MicroRNA-mRNA functional pairs for cisplatin resistance in ovarian cancer cells

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Abstract
Ovarian cancer is the leading cause of death in women worldwide. Cisplatin is the core of first-line chemotherapy for patients with advanced ovarian cancer. Many patients eventually become resistant to cisplatin, diminishing its therapeutic effect. MicroRNAs (miRNAs) have critical functions in diverse biological processes. Using miRNA profiling and polymerase chain reaction validation, we identified a panel of differentially expressed miRNAs and their potential targets in cisplatin-resistant SKOV3/DDP ovarian cancer cells relative to cisplatin-sensitive SKOV3 parental cells. More specifically, our results revealed significant changes in the expression of 13 of 663 miRNAs analyzed, including 11 that were up-regulated and 2 that were down-regulated in SKOV3/DDP cells with or without cisplatin treatment compared with SKOV3 cells with or without cisplatin treatment. miRNA array and mRNA array data were further analyzed using Ingenuity Pathway Analysis software. Bioinformatics analysis suggests that the genes ANKRD17, SMC1A, SUMO1, GTF2H1, and TP73, which are involved in DNA damage signaling pathways, are potential targets of miRNAs in promoting cisplatin resistance. This study highlights candidate miRNA-mRNA interactions that may contribute to cisplatin resistance in ovarian cancer.

Key words. Ovarian cancer, cisplatin resistance, miRNA, TP73

Ovarian cancer is the deadliest cancer of the female reproductive system[1]. Although advances in platinum-based chemotherapy have resulted in improved survival, patients typically experience disease relapse within 2 years of initial treatment and develop drug resistance[2]. Chemoresistance remains a major hurdle to successful therapy. The most commonly employed chemotherapeutic drug for ovarian cancer treatment is cisplatin. Cisplatin reacts with DNA to induce distinctive biological changes. Evidence suggests that the mechanisms responsible for platinum resistance in ovarian cancer may include reduced drug accumulation, increased levels of glutathione and metallothionein, and enhanced DNA repair[3-5]. Increasing tumor cell sensitivity to chemotherapeutic agents and predicting the effectiveness of chemotherapeutic agents while avoiding the development of drug resistance in patients are attractive goals for improving the clinical management of this cancer.

Recently, microRNAs (miRNAs) have been found to play an important role in cancer cell resistance to chemotherapeutic agents[6, 7]. miRNAs are a class of single-stranded RNA molecules, 17–24 bases in length, that are expressed in a broad range of organisms, from plants to animals[8]. miRNAs repress protein expression at the post-transcriptional level through imperfect base pairing with the 3' untranslated region (UTR) of target mRNA, thereby reducing translation and/or inducing degradation of the mRNA. miRNAs are involved in cell differentiation, proliferation, and death[9]. Recent evidence suggests that drug-induced dysregulation of miRNA function may modulate the sensitivity of cancer cells to chemotherapeutic agents[10, 11]. Therefore, the effect of miRNAs on chemotherapy was systematically studied as part of the Molecular Targets Program aimed at elucidating molecular targets and understanding mechanisms of chemosensitivity and chemoresistance[12].

Chemoresistance is a biological trait of tumor malignancy that directly impacts patient prognosis. Our study was designed to identify miRNAs that are associated with cisplatin resistance and to highlight candidate miRNA-mRNA interactions that might drive the formation or progression of ovarian carcinoma.

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Material and Methods

Cell culture, RNA isolation, and semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR)

SKOV3 cells and cisplatin-resistant SKOV3/DDP cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and maintained in an atmosphere containing 5% CO₂. Total RNA was isolated from cultured cells using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) and reversely transcribed to cDNA with M-MLV Reverse Transcriptase (Promega, Madison, WI, USA). RT-PCR was performed as follows: 95°C for 10 s and 60°C for 30 s (30 cycles). TaqMan real-time PCR miRNA array

Both SKOV3 and SKOV3/DDP cells were treated with cisplatin (4 μg/mL) for 48 h or not treated. Cells were subsequently harvested and washed in cold sterile phosphate-buffered saline (PBS). miRNA was then isolated using an mirVana RNA isolation kit (Ambion). Stem-loop RT-PCR based TaqMan MicroRNA Arrays (Applied Biosystems, Foster City, CA), which included 663 mature miRNAs in a two-card set of arrays (Array A and Array B), were used. Each array contains positive controls and one negative control. Array A focuses on more highly characterized miRNAs, whereas Array B contains many of the more recently discovered miRNAs along with the miR* sequences. RT-PCR reactions were performed according to the manufacturer's instructions. All reagents were obtained from Applied Biosystems. The Ct value of an endogenous control gene (MammU6) was subtracted from the corresponding Ct value of the target gene, resulting in the ΔCt value that was used for relative quantification of miRNA expression. Clustering analysis was performed using a hierarchical method and average linkage [13].

miRNA-specific quantitative real-time RT-PCR

For miRNA analysis from cultured cells, miRNA was isolated using an mirVana RNA isolation kit (Ambion). Reverse transcription and real-time PCR were performed as previously described [14] using miRNA-specific quantitative real-time RT-PCR (Applied Biosystems, CA). The small nuclear RNA RNU6 was used as an internal control for normalization. Real-time RT-PCR was performed using an ABI 7500 Sequence Detection System and fold changes in gene expression were calculated using the 2^{-ΔΔCt} method [15]. The mean miRNA level from three quantitative real-time PCR experiments was calculated for each case.

MTT assay

SKOV3 and SKOV3/DDP cells were plated at 2 × 10⁴ per well in 96-well plates and treated with cisplatin at indicated concentrations (0–64 μg/mL) for 48 h. The cells were plated in 4 wells in each condition, with media only wells used as controls. At 4 h before the end of the incubation, 20 μL MTT (5 mg/mL) was added to each well, and at the end of 48 h, 150 μL DMSO was added to stop the reaction. Viable cell numbers were measured at a wavelength of 570 nm with the Model 680 Microplate Reader (BIO-RAD, USA). Three independent experiments were performed.

Fluorescence-activated cell sorting (FACS) analysis

Both cell lines were seeded into a six-well tissue culture plate and treated with cisplatin (4 μg/mL). The cells were harvested and washed in cold sterile PBS 48 h later. Annexin V and propidium iodide (PI) staining were performed using the Annexin V-FITC Apoptosis Detection Kit (BD Biosciences) according to the manufacturer's protocol, and flow cytometric analysis of cells followed. Analyses of apoptosis profiles were performed with Coulter Elite 4.5 Multicycle software.

Human DNA damage signaling pathway

RT² Profiler™ PCR Array

Both SKOV3 and SKOV3/DDP cells with or without cisplatin treatment (4 μg/mL, 48 h) were harvested and washed in cold sterile PBS. Then 1 mL TRizol Reagent (Invitrogen, Carlsbad, CA, USA) was added. Total RNA preparation, cDNA synthesis, and real-time PCR were performed by KangChen Bio-tech Inc. (Shanghai, China) according to the manufacturer’s protocol (PAHS-029A, SABiosciences, CA, USA). The array contained 84 functionally well-characterized genes associated with the DNA damage response. β-actin was used as a control. Fold changes in gene expression were calculated using the 2^{-ΔΔCt} method [15]. The results were confirmed by RT-PCR. The primers used for RT-PCR are listed in Table 1.

Bioinformatics analysis and target prediction

Predicted targets of the miRNAs in the miRNA array were analyzed using the algorithms TargetScan [18], TarBase [17], and miRecords [19]. For miRNAs that were up-regulated in SKOV3/DDP compared with SKOV3, we searched for targeting miRNAs that were down-regulated, and vice versa. For this purpose, we used the Ingenuity Pathway Analysis (IPA) software. IPA identified the putative targets for the input miRNAs and then developed a network of the genes/targets.

Statistical analysis

SPSS 16.0 for Windows (SPSS Inc.) was used for statistical analysis. Differences in miRNA and mRNA expression between SKOV3 and SKOV3/DDP cells were analyzed using the unpaired Student’s t-test. P values were determined using two-tailed tests, and values of P < 0.05 were considered statistically significant.
**Results**

Cisplatin-induced cytotoxicity and apoptosis in resistant and sensitive cell lines

The MTT assay was used to examine comparatively how sensitive SKOV3 and SKOV3/DDP cells were to cisplatin. As shown in Figure 1A, SKOV3/DDP cells were significantly less sensitive to cisplatin compared with SKOV3 cells. A 4-fold higher concentration of cisplatin was required to induce a change in viability, as indicated by half maximal inhibitory concentration (IC_{50}) value, in SKOV3/DDP cells compared with SKOV3 cells. By flow cytometry, we observed that cisplatin treatment induced more apoptosis in SKOV3 cells as compared with SKOV3/DDP cells (Figure 1B).

| Gene symbol | Sequences of primers | Amplicon length |
|-------------|----------------------|-----------------|
| Beta-actin  | Forward: 5′-GGCGGCCACCATGTAACCT-3′  
Revert: 5′-AGGGGGCTGTCGCTAATC-3′ | 202 bp |
| TP73        | Forward: 5′-CGGGAGGGACTTCAACGA-3′  
Revert: 5′-CAGGGTGATGATGAGGAGATG-3′ | 235 bp |
| GTF2H2      | Forward: 5′-GACACATCTCATCTTCAATG-3′  
Revert: 5′-ATATCCCTGACATCATAAC-3′ | 100 bp |
| GTF2H1      | Forward: 5′-CAGCCAAGCAGACAAACAG-3′  
Revert: 5′-AACAGGGAACGAGCAGCAG-3′ | 112 bp |
| SMC1A       | Forward: 5′-CAGGGAAAGGGCAAGGCTGATG-3′  
Revert: 5′-TCAGGTAGTAAGCAGAGCAG-3′ | 239 bp |
| SUMO1       | Forward: 5′-ACTGGGAATGGAGGAAGA-3′  
Revert: 5′-TCACCCAAAGCTGAAA-3′ | 356 bp |
| ANKRD17     | Forward: 5′-GGAGGGACTTCAACGA-3′  
Revert: 5′-ATATCCCTGACATCATAAC-3′ | 421 bp |
| GADD45A     | Forward: 5′-CAGCAAGGACTTCAACGA-3′  
Revert: 5′-CACACAGGGAACGAGCAGCAG-3′ | 234 bp |
| CCNH        | Forward: 5′-GAGCTCACCTTCACTGACTAA-3′  
Revert: 5′-ATATCCCTGACATCATAAC-3′ | 444 bp |
| DMC1        | Forward: 5′-CAGCGAACAGAGATAATG-3′  
Revert: 5′-TCACCCAAAGCTGAAA-3′ | 203 bp |
| ATM         | Forward: 5′-CAGCAAGGACTTCAACGA-3′  
Revert: 5′-CACACAGGGAACGAGCAGCAG-3′ | 446 bp |
| SESN1       | Forward: 5′-CAGGGAAAGGGCAAGGCTGATG-3′  
Revert: 5′-TCAGGTAGTAAGCAGAGCAG-3′ | 134 bp |
| ATRX        | Forward: 5′-CAGGGWGAGGTGAATTTTACT-3′  
Revert: 5′-ATATCCCTGACATCATAAC-3′ | 130 bp |
| RAD1        | Forward: 5′-CAGCGAACAGAGATAATG-3′  
Revert: 5′-TCACCCAAAGCTGAAA-3′ | 153 bp |
| MRE11A      | Forward: 5′-CAGGGAAAGGGCAAGGCTGATG-3′  
Revert: 5′-TCAGGTAGTAAGCAGAGCAG-3′ | 84 bp |
| MAP2K6      | Forward: 5′-CAGGGAAAGGGCAAGGCTGATG-3′  
Revert: 5′-TCAGGTAGTAAGCAGAGCAG-3′ | 130 bp |
| BRCA1       | Forward: 5′-CAGGGAAAGGGCAAGGCTGATG-3′  
Revert: 5′-TCAGGTAGTAAGCAGAGCAG-3′ | 278 bp |
| ERCC1       | Forward: 5′-CAGGGAAAGGGCAAGGCTGATG-3′  
Revert: 5′-TCAGGTAGTAAGCAGAGCAG-3′ | 382 bp |

**miRNA expression profiles in SKOV3 and SKOV3/DDP cells**

miRNAs isolated from SKOV3 and SKOV3/DDP cells with or without cisplatin treatment (4 μg/mL, 48 h) were screened with miRNA
miRNA expression patterns were generally similar among untreated and treated SKOV3 cells as well as untreated and treated SKOV3/DDP cells. Among the 663 miRNAs analyzed, 13 miRNAs were significantly differentially expressed between the two sample groups, with fold change > 2 and P < 0.05. Of those 13 miRNAs, 11 were up-regulated and 2 were down-regulated in SKOV3/DDP cells as compared to SKOV3 cells (Table 2). The up-regulated miRNAs were hsa-miR-100, hsa-miR-125b, hsa-miR-7c, hsa-miR-10a, hsa-miR-133a, hsa-miR-27b, hsa-miR-34a, hsa-miR-486-3p, hsa-miR-181c*, hsa-miR-100*, and hsa-miR-33a*. The down-regulated miRNAs were hsa-miR-139-3p and hsa-miR-383. We used hierarchical clustering to classify the changes in expression of miRNAs that were significantly differentially expressed in Array A (Figure 2A) and Array B (Figure 2B). The miRNA expression profiles of SKOV3 and SKOV3/DDP cells were confirmed with miRNA-specific quantitative real-time RT-PCR. Eight miRNAs were tested and the results were concordant with the miRNA array data (data not shown).

Bioinformatics and preliminary functional analysis

Cisplatin reacts with DNA to induce DNA damage and initiate the irreversible apoptotic process[19]. Evidence to date suggests that enhanced DNA repair is one of the mechanisms responsible for platinum resistance in ovarian cancer. Human DNA damage signaling pathway arrays were performed to identify genes differentially expressed between SKOV3 cells and SKOV3/DDP cells with or without cisplatin treatment (4 μg/mL, 48 h). Using a filtering criterion of a two-fold or greater change in expression, 8 genes were found to be up-regulated and 26 genes down-regulated in SKOV3/DDP cells treated with or without cisplatin compared with SKOV3 cells treated with or without cisplatin (Table 3). The clustering tree of the 34 genes was shown in Figure 2C. The mRNA expression profiles of SKOV3 and SKOV3/DDP cells were confirmed with RT-PCR. Of the 34 genes that met our criterion, 17 mRNAs had RT-PCR results that were concordant with the miRNA array data, indicating a concordance rate of 82.4% (14/17), and 3 genes showed no change in expression (RAD1, ATRX, and MRE11A) (Figure 3).

To identify putative miRNA-mRNA functional pairs, we integrated miRNA and mRNA profiles using IPA. For miRNAs that were up-regulated in SKOV3/DDP cells with or without cisplatin treatment compared with SKOV3 cells with or without cisplatin treatment, we searched for potential target mRNAs that were down-regulated, and vice versa. This approach allowed us to focus on interactions that might be especially relevant to our model system. Of the 13 miRNAs

Table 2. MicroRNAs differentially expressed in SKOV3/DDP cells compared to SKOV3 cells

| MicroRNA   | Fold-change | P value |
|------------|-------------|---------|
| let-7c     | 22.76       | 0.018   |
| miR-100    | 106.34      | 0.005   |
| miR-10a    | 5.75        | 0.039   |
| miR-125b   | 136.32      | 0.002   |
| miR-133a   | 1,759.01    | 0.050   |
| miR-139-3p | -2.65       | 0.014   |
| miR-27b    | 23.27       | 0.012   |
| miR-34a    | 24.47       | 0.030   |
| miR-383    | -3.86       | 0.011   |
| miR-486-3p | 7.86        | 0.047   |
| miR-181c   | 22.86       | < 0.001 |
| miR-100    | 22.41       | 0.017   |
| miR-33a    | 5.39        | 0.031   |

*, miRNA identity (ID).
that were significantly differentially expressed (fold change > 2, \( P < 0.05 \)) between SKOV3/DDP cells and SKOV3 cells, 8 miRNAs had target information that was filtered by the mRNA array dataset. The association between the miRNAs and their possible target genes is listed in Table 4. A gene network generated by IPA of the 6 miRNAs and 5 mRNAs is shown in Figure 4, based on high confidence from IPA software calculations or experimental observations.

**Discussion**

Ovarian cancer is the most fatal gynecologic malignancy in women[20]. Roles of miRNA have been reported in different cancers, including ovarian cancer[21-23]. Furthermore, some studies have indicated that miRNA expression patterns were significantly different between chemotherapy-sensitive and -resistant ovarian cancer cell lines and tissues[24-30]. Thus, targeting these miRNAs might offer novel strategies for early detection, diagnosis, and treatment of this disease. In this paper, for the first time, we used microarray to identify the miRNA signature associated with cisplatin-resistant SKOV3/DDP cells compared with parental SKOV3 cells. Our results demonstrated that miRNA expression patterns were generally similar among SKOV3 cells with or without cisplatin treatment and among SKOV3/DDP cells with or without cisplatin treatment. However, we identified 13 miRNAs (11 up-regulated and 2 down-regulated) that were differentially expressed in SKOV3/DDP cells compared with SKOV3 cells, including miR-10a, miR-27b, miR-125b, and miR-100. Our
study also demonstrated that the dysregulation of miRNA expression is associated with the cisplatin-resistant phenotype in SKOV3/DDP cells. Specifically, we identified miRNA changes associated with altered DNA repair. Current bioinformatics methods for predicting miRNA targets provide large numbers of candidate genes, many of which are most likely false positive results. To narrow down the list of candidates, we first looked for target genes whose mRNA levels were altered in the opposite direction as their corresponding targeting miRNA (i.e., miRNA levels up, mRNA levels down; or miRNA levels down, mRNA levels up). We then chose candidates based on high confidence from IPA software calculations or experimental observations. Based on the filtering criterion, our study identified a small collection of putative miRNA-mRNA interactions. These candidate genes included ANKRD17, SMC1A, SUMO1, TP73 (p73), and GTF2H1. The tumor suppressor p73, which is a target of miR-34a, miR-486-3p and, with lesser confidence, miR-125b, plays critical roles in multiple molecular mechanisms underlying chemoresistance in tumor cells. Our bioinformatics analyses led us to hypothesize that chemotherapy induces the expression of the miRNAs, thereby limiting chemosensitivity due to miRNA-mediated feedback inhibition of p73. SUMO1 can bind to target proteins as part of a sumolation modification system. As shown in Figure 5, miR-133a negatively regulated SUMO1 mRNA. The SUMO1 3'UTR element has a predicted site for miR-133a, and this site is conserved among

### Table 3. Genes differentially expressed in SKOV3/DDP cells compared to SKOV3 cells

| Gene symbol | Accession number | Fold change | P value |
|-------------|-----------------|-------------|---------|
| ATRX        | NM_000489       | 2.20        | 0.062   |
| CDK7        | NM_001799       | -4.08       | 0.165   |
| CHEK1       | NM_001274       | -4.34       | 0.188   |
| CIDEA       | NM_001279       | 2.02        | 0.305   |
| CRY1        | NM_004075       | -2.27       | 0.516   |
| DMC1        | NM_007068       | -192.31     | 0.373   |
| FANCG       | NM_004629       | -2.25       | 0.209   |
| GADD45A     | NM_001924       | -3.47       | 0.510   |
| GADD45G     | NM_006705       | -2.22       | 0.083   |
| GML         | NM_002066       | -3.36       | 0.310   |
| GTF2H2      | NM_001515       | -3.17       | 0.270   |
| MAP2K6      | NM_002758       | 137.56      | 0.258   |
| MLH3        | NM_014381       | -2.48       | 0.308   |
| MPG         | NM_002434       | -2.15       | 0.082   |
| MRE11A      | NM_005590       | 4.71        | 0.050   |
| MSH2        | NM_000251       | -2.02       | 0.210   |
| MUTYH       | NM_012222       | -2.60       | 0.253   |
| N4BP2       | NM_018177       | -2.44       | 0.461   |
| NBN         | NM_002485       | -4.06       | 0.180   |
| PCBP4       | NM_020418       | 5.57        | 0.377   |
| PCNA        | NM_182649       | -3.26       | 0.385   |
| PMS1        | NM_000534       | -3.84       | 0.393   |
| PMS2L3      | NM_005395       | -2.14       | 0.447   |
| PPP1R15A    | NM_014330       | -3.42       | 0.469   |
| RAD1        | NM_002853       | 2.21        | 0.392   |
| RAD21       | NM_006266       | -2.11       | 0.481   |
| RAD51L1     | NM_135509       | -5.05       | 0.086   |
| RBBP8       | NM_002945       | -5.40       | 0.263   |
| RPA1        | NM_002945       | -2.00       | 0.371   |
| SEMA4A      | NM_022367       | 4.11        | 0.399   |
| SESN1       | NM_014454       | 2.87        | 0.395   |
| SUMO1       | NM_003352       | -4.30       | 0.294   |
| XRCC2       | NM_005431       | -5.91       | 0.454   |
| XRCC3       | NM_005432       | -2.78       | 0.083   |
mammals. The mRNA levels of SMC1A, ANKRD17, and GTF2H1 did not significantly change, as determined with RT-PCR.

Interestingly, many of the miRNAs identified in our study have been previously reported to play a role in resistance to chemotherapy. For example, previous results also showed that miR-125b was up-regulated in A2780CIS, A2780TC1, and A2780TC3 ovarian cancer cells\(^{27}\). miR-10a is one of the three most up-regulated miRNAs in MCF-7/DDP cells, and it targets the gene HOXD10\(^{23}\). A recent study showed that resistance to vincristine and daunorubicin was characterized by an approximate 20-fold up-regulation of miR-125b, miR-99a, and miR-100 in pediatric acute lymphoblastic leukemia\(^{34}\). Additionally, miR-27a was up-regulated in a multidrug resistant (MDR) ovarian cancer cell line compared with its parental (A2780) cell line\(^{33}\). Furthermore, treatment with miR-27a antagomirs decreased

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**Table 4. List of predicted miRNA-mRNA functional pairs**

| miRNA      | Fold change | Gene symbol | mRNA fold change | Confidence (experimentally observed/predicted) | Algorithms                |
|------------|-------------|-------------|------------------|------------------------------------------------|--------------------------|
| let-7c     | 22.76       | SMC1A       | −1.06            | Experimentally observed, high                  | TargetScan, TarBase, miRecords |
| miR-10a    | 5.75        | GTF2H1      | −1.07            | High                                           | TargetScan               |
| miR-125b   | 136.33      | DMC1        | −192.31          | Moderate                                       | TargetScan               |
|            |             | IGHMBP2     | −1.63            | Moderate                                       | TargetScan               |
|            |             | TP73        | −1.27            | Moderate                                       | TargetScan               |
| miR-133a   | 1,759.01    | SUMO1       | −4.30            | High                                           | TargetScan               |
| miR-27b    | 23.27       | ANKRD17     | −1.74            | High                                           | TargetScan               |
|            |             | GTF2H2      | −3.17            | Moderate                                       | TargetScan               |
| miR-34a    | 24.47       | TP73        | −1.27            | High                                           | TargetScan               |
| miR-383    | 7.86        | HUS1        | 1.14             | Moderate                                       | TargetScan               |
| miR-486-3p | −3.39       | TP73        | −1.27            | High                                           | TargetScan               |
|            |             | IGHMBP2     | −1.63            | Moderate                                       | TargetScan               |
|            |             | MLH3        | −2.48            | Moderate                                       | TargetScan               |
|            |             | XRCC3       | −2.78            | Moderate                                       | TargetScan               |

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**Figure 3.** Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of genes in SKOV3 and SKOV3/DDP cells. Beta-actin was used as an internal control. Of the 17 genes, the alterations of 8 genes (RAD1, ATRX, and MRE11A) were not consistent with the mRNA array data.
Figure 4. Gene network generated by Ingenuity Pathway Analysis of 5 genes identified to be predicted targets of miRNAs associated with *in vitro* cisplatin resistance (fold change > 2, *P* < 0.05) in the current study. The other 3 miRNAs have no direct association with the 34 genes. Orange lines means the miRNA or gene can regulate the other gene expression directly. Fx, function.

Figure 5. *SUMO1* is a predicted target of miR-133a. A, predicted duplex formation between human *SUMO1* 3'UTR and miR-133a. Lower panel. Sequence of miR-133a conserved binding site within the *SUMO1* 3'UTR of human, mouse, rat, rabbit, and dog. B, miR-133a was detected with RT-PCR in SKOV3 and SKOV3/DDP cells treated with or without cisplatin (4 μg/mL, 48 h). U6 was used as an internal control for normalization (mean ± standard deviation, n = 3). C, RT-PCR analysis of *SUMO1* in SKOV3 and SKOV3/DDP cells both treated with or without cisplatin (4 μg/mL, 48 h). Beta-actin was used as an internal control.
the expression of P-glycoprotein (P-gp) and MDR1 mRNA, leading
to enhanced sensitivity to cytotoxic drugs due to their intracellular
accumulation. This suggests an alternative mechanism for the effect
of miR-27a on chemoresistance. As we know, miR-27b and miR-
27a bear the same “seed” sequence; thus, miR-27b may have
functions or gene targets similar to miR-27a[36]. Previously reported
mechanisms of platinum resistance have also shown that BRCA1 and
annexin A3 are up-regulated in SKOV3/DDP cells[37, 38]. In this study,
we also found that BRCA1 levels were slightly elevated in SKOV3/
DDP cells compared with SKOV3 cells. Although this study has
provided some insight into miRNAs and their potential targets that
play a role in cellular response to cisplatin, the in-depth mechanisms
of these miRNA-mRNA pairs need further study.

In summary, our study found a signature of 13 miRNAs that are
associated with response to cisplatin in ovarian cancer cells. Some
of these miRNAs could potentially target TP73 and SUMO1. Our
results uncover a new means of eliciting specific p73 down-regulation
through up-regulation of specific miRNA. The results also suggest
that a particular miRNA signature may represent a prognostic tool
to monitor the outcome of chemotherapy. Inhibiting specific miRNAs
may provide a new therapeutic opportunity for patients with cisplatin-
resistant ovarian cancer.

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