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Original Research

miR-34a-5p enhances the sensitivity of cervical cancer cells to oxaliplatin chemotherapy via targeting *MDM4*

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Abstract

Background: Cervical cancer is a common gynecologic malignancy worldwide, mainly developing in women aged about 50 years old. Currently, oxaliplatin (L-OHP) was widely used as a first-line chemotherapeutic drug to treat various tumors, including cervical cancer. The emergence of L-OHP resistance during chemotherapy has largely limited the clinical efficacy of chemotherapy. Numerous studies have demonstrated that abnormal expression of microRNAs (miRNAs) was associated with tumorigenesis and the development of cancer drug resistance. **Methods**: miR-34a-5p and Murine Double Minute 4 (*MDM4*) in cervical cancer cells was detected via RT-qPCR and Western blot assay. Cell proliferation and apoptosis were observed via ov-MDM4 and si-MDM4, Cell counting kit (CCK)-8 and flow cytometry analysis after transfection with miR-34a-5p inhibitor, miR-34a-5p mimics, respectively. Dual-luciferase reporter assay was utilized to confirm the associativity between miR-34a-5p and *MDM4*. **Results**: miR-34a-5p was significantly down-regulated whereas *MDM4* was increased in cervical cancer tumor tissues and cells. Compared with Hela cells, miR-34a-5p was further decreased and *MDM4* was elevated in Hela/L-OHP cells. miR-34a-5p significantly inhibited Hela/L-OHP cell viability and promoted apoptosis. Similar to the effects of miR-34a-5p, *MDM4* knockdown inhibited Hela/L-OHP cell proliferation, but induced apoptosis. miR-34a-5p directly targeted *MDM4* and could improve sensitivity of cervical cancer cells to L-OHP chemosensitivity by targeting *MDM4* expression *in vitro*. **Conclusions**: miR-34a-5p can improve sensitivity of cervical cancer cells to L-OHP chemosensitivity, serving as a potential curative target for cervical cancer chemotherapy.

Keywords: Cervical cancer; miR-34a-5p; *MDM4*; Oxaliplatin

1. Introduction

Cervical cancer is a common malignancy in women, of which squamous cell carcinoma accounted for about 85%, adenocarcinoma for about 10–15%, and squamous adenocarcinoma for 3% [1]. It is currently believed that the leading risk factor for cervical cancer is persistent infection with human papillomavirus. However, the mechanisms of tumor progression are intricate, and the molecular mechanisms of cervical carcinogenesis and development are still not fully understood [2]. Surgery, radiotherapy, as well as chemotherapy are the three main treatments for cervical cancer [3]. Early-stage patients have better outcomes due to surgery or chemotherapy, while advanced and recurrent patients have limited treatment options and a relatively poor prognosis. Therefore, exploring and revealing the cellular and molecular mechanisms of cervical cancer development, finding novel molecular standards that can predict its progression could provide new insights into clinical treatment.

Oxaliplatin (L-OHP) is a compound containing platinum atoms with a similar structure to cisplatin and belongs to a class of anti-tumor drugs that antagonized DNA [4–6]. L-OHP is generally used in combination with 5-fluorouracil and folinic acid [7]. As a platinum chemotherapeutic drug, L-OHP has been widely used clinically, serving as a first-line drug for treating various cancers [8–10]. However, the drug resistance problems hinder the therapeutic efficacy, leading to chemotherapy failure.

MicroRNAs (miRNAs) are non-coding RNAs binding complementarily to target messenger RNAs (mRNAs) at the base through the seed sequences of miRNAs, resulting in degradation or inhibition of target mRNAs [11]. It has been shown that abnormalities in miRNAs are markers of tumorigenesis in various cancers, including cervical cancer. For instance, studies revealed that miR-149 and miR-186 functioned as either onco-miRs or tumor suppressors in various cancers [12,13]. Gao *et al.* [14] regarded miR-188, miR-223, miR-99a, and miR-125b aberrant expressions were correlated with cervical cancer prognosis. Zheng *et al.* [15] observed higher expression of miR-31 in cervical cancer patients, and miR-31 was strongly correlated with lymph node metastasis and HPV status. Another research by Li *et al.* [16] illustrated miR-486-5p was highly expressed in cervical cancer tissues and serum samples, playing as an oncogene via activating PI3K/Akt pathway in cervical cancer. Increasing evidence proved that abnormal regulation of miRNAs was closely associated with acquired drug resistance in human tumors as well [17–19]. Due to the current emergence of chemotherapy resistance

Table 1. Clinicopathological information of 38 cervical cancer patients.

Parameters	Cases	miR-34a-5p level	*P* value
Age			
<50	11	0.58 ± 0.23	0.1973
≥50	27	0.69 ± 0.13	
Histological type			
Adenocarcinoma	7	0.52 ± 0.13	0.6523
Squamous cell carcinoma	31	0.74 ± 0.28	
Tumor size (cm)			
<4	26	0.34 ± 0.06	0.0010
≥4	12	0.93 ± 0.31	
FIGO stage			
I–II	29	0.86 ± 0.25	0.0012
III	9	0.26 ± 0.15	
Lymph node metastasis			
Yes	17	0.53 ± 0.20	0.0063
No	21	0.82 ± 0.29	
HPV status			
Positive	32	0.17 ± 0.05	<0.001
Negative	6	0.96 ± 0.26	

Note: FIGO, Federation of Gynecology and Obstetrics; HPV, human papillomavirus.

problems in various cancers, the relationship between tumor drug resistance and miRNA abnormalities has become a hot topic. With the continuous in-depth research on tumors and miRNAs, much evidence proved that miRNAs were related to tumorigenesis and had an inseparable relationship with tumor drug resistance, which largely affected the clinical outcomes of patients.

We investigated aberrant miR-34a-5p expression in L-OHP resistant cervical cancer cells in this work. Moreover, the role miR-34a-5p plays in regulating the chemosensitivity of cervical cancer cells to L-OHP were detected by in *in vitro* experiments.

2. Materials and methods

2.1 Specimen collection and cell culture

Our study has got the Ethics board approval Committee of Wuhan No.1 Hospital (approval number: wj6911) following the Declaration of Helsinki. Each participant signed an informed consent for inclusion before they participated in the study. 38 cervical cancer patients who underwent surgical section in Wuhan No.1 Hospital between December 2016 and April 2020 were enrolled (Table 1). All patients did not receive any chemotherapy or adjuvant therapy before surgical section. Tumor tissues and non-tumor adjacent (>2 cm away from tumor tissues) were collection under surgical treatment and preserved in –80 °C until use.

Human cervical cell line Hela, L-OHP resistant cervical Hela/L-OHP cell line, human normal cervical epithelial cell line H8, and HEK-293 cells were from the Chinese Academy of Sciences (Shanghai). All cells received culture in Dulbecco's Modified Eagle Medium (DMEM) (Hyclone, Logan, UT, USA) supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (Gibco, Grand Island, NY, USA) in a 5% CO_2 humidified incubator at room temperature.

2.2 L-OHP treatment and cell transfection

After preparing cell suspensions, we added 5, 10, 15, 20, 25, and 30 μM concentrations of L-OHP (Sigma-Aldrich, San Francisco, CA, USA) cell medium. Cells were treated with L-OHP for 48 hours and changed into fresh medium. Hela/L-OHP cells were cultured with the 50% maximal inhibitory concentration (IC50) of transfected cells against L-OHP for 3 hours to assess the chemical sensitivity of the drug. After treatment with drug, cells were subjected to further experiments.

miR-34a-5p mimics, mimics-NC, miR-34a-5p inhibitor, and inhibitor-NC were purchased from Taitool Bioscience (Shanghai, China). si-NC, si-MDM4, ov-NC, and ov-MDM4 were designed and synthesized by Bio-Rad (Hercules, CA, USA). Then, Hela/L-OHP cells were transfected with miR-34a-5p mimics, mimics-NC, miR-34a-5p inhibitor, inhibitor-NC, si-NC, si-MDM4, ov-NC, and ov-MDM4 by using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) as per instructions.

2.3 RNA extraction and RT-qPCR analysis

Total RNA extraction from cells was done via TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Afterward, cDNA was synthesized from RNA by using a Reverse Transcription Kit (Invitrogen, Carlsbad, CA, USA) following the protocol. Then, miScript SYBR Green PCR Kit (Qiagen, Shanghai, China) and ABI 7500 Fast PCR system (Thermo Fisher Scientific, Waltham, MA, USA) were applied to conduct PCR analysis. The cycling conditions included 95 °C for 10 minutes, 40 cycles of 94 °C for 10 seconds, and 62 °C for 20 seconds. Relative expressions of miR-34a-5p and *MDM4* were normalized based on U6 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as analysis standardization via $2^{-\Delta\Delta CT}$ method, respectively. Primers sequences were presented in the following:

Gene	Primers sequences
MiR-34a-5p	Forward: 5′-TGGCAGTGTCTTAGCTGGTTGT-3′
	Reverse: 5′-GCGAGCACAGAATTAATACGAC-3′
U6	Forward: 5′-CTCGCTTCGGCAGCACA-3′
	Reverse: 5′-AACGCTTCACGAATTTGCGT-3′
MDM4	Forward: 5′-CTAAGTCCTTAAGTGATGATACCGATGT-3′
	Reverse: 5′-AACTTTGAACAATCTGAATACCAATCC-3′
GAPDH	Forward: 5′-GGACCTGACCTGCCGTCTAG-3′
	Reverse: 5′-GAGGAGTGGGT GTCGCTGTT-3′

Notes: Primer sequences of genes used in the study are provided as above.

2.4 Cell viability determined by CCK-8 assay

The Cell Counting Kit-8 (Beyotime Biotechnology, Shanghai, China) and was used to assess the cultured cell viability and proliferation. 1×10^4 cells/well cells were seeded in 96-well plates and cultured at 5% CO_2, 37 °C. At 0, 12, 24, and 48 hours, cells were processed with 10 μL CCK-8 reagent and cultured for another 4 hours avoid lights. Finally, a microplate reader (PerkinElmer, Waltham, MA, USA). was utilized to measure the OD value of each sample at 450 nm.

2.5 Cell apoptosis evaluated by flow cytometry assay

Cell apoptosis rates were determined via Apoptosis Assay Kit (Takara, Shiga, Japan) according to instructions. After inoculation in 6-well plates for 24 hours, cells were collected, washed with cold phosphate buffer saline (PBS), and double-stained with Annexin V-fluorescein isothiocyanate (0.25 mg/mL) and propidium iodide (PI) for 15 minutes at room temperature in the dark. Finally, stained cells were measured by a Flow Cytometry system (BD Bioscience, San Jose, CA, USA) to analyze with FCS Express V4 software (Glendale, CA, USA).

2.6 Dual-luciferase reporter assay

Wild and mutant *MDM4* were subcloned into pGL4 luciferase vector (Promega, Madison, WI, USA) to construct WT-MDM4 and mut-MDM4 following the protocol. Then, HEK-293 cells and Hela/L-OHP cells received transfection with miR-34a-5p mimics or mimics-NC together with WT-MDM4 or mut-MDM4 via Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Luciferase activity measurement was done via Firefly Luciferase Assay Kit (Promega, Madison, WI, USA) and normalized to Renilla luciferase activity.

2.7 Western blot assay

Total proteins received separation with Radio-Immunoprecipitation assay (RIPA) lysis buffer and determined by BCA protein kit (Pierce, Rockford, IL, USA). Then, 25 μg proteins were loaded by 12% sulfate polyacrylamide gel electropheresis (SDS-PAGE) and transferred onto Polyvinylidene fluoride (PVDF) films. Following bloc in 5% skim milk for 50 min, films were probed with primary antibodies (rabbit anti-MDM4, 1:1000, ab16058, rabbit anti-GAPDH, 1:1000, ab9485, Abcam, Cambridge, MA, USA) at 4 °C overnight to incubate with HPR-conjugated secondary antibody at room temperature for 1 hour. Finally, bands were detected via enhanced ECL kit (Pierce, Rockford, IL, USA), and GAPDH served as the internal control.

2.8 Statistical analysis

All experiments were repeatedly done three times. Data presentation were depicted as mean $\pm$ SD. GraphPad Prism 8.0 software (GraphPad, San Diego, CA, USA) was run to analyze all the data. Significance between two or more experimental groups was analyzed using Wilcoxon's test or one-way ANOVA with Tukey's post hoc test. $P < 0.05$ reminded statistical significance.

3. Results

3.1 miR-34a-5p is prominently down-regulated in cervical cancer tumor tissues, Hela and Hela/L-OHP cells

To detect the mechanisms of miR-34a-5p in cervical cancer, we detect miR-34a-5p expression in cervical cancer tissues, human cervical epithelial cell H8 and cervical cancer cell line Hela. As depicted in Fig. 1A, miR-34a-5p was visually diminished in tumor tissues vs. non-tumor tissues, which had a similarly decrease in Hela cells compared with H8 cells (Fig. 1B). Furthermore, compared with Hela cells, miR-34a-5p was also down-regulated in L-OHP resistant cervical cancer cell line Hela/L-OHP cells (Fig. 1C). IC50 of L-OHP in Hela and Hela/L-OHP cells were 10 and 20 μM, respectively (Fig. 1D). Finally, after exposing Hela/L-OHP cells to different concentrations of L-OHP, expressions of miR-34a-5p were detected accordingly. As demonstrated in Fig. 1E, miR-34a-5p decreased gradually in Hela/L-OHP with the increase of L-OHP dose.

3.2 miR-34a-5p enhances chemosensitivity of cervical cancer cells to L-OHP

We transfected miR-34a-5p mimics and miR-34a-5p inhibitor in Hela/L-OHP cells, and confirmed the transfection efficacy subsequently. miR-34a-5p was significantly increased in the mimics group while down-regulated in the inhibitor group, indicating the transfection was successful in Fig. 2A. Then, Fig. 2B revealed over-expressing miR-34a-5p could suppress Hela/L-OHP cell viability while miR-34a-5p inhibitor presented opposite effects. On the contrary, the apoptosis results in Fig. 2C–H unveiled that miR-34a-5p mimics promote cell apoptosis of Hela/L-OHP while miR-34a-5p silencing presented opposite effects. Collectively, these results suggested miR-34a-5p promotes the chemosensitivity of cervical cancer cells to L-OHP.

3.3 MDM4 hinders the chemosensitivity of cervical cancer cells to L-OHP

Furthermore, we exploited underlying roles of *MDM4* in regulating Hela/L-OHP cell development. As shown in Fig. 3A, *MDM4* prominently increased in tumor tissues from cervical cancer patients compared with non-tumor tissues. Meanwhile, in Fig. 3B,C, *MDM4* was remarkably increased in Hela cells and Hela/L-OHP cells compared with H8 or Hela cells. These results implied that *MDM4* was implicated in the carcinogenesis of cervical cancer. Afterward, we found that the *MDM4* level was gradually increased after adding L-OHP concentration in a dose-dependent manner (Fig. 3D). We constructed *MDM4* silencing or overexpressing Hela/L-OHP cell lines. As depicted in Fig. 3E, *MDM4* was markedly increased in ov-

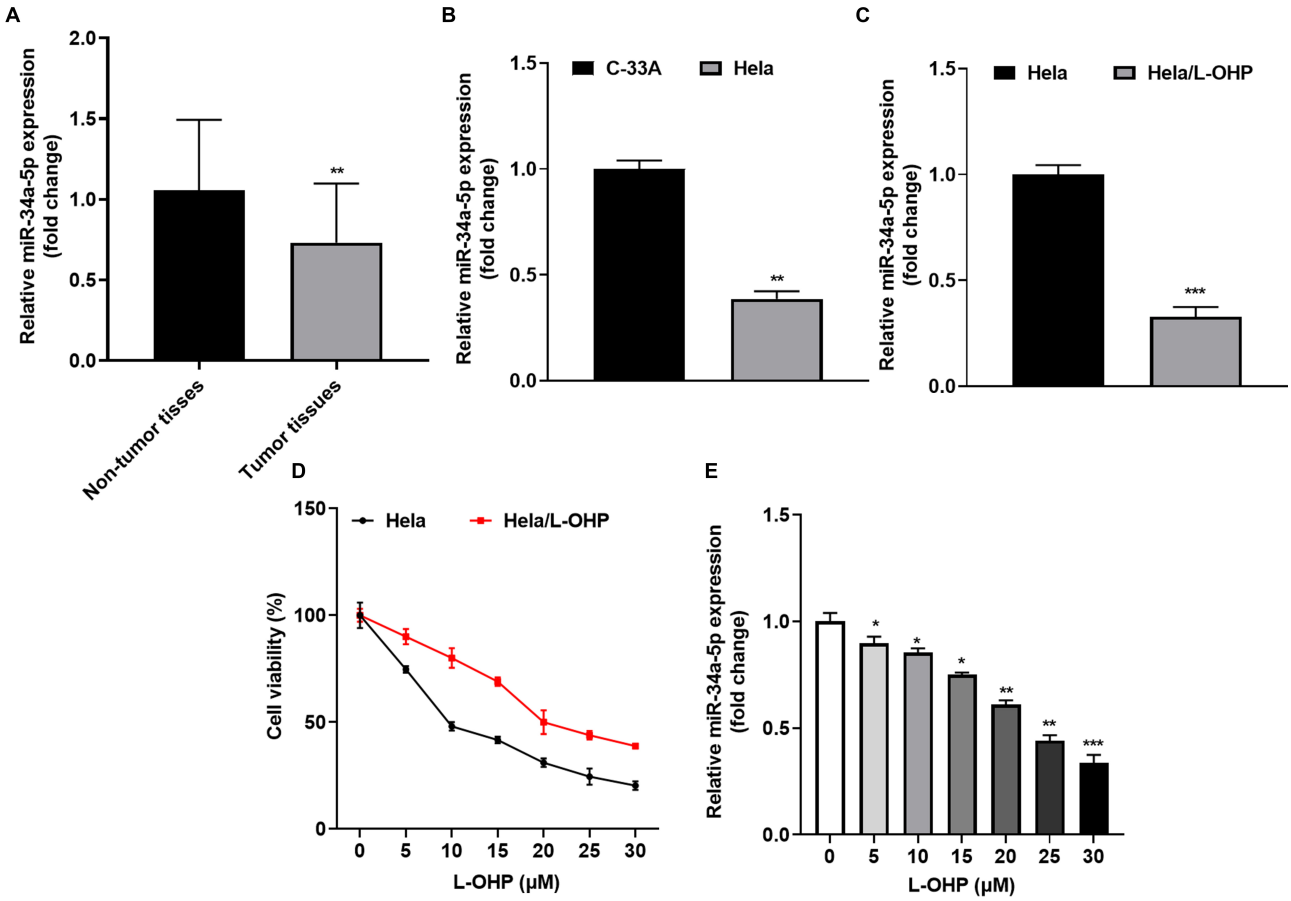

Fig. 1. miR-34a-5p was significantly down-regulated in Hela and Hela/L-OHP cell lines. (A) miR-34a-5p expression in tumor tissues and non-tumor tissues from 38 cervical cancer patients. $^{**}P = 0.0072$, tumor tissues vs. non-tumor tissues. (B) miR-34a-5p in C-33A and Hela cell lines. $^{**}P = 0.0068$, Hela vs. C-33A. (C) miR-34a-5p in Hela and Hela/L-OHP cells. $^{**}P = 0.0057$, Hela/L-OHP vs. Hela. (D) IC50 of miR-34a-5p in Hela and Hela/L-OHP cells measured by CCK-8 assay. (E) Changes of miR-34a-5p levels under different concentrations of L-OHP in Hela/L-OHP cells. $^{*}P = 0.038$, $^{**}P = 0.0082$, $^{***}P < 0.001$ vs. 0 μM group.

MDM4 while reduced in the si-MDM4 group, suggesting the transfection was successful. Cell viability and apoptosis results in Fig. 3F–L demonstrated that *MDM4* over-expressing promoted Hela/L-OHP cell viability but inhibit apoptosis; however, *MDM4* silencing presented the opposite effects on Hela/L-OHP cell progression.

3.4 MDM4 is a direct targeting site of miR-34a-5p

Through TargetScan online tool, we hypothesized that *MDM4* was a potential target of miR-34a-5p (Fig. 4A). Afterward, the dual-luciferase reporter assay confirmed co-transfection with miR-34a-5p mimics reduce the luciferase activity in WT-MDM4 groups; however, there were no significant differences in Mut-MDM4 groups (Fig. 4B,C). Furthermore, transfection with miR-34a-5p mimics could impede *MDM4* expression; whereas, co-transfection with ov-MDM4 could partially reverse the decline of *MDM4* induced by miR-34a-5p mimics. Similarly, miR-34a-5p inhibitor could increase *MDM4* expression in Hela/L-OHP cells; however, the increase could be partially counteracted by co-transfection si-MDM4 (Fig. 4D,E). These results suggested miR-34a-5p directly targeted *MDM4*.

3.5 miR-34a-5p improves chemosensitivity of cervical cancer cells to L-OHP by targeting MDM4

After confirming the targeted relationship between miR-34a-5p and *MDM4*, we further elucidated the role of miR-34a-5p/*MDM4* axis in Hela/L-OHP cell progression. miR-34a-5p mimics decrease Hela/L-OHP cell viability but promote cell apoptosis in Fig. 5A–G; however, the effects of miR-34a-5p over-expressing on Hela/L-OHP cell development could be counteracted by co-transfection with ov-MDM4. Meanwhile, miR-34a-5p silencing could promote Hela/L-OHP viability and suppress apoptosis; whereas, the *MDM4* knockdown could counteract impacts of the miR-34a-5p inhibitor on Hela/L-OHP cells. Finally, in Fig. 5H,I, we found compared with the control group, protein expression of *MDM4* showed a decrease in miR-34a-5p mimics group, while increase in the miR-34a-5p inhibitor group. However, ov-MDM4 or si-MDM4 partially antagonized miR-34a-5p analogs or inhibitor-induced differences.

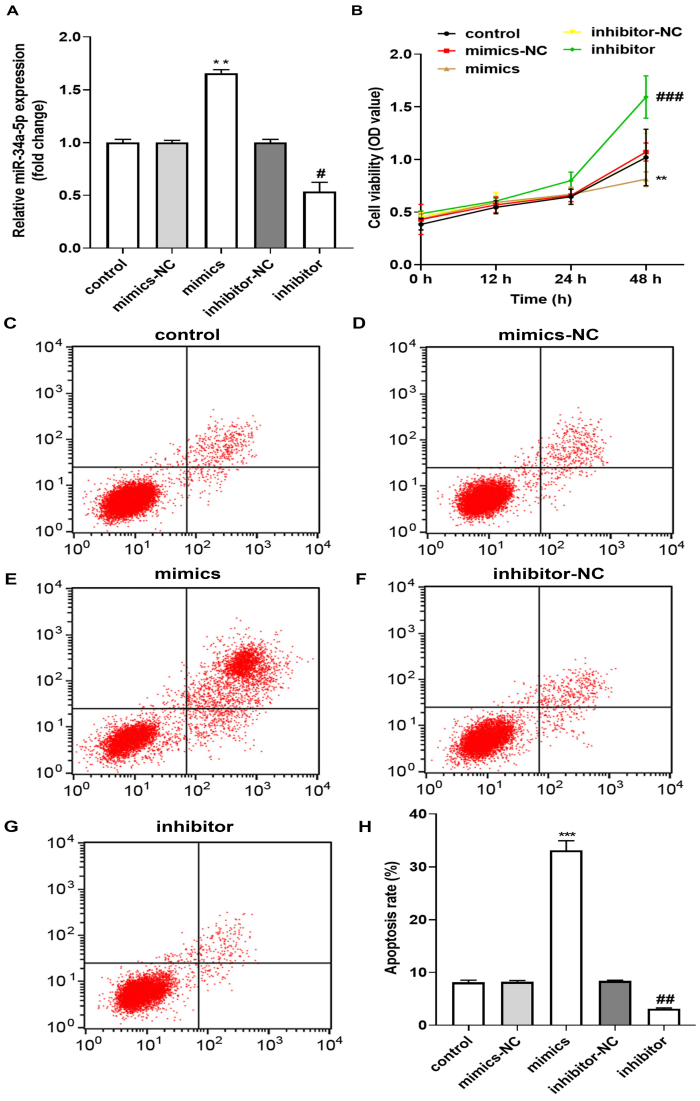

Fig. 2. miR-34a-5p increased the chemosensitivity of cervical cancer cells to L-OHP. (A) Transfection efficacy of miR-34a-5p. $^{**}P = 0.0064$, mimics vs. mimics-NC; $^{\#}P = 0.045$, inhibitor vs. inhibitor-NC. (B) Cell viability in Hela/L-OHP cells after transfection with miR-34a-5p mimics or miR-34a-5p inhibitor. $^{**}P = 0.0057$, mimics vs. mimics-NC; $^{\#\#\#}P < 0.001$, inhibitor vs. inhibitor-NC. (C–H) Cell apoptosis in Hela/L-OHP cells after evaluated by flow cytometry analysis. $^{***}P < 0.001$, mimics vs. mimics-NC; $^{\#\#}P = 0.0084$, inhibitor vs. inhibitor-NC.

4. Discussion

miRNAs are approximately 21–23 nt long non-coding RNAs, which are widely found in various organisms from bacteria to humans, and are able to bind to target mRNAs to downregulate target gene expressions [11]. The miR-34 family, commonly found in mammals, is highly conserved. Recent studies have found that p53 directly transcribed miR-34 family expression [20]. Meanwhile, miR-34a can promote cell cycle arrest, inhibit cell proliferation, activate apoptosis, and suppress epithelial-mesenchymal transition and metastasis by blocking the p53 pathway [21–23]. miR-34a negatively regulates gene expression at the post-transcriptional level via targeting 3'UTR of various cycle- and apoptosis-related proteins (Cyclin D1, CDK4, c-myc, and Bcl-2), causing cell cycle transition to S-phase, inhibiting cell proliferation, promoting apoptosis, and thus suppressing tumorigenesis [24–27].

The molecular mechanisms of chemotherapeutic drug resistance are complex and diverse, among which abnormal miRNAs expression is closely relative to tumorigenesis and drug resistance. Among these miRNAs, miR-34a-5p has been reported as a promising predictive marker in a variety of cancers. For instance, Gao *et al.* [28] elucidated that miR-34a-5p was down-regulated in colorectal cancer, inhibited cell growth and development. Sun *et al.* [29] validated that miR-34a-5p was reduced in pancreatic tissues and cell lines, serving as a tumor-suppressive role. Down-regulated miR-34a-5p was also found in cervical cancer,

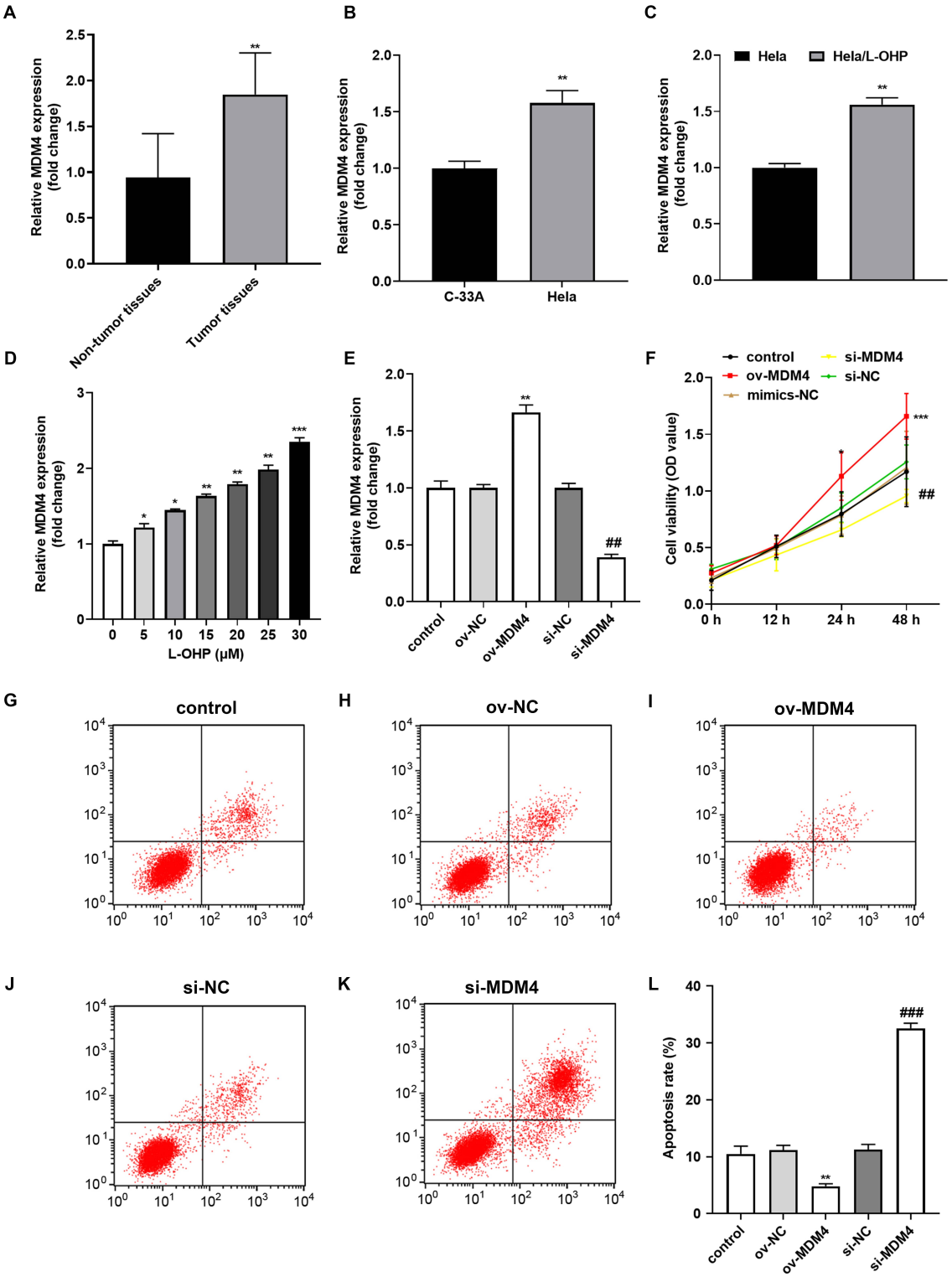

Fig. 3. *MDM4* was up-regulated in Hela/L-OHP cells and suppressed the chemosensitivity of cervical cancer cells to L-OHP. (A) Expression level of *MDM4* in tumor tissues and non-tumor adjacent tissues from cervical cancer patients. $^{**}P = 0.0059$, tumor tissues vs. non-tumor tissues. (B) *MDM4* was elevated in Hela cells, vs. C-33A cells. $^{**}P = 0.0043$, Hela vs. C-33A. (C) Highly expressed *MDM4* was found in Hela/L-OHP cells. $^{**}P = 0.0062$, Hela/L-OHP vs. Hela. (D) Expression levels of *MDM4* after adding different doses of L-OHP in Hela/L-OHP cells. $^{*}P = 0.042$, $^{**}P = 0.0036$, $^{***}P < 0.001$ vs. 0 μM group. (E) Transfection efficiency of *MDM4*. (F) CCK-8 assay revealed the Hela/L-OHP cell viability after transfection. $^{**}P = 0.0021$, ov-MDM4 vs. ov-NC; $^{\#\#}P = 0.0033$, si-MDM4 vs. si-NC. (G–L) Cell apoptosis in Hela/L-OHP cells. $^{***}P < 0.001$, ov-MDM4 vs. ov-NC; $^{\#\#}P = 0.0041$, si-MDM4 vs. si-NC.

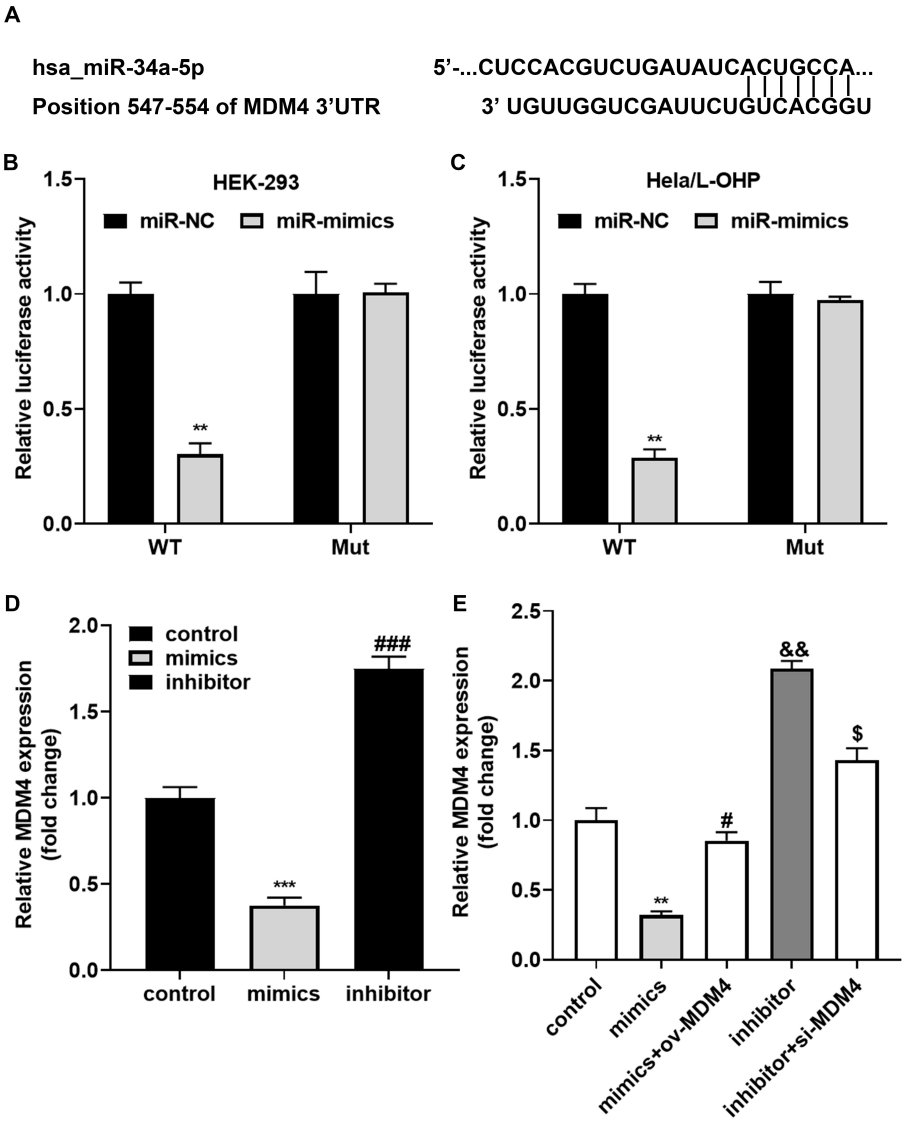

Fig. 4. miR-34a-5p directly targeted *MDM4*. (A) Potential binding sites of miR-34a-5p and *MDM4*. (B,C) Dual-luciferase reporter assay. $^{**}P = 0.0022$, miR-mimics vs. miR-NC. (D) Expression levels of *MDM4* after transfection with miR-34a-5p mimics or miR-34a-5p inhibitor. $^{***}P < 0.001$, mimics vs. control; $^{\#\#\#}P < 0.001$, inhibitor vs. control. (E) *MDM4* expressions after co-transfection with miR-34a-5p and *MDM4*. $^{**}P = 0.0016$, mimics vs. control; $^{\#}P = 0.036$, mimics + ov-MDM4 vs. mimics; $^{\&\&}P = 0.0035$, inhibitor vs. control; $^{\$}P = 0.027$, inhibitor + si-MDM4 vs. inhibitor.

and regarded as a diagnostic biomarker [30–32]. Moreover, the roles of miR-34a-5p in regulating the chemosensitivity in cancer cells to chemotherapy drugs are validated as well. Zuo *et al*. and Luo *et al*. [33,34] revealed that miR-34a-5p was decreased in cisplatin-resistant ovarian cancer cells or non-small cell lung cancer cells, and over-expressing miR-34a-5p could attenuate cisplatin chemoresistance. In addition, miR-34a-5p could also attenuate the chemoresistance of colorectal cancer cells to L-OHP [35–37]. However, the mechanism of miR-34a-5p in regulating chemoresistance of cervical cancer cells to L-OHP is still unknown. Therefore, this project aims to investigate the relationship between miR-34a-5p and oxaliplatin acquired resistance in cervical cancer. From the results, we found that miR-34a-5p was prominently decreased in Hela cells vs. normal cervical epithelial cells. Moreover, compared with Hela cells, miR-34a-5p was further lowered in Hela/L-OHP cells; with the augment of L-OHP concentrations, miR-34a-5p was gradually lowered in Hela/L-OHP cells as well. In *in vitro* experiments further validated that miR-34a-5p could enhance Hela/L-OHP cell apoptosis but inhibit proliferation, suggesting that miR-34a-5p can promote chemosensitivity in cervical cancer cells to L-OHP.

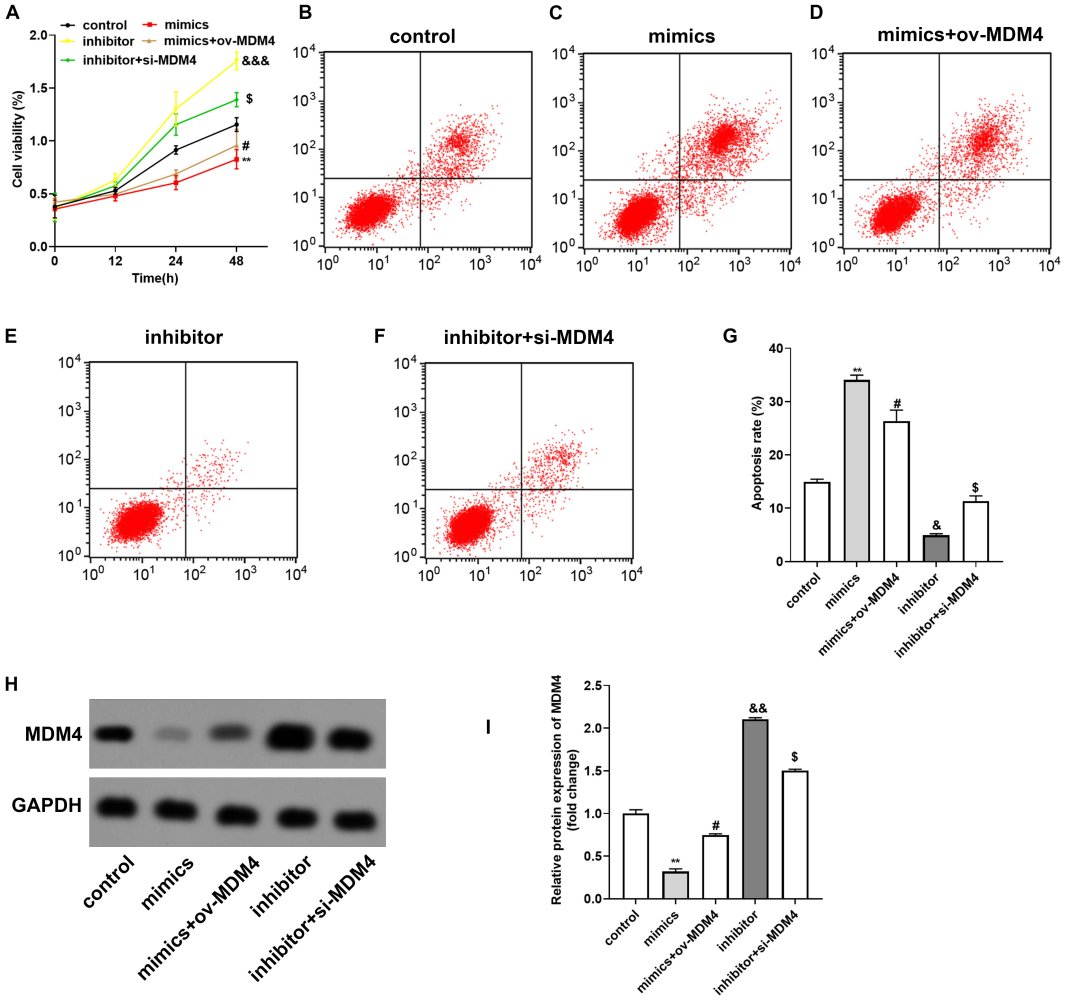

Fig. 5. miR-34a-5p enhanced the chemosensitivity of cervical cancer cells to L-OHP by targeting *MDM4*. (A) CCK-8 assays unveiled the Hela/L-OHP cell viability. $^{**}P = 0.0037$, mimics vs. control; $^{\#}P = 0.028$, mimics + ov-MDM4 vs. mimics; $^{\&\&\&}P < 0.001$, inhibitor vs. control; $^{\$}P = 0.043$, inhibitor + si-MDM4 vs. inhibitor. (B–G) Cell apoptosis measurement done by flow cytometry assay. $^{**}P = 0.0049$, mimics vs. control; $^{\#}P = 0.025$, mimics + ov-MDM4 vs. mimics; $^{\&}P = 0.017$, inhibitor vs. control; $^{\$}P = 0.015$, inhibitor + si-MDM4 vs. inhibitor. (H,I) Protein expressions of *MDM4* after co-transfection with miR-34a-5p and *MDM4*. $^{**}P = 0.0053$, mimics vs. control; $^{\#}P = 0.038$, mimics + ov-MDM4 vs. mimics; $^{\&\&}P = 0.0064$, inhibitor vs. control; $^{\$}P = 0.041$, inhibitor + si-MDM4 vs. inhibitor.

Mouse double minute 4 (*MDM4*), one of the significant endogenous negative regulators of p53 [38], was overexpressed in a variety of human tumors, functioning as an oncogene [39]. *MDM4* bonded directly to p53 and repressed its transcriptional activity [40], and promoted the degradation of p53 by enhancing the function of the E3 ubiquitin ligase of MDM2 to promote p53 degradation [41]. Recent studies identified the potential of *MDM4* to predict the clinical outcome of tumors [42–44] and to be a potential therapeutic target for p53 reactivation in anti-cancer therapy [45,46]. In the present study, we observed a significantly increased level of *MDM4* in Hela cells, which is consistent with a previous study [47]. Compared with Hela cells, *MDM4* was further increased in Hela/L-OHP cells. With the increase of L-OHP, the expression of *MDM4* gradually increased in an L-OHP dose-dependent way. After transfected *MDM4* silencing or over-expressing into Hela/L-OHP cells, it was shown that *MDM4* could promote Hela/L-OHP cell viability but inhibit apoptosis. Dual-luciferase reporter assay confirmed *MDM4* was a downstream target of miR-34a-5p and received negative regulation of miR-34a-5p. We also found that miR-34a-5p could enhance the chemosensitivity of cervical cancer cells to L-OHP by targeting *MDM4*.

5. Conclusions

In conclusion, miR-34a-5p/*MDM4* axis has positive feedback effects on the sensitivity of cervical cancer cells to L-OHP chemotherapy, which is expected to be a new target for L-OHP chemotherapy in cervical cancer.

Author contributions

PH, XLL and LRC conceived the study. PH and XLL carried out the experiments and the data analysis. YQL and SXG provided help and advice on the flow cytometry assay. SXG contributed to sample preparation. PH wrote the manuscript in consultation with XLL and LRC who supervised the project. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The experiment was often approved by the Ethics Committee of Wuhan No.1 Hospital (approval number: wj6911), and all patients participating in this study provided written informed consent in accordance with the "Helsinki Declaration".

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Conflict of interest

The authors declare no conflict of interest.

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