Upregulation of miRNA-450b-5p targets ACTB to affect drug resistance and prognosis of ovarian cancer via the PI3K/Akt signaling pathway

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Background: Ovarian cancer (OC) is the most malignant gynecologic cancer, and chemoresistance is a major cause of treatment failure in patients with OC. The understanding of microRNA (miRNA) in cancer is limited, and the role of miRNA (miR)-450b-5p in cancer drug resistance is unknown. In this study, we aim to evaluate the role of miR-450b-5p in drug-resistant OC and its underlying mechanisms.

Methods: MiR-450b-5p expression was assessed in drug-sensitive and resistant OC cells via quantitative real-time polymerase chain reaction. Cell viability was evaluated using the Cell Counting Kit-8 assay. Progression-free survival (PFS) and overall survival (OS) curves were generated using the Kaplan-Meier method and the log-rank test. Target genes of miR-450b-5p were identified from the Cancer MIRNome database. Co-expressed genes were obtained from The Cancer Genome Atlas and Cancer Genome cBioportal for pathway enrichment and functional clustering analysis.

Results: The miRNA-450b-5p expression was significantly increased in A2780 and SKOV3 OC-resistant cells and significantly increased by 17-fold in the A2780-CBP-Lv-miR-450b-5p cells compared to A2780-CBP and A2780-CBP-Lv-NC cells. The up-regulated expression of miR-450b-5p increased the cell viability and half maximal inhibitory concentration (IC_{50}) of A2780 platinum-resistant cells and was associated with poor OS. We obtained 33 potential target genes of miR-450b-5p and beta-actin (ACTB) might be a potential target of miR-450b-5p. Low expression of ACTB predicted poor OS and PFS. We obtained 362 common genes co-expressed with ACTB, which involved 4 critical pathways. PI3K acted as an upstream pathway of the other three pathways, which ultimately responded to drug resistance regulation in OC. The genes enriched in four pathways were cross-analyzed and 13 overlapping genes were obtained. These 13 genes were also significantly and positively co-expressed with ACTB at both protein and mRNA levels.

Conclusions: High expression of miRNA-450b-5p might affect drug resistance and prognosis in OC by targeting 13 co-expressed genes of ACTB directly through the PI3K/Akt signaling pathway. Thus, miR-450b-5p might provide a new therapeutic target for drug resistance in OC.

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Introduction

Globally, ovarian cancer (OC) is the eighth most common cancer in women (1), and it seriously endangers the health of women worldwide. The current first-line treatment for OC is surgical resection combined with platinum and paclitaxel chemotherapy (2). However, chemoresistance is the most frequent reason for chemotherapy failure in patients with OC. Therefore, novel therapeutic targets to overcome the issue of drug resistance in OC are urgently needed.

Drug resistance is a complex process involving multiple factors, including multiple genes and a variety of biological events, such as events related to the drug efflux, and the regulation of tumor suppressor gene/oncogene expression (3,4). Tumor drug resistance-related genes participate in regulating tumor resistance through multiple pathways. For example, microRNA (miRNA)-21 enhances resistance in OC cells by promoting M2 macrophage polarization to regulate tumor immune infiltration (5). In OC patients with BRCA mutations, high tumor mutation burden and alterations in drug efflux-related genes are associated with differences in progression-free survival (PFS) on PARP inhibitors (6). The increased expression of miR-181a/135a/302c promotes sensitivity of microsatellite instability (MSI) colorectal cancer cells to 5-fluorouracil treatment (7).

MiRNAs are important members of non-coding RNA that have the potential to serve as biomarkers in cancer diagnosis and prognosis and they also play a critical role in regulating cancer drug resistance (8). Research has shown that miR-139-5p and MAPK pathway inactivation can overcome cisplatin resistance in OC by inhibiting RNF2 (9). MiR-590-5p promotes cisplatin resistance in OC by negatively regulating hMSH2 expression (10). Overexpression of miR-296-3p significantly enhances proliferation, migration, invasion, and drug resistance of OC cells *in vitro*, as well as tumor growth *in vivo* (11). These studies suggest a critical regulatory role of miRNA expression in drug-resistant OC.

MiR-450b-5p is a miRNA that contributes to the development of malignant tumors, including lung squamous cell carcinoma (12), cervical cancer (13) and glioblastoma (14). However, most studies of miR-450b-5p have focused on ischemia/reperfusion (15,16), and studies on its involvement in tumor drug resistance and OC is scarce. We herein hope to investigate the role of miR-450b-5p in drug-resistant OC and its potential mechanisms through experimental exploration, integrated bioinformatics analyses, and big data analysis. Our study represents the inaugural investigation the role of miR-450b-5p in drug-resistant OC. Additionally, through integrating bioinformatics and big data analysis, we predicted ACTB as a potential target of miR-450b-5p. ACTB is traditionally considered an endogenous housekeeping gene and has been widely used as a reference gene/protein to quantify tumor expression levels. However, ACTB as a target of miRNA regulation has rarely been reported and its regulatory role in cancer drug resistance remains unclear. Our study analyzed the possibility of miR-450b-5p/ACTB/PI3K/Akt axis, which is expected to provide a reference for future studies on the role of ACTB in cancer and the mechanisms by which other miRNAs regulate tumor drug resistance. Through this research, we aim to provide

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Highlight box

Key findings
• Upregulation of miR-450b-5p affects drug resistance and prognosis in ovarian cancer (OC).

What is known and what is new?
• MiR-450b-5p has been found to promote the progression and drug resistance of colorectal cancer and hepatocellular carcinoma. Additionally, miR-450b-5p plays a significant role in ameliorating liver ischemia/reperfusion injury and acute myocardial infarction.
• The effect of miR-450b-5p in drug-resistant OC and its possible mechanism remains unclear. Our study observed for the first time that high expression of miR-450b-5p promotes cisplatin resistance in OC cells. Furthermore, we predicted that beta-actin (ACTB) may be a potential target of miR-450b-5p and conducted the first analysis of the potential miR-450b-5p/ACTB/PI3K/Akt axis. This study provides further insights into the role of miR-450b-5p and ACTB in drug-resistant OC.

What is the implication, and what should change now?
• MiR-450b-5p may serve as a new therapeutic target for OC treatment and a new biomarker for prognosis.

support for the potential clinical application of miR-450b-5p in targeted therapy and to offer new treatment targets and strategies for overcoming drug resistance in OC. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-292/rc).

Methods

Cell lines and cell culture

OC cell lines A2780 and SKOV3 were stored in our laboratory. The carboplatin-resistant cells (A2780-CBP and SKOV3-CBP) and the cisplatin-resistant cells (A2780-DDP and SKOV3-DDP) were established from A2780 and SKOV3 cells by treating with increasing concentrations of carboplatin and cisplatin. The cell lines were maintained in RPMI-1640 medium (Wisent corporation, Nanjing, China) containing 10% fetal bovine serum (10099141, Thermo Fisher Scientific, Thornton, NSW, Australia) at 37 ℃ with 5% CO_2. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Lentiviral vectors and cell transfection

MiR-450b-5p-overexpressing lentiviral vectors LV-3 (pGLVH1/GFP+Puro) and miR-negative control (miR-NC) were obtained from GenePharma (C06011, Suzhou, China). A2780-CBP cells were seeded into six-well plates with 1.5×10^5 cells per well. The cell density was 30–50%. The medium was removed before transfection and washed gently twice with PBS. Polybrene was mixed with antibiotic-free RPMI-1640 medium at a final concentration of 5 μg/mL; 900 μL of the mixture was used and mixed with 100 μL of virus solution, added to six-well plates with two replicate wells each and replaced after 24 h with medium containing 2 μg/mL of puromycin, and the culture was continued for 72 h to observe. The remaining cells continued to be screened with puromycin, and the cells were promptly changed and passaged according to the cell growth to ensure good cell growth.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Complementary DNA (cDNA) synthesis and measurement of miR-450b-5p by qRT-PCR were performed using the All-in-One miRNA qRT-PCR Detection Kit (AOMD-Q050, GeneCopoeia, Guangzhou, China). Total RNA of A2780-CBP and -DDP, SKOV3-CBP and -DDP cells was extracted using TRIzol (15596026, Thermo Fisher Scientific, Carlsbad, CA, USA) and quantified using NanoDrop 2000 spectrophotometer (NanoDrop 2000, Thermo Scientific, Waltham, MA, USA). The total RNA (2 μg) was reverse transcribed, and qRT-PCR was performed with a total volume of 20 μL using the Mx3000P qPCR system (MxPro 3000p, Agilent, Palo Alto, USA). All primers were supplied by GeneCopoeia (The 3000p, Agilent, Palo Alto, USA). All primers were supplied by GeneCopoeia: 5'-CGTTTTGCAATATGTTCCTGAATA-3' for miR-450b-5p; 5'-CAAGGATGACACGCAAATTCG-3' for U6 (used as the internal standard). The qRT-PCR experiments were performed in triplicate.

Cell proliferation assay

Cell proliferation was determined with Cell Counting Kit-8 (CCK-8) assay. The miR-450b-5p overexpressed OC cells, negative and controls cells were cultured in 96-well plates of 1,000 per well. After incubation for 16–18 h, the cells were treated with varied concentrations of carboplatin and incubated for 72 h. Then, a volume of 10 μL of CCK-8 (Solarbio, Beijing, China) was added to each well for 2 h in a constant temperature incubator protected from light, and the absorbance values at 450 nm were detected by Multiskan GO Microplate Spectrophotometer (Thermo Fisher Scientific). Cell proliferation was observed in triplicate for each experiment.

Data acquisition

The starBase (ENCORI) database (http://starbase.sysu.edu.cn/) (17) was utilized to acquire the prognostic data for miR-450b-5p in 374 OC tissue samples. Genes co-expressed with ACTB in 558 OC tissue samples, 174 protein levels, and 90 drug-resistant OC tissue samples from The Cancer Genome Atlas (TCGA) (18) ovarian cohort were downloaded from the Cancer Genome cBioportal database (https:// www.cbioportal.org/) (19). The Cancer MIRNome database (http://bioinfo.jialab-ucr.org/CancerMIRNome) (20) was used to predict the targets of miR-450b-5p and to analyze their correlations with miR-450b-5p.

Statistical analysis
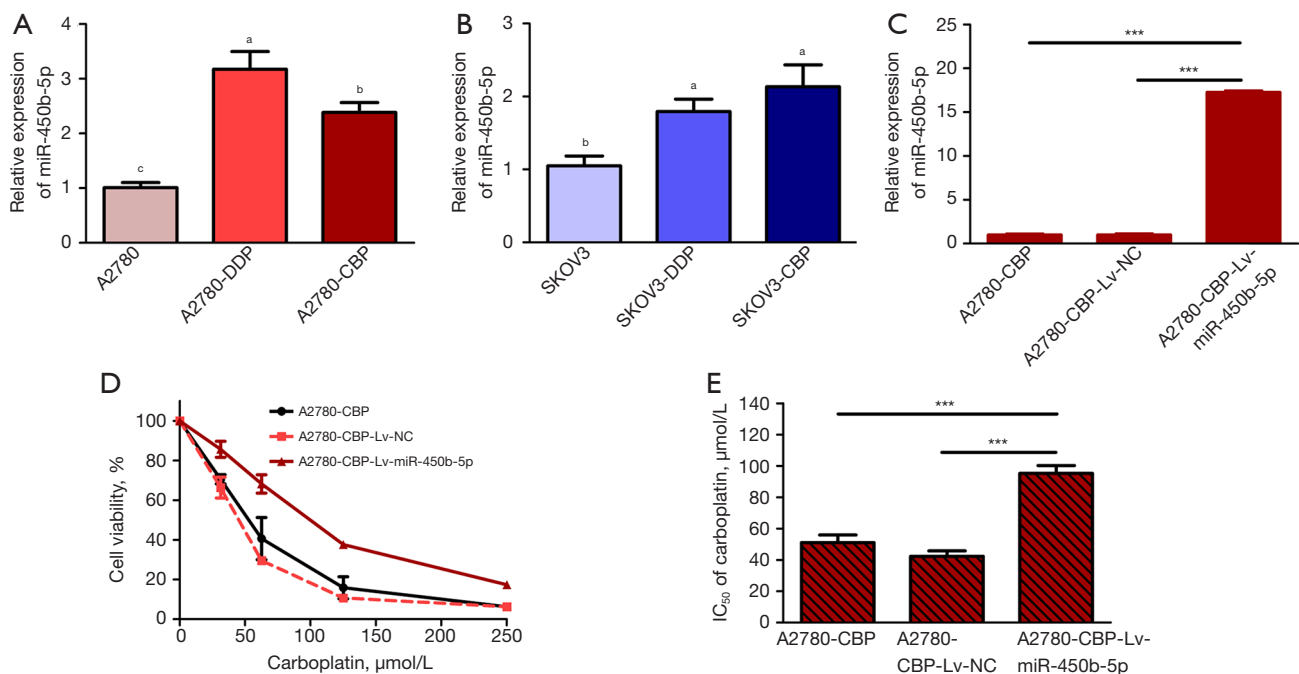
SPSS v23.0 software (IBM, Armonk, NY, USA) and

Figure 1 Overexpression of miR-450b-5p increased the resistance of OC cells to carboplatin. (A,B) qRT-PCR was used to measure the expression of miR-450b-5p in drug-sensitive and drug-resistant OC cells. The lowercase letters above the columns indicate significant differences among the groups (P<0.05 by one-way ANOVA and the LSD test). (C) qRT-PCR confirmed miR-450b-5p expression in A2780-carboplatin cells transfected with Lv-NC or Lv-miR-450b-5p (***, P<0.001). (D,E) Effect of Lv-NC or Lv-miR-450b-5p on cell viability in A2780-carboplatin cell lines according to the CCK-8 assay and carboplatin IC$_{50}$ (***, P<0.001). A2780-CBP, carboplatin-resistant A2780 cells; A2780-DDP, cisplatin-resistant A2780 OC cells; SKOV3-CBP, carboplatin-resistant SKOV3 cells; SKOV3-DDP, cisplatin-resistant SKOV3 OC cells; A2780-CBP-Lv-NC, negative A2780-carboplatin OC cells; A2780-CBP-Lv-miR-450b-5p, miR-450b-5p-overexpressing A2780 carboplatin cells; IC50, half maximal inhibitory concentration; OC, ovarian cancer; ANOVA, analysis of variance; LSD, least significance difference; qRT-PCR, quantitative real-time polymerase chain reaction; CCK-8, Cell Counting Kit-8.

GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA) were used for the statistical analyses. All data were presented as the mean ± standard deviation. The *t*-test and one-way analysis of variance (ANOVA) followed by the least significance difference (LSD) test were used to determine statistically significant differences. The Kaplan-Meier method was used to construct PFS and overall survival (OS) curves. The receiver operating characteristic (ROC) curve analysis was used to analyze the role of each gene in predicting OC cell drug resistance. Pearson's χ² test was used for the correlation analysis. KOBAS-i (KOBAS 3.0) (http://bioinfo.org/kobas) was used for pathway enrichment and functional clustering of genes that were co-expressed with ACTB. The level of statistical significance was set at P<0.05 (*, P<0.05; **, P<0.01; ***, P<0.001) for the *t*-test. The different lowercase letters above the columns indicate significant differences among groups (P<0.05 for one-way

ANOVA and the LSD test).

Results

High expression of miR-450b-5p enhanced drug resistance of OC cells to carboplatin and predicted short OS

We initially examined the expression of miR-450b-5p in drug-sensitive and drug-resistant OC cells. The expression of miR-450b-5p was significantly increased in both carboplatin- and cisplatin-resistant A2780 (*Figure 1A*) and SKOV3 (*Figure 1B*) cells compared with carboplatin- and cisplatin-sensitive cells (P<0.05), and miRNA expression in carboplatin-resistant cells was more stable and consistent than in cisplatin-resistant cells. To explore the impact of miR-450b-5p on carboplatin resistance in OC cells, the miR-450b-5p-overexpressing A2780-carboplatin cell line was constructed. MiRNA was significantly upregulated
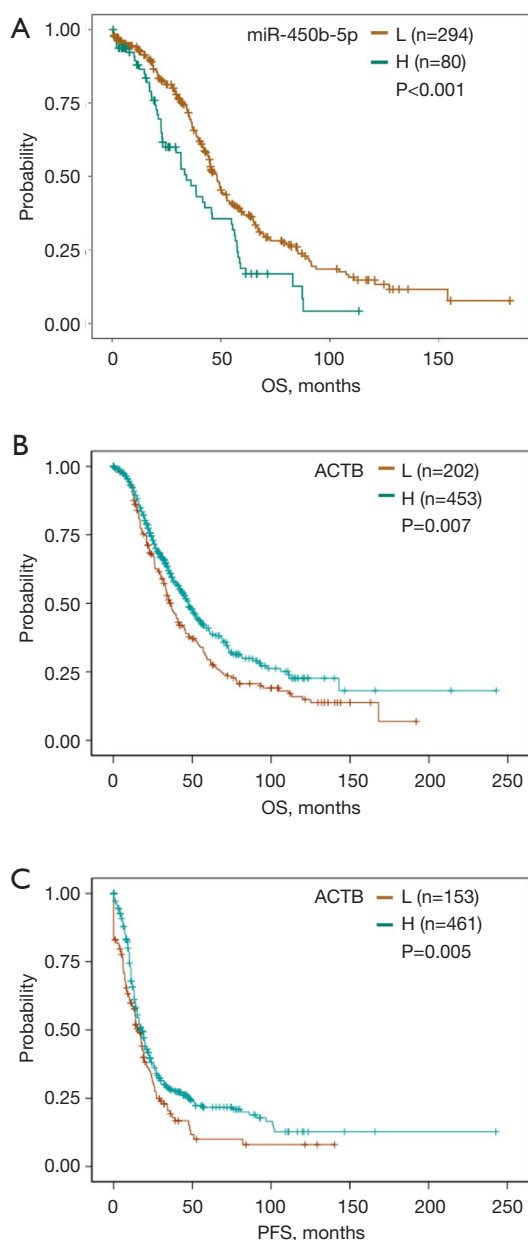
Figure 2 Progression analysis of RNA expression in association with OS and PFS in 374 and 655 patients with OC. (A) High miR-450b-5p expression predicted poor OS by ENCORI from TCGA database. (B) Low ACTB expression predicted poor OS according to the Kaplan–Meier method. (C) Low ACTB expression predicted poor PFS according to the Kaplan–Meier method. The Kaplan–Meier method dichotomized RNA expression into the high group (H) and low group (L) according to the best cut-off value, which was selected automatically. H, high RNA expression; L, low RNA expression; OS, overall survival; PFS, progression-free survival; OC, ovarian cancer; TCGA, The Cancer Genome Atlas; ACTB, beta-actin.

17-fold in the A2780-carboplatin-Lv-miR-450b-5p cells compared with A2780-carboplatin and A2780-carboplatin-Lv-NC cells (*Figure 1C*) (P<0.001). Subsequent assays revealed that miR-450b-5p overexpression significantly enhanced the viability (*Figure 1D*) and half-maximal inhibitory concentration (IC$_{50}$) (*Figure 1E*) of A2780-carboplatin cells to carboplatin (P<0.001). Furthermore, the relationship of the miRNA with the survival of patients with OC was investigated, and the results suggested that high miR-450b-5p expression was related to short OS based on 374 OC samples (P<0.001; *Figure 2A*).

ACTB may serve as a target of miR-450b-5p to affect drug resistance and prognosis

We investigated the potential targets of miR-450b-5p to reveal the possible mechanism of this miRNA in the regulation of OC progression. There were 33 genes identified as potential targets of miR-450b-5p. Among these 33 genes, low ACTB expression was significantly correlated with poor OS (P=0.007; *Figure 2B*) and PFS (P=0.005; *Figure 2C*). Through further analysis, we found that miR-450b-5p was significantly and negatively co-expressed with these 33 genes in OC tissues (P<0.01; *Table 1*; *Figure 3*). As high expression of miR-450b-5p was associated with drug resistance (*Figure 1*), the relationship between the low expression of the 33 target genes and drug resistance was investigated. Among the 33 genes, the expression of three genes, including *ACTB*, *RPL30*, and *TMEM59*, was decreased in multi-drug-resistant tissues and platin- and taxane-resistant tissues. Low expression of these three genes could effectively predict OC resistance to multiple drugs (resistance to any drug), platin, and taxane, respectively [area under the curve (AUC) >0.6] (*Figure 4*).

ACTB might affect OC drug resistance and prognosis through PI3K/Akt signaling

A pathway enrichment analysis of genes co-expressed with ACTB was performed to explore the possible molecular mechanisms underpinning the effects of ACTB on OC drug resistance and prognosis. A total of 5,253 genes that were co-expressed with ACTB at the mRNA level in 558 OC tissues were retrieved (Q<0.001). Of them, 3,241 were positively co-expressed and 2,012 were negatively co-expressed. A total of 1,485 genes that were significantly co-expressed with ACTB at the protein level in 174 OC tissues

Table 1 MiR-450b-5p potentially negatively regulated 33 target genes in OC tissue

Target ensemble	Target symbol[†]	Correlation[‡]	P value	BH.Adj.P
ENSG00000156482	RPL30	−0.137	8.42e−03	5.26e−02
ENSG00000075624	ACTB	−0.152	3.35e−03	3.36e−02
ENSG00000116209	TMEM59	−0.213	3.55e−05	8.91e−04
ENSG00000196743	GM2A	−0.281	3.50e−08	3.52e−06
ENSG00000103490	PYCARD	−0.244	2.02e−06	1.36e−04
ENSG00000015475	BID	−0.219	2.13e−05	7.15e−04
ENSG00000169021	UQCRFS1	−0.216	2.73e−05	7.85e−04
ENSG00000215193	PEX26	−0.185	3.38e−04	7.24e−03
ENSG00000124098	FAM210B	−0.161	1.84e−03	2.05e−02
ENSG00000113384	GOLPH3	−0.153	3.05e−03	3.23e−02
ENSG00000149547	EI24	−0.150	3.80e−03	3.64e−02
ENSG00000181649	PHLDA2	−0.149	4.01e−03	3.67e−02
ENSG00000053371	AKR7A2	−0.145	5.24e−03	4.21e−02
ENSG00000205423	CNEP1R1	−0.143	5.76e−03	4.28e−02
ENSG00000180233	ZNRF2	−0.142	5.98e−03	4.30e−02
ENSG00000173200	PARP15	−0.140	7.05e−03	4.73e−02
ENSG00000070761	CFAP20	−0.140	7.06e−03	4.73e−02
ENSG00000198160	MIER1	−0.137	8.32e−03	5.26e−02
ENSG00000149084	HSD17B12	−0.135	9.02e−03	5.26e−02
ENSG00000134954	ETS1	−0.135	9.17e−03	5.26e−02
ENSG00000118263	KLF7	−0.134	9.91e−03	5.45e−02
ENSG00000181061	HIGD1A	−0.131	1.18e−02	6.10e−02
ENSG00000006638	TBXA2R	−0.124	1.67e−02	7.62e−02
ENSG00000158042	MRPL17	−0.124	1.71e−02	7.64e−02
ENSG00000189306	RRP7A	−0.123	1.80e−02	7.71e−02
ENSG00000078401	EDN1	−0.122	1.87e−02	7.85e−02
ENSG00000111832	RWDD1	−0.120	2.11e−02	8.33e−02
ENSG00000119878	CRIPT	−0.114	2.85e−02	1.02e−01
ENSG00000243678	NME2	−0.112	3.05e−02	1.04e−01
ENSG00000181449	SOX2	−0.111	3.26e−02	1.07e−01
ENSG00000111816	FRK	−0.109	3.52e−02	1.12e−01
ENSG00000169860	P2RY1	−0.105	4.42e−02	1.33e−01
ENSG00000066557	LRRC40	−0.103	4.64e−02	1.37e−01

[†], names of 33 target genes potentially regulated by miR-450b-5p; [‡], expression correlation between miR-450b-5p and 33 target genes. OC, ovarian cancer; BH.Adj.P, Benjamini-Hochberg adjusted P value; ACTB, beta-actin.
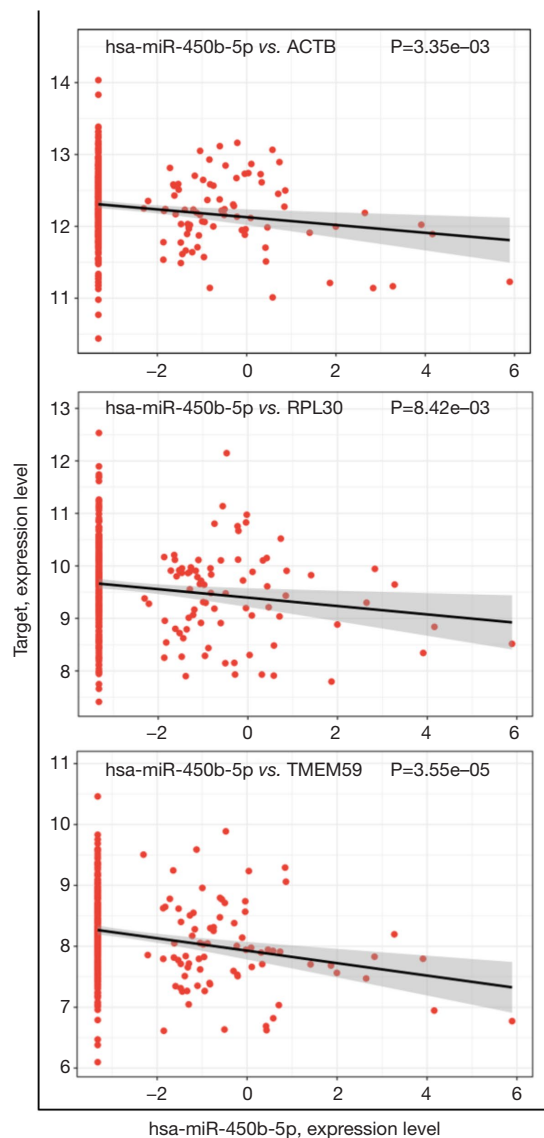
Figure 3 Correlation analysis between miR-450b-5p expression and the mRNA expression of its potential target genes (*ACTB*, *RPL30*, and *TMEM59*) in OC tissues. The expression of the three genes in OC tissues from the TCGA cohort according to the Cancer MIRNome database (P<0.01). OC, ovarian cancer; TCGA, The Cancer Genome Atlas; ACTB, beta-actin; RPL30, ribosomal protein L30; TMEM59, transmembrane protein 59.

were retrieved (Q<0.001). Of them, 845 were positively co-expressed and 640 were negatively co-expressed. The intersection of those genes identified a total of 680 overlapping genes that ACTB was co-expressed with at both the mRNA and protein levels, including 559 positively co-expressed genes and 121 negatively co-expressed genes.

Further, in 90 platinum-resistant tissues (a subgroup of 558 OC tissues), ACTB was co-expressed with 362 of 680 genes at the mRNA level. Collectively, ACTB was co-expressed with 362 genes in 90 platinum-resistant tissues and 558 OC tissues at the mRNA level, as well as in 174 OC tissues at the protein level. The 362 genes that were co-expressed with ACTB were enriched in 241 pathways, and these pathways were grouped into seven functional clusters (C1–C7). Among these functional clusters, the C2 cluster covered PI3K/Akt signaling, focal adhesion, extracellular matrix (ECM)-receptor interaction, and human papillomavirus infection (*Figure 5*), which are all typical pathways implicated in OC development and tumor progression. PI3K/Akt signaling is a downstream effector of the other three pathways. Specifically, these three upstream pathways converge to the downstream PI3K/Akt pathway according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in cancer (map04151).

To investigate how ACTB was involved in the four pathways, the intersection of the member genes in the four pathways was evaluated. Among the 362 genes co-expressed with ACTB, 16, 14, 25, and 16 genes were enriched in the PI3K/Akt pathway, focal adhesion, ECM-receptor interaction, and human papillomavirus infection, respectively, and 13 genes were involved in all four of these pathways, including *COL1A1*, *COL1A2*, *COL6A1*, *COL6A2*, *COL6A3*, *COMP*, *FN1*, *ITGA11*, *ITGA5*, *ITGB5*, *LAMA4*, *THBS1*, and *THBS2* (*Figure 6*). ACTB was positively co-expressed with these 13 overlapping genes in 558 OC tissues and 90 drug-resistant tissues at the mRNA level, as well as in 174 OC tissues at the protein level (*Figure 7*). Therefore, the results suggest a strong axis of 13 genes associated with ACTB in PI3K/Akt signaling in OC.

Discussion

Currently, chemoresistance is the brief reason for chemotherapy failure in OC. Hence, identifying target molecules that affect chemoresistance and prognosis in OC is of great significance for the treatment. MiRNAs play a crucial role in cancer drug resistance (21), prognosis (22), gene regulation (23) and tumor microenvironment (24), which have potential clinical implications for tumor therapy. For example, a study indicated that miRNAs have prominent diagnostic accuracy (AUC =0.99) in patients with stage I high-grade serous OC (25). Similarly, a study showed that serum miR-125b has potential as a diagnostic and prognostic biomarker in OC (26). Additionally,
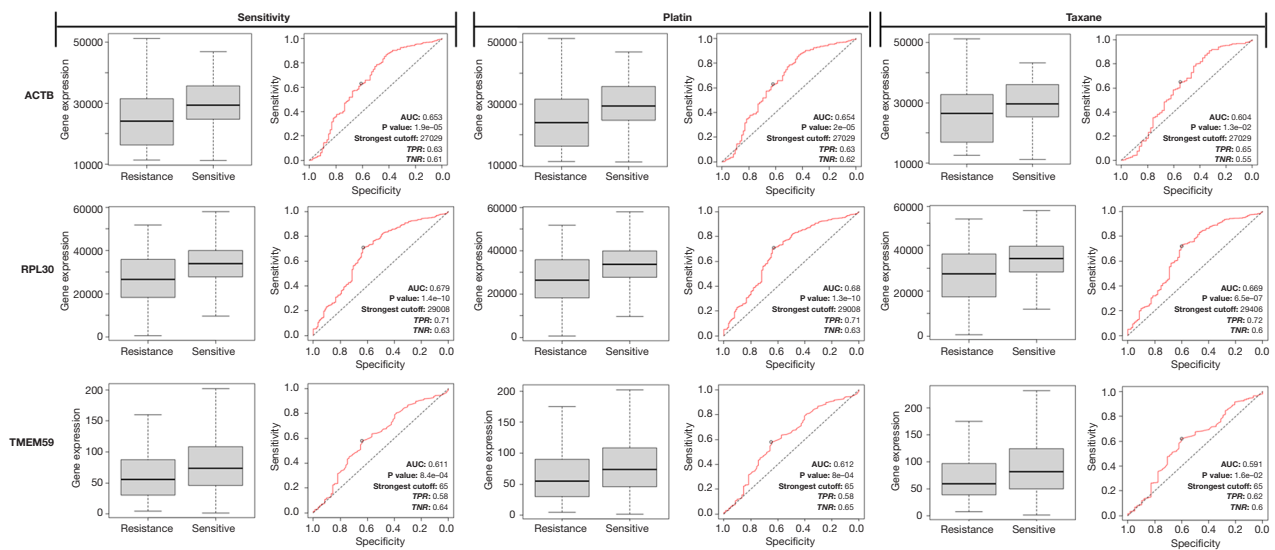
Figure 4 ROC curve analysis of the influence of potential target genes of miR-450b-5p, including *ACTB*, *RPL30*, and *TMEM59*, on drug resistance in OC. The expression of *ACTB*, *RPL30*, and *TMEM59* was compared in drug-resistant (non-responder) and sensitive (responder) OC tissues. Recurrence at 6 months after chemotherapy was used as the basis to determine sensitivity or resistance. For the grouping of chemotherapy drugs, three groups were included, namely any drug group, the platin group, and the taxane group. AUC, area under the curve; TPR, true positive rate; TNR, true negative rate; OC, ovarian cancer; ROC, receiver operating characteristic; ACTB, beta-actin; RPL30, ribosomal protein L30; TMEM59, transmembrane protein 59.

recent reviews have elucidated that miRNA promotes OC resistance by modulating ATP-binding cassette transporter (ABC)-mediated drug efflux, epithelial-mesenchymal transition or inhibiting apoptosis (27,28). One of the characteristic features of the tumor microenvironment in OC is the enrichment of cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) (29). MiR-141 can reprograms stromal fibroblasts into pro-inflammatory CAFs, promoting metastatic colonization in OC (30). The above studies suggest that exploring the potential mechanisms of miRNAs may provide a theoretical basis for the clinical treatment of OC-resistant. In the present study, we found that miR-450b-5p was highly expressed in OC platinum-resistant cells (*Figure 1A,1B*), and overexpression of this miRNA increased the resistance of OC cells to carboplatin (*Figure 1D,1E*). Moreover, high expression of miR-450b-5p predicted short OS (*Figure 2A*), suggesting that regulating miR-450b-5p expression may have potential implications for improving OS and providing clinical treatment strategies in OC patients.

Further, we predicted that ACTB, which is negatively co-expressed with miR-450b-5p, may be a potential target of miR-450b-5p (*Table 1*; *Figure 3*). We found that ACTB was decreased in multi-drug-resistant tissues and platin- and taxane-resistant OC tissues, and low expression of ACTB effectively predicted the occurrence of chemoresistance (*Figure 4*). In addition, low ACTB expression predicted shorter OS and PFS in patients with OC (*Figure 2B*). All of these results theoretically support the idea that ACTB might serve as a negative regulatory target of miR-450b-5p to affect drug resistance and prognosis. ACTB is a highly conserved cytoskeletal structural protein associated with cell growth and migration (31), but recent studies have shown that differential expression of ACTB plays a key role in a variety of tumors (32,33). It has also been reported that ACTB is decreased in OC tissues compared with normal tissues (34) and that it plays a critical role in OC progression (35), which is consistent with the results of our study.

We further indicated that miR-450b-5p may potentially regulate PI3K/Akt signaling in OC via their interactions with ACTB. PI3K/Akt signaling plays a crucial role in tumorigenesis and chemoresistance of OC and it is one of the essential signaling pathways in OC therapeutic interventions. For example, miR-126-5p promotes OC progression by targeting phosphatase and tensin homolog (PTEN) to activate the PI3K/Akt/mTOR pathway (35). MiR-181c increases paclitaxel sensitivity in OC cells by
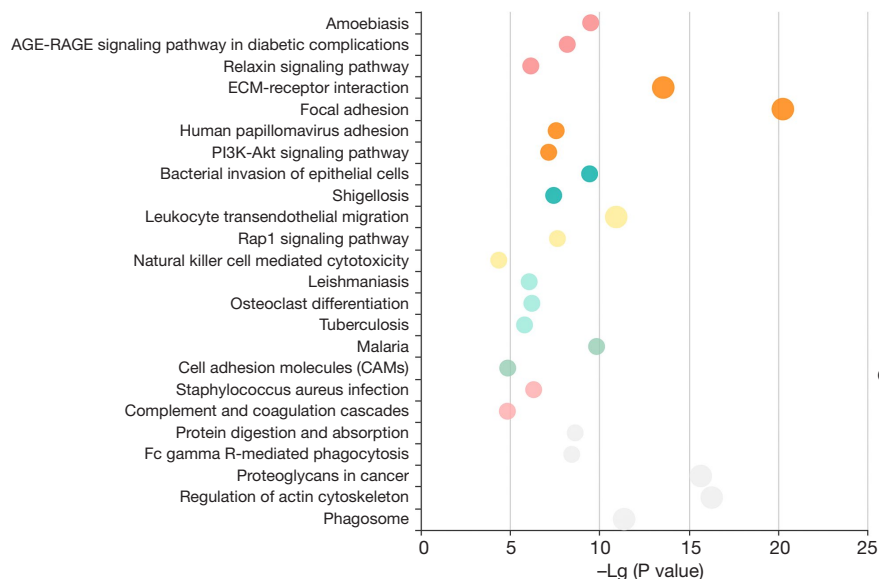
Figure 5 Pathway enrichment and functional clustering analysis of genes that were co-expressed with ACTB in 558 OC tissues and 90 platinum-resistant tissues from TCGA cohort downloaded from cBioPortal, as well as in 174 OC tissues at the protein level according to the CPTAC. Among them, 362 genes that were co-expressed with ACTB were enriched in 241 pathways and functionally clustered into seven functional categories (C1–C7). Of these, the C2 cluster contained the PI3K/Akt signaling pathway, ECM−receptor interaction, focal adhesion, and human papillomavirus infection. In the pathway enrichment analysis, Q<0.05 was considered to indicate significant enrichment. ACTB, beta-actin; OC, ovarian cancer; TCGA, The Cancer Genome Atlas; CPTAC, Clinical Proteomic Tumor Analysis Consortium; ECM, extracellular matrix.
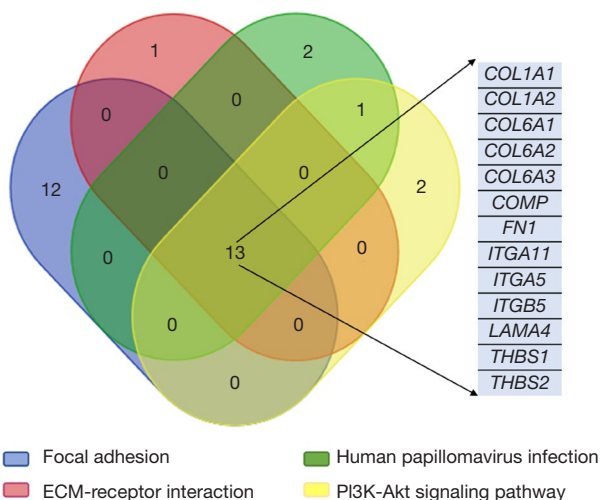

Figure 6 Venn diagram contained 13 overlapping genes co-expressed with ACTB that were enriched in PI3K/Akt signaling, human papillomavirus infection, focal adhesion and ECM-receptor interaction. The above four pathways were enriched with 26, 15, 17 and 17 co-expressed genes with ACTB; finally, 13 overlapping genes co-expressed with ACTB were obtained by cross-tabulation analysis of the four groups of genes. ACTB, beta-actin; ECM, extracellular matrix.

targeting GRP78 through the PI3K/Akt pathway (36). Despite the availability of strong pre-clinical and clinical data of PI3K/Akt/mTOR pathway inhibitors in OC, there is no Food and Drug Administration (FDA) approved inhibitor available for the treatment of OC (37). However, the above studies indicate the prominent role of the PI3K/Akt pathway in OC resistance and its great potential for clinical application in the treatment of OC. In this study, as a potential target of miR-450b-5p, ACTB positively co-expressed with 13 genes *COL1A1*, *COL1A2*, *COL6A1*, *COL6A2*, *COL6A3*, *COMP*, *FN1*, *ITGA11*, *ITGA5*, *ITGB5*, *LAMA4*, *THBS1* and *THBS2* in 558 OC samples and 90 drug-resistant samples at mRNA levels, and co-expressed in 174 OC tissues at protein levels (*Figure 7*), indicating that ACTB might be implicated in drug resistance and tumor progression via strong interactions with those 13 genes. Consistent with our findings, a strong interaction between COL1A1 and ACTB in breast cancer cell has also been identified (38). The above mentioned 13 genes are all the members of four pathways including focal adhesion, ECM-receptor interaction, PI3K/Akt signaling and human papillomavirus infection (39-41) (*Figure 5*). The four
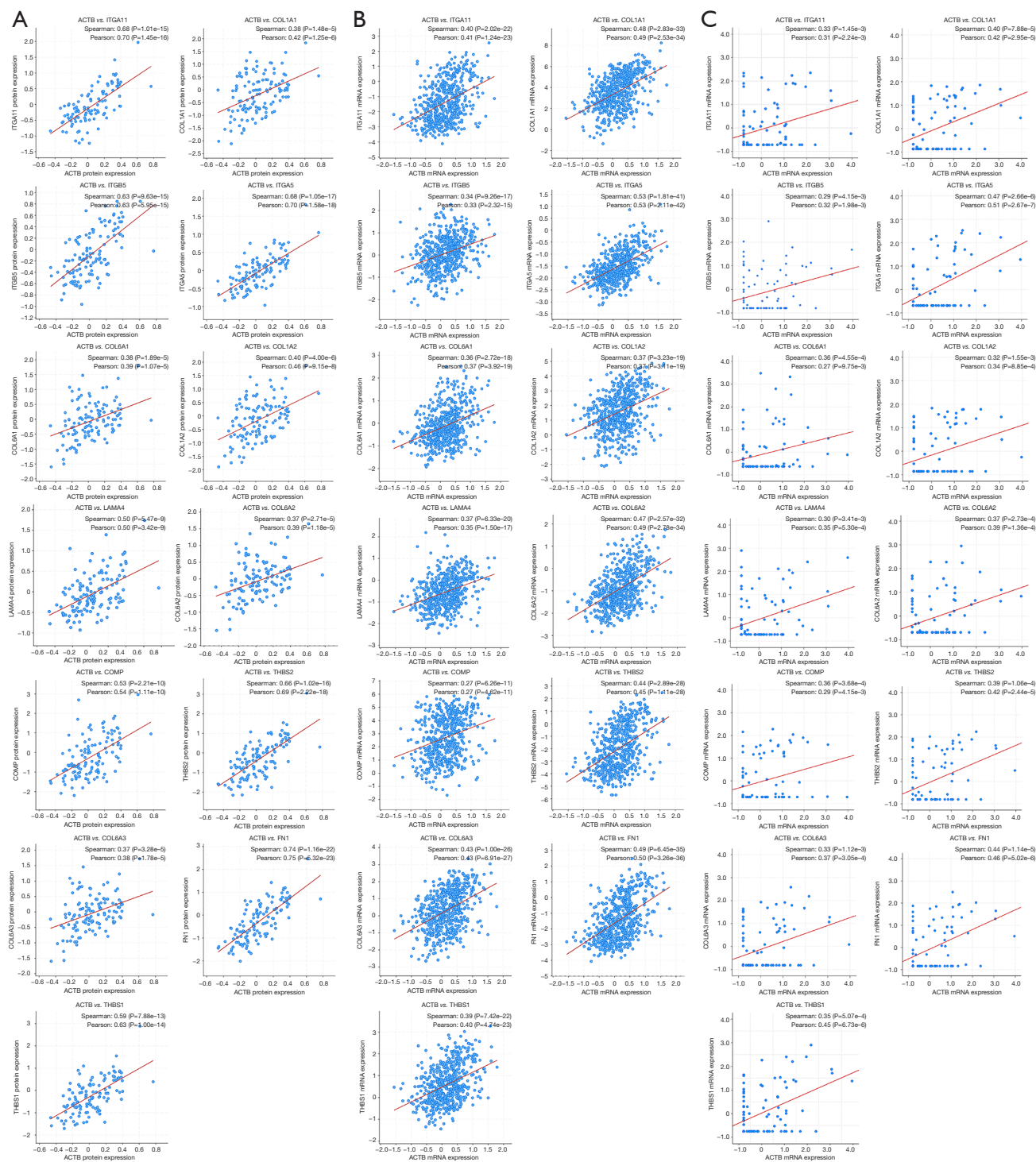
Figure 7 Correlations between ACTB and its co-expressed genes determined by the bivariate correlation method. (A) Correlations between ACTB and the 13 co-expressed genes at the level of protein expression in 174 OC tissues. (B, C) Correlations between ACTB and the 13 co-expressed genes at the level of mRNA expression in 558 OC tissues and 90 platinum-resistant tissues (P<0.05). ACTB, beta-actin; OC, ovarian cancer.

pathways potentially interacted with each other. Beside the overlapping genes they shared together, focal adhesion, ECM-receptor interaction and human papillomavirus infection were also the three upstream pathways and they converge to the downstream pathway PI3K/Akt according to KEGG pathways in cancer (map04151). All the data above composed a strong axis ACTB-13 genes-PI3K/Akt signaling in OC. Previous reports showed that miR-450-5p inhibited the progression of hepatocellular cancer cells through KIF26B/PI3K/Akt axis (42). Long non-coding RNA (lncRNA) SNHG1 mediates apoptosis of AC16 cardiomyocytes and reduces oxidative stress levels by regulating the miR-450b-5p/IGF1 axis and activating the PI3K/Akt pathway (43). Activation of PI3K/Akt prevents ferroptosis in cardiomyocytes, while miR-450b-5p increases myocardial cell tolerance to hypoxia/reoxygenation injury by inhibiting ferroptosis (44). Meanwhile, as a gene that ACTB strongly co-expressed with it, COL1A1 inhibited apoptosis of cancer cells by affecting the caspase-3/PI3K/Akt pathway (45), and low level of COL1A1 predicted short survival (46). Thus, miR-450b-5p might be involved in regulating PI3K/Akt signaling pathway by targeting ACTB to affect OC drug resistance and survival, although their mechanisms remain to be further investigated. In summary, the results observed in this study are expected to lay the foundation for further investigation into the miR-450b-5p/ACTB/PI3K/Akt axis, offering additional possibilities for research into therapeutic targets for OC, for example, exploring targeted therapies aimed at regulating the PI3K/Akt signaling pathway through miR-450b-5p (for example, the development of miR-450b-5p inhibitors), or investigating combined therapies targeting miR-450b-5p with conventional chemotherapy or immunotherapy.

Conclusions

In conclusions, the expression of miRNA-450b-5p was significantly increased in drug-resistant OC cells. Meanwhile, overexpression of miRNA-450b-5p affected OC drug resistance and predicted short OS. The effect of miRNA-450b-5p on drug resistance of OC cells may be related to regulation PI3K/Akt signaling pathway by targeting ACTB.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-292/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

References

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024;74:229-63.

2. Vergote I, Gonzalez-Martin A, Lorusso D, et al. Clinical research in ovarian cancer: consensus recommendations from the Gynecologic Cancer InterGroup. Lancet Oncol 2022;23:e374-84.

3. Norouzi-Barough L, Sarookhani MR, Sharifi M, et al. Molecular mechanisms of drug resistance in ovarian cancer. J Cell Physiol 2018;233:4546-62.

4. Bukowski K, Kciuk M, Kontek R. Mechanisms of Multidrug Resistance in Cancer Chemotherapy. Int J Mol Sci 2020;21:3233.

5. An Y, Yang Q. MiR-21 modulates the polarization of macrophages and increases the effects of M2 macrophages on promoting the chemoresistance of ovarian cancer. Life Sci 2020;242:117162.

6. Kim YN, Shim Y, Seo J, et al. Investigation of PARP Inhibitor Resistance Based on Serially Collected Circulating Tumor DNA in Patients With BRCA-Mutated Ovarian Cancer. Clin Cancer Res 2023;29:2725-34.

7. Shi L, Li X, Wu Z, et al. DNA methylation-mediated repression of miR-181a/135a/302c expression promotes the microsatellite-unstable colorectal cancer development and 5-FU resistance via targeting PLAG1. J Genet Genomics 2018;45:205-14.

8. Kim T, Croce CM. MicroRNA: trends in clinical trials of cancer diagnosis and therapy strategies. Exp Mol Med 2023;55:1314-21.

9. Chen Y, Cao XY, Li YN, et al. Reversal of cisplatin resistance by microRNA-139-5p-independent RNF2 downregulation and MAPK inhibition in ovarian cancer. Am J Physiol Cell Physiol 2018;315:C225-35.

10. Li B, Xu X, Zheng L, et al. MiR-590-5p promotes cisplatin resistance via targeting hMSH2 in ovarian cancer. Mol Biol Rep 2023;50:6819-27.

11. Sun L, Ke M, Yin M, et al. Extracellular vesicle-encapsulated microRNA-296-3p from cancer-associated fibroblasts promotes ovarian cancer development through regulation of the PTEN/AKT and SOCS6/STAT3 pathways. Cancer Sci 2024;115:155-69.

12. Ye P, Lv X, Aizemaiti R, et al. H3K27ac-activated LINC00519 promotes lung squamous cell carcinoma progression by targeting miR-450b-5p/miR-515-5p/YAP1 axis. Cell Prolif 2020;53:e12797.

13. Han H, Shao Q, Liu X. LINC00441 promotes cervical cancer progression by modulating miR-450b-5p/RAB10 axis. Cancer Cell Int 2020;20:368.

14. Zhang J, Furuta T, Sabit H, et al. Gelsolin inhibits malignant phenotype of glioblastoma and is regulated by miR-654-5p and miR-450b-5p. Cancer Sci 2020;111:2413-22.

15. Huang Z, Mou T, Luo Y, et al. Inhibition of miR-450b-5p ameliorates hepatic ischemia/reperfusion injury via targeting CRYAB. Cell Death Dis 2020;11:455.

16. Qin Z, Wang H, Dou Q, et al. Protective effect of fluoxetine against oxidative stress induced by renal ischemia-reperfusion injury via the regulation of miR-450b-5p/Nrf2 axis. Aging (Albany NY) 2022;15:15640-56.

17. Li JH, Liu S, Zhou H, et al. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res 2014;42:D92-7.

18. Integrated genomic analyses of ovarian carcinoma. Nature 2011;474:609-15.

19. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401-4.

20. Li R, Qu H, Wang S, et al. CancerMIRNome: an interactive analysis and visualization database for miRNome profiles of human cancer. Nucleic Acids Res 2022;50:D1139-46.

21. Palma GBH, Kaur M. miRNA-128 and miRNA-223 regulate cholesterol-mediated drug resistance in breast cancer. IUBMB Life 2023;75:743-64.

22. Song Y, Kelava L, Kiss I. MiRNAs in Lung Adenocarcinoma: Role, Diagnosis, Prognosis, and Therapy. Int J Mol Sci 2023;24:13302.

23. Yu B, Li X, Yan W, et al. Post-transcriptional regulation of tumor suppressor gene lncRNA CARMN via m(6)A modification and miRNA regulation in cervical cancer. J Cancer Res Clin Oncol 2023;149:10307-18.

24. Kousar K, Ahmad T, Abduh MS, et al. miRNAs in Regulation of Tumor Microenvironment, Chemotherapy Resistance, Immunotherapy Modulation and miRNA Therapeutics in Cancer. Int J Mol Sci 2022;23:13822.

25. Kandimalla R, Wang W, Yu F, et al. OCaMIR-A Noninvasive, Diagnostic Signature for Early-Stage Ovarian Cancer: A Multi-cohort Retrospective and Prospective Study. Clin Cancer Res 2021;27:4277-86.

26. Zhu T, Gao W, Chen X, et al. A Pilot Study of Circulating MicroRNA-125b as a Diagnostic and Prognostic

Biomarker for Epithelial Ovarian Cancer. Int J Gynecol Cancer 2017;27:3-10.

27. Saburi A, Kahrizi MS, Naghsh N, et al. A comprehensive survey into the role of microRNAs in ovarian cancer chemoresistance; an updated overview. J Ovarian Res 2022;15:81.

28. Moghbeli M. MicroRNAs as the critical regulators of Cisplatin resistance in ovarian cancer cells. J Ovarian Res 2021;14:127.

29. Zhang Q, Ding J, Wang Y, et al. Tumor microenvironment manipulates chemoresistance in ovarian cancer (Review). Oncol Rep 2022;47:102.

30. Mo Y, Leung LL, Mak CSL, et al. Tumor-secreted exosomal miR-141 activates tumor-stroma interactions and controls premetastatic niche formation in ovarian cancer metastasis. Mol Cancer 2023;22:4.

31. Suresh R, Diaz RJ. The remodelling of actin composition as a hallmark of cancer. Transl Oncol 2021;14:101051.

32. Liu K, Gao R, Wu H, et al. Single-cell analysis reveals metastatic cell heterogeneity in clear cell renal cell carcinoma. J Cell Mol Med 2021;25:4260-74.

33. Li G, Samuel S, Ul Haq SE, et al. Characterizing the oncogenic importance and exploring gene-immune cells correlation of ACTB in human cancers. Am J Cancer Res 2023;13:758-77.

34. Gu Y, Tang S, Wang Z, et al. A pan-cancer analysis of the prognostic and immunological role of β-actin (ACTB) in human cancers. Bioengineered 2021;12:6166-85.

35. Bi X, Lv X, Liu D, et al. METTL3-mediated maturation of miR-126-5p promotes ovarian cancer progression via PTEN-mediated PI3K/Akt/mTOR pathway. Cancer Gene Ther 2021;28:335-49.

36. Zhang LY, Yu JY, Leng YL, et al. MiR-181c sensitizes ovarian cancer cells to paclitaxel by targeting GRP78 through the PI3K/Akt pathway. Cancer Gene Ther 2022;29:770-83.

37. Ediriweera MK, Tennekoon KH, Samarakoon SR. Role of the PI3K/AKT/mTOR signaling pathway in ovarian cancer: Biological and therapeutic significance. Semin Cancer Biol 2019;59:147-60.

38. Sahana J, Cortés-Sánchez JL, Sandt V, et al. Long-Term Simulation of Microgravity Induces Changes in Gene Expression in Breast Cancer Cells. Int J Mol Sci 2023;24:1181.

39. Bao Y, Wang L, Shi L, et al. Transcriptome profiling revealed multiple genes and ECM-receptor interaction pathways that may be associated with breast cancer. Cell Mol Biol Lett 2019;24:38.

40. Zhang P, Cao X, Guan M, et al. CPNE8 Promotes Gastric Cancer Metastasis by Modulating Focal Adhesion Pathway and Tumor Microenvironment. Int J Biol Sci 2022;18:4932-49.

41. Yang Q, Jiang W, Hou P. Emerging role of PI3K/AKT in tumor-related epigenetic regulation. Semin Cancer Biol 2019;59:112-24.

42. Li H, Shen S, Chen X, et al. miR-450b-5p loss mediated KIF26B activation promoted hepatocellular carcinoma progression by activating PI3K/AKT pathway. Cancer Cell Int 2019;19:205.

43. Zhan J, Yin Q, Zhao P, et al. Role and mechanism of the lncRNA SNHG1/miR-450b-5p/IGF1 axis in the regulation of myocardial ischemia reperfusion injury. Mol Med Rep 2022;25:176.

44. Ryabov VV, Maslov LN, Vyshlov EV, et al. Ferroptosis, a Regulated Form of Cell Death, as a Target for the Development of Novel Drugs Preventing Ischemia/Reperfusion of Cardiac Injury, Cardiomyopathy and Stress-Induced Cardiac Injury. Int J Mol Sci 2024;25:897.

45. Liu S, Liao G, Li G. Regulatory effects of COL1A1 on apoptosis induced by radiation in cervical cancer cells. Cancer Cell Int 2017;17:73.

46. Hayashi M, Nomoto S, Hishida M, et al. Identification of the collagen type 1 α 1 gene (COL1A1) as a candidate survival-related factor associated with hepatocellular carcinoma. BMC Cancer 2014;14:108.