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Original Article

Analysis of microarray-identified genes and microRNAs associated with drug resistance in ovarian cancer

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Abstract: The aim of this study was to identify potential microRNAs and genes associated with drug resistance in ovarian cancer through web-available microarrays. The drug resistant-related microRNA microarray dataset GS54665 and mRNA dataset GSE33482, GSE28646, and GSE15372 were downloaded from the Gene Expression Omnibus database. Dysregulated microRNAs/genes were screened with GEO2R and were further identified in SKOV3 (SKOV3/DDP) and A2780 (A2780/DDP) cells by real-time quantitative PCR (qRT-PCR), and then their associations with drug resistance was analyzed by comprehensive bioinformatic analyses. Nine microRNAs (microRNA-199a-5p, microRNA-199a-3p, microRNA-199b-3p, microRNA-215, microRNA-335, microRNA-18b, microRNA-363, microRNA-645 and microRNA-141) and 38 genes were identified to be differentially expressed in drug-resistant ovarian cancer cells, with seven genes (NHSL1, EPHA3, USP51, ZSCAN4, EPHA7, SNCA and PI15) exhibited exactly the same expression trends in all three microarrays. Biological process annotation and pathway enrichment analysis of the 9 microRNAs and 38 genes identified several drug resistant-related signaling pathways, and the microRNA-mRNA interaction revealed the existence of a targeted regulatory relationship between the 9 microRNAs and most of the 38 genes. The expression of 9 microRNAs and the 7 genes by qRT-PCR in SKOV3/DDP and A2780/DDP cells indicating a consistent expression profile with the microarrays. Among those, the expression of EPHA7 and PI15 were negatively correlated with that of microRNA-141, and they were also identified as potential targets of this microRNA via microRNA-mRNA interaction. We thus concluded that microRNA-141, EPHA7, and PI15 might jointly participate in the regulation of drug resistance in ovarian cancer and serve as potential targets in targeted therapies.

Keywords: Ovarian cancer, drug resistance, expression profiling microarray, gene, microRNA, bioinformatics

Introduction

Ovarian cancer (epithelial carcinoma) is one of the three major malignancies of the female reproductive system. The mortality rate of ovarian cancer is the highest among all gynecological malignancies, and the prognosis of ovarian cancer is generally poor [1]. Currently, surgery is the primary treatment for ovarian cancer, and this treatment is usually supplemented with chemotherapy to deliver a comprehensive therapy. Platinum-based combination chemotherapy improves the overall response rate, the clinical remission rate, and the median survival rate in patients with ovarian cancer. However, primary and acquired multidrug resistance (MDR) remains the main obstacles to the success of chemotherapeutic treatment in clinical practice [2]. Therefore, an understanding of the mechanisms underlying the development and reversal of drug resistance in ovarian cancer patients is of great clinical significance for enhancing the survival rates and improving the prognosis of these patients.

In recent years, data mining has been conducted at the molecular level using bioinformatic approaches such as biochip data extraction, sequence alignment, statistical analysis, visual mapping, biological data clustering, pathway analysis, and promoter prediction, and these analyses provide novel research ideas for studying the molecular pathogenesis of various diseases, including tumors. For example, through a comprehensive bioinformatic analysis, Yin et al. [3] identified 25 genes (including

AKT1/2D) that are associated with drug resistance in ovarian cancer. In the present study, microarray data detailing the messenger RNA (mRNA) and microRNA expression profiles in drug-sensitive and drug-resistant ovarian cancer cells were retrieved from the Gene Expression Omnibus (GEO) database [4] and were analyzed to identify differentially expressed genes and microRNAs. The correlation between differentially expressed genes and microRNAs in drug-resistant and drug-sensitive ovarian cancer was examined through comprehensive bioinformatics analysis including biological process annotation, biological pathway enrichment, protein/gene interaction analysis, and mRNA-microRNA interaction analysis. In addition, text mining was conducted to obtain clues and ideas for further experimental research regarding the mechanisms underlying drug resistance in ovarian cancer.

Materials and methods

Acquisition and analysis of datasets

Microarray data published prior to December 2014 detailing ovarian cancer chemoresistance-related microRNA and mRNA expression profiles were retrieved and downloaded from the National Center for Biotechnology Information (NCBI) GEO database (http://www.ncbi.nlm Nih.gov/geo). Queries were performed using "ovarian cancer" as a keyword. The search was restricted to the following specific fields: study type, expression profiling by array, and species: Homo sapiens. We downloaded microRNA expression microarray datasets GSE54665 and mRNA expression microarray datasets GSE33482, GSE28646 [5] and GSE15372 [6].

Inclusion criteria for differentially expressed microRNAs and genes

Differentially expressed microRNAs and genes were screened using the GEO2R tool in the GEO database. GEO2R is an R programming language-based dataset analysis tool that was launched in 2012. This tool is based on a t-test or analysis of variance (ANOVA) and is useful for comparing two groups of samples under the same experimental conditions to identify differentially expressed genes or microRNAs [7].

In the present study, microRNAs and genes that were differentially expressed between drug-resistant and drug-sensitive ovarian cancer cells were screened using an adjusted p-value (adj. P) of less than 0.05 and a fold change of at least 2 (> 2-fold change) as thresholds. MicroRNAs or genes that were common to at least two expression profile datasets were selected using the Bioinformatics & Research Computing website (http://jura.wi.mit.edu/bioc/tools/compare.php). Bioinformatic analysis of differentially expressed genes and microRNAs. Pathway enrichment analysis of differentially expressed microRNAs was performed using the DIANA miRPath (http://diana.imis.athenainnovation.gr/DianaTools/index.php? r=mirpath/index) [8]. DAVID (http://david.abcc.ncifcrf.gov/) was used to analyze the pathway enrichment of differentially expressed genes [9]. Biological process annotation of differentially expressed genes was performed using ToppGene (http://toppgene.cchmc.org/) [10]. Protein/gene interactions were analyzed using GeneMANIA (http://www.genem ania.org/) [11] and mRNA-microRNA interactions were analyzed using the miRanda (http://www.microrna. org/microrna/home.do) [12], miRBase (http://www.mirbase.org/) [13], TargetScan (http://www. targetscan.org/) [14], miRWalk (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk) [15] and RNA22 (http://cbc-srv.watson.ibm.com/ma 22 .html) [16] tools.

Cell culture

The human ovarian cancer cell lines SKOV3 and A2780 were generated in our lab, and A2780/DDP was established from A2780 as previously described A stable cisplatin-resistant cell line, SKOV3/DDP, was established from SKOV3 by continuous exposure of the cells to increasing concentrations of DDP [17], and routinely maintained in 1640 (CORNING, USA) supplemented with 10% fetal bovine serum (FBS) (CORNING, USA), 2 μmol/L L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco BRL, Grand Island, NY) at 37°C in a humidified atmosphere containing 5% CO_2.

Real-time quantitative PCR

MicroRNA: RNA was extracted from A2780, A2780/DDP, SKOV3, and SKOV3/DDP cells using an RNeasy® Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. cDNA was then obtained using a miScript II RT kit (QIAGEN, Germany) in accordance with the

Table 1. microRNA and gene expression microarray datasets related to the chemotherapy response in epithelial ovarian cancer

	Accession number of the dataset	Organization name	Platforms	Design	Response to chemotherapy	
					Resistance	Sensitive
microRNA	GSE54665	Erasmus University Medical Center	GPL16851	A2780/DDP AND A27800	3	3
mRNA	GSE33482	Queen's University	GPL6480	A2780/cis AND A2780	6	6
	GSE28646	Imperial College London	GPL570	A2780/CP70 AND A2780	3	3
	GSE15372	Indiana University	GPL570	A2780-DDP AND A2780	5	5

Table 2. Screening for differentially expressed microRNAs and their taget gens in the GSE54665 dataset

Accession number of the dataset	differentially expressed microRNAs	LogFC*	Target genes
GSE54665	microRNA-199a-5p	5.244	C1orf21, DCLK1, EPHA7, IL20RB, MAB21L1, PI15
	microRNA-199a-3p	4.103	C1orf21, DCLK1, IL20RB
	microRNA-199b-3p	4.103	C1orf21, DCLK1, IL20RB
	microRNA-335	3.319	C1orf21, CCR5, TPM1
	microRNA-141	-3.083	C1orf21, DACT1, DCLK1, EPHA3, IL20RB, FOXP2, PI15
	microRNA-215	3.02	C1orf21, CD55, CNN1, GSG1 TSLP
	microRNA-363	2.389	C1orf21, DCLK1
	microRNA-18b	2.069	EPHA7, PI15
	microRNA-645	1.729	C1orf21, FILIP1

*A positive logFC value indicates that the miRNA expression level was downregulated in the drug-resistant group, whereas a negative logFC value indicates that the miRNA expression level was upregulated in the drug-resistant group, Note: Thresholds used in the GEO2R analysis: adj. $P < 0.05$, fold > 2.

manufacturer's instructions. Real-time quantitative PCR (qRT-PCR) was completed with FastStart Universal SYBR Green PCR Master (ROX) in a total volume of 10 μL on an ABI 7500 (Applied Biosystems, USA). The conditions were as follows: 40 cycles of three-step PCR (95°C for 15 sec, 55°C for 30 sec, and 72°C for 30 sec) following an initial denaturation at 95°C for 10 min. The microRNA sequences were analyzed using miRBase (http://microrna.sanger.ac.uk/sequences/). Details regarding the primers used to measure gene expression are listed in Supplementary Table 1. All primers were supplied by BGI. The $2^{-\Delta\Delta}$ CT method was used for data analysis [18].

mRNA: Total RNA was isolated as described above. First-strand cDNA was synthesized from 1 μg of total RNA using the Transcriptor First Strand cDNA Synthesis kit (Roche, Germany) as instructed by the manufacturer. qRT-PCR reactions were performed on an ABI 7500 real-time PCR system (Applied Biosystems, USA) using the following procedure: 95°C for 10 min, followed by 40 cycles of 95°C for 30 sec and 60°C for 1 min. To create a qRT-PCR standard, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. The sequences of the PCR primers are listed in Supplementary Table 2. The $2^{-\Delta\Delta}$ CT method was used for data analysis [18].

Results

Microarray datasets that met the inclusion criteria

In the present study, the microRNA expression profiling datasets were GSE54665, which was generated by the Erasmus University Medical Center using the GPL16851 platform. The samples used to generate the GSE54665 dataset were cisplatin-resistant A2780 ovarian cancer cells and parental A2780 cells (three sets each). The mRNA expression profiling datasets studied were GSE33482, GSE28646, and GSE15372. GSE33482 was generated by Queen's University using the GPL640 microarray platform. The data were derived from cisplatin-resistant A2780 ovarian cancer cells (A2780-cis) and parental A278 cells (six repeats of each). GSE28646 was generated by

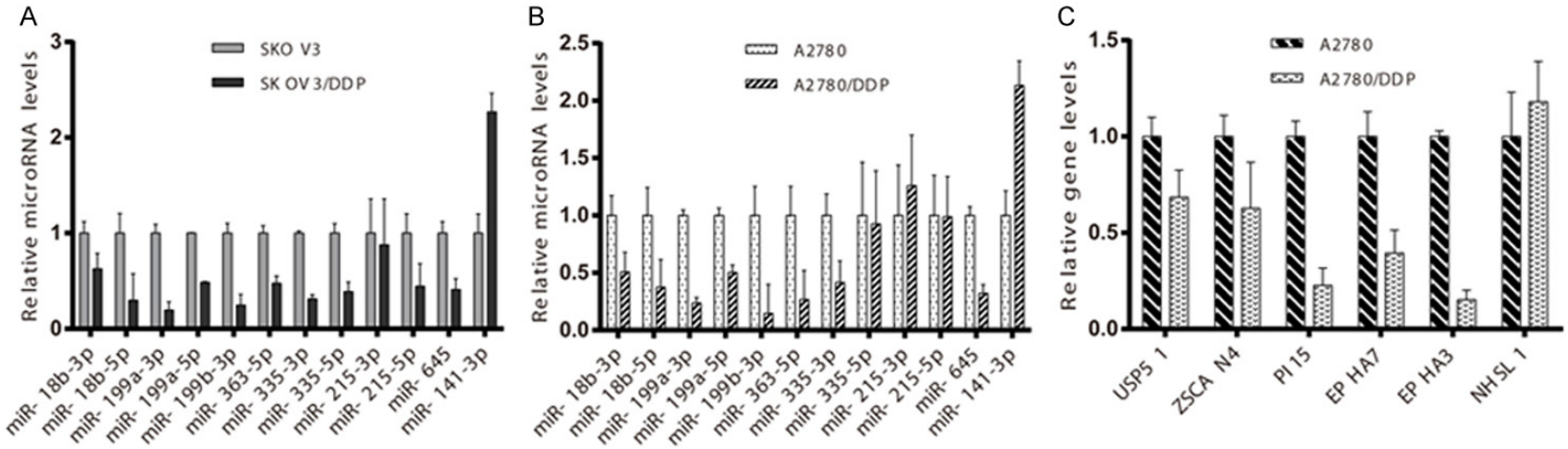

Figure 1. Relative expression levels of microRNAs and genes in SKOV3/DDP vs. SKOV3 cells and A2780/DDP vs. A2780 cells. A. Relative expression levels of microRNAs in SKOV3/DDP vs. SKOV3 cells. B. Relative expression levels of microRNAs in A2780/DDP vs. A2780 cells. C. Relative expression levels of genes in A2780/DDP vs. A2780 cells.

Table 3. microRNAs and pathways related to drug resistance in ovarian cancer

	Pathway	Numbers of microRNAs	*P* value	Associations with drug resistance in ovarian cancer (Ref.)
microRNA199a-5p	Focal adhesion	8	2.58E-13	YES [21]
microRNA199a-3p	PI3K-Akt Signaling pathway	7	2.24E-12	YES [22]
microRNA199b-3p	Ubiquitin mediated proteolysis	7	4.76E-12	YES [23]
microRNA215	ErbB signaling pathway	9	1.78E-09	YES [24]
microRNA363	Regulation of actin cytoskeleton	8	2.74E-09	-
microRNA335	Long-term potentiation	6	2.74E-09	-
microRNA18b	Gap junction	8	2.74E-09	YES [25]
microRNA141	Ascorbate and aldarate metabolism	3	9.60E-09	-
microRNA-645	Calcium signaling pathway	8	9.60E-09	YES [26]
	MAPK signaling pathway	9	4.30E-07	YES [27]

-indicates no reports or an inability to retrieve relevant reports.

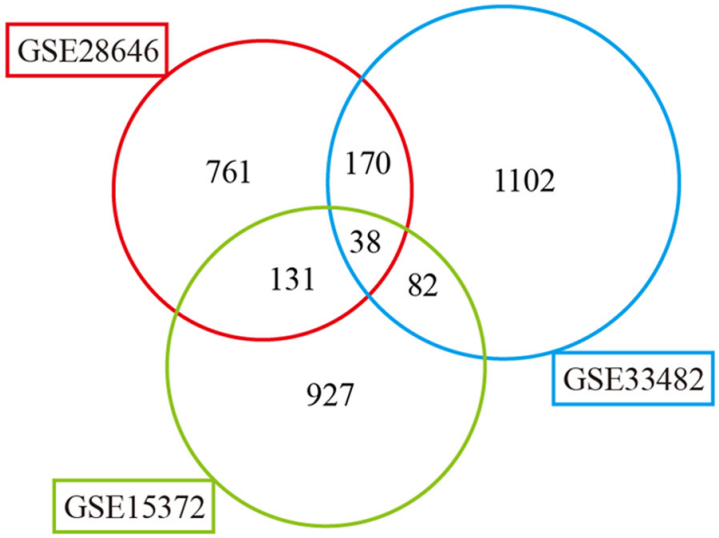

Figure 2. Analysis of the differentially expressed genes in the three mRNA expression profiling datasets using the GEO2R tool. Note: Thresholds used in the GEO2R analysis: adj. P < 0.05, fold > 2.

the Imperial College of London using the GPL570 microarray platform. The data were derived from cisplatin-resistant A2780 ovarian cancer cells (A2780-CP70) and parental A2780 cells (three repeats of each). GSE15372 originated from the Indiana University School of Medicine using the GPL570 microarray platform. The data were derived from cisplatin-resistant A2780 ovarian cancer cells (Round5-A2780) and parental A2780 cells (five repeats of each) (**Table 1**).

Differentially expressed microRNAs and pathway enrichment

The drug-resistant A2780 ovarian cancer cells and the parental A2780 cells used to generate the GSE54665 expression profiling datasets served as the experimental group and the control group, respectively, and these groups were screened for differentially expressed microRNAs using the GEO2R tool. As shown in **Table 2**, after rigorous screening using adj. P < 0.05 and fold > 2 as thresholds, a total of nine differentially expressed microRNAs were identified from the GSE54665 microarray dataset. One microRNA (microRNA-141) was significantly upregulated in drug-resistant cells, whereas the expression levels of the remaining eight microRNAs were decreased. We evaluated the nine differentially expressed microRNAs through qRT-PCR and found that the expression levels of microRNA-199a-5p, microRNA199a-3p, microRNA199b-3p, microRNA-645, microRNA-335, microRNA-18b, and microRNA-141 in SKOV3/DDP and A2780/DDP cells were in agreement with the results from the microRNA chip (**Figure 1A**, **1B**). Among these differentially expressed microRNAs, microRNA-335 [19] and microRNA-141 [20] have been proven to be associated with the regulation of drug resistance in ovarian cancer. Pathway enrichment analysis was performed on these nine differentially expressed microRNAs using DIANA miRPath [8]. A total of 67 signaling pathways were identified (P < 0.05), and the 10 most significantly enriched pathways were selected according to their *P* values (**Table 3**).

Table 4. The seven differentially expressed genes that exhibited exactly the same expression pattern trends in all three microarray datasets

Gene name	LogFC*			Literature related to drug resistance in tumors
	GSE15372	GSE28646	GSE33482	
NHSL1	-2.01	-2.79	-2.07	-
EPHA3	3.87	3.25	2.01	Related to drug resistance in melanoma [28]
EPHA7	2.84	4.72	7.77	Relate to chemosensitivity in colon cancer [29]
PI15	2.76	3.85	4.78	-
SNCA	3.2	5.51	10.57	-
USP51	2.89	2.81	2.165	-
ZSCAN4	2.03	2.14	5.02	-

*A positive logFC value indicates that the gene is downregulated in the drug-resistant group, and a negative logFC value indicates that the gene is upregulated in the drug-resistant group.

Screening for differentially expressed genes in three sets of mRNA microarrays and analysis of the correlation between differentially expressed genes and drug resistance in ovarian cancer

In the present study, drug-resistant A2780 ovarian cancer cells and parental A2780 cells were used as the experimental group and the control group, respectively. The GSE33482, GSE28646, and GSE15372 datasets were screened using the GEO2R tool to identify genes that were differentially expressed between the experimental and control groups, and 1102, 761, and 927 differentially expressed genes were identified, respectively. A total of 38 genes were found to be significantly differentially expressed in all three microarray sets (**Figure 2**). (The list of the 38 differentially expressed genes is shown in Supplementary Table 3) Seven of the 38 genes, NHS-like 1 (NHSL1), ephrin type-A receptor 3 (EPHA3), ephrin type-A receptor 7 (EPHA7), peptidase inhibitor 15 (PI15), synuclein alpha (SNCA), ubiquitin-specific peptidase 51 (USP51), and zinc finger and SCAN domain-containing protein 4 (ZSCAN4), exhibited exactly the same expression trends in all three microarray sets (**Table 4**), and qRT-PCR analysis showed that the expression of NHSL1, EPHA3, USP51, ZSCAN4, EPHA7, and PI15 in A2780/DDP cells coincided with results from mRNA chips (these genes were not expressed in SKOV3/DDP or SKOV3 cells) (**Figure 1C**).

To reveal the biological significance of the commonly differentially expressed genes in the regulation of ovarian cancer drug resistance at the unitary level, biological pathway enrichment and biological process annotation were performed on the above-described 38 genes using DAVID and ToppGene, respectively. DAVID-based signaling pathway enrichment analysis identified cytokine-cytokine receptor interaction pathways, whereas the ToppGene-based analysis identified a total of 32 biological processes that were significantly related to the 38 genes ($P < 0.05$). Among the 32 biological processes, cell adhesion and immunomodulatory responses were found to be related to the regulation of drug resistance in ovarian cancer (**Figure 3A**). To further demonstrate the relationship between the 38 commonly differentially expressed genes and the regulation of drug resistance in ovarian cancer, text mining was conducted using the COREMINE tool. Co-occurrence analysis of the literature was conducted using the gene names and "Ovarian Neoplasms", "Drug Resistance", and "Drug Resistance, Neoplasm" as keywords. The results revealed that 22 genes were present in the text-mining networks. Among these 22 genes, 13 genes were related to ovarian cancer; 11 co-occurred with tumor drug resistance and multidrug resistance in the literature; and nine (aldehyde dehydrogenase 1 family, member A1 (ALDH1A1), JUN, C-C chemokine receptor type 5 (CCR5), SNCA, lysyl oxidase (LOX), cluster of differentiation 55 (CD55), tropomyosin 1 (TPM1), filamin A-interacting protein 1 (FILIP1), and transforming growth factor beta 2 (TGFB2)) were significantly related to ovarian cancer, multidrug resistance, and tumor drug resistance. In addition to significant correlations with "Ovarian Neoplasms", "Drug Resistance", and "Drug Resistance, Neoplasm", the genes were also significantly related to each other, with the relationship between

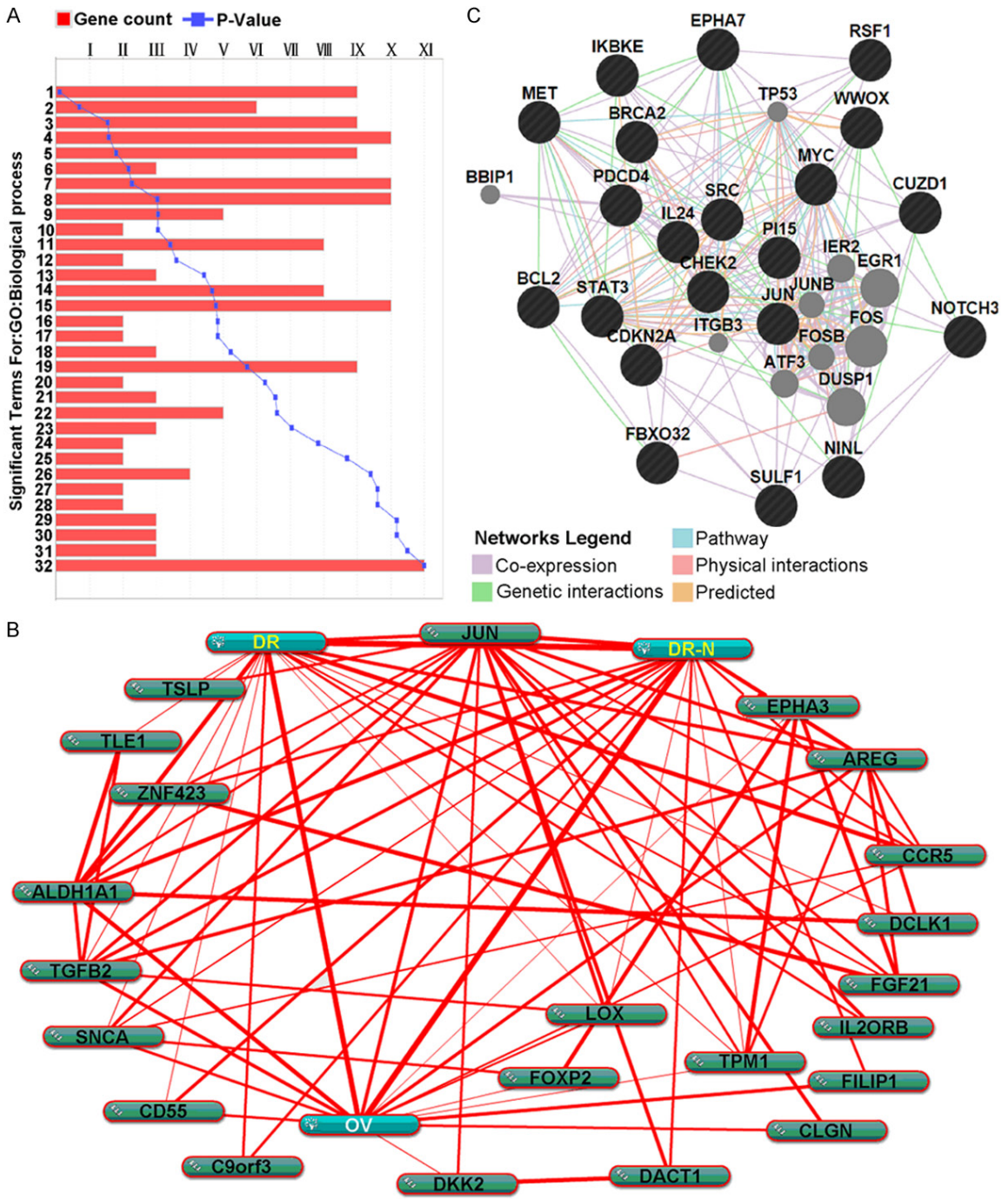

Figure 3. Bioinformatic analysis of different expressed genes. A. Biological processes of different genes related to ovarian cancer. B. Diagram of the linear relationship between the differentially expressed genes retrieved using COREMINE and drug resistance and the linear relationships between differentially expressed genes (Note: DR: drug resistance; DR-N: drug resistance, Neo~; OV: ovarian carcinoma.) C. Diagram of EPHA7 protein/gene and PI15 protein/gene interactions. note: 1. response to lipid, 2. regulation of cell adhesion, 3. neuron development, 4. neruon differentiation, 5. enzyme linked receptor protein signaling, 6. positive regulation of neuron apoptotic process, 7. response to oxygen-containing compound, 8. generation of neurons, 9. neuron death, 10. dopamine biosynthetic process, 11. neuron projection development, 12. regulation of acyl-CoA biosynthetic process, 13. positive regulation of neuron death, 14. organ morphogenesis, 15. neurogenesis, 16. regulation of cafactor metabolic process, 17. regulation of coenzyme metabolic process, 18. regulation of acute inflammatory response, 19. cell projection organization, 20. regulation of swlfur metabolic process, 21. negative regulation of immune response, 22. eye development, 23. negative regulation of immune effector process, 24. microglial cell activation, 25. SMAD protein import into nucleus, 26. regulation of neuron apoptotic process, 27. catecholamine biosynthetic process,

28. catechol-containing compound biosynthetic process, 29. negative regulation of sequestering of calcium ion, 30. release of sequestered calcium ion into cytosol, 31. regulation of sequestering of calcium ion, 32. regulation of phosphorus metabolic process.

ALDH1A1 and JUN presented as an example **Figure 3B**.

Analysis of the correlation between differentially expressed microRNAs and the differentially expressed genes associated with drug resistance in ovarian cancer

The correlation between the 9 dysregulated microRNAs (**Table 2**) and the 38 differentially expressed genes listed in Supplementary Table 3 was analyzed using five microRNA-mRNA interaction tools including miRanda, miRBase, TargetScan, miRWalk, and RNA22. Genes identified by at least three prediction tools were selected as candidate targets, and the intersections of the candidate target genes with the 38 common differentially expressed genes in the three microarray datasets were determined. As shown in **Table 2**, C1orf21 was predicted as the target of the nine microRNAs, PI15 and interleukin 20 receptor beta (IL20RB) were predicted as the target of five microRNAs including microRNA199a-5p, microRNA199a-3p, microRNA199b-3p, microRNA-141 and microRNA-363. In particular, EPHA7 and PI15 were identified as the potential target of microRNA-141, for which their expressions were negatively correlated in drug resistant ovarian cancer cells

Analysis of interactions between either EPHA7 or PI15 and the protein-coding genes associated with drug resistance in ovarian cancer

The relationships between EPHA7 or PI15 and drug resistance-related oncogenes or tumor-suppressor genes in ovarian cancer were analyzed using the GeneMANIA tool (http://www.genemania.org/). Among the 15 tumor suppressor genes [3] and 25 oncogenes [30] that have been reported to be related to the regulation of drug resistance in ovarian cancer, 19 were found to directly interact with PI15 and EPHA7 through co-expression, genetic interaction, physical interaction, and joint participation in signaling pathways (**Figure 3C**). Among the 19 directly interacting genes, 10 were found to directly interact with PI15, whereas nine directly interacted with EPHA7. The oncogene CUB and zona pellucida-like domain-containing protein 1 (CUZD1) were found to directly interact with both EPHA7 and PI15. In addition, in protein networks, tumor protein p53 (TP53) [31], early growth response 1 (EGR1) [32], FOS [33], and other genes were associated with the regulation of drug resistance in ovarian cancer.

Discussion

Ovarian cancer is a malignancy that poses a serious threat to women's health. Ovarian cancer has the highest degree of malignancy of all gynecological cancers [34]. Postoperative chemotherapy is the main approach employed for the treatment of ovarian cancer, and multidrug resistance is an important reason for the failure of chemotherapy [2]. Previous studies have shown that multidrug resistance in ovarian cancer is related to various factors, including differential expression of microRNAs, differential expression of genes, and microRNA-controlled differential gene expression [35, 36]. Therefore, screening for and identifying microRNAs and genes that are differentially expressed between drug-resistant and drug-sensitive cells and investigating the correlations between differentially expressed microRNAs and genes may shed light on the molecular mechanisms underlying drug resistance in ovarian cancer and may provide guidance for clinicians when choosing chemotherapy regimens and predicting prognosis.

GEO2R [7] (http://www.ncbi.nlm.nih.gov/geo/geo2r/) is an R programming language-based analytical tool for studying differentially expressed genes. In the present study, microRNA expression microarray data for platinum-resistant and platinum-sensitive ovarian cancer cells (GSE54665) were analyzed, and nine differentially expressed microRNAs were identified. Eight of these microRNAs were downregulated, whereas one microRNA (microRNA-141) was upregulated (**Table 2**). We identified nine differentially expressed microRNAs through qRT-PCR and found that the results of microRNA199a-5p, microRNA199a-3p, microRNA199b3p, microRNA-645, microRNA-335, microR-

NA-18b, and microRNA-141 expression analysis in SKOV3/DDP and A2780/DDP cells agreed with the results obtained from the microRNA chip (**Figure 1A**, **1B**). Among the top 10 signaling pathways regulated by the nine identified microRNAs, at least seven were related to the regulation of drug resistance in ovarian cancer, including the focal adhesion pathway [21], the phosphatidylinositol 3-kinase (PI3K-Akt) signaling pathway [22], the erythroblastosis oncogene B (ErbB) signaling pathway [24], the mitogen-activated protein kinase (MAPK) signaling pathway [27]. These findings indicate that the nine microRNAs that are differentially expressed in drug-resistant ovarian cancer cells may be associated with the regulation of drug resistance in ovarian cancer. Previous studies have shown that microRNA199a-3p promotes the sensitivity of hepatoma cells to doxorubicin by targeting mammalian target of rapamycin (mToR) and c-Met [36], microRNA199a-5p is related to multidrug resistance in colon cancer [37] and cisplatin resistance in liver cancer [38]. MicroRNA-215 is related to chemoresistance and the loss of sensitivity to radiotherapy in colon cancer [39], and microRNA-141 participates in the regulation of drug resistance in ovarian cancer by regulating Kelch-like ECH-associated protein 1 (KEAP1) [20].

Three sets of mRNA expression profiles were analyzed, and a total of 38 genes were identified as differentially expressed in drug-resistant ovarian cancer cells. Enrichment analysis of biological processes and signaling pathways showed that the above-described 38 differentially expressed genes are significantly related to a series of biological processes such as cell adhesion, immunomodulation, and cytokine-cytokine receptor interactions. These processes have been proven to be closely related to the regulation of drug resistance in ovarian cancer. A previous study has demonstrated that cell adhesion is a critical step leading to the occurrence of peritoneal metastasis [40]. Peritoneal metastasis indicates that ovarian cancer is already at an advanced stage, which is frequently associated with a lack of sensitivity to chemotherapy. Cytokine-cytokine receptor interaction pathways are involved in cell growth, differentiation, and death as well as blood vessel formation, and further study had indicated that the involvement of cytokine-cytokine receptor interactions in drug resistance would proceed via tumor-stroma communication [41].

These results reveal that the 38 genes that are significantly differentially expressed in all three drug-resistant ovarian cancer cell-derived gene expression profile datasets may be involved in the regulation of drug resistance in ovarian cancer through participating in processes such as cell adhesion, immunomodulatory responses, and cytokine-cytokine receptor interactions.

Text mining was performed on the 38 genes using the COREMINE search and analysis tool. We discovered that ALDH1A1, JUN, CCR5, SNCA, LOX, CD55, TPM1, FILIP1, and TGFB2 are significantly correlated with ovarian cancer, drug resistance, and multidrug resistance in tumors. These results indicate that the above-mentioned genes may be involved in the regulation of drug resistance in ovarian cancer, which is consistent with the findings of previous studies. Four of the nine genes described above, ALDH1A1, JUN, LOX, and CD55, have been shown to be related to drug resistance in ovarian cancer [42-45].

Epigenetic regulation of microRNAs plays an important role in the regulation of drug resistance in ovarian cancer. Aberrant gene expression due to the action of microRNAs is an important mechanism underlying drug resistance in ovarian cancer. Therefore, in the present study, microRNA-mRNA interaction analysis was conducted using microRNA and mRNA expression profiles to obtain additional information related to the regulation of drug resistance in ovarian cancer. By analyzing interactions between differentially expressed microRNAs and differentially expressed genes in drug-resistant and drug-sensitive ovarian cancer cells, we discovered that nine microRNAs may have regulatory effects on nearly half of the 38 identified genes. These findings indicate that the differentially expressed microRNAs and genes described above may act in concert to regulate drug resistance in ovarian cancer. MicroRNA-target relationships existed between C1orf21 and all nine microRNAs as well as between PI15 or IL20RB and three of the nine microRNAs.

Among the 38 genes that were identified to be associated with drug resistance in ovarian can-

cer, EPHA7 and PI15 might significantly correlate with drug resistance in ovarian cancer. Firstly, only seven (NHSL1, EPHA3, EPHA7, PI15, SNCA, USP51, and ZSCAN4) of the 38 genes exhibited exactly the same expression trends in the three mRNA microarrays, from which the EPHA7 and PI15 were included. The expression of EPHA7 and PI15 was significantly downregulated in the three independent drug-resistant A2780 ovarian cancer cells (a fold change of at least 2.7 was noted in both genes), and our qRT-PCR data showed that the expression of six genes, including NHSL1, EPHA3, USP51, ZSCAN4, EPHA7, and PI15, in A2780/DDP cells was consistent with data from mRNA chips (these genes were not expressed in SKOV3/DDP or SKOV3 cells) (**Figure 1C**). Secondly, the negative regulatory relationships between microRNA-141 and the two genes were predicted. MicroRNA-141 was found to be highly expressed in drug-resistant A2780 ovarian cancer cells. MicroRNA-mRNA interaction analysis showed that EPHA7 and PI15 are potential target genes of microRNA-141. Additionally, a previous study showed that microRNA-141 is significantly related to the regulation of drug resistance in ovarian cancer [20]. Third, protein/gene interaction analysis showed that PI15 and EPHA7 directly interact with multiple genes related to drug resistance in ovarian cancer through genetic co-expression, genetic interaction, physical interaction, and joint participation in signaling pathways. In a protein/gene interaction network composed of 31 genes, PI15 directly interacts with a total of 11 genes. Among these 11 genes, five are oncogenes (inhibitor of nuclear factor kappa-B kinase subunit epsilon (IKBKE), ninein-like protein (NINL), neurogenic locus notch homolog protein 3 (NOTCH3), signal transducer and activator of transcription 3 (STAT3), and CUZD1), and six are tumor-suppressor genes (cyclin-dependent kinase inhibitor 2A (CDKN2A), F-box protein 32 (FBXO32), interleukin 24 (IL24), programmed cell death protein 4 (PDCD4), sulfatase 1 (SULF1), and WW domain-containing oxidoreductase (WWOX)). EPHA7 was found to directly interact with a total of nine genes, among which seven are oncogenes (B-cell lymphoma 2 (BCL2), CUZD1, JUN, MET, MYC, remodeling and spacing factor 1 (RSF1), and SRC), and two are tumor-suppressor genes (breast cancer 2 early onset (BRCA2), and checkpoint kinase 2 (CHEK2)). The oncogene CUZD1 was found to directly interact with both PI15 and EPHA7. The above-mentioned oncogenes and proto-oncogenes have been proven to be related to the regulation of multidrug resistance in ovarian cancer [3, 30]. For example, the STAT3 gene is related to platinum and paclitaxel resistance in ovarian cancer [35].

These results indicate that PI15 and EPHA7, which are significantly correlated with the 22 drug resistance-related protein-coding genes in ovarian cancer, are also likely to be associated with the regulation of drug resistance in ovarian cancer. Fourth, through a query of signaling pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG), we found that EPHA7 is upstream of the MAPK pathway, which is a critical signaling pathway that regulates drug resistance in ovarian cancer. In summary, PI15 and EPHA7 are important candidate protein-coding genes that may be involved in the regulation of drug resistance in ovarian cancer. The exact roles of PI15 and EPHA7 will require further in-depth study.

Disclosure of conflict of interest

None.

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Supplementary Table 1. Primer list 1

Primer	sequences (5'- > 3')
microRNA-141-5p	TCCAACACTGTACTGGAAGATG
microRNA-141-3p	CCATCTTTACCAGACAGTGTTAGC
microRNA-199a-5p	GAAACAGGTAGTCTGAACACTGGG
microRNA-199a-3p	CTAACCAATGTGCAGACTACTGT
microRNA-199b-3p	TAACCAATGTGCAGACTACTGT
microRNA-335-5p	ACATTTTTCGTTATTGCTCTTGAGC
microRNA-335-3p	GGTCAGGAGCAATAATGAAAAAGC
microRNA-215-5p	GTCTGTCAATTCATAGGTCATGC
microRNA-215-3p	TATTGGCCTAAAGAAATGACAGAGC
microRNA-363-5p	GAATTGCATCGTGATCCACCCG
microRNA-363-3p	TACAGATGGATACCGTGCAATTGC
microRNA-18b-5p	CTAACTGCACTAGATGCACCTTAG
microRNA-18b-3p	AGAAGGGGCATTTAGGGCAG
microRNA-645	TCAGCAGTACCAGCCTAGAG
U6	CAAGGATGACACGCAAATTCG

Supplementary Table 2. Primer list 2

	Primer	sequences (5'- > 3')
GAPDH	Forward primer	GTCAAGGCTGAGAACGGGA
	Reverse primer	AAATGAGCCCCAGCCTTCTC
NHSL1	Forward primer	TGAGGGAATGCGATAAGTTGC
	Reverse primer	TGGTCCAGTTCGTCTGAGTATT
EPHA3	Forward primer	AGCTGGGCTGGATCTCTTATC
	Reverse primer	GGTAAGTCCTGATGGGTGTGT
SNCA	Forward primer	TGGTGAGCGAAACAGAAGCC
	Reverse primer	CCATAGCAACCTGCGTAATGAA
USP51	Forward primer	CCAGGTTCGAGAAACTTCTTTGC
	Reverse primer	TCACGCTCTTGTAATGGCTCC
ZSCAN4	Forward primer	TTTCAGTGTGAACCATCCGAG
	Reverse primer	AGCACCATTCTTGAGAACTCAG
EPHA7	Forward primer	AGAACTATACCCCGATACGAACA
	Reverse primer	TGGAAATCCAGTTAGTCCGCA
PI15	Forward primer	CAAGTACCGTCGTCCTACTCA
	Reverse primer	TCCGCTGAATCTAATTGTGCTT

Supplementary Table 3. 38 differentially expressed genes

Gene name	Gene ID	Gene name	Gene ID
ALDH1A1	216	JUN	3725
AREG	374	LOX	4015
ASXL3	80816	MAB21L1	4081
C1orf21	81563	OLFM3	118427
81563	84909	OTUD1	220213
CCDC178	374864	PDK3	5165
CCR5	1234	RTN1	6252
CD55	1604	TGFB2	7042
CLGN	1047	TLE1	7088
CNN1	1264	TPM1	7168
DACT1	51339	TSLP	85480
DCLK1	9201	NHSL1	57224
DKK2	27123	PI15	51050
FGF21	26291	SNCA	6622
FILIP1	27145	USP51	158880
FOXP2	93986	EPHA3	2042
GSG1	83445	EPHA7	2045
IL20RB	53833	ZNF423	23090
JAM2	58494	ZSCAN4	201516