Differential expression of SERTAD4 antisense RNA 1 in human epithelial ovarian cancer.

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Epithelial ovarian cancer (EOC) is the most lethal gynecologic cancer (1). We performed discovery of genes associated with epithelial ovarian cancer and of the high-grade serous ovarian cancer (HGSC) subtype, using published microarray data (2, 3) to compare global gene expression profiles of normal ovary or fallopian tube with that of primary tumors from women diagnosed with epithelial ovarian cancer or HGSC. We identified both coding and non-coding SERTAD4 transcripts as among those whose expression was most different in epithelial ovarian cancer as compared to the normal fallopian tube or ovary. Expression of a SERTAD4 antisense transcript, SERTAD4-AS1, was significantly lower in high-grade serous ovarian tumors relative to normal fallopian tube. SERTAD4-AS1 expression correlated with progression-free survival in patients with ovarian cancer. These data indicate that expression of SERTAD4-AS1 is perturbed in epithelial ovarian cancers broadly and in ovarian cancers of the HGSC subtype. SERTAD4 coding and non-coding RNAs may be relevant to pathways underlying ovarian cancer initiation (transformation) or progression.

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

The five-year survival rate for women diagnosed with high-grade serous ovarian cancer is between 30-40% and has not changed significantly in decades (4, 5). The development of novel, targeted therapeutics to treat HGSC can be facilitated by an enhanced understanding of the transcriptional behavior of ovarian tumors relative to that of the normal ovary. We mined published microarray data (2, 3) to compare global gene expression profiles between human ovarian tumors, including that of the HGSC subtype, and that of normal ovarian and fallopian tissue. We identified the gene encoding SERTAD4 antisense RNA 1, SERTAD4-AS1, as among the most differentially expressed in HGSC tumors of the ovary and in epithelial ovarian cancer broadly. SERTAD4-AS1 may be a gene of interest when prioritizing the study of target genes and pathways for the development of novel therapeutic interventions in epithelial ovarian cancer and specifically in high-grade serous ovarian cancers.

Methods

We used microarray data from datasets GSE124766 (2) and GSE146556 (3) for this differential gene expression analysis of epithelial ovarian cancer in conjunction with GEO2R. The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of statistical significance of global differential expression. Log transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. GSE124766 (2) was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=3$ normal fallopian tube tissue (control) and $n=8$ for tumors from patients with high-grade serous ovarian cancer. GSE146556 (3) was generated using Affymetrix Human Gene 1.0 ST Array with $n=3$ for normal ovarian tissue and $n=40$ for tumors from patients with epithelial ovarian cancer: $n=26$ high-grade serous ovarian cancer; $n=3$ carcinosarcoma, $n=2$ clear cell, $n=1$ endometrioid, $n=1$ granulosa cell, $n=1$ immature teratoma, $n=4$ mixed, and $n=2$ mucinous; analysis was performed using platform GPL6244. GEO2R provides mRNA expression levels only for the top 250 most differentially expressed genes transcriptome-wide.

A statistical test was performed to evaluate whether SERTAD4-AS1 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. We used PRISM for all statistical analyses (Version 8.4.0)(455). For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool (6) for correlation of SERTAD4-AS1 mRNA expression levels with progression-free survival (PFS) in $n=614$ ovarian cancer patients.

Results

We mined published microarray data (2, 3) to identify differentially expressed genes in epithelial ovarian cancer and specifically 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.

SERTAD4-AS1 is differentially expressed in ovarian tumors from women diagnosed with HGSC

We identified SERTAD4-AS1 as among the genes whose expression was most different when comparing primary HGSC tumors to normal fallopian tube tissue (2) (Chart 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal fallopian tube, in this data set, SERTAD4-AS1 ranked 153 out of 40481 total transcripts (Chart 1), equating to 99.6% differential expression. Differential expression of SERTAD4-AS1 in HGSC tumors was statistically significant (Chart 1; $p=6.93E-06$).

We analyzed a second microarray dataset (3) generated using normal ovarian tissues and tumors from women diagnosed with epithelial ovarian cancer (EOC) to determine whether differential expression of SERTAD4-AS1 could be observed in tumors from a different group of patients, which included HGSC tumors.

We found that SERTAD4 was also differentially expressed in epithelial ovarian cancers broadly (Chart 2). When sorting all of the transcripts measured by microarray based on change in expression between tumors from patients with epithelial ovarian cancers and the normal ovary, SERTAD4 ranked 14456 out of 29088 total transcripts (Chart 2), equating to 50.3% differential expression. Differential expression of SERTAD4-AS1 in EOC was not deemed statistically significant (Chart 2; $p=2.42E-01$). Thus, both coding and non-coding SERTAD4 transcripts are differentially expressed in human epithelial ovarian cancer.

SERTAD4-AS1 is expressed at significantly lower levels in HGSC when compared to the normal fallopian tubes.

We then extracted exact mRNA expression levels for the differentially expressed SERTAD4-AS1 transcript from both normal fallopian tube tissue and from epithelial ovarian tumors. SERTAD4-AS1 was expressed at significantly lower levels in high-grade serous ovarian cancers when compared to the normal fallopian tube (Figure 1; $p<0.0001$). We calculated a mean fold change of 0.75 in SERTAD4-AS1 expression when comparing HGSC tumors to normal fallopian tissue, as SERTAD4-AS1 was expressed at 12.82 ± 0.41 arbitrary units (AU) in the fallopian tubes but at 9.60 ± 0.72 AU in tumors of the ovary.

SERTAD4-AS1 tumor expression is correlated with survival outcomes in patients with ovarian cancer.

We performed Kaplan Meier survival analysis (6) to evaluate whether SERTAD4-AS1 tumor expression correlated with survival outcomes in patients with ovarian cancer. SERTAD4-AS1 primary tumor expression was correlated with progression-free survival (PFS) in patients with ovarian cancer, in the lower quartile, in the lower tertile, in the upper tertile and in the upper quartile (Figure 2).

SERTAD4-AS1 expression was a negative prognostic indicator in patients with ovarian cancer, in the lower quartile. While median PFS was 20 months for ovarian cancer patients with low tumor expression of SERTAD4-AS1, median PFS was 16.07 months for ovarian cancer patients with high tumor expression of SERTAD4-AS1 (Chart 3). Correlation of PFS with SERTAD4-AS1 tumor expression in patients with ovarian cancer, in the lower quartile, was statistically significant (Figure 2: log rank p-value: 0.0056; hazard ratio: 1.37 (1.09-1.71)).

SERTAD4-AS1 expression was also a negative prognostic indicator in patients with ovarian cancer, in the lower tertile. While median PFS was 20 months for ovarian cancer patients with low tumor expression of SERTAD4-AS1, median PFS was 16 months for ovarian cancer patients with high tumor expression of SERTAD4-AS1 (Chart 3). Correlation of PFS with SERTAD4-AS1 tumor expression in patients with ovarian cancer, in the lower tertile, was statistically significant (Figure 2: log rank p-value: 0.00089; hazard ratio: 1.41 (1.15-1.73)).

SERTAD4-AS1 expression was also a negative prognostic indicator in patients with ovarian cancer, in the upper tertile. While median PFS was 18.79 months for ovarian cancer patients with low tumor expression of SERTAD4-AS1, median PFS was 14 months for ovarian cancer patients with high tumor expression of SERTAD4-AS1 (Chart 3). Correlation of PFS with SERTAD4-AS1 tumor expression in

1 patients with ovarian cancer, in the upper tertile, was statistically significant (Figure 2: log rank p-value:
2 0.0019; hazard ratio: 1.4 (1.13-1.73)).

3 SERTAD4-AS1 primary tumor expression was not correlated with progression-free survival in the
4 upper tertile (Figure 2: log rank p-value: 0.15; hazard ratio: 1.16 (0.95-1.41)).

5 Thus, we found using published microarray data (2, 3) that SERTAD4-AS1 was among the genes
6 most differentially expressed in tumors from women with high-grade serous carcinomas, and in epithelial
7 ovarian cancer broadly. SERTAD4-AS1 expression was significantly lower in HGSC tumors when
8 compared to the normal fallopian tubes, and SERTAD4-AS1 tumor expression was correlated with
9 progression-free survival in patients with ovarian cancer.

10 **Discussion**

11 Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic analysis
12 classified HGSC into four subtypes based on gene clustering: immunoreactive, differentiated,
13 proliferative, and mesenchymal (7). We sought to describe the transcriptional landscape of epithelial
14 ovarian cancers in an unbiased fashion by using published microarray data from primary tumors of women
15 diagnosed with HGSC compared to transcriptome data from normal ovarian and fallopian tube tissue (2,
16 3). In both datasets analyzed (2, 3), SERTAD4-AS1 was among the genes whose expression changed most
17 significantly when comparing normal gynecological tissue to ovarian tumors, from women with epithelial
18 ovarian cancer as well as specifically from women with HGSC. SERTAD4-AS1 may be a molecule of
19 relevance in understanding transformation or disease progression in human epithelial ovarian cancer.
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Rank: 153
Probe ID: A_32_P207169
p-value: 6.93E-06
t: 7.6243466
B: 4.22174
Gene: SERTAD4-AS1
Gene name: SERTAD4 antisense RNA 1

Chart 1: SERTAD4-AS1 is differentially expressed in high-grade serous ovarian carcinomas (HGSC).

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 gene and gene name are listed in this chart.

Rank: 14456
Probe ID: 7909503
p-value: 2.42E-01
t: 1.186762
B: -5.8412009
Gene: SERTAD4-AS1
Gene name: SERTAD4 antisense RNA 1

Chart 2: SERTAD4-AS1 is differentially expressed in epithelial ovarian cancers, including high-grade serous ovarian carcinomas (HGSC).

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 gene and gene name are listed in this chart.

Figure 1: SERTAD4-AS1 is expressed at significantly lower levels in HGSC tumors when compared to normal fallopian tube.

The mRNA expression of SERTAD4-AS1 in normal fallopian tube tissues (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 SERTAD4-AS1 expression between the normal fallopian tube and primary tumors from patients with HGSC, a *p*-value, listed above.

Figure 2: SERTAD4-AS1 tumor expression correlates with progression-free survival in patients with ovarian cancer.

Depicted in this Kaplan-Meier plot is the probability of progression-free survival (PFS) for n=614 patients, in the lower quartile (top left), in the lower tertile (top right), in the upper tertile (bottom left), and in the upper quartile (bottom right), based on primary tumor expression of SERTAD4-AS1 (low and high). The log rank *p*-value denoting statistical significance of difference in progression-free survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on SERTAD4-AS1 expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Lower quartile

Low SERTAD4-AS1 expression: 20 months

High SERTAD4-AS1 expression: 16.07 months

Lower tertile

Low SERTAD4-AS1 expression: 20 months

High SERTAD4-AS1 expression: 16 months

Upper quartile

Low SERTAD4-AS1 expression: 18.79 months

High SERTAD4-AS1 expression: 14 months

Chart 3: Progression-free survival is superior in ovarian cancer patients with low tumor expression of SERTAD4-AS1.

The median progression-free survival of $n=614$ ovarian cancer patients based on stratification into low or high tumor expression of SERTAD4-AS1 is listed in this chart, in the lower quartile, in the lower tertile, in the upper quartile and in the upper tertile.