STARD9 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 cancer¹⁻³. 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 the steroidogenic acute regulatory protein-related lipid transfer (START) domain containing protein STARD9⁶ as among the genes whose expression was most different in HGSC ovarian tumors. STARD9 expression was significantly lower in ovarian tumors relative to normal ovary. These data reveal perturbed expression of a mitotic kinesin and the only kinesin located at the daughter centriole⁶ in high-grade serous ovarian cancers.

Keywords: STARD9, 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 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^{4,5} to compare global gene expression profiles between HGSC
6 ovarian tumors and that of normal ovarian tissue. We identified the gene encoding STARD9 as
7 among the most differentially expressed in HGSC tumors of the ovary. STARD9 may be a gene
8 of interest when prioritizing the study of target genes and pathways for the development of novel
9 therapeutic interventions in high-grade serous ovarian cancers.
10

11 12 **Methods**

13
14 We used microarray data from datasets GSE146556⁴ and GSE124766⁵ 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 was generated using Affymetrix Human Gene 1.0 ST Array
20 with n=3 for normal ovarian tissue and n=40 for tumors from patients with high-grade serous
21 ovarian cancer. GSE124766 was generated using Agilent-014850 Whole Human Genome
22 Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors from
23 patients with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels only
24 for the top 250 most differentially expressed genes.
25

26 A statistical test was performed to evaluate whether STARD9 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.

STARD9 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified STARD9 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, STARD9 ranked 190 out of 29088 total transcripts (Table 1). Differential expression of STARD9 in HGSC tumors was statistically significant (Table 1; $p=1.4E-07$).

We analyzed a second microarray dataset generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of STARD9 could be observed in tumors from a different group of patients. We again found that STARD9 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, STARD9 ranked 216 out of 41093 total transcripts (Table 2). Differential expression of STARD9 in HGSC tumors was statistically significant (Table 2; $p=1.43E-05$).

STARD9 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 STARD9 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. STARD9 was expressed at significantly lower levels in high-grade serous ovarian cancers when

1 compared to the normal ovary (Figure 1; $p=0.0228$). We calculated a mean fold change of
2 0.3723 ± 0.1093 in STARD9 expression when comparing HGSC tumors to normal ovarian
3 tissue (Table 1).

4 Analysis of STARD9 differential expression in a separate dataset demonstrated that
5 STARD9 was again expressed at significantly lower levels in primary tumors from women
6 diagnosed with high-grade serous ovarian cancer when compared to normal ovarian tissue
7 (Figure 2; $p=0.0002$). We calculated a mean fold change of 0.7680 ± 0.0527 in STARD9
8 expression when comparing HGSC tumors to normal ovarian tissue (Table 2).

9 Thus, we found using published microarray data that STARD9 was among the genes
10 most differentially expressed in tumors from women with high-grade serous carcinomas and that
11 STARD9 expression was significantly lower in HGSC tumors when compared to the normal
12 ovary.
13

14 15 Discussion

16
17 Transcriptional profiling of HGSC in a Cancer Genome Atlas integrated genomic analysis
18 classified HGSC into four subtypes based on gene clustering: immunoreactive, differentiated,
19 proliferative, and mesenchymal⁷. We sought to continue to describe the transcriptional
20 landscape of high-grade serous ovarian cancers⁷ and identify genes whose differential
21 expression was associated with HGSC by using published microarray data from primary tumors
22 of women diagnosed with HGSC compared to transcriptome data from normal ovarian tissue^{4, 5}.
23 In both datasets analyzed, STARD9 was among the genes whose expression changed most
24 significantly when comparing the normal ovary to primary HGSC tumors.

25
26 STARD9 appears to function as a regulator of spindle pole assembly as a mitotic
27 kinesin^{6, 8-10}. During the process of cell division, chromosomes align at the metaphase plate,
28 and an apparatus known as the mitotic spindle is utilized to bind chromosomes at the
kinetochore; the spindle is composed of microtubules¹¹. The centrosome functions as a

1 microtubule-organizing and nucleation center to ensure faithful transmission of one
2 chromosome each to mother and daughter cell. Two barrel-like structures are formed at the
3 centrosome known as centrioles⁸. Kinesins are molecular motors that use energy in the form of
4 ATP to perform work, like that required for the transport of vesicles⁸. In humans, there are 45
5 kinesins, and STARD9 is the only kinesin enriched at the daughter centriole⁶. Centrioles are
6 surrounded by a matrix of amorphous protein, composed of pericentrin and CG-NAP, known as
7 the pericentriolar material (PCM)^{6, 8}. STARD9 may be important for the cohesion of the PCM
8 early in mitosis⁶. STARD9 associates with centrioles only from early in mitosis until late
9 anaphase⁶. A study identifying mitotic microtubule associating proteins found STARD9 in
10 association with mitotic microtubule asters⁶. STARD9 contains a forkhead-associated domain
11 (FHA) and motor domain conserved among all Kinesin-3 family members, as well as a carboxy-
12 terminal START domain used to bind phospholipids and/or sterols, similar to the pleckstrin
13 homology domain found in KIF1A/KIF1B⁶. STARD9 has broad expression across tissues at
14 low-levels including the brain, heart and skin⁶. Unlike other kinesins, STARD9 possesses a loop
15 in its motor domain; STARD9 associates with Plk1 through this region¹⁰. Phosphorylation of
16 STARD9 by Plk1 at serine 132 regulates its stability¹⁰.

17
18 Depletion of STARD9 in a panel of cancer cell lines including H460, HCT116, and M-395
19 using small interfering RNAs leads to fragmentation of the PCM⁶. Cells depleted of STARD9
20 share phenotypic similarities to cells depleted of Kizuna, a centrosomal protein that functions in
21 tethering the PCM to the centriole⁶. The spindle assembly checkpoint (SAC) is used to monitor
22 attachment between microtubules and kinetochore and tension between kinetochores⁶. Cells
23 depleted of STARD9 manifest signs of an activated SAC, including arrest early in mitosis and
24 undergo mitotic cell death, suggesting that STARD9 regulates assembly of the spindle pole⁶.
25 Though microtubule-associated proteins are intuitively targets in cancer¹²⁻¹³, and though
26 depletion of STARD9 in a variety of cancer cell lines induces mitotic arrest and cell death^{6,8},
27 there is otherwise limited information regarding STARD9 in human cancers.
28

Differential expression of STARD9 in HGSC should be validated in larger and separate cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian cancers¹⁴⁻¹⁶ can be utilized to assess the effect of conditional expression of STARD9 on tumor cell proliferation and cancer progression *in vivo*, as well as on tumor initiation. We found here that a mitotic kinesin that interacts with microtubules and regulates spindle pole assembly was among the genes most differentially expressed in tumors from women diagnosed with the most lethal gynecological cancer.

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. 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.
5. 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.
6. Torres, J.Z., Summers, M.K., Peterson, D., Brauer, M.J., Lee, J., Senese, S., Gholkar, A.A., Lo, Y.C., Lei, X., Jung, K. and Anderson, D.C., 2011. The STARD9/Kif16a kinesin associates with mitotic microtubules and regulates spindle pole assembly. *Cell*, 147(6), pp.1309-1323.
7. Cancer Genome Atlas Research Network, 2011. Integrated genomic analyses of ovarian carcinoma. *Nature*, 474(7353), p.609.
8. Torres, J.Z., 2012. STARD9/Kif16a is a novel mitotic kinesin and antimitotic target. *Bioarchitecture*, 2(1), pp.19-22.
9. Srivastava, S. and Panda, D., 2018. A centrosomal protein STARD9 promotes microtubule stability and regulates spindle microtubule dynamics. *Cell Cycle*, 17(16), pp.2052-2068.
10. Senese, S., Cheung, K., Lo, Y.C., Gholkar, A.A., Xia, X., Wohlschlegel, J.A. and Torres, J.Z., 2015. A unique insertion in STARD9's motor domain regulates its stability. *Molecular biology of the cell*, 26(3), pp.440-452.
11. Walczak, C.E. and Heald, R., 2008. Mechanisms of mitotic spindle assembly and function. In *International review of cytology* (Vol. 265, pp. 111-158). Academic Press.

12. Dumontet, C. and Jordan, M.A., 2010. Microtubule-binding agents: a dynamic field of cancer therapeutics. *Nature reviews Drug discovery*, 9(10), pp.790-803.
13. Kavallaris, M., 2010. Microtubules and resistance to tubulin-binding agents. *Nature Reviews Cancer*, 10(3), pp.194-204.
14. Harrington, B.S., He, Y., Davies, C.M., Wallace, S.J., Adams, M.N., Beaven, E.A., Roche, D.K., Kennedy, C., Chetty, N.P., Crandon, A.J. and Flatley, C., 2016. Cell line and patient-derived xenograft models reveal elevated CDCP1 as a target in high-grade serous ovarian cancer. *British journal of cancer*, 114(4), pp.417-426.
15. Perets, R., Wyant, G.A., Muto, K.W., Bijron, J.G., Poole, B.B., Chin, K.T., Chen, J.Y.H., Ohman, A.W., Stepule, C.D., Kwak, S. and Karst, A.M., 2013. Transformation of the fallopian tube secretory epithelium leads to high-grade serous ovarian cancer in Brca; Tp53; Pten models. *Cancer cell*, 24(6), pp.751-765.
16. 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
190	7983132	1.4E-07	-6.2511792	7.3853274	0.3723 ± 0.1093	STARD9	StAR related lipid transfer domain containing 9

Table 1: STARD9 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 STARD9 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
216	A_32_P130641	1.43E-05	-7.0821224	3.52198	0.7680 ± 0.0527	STARD9	StAR related lipid transfer domain containing 9

Table 2: STARD9 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 STARD9 in the patient population when compared to control subjects, the gene and gene name are listed in this chart.

Figure 1: STARD9 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of STARD9 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 STARD9 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.

Figure 2: STARD9 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of STARD9 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 STARD9 expression between the ovary of control subjects and primary tumors from patients with HGSC, a p-value, listed above.