Pathology - Research and Practice 215 (2019) 152606

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

MiR-1307 influences the chemotherapeutic sensitivity in ovarian cancer cells through the regulation of the CIC transcriptional repressor

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ARTICLE INFO

Keywords:
miR-1307
CIC
RTK signaling
Ovarian cancer
Chemoresistant
Chemosensitive

ABSTRACT

Background: Extended from our previously observation that expression of miR-1307 in chemoresistant primary ovarian cancer tissues is elevated, here we are aiming to dissect the function of miR-1307 and its predicted target gene, CIC (capicua transcriptional repressor), in ovarian cancer chemotherapy.

Methods: We evaluated the expression of miR-1307 and CIC in chemoresistant and chemosensitive ovarian cancer tissues and cells by real time-PCR and western blot. We used chemoresistant/chemosensitive cells with miR-1307 suppression/overexpression to study the biological effects of miR-1307 by MTT and flow cytometer. Dual luciferase reporter gene assay was used to validate direct binding between miR-1307 and the 3′-UTR of CIC. Real-time PCR and western blot analyses, MTT and flow cytometry were used to reveal the biological effects of miR-1307 and CIC, as well as their regulation.

Results: We found that miR-1307 affects cell cycle dynamics, cell viability in ovarian cancer cells. In addition, its expression level can influence chemosensitivity to paclitaxel in ovarian cancer cells. We also validate that CIC is a downstream target of miR-1307 via its regulation on 3'-UTR of CIC gene and ETV4 and ETV5 are also regulated by miR-1307/CIC axis.

Conclusions: Our data suggested that miR-1307 may be involved in the resistance of ovarian cancer to chemotherapy drugs via regulation of CIC, and should be further explored as a potential therapeutic target.

1. Introduction

The mortality rate of the ovarian cancer ranks highest amongst all cancer types in the female reproductive system, which may due to the drug resistance in cancer chemotherapy that influences treatment efficacy and prognosis. Numerous factors contribute to the development of resistance to cancer chemotherapy and recently, miRNAs have been shown to be involved in the regulation of chemotherapeutic resistance in ovarian cancer in several studies [1–5].

It is well perceived that miRNAs may function to influence cancer development via regulating oncogenes or tumor suppressor genes [6]. The miR-1307, which was originally discovered in human embryonic stem cells and embryoid bodies [7], was found to be specifically expressed in Epstein-Barr Virus (EBV) infected nasopharyngeal carcinomas [8] and peripheral blood mononuclear cells (PBMCs) of diabetic patients [9]. In addition, its serum level has been reported valuable for early detection of breast cancer [10,11]. We previously found upregulated expression of miR-1307 in the chemoresistant ovarian cancers compared to the chemosensitive counterparts [12–14], suggesting that miR-1307 may play a role in the development of chemotherapeutic resistance in ovarian cancer.

In this study, we aimed to utilize the chemoresistant (SKOV3-TR30) and chemosensitive (SKOV3) ovarian cancer cell lines to test the hypothesis that miR-1307 activity affects the chemosensitivity of ovarian cancers by studying the roles of miR-1307 in cell cycle dynamics, chemotherapy drug (paclitaxel) sensitivity, and the regulation on downstream genes, including capicua transcriptional repressor (*CIC*) gene and the transcriptional effectors of receptor tyrosine kinase (RTK) signaling in ovarian cancer cells. Our results will provide theoretical and experimental basis for studying chemotherapy drug resistance and

Abbreviations: CIC, capicua transcriptional repressor; miRNAs, Micro RNAs; PCR, polymerase chain reaction; PI, propidium iodide; RISC, RNA-induced silencing complex

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https://doi.org/10.1016/j.prp.2019.152606
Received 9 April 2019; Received in revised form 6 August 2019; Accepted 18 August 2019

reversal therapy in ovarian cancer.

2. Materials and methods

2.1. Vector construction

MiR-1307 coding DNA sequence (CDS) region was amplified by polymerase chain reaction (PCR) (using Forward Primer: GCTGAAGC TTGCCTAAGGAAGTTTGGATT; Reverse Primer: ATTGGATCCGGCAGC TTAATCTATAGTACCT) and cloned into pUM-T simple vector (BioTeke, China) by TA (Original TA Cloning Kit, Invitrogen, US) cloning. The sequence was ligated with pcDNA3.1 (+) and then transformed and the positive clones were selected and over-expression of miR-1307 was confirmed by sequencing analysis and PCR.

The seed region of miR-1307 was CUCGGCG in mirBASE. Oligo Designer 3.0 was used to design the sponges for miR-1307. TTGGCCA CTGACT was used in the bulge to prevent the formation of the transcription terminal signal. AATTC was added to the 5′ end of the sense strand template, and CCGGT was added to the 5′ end of the antisense strand template.

The resulting DNA oligo annealed shRNA template and linearized pPG/miR/eGFP/Blasticidin vector (Promega, US) were ligated to construct an expression vector with miR-1307 suppression. The plasmids were transformed into competent bacteria. The positive clones were selected, and miR-1307 suppression was confirmed by sequencing analysis.

2.2. Dual-luciferase reporter assay

Semi-nested PCR was used to obtain part of the 3'-UTR sequences of CIC (NC_000019) containing miR-1307 binding site. The primers have been shown in Table 1. The sequence was cloned into the pMIR-REPORT vector (Promega) to build pMIR-REPORT-CIC and a site-directed mutagenesis kit (Beyotime, China) was used to induce 5 nucleotide mutations in the pMIR-REPORT-CIC-mut. The mutation on CIC was confirmed by DNA sequencing (from CGCCGAG to CATTCTG). pMIR-REPORT-CIC and pMIR-REPORT-CIC-mut were then transfected into HEK293 cells with Renilla luciferase normalization plasmid (pRL-TK) and miR-1307 mimic or control. Luciferase reporter activity was measured 24 h after transfection and normalized by Renilla reporter signals (Promega).

2.3. Cell culture

Human ovarian cancer cell lines SKOV3 and SKOV3-TR30 are sensitive and resistant to chemotherapeutic drugs such as paclitaxel, respectively. These cell lines were obtained from the Women's Hospital of the Zhejiang University School of Medicine and authenticated in previous stduies14. SKOV3 cells were maintained in RPMI-1640 medium (Corning, US) supplemented with 10% FBS (Corning) and 1% Penicillin-Streptomycin Solution (Corning). SKOV3-TR30 cells were maintained in RPMI-1640 medium containing 10% FBS, 1% Penicillin-Streptomycin Solution, and 30 nmol/L paclitaxel (Selleck, US). All cells were incubated at 37 °C with 5% CO_2.

2.4. Real-time quantitative RT-PCR

Total RNA was extracted from transfected SKOV3 and SKOV3-TR30 cells using Trizol (Invitrogen). Reverse transcription for miR-1307 and CIC were performed according to manufacturer's instruction of TaqMan® Small RNA Assays and Primescript™ RT reagent Kit (TaKaRa, China), respectively. The TaqMan microRNA kit (TaKaRa) and The SYBR® Premix Ex Taq II (Tli RNaseH Plus, TaKaRa) were used to assess the expression levels of miR-1307 and CIC mRNA by real-time RT-PCR, respectively (Table 2). The experiments were performed independently three times. The relative target gene expression was normalized with the housekeeping gene and quantified using the ΔΔCt method.

Table 1
Primers for semi-nested PCR for obtaining part of the 3'-UTR sequences of CIC.

Outer primer	F	5′-AGCCATTTCGTCCTCTCCAGTT -3′
Internal primer	F	5′-**GGACTAGT**TAACCTGGAGCGTGTGACCTTC-3′
	R	5′-**CCCAAGCTT**CGGCTTCTCGGCACAGTAAGAA-3′

Table 2
Primers for real-time quantitative PCR.

Gene	Primers	
miR-1307	F	5′-AACTCGGCGTGGC-3′
	R	5′-GAGCAGGCTGGAGAA-3′
U6	F	5′-GCTTCGGCAGCACATATACTAAAAT-3′
	R	5′-CGCTTCACGAATTTGCGTGTCAT-3′
CIC	F	5′-TGTCACCACCACCCAAGTCT-3′
	R	5′-CCCTTCTTGTACTTCTGCTG-3′
β-actin	F	5′-CTGTGCCCATCTACGAGGGCTAT-3′
	R	5′-TTTGATGTCACGCACGATTTCC-3′

2.5. Western blot

Cellular proteins were extracted from each group using cell lysis buffer (Beyotime, China), and quantified using the Bradford assay. Equal amounts of protein samples (40 μg/20 μl) were loaded onto SDS-PAGE gel, then transferred onto PVDF (polyvinylidene fluoride) membranes, blocked with 5% skim milk and incubated with primary antibodies (anti-CIC antibody diluted 1:500; anti-β-actin antibody diluted 1:1000, Abcam) and secondary antibody (IgG-HRP diluted 1:5000, Abcam). The bound proteins were detected by ECL system (ECL Western Blotting Substrate; 7sea biotech, China). The relative band intensities were calculated by Gel-Pro-Analyzer densitometric analysis.

2.6. MTT assay

The transfected and the control group were collected in the logarithmic phase, and plated into 96-well plates at a density of 1000–10,000/well to reach a confluent cell monolayer. Paclitaxel was added to each well at different concentrations, and the cells were cultured for 24 h, 48 h, 72 h, and 96 h. The MTT (2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Beyotime, China) substrate was then added to the cells. The number of viable cells was measured by recording changes in OD (optical density) values at 490 nm using a microplate reader. The following formulae were used to calculate (a) the cell viability after treated with paclitaxel (%) = OD (A experiment – A blank)/OD (A control − A blank) × 100%. All experiments were repeated for at least 3 times.

2.7. Apoptosis and cell cycle

The transfected and control cells were plated and grown to 90% confluence. The cells were then equally divided into 4 groups. 5 μl of FITC Annexin V (Abcam, UK) and then 5 μl propidium iodide (PI, Abcam, UK) were added to the cells. The remaining 3 groups were controls: (1) Cells only; (2) FITC Annexin V only; and (3) PI only. Cell apoptosis was then detected by a flow cytometer. For cell cycle detection by the flow cytometer, cells were fixed in 70% ice-cold ethanol and then stained with PI. All experiments were repeated for at least 3 times.

2.8. Statistical analysis

Data analysis was performed using SPSS 16.0 (IBM) statistical software. Numerical data were presented as mean ± standard deviation (SD), and categorical data were presented as percentage. To

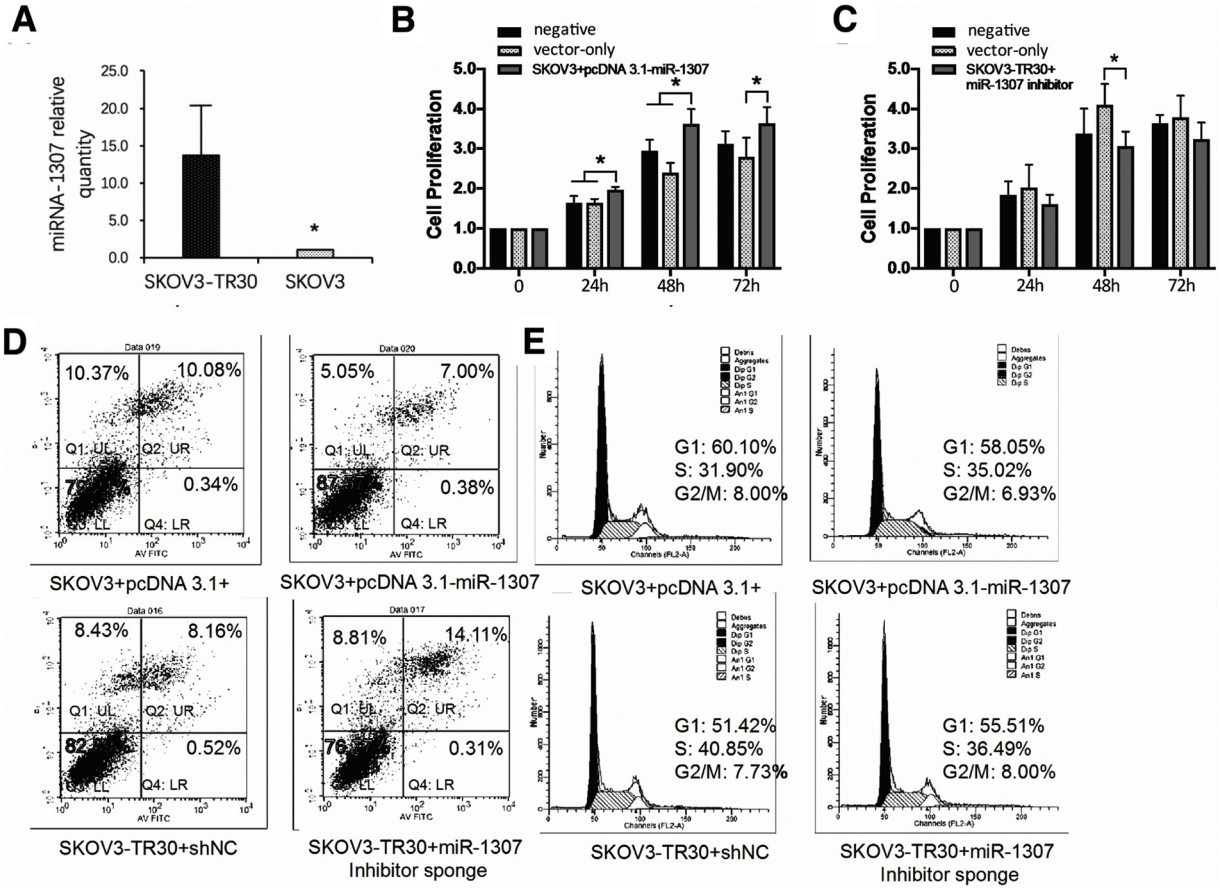

Fig. 1. Changes in the expression of miR-1307 affect the proliferation and apoptosis in SKOV3 and SKOV3-TR30 cells.
(A) RT-PCR analysis of miR-1307 expression in chemosensitive SKOV3 and chemoresistant SKOV3-TR30 ovarian cancer cells. The relative expression levels of miR-1307 between chemosensitive and chemoresistant cells were quantified using U6 housekeeping genes, respectively. Cell proliferation was evaluated by the MTT assay in SKOV3 and SKOV3-TR30 cells transfected with pcDNA 3.1-miR-1307 and miR-1307 inhibitor sponge, respectively. Cell proliferation in (B) transfected SKOV3 cells overexpressing miR-1307, and (C) transfected SKOV3-TR30 cells with miR-1307 inhibited, * indicates $P < 0.05$. (D) Cell apoptosis was evaluated by flow cytometry in SKOV3 and SKOV3-TR30 cells transfected with pcDNA 3.1-miR-1307 and miR-1307 inhibitor sponge, respectively, and stained with Annexin-V-FITC and PI. (E) Cells stained with PI were analyzed for cell cycle phase using flow cytometry. Abbreviation: Each quadrant (Q) is labeled and interpreted as follows: (Q1) PI positive and Annexin-V-FITC negative stained cells represent necrotic cells. (Q2) PI positive and Annexin-V-FITC positive stained cells represent late apoptotic cells. (Q3) Stained cells with negative PI and Annexin-V-FITC represent live cells. (Q4) PI negative and Annexin-V-FITC positive stained cells represent early apoptotic cells. G1: gap 1, S: synthesis phase, G2: gap 2 and M: mitosis phase. Negative: non-transfected control SKOV3 in (B) or SKOV3-TR30 in.(C), vector-only: transfect empty or scramble construct pcDNA 3.1 in.(B) and inhibitor negative control in (C).

compare the mean values of two related groups, homogeneity of variance test and unpaired samples *t*-test were performed. The Bonferroni test was used for pairwise comparisons of three data sets. $P < 0.05$ or $P < 0.01$ was considered statistically significant.

3. Results

3.1. The expression of miR-1307 is upregulated in paclitaxel-resistant ovarian cancer cell line

Given the limited resource and intrinsic difficulties to manipulate primary ovarian cancer cells, we set to search alternative cell line models in order to examine the regulatory relationships between miR-1307 and its downstream targets. One of the candidate models is a chemoresistant ovarian carcinoma cell line, SKOV3-TR30, which is derived from its parental SKOV3 line, and has been shown to be resistant to anti-cancer drug, paclitaxel [15].

The real-time RT-PCR analysis showed that there was significantly higher expression (13.69 ± 3.25 folds; $p < 0.05$) of miR-1307 in SKOV3-TR30 cells compared to SKOV3 cells (Fig. 1A). This result was well correlated to our previous finding that showing miR-1307 exhibited a higher expression level in chemoresistant ovarian cancer primary tissues than in those are chemosensitive [14]. Therefore, we determined that these two cell lines are suitable ovarian cancer model to study gene regulation of miR-1307 and its involvement in the development of chemoresistance.

3.2. Expression level of miR-1307 affects the viability and the cell cycle dynamics of SKOV3-TR30 and SKOV3 cell lines

Using the MTT assay, we showed that overexpressing miR-1307 significantly increased proliferation in SKOV3 cells, particulary at 24 and 48 h after seeding, when compared to both non-transfected and vector-only controls ($P < 0.05$; Fig. 1B). In contrast, suppression of miR-1307 in SKOV3-TR30 cells significantly reduced cell proliferation at 48 h after seeding, compared with negative controls ($P < 0.05$; Fig. 1C).

In addition, overexpression of miR-1307 in SKOV3 cells reduced the apoptosis, compared to vector only control (Fig. 1D, combining Q2 and Q4, late and early apoptotic phase respectively), while the total percentage of cells in S and G2/M phase increased (Fig. 1D), as revealed by the flow cytometric analysis.

On the other hand, in miR-1307 inhibited SKOV3-TR30 cells, we observed an opposite trend as the result indicated a significant increase

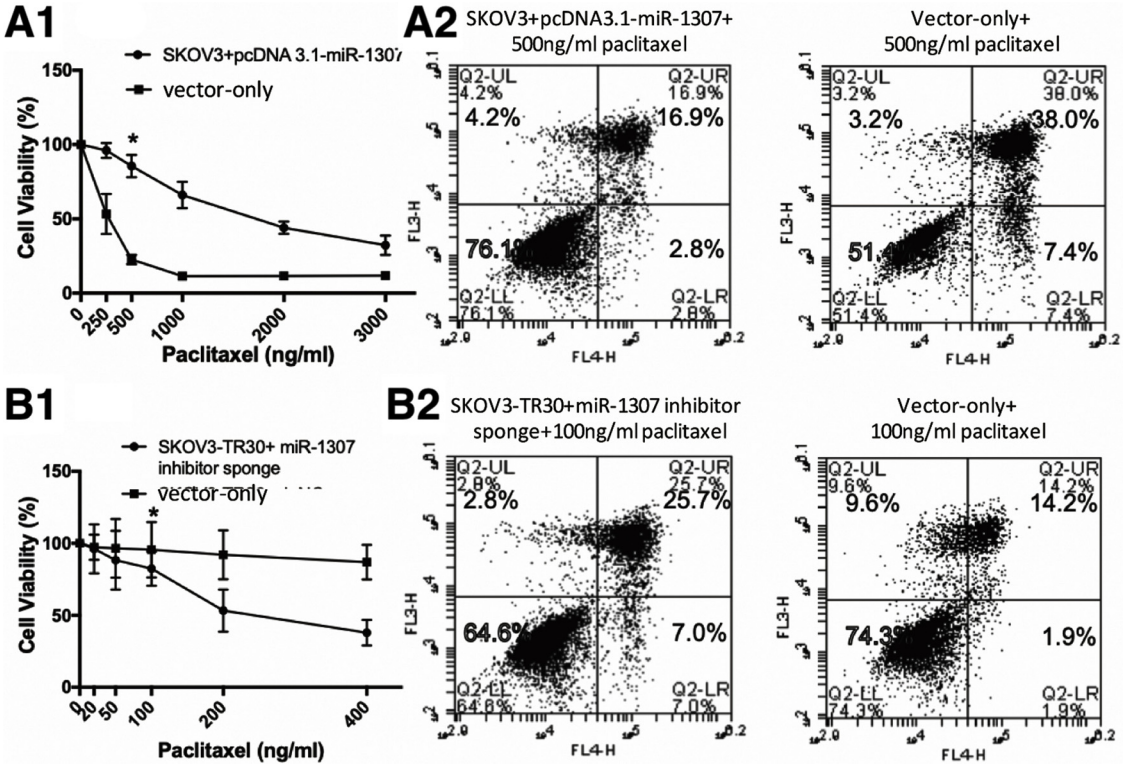

Fig. 2. Expression of miR-1307 influences the susceptibility of ovarian cancer cells to paclitaxel.
Cell viability and apoptosis were evaluated by MTT assay and flow cytometry, respectively, in transfected cells treated with different concentrations of paclitaxel for 24–96 h. Left panels: Cell viability (%) in (A1) SKOV3 cells transfected with miR-1307 overexpressing vector and (B1) SKOV3-TR30 cells transfected with miR-1307 inhibitor sponge were treated with different doses of paclitaxel, and detected the OD value in 24 h. Right Panels: Apoptosis in (A2) transfected SKOV3 cells and (B2) transfected SKOV3-TR30 cells treated with 500 ng/ml and 100 ng/ml paclitaxel, respectively, for 48 h. * indicates P < 0.05. Vector-only: transfect empty or scramble construct pcDNA 3.1 in.(A) and inhibitor negative control in (B).

in the percentage of apoptotic cells (Fig. 1E, combining Q2 and Q4, late and early apoptotic phase respectively) and decrease percentage of cells in S and G2/M phase (Fig. 1E).

3.3. Expression of miR-1307 influences the susceptibility of ovarian cancer cells to paclitaxel

As we have shown that miR-1307 is highly expressed in SKOV3-TR30 cells (Fig. 1A) and its expression level affects the biological properties of SKOV3 and SKOV3-TR30 cell lines (Fig. 1B–E), we hypothesize that miR-1307 would play a key role in paclitaxel resistance.

The miR-1307 overexpressed SKOV3 cells has shown significant resistance toward paclitaxel (IC50 of 908.5 ng/ml) when compared to vector-only control cells (IC50 of vs 294.6 ng/ml) as demonstrated in drug dose-response curve (Fig. 2A1, $P < 0.05$). Conversely, we observed significantly increased susceptibility to paclitaxel treatment in SKOV3-TR30 cells when miR-1307 was inhibited (IC50 of 138.4 ng/ml), compared to the null vector control (IC50 of 3752.7 ng/ml; Fig. 2B1, $P < 0.05$).

Using flow cytometry, we also examined if miR-1307 alters the paclitaxel treatment responses in SKOV3 cell lines. Indeed, we showed that miR-1307 overexpressed SKOV3 cells significantly enhanced its viability compared to control group (500 ng/ml paclitaxel, Fig. 2A2, P < 0.05). Conversely, the SKOV3-TR30 cells significantly reduce the viability when miR-1307 was inhibited (100 ng/ml paclitaxel, Fig. 2B2, P < 0.05). Taken together, these results were consistent with our hypothesis that miR-1307 expression level is correlated with the paclitaxel resistance in SKOV3-TR30 cell line.

3.4. CIC transcription repressor is regulated by miR-1307 in ovarian cancer cells

Based on our preliminary *in silico* analyses (data not shown), one of the potential downstream targets of miR-1307 is the CIC gene, which is a member of the high mobility group (HMG)-box superfamily of transcriptional repressors and often associated with tumor formation [16,17].

To examine if miR-1307 could regulate CIC, we first overexpressed miR-1307 in SKOV3 cells and found that both mRNA and protein levels of CIC gene significantly decreased (Fig. 3C, D). Moreover, when miR-1307 was suppressed in SKOV3-TR30 cells, both CIC mRNA and protein levels increased significantly (Fig. 3C, D, $P < 0.05$). In addition to the exogenous manipulation of miR-1307 level, we observed that the endogenous CIC protein levels in SKOV3-TR30 cells were significantly lower than those in SKOV3 cells (Fig. 3B; $P < 0.05$), even though the real-time RT-PCR analysis did not show significantly difference in mRNA expression (0.96 ± 0.02 folds; $p > 0.05$) of CIC gene between SKOV3-TR30 cells and SKOV3 cells (Fig. 3A). Again, this remained consistent with miR-1307 expression level is higher in SKOV3-TR30 cells (Fig. 1A) and that miR-1307 regulates CIC protein expression level.

Next, we set to test if the miR-1307 putative binding sites located at the 3'-UTR of the CIC transcript could be regulated by miR-1307. Using the luciferase reporter assay, we observed a significant decrease of the luciferase activity in HEK293 cells co-transfected with miR-1307 and wild type reporter construct (Fig. 4A, $P < 0.05$), while the reporter construct contained mutated 3'-UTR failed to show any effect on its luciferase activity when co-transfected with miR-1307. This data suggested that miR-1307 regulates CIC gene through the binding of 3'-UTR sequence of CIC transcript.

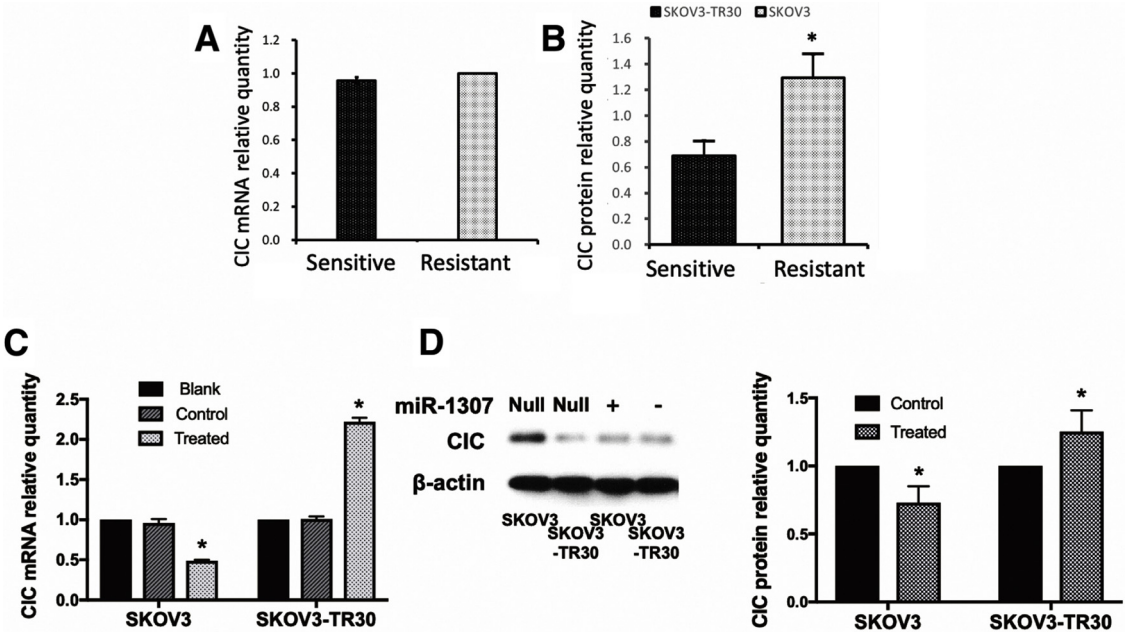

Fig. 3. CIC expression is regulated by miR-1307 in transfected SKOV3 and SKOV3-TR30 cells.
(A) RT-PCR analysis of CIC mRNA expression in chemosensitive SKOV3 and chemoresistant SKOV3-TR30 ovarian cancer cells. The relative expression levels of CIC mRNA between chemosensitive and chemoresistant cells were quantified using GAPDH housekeeping genes, $P > 0.05$. (B) The relative expression levels of CIC protein between chemosensitive and chemoresistant cells were quantified and normalized using β-actin housekeeping gene, $P < 0.05$. The changes of CIC expression after transfected were determined by real-time PCR (C) and western blot (D). The blank cell represented wild type SKOV3 and SKOV3-TR30 without any treatment. The control cells in SKOV3 and SKOV3-TR30 group represented cells transfected null vectors only. And the treated cells in the two groups represented as "miR-1307 overexpressed" in SKOV3 and "miR-1307 suppressed" in SKOV3-TR30, respectively. *Indicates $P < 0.05$.

As shown in several previous studies, CIC gene is part of the MAPK signaling regulation [18], so we focused on PEA3/ETS transcription factors as the downstream targets of CIC gene. When miR-1307 was overexpressed in SKOV3 cells, we observed significantly increased protein levels of ETV4 and ETV5 compared to vector-only controls (Fig. 4B, C, $P < 0.05$). Conversely, the protein levels of both ETV genes reduced significantly in SKOV3-TR30 cells when miR-1307 was inhibited compared to controls (Fig. 4B, C, $P < 0.05$). These results suggested the presence of regulatory axis of miR-1307/CIC/ETV genes in ovarian cancer cells.

3.5. The expression of CIC regulates paclitaxel sensitivity in ovarian cancer cells

If CIC gene is indeed regulated by miR-1307, as we demonsated above (Fig. 4), it is possible that CIC gene may also play a role in chemotherapeutic resistance. Similar to the experimental setting for miR-1307, we found that cell proliferation was significantly increased in CIC siRNA transfected SKOV3 cells at 48 h and 72 h compared to cells transfected negative control (Fig. 5A, $P < 0.05$). In addition, the cell viability in CIC siRNA-1-transfected cells (IC50: 620.7 ng/ml) was

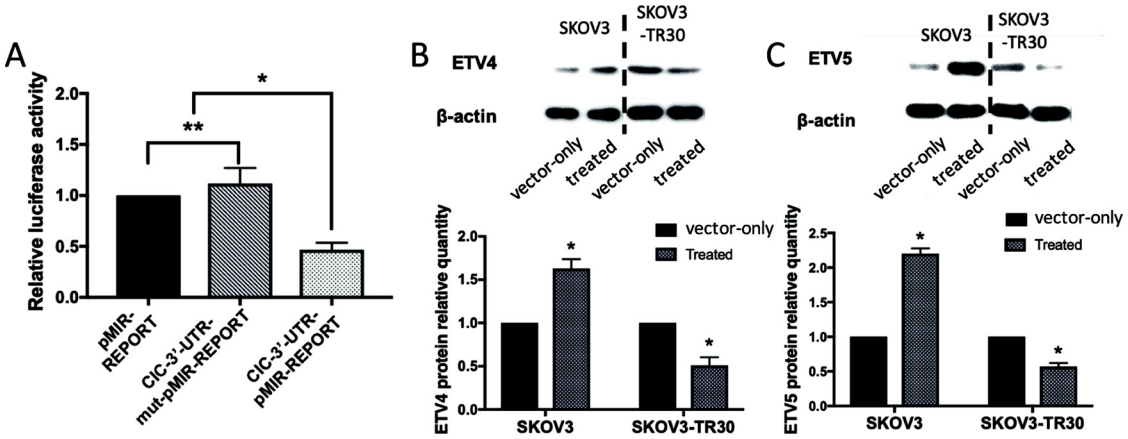

Fig. 4. The miR-1307 regulates CIC via its 3'UTR and associated RTK signaling effectors.
Wild-type and mutant containing miR-1307 binding sites of CIC 3′-UTR were constructed and co-transfected with pRL-TK and miR-1307 mimic or control. Results of dual-luciferase reporter assay for wild CIC, CIC-mut and pMIR-REPORT are shown in (A). The changes of ETV4 (B) and ETV5 (C) expression after transfected were determined by real-time PCR and western blot. The vector-only control cells in SKOV3 and SKOV3-TR30 group represented cells transfected null vectors only. And the treated cells in the two groups served as miR-1307 overexpressed SKOV3 and suppressed SKOV3-TR30, respectively. The relative expression of protein was quantified by Gel-Pro-Analyzer after western blot, and the levels were normalized by β-actin loading control. Relative changes in protein intensities were presented as histogram, control was standardized as 1-fold. Results were expressed as mean ± SD of three independent assays (n = 3), and * indicates $P < 0.05$ suggesting significant difference compared with control cells.

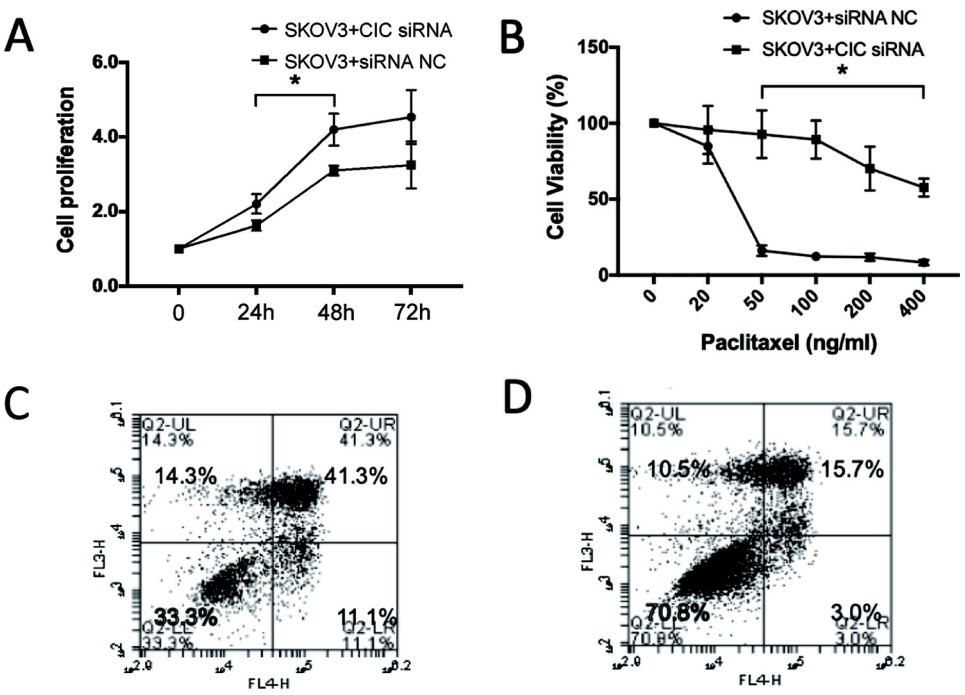

Fig. 5. CIC knockdown in SKOV3 cells enhances the resistance to paclitaxel.
Cell proliferation and apoptosis were evaluated by MTT assay and flow cytometry, respectively, IC50 values in CIC siRNA and null control transfected cells were calculated by treating cells with different concentrations of paclitaxel. (A) Cell proliferation (%) in transfected SKOV3 cells cultured for 0–72 h. (B) cell viability (%) transfected SKOV3 cells treated with different concentrations of paclitaxel for 48 h. Apoptosis and cell cycle in null control (C) and CIC siRNA transfected SKOV3 cells (D) treated with 50 ng/ml paclitaxel for 48 h. * indicates $P < 0.05$.

significantly higher than that in control cells (IC50: 33.20 ng/ml) after treated with 50 ng/ml or higher concentration of paclitaxel for 48 h (Fig. 5B, $P < 0.05$). Moreover, flow cytometric analysis showed that CIC siRNA-transfected SKOV3 cells treated with 50 ng/ml paclitaxel had significantly reduced apoptotic rate compared to controls (Fig. 5C, D).

4. Discussion

Several studies have found an association between miR-1307 expression and the occurrence and development of various human tumors, including colorectal carcinogenesis [19] and prostate cancer proliferation [20]. Moreover, the involvement of miRNAs in the development of chemotherapy drug resistance in ovarian cancer via inhibition of pro-apoptotic signaling pathway has been previously demonstrated [21]. Shimomura A. et al. reported that a combination of specific miRNAs with miR-1307 could be used for early stage detection of breast cancer with higher accuracy and sensitivity [10]. Even though miR-1307 clearly plays a role in cancer development and drug resistance, its underlying molecular events remain unclear. In this study, we aimed to provide a new line of evidence showing that miR-1307 expression impacts the susceptibility of chemotherapeutic drug in ovarian cancers by studying the regulatory relationship of miR-1307 and one of its downstream targets, CIC transcriptional repressor.

Benefited from the fact that SKOV3-TR30 cell line, which is derived from the parental SKOV3 line, exhibits higher level of miR-1307 (Fig. 1A), it works perfectly as a substitute cell model for studying chemoresistant ovarian cancers, without the drawback of using primary tissues/cells. By manipulating miR-1307 level in SKOV3 and SKOV3-TR30 lines, we demonstrated that the expression of miR-1307 strongly influences the balance of cell proliferation and cell death, as well as the dynamics of cell cycle (Fig. 1). Importantly, the expression of miR-1307 in SKOV3 ovarian cancer cells is also strongly correlated with the sensitivity to paclitaxel, a microtubule inhibitor and a first-line chemotherapeutic drug for ovarian serous cancer. Our miR-1307 result is interesting because it contrasts with the roles of some known miRNAs on chemoresistance of tumor cells, particularly the miR-200 family, which has been shown to confer the chemosensitivity to microtubule-targeting chemotherapeutics in various cancers [22]. For instance, miR-200c sensitizes breast cancer cells to doxorubicin treatment [23]. However, our result is not unusual as the function of a particular microRNA is largely dependent on its downstream targets and their roles in chemoresistance.

As a proof-of-principle, we focused on one of the downstream targets, CIC gene, which was identified first in a study of gene mutation affecting the development of head and tail in Drosophila embryo [24]. Identified via our *in silico* target search, we confirmed here that CIC is regulated by miR-1307 and its expression could explain, at least partly, how miR-1307 regulates the resistance of ovarian cancers to paclitaxel (Figs. 2–4). Furthermore, we also identified a “double negative” regulation by miR-1307/CIC axis on RTK signaling effectors [16], such as ETV4 and ETV5 genes (Fig. 5), that could in turn contributes significantly to the acquisitions of chemoresistant characteristics in certain ovarian cancers. This is highly consistent with published literatures showing that overexpression of ETV4 and ETV5 has been reported in ovarian cancer, where ETV4 bound the promoter region of the multidrug resistance protein 1 (MDR1), and upregulated its transcription [25,26]. It would be of interest to check if other members of the MDR/ABCB family genes are regulated by miR-1307/CIC axis in ovarian cancers.

In conclusion, our study not only provide a new line of research but also offer a new basis for individualized treatment of serous ovarian cancer as well as guidance for clinical treatment to improve prognosis of patients. The expression level of miR-1307 may be used to effectively predict the prognosis of chemotherapy for serous ovarian cancer, to assess the effectiveness of chemotherapy and to direct individualized clinical treatment regimens. Further detailed studies concerning the precise role of miR-1307 and its regulatory mechanism on downstream targets on the development of drug resistance in ovarian cancer may pave a way towards new theoretical therapeutic bases for the reversal of drug resistance in ovarian cancer.

Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Funding

This study is supported by the program of Outstanding Scientific Fund of Shengjing Hospital, grant No. 201705.

Author contributions

YZ (Zhou) participated in molecular biology studies, bioinformatics analysis and drafted the manuscript. T S participated in the molecular biology studies. YL participated in the statistical analyses and bioinformatics analysis. YZ (Zhang) and CS participated in the bioinformatics analysis. MW is the corresponding author and participated in the research design, quality control and provided research grant. All authors read and approved the final manuscript.

Declaration of Competing Interest

All the authors declare that they have no conflict of interest.

Acknowledgment

None.

References

[1] X. Zhu, Y. Li, C. Xie, X. Yin, Y. Liu, Y. Cao, et al., miR-145 sensitizes ovarian cancer cells to paclitaxel by targeting Sp1 and Cdk6, Int. J. Cancer 135 (2014) 1286–1296.
[2] C. Zong, J. Wang, T.M. Shi, MicroRNA 130b enhances drug resistance in human ovarian cancer cells, Tumour Biol. 35 (2014) 12151–12156.
[3] V. Lopes-Rodrigues, H. Seca, D. Sousa, E. Sousa, R.T. Lima, M.H. Vasconcelos, The network of P-glycoprotein and microRNAs interactions, Int. J. Cancer 135 (2014) 253–263.
[4] H. Zhao, S. Liu, G. Wang, X. Wu, Y. Ding, G. Guo, et al., Expression of miR-136 is associated with the primary cisplatin resistance of human epithelial ovarian cancer, Oncol. Rep. 33 (2015) 591–598.
[5] C. Denoyelle, B. Lambert, M. Meryet-Figuiere, N. Vigneron, E. Brotin, C. Lecerf, et al., miR-491-5p-induced apoptosis in ovarian carcinoma depends on the direct inhibition of both BCL-XL and EGFR leading to BIM activation, Cell Death Dis. 5 (2014) e1445.
[6] M.V. Iorio, C.M. Croce, MicroRNAs in cancer: small molecules with a huge impact, J. Clin. Oncol. 27 (2009) 5848–5856.
[7] R.D. Morin, M.D. O'Connor, M. Griffith, F. Kuchenbauer, A. Delaney, A.L. Prabhu, et al., Application of massively parallel sequencing to microRNA profiling and discovery in human embryonic stem cells, Genome Res. 18 (2008) 610–621.
[8] J.Y. Zhu, T. Pfuhl, N. Motsch, S. Barth, J. Nicholls, F. Grasser, et al., Identification of novel Epstein-Barr virus microRNA genes from nasopharyngeal carcinomas, J. Virol. 83 (2009) 3333–3341.
[9] C.V. Collares, A.F. Evangelista, D.J. Xavier, D.M. Rassi, T. Arns, M.C. Foss-Freitas, et al., Identifying common and specific microRNAs expressed in peripheral blood mononuclear cell of type 1, type 2, and gestational diabetes mellitus patients, BMC Res. Notes 6 (2013) 491.
[10] A. Shimomura, S. Shiino, J. Kawauchi, S. Takizawa, H. Sakamoto, J. Matsuzaki, et al., Novel combination of serum microRNA for detecting breast cancer in the early stage, Cancer Sci. 107 (2016) 326–334.
[11] F. Cordero, G. Ferrero, S. Polidoro, G. Fiorito, G. Campanella, C. Sacerdote, et al., Differentially methylated microRNAs in prediagnostic samples of subjects who developed breast cancer in the European Prospective Investigation into Nutrition and Cancer (EPIC-Italy) cohort, Carcinogenesis 36 (2015) 1144–1153.
[12] W.M. Tao, Screening of differentially expressed microrna in chemoresistant and sensitive ovarian cancer cells and the identification of the tissues, J. China Med. Univ. 42 (2013) 4.
[13] T. Shuang, J. Wu, Y. Zhu, C. Shi, P. Zhang, Z. Jie, et al., Differentially microRNA expression detection between chemoresistance and sensitive ovarian cancer tissue and preliminary analysis, China J. Prac. Gynecol. Obst. 29 (2013) 33–37.
[14] J. Qiu, Y.F. Fu, Q. Cheng, X.D. Cheng, X. Xie, W.G. Lü, Reversing paclitaxel-resistance of SKOV3-TR30 cell line by curcumin, Zhonghua Yi Xue Za Zhi 92 (2012) 1926–1928.
[15] Y. Fu, D. Ye, H. Chen, W. Lu, F. Ye, X. Xie, Weakened spindle checkpoint with reduced BubR1 expression in paclitaxel-resistant ovarian carcinoma cell line SKOV3-TR30, Gynecol. Oncol. 105 (2007) 66–73.
[16] V.G. LeBlanc, M. Firme, J. Song, S.Y. Chan, M.H. Lee, S. Yip, et al., Comparative transcriptome analysis of isogenic cell line models and primary cancers links capicua (CIC) loss to activation of the MAPK signalling cascade, J. Pathol. 242 (2017) 206–220.
[17] R.A. Okimoto, F. Breitenbuecher, V.R. Olivas, W. Wu, B. Gini, M. Hofree, et al., Inactivation of Capicua drives cancer metastasis, Nat. Genet. 49 (2017) 87–96.
[18] G. Jimenez, S.Y. Shvartsman, Z. Paroush, The Capicua repressor – a general sensor of RTK signaling in development and disease, J. Cell. Sci. 125 (Pt 6) (2012) 1383–1391.
[19] R. Tang, Q. Qi, R. Wu, X. Zhou, D. Wu, H. Zhou, et al., The polymorphic terminal-loop of pre-miR-1307 binding with MBNL1 contributes to colorectal carcinogenesis via interference with Dicer1 recruitment, Carcinogenesis 36 (2015) 867–875.
[20] X. Qiu, Y. Dou, miR-1307 promotes the proliferation of prostate cancer by targeting FOXO3A, Biomed. Pharmacother. 88 (2017) 430–435.
[21] S. Kumar, A. Kumar, P.P. Shah, S.N. Rai, S.K. Panguluri, S.S. Kakar, MicroRNA signature of cis-platin resistant vs. cis-platin sensitive ovarian cancer cell lines, J. Ovarian Res. 4 (2011) 17.
[22] B. Humphries, C. Yang, The microRNA-200 family: small molecules with novel roles in cancer development, progression and therapy, Oncotarget 6 (2015) 6472–6498.
[23] F. Kopp, P.S. Oak, E. Wagner, Roidl A. miR-200c sensitizes breast cancer cells to doxorubicin treatment by decreasing TrkB and Bmi1 expression, PLoS One 7 (2012) e50469.
[24] G. Jimenez, A. Guichet, A. Ephrussi, J. Casanova, Relief of gene repression by torso RTK signaling: role of capicua in Drosophila terminal and dorsoventral patterning, Genes Dev. 14 (2000) 224–231.
[25] Y. Zhao, J. Liu, Q. Hong, C. Yang, L. Chen, Y. Chen, et al., Involvement of MyoD and PEA3 in regulation of transcription activity of MDR1 gene, Acta Biochim. Biophys. Sin. (Shanghai) 42 (2010) 900–907.
[26] S. L'Esperance, I. Popa, M. Bachvarova, M. Plante, N. Patten, L. Wu, et al., Gene expression profiling of paired ovarian tumors obtained prior to and following adjuvant chemotherapy: molecular signatures of chemoresistant tumors, Int. J. Oncol. 29 (2006) 5–24.