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Metadata Analysis to Get Insight into Drug Resistant Ovarian Cancer

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ABSTRACT

The most prevalent kind of ovarian cancer is high-grade serous ovarian cancer. Drug resistance is the major issue in this cancer. Transcriptional fusions involving SLC25A40-ABCB1 is a leading cause of this cancer. To understand the phenotypic consequences, transcriptional profile was studied using high throughput sequencing technologies. Here we have used that data to understand co-expressed genes and their functional role in two different cell types, fusion positive and fusion negative using WGCNA analysis. The major biological processes which are correlated with fusion positive cells are extracellular structure organization, external encapsulating structure organization, regulation of cell migration and axon guidance etc. In addition to these investigations, gene expression data of a PARPi-sensitive cell line and resistance was analyzed to determine the role and capabilities of PARP-inhibitors in controlling drug-resistant High-grade serous ovarian cancer. This investigation also shed light on the possible mechanism of PARPi resistant cases and concluded that the resistance comes from the dynamics of four biological processes like regulation of cell junction assembly, cell-cell adhesion, tissue morphogenesis, neuron projection development and negative regulation of cellular component organization. Further analysis with different Gene Set Enrichment analysis illustrates that four processes, negative regulation of lens fiber cell differentiation, sarcoplasmic reticulum lumen, presynaptic membrane assembly and nitrobenzene metabolic process are activated in PARPi resistance. These processes are connected to each other through an important kinase protein ERBB2 which is interpreted as a key protein in PARPi resistance.

1. INTRODUCTION

The deadliest kind of gynaecological cancer is ovarian cancer [1]. Seventy percent of these patients have advanced high-grade serous ovarian cancer [2]. Surgery followed by platinum/taxane chemotherapy is the standard course of action for HGSOC. Because of this, there is still no hope for recovery. 25% of HGSOCs return in the first six months of treatment, and a 5-year overall survival rate of 31% has been recorded [3]. Resistance develops to the existing chemotherapeutic drug, resulting in a poor success rate [4]. In recurrent ovarian cancer that is resistant to treatment, ABCB1 fusions are common [5]. After therapy, high-grade serous ovarian cancer (HGSC) samples underwent whole-genome analysis, which revealed a transcriptional fusion between ABCB1 and the upstream gene SLC25A40 linked to an increase in ABCB1 expression and eventually responsible for drug resistance. Chromosomal closeness to ABCB1 are major determinants in being involved in a productive fusion event. SLC25A40-ABCB1 Fusion was examined by Pishas et al. [6] using high-throughput sequencing technologies and data was deposited in the GEO database for further analysis.

We've learned more about the mechanism of resistance using the weighted gene co-expression analysis method. The phenomenon of medication resistance is extraordinarily nuanced. Understanding the genes and signaling pathways involved in chemo-resistance processes and identifying innovative and effective medication targets as well as drug discovery to improve therapeutic results are essential.

Cellular processes such as transcription, apoptosis, and the DNA damage response are all controlled by the Poly (ADP-ribose) polymerase family (PARP). The first approved cancer treatments that precisely target DNA damage response were PARP inhibitors (PARPi) [7]. Drug resistant HGSOC can be treated with PARP inhibitors, although this therapeutic strategy imposes an unacceptable burden of DNA damage repair failure and may cause cell death. Resistance to PARPi, on the other hand, is lethal. The sensitivity of PARPi was tested using a functional assay. To better understand how PARPis function in a resistant cell line, this gene expression data was gathered. Fusion positive cells in HGSOC, as well as the gene expression data from PARPi-treated cells, were

compared and integrated to examine the potential of PARPi to control resistant HGSOC.

Pishas et al. [6] found that the NABA matrisome-associated pathway, exterior encapsulation structure organization, and regulation of cell adhesion were the most significantly enriched pathways in fusion-positive clones. Here we have extended our analysis using weighted co-expression gene network analysis and integrated and compared with PARPi treated expression data analysis to understand the benefit of PARPi in the drug resistant HGSOC [8].

In a process known as epithelial-to-mesenchymal transition (EMT), epithelial cells lose cell-cell adhesion and cell polarity while developing mesenchymal traits. Homeostasis depends on cell-cell adhesion and interactions with the extracellular matrix. Cancer cells are able to defy social order by reducing intercellular adhesion, which results in the breakdown of histological structure, a hallmark of malignant tumors [9]. There are many ways in which the extracellular matrix (ECM) influences cancer cell proliferation and development. Proteomes encoded by genes for ECM and ECM-associated proteins make up the matrisome. ECM and matrisome in cancer cells are highly variable [10]. A crucial mechanism in drug-resistant ovarian cancer has been identified as the heterogeneity and dynamism of cell adhesion. Chemotherapy itself increases resistance by altering cancer cell adhesion signals and the extracellular matrix (ECM) surrounding the cells. The aggressiveness of HGSCs is driven by an adaptive response called matrix adhesion, which has only recently been discovered [11]. We have highlighted these dynamics together with other dynamical biological processes, such as cell junction construction, tissue morphogenesis, neuron projection development, and regulation of cellular component organization, using integrated data analysis.

2. METHODOLOGY

2.1 Weighted Gene Co-expression Network Analysis (WGCNA)

WGCNA is primarily intended for use with microarray datasets or count data of RNA-seq, particularly transcriptomic data from the Gene Expression Omnibus (GEO) in order to construct a correlation network [12]. It is a powerful method for finding clusters (modules) of strongly associated genes in biological investigations. These modules are then used to scan genes for prospective biomarkers and medicinal targets. Genes with similar expression patterns are grouped into modules by WGCNA (GSE183210) using a systematic approach to clustering genes [13]. Cells with drug resistance due to fusion (SLC25A40-ABCB1 fusion) were subjected to transcriptome comparisons with counterparts with drug resistance due to non-fusion (fusion negative) cells. Data reduction and unsupervised classification are two features of the Weighted Correlation Network Analysis (WGCNA). If there is an association between the expression of two genes, the network will create a link between them. Based on the correlation value, genes may be more or less linked (the weights). Soft thresholding parameter (β) for network development was used in this package, which was set at 0.8 for scale-free topology model fit. Using R software, the WGCNA package was used to analyze the data. Different coloured modules were depicted in a cluster dendrogram. The Pearson correlation analysis was used to examine the relationship between gene modules in order to identify the module with the strongest association with genes that were fusion positive. There were also generated values for the gene significance (GS) and module membership (MM). GS>0.85 and MM>0.85 were chosen as critical selection criteria for key genes.

2.2 Enrichment analysis

Metascape and Enrichr [14] were used to analyze the GO and KEGG pathway enrichment of module genes to investigate their potential biological roles [15]. It is a search engine and a resource for curated gene sets that gathers biological information for the purpose of facilitating further biological discoveries. For experimental biologists, Metascape is a useful and efficient tool for the analysis and interpretation of OMICs-based investigations in the era of big data. GSEA (gene set enrichment analysis) is a tool for identifying classes of genes or proteins that are over-represented in a large set of genes and may be associated with phenotypes or disease characteristics. The strategy employs statistical methodologies to identify gene groups that are significantly enriched or depleted R package, Cluster Profiler was used for GSEA analysis [16].

2.3 Analysis of differentially expressed genes

In sensitive cell lines, PARP inhibition can prevent the development of an EMT phenotype; however, in resistant cell lines, this does not happen. The GEO2R software package was used to analyze the difference in gene expression between the sensitive and resistant cell lines treated with PARPi (Smyth G.K 2005, Sean D and Paul S.M., 2007). P value (0.05) was used to identify the DEGs (differentially expressed genes).

3. RESULTS

We integrated and compared the WGCNA result of drug resistant fusion positive cell line (GSE183210) and differentially expressed genes in PPARi resistant case (GSE149940).

3.1 Analysis of resistant HGSOC

HGSOC cells with SLC25A40-ABCB1 fusions were studied by transcriptome analysis of fusion-positive cells versus fusion-negative cells for the phenotypic consequences of these fusions in High Grade Serous Ovarian Cancer (HGSOC). Dendrograms from WGCNA analysis show that the Turquoise module is strongly correlated with the trait. Figure 1 and 2 show the module's dendrogram and their correlation.

Total 1460 genes were found in the Turquoise module. Those genes were studied for gene ontology and functional enrichment analysis using both Metascape and Enrichr. Enrichr Pathway enrichment analysis portrays that Axon guidance, cell adhesion molecules, ECM receptor interaction, Proteoglycan in cancer and pathways in Cancer play a major role in the transcriptome of the fusion positive High Grade Serous Ovarian Cancer. The biological process involved extracellular matrix organization, extracellular structure organization, external encapsulating structure organization and regulation of cell migration etc. (Figure 3). Metascape, on the other hand, proposes four biological processes: cell

adhesion regulation, cell junction organization, cell projection organization regulation, and positive regulation of cell component biogenesis (Figure 4).

Total 1124 genes were found in blue genes that are downregulated in fusion positive cells. The most prevalent downregulated pathways were nervous system development and Cancer pathway. Involvement of nervous system involvement matters in the tumor microenvironment but in this case in the transcriptome of fusion positive cloned cell we cannot conclude anything about that. Few Pathways of Cancer are diminished in fusion positive cells also. Detailed analysis is required to investigate the cancer pathway in both the cases.

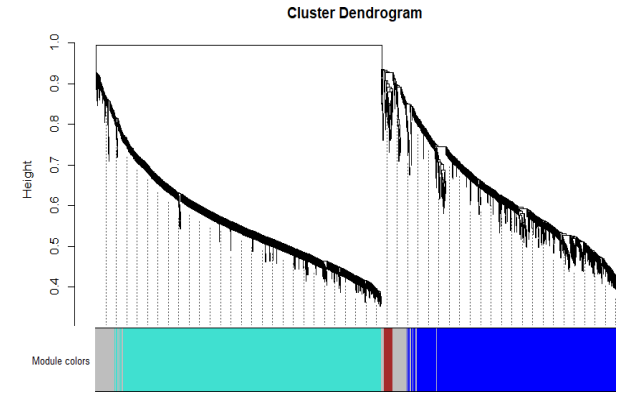


Figure 1. Modules generated by WGCNA using the data, GSE183210. Phenotypic consequences of SLC25A40-ABCB1 fusions in High Grade Serous OvarianCancer (HGSOC)

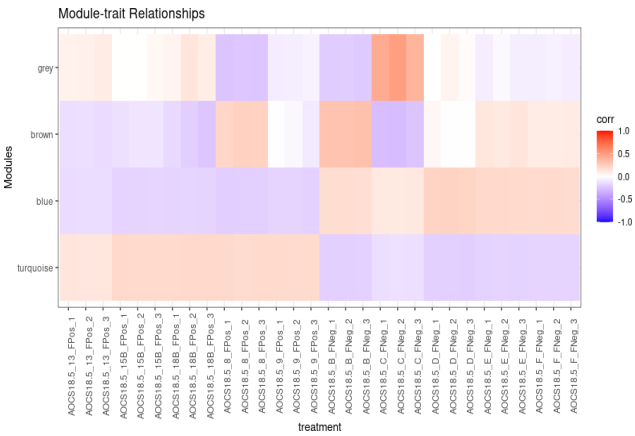
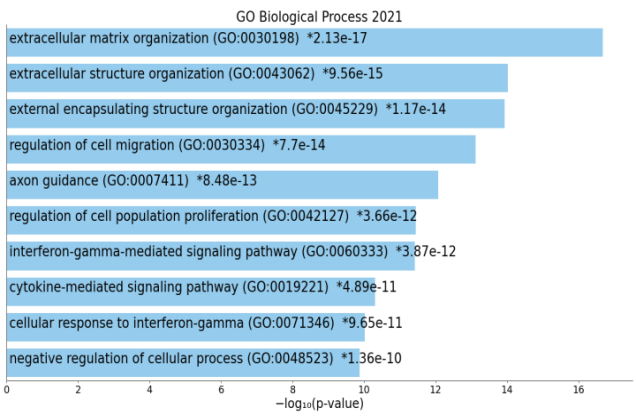
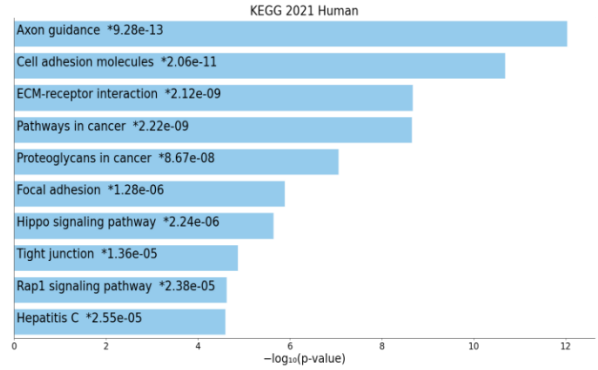


Figure 2. Module Trait relationship in fusion positive and fusion negative HGSOC. Violet color shows anti correlation light brown shows correlation

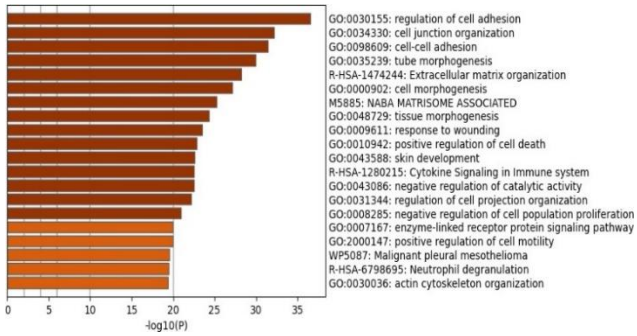


(a) Biological process

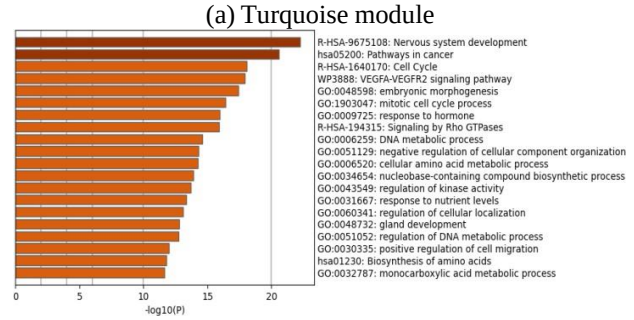


(b) KEGG pathway

Figure 3. GSEA analysis of the Turquoise module using Enrichr. (a) Biological process; (b) KEGG pathways



(a) Turquoise module



(b) Blue module

Figure 4. Functional enrichment analysis using Metascape, (a) Turquoise module; (b) Blue module

3.2 Analysis of PARPi treated cells

To understand the potential of PARPi to treat HGSOC ovarian cancer we have compared the phenotypic consequence data of fusion positive HGSOC cell transcriptome data with gene expression data of PARPi sensitive.

3.3 Differentially expressed genes

The GEO database was used to obtain the dataset (GSE149940) containing the gene expression profiles of six AsPCs resistant to PARP inhibitors and six AsPCs sensitive to it. These findings were made possible thanks to the GPL4133 platform (Agilent-014850 Whole Human Genome Microarray 4x44K G4112F) [17]. DEGs between PARP-inhibitor resistant and sensitive AsPC were identified using the publicly available GEO2R tool based on the cutoff values of | log2FC| ≥1.1 and p-values<0.05, which were calculated using the inbuilt R/Bioconductor and limma packages v3.26.8 from the GEO2R tool. For further investigation, the top 135

differentially expressed genes (DEGs) (32 up-regulated and 103 down-regulated genes) were found after removing the duplicates.

3.4 Functional enrichment analysis

Functional enrichment analysis shown in Figure 5, shows PARP-inhibitor able to downregulate many pathways like epithelial cell differentiation, regulation of epithelial cell proliferation, prostaglandin synthesis and regulation, skin development, negative regulation of cell differentiation and many more which are the cause of anticancer activity in fusion positive HGSOC.

PARPi resistance is the deadliest cancer. So, understanding the mechanism of PARPi resistant is crucial.

Potential gene biomarkers for PARPis sensitivity and resistance have been identified by Razan Sheta and colleagues. Here we have mainly focused on understanding the biological process involved in PARPi resistance. Few biological processes like regulation of cell junction assembly, cell-cell adhesion, tissue morphogenesis, neuron projection development and negative regulation of cellular component organization are both downregulated and upregulated. This analysis depicts that PARPi has the ability to suppress most of the cancer-causing pathways shown in the previous figure but the resistance comes from the dynamics of the above-mentioned five processes.

Networks of those biological processes are shown in Figure 5 that enable us to identify functional modules. The detailed analysis of genes involved in the process may lead to identifying the actual dynamical process involved in the PARPi resistance.

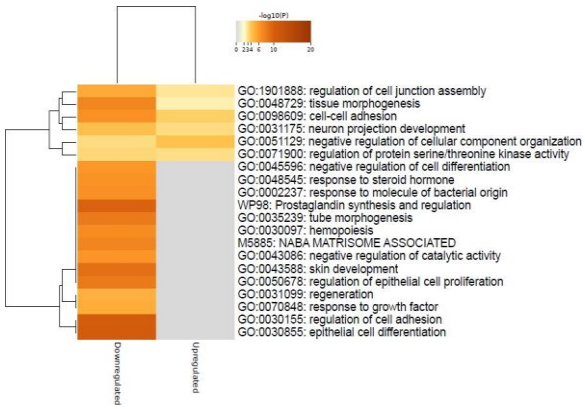
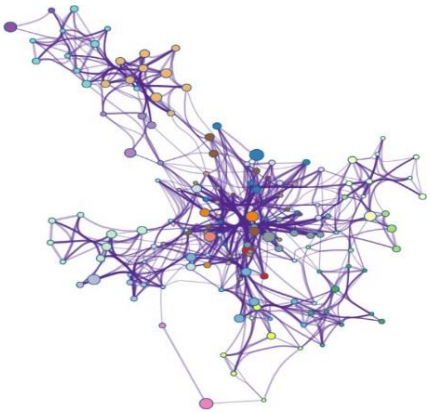


Figure 5. (a) Metascape analysis of DEG from gene expression profiles of PARP-inhibitor resistant AsPC and sensitive AsPC



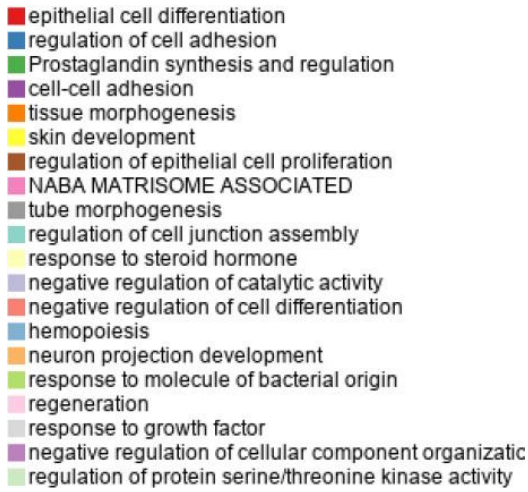
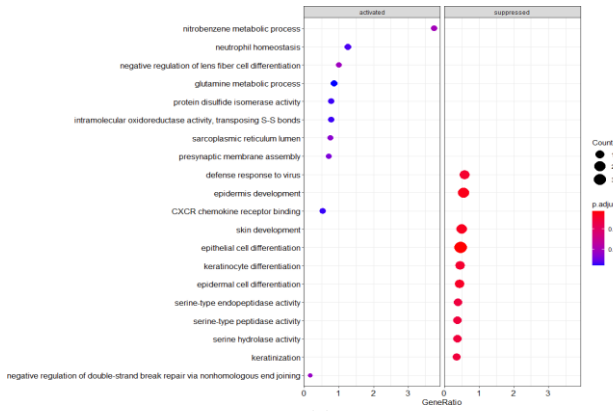
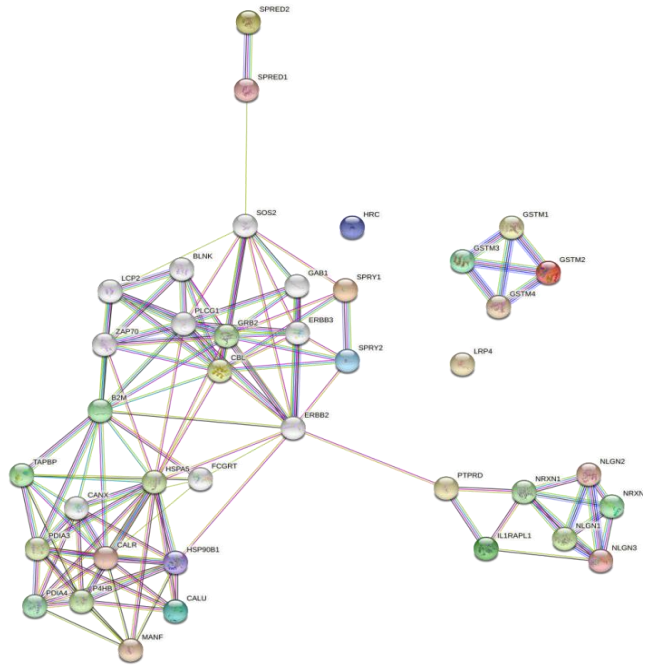


Figure 5. (b) Functional network

3.5 GSEA with cluster profiler analysis



(a)



(b)

Figure 6. (a) GSEA analysis of PARP-inhibitor resistantdata (GSE149940) using cluster profiler; (b) String analysis of genes involved in four processes negative regulation of lens fibre cell differentiation, sarcoplasmic reticulum lumen, presynaptic membrane assembly and nitrobenzene metabolic process

GSEA analysis of PARPi resistant vs sensitive is shown in Figure 6. According to P adjust value, it was found that negative regulation of lens fibre cell differentiation, sarcoplasmic reticulum lumen, presynaptic membrane assembly and nitrobenzene metabolic process get activated in PARPi resistant cases. We investigated the names of the genes involved in these processes and analyzed them with the STRING database. We added three layers of nodes to get the relation between these apparently isolated processes. The result shows that SPRY2 of Negative regulation of lens fibre cell differentiation is connected with PTPRD of presynaptic membrane assembly and HSP90B1 of sarcoplasmic reticulum lumen through the node ERBB2. So, we conclude that ERBB2 is highly important in the PARPi resistant process.

4. DISCUSSION AND CONCLUSION

High Grade Serous Ovarian Cancer (HGSOC) is mostly homologous recombination repair (HRR)-deficient. Hence, they are susceptible to PARP inhibition. We compared the result of transcriptome analysis of fusion positive cells (SLC25A40-ABCB1 fusions) in drug resistant HGSOC with differentially expressed genes in PARPi resistant and sensitive AsPC cell line to understand whether PPAR inhibitor can control the Cancer Pathways in HGSOC. It was found (Figure 1 and Figure 5) that PPARi can downregulate most of the major cancer causing pathways in drug resistant HGSOC. Homologous Recombination restoration (HRR) and DNA replication fork protection, have been found as potential mechanisms of PARPi resistance [18] It is reported that loss of PTEN leads to HRD, increased genomic instability and replication fork collapse [19]. Likewise, there are lots of conflicting results also [20, 21]. But clinical studies showed that the PARPi had increased progression-free survival regardless of the presence or absence of Homologous recombination repair deficient (HRD). As a result, we must thoroughly understand how PARPi acts, particularly how the roles of PARPi in processes unrelated to DNA repair impact the anti-cancer effect of PARPi, which will aid in understanding the development of resistance.

Here we found the dynamics of regulation of cell junction assembly, cell-cell adhesion, tissue morphogenesis, neuron projection development and negative regulation of cellular component organization plays a major role in PPARi resistance. From this integrative and comparative analysis in drug resistant HGSOC we can conclude that cross talk and dynamics between Axon development, cell adhesion molecules, tissue morphogenesis and regulation of serine threonine kinase activity are responsible for PARPi resistance.

Through the GSEA analysis by Cluster Profiler and STRING database we conclude the four processes negative regulation of lens fibre cell differentiation, sarcoplasmic reticulum lumen, presynaptic membrane assembly and nitrobenzene metabolic process are activated in PARPi resistant. Here we found that the nitrobenzene metabolic process is an isolated process but other processes are connected by an important protein ERBB2. It functions via phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling pathways. One recent study emphasizes the role of Akt activation in PARPi resistance [22]. Our extrapolated STRING analysis fetches ERBB2 as an important node which couldn't be captured in the usual analysis of transcriptome.

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REFERENCES

[1] Hao, Q., Li, J.J., Zhang, Q.H., Xu, F., Xie, B.X., Lu, H., Wu, X.H., Zhou, X. (2021). Single-cell transcriptomes reveal heterogeneity of high-grade serous ovarian carcinoma. Clinical and Translational Medicine, 11(8): e500. https://doi.org/10.1002/ctm2.500

[2] Koonings, P.P., Campbell, K., Mishell Jr, D.R., Grimes, D.A. (1989). Relative frequency of primary ovarian neoplasms: A 10-year review. Obstetrics and Gynecology, 74(6): 921-926. https://doi.org/10.1016/0020-7292(90)90378-X

[3] Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Thun, M.J. (2009). Cancer statistics. CA: A Cancer Journal for Clinicians, 59(4): 225-249. https://doi.org/10.3322/caac.20006

[4] Eismann, J., Heng, Y.J., Waldschmidt, J.M., Vlachos, I.S., Gray, K.P., Matulonis, U.A., Konstantinopoulos, P.K., Murphy, C.J., Nabavi, S., Wulf, G.M. (2020). Transcriptome analysis reveals overlap in fusion genes in a phase I clinical cohort of TNBC and HGSOC patients treated with buparlisib and olaparib. Journal of Cancer Research and Clinical Oncology, 146(2): 503-514. https://doi.org/10.1007/s00432-019-03078-9

[5] Christie, E.L., Pattnaik, S., Beach, J., Copeland, A., Rashoo, N., Fereday, S., Hendley, J., Alsop, K., Brady, S.L., Lamb, G., Pandey, A., deFazio, A., Thorne, H., Bild, A., Bowtell, D.D.L. (2019). Multiple ABCB1 transcriptional fusions in drug resistant high-grade serous ovarian and breast cancer. Nature Communications, 10: 1295. https://doi.org/10.1038/s41467-019-09312-9

[6] Pishas, K.I., Cowley, K.J., Pandey, A., Hoang, T., Beach, J.A., Luu, J., Vary, R., Smith, L.K., Shembrey, C.E., Rashoo, N., White, M.O., Simpson, K.J., Bild, A., Griffiths, J.I., Cheasley, D., Campbell, I., Bowtell, D.D.L., Christie, E.L. (2021). Phenotypic consequences of SLC25A40-ABCB1 fusions beyond drug resistance in high-grade serous ovarian cancer. Cancers, 13(22): 5644. https://doi.org/10.3390/cancers13225644

[7] Wang, G.X., Li, Y., Wang, P., Liang, H., Cui, M., Zhu, M.L., Guo, L.M., Su, Q., Sun, Y.J., McNutt, M.A., Yin, Y.X. (2015). PTEN regulates RPA1 and protects DNA replication forks. Cell Research, 25(11): 1189-1204. https://doi.org/10.1038/cr.2015.115

[8] Chiappa, M., Guffanti, F., Bertoni, F., Colombo, I., Damia, G. (2021). Overcoming PARPi resistance: Preclinical and clinical evidence in ovarian cancer. Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy, 55: 100744. https://doi.org/10.1016/j.drup.2021.100744

[9] Janiszewska, M., Primi, M.C., Izard, T. (2020). Cell

adhesion in cancer: Beyond the migration of single cells. Journal of Biological Chemistry, 295(8): 2495-2505. https://doi.org/10.1074/jbc.rev119.007759

[10] Janysek, D.C., Kim, J., Duijf, P.H.G., Dray, E. (2021). Clinical use and mechanisms of resistance for PARP inhibitors in homologous recombination-deficient cancers. Translational Oncology, 14(3): 101012. https://doi.org/10.1016/j.tranon.2021.101012

[11] Pietilä, E.A., Gonzalez-Molina, J., Moyano-Galceran, L., Jamalzadeh, S., Zhang, K.Y., Lehtinen, L., Turunen, S.P., Martins, T.A., Gultekin, O., Lamminen, T., Kaipio, K., Joneborg, U., Hynninen, J., Hietanen, S., Grenman, S., Lehtonen, R., Hautaniemi, S., Carpen, O., Carlson, J.W., Lehti, K. (2021). Co-evolution of matrisome and adaptive adhesion dynamics drives ovarian cancer chemoresistance. Nature Communications, 12: 3904. https://doi.org/10.1038/s41467-021-24009-8

[12] Davis, S., Meltzer, P.S. (2007). GEOquery: A bridge between the Gene Expression Omnibus (GEO) and BioConductor. Bioinformatics, 23(14): 1846-1847. https://doi.org/10.1093/bioinformatics/btm254

[13] Langfelder, P., Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. BMC Bioinformatics, 9: 559. https://doi.org/10.1186/1471-2105-9-559

[14] Zhou, Y.Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A.H., Tanaseichuk, O., Benner, C., Chanda, S.K. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nature Communications, 10(1): 1523. https://doi.org/10.1038/s41467-019-09234-6

[15] Chen, E.Y., Tan, C.M., Kou, Y., Duan, Q.N., Wang, Z.C., Meirelles, G.V., Clark, N.R., Ma'ayan, A. (2013). Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics, 14: 128. https://doi.org/10.1186/1471-2105-14-128

[16] Wallace, R., Leonova, I., Gochhait, S. (2022). On the inherent instability of biocognition: Toward new probability models and statistical tools. Entropy, 24(8): 1070. http://dx.doi.org/10.3390/e24081070

[17] Smyth, G.K. (2004). Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Statistical Applications in Genetics and Molecular Biology, 3(1): Article 3. https://doi.org/10.2202/1544-6115.1027

[18] Gomez, M.K., Illuzzi, G., Colomer, C., Churchman, M., Hollis, R.L., O'Connor, M.J., Gourley, C., Leo, E., Melton, D.W. (2020). Identifying and overcoming mechanisms of PARP inhibitor resistance in homologous recombination repair-deficient and repair-proficient high grade serous ovarian cancer cells. Cancers, 12(6): 1503. https://doi.org/10.3390/cancers12061503

[19] Smyth, G.K. (2005). Limma: Linear models for microarray data. In R. Gentleman, V. Carey, S. Dudoit, R. Irizarry & W. Huber (Eds.), Bioinformatics and Computational Biology Solutions Using R and Bioconductor, pp. 397-420. https://doi.org/10.1007/0-387-29362-0_23

[20] Bian, X., Gao, J., Luo, F., Rui, C., Zheng, T., Wang, D., Wang, Y., Roberts, T.M., Liu, P., Zhao, J.J., Cheng, H. (2018). PTEN deficiency sensitizes endometrioid endometrial cancer to compound PARP-PI3K inhibition but not PARP inhibition as monotherapy. Oncogene, 37(3): 341-351. https://doi.org/10.1038/onc.2017.326

[21] Fraser, M., Zhao, H., Luoto, K.R., Lundin, C., Coackley, C., Chan, N., Joshua, A.M., Bismar, T.A., Evans, A., Helleday, T., Bristow, R.G. (2012). PTEN deletion in prostate cancer cells does not associate with loss of RAD51 function: Implications for radiotherapy and chemotherapy. Clinical Cancer Research, 18(4): 1015-1027. https://doi.org/10.1158/1078-0432.ccr-11-2189

[22] Gallyas Jr, F., Sumegi, B., Szabo, C. (2020). Role of akt activation in PARP inhibitor resistance in cancer. Cancers, 12(3): 532. https://doi.org/10.3390/cancers12030532