Elucidating Differentially Expressed miRNA-mRNA Regulatory Networks in Ovarian Cancer: Insights into Pathogenesis, Prognosis, and Drug Resistance

By

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A thesis submitted to the Department of Mathematics & Natural Sciences in partial fulfillment of the requirements for the degree of
Bachelor of Science in Microbiology

Department of Mathematics and Natural Sciences
Brac University
June 2025

Declaration

It is at this moment declared that.

1. The thesis submitted titled "**Elucidating Differentially Expressed miRNA-mRNA Regulatory Networks in Ovarian Cancer: Insights into Pathogenesis, Prognosis, and Drug Resistance**" is our original work while completing our degree at BRAC University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.

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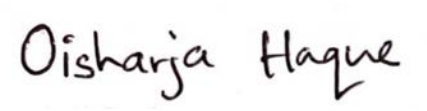

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Approval

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Abstract

Ovarian cancer has one of the highest mortality rates in gynecological malignancies worldwide, with frequent treatment fallouts while limiting improvements, mostly due to recurrence and development of drug resistance. MicroRNAs, a group of small non-coding RNAs which are known to regulate gene expression post-transcriptionally, have been linked to induce various aspects of malignancies, including ovarian cancer. This study tries to shed light on a comprehensive analysis of the differentially expressed miRNA-mRNA regulatory networks in OC, also elucidating exceptional expression landscapes to gain insights into its pathogenesis, prognosis, and drug resistance mechanisms. By integrating high-throughput microarray data from publicly available databases (GEO) and leveraging in-silico approaches with limma package in R software, we classify key miRNAs and their target mRNAs that are dysregulated in ovarian cancer tissues and drug resistant cell lines compared to normal controls. Our results reveal differentiated miRNA and their cognate regulated mRNAs in OC tissues and cell lines; as well as suggests novel regulatory pathways involving miRNAs such as hsa-miR-let7 family, hsa-miR-132-3p, hsa-miR-221-3p and hsa-miR-200c-3p; as well as targeted genes (LOX, LGR5, COL11A1, DUSP1, CXCL2, ALDH1A1); which are found to be associated with cell cycle regulation, apoptosis, and chemoresistance through further functional role analyses. This research not only deepens our understanding of the molecular mechanisms underlying ovarian cancer but also gives perspectives into complex and exceptional regulatory landscapes, and detects potential biomarkers and therapeutic targets for better patient outcomes.

Keywords:

Ovarian cancer; miRNAs; Regulatory networks; Drug resistance; Biomarkers; Therapeutic targets

Acknowledgment

I would like to start by expressing my deepest gratitude to my Almighty Allah for allowing me to pursue and complete this research and to my friends and family for supporting me from the very beginning till the end.

I am grateful to the Chairperson of the Department of MNS**, Md. Firoze H. Haque** Sir and to all the teachers of BRAC University for always supporting me. Special thanks to my supervisors, **Mohammad Sayem**, lecturer, Department of Biochemistry and Molecular Biology, Dhaka University and **Dr. Nadia Sultana Deen**, Associate Professor, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University whose guidance, insightful feedback, and endless support were instrumental when it came to the success of this research. The research would never have reached its potential if my supervisors had not given dedication and constructive criticism at times.

Finally, I would like to dedicate this research to all those who have believed in themselves and pursued independent research with perseverance and determination. Your self-belief and commitment to yourselves continue to inspire.

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List of Acronyms

OC	Ovarian Cancer
miR	Micro-RNA
DEG	Differentially Expressed Gene
GEO	Gene Expression Omnibus
p-value	Probability Value
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
PFS	Progression Free Survival
KM plot	Kaplan-Meier plot

Chapter 1

Introduction

1.1 Background

Ovarian cancer (OC) ranks as one of the most lethal gynecologic malignancies, the seventh most common occurrence and the eighth leading cause of cancer deaths in women globally (Zhao et al., 2022). In accordance with Globocan 2022, global cases of OC are predicted to rise 55% to 503,448 by 2050, and mortality would rise nearly 70% to 350,956. Over eight million women globally are likely to die from the condition if current trends persist, 70% of them in low- and middle-income countries- like Bangladesh, emphasizing the need for improved interventions (Everhobbes, n.d.). Further, platinum-based chemotherapy with paclitaxel being the typical treatment for ovarian cancer remains consistent for decades. While it is initially successful, ~80% of patients eventually relapse due to platinum resistance, demonstrating the urgency of understanding its molecular mechanism to pave an improved way to deal with this malignancy (Ortiz et al., 2022). Pujade-Lauraine and his team (2019) revealed that nearly 25% of patients with metastatic OC develop recurrence or progression of the disease within six months of the initial procedure, usually debulking surgery with platinum-based chemotherapy. Molecular expressions with diverse factors of biological processes control at least one or more of the prespecified contexts.

MicroRNAs (miRNAs) are small non-coding RNAs of about 20–25 nucleotides length-wise; they regulate eukaryotic gene expression by degrading or inhibiting translation of target mRNAs.

A single miRNA can regulate multiple genes, and they have a pivotal role in many disease development and progression, including cancer (Zhao et al., 2022). In cancer biology, miRNAs are largely classified as tumor-suppressive miRs and oncomiRs regulating genes post-transcriptionally which aid cancer pathogenesis and even drug resistance. Similarly, miRs play crucial roles in ovarian cancer progression, development and treatment resistance (Fasoulakis et al., 2023).

1.2 miRNAs in ovarian cancer pathogenesis and progression

miRNAs can act as either oncogenes or tumor suppressors when they are dysregulated in ovarian cancer. As a consequence, the role of microRNA expression in OC has been extensively studied (Srivastava et al., 2017) and those researches have shown the function of microRNAs in disease progression, chemoresistance, metastatic potentials, EMT, and CSC regulation. One of the examples would be miR-195-5p a member of the miR-16 family, it's usually known to be a tumor suppressor by targeting genes involved in cell cycle regulation and cell division but it shows a decreased expression in OC; consequently target genes like CCNE1, CEP55, HMGA1, and WWC1 and help promote aggressive cancer behaviour (Singh, 2021). Moreover, hsa-miR-424-5p functions as a tumor suppressor in ovarian cancer inhibiting proliferation, migration, and invasion of cancer cells. While Liu with his team (2017) showed that it is downregulated in OC cells while suppressing the E2F1-pRb pathway and arrest the cell cycle at the G1/G0 phase by specifically targeting CCNE1 along with HMGA1 and WWC1; although the precise mechanisms governing HMGA1 and WWC1 in OC are less clear. The microRNA hsa-miR-125b-5p is suppressed in ovarian cancer. The suppression leads to the overexpression of its target genes, E2F2 and SAMD10, by eliminating the inhibitory influence that

hsa-miR-125b-5p otherwise exerts on their mRNA. E2F2 regulates the expression of MCM4, CCNE2, and WHSC1. Elevated E2F2 expression in OC cells greatly increases the expression of these genes, which promote tumor growth (Xie et al., 2017).

1.3 miRNAs behind drug resistance in ovarian cancer

Combative behavior of ovarian cancer is widely thought to be linked with the development of resistance to chemotherapeutic treatments that patients are exposed to throughout the therapy sessions. Drug resistance is a complex phenomenon and cannot be solved by focusing on just one element or route because there are many factors and processes that affect how susceptible cells are to drugs; for example, increased membrane transporter activity, apoptosis dysregulation, drug efflux, cancer stem cells, autophagy, epigenetics, and the epithelial-mesenchymal transition (EMT) are the one of most significant resistance mechanisms in ovarian cancer (Saburi et al., 2022). Notably, miR-200c-3p has been observed to play a paradoxical role in ovarian cancer; despite its conventional function as a metastasis suppressor, hsa-miR-200c-3p is strongly overexpressed in ovarian cancer tissues and cell lines, where it increases treatment resistance and aggressive tumor phenotype through targeting genes like ZEB1, ZEB2, and TUBB3 (Oliveira et al., 2019). Similarly, overexpression of hsa-miR-141-3p in OC also contributes to cisplatin resistance by activating the Keap1/Nrf2 pathway, which targets Keap1 and promotes a tumor-protective microenvironment; this pathway is often correlated with advanced stages of the disease (Zhang et al., 2024). Furthermore, Amini-Farsani and team (2017) has demonstrated that hsa-miR-221-3p promotes cisplatin resistance by bypassing apoptosis through targeting PTEN, resulting in activation of the PI3K/Akt pathway and cancer cell survival in resistant cells.

1.4 Research gaps

Despite extensive studies on miRNAs and their target genes in ovarian cancer progression, recurrence and drug resistance, there remains a knowledge gap in comprehending inconsistent expression patterns and how miRNA regulatory networks adjust and evolve across different disease contexts; we believe this study is a notable step forward. In this research, we intend to bridge this gap by comprehensively analyzing differentially expressed miR landscape and their cognate regulated genes; integrating significant expressions, pathways, and survival information to understand ovarian cancer pathogenesis, prognosis, and the mechanism of drug resistance.

1.5 Objectives and hypothesis

To understand the differentially expressed miRNA and cognate regulated mRNA in ovarian cancer pathogenesis, prognosis, and drug resistance mechanism.

The study displayed a complex miRNA-mRNA regulatory network defined by contradictory expression patterns and involvement of viral miRNA, which may contribute in pathogenesis, induce prognosis, and mediate drug resistance through context-sensitive mechanisms potentially regulated by adaptive metabolic reprogramming, cellular heterogeneity, and epigenetic modifications.

Chapter 2

Materials and Methods

2.1 Dataset collection

NCBI hosted database Gene Expression Omnibus (GEO) was utilized to retrieve datasets investigating miRNA and mRNA expression of ovarian cancer tissues in human samples and human cell lines. Intending this, GSE133859 dataset was downloaded; which used Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 platform to characterize gene expression between ovarian cancer tissue and normal ovarian tissue. Also for miRNA expression analysis, GSE83693 dataset was downloaded which used Agilent-031181 Unrestricted_Human_miRNA_V16.0_Microarray (miRNA_107_Sep09) platform; profiling miRNA expression of recurrent high grade ovarian carcinoma compared to primary ovarian cancer and normal ovary. Besides, we aimed to pin down datasets that studied miRNA and mRNA expression profiles in drug resistant and drug sensitive samples. For this reason, GSE161784 was chosen to profile miRNA of cisplatin-resistant and cisplatin-sensitive ovarian cancer cells; this dataset used [miRNA-4] Affymetrix Multispecies miRNA-4 Array platform for miRNA expression profile. For mRNA expression analysis in drug resistant cells, GSE15372 dataset applying [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array platform had been incorporated in the process; it assessed gene expression data from A2780 (cisplatin-sensitive) and Round5 A2780 (cisplatin-resistant) cell lines.

2.2 Differential expression analysis

The limma package from R (version 4.4.2) software (Ritchie et al., 2015) was leveraged to characterize differentially expressed miRNAs and genes in four different datasets from affymetrix and agilent platforms Preprocessing involved background correction and data normalization. RMA (Robust Multi-array Average) method offered by the "affy" package was used for background correction, data normalization and probe summarization. And for agilent data, the "normalizeBetweenArrays()" function from the limma package was applied for quantile normalization across arrays. In DEG analysis, the following experimental groups were determined- primary ovarian cancer vs. normal, recurrent ovarian cancer vs. normal drug-resistant vs. drug-sensitive and they were reflected in the design matrix. Empirical Bayes moderation by the limma package was applied to the standard errors of the predicted log-fold changes using data from all the genes for more reliable variance estimation. Following that, differential expression analysis progressed using the Benjamini and Hochberg method with threshold of $|\log2FC| > 1$ and adjusted P-value < 0.05 for the miRNA or mRNA to be significantly differentiated. For visualizing the DEG or DE miR findings, volcanoplots and heatmaps were generated with ggplot2, ggrepel, and pheatmap packages. To identify potential regulatory relationships, target genes of DE miRNAs were predicted using the miRDB database (Chen & Wang, 2019) (prediction score > 80) followed by intersection with DEGs to separate genes likely regulated by the DE miRNAs.

2.3 Pathway enrichment analysis

In the interest of finding gene functions of the DEGs, pathway enrichment analyses were conducted using the Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) via clusterProfiler package in R-studio (Yu et al., 2012). To assess whether any GO or KEGG pathways were statistically enriched in the list of significant DEGs relative to a background of all genes expressed, overrepresentation analysis was performed using the Benjamini–Hochberg ($p < 0.05$) method. At the end of analysis, dot and bar plots were used to display terms that were deemed significant when their adjusted p-value was less than 0.05.

2.4 Survival analysis

Kaplan-Meier survival analyses were conducted with the top 10 genes from each dataset; both upregulated and downregulated genes with the highest strength scores (|miRlogFC| + |mRNAlogFC|) to assess the prognostic significance of the selected DEGs. The median expression was used to categorize patients, and a log-rank test ($p < 0.05$) revealed significant variations in survival rates.

Chapter 3

Results

3.1 Identifying differentially expressed genes, miRNAs and their targets

The microarray expression values from all the datasets were normalized using quantile normalization to account for the technical differences between experiments. After this preprocessing step, differential expression analysis was carried out leveraging the *limma* package in R studio to identify differentially expressed miRs and genes between ovarian cancer and normal tissue, and cisplatin sensitive (A2780) and cisplatin resistant ovarian cancer cell lines (CP20 and CIS). The identification of significant miRs and genes were performed upon setting the threshold of adjusted p-value (padj) < 0.05 and absolute logarithmic fold change (|log2fc|) > 1. (Fig. 1 & 2 respectively)

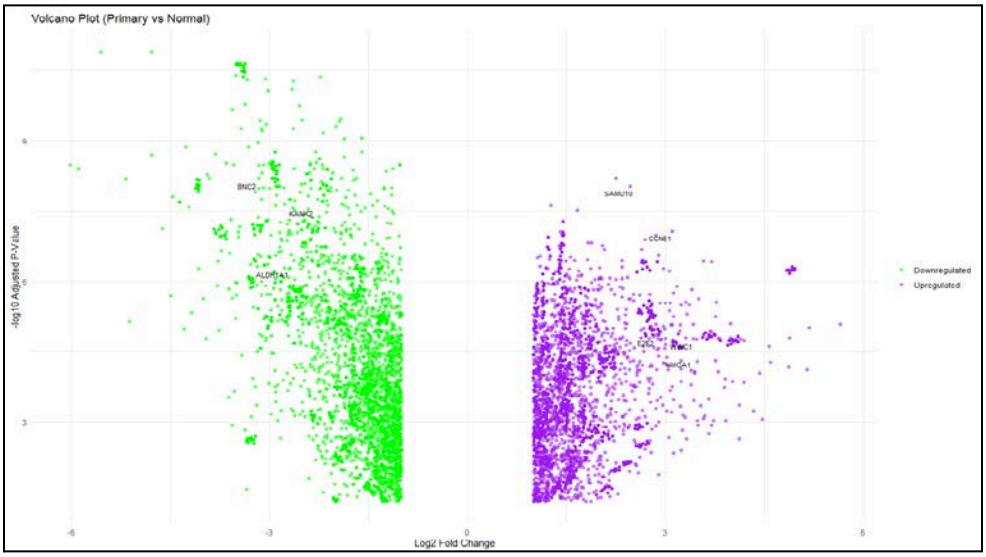
- **DEG from Ovarian cancer vs normal ovarian tissue (GSE133859):** DEG analysis featuring HGSOC tissue and normal ovarian tissue revealed a total of 1170 significantly differentiated genes, among 5832 upregulated and 5868 downregulated.
- **DE miR from Primary ovarian cancer vs normal ovarian tissue (GSE83693):** 1600 DE miRs were found, number of upregulated and downregulated miRs being 727 and 873 respectively.
- **DE miR from Recurrent ovarian cancer vs normal ovarian tissue (GSE83693):** 2430 DE miRNAs, 723 and 1707 upregulated and downregulated miRs respectively.
- **DEG from drug sensitive vs drug resistant cell lines (GSE161784):** A total of 1108 differentially expressed genes; 447 upregulated and 661 downregulated genes.

- **DE miR from drug sensitive vs drug resistant cell lines (GSE15372):** 10 miRNAs were differentially expressed; 3 upregulated and 7 downregulated miRNAs.

Thereafter, miRBD website was used to predict the target genes of DE miRNAs (prediction score > 60). The predicted targets were then intersected with the differentially expressed genes across all the datasets to identify common genes that were both DEGs and miRNA targets.

Figure 1

A)



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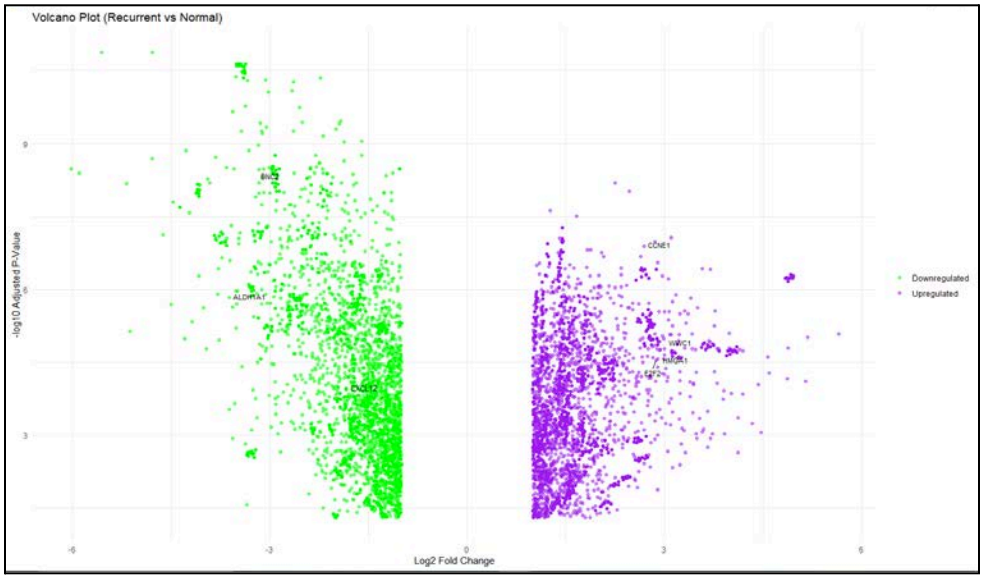


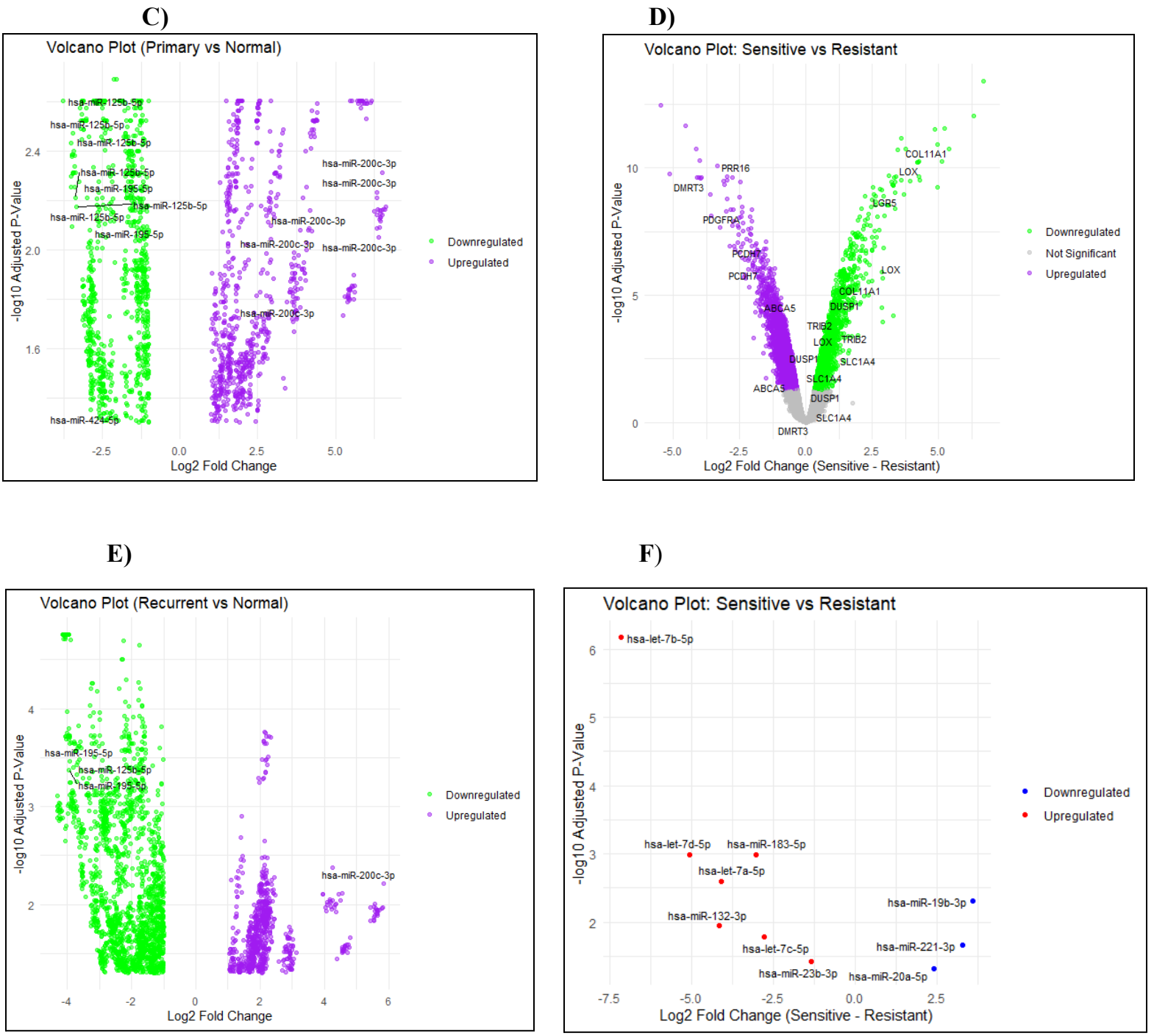
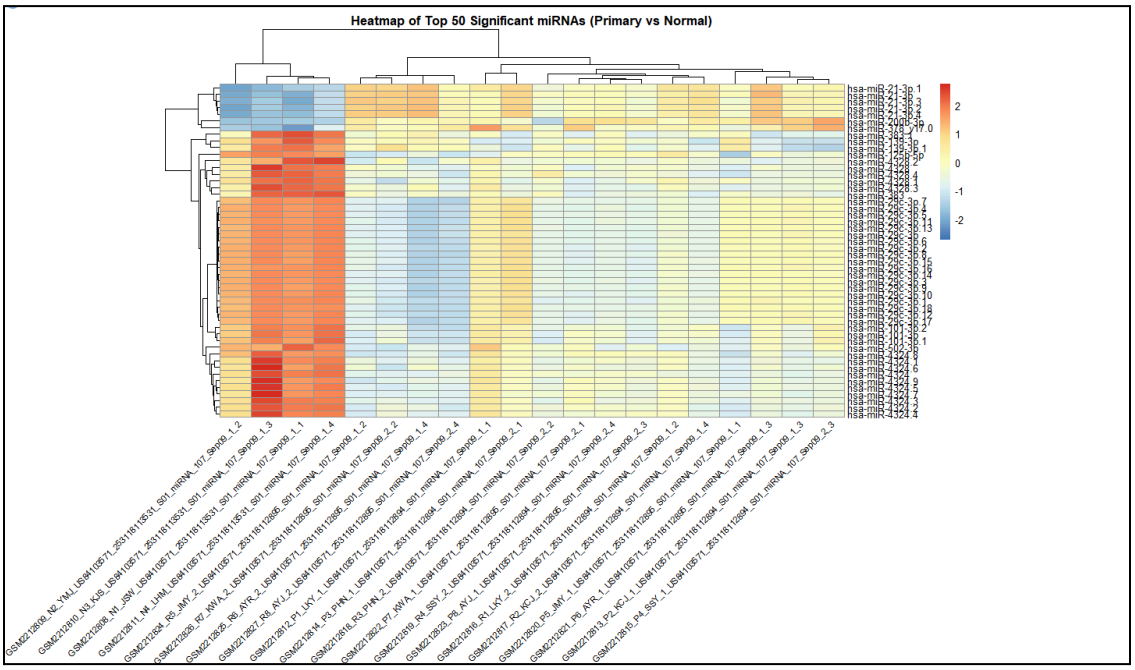


Figure 1. Volcano plots of differentially expressed miRNAs and genes from the 4 datasets included with the cut-off of adjusted P-value < 0.05 and |log2FC| ≥ 1. **A)** DEGs of primary ovarian cancer vs normal ovary. **B)** DEGs of recurrent ovarian cancer vs normal ovary. **C)** DE miRs of primary ovarian cancer vs normal ovary. **D)** DE miRs of recurrent ovarian cancer vs

normal ovary. **E)** DE miRs of sensitive vs resistant OC cell lines. **F)** DEGs of sensitive vs resistant OC cell lines.

Figure 2

A)



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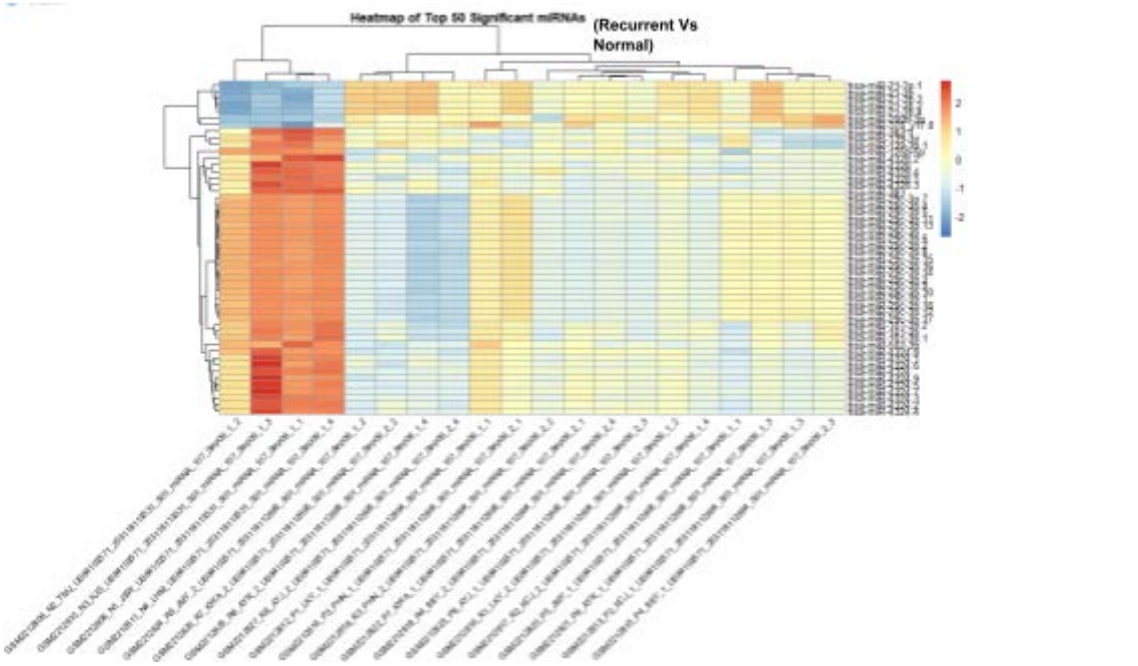

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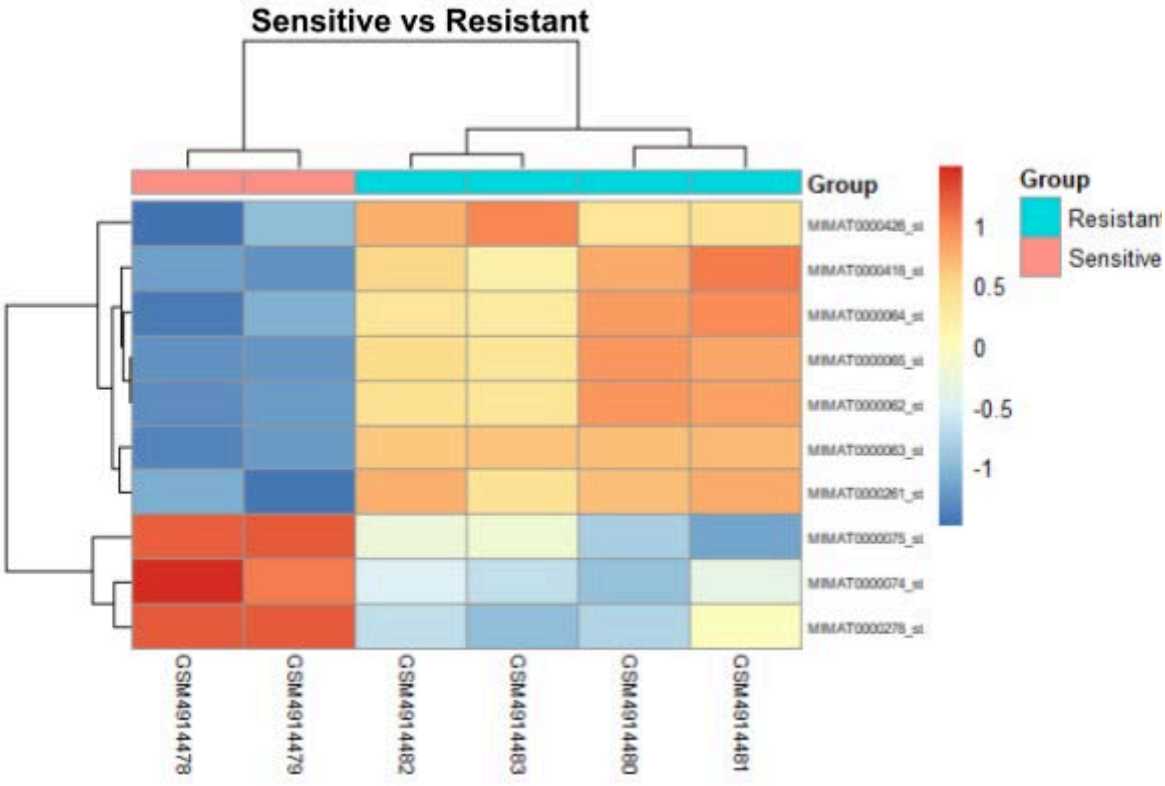

Figure 2. The heatmaps of DE miRNA expression patterns between **A)** Primary ovarian cancer and normal ovary, **B)** Recurrent ovarian cancer and normal ovary, **C)** Cisplatin sensitive and resistant miRNAs

To focus on the most significant attributes, top 5 upregulated and top 5 downregulated DEGs and DE miRNAs for each comparison based on their fold change strength score (Strength Score = |log2FCmiRNA| + |log2FCmRNA|) and statistical significance were selected (Tables 1).

These top candidates were further analyzed to explore their potential regulatory roles.

It was found that hsa-miR-125b-5p, hsa-miR-195-5p, hsa-miR-424-5p, and hsa-miR-125b-5p are significantly downregulated in primary ovarian cancer tissue samples; and they target E2F2, WWC1, SAMD10, CCNE1, HMGA1 genes; as a result these genes are significantly upregulated in malignant samples. Further, hsa-miR-200c-3p was found to be significantly upregulated in primary ovarian cancer tissues and targets multiple genes simultaneosly, that are upregulated in cancer tissues, these genes are BNC2, NDN, ALDH1A1, RUNX1T1, KANK2. Interestingly, similar patterns emerged in recurrent ovarian cancer tissues with hsa-miR-195-5p and hsa-miR-125b-5p being downregulated and targeting upregulated WWC1, CCNE1, HMGA1, CEP55, and E2F2 genes. The upregulated hsa-miR-200c-3p and hsa-miR-141-3p miRs consistently targets BNC2, NDN, ALDH1A1, RUNX1T1, and CXCL12; as a result they are downregulated in recurrent tumor samples. The results have also indicated that in drug resistant datasets, hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7c-5p, hsa-let-7d-5p, hsa-miR-183-5p, hsa-miR-23b-3p and hsa-miR-132-3p miRs are upregulated and are predicted to regulate the expression of LRIG3, TRIB2, DUSP1, LOX, LGR5, COL11A1; as a result these genes are downregulated in resistant samples potentially contributing to resistance mechanism. Downregulated miRs like hsa-miR-19b-3p, hsa-miR-20a-5p, and hsa-miR-221-3p were linked to upregulated targets including ABCA5 , PCDH7, PRR16, PDGFRA and DMRT3. (Tables 1).

Table 1

Differentially expressed miRNAs and their DEGs in ovarian cancer tissues and cell lines.

Primary ovarian cancer vs normal	miRNA	miRNA Expression in Resistance	Target Gene	Gene Expression in Resistance
	hsa-miR-125	Downregulated	E2F2	Upregulated

ovarian tissue	b-5p			
	hsa-miR-424-5p	Downregulated	WWC1	Upregulated
	hsa-miR-125b-5p	Downregulated	SAMD10	Upregulated
	hsa-miR-195-5p	Downregulated	CCNE1	Upregulated
	hsa-miR-424-5p	Downregulated	HMGA1	Upregulated
	hsa-miR-200c-3p	Upregulated	BNC2	Downregulated
	hsa-miR-200c-3p	Upregulated	NDN	Downregulated
	hsa-miR-200c-3p	Upregulated	ALDH1A1	Downregulated
	hsa-miR-200c-3p	Upregulated	RUNX1T1	Downregulated
	hsa-miR-200c-3p	Upregulated	KANK2	Downregulated
Recurrent ovarian cancer vs normal ovarian tissue	hsa-miR-195-5p	Downregulated	WWC1	Upregulated
	hsa-miR-195-5p	Downregulated	CCNE1	Upregulated
	hsa-miR-195-5p	Downregulated	HMGA1	Upregulated
	hsa-miR-195-5p	Downregulated	CEP55	Upregulated

	hsa-miR-125b-5p	Downregulated	E2F2	Upregulated
	hsa-miR-200c-3p	Upregulated	BNC2	Downregulated
	hsa-miR-200c-3p	Upregulated	NDN	Downregulated
	hsa-miR-200c-3p	Upregulated	ALDH1A1	Downregulated
	hsa-miR-200c-3p	Upregulated	RUNX1T1	Downregulated
	hsa-miR-141-3p	Upregulated	CXCL12	Downregulated
Drug sensitive vs drug resistant cell lines	hsa-let-7a-5p	Upregulated	LRIG3 TRIB2 DUSP1	Downregulated
	hsa-let-7b-5p	Upregulated		
	hsa-let-7c-5p	Upregulated		
	hsa-let-7d-5p	Upregulated		
	hsa-miR-183-5p	Upregulated	LOX	Downregulated
	hsa-miR-23b-3p	Upregulated	LGR5	Downregulated
	hsa-miR-132-3p	Upregulated	COL11A1	Downregulated
	hsa-miR-19b-3p	Downregulated	ABCA5	Upregulated
			PCDH7	Upregulated
	hsa-miR-20a-5p	Downregulated	PRR16	Upregulated
			PDGFRA	Upregulated

	miR-221-3p	Downregulated	DMRT3	Upregulated

3.2 Function and pathway enrichment analysis of DEGs

GO and KEGG enrichment analyses were conducted to interpret the biological functions and pathways associated with the significant DEGs so that molecular mechanisms underlying ovarian cancer progression and drug resistance are uncovered.

- **GO and KEGG analysis in primary ovarian cancer:** Results of GO analysis in primary ovarian cancer tissues exhibited that epithelial cell proliferation, muscle system process, Wnt signaling pathway, muscle tissue development, positive regulation of MAPK cascade, muscle cell differentiation 1, striated muscle tissue development, response to oxygen levels, respiratory tube development, lung development were the top 10 significant biological processes of the significant DEGs (Figure 3(A)). Further, KEGG pathway analysis highlighted focal adhesion, PI3K-Akt signaling pathway, AGE-RAGE signaling pathway in diabetic complications, MAPK signaling pathway 11, breast cancer, Wnt signaling pathway, gastric cancer, dilated cardiomyopathy, cytoskeleton in muscle cells 1, human papillomavirus infection as the top 10 enriched pathways (Figure 3 (C))
- **GO and KEGG analysis in recurrent ovarian cancer:** Similar patterns as primary ovarian cancer emerged while conducting GO and KEGG analysis in recurrent samples. Top 10 significant GO terms for BP additionally includes mesenchyme development, mesenchymal cell differentiation and respiratory system development lung development (Figure 3 (B)) and additional enriched KEGG pathways in recurrent samples are chemical carcinogenesis --receptor activation, prostate cancer, and melanoma (Figure 3 (D))

- **GO and KEGG analysis in cisplatin resistant ovarian cancer:** GO analysis of cisplatin resistant genes demonstrated significantly enriched BP terms including small GTPase-mediated signal transduction. Wnt signaling pathway, axonogenesis, regulation of neuron projection development, positive regulation of cell projection organization, regulation of small GTPase mediated signal transduction, response to transforming growth factor beta, regulation of synapse organization, regulation of synapse or activity, and cellular response to transforming growth factor beta stimulus (Figure 3 (E)). KEGG pathway analysis pinpointed axon guidance 1, mTOR signaling pathway 1, proteoglycans in cancer 1, FoxO signaling pathway, growth hormone synthesis, secretion and action, MAPK signaling pathway, polycomb repressive complex, prolactin signaling pathway, AGE-RAGE signaling pathway in diabetic complications, and EGFR tyrosine kinase inhibitor resistance as the top 10 enriched pathways ((Figure 3 (F)).

Figure 3

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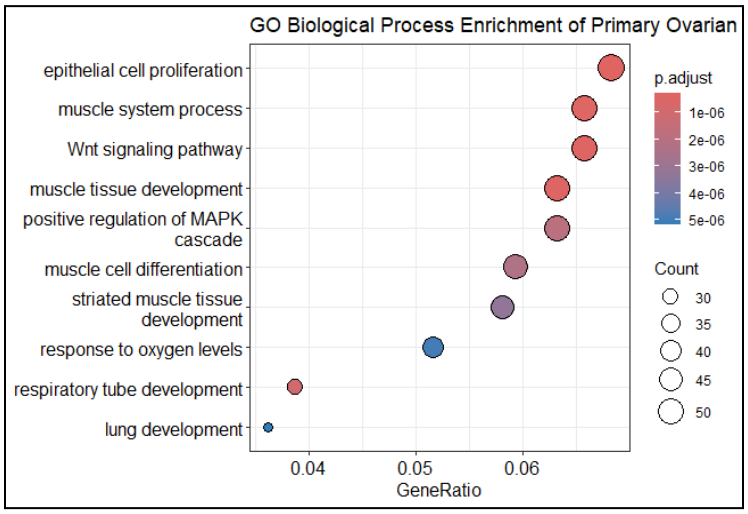

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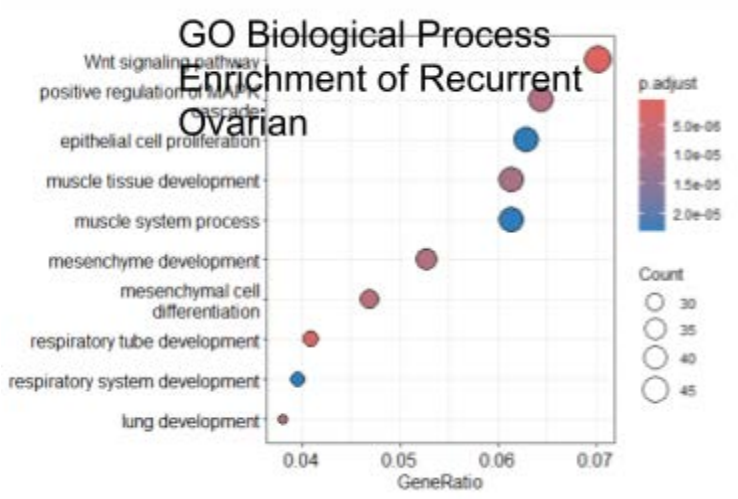

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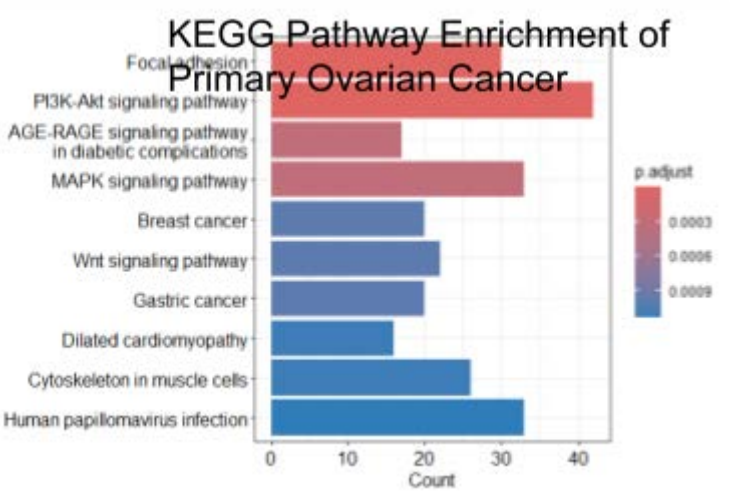

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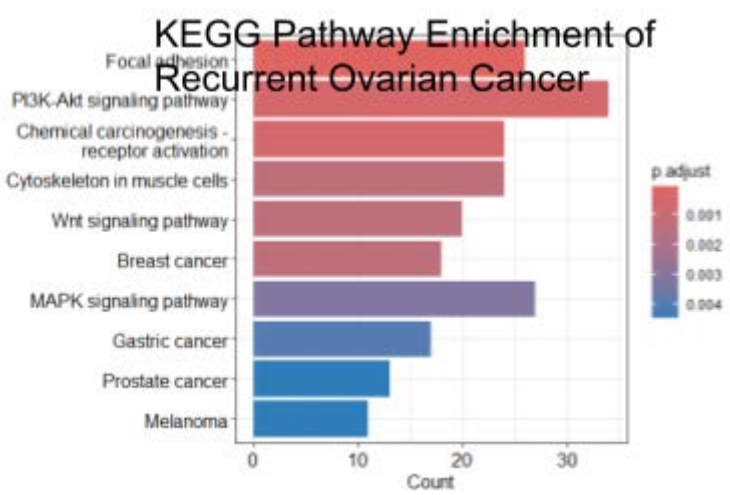

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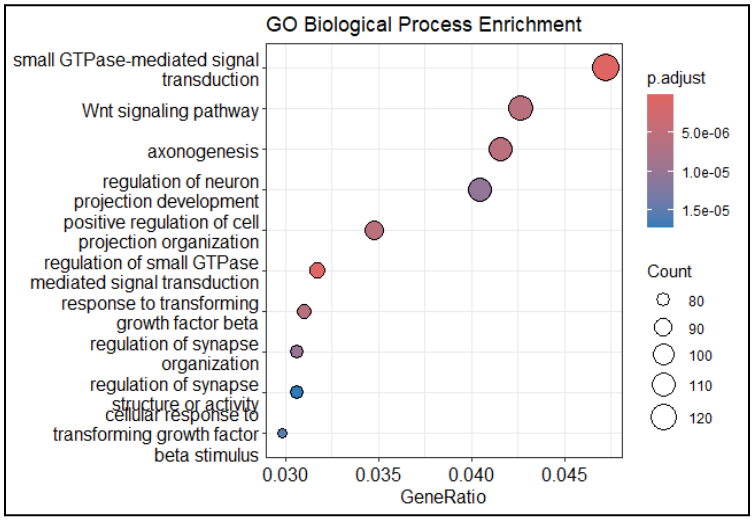

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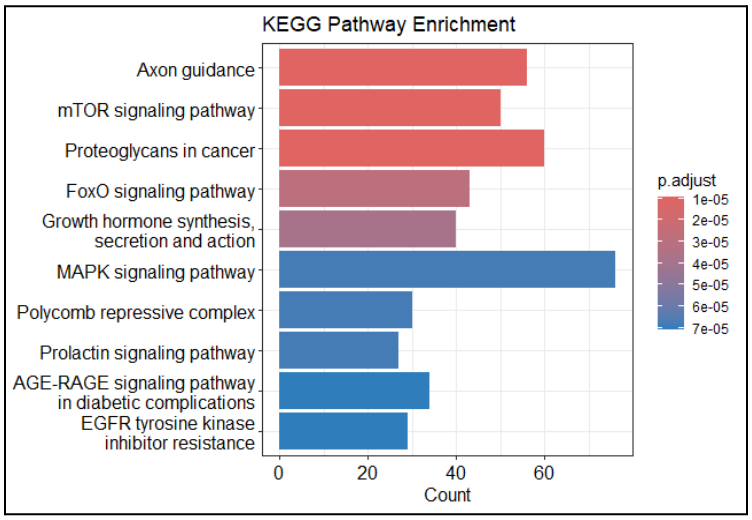

Figure 3. Bubble chart and bar chart of GO and KEGG enrichment. Horizontal axis: gene ratios or gene counts; vertical axis: enrichment results. The color of each point correlates to the value of p adjust, and the size of each point corresponds to the number of DGEs under GO and KEGG

entries. **A)** Enriched GO terms in primary ovarian cancer. **B)** Enriched GO terms of recurrent ovarian cancer. **C)** KEGG pathways in primary ovarian cancer. **D)** KEGG pathways in recurrent ovarian cancer. **E)** Enriched GO terms in cisplatin resistance. **F)** KEGG pathways in cisplatin resistance.

3.3 Survival analysis of top DEGs

Prognostic significance of 23 genes was validated with the Kaplan-Meier Plotter database for progression-free survival (PFS) in patients with ovarian cancer. Among them, 14 genes had significant associations with PFS ($p < 0.05$). Among them, increased expression of BNC2, CCNE1, CEP55, COL11A1, CXCL12, E2F2, KANK2, LOX, LRIG3, PCDH7, PDGFRA, RUNX1T1, and SAMD10 (HR > 1) is associated with poorer progression-free survival (PFS). In contrast, decreased expression of ABCA5 (HR = 0.83, < 1) is associated with poorer PFS (Table 2, Figure 4)

Figure 4

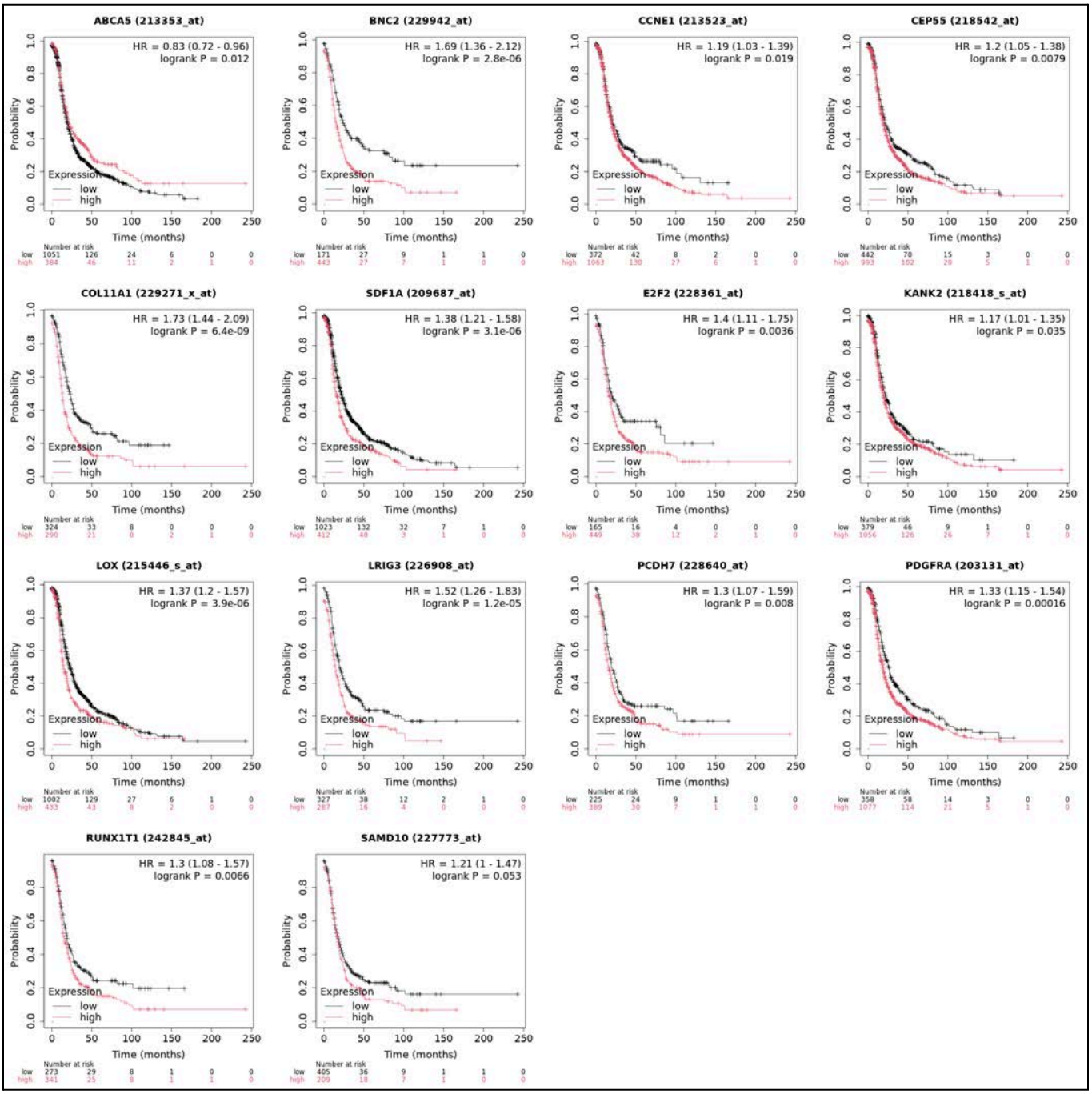

Figure 4. Kaplan-Meier survival curves for assessing progression free survival (PFS) of module genes in OC patients. Survival curves are based on the high and low expression of the regulated genes. Log-rank $P < 0.05$ was considered statistically significant.

Table 2

Prognostic Significance of Differentially Expressed Genes in Ovarian Cancer (PFS)

Gene	Expression in resistant sample	P-value	Hazard ratio (HR)	95% CI
ABCA5	Upregulated	0.012	0.83	0.72-0.96
ALDHA1A	Downregulated	0.29	0.93	0.8-1.07
BNC2	Downregulated	2.80E-06	1.69	1.36-2.12
CCNE1	Upregulated	0.019	1.19	1.03-1.39
CEP55	Upregulated	0.0079	1.2	1.05-1.38
COL11A1	Downregulated	6.40E-09	1.73	1.44-2.09
CXCL12	Downregulated	3.10E-06	1.38	1.21-1.58
DMRT3	Upregulated	0.095	1.22	0.97-1.53
DUSP1	Downregulated	0.086	1.12	0.98-1.28
E2F2	Upregulated	0.0036	1.4	1.11-1.75
HMGA1	Upregulated	0.25	0.93	0.82-1.05
KANK2	Downregulated	0.035	1.17	1.01-1.35
LGR5	Downregulated	0.092	0.88	0.77-1.02
LOX	Downregulated	3.90E-06	1.37	1.2-1.57
LRIG3	Downregulated	1.20E-05	1.52	1.26-1.83
NDN	Downregulated	0.12	1.11	0.97-1.26
PCDH7	Upregulated	0.008	1.3	1.07-1.59
PDGFRA	Upregulated	0.00016	1.33	1.15-1.54

PRR16	Upregulated	0.43	0.95	0.84-1.08
RUNX1T1	Downregulated	0.0066	1.3	1.08-1.57
SAMD10	Upregulated	0.053	1.21	1-1.47
TRIB2	Downregulated	0.12	1.13	0.97-1.31
WWC1	Upregulated	0.17	1.09	0.96-1.24

3.4 Differentially expressed viral miRNA

Two viral miRNA, ebv-miR-BART12 and hcmv-miR-UL70-3p were found among differentially expressed miR lists while analyzing primary ovarian cancer compared to normal controls. However, no human gene targets were detected for the viral miRs.

Table 3

Differentially expressed viral miRNAs

Viral miRNA	LogFC	Adjusted P-Value
ebv-miR-BART12	-1.54430668216875	0.0011944249169939
hcmv-miR-UL70-3p	-1.2454958541166	0.00153164465837346

Chapter 4

Discussion & Conclusion

There has been significant progress in comprehending ovarian cancer development, yet the complex interplay of microRNAs and their target genes in pathogenesis, prognosis, and drug resistance remains insufficiently explored, restricting a comprehensive understanding of the disease's heterogeneity. This in-silico study delved into a landscape of differentially expressed miRNA and their cognate regulated genes in ovarian cancer; and analyzed primary, recurrent, and cisplatin-resistant OC samples in human tissues and cell lines. The analysis identified key DE miRs, for instance- hsa-miR-200c-3p, hsa-miR-23b-3p, hsa-miR-141-3p, hsa-miR-195-5p, hsa-let-7a-5p and many more, including their targets such as CCNE1, HMGA1, BNC2, TRIB2 and LGR5. Additionally, two viral miRNAs, ebv-miR-BART12 and hcmv-miR-UL70-3p were found in the list of DE miRs. The following discusses the significance of these findings in OC pathogenesis, prognosis, and drug resistance.

Differential expression analysis across four microarray datasets indicated significant dysregulation in ovarian cancer molecular expressions and mechanisms. Fascinatingly, our data showed that miRNA and corresponding controlled genes show similar expression profiles across primary and recurrent ovarian cancer cells and the reasons behind this pattern strikes an interesting research aspect that is yet to elucidate (Saburi et al., 2022). Nevertheless, miiR-141 is upregulated in recurrent samples and regulates tumor progression through the Keap1/Nrf2 pathway by promoting M2 macrophage polarization and affects the tumor microenvironment via CXCL12 signaling (J. Zhao et al., 2023). Our analysis further revealed that miR-125b-5p targets

proliferation regulators like E2F2 impacting cell cycle control and DNA repair through interactions with key proteins such as Rb, p53, and BRCA1 (Gao et al., 2024). miR-195-5p targets CCNE1, CEP55, HMGA1, and WWC1, suggesting shared functions with miR-424-5p in cancer cell stemness, cell cycle progression and chromatin remodeling. It has been recorded by Liu et al. (2017) that miR-424-5p suppresses the E2F1-pRb pathway and arrests the cell cycle at the G1/G0 phase but the function gets impaired as this tumor suppressor is observed downregulated; although specific mechanism behind HMGA1 and WWC1 regulation in OC are less detailed. Moreover, CCNE1 with CDK2 controls the cell cycle's G1/S transition. When CCNE1 is overexpressed, it becomes an especially critical target because of its function as a G1/S transition regulator (Zheng et al., 2023). This study also showed that miR-200c-3p impacts OC progression by modulating targets such as BNC2, NDN, ALDH1A1, RUNX1T1, and KANK2. Several studies strengthen the findings correlating them in OC progression, for instance, BNC2 exhibits tumor-suppressive attributes but might correlate with poor prognosis in different cancers, reflecting its context-specific role in malignancy (Cesaratto et al., 2016). NDN is a tumor suppressor that induces apoptosis via Bcl-2 inhibition; being downregulated in OC enhances ovarian cancer cell survival and metastasis (Yang et al., 2015). Also, ALDH1A1 controls cell cycle progression, DNA repair and ABC transporter expression to induce stemness and platinum resistance in OC (Meng et al., 2014); through TGFβ/SMAD4 signaling, RUNX1T1 inhibits invasion and proliferation; its epigenetic silencing aids in the development of ovarian cancer (Hu et al., 2022). Lastly, the exact impact of KANK2 in ovarian cancer is less defined.

Additionally, drug resistance in ovarian cancer continues to be a significant obstacle to successful treatment, as our results show a variety of molecular processes including genes and

microRNAs involved in this phenomena. Our data recognised let-7a-5p, let-7b-5p, let-7c-5p, let-7d-5p, miR-183-5p, miR-23b-3p and miR-132-3p as oncomiRs. The let-7 family downregulates LRIG3, TRIB2, and DUSP1 expressions that contributes to drug resistance mechanisms, for instance, TRIB2 downregulation impede the focus from nucleotide excision repair (NER) to mismatch repair (MMR), allowing cells to dodge cisplatin-induced DNA damage and apoptosis (Kritsch et al., 2017). Another let-7 target, LRIG3, may increase MRP-1 and Bcl-2 levels in resistant cells, assisting drug efflux and better cell survival, thus contributing to resistance against drugs like etoposide or cisplatin. (Yang et al., 2016). Then, Kang and team (2016) speculated that DUSP1 works through PI3K/Akt signaling or reducing autophagy to promote resistance, though its contribution appears to be context-dependent as given in conflicting literature. Meanwhile, upregulated by hsa-miR-19b-3p in resistant cells, ABCA5 induces drug efflux, decreasing intracellular cisplatin levels, and possibly boosts epithelial-mesenchymal transition (EMT) through wnt signaling to further drug resistance (Wani et al., 2025); and PCDH7 might impede chemotherapy-induced apoptosis, again contributing to resistance, although its contribution in OC is less studied (H. Li et al., 2023). Tumor suppressor miR-20a-5p was discovered to regulate expressions of both PRR16 and PDGFRA, despite these two genes being candidates for further research in OC resistance. Finally, miR-221-3p regulated DMRT3 was associated with EGFR/ErbB2 signaling, a pathway responsible for apoptosis and hence driving resistance (L. Yang et al., 2020).

Moreover, pathway enrichment analyses utilizing Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) demonstrated a functional lens to interpret the biological relevance of the DEGs identified in primary, recurrent, and cisplatin-resistant ovarian cancer.

Enriched in both of the primary and recurrent OC, the top GO biological processes (BPs) revealed several hallmarks of OC pathogenesis and progression, for instance, enrichment of Wnt and MAPK signaling which promote cell and CSC proliferation, recurrence and resistance; suggesting DEGs such as CCNE1 or LGR5 may promote uncontrollable growth (W. Wang et al., 2025). Moreover, PI3K-Akt signaling promotes cell survival and is frequently activated in OC, potentially associated with DEG in this study like CEP55; often linked to chromosomal instability advocating for aggressive cancer cell populations (Carden et al., 2012). Other pathways included focal adhesion that regulates cell-matrix interactions, facilitating metastasis, several muscle-related terms that are intriguing and require further studying, alongside cancer specific pathways like breast and gastric cancer and human papillomavirus infection. However, unique pathways were observed in recurrent OC that do not overlap with primary OC pathways, which are mesenchyme development and mesenchymal cell differentiation. They are hallmarks of epithelial-mesenchymal transition (EMT), a process inducing metastasis and therapy resistance in recurrent OC (Wani et al., 2025).

In cisplatin resistant cancer cell lines, the most enriched GO processes and KEGG pathways include several interesting insights corroborating the DEGs possible contribution in resistance mechanisms. For example, small GTPases signal transduction regulates Ras initiated downstream cascades that activate both the MAPK and PI3K/AKT pathways as seen in LRIG3, DUSP1 and COLA11A mediated pathways, thereby facilitating increased anti-apoptotic proteins and promoting EMT, a phenotypic expression frequently linked to cisplatin-resistance (Meng et al., 2014). Mechanistically, hyperactivation of mTOR signaling in OC activates the PI3K/AKT/mTOR axis and increases the expression of proteins like CHK1, BRCA1, and

anti-apoptotic factors that combinedly enable tumor cells to repair platinum caused DNA damage and bypass apoptosis hence linking to cisplatin resistance (Deng et al., 2019). Interestingly, FoxO signaling acts as a double edged sword contributing in both cisplatin sensitivity and resistance. Only inactivation of this signal through Akt-mediated phosphorylation results in FoxO1 nuclear export, which has been linked with diminished apoptotic outcome when OC cells are exposed to cisplatin; enhancing resistance (Beretta et al., 2019). In ovarian cancer, both Wnt and MAPK pathways work as a network; the Wnt/β-catenin pathway induces chemoresistance by promoting EMT and oncogenic gene transcription and diminishing cisplatin-induced apoptosis (Kielbik et al., 2018). Also, Huang et al. (2016) revealed that MAPK/ERK pathway drives cell cycle progression, bypasses apoptosis, and sustains anti-apoptotic outcomes despite cisplatin-induced DNA damage; reinforcing a robust resistant phenotype.

Although axon guidance pathways like Netrin is involved in cell migration, insufficient evidence is out there to elucidate the role of such neural pathways in ovarian cancer chemoresistance. It might reflect pathway overlap rather than neural function. Similarly, muscle-related pathways are unexpected; both of the findings strike intriguing research avenues.

In order to explore the prognostic inference of the analyzed DEGs, Kaplan-Meier Plotter database was leveraged to generate KM plots for progression-free survival (PFS) in ovarian cancer patients. The plots revealed that 14 out of 23 DEGs were significantly linked to PFS, with 13 genes (BNC2, CCNE1, CEP55, COL11A1, CXCL12, E2F2, KANK2, LOX, LRIG3, PCDH7, PDGFRA, RUNX1T1, and SAMD10) associated with poorer consequence when overexpressed and one gene, ABCA5, showed worse PFS when underexpressed. The significance here is that they highlight distinct molecular roles to patient outcomes; offering further understanding of ovarian cancer development and tentative avenues for targeted therapies.

Our analyses revealed contradictory DEG and DE miR expression patterns, particularly between miRNAs such as let-7, miR-132, and miR-221 and distinct miRNA targets (e.g., LOX, LGR5, COL11A1, DUSP1, CXCL2, ALDH1A1) in ovarian cancer. They should not be dismissed just as anomalies or experimental blunder; rather, such inconsistencies most likely indicate fundamental biological complexities. The first and important assumption is that miRs are involved in a complex regulatory network suggesting that a single miRNA has the ability to target multiple mRNAs, with effects depending on cellular context (Gurtan & Sharp, 2013). In addition to that, contradictory findings could be an outcome of epigenetic changes, for example, DNA methylation and histone modifications (Ivan et al., 2012). This is particularly important for change of expression like LOX or LGR5, where the impact of the tumor microenvironment on gene expression might disguise miRNA-mediated repression directly. Additionally, Müller and his team (2018) revealed that RNA-binding proteins (RBPs) can regulate miRNA-mRNA network by binding to sites within the 3′ untranslated regions hence protecting the genes from degradation and altering miRNA efficacy. For example, RBPs like IGF2BP1 and HuR; especially, modification of miR-200c by HuR can cause paradoxical gene upregulation, as seen in our results, likely clarifying contradictory expression patterns for miRs like let-7, miR-132, and miR-221 (Prislei et al., 2013). Moreover, cellular heterogeneity in OC and dynamic changes during pathogenesis and treatment can obscure miRNA-mRNA connections in bulk tissue analyses (X. Meng et al., 2015). Another critical aspect to the paradoxical expression pattern is that cancer cells adaptively interchange between glycolysis and oxidative phosphorylation for energy conservation and as a treatment stress response; this phenomenon is known as reprogramming of cellular energy metabolism aka “smart metabolism” (X. Chen et al., 2020; S. Li et al., 2018). This could potentially explain that let-7, miR-132, and miR-221 may be

engaging in a sophisticated regulatory rebalancing in chemoresistant ovarian cancer while triggering unexpected expression patterns. Lastly, taking account of technical variability and analytical contrast like distinct platforms, sensitivity of detection process, and computational pipelines used in the DEG analysis could be an important source for explaining such paradoxical expression patterns (Kasavi, 2022; Teng et al., 2016).

An interesting finding in OC tissues was the significant differential expression of two viral miRNAs, ebv-miR-BART12 and hcmv-miR-UL70-3p. Although no human gene targets were detected, their presence strikes an intriguing potential role in OC pathogenesis. There are records of Epstein-Barr virus (EBV)-encoded miRNAs in the induction of viral latency and regulating host immune responses in malignancies like nasopharyngeal carcinoma (Ko, 2015). In the same way, human cytomegalovirus (HCMV) miRNAs, such as hcmv-miR-UL70-3p, was recorded to contribute in GBM invasiveness and cell migration suggesting influence in oncogenesis (Fernández-Moreno et al., 2021). Presence of these viral miRNAs in OC could pave way for a novel research direction, warranting future investigation to detect their targets and functional roles.

In conclusion, this research portrays a complex DE miR and DEG landscape in ovarian cancer, suggesting key regulatory networks inducing pathogenesis, prognosis, and drug resistance. The findings of contradictory molecular expression patterns and viral miRNAs highlight ovarian cancer's molecular diversity and potentially unfold novel research avenues. These findings

would facilitate the way to sophisticated personalized therapeutic strategies to improve ovarian cancer patient outcomes, particularly in high-burden regions like Bangladesh.

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