GADD45G is differentially expressed in high-grade serous ovarian cancers.

Shahan Mamoor¹

¹shahanmamoor@gmail.com

East Islip, NY 11730

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 GADD45G 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, GADD45G.

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^{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 GADD45G as
7 among the most differentially expressed in HGSC tumors of the ovary. GADD45G 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.
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11 12 **Methods**

13 We used microarray data from datasets GSE146556² and GSE124766³ for this
14 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
15 and Hochberg method of p-value adjustment was used for ranking of differential expression but
16 raw p-values were used for assessment of statistical significance of global differential
17 expression. Log-transformation of data was auto-detected, and the NCBI generated category of
18 platform annotation was used. GSE124766³ was generated using Agilent-014850 Whole
19 Human Genome Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for
20 tumors from patients with high-grade serous ovarian cancer. GSE146556² was generated using
21 Affymetrix Human Gene 1.0 ST Array with n=3 for normal ovarian tissue and n=40 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.
24

25 A statistical test was performed to evaluate whether GADD45G 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.

GADD45G is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified GADD45G as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, GADD45G ranked 217 out of 41093 total transcripts (Table 1). Differential expression of GADD45G in HGSC tumors was statistically significant (Table 1; $p=2.48E-07$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of GADD45G could be observed in tumors from a separate group of patients. We again found that GADD45G 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, GADD45G ranked 4439 out of 29088 total transcripts (Table 2). Differential expression of GADD45G in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=1.12E-02$).

GADD45G is expressed at lower levels in HGSC when compared to the normal ovary.

We obtained exact mRNA expression levels for the differentially expressed GADD45G transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. GADD45G was expressed at lower levels in high-grade serous ovarian cancers when compared to the normal ovary; this difference was not statistically significant (Figure 1; $p=0.1143$). We

1 calculated a mean fold change of 0.4922 ± 0.1004 in GADD45G expression when comparing
2 HGSC tumors to normal ovarian tissue (Table 1).

3 Thus, we found using published microarray data that GADD45G was among the genes
4 most differentially expressed in tumors from women with high-grade serous carcinomas and that
5 GADD45G expression was lower in HGSC tumors when compared to the normal ovary.
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7 **Discussion**

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9 Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic analysis
10 classified HGSC into four subtypes based on gene clustering: immunoreactive, differentiated,
11 proliferative, and mesenchymal⁶. We sought to continue to describe the transcriptional
12 landscape of high-grade serous ovarian cancers⁶ and identify genes whose differential
13 expression was associated with HGSC by using published microarray data from primary tumors
14 of women diagnosed with HGSC compared to transcriptome data from normal ovarian tissue^{2,3}.
15 In both datasets analyzed, GADD45G was among the genes whose expression changed most
16 significantly when comparing the normal ovary to primary HGSC tumors.
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18 A subset of clones identified as DNA-damage inducible transcripts in a study utilizing UV
19 irradiation of Chinese hamster cells was found to be growth arrest and DNA damage inducible
20 and thus termed *gadd* genes^{7,8}. Induction of *gadd45* could be observed at X-ray doses starting
21 at 2 Gy, and unlike all other X-ray inducible genes in mammalian cells, its induction was
22 independent of protein kinase C⁹. GADD45G was found to be a MEKK4-interacting protein in a
23 yeast two-hybrid screen¹⁰. A second study identified GADD45G (also known as CR6) based on
24 homology to GADD45A (GADD45) and GADD45B (MyD118); CR6 was initially cloned as an
25 immediate-early gene in response to interleukin-2 (IL-2)¹¹.
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27 GADD45G can interact with the CDK1/Cyclin B1 complex. Interestingly, the interaction
28 of each of GADD45A, GADD45B, and GADD45C inhibits the kinase activity of CDK1/Cyclin B,
kinase inhibition by GADD45A and GADD45B involved disruption of complex formation, while

1 kinase inhibition by GADD45G did not affect complex formation¹². GADD45G is the only
2 GADD45 family member whose expression is IL2-inducible. The expression of GADD45G is
3 dependent on signaling through JAK3 and STAT5A/STAT5B. GADD45G deficient mice are
4 fertile, born in normal mendelian ratios, and checkpoint activation assessed by mitotic index in
5 response to UV irradiation in proliferating T-lymphocytes from GADD45G deficient mice was
6 unaltered¹³.

7
8 T_H1 cells from GADD45 γ $-/-$ mice fail to activate p38 MAP kinase and JNK signaling in
9 response to TCR ligation, and fail to secrete normal amounts of the T_H1 cytokine interferon- γ ¹⁴.

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11 Treatment of CD150⁺ CD48⁻ CD34^{lo} CD117⁺ Sca1⁺ lineage- long-term murine hematopoietic
12 stem cells (HSC) with thrombopoietin (TPO) results in a substantial increase in expression of
13 GADD45G. In multipotent progenitors (MPPs), GADD45G expression can be induced by
14 treatment with either TPO or IL-6¹⁵. Experiments using lentiviral expression of GADD45G in
15 purified LSK cells demonstrated that GADD45G expression was simply incompatible with the
16 megakaryocyte-erythroid lineage and thus its expression selected for lymphoid and myeloid
17 lineages¹⁵. Deletion of GADD45G in mice has no effect on steady-state hematopoiesis but
18 engraftment of GADD45G $-/-$ bone marrow cells is significantly enhanced upon secondary
19 transplantation, indicating that GADD45G controls self-renewal of HSC *in vivo*¹⁵. GADD45G
20 functioned in HSC differentiation through a MAP3K4-MKK6-p38 signaling pathway¹⁵.

21
22 Repression of GADD45G gene expression in conjunction with hypermethylation of the
23 GADD45G promoter was observed in 11/13 (85%) non-Hodgkin's lymphomas, 3/6 (50%)
24 Hodgkin's lymphomas, 8/11 (73%) of nasopharyngeal carcinomas, 2/4 (50%) of cervical
25 carcinomas, 5/17 (29%) esophageal carcinomas, and 2/5 (40%) lung carcinomas, but not in any
26 other cell line or normal tissue¹⁶. Evidence supporting an epigenetic mechanism for silencing of
27 GADD45G was reversal of silencing by 5-aza-2'-deoxycytidine or deletion of the DNA
28 methyltransferases DNMT1 and DNMT3B¹⁶. In cell lines where the promoter was methylated,
heat shock or UV irradiation induction of GADD45G was inhibited¹⁶. Together with data showing

1 that ectopic expression of GADD45G could inhibit colony formation of CNE-1, HK1, HepG2,
2 EC109, and HEPG2 cancer cell lines, GADD45G was deemed to be a tumor suppressor¹⁶.

3 Investigators using a model where mice were vaccinated with irradiated B16 melanoma
4 cells and challenged 90 days post-vaccination with live B16 cells to assess whether GADD45B
5 and GADD45G were important for adaptive anti-tumor responses found that tumors grew in
6 70% of vaccinated GADD45B/GADD45G double knock-out mice; no tumor nodules were
7 observed in wild-type vaccinated mice¹⁷. GADD45G is silenced in a number of human cancers.
8 GADD45G but not GADD45A or GADD45B was found to be frequently silenced in esophageal
9 squamous cell carcinoma (ESCC) tissues, and promoter methylation and protein expression of
10 GADD45G were both associated with patient survival in ESCC¹⁸. In gastric cardia
11 adenocarcinoma, tumor expression of GADD45G was significantly decreased as compared to
12 normal tissues, and GADD45 expression was significantly associated with TNM tumor staging.
13 Positive GADD45G expression was associated with significantly enhanced cumulative patient
14 survival in GCA¹⁹.

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16 GADD45 downregulation has also been observed in hepatocellular carcinoma (HCC)²⁰.
17 Ectopic expression of GADD45g inhibited tumor growth *in vivo* and suppressed proliferation of
18 HCC cells *in vitro*; induction of senescence through GADD45G was independent of p53 and
19 pRb. Investigators found that Smad-interacting protein-1 (SIP1) was important for induction of
20 senescence in tumors through GADD45 through mechanisms involving the proteasome; JNK
21 but not p38 was important for induction of SIP1 through GADD45G²¹.

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23 Interestingly, GADD45G mutant mice undergo male to female sex reversal - while
24 genotyping of GADD45G homozygous mutant mice indicates that males and females are born
25 in normal mendelian ratios, only 3.5% of GADD45G deficient mice were males based on the
26 appearance of external genitalia²². Both the internal reproductive organs and the external
27 genitalia of GADD45G XY ^{-/-} mice are indistinguishable from that of female mice²². At E11.5
28 and E12.5, when gene expression is sexually dimorphic, GADD45^{-/-} XY mutant gonads express
female marker genes Wnt4 and Rspo1²². p38 signaling is impaired in GADD45G mutant mice;

1 as GATA4 is required for Sry expression, and since GATA4 bound the Sry promoter in a MAP
2 kinase-dependent manner, authors concluded that GADD45G-induced activation of p38 MAP
3 kinase licensed GATA4 occupancy of SRY to promote male sex determination²².

4 Differential expression of GADD45G in HGSC should be validated in larger and separate
5 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
6 cancers²³⁻²⁵ can be utilized to assess the effect of conditional expression of GADD45G in HGSC
7 tumors growth post-development and on survival *in vivo*. We found here that a DNA damage-
8 inducible gene with tumor suppressive and growth control properties, implicated in adaptive
9 anti-tumor responses was among the genes most differentially expressed in tumors from
10 women diagnosed with high-grade serous ovarian carcinoma.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
217	8156309	2.48E-07	-6.0818219	6.8319349	0.4922 ± 0.1004	GADD45G	growth arrest and DNA damage inducible gamma

Table 1: GADD45G 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 GADD45G 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
4439	A_24_P120934	1.12E-02	-3.0044553	-3.05273	GADD45G	growth arrest and DNA damage inducible gamma

Table 2: GADD45G 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 GADD45G in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: GADD45G is expressed at lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of GADD45G 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 GADD45G expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.