Differential expression of HOXA cluster antisense RNA 3 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 and public 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 the gene encoding HOXA cluster antisense RNA 3, HOXA-AS3, as among the genes whose expression was most different in epithelial ovarian cancer as compared to the normal fallopian tube. HOXA-AS3 expression was significantly lower in high-grade serous ovarian tumors relative to normal fallopian tube. HOXA-AS3 expression correlated with overall survival in patients with ovarian cancer. These data indicate that expression of HOXA-AS3 is perturbed in epithelial ovarian cancers broadly and in ovarian cancers of the HGSC subtype. HOXA-AS3 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 and public 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 HOXA cluster antisense RNA 3, HOXA-AS3, as among the most differentially expressed in HGSC tumors of the ovary and in epithelial ovarian cancer broadly. HOXA-AS3 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 GSE126308 (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. GSE126308 (3) was generated using Affymetrix Human Transcriptome Array 2.0 with $n=20$ tumors from high-grade serous adenocarcinoma (HGSC) patients classified as early in disease progression and $n=30$ for tumors from HGSC patients classified as “late” in disease progression; analysis was performed using platform GPL17586.

A statistical test was performed to evaluate whether HOXA-AS3 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 HOXA-AS3 mRNA expression levels with overall survival (OS) in $n=655$ ovarian cancer patients.

Results

We mined published and public 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.

HOXA-AS3 is differentially expressed in ovarian tumors from women diagnosed with HGSC

We identified HOXA-AS3 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, HOXA-AS3 ranked 28 out of 40481 total transcripts (Chart 1), equating to 99.9% differential expression. Differential expression of HOXA-AS3 in HGSC tumors was statistically significant (Chart 1; $p=7.25E-08$).

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 we could validate differential expression of HOXA-AS3 using independent microarray data.

We found that another HOXA antisense RNA transcript, HOXA-AS4 was differentially expressed in epithelial ovarian cancers when analyzing based on disease progression (Chart 2). When sorting all of the transcripts measured by microarray based on change in expression between tumors from patients early in disease progression and tumors from patients late in disease progression, HOXA-AS4 ranked 11342 out of 70523 total transcripts (Chart 2), equating to 83.9% differential expression. Differential expression of HOXA-AS4 in EOC based on disease progression was statistically significant (Chart 2; $p=4.72E-02$).

HOXA-AS3 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 HOXA-AS3 transcript from both normal fallopian tube tissue and from epithelial ovarian tumors. HOXA-AS3 was expressed at significantly lower levels in high-grade serous ovarian cancers when compared to the normal fallopian tube (Figure 1; $=0.0006$). We calculated a mean fold change of 0.63 in HOXA-AS3 expression when comparing HGSC tumors to normal fallopian tissue, as HOXA-AS3 was expressed at 8.75 ± 0.39 arbitrary units (AU) in the fallopian tubes but at 5.55 ± 0.37 AU in tumors of the ovary.

HOXA-AS3 tumor expression is correlated with survival outcomes in patients with ovarian cancer.

We performed Kaplan Meier survival analysis (6) to evaluate whether HOXA-AS3 tumor expression correlated with survival outcomes in patients with ovarian cancer. HOXA-AS3 primary tumor expression was correlated with overall survival (OS) in patients with ovarian cancer, in the upper tertile (Figure 2)

HOXA-AS3 expression was a negative prognostic indicator in patients with ovarian cancer, in the upper tertile. While median OS was 46 months for ovarian cancer patients with low tumor expression of HOXA-AS3, median OS was 37.47 months for ovarian cancer patients with high tumor expression of HOXA-AS3 (Chart 3). Correlation of OS with HOXA-AS3 tumor expression in patients with ovarian cancer, in the upper tertile, approached statistical significance (Figure 2: log rank p-value: 0.062; hazard ratio: 1.22 (0.99-1.5)).

HOXA-AS3 primary tumor expression was not correlated with overall survival in the lower quartile (Figure 2: log rank p-value: 0.48; hazard ratio: 1.1 (0.85-1.41)), in the lower tertile (Figure 2: log rank p-value: 0.34; hazard ratio: 1.12 (0.89-1.4)), or in the upper quartile (Figure 2: log rank p-value: 0.23; hazard ratio: 1.15 (0.91-1.44)).

Thus, we found using published and public microarray data (2, 3) that a HOXA antisense transcript, HOXA-AS3 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas, and in epithelial ovarian cancer broadly. HOXA-AS3 expression was significantly lower in HGSC tumors when compared to the normal fallopian tubes, and HOXA-AS3 tumor expression was correlated with overall survival in patients with ovarian cancer.

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 mesenchymal (7). We sought to describe the transcriptional landscape of epithelial

ovarian cancers in an unbiased fashion by using published and public microarray data from primary tumors of women diagnosed with HGSC compared to transcriptome data from normal ovarian and fallopian tube tissue (2, 3). In both datasets analyzed (2, 3), HOXA-AS3 was among the genes whose expression changed most significantly when comparing normal gynecological tissue to ovarian tumors, from women with epithelial ovarian cancer as well as specifically from women with HGSC. HOXA-AS4 was identified as differentially expressed when comparing stage I tumors to stage III and stage IV epithelial ovarian cancers (differentially expressed based on disease progression). HOXA antisense transcription may be of relevance in understanding transformation or disease progression in human epithelial ovarian cancer.

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Rank: 28

Probe ID: A_24_P829209

p-value: 7.25E-08

t: 11.7864073

B: 8.4337

Gene: HOXA-AS3

Gene name: HOXA cluster antisense RNA 3

Chart 1: HOXA-AS3 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: 11342
Probe ID: TC07000168.hg.1
p-value: 4.72E-02
t: -2.03
B: -3.95688
Gene: HOXA-AS4
Gene name: HOXA cluster antisense RNA 4

Chart 2: HOXA-AS4 is differentially expressed in epithelial ovarian cancers when comparing tumors based on degree of disease progression - "early" versus "late".

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: HOXA-AS3 is expressed at significantly lower levels in HGSC tumors when compared to normal fallopian tube.

The mRNA expression of HOXA-AS3 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 HOXA-AS3 expression between the normal fallopian tube and primary tumors from patients with HGSC, a *p*-value, listed above.

Figure 2: HOXA-AS3 tumor expression correlates with overall survival in patients with ovarian cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for n=655 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 HOXA-AS3 (low and high). The log rank *p*-value denoting statistical significance of difference in overall 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 HOXA-AS3 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.

Upper tertile

Low HOXA-AS3 expression: 46 months

High HOXA-AS3 expression: 37.47 months

Chart 3: Overall survival is superior in ovarian cancer patients with low tumor expression of HOXA-AS3.

The median overall survival of $n=655$ ovarian cancer patients based on stratification into low or high tumor expression of HOXA-AS3 is listed in this chart, in the upper tertile.