Carboxypeptidase Z is differentially expressed in high-grade serous ovarian cancers.

Shahan Mamoor¹

¹shahanmamoor@gmail.com

East Islip, NY 11730

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 (1, 2). We identified carboxypeptidase Z (CPZ) (3) as among the genes whose expression was most different in HGSC ovarian tumors. CPZ expression was significantly lower in ovarian tumors relative to normal ovary. CPZ may be relevant to the biology 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 (1, 2) to compare global gene expression profiles between HGSC
6 ovarian tumors and that of normal ovarian tissue. We identified the gene encoding the
7 carboxypeptidase Z as among the most differentially expressed in HGSC tumors of the ovary.
8 CPZ may be a gene of interest when prioritizing the study of target genes and pathways for the
9 development of novel therapeutic interventions in high-grade serous ovarian cancers.
10

11 12 **Methods**

13 We used microarray data from datasets GSE146556 (1) and GSE124766 (2) 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 (1) 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 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 CPZ 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 (1, 2) to identify differentially expressed genes in high-grade serous ovarian cancer (HGSC).

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

We identified carboxypeptidase Z, CPZ, as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue. When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, CPZ ranked 59 out of 29088 total transcripts. Differential expression of CPZ in HGSC tumors was statistically significant (Table 1; $p=7.39E-10$).

By mining a second microarray dataset generated using normal ovarian tissues and tumors from women diagnosed with HGSC, we again found that CPZ was differentially expressed in primary HGSC tumors. When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, in this data set, CPZ ranked 2907 out of 41093 total transcripts. The change in expression of CPZ in primary tumors from women diagnosed with HGSC as compared to the normal ovary was greater than 93% of the measured HGSC transcriptome. Differential expression of CPZ in HGSC tumors was statistically significant (Table 2; $p=4.31E-03$).

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

We then extracted exact mRNA expression levels for the differentially expressed CPZ transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. CPZ was expressed at significantly lower levels in high-grade serous ovarian cancers when

1 compared to the normal ovary (Figure 1; $p < 0.0001$). We calculated a mean fold change of
2 0.02802 ± 0.08991 in CPZ expression when comparing HGSC tumors to normal ovarian tissue
3 (Table 1).

4
5 Thus, we found using published microarray data that carboxypeptidase Z (CPZ) was
6 among the genes most differentially expressed in tumors from women with high-grade serous
7 carcinomas and that CPZ expression was significantly lower in HGSC tumors when compared
8 to the normal ovary.
9

10 11 Discussion

12 A Cancer Genome Atlas integrated genomic analysis of ovarian cancers found that
13 nearly all ovarian cancers displayed mutation in the p53 gene, with nine other genes displaying
14 less frequent but recurrent mutation in ovarian cancers; these genes include NF1, BRCA1,
15 BRCA2, RB1 and CDK12 (6). Transcriptional profiling of HGSC in this same integrated
16 genomic analysis classified HGSC into four subtypes based on gene clustering:
17 immunoreactive, differentiated, proliferative, and mesenchymal (6). We sought to continue to
18 describe the transcriptional landscape of high-grade serous ovarian cancers (6) and identify
19 genes whose differential expression was associated with HGSC by using published microarray
20 data from primary tumors of women diagnosed with HGSC compared to transcriptome data
21 from normal ovarian tissue (1, 2). In both datasets analyzed (1, 2), carboxypeptidase Z (CPZ)
22 was among the genes whose expression changed most significantly when comparing the
23 normal ovary to primary HGSC tumors.
24

25
26 The general function of carboxypeptidases is the proteolytic digestion of substrate
27 proteins at the carboxy-terminus; for example, Carboxypeptidase A1, A2 and B are pancreatic
28 enzymes involved in the proteolysis of ingested goods (7). Carboxypeptidase Z removes
carboxy-terminal arginine residues from substrate proteins using a metallopeptidase domain

common to the carboxypeptidases (7). CPZ, unlike other carboxypeptidases, contains an amino-terminal cysteine-rich Frizzled (Fz) domain characteristic of molecules that interact with Wnt proteins (7). CPZ is expressed in the placenta and in embryonic tissues. CPZ can be found in the extracellular milieu of human placental invasive trophoblasts and in the extracellular matrix of multiple cancer cell lines including PC12 and AtT-20 (8). Physical interaction between heparin and CPZ has been demonstrated (8). In chicken, CPZ is a modulator of Wnt signaling in the skeletal system, consistent with the presence of a Frizzled domain at its amino-terminus (9). *In vitro*, CPZ could support Wnt-dependent homeobox Cdx1 gene expression, and the cysteine-rich domain of CPZ bound Wnt4 (9). A study investigating CPZ expression during murine development found that the peak of CPZ expression during embryonic development was between embryonic days E9 and E12 (10). The expression pattern of CPZ displayed overlap with that of Wnt pathway components, suggesting a role for CPZ in Wnt pathway signaling (10). CPZ expression was also found in cochlear epithelial cells, in amnion cells, in the ventricular lining cells of the brain and in connective tissues that surround the ribs (10).

We could not identify substantial published information describing a relationship between CPZ expression and high-grade serous ovarian cancers. CPZ differential expression should be validated in larger and separate cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian cancers (11) can be utilized to assess the effect of enforced expression of CPZ in HGSC tumors on tumor cell proliferation and cancer progression *in vivo*.

References

1. 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.
2. 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.
3. Song, L. and Fricker, L.D., 1997. Cloning and expression of human carboxypeptidase Z, a novel metallocarboxypeptidase. *Journal of Biological Chemistry*, 272(16), pp.10543-10550.
4. 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.
5. 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.
6. Cancer Genome Atlas Research Network, 2011. Integrated genomic analyses of ovarian carcinoma. *Nature*, 474(7353), p.609.
7. Reznik, S.E. and Fricker, L.D., 2001. Carboxypeptidases from A to Z: implications in embryonic development and Wnt binding. *Cellular and Molecular Life Sciences CMLS*, 58(12-13), pp.1790-1804.
8. Novikova, E.G., Reznik, S.E., Varlamov, O. and Fricker, L.D., 2000. Carboxypeptidase Z is present in the regulated secretory pathway and extracellular matrix in cultured cells and in human tissues. *Journal of Biological Chemistry*, 275(7), pp.4865-4870.
9. Moeller, C., Swindell, E.C., Kispert, A. and Eichele, G., 2003. Carboxypeptidase Z (CPZ) modulates Wnt signaling and regulates the development of skeletal elements in the chicken. *Development*, 130(21), pp.5103-5111.
10. Novikova, E., Fricker, L.D. and Reznik, S.E., 2001. Metallocarboxypeptidase Z is dynamically expressed in mouse development. *Mechanisms of development*, 102(1-2), pp. 259-262.
11. 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 title
59	8094101	7.39E-10	-7.800621	12.4201768	0.02802 ± 0.08991	CPZ	carboxypeptidase Z

Table 1: Carboxypeptidase Z 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 CPZ 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	Gene	Gene name
2907	A_23_P58251	4.31E-03	3.5254948	-2.11869	CPZ	carboxypeptidase Z

Table 2: Carboxypeptidase Z 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 CPZ in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

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

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