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An Exploratory Bioinformatics Investigation of microRNA Signatures in Cisplatin-Resistant Gastric Cancer Cell Lines

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Abstract

Background & Objective: Gastric cancer (GC) remains one of the most prevalent and lethal malignancies worldwide, with cisplatin serving as a cornerstone in its chemotherapeutic regimen. However, the frequent and often rapid development of cisplatin resistance significantly compromises therapeutic efficacy. *MicroRNAs (miRNAs)* are key post-transcriptional regulators that modulate critical cellular mechanisms underlying chemoresistance, including apoptosis, DNA repair, drug efflux, and proteostasis. This study aimed to identify candidate miRNAs and molecular pathways associated with cisplatin resistance through an integrated bioinformatics approach.

Materials & Methods: *miRNA expression profiles* from the GEO dataset *GSE86195*, comprising cisplatin-sensitive and cisplatin-resistant GC cell lines, were analyzed. Differential expression analysis was conducted using *limma*, followed by functional enrichment analysis of validated miRNA targets via *clusterProfiler*. Family-level aggregation, *Weighted Gene Co-expression Network Analysis (WGCNA)*, and *Random Forest* feature ranking were subsequently applied to identify potential hub and predictive miRNAs.

Results: Although no miRNAs survived false discovery rate (FDR) correction, an exploratory nominal p-value threshold of < 0.05 revealed 957 candidate differentially expressed miRNAs (538 upregulated and 416 downregulated). Enrichment analysis indicated the involvement of pathways related to nucleocytoplasmic transport, RNA splicing, ubiquitin-mediated proteolysis, and platinum drug resistance. A coordinated dysregulation of the *miR-346*, *miR-421*, and *miR-139-5p* families was identified. *Machine learning* further highlighted *hsa-let-7e* and *hsa-miR-20a-star* as top-ranked predictive candidates, although both WGCNA and Random Forest findings should be interpreted cautiously due to the limited sample size ($n = 4$).

Conclusion: This exploratory bioinformatics analysis identifies candidate miRNAs and signaling pathways that may underlie cisplatin resistance in *GC*. The findings should be considered hypothesis-generating and warrant validation in larger cohorts (e.g., *TCGA*) as well as experimental confirmation through functional assays prior to clinical translation.

Keywords: Gastric Cancer, Cisplatin Resistance, microRNA, Bioinformatics, Biomarkers

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Introduction

Gastric cancer (GC) remains a significant global health challenge, with approximately 1.1 million new cases and 770,000 deaths reported worldwide in 2020 (1). Despite gradual declines in incidence in some high-income countries, the overall burden is projected to increase, reaching 1.8 million new cases and 1.3 million deaths by 2040 (2). Eastern Asia, including China, Japan, and the Republic of Korea, continues to exhibit

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the highest incidence and mortality rates (3). The etiology of GC is multifactorial, encompassing genetic predispositions, chronic infection with *Helicobacter pylori*, and environmental or lifestyle factors such as smoking, high salt intake, and obesity (4, 5). Notably, over 15 million individuals born between 2008 and 2017 are projected to develop GC during their lifetimes, with the majority of risk attributable to *H. pylori* infection (6).

For patients with advanced GC, chemotherapy constitutes the primary systemic treatment. Cisplatin (DDP) has long been a central component of first-line regimens. However, the frequent emergence of cisplatin resistance substantially undermines therapeutic efficacy and contributes to poor survival outcomes (7). Resistance arises through multiple mechanisms, including enhanced DNA damage repair, suppression of apoptosis, increased drug efflux, activation of autophagy, and epithelial–mesenchymal transition (EMT) (7, 8). Therefore, elucidating the molecular basis of cisplatin resistance is critical for identifying predictive biomarkers and developing strategies to overcome therapeutic failure.

MicroRNAs (miRNAs), small noncoding RNAs of approximately 22 nucleotides, have emerged as pivotal regulators of cisplatin resistance in GC. Functioning as oncogenes or tumor suppressors, miRNAs exert post-transcriptional control over gene expression by binding to complementary sequences in target mRNAs. Their dysregulation affects numerous processes linked to chemoresistance, including apoptosis, DNA repair, drug efflux, and cell cycle regulation (8, 9). For instance, *miR-25* promotes cisplatin resistance by suppressing *FOXO3a*, a critical regulator of apoptosis and the cell cycle (10), whereas *miR-129* enhances sensitivity by targeting P-glycoprotein (11).

Beyond these examples, experimental studies have demonstrated that several miRNAs directly modulate cisplatin response in GC. A well-established case is *miR-21*, which is consistently upregulated in resistant GC cells. Overexpression of *miR-21* attenuates apoptosis following cisplatin exposure, whereas its inhibition restores drug sensitivity. Mechanistically, *miR-21* targets *PTEN*, leading to activation of the PI3K/Akt survival pathway (12). Another critical regulator is *miR-223*, which is overexpressed in resistant GC cells and promotes resistance by targeting *FBXW7*, a tumor suppressor that governs cell cycle progression. Knockdown of *miR-223* enhances cisplatin sensitivity and induces apoptosis, highlighting its functional significance (13). Conversely, *miR-143* functions as a tumor suppressor in this context. Its expression is diminished in resistant GC cells, and restoring *miR-143* expression re-sensitizes cells to cisplatin. Direct targeting of *IGF1R* and *BCL2* links *miR-143* to both survival and apoptotic pathways, underscoring its role as a mediator of chemosensitivity (14). Collectively, these studies demonstrate that miRNAs not only modulate cisplatin response but also serve as potential biomarkers and therapeutic targets in GC.

Recent advances in transcriptomics and computational biology now enable systematic profiling of miRNAs and their regulatory networks in resistant versus sensitive GC cells. Integrative approaches combining differential expression analysis, pathway enrichment, and network modeling provide deeper insights into convergent resistance mechanisms. Nevertheless, the translational potential of these findings depends on careful interpretation, given the limited sample sizes and frequent lack of experimental validation in publicly available datasets (15, 16). In light of these challenges, the present study utilizes a transcriptomic dataset comprising cisplatin-sensitive and -resistant GC cell lines to identify candidate miRNAs and associated pathways. By integrating expression profiles with functional enrichment, co-expression analysis, and machine learning, this work aims to highlight hypothesis-

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generating miRNA signatures that may guide future biomarker validation and therapeutic development.

Materials & Methods

Data Acquisition and Overview

MicroRNA (miRNA) expression data were obtained from the Gene Expression Omnibus (GEO) under accession number GSE86195 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE86195).

This dataset profiles two GC cell lines with acquired cisplatin resistance (SGC-7901/DDP and BGC-823/DDP) along with their parental, cisplatin-sensitive counterparts (SGC-7901 and BGC-823). Expression measurements were generated using the Affymetrix Multispecies miRNA-2 Array (GPL14613). Importantly, this dataset includes only four samples without biological replicates. While this limits statistical power and precludes definitive conclusions, it provides a valuable exploratory resource for generating hypotheses regarding miRNA dysregulation in cisplatin resistance. All analyses should therefore be interpreted as exploratory and hypothesis-generating rather than conclusive.

Data Preprocessing and Normalization

Raw microarray text files were downloaded and processed in R (version 4.4.2) (Table 1). Data import was performed using *read.delim*, and probes lacking expression values were excluded (17). To stabilize variance across probes, expression intensities were log2-transformed. Probes with average expression values greater than 4 across the four samples were retained to ensure inclusion of reliably detected miRNAs. Quality control was conducted using principal component analysis (PCA) and correlation heatmaps, confirming consistency and reproducibility of expression profiles across samples.

Experimental Grouping and Design Matrix

Samples were stratified into two groups: cisplatin-sensitive (Control: SGC-7901, BGC-823) and cisplatin-resistant (DDP: SGC-7901/DDP, BGC-823/DDP). Group assignments were encoded as factors within a design matrix constructed for downstream modeling. Given the absence of replicates, all comparisons represent pooled assessments between resistant and sensitive cell lines.

Exploratory Quality Control

To evaluate global expression patterns, PCA was performed using the prcomp function (17, 18). PCA plots were generated with ggplot2 and annotated using ggrepel to enhance clarity. This approach facilitated visualization of variance between resistant and parental groups. Additionally, Pearson correlation heatmaps were produced using the pheatmap package, confirming high within-group similarity and clear separation between resistant and sensitive lines. These steps provided confidence that, despite the limited sample size, broad expression differences could be reliably detected.

Differential Expression Analysis

Differential expression was assessed using

Table 1. Key R packages utilized in the analysis pipeline, their purposes, versions, and references.

Package	Version	Purpose in the Study	Reference
tidyverse	2.0.0	Data manipulation, transformation, and visualization	(18)
ggplot2	3.5.2	Visualization of PCA, volcano, and bar plots	(19)
pheatmap	1.0.13	Generation of correlation and expression heatmaps	(20)
limma	3.62.2	Differential expression analysis using linear modeling	(21)
multiMiR	1.28.0	Retrieval of validated miRNA–mRNA target interactions	(22)
clusterProfiler	4.14.6	Gene Ontology and KEGG pathway enrichment analysis	(23)
WGCNA	1.73	Weighted gene co-expression network analysis	(24)
caret	7.0-1	Machine learning modeling and variable importance estimation	(25)

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the limma package, which fits linear models to expression data. Contrasts were defined as DDP versus Control, and empirical Bayes moderation was applied to shrink standard errors. Given the small sample size and absence of replicates, no miRNAs survived false discovery rate (FDR) correction. Therefore, a nominal cutoff of $p < 0.05$ and absolute log2 fold change > 1 was applied to identify candidate differentially expressed miRNAs (DEMs). This unadjusted threshold was chosen to maximize hypothesis generation. Importantly, all findings are presented as exploratory rather than statistically definitive. Results were visualized using volcano plots to highlight up- and downregulated miRNAs, along with heatmaps of the top 20 DEMs to illustrate expression clustering between resistant and sensitive groups.

Target Prediction and Functional Enrichment

Validated mRNA targets of the top 200 DEMs were retrieved using the multiMiR package, which integrates multiple databases of experimentally confirmed interactions. Only human miRNAs (prefix "hsa-") were retained, and duplicates or non-targeting entries were filtered out to refine the candidate target list. Functional annotation was performed using clusterProfiler. Gene Ontology (GO) enrichment focused on Biological Process terms, whereas Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment highlighted cancer-related and drug resistance pathways. Adjusted p-values ≤ 0.05 were considered significant. Visualization was conducted using dot plots to represent enrichment significance and gene ratios.

miRNA Family-Level Analysis

To assess coordinated regulation, DEMs were mapped to families using TargetScanHuman 8.0 (26). Family-level summaries were calculated, including mean log2 fold change and the number of members per family. Enriched families were visualized using bar plots, emphasizing potential cooperative effects of miRNA clusters in drug resistance.

Machine Learning for Feature Selection

In addition to Random Forest classification, co-expression analysis was initially performed using Weighted Gene Co-Expression Network Analysis (WGCNA) to detect co-expressed miRNA modules based on scale-free topology (soft threshold $\beta = 8$). The modules identified by WGCNA provided a biologically informed background for subsequent machine learning. The Random Forest classifier was trained using the caret package with default parameters. Given the very small sample size ($n = 4$), these results should be interpreted as exploratory and hypothesis-generating rather than confirmatory. Variable importance was extracted, and the 15 most significant miRNAs contributing to classification were depicted in horizontal bar plots. These miRNAs, highlighted both by co-expression patterns and machine learning ranking, represent candidate predictive biomarkers of cisplatin resistance.

Results

Global Expression Profiles and Quality Assessment

Microarray profiling of four GC cell lines—two cisplatin-sensitive parental lines (*SGC-7901* and *BGC-823*) and their resistant derivatives (*SGC-7901/DDP* and *BGC-823/DDP*)—yielded expression values for 16,176 miRNAs following log2 transformation and filtering (mean expression > 4). Mean expression values ranged narrowly (4.951–4.961), with stable medians (4.26) across all cell lines. Resistant samples exhibited slightly higher minimum expression values (3.25–3.26) than parental lines (2.94–3.20), whereas maximum values were comparable (15.13–15.20). These findings suggest that cisplatin resistance is not accompanied by global transcriptomic reprogramming but instead involves selective deregulation of specific miRNAs (Table 2).

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Table 2. Summary statistics of log2-transformed miRNA expression values across four gastric cancer cell lines.

Cell Line	Mean Expression	Median Expression	Min Expression	Max Expression
SGC-7901	4.961	4.26	2.94	15.18
BGC-823	4.957	4.26	3.20	15.13
SGC-7901/DDP	4.960	4.25	3.25	15.20
BGC-823/DDP	4.951	4.26	3.26	15.15

Table 3. Representative top upregulated and downregulated miRNAs in cisplatin-resistant gastric cancer cell lines.

miRNA	logFC	p-value	Regulation
ppy-miR-1301	2.40	4.6×10^{-5}	Upregulated
miR-99a orthologs	1.88–2.13	<0.001	Upregulated
miR-125b-star	1.8	7.1×10^{-4}	Upregulated
miR-505/miR-505-star	2.0	<0.001	Upregulated
miR-424-star	–2.49 to –2.39	<0.001	Downregulated
miR-503	–2.24 to –2.06	<0.005	Downregulated
miR-181 family	–1.8 to –1.7	<0.02	Downregulated

PCA revealed that PC1 and PC2 accounted for 35.7% and 32.8% of the total variance, respectively, and fully separated resistant from sensitive groups. Hierarchical clustering initially segregated samples by treatment status (resistant versus parental) and subsequently by cell line origin. Pearson correlation coefficients ranged from 0.9727 to 0.9792, confirming high internal reproducibility. While these multivariate patterns support the existence of resistance-associated signatures, the absence of biological replicates (n = 1 per condition) and the small sample size limit robustness and necessitate cautious interpretation.

Differential Expression Analysis

Differential expression testing using *limma* identified no miRNAs meeting the false discovery rate (FDR) threshold of 0.05. Consequently, an exploratory nominal cutoff ($p < 0.05$) was applied, yielding 957 candidate differentially expressed miRNAs (DEMs): 538 upregulated and 416 downregulated in resistant cells. A volcano plot illustrated these candidates, while a heatmap of the top 20 DEMs demonstrated consistent clustering of resistant versus sensitive lines (Table 3, Figure 1).

Functional Annotation and Enrichment Analyses

Gene Ontology (GO) enrichment of validated targets of the top 200 DEMs revealed significant overrepresentation of nucleocytoplasmic transport (GO:0006913, fold enrichment ≈ 1.99, adjusted $p = 4.69 \times 10^{-22}$) and nuclear transport (GO:0051169), both critical for the regulated transport of transcription factors and DNA repair proteins. Additional enriched processes included RNA splicing (GO:0008380), proteasome-mediated ubiquitin-dependent protein catabolic process (GO:0043161), and small GTPase-mediated signal transduction (GO:0007264).

KEGG pathway analysis highlighted several major enriched pathways: nucleocytoplasmic transport (hsa03013, fold enrichment = 2.22, adj. $p = 3.69 \times 10^{-11}$), endocytosis (hsa04144), and proteoglycans in cancer (hsa05205). Resistance-associated networks, including EGFR tyrosine kinase inhibitor resistance (hsa01521, fold enrichment = 2.11, adj. $p = 8.19 \times 10^{-8}$), endocrine resistance (hsa01522, fold enrichment = 1.99, adj. $p = 7.61 \times 10^{-8}$), insulin resistance (hsa04931, adj. $p = 2.84 \times 10^{-6}$), and platinum drug resistance (hsa01524, adj. p = 0.020), were among the most significantly enriched (Table 4, Figure 2).

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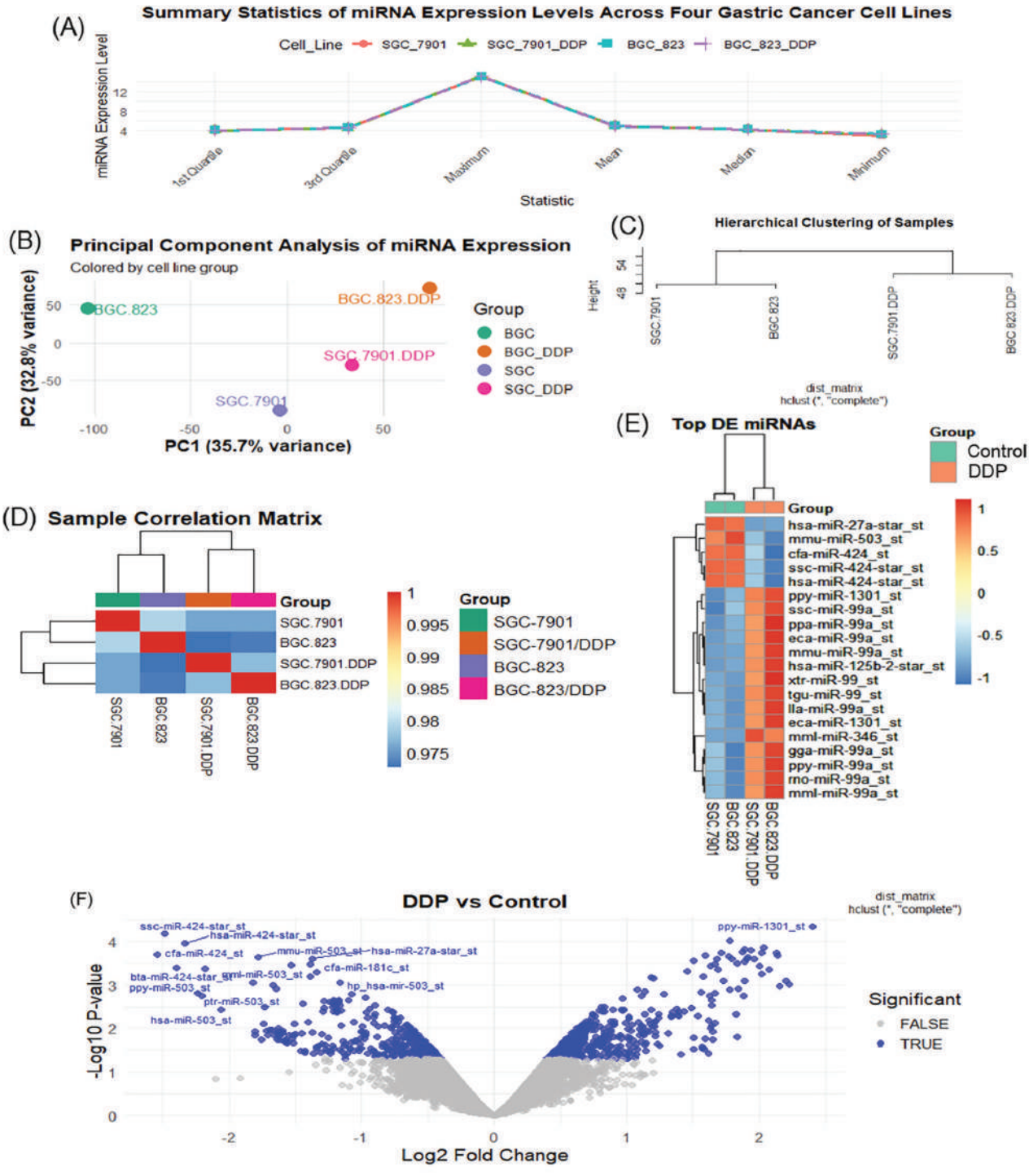

Figure 1. Global miRNA expression patterns and differential analysis in cisplatin-sensitive and cisplatin-resistant gastric cancer cell lines. (A) Summary statistics of log2-transformed miRNA expression with uniform distributions in all four cell lines. (B) PCA shows clear separation of resistant (DDP) and sensitive (Control) groups along PC1 (35.7%) and PC2 (32.8%). (C) Hierarchical clustering separates samples by treatment status first, then cell line origin. (D) Pearson correlation heatmap confirms high within-group reproducibility ($r > 0.975$). (E) Heatmap of top 20 DEMs (unadjusted $p < 0.05$) shows distinct resistant–sensitive clustering. (F) Volcano plot shows up- and downregulated candidate miRNAs for DDP vs. Control comparison.

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Table 4. Summary of significant enriched Gene Ontology (GO) biological processes and KEGG pathways from DEM target analysis.

Category	Term	Fold Enrichment	Adjusted p-value	Representative Genes
GO-BP	Nucleocytoplasmic transport	1.99	4.69×10^{-22}	XPO1, IPO7, RANBP2
GO-BP	Nuclear transport	1.99	4.69×10^{-22}	KPNB1, TNPO1, NUP98
GO-BP	RNA splicing	1.80	3.92×10^{-21}	SRSF1, SF3B1, HNRNPA1
KEGG	Platinum drug resistance	1.48	0.020	BCL2L1, TP53, ATP7A
KEGG	EGFR-TKI resistance	2.11	8.19×10^{-8}	EGFR، MTOR، MAP2K1
KEGG	Endocrine resistance	1.99	7.54×10^{-8}	PIK3R3, MAPK8, IGF1

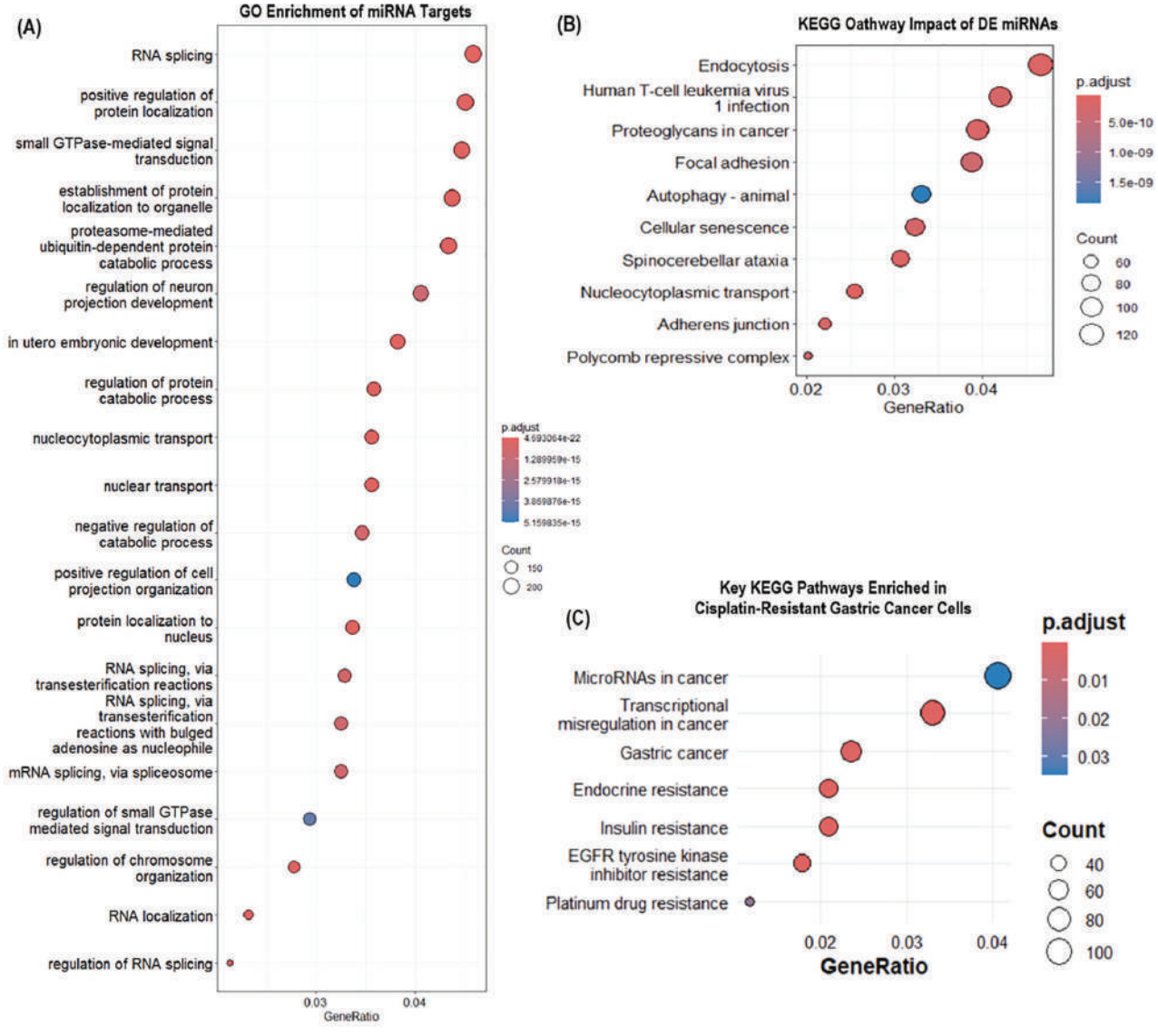

Figure 2. Functional enrichment of targets for differentially expressed miRNAs in cisplatin-resistant gastric cancer cell lines. (A) GO Biological Process enrichment shows overrepresentation of nucleocytoplasmic transport, nuclear transport, RNA splicing, and ubiquitin-mediated protein catabolism. (B) KEGG pathway analysis also shows significant enrichment in cancer-related and resistance-associated pathways, e.g., EGFR-TKI resistance, endocrine resistance, and insulin resistance. (C) Focused KEGG plot shows "Platinum drug resistance," "MicroRNAs in cancer," and "Gastric cancer" pathways as the primary mechanisms of cisplatin resistance.

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Family-Level miRNA Dysregulation

Mapping DEMs to miRNA families using *TargetScanHuman* identified 57 deregulated families. The most frequently represented were *miR-346* and *miR-421* (each n = 7 members, mean logFC = +1.68 and +1.19, respectively), followed by *miR-139-5p*, *miR-342-3p*, and *miR-324-3p/1913* (each n = 6). While *miR-346* and *miR-342-3p* were consistently upregulated, *miR-139-5p* exhibited coordinated downregulation (mean logFC = –1.11). These coordinated patterns suggest that family-level regulation may contribute to resistance phenotypes, although confirmatory studies are warranted (Figure 3).

Network and Machine Learning Analyses

Weighted Gene Co-Expression Network Analysis (WGCNA) did not achieve a reliable scale-free topology (β = 8), limiting confidence in the inferred modules. Nonetheless, exploratory signals indicated putative hub miRNAs, including mmu-miR-22-star_st, mml-miR-519b_st, and xtr-miR-199a-star_st. These should be interpreted as preliminary associations rather than validated hubs.

Random Forest analysis successfully distinguished resistant from sensitive samples; however, this analysis was based on only four samples without replicates, rendering the results highly susceptible to overfitting and statistically unreliable. The top-ranked features included dwi-miR-5_st (importance score = 100), mmu-miR-183_st, hsa-miR-4258_st, hp_hsa-mir-101.1_x_st (score = 87.5), cfa-miR-302a_st, zma-miR164h_st (score = 75), hsa-let-7e_st, and hsa-miR-20a-star_st (score = 50). These candidates should be considered preliminary, hypothesis-generating signals that require validation in independent datasets and functional experimental models (Figure 4).

Summary of Key Exploratory Findings

Collectively, the results suggest that cisplatin resistance in GC cells is shaped by selective miRNA deregulation rather than global transcriptomic alterations. Exploratory enrichment analyses highlight nucleocytoplasmic transport, RNA splicing, and ubiquitin-mediated proteolysis as processes of interest. Candidate miRNAs—including *miR-346*, *miR-421*, *miR-139-5p*, *let-7e*, and *miR-20a-star*—emerge as potential contributors. However, due to the limited sample size, absence of biological replicates, and failure of FDR correction, these findings should be interpreted solely as hypothesis-generating.

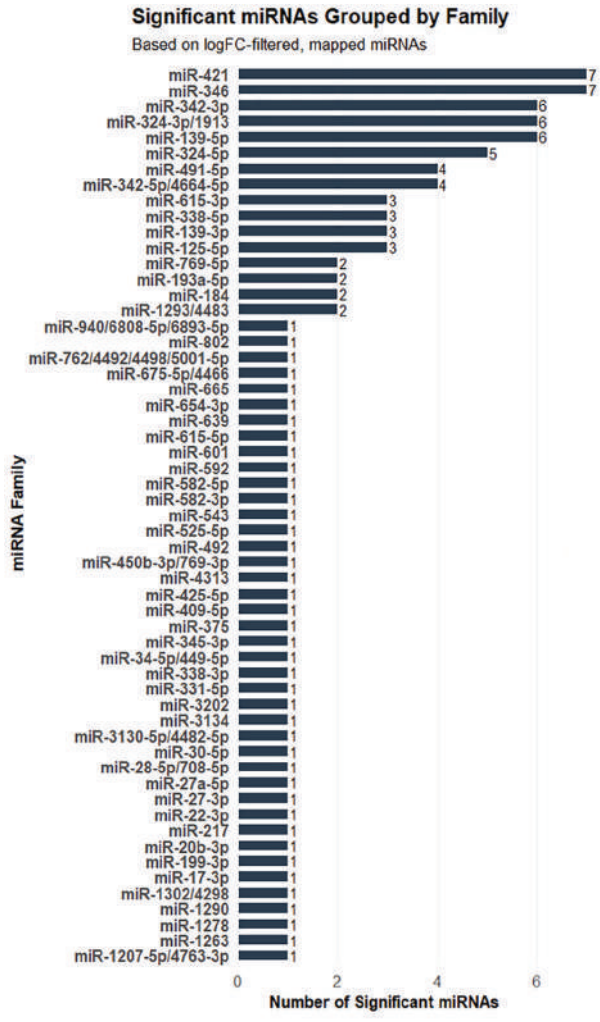

Figure 3. Family-level dysregulation and network features of miRNAs in cisplatin-resistant gastric cancer cell lines. Differentially expressed miRNAs were grouped by TargetScanHuman families, revealing coordinated up- or downregulation within significant families such as miR-346 and miR-421.

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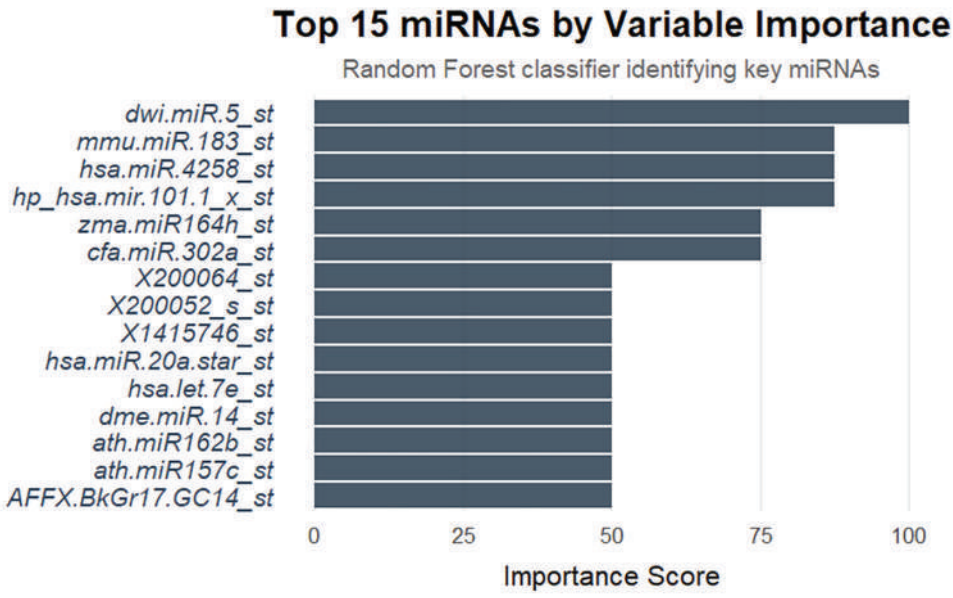

Figure 4. Machine learning-based identification of cisplatin resistance-predictive miRNAs in gastric cancer. Top 15 ranked miRNAs by variable importance from a Random Forest classifier distinguishing resistant and sensitive cell lines. Human-specific miRNAs (e.g., *hsa-let-7e_st*, *hsa-miR-20a-star_st*) are some of the top-ranked features, along with cross-species orthologs, suggesting putative biomarkers for therapeutic response prediction.

Discussion

Cisplatin remains the backbone of chemotherapy for advanced GC, but its clinical utility is often undermined by the frequent development of resistance (7, 27). Resistance arises through multifaceted mechanisms, including impaired apoptosis, dysregulated DNA repair, enhanced efflux transport, and altered proteostasis. *MicroRNAs (miRNAs)* act as critical post-transcriptional regulators of these processes, and their deregulation has increasingly been implicated in chemoresistance (7, 8). In this study, we integrated transcriptomic data, functional enrichment, network modeling, and exploratory machine learning to delineate miRNA-mediated mechanisms underlying cisplatin resistance in GC cell lines.

Our analyses revealed that resistance was characterized not by global shifts in miRNA expression but by the selective deregulation of specific candidates. Principal component analysis and hierarchical clustering clearly separated resistant from sensitive lines, supporting the presence of distinct resistance-associated signatures. Although no miRNAs met statistical significance after false discovery rate (FDR) correction, nominal p-values identified 957 differentially expressed miRNAs (DEMs). These exploratory candidates included the *miR-181* family, *miR-99a* orthologs, *miR-346*, and *miR-421*, many of which have previously been reported to regulate proliferation, migration, or drug response in GC and other tumor types (28–34). While the lack of FDR significance precludes definitive conclusions, the recurrence of these miRNAs across independent studies underscores their potential biological relevance.

Comparison with earlier studies reveals both concordances and divergences. Zhou et al. (2018) (35) described the *miR-497/MTHFD2* axis in cisplatin resistance; although this axis was not identified in our study, both analyses emphasize miRNA-mediated regulation of drug response. Ge et al. (2016) (36) reported *miR-421* as a key driver of resistance, consistent with our detection of its dysregulation. In contrast, findings on the *hsa_circ_0006427/miR-346/VGLL4* pathway in lung cancer (37) involve the same miRNA

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but differ in cancer type and functional context. Broader reviews (38) have shown that miRNAs regulate resistance via PI3K/AKT and Wnt/β-catenin signaling, which aligns with the enriched pathways observed in our results. Other studies (39) highlighted the tumor microenvironment as a major layer of miRNA regulation, whereas our investigation focused on cell lines. Additionally, circulating miRNA panels (40) have been proposed as diagnostic tools, supporting our suggestion that miRNAs may serve as candidate biomarkers. Overall, while several studies corroborate our findings, discrepancies across biological models underscore the complexity of miRNA-mediated cisplatin resistance.

Target enrichment analysis implicated nucleocytoplasmic transport, RNA splicing, and ubiquitin-mediated proteolysis, processes consistently associated with chemoresistance. Disrupted miRNA nuclear transport may impair regulatory feedback loops (41–44), aberrant splicing facilitates apoptosis evasion (45), and altered ubiquitination modulates the degradation of oncogenic or tumor-suppressive proteins (46–48). Notably, platinum drug resistance and EGFR-TKI resistance emerged as enriched pathways, indicating overlap with clinically observed resistance phenotypes (7, 27, 49).

At the family level, coordinated upregulation of *miR-346* and *miR-421* and downregulation of *miR-139-5p* suggest potential cooperative regulation. These observations align with reports that *miR-346* promotes tumor growth (33) and *miR-421* enhances cell proliferation (34), while *miR-139-5p* functions as a tumor suppressor in GC (50). Such convergence highlights the value of family-level analyses compared with individual miRNA evaluations.

Machine learning and network analyses provided additional, though preliminary, insights. *Random Forest* highlighted *hsa-let-7e* and *hsa-miR-20a-star* among the top-ranked features distinguishing resistant from sensitive cells, suggesting their potential as candidate biomarkers. However, these results must be interpreted with extreme caution, given the very small sample size (n = 4). Similarly, *Weighted Gene Co-expression Network Analysis (WGCNA)* did not achieve scale-free topology, limiting confidence in hub assignments. Accordingly, both approaches should be regarded as hypothesis-generating tools rather than confirmatory evidence.

Overall, the integrative framework applied here situates candidate miRNAs within functional networks underpinning cisplatin resistance. While exploratory, these findings provide a rationale for further validation and experimental interrogation of nuclear transport, ubiquitin–proteasome signaling, and selected miRNA families as potential therapeutic targets.

Limitations and Future Work

This study has several notable limitations. First, it relied exclusively on the GSE86195 dataset, which included only four samples without biological replicates, a major constraint that severely limits statistical power. Second, none of the differentially expressed miRNAs passed FDR correction, and all findings were derived from nominal p-values, necessitating cautious interpretation as exploratory, hypothesis-generating evidence rather than definitive conclusions. Third, the exclusive reliance on cell lines limits biological generalizability, as in vitro systems do not fully recapitulate the tumor microenvironment or patient heterogeneity. Fourth, network analysis (WGCNA) and machine learning (Random Forest) were applied to a dataset not statistically suited for such modeling; therefore, results should be considered preliminary indicators only. Finally, the study lacked experimental validation (e.g., qRT-PCR, knockdown, or luciferase assays), which is essential for confirming computational predictions.

Future studies should address these limitations. Validation of candidate miRNAs such as *hsa-let-7e*, *hsa-miR-20a-star*, *miR-346*,

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and *miR-421* in independent datasets (e.g., TCGA) and patient cohorts is critical. Integration with clinical outcomes would enable assessment of predictive and prognostic value. Functional assays should determine whether modulation of these miRNAs can restore cisplatin sensitivity in vitro or in vivo. Moreover, therapeutic strategies combining cisplatin with inhibitors of nuclear transport or the ubiquitin–proteasome pathway (47, 49, 51) could be explored. Ultimately, multi-institutional collaborations and patient-derived models will be required to establish the clinical translatability of these findings.

Conclusion

This study provides an exploratory transcriptomic and systems-level perspective on miRNA-mediated cisplatin resistance in GC. Although limited by small sample size, absence of FDR-significant results, and lack of experimental validation, the integrative approach identified candidate miRNAs and convergent pathways—including nuclear–cytoplasmic transport, ubiquitin-mediated proteolysis, and platinum drug resistance—that may contribute to therapy failure. These results should be regarded as hypothesis-generating and require rigorous validation in larger datasets and experimental studies. If confirmed, the highlighted miRNAs and pathways may serve as promising biomarkers and therapeutic targets, advancing precision oncology approaches to overcome cisplatin resistance in GC.

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During manuscript preparation, the authors utilized ChatGPT (OpenAI) to organize and refine the text. The authors subsequently reviewed and edited all content as needed and take full responsibility for the final publication.

Code of Ethics

N/A-2025-002 — This study used the publicly available GEO dataset (accession GSE86195) and therefore did not require ethical approval. A general code was assigned by the University of Tabriz.

Conflict of Interest

The authors declare no conflicts of interest.

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Ethical Consideration

Ethical approval was not required. All analyses were conducted in accordance with ethical guidelines for the use of publicly available datasets.

Authors' Contribution

Kianoush Mohammadi performed the analyses and drafted the manuscript, Reza Safaralizadeh conceived and designed the study, and Asadollah Asadi supervised the work and revised the manuscript. All authors read and approved the final version of the manuscript.

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