differentially expressed in a separate group in HGSC was also expressed at significantly lower levels in primary tumors from women diagnosed with HGSC when compared to normal ovarian tissue (Figure 2; $p=0.0302$). We calculated a mean fold change of 0.0641 ± 0.0214 in ZIM2/PEG3 expression when comparing HGSC tumors to normal ovarian tissue (Table 2). PEG3-AS1 was also found to be expressed at significantly lower levels in HGSC primary tumors when compared to the normal ovary (Figure 3; $p=0.0015$). We calculated a mean fold change of 0.6412 ± 0.0766 in PEG3-AS1 expression when comparing HGSC tumors to normal ovarian tissue (Table 3).

Thus, we found using published microarray data that PEG3 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that PEG3 expression was significantly lower in HGSC tumors when compared to the normal ovary.

Discussion

Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic analysis classified HGSC into four subtypes based on gene clustering: immunoreactive, differentiated, proliferative, and mesenchymal (7). We sought to continue to describe the transcriptional landscape of high-grade serous ovarian cancers (7) and identify genes whose differential expression was associated with HGSC by using published microarray data from primary tumors of women diagnosed with HGSC compared to transcriptome data from normal ovarian tissue (4, 5). In both datasets analyzed, PEG3 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

The PEG3 gene is imprinted, paternally expressed (6), and part of a broader locus controlled by an imprinting control region (9). Mutation of PEG3 in mice leads to death of offspring resulting from a deficits in maternal behavior, demonstrating that PEG3 plays a role in nurturing (10). An anti-sense transcript produced at the neighboring ZIM2 locus is primate-specific and occupies a homologous region in mammals containing olfactory receptor genes (11).

Dysregulated expression of PEG3 in cancers has been established (13-15). One study that performed microarray analyses of 40 tumors from women with varying types of ovarian cancers showed that 75% of cancers examined down-regulated PEG3 and that PEG3 was among the most frequently down-regulated genes in ovarian cancers (13). PEG3 expression was found to be lost in 5 out of 8 ovarian cancer cell lines examined in another study, with relatively low expression of PEG3 in the 3 other ovarian cancer cell lines (15). The same study found that all cervical and endometrial cancer cell lines examined had significantly decreased or

1 ablated expression of PEG3. In glioma, PEG3 was reported to act as an inhibitor Wnt signaling
2 by binding to beta-catenin and targeting it for degradation (14). In primary gliomas, the PEG3
3 promoter was hypermethylated and this corresponded to decreased PEG3 expression, which
4 correlated with the grade of the tumor. In CD133+ glioma stem cells, decreased PEG3 levels
5 lead to increased beta-catenin levels and enhanced cell proliferation (14). A study using a model
6 of apoptosis driven by both p53 and cMyc showed that PEG3 exerts an effect on cell
7 proliferation and cell death through the p53 pathway, interacting with the p53-inducible gene
8 Siah1, an E3 ubiquitin ligase (16).
9

10
11 Differential expression of PEG3 in HGSC should be validated in larger and separate
12 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
13 cancers (17) can be utilized to assess the effect of conditional expression of PEG3 in HGSC
14 tumor cell proliferation and cancer progression *in vivo*. We found here that coding and non-
15 coding transcripts produced at the paternally expressed, imprinted xsPEG3 locus are among
16 those whose expression changes most significantly in high-grade serous ovarian cancers.
17
18
19
20
21
22
23
24
25
26
27
28

References

1. Guppy, A.E., Nathan, P.D. and Rustin, G.J., 2005. Epithelial ovarian cancer: a review of current management. *Clinical Oncology*, 17(6), pp.399-411.
2. Bowtell, D.D., Böhm, S., Ahmed, A.A., Aspuria, P.J., Bast Jr, R.C., Beral, V., Berek, J.S., Birrer, M.J., Blagden, S., Bookman, M.A. and Brenton, J.D., 2015. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. *Nature reviews Cancer*, 15(11), pp.668-679.
3. Vaughan, S., Coward, J.I., Bast, R.C., Berchuck, A., Berek, J.S., Brenton, J.D., Coukos, G., Crum, C.C., Drapkin, R., Etemadmoghadam, D. and Friedlander, M., 2011. Rethinking ovarian cancer: recommendations for improving outcomes. *Nature Reviews Cancer*, 11(10), pp.719-725.
4. Zhang, W., Klinkebiel, D., Barger, C.J., Pandey, S., Guda, C., Miller, A., Akers, S.N., Odunsi, K. and Karpf, A.R., 2020. Global DNA hypomethylation in epithelial ovarian cancer: passive demethylation and association with genomic instability. *Cancers*, 12(3), p.764.
5. Hoffmann, K., Berger, H., Kulbe, H., Thillainadarasan, S., Mollenkopf, H.J., Zemojtel, T., Taube, E., Darb-Esfahani, S., Mangler, M., Sehouli, J. and Chekerov, R., 2020. Stable expansion of high-grade serous ovarian cancer organoids requires a low-Wnt environment. *The EMBO journal*, 39(6), p.e104013.
6. Kim, J., Ashworth, L., Branscomb, E. and Stubbs, L., 1997. The human homolog of a mouse-imprinted gene, Peg3, maps to a zinc finger gene-rich region of human chromosome 19q13. 4. *Genome research*, 7(5), pp.532-540.
7. Kim, J., Bergmann, A. and Stubbs, L., 2000. Exon sharing of a novel human zinc-finger gene, ZIM2, and paternally expressed gene 3 (PEG3). *Genomics*, 64(1), pp.114-118.
8. Cancer Genome Atlas Research Network, 2011. Integrated genomic analyses of ovarian carcinoma. *Nature*, 474(7353), p.609.
9. Kim, J., Ekram, M.B., Kim, H., Faisal, M., Frey, W.D., Huang, J.M., Tran, K., Kim, M.M. and Yu, S., 2012. Imprinting control region (ICR) of the Peg3 domain. *Human molecular genetics*, 21(12), pp.2677-2687.
10. Murphy, S.K., Wylie, A.A. and Jirtle, R.L., 2001. Imprinting of PEG3, the human homologue of a mouse gene involved in nurturing behavior. *Genomics*, 71(1), pp.110-117.
11. Huang, J.M., Yu, S. and Kim, J., 2009. Identification of an antisense transcript to ZIM2 in the primate lineage. *Gene*, 445(1-2), pp.1-6.

12. Li, L.L., Keverne, E.B., Aparicio, S.A., Ishino, F., Barton, S.C. and Surani, M.A., 1999. Regulation of maternal behavior and offspring growth by paternally expressed Peg3. *Science*, 284(5412), pp.330-334.
13. Feng, W., Marquez, R.T., Lu, Z., Liu, J., Lu, K.H., Issa, J.P.J., Fishman, D.M., Yu, Y. and Bast Jr, R.C., 2008. Imprinted tumor suppressor genes ARHI and PEG3 are the most frequently down-regulated in human ovarian cancers by loss of heterozygosity and promoter methylation. *Cancer*, 112(7), pp.1489-1502.
14. Jiang, X., Yu, Y., Yang, H.W., Agar, N.Y., Frado, L. and Johnson, M.D., 2010. The imprinted gene PEG3 inhibits Wnt signaling and regulates glioma growth. *Journal of Biological Chemistry*, 285(11), pp.8472-8480.
15. Dowdy, S.C., Gostout, B.S., Shridhar, V., Wu, X., Smith, D.I., Podratz, K.C. and Jiang, S.W., 2005. Biallelic methylation and silencing of paternally expressed gene 3 (PEG3) in gynecologic cancer cell lines. *Gynecologic oncology*, 99(1), pp.126-134.
16. Relaix, F., Wei, X.J., Li, W., Pan, J., Lin, Y., Bowtell, D.D., Sassoon, D.A. and Wu, X., 2000. Pw1/Peg3 is a potential cell death mediator and cooperates with Siah1a in p53-mediated apoptosis. *Proceedings of the National Academy of Sciences*, 97(5), pp.2105-2110.
17. Kim, J., Coffey, D.M., Creighton, C.J., Yu, Z., Hawkins, S.M. and Matzuk, M.M., 2012. High-grade serous ovarian cancer arises from fallopian tube in a mouse model. *Proceedings of the National Academy of Sciences*, 109(10), pp.3921-3926.

Rank	ID	p-value	t	B	Fold change	Gene	Gene name
84	A_23_P407583	1.52E-06	-8.8552692	5.66669	0.7223 ± 0.0351	PEG3	paternally expressed 3

Table 1: PEG3 is differentially expressed in high-grade serous ovarian carcinomas.

The rank of differential expression, the probe/transcript ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change of PEG3 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Fold change	Gene	Gene name
57	8039607	6.51E-10	-7.8385444	12.5416055	0.0641 ± 0.0214	ZIM2///PEG3	zinc finger imprinted 2/// paternally expressed 3

Table 2: A transcript encoded by sequences shared by the PEG3 and ZIM2 gene is among the most differentially expressed in tumors from patients with high-grade serous ovarian carcinomas.

The rank of differential expression, the probe/transcript ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change of ZIM2//PEG3 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Fold change	Gene	Gene name
146	A_32_P119830	6.5E-06	-7.6727651	4.28272	0.6412 ± 0.0766	PEG3-AS1	PEG3 antisense RNA 1

Table 3: PEG3-AS1, an antisense transcript encoded by the PEG3 gene, is differentially expressed in high-grade serous ovarian carcinomas.

The rank of differential expression, the probe/transcript ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change of PEG3 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Figure 1: PEG3 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of PEG3 in normal ovarian tissues from control subjects (left) and in the primary tumors of patients with HGSC (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in PEG3 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.

Figure 2: ZIM2//PEG3 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of PEG3 in normal ovarian tissues from control subjects (left) and in the primary tumors of patients with HGSC (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in ZIM2//PEG3 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.

Figure 3: PEG3 antisense transcript (PEG3-AS1) is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of PEG3 in normal ovarian tissues from control subjects (left) and in the primary tumors of patients with HGSC (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in PEG3-AS1 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.