The progesterone receptor is differentially expressed in epithelial ovarian cancer and its expression correlates with patient survival.

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Epithelial ovarian cancer (EOC) is the most lethal gynecologic cancer¹. We sought to identify genes associated with high-grade serous ovarian cancer (HGSC), the most common type of EOC 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 found significant differential expression of the gene encoding the progesterone receptor, PGR, in high-grade serous ovarian tumors and in epithelial ovarian cancer broadly. PGR was expressed at lower levels in tumors from patients with high-grade serous ovarian cancer as compared to the normal ovary, and EOC patients whose tumors expressed low levels of PGR possessed significantly shorter progression-free survival than did those whose tumors expressed high levels of PGR. Multiple studies have described potential roles for the progesterone receptor in ovarian cancer, but our analysis is the first to demonstrate differential, decreased expression of PGR in tumors from patients with ovarian cancer and specifically in HGSC, in conjunction with unfavorable progression-free survival outcomes for ovarian cancer patients with low tumor expression of PGR.

Keywords: ovarian cancer, epithelial ovarian cancer, EOC, high-grade serous ovarian cancer, HGSC, targeted therapeutics in ovarian cancer, systems biology of ovarian cancer, PGR, progesterone receptor.

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

11 **Methods**

12 We used microarray data from datasets and GSE124766² and GSE146556³ for this
13 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
14 and Hochberg method of p-value adjustment was used for ranking of differential expression but
15 raw p-values were used for assessment of statistical significance of global differential expression.
16 Log-transformation of data was auto-detected, and the NCBI generated category of platform
17 annotation was used. GSE124766 was generated using Agilent-014850 Whole Human Genome
18 Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors from
19 patients with high-grade serous ovarian cancer. GSE146556 (2) was generated using Affymetrix
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Human Gene 1.0 ST Array with $n=3$ for normal ovarian tissue and $n=40$ for tumors from patients with epithelial ovarian cancer: $n=26$ high-grade serous ovarian cancer; $n=3$ carcinosarcoma, $n=2$ clear cell, $n=1$ endometrioid, $n=1$ granulosa cell; $n=1$ immature teratoma; $n=4$ mixed, and $n=2$ mucinous. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of PGR mRNA expression levels with progression-free survival in $n=614$ patients with ovarian cancer.

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

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

We identified the progesterone receptor, PGR, 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

1 between HGSC and the normal ovary, PGR ranked 40 out of 41093 total transcripts, equating to
2 99.9% differential expression (Table 1). Differential expression of PGR in HGSC tumors was
3 statistically significant (Table 1; $p=0.5286 \pm 0.0815$).
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6 We analyzed a second microarray dataset³ generated using normal ovarian tissues and
7 tumors from women diagnosed with HGSC, as well as tumors from other types of EOC, to
8 determine whether differential expression of PGR could be observed in tumors from a separate
9 group of patients. We again found that PGR was differentially expressed in primary HGSC
10 tumors and in EOC broadly (Table 2). When sorting all of the transcripts measured by
11 microarray based on change in expression between HGSC and the normal ovary, in this data set,
12 PGR ranked 1618 out of 29088 total transcripts, equating to 94.4% differential expression (Table
13 2). Differential expression of PGR in HGSC tumors in this second microarray dataset was
14 statistically significant (Table 2; $p=4.55E-04$).
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19 PGR is expressed at lower levels in HGSC when compared to the normal ovary.

20 We obtained exact mRNA expression levels for the differentially expressed PGR
21 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. PGR was
22 expressed at lower levels in high-grade serous ovarian cancers when compared to the normal
23 ovary; this difference was statistically significant (Figure 1; $p<0.0001$).
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26 PGR expression in ovarian tumors significantly correlates with patient survival.

27 To determine whether expression of the progesterone receptor at the mRNA level in
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1 HGSC tumors was correlated with survival outcomes in EOC patients, we performed Kaplan-
2 Meier survival analysis, which stratified tumors from 614 ovarian cancer patients into low and
3 high expression groups, and utilized paired progression-free survival data from each patient to
4 evaluate a relationship between PGR expression in ovarian tumors and patient survival. We
5 found a significant relationship between PGR tumor expression and progression-free survival, as
6 patients with low tumor expression of PGR possessed significantly shorter progression-free
7 survival than did patients with high tumor expression of PGR (Table 3 and Figure 2). Median
8 PFS for patients in the low PGR expression group was 18.3 months, while median PFS for
9 patients in the high PGR expression group was 26.77 months (Table 3). The correlation between
10 tumor PGR expression and PFS in ovarian cancer patients was statistically significant (log rank
11 p -value: $1.2e-5$; hazard ratio: 0.73 (0.63 — 0.84)) (Figure 2).

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17 Thus, we found using published microarray data that PGR was among the genes most
18 differentially expressed in tumors from women with high-grade serous carcinomas and in EOC
19 broadly. PGR was expressed at significantly lower levels in HGSC tumors when compared to
20 the normal ovary, and ovarian cancer patients with low tumor expression of PGR possessed
21 significantly shorter progression-free survival than did patients with high tumor expression of
22 PGR.

23 Discussion

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27 Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic analysis
28 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 of women diagnosed with HGSC compared to transcriptome data from normal ovarian tissue^{2,3}. In both datasets analyzed, the progesterone receptor, PGR was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors. Multiple studies have described a role for the progesterone receptor (PGR, or PR) in patients with ovarian cancer of different histotypes, but with varying and at times contradictory conclusions. One study of 322 ovarian carcinomas found that PR expression could be detected in only 26.2% of cases and that its over-expression was associated with enhanced survival⁸. A study of 773 Danish patients, 582 of which had ovarian cancers and 191 of which had low malignancy potential (LMP) ovarian tumors, found that PR was expressed in 20% of LMP tumors and in 20% of ovarian tumors⁹. A third study measured PR proteins levels and found PR positivity in 35% of tumors, with estrogen receptor and progesterone receptor expression being correlated. However, these investigators found that in 113 patients with ovarian cancers of multiple histotypes, 83 of which were serous tumors, there was no significant correlation between tumors deemed “PR+” and “PR-” with respect to survival outcomes, both overall survival and progression-free survival¹⁰. In a pooled dataset comprised of genetic information from 12 patient groups worldwide, investigators found no significant correlation between genetic variation at the PGR locus - specifically, rs10895068, a +331C/T allele, rs1042838 (the

1 “PROGINS” allele) and rs608995, a 3’ variant - and risk of development of ovarian cancer¹¹.
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3 However, the PROGINS allele was significantly correlated with the risk of development of the
4 endometrioid subtype, but not the serous subtype¹¹. A fourth study described positivity for PR-A
5 and PR-B (A and B forms of the progesterone receptor) in 36.2% and 33.8% of 155 ovarian
6 cancers, respectively, and concluded that PR-B+ ovarian cancer patients possessed better
7 survival than PR-B- ovarian cancer patients, finding PR-B was an independent prognostic
8 marker for survival in patients with ovarian cancer¹². In the context of cancer progression,
9 concentration of PR was going to be significantly higher in stage I and stage II tumors as
10 compared to stage III and IV tumors¹³. Survival for patients with PR concentration in tumors >
11 40 fmol/mg was found to be significantly better than for patients with tumor PR concentration <
12 40 fmol/mg¹³.
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17 The A and B form of the progesterone receptor is present at 2:1 ratio in the granulosa
18 cells of the ovary and its expression is induced by human chorionic gonadotropin¹⁴. In most
19 ovarian cancer cells whose expression was surveyed in one study, the expression of PR was
20 down-regulated as compared to normal human ovarian surface epithelial cells, consistent with
21 our finding that PGR is expressed at significantly lower levels in EOC tumors in general and of
22 the HGSC type¹⁵.
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25 Differential expression of PGR in HGSC should be validated in larger and separate
26 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
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cancers¹⁶⁻¹⁸ can be utilized to assess the effect of conditional expression of PGR in HGSC tumor growth post-development and on survival *in vivo*. The progesterone receptor may be relevant to prognostic stratification or therapeutic strategies in patients with epithelial ovarian cancer.

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Rank	ID	p-value	t	B	FC	Gene	Gene name	% DE
40	A_32_P49199	2.53E-07	-10.4969508	7.31833	0.5286 ± 0.0815	PGR	progesterone receptor	99.9

Table 1: PGR 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 PGR in HGSC tumors when compared to the normal ovary, the gene, gene name, and percent differential expression (% DE) are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name	% DE
1618	7951165	4.55E-04	-3.7871137	-0.3323292	PGR	progesterone receptor	94.4

Table 2: PGR is differentially expressed in epithelial ovarian cancer.

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, gene name and percent differential expression (% DE) are listed in this chart.

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

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

Figure 2: Expression of PGR in epithelial ovarian cancers correlates with progression-free survival in patients with HGSC.

Depicted in this Kaplan-Meier plot is the probability of progression-free survival for $n=614$ patients with epithelial ovarian cancer stratified into two groups, based on low or high expression of the progesterone receptor (PGR) in patient tumors. The log rank p -value denoting statistical significance of difference in progression-free 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 PGR 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)
18.3	26.77

Table 3: Patients with low tumor expression of PGR possess significantly shorter median progression-free survival than patients with high tumor expression of PGR.

Median progression-free survival (in months) is listed for $n=614$ HGSC patients, after stratification into two groups: EOC patients with low tumor expression of PGR as compared to EOC patients with high tumor expression of PGR.