Differential expression of mex-3 RNA binding family member A in human epithelial ovarian cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

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 mex-3 RNA binding family member A, MEX3A, as among the genes whose expression was most different in epithelial ovarian cancer as compared to the normal fallopian tube. MEX3A expression was significantly higher in high-grade serous ovarian tumors relative to normal fallopian tube. MEX3A expression correlated with overall survival in patients with ovarian cancer. These data indicate that expression of MEX3A is perturbed in epithelial ovarian cancers broadly and in ovarian cancers of the HGSC subtype. MEX3A 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 mex-3 RNA binding family member A, MEX3A, as among the most differentially expressed in HGSC tumors of the ovary and in epithelial ovarian cancer broadly. MEX3A 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 and GSE124766 (2) and GSE103737 (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 $n=7$ for tumors from patients with high-grade serous ovarian cancer; analysis was performed using platform GPL6480. GSE103737 (3) was generated using Illumina HumanHT-12 V4.0 expression beadchip with $n=17$ for stage 1 ovarian tumors and $n=72$ stage 3 and 4 ovarian tumors; analysis was performed using platform GPL10904. GEO2R provides mRNA expression levels only for the top 250 most differentially expressed genes transcriptome-wide.

A statistical test was performed to evaluate whether MEX3A 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 MEX3A mRNA expression levels with overall survival (OS) in $n=1656$ 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.

MEX3A is differentially expressed in ovarian tumors from women diagnosed with HGSC

We identified MEX3A 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, MEX3A ranked 146 out of 40481 total transcripts (Chart 1), equating to ____% differential expression. Differential expression of MEX3A in HGSC tumors was statistically significant (Chart 1; $p=5.85E-06$).

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 MEX3A could be observed in tumors from a different group of patients, which included HGSC tumors.

We found that MEX3A was also differentially expressed during the course of epithelial ovarian cancers broadly (Chart 2). When sorting all of the transcripts measured by microarray based on change in expression between tumors from patients at stage I and tumors from patients at stage III and IV, MEX3A ranked 13318 of 29088 total transcripts (Chart 2), equating to 54.2% differential expression. Differential expression of MEX3A in EOC based on disease progression was statistically significant (Chart 2; $p=1.34E-01$).

MEX3A 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 MEX3A transcript from both normal fallopian tube tissue and from epithelial ovarian tumors. MEX3A was expressed at significantly higher levels in high-grade serous ovarian cancers when compared to the normal fallopian tubes (Figure 1; $p=0.0011$). We calculated a mean fold change of 1.38 in MEX3A expression when comparing HGSC tumors to normal fallopian tissue, as MEX3A was expressed at 7.25 ± 0.63 arbitrary units (AU) in the fallopian tubes but at 10.03 ± 0.58 AU in tumors of the ovary.

MEX3A tumor expression is correlated with survival outcomes in patients with ovarian cancer.

We performed Kaplan Meier survival analysis (6) to evaluate whether MEX3A tumor expression correlated with survival outcomes in patients with ovarian cancer. MEX3A primary tumor expression was correlated with overall survival (OS) in patients with ovarian cancer, in the upper quartile, the upper tertile, the lower quartile and in the lower tertile (Figure 2).

MEX3A expression was a negative prognostic indicator in patients with ovarian cancer, in the lower quartile. While median OS was 54.67 months for ovarian cancer patients with low tumor expression of MEX3A, median OS was 39.87 months for ovarian cancer patients with high tumor expression of MEX3A (Chart 3). Correlation of OS with MEX3A tumor expression in patients with ovarian cancer, in the lower quartile, was statistically significant (Figure 2: log rank p-value: 0.0062; hazard ratio: 1.41 (1.1-1.81)).

MEX3A expression was a negative prognostic indicator in patients with ovarian cancer, in the lower tertile. While median OS was 54 months for ovarian cancer patients with low tumor expression of MEX3A, median OS was 38.4 months for ovarian cancer patients with high tumor expression of MEX3A (Chart 3). Correlation of OS with MEX3A tumor expression in patients with ovarian cancer, in the lower tertile, was statistically significant (Figure 2: log rank p-value: 0.004; hazard ratio: 1.39 (1.11-1.74)).

MEX3A expression was a negative prognostic indicator in patients with ovarian cancer, in the upper tertile. While median OS was 46.52 months for ovarian cancer patients with low tumor expression of MEX3A, median OS was 37 months for ovarian cancer patients with high tumor expression of MEX3A (Chart 3). Correlation of OS with MEX3A tumor expression in patients with ovarian cancer, in the upper tertile, was statistically significant (Figure 2: log rank p-value: 0.04; hazard ratio: 1.25 (1.01-1.54)).

MEX3A expression was a negative prognostic indicator in patients with ovarian cancer, in the upper quartile. While median OS was 45.77 months for ovarian cancer patients with low tumor expression of MEX3A, median OS was 37.47 months for ovarian cancer patients with high tumor expression of MEX3A (Chart 3). Correlation of OS with MEX3A tumor expression in patients with ovarian cancer, in the upper quartile, approached statistical significance (Figure 2: log rank p-value: 0.063; hazard ratio: 1.24 (0.99-1.55)).

Thus, we found using published microarray data (2, 3) that MEX3A was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas, and in epithelial ovarian cancer broadly. MEX3A expression was significantly higher in HGSC tumors when compared to the normal fallopian tubes, and MEX3A 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), MEX3A was among the genes whose expression changed most significantly transcriptome-wide in an ovarian cancer, when comparing normal gynecological tissue to ovarian tumors, or when comparing ovarian tumors from human across disease progression. MEX3A 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. Transcriptional correlates of high norepinephrine content in ovarian carcinomas. GSE103737. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE103737>. Steve Cole. UCLA School of Medicine.
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: 146

Probe ID: A_32_P96036

p-value: 5.85E-06

t: -8.17

B: 4.43424

Gene: MEX3A

Gene name: mex-3 RNA binding family member A

Chart 1: MEX3A 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: 13318

p-value: 1.34E-01

t: -1.5122108

B: -4.9416953

Gene: MEX3A

Gene name: mex-3 RNA binding family member A

Chart 2: MEX3A is differentially expressed in epithelial ovarian cancers when comparing tumors based on tumor progression: stage I versus stage III and IV.

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: MEX3A is expressed at significantly higher levels in HGSC tumors when compared to normal fallopian tube.

The mRNA expression of MEX3A 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 MEX3A expression between the normal fallopian tube and primary tumors from patients with HGSC, a *p*-value, listed above.

Figure 2: MEX3A 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=1656 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 MEX3A (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 MEX3A 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 MEX3A expression: 54.67 months

High MEX3A expression: 39.87 months

Lower tertile

Low MEX3A expression: 54 months

High MEX3A expression: 38.4 months

Upper tertile

Low MEX3A expression: 46.52 months

High MEX3A expression: 37 months

Upper quartile

Low MEX3A expression: 45.77 months

High MEX3A expression: 37.47 months

Chart 3: Overall survival is superior in ovarian cancer patients with low tumor expression of MEX3A.

The median overall survival of $n=1656$ ovarian cancer patients based on stratification into low or high tumor expression of MEX3A is listed in this chart, in the lower quartile, lower tertile, upper tertile, and upper quartile.