Open reading frame 28 on Chromosome 14 (C14ORF28) is differentially expressed in high-grade serous ovarian cancers.

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Ovarian cancer is the most lethal gynecologic cancer (1-3). 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 (4, 5). We identified C14ORF28 (6, 7) as among the genes whose expression was most different in HGSC ovarian tumors. C14ORF28 expression was significantly lower in ovarian tumors relative to normal ovary. These data indicate that expression of C14ORF28 might be perturbed in high-grade serous ovarian cancers.

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

1 The five-year survival rate for women diagnosed with high-grade serous ovarian cancer
2 is between 30-40% and has not changed significantly in decades (2, 3). The development of
3 novel, targeted therapeutics to treat HGSC can be facilitated by an enhanced understanding of
4 the transcriptional behavior of ovarian tumors relative to that of the normal ovary. We mined
5 published microarray data (4, 5) to compare global gene expression profiles between HGSC
6 ovarian tumors and that of normal ovarian tissue. We identified the gene encoding C14ORF28
7 as among the most differentially expressed in HGSC tumors of the ovary. C14ORF28 may be a
8 gene of interest when prioritizing the study of target genes and pathways for the development of
9 novel therapeutic interventions in high-grade serous ovarian cancers.
10

11 12 **Methods**

13 We used microarray data from datasets GSE146556 (4) and GSE124766 (5) for this
14 differential gene expression analysis of high-grade serous carcinomas. The Benjamini and
15 Hochberg method of p-value adjustment was used for ranking of differential expression but raw
16 p-values were used for assessment of statistical significance of global differential expression.
17 Log-transformation of data was auto-detected, and the NCBI generated category of platform
18 annotation was used. GSE146556 was generated using Affymetrix Human Gene 1.0 ST Array
19 with n=3 for normal ovarian tissue and n=40 for tumors from patients with high-grade serous
20 ovarian cancer. 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. GEO2R provides mRNA expression levels only
23 for the top 250 most differentially expressed genes.
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26 A statistical test was performed to evaluate whether C14ORF28 expression was
27 significantly different when comparing normal ovarian tissue from control subjects and primary
28 tumors from women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's

correction. Only p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined published microarray data (4, 5) to identify differentially expressed genes 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.

C14ORF28 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified C14ORF28 as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue (Table 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, C14ORF28 ranked 237 out of 29088 total transcripts (Table 1). Differential expression of C14ORF28 in HGSC tumors was statistically significant (Table 1; $p=3.15E-07$).

We analyzed a second microarray dataset generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of C14ORF28 could be observed in tumors from a different group of patients. We again found that C14ORF28 was differentially expressed in primary HGSC tumors (Table 2). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, in this data set, C14ORF28 ranked 167 out of 41093 total transcripts (Table 2). Differential expression of C14ORF28 in HGSC tumors was statistically significant (Table 2; $p=7.92E-06$).

C14ORF28 is expressed at significantly lower levels in HGSC when compared to the normal ovary.

We then obtained exact mRNA expression levels for the differentially expressed C14ORF28 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. C14ORF28 was expressed at significantly lower levels in high-grade serous ovarian

1 cancers when compared to the normal ovary (Figure 1; $p < 0.0001$). We calculated a mean fold
2 change of 0.3177 ± 0.1240 in C14ORF28 expression when comparing HGSC tumors to normal
3 ovarian tissue (Table 1).

4 In the second dataset analyzed, we again found that C14ORF28 was expressed at
5 significantly lower levels in primary tumors from women diagnosed with high-grade serous
6 ovarian cancer when compared to normal ovarian tissue (Figure 2; $p < 0.0001$). We calculated a
7 mean fold change of 0.7705 ± 0.0487 in C14ORF28 expression when comparing HGSC tumors
8 to normal ovarian tissue (Table 2).

9 Thus, we found using published microarray data that C14ORF28 was among the genes
10 most differentially expressed in tumors from women with high-grade serous carcinomas and that
11 C14ORF28 expression was significantly lower in HGSC tumors when compared to the normal
12 ovary.
13

14 Discussion

15 Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic analysis
16 classified HGSC into four subtypes based on gene clustering: immunoreactive, differentiated,
17 proliferative, and mesenchymal (8). We sought to continue to describe the transcriptional
18 landscape of high-grade serous ovarian cancers (8) and identify genes whose differential
19 expression was associated with HGSC by using published microarray data from primary tumors
20 of women diagnosed with HGSC compared to transcriptome data from normal ovarian tissue (4,
21 5). In both datasets analyzed, C14ORF28 was among the genes whose expression changed
22 most significantly when comparing the normal ovary to primary HGSC tumors.
23

24 There is limited literature describing the functions of C14ORF28, also known as DRIP1
25 (dopamine receptor-interacting protein 1) (6). A search of UniProt Knowledgebase (UniProtKB)
26 revealed that the translated protein product of C14ORF28 is 310 amino acids in length, with one
27 potential isoform 280 amino acids long (9). A group using a yeast two-hybrid system to identify
28 dopamine receptor-interacting proteins found that expression of C14ORF28 was altered in the

1 pre-frontal cortex of patients with schizophrenia post-mortem (6). A survey of microRNA
2 expression in ovarian cancers found that the microRNA Let-7d was among the most
3 differentially expressed microRNAs in ovarian cancers (10); C14ORF28 has been reported as a
4 potential target of the microRNA let-7d (10). Multiple studies have documented a role for the
5 family of Let-7 microRNAs in modulation of gene expression in ovarian cancers (11-14). We
6 could only identify one report of a specific role for C14ORF28 in cancer (7). C14ORF28 was
7 found to be expressed at higher levels in colorectal cancer tumor tissues as compared to
8 neighboring benign tissues (7). Over-expression of C14ORF28 inhibited apoptosis and
9 supported the proliferative and invasive capacity of colorectal cancer cells (7). The same study
10 identified C14ORF28 as a target of the microRNA miR-519d (7).

12 Differential expression of C14ORF28 in HGSC should be validated in larger and
13 separate cohorts of women with HGSC. If validated, mouse models of high-grade serous
14 ovarian cancers (15) can be utilized to assess the effect of conditional expression of C14ORF28
15 on tumor cell proliferation and cancer progression *in vivo*. We found here that a relatively
16 uncharacterized open reading frame was among the genes most differentially expressed in
17 tumors from the most lethal gynecological cancer.

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Rank	ID	p-value	t	B	Fold change	Gene	Gene name
237	7974117	3.15E-07	-6.011265	6.6016149	0.3177 ± 0.1240	C14orf28	chromosome 14 open reading frame 28

Table 1: C14ORF28 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 C14ORF28 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Fold change	Gene	Gene name
167	A_24_P33048	7.92E-06	-7.5219692	4.09311	0.7705 ± 0.0487	C14orf28	chromosome 14 open reading frame 28

Table 2: C14ORF28 is among the most differentially expressed genes in a tumor dataset from patients with 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 C14ORF28 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Figure 1: C14ORF28 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of C14ORF28 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 C14ORF28 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.

Figure 2: C14ORF28 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of C14ORF28 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 C14ORF28 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.