Differential expression of cyclin dependent kinase inhibitor 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 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 cyclin dependent kinase inhibitor 3, CDKN3, as among the genes whose expression was most different in epithelial ovarian cancer as compared to the normal fallopian tube. CDKN3 expression was significantly higher in high-grade serous ovarian tumors relative to normal fallopian tube. CDKN3 expression correlated with overall survival in patients with ovarian cancer. These data indicate that expression of CDKN3 is perturbed in epithelial ovarian cancers broadly and in ovarian cancers of the HGSC subtype. CDKN3 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 cyclin dependent kinase inhibitor 3, CDKN3, as among the most differentially expressed in HGSC tumors of the ovary and in epithelial ovarian cancer broadly. CDKN3 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 CDKN3 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 CDKN3 mRNA expression levels with overall survival (OS) in $n=655$ 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.

CDKN3 is differentially expressed in ovarian tumors from women diagnosed with HGSC

We identified CDKN3 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, CDKN3 ranked 190 out of 40481 total transcripts (Chart 1), equating to 99.5% differential expression. Differential expression of CDKN3 in HGSC tumors was statistically significant (Chart 1; $p=1.59E-05$).

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 CDKN3 could be observed in tumors from a different group of patients, which included HGSC tumors.

We found that CDKN3 was 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, CDKN3 ranked 5011 out of 29088 total transcripts (Chart 2), equating to 82.8% differential expression. Differential expression of CDKN3 in EOC was statistically significant (Chart 2; $p=1.55E-02$).

CDKN3 is expressed at significantly higher levels in HGSC when compared to the normal fallopian tubes.

We then extracted exact mRNA expression levels for the differentially expressed CDKN3 transcript from both normal fallopian tube tissue and from epithelial ovarian tumors. CDKN3 was expressed at significantly higher levels in high-grade serous ovarian cancers when compared to the normal fallopian tube (Figure 1; $p=0.0052$). We calculated a mean fold change of 1.45 in CDKN3 expression when comparing HGSC tumors to normal fallopian tissue, as CDKN3 was expressed at 6.46 ± 0.68 arbitrary units (AU) in the fallopian tubes but at 9.40 ± 0.58 AU in tumors of the ovary.

CDKN3 tumor expression is correlated with survival outcomes in patients with ovarian cancer.

We performed Kaplan Meier survival analysis (6) to evaluate whether CDKN3 tumor expression correlated with survival outcomes in patients with ovarian cancer. CDKN3 primary tumor expression was correlated with overall survival (OS) in patients with ovarian cancer, in the lower quartile (Figure 2). CDKN3 expression was a negative prognostic indicator in patients with ovarian cancer. While median OS was 57 months for ovarian cancer patients with low tumor expression of CDKN3, median OS was 40.1 months for ovarian cancer patients with high tumor expression of CDKN3 (Chart 3). Correlation of OS with CDKN3 tumor expression in patients with ovarian cancer, in the lower quartile, was statistically significant (Figure 2: log rank p-value: 0.002; hazard ratio: 1.48 (1.15-1.91)).

CDKN3 primary tumor expression also correlated with overall survival (OS) in patients with ovarian cancer in the lower tertile (Figure 2). CDKN3 expression was a negative prognostic indicator in patients with ovarian cancer. While median OS was 50.03 months for ovarian cancer patients with low tumor expression of CDKN3, median OS was 41 months for ovarian cancer patients with high tumor expression of CDKN3 (Chart 3). Correlation of PFS with CDKN3 tumor expression in patients with ovarian cancer, in the lower tertile, approached statistical significance (Figure 2: log rank p-value: 0.065; hazard ratio: 1.23 (0.99-1.53)). CDKN3 primary tumor expression was not correlated with overall survival in the upper tertile (Figure 2: log rank p-value: 0.22; hazard ratio: 1.14 (0.92-1.41)), or in the upper quartile (Figure 2: log rank p-value: 0.34; hazard ratio: 1.12 (0.89)).

Thus, we found using published microarray data (2, 3) that CDKN3 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas, and in epithelial ovarian cancer broadly. CDKN3 expression was significantly higher in HGSC tumors when compared to the normal fallopian tubes, and CDKN3 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 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), CDKN3 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. CDKN3 may be a molecule of relevance in understanding transformation or disease progression in human epithelial ovarian cancer.

References

1. Guppy, A.E., Nathan, P.D. and Rustin, G.J., 2005. Epithelial ovarian cancer: a review of current management. *Clinical Oncology*, 17(6), pp.399-411.
2. Zhang, W., Klinkebiel, D., Barger, C.J., Pandey, S., Guda, C., Miller, A., Akers, S.N., Odunsi, K. and Karpf, A.R., 2020. Global DNA hypomethylation in epithelial ovarian cancer: passive demethylation and association with genomic instability. *Cancers*, 12(3), p.764.
3. Hoffmann, K., Berger, H., Kulbe, H., Thillainadarasan, S., Mollenkopf, H.J., Zemojtel, T., Taube, E., Darb-Esfahani, S., Mangler, M., Sehouli, J. and Chekerov, R., 2020. SChart expansion of high-grade serous ovarian cancer organoids requires a low-Wnt environment. *The EMBO journal*, 39(6), p.e104013.
4. Bowtell, D.D., Böhm, S., Ahmed, A.A., Aspuria, P.J., Bast Jr, R.C., Beral, V., Berek, J.S., Birrer, M.J., Blagden, S., Bookman, M.A. and Brenton, J.D., 2015. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. *Nature reviews Cancer*, 15(11), pp.668-679.
5. Vaughan, S., Coward, J.I., Bast, R.C., Berchuck, A., Berek, J.S., Brenton, J.D., Coukos, G., Crum, C.C., Drapkin, R., Etemadmoghadam, D. and Friedlander, M., 2011. Rethinking ovarian cancer: recommendations for improving outcomes. *Nature Reviews Cancer*, 11(10), pp.719-725.
6. Györffy, B., Lánckzy, A. and Szállási, Z., 2012. Implementing an online tool for genomewide validation of survival-associated biomarkers in ovarian-cancer using microarray data from 1287 patients. *Endocrine-related cancer*, 19(2), pp.197-208.
7. Cancer Genome Atlas Research Network, 2011. Integrated genomic analyses of ovarian carcinoma. *Nature*, 474(7353), p.609.

Rank: 190

Probe ID: A_23_P48669

p-value: 1.59E-05

t: -7.3956335

B: 3.4722

Gene: CDKN3

Gene name: cyclin dependent kinase inhibitor 3

Chart 1: CDKN3 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: 5011

Probe ID: 7974404

p-value: 1.55E-02

t: -2.5183708

B: -3.5712771

Gene: CDKN3

Gene name: cyclin dependent kinase inhibitor 3

Chart 2: CDKN3 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: CDKN3 is expressed at significantly higher levels in HGSC tumors when compared to normal fallopian tube.

The mRNA expression of CDKN3 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 CDKN3 expression between the normal fallopian tube and primary tumors from patients with HGSC, a *p*-value, listed above.

Figure 2: CDKN3 tumor expression correlates with overall survival in patients with ovarian cancer, in the lower quartile and lower tertile.

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 CDKN3 (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 CDKN3 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 CDKN3 expression: 57 months

High CDKN3 expression: 40.1 months

Lower tertile

Low CDKN3 expression: 50.03 months

High CDKN3 expression: 41 months

Chart 3: Overall survival is superior in ovarian cancer patients with low tumor expression of CDKN3.

The median overall survival of $n=655$ ovarian cancer patients based on stratification into low or high tumor expression of CDKN3 is listed in this chart, in the lower quartile and lower tertile.