KIAA0101 (p15^{PAF}) 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 KIAA0101, p15^{PAF}, 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, KIAA0101, p15^{PAF}.

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 KIAA0101 (p15^{PAF1})
7 as among the most differentially expressed in HGSC tumors of the ovary. KIAA0101 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.
10

11 12 **Methods**

13 We used microarray data from datasets GSE124766² and GSE146556³ 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. GSE124766² 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. GSE146556³ 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 KIAA0101 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
28

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.

KIAA0101 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified KIAA0101, also known as p15^{PAF}, as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 1). We found that KIAA0101 was differentially expressed in primary HGSC tumors (Table 1). When sorting all of the transcripts measured by microarray based on change in expression between HGSC and the normal ovary, KIAA0101 ranked 210 out of 41093 total transcripts (Table 1). Differential expression of KIAA0101 in HGSC tumors was statistically significant (Table 1; $p=1.35E-05$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of KIAA0101 could be observed in tumors from a separate group of patients. We again found that KIAA0101 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, KIAA0101 ranked 325 out of 29088 total transcripts (Table 2). Differential expression of KIAA0101 in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=1.28E-06$).

KIAA0101 is expressed at higher levels in HGSC when compared to the normal ovary.

We obtained exact mRNA expression levels for the differentially expressed KIAA0101 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors.

KIAA0101 was expressed at higher levels in high-grade serous ovarian cancers when compared to the normal ovary; this difference was statistically significant (Figure 1; $p=0.0219$). We calculated a mean fold change of 0.2751 ± 0.0220 in KIAA0101 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

Thus, we found using published microarray data that KIAA0101/p15^{PAF} was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that KIAA0101 expression was 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 mesenchymal⁶. We sought to continue to describe the transcriptional landscape of high-grade serous ovarian cancers⁶ 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, KIAA0101 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

KIAA0101 is also referred to in the literature as p15^{PAF}, and identified as an interacting protein of the proliferating cell nuclear antigen (PCNA) sliding clamp using a yeast two-hybrid screen. p15^{PAF} possesses a PCNA binding site ("PIP-box") similar to that of p21 and when over-expressed can compete with p21 for binding to PCNA⁷. The transcription factor ATF3 can transactivate expression of KIAA0101 and that co-expression of ATF3 and KIAA0101 was sufficient to trigger induction of DNA damage response machinery. KIAA0101 was implicated in the DNA damage response following UV exposure as KIAA0101 was important for PCNA function during DNA repair, and cells depleted of KIAA0101 manifested impaired DNA repair and were more susceptible to UV-induced cell death⁸. Only under conditions of UV irradiation

could KIAA0101 and PCNA be co-immunoprecipitated, and endogenous KIAA localized to both the nucleus during S phase but localization in the mitochondria has also been documented^{9,11}. Association of KIAA0101 with potential tumor suppressor protein p33ING1b was also detected⁹. Unlike other PCNA-interacting proteins, KIAA0101 could make contacts with the inside of the PCNA ring and pass through the inside. KIAA0101 is considered an intrinsically disordered protein¹⁰. Authors suggested that KIAA0101 acts as a “drag” to regulate PCNA sliding on DNA, supporting the switch from polymerase binding during replicative DNA synthesis (DNA replication during cell division) to that of polymerase binding during translesion DNA synthesis (DNA replication to repair DNA damage)¹⁰. Another structural examination of KIAA0101 suggested that it might function as a “belt”, anchoring DNA to the PCNA sliding clamp during replicative DNA synthesis by polymerase δ ¹¹. KIAA0101 function is regulated by ubiquitination at lysine 15 and lysine 24, and doubly monoubiquitinated KIAA0101 could interact with the DNA methyltransferase DNMT1, recruiting DNA methylation machinery to sites of DNA replication for maintenance of epigenetic marks¹².

KIAA0101 is implicated in a number of human cancers including cancers of the breast, the pancreas, the lungs, the liver, the adrenal glands, the soft tissue, the stomach, and in the ovary. A study identifying genes whose expression was most similar to that of BRCA1 and related interactors found that KIAA0101 was among the genes co-expressed most similarly to BRCA1, BRCA2, ATM and CHK2¹³. Modulation of KIAA0101 had a specific effect on centrosome number. KIAA0101 was over-expressed in aggressive breast cancers and over-expression of KIAA0101 was correlated with worse patient survival¹³. A separate study found that depletion of KIAA0101 in breast cancer cell culture impaired colony formation, cell proliferation and cell cycle transition between G1 and S phases, and resulted in decreased expression of cell cycle components CCNE2, CDK6, CDKN1A¹⁴. Data suggesting that KIAA0101 intersected with the p53 pathway was that depletion of p53 antagonized impairment of proliferation and cell cycle transition induced by KIAA0101 depletion¹⁴. In pancreatic cancer,

1 depletion of KIAA0101 in MIA-PaCa2 and KLM-1 cell lines resulted in significant decreases in
2 cell growth *in vitro*. Over-expression of KIAA0101, both in PK45P cells and in the non-
3 transformed NIH 3T3 cell lines resulted in tumor growth *in vivo* when transplanted into the flank
4 of nude mice. Importantly, a peptide modeled against the PIP-box, designed to disrupt the
5 interaction between KIAA0101 and PCNA impaired the growth of KLM-1 cancer cells but not
6 that of non-transformed NIH 3T3 cells¹⁵. High expression of KIAA0101 at the mRNA level was
7 found in 33.9% of non-small-cell lung cancers (121/357 tumors) and correlated with male
8 gender, metastasis to the lymph nodes, and smoking history. Survival was significantly shorter
9 in NSCLC patients whose tumors had high expression of KIAA0101¹⁶. In hepatocellular
10 carcinoma, KIAA0101 was over-expressed in 61% (131/164 tumors) HCCs, and this correlated
11 with higher tumor grade, tumor stage, lower 5-year survival, and early tumor recurrence¹⁷.
12 KIAA0101 was associated with microvascular invasion and experiments *in vitro* demonstrated
13 that KIAA0101 was a direct target of FoxM1. KIAA0101-FoxM1 axis was important for induction
14 of genes required for the epithelial mesenchymal transition, including N-Cadherin, Zeb1, TWIST
15 and Snail¹⁸.

17 KIAA0101 was over-expressed in adrenocortical tumors, and depletion of KIAA0101
18 impaired anchorage independent growth as well as invasion¹⁹. KIAA0101 was also found to be
19 over-expressed in gastric cancer, with elevated KIAA0101 expression associated with higher
20 recurrence and worse survival. Depletion of KIAA0101 in the AGS gastric cancer cell line
21 resulted in impaired cell viability suggested KIAA0101 could exert control over gastric cancer
22 cell proliferation²⁰. KIAA0101 was also observed to be over-expressed in esophageal cancers
23 (EC), correlating with TNM tumor stage and worse survival in EC patients. Mechanistically,
24 expression of KIAA0101 resulted in up-regulation of cell cycle components Cyclin A and Cyclin
25 B, and in Eca-109 and TE-1 cell lines, KIAA0101 expression was involved in resistance to
26 cisplatin chemotherapy by affecting cell death responses²¹. In the soft tissue sarcoma HT-1080
27 cell line, KIAA0101 was a target of the microRNA miR-429²². We could identify one report of
28 KIAA0101 involvement in epithelial ovarian cancer, with over-expression detected in metastatic

EOC tissues as compared to primary tissues. miR-429 control of KIAA0101 expression was also observed in EOC, with over-expression of miR-429 phenocopying depletion of KIAA0101 with respect to impairment of invasion and chemoresistance²³. Thus, KIAA0101/p15^{PAF} is over-expressed in a variety of human cancers and its expression is correlated with aggressive behavior (i.e., invasion and metastasis) and poor outcomes.

Differential expression of KIAA0101/p15^{PAF} in HGSC should be validated in larger and separate cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian cancers²⁴⁻²⁶ can be utilized to assess the effect of conditional expression of KIAA0101 in HGSC tumors growth post-development and on survival *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. Yu, P., Huang, B., Shen, M., Lau, C., Chan, E., Michel, J., Xiong, Y., Payan, D.G. and Luo, Y., 2001. p15 PAF, a novel PCNA associated factor with increased expression in tumor tissues. *Oncogene*, 20(4), pp.484-489.
8. Turchi, L., Fareh, M., Aberdam, E., Kitajima, S., Simpson, F., Wicking, C., Aberdam, D. and Virolle, T., 2009. ATF3 and p15 PAF are novel gatekeepers of genomic integrity upon UV stress. *Cell Death & Differentiation*, 16(5), pp.728-737.
9. Simpson, F., van Bueren, K.L., Butterfield, N., Bennetts, J.S., Bowles, J., Adolphe, C., Simms, L.A., Young, J., Walsh, M.D., Leggett, B. and Fowles, L.F., 2006. The PCNA-associated factor KIAA0101/p15PAF binds the potential tumor suppressor product p33ING1b. *Experimental cell research*, 312(1), pp.73-85.
10. De Biasio, A., De Opakua, A.I., Mortuza, G.B., Molina, R., Cordeiro, T.N., Castillo, F., Villate, M., Merino, N., Delgado, S., Gil-Cartón, D. and Luque, I., 2015. Structure of p15 PAF-PCNA complex and implications for clamp sliding during DNA replication and repair. *Nature communications*, 6(1), pp.1-12.

11. De March, M., Barrera-Vilarmau, S., Crespan, E., Mentegari, E., Merino, N., Gonzalez-Magaña, A., Romano-Moreno, M., Maga, G., Crehuet, R., Onesti, S. and Blanco, F.J., 2018. p15PAF binding to PCNA modulates the DNA sliding surface. *Nucleic acids research*, 46(18), pp.9816-9828.
12. González-Magaña, A., de Opakua, A.I., Merino, N., Monteiro, H., Diercks, T., Murciano-Calles, J., Luque, I., Bernadó, P., Cordeiro, T.N., Biasio, A.D. and Blanco, F.J., 2019. Double monoubiquitination modifies the molecular recognition properties of p15PAF promoting binding to the reader module of Dnmt1. *ACS chemical biology*, 14(10), pp.2315-2326.
13. Kais, Z., Barsky, S.H., Mathsvaraja, H., Zha, A., Ransburgh, D.J., He, G., Pilarski, R.T., Shapiro, C.L., Huang, K. and Parvin, J.D., 2011. KIAA0101 interacts with BRCA1 and regulates centrosome number. *Molecular Cancer Research*, 9(8), pp.1091-1099.
14. Lv, W., Su, B., Li, Y. and Geng, C., 2018. KIAA0101 inhibition suppresses cell proliferation and cell cycle progression by promoting the interaction between p53 and Sp1 in breast cancer. *Biochemical and biophysical research communications*, 503(2), pp.600-606.
15. Hosokawa, M., Takehara, A., Matsuda, K., Eguchi, H., Ohigashi, H., Ishikawa, O., Shinomura, Y., Imai, K., Nakamura, Y. and Nakagawa, H., 2007. Oncogenic role of KIAA0101 interacting with proliferating cell nuclear antigen in pancreatic cancer. *Cancer research*, 67(6), pp.2568-2576.
16. Kato, T., Daigo, Y., Aragaki, M., Ishikawa, K., Sato, M. and Kaji, M., 2012. Overexpression of KIAA0101 predicts poor prognosis in primary lung cancer patients. *Lung cancer*, 75(1), pp. 110-118.
17. Yuan, R.H., Jeng, Y.M., Pan, H.W., Hu, F.C., Lai, P.L., Lee, P.H. and Hsu, H.C., 2007. Overexpression of KIAA0101 predicts high stage, early tumor recurrence, and poor prognosis of hepatocellular carcinoma. *Clinical Cancer Research*, 13(18), pp.5368-5376.
18. Zhang, T., Guo, J., Gu, J., Chen, K., Wang, Z., Li, H., Wang, G. and Wang, J., 2019. KIAA0101 is a novel transcriptional target of FoxM1 and is involved in the regulation of hepatocellular carcinoma microvascular invasion by regulating epithelial-mesenchymal transition. *Journal of Cancer*, 10(15), p.3501.
19. Jain, M., Zhang, L., Patterson, E.E. and Kebebew, E., 2011. KIAA0101 is overexpressed, and promotes growth and invasion in adrenal cancer. *PloS one*, 6(11), p.e26866.
20. Zhu, K., Diao, D., Dang, C., Shi, L., Wang, J., Yan, R., Yuan, D. and Li, K., 2013. Elevated KIAA 0101 expression is a marker of recurrence in human gastric cancer. *Cancer science*, 104(3), pp.353-359.
21. Cheng, Y., Li, K., Diao, D., Zhu, K., Shi, L., Zhang, H., Yuan, D., Guo, Q., Wu, X., Liu, D. and Dang, C., 2013. Expression of KIAA0101 protein is associated with poor survival of

esophageal cancer patients and resistance to cisplatin treatment in vitro. *Laboratory investigation*, 93(12), pp.1276-1287.

22. Samantarrai, D. and Mallick, B., 2017. miR-429 inhibits metastasis by targeting KIAA0101 in Soft Tissue Sarcoma. *Experimental Cell Research*, 357(1), pp.33-39.
23. Chen, H., Xia, B., Liu, T., Lin, M. and Lou, G., 2016. KIAA0101, a target gene of miR-429, enhances migration and chemoresistance of epithelial ovarian cancer cells. *Cancer cell international*, 16(1), pp.1-11.
24. 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.
25. 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.
26. 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
210	2.64E-03	1.35E-05	7.1221363	3.57507	0.2751 ± 0.0220	KIAA0101	KIAA0101

Table 1: KIAA0101 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 KIAA0101 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
325	7989647	1.28E-06	5.5968448	5.2538103	KIAA0101	KIAA0101

Table 2: KIAA0101 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 KIAA0101 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: KIAA0101 is expressed at significantly higher levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of KIAA0101 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 KIAA0101 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.