ESPL1 (Separase) is differentially expressed in high-grade serous ovarian cancers.

Shahan Mamoor¹

¹shahanmamoor@gmail.com

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 Separase, ESPL1, 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, ESPL1, Separase.

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 ESPL1 (*extra spindle pole bodies like 1*)
7 as among the most differentially expressed in HGSC tumors of the ovary. ESPL1 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
13

14 **Methods**

15 We used microarray data from datasets GSE124766² and GSE146556³ for this
16 differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini
17 and Hochberg method of p-value adjustment was used for ranking of differential expression but
18 raw p-values were used for assessment of statistical significance of global differential expression.
19 Log-transformation of data was auto-detected, and the NCBI generated category of platform
20 annotation was used. GSE124766² was generated using Agilent-014850 Whole Human Genome
21 Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors from
22 patients with high-grade serous ovarian cancer. GSE146556³ was generated using Affymetrix
23 Human Gene 1.0 ST Array with n=3 for normal ovarian tissue and n=40 for tumors from patients
24 with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels only for the
25
26
27
28

top 250 most differentially expressed genes.

A statistical test was performed to evaluate whether ESPL1 expression was significantly different when comparing normal ovarian tissue from control subjects and primary 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.

ESPL1 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified ESPL1 as among the genes whose expression was most different when comparing primary HGSC tumors to normal ovarian tissue² (Table 1). We found that ESPL1 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, ESPL1 ranked 119 out of 41093 total transcripts (Table 1). Differential expression of ESPL1 in HGSC tumors was statistically significant (Table 1; $p=3.12E-06$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of

ESPL1 could be observed in tumors from a separate group of patients. We again found that ESPL1 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, ESPL1 ranked 2987 out of 29088 total transcripts (Table 2). Differential expression of ESPL1 in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=3.17E-03$).

ESPL1 is expressed at higher levels in HGSC tumors when compared to the normal ovary.

We obtained exact mRNA expression levels for the differentially expressed ESPL1 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. ESPL1 was expressed at higher levels in high-grade serous ovarian cancers when compared to the normal ovary; this difference was statistically significant (Figure 1; $p=0.0018$). We calculated a mean fold change of 1.599 ± 0.1156 in ESPL1 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

Thus, we found using published microarray data that ESPL1 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that ESPL1 expression was 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, ESPL1 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

ESPL1 encodes for a protease known as Separase which has functions in the division of somatic cells (mitosis) and gametes (meiosis). Sister chromatids are normally bound together by a complex known as cohesin, which includes the subunits SMC3, SMC1, SCC1 and SCC3. In anaphase, sister chromatids are separated; chromatid separation is induced by and requires cleavage of the SCC1/Rad21 cohesin subunit by Separase⁷. Separase activity is regulated by interactions with securin; securin prevents Separase function by sterically preventing binding to its substrates⁸. Separase can also be regulated in a securin-independent manner through phosphorylation in metaphase and dephosphorylation in anaphase, at serine 1121^{9,10}. In fission yeast, securin-Separase interactions are also important for DNA repair following ultraviolet, X-ray and gamma-ray irradiation; authors suggested Separase might support DNA repair through cohesin removal during interphase¹¹.

Separase has influences on spindle apparatus dynamics independent of cohesion cleavage. In yeast, the phosphatase Cdc14 regulates a complex composed of the inner centromere-like protein (INCENP) and Aurora B kinases (INCENP-Aurora) to regulate

1 chromosome segregation and spindle assembly. The yeast INCENP-Aurora complex Sli15-Ipl1
2 is regulated by interactions with and dephosphorylation by the phosphatase Cdc14; Cdc14
3 dephosphorylation of Sli15 induces localization of the INCENP-Aurora complex to spindles.
4 Separase, in turn, is a Cdc14 activator, and thus one way separase is able to exert control over
5 spindle assembly is by regulating phosphorylation status of the INCENP-Aurora complex via
6 activation of Cdc14¹².
7

8
9 During cell division of gametes, meiosis, sister centromeres are held together in
10 structures known as chiasmata; linkage of centromeres is mediated by sister chromatid
11 interactions through cohesin. Chiasma resolution in yeast meiosis I requires cleavage of the
12 cohesin subunit Rec8 by separase¹³. Deletion of separase in mouse oocytes prevents both Rec8
13 removal from chromosome arms as well as resolution of chiasma. Thus, separase is important for
14 Rec8 removal from chromosome arms and for chiasma resolution in mammalian oocytes during
15 meiosis¹³.
16
17

18
19 Separase can also influence cell cycle dynamics by regulating proteins involved in the
20 anaphase-metaphase transition. Anaphase is marked by high cyclin dependent kinase (CDK)
21 activity, and the CDK counter-acting protein phosphatase Cdc14 can antagonize this function to
22 promote mitotic exit by dephosphorylation of an activating component of the anaphase
23 promoting complex, Cdh1, as well as through direct dephosphorylation of CDK substrates¹⁴.
24 During metaphase, Cdc14 is inhibited through interactions with Net1, where it remains inactive
25 in the nucleolus. Phosphorylation of Net1 releases Cdc14 and permits its activation; the
26
27
28

1 PP2A^{Cdc55} phosphatase controls Net1 phosphorylation status and activation by dephosphorylating
2 Net1 during metaphase. Separase, in turn regulates PP2A^{Cdc55} activity; the mechanism by which
3 this inhibition occurs is unclear but may be a result of direct interactions with PP2A^{Cdc55}. Thus,
4 by curbing activity of the Net1 inhibitor PP2A^{Cdc55}, Separase can facilitate Net1 phosphorylation
5 to promote release of Cdc14 and mitotic exit¹⁴.
6
7

8 Literature describes a role for Separase in human cancers, and mouse models support an
9 active role for Separase in driving tumor development. In a panel of 30 breast tumors, separase
10 was over-expressed at the protein level between 2-90 fold in 70% of tumors. Over-expression of
11 separase in FSK mouse mammary cells was sufficient to induce aneuploidy *in vitro* on a p53
12 mutant background. Transplantation of FSK3 cells with tetracycline-inducible conditional
13 expression of separase into the mammary pad of wild-type mice resulted in generation of
14 mammary tumors within 3-4 weeks of induction. Scientists used xenografts engrafted with
15 tumor cells from Separase-induced tumors to assess the effect of Separase on chromosome
16 stability. DNA from two tumor clones revealed gain at chromosome 15 in both tumors, as well
17 as telomeric amplification on chromosome 8 and amplification on chromosome 8 and 11 in one
18 tumor each. Together these data provided strong evidence that Separase could function as an
19 oncogene *in vivo* and that Separase over-expression could induce aneuploidy and other structural
20 aberrations characteristic of chromosomal instability such as anaphase bridges¹⁵.
21
22

23 Another study using MMTV over-expression of Separase in the mammary glands of
24 C57BL/6 mice found that Separase over-expression resulted in the development of aneuploid,
25
26
27
28

1 estrogen receptor α -positive mammary adenocarcinomas with 80% penetrance, with both
2
3 luminal and basal features. Additional indications of chromosomal instability in Separase-driven
4 mammary adenocarcinomas were the presence of premature chromatid separation (PCS) and
5 lagging chromosomes¹⁶.
6

7 ESPL1 was over-expressed in 52% of a panel of 3,074 luminal breast cancers, with
8 higher frequency in luminal B breast cancers (83%) as opposed to luminal A breast cancers
9 (29%). Luminal breast cancers over-expressing ESPL1 *in vivo* manifested chromosome
10 instability and loss of p53 and Rb tumor suppressors. In patients with luminal B cancers,
11 expression of ESPL1 was significantly associated with worse prognosis criteria (lymph node
12 involvement) and with worse metastasis-free survival¹⁷.
13
14

15 A separate study demonstrated that Separase was over-expressed in a panel of 316 tumors
16 including tumors from patients with breast cancer, osteosarcoma, prostate cancer, and colon
17 cancer. Authors found a significant negative correlation between Separase expression levels and
18 survival, both disease-free and overall. Interestingly, nuclear localization of Separase was the
19 single most statistically significant factor studied that distinguished tumor tissue from normal
20 tissue¹⁸. Finally, in cell lines and in clinical samples from patients with chronic myelogenous
21 lymphoma (CML), the expression of c-MYB, a factor critical for the development of BCR-
22 ABL+ CMLs positively correlated that of Separase. Depletion of c-MYB using RNA
23 interference resulted in decreased expression of Separase at the protein level¹⁹. Thus, in multiple
24
25
26
27
28 cancers, over-expression of Separase in tumor tissues and negative correlation between

1 Separate tumor expression and patient survival outcomes has been documented.

2 Differential expression of ESPL1 in HGSC should be validated in larger and separate
3 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
4 cancers²⁰⁻²² can be utilized to assess the effect of conditional expression of ESPL1 in HGSC
5 tumor growth post-development and on survival *in vivo*. Along with ASPM and TPX2, the gene
6 encoding Separase, ESPL1, is now the third gene encoding a molecule with central functions at
7 the spindle apparatus that we observe to be among the most differentially expressed genes in
8 HGSC tumors, suggesting that the process by which sister chromatids are faithfully segregated
9 during mitosis may be compromised in high-grade serous ovarian cancer.
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

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. Hauf, S., Waizenegger, I.C. and Peters, J.M., 2001. Cohesin cleavage by separase required for anaphase and cytokinesis in human cells. *Science*, 293(5533), pp.1320-1323.
8. Hornig, N.C., Knowles, P.P., McDonald, N.Q. and Uhlmann, F., 2002. The dual mechanism of separase regulation by securin. *Current biology*, 12(12), pp.973-982.
9. Stemmann, O., Zou, H., Gerber, S.A., Gygi, S.P. and Kirschner, M.W., 2001. Dual inhibition of sister chromatid separation at metaphase. *Cell*, 107(6), pp.715-726.
10. Huang, X., Andreu-Vieyra, C.V., York, J.P., Hatcher, R., Lu, T., Matzuk, M.M. and Zhang, P., 2008. Inhibitory phosphorylation of separase is essential for genome stability and viability of murine embryonic germ cells. *PLoS Biol*, 6(1), p.e15.

11. Nagao, K., Adachi, Y. and Yanagida, M., 2004. Separase-mediated cleavage of cohesin at interphase is required for DNA repair. *Nature*, 430(7003), pp.1044-1048.
12. Pereira, G. and Schiebel, E., 2003. Separase regulates INCENP-Aurora B anaphase spindle function through Cdc14. *Science*, 302(5653), pp.2120-2124.
13. Kudo, N.R., Wassmann, K., Anger, M., Schuh, M., Wirth, K.G., Xu, H., Helmhart, W., Kudo, H., Mckay, M., Maro, B. and Ellenberg, J., 2006. Resolution of chiasmata in oocytes requires separase-mediated proteolysis. *Cell*, 126(1), pp.135-146.
14. Queralt, E., Lehane, C., Novak, B. and Uhlmann, F., 2006. Downregulation of PP2A^{Cdc55} phosphatase by separase initiates mitotic exit in budding yeast. *Cell*, 125(4), pp.719-732.
15. Zhang, N., Ge, G., Meyer, R., Sethi, S., Basu, D., Pradhan, S., Zhao, Y.J., Li, X.N., Cai, W.W., El-Naggar, A.K. and Baladandayuthapani, V., 2008. Overexpression of Separase induces aneuploidy and mammary tumorigenesis. *Proceedings of the National Academy of Sciences*, 105(35), pp.13033-13038.
16. Finetti, P., Guille, A., Adelaide, J., Birnbaum, D., Chaffanet, M. and Bertucci, F., 2014. ESPL1 is a candidate oncogene of luminal B breast cancers. *Breast cancer research and treatment*, 147(1), pp.51-59.
17. Mukherjee, M., Ge, G., Zhang, N., Edwards, D.G., Sumazin, P., Sharan, S.K., Rao, P.H., Medina, D. and Pati, D., 2014. MMTV-Espl1 transgenic mice develop aneuploid, estrogen receptor alpha (ER α)-positive mammary adenocarcinomas. *Oncogene*, 33(48), pp. 5511-5522.
18. Meyer, R., Fofanov, V., Panigrahi, A., Merchant, F., Zhang, N. and Pati, D., 2009. Overexpression and mislocalization of the chromosomal segregation protein separase in multiple human cancers. *Clinical Cancer Research*, 15(8), pp.2703-2710.
19. Prinzhorn, W., Stehle, M., Kleiner, H., Ruppenthal, S., Müller, M.C., Hofmann, W.K., Fabarius, A. and Seifarth, W., 2016. c-MYB is a transcriptional regulator of ESPL1/Separase in BCR-ABL-positive chronic myeloid leukemia. *Biomarker research*, 4(1), p.5.
20. Deng, J., Cheng, X. and Zhou, Y., 2019, August. AGRP and ESPL1 as Biomarkers of Brain-Metastasis in Lung Adenocarcinoma. In *Journal of Physics: Conference Series* (Vol. 1302, No. 4, p. 042040). IOP Publishing.

- 1 21. Harrington, B.S., He, Y., Davies, C.M., Wallace, S.J., Adams, M.N., Beaven, E.A., Roche,
2 D.K., Kennedy, C., Chetty, N.P., Crandon, A.J. and Flatley, C., 2016. Cell line and patient-
3 derived xenograft models reveal elevated CDCP1 as a target in high-grade serous ovarian
4 cancer. *British journal of cancer*, 114(4), pp.417-426.
- 5 22. Perets, R., Wyant, G.A., Muto, K.W., Bijron, J.G., Poole, B.B., Chin, K.T., Chen, J.Y.H.,
6 Ohman, A.W., Stepule, C.D., Kwak, S. and Karst, A.M., 2013. Transformation of the
7 fallopian tube secretory epithelium leads to high-grade serous ovarian cancer in Brca; Tp53;
8 Pten models. *Cancer cell*, 24(6), pp.751-765.
- 9 23. Kim, J., Coffey, D.M., Creighton, C.J., Yu, Z., Hawkins, S.M. and Matzuk, M.M., 2012.
10 High-grade serous ovarian cancer arises from fallopian tube in a mouse model. *Proceedings*
11 *of the National Academy of Sciences*, 109(10), pp.3921-3926.
- 12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

Rank	ID	p-value	t	B	FC	Gene	Gene name
119	A_23_P32707	3.12E-06	8.2536814	4.98481	1.599 ± 0.1156	ESPL1	extra spindle pole bodies like 1, separase

Table 1: ESPL1 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 ESPL1 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
2987	7955736	3.17E-03	3.1201335	-2.1377574	ESPL1	extra spindle pole bodies like 1, separase

Table 2: ESPL1 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 ESPL1 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: ESPL1 is expressed at significantly higher levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of ESPL1 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 ESPL1 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.