miR-519d-3p/HIF-2α axis increases the chemosensitivity of human cervical cancer cells to cisplatin via inactivation of PI3K/AKT signaling

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Abstract. Cisplatin (DDP)-based chemotherapy is a standard treatment for cervical cancer, although chemotherapy resistance remains a major concern. Hypoxia-inducible factor-2 α (HIF-2α) plays an important role in chemotherapy resistance. MicroRNAs (miRs) can inhibit gene expression by binding to the 3'-untranslated region of the target gene. The authors' previous study showed that miR-519d-3p plays an important role in the regulation of HIF-2α expression under hypoxic conditions in cervical cancer. However, the function and regulatory mechanisms of the miR-519d-3p/HIF-2α axis in DDP-resistance in cervical cancer are not fully understood. Therefore, the aim of the present study was to investigate whether the miR-519d-3p/HIF-2α axis increased DDP resistance by regulating the PI3K/AKT signaling pathway. It was found that the expression of miR-519d-3p was lower in DDP-resistant cervical cancer cells (CaSki/DDP and HeLa/DDP) compared with CaSki and HeLa cells under hypoxic conditions. Additionally, miR-519d-3p overexpression decreased the IC_{50} value in CaSki/DDP and HeLa/DDP cells, and inhibited HIF-2α protein expression and the PI3K/AKT signaling pathway under hypoxic conditions. Furthermore, it was demonstrated that HIF-2α overexpression reduced the effect of miR-519d-3p overexpression on HeLa/DDP and CaSki/DDP cells. Moreover, the present results suggested that HIF-2α overexpression increased the IC_{50} value in CaSki/DDP and HeLa/DDP cells. It was also found that HIF-2α overexpression reduced the effect of miR-519d-3p overexpression on the PI3K/AKT signaling pathway. Therefore, the present results indicated that the miR-519d-3p/HIF-2α axis increased DDP resistance of cervical cancer cells by suppressing the PI3K/AKT signaling pathway under hypoxic conditions.

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Key words: microRNAs, hypoxia-inducible factor-2 α, cervical cancer cells, phosphatidyl inositol 3 kinase/protein kinase B signaling pathway

Introduction

Cervical cancer is the 2nd most common malignancy and is the leading cause of cancer mortality in women in the United States (1). Cisplatin (DDP)-based chemotherapy is a standard treatment for cervical cancer. While DDP has shown efficacy for treating cervical cancer, numerous patients present with resistance to available chemotherapeutics prior to mortality, which is due to widespread metastasis and tumor relapse (2). Therefore, it is important to identify the molecular mechanisms underlying cervical cancer DDP resistance.

Hypoxia is a common phenomenon in the majority of solid tumors (3). Furthermore, hypoxia is associated with aggressive tumor progression and resistance to chemotherapy and radiation (4). Hypoxia-inducible factor-2 α (HIF-2α) is the oxygen-regulated α-subunit of HIF (5). HIF-2α has the ability to upregulate drug resistance-related gene expression and causes chemotherapy resistance in numerous tumors (6,7). However, the relationship between HIF-2α and chemotherapy resistance in cervical cancer cells remains elusive.

MicroRNAs (miRNAs/miRs), which are non-coding RNAs, are shown to post-transcriptionally regulate gene expression by binding to the seed region and sequences in the 3'-untranslated region of the target mRNA (8). Previous studies have revealed that miRNAs have a number of roles such as regulating the cell cycle, cell proliferation, migration, invasion, adhesion, angiopoiesis and apoptosis, as well as having an important role in cervical cancer occurrence and progression (9,10). Recent studies have shown that miR-519-3p regulates cell proliferation, migration and invasion in multiple cancer cells (11-13). The authors' previous study revealed that in cervical cancer, under hypoxic conditions miR-519d-3p plays an important role in the regulation of HIF-2α expression (14). However, the function and regulatory mechanisms of the miR-519d-3p/HIF-2α axis in cervical cancer DDP resistance remains unknown.

Therefore, the present study assessed the expression level of miR-519d-3p in cervical cancer cells and examined the effects of miR-519d-3p overexpression on DDP resistance in cervical cancer cells. Moreover, the present study analyzed the potential regulatory mechanism of the miR-519d-3p/HIF-2α axis in cervical cancer DDP resistance under hypoxic conditions.

Materials and methods

Cell culture and hypoxic exposure. Human cervical cancer cell lines, CaSki and HeLa, were purchased from the American Type Culture Collection. DDP-resistant cervical cancer cell lines, HeLa/DDP and CaSki/DDP, were purchased from Shenglong Biological Corporation. CaSki, HeLa, Hela/DDP and CaSki/DDP cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.), and supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin G and 100 μg/ml streptomycin (Sigma-Aldrich; Merck KGaA). Cells were maintained at 37˚C in a humidified 5% CO_2 incubator. To expose cells to hypoxic conditions, the cells were cultured for 24 h in a Billups-Rothenburg chamber with 94% N_2, 1% O_2 and 5% CO_2 at 37˚C.

miRNA mimic synthesis, HIF-2α overexpression plasmid construction and cell transfection. miR-519d-3p mimics (5'-CAAAGUGCCUCCCUUUAGAGUG-3') and negative-control (NC) miRNA mimics (5'-UUCUCCGAACGUGUCACGUTT-3') were purchased from Shanghai GenePharma Co., Ltd. To study the function of miR-519d-3p, HeLa/DDP and CaSki/DDP cells were plated into 96 well plates ($4x10^4$ cells/well) or 6-well plates ($5x10^5$ cells/well), and subsequently incubated at 37˚C for 6 h. Cells were then transfected with 50 nM miR-519d-3p or 50 nM NC using Lipofectamine 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. To examine the relationship between miR-519d-3p and HIF-2α, the full-length HIF-2α (NM_001430.5) was cloned and inserted into the pcDNA3.1 expression plasmid (Promega Corporation), referred to as 'pcDNA-HIF-2α'. Empty pcDNA3.1 plasmid was used as a control. Cells were plated into 96-well plates ($4x10^4$ cells/well) or 6-well plates ($5x10^5$ cells/well), and co-transfected with 50 nM miR-519d-3p, and 2 μg/ml empty pcDNA3.1 plasmid (miR-519d-3p + pcDNA) or pcDNA-HIF-2α (miR-519d-3p + pcDNA-HIF-2α), using Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.). After transfection for 48 h, MTT assays and western blotting were performed.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and RT to cDNA was performed using a miRcute miRNA first-strand cDNA synthesis kit at 42˚C for 60 min (Tiangen Biotech Co., Ltd.) and PrimeScript RT Reagent kit with gDNA Eraser (Tiangen Biotech Co., Ltd.). mRNA expression level was detected using qPCR with a miRcute miRNA qPCR detection kit (SYBR® Green; Tiangen Biotech Co., Ltd.). U6 was used as an internal reference. qPCR was performed on a 7500 RT PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) under the following conditions: Initial denaturation at 95˚C for 5 min, followed by 40 cycles at 95˚C for 15 sec and 60˚C for 60 sec. Gene expression was measured in triplicate using the $2^{-\Delta\Delta Cq}$ method (15). The product length of miR-519d-3p for the RT-qPCR was 73 bp and was amplified using the following primers: Forward, 5'-ACACTCCAGCTGGGCAAAGTGCCTCCCTTT-3' and reverse, 5'-CTCAACTGGTGTCGTGGA-3'. The product length of U6 for the RT-qPCR was 94 bp and was amplified using the following primers: Forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'.

MTT assay. After 24 h of seeding cells into 96-well plates (5,000 cells per well), cells were transfected with miR-519d-3p, miR-519d-3p + pcDNA, or miR-519d-3p + pcDNA-HIF-2α. Cells were treated with DDP (Merck KGaA) (0, 1, 2, 4, 6, 8, 16 or 32 μg/ml) at 24 h post-transfection 37˚C (16). Then, 48 h after transfection, 20 μl MTT (5 mg/ml; Sigma-Aldrich; Merck KGaA) was added and the cells were incubated for another 4 h in a humidified incubator. After the supernatant was discarded, 200 μl DMSO was added to dissolve the formazan. The optical density (OD) at 570 nm was measured. The growth inhibition rate was calculated as follows: Growth inhibition rate (%)=(average $OD_{570\ nm}$ of the control group-average $OD_{570\ nm}$ value of the experimental group)/average $OD_{570\ nm}$ value of the control group x100%. The IC_{50} was calculated based on growth inhibition rate using GraphPad Prism software, version 7.0 (GraphPad Software, Inc.).

Western blotting. Total cellular protein was extracted using pre-cooled RIPA buffer (Beyotime Institute of Biotechnology) with a protease inhibitor. Protein concentrations were determined using BCA Protein Assay kit (Beyotime Institute of Biotechnology). Equal amounts (30 μg) of protein samples were separated using 10% SDS-PAGE and transferred to PVDF membranes. After blocking in PBST (0.1% Tween-20) containing 5% non-fat milk at 25˚C for 2 h, the membrane was incubated with the corresponding primary antibody overnight at 4˚C. After washing with PBST, the membranes were incubated with the secondary antibody (1:10,000; Goat Anti-Mouse IgG1, Human ads-HRP; cat. no. 1070-05; SouthernBiotech) at 25˚C for 2 h. The membranes were rinsed with PBST and protein bands were visualized using an SuperSignal™ West Pico PLUS (Thermo Fisher Scientific, Inc.). GAPDH was used as the loading control. Membranes were incubated with the following primary antibodies: Anti-HIF-2α mouse monoclonal antibody (1:2,000; cat. no. sc-13596, Santa Cruz Biotechnology, Inc.), anti-human PI3K p85 polyclonal antibody (1:10,000; cat. no. sc-1637, Santa Cruz Biotechnology, Inc.), anti-human phosphorylated (p)-AKT1 polyclonal antibody (1:10,000; cat. no. sc-52940, Santa Cruz Biotechnology, Inc.), anti-human total (t)-AKT1 polyclonal antibody (1:4,000; cat. no. sc-5298, Santa Cruz Biotechnology, Inc.), anti-human p-mTOR polyclonal antibody (1:5,000; cat. no. sc-293133, Santa Cruz Biotechnology, Inc.), anti-human mTOR polyclonal antibody (1:5,000; cat. no. sc-517464, Santa Cruz Biotechnology, Inc.) and anti-human GAPDH polyclonal antibody (1:10,000; cat. no. ab8245, Abcam). The quantification of specific bands was performed using Image Pro-Plus 6.0 software (Media Cybernetics, Inc.). The relative expression of p-AKT1 or p-mTOR was referenced to both t-protein and

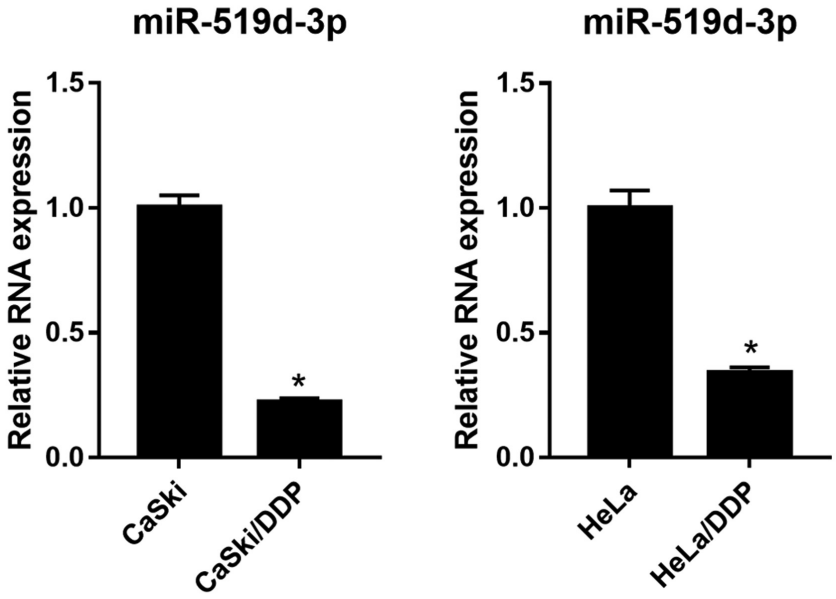

Figure 1. Expression of miR-519d-3p in cervical cancer cells under hypoxic conditions. miR-519d-3p is decreased in CaSki/DDP and HeLa/DDP cells, compared to CaSki and HeLa cells. *P<0.05, CaSki vs. CaSki/DDP, and HeLa vs. HeLa/DDP. miR, microRNA; DDP, cisplatin.

GAPDH. The relative expression of HIF-2α, AKT1 or mTOR was referenced to GAPDH.

Statistical analysis. All experiments were repeated three times. All statistical analysis was performed using SPSS version 19.0 software (IBM Corp.). Continuous variables are presented as the mean ± SD. An unpaired t-test was used to compare the differences between the two groups. P<0.05 was considered to indicate a statistically significant difference.

Results

miR-519d-3p expression decreases in CaSki/DDP and HeLa/DDP cells under hypoxic conditions. RT-qPCR was used to detect the expression level of miR-519d-3p in CaSki, HeLa, CaSki/DDP and HeLa/DDP cells under hypoxic conditions. It was found that miR-519d-3p expression level was significantly decreased in both CaSki/DDP and HeLa/DDP cells, compared with CaSki and HeLa cells under hypoxic conditions (Fig. 1).

miR-519d-3p overexpression decreases DDP resistance in HeLa/DDP and CaSki/DDP cells. To investigate the effect of miR-519d-3p overexpression on DDP-resistant cells, miR-519d-3p mimic or NC were transfected into HeLa/DDP and CaSki/DDP cells. RT-qPCR was used to evaluate the mRNA expression level of miR-519d-3p in HeLa/DDP and CaSki/DDP cells after transfection. It was demonstrated that miR-519d-3p expression levels were significantly increased in HeLa/DDP and CaSki/DDP cells transfected with miR-519d-3p mimics compared with the NC groups, under hypoxic conditions (Fig. 2A). Thus, the present results indicated that miR-519d-3p was successfully overexpressed in HeLa/DDP and CaSki/DDP cells. To examine the effect of miR-519d-3p overexpression on DDP resistance in HeLa/DDP and CaSki/DDP cells under hypoxic conditions, miR-519d-3p mimic or NC-transfected HeLa/DDP and CaSki/DDP cells were treated with different DDP doses. The growth inhibition rate of HeLa/DDP and CaSki/DDP cells transfected with miR-519d-3p mimic was increased compared with cells transfected with NC, under hypoxic conditions after DPP treatment (Fig. 2B). It was demonstrated that the DDP IC_{50} of HeLa/DDP and CaSki/DDP cells transfected with miR-519d-3p mimic was 9.95 and 11.13 μg/ml, respectively. Furthermore, the DDP IC_{50} of HeLa/DDP and CaSki/DDP cells transfected with NC was 20.52 and 23.24 μg/ml, respectively. Therefore, the present results suggested that miR-519d-3p overexpression decreased DDP resistance in HeLa/DDP and CaSki/DDP cells.

Overexpression of miR-519d-3p inhibits HIF-2α protein expression levels and PI3K/AKT signaling pathways under hypoxic conditions. Western blotting results showed that the protein expression levels of HIF-2α, PI3K p85, p-AKT1 and p-mTOR were significantly decreased in HeLa/DDP and CaSki/DDP cells transfected with miR-519d-3p mimic compared with the NC group (Fig. 3). However, the protein expression levels of AKT1 and mTOR were not significantly different (Fig. 3).

HIF-2α overexpression weakens the effect of miR-519d-3p overexpression on HeLa/DDP and CaSki/DDP cells under hypoxic conditions. Western blotting was used to assess HIF-2α expression in HeLa/DDP and CaSki/DDP cells transfected with pcDNA or pcDNA-HIF-2α. The present results indicated that HIF-2α expression was significantly increased both in HeLa/DDP and CaSki/DDP cells transfected with pcDNA-HIF-2α, compared with cells transfected with pcDNA, under hypoxic conditions (Fig. 4A and B).

To investigate whether HIF-2α overexpression reduces the effect of miR-519d-3p overexpression on DDP resistance, HeLa/DDP and CaSki/DDP cells were co-transfected with miR-519d-3p, and either pcDNA or pcDNA-HIF-2α. It was found that the growth inhibition rate of HeLa/DDP and CaSki/DDP cells transfected with miR-519d-3p + pcDNA-HIF-2α was lower

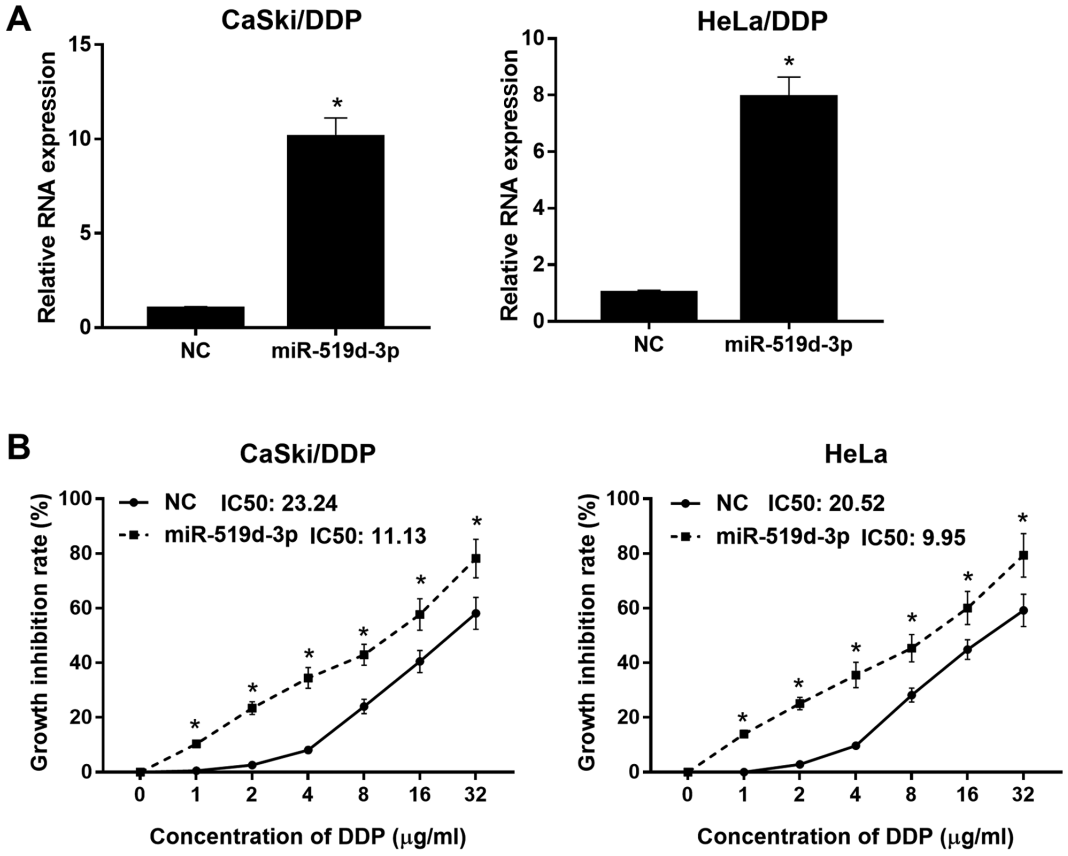

Figure 2. miR-519d-3p overexpression decreases the IC_{50} value of DDP in HeLa/DDP and CaSki/DDP cells under hypoxic conditions. (A) miR-519d-3p mRNA expression in HeLa/DDP and CaSki/DDP cells transfected with miR-519d-3p mimics or NC mimics under hypoxic conditions. (B) HeLa/DDP and CaSki/DDP cells transfected with miR-519d-3p mimics or NC were treated with a series of DDP doses, under hypoxic conditions. Viability was determined using MTT assays. *P<0.05. miR, microRNA; DDP, cisplatin; NC, negative control.

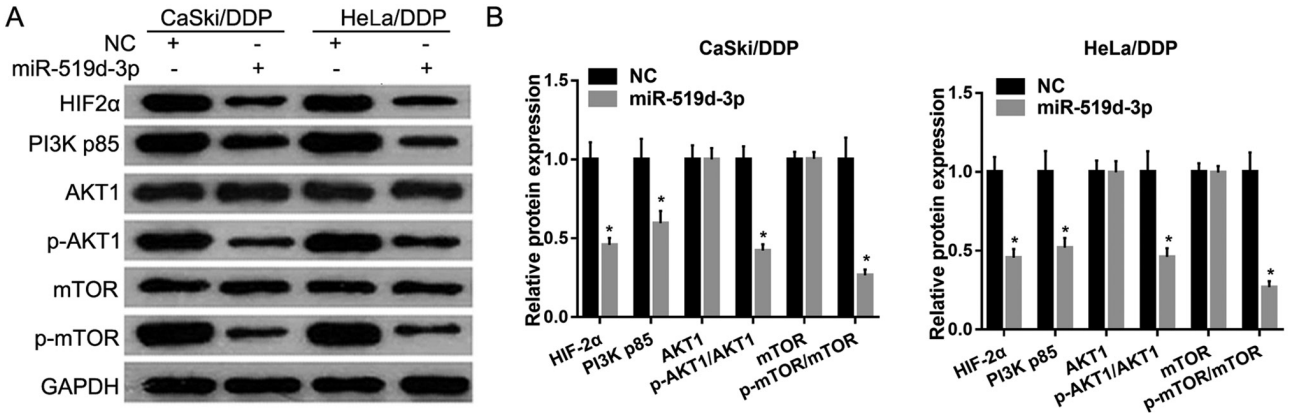

Figure 3. Overexpression of miR-519d-3p inhibits HIF-2α protein expression and the PI3K/AKT signaling pathway under hypoxic conditions. (A) Protein expression levels of HIF-2α, PI3K p85, p-AKT1, AKT1, mTOR and p-mTOR was measured by western blotting after transfection with miR-519d-3p mimics or NC mimics. (B) Relative protein expression levels of HIF-2α, PI3K p85, p-AKT1, AKT1, mTOR and p-mTOR. Data are presented as the mean ± SD. *P<0.05. miR, microRNA; DDP, cisplatin; NC, negative control; p-, phosphorylated; HIF-2α, hypoxia-inducible factor-2 α.

compared with cells transfected with miR-519d-3p + pcDNA, under hypoxic conditions after DDP treatment (Fig. 4C). Moreover, the DDP IC_{50} of HeLa/DDP and CaSki/DDP cells transfected with miR-519d-3p + pcDNA-HIF-2α was 27.38 and 29.21 μg/ml, respectively. The DDP IC_{50} of HeLa/DDP and CaSki/DDP cells transfected with miR-519d-3p + pcDNA was 9.51 and 12.88 μg/ml, respectively. Therefore, the present results indicated that HIF-2α overexpression could reduce the effect of miR-519d-3p overexpression on DDP resistance in HeLa/DDP and CaSki/DDP cells, under hypoxic conditions.

To examine whether HIF-2α overexpression reduces the effect of miR-519d-3p overexpression on the PI3K/AKT signaling pathway, HeLa/DDP and CaSki/DDP cells were co-transfected with miR-519d-3p, and either pcDNA or pcDNA-HIF-2α.

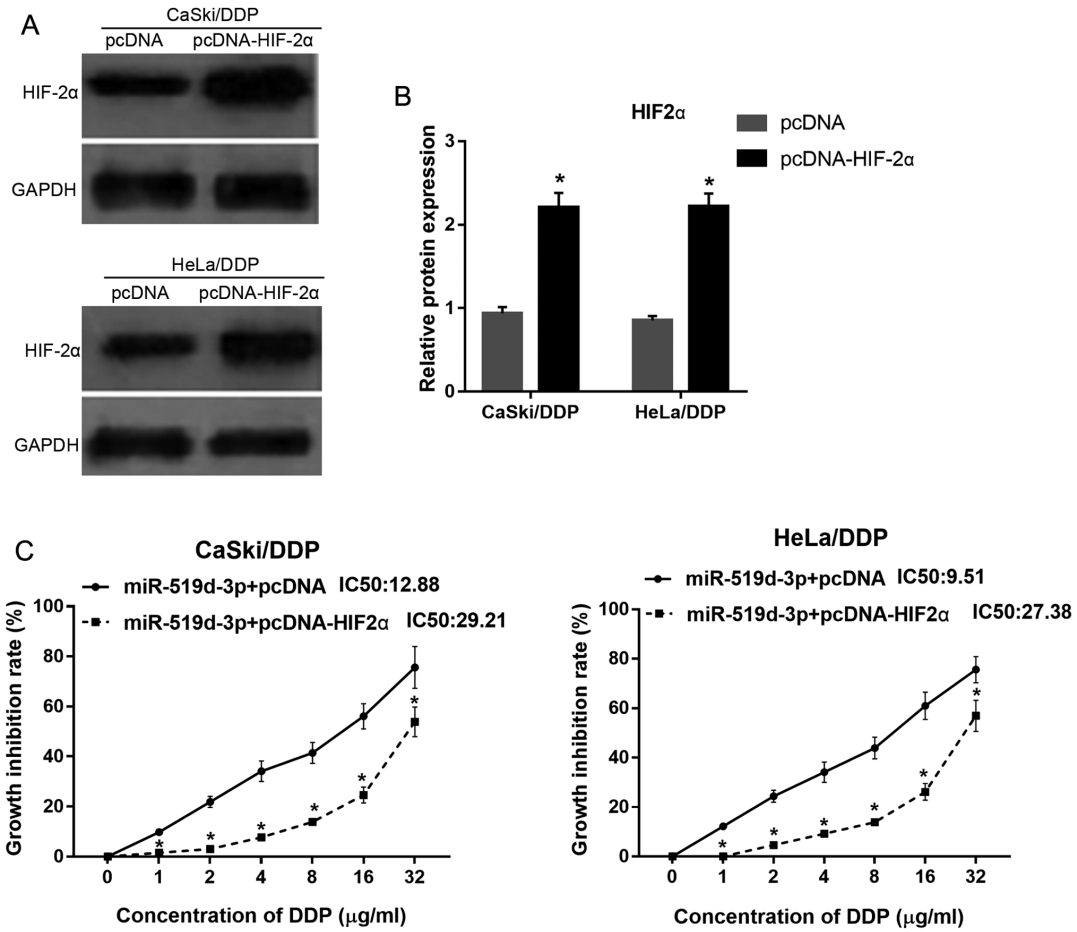

Figure 4. HIF-2α overexpression reduces the effect of miR-519d-3p overexpression on DDP resistance in HeLa/DDP and CaSki/DDP cells under hypoxic conditions. (A) Protein expression levels of HIF-2α in HeLa/DDP and CaSki/DDP cells transfected with pcDNA or pcDNA-HIF-2α under hypoxic conditions. (B) Relative HIF-2α protein expression presented as the mean ± SD. (C) After co-transfection with miR-519d-3p and pcDNA or pcDNA-HIF-2α, cells were treated with DDP under hypoxic conditions. The viability of the cells was determined using MTT assays. *P<0.05. pcDNA, empty pcDNA3.1 plasmid; pcDNA-HIF-2α, HIF-2α overexpressing pcDNA3.1 plasmid; miR, microRNA; DDP, cisplatin; NC, negative control; p-, phosphorylated; HIF-2α, hypoxia-inducible factor-2 α.

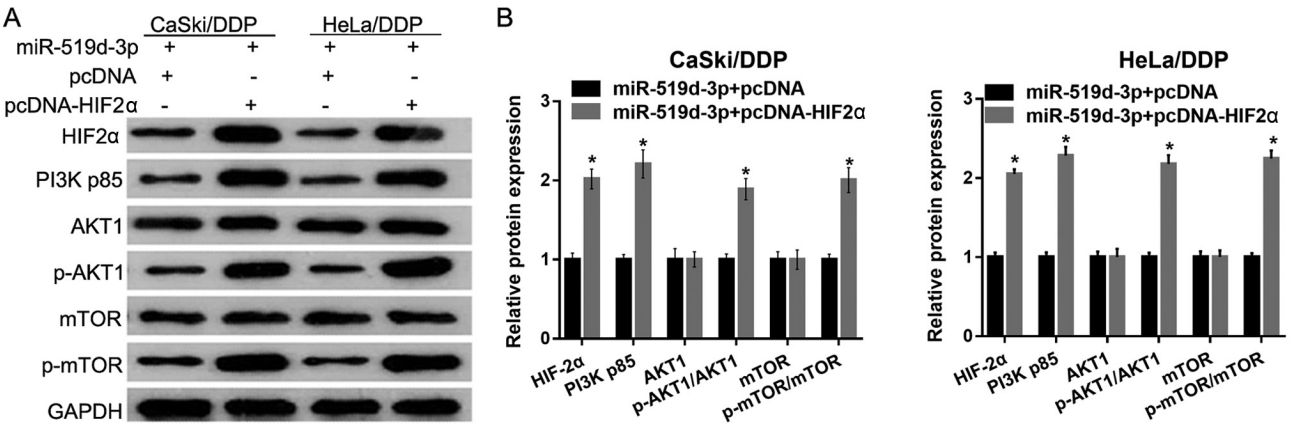

Figure 5. HIF-2α overexpression reduces the effect of miR-519d-3p overexpression on the PI3K/AKT signaling pathways under hypoxic conditions. (A) Protein expression levels of HIF-2α, PI3K p85, p-AKT1, AKT1, mTOR and p-mTOR were measured by western blotting after co-transfection with miR-519d-3p, and pcDNA or pcDNA-HIF-2α. (B) Relative protein expression levels of HIF-2α, PI3K p85, p-AKT1, AKT1, mTOR and p-mTOR are presented as the mean ± SD. *P<0.05. miR, microRNA; DDP, cisplatin; NC, negative control; p-, phosphorylated; HIF-2α, hypoxia-inducible factor-2 α; pcDNA, empty pcDNA3.1 plasmid; pcDNA-HIF-2α, HIF-2α overexpressing pcDNA3.1 plasmid.

Western blotting results demonstrated that the protein expression levels of HIF-2α, PI3K p85, p-AKT1 and p-mTOR were significantly increased in HeLa/DDP and CaSki/DDP cells transfected with miR-519d-3p + pcDNA-HIF-2α, compared with cells transfected with miR-519d-3p + pcDNA (Fig. 5). Furthermore, the present results suggested that the protein

expression levels of AKT1 and mTOR were not significantly different (Fig. 5).

Discussion

While DDP chemotherapy is one of the most widely used drugs for the treatment of cervical cancer, it eventually results in drug resistant cervical cancer cells, which leads to poor clinical outcomes (17,18). Therefore, improving the understanding of the underlying chemoresistance mechanism may facilitate the development of strategies to reverse drug resistance in cervical cancer and improve overall survival of patients with cervical cancer.

miRNAs can regulate multiple pathways involved in the cellular response to DDP (19). Wang *et al* (20) showed that miR-214 reduces cell survival and enhances DDP-induced cytotoxicity via downregulation of Bcl-2-like protein 2 in cervical cancer cells. Moreover, Lei *et al* (21) revealed that upregulated miR-155 reverses the epithelial-mesenchymal transition induced by epidermal growth factor and increases chemosensitivity to DDP in human CaSki cells. Several previous studies have investigated the mechanisms of drug resistance in human cervical cancer cells (17). However, evidence on the role of miR-519d-3p in cervical cancer DDP resistance is limited. Previous studies have demonstrated that miR-519d-3p functions as a tumor suppressor in several tumors (11-13). In gastric cancer, miR-519d-3p suppresses cell proliferation and invasion by downregulating B-cell lymphoma 6 (11). Furthermore, in colorectal cancer, miR-519d-3p inhibits oncogenicity and promotes apoptosis by targeting trophinin associated protein (12). The authors' previous study found that miR-519d-3p inhibits proliferation and promotes apoptosis by targeting HIF-2α in cervical cancer cells under hypoxic conditions (14). In the present study, it was found that miR-519d-3p expression was lower in CaSki/DDP and HeLa/DDP cells, compared with CaSki and HeLa cells under hypoxic conditions. Moreover, it was demonstrated that miR-519d-3p overexpression decreased the IC_{50} value in CaSki/DDP and HeLa/DDP cells. Therefore, the present results suggested that miR-519d-3p may be correlated with DDP resistance.

Hypoxia plays an important role in tumor chemoresistance (3). HIF-2α is associated with drug resistance-related gene expression and leads to chemotherapy resistance in numerous tumors (6,7). In the present study, miR-519d-3p overexpression was shown to inhibit HIF-2α protein expression. However, HIF-2α overexpression reduced the effect of miR-519d-3p overexpression on DDP resistance. Thus, the present results indicated that the miR-519d-3p/HIF-2α axis may be involved in DDP resistance regulation in cervical cancer cells. Therefore, the miR-519d-3p/HIF-2α axis could be a potential target for cervical cancer treatment in DDP resistance.

Previous studies have revealed that aberrant activation of the PI3K/AKT signaling pathway plays a pivotal role in malignant transformation and chemoresistance in cancer cells (22,23). The present study identified that miR-519d-3p overexpression decreased the expression levels of HIF-2α, PI3K p85, p-AKT1 and p-mTOR. However, HIF-2α overexpression reversed the effect of miR-519d-3p overexpression on the PI3K/AKT signaling pathway. Moreover, western blotting results demonstrated that HIF-2α overexpression increased PI3K p85, p-AKT1 and p-mTOR expression levels. Thus, the present results suggested that the miR-519d-3p/HIF-2α axis may regulate the PI3K/AKT signaling pathway.

However, there are certain limitations of the present study, which will need to be overcome in future studies. Firstly, miR-519d-3p expression was not detected in solid cervical cancer tissues from patients with DDP sensitivity or resistance. In addition, the function of miR-519d-3p and its regulatory mechanism were not investigated in an animal model.

In conclusion, it was found that miR-519d-3p was expressed at a low level in CaSki/DDP and HeLa/DDP cells. Furthermore, miR-519d-3p overexpression decreased DDP resistance in HeLa/DDP and CaSki/DDP cells, and inhibited HIF-2α protein expression and the PI3K/AKT signaling pathway. It was also demonstrated that HIF-2α overexpression reduced the effect of miR-519d-3p overexpression on CaSki/DDP and HeLa/DDP cells. Collectively, the present results suggested that the miR-519d-3p/HIF-2α axis is important in chemoresistance development and may be a novel target for human cervical cancer treatment. However, further studies, including both *in vivo* models and clinical trials, are required to verify the present results.

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Availability of data and materials

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

Authors' contributions

LJ was involved in study design and preparation of the manuscript. LJ, SS and FL performed the experiments. QS and TZ revised the manuscript and performed the western blotting. HZ and YX performed the cell culture. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2016. CA Cancer J Clin 66: 7-30, 2016.
2. Pectasides D, Kamposioras K, Papaxoinis G and Pectasides E: Chemotherapy for recurrent cervical cancer. Cancer Treat Rev 34: 603-613, 2008.
3. Li SH, Shin DH, Chun YS, Lee MK, Kim MS and Park JW: A novel mode of action of YC-1 in HIF inhibition: Stimulation of FIH-dependent p300 dissociation from HIF-1{alpha}. Mol Cancer Ther 7: 3729-3738, 2008.
4. Lou JJ, Chua YL, Chew EH, Gao J, Bushell M and Hagen T: Inhibition of hypoxia-inducible factor-1alpha (HIF-1alpha) protein synthesis by DNA damage inducing agents. PLoS One 5: e10522, 2010.
5. Wiesener MS, Jurgensen JS, Rosenberger C, Scholze CK, Hörstrup JH, Warnecke C, Mandriota S, Bechmann I, Frei UA, Pugh CW, *et al*: Widespread hypoxia-inducible expression of HIF-2alpha in distinct cell populations of different organs. FASEB J 17: 271-273, 2003.
6. Gao ZJ, Yuan WD, Yuan JQ, Yuan K and Wang Y: Downregulation of HIF-2α reverse the chemotherapy resistance of lung adenocarcinoma a549 cells to cisplatin. Med Sci Monit 24: 1104-1111, 2018.
7. Wang L, Xue M and Chung DC: c-Myc is regulated by HIF-2α in chronic hypoxia and influences sensitivity to 5-FU in colon cancer. Oncotarget 7: 78910-78917, 2016.
8. Pereira DM, Rodrigues PM, Borralho PM and Rodrigues CM: Delivering the promise of miRNA cancer therapeutics. Drug Discov Today 18: 282-289, 2013.
9. Li S, Yang F, Wang M, Cao W and Yang Z: miR-378 functions as an onco-miRNA by targeting the ST7L/Wnt/β-catenin pathway in cervical cancer. Int J Mol Med 40: 1047-1056, 2017.
10. Liang H, Luo R, Chen X, Zhao Y and Tan A: miR-187 inhibits the growth of cervical cancer cells by targeting FGF9. Oncol Rep 38: 1977-1984, 2017.
11. Li YY, Shao JP, Zhang SP, Xing GQ and Liu HJ: miR-519d-3p inhibits cell proliferation and invasion of gastric cancer by down-regulating B-cell lymphoma 6. Cytogenet Genome Res 154: 12-19, 2018.
12. Ye X and Lv H: MicroRNA-519d-3p inhibits cell proliferation and migration by targeting TROAP in colorectal cancer. Biomed Pharmacother 105: 879-886, 2018.
13. Ma L and Li J: MicroRNA-519d-3p inhibits cell proliferation and cell cycle G1/S transition in glioma by targeting CCND1. Biosci Biotechnol Biochem 84: 297-304, 2020.
14. Jiang L, Shi S, Shi Q, Zhang H, Xia Y and Zhong T: MicroRNA-519d-3p inhibits proliferation and promotes apoptosis by targeting HIF-2α in cervical cancer under hypoxic conditions. Oncol Res 26: 1055-1062, 2018.
15. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
16. Feng Y, Zou W, Hu C, Li G, Zhou S, He Y, Ma F, Deng C and Sun L: Modulation of CASC2/miR-21/PTEN pathway sensitizes cervical cancer to cisplatin. Arch Biochem Biophys 623-624: 20-30, 2017.
17. Zhu H, Luo H, Zhang W, Shen Z, Hu X and Zhu X: Molecular mechanisms of cisplatin resistance in cervical cancer. Drug Des Devel Ther 10: 1885-1895, 2016.
18. Small W Jr, Bacon MA, Bajaj A, Chuang LT, Fisher BJ, Harkenrider MM, Jhingran A, Kitchener HC, Mileshkin LR, Viswanathan AN and Gaffney DK: Cervical cancer: A global health crisis. Cancer 123: 2404-2412, 2017.
19. Drayton RM: The role of microRNA in the response to cisplatin treatment. Biochem Soc Trans 40: 821-825, 2012.
20. Wang F, Liu M, Li X and Tang H: MiR-214 reduces cell survival and enhances cisplatin-induced cytotoxicity via down-regulation of Bcl2l2 in cervical cancer cells. FEBS Lett 587: 488-495, 2013.
21. Lei C, Wang Y, Huang Y, Yu H, Huang Y, Wu L and Huang L: Up-regulated miR155 reverses the epithelial-mesenchymal transition induced by EGF and increases chemo-sensitivity to cisplatin in human Caski cervical cancer cells. PloS One 7: e52310, 2012.
22. Ma Q, Chang Z, Wang W and Wang B: Rapamycin-mediated mTOR inhibition reverses drug resistance to adriamycin in colon cancer cells. Hepatogastroenterology 62: 880-886, 2015.
23. Marklein D, Graab U, Naumann I, Yan T, Ridzewski R, Nitzki F, Rosenberger A, Dittmann K, Wienands J, Wojnowski L, *et al*: PI3K inhibition enhances doxorubicin-induced apoptosis in sarcoma cells. PLoS One 7: e52898, 2012.