TPX2 is differentially expressed in high-grade serous ovarian cancers.

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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 TPX2 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, TPX2.

1 The five-year survival rate for women diagnosed with high-grade serous ovarian cancer is
2 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 TPX2 as among the
7 most differentially expressed in HGSC tumors of the ovary. TPX2 may be a gene of interest
8 when prioritizing the study of target genes and pathways for the development of novel
9 therapeutic interventions in high-grade serous ovarian cancers.
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15 **Methods**

16 We used microarray data from datasets GSE124766² and GSE146556³ for this
17 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
18 and Hochberg method of p-value adjustment was used for ranking of differential expression but
19 raw p-values were used for assessment of statistical significance of global differential expression.
20 Log-transformation of data was auto-detected, and the NCBI generated category of platform
21 annotation was used. GSE124766² 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. GSE146556³ was generated using Affymetrix
24 Human Gene 1.0 ST Array with n=3 for normal ovarian tissue and n=40 for tumors from patients
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1 with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels only for the
2 top 250 most differentially expressed genes.

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4 A statistical test was performed to evaluate whether TPX2 expression was significantly
5 different when comparing normal ovarian tissue from control subjects and primary tumors from
6 women diagnosed with HGSC using a two-tailed, unpaired t-test with Welch's correction. Only
7 p-values less than 0.05 were considered statistically significant. We used PRISM for all
8 statistical analyses (Version 8.4.0)(455).
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10 11 **Results**

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13 We mined published microarray data^{2,3} to identify differentially expressed genes in high-
14 grade serous ovarian cancer (HGSC), the type of ovarian cancer responsible for 70-80% of
15 deaths resulting from the most lethal gynecologic malignancy.
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17 TPX2 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

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19 We identified TPX2 as among the genes whose expression was most different when
20 comparing primary HGSC tumors to normal ovarian tissue² (Table 1). We found that TPX2 was
21 differentially expressed in primary HGSC tumors (Table 1). When sorting all of the transcripts
22 measured by microarray based on change in expression between HGSC and the normal ovary,
23 TPX2 ranked 200 out of 41093 total transcripts (Table 1). Differential expression of TPX2 in
24 HGSC tumors was statistically significant (Table 1; $p=1.3E-05$).
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27 We analyzed a second microarray dataset³ generated using normal ovarian tissues and
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tumors from women diagnosed with HGSC to determine whether differential expression of TPX2 could be observed in tumors from a separate group of patients. We again found that TPX2 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, TPX2 ranked 495 out of 29088 total transcripts (Table 2). Differential expression of TPX2 in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=7.43E-06$).

TPX2 is expressed at significantly higher levels in HGSC when compared to the normal ovary.

We next obtained exact mRNA expression levels for the differentially expressed TPX2 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. TPX2 was expressed at significantly higher levels in high-grade serous ovarian cancers when compared to the normal ovary; this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a mean fold change of 1.7116 ± 0.1805 in TPX2 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

Thus, we found using published microarray data that TPX2 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that TPX2 expression was significantly higher 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, TPX2 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

TPX2 was identified in *Xenopus* as a 82.4 kilodalton protein with dynamic phosphorylation during mitosis dependent on microtubules⁷. In mitotic egg extracts depleted of TPX2, spindle poles disintegrated and were bipolar in structure; conversely, addition of exogenous TPX2 to spindle assembly reactions resulted in monopolar structures and spindle poles were enlarged⁷. The small GTPase Ran is required for the action of TPX2. The nuclear import receptor Importin α (Imp α) bound TPX2 and inactivated it; binding of RanGTP lead to disassembly of this complex⁸. A TPX2 mutant defective for Imp α binding was constitutively active with respect to induction of aster-like structures, and binding by Imp α prevented microtubule formation but not bundling by TPX2⁹. Thus, TPX2 functions in M phase of the cell cycle, in the induction of spindle bodies by chromatin involving microtubules, and this process is regulated by nuclear transport machinery.

Aurora-A kinase is another binding partner of TPX2¹⁰. TPX2 was phosphorylated by Aurora-A, and depletion of TPX2 using small interfering RNA (siRNA) resulted in loss of association of Aurora-A kinase with spindle poles, demonstrating that TPX2 was important for

1 recruitment of Aurora-A kinase to the spindle apparatus¹⁰. Degradation of TPX2 to regulate
2 TPX2 spindle assembly is performed by the anaphase promoting complex / cyclosome (APC/C)
3 activated by Cdh1 (APC/C^{Cdh1}). APC/C^{Cdh1} binding to TPX2 involved interactions through two
4 structural elements of TPX2: a KEN box, and amino-terminal residues 1-86 of TPX2¹¹. In
5 mouse oocytes, TPX2 control of spindle assembly involved phosphorylation of TACC3, a
6 molecule important for the regulation of the microtubule organizing center (MTOC)¹².

9 Another binding partner of TPX2 is the kinesin-5 motor Eg5. In cells expressing a TPX2
10 mutant that lacked Eg5 interaction sequences, spindles were disorganized and multiple poles
11 were present. TPX2 could reduce the speed with which Eg5-dependent microtubule gliding
12 occurred, resulting in inhibition of microtubule sliding¹³. A separate study demonstrated that
13 microtubule nucleation could occur not only from centrosomes, but also from pre-existing
14 microtubules at the spindle. Microtubules could nucleate from the sides of existing microtubules
15 in *Xenopus* egg extracts during meiosis; the molecules augmin and γ -tubulin were required for
16 this alternative form of microtubule nucleation known as branching microtubule nucleation¹⁴.

21 TPX2 is implicated in the biology of a number of cancers including cancers of the
22 esophagus, the cervix, the prostate, the pancreas and the colon. Mice engineered to express a
23 conditional null mutant of Tpx2 manifest defects in chromosome segregation and proper function
24 of the spindle apparatus. Haploinsufficiency of Tpx2 supported the development of aneuploidies
25 *in vivo* and resulted in increased susceptibility to development of lung tumors and lymphomas¹⁵.
26 In the EC9706 esophageal cancer cell line, TPX2 depletion resulted in impairment of
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proliferation *in vitro* and in metastasis in xenografts *in vivo*¹⁶. TPX2 was found to be over-expressed in cervical carcinomas when compared to normal cervical tissues; TPX2 expression was correlated with metastasis to the lymph nodes and with FIGO tumor stage. In HeLa cells, TPX2 depletion using siRNA resulted in cell cycle arrest in S-phase¹⁷.

An integrative study that combined results from expression analyses to identify genes over-expressed in prostate cancer with that of functional RNA interference viability assays in VCaP and LNCaP prostate cancer cell lines identified TPX2 as a potential therapeutic target in prostate cancers. TPX2 expression was associated with PSA failure, and TPX2 depletion resulted in decreased PSA expression; investigators concluded that TPX2 might have an important role in regulation of androgen signaling in prostate cancers¹⁸. A separate study demonstrated that depletion of TPX2 resulted in increased cell death (apoptosis) and impairment of tumor cell growth in the PC3/PPC1 prostate cancer cell line. Importantly, TPX2 expression levels in prostate tumors were positively correlated with Gleason score in a cohort of patients with prostate cancer¹⁹.

TPX2 has been identified as over-expressed in pancreatic cancer cell lines and in patient tumors. Depletion of TPX2 in the MIA PaCa-2 pancreatic cancer cell line resulted in increased cell death (apoptosis), as well as impaired tumor growth in soft agar *in vitro* and in nude mice *in vivo*²⁰. TPX2 was also identified as over-expressed in 60.8% (129/203) metastatic tissues from patients with colorectal cancer as compared to primary tumors and colonic mucosa. TPX2 positive expression in patient tumors was significantly associated with worse overall survival as

1 well as metastasis free survival²¹. Depletion of TPX2 in SW620 colon cancer cells resulted in
2 impaired proliferation and decreased migratory and invasive capacity of colon cancer cell lines.
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4 Investigators identified changes in matrix metalloproteinase 2 (MMP2) activity mediated by
5 protein kinase AKT as important for inhibition of migration and invasion observed subsequent to
6 TPX2 depletion²¹. Thus, TPX2 is over-expressed in multiple cancer types, and over-expression
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8 of TPX2 in patient tumors has been associated with worse outcomes in multiple cancer types.

9   Differential expression of TPX2 in HGSC should be validated in larger and separate
10 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
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12 cancers²²⁻²⁴ can be utilized to assess the effect of conditional expression of TPX2 in HGSC tumor
13 growth post-development and on survival *in vivo*. In conjunction with ASPM²⁵, TPX2 is the
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15 second piece of machinery functioning in regulation of the spindle apparatus and centrosome that
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17 we observe among the most differentially expressed genes in high-grade serous tumors of the
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19 ovary at the transcriptome level, suggesting that the process by which sister chromatids are
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21 faithfully segregated during cell division might be perturbed in HGSC.
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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., Schouli, 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. Wittmann, T., Wilm, M., Karsenti, E. and Vernos, I., 2000. TPX2, A novel xenopus MAP involved in spindle pole organization. *Journal of Cell Biology*, 149(7), pp.1405-1418.
8. Gruss, O.J., Carazo-Salas, R.E., Schatz, C.A., Guarguaglini, G., Kast, J., Wilm, M., Le Bot, N., Vernos, I., Karsenti, E. and Mattaj, I.W., 2001. Ran induces spindle assembly by reversing the inhibitory effect of importin α on TPX2 activity. *Cell*, 104(1), pp.83-93.
9. Schatz, C.A., Santarella, R., Hoenger, A., Karsenti, E., Mattaj, I.W., Gruss, O.J. and Carazo-Salas, R.E., 2003. Importin α -regulated nucleation of microtubules by TPX2. *The EMBO journal*, 22(9), pp.2060-2070.

10. Kufer, T.A., Silljé, H.H., Körner, R., Gruss, O.J., Meraldi, P. and Nigg, E.A., 2002. Human TPX2 is required for targeting Aurora-A kinase to the spindle. *The Journal of cell biology*, 158(4), pp.617-623.
11. Stewart, S. and Fang, G., 2005. Anaphase-promoting complex/cyclosome controls the stability of TPX2 during mitotic exit. *Molecular and cellular biology*, 25(23), pp. 10516-10527.
12. Brunet, S., Dumont, J., Lee, K.W., Kinoshita, K., Hikal, P., Gruss, O.J., Maro, B. and Verlhac, M.H., 2008. Meiotic regulation of TPX2 protein levels governs cell cycle progression in mouse oocytes. *PloS one*, 3(10), p.e3338.
13. Ma, N., Titus, J., Gable, A., Ross, J.L. and Wadsworth, P., 2011. TPX2 regulates the localization and activity of Eg5 in the mammalian mitotic spindle. *Journal of Cell Biology*, 195(1), pp.87-98.
14. Petry, S., Groen, A.C., Ishihara, K., Mitchison, T.J. and Vale, R.D., 2013. Branching microtubule nucleation in Xenopus egg extracts mediated by augmin and TPX2. *Cell*, 152(4), pp.768-777.
15. Aguirre-Portolés, C., Bird, A.W., Hyman, A., Cañamero, M., de Castro, I.P. and Malumbres, M., 2012. Tpx2 controls spindle integrity, genome stability, and tumor development. *Cancer research*, 72(6), pp.1518-1528.
16. Liu, H.C., Zhang, G.H., Liu, Y.H., Wang, P., Ma, J.F., Su, L.S., Li, S.L., Zhang, L. and Liu, J.W., 2014. TPX2 siRNA regulates growth and invasion of esophageal cancer cells. *Biomedicine & pharmacotherapy*, 68(7), pp.833-839.
17. Chang, H., Wang, J., Tian, Y., Xu, J., Gou, X. and Cheng, J., 2012. The TPX2 gene is a promising diagnostic and therapeutic target for cervical cancer. *Oncology reports*, 27(5), pp. 1353-1359.
18. Vainio, P., Mpindi, J.P., Kohonen, P., Fey, V., Mirtti, T., Alanen, K.A., Perälä, M., Kallioniemi, O. and Iljin, K., 2012. High-throughput transcriptomic and RNAi analysis identifies AIM1, ERGIC1, TMED3 and TPX2 as potential drug targets in prostate cancer. *PloS one*, 7(6), p.e39801.
19. Pan, H.W., Su, H.H., Hsu, C.W., Huang, G.J. and Wu, T.T.L., 2017. Targeted TPX2 increases chromosome missegregation and suppresses tumor cell growth in human prostate cancer. *OncoTargets and therapy*, 10, p.3531.

- 1 20. Warner, S.L., Stephens, B.J., Nwokenkwo, S., Hostetter, G., Sugeng, A., Hidalgo, M., Trent,
2 J.M., Han, H. and Von Hoff, D.D., 2009. Validation of TPX2 as a potential therapeutic target
3 in pancreatic cancer cells. *Clinical Cancer Research*, 15(21), pp.6519-6528.
- 4 21. Wei, P., Zhang, N., Xu, Y., Li, X., Shi, D., Wang, Y., Li, D. and Cai, S., 2013. TPX2 is a
5 novel prognostic marker for the growth and metastasis of colon cancer. *Journal of*
6 *translational medicine*, 11(1), pp.1-11.
- 7 22. Harrington, B.S., He, Y., Davies, C.M., Wallace, S.J., Adams, M.N., Beaven, E.A., Roche,
8 D.K., Kennedy, C., Chetty, N.P., Crandon, A.J. and Flatley, C., 2016. Cell line and patient-
9 derived xenograft models reveal elevated CDCP1 as a target in high-grade serous ovarian
10 cancer. *British journal of cancer*, 114(4), pp.417-426.
- 11 23. Perets, R., Wyant, G.A., Muto, K.W., Bijron, J.G., Poole, B.B., Chin, K.T., Chen, J.Y.H.,
12 Ohman, A.W., Stepule, C.D., Kwak, S. and Karst, A.M., 2013. Transformation of the
13 fallopian tube secretory epithelium leads to high-grade serous ovarian cancer in Brca; Tp53;
14 Pten models. *Cancer cell*, 24(6), pp.751-765.
- 15 24. Kim, J., Coffey, D.M., Creighton, C.J., Yu, Z., Hawkins, S.M. and Matzuk, M.M., 2012.
16 High-grade serous ovarian cancer arises from fallopian tube in a mouse model. *Proceedings*
17 *of the National Academy of Sciences*, 109(10), pp.3921-3926.
- 18 25. Mamoor, S., 2020. ASPM is differentially expressed in high-grade serous ovarian cancers.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
200	A_23_P68610	1.3E-05	7.1501481	3.61209	1.7116 ± 0.1805	TPX2	TPX2, microtubule nucleation factor

Table 1: TPX2 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 TPX2 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
495	8061579	7.43E-06	5.0730844	3.5721001	TPX2	TPX2, microtubule nucleation factor

Table 2: TPX2 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 TPX2 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: TPX2 is expressed at significantly higher levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of TPX2 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 TPX2 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.