Chain A, B, and C of the complement component C1q are differentially expressed 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 each of the genes encoding the subunits of the complement component C1q - C1QA, C1QB, and C1QC - as among the genes whose expression was most different in epithelial ovarian cancer as compared to the normal fallopian tube. C1QA, C1QB, and C1QC expression was significantly higher in high-grade serous ovarian tumors relative to normal fallopian tube. C1QA, C1QB, and C1QC expression correlated with progression-free survival in patients with ovarian cancer. These data indicate that expression of C1QA, C1QB, and C1QC is perturbed in epithelial ovarian cancers broadly and in ovarian cancers of the HGSC subtype. C1q and the complement pathway may be relevant to 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, C1q, C1QA, C1QB, C1QC.

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 genes that encode the A, B, and C chains of the complement component C1q - C1QA, C1QB, and C1QC - as among the most differentially expressed in HGSC tumors of the ovary and in epithelial ovarian cancer broadly. C1QA, C1QB, and C1QC may be genes 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 C1QA, C1QB, and C1QC expression was significantly different when comparing control fallopian tissue 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 C1QA, C1QB, and C1QC mRNA expression levels with progression-free survival (PFS) in $n=1435$ or $n=614$ 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.

C1QA is differentially expressed in ovarian tumors from women diagnosed with HGSC

We identified complement C1q A chain, encoded by C1QA, 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, C1QA ranked 155 out of 40481 total transcripts (Chart 1), equating to 99.6% differential expression. Differential expression of C1QA in HGSC tumors was statistically significant (Chart 1; $p=9.60E-06$).

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 C1QA could be observed in tumors from a different group of patients, which included HGSC tumors.

We found that C1QA 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, C1QA ranked 5689 of 29088 total transcripts (Chart 2), equating to 80.4% differential expression. Differential expression of C1QA in EOC was statistically significant (Chart 2; $p=2.20E-02$).

C1QB is differentially expressed in ovarian tumors from women diagnosed with HGSC

We also identified the complement C1q B chain, encoded by C1QB, as among the genes whose expression was most different when comparing primary HGSC tumors to normal fallopian tube tissue (2) (Chart 3). 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, C1QB ranked 152 out of 40481 total transcripts (Chart 3), equating to 99.6% differential expression. Differential expression of C1QB in HGSC tumors was statistically significant (Chart 3; $p=9.24E-06$).

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 C1QB could be observed in tumors from a different group of patients, which included HGSC tumors.

We found that C1QB was also differentially expressed in epithelial ovarian cancers broadly (Chart 4). 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, C1QB ranked 3979 of 29088 total transcripts (Chart 4), equating to 86.3% differential expression. Differential expression of C1QB in EOC was statistically significant (Chart 4; $p=7.78E-03$).

C1QC is differentially expressed in ovarian tumors from women diagnosed with HGSC

We also identified the complement C1q C chain, encoded by C1QC, as among the genes whose expression was most different when comparing primary HGSC tumors to normal fallopian tube tissue (2) (Chart 5). 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, C1QC ranked 244 out of 40481 total transcripts (Chart 5), equating to 99.4% differential expression. Differential expression of C1QC in HGSC tumors was statistically significant (Chart 5; $p=2.69E-05$).

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 C1QC could be observed in tumors from a different group of patients, which included HGSC tumors.

We found that C1QC was also differentially expressed in epithelial ovarian cancers broadly (Chart 6). 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, C1QC ranked 5723 of 29088 total transcripts (Chart 6), equating to 80.3% differential expression. Differential expression of C1QC in EOC was statistically significant (Chart 6; $p=2.24E-02$).

C1QA, C1QB, and C1QC are 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 C1QA, C1QB, and C1QC transcripts from both normal fallopian tube tissue and from epithelial ovarian tumors.

C1QA was expressed at significantly higher levels in high-grade serous ovarian cancers when compared to the normal fallopian tube (Figure 1; $p=0.0047$). We calculated a mean fold change of 1.21 in C1QA expression when comparing HGSC tumors to normal fallopian tissue, as C1QA was expressed at 7.48 ± 0.53 arbitrary units (AU) in the fallopian tubes but at 9.90 ± 0.44 AU in tumors of the ovary.

C1QB was expressed at significantly higher levels in high-grade serous ovarian cancers when compared to the normal fallopian tube (Figure 2; $p<0.0001$). We calculated a mean fold change of 1.21 in C1QB expression when comparing HGSC tumors to normal fallopian tissue, as C1QB was expressed at 9.05 ± 0.11 arbitrary units (AU) in the fallopian tubes but at 10.91 ± 0.33 AU in tumors of the ovary.

C1QC was expressed at significantly higher levels in high-grade serous ovarian cancers when compared to the normal fallopian tube (Figure 3; $p<0.0001$). We calculated a mean fold change of 1.56 in C1QC expression when comparing HGSC tumors to normal fallopian tissue, as C1QC was expressed at 6.38 ± 0.36 arbitrary units (AU) in the fallopian tubes but at 9.94 ± 0.91 AU in tumors of the ovary.

C1QA, C1QB, and C1QC tumor expression is correlated with survival outcomes in patients with ovarian cancer.

We performed Kaplan Meier survival analysis (6) to evaluate whether C1QA, C1QB, and C1QC tumor expression correlated with survival outcomes in patients with ovarian cancer. C1QA, C1QB, and C1QC primary tumor expression were each correlated with progression-free survival (PFS) in patients with ovarian cancer (Figures 4-6).

C1QA primary tumor expression was correlated with progression-free survival (PFS) in patients with ovarian cancer, in the upper quartile.

C1QA expression was a negative prognostic indicator in patients with ovarian cancer, in the upper quartile. While median OS was 21 months for ovarian cancer patients with low tumor expression of C1QA, median PFS was 17.41 months for ovarian cancer patients with high tumor expression of C1QA (Chart 7). Correlation of PFS with C1QA tumor expression in patients with ovarian cancer, in the upper quartile, was statistically significant (Figure 4: log rank p -value: 0.027; hazard ratio: 1.17 (1.02-1.35)).

C1QA primary tumor expression was not correlated with progression-free survival in the lower quartile (Figure 4: log rank p -value: 0.74; hazard ratio: 0.98 (0.84-1.13)), in the lower tertile (Figure 4: log rank p -value: 0.46; hazard ratio: 1.05 (0.92-1.2)), or in the upper tertile (Figure 4: log rank p -value: 0.31; hazard ratio: 1.07 (0.94-1.22)).

C1QB primary tumor expression was also correlated with progression-free survival (PFS) in patients with ovarian cancer: in the upper quartile and in the upper tertile.

C1QB expression was a negative prognostic indicator in patients with ovarian cancer, in the upper quartile. While median OS was 21 months for ovarian cancer patients with low tumor expression of C1QB, median PFS was 17.41 months for ovarian cancer patients with high tumor expression of C1QB

(Chart 7). Correlation of PFS with C1QB tumor expression in patients with ovarian cancer, in the upper quartile, was statistically significant (Figure 5: log rank p-value: 0.034; hazard ratio: 1.17 (1.01-1.35)).

C1QB expression was a negative prognostic indicator in patients with ovarian cancer, in the upper tertile. While median OS was 21.47 months for ovarian cancer patients with low tumor expression of C1QB, median PFS was 18 months for ovarian cancer patients with high tumor expression of C1QB (Chart 7). Correlation of PFS with C1QB tumor expression in patients with ovarian cancer, in the upper tertile, was statistically significant (Figure 5: log rank p-value: 0.015; hazard ratio: 1.18 (1.03-1.35)).

C1QB primary tumor expression was not correlated with progression-free survival in the lower quartile (Figure 5: log rank p-value: 0.16; hazard ratio: 1.11 (0.96-1.28)), or in the lower tertile (Figure 5: log rank p-value: 0.19; hazard ratio: 1.09 (0.96-1.25)).

C1QC primary tumor expression was also correlated with progression-free survival (PFS) in patients with ovarian cancer: in the lower quartile, the lower tertile, the upper tertile, and the upper quartile.

C1QC expression was a negative prognostic indicator in patients with ovarian cancer, in the lower quartile. While median OS was 23 months for ovarian cancer patients with low tumor expression of C1QC, median PFS was 15.87 months for ovarian cancer patients with high tumor expression of C1QC (Chart 7). Correlation of PFS with C1QC tumor expression in patients with ovarian cancer, in the lower quartile, was statistically significant (Figure 6: log rank p-value: 0.00066; hazard ratio: 1.48 (1.18-1.85)).

C1QC expression was a negative prognostic indicator in patients with ovarian cancer, in the lower tertile. While median OS was 21.59 months for ovarian cancer patients with low tumor expression of C1QC, median PFS was 15.87 months for ovarian cancer patients with high tumor expression of C1QC (Chart 7). Correlation of PFS with C1QC tumor expression in patients with ovarian cancer, in the lower tertile, was statistically significant (Figure 6: log rank p-value: 0.0014; hazard ratio: 1.4 (1.14-1.71)).

C1QC expression was a negative prognostic indicator in patients with ovarian cancer, in the upper tertile. While median OS was 19 months for ovarian cancer patients with low tumor expression of C1QC, median PFS was 14 months for ovarian cancer patients with high tumor expression of C1QC (Chart 7). Correlation of PFS with C1QC tumor expression in patients with ovarian cancer, in the upper tertile, was statistically significant (Figure 6: log rank p-value: 0.0061; hazard ratio: 1.31 (1.08-1.6)).

C1QC expression was a negative prognostic indicator in patients with ovarian cancer, in the upper quartile. While median OS was 18.27 months for ovarian cancer patients with low tumor expression of C1QC, median PFS was 13 months for ovarian cancer patients with high tumor expression of C1QC (Chart 7). Correlation of PFS with C1QC tumor expression in patients with ovarian cancer, in the upper quartile, was statistically significant (Figure 6: log rank p-value: 0.007; hazard ratio: 1.33 (1.08-1.65)).

Thus, we found using published microarray data (2, 3) that each of the three subunits of the complement component C1q - C1QA, C1QB, and C1QC - were among the genes most differentially expressed in tumors from women with high-grade serous carcinomas, and in epithelial ovarian cancer broadly. C1QA, C1QB, and C1QC expression was significantly higher in HGSC tumors when compared to the normal fallopian tubes, and C1QA, C1QB, and C1QC tumor expression was correlated with progression-free 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), C1QA, C1QB, and C1QC were 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. C1q appears to be a molecule of relevance in understanding transformation or disease progression in human epithelial ovarian cancer.

References

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Rank: 155
Probe ID: A_24_P222655
p-value: 9.60E-06
t: -7.8100856
B: 3.961075
Gene: C1QA
Gene name: complement C1q A chain

Chart 1: C1QA 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: 5689
Probe ID: 7898793
p-value: 2.20E-02
t: -2.3745303
B: -3.8809051
Gene: C1QA
Gene name: complement C1q A chain

Chart 2: C1QA 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.

Rank: 152

Probe ID: A_23_P137366

p-value: 9.24E-06

t: -7.8430023

B: 3.998403

Gene: C1QB

Gene name: complement C1q B chain

Chart 3: C1QB 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: 3979
Probe ID: 7898805
p-value: 7.78E-03
t: -2.7882494
B: -2.9545792
Gene: C1QB
Gene name: complement C1q B chain

Chart 4: C1QB 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.

Rank: 244

Probe ID: A_23_P125977

p-value: 2.69E-05

t: -6.9743025

B: 2.964943

Gene: C1QC

Gene name: complement C1q C chain

Chart 5: C1QC 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: 5723
Probe ID: 7898799
p-value: 2.24E-02
t: -2.366783
B: -3.8971873
Gene: C1QC
Gene name: complement C1q C chain

Chart 6: C1QC 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: C1QA is expressed at significantly higher levels in HGSC tumors when compared to normal fallopian tube.

The mRNA expression of C1QA 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 C1QA expression between the normal fallopian tube and primary tumors from patients with HGSC, a *p*-value, listed above.

Figure 2: C1QB is expressed at significantly higher levels in HGSC tumors when compared to normal fallopian tube.

The mRNA expression of C1QB 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 C1QB expression between the normal fallopian tube and primary tumors from patients with HGSC, a *p*-value, listed above.

Figure 3: C1QC is expressed at significantly higher levels in HGSC tumors when compared to normal fallopian tube.

The mRNA expression of C1QC 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 C1QC expression between the normal fallopian tube and primary tumors from patients with HGSC, a *p*-value, listed above.

Figure 4: C1QA 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=1435 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 C1QA (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 C1QA 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.

Figure 5: C1QB 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=1435 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 C1QB (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 C1QB 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.

Figure 6: C1QC 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 C1QC (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 C1QC 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.

C1QA

Upper quartile

Low C1QA expression: 21 months

High C1QA expression: 17.41 months

C1QB

Upper tertile

Low C1QA expression: 21.47 months

High C1QA expression: 18 months

Upper quartile

Low C1QA expression: 21 months

High C1QA expression: 17.41 months

C1QC

Lower quartile

Low C1QA expression: 23 months

High C1QA expression: 15.87 months

Lower tertile

Low C1QA expression: 21.59 months

High C1QA expression: 15.87 months

Upper tertile

Low C1QA expression: 19 months

High C1QA expression: 14 months

Upper quartile

Low C1QA expression: 18.27 months

High C1QA expression: 13 months

Chart 7: Progression-free survival is superior in ovarian cancer patients with low tumor expression of C1QA, C1QB, and C1QC.

The median progression-free survival of $n=1435$ (C1QA and C1QB) or $n=614$ (C1QC) ovarian cancer patients based on stratification into low or high tumor expression of C1QA, C1QB, and C1QC is listed in this chart.