1 **CASP12 is differentially expressed in high-grade serous ovarian cancers.**

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5 Ovarian cancer is the most lethal gynecologic cancer (1). To identify genes associated with
6 high-grade serous ovarian cancer (HGSC), we used published microarray data (2, 3) to
7 compare global gene expression profiles of the normal ovary with that of primary tumors from
8 women diagnosed with HGSC. We identified caspase-12 (CASP12) (4) as among the genes
9 whose expression was most quantitatively different in HGSC ovarian tumors. CASP12
10 expression was decreased in ovarian tumors when compared to normal ovary. These data
11 indicate that CASP12 might be relevant to the biology of high-grade serous ovarian cancers.

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27 Keywords: ovarian cancer, high-grade serous ovarian cancer, HGSC, targeted therapeutics in
28 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 (5, 6). 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 (2, 3) to compare global gene expression profiles between HGSC
6 ovarian tumors and that of normal ovarian tissue. We identified the CASP12 gene as among
7 the most differentially expressed in HGSC tumors of the ovary. CASP12 may be a gene of
8 interest when prioritizing the study of target genes and pathways for the development of novel
9 therapeutic interventions in high-grade serous ovarian cancers.
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11 12 **Methods**

13 We used microarray data from datasets GSE146556 (2) and GSE124766 (3) 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 (2) was generated using Affymetrix Human Gene 1.0 ST
19 Array with n=3 for normal ovarian tissue and n=40 for tumors from patients with high-grade
20 serous ovarian cancer. GSE124766 (3) was generated using Agilent-014850 Whole Human
21 Genome Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors
22 from patients with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels
23 only for the top 250 most differentially expressed genes.
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25 A statistical test was performed to evaluate whether CASP12 expression was
26 significantly different when comparing normal ovarian tissue from control subjects and primary
27 tumors from women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's
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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 (2, 3) 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.

CASP12 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified caspase-12 (CASP12) 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, CASP12 ranked 3 out of 29088 total transcripts (Table 1). Differential expression of CASP12 in HGSC tumors was statistically significant (Table 1; $p=3.76E-17$).

We analyzed a second microarray dataset generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of CASP12 could be observed in tumors from a different group of patients. We again found that CASP12 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, CASP12 ranked 2209 out of 41093 total transcripts (Table 2). Differential expression of CASP12 in HGSC tumors was statistically significant (Table 2; $p=2.31E-03$).

CASP12 is expressed at significantly lower levels in HGSC when compared to the normal ovary.

We then extracted exact mRNA expression levels for the differentially expressed CASP12 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. CASP12 was expressed at lower levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 1). This difference in caspase-12 expression approached statistical significance (Figure 1; $p=0.0582$). We calculated a mean fold change of 0.20877 ± 0.03803 in CASP12 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

Thus, we found using published microarray data that CASP12 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that CASP12 expression was decreased in primary HGSC tumors when compared to the normal ovary.

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 continue to describe the transcriptional landscape of high-grade serous ovarian cancers (7) 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, CASP12 was among the genes whose expression changed most significantly when comparing primary HGSC tumors to the normal ovary, with decreased expression of CASP12 in HGSC tumors compared to the ovary.

Caspases are a family of cysteine proteases that have critical roles in development, in cell fate decisions and in immunity and share as a family the ability to cleave peptides bonds following aspartic acid residues (8-11). Caspase-12 is phylogenetically most similar to caspase-1 and caspase-11 (4) and has been described as important for cytotoxicity and

1 apoptosis in response to amyloid beta involving the endoplasmic reticulum (ER) stress pathway
2 (12). Caspase-12 can be activated by calpain during apoptotic responses, demonstrating
3 intersection of caspase-12 with other cysteine protease pathways (13). In neuroblastoma cells,
4 apoptosis resulting from pharmacological induction of ER stress requires caspase-12 activation
5 of caspase 3 (14). In addition to its roles in apoptotic responses, caspase-12 has functions in
6 the immune system. Caspase-12 expression can be induced, in certain cell types, by interferon
7 gamma but not by IFN-alpha or IFN-beta; this induction is enhanced by the addition of TNF-
8 alpha, lipopolysaccharide (endotoxin; LPS), or double stranded RNA (15). Polymorphisms in
9 the caspase-12 gene are associated with differential immune responses to endotoxin in humans
10 (16), and CASP12-deficient mice displayed increased resistance in an experimental sepsis
11 model (17). Caspase-12 has also been reported as a regulator of NOD signaling and important
12 for immunity at mucosal surfaces (18).

14 There is limited data describing a role for caspase-12 in the ovary, in cancer, or
15 specifically in ovarian cancer. Caspase-2 and caspase-3 are important for the apoptotic in, but
16 Caspase-12 can compensate for loss of caspase-2 and caspase 3 in apoptosis of female germ
17 cells (19). Caspase-12 deficient mice, with enhanced inflammasome responses, were protected
18 from dextran sodium sulfate (DSS)-induced colitis, while significantly more prone to developing
19 azoxymethane/DSS-induced colorectal cancer (20), demonstrating nuanced functions of a
20 caspase in the immune system versus cell death that are not straightforward or intuitive.

22 Differential expression of CASP12 in HGSC should be validated in larger and separate
23 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
24 cancers (21) can be utilized to assess the effect of conditional expression of CASP12 in HGSC
25 tumors post-development and on tumor cell proliferation and cancer progression *in vivo*.

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Rank	ID	p-value	t	B	Fold change	Gene	Gene name
3	7951363	3.76E-17	-13.3565731	28.2612292	0.20877 ± 0.03803	CASP12	caspase 12 (gene/pseudogene)

Table 1: CASP12 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 CASP12 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	Gene	Gene name
2209	A_23_P13102	2.31E-03	-3.8691828	-1.5059	CASP12	caspase 12 (gene/ pseudogene)

Table 2: CASP12 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 CASP12 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Figure 1: CASP12 expression is decreased in HGSC tumors when compared to the normal ovary.

The mRNA expression of CASP12 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 CASP12 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.