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Involvement of microRNA-93, a new regulator of PTEN/Akt signaling pathway, in regulation of chemotherapeutic drug cisplatin chemosensitivity in ovarian cancer cells

Xin Fu [a,b], Jing Tian [a], Lei Zhang [a], Ying Chen [a], Quan Hao [a,b,*]

[a] Department of Gynecology Cancer, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China
[b] Key Laboratory of Cancer Prevention and Therapy, Tianjin, China

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ABSTRACT

The mechanisms underlying ovarian cancer cell resistance to cisplatin (CDDP) are not fully understood. MicroRNAs (miRNAs) play important roles in tumorigenesis and drug resistance. In this paper, we utilized microRNA array and real-time PCR to show that miR-93 is significantly up-regulated in cisplatin-resistant ovarian cancer cells. In vitro assays show that over-expression and knock-down of miR-93 regulate apoptotic activity, and thereby cisplatin chemosensitivity, in ovarian cells. Furthermore, we found that miR-93 can directly target PTEN, and participates in the regulation of the AKT signaling pathway. MiR-93 inversely correlates with PTEN expression in CDDP-resistant and sensitive human ovarian cancer tissues. These results may have implications for therapeutic strategies aiming to overcome ovarian cancer cell resistance to cisplatin.

1. Introduction

Ovarian cancer is the leading cause of death from gynecologic malignancies and the fourth most common cause of death due to cancer among women. The regular treatment for ovarian cancer is surgical intervention followed by combination chemotherapy [1]. The main clinical obstacle is that the initial response rate is poor, and most of the patients will experience disease recurrence. Cisplatin is one of the most effective cell cycle non-specific drugs for the treatment of ovarian cancer and the mechanism involved in the process of its cytotoxicity include survival inhibition and apoptosis [2]. The poor five-year survival rates in ovarian cancer are partly due to the development of platinum resistance [3]. Until now, CDDP chemoresistance still remains a major obstacle for the successful treatment of ovarian cancer.

The characteristics of chemoresistance in cancer cells are linked to mutational events (genetic hypothesis) and to non-mutational alterations of gene function (epigenetic hypothesis). Although focusing on known genes has already yielded new information, previously unknown noncoding RNAs, such as microRNAs (miRNAs), may also lead insight into the biology of chemoresistance [4]. MiRNAs, which are approximately 22 nt in length, are shown to function as post-transcriptional regulators by binding to complementary sites in the 3′untranslated regions (3′UTRs) of the target mRNAs [5]. Due to the wide range of target genes, miRNAs are involved in various physiological and pathological processes, including CDDP chemoresistance. Some miRNAs were found to be up- or down-regulated in cancer cells characteristic of a CDDP resistant phenotype, such as germ cell tumors [6], tongue squamous cell carcinoma [7] and MCF-7 breast cancer cells [8]. Up-regulation of miR-451 increases CDDP sensitivity of non-small cell lung cancer cell line A549 [9], and miR-148a could sensitize esophageal cancer cell lines to CDDP [10]. On the contrary, miR-141 could counteract the CDDP-induced apoptosis in esophageal squamous cell carcinoma by targeting YAP1 [11]. MiR-200c induces CDDP chemoresistance in esophageal cancers through interacting with the AKT signaling pathway [12]. Moreover, miR-214 could enhance ovarian cancer cell survival and induce CDDP resistance primarily through targeting the PTEN/AKT pathway [13]. These studies together highlight the need to study miRNAs that are involved in CDDP chemoresistance in various cancers. However, whether there are other unknown miRNAs that are involved in the function and mechanism of CDDP chemosensitivity in ovarian cancer is still largely unknown.

In the present study, we induced CDDP-resistant OVCAR3 and SKOV3 human ovarian cancer cells, and then detected the

* Corresponding author at: Department of Gynecology Cancer, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China. Fax: +86 22 23521569.
E-mail address: Haoquandoctor_2011@yahoo.cn (Q. Hao).

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dysregulated miRNAs in the CDDP-resistant cells compared with the parent cancer cells. MiR-93 was found to be up-regulated in both of the two CDDP-resistant ovarian cancer cell lines, and the tumor suppressor gene phosphatase and tensin homolog (PTEN) was confirmed to be a direct target gene of miR-93. Overexpression of miR-93 could suppress PTEN expression and induce CDDP chemoresistance in parent OVCAR3 and SKOV3 cells, which could be partly alleviated by ectopic expression of PTEN. Conversely, suppression of miR-93 led to the increase of PTEN expression and CDDP sensitization in CDDP-resistant OVCAR3 and SKOV3 cells, which was partly alleviated by PTEN siRNA. Furthermore, miR-93 mediated suppression of PTEN could induce phosphorylation of AKT1, which was indicated to facilitate cell survival and reduction of apoptosis. Taken together, miR-93 plays a critical role in regulating CDDP chemosensitivity through suppression of PTEN expression, and it may serve as a potential target for overcoming CDDP resistance in human ovarian cancer.

2. Materials and methods

2.1. Ovarian cancer tissue samples, cell culture, transfection and RNA extraction

The CDDP-sensitive and -resistant ovarian cancer tissue samples were obtained from Tianjin Medical University Cancer Hospital with patients' informed consent. All the samples were snap-frozen in liquid nitrogen and stored at −80 °C until use. Human ovarian cancer cell line OVCAR3 and SKOV3 were maintained in RPMI-1640 (Invitrogen) supplemented with 10% fetal bovine serum (FBS), 100 IU/ml of penicillin, 100 μg/ml of streptomycin, and variant concentration of CDDP (Wako) if needed. Cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. Transfection was performed using Lipofectamine 2000 Reagent (Invitrogen) following the manufacturer's instructions. RNA extraction was performed using mirVana™ miRNA Isolation Kit (Ambion) according to the manufacturer's protocol.

2.2. Induction of CDDP-resistant ovarian cancer cell lines

The CDDP-resistant ovarian cancer cell lines were induced using progressive concentration of CDDP. Briefly, the OVCAR3 and SKOV3 cells in logarithmic growth were treated with 0.5 μmol/L of CDDP. After 48h, CDDP was withdrawn and cells were cultured without CDDP until they recovered. Then, the same treatment was performed, and when the cells were resistant to the current concentration, the CDDP concentration was gradually increased to 1, 2, 3, 4 and finally to 6 μmol/L. When the induced cells survived in 6 μmol/L of CDDP for about 2 months with a normal activity, and withdrawn of CDDP, they still keep the same activity and CDDP resistance characteristic with the condition of 6 μmol/L CDDP drugs in the culture medium, the cells were confirmed to be CDDP-resistant and named OVCAR3/CDDP and SKOV3/CDDP.

2.3. IC_{50} analysis

Cytotoxicity of CDDP was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The logarithmically growing ovarian cancer cells were plated in 96-well plates with 0, 20, 40, 60, 80, or 100 μmol/L of CDDP. At 48 h after CDDP treatment, MTT was added into cells to a final concentration of 0.5 mg/ml. Four hours later, the reduced insoluble MTT was removed and the formazan was solubilized in 100 ml dimethyl sulfoxide. The absorbance of each well was determined at 490 nm using a μQuant Universal Microplate Spectrophotometer (Bio-Tech Instruments, Winooski, USA). The concentration of CDDP that caused 50% inhibition of ovarian cancer cell activity were defined as IC_{50}.

2.4. MiRNA microarray

The miRNA microarray analysis was performed as previously described [14]. Briefly, 30 μg of purified small RNA was fractionated on urea denaturing 15% polyacrylamide gel, and 18–26 nt of RNAs were recovered and purified. Two oligonucleotide adaptors were then ligated to the 5′ and 3′ ends of the retrieved RNA using T4 RNA ligase (Fermentas). A cDNA library was generated by reverse transcription using M-MLV reverse transcriptase (Promega) and the product was amplified using asymmetric PCR and labeled with Cy3 or Cy5. The samples were hybridized to the microarray containing 640 human mature miRNA probes at 42 °C overnight Then the slide was washed and scanned. The miRNAs displayed up- or down-regulation for more than threefold was picked out to be the dysregulated miRNAs.

2.5. Quantitative RT-PCR

For the detection of miR-93 level, the stem-loop quantitative RT-PCR [15] was performed. SYBR Premix Ex TaqTM Kit (TaKaRa) was used following the manufacturer's instructions, and the real-time PCR was performed and analyzed by iQ5 Real-Time PCR Detection System (Bio-Rad). PCR cycles were as follows: 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s.

2.6. Bioinformatics

The target genes of miRNA were predicted by the following two computer-aided algorithms: TargetScan Release 5.2 (http://www.targetscan.org) and PicTar (http://pictar.mdc-berlin.de/cgi-bin/new_PicTar_vertebrate.cgi).

2.7. EGFP reporter assay

To confirm the direct interaction of miRNA and the target mRNA, the ovarian cancer cells were plated in 48-well plates and transfected with the EGFP reporter vectors along with the miR-93 expression vector or the miR-93 blockage (2′-O-methyl miR-93 ASO, 5′-cuaccugcacgaacagcacuuug-3′). An accordant amount of red fluorescence protein (RFP) expression vector pDsRed2-N1 was co-transfected into each group to be used for normalization. At 48 h post-transfection, cells were lyzed with ratio immunoprecipitation assay lysis buffer (RIPA lysis buffer, 50 mM Tris–HCl pH 7.2, 150 mM NaCl, 1% Triton X–100, 0.1% SDS) and total proteins were harvested. The intensity of EGFP and RFP fluorescence were detected with a Fluorescence Spectrophotometer F-4500 (HITACHI).

2.8. Western blot

At 72 h after transfection, the ovarian cancer cells were lyzed with RIPA lysis buffer and proteins were harvested. The protein of tissue samples were extracted using TRIzol Reagent (Invitrogen). Proteins were resolved on an SDS denatured polyacrylamide gel and then transferred onto a nitrocellulose membrane. Antibodies to PTEN, AKT1, phosphorylated AKT1 or the endogenous control GAPDH were incubated with the blot overnight at 4 °C. Membranes were washed and incubated with horseradish peroxidase (HRP) conjugated secondary antibody. Protein expression was assessed by enhanced chemiluminescence and exposure to chemiluminescent film. LabWorks™ Image Acquisition and Analysis Software (UVP) was used to quantify band intensities. The antibodies were purchased from Saier (Tianjin, China).

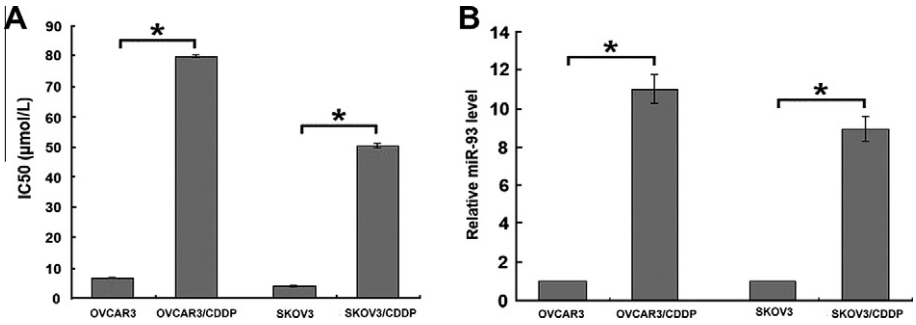

Fig. 1. miR-93 is up-regulated in CDDP-resistant ovarian cancer cell lines. (A) Gradually increased CDDP was added into OVCAR3 and SKOV3 cells to induce the CDDP-resistant ovarian cancer cell lines. The sensitivity of the induced cells to CDDP was measured using MTT assay, and the IC50 was calculated as described in the Section 'Materials and methods' part. (B) The miR-93 expression level in the finally induced CDDP-resistant cells was measured using quantitative RT-PCR assay. U6 snRNA was regarded as the endogenous normalizer and the relative miR-93 expression level is shown (*$p < 0.05$).

Table 1
Dysregulated miRNAs in OVCAR3/CDDP and SKOV3/CDDP cells by miRNA microarray.

miRNA ID	Fold change	
	OVCAR3/CDDP: OVCAR3	SKOV3/CDDP: SKOV3
hsa-miR-15a	3.24	8.71
hsa-miR-19a	12.78	4.87
hsa-miR-21	3.57	7.62
hsa-miR-204	3.16	5.59
hsa-miR-93	9.47	7.28
hsa-miR-96	4.74	6.21
hsa-miR-22	0.19	0.22
hsa-miR-489	0.27	0.16

The fold change of dysregulated miRNAs in CDDP-resistant ovarian cancer cells compared to the parent cells is shown.

2.9. Annexin V and 7-aminoactinomycin D staining

Enumeration of apoptotic cells was done by using FITC conjugated Annexin V (BD Pharmingen, San Jose, CA) and 7-aminoactinomycin D (7-AAD). Cells were washed twice in cold 1× PBS and resuspended in Annexin V–binding buffer (BD Pharmingen) at a concentration of 3×10^6 per ml. This suspension (100 μl) was stained with 5 μl of Annexin V-FITC and 5 μl 7-AAD. 7-AAD (BD Pharmingen) is a nucleic acid dye and used for exclusion of nonviable cells. These cells were gently vortexed and incubated for 15 min at room temperature in the dark. After addition of 400 μl of binding buffer to each tube, cells were analyzed by flow cytometry.

2.10. Statistical analysis

All the experiments were carried out in triplicate and data were analyzed using SPSS v11.0. Quantitative values were expressed as means ± SD, and statistical analysis utilized two-tailed Student's *t* test. Statistical significance was set as $p < 0.05$.

3. Results

3.1. The expression level of miR-93 was up-regulated in CDDP-resistant ovarian cancer cell lines OVCAR3/CDDP and SKOV3/CDDP

To seek the miRNAs associated with CDDP resistance in ovarian cancer cells, we first established the CDDP-resistant OVCAR3/CDDP and SKOV3/CDDP cells by progressive concentration of CDDP from 0.5 μmol/L to 6 μmol/L. Using MTT assay, we found that the IC_{50} of

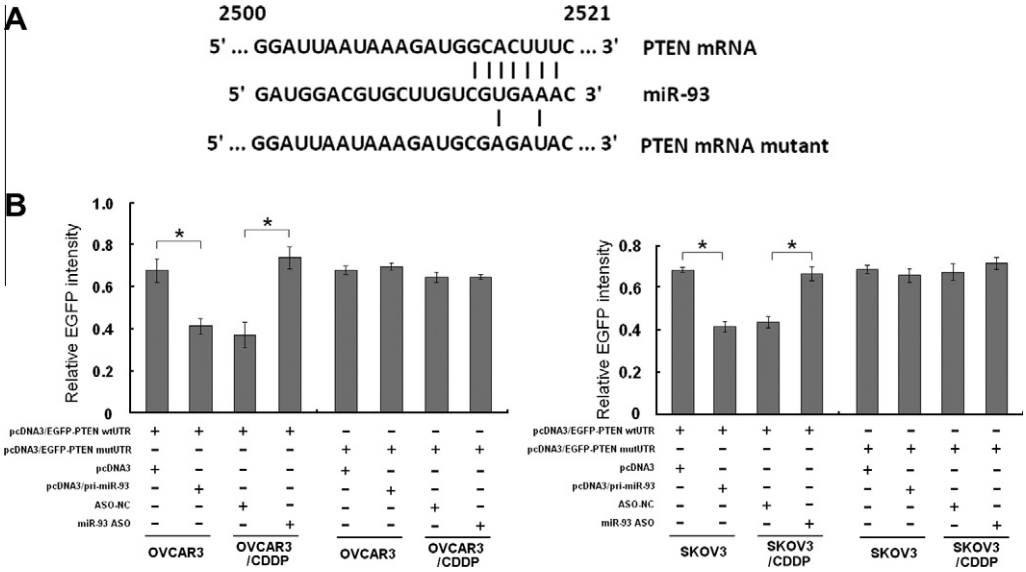

Fig. 2. PTEN is a direct target gene of miR-93. (A) As is predicted in TargetScan database, the PTEN 3′UTR carries a potential miR-93 binding site. The sequence of mutated PTEN 3′UTR is also shown. (B) EGFP reporter assay was performed in the parental and CDDP-resistant OVCAR3 and SKOV3 cells. Cells were transfected with the reporter vector with wild-type or mutated PTEN 3′UTR fragment, along with the miR-93 ectopic expression vector (pcDNA3/pri-miR-93) or the miR-93 blockage (miR-93 ASO). An accordant amount of RFP expression vector pDsRed2-N1 was co-transfected into each group to be used for normalization. At 48 h post transfection the cells were lysed and the intensity of EGFP and RFP fluorescence was detected. The normalized EGFP intensity is shown (*$p < 0.05$).

OVCAR3/CDDP and SKOV3/CDDP cells increased to 11.89 and 12.51 times, respectively, compared with the parent cancer cells (Fig. 1A). Next, we used miRNA microarray analysis to screen the dysregulated miRNAs in the ovarian cancer cell lines. As a result, six miRNAs were showed significantly up-regulation, and two miRNAs were showed significantly down-regulation in both the OVCAR3/CDDP and SKOV3/CDDP cells (Table 1). The microarray datas were verified by the quantitative RT-PCR assay, in which miR-93 showed completely coincidence trend with the microarray (Fig. 1B). Thus, we chose miR-93 as a potential regulator in the process of ovarian cancer cell chemosensitivity adjustment to CDDP in the further study.

3.2. MiR-93 directly targets PTEN 3′UTR and negatively regulates its expression

The cellular functions of miRNAs are revealed through their target genes. With the help of PicTar-Vert and Target Scan databases, we hypothesized PTEN, a tumor suppressor gene with phosphorylase activity, to be a potential target gene of miR-93, and found the target site of miR-93 within the PTEN 3′UTR (Fig. 2A). Next, we used EGFP reporter assay to confirm the direct regulation of PTEN by miR-93 in the parent and CDDP-resistant ovarian cancer cell lines. When the EGFP reporter vector with the wild type PTEN 3′UTR was used, overexpression of miR-93 in the parent OVCAR3 and SKOV3 cells

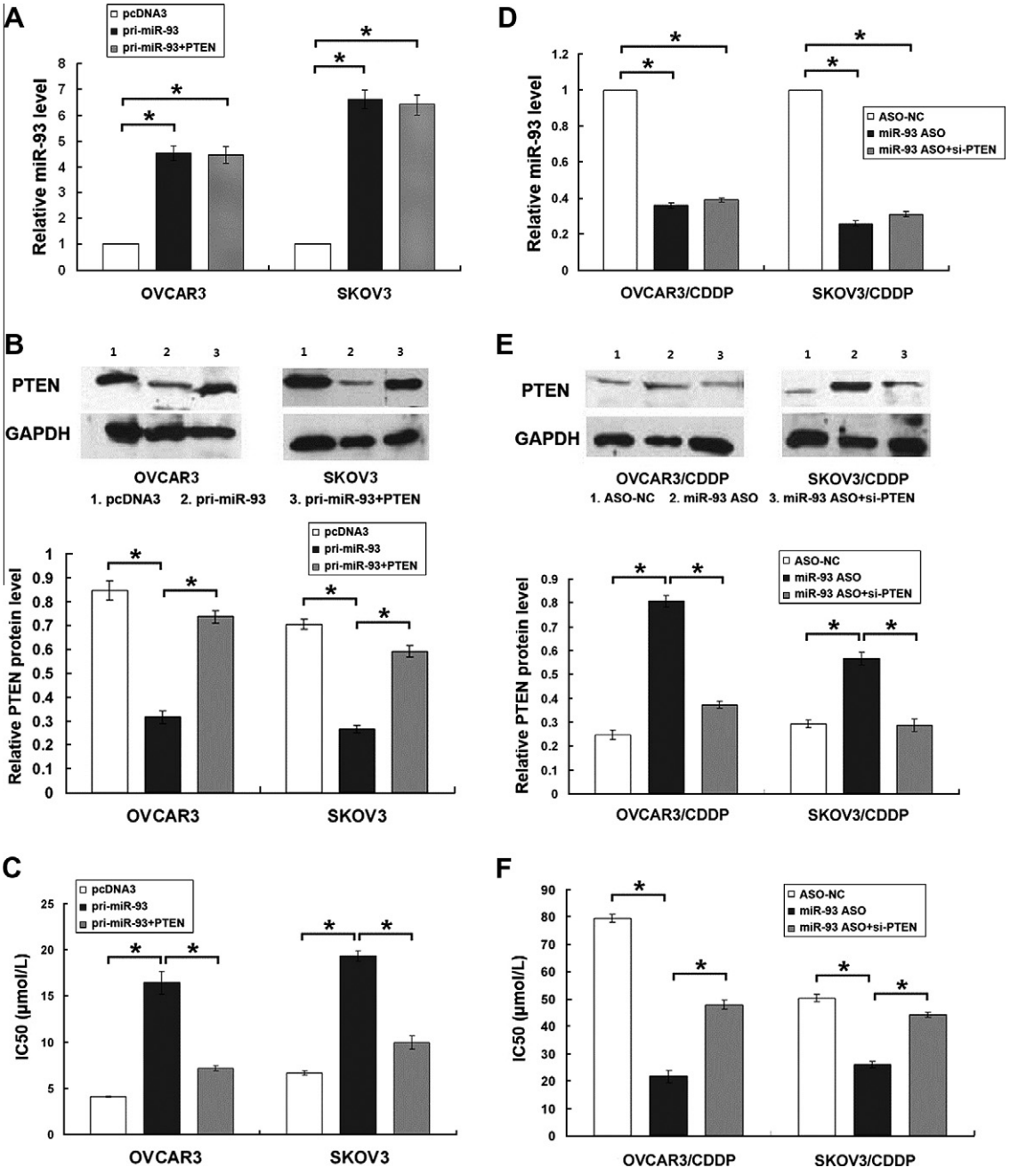

Fig. 3. MiR-93 regulate cisplatin chemosensitivity in OVCAR3 and SKOV3 cells by targeting PTEN. (A) OVCAR3 and SKOV3 cells were transfected with miR-93 expression vector (pcDNA3/pri-miR-93) with or without a PTEN ectopic expression vector (PTEN), and the miR-93 level was detected using quantitative RT-PCR assay. (B) The PTEN protein level in the transfected OVCAR3 and SKOV3 cells was measured using Western blot assay. GAPDH was used as the endogenous normalizer, and the histogram shows the relative PTEN protein level. (C) The sensitivity of the transfected cells to CDDP was measured using IC50 index. (D-F) OVCAR3/CDDP and SKOV3/CDDP cells were transfected with miR-93 blockage (miR-93 ASO) with or without a PTEN siRNA expression vector (si-PTEN), and the same experiments as described above was performed (*$p < 0.05$).

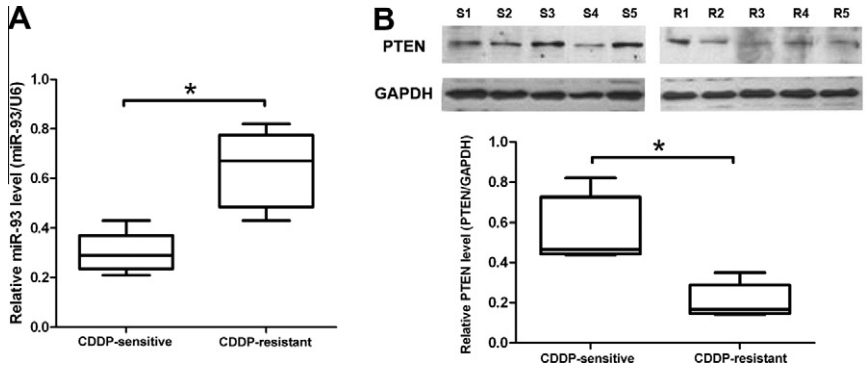

Fig. 4. miR-93 exhibits opposite alteration to PTEN in CDDP-resistant human ovarian cancer tissues. Five CDDP-resistant and five CDDP-sensitive human ovarian cancer tissues were collected, and the expression of miR-93 and PTEN was detected at the mRNA and protein levels by quantitative RT-PCR assay (A) and Western blot assay (B), respectively (*$p < 0.05$).

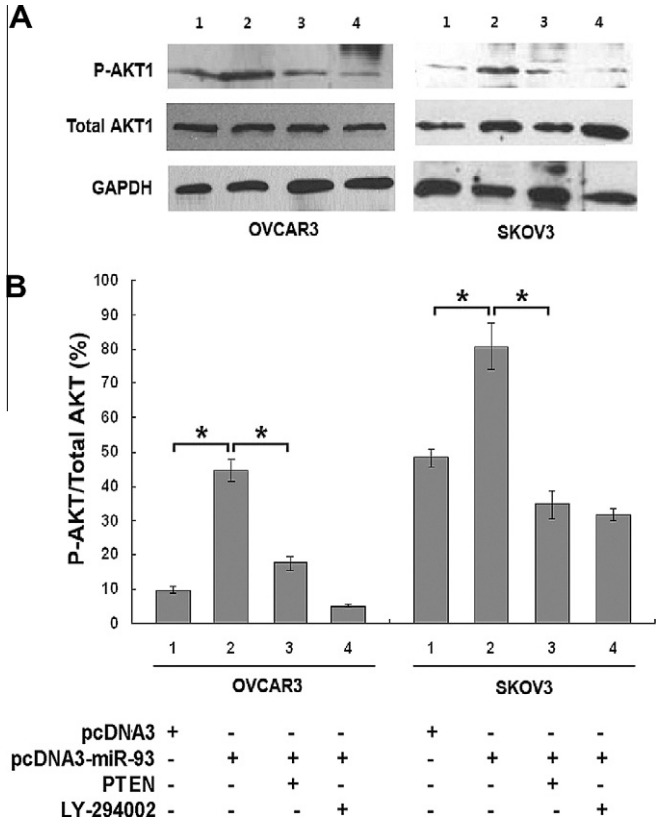

Fig. 5. Suppression of PTEN expression by miR-93 could induce phosphorylation of AKT1. Parental OVCAR3 and SKOV3 cells were transfected with the miR-93 ectopic expression vector (pcDNA3/pri-miR-93) with or without a PTEN ectopic expression vector (PTEN). Then, the levels of phosphorylated AKT1 and total AKT1 were measured using Western blot assay. LY-294002 is a potent inhibitor of phosphoinositide 3-kinases (PI3Ks), which served here as a reference of the dephosphorylated AKT1 (*$p < 0.05$).

could suppress EGFP intensity, and suppression of miR-93 in the CDDP-resistant OVCAR3/CDDP and SKOV3/CDDP cells led to an increase of EGFP expression (Fig. 2B and C). The binding was specific, because the EGFP reporter vector with the mutated PTEN 3′UTR was not affected by the change of miR-93 (Fig. 2B and C). These data indicated that PTEN is directly and negatively regulated by miR-93.

3.3. MiR-93 could regulate cisplatin chemosensitivity in OVCAR3 and SKOV3 cells by targeting PTEN

Given that miR-93a showed a high expression level in CDDP-resistant ovarian cancer cells, we explored that if miR-93a may contribute to the CDDP chemoresistance in ovarian cancer. We used the pcDNA3 vector to express miR-93 and PTEN cDNA coding sequence without 3′UTR. The miR-93 was over-expressed in the parent OVCAR3 and SKOV3 cells when transfected with pri-miR-93 but without subject to the effects of PTEN expression (Fig. 3A). Over expression of miR-93 could suppress the PTEN protein expression level, which could be partly restored by the ectopic PTEN expression vector because of the effect of the miR-93 regulatory site (Fig. 3B). In the cellular activity assays, we found that ectopic expression of miR-93 could enhance the CDDP resistance in parent OVCAR3 and SKOV3 cells. Furthermore, when PTEN was ectopic expressed, the miR-93 enhanced chemoresistance was partly alleviated (Fig. 3C). On the other hand, miR-93 antisense oligonucleotides (ASO) were used to suppress miR-93 activity, and PTEN small interfering RNA (siRNA) technology to inhibit PTEN expression in CDDP-resistant OVCAR3 and SKOV3 cells (Fig. 3D). As is presumed, suppression of miR-93 could lead to increase of PTEN expression level and enhance the CDDP chemosensitivity, both of which could be partly alleviated by the PTEN siRNA (Fig. 3E and F). We also compared the miR-93 and PTEN expression level in clinical fresh ovarian cancer tissue samples with different chemosensitivity to CDDP determined by in vitro drug resistance assay [16]. MiR-93 was showed about 2.5-fold higher expression level (Fig. 4A), while PTEN was showed about 0.22-fold lower level (Fig. 4B) in CDDP-resistant ovarian cancer tissues compared with CDDP-sensitive ovarian cancer tissues. We concluded that miR-93 may regulate CDDP chemosensitivity in ovarian cancer cells by directly targeting PTEN.

3.4. Suppression of PTEN expression by miR-93 could induce phosphorylation of AKT1

AKT is one of the major cell survival pathway molecules and its activation form phospho-Akt plays a key role in multiple drug resistance, including cisplatin [17,18], so we measured the total AKT1 and phosphorylated AKT1 expression level in the transfected ovarian cancer cells. In both the OVCAR3 and SKOV3 cells, overexpression of miR-93 could increase the ratio of the phosphorylated AKT1/total AKT, and this could be partly alleviated by the PTEN expression vector (Fig. 5). This result showed that miR-93 can regulate PTEN/AKT1 signaling pathway through directly targeting PTEN.

3.5. MiR-93 regulates anti-apoptosis activity by targeting PTEN in both resistant- and sensitive ovarian cancer cells

To demonstrate that if the effect of miR-93 in regulating cisplatin chemosensitivity is via reduction of apoptosis activity in

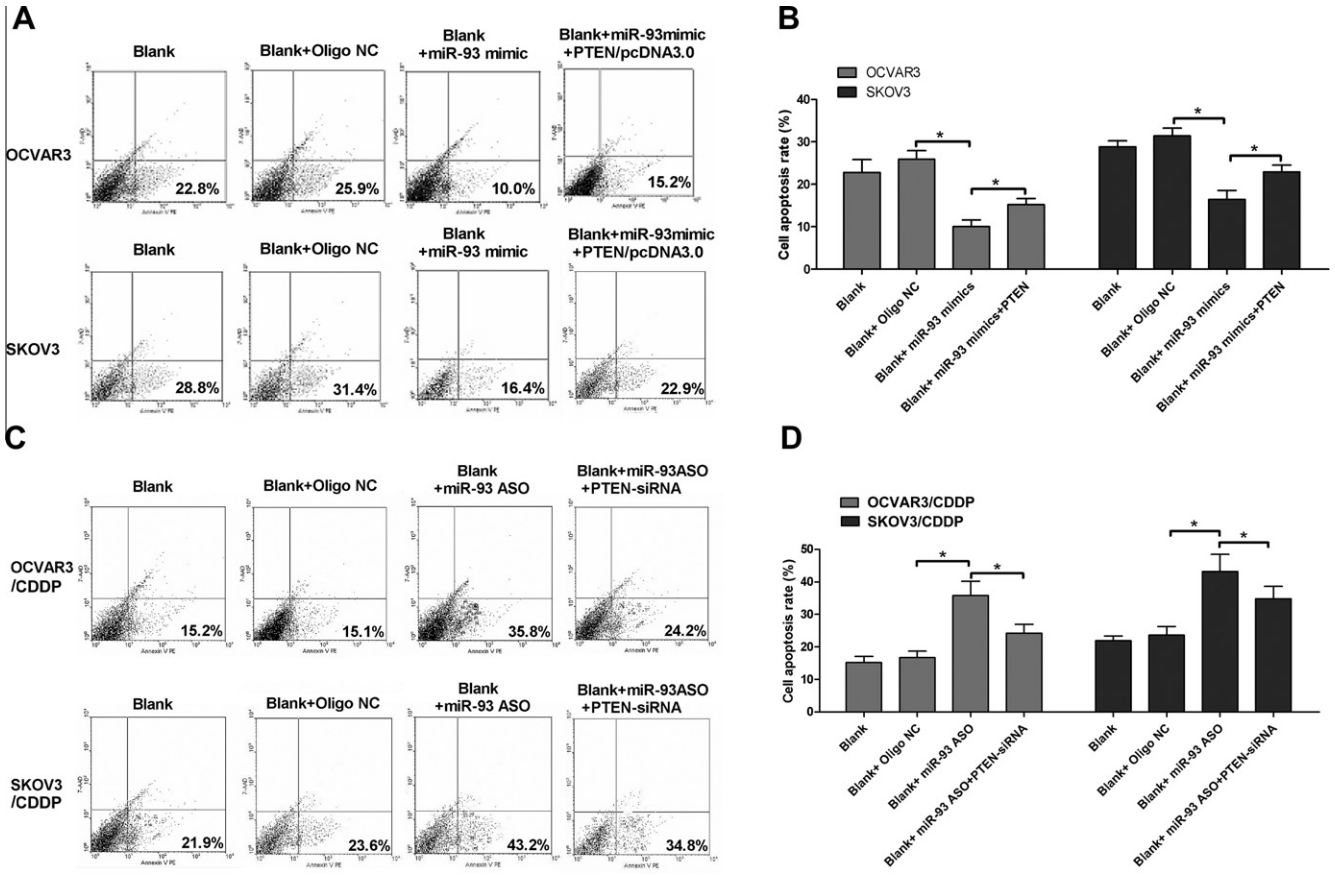

Fig. 6. miR-93 regulates anti-apoptosis activity by targeting PTEN in both resistant and sensitive colon cancer cells. (A and B) OVCAR3 and SKOV3 cells were transfected with different oligos and plasmids. 48 h after the treatment, cells were labeled with Annexin V and analyzed by flow cytometry. The representive images show that the apoptotic rate was reduced by transfection with miR-93 minics and rescued with PTEN expression vectors. Three independent experiments were done with two breast cancer cell lines in each group. (C and D) The similar treatment was done in OCVAR3/CDDP and SKOV3/CDDP cells and then FACS assays was performed the same way as (A and B).

ovarian cancer cells, FACS assays were performed in different groups of transfected ovarian cancer cells. As shown in Fig. 6, in sensitive ovarian cancer cells OVCAR3 and SKOV3, the apoptotic rates were reduced by about 60% and 48%, respectively in miR-93 mimics group compared with the Oilgo NC group and almost rescued by 33% and 43% by PTEN expression (Fig. 6A). The similar result was obtained in resistant ovarian cancer cells OVCAR3/CDDP and SKOV3/CDDP treated with miR-93 ASO, PTEN siRNA and control oligos (Fig. 6B). These results suggest that miR-93 may play an important role in regulating cisplatin chemosensitivity directly through adjustment of anti-apoptosis activity by targeting PTEN in ovarian cancer cells.

4. Discussion

MiRNAs are a class of small, noncoding RNAs, about 22 nt in length, which regulate gene expression by targeting mRNAs for translational repression, mRNA degradation or both. Recently, a larger number of miRNAs that were deregulated in different types of human malignancy were identified [19–21]. These miRNAs play an important regulatory role in pathogenesis of tumor in human involved in cell proliferation, differentiation, apoptosis, metastasis, and drug resistance [22–25]. Thus far, there are several published miRNAs that were reported to involved in the process of cisplantin resistance in varies tumors include miR-451, miR-21, miR-214, miR-23a and miR-141 [7,11,13]. Base on these findings, we performed global miRNA expression profiling in human ovarian cisplatin-resistance and parental cancer cells to find that up-regulation of miR-15a, miR-19a, miR-21, miR-204, miR-93, miR-96 and down-regulation of miR-22, miR-489 are recurrent events. This findings suggest that dysregulation of these miRNAs may represent CDDP-induced ones without any chemosensitivity adjustment function or have some certain contribution to regulate cisplatin chemosensitivity in ovarian cancer.

MiR-93 is over-expressed with the similar folds in the cisplatin-resistant OVCR3/CDDP and SKOV3/CDDP cells compared with its corresponding cisplatin-sensitive parental cell lines (Table 1), and subsequent real-time PCR experiment confirmed this result (Fig. 1). Knockdown of miR-93 enhance cisplatin chemosensitivity in cisplatin-resistant OVCR3/CDDP and SKOV3/CDDP cells, while ectopic expression of miR-93 renders OVCR3 and SKOV3 cells more resistant to cisplatin-induced apoptosis. It has been well reported that constitutive activation of AKT contributes to chemoresistance in different types of tumors, including ovarian carcinoma [17]. Recent studies have demonstrated BIM as a key regulator participating in the AKT pathway in Cisplatin-sensitive and -resistant ovarian cancer cells [26]. Yang et al. have reported that miR-214 induces cisplatin resistance by targeting PTEN [13]. In our study, we confirmed that miR-93, a new family member of PTEN regulator, blocks PTEN translation leading to activation of the AKT pathway and played an important role in regulating cisplatin chemosensitivity pathway in ovarian cancer.

MiR-93, a typical member of miR-106b-25 clusters, is participating in many kinds of cellular biological processes. One report showed that miR-93 affects the proliferation and survival of HTLV-1-infected/transformed cells through target TP53INP1 [27],

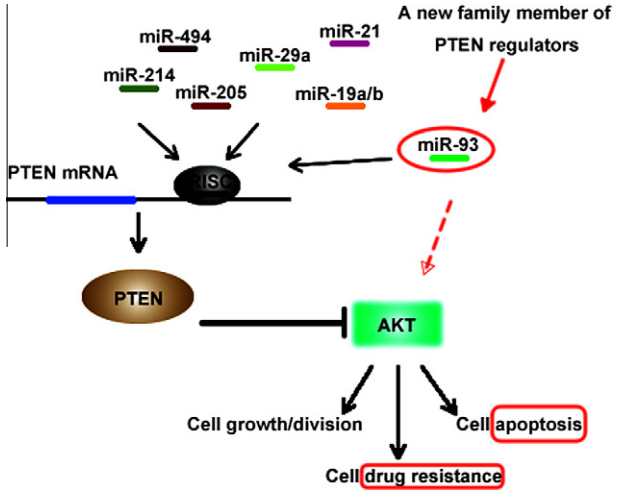

Fig. 7. Schematic representation showing the sequence of steps by which, miR-93, a new family member of PTEN regulator, regulates PTEN and phospho-Akt expression, anti-apoptosis activity and finally regulates CDDP chemo-sensitivity.

while Li et al. recently demonstrated miR-93 and its family members directly target TGF-β receptor II to enhance iPSC generation [28]. In hyperglycemic conditions, miR-93 could participate in VEGF signaling pathway and down-regulate the expression of VEGF-A in the progression of diabetic nephropathy [29]. To the best of our knowledge, however, the definitive effect of miR-93 on other anti-cancer drugs chemosensitivity such as docetaxel, paclitaxel, and doxorubicin/adriamycin has not been reported, only given the evidences showing that the miR-93 is dysregulated in drug-resistant cell lines in microRNA expression profiling study. A typical example is that miR-93 is down-regulated in doxorubicin resistant cell lines MCF/ADR [30,31]. In another study gives an controversial results that, although following the same concentration of oxaliplatin (the derivatives of cisplatin), evaluation of miR-93 was found to be up-regulated 3 times in HCT-116 colon cancer cell line, while down-regulated by 50% in another colon cancer cell line HCT-8 [32].

Although the classic PTEN pathway illustrates that PTEN can decrease the AKT activity through dephosphorylate PIP3 [33–35], the upstream regulators of PTEN were not fully understood. Previous studies show that some miRNAs such as miR-19a/b, miR-29a, miR-214, miR-205 and miR-494, can directly regulate the expression of PTEN in a post-transcription regulation manner, through interacting with its three-untranslated region in various cell lines [36–40]. In the present study, we showed that PTEN is negatively regulated by miR-93 at the protein level and the expression levels of PTEN negatively correlates with miR-93 in both the ovarian cancer cell lines OVCR3, SKOV3 and freshly surgical removed ovarian cancer specimens. The cisplatin-resistant ovarian cancer specimens showed a relative higher expression of miR-93, and lower PTEN expression compared with cisplatin-sensitive ones (Fig. 4). Therefore, these data indicate that miR-93 could be a new member of the miRNAs that was the upstream regulator of PTEN, which was involved in AKT signal pathway through targeting PTEN. But it is not clear whether and how these confirmed miRNAs such as miR-214, miR-494, miR-205 directly targeting PTEN co-regulate the PTEN/Akt pathway, further influences the CDDP drug resistance and other biological function in a collaborative or antagonistic manner (Fig. 7).

In conclusion, we have shown that miR-93 has a modulator effect on PTEN expression and its downstream AKT signal pathway, anti-apoptosis activity, which play important roles in regulating cisplatin chemosensitivity in ovarian cancer. These results provide a strong rationale for the development of miRNA-based therapeutic strategies aiming to overcome ovarian cancer cell CDDP resistance. Further studies are under way to characterize if miR-93 can regulate other anti-cancer drug chemosensitivity, serve as prognostic and/or diagnostic markers as well as its in vivo role in ovarian tumor cisplatin resistance by creating nude mice model.

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