1 **Thyrotroph embryonic factor (TEF) is differentially expressed in high-grade serous**
2 **ovarian cancers.**

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6 Ovarian cancer is the most lethal gynecologic cancer¹. We sought to identify genes associated
7 with high-grade serous ovarian cancer (HGSC) by comparing global gene expression profiles of
8 normal ovary with that of primary tumors from women diagnosed with HGSC using published
9 microarray data^{2,3}. We previously reported differential expression of the PAR-bZIP transcription
10 factor HLF in HGSC⁴. Here, we report significant differential expression of a second PAR-bZIP
11 transcription factor, thyrotroph embryonic factor (TEF)⁵ in high-grade serous ovarian tumors.

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

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 the gene encoding the bZIP-PAR
7 transcription factor TEF as among the most differentially expressed in HGSC tumors of the
8 ovary. TEF, and bZIP-PAR transcription factors in general, may be genes of interest when
9 prioritizing the study of target genes and pathways for the development of novel therapeutic
10 interventions in high-grade serous ovarian cancers.
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12 13 **Methods**

14 We used microarray data from datasets and GSE146556² and GSE124766³ for this
15 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
16 and Hochberg method of p-value adjustment was used for ranking of differential expression but
17 raw p-values were used for assessment of statistical significance of global differential
18 expression. Log-transformation of data was auto-detected, and the NCBI generated category of
19 platform annotation was used. GSE124766³ was generated using Agilent-014850 Whole
20 Human Genome Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for
21 tumors from patients with high-grade serous ovarian cancer. GSE146556² was generated using
22 Affymetrix Human Gene 1.0 ST Array with n=3 for normal ovarian tissue and n=40 for tumors
23 from patients with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels
24 only for the top 250 most differentially expressed genes.
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26 A statistical test was performed to evaluate whether TEF expression was significantly
27 different when comparing normal ovarian tissue from control subjects and primary tumors from
28 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.

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

We identified TEF 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, TEF ranked 93 out of 41093 total transcripts (Table 1). Differential expression of TEF in HGSC tumors was statistically significant (Table 1; $p=4.1E-09$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of TEF could be observed in tumors from a separate group of patients. We again found that TEF 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, TEF ranked 6379 out of 29088 total transcripts (Table 2), indicating that the change in TEF expression in this dataset when comparing tumor to ovary was greater than 78.07% of the measured transcriptome. Differential expression of TEF in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=2.57E-02$).

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

We obtained exact mRNA expression levels for the differentially expressed TEF transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. TEF was expressed at significantly lower levels in high-grade serous ovarian cancers when

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1 compared to the normal ovary (Figure 1; $p=0.0236$). We calculated a mean fold change of
2 0.5660 ± 0.0697 in TEF expression when comparing HGSC tumors to normal ovarian tissue
3 (Table 1).

4 Thus, we found using published microarray data that TEF was among the genes most
5 differentially expressed in tumors from women with high-grade serous carcinomas and that TEF
6 expression was significantly lower in HGSC tumors when compared to the normal ovary.

7 8 **Discussion**

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10 Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic analysis
11 classified HGSC into four subtypes based on gene clustering: immunoreactive, differentiated,
12 proliferative, and mesenchymal⁷. We sought to continue to describe the transcriptional
13 landscape of high-grade serous ovarian cancers⁷ and identify genes whose differential
14 expression was associated with HGSC by using published microarray data from primary tumors
15 of women diagnosed with HGSC compared to transcriptome data from normal ovarian tissue^{2,3}.
16 In both datasets analyzed, TEF was among the genes whose expression changed most
17 significantly when comparing the normal ovary to primary HGSC tumors.

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19 TEF was identified in a screen for trans-acting factors with the ability to regulate gene
20 expression in the pituitary gland, possessing homology in the PAR (proline and acid-rich) region,
21 DNA-binding domain, and in the leucine zipper region to DBP (albumin D-box binding protein),
22 another bZIP (leucine zipper) transcription factor. TEF expression in mice is developmental
23 stage-specific, with expression restricted to pituitary thyrotrophs during development⁵.
24 Expression of TEF coincides with the start of expression of the beta subunit of the thyroid-
25 stimulating hormone (TSH β)⁵. TEF could bind the promoters of TSH β , prolactin, and growth
26 hormone receptors, and TEF could trans-activate a reporter construct under the control of the
27 TSH β promoter⁵. Although first identified as expressed in the anterior pituitary gland, TEF has
28 since been found to be expressed in the spleen, lymph nodes, thymus, and fetal liver, with little

1 to no expression in peripheral blood leukocytes and in the bone marrow⁹. In murine
2 interleukin-3 (IL-3)-dependent FL5.12 cells, TEF repressed expression of the IL-3 promoter and
3 supported survival of FL5.12 cells in the absence of IL-3, with a block in proliferation and cells
4 arrested in G₀/G₁⁹. This suggested that though TEF expression had anti-apoptotic properties, it
5 could also inhibit cell growth in certain contexts⁹.

6 While 50% of mice deficient in three bZIP transcription factors, HLF, TEF and DBP die
7 within two months of birth, the rest are prone to epileptic seizures triggered by sound
8 (audiogenic) and spontaneously¹⁰. Interestingly, investigators noted that more animals died on
9 Mondays and Thursdays - days on which the vacuum cleaner was utilized in the animal
10 facility¹⁰. Closer observation revealed that nearly half of triple knockout mice experienced tonic-
11 clonic seizures when placed in close proximity to an operating vacuum, suggestive of
12 audiogenic seizures¹⁰. PAR bZIP-deficient mice possessed lower levels of serotonin, dopamine,
13 and pyridoxal pyrophosphate, and microarray analysis of triple knockout mice demonstrated that
14 among the transcriptional targets of PAR bZIP proteins in the brain and in the liver was pyridoxal
15 kinase (Pdxk), a molecule important for the conversion of vitamin B6 derivatives into pyridoxal
16 phosphate, a coenzyme involved in neurotransmitter metabolism¹⁰. Mice deficient in bZIP-PAR
17 transcription factors are sensitive to xenobiotics, as PAR bZIP proteins control the expression of
18 multiple enzymes important for detoxification, including cytochrome p450 enzymes and
19 carboxylesterases¹¹. Triple knockout bZIP-PAR (HLF^{-/-}, TEF^{-/-}, DBP^{-/-}) mice manifest
20 significantly increased liver mass¹¹. Investigators concluded that though some of the
21 transcriptional targets dysregulated in triple knockout mice were probably direct bZIP-PAR
22 transcriptional targets, others were probably indirect targets of the constitutive androstane
23 receptor (CAR), itself a transcriptional target of bZIP-PAR transcription factors¹¹.

26 TEF can trans-activate expression of the bcl-gS gene, a shorter splice variant of the pro-
27 apoptotic bcl-g molecule with testis-specific expression¹². Etoposide and cisplatin
28 chemotherapy could induce bcl-gS transcriptional pathway in HCT-116 colorectal cancer cells
and this was accompanied by induction of TEF; silencing of TEF reduced cell death caused by

etoposide¹². In fibroblasts from PAR bZIP triple knockout mice, the pro-apoptotic molecule bik is the only BH3-only gene down-regulated, and fibroblasts from PAR bZIP (-/-) mice undergo apoptosis in response to oxidative stress at significantly lower levels than wild-type fibroblasts. TEF occupancy at the bik promoter was demonstrated, together suggesting TEF functioned to transactivate bik under conditions of oxidative stress, inducing apoptosis in that context¹³.

Differential expression of TEF in HGSC should be validated in larger and separate cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian cancers¹²⁻¹⁴ can be utilized to assess the effect of conditional expression of TEF in HGSC tumors post-development and on tumor cell proliferation and cancer progression *in vivo*. TEF now joins HLF as the second bZIP-PAR transcription factor found to be differentially expressed and down-regulated in high-grade serous ovarian cancers.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
93	8073422	4.1E-09	-7.2899714	10.7739576	0.5660 ± 0.0697	TEF	TEF, PAR bZIP transcription factor

Table 1: TEF 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 TEF 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
6379	A_23_P91757	2.57E-02	-2.5526969	-3.84939	TEF	TEF, PAR bZIP transcription factor

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

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

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