C2 is differentially expressed in high-grade serous ovarian cancers.

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Ovarian cancer is the most lethal gynecologic cancer¹. We sought to identify genes associated with high-grade serous ovarian cancer (HGSC) by comparing global gene expression profiles of normal ovary with that of primary tumors from women diagnosed with HGSC using published microarray data^{2,3}. We found significant differential expression of the gene encoding the complement component C2 in high-grade serous ovarian tumors.

Keywords: ovarian cancer, high-grade serous ovarian cancer, HGSC, targeted therapeutics in ovarian cancer, systems biology of ovarian cancer, C2.

1 The five-year survival rate for women diagnosed with high-grade serous ovarian cancer is
2 between 30-40% and has not changed significantly in decades^{4,5}. The development of novel,
3 targeted therapeutics to treat HGSC can be facilitated by an enhanced understanding of the
4 transcriptional behavior of ovarian tumors relative to that of the normal ovary. We mined
5 published microarray data^{2,3} to compare global gene expression profiles between HGSC ovarian
6 tumors and that of normal ovarian tissue. We identified the gene encoding complement
7 component C2 as among the most differentially expressed in HGSC tumors of the ovary. C2 may
8 be a gene of interest when prioritizing the study of target genes and pathways for the
9 development of novel therapeutic interventions in high-grade serous ovarian cancers.
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14 **Methods**

15 We used microarray data from datasets GSE124766² and GSE146556³ for this
16 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
17 and Hochberg method of p-value adjustment was used for ranking of differential expression but
18 raw p-values were used for assessment of statistical significance of global differential expression.
19 Log-transformation of data was auto-detected, and the NCBI generated category of platform
20 annotation was used. GSE124766 was generated using Agilent-014850 Whole Human Genome
21 Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors from
22 patients with high-grade serous ovarian cancer. GSE146556 was generated using Affymetrix
23 Human Gene 1.0 ST Array with n=3 for normal ovarian tissue and n=40 for tumors from patients
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1 with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels only for the
2 top 250 most differentially expressed genes.
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4 A statistical test was performed to evaluate whether C2 expression was significantly
5 different when comparing normal ovarian tissue from control subjects and primary tumors from
6 women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's correction. Only
7 p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical
8 analyses (Version 8.4.0)(455).
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11 **Results**

12 We mined published microarray data^{2,3} to identify differentially expressed genes in high-
13 grade serous ovarian cancer (HGSC), the type of ovarian cancer responsible for 70-80% of
14 deaths resulting from the most lethal gynecologic malignancy.
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17 C2 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

18 We identified C2 as among the genes whose expression was most different when
19 comparing primary HGSC tumors to normal ovarian tissue² (Table 1). We found that C2 was
20 differentially expressed in primary HGSC tumors (Table 1). When sorting all of the transcripts
21 measured by microarray based on change in expression between HGSC and the normal ovary,
22 C2 ranked 127 out of 41093 total transcripts (Table 1). Differential expression of C2 in HGSC
23 tumors was statistically significant (Table 1; $p=4.56E-06$).
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1 We analyzed a second microarray dataset³ generated using normal ovarian tissues and
2 tumors from women diagnosed with HGSC to determine whether differential expression of C2
3 could be observed in tumors from a separate group of patients. We again found that C2 was
4 differentially expressed in primary HGSC tumors (Table 2). When sorting all of the transcripts
5 measured by microarray based on change in expression between HGSC and the normal ovary, in
6 this data set, C2 ranked 3270 out of 29088 total transcripts (Table 2). Differential expression of
7 C2 in HGSC tumors in this second microarray dataset was statistically significant (Table 2;
8 $p=4.24E-03$).

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13 C2 is expressed at higher levels in HGSC when compared to the normal ovary.

14 We obtained exact mRNA expression levels for the differentially expressed C2 transcript
15 from both normal ovarian tissue and from high-grade serous ovarian tumors. C2 was expressed
16 at higher levels in high-grade serous ovarian cancers when compared to the normal ovary; this
17 difference was statistically significant (Figure 1; $p=0.0196$). We calculated a mean fold change of
18 1.5100 ± 0.0793 in C2 expression when comparing HGSC tumors to normal ovarian tissue
19 (Table 1).

22 Thus, we found using published microarray data that C2 was among the genes most
23 differentially expressed in tumors from women with high-grade serous carcinomas and that C2
24 expression was higher in HGSC tumors when compared to the normal ovary.
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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 mesenchymal⁶. We sought to continue to describe the transcriptional landscape of high-grade serous ovarian cancers⁶ and identify genes whose differential expression was associated with HGSC by using published microarray data from primary tumors of women diagnosed with HGSC compared to transcriptome data from normal ovarian tissue^{2,3}. In both datasets analyzed, C2 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

Complement proteins comprise nearly 15% of the globulin fraction and function in diverse innate immune responses including opsonization of infected cells and lysis of bacteria through membrane attack complexes⁷. Activation of the complement pathway can follow three routes, including the classical, alternative and lectin pathways; C2 functions in the classical pathway along with components C1q, C1s, C1r, C4 and C3^{7,8}. Activation of complement cascades frequently involves proteolytic cleavage of complement proteins in sequential fashion. Activation of the classical pathway starts with binding of the C1 complex (C1q, C1s, and C1r) to antibody bound to the bacterial plasma membrane. C1s cleavage of C4 results in similar binding of C4 to the bacterial surface and subsequent cleavage of C2; this results in formation of the classical pathway C3 convertase from cleaved components of C4 and C2 - C4b2a⁷. C4b2a can

1 then cleave C3, producing C3b, which then attaches near the site of complement activation. C3b
2 can interact with C4b, producing a C5 convertase; C5 cleavage produces C5a and C5b. C5b, in
3 conjunction with C6, C7 and C8 lead to activation of the membrane attack complex^{7,8}. The
4 lectin pathway of complement activation also utilizes C2, wherein the C3 convertase can also be
5 generated by cleavage of C2 and C4 by the MBL-associated serine protease 2 (MBL-2)⁸.
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9 cDNA for the C2 complement component was isolated in 1984⁹. C2 deficiency^{10,11} is the
10 most common hereditary complement deficiency, and 10-30% of patients with C2 deficiency are
11 also diagnosed with systemic lupus erythematosus (SLE)¹². We could identify functions for
12 complement proteins in benign cells alluding to the potential for these molecules to exert pro-
13 tumorigenic functions in cancer^{13,14}, including anti-apoptotic properties of C5 through inhibition
14 of caspase-3¹⁵ and support of invasion through degradation of cartilage collagen by C1s¹⁶.
15 Studies have also documented direct roles for complement components in cancer^{13, 14}. Ovarian
16 cancer cell lines SKOV3ip1, ID8, ID8-VEGF, and IG10 secreted significantly higher levels of
17 C3 than did murine ovarian endothelial cells, suggesting ovarian tumors secrete C3¹⁶. In SKOV3
18 and OVCAR5 ovarian cancer cell lines, TWIST1 expression correlated with C3 expression, and
19 depletion of TWIST1 in cell culture using small interfering RNA resulted in significantly
20 decreased C3 expression, suggesting that the epithelial mesenchymal transition transcription
21 factor TWIST might exert control over C3 expression in ovarian cancers¹⁷. Depletion of C3 in
22 tumors derived from OVCAR5 cells in ovarian cancer murine xenograft models resulted in
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1 impairment of tumor growth, demonstrating that C3 promoted tumor growth *in vivo*¹⁷.

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3 We were able to identify one study that described a relationship between the C2 molecule
4 and cancer cells, wherein investigators collected human sera from 1000 healthy donors and used
5 KATO III stomach cancer cells to screen sera for cancer-killing activity, finding that sera from
6 35.4% of compatible individuals was able to induce cell death in KATO III cells (termed
7 “positive human serum”, or PHS)¹⁸. By heat-inactivating serum and re-introducing C2,
8 investigators demonstrated that the ability of PHS to induce lysis of cancer cell lines MCF-7,
9 HT-29 and COLO 205 was dependent on C2¹⁸. However, we find that C2 is produced at higher
10 levels in HGSC tumors, counterintuitive with the findings highlighted¹⁸ suggesting that the
11 tumor lysing capacity of positive human serum was at least in part dependent on C2-mediated
12 mechanisms.
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17 Differential expression of C2 in HGSC should be validated in larger and separate cohorts
18 of women with HGSC. If validated, mouse models of high-grade serous ovarian cancers¹⁹⁻²¹ can
19 be utilized to assess the effect of conditional expression of C2 in HGSC tumor growth post-
20 development and on survival *in vivo*.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
127	A_23_P133916	4.56E-06	7.9496706	4.62292	1.5100 ± 0.0793	C2	complement component 2

Table 1: C2 is differentially expressed in high-grade serous ovarian carcinomas.

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 fold change of C2 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
3270	8118324	4.24E-03	3.01486	-2.4033928	C2	complement component 2

Table 2: C2 is differentially expressed in high-grade serous ovarian carcinomas.

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 fold change of C2 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: C2 is expressed at significantly higher levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of C2 in normal ovarian tissues from control subjects (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 C2 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.