Differential expression of PAGE family member 4 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 PAGE family member 4, PAGE4, as among the genes whose expression was most different in epithelial ovarian cancer as compared to the normal fallopian tube. PAGE4 expression was significantly lower in high-grade serous ovarian tumors relative to normal fallopian tube. PAGE4 expression correlated with progression-free survival in patients with p53 mutant ovarian cancer. These data indicate that expression of PAGE4 is perturbed in epithelial ovarian cancers broadly and in ovarian cancers of the HGSC subtype. PAGE4 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 PAGE family member 4, PAGE4, as among the most differentially expressed in HGSC tumors of the ovary and in epithelial ovarian cancer broadly. PAGE4 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 PAGE4 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 PAGE4 mRNA expression levels with progression-free survival (PFS) in $n=483$ 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.

PAGE4 is differentially expressed in ovarian tumors from women diagnosed with HGSC

We identified PAGE4 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, PAGE4 ranked 69 out of 40481 total transcripts (Chart 1), equating to 99.8% differential expression. Differential expression of PAGE4 in HGSC tumors was statistically significant (Chart 1; $p=7.28E-07$).

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 PAGE4 could be observed in tumors from a different group of patients, which included HGSC tumors.

We found that PAGE4 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, PAGE4 ranked 4009 out of 70523 total transcripts (Chart 2), equating to 94.3% differential expression. Differential expression of PAGE4 in EOC based on disease progression was statistically significant (Chart 2; $p=1.53E-02$).

PAGE4 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 PAGE4 transcript from both normal fallopian tube tissue and from epithelial ovarian tumors. PAGE4 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 0.52 in PAGE4 expression when comparing HGSC tumors to normal fallopian tissue, as PAGE4 was expressed at 10.80 ± 1.02 arbitrary units (AU) in the fallopian tubes but at 5.61 ± 0.84 AU in tumors of the ovary.

PAGE4 tumor expression is correlated with survival outcomes in patients with ovarian cancer.

We performed Kaplan Meier survival analysis (6) to evaluate whether PAGE4 tumor expression correlated with survival outcomes in patients with ovarian cancer. PAGE4 primary tumor expression was correlated with progression-free survival (PFS) in patients with p53 mutant ($p53^{mut}$) ovarian cancer, in the lower tertile, in the upper tertile, and in the upper quartile (Figure 2)

PAGE4 expression was a negative prognostic indicator in patients with $p53^{mut}$ ovarian cancer, in the lower tertile. While median PFS was 19.8 months for ovarian cancer patients with low tumor expression of PAGE4, median PFS was 16.93 months for ovarian cancer patients with high tumor expression of PAGE4 (Chart 3). Correlation of PFS with PAGE4 tumor expression in patients with $p53^{mut}$ ovarian cancer, in the lower tertile, was statistically significant (Figure 2: log rank p-value: 0.02; hazard ratio: 1.33 (1.05-1.69)).

PAGE4 expression was a negative prognostic indicator in patients with $p53^{mut}$ ovarian cancer, in the upper tertile. While median PFS was 18.27 months for ovarian cancer patients with low tumor expression of PAGE4, median PFS was 16.07 months for ovarian cancer patients with high tumor expression of PAGE4 (Chart 3). Correlation of PFS with PAGE4 tumor expression in patients with $p53^{mut}$ ovarian cancer, in the upper tertile, approached statistical significance (Figure 2: log rank p-value: 0.064; hazard ratio: 1.24 (0.99-1.57)).

PAGE4 expression was a negative prognostic indicator in patients with $p53^{mut}$ ovarian cancer, in the upper quartile. While median PFS was 18.27 months for ovarian cancer patients with low tumor expression of PAGE4, median PFS was 15.77 months for ovarian cancer patients with high tumor expression of PAGE4 (Chart 3). Correlation of PFS with PAGE4 tumor expression in patients with $p53^{mut}$ ovarian cancer, in the upper quartile, trended towards statistical significance (Figure 2: log rank p-value: 0.084; hazard ratio: 1.24 (0.97-1.59)).

PAGE4 primary tumor expression was not correlated with progression-free survival in the lower quartile (Figure 2: log rank p-value: 0.15; hazard ratio: 1.2 (0.93-1.55)) in patients with p53 mutant ovarian cancer.

1 Thus, we found using published and public microarray data (2, 3) that PAGE4 was among the
2 genes most differentially expressed in tumors from women with high-grade serous carcinomas, and in
3 epithelial ovarian cancer broadly. PAGE4 expression was significantly lower in HGSC tumors when
4 compared to the normal fallopian tubes, and PAGE4 tumor expression was correlated with
progression-free survival in patients with p53 mutant ovarian cancer.

5 **Discussion**

6 Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic analysis
7 classified HGSC into four subtypes based on gene clustering: immunoreactive, differentiated,
8 proliferative, and mesenchymal (7). We sought to describe the transcriptional landscape of epithelial
9 ovarian cancers in an unbiased fashion by using published and public microarray data from primary
10 tumors of women diagnosed with HGSC compared to transcriptome data from normal ovarian and
11 fallopian tube tissue (2, 3). In both datasets analyzed (2, 3), PAGE4 was among the genes whose
12 expression changed most significantly when comparing normal gynecological tissue to ovarian tumors,
13 from women with epithelial ovarian cancer as well as specifically from women with HGSC. PAGE4 may
14 be a molecule of relevance in understanding transformation or disease progression in human epithelial
15 ovarian cancer.
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Rank: 69
Probe ID: A_24_P254506
p-value: 7.28E-07
t: 9.5038898
B: 6.35518
Gene: PAGE4
Gene name: PAGE family member 4

Chart 1: PAGE4 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: 4009
Probe ID: TC0X000276.hg.1
p-value: 1.53E-02
t: -2.51
B: -3.05427
Gene: PAGE4
Gene name: PAGE family member 4

Chart 2: PAGE4 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: PAGE4 is expressed at significantly lower levels in HGSC tumors when compared to normal fallopian tube.

The mRNA expression of PAGE4 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 PAGE4 expression between the normal fallopian tube and primary tumors from patients with HGSC, a *p*-value, listed above.

Figure 2: PAGE4 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=483 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 PAGE4 (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 PAGE4 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 tertile

Low PAGE4 expression: 19.8 months

High PAGE4 expression: 16.93 months

Upper tertile

Low PAGE4 expression: 18.27 months

High PAGE4 expression: 16.07 months

Upper quartile

Low PAGE4 expression: 18.27 months

High PAGE4 expression: 15.77 months

Chart 3: Progression-free survival is superior in ovarian cancer patients with low tumor expression of PAGE4.

The median progression-free survival of $n=483$ ovarian cancer patients based on stratification into low or high tumor expression of PAGE4 is listed in this chart, in the lower tertile, upper tertile and upper quartile.