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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% CO_2. 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 150 s (one cycle), followed by 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 $2^{-\Delta\Delta Ct}$ 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 2×10^4 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 RT^2 Profiler^{TM} 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 $2^{-\Delta\Delta Ct}$ 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[16], TarBase[17], and miRecords[18]. For mRNAs 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.

Table 1. Primers used for polymerase chain reaction amplification of the genes

Gene symbol	Sequences of primers	Amplicon length
Beta-actin	Forward: 5′-GGCGGCACCACCATGTACCCT-3′ Reverse: 5′-AGGGGCCGGACTCGTCATACT-3′	202 bp
TP73	Forward: 5′-CGGGAGGGACTTCAACGA-3′ Reverse: 5′-CAGGGTGATGATGATGAGGATG-3′	235 bp
GTF2H2	Forward: 5′-GCACGGTCTTACCATCATTTG-3′ Reverse: 5′-ATTCCCCCTGACATCCATAAC-3′	100 bp
GTF2H1	Forward: 5′-ACACAGCAAGCCATAAACCAG-3′ Reverse: 5′-TAACAGGAAAGCAGGACCAGA-3′	112 bp
SMC1A	Forward: 5′-CAGCGAAAGGCAGAGATAATG-3′ Reverse: 5′-TCCAGGTAGTCAAGAGGCAAG-3′	239 bp
SUMO1	Forward: 5′-ACTGGGAATGGAGGAAGA-3′ Reverse: 5′-TCACCACAAGCCTGAAAA-3′	356 bp
ANKRD17	Forward: 5′-GGAGCGAATGTGAATAGA-3′ Reverse: 5′-TGTGGGTAGGAGTGTTTG-3′	421bp
GADD45A	Forward: 5′-CCGAAAGGATGGATAAGGTG-3′ Reverse: 5′-GCAGGATGTTGATGTCGTTCT-3′	234 bp
CCNH	Forward: 5′-GGCTTCCTCATCGACTTAAAGA-3′ Reverse: 5′-TCATAGCCTTTCCTCTTCTTCG-3′	444 bp
DMC1	Forward: 5′-AAGAGGCAGCGAACAAACTAA-3′ Reverse: 5′-CACACAGAGGGTATGAGAAAGC-3′	203 bp
ATM	Forward: 5′-TGCATACTTGAAAGCTCAGGAA-3′ Reverse: 5′-TGGACTTCACCTCATCAAAATG-3′	446 bp
SESN1	Forward: 5′-AATGAAGTGAGATGGGATGGAC-3′ Reverse: 5′-GATGGACGATGAGGTGTTTCTT-3′	134 bp
ATRX	Forward: 5′-CAGGTGGAGCGTCATTTTACT-3′ Reverse: 5′-GTATGGTATCCTTTGGCAGCA-3′	130 bp
RAD1	Forward: 5′-CCCACCTTGACTATCCCAAAG-3′ Reverse: 5′-AGCCTCTGTTATCTGTCCGAAT-3′	153 bp
MRE11A	Forward: 5′-GAAGATGATGAAGTCCGTGAGG-3′ Reverse: 5′-AGCACTAAAGGCAGAAGCAGAC-3′	84 bp
MAP2K6	Forward: 5′-ATTTGGAGTCTGGGCATCAC-3′ Reverse: 5′-ACTTGTCTGCTGGGAGTTGTG-3′	130 bp
BRCA1	Forward: 5′-AAAAGACATGACAGCGATAC-3′ Reverse: 5′-CTTTCCTGAGTGCCATAA-3′	278 bp
ERCC1	Forward: 5′-GTAATTCCCGACTATGTGCT-3′ Reverse: 5′-GGGTCTGACTGTCCGTTT-3′	382 bp

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**).

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

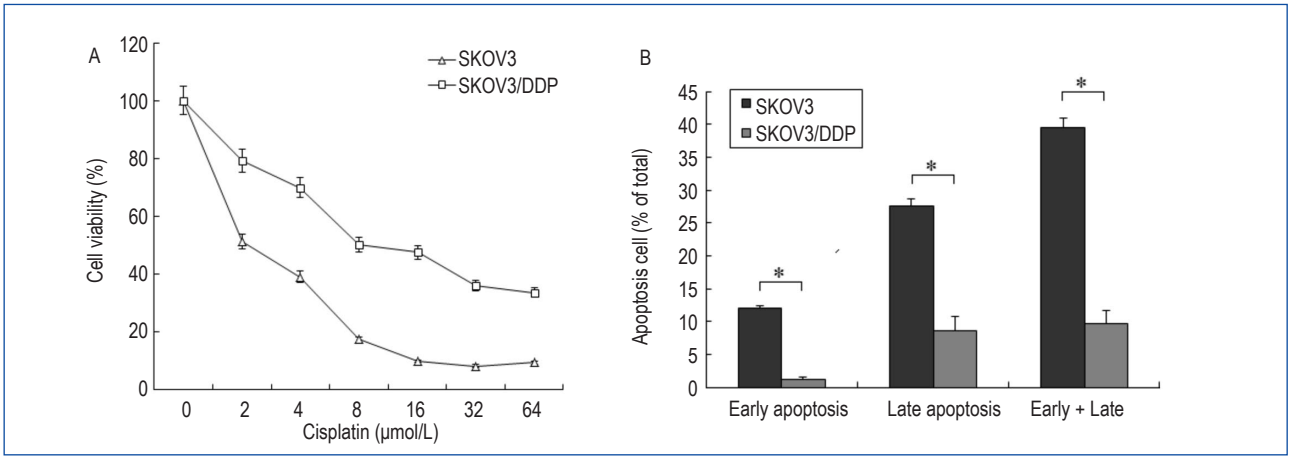

Figure 1. **Responses of SKOV3 and SKOV3/DDP cells to cisplatin. A, SKOV3/DDP cells were less sensitive to cisplatin than SKOV3. SKOV3 and SKOV3/DDP cells were plated at 2 × 10^4 per well in 96-well plates and treated with cisplatin at the indicated concentration (0–64 μg/mL) for 48 h. Cell viability is presented as mean ± standard deviation (SD) (n = 3) and was assessed using the MTT assay. B, cisplatin induced apoptosis in SKOV3 and SKOV3/DDP cells. SKOV3 and SKOV3/DDP cells 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 phosphate buffered saline (PBS) 48 h later. Then, cells were harvested and stained with Annexin V and propidium iodide (PI), followed by fluorescence-activated cell sorting (FACS) analysis. The percentage of apoptotic cells is presented as mean ± SD (n = 3). *P < 0.01.**

microarray. As shown in **Figure 2**, 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-let-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).

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).

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 mRNA 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

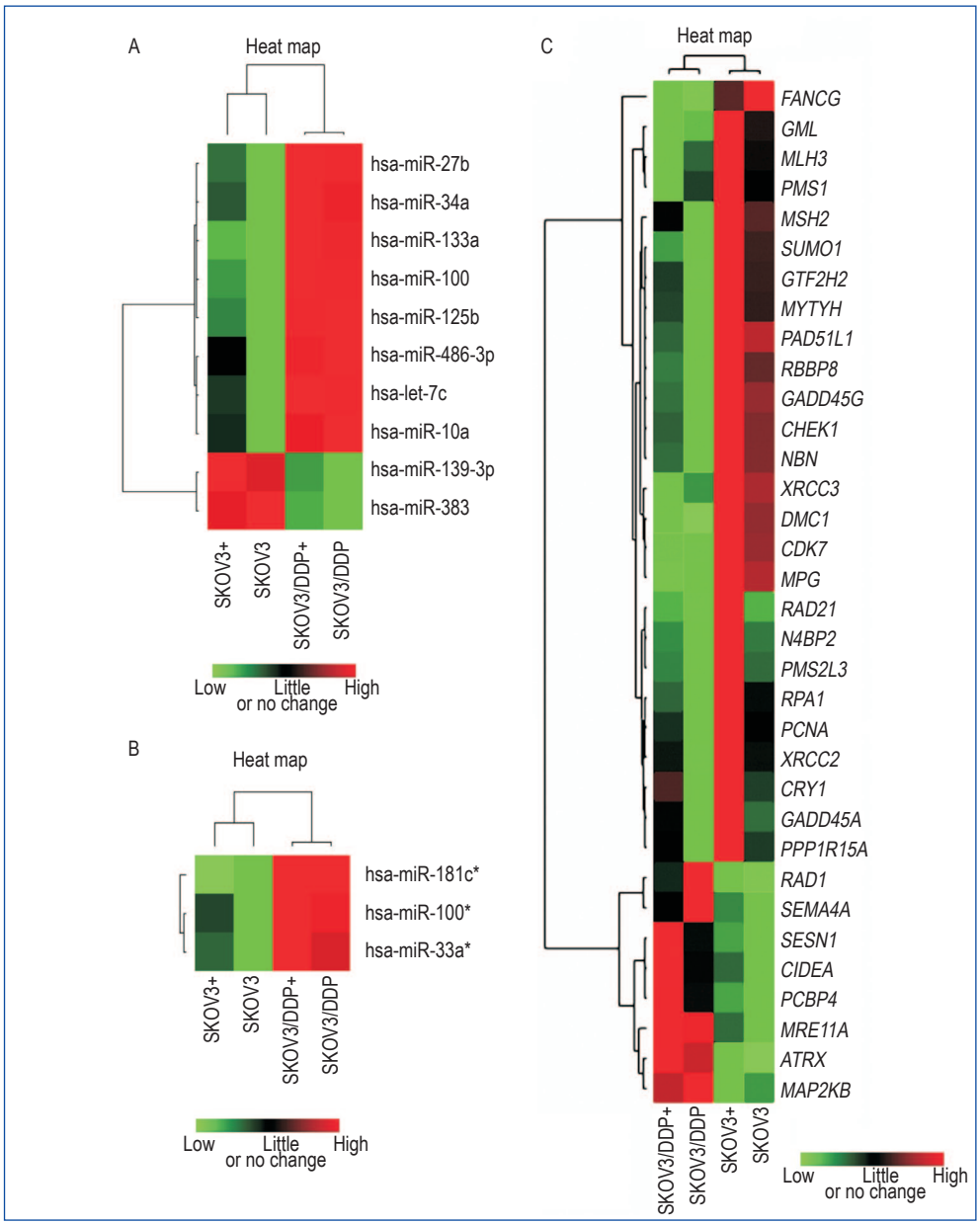

Figure 2. Hierarchical clustering of 13 miRNAs and 34 genes with different expression in SKOV3 with or without cisplatin treatment and SKOV3/DDP cells with or without cisplatin treatment, respectively. Each row represents an miRNA or a gene, and each column represents a sample. The color red indicates up-regulation, with a ΔCt value below the average level, and the color green indicates down-regulation, with a ΔCt value above the average level. A, heat map representation of 10 miRNAs (fold change > 2, $P < 0.05$) examined in Array A overexpressed (red) and underexpressed (green) in SKOV3/DDP cells compared with SKOV3 cells with or without cisplatin treatment (4 μg/mL, 48 h), respectively. B, heat map representation of 3 miRNAs (fold change > 2, $P < 0.05$) examined in Array B overexpressed (red) in SKOV3/DDP cells compared with SKOV3 cells with or without cisplatin treatment (4 μg/mL, 48 h), respectively. C, heat map of 34 genes that showed differential expression (fold change > 2) in SKOV3 cells and SKOV3/DDP cells with or without cisplatin treatment (4 μg/mL, 48 h), respectively. +: cells treated with cisplatin (4 μg/mL, 48 h).

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

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_006265	–2.11	0.481
RAD51L1	NM_133509	–5.05	0.086
RBBP8	NM_002894	–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

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[31]. 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[32]. 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

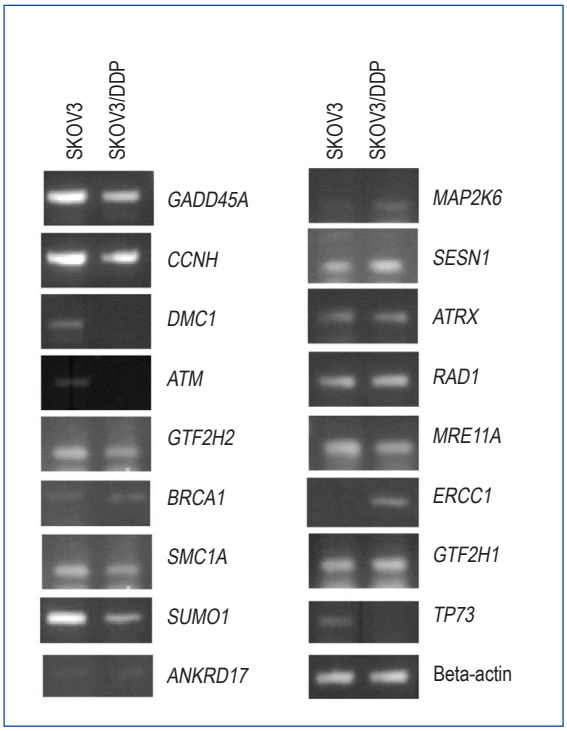

Figure 3. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of genes in SKOV3 and SKOV3/DDP cells. Beta-actin was used as an internal control. Of the 17 genes, the alterations of 3 genes (*RAD1*, *ATRX*, and *MRE11A*) were not consistent with the mRNA array data.

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	–3.39	*HUS1*	1.14	Moderate	TargetScan
miR-486-3p	7.86	*TP73*	–1.27	High	TargetScan
		IGHMBP2	–1.63	Moderate	TargetScan
		MLH3	–2.48	Moderate	TargetScan
		XRCC3	–2.78	Moderate	TargetScan

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*[33]. 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[35]. Furthermore, treatment with miR-27a antagomirs decreased

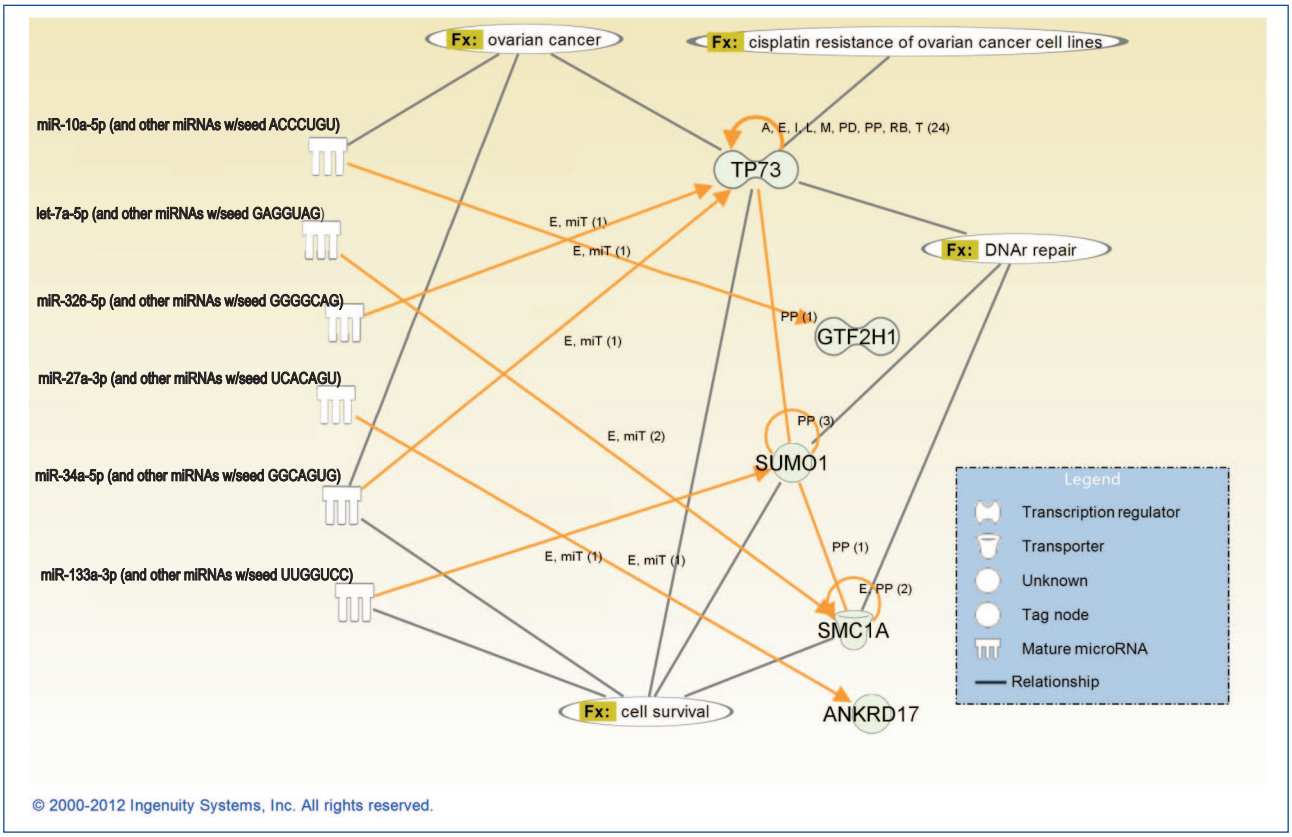

Figure 4. **Gene network generated by Ingenuity Pathway Analysis of 5 genes identified to be predicted targets of miRNAs associated with *in vitro* cisplatin resistance (fold change > 2, $P < 0.05$) in the current study. The other 3 miRNAs have no direct association with the 34 genes. Orange lines means the miRNA or gene can regulate the other gene expression directly. Fx, function.**

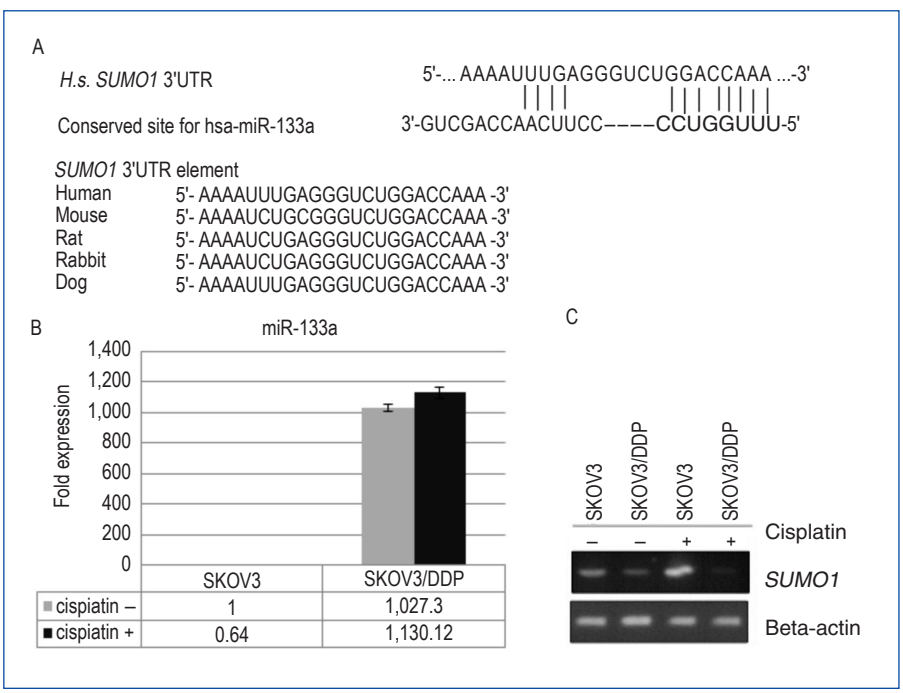

Figure 5. ***SUMO1* is a predicted target of miR-133a. A, predicted duplex formation between human *SUMO1* 3'UTR and miR-133a. Lower panel. Sequence of miR-133a conserved binding site within the *SUMO1* 3'UTR of human, mouse, rat, rabbit, and dog. B, miR-133a was detected with RT-PCR in SKOV3 and SKOV3/DDP 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.

Acknowledgments

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