The CCHC-type zinc finger ZCCHC18 is differentially expressed in high-grade serous ovarian cancers.

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Ovarian cancer is the most lethal gynecologic cancer (1). 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 (2, 3). We identified the zinc finger CCHC-type containing 18 (ZCCHC18) as among the genes whose expression was most different in HGSC ovarian tumors. ZCCHC18 expression was significantly lower in ovarian tumors relative to normal ovary. These data indicate that significant loss of ZCCHC18 expression 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.

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 (4, 5). 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 (2, 3) to compare global gene expression profiles between HGSC
6 ovarian tumors and that of normal ovarian tissue. We identified the gene encoding ZCCHC18
7 as among the most differentially expressed in HGSC tumors of the ovary. ZCCHC18 may be a
8 gene of interest when prioritizing the study of target genes and pathways for the development of
9 novel therapeutic interventions in high-grade serous ovarian cancers.
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11 12 **Methods**

13 We used microarray data from datasets GSE146556 (2) and GSE124766 (3) 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 (2) 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 (3) 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.
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25 A statistical test was performed to evaluate whether ZCCHC18 expression was
26 significantly different when comparing normal ovarian tissue from control subjects and primary
27 tumors from women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's
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correction. Only 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 (2, 3) 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.

ZCCHC18 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified ZCCHC18 as among the genes whose expression was quantitatively most different when comparing primary HGSC tumors to normal ovarian tissue (Table 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC tumors and the normal ovary, ZCCHC18 ranked 58 out of 29088 total transcripts (Table 1). Differential expression of ZCCHC18 in HGSC tumors was statistically significant (Table 1; $p=6.6E-10$).

We analyzed a second microarray dataset generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of ZCCHC18 could be observed in a separate group of patients. We again found that ZCCHC18 was differentially expressed in primary HGSC tumors (Table 2). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, in this data set, ZCCHC18 ranked 6 out of 41093 total transcripts (Table 2). Differential expression of ZCCHC18 in HGSC tumors was statistically significant (Table 2; $1.56E-09$).

ZCCHC18 is expressed at significantly lower levels in HGSC when compared to the normal ovary.

We then obtained exact mRNA expression levels for the differentially expressed ZCCHC18 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. ZCCHC18 was expressed at significantly lower levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 1; $p=0.0185$). We calculated a mean fold change of $.34076 \pm 0.08772$ in ZCCHC18 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

In the second dataset analyzed, we again found that ZCCHC18 was expressed at significantly lower levels in primary tumors from women diagnosed with high-grade serous ovarian cancer when compared to normal ovarian tissue (Figure 2; $p=0.0072$). We calculated a mean fold change of 0.50091 ± 0.02653 in ZCCHC18 expression when comparing HGSC tumors to normal ovarian tissue (Table 2).

Thus, we found using published microarray data that ZCCHC18 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that ZCCHC18 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 (6). We sought to continue to describe the transcriptional landscape of high-grade serous ovarian cancers (6) 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 (2, 3). In both datasets analyzed (2, 3), ZCCHC18 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

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2 ZCCHC-containing proteins possess 18 amino acid long zinc knuckle-type zinc fingers
3 that are characterized by the motif CX₂CX₄HX₄C (7). ZCCHC18, also known as PNMA7B, is a
4 member of one of five families of “domesticated” genes that are derived from Metaviridae long
5 terminal repeat (LTR) retrotransposons (8). PNMA, or paraneoplastic gene family evolved from
6 the Metaviridae Gag gene, which encodes the viral matrix, capsid, and nucleocapsid (8). A study
7 that analyzed tissue expression patterns of the “ignorome”, or set of genes that has no ascribed
8 function, showed that ZCCHC18 has pan-neuronal expression in C57BL/6J mice (9). Homo
9 sapiens ZCCHC18 is 403 amino acids long and shares 92% amino acid identity with
10 chimpanzee ZCCHC18 and 68% amino acid identity with mouse ZCCHC18 (10). The
11 ZCCHC18 gene is encoded by the X chromosome and is composed of two exons and one
12 intron (10). ZCCHC18/PNMA7B shares with the rest of the PNMA family a central conserved
13 domain (CCD) and, except for PNMA8B and PNMA8C, a basic region rich in lysine and arginine
14 residues (10). PNMA7B also possesses a nuclear localization sequence and a carboxy-
15 terminal zinc finger (10). PNMA genes were in part identified based on the presence of neuronal
16 auto antigens using sera from patients with paraneoplastic neurological syndromes (11). There
17 is little published data describing a role for PNMA7B in the ovary or in ovarian cancer.
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21 Differential expression of ZCCHC18 in HGSC should be validated in larger and
22 separate cohorts of women with HGSC. If validated, mouse models of high-grade serous
23 ovarian cancers (12) can be utilized to assess the effect of conditional expression of ZCCHC18
24 in HGSC tumor cell proliferation and cancer progression *in vivo*.
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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. 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.
5. 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.
6. Cancer Genome Atlas Research Network, 2011. Integrated genomic analyses of ovarian carcinoma. *Nature*, 474(7353), p.609.
7. Aceituno-Valenzuela, U., Micol-Ponce, R. and Ponce, M.R., 2020. Genome-wide analysis of CCHC-type zinc finger (ZCCHC) proteins in yeast, Arabidopsis, and humans. *Cellular and Molecular Life Sciences*, pp.1-24.
8. Kordiš, D., 2017. The life history of domesticated genes illuminates the evolution of novel mammalian genes. In *Evolutionary Biology: Self/Nonself Evolution, Species and Complex Traits Evolution, Methods and Concepts* (pp. 147-162). Springer, Cham.
9. Pandey, A.K., Lu, L., Wang, X., Homayouni, R. and Williams, R.W., 2014. Functionally enigmatic genes: a case study of the brain ignorome. *PloS one*, 9(2).
10. Pang, S.W., Lahiri, C., Poh, C.L. and Tan, K.O., 2018. PNMA family: Protein interaction network and cell signalling pathways implicated in cancer and apoptosis. *Cellular signalling*, 45, pp.54-62.

11. Schüller, M., Jenne, D. and Voltz, R., 2005. The human PNMA family: novel neuronal proteins implicated in paraneoplastic neurological disease. *Journal of neuroimmunology*, 169(1-2), pp.172-176.
12. 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
58	8169083	6.6E-10	-7.8343807	12.5282798	.34076 ± 0.08772	ZCCHC18	zinc finger CCHC-type containing 18

Table 1: ZCCHC18 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 ZCCHC18 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
6	A_32_P116989	1.56E-09	-16.6410665	11.5448	0.50091 ± 0.02653	ZCCHC18	zinc finger CCHC-type containing 18

Table 2: ZCCHC18 is among the most differentially expressed gene in a tumor dataset 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 ZCCHC18 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Figure 1: ZCCHC18 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of ZCCHC18 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 ZCCHC18 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.

Figure 2: ZCCHC18 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of ZCCHC18 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 ZCCHC18 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.