CBX7 is differentially expressed in high-grade serous ovarian cancers.

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Ovarian cancer is the most lethal gynecologic cancer¹⁻³. 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^{4,5}. We identified the chromobox homolog 7 (CBX7)⁶ as among the genes whose expression was most different in HGSC ovarian tumors. CBX7 expression was significantly lower in ovarian tumors relative to normal ovary. These data reveal perturbed expression of a tumor suppressor⁷ with epigenetic activity at lysine 27 of histone H3⁸ (H3K27) in high-grade serous ovarian cancers.

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

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^{2,3}. 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^{4,5} to compare global gene expression profiles between HGSC
6 ovarian tumors and that of normal ovarian tissue. We identified the gene encoding CBX7 as
7 among the most differentially expressed in HGSC tumors of the ovary. CBX7 can promote or
8 repress tumor growth in a context-dependent manner^{6,9}. CBX7 may be a gene 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.
11

12 13 **Methods**

14 We used microarray data from datasets GSE146556⁴ and GSE124766⁵ for this
15 differential gene expression analysis of high-grade serous carcinomas. The Benjamini and
16 Hochberg method of p-value adjustment was used for ranking of differential expression but raw
17 p-values were used for assessment of statistical significance of global differential expression.
18 Log-transformation of data was auto-detected, and the NCBI generated category of platform
19 annotation was used. GSE146556 was generated using Affymetrix Human Gene 1.0 ST Array
20 with n=3 for normal ovarian tissue and n=40 for tumors from patients with high-grade serous
21 ovarian cancer. GSE124766 was generated using Agilent-014850 Whole Human Genome
22 Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors from
23 patients with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels only
24 for the top 250 most differentially expressed genes.
25

26 A statistical test was performed to evaluate whether CBX7 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^{4,5} 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.

CBX7 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified CBX7 as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue (Table 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, CBX7 ranked 63 out of 29088 total transcripts (Table 1). Differential expression of CBX7 in HGSC tumors was statistically significant (Table 1; $p=8.53E-10$).

We analyzed a second microarray dataset generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of CBX7 could be observed in tumors from a different group of patients. We again found that CBX7 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, CBX7 ranked 227 out of 41093 total transcripts (Table 2). Differential expression of CBX7 in HGSC tumors was statistically significant (Table 2; $p=1.63E-05$).

CBX7 is expressed at significantly lower levels in HGSC when compared to the normal ovary.

We then obtained exact mRNA expression levels for the differentially expressed CBX7 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. CBX7 was expressed at significantly lower levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 1; $p<0.0001$). We calculated a mean fold change of

0.3824 ± 0.0855 in CBX7 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

Analysis of CBX7 differential expression in a separate dataset demonstrated that CBX7 was again expressed at significantly lower levels in primary tumors from women diagnosed with high-grade serous ovarian cancer when compared to normal ovarian tissue (Figure 2; $p < 0.0001$). We calculated a mean fold change of 0.7819 ± 0.0538 in CBX7 expression when comparing HGSC tumors to normal ovarian tissue (Table 2).

Thus, we found using published microarray data that CBX7 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that CBX7 expression was significantly lower in HGSC tumors when compared to the normal ovary.

Discussion

Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic analysis classified HGSC into four subtypes based on gene clustering: immunoreactive, differentiated, proliferative, and mesenchymal¹⁰. We sought to continue to describe the transcriptional landscape of high-grade serous ovarian cancers¹⁰ 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^{4, 5}. In both datasets analyzed, CBX7 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

Chromobox 7 is a protein with epigenetic activity⁷ that is important for the maintenance of pluripotency in embryonic stem cells (ESC)^{11,12}. CBX7 is the main CBX component of the polycomb repressive complex 1 (PRC1)¹² and has the ability to read tri-methylation of lysine 27 on histone H3 (H3K27me3) by interactions through its chromodomain¹³. CBX7 might also be involved in reading of tri-methylation of lysine 9 on histone H3 (H3K9me3)¹⁴. CBX7 has RNA-binding properties, and binding of the long non-coding RNA (lncRNA) ANRIL by CBX7 is important for epigenetic regulation of gene expression at the INK4b/ARF/INK4a tumor

1 suppressor locus⁸. Disrupting the interaction between the CBX7 chromodomain and either RNA
2 or H3K27me3 by single point mutation was sufficient to interfere with the ability of CBX7 to
3 inhibit gene expression at the INK4/ARF locus and control senescence⁸. The authors
4 suggested that ANRIL functions in the recruitment of PRC1 to the INK4b/ARF/INK4a locus
5 through interactions between ANRIL and CBX1, mirroring *cis* recruitment of PRC2 to the HOXC
6 locus by interactions between the lncRNA HOTAIR and EZH2⁸. Activity of CBX7 at the INK4A/
7 ARF locus is important for the ability of CBX7 to extend the lifespan of cultured cells⁶. A study
8 assessing the overlapping and distinct functions of the CBX family found that CBX7 was
9 important for the maintenance of pluripotency of ESC, sharing occupancy with Ring1B at 96.7%
10 of target genes¹¹. CBX proteins functioned in a regulatory loop in orchestrating the balance
11 between pluripotency and differentiation, as CBX2 and CBX4 were required for lineage
12 specification, and CBX7 repressed expression of CBX2 and CBX4 to PRC1 target genes to
13 maintain a pluripotent cell state¹¹. A specific requirement for CBX7 in hematopoietic stem cell
14 differentiation has also been demonstrated¹⁵. Genes targeted by CBX7 were repressed in HSC
15 but activated in progenitor cells, suggesting that CBX7 preserved HSC identity by repressing
16 activation of progenitor cell-specific gene expression¹⁵.

17
18 CBX7 has been demonstrated to be a tumor suppressor⁶. However, the role of CBX7 in
19 cancer is not straightforward and appears to be context dependent as in certain tissues, over-
20 expression rather than loss of CBX7 promotes tumor growth⁹. CBX7 is extensively implicated in
21 cancers across tissue type: in thyroid cancer, prostate cancer, glioblastoma, pancreatic cancer,
22 and in ovarian clear cell adenocarcinoma¹⁶⁻²². Mice deficient in CBX7 develop lung and liver
23 carcinomas in adulthood⁷. Authors demonstrated that CBX7 occupied the promoter of Cyclin E1
24 (CCNE1) along with HDAC2 and that in lung carcinomas, loss of CBX7 protein correlated with
25 increased expression of CCNE1⁷. Conversely, enforced expression of CBX7 in the lymphoid
26 compartment results in development of mature T-cell lymphomas and cooperates with Myc in
27 Eμ-myc transgenic mice to produce B-cell lymphomas⁷. CBX7 expression is increased in
28 germinal center lymphocytes and germinal center-derived follicular lymphomas⁷. Increased

1 expression of CBX7 correlated with higher expression of cMyc⁷. One recent study showed that
2 in secondary epithelial ovarian cancer cells (sEOC), CBX7 could prevented the binding of
3 transcriptional activator TWIST to E-box motifs at transcriptional targets, and deletion of CBX7
4 in sEOC allowed activation of the TWIST transcriptional network and transition to a
5 mesenchymal cell state, supporting tumor growth *in vivo*¹⁷. A separate study analyzing tumors
6 from patients with ovarian clear cell adenocarcinoma (OCCA) found that patients with CBX7-
7 positive tumors, as assessed by immunohistochemistry, had significantly shorter progression-
8 free and overall survival¹⁶. Knocking down CBX7 in two OCCA cell lines resulted in increased
9 cell death through apoptosis. Cells depleted of CBX7 manifested increased levels of
10 messenger RNA for genes that encode proteins involved in apoptosis and in particular, the
11 tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)¹⁶.

13 Differential expression of CBX7 in HGSC should be validated in larger and separate
14 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
15 cancers²³⁻²⁵ can be utilized to assess the effect of conditional expression of CBX7 on tumor cell
16 proliferation and cancer progression *in vivo*. We found here that a tumor suppressor with
17 epigenetic activity was among the genes most differentially expressed in tumors from women
18 diagnosed with the most lethal gynecological cancer.

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Rank	ID	p-value	t	B	Fold change	Gene	Gene name
63	8076185	8.53E-10	-7.7576979	12.2825886	0.3824 ± 0.0855	CBX7	chromobox 7

Table 1: CBX7 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 CBX7 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Fold change	Gene	Gene name
227	A_23_P250735	1.63E-05	-6.9856384	3.39302	0.7819 ± 0.0538	CBX7	chromobox 7

Table 2: CBX7 is among the most differentially expressed genes in a tumor dataset from patients with 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 CBX7 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Figure 1: CBX7 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of CBX7 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 CBX7 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.

Figure 2: CBX7 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of CBX7 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 CBX7 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.