DUSP26 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 dual specificity phosphatase DUSP26 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, DUSP26.

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^{6,7}. 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 the dual specificity
7 phosphatase 26 (DUSP26) as among the most differentially expressed in HGSC tumors of the
8 ovary. DUSP26 may be a gene of interest when prioritizing the study of target genes and
9 pathways for the development of novel therapeutic interventions in high-grade serous ovarian
10 cancers.
11

12 13 **Methods**

14 We used microarray data from datasets GSE146556² and GSE124766³ for this
15 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
16 and Hochberg method of p-value adjustment was used for ranking of differential expression but
17 raw p-values were used for assessment of statistical significance of global differential
18 expression. Log-transformation of data was auto-detected, and the NCBI generated category of
19 platform annotation was used. GSE146556² was generated using Affymetrix Human Gene 1.0
20 ST Array with n=3 for normal ovarian tissue and n=40 for tumors from patients with high-grade
21 serous ovarian cancer. GSE124766³ 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.
25

26 A statistical test was performed to evaluate whether DUSP26 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^{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.

DUSP26 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified DUSP26 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, DUSP26 ranked 44 out of 29088 total transcripts (Table 1). Differential expression of DUSP26 in HGSC tumors was statistically significant (Table 1; $p=8.4139177$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of DUSP26 could be observed in tumors from a separate group of patients. We again found that DUSP26 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, DUSP26 ranked 141 out of 41093 total transcripts (Table 2). Differential expression of DUSP26 in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=6.11E-06$).

DUSP26 is expressed at significantly lower levels in HGSC when compared to the normal ovary.

We obtained exact mRNA expression levels for the differentially expressed DUSP26 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. DUSP26 was expressed at significantly lower levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 1; $p<0.0001$). We calculated a mean fold change of

0.4340 ± 0.0699 in DUSP26 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

We then obtained exact mRNA expression levels for the differentially expressed DUSP26 transcript from both normal ovarian tissue and in high-grade serous ovarian tumors from the second microarray dataset used to identify differential expression of DUSP26 in HGSC tumors. DUSP26 was again expressed at significantly lower levels in high-grade serous ovarian cancers when compared to the normal ovary (Figure 1; $p < 0.0001$). We calculated a mean fold change of 0.6486 ± 0.0801 in DUSP26 expression when comparing HGSC tumors to normal ovarian tissue (Table 2).

Thus, we found using published microarray data that DUSP26 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that DUSP26 expression was significantly lower in 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 mesenchymal⁸. We sought to continue to describe the transcriptional landscape of high-grade serous ovarian cancers⁸ 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, DUSP26 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors

Dual specificity kinases (DUSPs) are named for their ability to dephosphorylate both serine/threonine residues as well as tyrosine residues⁷. DUSP26 was identified as an inhibitor of p38 MAP kinase signaling, acting through dephosphorylation of p38 kinase⁸. Other substrates of DUSP26 include p53⁹ and the fas-associated death domain protein FADD¹⁰.

DUSP26 can associate with the motor protein KIF3 and promote cell-cell adhesion through N-cadherin¹¹.

In developmental processes, DUSP26 can suppress receptor tyrosine kinases and regulate neuronal development in zebrafish¹². In rat pheochromocytoma cell line PC12, DUSP26 (also known as neuroendocrine-associated phosphatase [NEAP]) can control the expression of epidermal growth factor receptor (EGFR) and inhibit differentiation of PC12 induced by nerve growth factor (NGF)¹³. There are limited studies describing roles for DUSP26 in the ovary. Investigators examining gene expression differences in mice deficient for Foxl2, Wnt4, and Kit found that DUSP26 was among the genes most significant up-regulated in the ovaries of triple-knockout mice¹⁴.

At least one study has demonstrate decreased expression of DUSP26 in ovarian cancer, showing that depletion of DUSP26 in the HOSE17.1 human ovarian surface epithelial cell line enhanced colony formation and proliferation *in vitro*¹⁵. DUSP26 is implicated in the biology of a number of other cancers. In anaplastic thyroid cancer, DUSP26 promotes cell growth by inhibition of p38 MAP kinase¹⁶. In neuroblastoma, DUSP26 functions as a tumor suppressor, acting as a phosphatase of the p53 tumor suppressor¹⁷; a specific inhibitor of DUSP26 displayed efficacy in a murine model of neuroblastoma, acting through induction of p53-mediated apoptosis¹⁸. A complex of DUSP6 and its activator adenylate kinase 2 (AK2) can dephosphorylate FADD; depletion of AK2 in MCF-7 breast cancer and C33A cervical cancer cell lines inhibited tumor growth in xenografts *in vivo*¹⁰.

Differential expression of DUSP26 in HGSC should be validated in larger and separate cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian cancers¹⁹⁻²¹ can be utilized to assess the effect of conditional expression of DUSP26 in HGSC tumors post-development and on tumor cell proliferation and cancer progression *in 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. 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.
3. 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.
4. 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.
5. 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.
6. Cancer Genome Atlas Research Network, 2011. Integrated genomic analyses of ovarian carcinoma. *Nature*, 474(7353), p.609.
7. Tonks, N.K., 2006. Protein tyrosine phosphatases: from genes, to function, to disease. *Nature reviews Molecular cell biology*, 7(11), pp.833-846.
8. Vasudevan, S.A., Skoko, J., Wang, K., Burlingame, S.M., Patel, P.N., Lazo, J.S., Nuchtern, J.G. and Yang, J., 2005. MKP-8, a novel MAPK phosphatase that inhibits p38 kinase. *Biochemical and biophysical research communications*, 330(2), pp.511-518.
9. Shang, X., Vasudevan, S.A., Yu, Y., Ge, N., Ludwig, A.D., Wesson, C.L., Wang, K., Burlingame, S.M., Zhao, Y.J., Rao, P.H. and Lu, X., 2010. Dual-specificity phosphatase 26 is a novel p53 phosphatase and inhibits p53 tumor suppressor functions in human neuroblastoma. *Oncogene*, 29(35), pp.4938-4946.
10. Kim, H., Lee, H.J., Oh, Y., Choi, S.G., Hong, S.H., Kim, H.J., Lee, S.Y., Choi, J.W., Hwang, D.S., Kim, K.S. and Kim, H.J., 2014. The DUSP26 phosphatase activator adenylate kinase 2 regulates FADD phosphorylation and cell growth. *Nature communications*, 5(1), pp.1-11.
11. Tanuma, N., Nomura, M., Ikeda, M., Kasugai, I., Tsubaki, Y., Takagaki, K., Kawamura, T., Yamashita, Y., Sato, I., Sato, M. and Katakura, R., 2009. Protein phosphatase Dusp26

associates with KIF3 motor and promotes N-cadherin-mediated cell–cell adhesion. *Oncogene*, 28(5), pp.752-761.

12. Yang, C.H., Yeh, Y.J., Wang, J.Y., Liu, Y.W., Chen, Y.L., Cheng, H.W., Cheng, C.M., Chuang, Y.J., Yuh, C.H. and Chen, Y.R., 2017. NEAP/DUSP26 suppresses receptor tyrosine kinases and regulates neuronal development in zebrafish. *Scientific reports*, 7(1), pp.1-9.
13. Wang, J.Y., Yang, C.H., Yeh, C.L., Lin, C.H. and Chen, Y.R., 2008. NEAP causes down-regulation of EGFR, subsequently induces the suppression of NGF-induced differentiation in PC12 cells. *Journal of neurochemistry*, 107(6), pp.1544-1555.
14. Garcia-Ortiz, J.E., Pelosi, E., Omari, S., Nedorezov, T., Piao, Y., Karmazin, J., Uda, M., Cao, A., Cole, S.W., Forabosco, A. and Schlessinger, D., 2009. Foxl2 functions in sex determination and histogenesis throughout mouse ovary development. *BMC developmental biology*, 9(1), p.36.
15. Patterson, K.I., Brummer, T., Daly, R.J. and O'Brien, P.M., 2010. DUSP26 negatively affects the proliferation of epithelial cells, an effect not mediated by dephosphorylation of MAPKs. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1803(9), pp.1003-1012.
16. Yu, W., Imoto, I., Inoue, J., Onda, M., Emi, M. and Inazawa, J., 2007. A novel amplification target, DUSP26, promotes anaplastic thyroid cancer cell growth by inhibiting p38 MAPK activity. *Oncogene*, 26(8), pp.1178-1187.
17. Ma, I.T., Vasudevan, S.A., Patel, R.H., Yang, J., Shohet, J.M., Chen, Z. and Fan, Y., 2013. DUSP26 is a transcriptional target of MYCN in neuroblastoma. *Journal of the American College of Surgeons*, 217(3), pp.S81-S82.
18. Shi, Y., Ma, I.T., Patel, R.H., Shang, X., Chen, Z., Zhao, Y., Cheng, J., Fan, Y., Rojas, Y., Barbieri, E. and Yu, Y., 2015. NSC-87877 inhibits DUSP26 function in neuroblastoma resulting in p53-mediated apoptosis. *Cell death & disease*, 6(8), pp.e1841-e1841.
19. 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.
20. 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.

21. 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	FC	Gene	Gene name
44	8150197	9.68E-11	-8.4139177	14.367052	0.4340 ± 0.0699	DUSP26	dual specificity phosphatase 26 (putative)

Table 1: DUSP26 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 DUSP26 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	FC	Gene	Gene name
141	A_23_P146134	6.11E-06	-7.7211548	4.34291	0.6486 ± 0.0801	DUSP26	dual specificity phosphatase 26 (putative)

Table 2: DUSP26 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 DUSP26 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: DUSP26 is expressed at significantly lower levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of DUSP26 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 DUSP26 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.