Prepronociceptin is differentially expressed in epithelial ovarian cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY 11730

Epithelial ovarian cancer (EOC) is the most lethal gynecologic cancer¹. We sought to identify genes associated with epithelial ovarian cancer and the high-grade serous ovarian cancer (HGSC) subtype by comparing global gene expression profiles of normal ovary with that of primary tumors from women diagnosed with epithelial cancer and HGSC in specific using published microarray data^{2,3}. We identified the gene encoding prepronociceptin, PNOC, as among the genes whose expression was most different in epithelial ovarian cancer and in HGSC ovarian tumors. PNOC expression was significantly higher in high-grade serous ovarian tumors relative to normal ovary. PNOC expression correlated with overall survival in patients with ovarian cancer. These data indicate that expression of PNOC is perturbed in epithelial ovarian cancers broadly and in ovarian cancers of the HGSC subtype. PNOC may be relevant to pathways underlying ovarian cancer progression.

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

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 novel,
3 targeted therapeutics to treat HGSC can be facilitated by an enhanced understanding of the
4 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
7 prepronociceptin, PNOC, as among the most differentially expressed in HGSC tumors of the
8 ovary and in epithelial ovarian cancer broadly. PNOC may be a gene of interest when
9 prioritizing the study of target genes and pathways for the development of novel therapeutic
10 interventions in epithelial ovarian cancer and specifically in high-grade serous ovarian cancers.

11 **Methods**

12 We used microarray data from datasets and GSE124766² and GSE146556³ for this
13 differential gene expression analysis of epithelial ovarian cancer. The Benjamini and Hochberg
14 method of *p*-value adjustment was used for ranking of differential expression but raw *p*-values
15 were used for assessment of statistical significance of global differential expression. Log-
16 transformation of data was auto-detected, and the NCBI generated category of platform
17 annotation was used. GSE124766² was generated using Agilent-014850 Whole Human
18 Genome Microarray 4x44K G4112F with *n*=3 of for normal ovarian tissue and *n*=8 for tumors
19 from patients with high-grade serous ovarian cancer. GSE146556³ was generated using
20 Affymetrix Human Gene 1.0 ST Array with *n*=3 for normal ovarian tissue and *n*=40 for tumors
21 from patients with epithelial ovarian cancer: *n*=26 high-grade serous ovarian cancer; *n*=3
22 carinosarcoma, *n*=2 clear cell, *n*=1 endometrioid, *n*=1 granulosa cell, *n*=1 immature teratoma,
23 *n*=4 mixed, and *n*=2 mucinous. GEO2R provides mRNA expression levels only for the top 250
24 most differentially expressed genes.

25 A statistical test was performed to evaluate whether PNOC 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). For Kaplan-Meier survival analysis, we used the
Kaplan-Meier plotter online tool⁶ for correlation of PNOC mRNA expression levels with overall
survival (OS) in *n*=1656 ovarian cancer patients.

Results

We mined published microarray data^{2,3} to identify differentially expressed genes in epithelial ovarian cancer and specifically 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.

PNOC is differentially expressed in ovarian tumors from women diagnosed with HGSC

We identified PNOC as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, in this data set, PNOC ranked 111 out of 41093 total transcripts (Table 1), equating to 99.7% differential expression. Differential expression of PNOC in HGSC tumors was statistically significant (Table 1; $p=2.76E-06$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with epithelial ovarian cancer (EOC) to determine whether differential expression of PNOC could be observed in tumors from a different group of patients, which included HGSC tumors. We found that PNOC was differentially expressed in epithelial ovarian cancers broadly (Table 2). When sorting all of the transcripts measured by microarray based on change in expression between tumors from patients with epithelial ovarian cancers and the normal ovary, PNOC ranked 7163 out of 29088 total transcripts (Table 2), equating to 75.4% differential expression. Differential expression of PNOC in EOC tumors was statistically significant (Table 2; $p=4.21E-02$).

PNOC is expressed at significantly higher levels in HGSC when compared to the normal ovary.

We then extracted exact mRNA expression levels for the differentially expressed PNOC transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. PNOC was expressed at significantly higher levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 1; $p<0.0001$). We calculated a mean fold change of 2.079 ± 0.239 in PNOC expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

PNOC tumor expression is significantly correlated with survival outcomes in patients with ovarian cancer.

We performed Kaplan Meier survival analysis⁶ to evaluate whether PNOC tumor expression correlated with survival outcomes in patients with ovarian cancer. PNOC tumor expression was significantly correlated with overall survival (OS) in patients with ovarian cancer (Figure 2). High PNOC expression was a negative prognostic indicator in patients with ovarian cancer. While median OS was 47 months for ovarian cancer patients with low tumor expression of PNOC, median OS was 39.93 months for ovarian cancer patients with high tumor expression of PNOC (Table 3). Correlation of PFS with PNOC tumor expression in patients with ovarian cancer was statistically significant (Figure 2: log rank p -value: $4e-04$; hazard ratio: 1.29 (1.12 - 1.49)).

Thus, we found using published microarray data^{2,3} that PNOC was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas, and in epithelial ovarian cancer broadly. PNOC expression was significantly higher in HGSC tumors when compared to the normal ovary, and PNOC tumor expression was significantly correlated with overall survival in patients with ovarian cancer.

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 mesenchymal⁷. We sought to continue to describe the transcriptional landscape of epithelial ovarian cancers as well as ovarian cancers of the high-grade serous type, identifying genes whose differential expression was associated with EOC and 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}, PNOC was among the genes whose expression changed most significantly when comparing the normal ovary to ovarian tumors, from women with epithelial ovarian cancer as well as specifically from women with HGSC.

Prepronociceptin is the precursor of the neuropeptide nociceptin, also known as orphanin FQ, a ligand of the opioid receptor-like receptor (ORL1)⁸. Prepronociceptin displays similarity to the endogenous opioid peptide precursors preproenkephalin, preprodynorphin, and preproopiomelanocortin⁸. Prepronociceptin is expressed in the brain, the spinal cord, and in the ovary⁸. The regulatory elements upstream of the prepronociceptin open reading frame contain binding sites for TF-IID, the glucocorticoid receptor, the estrogen receptor, and the cyclic AMP (cAMP) response element binding protein (CREB)⁹. Accordingly, increase in cAMP resulting from forskolin stimulation results in activation of prepronociceptin transcription⁹. While estrogen stimulation induces prepronociceptin transcription, glucocorticoid stimulation

represses prepronociceptin transcription⁹. Transcription of prepronociceptin appears to be regulated by histone acetylation as trichostatin A, an inhibitor of histone deacetylases, results in increased CREB occupancy at the PNOC promoter and increased levels of PNOC mRNA¹⁰. In rat hippocampal progenitor cells (AH94), induction of differentiation with defined components including dbcAMP, retinoic acid or serum concomitantly results in induction of PNOC mRNA, suggesting a link between cellular differentiation and PNOC transcription in cells of the neural lineage¹⁰. Neurons in the central amygdala that expression prepronociceptin are important for feeding behaviors in mice: specifically, the consumption of palatable foods - PNOC expression neurons in the central amygdala are activated upon consumption of palatable foods, and these neurons project to areas of the brain including the stria terminalis, parabrachial nucleus, and nucleus of the solitary tract to promote reward¹¹. Other neurons that express prepronociceptin include those of the bed nucleus of the stria terminalis, which can modulate arousal, such as changes in size of pupils, in response to stimulus associated with motivational salience such as rewarding odors¹². We could not identify literature describing differential gene expression of PNOC, up-regulation of PNOC mRNA in HGSC tumors, and correlation of PNOC expression with survival outcomes in patients with ovarian cancer, but one study described over-expression of prepronociceptin in pediatric patients with brainstem gangliogliomas (GGs)¹³. Microarray analysis of pediatric brainstem gangliogliomas as compared to non-brainstem gangliogliomas revealed 256-fold up-regulation of PNOC in pediatric brainstem GGs, the single most up-regulated gene. Expression of PNOC in pediatric brainstem GGs at the protein level was confirmed by immunohistochemistry¹³.

We found that prepronociceptin, encoded by PNOC, was among the genes whose expression was most significantly different transcriptome-wide when comparing primary tumors from women with epithelial ovarian cancer and high-grade serous ovarian cancer with the normal ovary. PNOC was expressed at higher levels in HGSC tumors as compared the normal ovary, and high tumor expression of PNOC in patients with ovarian cancer was associated with inferior overall survival. Differential expression of PNOC in EOC and HGSC should be validated in larger and separate cohorts of women. If validated, mouse models of high-grade serous ovarian cancers¹⁴ can be utilized to assess the effect of conditional deletion of PNOC in HGSC tumors post-development and on tumor cell proliferation and cancer progression *in vivo*.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
111	A_23_P253321	2.76E-06	8.353288	5.1008	2.079 ± 0.239	PNOC	prepronociceptin

Table 1: PNOC is differentially expressed in high-grade serous ovarian carcinomas (HGSC).

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 PNOC 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
7163	8145603	4.21E-02	2.0929514	-4.4455969	PNOC	prepronociceptin

Table 2: PNOC is differentially expressed in epithelial ovarian cancers, including high-grade serous ovarian carcinomas (HGSC).

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 gene and gene name are listed in this chart.

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

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

Figure 2: PNOC tumor expression correlates with overall survival in patients with ovarian cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=1656$ patients. The log rank p -value denoting statistical significance of difference in overall survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on PNOC expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Low expression cohort (months)	High expression cohort (months)
47	39.93

Table 3: Overall survival is greater in ovarian cancer patients with low tumor expression of PNOc.

The median overall survival of $n=1656$ ovarian cancer patients based on stratification into low or high tumor expression of PNOc is listed in this chart, for patients in the upper survival quartile.