Multiple members of the transcription factor elongation A like (TCEAL) family 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}. Perturbed expression of TCEAL7 has previously been reported in epithelial ovarian cancers⁴. Here we find significant differential expression of multiple members of the TCEAL gene family 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, TCEAL1, TCEAL3, TCEAL4, TCEAL5, TCEAL6.

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 multiple members of the TCEAL gene
7 family as among the most differentially expressed genes in HGSC tumors of the ovary. The
8 TCEAL family may be relevant for the study of target genes and pathways and for the
9 development of novel therapeutic interventions in high-grade serous ovarian cancers.
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14 **Methods**

15 We used microarray data from datasets and GSE124766² and GSE146556³ for this
16 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
17 and Hochberg method of p-value adjustment was used for ranking of differential expression but
18 raw p-values were used for assessment of statistical significance of global differential
19 expression. Log-transformation of data was auto-detected, and the NCBI generated category of
20 platform annotation was used. 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. GSE146556 was generated using
23 Affymetrix Human Gene 1.0 ST Array with n=3 for normal ovarian tissue and n=40 for tumors
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1 from patients with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels
2 only for the top 250 most differentially expressed genes.
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4 A statistical test was performed to evaluate whether TCEAL gene expression was
5 significantly different when comparing normal ovarian tissue from control subjects and primary
6 tumors from women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's
7 correction. Only p-values less than 0.05 were considered statistically significant. We used
8 PRISM for all statistical analyses (Version 8.4.0)(455).
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11 **Results**

12 We mined published microarray data^{2,3} to identify differentially expressed genes in high-
13 grade serous ovarian cancer (HGSC), the type of ovarian cancer responsible for 70-80% of
14 deaths resulting from the most lethal gynecologic malignancy.
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17 TCEAL1 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

18 We identified the gene encoding TCEAL1 as among the genes whose expression was
19 most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 1).
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21 When sorting all of the transcripts measured by microarray based on change in expression
22 between HGSC and the normal ovary, TCEAL1 ranked 169 out of 41093 total transcripts (Table
23 1). Differential expression of TCEAL1 in HGSC tumors was statistically significant (Table 1;
24 p=8.05E-06).
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1 We analyzed a second microarray dataset³ generated using normal ovarian tissues and
2 tumors from women diagnosed with HGSC to determine whether differential expression of
3 TCEAL1 could be observed in tumors from a separate group of patients. We again found that
4 TCEAL1 was differentially expressed in primary HGSC tumors (Table 1). When sorting all of
5 the transcripts measured by microarray based on change in expression between HGSC and the
6 normal ovary, in this data set, TCEAL1 ranked 193 out of 29088 total transcripts (Table 1).
7 Differential expression of TCEAL1 in HGSC tumors in this second microarray dataset was
8 statistically significant (Table 1; $p=1.52E-07$).
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13 TCEAL3 is differentially expressed in ovarian tumors from women diagnosed with HGSC.
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15 We also identified the gene encoding TCEAL3 as among the genes whose expression
16 was most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 2).
17 When sorting all of the transcripts measured by microarray based on change in expression
18 between HGSC and the normal ovary, TCEAL3 ranked 63 out of 41093 total transcripts (Table
19 2). Differential expression of TCEAL3 in HGSC tumors was statistically significant (Table 2;
20 $p=6.41E-07$).
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24 We analyzed a second microarray dataset³ generated using normal ovarian tissues and
25 tumors from women diagnosed with HGSC to determine whether differential expression of
26 TCEAL3 could be observed in tumors from a separate group of patients. We again found that
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1 TCEAL3 was differentially expressed in primary HGSC tumors (Table 2). When sorting all of
2 the transcripts measured by microarray based on change in expression between HGSC and the
3 normal ovary, in this data set, TCEAL3 ranked 5405 out of 29088 total transcripts (Table 2).
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5 Differential expression of TCEAL3 in HGSC tumors in this second microarray dataset was
6 statistically significant (Table 2; $p=1.92E-02$).
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8 TCEAL4 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

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10 We identified the gene encoding TCEAL4 as among the genes whose expression was
11 most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 3).
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13 When sorting all of the transcripts measured by microarray based on change in expression
14 between HGSC and the normal ovary, TCEAL4 ranked 48 out of 41093 total transcripts (Table
15 3). Differential expression of TCEAL4 in HGSC tumors was statistically significant (Table 3;
16 $p=3.78E-07$).
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18 We analyzed a second microarray dataset³ generated using normal ovarian tissues and
19 tumors from women diagnosed with HGSC to determine whether differential expression of
20 TCEAL4 could be observed in tumors from a separate group of patients. We again found that
21 TCEAL4 was differentially expressed in primary HGSC tumors (Table 3). When sorting all of
22 the transcripts measured by microarray based on change in expression between HGSC and the
23 normal ovary, in this data set, TCEAL4 ranked 630 out of 29088 total transcripts (Table 3).
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25 Differential expression of TCEAL4 in HGSC tumors in this second microarray dataset was
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1 statistically significant (Table 3; $p=1.71E-05$).

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3 TCEAL5 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

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5 We also identified TCEAL5 as among the genes whose expression was most different
6 when comparing primary HGSC tumors to normal ovarian tissue² (Table 4). When sorting all of
7 the transcripts measured by microarray based on change in expression between HGSC and the
8 normal ovary, TCEAL5 ranked 65 out of 41093 total transcripts (Table 4). Differential
9 expression of TCEAL5 in HGSC tumors was statistically significant (Table 4; $p=6.72E-07$).
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12 We analyzed a second microarray dataset³ generated using normal ovarian tissues and
13 tumors from women diagnosed with HGSC to determine whether differential expression of
14 TCEAL5 could be observed in tumors from a separate group of patients. We again found that
15 TCEAL5 was differentially expressed in primary HGSC tumors (Table 4). When sorting all of
16 the transcripts measured by microarray based on change in expression between HGSC and the
17 normal ovary, in this data set, TCEAL5 ranked 7592 out of 29088 total transcripts (Table 4).
18 Differential expression of TCEAL5 in HGSC tumors in this second microarray dataset was
19 statistically significant (Table 4; $p=4.9E-02$).
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24 TCEAL6 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

25 In addition to TCEAL1, TCEAL3, TCEAL4, and TCEAL5, we also identified TCEAL6
26 as among the genes whose expression was most different when comparing primary HGSC
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1 tumors to normal ovarian tissue² (Table 5). When sorting all of the transcripts measured by
2
3 microarray based on change in expression between HGSC and the normal ovary, TCEAL6
4 ranked 47 out of 41093 total transcripts (Table 5). Differential expression of TCEAL6 in HGSC
5 tumors was statistically significant (Table 5; $p=3.72E-07$).
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7 We analyzed a second microarray dataset³ generated using normal ovarian tissues and
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9 tumors from women diagnosed with HGSC to determine whether differential expression of
10 TCEAL6 could be observed in tumors from a separate group of patients. Here we found that a
11 transcript representing either TCEAL6, TCEAL2 or TCEAL3 was differentially expressed in
12 primary HGSC tumors (Table 5). When sorting all of the transcripts measured by microarray
13 based on change in expression between HGSC and the normal ovary, the TCEAL6/TCEAL2/
14 TCEAL3 transcript ranked 699 out of 29088 total transcripts (Table 5). Differential expression
15 of TCEAL6 in HGSC tumors in this second microarray dataset was statistically significant
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17 (Table 5; $p=2.57E-05$).
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20 Expression of TCEAL1, TCEAL3, TCEAL4, TCEAL5 and TCEAL6 is significantly decreased
21 in HGSC tumors.
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23 We obtained exact mRNA expression levels for each of the differentially expressed
24 TCEAL transcripts from the ovary and from high-grade serous ovarian tumors to directly
25 compare TCEAL expression between HGSC tumors and the ovary. TCEAL1 was expressed at
26 lower levels in high-grade serous ovarian cancers when compared to the normal ovary; this
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1 difference was statistically significant (Figure 1A; $p < 0.0001$). We calculated a mean fold change
2 of 0.8165 ± 0.0395 in TCEAL1 expression when comparing HGSC tumors to normal ovarian
3 tissue (Table 1). In the second microarray dataset examined, TCEAL1 was again expressed at
4 significantly lower levels in HGSC tumors when compared to the ovary (Figure 1B; $p = 0.0417$).
5 We calculated a mean fold change of 0.2760 ± 0.1730 in TCEAL1 expression when comparing
6 HGSC tumors to normal ovarian tissue.
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10 TCEAL3 was expressed at lower levels in high-grade serous ovarian cancers when
11 compared to the normal ovary; this difference was statistically significant (Figure 2; $p < 0.0001$).
12 We calculated a mean fold change of 0.8581 ± 0.0180 in TCEAL3 expression when comparing
13 HGSC tumors to normal ovarian tissue (Table 2).
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16 TCEAL4 was expressed at lower levels in high-grade serous ovarian cancers when
17 compared to the normal ovary; this difference was statistically significant (Figure 3; $p = 0.0005$).
18 We calculated a mean fold change of 0.8579 ± 0.0166 in TCEAL4 expression when comparing
19 HGSC tumors to normal ovarian tissue (Table 3).
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22 TCEAL5 was expressed at lower levels in high-grade serous ovarian cancers when
23 compared to the normal ovary; this difference was statistically significant (Figure 4; $p = 0.0048$).
24 We calculated a mean fold change of 0.8527 ± 0.0152 in TCEAL5 expression when comparing
25 HGSC tumors to normal ovarian tissue (Table 4).
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28 TCEAL6 was expressed at lower levels in high-grade serous ovarian cancers when

1 compared to the normal ovary; this difference was statistically significant (Figure 5; $p=0.0001$).
2 We calculated a mean fold change of 0.8415 ± 0.0199 in TCEAL5 expression when comparing
3 HGSC tumors to normal ovarian tissue (Table 1).
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6 Thus, we found using published microarray data that TCEAL1, TCEAL3, TCEAL4,
7 TCEAL5 and TCEAL6 were all among the genes most differentially expressed in tumors from
8 women with high-grade serous carcinomas, and that each of these five TCEAL genes were
9 expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.
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11 **Discussion**

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13 Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic
14 analysis classified HGSC into four subtypes based on gene clustering: immunoreactive,
15 differentiated, proliferative, and mesenchymal⁷. We sought to continue to describe the
16 transcriptional landscape of high-grade serous ovarian cancers⁷ and identify genes whose
17 differential expression was associated with HGSC by using published microarray data from
18 primary tumors of women diagnosed with HGSC compared to transcriptome data from normal
19 ovarian tissue^{2,3}. In both datasets analyzed, TCEAL1, TCEAL3, TCEAL4, TCEAL5 and
20 TCEAL6 were among the genes whose expression changed most significantly when comparing
21 the normal ovary to primary HGSC tumors.
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26 The TCEAL gene family is related to transcriptional machinery TCEA/TFIIS which
27 functions in “pause-release” of RNA Polymerase II, allowing the enzyme to bypass stalling of
28 transcription during transcriptional elongation^{8,9}. Stable expression of TCEAL5 in a pituitary

1 progenitor cell line could drive cell proliferation, demonstrating that TCEAL5 could support
2 proliferative capacity of cells and potentially acting as a proto-oncogene in that cellular
3 context¹⁰; this is different from the apparent tumor-suppressive function of TCEAL7 in ovarian
4 cancers⁴. TCEAL5 possesses ~80% homology to TCEAL3 and TCEAL6 but only 20-30%
5 homology to TCEAL1, TCEAL7 and TCEAL8. Pro-apoptotic and regulatory domain BEX3 is
6 conserved among mammalian TCEAL5 proteins¹⁰. TCEAL1, also known as p21/SIIR, is
7 expressed broadly across tissues as a 1.2kb transcript and expressed in heart and skeletal muscle
8 as a roughly 7kb transcript¹¹. In 2005, a study demonstrated significantly decreased expression
9 of TCEAL1 in esophageal squamous cell carcinoma, suggesting that loss of TCEAL1
10 expression might be an early event in tumorigenesis as even superficial cancers manifested
11 decreased TCEAL1 expression¹². In prostate cancer cells, loss of TCEAL1 expression is
12 associated with gain of response to the chemotherapy drug docetaxel¹³. A study using
13 expression data to determine top 100 marker genes for non-small cell lung cancer (NSCLC)
14 types adenocarcinoma, squamous cell, large cell and carcinoid using nearest neighbor analysis
15 found that TCEAL1 was the 13th ranked marker gene in carcinoid NSCLC¹⁴. In anaplastic
16 thyroid cancers, investigators documented down-regulation of TCEAL4 in all 11 ATC tumors
17 examined and the TCEAL4 promoter was uniformly hemi-methylated¹⁵. An investigation of 5
18 ovarian cancer cell lines (CAOV-3, SK-OV-3, OVCAR-3, OV-1063 and OVK18), 7 cervical
19 cancer cell lines (SIHA, HT-3, D98-AH2, HeLa TG, HeLa, CaSki, Me-180 and HeLa.P3), 7
20 lung cancer cell lines (RERF-LC AI, PC-14, A549, EBC-1, LU65, LU99 and LK-2), 3 bladder
21 cancer cell lines (5637, T24 and EJ-1), and two neuroblastoma cell lines (IMR-32 and
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1 SCCH-26), using semi-quantitative polymerase chain reaction demonstrated relatively
2 decreased expression of TCEAL4 in cancer cells¹⁵. Thus, TCEAL gene expression is perturbed
3 across tissues in human cancers but its role as supporting or restricting tumorigenesis appears to
4 be context dependent.
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7 Differential expression of the TCEAL gene family in HGSC should be validated in
8 larger and separate cohorts of women with HGSC. If validated, mouse models of high-grade
9 serous ovarian cancers¹⁶⁻¹⁸ can be utilized to assess the effect of conditional expression of
10 TCEAL genes in HGSC tumor growth post-development and on survival *in vivo*. We previously
11 reported TCEAL7 expression was associated with progression-free survival in patients with
12 high-grade serous ovarian cancer¹⁹; we find here that TCEAL1, TCEAL3, TCEAL4, TCEAL5
13 and TCEAL6 are uniformly differentially expressed and down-regulated in tumors from patients
14 with HGSC. Using technology like 4sUDRB-seq to probe the effect of TCEAL down-
15 regulation on transcriptional dynamics can provide important information regarding rate of
16 transcriptional elongation at nascent RNA genome-wide²⁰.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
169	A_23_P73801	8.05E-06	-7.5091766	4.07688	0.8165 ± 0.0395	TCEAL1	transcription elongation factor A like 1
193	8169049	1.52E-07	-6.2255033	7.301391	0.2760 ± 0.1730	TCEAL1	transcription elongation factor A like 1

Table 1: TCEAL1 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 TCEAL1 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart. Data in this table are from GSE124766 (ID: A_23_P73801) and GSE146553 (ID: 8169049).

Rank	ID	p-value	t	B	FC	Gene	Gene name
63	A_23_P434442	6.41E-07	-9.6183451	6.47035	0.8581 ± 0.0180	TCEAL3	transcription elongation factor A like 3
5405	8169044	1.92E-02	-2.4293044	-3.7646269		TCEAL3	transcription elongation factor A like 3

Table 2: TCEAL3 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 TCEAL3 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart. Data in this table are from GSE124766 (ID: A_23_P434442) and GSE146553 (ID: 8169044).

Rank	ID	p-value	t	B	FC	Gene	Gene name
48	A_23_P259166	3.78E-07	-10.1103439	6.95487	0.8579 ± 0.0166	TCEAL4	transcription elongation factor A like 4
630	8169035	1.71E-05	-4.8203669	2.7743574		TCEAL4	transcription elongation factor A like 4

Table 3: TCEAL4 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 TCEAL4 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart. Data in this table are from GSE124766 (ID:A_23_P259166) and GSE146553 (ID:8169035).

Rank	ID	p-value	t	B	FC	Gene	Gene name
65	A_32_P18470	6.72E-07	-9.5755656	6.42701	0.8527 ± 0.0152	TCEAL5	transcription elongation factor A like 5
7592	8174235	4.9E-02	-2.0245826	-4.5740069		TCEAL5	transcription elongation factor A like 5

Table 4: TCEAL5 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 TCEAL5 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart. Data in this table are from GSE124766 (ID:A_32_P18470) and GSE146553 (ID:8174235).

Rank	ID	p-value	t	B	FC	Gene	Gene name
47	A_32_P192545	3.72E-07	-10.1274611	6.97128	0.8415 ± 0.199	TCEAL6	transcription elongation factor A like 6
699	8174134	2.57E-05	-4.6962777	2.3869265		TCEAL6 /// TCEAL2 /// TCEAL3	transcription elongation factor A like 6/// transcription elongation factor A like 2/// transcription elongation factor A like 3

Table 5: TCEAL6 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 TCEAL6 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart. Data in this table are from GSE124766 (ID:A_32_P192545) and GSE146553 (ID:8174134).

Figure 1: TCEAL1 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of TCEAL1 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 TCEAL1 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above. Data in this table are from GSE124766 (A) and GSE146553 (B).

Figure 2: TCEAL3 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of TCEAL3 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 TCEAL3 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.

Figure 3: TCEAL4 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of TCEAL4 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 TCEAL4 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.

Figure 4: TCEAL5 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of TCEAL5 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 TCEAL5 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.

Figure 5: TCEAL6 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of TCEAL6 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 TCEAL6 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.