Sense and anti-sense transcripts at the PGM5 locus are differentially expressed in high-grade serous ovarian cancers.

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Ovarian cancer is the most lethal gynecologic cancer¹. 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}. Here, we report significant differential sense and anti-sense transcription at the PGM locus in high-grade serous ovarian tumors.

Keywords: ovarian cancer, high-grade serous ovarian cancer, HGSC, targeted therapeutics in ovarian cancer, systems biology of ovarian cancer, PGM5, PGM5-AS1.

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^{5,6}. 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 ovarian
6 tumors and that of normal ovarian tissue. We identified coding and non-coding transcripts
7 transcribed at the PGM5 locus as among the most differentially expressed in HGSC tumors of
8 the ovary. PGM5 may be a gene of interest when prioritizing the study of target genes and
9 pathways for the development of novel therapeutic interventions in high-grade serous ovarian
10 cancers.
11

12 **Methods**

13 We used microarray data from datasets GSE146556² and GSE124766³ for this
14 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
15 and Hochberg method of p-value adjustment was used for ranking of differential expression but
16 raw p-values were used for assessment of statistical significance of global differential
17 expression. Log-transformation of data was auto-detected, and the NCBI generated category of
18 platform annotation was used. GSE124766³ was generated using Agilent-014850 Whole
19 Human Genome Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for
20 tumors from patients with high-grade serous ovarian cancer. GSE146556² was generated using
21 Affymetrix Human Gene 1.0 ST Array with n=3 for normal ovarian tissue and n=40 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 PGM5 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
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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.

PGM5 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified PGM5 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, PGM5 ranked 23 out of 41093 total transcripts (Table 1). Differential expression of PGM5 in HGSC tumors was statistically significant (Table 1; $p=3.76E-08$). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, a second PGM5 transcript ranked 24 out of 41093 total transcripts (Table 1). Differential expression of this second PGM5 transcript in HGSC tumors was statistically significant (Table 1; $p=4.49E-08$). Finally, we observed differential expression of a third PGM5 transcript in HGSC tumors (Table 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, a third PGM5 transcript ranked 85 out of 41093 total transcripts (Table 1). Differential expression of this third PGM5 transcript in HGSC tumors was statistically significant (Table 1; $p=1.53E-06$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of PGM5 could be observed in tumors from a separate group of patients. We again found that PGM5 was differentially expressed in primary HGSC tumors (Table 3), detecting three separate transcripts produced at the PGM5 locus; only one of these transcripts was unambiguously a coding transcript. When sorting all of the transcripts measured by microarray based on change

in expression between HGSC and the normal ovary, in this data set, PGM5 mRNA ranked 6254 out of 29088 total transcripts (Table 3). Differential expression of PGM5 in HGSC tumors in this second microarray dataset was statistically significant (Table 3; $p=2.88E-02$). As indicated, we also detected two differentially expressed PGM5 transcripts which could correspond to either coding or non-coding PGM5 pseudogene transcripts (Table 3). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, in this data set, PGM5P2/PGM5 ranked 910 and 1798 out of 29088 total transcripts (Table 3). Differential expression of both of these PGM5P2/PGM5 transcripts in this second microarray dataset was statistically significant (Table 3; $p=6.46E-05$ and $p=6.66E-04$, respectively).

PGM5 is expressed at significantly lower levels in HGSC when compared to the normal ovary.

We obtained exact mRNA expression levels for the differentially expressed PGM5 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. PGM5 was expressed at significantly lower levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 1A; $p=0.0005$); this was the case for the second (Figure 1B; $p<0.0001$) and the third (Figure 1C; $p=0.0115$) differentially expressed PGM5 transcript. We calculated a mean fold change of 0.5502 ± 0.0560 in PGM5 expression when comparing HGSC tumors to normal ovarian tissue (Table 1) for the most differentially expressed PGM5 transcript. For the second differentially expressed PGM5 transcript, we calculated a mean fold change of 0.6005 ± 0.0528 in PGM expression when comparing HGSC tumors to normal ovarian tissue (Table 1). Finally, for the third differentially expressed PGM5 transcript, we calculated a mean fold change of 0.6144 ± 0.0591 in PGM5 expression when comparing GSC tumors to normal ovarian tissue (Table 1).

PGM5-AS1 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

During our analysis we also observed differential expression of an anti-sense transcript at the PGM locus, PGM5-AS1, 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, PGM5-AS1 ranked 91 out of 41093 total transcripts (Table 1). Differential expression of PGM5-AS1 in HGSC tumors was statistically significant (Table 1; $p=1.86E-06$).

PGM5-AS1 is expressed at significantly lower levels in HGSC when compared to the normal ovary.

We obtained exact mRNA expression levels for the differentially expressed PGM5-AS1 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. PGM5-AS1 was expressed at significantly lower levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 2; $p=0.0030$). We calculated a mean fold change of 0.5761 ± 0.0776 in PGM5-AS1 expression when comparing HGSC tumors to normal ovarian tissue (Table 2).

Thus, we found using published microarray data that PGM5 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas, and that both sense and anti-sense transcripts could be detected as differentially expressed in independent datasets; PGM5 mRNA and non-coding RNA 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 mesenchymal⁶. We sought to continue to describe the transcriptional landscape of high-grade serous ovarian cancers⁶ and identify genes whose differential expression was associated with HGSC by using published microarray data from primary tumors

1 of women diagnosed with HGSC compared to transcriptome data from normal ovarian tissue^{2,3}.
2 In both datasets analyzed, sense and anti-sense transcripts encoded by the PGM5 gene were
3 among the genes whose expression changed most significantly when comparing the normal
4 ovary to primary HGSC tumors.

5 Phosphoglucomutases function in metabolic reactions by catalyzing the conversion of
6 glucose-1-phosphate to glucose-6-biphosphate⁷. Phosphoglucomutase 5 (PGM5) was cloned
7 as a novel member of the PGM family in 1995⁸. However, PGM5 (also known as aciculin) has
8 been identified as a cytoskeletal protein, and purified 60kDa and 63kDa PGM5 proteins had no
9 phosphoglucomutase activity⁹. In vascular smooth muscle cells, expression of PGM5 is
10 developmentally regulated, correlating with the ability of differentiated cells to contract^{9,10}. In
11 epithelial cells, expression of the 63 kDa isoform was found in adherens junctions of
12 keratinocytes⁹. Expression of aciculin increased during chicken development, with expression
13 in the thigh detectable by day 16 in embryos¹⁰. In C2C12 myotube culture, association of
14 aciculin with myotubes increased through development, culminating in localization in striations
15 aligned with sarcomere Z-discs; authors concluded that aciculin was a component of skeletal
16 muscle costameres¹⁰. A study using C2C12 muscle cells to identify aciculin interacting proteins
17 found that aciculin (PGM5) interacted with dystrophin¹¹. A more recent study demonstrated
18 PGM5 interacts with Filamin C (FLNc) and Xin, an actin-binding repeat-containing protein
19 (XIRP)¹². Knockdown of PGM5 in myotubes resulted in a decrease in myofibrils, and failures in
20 myofibril assembly, alignment, and attachment. In cardiac muscle and in myotendinous
21 junctions of skeletal muscle, FLNc, PGM5 and Xin co-localized to intercalated discs¹².

24 PGM5 is implicated in the biology of a number of cancers. A genome-wide study of
25 transcription in clear cell renal cell carcinomas found that while ccRCCs expressed significantly
26 lower levels of PGM5-AS1 than normal tissues, high expression of PGM5-AS1 was significantly
27 correlated with lymph node metastasis as well as the presence of distant metastasis¹³.

28 However, patients with low tumor expression of PGM5-AS1 possessed significantly higher
overall survival than patients whose tumors expressed high levels of PGM5-AS1. Thus, while

1 this tumor antisense transcript was lower in tumors than in neighboring tissue, higher
2 expression of the antisense transcript was associated with metastatic events, and patients with
3 higher expression of the antisense transcript, overall, had better survival outcomes¹³.

4 In colorectal cancers, PGM5-AS1 was found to be up-regulated in tumor tissue and cell
5 lines¹⁴. Investigators found microRNA-484 as a predicted target of PGM5-AS1, and down-
6 regulation of PGM5-AS1 lead to partial correction of elevated invasion and cell growth induced
7 by miR-484 inhibition in SW480 and HCT 116 cell lines¹⁴.

8 PGM5-AS1 has also been found to be down-regulated in esophageal squamous cell
9 carcinomas - in tumor tissues, plasma, and in ESCC cell lines¹⁵. Transcription of PGM5-AS1
10 could be activated by the tumor suppressor p53 and antisense PGM5 transcript directly
11 interacted with microRNA 466, leading to elevated PTEN expression and thus inhibition of
12 ESCC progression¹⁵.

13 Dysregulated expression of PGM5 has also been reported in liver cancer¹⁶. Expression
14 of the sense transcript of PGM5 was found to be significantly lower in cancerous tissues than in
15 the liver¹⁶. Patients with low expression of PGM5 had worse survival outcomes, both overall
16 survival and relapse-free survival, and expression of PGM5 in the liver was positively correlated
17 with survival, but negatively correlated with advanced histological grade, advanced T
18 classification, and advanced stage¹⁶. Thus, dysregulated expression of PGM5 sense and anti-
19 sense transcripts has been reported in a variety of cancers; we could not identify published
20 literature describing differential expression of coding or non-coding RNAs in PGM5 in ovarian
21 cancer. However, a study assessing microRNA expression in human ovaries reported that
22 PGM5 was among the predicted gene expression targets of the top 10 most abundantly
23 microRNAs in the ovary¹⁷.

24 Differential expression of PGM5 in HGSC should be validated in larger and separate
25 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
26 cancers¹⁸⁻²⁰ can be utilized to assess the effect of conditional expression of PGM5 in HGSC
27 tumor growth post-development and in survival outcomes *in vivo*. We found here, by mining
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published microarray data, in an unbiased and systematic fashion, that multiple coding and non-coding transcripts produced at the PGM5 locus were among the most differentially expressed in tumors from women with HGSC.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
23	A_24_P120907	3.76E-08	-12.512821	8.99501	0.5502 ± 0.0560	PGM5	phosphoglucomutase 5
24	A_23_P10956	4.49E-08	-12.3138379	8.84437	0.6005 ± 0.0528	PGM5	phosphoglucomutase 5
85	A_24_P144423	1.53E-06	-8.8481179	5.65884	0.6144 ± 0.0591	PGM5	phosphoglucomutase 5

Table 1: PGM5 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 PGM5 transcripts in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
97	A_32_P157208	1.86E-06	-8.6831835	5.47615	0.5761 ± 0.0776	PGM5-AS1	PGM5 antisense RNA 1

Table 2: PGM5-AS1 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 PGM5-AS1 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
910	8044640	6.46E-05	-4.4118786	1.5116328	PGM5P2/// PGM5	phosphoglucomutase 5 pseudogene 2/// phosphoglucomutase 5
1798	8161520	6.66E-04	-3.6605271	-0.6896698	PGM5P2/// PGM5	phosphoglucomutase 5 pseudogene 2/// phosphoglucomutase 5
6254	8155665	2.88E-02	-2.2602458	-4.1168882	PGM5	phosphoglucomutase 5

Table 3: Differential expression of PGM5 transcripts observed in an independent high-grade serous ovarian carcinoma microarray dataset.

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 PGM5 transcripts in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: PGM5 transcripts are expressed at significantly lower levels in HGSC tumors than in the normal ovary.

The mRNA expression of PGM5 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 PGM5 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.

Figure 2: PGM5-AS1 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of PGM5-AS1 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 PGM5 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.