Over-expression of TIGAR in high-grade serous ovarian cancers.

Shahan Mamoor¹

¹shahanmamoor@gmail.com

East Islip, NY 11730

Ovarian cancer is the most lethal gynecologic malignancy and 70-80% of ovarian cancers are of the high-grade serous type (1-3). To identify the most significant changes in gene expression in high-grade serous ovarian cancer (HGSC), we compared global gene expression profiles of tumors from patients with HGSC to that of normal ovary using published microarray datasets (4, 5). We found that the TP53-inducible glycolysis and apoptosis regulator, or TIGAR (6), was among the genes whose expression quantitatively changed most significantly when comparing HSGC tumors to the ovary.

Keywords: TIGAR, ovarian cancer, HGSC, high-grade serous ovarian cancer, systems biology of ovarian cancer, targeted therapeutics in 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 (1-3). 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 (7). 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 TP53-
7 induced glycolysis and apoptosis regulator, TIGAR, as among the most differentially expressed
8 in HGSC tumors of the ovary. TIGAR may be a gene of interest when prioritizing the study of
9 target genes and pathways for the development of novel therapeutic interventions in high-grade
10 serous ovarian cancers.

13 **Methods**

14 We used microarray data from datasets GSE146556 (4) and GSE124766 (5) for this
15 differential gene expression analysis of high-grade serous carcinomas. The Benjamini and
16 Hochberg method of p-value adjustment was used for ranking of differential expression but raw
17 p-values were used for assessment of statistical significance of global differential expression.
18 Log-transformation of data was auto-detected, and the NCBI generated category of platform
19 annotation was used. GSE146556 (4) was generated using Affymetrix Human Gene 1.0 ST
20 Array with n=3 for normal ovarian tissue and n=40 for tumors from patients with high-grade
21 serous ovarian cancer. GSE124766 (5) was generated using Agilent-014850 Whole Human
22 Genome Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors
23 from patients with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels
24 only for the top 250 most differentially expressed genes.

26 A statistical test was performed to evaluate whether TIGAR expression was significantly
27 different when comparing normal ovarian tissue from control subjects and primary tumors from
28 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.

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

We identified TIGAR as among the genes whose expression was quantitatively most different in a published microarray dataset (4) 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 tumors and the normal ovary, TIGAR ranked 233 out of 29088 total transcripts (Table 1). Differential expression of TIGAR in HGSC tumors was statistically significant (Table 1; $p=3.09E-07$).

We analyzed a second microarray dataset generated using normal ovarian tissues and tumors from women diagnosed with HGSC (5) to determine whether differential expression of TIGAR could be observed in a separate group of patients. We again found that TIGAR 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, TIGAR ranked 4939 out of 41093 total transcripts (Table 2). Differential expression of TIGAR in HGSC tumors was statistically significant (Table 2; $1.43E-02$).

TIGAR is expressed at significantly higher levels in HGSC when compared to the normal ovary.

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

1 compared to the normal ovary (Figure 1; $p < 0.0001$). We calculated a mean fold change of 5.13 ± 2.50 in TIGAR expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

2
3 Thus, we found using published microarray data (4, 5) that TIGAR was among the genes
4 most differentially expressed in tumors from women with high-grade serous carcinomas and that
5 TIGAR expression was significantly higher in HGSC tumors when compared to the normal
6 ovary.

7 8 **Discussion**

9
10 TIGAR is a fructose-2,6-bisphosphatase, an inhibitor of glycolysis, and supports
11 production of NADPH through activation of the pentose phosphate pathway (6, 8). Increases in
12 cellular NADPH caused by TIGAR have antioxidant functions, serving to limit reactive oxygen
13 species (ROS) (6, 8). Human TIGAR display homology at the genetic level to the
14 bisphosphatase domain of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB).
15 TIGAR enzymatic function leads to reduced levels of fructose-2,6-bisphosphate (Fru-2,6-P₂)
16 and 2,3-bisphosphoglycerate (6, 8). Fru-2,6-P₂ is an allosteric activator of
17 phosphofructokinase-1 (PFK-1), which catalyzes a key step in the glycolytic pathway, the
18 conversion of fructose-6-phosphate to fructose-1,6-bisphosphate (6, 8). Thus, by decreasing
19 levels of a PFK-1 allosteric inhibitor, Fru-2,6-P₂, TIGAR is able to limit activation of an enzyme
20 important for glycolysis, PFK-1, and thereby exert its control over glycolysis.

21
22 While both production and limitation of ROS are suggested to function to support
23 tumorigenesis (9), there are multiple examples of human cancers limiting cellular exposure to
24 ROS. One mechanism by which tumor cells can support growth by limiting ROS exposure is
25 the up-regulation of TIGAR. Treating liver and lung cancer cells *in vitro* with epirubicin resulted
26 in increased expression of TIGAR (8). Genetic depletion of TIGAR *in vitro* lead to increased cell
27 death and autophagy resulting from epirubicin drug treatment; this phenotype could be rescued
28 by addition of exogenous NADPH (8). TIGAR is important for tumorigenesis in murine cancer

models of the pancreas and the intestine (10, 11). In a mouse model of pancreatic ductal adenocarcinoma, a double TIGAR expression gradient was documented wherein increased expression of TIGAR was found in tumor cells, with a decreased TIGAR expression in invasive cancer cells, but with higher TIGAR expression again found in metastases (10). These data suggested that while limiting ROS was beneficial during the premalignant state, early in cancer progression, that ROS exposure was beneficial to cancer during metastasis. Under hypoxic conditions, a fraction of TIGAR protein re-localized to the mitochondria where it interacted with hexokinase 2 (HK2); this was dependent on HIF-1a activity (12). Mitochondrial re-localization of TIGAR lead to decreases in both mitochondrial ROS exposure and cell death (12).

Just as increased expression of TIGAR appears to function in a pro-tumorigenic manner through supporting cell survival through increased levels of NADPH, one study demonstrated that inhibition of c-Met lead to decreased TIGAR expression, decreased NADPH levels, and increased cancer cell death (13). TIGAR is closely tied to the metabolic state of cancer cells, as one study documented that cancer cells over-expressing TIGAR displayed higher levels of ATP in the presence of glutamine and/or lactate, as well as increased oxygen consumption rates (14). Co-culturing fibroblasts with these TIGAR-over-expressing cells in vitro lead to HIF-1a induction and increases in lactate dehydrogenase in fibroblasts, suggesting TIGAR in carcinoma cells can modulate the metabolism of neighboring stroma (14). TIGAR may also influence the DNA damage response pathway by modulating the phosphorylation of ATM via Cdk5 (15).

We found here that the TP53-induced regulatory phosphatase TIGAR was expressed at significantly higher levels in tumors from a group of patients with high-grade serous ovarian cancer when compared to the normal ovary, using published microarray data (4, 5). This change in TIGAR expression was among the highest transcriptome-wide when comparing HGSC tumors to benign ovarian tissue. TIGAR is important for cancer cell survival and progression in multiple solid tumors (6-8, 10, 11, 13-15). TIGAR may be relevant to the biology of high-grade serous ovarian cancers.

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. 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.
3. 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.
4. 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.
5. 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.
6. Bensaad, K., Tsuruta, A., Selak, M.A., Vidal, M.N.C., Nakano, K., Bartrons, R., Gottlieb, E. and Vousden, K.H., 2006. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. *Cell*, 126(1), pp.107-120.
7. Cancer Genome Atlas Research Network, 2011. Integrated genomic analyses of ovarian carcinoma. *Nature*, 474(7353), p.609.
8. Xie, J.M., Li, B., Yu, H.P., Gao, Q.G., Li, W., Wu, H.R. and Qin, Z.H., 2014. TIGAR has a dual role in cancer cell survival through regulating apoptosis and autophagy. *Cancer research*, 74(18), pp.5127-5138.
9. Chio, I.I.C. and Tuveson, D.A., 2017. ROS in cancer: the burning question. *Trends in molecular medicine*, 23(5), pp.411-429.
10. Cheung, E.C., DeNicola, G.M., Nixon, C., Blyth, K., Labuschagne, C.F., Tuveson, D.A. and Vousden, K.H., 2020. Dynamic ROS control by TIGAR regulates the initiation and progression of pancreatic cancer. *Cancer cell*, 37(2), pp.168-182.
11. Cheung, E.C., Athineos, D., Lee, P., Ridgway, R.A., Lambie, W., Nixon, C., Stratthdee, D., Blyth, K., Sansom, O.J. and Vousden, K.H., 2013. TIGAR is required for efficient intestinal regeneration and tumorigenesis. *Developmental cell*, 25(5), pp.463-477.

- 1
- 2
- 3 12. Cheung, E.C., Ludwig, R.L. and Vousden, K.H., 2012. Mitochondrial localization of TIGAR
- 4 under hypoxia stimulates HK2 and lowers ROS and cell death. *Proceedings of the National Academy of Sciences*, 109(50), pp.20491-20496.
- 5 13. Lui, V.W.Y., Wong, E.Y.L., Ho, K., Ng, P.K.S., Lau, C.P.Y., Tsui, S.K.W., Tsang, C.M., Tsao,
- 6 S.W., Cheng, S.H., Ng, M.H.L. and Ng, Y.K., 2011. Inhibition of c-Met downregulates TIGAR
- 7 expression and reduces NADPH production leading to cell death. *Oncogene*, 30(9), pp.
- 8 1127-1134.
- 9 14. Ko, Y.H., Domingo-Vidal, M., Roche, M., Lin, Z., Whitaker-Menezes, D., Seifert, E.,
- 10 Capparelli, C., Tuluc, M., Birbe, R.C., Tassone, P. and Curry, J.M., 2016. TP53-inducible
- 11 glycolysis and apoptosis regulator (TIGAR) metabolically reprograms carcinoma and
- 12 stromal cells in breast cancer. *Journal of biological chemistry*, 291(51), pp.26291-26303.
- 13 15. Yu, H.P., Xie, J.M., Li, B., Sun, Y.H., Gao, Q.G., Ding, Z.H., Wu, H.R. and Qin, Z.H., 2015.
- 14 TIGAR regulates DNA damage and repair through pentosephosphate pathway and Cdk5-
- 15 ATM pathway. *Scientific reports*, 5, p.9853.
- 16
- 17
- 18
- 19
- 20
- 21
- 22
- 23
- 24
- 25
- 26
- 27
- 28

Rank	ID	p-value	t	B	Fold change	Gene	Gene name
233	7953211	3.09E-07	6.0168587	6.6198681	5.13 ± 2.50	TIGAR	TP53 induced glycolysis regulatory phosphatase

Table 1: TIGAR 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 TIGAR 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
4939	A_23_P128486	1.43E-02	2.8732518	-3.2864	TIGAR	TP53 induced glycolysis regulatory phosphatase

Table 2: TIGAR 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 gene and gene name are listed in this chart.

Figure 1: TIGAR expression is significantly elevated in HGSC tumors.

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