

Hsa_circ_0009910 Regulates Cisplatin Sensitivity of Ovarian Cancer Cells by Targeting miR-455-5p /PAX2

Lei Jiang, Yi Huang, Runfeng Yang, Xiaohui Jiang* and Hao Yan

Gynomaceae, Hubei Cancer Hospital, Wuhan, 430079, China

ABSTRACT

The objective of this study was to investigate whether Hsa_circ_0009910 regulates cisplatin sensitivity of ovarian cancer cells by regulating the expression of miR-455-5p/PAX2. The expressions of circ_0009910, miR-455-5p and PAX2 mRNA in HOSEPiCs and ovarian cancer cells SKOV3 and SKOV3/DDP were detected by qRT-PCR. SKOV3/DDP cells were grouped according to the transfection of different genes. The sensitivity of SKOV3/DDP cells to cisplatin was detected by CCK8 assay, and the apoptosis of SKOV3/DDP cells induced by cisplatin was detected by flow cytometry. Western blot was used to detect the expressions of apoptotic proteins Bax, Bcl-2, cleaved caspase and PAX2 in SKOV3/DDP cells, and the targeted relationship between circ_0009910, miR-455-5p and PAX2 was verified by double luciferase. Our results showed that circ_0009910 and PAX2 genes were significantly up-regulated in SKOV3/DDP cells, and miR-455-5p was significantly down-regulated in SKOV3/DDP cells. circ_0009910 could enhance cisplatin sensitivity of SKOV3/DDP cells. circ_0009910 targeted miR-455-5p, and knockdown of circ_0009910 up-regulated the expression of miR-455-5p in SKOV3/DDP cells. Overexpression of miR-455-5p can enhanced cisplatin sensitivity of SKOV3/DDP cells. miR-455-5p targeted PAX2 and overexpressed miR-455-5p down-regulated the expression of PAX2 gene in SKOV3/DDP cells. Inhibition of miR-455-5p or overexpression of PAX2 reversed the enhanced cisplatin sensitivity of knockdown circ_0009910 on SKOV3/DDP cells. It is concluded that circ_0009910 enhanced cisplatin sensitivity of ovarian cancer cells SKOV3/DDP by regulating miR-455-5p /PAX2 axis.

Article Information

Received 31 October 2020

Revised 17 November 2020

Accepted 04 December 2020

Available online 30 September 2022 (early access)

Published 18 March 2023

Authors' Contribution

LJ and YH collected the samples. LJ, RY and XJ analysed the data. XJ and HY conducted the experiments and analysed the results. All authors discussed the results and wrote the manuscript.

Key words

Ovarian cancer, Cisplatin sensitivity, circ_0009910, miR-455-5p, PAX2, Apoptosis

INTRODUCTION

Ovarian cancer is a gynecological malignant tumor with the highest fatality rate, which features early-onset metastasis and poor prognosis. According to statistics, the 5-year average survival rate of patients with ovarian cancer is less than 50%, while that of advanced patients is less than 30% (Pearl *et al.*, 2014; Chay *et al.*, 2010). Ovarian cancer is mainly treated by adjuvant chemotherapy after surgery. Platinum-based chemotherapy drugs are regarded as the standard chemotherapy regimen for ovarian cancer, but drug resistance which gradually develops during chemotherapy will eventually lead to treatment failure and seriously affect patients' prognosis (Schmid and Oehler, 2014; Raspagliesi *et al.*, 2006). Therefore, understanding the drug resistance mechanism of ovarian cancer carries

great significance for improving prognosis of ovarian cancer patients.

Circular RNA (circRNA) is a type of non-coding RNA with a closed loop structure. Due to expression stability, and tissue and cell specificity, it exists extensively in the human body, participating in the occurrence and progression of various diseases such as tumors, cardiovascular and cerebrovascular diseases, arthritis (Ma *et al.*, 2019; Wang *et al.*, 2018; Zheng *et al.*, 2017). Hsa_circ_0009910 is a circRNA which promotes tumor growth and metastasis. In addition, it has been confirmed to be highly expressed in chronic myeloid leukemia to promote its chemotherapy resistance (Li *et al.*, 2020; Cao *et al.*, 2020). However, whether circ_0009910 is related to drug resistance of ovarian cancer has not been reported. Therefore, this study investigated the role of circ_1119910 in cisplatin sensitivity of ovarian cancer cells.

MATERIALS AND METHODS

Cell culture

Normal human ovarian epithelial cell line HOSEPiCs, cisplatin-sensitive human ovarian cancer cell line SKOV3, and cisplatin-resistant cell line SKOV3/DDP were purchased from Shanghai Cell Bank, Chinese Academy of

* Corresponding author: dr.med.jiang@gmail.com
0030-9923/2023/0003-1285 \$ 9.00/0



Copyright 2023 by the authors. Licensee Zoological Society of Pakistan.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Sciences. The cells were inoculated in RPMI1640 culture medium containing 10% fetal bovine serum, and cultured at 37°C with 5% CO₂. When the cell fusion reached 90%, the cells were subcultured.

Cell transfection and grouping

SKOV3/DDP cells grown to logarithmic phase, were transfected with (i) small interfering RNA (siRNA) (negative control group), (ii) siRNA circ_0009910, miRNA mimics, miR-455-5p mimics into cells (as si-NC group), (iii) si-circ_0009910 group, (iv) miR-NC group, (v) miR-455-5p group. In addition, siRNA circ_0009910 was transfected into cells with miRNA inhibitor, miR-455-5p inhibitor, pcDNA3.1 empty vector, and pcDNA3.1-PAX2, (vi) si-circ_0009910+anti-NC group, (vii) si-circ_0009910+anti-miR-455-5p group, (viii) si-circ_0009910+pcDNA group and (ix) si-circ_0009910+pcDNA-PAX2 group. For the transfection Lipofectamine 3000 reagent manual was followed. After 48h of transfection, transfection efficiency was detected by qRT-PCR.

qRT-PCR experiment

Total cell RNA was extracted using TRIzol method, cDNA was reverse transcribed by Takara reverse transcription kit, and qRT-PCR was used to detect the expression of circ_0009910, miR-455-5p and PAX2 mRNA. circ_0009910 Primer sequence: forward 5'-TGAGAGGCATCAGTGAGGTG-3', reverse 5'-AAGTGCTTAAGTGGGGATGC-3', internal reference GAPDH primer sequence: forward 5'-GTCAACGGATTTGGTCTGTATT-3', reverse 5'-AGTCTTCTGGGTGGCAGTGAT-3'; miR-455-5p primer sequence: forward 5'-GAACTGCAGTCCATGGGGATA-3', reverse 5'-GCAGGGTCCGAGGTATTC-3', internal reference U6 primer sequence: forward 5'-CTCGCTTCGGCAGCAC-3', reverse 5'-AACGCTTCACGAATTTGCGT-3'. The relative expression level of genes was calculated by 2^{-ΔΔCT} method.

Detection of cisplatin sensitivity of cells by CCK8 test

SKOV3/DDP cells of different transfection groups were prepared into single cell suspension, and seeded in 96-well cell culture plate at 100 μL per well. Five replicate wells were set for each group. After cisplatin treatment for 72h at concentrations of 0, 1.25, 2.5, 5, 10, 20, 40, 80 μM, respectively, 10 μL of CCK8 solution was added to each well for 4h incubation. The absorbance value was detected using a microplate reader at 450nm wavelength. The cell survival curve was plotted, and Cisplatin 50% inhibition concentration (IC₅₀) was calculated.

Apoptosis detection by flow cytometry

SKOV3/DDP cells from different transfection groups

were trypsin digested and collected to prepare single cell suspension with a cell density of 1×10⁶ cells/mL. Cell suspension was transferred to a flow tube to which 5 μL Annexin-V FITC and 5 μL PI was added to each tube for 15 min, incubated at room temperature in the dark. Flow cytometry was used to detect the apoptosis rate.

Western blot experiment

The total protein of the cells was extracted by RIPA pyrolysis, subjected to SDS-PAGE electrophoresis, electro-transferred to PVDF membrane, placed in 5% skimmed milk powder for one h. The membranes were washed with PBS and then soaked in primary antibody dilution PAX2 (1:500), Bax (1:1000), Bcl-2 (1:1000), cleaved-caspase (1:1000), β-actin (1:1000) at 4°C overnight. The membranes were washed with PBS and then horseradish peroxidase-labeled secondary antibody dilution was added and incubated for 1 h at room temperature. The membranes were developed in a super-sensitive chemiluminescent solution and exposed for photography under a microscope. With β-actin as an internal reference, gray value of the target protein was analyzed using Image J software and relative expression of the target protein was calculated.

Dual luciferase experiment

Dual-luciferase reporter gene vectors were constructed using circ_0009910 wild type sequence (circ_0009910 WT) containing miR-455-5p binding sequence and mutant sequence (circ_0009910 MUT), PAX2 3'UTR wild type sequence (PAX2 3'UTR WT), mutant sequence (PAX2 3'UTR MUT) and SKOV3/DDP cells were co-transfected with miRNA mimics and miR-455-5p mimics. At 48h after transfection, Dual-Glo® dual luciferase reporter gene detection system (US Promega) was used to detect luciferase activity.

Statistical analysis

The above experiments were repeated three times in this study. Statistical software SPSS21.0 and GraphPad Prism 7.0 were used to analyze the data and plot graphs. The measurement data were expressed as mean ± standard deviation. Comparison among groups was performed by Student's t test, one-way ANOVA test or Tukey's test. *P*<0.05 indicates statistically significant difference.

RESULTS

Expression of circ_0009910, miR-455-5p and PAX2 in ovarian cancer cells

Compared with HOSEPiCs cells, SKOV3 cells and SKOV3/DDP cells have significantly increased relative expression levels of circ_0009910, PAX2 mRNA and protein (*P*<0.05), and significantly reduced relative

expression level of miR-455-5p ($P<0.05$). Compared with SKOV3 cells, SKOV3/DDP cells have significantly increased relative expression levels of circ_0009910, PAX2 mRNA and protein ($P<0.05$), and significantly reduced relative expression level of miR-455-5p ($P<0.05$), as shown in Figure 1 and Table I.

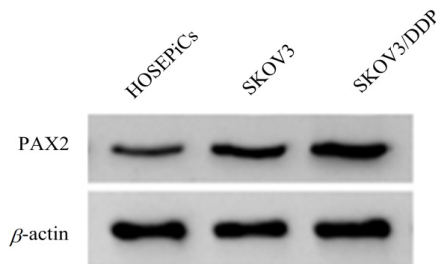


Fig. 1. Western bolt analysis of expression of PAX2 protein in HOSEPiCs, SKOV3 and SKOV3/DDP cell lines.

Knocking out circ_0009910 promotes SKOV3/DDP sensitivity to DDP

The knockdown efficiency of circ_0009910 was detected by qRT-PCR. Compared with si-NC group, si-circ_0009910 group had significantly reduced relative expression of circ_0009910 in SKOV3/DDP cells ($P<0.05$), indicating successful transfection. CCK experiment results showed that, compared with si-NC group, si-circ_0009910 group had significantly reduced IC_{50} value of SKOV3/DDP cells ($P<0.05$). The cells in si-NC and si-circ_0009910 groups were all treated with 20 μ M DDP, followed by apoptosis rate detection by flow cytometry. It turned out that compared with si-NC group, si-circ_0009910 group had significantly increased apoptosis rate ($P<0.05$), indicating that knocking down circ_0009910 could enhance SKOV3/DDP cell sensitivity to cisplatin. See Figure 2 and Table II.

Table I. Expression of circ_0009910, miR-455-5p and PAX2 in HOSEPiCs, SKOV3, SKOV3/DDP cell lines as detected by q RT-PCR.

Cell line	Relative expression of			
	circ_0009910	miR-455-5p	PAX2 mRNA	PAX2 protein
HOSEPiCs	1.00±0.09	0.99±0.10	1.00±0.07	1.01±0.05
SKOV3	2.85±0.31*	0.46±0.06*	2.17±0.25*	1.81±0.21*
SKOV3/DDP	3.91±0.45*#	0.28±0.03*#	2.94±0.33*#	2.53±0.28*#

Note: Compared with HOSEPiCs, * $P<0.05$; compared with SKOV3, # $P<0.05$.

Table II. Effect of circ_0009910 knockout on SKOV3/DDP cell sensitivity to cisplatin as detected by qRT-PCR.

Group	Relative expression of circ_0009910	IC_{50}	Cell apoptosis rate (%)	Relative expression of	
				Bax/Bcl-2 protein	Cleaved-caspase3 protein
si-NC	1.00±0.03	34.26±4.15	9.11±1.17	1.01±0.08	0.98±0.07
si-circ_0009910	0.24±0.04*	13.08±2.87*	25.95±3.24*	1.94±0.22*	2.39±0.28*

Note: Compared with si-NC group, * $P<0.05$.

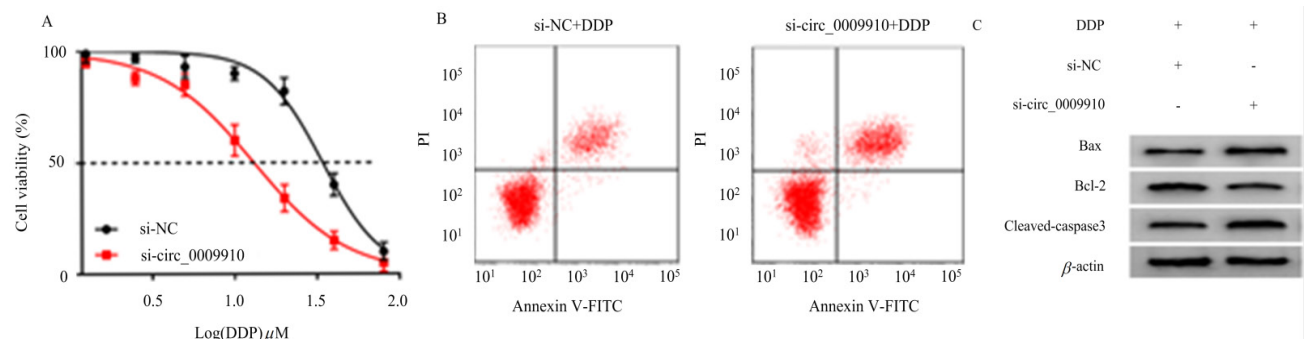


Fig. 2. Effect of circ_0009910 knockout on viability and apoptosis of SKOV3/DDP.

Note: A, CCK8 detects the cell sensitivity to cisplatin; B, Flow cytometry detects cell apoptosis; C, Western bolt detects apoptosis-related protein expression.

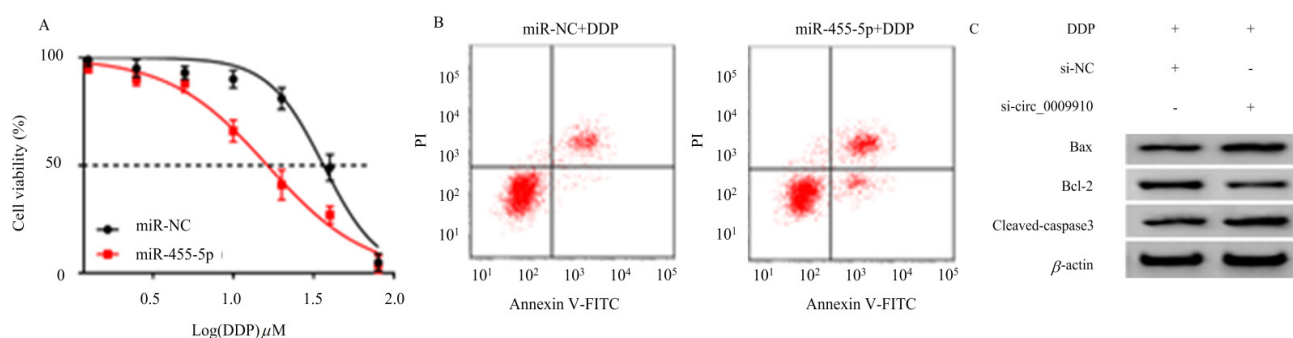


Fig. 3. Effect of overexpression of miR-455-5p viability and apoptosis of SKOV3/DDP.

Note: A, CCK8 detects cell sensitivity to cisplatin; B, Flow cytometry detects cell apoptosis; C, Western bolt detects apoptosis-related protein expression.

Targeted inhibition of miR-455-5p expression in SKOV3/DDP cells by circ_0009910

The bioinformatics website Starbase predicts there are targeted binding sites between circ_0009910 and miR-455-5p (see Table III). Dual luciferase reporter gene assay results show that, compared with miR-NC+circ_0009910 WT co-transfection, co-transfection of miR-455-5p+circ_0009910 WT has significantly reduced luciferase activity in SKOV3/DDP cells ($P<0.05$, see Table IV). qRT-PCR results show that, compared with si-NC group, si-circ_0009910 group has significantly increased relative expression level of miR-455-5p in SKOV3/DDP cells ($P<0.05$, see Table V).

Table III. Targeted binding sites between circ_0009910 and miR-455-5p.

circ_0009910 MUT: 5'-AGGUGCUGGCUCGGUCCGUGUAG-3'
circ_0009910 WT: 5'-AGGUGCUGGCUCGGUCCGUGUAG-3'
miR-455-5p: 5'-GCUACAUCAGGUUCCGUGUAU-3'

Overexpression of miR-455-5p promotes SKOV3/DDP sensitivity to DDP

Compared with miR-NC group, miR-455-5p group has significantly increased relative expression of miR-455-5p in SKOV3/DDP cells ($P<0.05$), indicating that miR-455-5p is overexpressed and transfected successfully. Compared with miR-NC group, miR-455-5p group has

significantly reduced IC₅₀ value of SKOV3/DDP cells ($P<0.05$), and significantly increased cisplatin-induced apoptosis rate ($P<0.05$), indicating that overexpression of miR-455-5p can enhance SKOV3/DDP cell sensitivity to cisplatin, as shown in Figure 3 and Table VI.

Table IV. Effect of cotransfection of miR-455-5p+ circ_0009910 WT on luciferase activity in SKOV3/DDP cells.

Group	circ_0009910 WT	circ_0009910 MUT
miR-NC	0.99±0.03	0.98±0.02
miR-455-5p	0.41±0.04*	0.95±0.07

Note: Compared with miR-NC, * $P<0.05$.

Table V. circ_0018289 knockout up-regulates the expression of miR-455-5p.

Group	relative expression of miR-455-5p
si-NC	1.00±0.06
si-circ_0009910	3.55±0.42*

Note: Compared with si-NC group, * $P<0.05$.

miR-455-5p targets PAX2

The bioinformatics website Starbase predicts there is a targeted binding site between miR-455-5p and PAX2 at 3'UTR (see Table VII). Double luciferase reporter gene assay results show that, compared with miR-NC+ PAX2

Table VI. Effect of miR-455-5p overexpression on SKOV3/DDP cell sensitivity to cisplatin as shown by qRT-PCR.

Group	Relative expression of miR-455-5p	IC ₅₀	Cell apoptosis rate (%)	Relative expression of	
				Bax/Bcl-2 protein	Cleaved-caspase3 protein
miR-NC	1.01±0.13	36.09±5.02	8.65±0.97	1.00±0.07	1.00±0.10
miR-455-5p	8.86±0.94	16.33±2.33	23.17±2.54	1.73±0.19	2.05±0.24

Note: Compared with miR-NC group, * $P<0.05$.

3'UTR WT co-transfection, luciferase activity of SKOV3/DDP cells is significantly reduced in miR-455-5p+PAX2 3'UTR WT co-transfection ($P<0.05$, see Table VIII). qRT-PCR results indicate that, compared with miR-NC group, miR-455-5p group has significantly reduced relative expression levels of PAX2 mRNA and protein in SKOV3/DDP cells ($P<0.05$, Fig. 4, Table IX).

Table VII. Binding site between miR-455-5p and PAX2 at 3'UTR.

PAX2 3'UTR MUT: 5'-UUCUUUUUUCUUUUGUCGUGUAAA-3'
PAX2 3'UTR WT: 5'-UUCUUUUUUCUUUUGUGCAUA-3'
miR-455-5p:5'-GCUACAUCAGGUUCCGUGUAU-3'

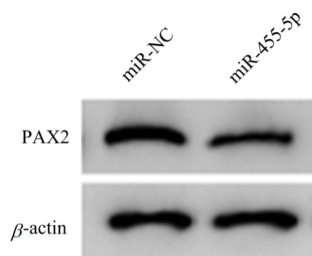


Fig. 4. Western bolt detection of PAX2 protein expression.

Table VIII. Luciferase activity.

Group	PAX2 3'UTR WT	PAX2 3'UTR MUT
miR-NC	1.00±0.02	0.99±0.04
miR-455-5p	0.47±0.06*	1.01±0.03

Note: Compared with miR-NC group, * $P<0.05$.

miR-455-5p or PAX2 can reverse the role of circ_0009910 knockdown in enhancing SKOV3/DDP sensitivity to cisplatin

Compared with si-circ_0009910+anti-NC group, si-circ_0009910+anti-miR-455-5p group has significantly increased IC₅₀ value of SKOV3/DDP cells ($P<0.05$), significantly reduced cisplatin-induced apoptosis rate ($P<0.05$), significantly reduced relative expression of Bax and cleaved-caspase3 protein in cells ($P<0.05$), and

significantly increased relative expression of Bcl-2 protein ($P<0.05$). Compared with si-circ_0009910+pcDNA group, si-circ_0009910+pcDNA-PAX2 has significantly increased IC₅₀ value of SKOV3/DDP cells ($P<0.05$), significantly reduced cisplatin-induced apoptosis rate ($P<0.05$), significantly decreased relative expression of Bax and Cleaved-caspase 3 protein in the cells ($P<0.05$), and significantly increased relative expression of Bcl-2 protein ($P<0.05$). This indicates that inhibition of miR-455-5p or overexpression of PAX2 can reverse the effect of circ_0009910 knockdowns, as shown in Figure 5 and Table X.

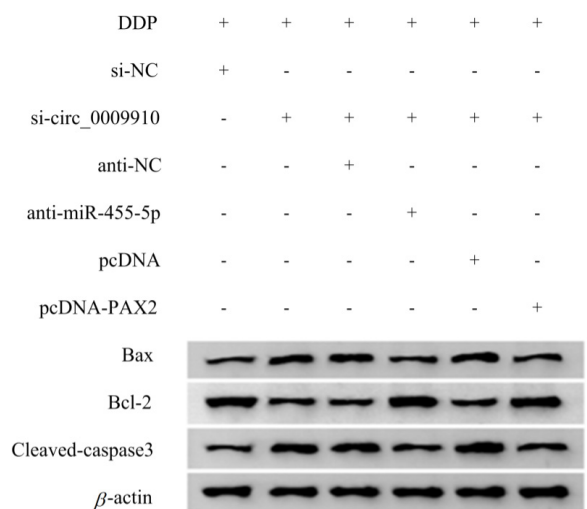


Fig. 5. Western bolt detection of apoptosis-related protein expression.

Table IX. Overexpression of miR-455-5p inhibits PAX2 gene expression.

Group	Relative expression of	
	PAX2 mRNA	PAX2 protein
miR-NC	1.00±0.05	1.01±0.03
miR-455-5p	0.44±0.05*	0.57±0.06

Note: Compared with miR-NC group, * $P<0.05$.

Table X. Effect of circ_0009910 knockout on SKOV3/DDP cell sensitivity to cisplatin.

Group	IC ₅₀	Apoptosis rate (%)	Relative expression of	
			Bax/Bcl-2 protein	Cleaved-caspase3protein
si-NC	35.61±5.14	9.74±1.58	1.00±0.11	1.01±0.09
si-circ_0009910	13.89±3.01*	24.78±2.96*	2.01±0.19*	2.28±0.25*
si-circ_0009910+anti-NC	13.22±2.49	25.34±3.11	1.96±0.21	2.33±0.27
si-circ_0009910+anti-miR-455-5p	30.87±4.26 [#]	12.52±2.07 [#]	1.13±0.15 [#]	1.32±0.16 [#]
si-circ_0009910+pcDNA	14.01±2.77	24.43±2.69	1.88±0.17	2.19±0.23
si-circ_0009910+pcDNA-PAX2	28.64±3.51 [#]	15.06±1.74 ^{and}	1.27±0.14 ^{and}	1.51±0.18 ^{and}

Note: Compared with si-NC group, * $P<0.05$; compared with si-circ_0009910+anti-NC group, [#] $P<0.05$; compared with si-circ_0009910+pcDNA group, ^{and} $P<0.05$.

DISCUSSION

In recent years, several studies have found that circRNA can participate in post-transcriptional regulation by adsorbing microRNA (miRNA) and RNA binding proteins, thereby affecting proliferation and apoptosis in the process of tumor resistance, and mediating the resistance of tumor-resistant cells. This suggests that circRNA may be a new target for anti-tumor resistance (Geng *et al.*, 2020; Gao *et al.*, 2017). miR-455-5p is a cancer-related miRNA. Studies have shown that miR-455-5p is down-regulated in epithelial ovarian cancer cells and participates in the proliferation, migration and apoptosis of tumor cells by regulating the expression of its target genes (Hu *et al.*, 2019). The proteins encoded by PAX gene family are a group of very important transcriptional regulators, which play an important role in regulating cell proliferation, promoting cell self-renewal, changing differentiation direction of specific cell lines, and inducing directional transfer of precursor cells (Al-Hujaily *et al.*, 2015). PAX2, as one of the most characteristic genes of the PAX gene family, has been proved to be highly expressed in various tumors such as human ovarian cancer, breast cancer, liver cancer, etc., thereby promoting tumor progression (Song *et al.*, 2017; Wang *et al.*, 2019). In addition, PAX2 also plays a role in the chemotherapy sensitivity of ovarian cancer (Yang *et al.*, 2019).

This study first demonstrates that circ_0009910 and PAX2 proteins are highly expressed in cisplatin-resistant ovarian cancer cell line SKOV3/DDP, while miR-455-5p is lowly expressed in SKOV3/DDP. The expression of circ_0009910 was knocked out in SKOV3/DDP cells. The effect of cisplatin in different concentrations on cell proliferation was tested by CCK8 experiment. It was found that after circ_0009910 knockouts, the cisplatin-induced cell proliferation activity was significantly reduced, and IC50 value of the cells was significantly reduced. Flow cytometry and Western blot were used to detect cisplatin-induced apoptosis and apoptosis pathway-associated protein expression. It was found that after circ_0009910 knockout, the cisplatin-induced apoptosis rate increased significantly, and the pro-apoptotic proteins Bax and Cleaved-Caspase3 protein had significantly increased expression, while anti-apoptotic protein Bcl-2 had significantly reduced expression, indicating that circ_0009910 knockout can enhance SKOV3/DDP cell sensitivity to cisplatin.

miRNA regulates various signaling pathways of cells in epigenetics, and plays an important role in the formation, development and reversal of tumor drug resistance. Therefore, reversing the expression of related miRNA may become an important way to change drug

resistance of tumor cells (Lin and Xu, 2014). In this study, the bioinformatics website Starbase predicts there is targeted binding site between circ_0009910 and miR-455-5p. Dual-luciferase reporter gene assay confirms that miR-455-5p is the targeted miRNA of circ_0009910. The qRT-PCR experiment confirms that miR-455-5p expression is significantly up-regulated in SKOV3/DDP cells knocked out of circ_0009910. It is speculated that circ_0009910 knockout may enhance SKOV3/DDP cell sensitivity to cisplatin through sponge adsorption of miR-455-5p and up-regulation of miR-455-5p expression. However, it is unclear whether miR-455-5p has relation to cisplatin resistance of ovarian cancer cell, so this study overexpressed miR-455-5p expression in SKOV3/DDP cells. It was found that after overexpression of miR-455-5p, SKOV3/DDP cell had increased sensitivity to cisplatin, which was basically consistent with the above speculation.

miRNA regulates tumor resistance by negatively regulating expression of its target genes. MiRNA regulates downstream genes by complementary binding to complete or partial base sequence of the target gene at 3'UTR, thereby inducing mRNA degradation or inhibiting mRNA translation (Zhu *et al.*, 2008). In this study, the bioinformatics website Starbase predicts there is binding site between miR-455-5p and PAX2 at 3'UTR. As confirmed by dual luciferase reporter gene assay and qRT-PCR, PAX2 is a downstream target gene of miR-455-5p, and overexpression of miR-455-5p in SKOV3/DDP cells can down-regulate PAX2 gene expression. In this study, we further inhibited the expression of miR-455-5p or overexpressed PAX2 gene in SKOV3/DDP cells knocked out of circ_0009910 in functional recovery experiment, finding that after inhibiting miR-455-5p or overexpressing PAX2, knockdown of circ_0009910 played a weakened role in enhancing SKOV3/DDP cell sensitivity to cisplatin. It suggests that miR-455-5p/PAX2 is the downstream pathway to enable action of circ_0009910 (Yang *et al.*, 2020).

CONCLUSION

In summary, circ_0009910 is highly expressed in cisplatin-resistant ovarian cancer cell SKOV3/DDP. By knocking down circ_0009910, it is possible to up-regulate miR-455-5p, inhibit PAX2 gene expression, promote cisplatin-induced apoptosis, and enhance cisplatin sensitivity of ovarian cancer cell SKOV3/DDP. This study provides a new therapeutic target for overcoming cisplatin resistance in ovarian cancer (Wu *et al.*, 2020). circ_0009910 and PAX genes are highly expressed in cisplatin-resistant ovarian cancer cell SKOV3/DDP; miR-455-5p is lowly expressed in cisplatin-resistant ovarian cancer cell SKOV3/

DDP; circ_0009910 knockdown enhances the sensitivity of SKOV3/DDP cells to cisplatin; circ_0009910 targets miR-455-5p. By knocking down circ_0009910, it is possible to up-regulate miR-455-5p expression in SKOV3/DDP cells; Overexpression of miR-455-5p enhances the sensitivity of SKOV3/DDP cells to cisplatin; miR-455-5p targets PAX2. Overexpression of miR-455-5p inhibits PAX2 gene expression in SKOV3/DDP cells; miR-455-5p inhibition or PAX2 overexpression can reverse the role of circ_0009910 knockdown in enhancing cisplatin sensitivity of SKOV3/DDP cells; circ_0009910 regulates the miR-455-5p/PAX2 axis to regulate cisplatin sensitivity of cisplatin-resistant ovarian cancer cell SKOV3/DDP.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

- Al-Hujaily, E.M., Tang, Y., Yao, D.-S., Carmona, E., Garson, K. and Vanderhyden, B.C., 2015. Divergent roles of PAX2 in the etiology and progression of ovarian cancer. *Cancer Prev. Res.*, **8**: 1163-1173. <https://doi.org/10.1158/1940-6207.CAPR-15-0121-T>
- Cao, H.-X., Miao, C.-F., Sang, L.-N., Huang, Y.-M., Zhang, R., Sun, L. and Jiang, Z.-X., 2020. Circ_0009910 promotes imatinib resistance through ULK1-induced autophagy by sponging miR-34a-5p in chronic myeloid leukemia. *Life Sci.*, **243**: 117255. <https://doi.org/10.1016/j.lfs.2020.117255>
- Chay, D., Cho, H., Lim, B.J., Kang, E.S., Oh, Y.J., Choi, S.M., Kim, B.W., Kim, Y.T. and Kim, J.-H., 2010. ER-60 (PDIA3) is highly expressed in a newly established serous ovarian cancer cell line, YDOV-139. *Int. J. Oncol.*, **37**: 399-412. <https://doi.org/10.3892/ijo.00000688>
- Gao, D., Zhang, X., Liu, B., Meng, D., Fang, K., Guo, Z. and Li, L., 2017. Screening circular RNA related to chemotherapeutic resistance in breast cancer. *Epigenomics*, **9**: 1175-1188. <https://doi.org/10.2217/epi-2017-0055>
- Geng, X., Jia, Y., Zhang, Y., Shi, L., Li, Q., Zang, A. and Wang, H., 2020. Circular RNA: Biogenesis, degradation, functions and potential roles in mediating resistance to anticarcinogens. *Epigenomics*, **12**: 267-283. <https://doi.org/10.2217/epi-2019-0295>
- Hu, Y., Yang, Z., Bao, D., Ni, J.-S. and Lou, J., 2019. miR-455-5p suppresses hepatocellular carcinoma cell growth and invasion via IGF-1R/AKT/GLUT1 pathway by targeting IGF-1R. *Pathol. Res. Pract.*, **215**:152674. <https://doi.org/10.1016/j.prp.2019.152674>
- Li, Y., Lin, S. and An, N., 2020. Hsa_circ_0009910: Oncogenic circular RNA targets microRNA-145 in ovarian cancer cells. *Cell Cycle*, **19**: 1857-1868. <https://doi.org/10.1080/15384101.2020.1731650>
- Lin, G. and Xu, K., 2014. Advances in tumor chemoresistance regulated by MicroRNA. *Chinese J. Lung Cancer*, **17**: 741-749.
- Ma, Y., Zhang, X., Wang, Y.-Z., Tian, H. and Xu, S., 2019. Research progress of circular RNAs in lung cancer. *Cancer Biol. Ther.*, **20**: 123-129. <https://doi.org/10.1080/15384047.2018.1523848>
- Pearl, M.L., Zhao, Q., Yang, J., Dong, H., Tulley, S., Zhang, Q., Golightly, M., Zucker, S. and Chen, W.-T. 2014. Prognostic analysis of invasive circulating tumor cells (iCTCs) in epithelial ovarian cancer. *Gynecol. Oncol.*, **134**: 581-590. <https://doi.org/10.1016/j.ygyno.2014.06.013>
- Raspagliesi, F., Kusamura, S., Torres, J.C., De Souza, G., Ditto, A., Zanaboni, F., Younan, R., Baratti, D., Mariani, L. and Laterza, B. 2006. Cytoreduction combined with intraperitoneal hyperthermic perfusion chemotherapy in advanced/recurrent ovarian cancer patients: The experience of National Cancer Institute of Milan. *Eur. J. Surg. Oncol.*, **32**: 671-675. <https://doi.org/10.1016/j.ejso.2006.03.011>
- Schmid, B.C. and Oehler, M.K., 2014. New perspectives in ovarian cancer treatment. *Maturitas*, **77**: 128-136. <https://doi.org/10.1016/j.maturitas.2013.11.009>
- Song, Y., Huang, X., Shen, G., Liu, X. and Zhang, X., 2017. Comparison of paired box genes 8 and 2 expression in epithelium tissues and the related tumors. *Chinese J. Oncol.*, **39**: 424-428.
- Wang, G., Zhao, W., Wang, H., Qiu, G., Jiang, Z., Wei, G. and Li, X., 2019. Exosomal MiR-744 inhibits proliferation and sorafenib chemoresistance in hepatocellular carcinoma by targeting PAX2. *Med. Sci. Monit. Int. med. J. exp. clin. Res.*, **25**: 7209. <https://doi.org/10.12659/MSM.919219>
- Wang, L., Meng, X., Li, G., Zhou, Q. and Xiao, J., 2018. Circular RNAs in cardiovascular diseases. *Circular RNAs*. Springer. https://doi.org/10.1007/978-981-13-1426-1_15
- Wu, Z., Liu, Y. and Jia, X., 2020. A novel hierarchical secret image sharing scheme with multi-group joint management. *Mathematics*, **8**: 448. <https://doi.org/10.3390/math8030448>
- Yang, F., Yang, F., Wang, G., Kong, T., Wang, H. and Zhang, C., 2020. Effects of water temperature on tissue depletion of florfenicol and its metabolite

- florfenicol amine in crucian carp (*Carassius auratus gibelio*) following multiple oral doses. *Aquaculture*, **515**: 734542. <https://doi.org/10.1016/j.aquaculture.2019.734542>
- Yang, S., Yang, R., Lin, R. and Si, L., 2019. MicroRNA-375 inhibits the growth, drug sensitivity and metastasis of human ovarian cancer cells by targeting PAX2. *J. Buon*, **24**: 2341-2346.
- Zheng, F., Yu, X., Huang, J. and Dai, Y., 2017. Circular RNA expression profiles of peripheral blood mononuclear cells in rheumatoid arthritis patients, based on microarray chip technology. *Mol. Med. Rep.*, **16**: 8029-8036. <https://doi.org/10.3892/mmr.2017.7638>
- Zhu, H., Wu, H., Liu, X., Evans, B.R., Medina, D.J., Liu, C.-G. and Yang, J.-M., 2008. Role of MicroRNA miR-27a and miR-451 in the regulation of MDR1/P-glycoprotein expression in human cancer cells. *Biochem. Pharmacol.*, **76**: 582-588. <https://doi.org/10.1016/j.bcp.2008.06.007>