TBX3 is 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 expression the T-box transcription factor TBX3 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, TBX3.

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^{4,5}. 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 T-box
7 transcription factor TBX3 as among the most differentially expressed in HGSC tumors of the
8 ovary. TBX3 may be a gene of interest when prioritizing the study of target genes and pathways
9 for the development of novel therapeutic interventions in high-grade serous ovarian cancers.
10

11 **Methods**

12 We used microarray data from datasets GSE124766³ 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
16 expression. Log-transformation of data was auto-detected, and the NCBI generated category of
17 platform annotation was used. GSE146556 was generated using Affymetrix Human Gene 1.0
18 ST Array with $n=3$ for normal ovarian tissue and $n=40$ for tumors from patients with high-grade
19 serous ovarian cancer. GSE124766 was generated using Agilent-014850 Whole Human
20 Genome Microarray 4x44K G4112F with $n=3$ of for normal ovarian tissue and $n=8$ for tumors
21 from patients with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels
22 only for the top 250 most differentially expressed genes.
23

24 A statistical test was performed to evaluate whether TBX3 expression was significantly
25 different when comparing normal ovarian tissue from control subjects and primary tumors from
26 women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's correction. Only
27
28

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.

TBX3 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified TBX3 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, in this data set, TBX3 ranked 92 out of 29088 total transcripts (Table 2). Differential expression of TBX3 in HGSC tumors in this second microarray dataset was statistically significant (Table 1; $p=3.6E-09$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of TBX3 could be observed in tumors from a separate group of patients. We again found that TBX3 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, TBX3 ranked 2437 out of 41093 total transcripts (Table 1). Differential expression of TBX3 in HGSC tumors was statistically significant (Table 1; $p=2.81E-03$).

TBX3 is expressed at lower levels in HGSC when compared to the normal ovary.

We obtained exact mRNA expression levels for the differentially expressed TBX3 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. TBX3 was expressed at lower levels in high-grade serous ovarian cancers when compared to the normal ovary; this difference approached statistical significance (Figure 1; $p=0.0593$). We

1 calculated a mean fold change of 0.3436 ± 0.0856 in TBX3 expression when comparing HGSC
2 tumors to normal ovarian tissue (Table 1).

3 Thus, we found using published microarray data that TBX3 was among the genes most
4 differentially expressed in tumors from women with high-grade serous carcinomas and that
5 TBX3 was expressed at lower levels in HGSC tumors when compared to the normal ovary.
6

7 **Discussion**

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9 Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic analysis
10 classified HGSC into four subtypes based on gene clustering: immunoreactive, differentiated,
11 proliferative, and mesenchymal⁶. We sought to continue to describe the transcriptional
12 landscape of high-grade serous ovarian cancers⁶ and identify genes whose differential
13 expression was associated with HGSC by using published microarray data from primary tumors
14 of women diagnosed with HGSC compared to transcriptome data from normal ovarian tissue^{2,3}.
15 In both datasets analyzed, TBX3 was among the genes whose expression changed most
16 significantly when comparing the normal ovary to primary HGSC tumors.
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18 TBX3 is important for developmental processes in a variety of tissues and cell types⁷.
19 TBX3 (T8X3) is mutated in human ulnar mammary syndrome, a condition affecting development
20 of limb, apocrine, and genitals⁸. Mice deficient in Tbx3 (Tbx3 ^{-/-}) manifest defects in the
21 mammary glands, the limbs and the yolk sac; defects similar to those found in patients with
22 human ulnar mammary syndrome⁹. The fact that the mutations were predicted to give rise to
23 haploinsufficient states suggested that a threshold of Tbx3 expression was required for its
24 proper developmental function. In the atria of the heart, rate and rhythm are controlled by the
25 sinoatrial node. Expression of TBX3 marks the sinoatrial node region; deficiency of Tbx3 results
26 in partial loss of sinoatrial node-specific gene expression and over-expression of Tbx3 results in
27 repression of the atrial phenotype with cells of the atria assuming a pacemaker phenotype¹⁰. A
28 study investigating positioning of the mammary gland found Tbx3 was co-expressed along with

1 Tbx15 in the mammary gland-forming region¹¹. While Tbx3 over-expression resulted in
2 extension of the mammary gland across the dorsoventral axis, Bmp4 over-expression resulted
3 in repression of both Tbx3 and Tbx15¹¹. Authors concluded that Tbx3 and Bmp4 controlled
4 formation of the mammary gland and establishment of the dorsoventral boundary during early
5 embryogenesis. Fascinating developmental studies in chicken demonstrated Tbx3 controlled
6 specification of digit III in the four-digit chick leg¹². In conjunction with Tbx2, Tbx3 also
7 controlled specification of digit IV¹². Thus, Tbx3 is important for development and specification
8 of positioning of mammary glands, the heart, of limbs and of digits.

9
10 ET is the *Xenopus* ortholog of TBX3; ET can both activate and repress transcription¹³.
11 The T domain of T-box proteins functions as a DNA-binding domain for transactivation; ET
12 possesses a carboxy-terminal repressor domain that is conserved in human TBX2 and TBX3
13 and used for transcriptional repression¹³. TBX3 has roles in execution of pluripotency as well.
14 TBX3 could enhance the induction of pluripotency by Oct4, Sox2, Nanog (OSK), termed OSKT
15 iPS cells¹⁴. Genome-wide analysis of TBX3 binding sites revealed that Tbx3 bound both unique
16 genomic sites involved in pluripotency and reprogramming, as well as gene expression targets
17 shared with Oct4, Sox2, Nanog and Smad1¹⁴. Global gene expression profiling was unable to
18 distinguish between OSK and OSKT iPS cells¹⁴.

19
20 TBX3 is able to exert control over cell cycle, proliferation and senescence through
21 multiple mechanisms. Expression of Tbx3 in conjunction with Myc and an oncogenic Ras
22 variant, H-Ras^{Val17}, could induce transformation of mouse embryo fibroblasts¹⁵. The ability of
23 Tbx3 to act in an oncogenic fashion was found to be a result of suppression of p53 and p19^{ARF}
24 induction caused by Myc and Ras over-expression. The ability of Tbx3 to interfere with Myc-
25 induced apoptosis was localized to a carboxy-terminal repression domain. The authors
26 concluded that Tbx3 might had oncogenic potential. Regulation of cell cycle dynamics and
27 sequence by TBX3 might also be attributed to its ability to repress expression of p21^{WAF1} and
28 p14^{ARF}. TBX3 can repress transcription of p21^{WAF1} through interactions with a T-box element at
proximal to the p21^{WAF1} initiator; repression of p21^{WAF1} by Tbx3 was important for support of

1 chondrosarcoma cell proliferation in the SW1353 cell line¹⁶. In a study of breast cancers,
2 investigators found that TBX3 could interact with histone deacetylases HDAC1, HDAC2,
3 HDAC3 and HDAC5; TBX3 control of p14^{ARF} expression was mediated by interactions with
4 histones, as histone inhibition was able to reverse this control in a dose-dependent fashion¹⁶.
5 The authors suggested Tbx3 might function by recruitment of HDACs to a T-box binding site in
6 the promoter of p14^{ARF}(17).

7
8 TBX3 is implicated a number of human cancers. TBX3 is reported as over-expressed in
9 breast cancers¹⁷. Loss of E-cadherin expression is associated with invasiveness and the
10 epithelial to mesenchymal transition in cancer^{18,19}. In human melanomas, Tbx3 could occupy
11 the E-cadherin promoter and repress expression thereby, establishing that Tbx3 could directly
12 repress E-cadherin gene expression²⁰. TBX3 was expressed in a variety of melanoma cell
13 lines, and Tbx3 expression was relatively highest in metastatic tissues when compared to
14 dysplastic nevi or to primary melanotic tumors²⁰. In human and murine liver cancers, TBX3
15 expression could be activated by dysregulated Wnt signaling, and could be directly induced by
16 beta-catenin²¹. TBX3 was required for proliferation of HepG2 liver cancer cells *in vivo*, and
17 TBX3 expression was protective against apoptosis induced by doxorubicin chemotherapy²¹.
18 Though we found decreased expression of TBX3 in the tumors of patients with HGSC as
19 compared to normal ovarian tissue, one study found that expression of a truncated TBX3 variant
20 was increased in serum from patients with ovarian cancer²².
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23 Differential expression of TBX3 in HGSC should be validated in larger and separate
24 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
25 cancers²³⁻²⁵ can be utilized to assess the effect of conditional expression of TBX3 in HGSC
26 tumors post-development and on tumor cell proliferation and cancer progression *in vivo*. It is
27 not clear at this time why we observe decreased rather increased expression of TBX3 in high-
28 grade serous ovarian carcinomas.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
92	7966690	3.6E-09	-7.3290978	10.9007493	0.3436 ± 0.0856	TBX3	T-box 3

Table 1: TBX3 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 TBX3 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
2437	A_23_P13772	2.81E-03	-3.7608191	-1.69836	TBX3	T-box 3

Table 2: TBX3 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 TBX3 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: TBX3 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of TBX3 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 TBX3 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.