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*Original Article*

# Upregulation of miR-494 promotes cervical cancer cell proliferation, migration and invasion via the MAPK/ERK pathway

Nina Yu[1], Jing Jin[2], Jing Liu[2], Ming Ma[3]

*Departments of [1]Gynecology, [3]Oncology, Linyi People's Hospital, Linyi, Shandong, China; [2]Department of Gynaecology, The Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China*

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**Abstract:** Cervical cancer is a common malignant tumor in women, and aberrant miR-494 expression has been identified to be associated with the initiation and progression of various cancers. However, the functional role of miR-494 in cervical cancer remains unknown to a large extent. The aim of this study was to investigate the biological function and potential mechanism of miR-494 in cervical cancer. miR-494 expression levels in plasma of healthy control subjects and cervical cancer patients were measured by qRT-PCR. miR-494 mimic, inhibitor and negative control were transfected with lipofectamine 2000. Cell proliferation was detected by CCK-8 assay and cell migration and invasion were performed by wound healing and transwell assay, respectively. Western blotting was adopted to determine pERK1/2, tERK1/2 and SOX9 levels. Dual luciferase assay was carried out to verify the target of miR-494. miR-494 was up-regulated in cervical cancer plasma and correlated with invasion degree. Increased miR-494 expression promoted HeLa cell proliferation, migration and invasion, while decreased miR-494 expression inhibited HT-3 cell proliferation, migration and invasion. Besides, the MAPK/ERK pathway was involved in miR-494-induced HeLa cell proliferation, migration and invasion. Dual Luciferase assay indicated that SOX9 was a target of miR-494. To summarize, these results indicate that upregulation of miR-494 promotes cell proliferation, migration and invasion through the MAPK/ERK pathway, and SOX9 is a direct target of miR-494.

**Keywords:** miR-494, proliferation, migration, invasion, SOX9, cervical cancer

## Introduction

Cervical cancer is the fourth most commonly diagnosed cancer in women, and there were 528,000 new cases occurred worldwide in 2012 [1]. In China, cervical cancer accounts for 5% of the cancer incidence and 3.8% of the mortality in women [2]. Numerous studies have suggested that aberrant miRNAs expression contributes to tumorigenesis [3], and thus investigation concerning cancer-related miRNAs is needed.

miRNAs are small, noncoding RNAs that play important roles in post-transcriptional regulation [4], and aberrant miRNAs expression is one of the most common changes observed in human cancers [5, 6]. The expression of mirR-494 in cancer has been reported to be tissue-dependent. Decreased expression of mir-494 has been found in gastric carcinoma [7], cholangiocarcinoma [8], whereas miR-494 up-regulation has been observed in retinoblastoma [9], gastrointestinal stromal tumors [10]. By targeting different genes, miR-494 may act as an oncogene or tumor suppressor gene and regulate cell proliferation, apoptosis, migration and invasion of numerous cancers [10-15]. However, the biological role of mir-494 is still needed to explore in cervical cancer.

In this study, we detected miR-494 expression levels in cervical cancer plasma, and evaluated the functional role and potential molecular mechanism of miR-494 in cervical cancer cell lines. It was initially showed that miR-494 was up-regulated in cervical cancer plasma. Furthermore, MAPK/ERK pathway was involved in miR-494-induced cervical cancer cell proliferation, migration and invasion, and SOX9 was a direct target of miR-494.

## Materials and methods

### *Blood samples and cell culture*

This study was approved by the Committee for the Ethical Review of Research at the Linyi People's Hospital. Blood of 40 cervical cancer patients and 40 healthy control subjects were collected, and consent forms were obtained.

The cervical cancer cell lines were purchased in Chinese Academy of Sciences Institute of Cell Resource Center (Shanghai, China). HeLa cell line was cultured in the Eagle's Minimum Essential Medium (Gibco-BRL, Invitrogen Life Technologies, Carlsbad, CA, USA), and HT-3 cell line was cultured in the McCoy's 5a Medium (Gibco-BRL, Invitrogen Life Technologies). All the mediums contain 10% FBS (Hyclone, Logan, UT, USA) and 1% penicillin/streptomycin (Gibco-BRL, Invitrogen Life Technologies), and all the cells were cultured in a humidified incubator with 5% $CO_2$ at 37°C.

### *Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)*

Blood samples were first centrifuged at 3000 rpm for 5 min to isolate the plasma, and then RNA was extracted using TRIzol LS Reagent (Invitrogen). RNA from cells was extracted using TRIzol Reagent (Invitrogen; Thermo Fisher Scientific, Inc.). 1 μg RNA was reverse-transcribed using PrimeScript™ RT reagent Kit (Takara, Japan) according to the manufacture's instruction. qRT-PCR was performed on ABI StepOne Plus using the SYBR Premix Ex Taq Kit (Takara, Japan). U6 or β-Actin was identified as the internal control. Primer sequences of miR-494 were: F 5'-UGAAACAUACACGGGAAACCUC-3' and R 5'-GGUUUCCCGUGUAUGUUUCAUU-3'. Primer sequences of U6 were: F 5'-CGCTTCGGCAGCACATATACTAAAATTGGAAC-3' (sense) and R 5'-GCTTCACGAATTTGCGTGTCATCCTTGC-3'. Primer sequences of SOX9 were: F 5'-ACGGCTCCAGCAAGAACAAG-3' and R 5'-CCCGTTCTTCACCGACTTCC-3'. Primer sequences of β-actin were: F 5'-AAAGACCTGTACGCCAACAC-3' and R 5'-GTCATACTCCTGCTTGCTGAT-3'.

### *Western blotting*

Cells were harvested using trypsin-EDTA solution (Gibco-BRL, Invitrogen Life Technologies), and lysed with the RIPA lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China). The protein was quantified using Bicinchoninic Acid Protein Assay kit (Beyotime Institute of Biotechnology). 30 μg proteins were separated by 10% SDS-PAGE and transferred into the PVDF memberane (EMD Millipore, Billerica, MA, USA). After blocking in 5% non-fat milk for 1 h, the membranes were incubated with primary antibodies including pERK1/2 (#4370, 1:2000, CST, MA, USA), tERK1/2 (#9102, 1:1000, CST, MA, USA), SOX9 (ab76997, 1:500, Abcam, Cambridge, UK) and β-actin (A5441, 1:8000, sigma, MO, USA) overnight. Subsequent to washing with TBST for 6×10 min, the membranes were incubated with a goat anti-mouse secondary antibody (BA1001; 1:5,000; Wuhan Boster Biological Technology, Ltd., Wuhan, China) or a goat anti-rabbit secondary antibody (BA1001; 1:5,000; Wuhan Boster Biological Technology) at 37°C for 2 h. After washing with TBST for 9×10 min, the protein bands were detected using BeyoECL Star kit (Beyotime Institute of Biotechnology) with exposure to X-ray films (Eastman Kodak, Rochester, NY, USA) on dark room. β-Actin was used as an the internal control.

### *Cell transfection*

Cells were planted into six-well plates at the density of $3\times10^5$, and transfected using lipofectamine 2000 (Invitrogen Life Technologies) after reaching to 70%-90% confluence according to the manufacturer's instructions. The concentration for transfection of miRNAs mimic was 100 nM, for miRNAs inhibitor was 200 nM and for plasmids was 2 μg. The RNA and protein were extracted at 48 h and 72 h post transfection, respectively.

### *CCK-8 assay*

Cell proliferation was assessed using Cell Counting Kit-8 (CCK-8, Beyotime, China). Cells were seeded in 96-well plates at the density of $5\times10^3$. 24 h later after transfection, the medium was replaced by the mixture of 10 μl CCK-8 and 100 μl fresh medium at 0 h, 24 h, 48 h, 72 h. Then the plate was incubated at 37°C for 2 h, and the absorbance of each well was measured at 450 nm on a microplate reader (Bio-Rad, USA).

### *Wound healing assay*

$3\times10^5$ cells were planted into six-well plate and transfected as described above. 24 h later, 20-μl pipette tips were used to scratch the wounds. The images were captured under a microscope (IX3; Olympus Co., Tokyo, Japan) at 0 and 48 h after the scratch.

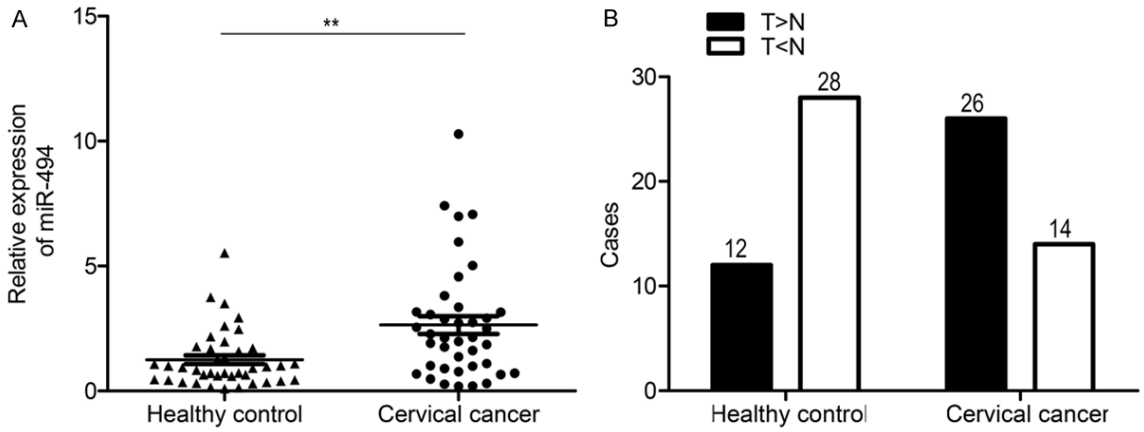


**Figure 1.** miR-494 is up-regulated in cervical cancer plasma. A. Scatter plots of miR-494 fold change in healthy control subjects and cervical cancer patients. B. Histogram showes the cases of miR-494 expression in healthy control subjects and cervical cancer patients. T, Tumor; N, Non-tumor, **P<0.01.

**Table 1.** Relationship between miR-494 expression and clinicopathologic features of cervical cancer patients

| Feature | Relative miR-494 expression | | *P* value |
|---|---|---|---|
| | Low | High | |
| Age | | | |
| <60 | 10 | 19 | 1 |
| ≥60 | 4 | 7 | |
| Differentiation | | | |
| Poorly | 5 | 8 | 0.75 |
| Moderately/Well | 9 | 18 | |
| Invasion degree | | | |
| Early stage | 9 | 7 | 0.021* |
| Progression | 5 | 19 | |
| Lymph node metastasis | | | |
| Yes | 6 | 19 | 0.06 |
| No | 8 | 7 | |

*P<0.05.

*Cell invasion analysis*

Cell invasion was detected using Transwell chambers (Costar, USA). 24 h after transfection, cells were starved for 8 h. $3\times10^5$ cells suspended in 100 μl serum-free medium were planted into the upper chamber pre-coated with matrigel, and 600 μl complete medium was added to the lower chamber. After 48 h incubation, cells invaded to the lower surface of the chamber were stained with crystal violet (Sunshine, China) for 20 min and photographed under an Olympus microscope.

*Dual luciferase assay*

The wild type and mutant 3'-UTR of SOX9 were cloned into the luciferase reporter vector pGL3 (Promega, WI, USA), respectively. HeLa cells were seeded into the 24-well plates, pGL3-SOX9-3'UTR-WT or pGL3-SOX9-3'UTR-Mut together with miR-494 mimics or negative control (NC) were co-transfected. After 48 h, cells were harvested and the luciferase activities were analyzed with the dual-luciferase reporter assay system (Promega). Renilla luciferase activities were served as the internal control.

*Statistical analysis*

All the data were analyzed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). Data were expressed as mean ± SD. Two-tailed Student's T-test was employed to compare group differences. The correlations between miR-494 expression and pathological features of cervical cancer patient were analyzed by $\chi^2$ test. P<0.05 was considered to be statistically significant difference.

## Results

*miR-494 is up-regulated in cervical cancer plasma*

To investigate the possible role of miR-494 in cervical cancer, the expression levels of miR-494 in cervical cancer plasma were measured by qRT-PCR. The data showed that the expression level of miR-494 was much higher in cervi-

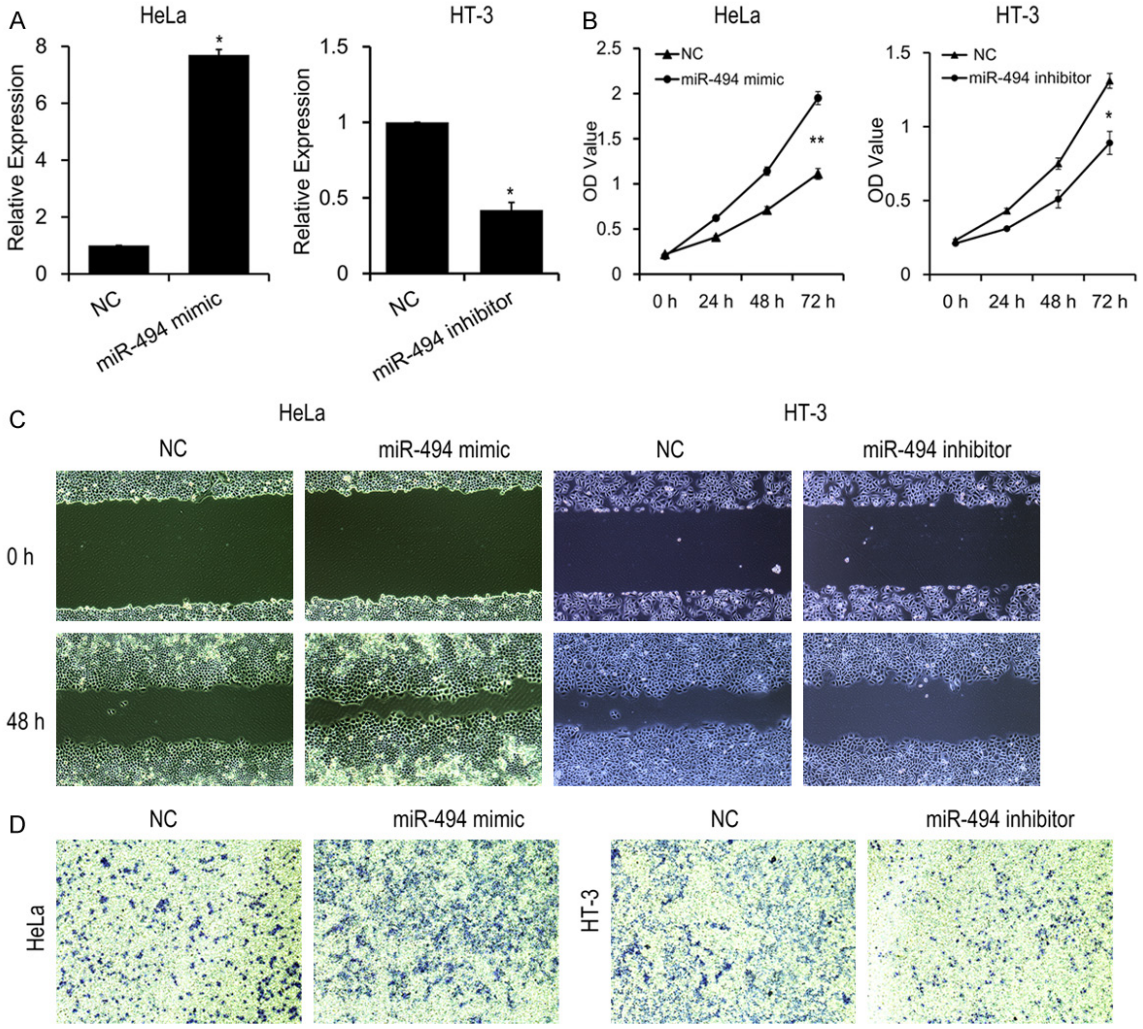


**Figure 2.** miR-494 promotes cervical cancer cell proliferation, migration and invasion of. (A) The expression of miR-494 was up-regulated after the transfection of mir-494 mimic and down-regulated after transfection of mir-494 inhibitor. miR-494 overexpression promoted the proliferation (B), migration (C) and invasion (D) ability of HeLa cells, while miR-494 downexpression inhibited the proliferation (B), migration (C) and invasion (D) ability of HT-3 cells. NC, Negative control, **P<0.01, when compared with NC; *P<0.05, when compared with NC.

cal cancer group than that in the healthy control group (**Figure 1A**). The frequency of miR-494 up-regulation was 65% in cervical cancer group (26/40) and 30% (12/40) in the healthy control group (**Figure 1B**). Subsequently, the relationship between miR-494 expression pattern and clinicopathological features of cervical cancer patients were explored and 40 patients were divided into two groups according to miR-494 expression levels (≥1.5 folds). The analysis revealed that high miR-494 expression level was correlated with invasion degree (P=0.021, **Table 1**). These results provided evidences that miR-494 might participate in cervical cancer progression.

*miR-494 promotes cervical cancer cell proliferation, migration and invasion*

The effects of miR-494 on cell proliferation, migration and invasion were detected to analyze the biological function of miR-494 in cervical cancer cells. miR-494 was over-expressed in HeLa cells and knocked down in HT-3 cells, respectively. The expression levels of miR-494 were examined by qRT-PCR (**Figure 2A**). The CCK-8 assay showed that miR-494 overexpression accelerated the growth of HeLa cells and knockdown of miR-494 suppressed the growth of HT-3 cells (**Figure 2B**). The wound healing results revealed that forced expression of miR-

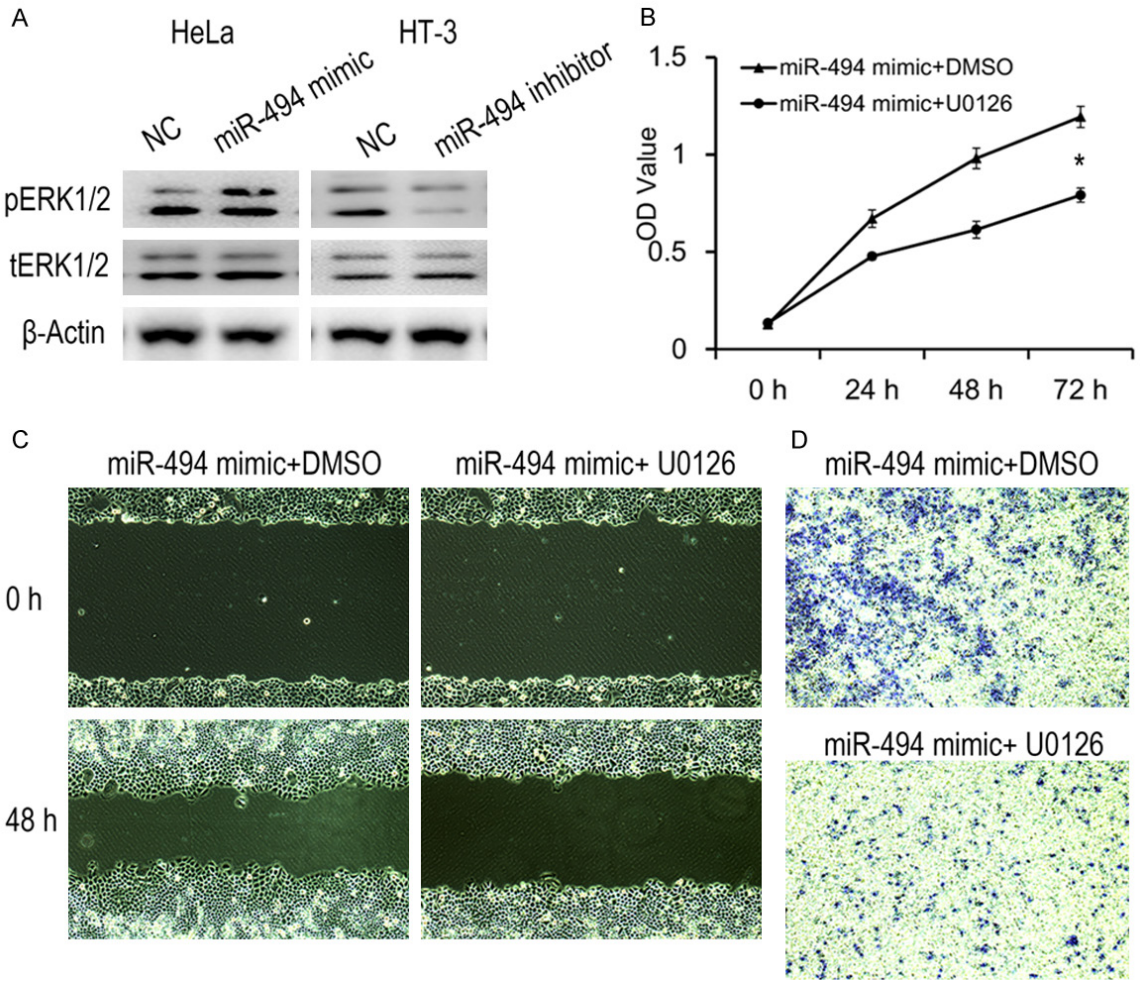


**Figure 3.** MAPK/ERK pathway is involved in miR-494-induced cells proliferation, migration and invasion. (A) The expression of pERK1/2 was up-regulated in miR-494 overexpression HeLa cells and down-regulated in miR-494 downexpression HT-3 cells. MAPK/ERK pathway blockage inhibited miR-494-induced HeLa cells proliferation (B), migration (C) and invasion (D). NC, Negative control; *P<0.05, when compared with miR-494+DMSO.

494 promoted HeLa cells migration, and reduced expression of miR-494 inhibited HT-3 cells migration (**Figure 2C**). Cell invasion assay indicated the invasive ability of HeLa cells transfected with miR-494 mimics was higher than that transfected with negative control, while there were adverse results in cells transfected with miR-494 inhibitor and negative control (**Figure 2D**).

*MAPK/ERK pathway is involved in miR-494-mediated cell proliferation, migration and invasion*

The involvement of MAPK/ERK pathway in miR-494-mediated cell behavior changes was further investigated and miR-494 overexpression was found to significantly increase pERK1/2 protein level. Adverse results were found in miR-494 down-expressed cells. Suppressed miR-494 expression led to decreased p-ERK1/2 expression (**Figure 3A**). To better confirm the results, miR-494 over-expressed HeLa cells were treated with ERK inhibitor U0126 or DMSO for 1 h, and then subjected to proliferation, migration and invasion assays. Results showed that ERK inhibition significantly suppressed cell proliferation, migration and invasion (**Figure 3B-D**). These results indicated that MAPK/ERK pathway was involved in miR-494-induced cell proliferation, migration and invasion.

*SOX9 is a target of miR-494*

According to predictions of TargetScan (www.targetscan.org), Pictar (pictar.mdc-berlin.de/) and miRdb (http:// www.mirdb.org/), SOX9 con-

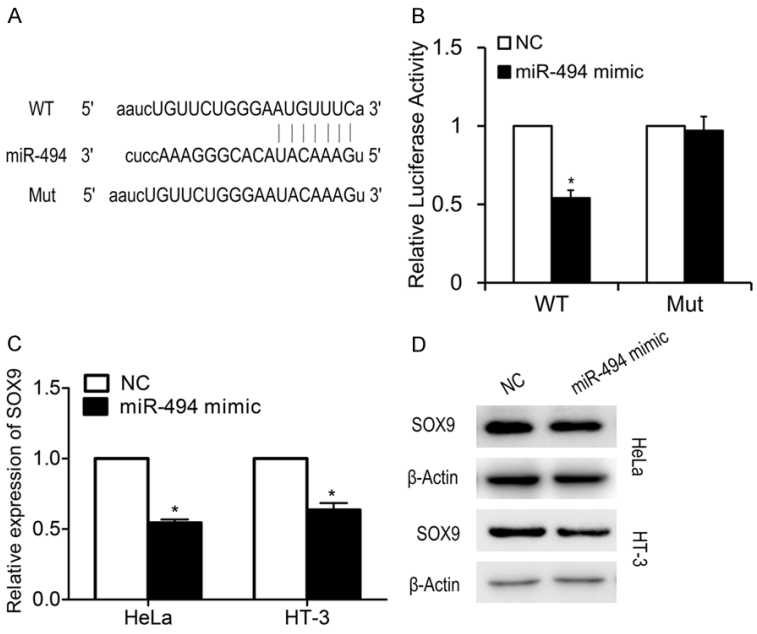


**Figure 4.** SOX9 is a target of miR-494. (A) The binding sequence of miR-494 in SOX9 3'UTR. (B) The luciferase activity was significantly decreased in cells co-transfected with miR-494 mimics and SOX9-3'UTR-WT in HeLa cell lines. The mRNA (C) and protein (D) level of SOX9 was down-regulated after transfection of miR-494 mimic in HeLa and HT-3 cells. NC: Negative control; WT: Wild type; Mut: Mutant; *P<0.05, compared with NC.

tains binding site to the seed sequence of miR-494. Therefore, luciferase reporter assay was performed to confirm the target of miR-494. The wild type and mutant binding site of miR-494 in SOX9 were cloned into the pGL3 vector, respectively (**Figure 4A**), and transfected into HeLa cells. The data showed that miR-494 decreased the luciferase activity of the pGL3-SOX9-3'UTR-WT instead of pGL3-SOX9-3'UTR-Mut (**Figure 4B**). Furthermore, we transfected miR-494 mimics and negative control into HeLa and HT-3 cells to analyze the regulatory effect of miR-494 on endogenous SOX9 expression and miR-494 was found to significantly decrease SOX9 expression level (**Figure 4C** and **4D**). In conclusion, these results suggested that SOX9 was a direct target of miR-494.

## Discussion

Aberrant expression of miRNAs has been regularly found in cervical cancer, which is associated with tumor initiation and progression [16, 17]. Therefore, investigation about miRNAs is beneficial to uncover the molecular mechanism of tumorgenesis and develop new diagnosis methods.

miR-494 has been reported to play significant roles in various cancers. Previous studies have shown that down-regulation of miR-494 functioned as an independent prognostic factor in nasopharyngeal carcinoma [18] and overexpression of miR-494 suppressed breast cancer progression by regulating the Wnt/β-catenin pathway [15]. In addition, miR-494 activated the Akt/eNOS pathway to promote angiogenesis in non-small cell lung cancer [14]. In the present study, we found miR-494 was up-regulated in plasma of 40 cervical cancer patients and high miR-494 level was correlated with invasion degree, suggesting that miR-494 might participate in cervical cancer progression.

In addition, the role of miR-494 in cervical cancer cell lines was further studied. Cellular experiments indicated that miR-494 promoted cervical cancer cells proliferation, migration and invasion. MAPK/ERK pathway modulated a number of cellular functions and played important roles in tumorgenesis [19]. Therefore, whether MAPK/ERK pathway is involved in miR-494-induced cell proliferation, migration and invasion was identified. The results indicated that overexpression of miR-494 could increase p-ERK1/2 expression level to activate the MAPK/ERK pathway. Furthermore, cell proliferation, migration and invasion were decreased in miR-494 over-expressed cells when treated with ERK inhibitor U0126. These data suggested that miR-494 promoted cell proliferation, migration and invasion by activating that MAPK/ERK pathway.

miRNAs participated in cell biological processes by regulating their targets, thus luciferase assay was performed to verify the target of miR-494. The results indicated that SOX9 was a direct target of miR-494 and miR-494 could negatively regulate endogenous SOX9 expression of in HeLa and HT-3 cells. As a transcription factor, SOX9 is essential to neurogenesis and the development of cartilage, male gonad

and neural crest [20-26]. Previous studies demonstrated that SOX9 functioned as the oncogene or tumor suppressor to regulate malignant behaviors, such as metastasis and tumorigenicity [20, 27]. Wang HY et al found that SOX9 was a tumor suppressor gene and may serve as a potential therapeutic target in cervical cancer [24]. Whether miR-494 regulates cell proliferation, migration and invasion by targeting SOX9 will be studied in the future.

In conclusion, this study demonstrates that miR-494 is up-regulated in cervical cancer plasma. miR-494 promotes cell proliferation, migration and invasion through the MAPK/ERK pathway and SOX9 is a direct target of miR-494.

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## Disclosure of conflict of interest

None.

**Address correspondence to:** Ming Ma, Department of Oncology, Linyi People's Hospital, 27 Jiefang Road, Linyi, Shandong, China. Tel: 0086-539-80-78518; E-mail: mamingmax@126.com

## References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.

[3] Hanahan D and Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57-70.

[4] Fabian MR, Sonenberg N and Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem 2010; 79: 351-379.

[5] Zhang H, Li Y and Lai M. The microRNA network and tumor metastasis. Oncogene 2010; 29: 937-948.

[6] Cho WC. OncomiRs: the discovery and progress of microRNAs in cancers. Mol Cancer 2007; 6: 60.

[7] He W, Li Y, Chen X, Lu L, Tang B, Wang Z, Pan Y, Cai S, He Y and Ke Z. miR-494 acts as an anti-oncogene in gastric carcinoma by targeting c-myc. J Gastroenterol Hepatol 2014; 29: 1427-1434.

[8] Olaru AV, Ghiaur G, Yamanaka S, Luvsanjav D, An F, Popescu I, Alexandrescu S, Allen S, Pawlik TM, Torbenson M, Georgiades C, Roberts LR, Gores GJ, Ferguson-Smith A, Almeida MI, Calin GA, Mezey E and Selaru FM. MicroRNA down-regulated in human cholangiocarcinoma control cell cycle through multiple targets involved in the G1/S checkpoint. Hepatology 2011; 54: 2089-2098.

[9] Zhao JJ, Yang J, Lin J, Yao N, Zhu Y, Zheng J, Xu J, Cheng JQ, Lin JY and Ma X. Identification of miRNAs associated with tumorigenesis of retinoblastoma by miRNA microarray analysis. Childs Nerv Syst 2009; 25: 13-20.

[10] Kim WK, Park M, Kim YK, Tae YK, Yang HK, Lee JM and Kim H. MicroRNA-494 downregulates KIT and inhibits gastrointestinal stromal tumor cell proliferation. Clin Cancer Res 2011; 17: 7584-7594.

[11] Kwak SY, Yang JS, Kim BY, Bae IH and Han YH. Ionizing radiation-inducible miR-494 promotes glioma cell invasion through EGFR stabilization by targeting p190B rhoGAP. Biochim Biophys Acta 2014; 1843: 508-516.

[12] Shen PF, Chen XQ, Liao YC, Chen N, Zhou Q, Wei Q, Li X, Wang J and Zeng H. MicroRNA-494-3p targets CXCR4 to suppress the proliferation, invasion, and migration of prostate cancer. Prostate 2014; 74: 756-767.

[13] Zhang R, Chen X, Zhang S, Zhang X, Li T, Liu Z, Wang J, Zang W, Wang Y, Du Y and Zhao G. Upregulation of miR-494 Inhibits Cell Growth and Invasion and Induces Cell Apoptosis by Targeting Cleft Lip and Palate Transmembrane 1-Like in Esophageal Squamous Cell Carcinoma. Dig Dis Sci 2015; 60: 1247-1255.

[14] Mao G, Liu Y, Fang X, Liu Y, Fang L, Lin L, Liu X and Wang N. Tumor-derived microRNA-494 promotes angiogenesis in non-small cell lung cancer. Angiogenesis 2015; 18: 373-382.

[15] Song L, Liu D, Wang B, He J, Zhang S, Dai Z, Ma X and Wang X. miR-494 suppresses the progression of breast cancer in vitro by targeting CXCR4 through the Wnt/beta-catenin signaling pathway. Oncol Rep 2015; 34: 525-531.

[16] Safari A, Seifoleslami M, Yahaghi E, Sedaghati F and Khameneie MK. Upregulation of miR-20a and miR-10a expression levels act as potential biomarkers of aggressive progression and poor prognosis in cervical cancer. Tumour Biol 2015; [Epub ahead of print].

[17] Fan Z, Cui H, Xu X, Lin Z, Zhang X, Kang L, Han B, Meng J, Yan Z, Yan X and Jiao S. MiR-125a suppresses tumor growth, invasion and metastasis in cervical cancer by targeting STAT3. Oncotarget 2015; 6: 25266-25280.

[18] Dadpay M, Zarea M, Rabati RG, Rezakhaniha B, Barari B, Behnod V and Ziari K. Upregulation of miR-21 and downregulation of miR-494 may serve as emerging molecular biomarkers for prediagnostic samples of subjects who developed nasopharyngeal carcinoma associates with lymph node metastasis and poor prognosis. Tumour Biol 2015; [Epub ahead of print].

[19] Kun D, Xiang-Lin C, Ming Z and Qi L. Chlamydia inhibit host cell apoptosis by inducing Bag-1 via the MAPK/ERK survival pathway. Apoptosis 2013; 18: 1083-1092.

[20] Hong Y, Chen W, Du X, Ning H, Chen H, Shi R, Lin S, Xu R, Zhu J, Wu S and Zhou H. Upregulation of sex-determining region Y-box 9 (SOX9) promotes cell proliferation and tumorigenicity in esophageal squamous cell carcinoma. Oncotarget 2015; 6: 31241-31254.

[21] Gao J, Zhang JY, Li YH and Ren F. Decreased expression of SOX9 indicates a better prognosis and inhibits the growth of glioma cells by inducing cell cycle arrest. Int J Clin Exp Pathol 2015; 8: 10130-10138.

[22] Wang X, Ju Y, Zhou MI, Liu X and Zhou C. Upregulation of SOX9 promotes cell proliferation, migration and invasion in lung adenocarcinoma. Oncol Lett 2015; 10: 990-994.

[23] Li XL, Chen XQ, Zhang MN, Chen N, Nie L, Xu M, Gong J, Shen PF, Su ZZ, Weng X, Tan JY, Zhao T, Zeng H and Zhou Q. SOX9 was involved in TKIs resistance in renal cell carcinoma via Raf/MEK/ERK signaling pathway. Int J Clin Exp Pathol 2015; 8: 3871-3881.

[24] Wang HY, Lian P and Zheng PS. SOX9, a potential tumor suppressor in cervical cancer, transactivates p21WAF1/CIP1 and suppresses cervical tumor growth. Oncotarget 2015; 6: 20711-20722.

[25] Wegner M. All purpose Sox: The many roles of Sox proteins in gene expression. Int J Biochem Cell Biol 2010; 42: 381-390.

[26] Pritchett J, Athwal V, Roberts N, Hanley NA and Hanley KP. Understanding the role of SOX9 in acquired diseases: lessons from development. Trends Mol Med 2011; 17: 166-174.

[27] Shen Z, Deng H, Fang Y, Zhu X, Ye GT, Yan L, Liu H and Li G. Identification of the interplay between SOX9 and S100P in the metastasis and invasion of colon carcinoma. Oncotarget 2015; 6: 20672-20684.