The transcription elongation factor A like 7 (TCEAL7) is differentially expressed in high-grade serous ovarian cancers and its expression associates with patient survival.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

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}. We found significant differential expression of the gene encoding the transcription elongation factor A like 7 (TCEAL7) in high-grade serous ovarian tumors, and TCEAL7 expression associated with progression-free survival in patients with HGSC.

Keywords: ovarian cancer, high-grade serous ovarian cancer, HGSC, targeted therapeutics in ovarian cancer, systems biology of ovarian cancer, TCEAL7.

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 TCEAL7 as among the most
7 differentially expressed genes in HGSC tumors of the ovary. In a microarray dataset generated
8 with tumors from patients enrolled in the ICON7 trial⁴, TCEAL7 was among the genes most
9 differentially expressed when comparing tumors based on patient survival. TCEAL7 may be a
10 gene of interest when prioritizing the study of target genes and pathways for the development of
11 novel therapeutic interventions in high-grade serous ovarian cancers.
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17 **Methods**

18 We used microarray data from datasets GSE146556², GSE124766³, and GSE140082⁴ for
19 this differential gene expression analysis of high-grade serous ovarian carcinomas. The
20 Benjamini and Hochberg method of p-value adjustment was used for ranking of differential
21 expression but raw p-values were used for assessment of statistical significance of global
22 differential expression. Log-transformation of data was auto-detected, and the NCBI generated
23 category of platform annotation was used. GSE146556 was generated using Affymetrix Human
24 Gene 1.0 ST Array with $n=3$ for normal ovarian tissue and $n=40$ for tumors from patients with
25 high-grade serous ovarian cancer. GSE124766 was generated using Agilent-014850 Whole
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Human Genome Microarray 4x44K G4112F with $n=3$ of for normal ovarian tissue and $n=8$ for tumors from patients with high-grade serous ovarian cancer. GSE140082 was generated using Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip technology with $n=30$ for tumors from patients with progression-free survival less than 200 days and $n=36$ for tumors from patients with progression-free survival greater than 1000 days. GEO2R provides mRNA expression levels only for the top 250 most differentially expressed genes.

A statistical test was performed to evaluate whether TCEAL7 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. The same statistical test was used to compare whether expression of TCEAL7 was significantly different between HGSC patients with progression-free survival (PFS) less than 200 days or HGSC patients with PFS greater than 1000 days. 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.

TCEAL7 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified the gene encoding the transcription elongation factor A like 7, TCEAL7,

as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 1). We found that TCEAL7 was differentially expressed in primary HGSC tumors (Table 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, TCEAL7 ranked 155 out of 29088 total transcripts (Table 1). Differential expression of TCEAL7 in HGSC tumors was statistically significant (Table 1; $p=5.08E-08$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of TCEAL7 could be observed in tumors from a separate group of patients. We again found that TCEAL7 was differentially expressed in primary HGSC tumors (Table 2). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, in this data set, TCEAL7 ranked 2228 out of 41093 total transcripts (Table 2). Differential expression of TCEAL7 in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=2.34E-03$).

TCEAL7 is expressed at lower levels in HGSC when compared to the normal ovary.

We obtained exact mRNA expression levels for the differentially expressed TCEAL7 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. TCEAL7 was expressed at lower levels in high-grade serous ovarian cancers when compared to the normal ovary; this difference was statistically significant (Figure 1; $p=0.0338$). We calculated a

mean fold change of 0.489 ± 0.092 in TCEAL7 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

TCEAL7 expression in HGSC associates with survival outcomes.

We used a third dataset to identify genes associated with greater patient survival in ovarian cancer, comparing tumors from patients with progression-free survival (PFS) less than 200 days to that of patients with PFS greater than 1000 days, using microarray data obtained from tumors of patients enrolled in the ICON7 trial (6). We again found that TCEAL7 was among the genes most differentially expressed in HGSC, but in this comparison, based on survival outcomes (Table 3 and Figure 2). Differential expression of TCEAL7 in HGSC tumors based on patient survival less than 200 days or greater than 1000 days was statistically significant (Table 3; $p=0.00083699$). When sorting all genes based on degree of change in expression between tumors of patients with PFS < 200 days and tumors of patients with PFS > 1000 days, TCEAL7 ranked 214 out of 29377 total transcripts. TCEAL7 was expressed at significantly lower levels in primary HGSC tumors from patients with PFS greater than 1000 days when compared to that of patients with PFS less than 200 days (Figure 2; $p=0.0013$). We calculated a mean fold change of 0.9293 ± 0.0682 in TCEAL7 expression when comparing tumors from patients with PFS > 1000 days to that of tumors from patients with PFS < 200 days (Table 1).

Thus, we found using published microarray data that TCEAL7 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that

1 TCEAL7 expression was lower in HGSC tumors when compared to the normal ovary.

3 **Discussion**

4 Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic
5 analysis classified HGSC into four subtypes based on gene clustering: immunoreactive,
6 differentiated, proliferative, and mesenchymal⁶. We sought to continue to describe the
7 transcriptional landscape of high-grade serous ovarian cancers⁶ and identify genes whose
8 differential expression was associated with HGSC by using published microarray data from
9 primary tumors of women diagnosed with HGSC compared to transcriptome data from normal
10 ovarian tissue^{2,3}. In both datasets analyzed, TCEAL7 was among the genes whose expression
11 changed most significantly when comparing the normal ovary to primary HGSC tumors.
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16 Transcription elongation factor A like 7, or TCEAL7 is one member of a family of genes
17 with sequence similarity to brain-expressed (Bex) proteins⁸. A related protein, TCEA/TFIIS
18 functions in “pause-release” of RNA Polymerase II, allowing the enzyme to bypass pausing of
19 transcription during transcriptional elongation⁹. TCEAL7 is a negative regulator of NF-κB
20 signaling. Depletion of TCEAL7 in OSE cell results in activation of NF-κB signaling *in vitro*,
21 and ectopic expression of TCEAL7 impairs NF-κB activation in multiple ovarian cancer cell
22 lines, including OVCAR8, SKOV3ip, and DOV13¹⁰. Increased recruitment of p65 and p300
23 was observed at the promoters of IL-6 and IL-8 in cells depleted of TCEAL7¹⁰. Investigators
24 studying mechanisms by which cancer cells bypass replicative senescence, including human
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1 telomerase (hTERT) or an alternative lengthening of telomeres (ALT) mechanism found that
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3 changes in TCEAL7 expression could affect the expression of hTERT. TCEAL7 has been
4 described as a competitive inhibitor of c-Myc; thus authors concluded that cMyc inhibition by
5 TCEAL7 could be involved in an ALT mechanism of replicative senescence bypass¹¹. In
6 cardiotoxin-injured mice, TCEAL7 was among the genes in the skeletal muscle whose
7 expression was regulated during muscle regeneration. Ectopic expression of TCEAL7 in
8 C2C12 myoblasts impaired cell proliferation while supporting differentiation. TCEAL7
9 expression has been reported as restricted to the skeletal muscle during embryogenesis; the
10 TCEAL7 promoter contains evolutionarily conserved E-box motifs and can be occupied by
11 myogenic regulatory factors such as Myf5, Myf6 and Myod¹², suggesting TCEAL7 might
12 function in differentiation of muscle during development.
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17 TCEAL7 down-regulation in ovarian cancer has previously been described. Decreased
18 expression of TCEAL7 was found in 7 epithelial ovarian cancer cell lines and in 21/24 primary
19 ovarian cancers¹³. Down-regulation of TCEAL7 in immortalized, non-transformed ovarian
20 surface epithelial cells (OSEtsT/hTERT) supported anchorage-independent cell growth *in vitro*,
21 and TCEAL7 could associate with the Cyclin D1 promoter to repress Cyclin D expression.
22 Decreased expression of Cyclin E and Cyclin A was also observed in OSEtsT/hTERT cells over-
23 expressing TCEAL7. Further, depletion of TCEAL7 in OSEtsT/hTERT cells resulted in
24 promoter activation of Myc target gene ornithine decarboxylase (ODC), whereas ectopic
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1 expression of TCEAL7 impaired ODC promoter activity induced by Myc¹³. Thus, TCEAL7
2 might modulate promoter activity of the Myc transcription factor. A separate study
3 demonstrated loss of TCEAL7 expression in all ovarian cancer cell lines and in a majority of
4 ovarian tumors examined; TCEAL7 expression could be activated by treatment of OV207 cells
5 with 5-aza-2'-deoxycytidine, suggesting that DNA methylation of TCEAL7 regulatory elements
6 is important for its repression¹⁴. CpG methylation at an SmaI site (site I) at the TCEAL7
7 promoter was important for repression of promoter activity and TCEAL7 gene expression¹⁴. In
8 multiple ovarian tumor samples, investigators observed biallelic methylation of site I. Ectopic
9 expression of TCEAL7 resulted in significant increases in cell death of SKOV3 ovarian cancer
10 cells and decreased colony formation of OV202 cells *in vitro*¹⁴. Moreover, a study of 930
11 ovarian cancers indicated that polymorphisms in TCEAL7 may be associated with the risk of
12 development of invasive serous ovarian cancer¹⁵. Together, published data clearly demonstrate
13 down-regulation of TCEAL7 in ovarian cancers.

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19 TCEAL7 is implicated in human cancers outside of the ovary. In non-small cell lung
20 cancer (NSCLC), decreased expression of TCEAL7 was observed in 68% of NSCLC tissues,
21 and decreased expression of TCEAL7 significantly correlated with increased Cyclin D1
22 expression¹⁶. In a survey of cancers across tissue encompassing 158 samples, TCEAL7 down-
23 regulation was observed in greater than 90% of cancers. Consistent with other findings,
24 TCEAL7 associated with the promoter of Cyclin D1 at Myc-binding E-box sequences, and
25 ectopic expression of TCEAL7 resulted in decreased expression of Cyclin D1 and ornithine
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1 decarboxylase, two Myc target genes¹⁷. In gastric adenocarcinoma, immunohistochemical
2 analysis demonstrated decreased protein expression of TCEAL7 in 43.3% of tumor tissues
3 sampled, and lower expression of TCEAL7 was significantly associated with worse prognosis in
4 gastric adenocarcinoma patients¹⁸. TCEAL7 expression was correlated with higher tumor
5 histological grade and larger tumor size in gastric adenocarcinoma¹⁸. In endometrial carcinoma,
6 investigators found that microRNA miR-182 could bind an 8 basepair sequence in the 3' UTR
7 (untranslated region) of the TCEAL7 promoter. Depletion of miR-182 resulted in increased
8 TCEAL7 expression, and impaired cell proliferation and colony forming potential *in vitro*¹⁹.
9 Thus, decreased expression of TCEAL7 can be observed in multiple human cancers, appears to
10 regulate Cyclin D1 expression, and its down-regulation can be observed in cancers across
11 tissues, including epithelial ovarian cancer¹⁹.
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17 Differential expression of TCEAL7 in HGSC should be validated in larger and separate
18 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
19 cancers²⁰⁻²² can be utilized to assess the effect of conditional expression of TCEAL7 on HGSC
20 tumor growth post-development and on survival *in vivo*. We found that TCEAL7 was among
21 the genes whose expression was most significantly different among high-grade serous ovarian
22 cancer patients with enhanced survival outcomes, expressed at significantly lower levels in
23 patients with progression-free survival greater than 1000 days.
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References

1. Guppy, A.E., Nathan, P.D. and Rustin, G.J., 2005. Epithelial ovarian cancer: a review of current management. *Clinical Oncology*, 17(6), pp.399-411.
2. Zhang, W., Klinebiel, 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. Hoffmann, K., Berger, H., Kulbe, H., Thillainadarasan, S., Mollenkopf, H.J., Zemojtel, T., Taube, E., Darb-Esfahani, S., Mangler, M., Sehoul, 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.
4. GSE140082. Sean Dowdy; Winterhoff K, Kommoss S. Mayo Clinic, Obstetrics and Gynecology. Rochester, MN. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE140082>.
5. 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.
6. 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.
7. Cancer Genome Atlas Research Network, 2011. Integrated genomic analyses of ovarian carcinoma. *Nature*, 474(7353), p.609.
8. Kazi, J.U., Kabir, N.N. and Rönstrand, L., 2015. Brain-Expressed X-linked (BEX) proteins in human cancers. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, 1856(2), pp. 226-233.
9. Jeon, C. and Agarwal, K., 1996. Fidelity of RNA polymerase II transcription controlled by elongation factor TFIIS. *Proceedings of the National Academy of Sciences*, 93(24), pp. 13677-13682.
10. Rattan, R., Narita, K., Chien, J., Maguire, J.L., Shridhar, R., Giri, S. and Shridhar, V., 2010. TCEAL7, a putative tumor suppressor gene, negatively regulates NF- κ B pathway. *Oncogene*, 29(9), pp.1362-1373.

- 11.Lafferty-Whyte, K., Bilsland, A., Hoare, S.F., Burns, S., Zaffaroni, N., Cairney, C.J. and Keith, W.N., 2010. TCEAL7 inhibition of c-Myc activity in alternative lengthening of telomeres regulates hTERT expression. *Neoplasia*, 12(5), pp.405-IN6.
- 12.Shi, X. and Garry, D.J., 2010. Myogenic regulatory factors transactivate the Tceal7 gene and modulate muscle differentiation. *Biochemical Journal*, 428(2), pp.213-221.
- 13.Chien, J., Narita, K., Rattan, R., Giri, S., Shridhar, R., Staub, J., Belefors, D., Lai, J., Roberts, L.R., Molina, J. and Kaufmann, S.H., 2008. A role for candidate tumor-suppressor gene TCEAL7 in the regulation of c-Myc activity, cyclin D1 levels and cellular transformation. *Oncogene*, 27(58), pp.7223-7234.
- 14.Chien, J., Staub, J., Avula, R., Zhang, H., Liu, W., Hartmann, L.C., Kaufmann, S.H., Smith, D.I. and Shridhar, V., 2005. Epigenetic silencing of TCEAL7 (Bex4) in ovarian cancer. *Oncogene*, 24(32), pp.5089-5100.
- 15.Peedicayil, A., Vierkant, R.A., Shridhar, V., Schildkraut, J.M., Armasu, S., Hartmann, L.C., Fridley, B.L., Cunningham, J.M., Phelan, C.M., Sellers, T.A. and Goode, E.L., 2009. Polymorphisms in TCEAL7 and risk of epithelial ovarian cancer. *Gynecologic oncology*, 114(2), pp.260-264.
- 16.Orhan, C., Bulut, P., Dalay, N., Ersen, E. and Buyru, N., 2019. Downregulation of TCEAL7 expression induces CCND1 expression in non-small cell lung cancer. *Molecular biology reports*, 46(5), pp.5251-5256.
- 17.Jeremy, C., Rattan, R., Narita, K., Giri, S., Staub, J., Belefors, D., Lai, J., Molina, J., Kaufmann, S., Prendergast, G. and Shridhar, V., 2008. Identification of TCEAL7 as a regulator of c-Myc and neoplastic transformation.
- 18.Huang, C.Y., Chen, Y.M., Zhao, J.J., Chen, Y.B., Jiang, S.S., Yan, S.M., Zhao, B.W., Pan, K., Wang, D.D., Lv, L. and Li, Y.F., 2013. Decreased expression of transcription elongation factor A-like 7 is associated with gastric adenocarcinoma prognosis. *PLoS One*, 8(1), p.e54671.
- 19.Guo, Y., Liao, Y., Jia, C., Ren, J., Wang, J. and Li, T., 2013. MicroRNA-182 promotes tumor cell growth by targeting transcription elongation factor A-like 7 in endometrial carcinoma. *Cellular physiology and biochemistry*, 32(3), pp.581-590.
- 20.Harrington, B.S., He, Y., Davies, C.M., Wallace, S.J., Adams, M.N., Beaven, E.A., Roche, D.K., Kennedy, C., Chetty, N.P., Crandon, A.J. and Flatley, C., 2016. Cell line and patient-derived xenograft models reveal elevated CDCP1 as a target in high-grade serous ovarian cancer. *British journal of cancer*, 114(4), pp.417-426.

- 1 21.Perets, R., Wyant, G.A., Muto, K.W., Bijron, J.G., Poole, B.B., Chin, K.T., Chen, J.Y.H.,
2 Ohman, A.W., Stepule, C.D., Kwak, S. and Karst, A.M., 2013. Transformation of the
3 fallopian tube secretory epithelium leads to high-grade serous ovarian cancer in Brca; Tp53;
4 Pten models. *Cancer cell*, 24(6), pp.751-765.
- 5 22.Kim, J., Coffey, D.M., Creighton, C.J., Yu, Z., Hawkins, S.M. and Matzuk, M.M., 2012.
6 High- grade serous ovarian cancer arises from fallopian tube in a mouse model. *Proceedings*
7 *of the National Academy of Sciences*, 109(10), pp.3921-3926.

Rank	ID	p-value	t	B	FC	Gene	Gene name
155	8169015	5.08E-08	-6.5480077	8.3559961	0.489 ± 0.092	TCEAL7	transcription elongation factor A like 7

Table 1: TCEAL7 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 TCEAL7 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
2228	A_23_P125788	2.34E-03	-3.8619486	-1.51872	TCEAL7	transcription elongation factor A like 7

Table 2: TCEAL7 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 TCEAL7 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: TCEAL7 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of TCEAL7 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 TCEAL7 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.

Rank	ID	p-value	t	B	FC	Gene	Gene name
214	ILMN_2084043	0.00083699	-3.5000112	-0.64917	0.9293 ± 0.0682	TCEAL7	transcription elongation factor A like 7

Table 3: TCEAL7 is differentially expressed in high-grade serous ovarian carcinomas when comparing tumors based on patient progression-free survival.

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 TCEAL7 in HGSC tumors when comparing patients with progression-free survival (PFS) less than 200 days with patients with PFS greater than 1000 days, the gene and gene name are listed in this chart.

Figure 2: TCEAL7 expression in HGSC tumors associates with patient survival outcomes.

The mRNA expression of TCEAL7 in tumors from patients with progression-free survival (PFS) < 200 days (left) and in the HGSC tumors from patients with PFS > 1000 days (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in TCEAL7 expression between the groups, a p-value, listed above.