Traf5 is differentially expressed in high-grade serous ovarian cancer and based on patient survival.

Shahan Mamoor¹

¹shahanmamoor@gmail.com

East Islip, NY 11730

High-grade serous ovarian cancer (HGSC) is the most common type of the most lethal gynecologic malignancy (1). To identify genes whose expression was specifically perturbed in HGSC, we used published microarray data (2, 3) to compare the global gene expression profiles of primary HGSC tumors to normal ovarian tissue. We found that the TNF receptor associated factor 5 (TRAF5) (4, 5) was among the genes most differentially expressed in HGSC tumors relative to the normal ovary. In a separate dataset from patients enrolled in the ICON7 trial (6), the Traf5 gene was among those most differentially expressed when comparing HGSC tumor gene expression based on patient survival outcomes. Traf5 may be relevant to the biology of high-grade serous ovarian cancers.

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

High-grade serous ovarian cancer (HGSC) is the most prevalent type of the most lethal gynecologic malignancy: ovarian cancer (1, 7, 8). 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 (7, 8). Understanding how the gene expression of tumors differs from the organ from which it is derived can provide insight into the mechanisms by which cancers are initiated and maintained. We used published microarray data (2, 3) to compare global gene expression profiles of HGSC tumors to the normal ovary. The TNF receptor associated factor 5 (TRAF5) emerged as among the genes most differentially expressed in HGSC tumors, both when comparing tumor to control ovarian tissue and when comparing patient tumor global gene expression profiles based on survival outcomes.

Methods

We used microarray data from datasets GSE146556 (2), GSE124766 (3) and GSE140082 (6) for this differential gene expression analysis of high-grade serous carcinomas. 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. GSE146556 (2) 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 high-grade serous ovarian cancer. GSE124766 (3) was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors from patients with high-grade serous ovarian cancer. GSE140082 was generated using Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip technology with n=31 for tumors from patients with progression-free survival less than 200 days and n=36 for tumors from patients with progression-free survival greater than 1000 days. GEO2R provides mRNA expression levels only for the top 250 most differentially expressed genes.

1 A statistical test was performed to evaluate whether Traf5 expression was significantly
2 different when comparing normal ovarian tissue from control subjects and primary tumors from
3 women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's correction. Only
4 p-values less than 0.05 were considered statistically significant. We used PRISM for all
5 statistical analyses (Version 8.4.0)(455).
6

7 **Results**

8 We mined published microarray data (2, 3) to identify differentially expressed genes in
9 high-grade serous ovarian cancer (HGSC), the type of ovarian cancer responsible for 70-80% of
10 deaths resulting from the most lethal gynecologic malignancy.
11

12 Traf5 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

13
14 We identified Traf5 as among the genes whose expression was most different when
15 comparing primary HGSC tumors to normal ovarian tissue (Table 1). When sorting all of the
16 transcripts measured by microarray based on change in expression between HGSC and the
17 normal ovary, Traf5 ranked 154 out of 29088 total transcripts (Table 1). Differential expression
18 of Traf5 in HGSC tumors was statistically significant (Table 1; 6.97E-06).
19

20 We analyzed a second microarray dataset generated using normal ovarian tissues and
21 tumors from women diagnosed with HGSC to determine whether differential expression of Traf5
22 could be observed in tumors from a different group of patients. We again found that Traf5 was
23 differentially expressed in primary HGSC tumors (Table 2). When sorting all of the transcripts
24 measured by microarray based on change in expression between HGSC and the normal ovary,
25 in this data set, Traf5 ranked 3632 out of 41093 total transcripts (Table 2). Differential
26 expression of Traf5 in HGSC tumors was statistically significant (Table 2; p=5.92E-03).
27
28

Traf5 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 Traf5 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. Traf5 was expressed at significantly lower levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 1; $p=0.0004$). We calculated a mean fold change of 0.81666 ± 0.03585 in Traf5 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

Traf5 expression in HGSC associates with survival outcomes.

We used a third dataset to identify genes associated with greater patient survival in ovarian cancer, comparing tumors from patients surviving less than 200 days to that of patients surviving greater than 1000 days. This data was obtained from public microarray data generated using tumors from patients enrolled in the ICON7 trial (6). We again found that Traf5 was among the genes most differentially expressed in HGSC, but in this comparison, based on survival outcomes (Table 3 and Figure 2). Differential expression of Traf5 in HGSC tumors based on patient survival less than 200 days or greater than 1000 days was statistically significant (Table 3; $p=0.00023573$). Though Traf5 was expressed at significantly lower levels in tumors when compared to the normal ovary, Traf5 was also expressed at significantly lower levels in primary HGSC tumors from patients with survival greater than 1000 days when compared to that of patients surviving less than 200 days (Figure 2; $p=0.0003$).

Thus, we found using published microarray data that Traf5 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that Traf5 expression was significantly lower in HGSC tumors when compared to the normal ovary. Moreover, Traf5 was among the most differentially expressed genes when comparing primary HGSC tumors based on patient survival, expressed at significantly lower levels in the tumors of

1 patients surviving greater than 1000 days when compared to that of patients surviving less than
2 200 days.

3 4 **Discussion**

5 Here we used published microarray data (2, 3) to compare system-wide gene
6 expression in the normal ovary and HGSC tumors. Traf5 emerged as differentially expressed in
7 separate patient datasets when comparing HGSC primary tumor tissue to the normal ovary. In
8 a third dataset (6), Traf5 was found to be significantly associated with patient survival,
9 differentially expressed when comparing patient tumor tissue based on survival time.

10 Traf5 is an intracellular adaptor for signal transduction in TNF-alpha receptor-mediated
11 signaling pathways, linking, in this case, extracellular ligand-binding by CD40 to recruitment of
12 TRAF5 to the intracellular domains of CD40 (4, 9). In addition to its role in CD40 signaling,
13 Traf5 was originally described as a component of CD27 signaling (9) and as a component of
14 lymphotoxin beta signaling (5). As a negative regulator of IL6-mediated signaling in CD4+
15 helper T-lymphocytes, Traf5 functions to limit differentiation of T-lymphocytes into the
16 inflammatory phenotype (10). Consistent with a role for Traf5 in restraining type I T-lymphocyte
17 responses, T_H17-associated experimental autoimmune encephalitis (EAE), an animal model of
18 multiple sclerosis, was significantly worsened in Traf5-deficient mice (10). Traf5 has also been
19 described as a negative regulator of TLR signaling in B-cells (11) and involved in NF-kB
20 activation through OX40 (12).
21

22 Traf5 has been implicated as important in human cancers but this data is limited. In
23 Hodgkin-Reed Sternberg cells associated with Hodgkin's lymphoma, Traf2 and Traf5 were
24 found to form cytoplasmic aggregates (13). Traf5 also localized proximal to the cell membrane.
25 This phenomena was not observed in other lymphoma cell types studied (13). The authors
26 concluded that this reflected the constitutively active CD30 signaling found in patients with
27 Hodgkin's lymphoma. In addition, TRAF5 has been suggested to play a role in esophageal
28 squamous cell cancer (14), colorectal cancer (15), and in gastric cancer (16) through microRNA

1 regulatory loops involving microRNA 26b, microRNA 873, and microRNA 135a, respectively.

2 Thus, Traf5 has roles in both immunity and cancer.

3
4 Differential expression of Traf5 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 cancers (17) in conjunction with conditional expression of Traf5 can be utilized to assess the
7 effect of a gradient of Traf5 transcript levels on tumor cell proliferation and cancer progression *in*
8 *vivo*.

References

1. Guppy, A.E., Nathan, P.D. and Rustin, G.J., 2005. Epithelial ovarian cancer: a review of current management. *Clinical Oncology*, 17(6), pp.399-411.
2. Hoffmann, K., Berger, H., Kulbe, H., Thillainadarasan, S., Mollenkopf, H.J., Zemojtel, T., Taube, E., Darb-Esfahani, S., Mangler, M., Sehouli, J. and Chekerov, R., 2020. Stable expansion of high-grade serous ovarian cancer organoids requires a low-Wnt environment. *The EMBO journal*, 39(6), p.e104013.
3. Zhang, W., Klinkebiel, D., Barger, C.J., Pandey, S., Guda, C., Miller, A., Akers, S.N., Odunsi, K. and Karpf, A.R., 2020. Global DNA hypomethylation in epithelial ovarian cancer: passive demethylation and association with genomic instability. *Cancers*, 12(3), p.764.
4. Ishida, T.K., ToJo, T.A.D.A.S.H.I., Aoki, T., Kobayashi, N., Ohishi, T., Watanabe, T., Yamamoto, T. and Inoue, J.I., 1996. TRAF5, a novel tumor necrosis factor receptor-associated factor family protein, mediates CD40 signaling. *Proceedings of the National Academy of Sciences*, 93(18), pp.9437-9442.
5. Nakano, H., Oshima, H., Chung, W., Williams-Abbott, L., Ware, C.F., Yagita, H. and Okumura, K., 1996. TRAF5, an activator of NF- κ B and putative signal transducer for the lymphotoxin- β receptor. *Journal of Biological Chemistry*, 271(25), pp.14661-14664.
6. GSE140082. Sean Dowdy; Winterhoff K, Kommoss S. Mayo Clinic, Obstetrics and Gynecology. Rochester, MN. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE140082>.
7. Bowtell, D.D., Böhm, S., Ahmed, A.A., Aspuria, P.J., Bast Jr, R.C., Beral, V., Berek, J.S., Birrer, M.J., Blagden, S., Bookman, M.A. and Brenton, J.D., 2015. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. *Nature reviews Cancer*, 15(11), pp.668-679.
8. Vaughan, S., Coward, J.I., Bast, R.C., Berchuck, A., Berek, J.S., Brenton, J.D., Coukos, G., Crum, C.C., Drapkin, R., Etemadmoghadam, D. and Friedlander, M., 2011. Rethinking ovarian cancer: recommendations for improving outcomes. *Nature Reviews Cancer*, 11(10), pp.719-725.
9. Nakano, H., Sakon, S., Koseki, H., Takemori, T., Tada, K., Matsumoto, M., Munechika, E., Sakai, T., Shirasawa, T., Akiba, H. and Kobata, T., 1999. Targeted disruption of Traf5 gene causes defects in CD40-and CD27-mediated lymphocyte activation. *Proceedings of the National Academy of Sciences*, 96(17), pp.9803-9808.
10. Nagashima, H., Okuyama, Y., Asao, A., Kawabe, T., Yamaki, S., Nakano, H., Croft, M., Ishii, N. and So, T., 2014. The adaptor TRAF5 limits the differentiation of inflammatory CD4+ T cells by antagonizing signaling via the receptor for IL-6. *Nature immunology*, 15(5), p.449.

11. Buchta, C.M. and Bishop, G.A., 2014. TRAF5 negatively regulates TLR signaling in B lymphocytes. *The Journal of Immunology*, 192(1), pp.145-150.
12. Kawamata, S., Hori, T., Imura, A., Takaori-Kondo, A. and Uchiyama, T., 1998. Activation of OX40 signal transduction pathways leads to tumor necrosis factor receptor-associated factor (TRAF) 2-and TRAF5-mediated NF- κ B activation. *Journal of Biological Chemistry*, 273(10), pp.5808-5814.
13. Horie, R., Watanabe, T., Ito, K., Morisita, Y., Watanabe, M., Ishida, T., Higashihara, M., Kadin, M. and Watanabe, T., 2002. Cytoplasmic aggregation of TRAF2 and TRAF5 proteins in the Hodgkin-Reed-Sternberg cells. *The American journal of pathology*, 160(5), pp. 1647-1654.
14. Chen, Z., Zhao, L., Zhao, F., Yang, G. and Wang, J., 2016. MicroRNA-26b regulates cancer proliferation migration and cell cycle transition by suppressing TRAF5 in esophageal squamous cell carcinoma. *American journal of translational research*, 8(5), p.1957.
15. Gong, H., Fang, L., Li, Y., Du, J., Zhou, B., Wang, X., Zhou, H., Gao, L., Wang, K. and Zhang, J., 2018. miR-873 inhibits colorectal cancer cell proliferation by targeting TRAF5 and TAB1. *Oncology reports*, 39(3), pp.1090-1098.
16. Xie, Y., Li, F., Li, Z. and Shi, Z., 2019. miR-135a suppresses migration of gastric cancer cells by targeting TRAF5-mediated NF- κ B activation. *OncoTargets and therapy*, 12, p.975.
17. Kim, J., Coffey, D.M., Creighton, C.J., Yu, Z., Hawkins, S.M. and Matzuk, M.M., 2012. High-grade serous ovarian cancer arises from fallopian tube in a mouse model. *Proceedings of the National Academy of Sciences*, 109(10), pp.3921-3926.

Rank	ID	p-value	t	B	Fold change	Gene	Gene name
154	A_23_P201731	6.97E-06	-7.6193799	4.21595	0.81666 ± 0.03585	TRAF5	TNF receptor associated factor 5

Table 1: Traf5 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 Traf5 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
3632	7909545	5.92E-03	2.8911954	-2.707781	TRAF5	TNF receptor associated factor 5

Table 2: Traf5 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 Traf5 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Figure 1: Traf5 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of Traf5 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 Traf5 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.

Rank	ID	p-value	t	B	Gene
88	ILMN_1740493 / NM_004619.3 / 77404345	0.00023573	-3.885656	0.439468	TRAF5

Table 3: Traf5 is differentially expressed in the tumors of patients with high-grade serous ovarian carcinomas surviving greater than 1000 days when compared to that of patients surviving less than 200 days.

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 Traf5 in the tumors of patients surviving great than 1000 days when compared to that of patients surviving less than 200 days, and the gene are listed in this chart.

Figure 2: Traf5 is expressed at significantly lower levels in HGSC tumors of patients surviving greater than 1000 days when compared to that of patients surviving less than 200 days.

The mRNA expression of Traf5 in tumors from HGSC patients surviving less than 200 days (left) and in the primary tumors of HGSC patients surviving greater than 1000 days (right) is represented with mean mRNA expression level marked and the result of a statistical test, a p-value, listed above.