Pyrroline-5-carboxylate reductase (PYCR1) 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 pyrroline-5-carboxylate reductase (PYCR1) 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, PYCR1.

The five-year survival rate for women diagnosed with high-grade serous ovarian cancer is between 30-40% and has not changed significantly in decades^{6,7}. The development of novel, targeted therapeutics to treat HGSC can be facilitated by an enhanced understanding of the transcriptional behavior of ovarian tumors relative to that of the normal ovary. We mined published microarray data^{2,3} to compare global gene expression profiles between HGSC ovarian tumors and that of normal ovarian tissue. We identified the gene encoding pyrroline-5-carboxylate reductase (PYCR1) as among the most differentially expressed in HGSC tumors of the ovary. PYCR1 may be a gene of interest when prioritizing the study of target genes and pathways for the development of novel therapeutic interventions in high-grade serous ovarian cancers.

Methods

We used microarray data from datasets GSE124766² and GSE146556³ for this differential gene expression analysis of high-grade serous ovarian carcinomas. The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. GSE124766² was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with n=3 of for normal ovarian tissue and n=8 for tumors from patients with high-grade serous ovarian cancer. GSE146556³ was generated using Affymetrix Human Gene 1.0 ST Array with n=3 for normal ovarian tissue and n=40 for tumors from patients with high-grade serous ovarian cancer. GEO2R provides mRNA expression levels only for the top 250 most differentially expressed genes.

A statistical test was performed to evaluate whether PYCR1 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.

PYCR1 is differentially expressed in ovarian tumors from women diagnosed with HGSC.

We identified PYCR1 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, PYCR1 ranked 123 out of 41093 total transcripts (Table 1). Differential expression of PYCR1 in HGSC tumors was statistically significant (Table 1; $p=3.62E-06$).

We analyzed a second microarray dataset³ generated using normal ovarian tissues and tumors from women diagnosed with HGSC to determine whether differential expression of PYCR1 could be observed in tumors from a separate group of patients. We again found that PYCR1 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, PYCR1 ranked 3711 out of 29088 total transcripts (Table 2). Differential expression of PYCR1 in HGSC tumors in this second microarray dataset was statistically significant (Table 2; $p=6.33E-03$).

PYCR1 is expressed at significantly higher levels in HGSC when compared to the normal ovary.

We obtained exact mRNA expression levels for the differentially expressed PYCR1 transcript from both normal ovarian tissue and from high-grade serous ovarian tumors. PYCR1 was expressed at significantly higher 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

1.2917 $\pm$ 0.0594 in PYCR1 expression when comparing HGSC tumors to normal ovarian tissue (Table 1).

Thus, we found using published microarray data that PYCR1 was among the genes most differentially expressed in tumors from women with high-grade serous carcinomas and that PYCR1 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, PYCR1 was among the genes whose expression changed most significantly when comparing the normal ovary to primary HGSC tumors.

The enzymatic activity of pyrroline-5-carboxylate reductase supports biosynthesis of the amino acid proline by converting the intermediate metabolite P5C to proline. A recent study of the Kaposi's sarcoma herpesvirus (KSHV) K1 oncoprotein demonstrated the importance of PYCR1 in tumorigenesis⁷. The K1 oncoprotein interacted with PYCR1, leading to activation of PYCR1 and subsequent increases in proline biosynthesis⁷. PYCR1 was required in 3-dimensional (3D) but not 2-dimensional (2D) cell culture for K1-induced tumor growth⁷. One study demonstrated that 24.7% of luminal B breast cancers contained copy number gains in the PYCR1 gene⁸. The Myc oncoprotein can reprogram cellular metabolism to support cancer cell growth⁹. Expression of Myc is sufficient to increase levels of intracellular proline, and Myc is a transcriptional activator of PYCR1⁹. One study reported that PYCR1 expression could support

1 proliferation of non-small cell lung cancer cells¹⁰, another citing mechanisms involving regulatory
2 interactions with the microRNA miR-488¹¹. The isocitrate dehydrogenase 1 (IDH1) R132H
3 mutation, modeled in the human anaplastic oligodendroglioma cell, was sufficient to induce
4 biosynthesis of proline from glutamine¹². Proline biosynthesis in IDH1 mutant cells was found to
5 be mediated by increased activity of PYCR1¹². Contrarily, a study of breast cancer metastasis
6 using the 4T1 and EMT6.5 orthotopic mouse models demonstrated that the enzyme that
7 antagonizes PYCR1 function, proline dehydrogenase (PRODH), supported tumor growth *in*
8 *vivo*, and inhibition of PRODH impaired metastasis to lung in these orthotropic models¹³.
9 However, a separate study utilizing a mouse model of invasive breast carcinoma demonstrated
10 that deletion of PYCR1 blocked tumor growth *in vivo*, suggesting that different cancer types and
11 cancer models may have different requirements for proline metabolism¹⁴. The genetic condition
12 cutis laxa, a condition of the skin resulting in loose, hypo-elastic and redundant tissue is caused
13 by mutations in PYCR1¹⁵.

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16 Differential expression of PYCR1 in HGSC should be validated in larger and separate
17 cohorts of women with HGSC. If validated, mouse models of high-grade serous ovarian
18 cancers¹⁶⁻¹⁸ can be utilized to assess the effect of conditional expression of PYCR1 in HGSC
19 tumors post-development and on tumor cell proliferation and cancer progression *in vivo*.
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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., 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. Choi, U.Y., Lee, J.J., Park, A., Zhu, W., Lee, H.R., Choi, Y.J., Yoo, J.S., Yu, C., Feng, P., Gao, S.J. and Chen, S., 2020. Oncogenic human herpesvirus hijacks proline metabolism for tumorigenesis. *Proceedings of the National Academy of Sciences*, 117(14), pp.8083-8093.
8. Craze, M.L., Cheung, H., Jewa, N., Coimbra, N.D., Soria, D., El-Ansari, R., Aleskandarany, M.A., Cheng, K.W., Diez-Rodriguez, M., Nolan, C.C. and Ellis, I.O., 2018. MYC regulation of glutamine-proline regulatory axis is key in luminal B breast cancer. *British journal of cancer*, 118(2), pp.258-265.
9. Liu, W., Le, A., Hancock, C., Lane, A.N., Dang, C.V., Fan, T.W.M. and Phang, J.M., 2012. Reprogramming of proline and glutamine metabolism contributes to the proliferative and metabolic responses regulated by oncogenic transcription factor c-MYC. *Proceedings of the National Academy of Sciences*, 109(23), pp.8983-8988.
10. Wang, D., Wang, L., Zhang, Y., Yan, Z., Liu, L. and Chen, G., 2019. PYCR1 promotes the progression of non-small-cell lung cancer under the negative regulation of miR-488. *Biomedicine & Pharmacotherapy*, 111, pp.588-595.

11. Cai, F., Miao, Y., Liu, C., Wu, T., Shen, S., Su, X. and Shi, Y., 2018. Pyrroline-5-carboxylate reductase 1 promotes proliferation and inhibits apoptosis in non-small cell lung cancer. *Oncology letters*, 15(1), pp.731-740.
12. Hollinshead, K.E., Munford, H., Eales, K.L., Bardella, C., Li, C., Escribano-Gonzalez, C., Thakker, A., Nonnenmacher, Y., Kluckova, K., Jeeves, M. and Murren, R., 2018. Oncogenic IDH1 mutations promote enhanced proline synthesis through PYCR1 to support the maintenance of mitochondrial redox homeostasis. *Cell reports*, 22(12), pp.3107-3114.
13. Loayza-Puch, F., Rooijers, K., Buil, L.C., Zijlstra, J., Vrielink, J.F.O., Lopes, R., Ugalde, A.P., van Breugel, P., Hofland, I., Wesseling, J. and van Tellingen, O., 2016. Tumour-specific proline vulnerability uncovered by differential ribosome codon reading. *Nature*, 530(7591), pp.490-494.
14. Elia, I., Broekaert, D., Christen, S., Boon, R., Radaelli, E., Orth, M.F., Verfaillie, C., Grünewald, T.G. and Fendt, S.M., 2017. Proline metabolism supports metastasis formation and could be inhibited to selectively target metastasizing cancer cells. *Nature communications*, 8(1), pp.1-11.
15. Reversade, B., Escande-Beillard, N., Dimopoulou, A., Fischer, B., Chng, S.C., Li, Y., Shboul, M., Tham, P.Y., Kayserili, H., Al-Gazali, L. and Shahwan, M., 2009. Mutations in PYCR1 cause cutis laxa with progeroid features. *Nature genetics*, 41(9), pp.1016-1021.
16. 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.
17. 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.
18. 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.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
123	A_23_P130194	3.62E-06	8.1347916	4.84471	1.2917 ± 0.0594	PYCR1	pyrroline-5-carboxylate reductase 1

Table 1: PYCR1 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 PYCR1 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
3711	8019316	6.33E-03	2.8663105	-2.7679987	PYCR1	pyrroline-5-carboxylate reductase 1

Table 2: PYCR1 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 PYCR1 in HGSC tumors when compared to the normal ovary, the gene and gene name are listed in this chart.

Figure 1: PYCR1 is expressed at significantly higher levels in HGSC tumors when compared to the normal ovary.

The mRNA expression of PYCR1 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 PYCR1 expression between the normal ovary and primary tumors from patients with HGSC, a p-value, listed above.