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# Potential suppressive functions of microRNA-504 in cervical cancer cells malignant process were achieved by targeting PAICS and regulating EMT

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## Abstract

**Purpose** The present study aimed to investigate the effects of miR-504 in cervical cancer.

**Methods** Normal and cervical cancer tissue specimens derived from TCGA and GTEx databases were employed to analyze the miR-504 and PAICS (one of potential target gene of miR-504) expression. Kaplan–Meier strategy was applied to analyze the prognostic powers of miR-504 and PAICS. The proliferation, clonogenic ability, invasion, and migration of cervical cancer cells (C-33A and HeLa) were detected using Cell Counting Kit 8, colony formation, and transwell assays. Pearson correlation analysis was used to assess the correlation between miR-504 and PAICS, which was confirmed using luciferase reporter assay. The mRNA and protein levels were detected by qRT-PCR and western blot, respectively.

**Results** TCGA data revealed that miR-504 expression might be decreased in cervical cancer, which was correlated with unfavorable prognosis. Further experiments exhibited that abnormal miR-504 expression negatively affected malignant cellular behaviors in cervical cancer, including proliferation, colony formation, invasion, and migration. PAICS was identified as a putative target of miR-504, and negatively related with miR-504 expression. PAICS expression was increased in cervical cancer and its high-regulation-induced worse outcomes of patients with cervical cancer. Rescue experiments indicated that PAICS restricted the impacts of miR-504 in cervical cancer cells. Analysis of western blot suggested that overexpression of PAICS overturned the miR-504-induced EMT inactivation.

**Conclusion** Our observations elucidated that miR-504, acting as a suppressor for the progression of cervical cancer, inhibits cell proliferation, invasion and migration, and mediates EMT via negatively regulating PAICS.

**Keywords** Cervical cancer · miR-504 · Proliferation · Invasion · Migration · PAICS · EMT

## Introduction

Cervical cancer is the fourth most frequent cancer among female worldwide [1] and its occurrence is mainly related with the persistent infection of human papillomavirus [2]. According to statistics, there are 570,000 novel cases in 2018, accounting for approximately 6.6% female cancers [3].

Although substantial progress has been obtained in therapeutic strategy, the prognosis of cervical cancer patients still remains unsatisfactory [4]. Therefore, investigating the novel effective therapeutic therapies for cervical cancer is still considered as a public health concern in the world.

MicroRNAs (miRNAs) are a type of non-protein-coding and small single-stranded RNAs that are 22–25 nucleotides in length [5]. Accumulating evidence has revealed that miRNAs can repress key protein translation through binding to the 3′-untranslated regions (3′-UTRs) of the corresponding target genes [6, 7], and affect the epigenetic molecular mechanism [8]. More interestingly, miRNAs serve as either oncogenic factors or tumor suppressors in the pathogenesis of cervical cancer. For example, miR-21 was up-regulated in cervical cancer and promoted proliferation, invasion, and migration of cervical cancer cells via regulating tissue inhibitor of metalloproteinase 3 (TIMP3) [9]. MiR-3647,

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mediated by TP53, has been verified to repress cervical cancer cell viability and strengthen cell apoptosis [10]. Recent investigations have elaborated that dysregulated miR-504 plays crucial roles in various kinds of human tumors, including hepatocellular carcinoma [11], non-small cell lung cancer (NSCLC) [12], and osteosarcoma [13]. However, the expression level of miR-504 and its underlying molecular mechanism in cervical cancer have not been clearly understood.

Phosphoribosylaminoimidazole carboxylase (PAICS), a bifunctional enzyme, is encoded by phosphoribosylaminoimidazole succinocarboxamide synthetase (*PAICS*) gene [14]. It has been identified as a potential effective target for cancer treatments due to its important roles in de novo purine biosynthesis pathway [15]. Several previous reports have determined that PAICS might act as an oncogenic factor in various cancers, including breast cancer [16], lung adenocarcinoma [17], and prostate cancer [18]. These publications indicated that high-regulated PAICS was related with poor outcomes of cancer patients. However, the impacts of PAICS in cervical cancer still remain limited.

In our present exploration, we explored the functional role and prognostic importance of miR-504 in cervical cancer. Bioinformatics analysis was performed to predict the putative target of miR-504 and luciferase reporter assay was used to verify their correlation. Further analyses were conducted to examine whether the effects of miR-504/PAICS axis in cervical cancer progression are associated with the EMT process. To sum up, these observations determined that miR-504, acting as a tumor suppressor, regulated the cervical cancer progression through mediating EMT process by targeting PAICS.

## Materials and methods

### Clinical data collection

The publicly available data of cervical cancer clinical samples were downloaded from the Cancer Genome Atlas (TCGA) database (https://cancergenome.nih.gov/) and the Genotype-Tissue Expression (GTEx) database (https://gtexportal.org/) to assess the miR-504 and PAICS expression levels.

### Cell transient transfection

Human cervical cancer cell lines (SiHa, CaSki, HeLa, and C-33A) were purchased from Cell Biology of the Chinese Academy of Sciences (Shanghai, China) and normal ectocervical cell line Ect1/E6E7 was obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). They were all inoculated in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with fetal bovine serum (FBS, 10%) and antibiotics (penicillin, 100 U/mL and streptomycin, 0.1 mg/mL) at 37 °C with 5% $CO_2$.

Abnormal miR-504 expression was implemented using miR-504 mimic/inhibitor (100 pmol) synthesized by the GenePharma Co., Ltd. (Shanghai, China). pcDNA3.1-PAICS (4 μg) and a PAICS small interfering RNA (si-PAICS, 100 pmol) were applied to high-regulate or down-regulate PAICS expression, respectively. The sequences of si-PAICS and its negative control (si-con) were as follows: si-PAICS, 5′-GTGGCAATGAAAGTAGTTAAA-3′; si-con, 5′-CGAACUCACUGGUCUGACC-3′. For transfection, cells were cultured until the confluence was over 80% and then transfected using Lipofectamine 3000 (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) based on the manufacturers' protocols. After 48-h transfection, the qRT-PCR was performed to study whether the transfection is successful.

### QRT-PCR analysis

Total RNA was isolated from cervical cancer cells using TRIzol solutions as the manufacturers' protocols. Reversed-transcribed miR-504 complementary DNA (cDNA) was performed using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) and qRT-PCR was implemented by TaqMan MicroRNA PCR kit (Applied Biosystems). For PAICS mRNA expression level analysis, the cDNA of PAICS was reversely transcribed with PrimeScript RT kit (Takara biomedical Technology Co., Ltd., Beijing, China) followed by qRT-PCR using SYBR Premix Ex Taq II (Takara biomedical Technology Co., Ltd.). Relative expression levels of miR-504 and PAICS were normalized to U6 or GAPDH, and quantified with $2^{-\Delta\Delta Ct}$ method. Specific primer sequences were as follows:

MiR-504 F, 5′- CCTGGTCTGCACTCTAT-3′,
R, 5′-GAACATGTCTGCGTATCTC-3′;
U6 F, 5′-CTCGCTTCGGCAGCA CATATACT-3′,
R, 5′-ACGCTTCACGAATTTGCGTGTC-3′;
PAICS F, 5′-ACCACCTGGAAGGAAAAGCTGC-3′
R, 5′-CGGTGCAATGAAAGCTGTCTCC-3′;
GAPDH F, 5′-TGTGTCCGTCGTGGATCTGA-3′,
R, 5′-CCTGCTTCACCACCTTCTTGA-3′.

### Western blot assay

Transfected cells were lysed and the total proteins were isolated utilizing RIPA lysis. The BCA kit was employed to assess the concentration of protein. Proteins (20 μg) denatured at 95 °C were loaded in 12% SDS-PAGE and transferred onto PVDF membranes. After blocked with 5% non-fat milk powder for 1 h, the PVDF membranes were incubated with primary antibodies (Cell Signaling

Technology, Danvers, MA, USA) at 4 °C overnight against PAICS, E-cadherin, N-cadherin, Vimentin, Snail and GAPDH. Subsequently, the PVDF membrane was washed using PBS for three times and incubated with the specific secondary antibodies (Cell Signaling Technology) at 25 °C for 1 h. The protein signals were observed by enhanced chemiluminescence (Thermo Fisher Scientific, Inc.) and analyzed using QUANTITY ONE software (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

## Cell counting kit-8 analysis

Cell proliferation of C-33A and HeLa cells was explored utilizing CCK-8 assay. The 48 post-transfected cervical cancer cells were resuspended and cultivated into 96-well plates with the density of 1000 cells per well at 37 °C with 5% $CO_2$ for 0 h, 24 h, 48 h and 72 h. At the specified time points, the optical density (OD) value at the wavelength of 450 nm was measured after adding 10 μL of CCK-8 reagent in per well and additional incubation for 1.5 h. The proliferative curves were plotted on the basis of the OD values.

## Colony formation analysis

After 48-h transfection, cells were inoculated in 60 mm dishes with 500 cells per dish at 37 °C with 5% $CO_2$. When the colonies were visible (about 2 weeks) by naked eyes, 4% paraformaldehyde and 0.1% crystal violet were employed to fix and dye these colonies, respectively. The number of colonies in each group was calculated under the microscope.

## Transwell invasion and migration assays

The 24-well chambers were utilized to describe the migratory and invasive abilities of transfected cells. The upper chamber with 8-μm pore size membranes with (for invasion) or without (for migration). Matrigel was prepared and 500 μL of serum-free medium was put in the lower surface of transwell chamber to hydrate the basement membrane. Then, transfectants ($1 \times 10^5$ cells/well for invasion and 5000 cells/well for migration) were placed in the upper chamber, and bottom chamber was supplemented with 500-μL complete medium. After overnight, cells on the upper chamber were wiped out with cotton swabs while the migratory or invasive cells on the lower chamber were fixed with 4% paraformaldehyde for 30 min and dyed using 0.1% crystal violet for 20 min. The stained cells were ultimately photographed and the mean number of migratory or invasive cells was calculated artificially.

## Dual-luciferase reporter gene assay

A fragment comprising the putative binding site between PAICS 3′UTR and miR-504 (WT PAICS) was cloned into the pMIR-reporter vector (Promega, Madison, WI, USA). A second construct without the above fragment (MUT PAICS) was also established. They were used to identify whether PAICS was the potential target gene of miR-504. Cervical cancer cells were inoculated in 96-well plates at a density of $1 \times 10^4$ cells per well for 24 h and subsequently, co-transfected with miR-504 mimic negative control (NC; 50 pmol) or miR-504 mimic (50 pmol) in combination with WT PAICS (0.2 μg) or MUT PAICS (0.2 μg) using Lipofectamine3000. After 48-h co-transfection, relative luciferase activity was assessed by the Dual-luciferase Reporter Assay Kit (Promega).

## Statistical analysis

A Student's *t* test and one-way analysis of variance with Dunnett or Bonferroni post hoc test were conducted to compare the difference in two groups or multiple groups. The curve of overall survival was plotted by Kaplan–Meier strategy and log-rank test and analyzed by dividing the cervical cancer patients into two groups on the basis of median value of miR-504 or PAICS expression in cervical cancer patients. Pearson analysis was performed to identify the correlations of miR-504 and PAICS. All the data were analyzed by SPSS 22.0 (SPSS, Chicago, IL, USA) and plotted by GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). All experiments were performed at least in triplicate and results were expressed as mean ± standard deviation (SD). The value of *P* less than 0.05 was considered statistically significant.

## Results

### MiR-504 expression might be inhibited and correlated with prognosis of cervical cancer

To explore the expressional pattern of miR-504 in cervical cancer, miR-504 expression was analyzed using normal tissues ($n = 3$) and cervical cancer tissues ($n = 309$). In comparison to the normal group, miR-504 expression was reduced in cervical cancer cases (Fig. 1a). To detect the prognostic value of miR-504 in cervical cancer, overall survival curve was plotted and revealed that down-regulated miR-504 expression was linked with poor prognosis whilst high miR-504 expression was related with better outcome ($P = 0.0228$; Fig. 1b). MiR-504 expression was also examined in four cervical cancer cell lines (SiHa, CaSki, HeLa, and C-33A), which were relative to miR-504 expression in
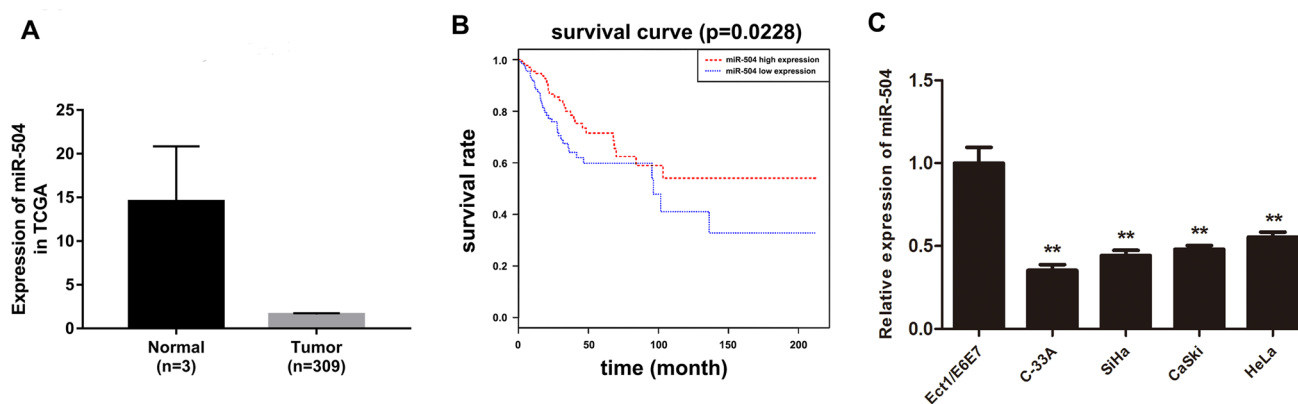
**Fig. 1** MiR-504 was down-regulated in cervical cancer tissue samples and indicated cell lines, and its down-regulation was correlated with unfavorable prognosis of patients with cervical cancer. **a** MiR-504 expression was decreased in cervical cancer cases ($n=309$) compared with normal specimens ($n=3$). **b** The survival rate curve performed by Kaplan–Meier method uncovered the prognosis of cervical cancer patients with high or low miR-504 expression, $P=0.0228$. **c** Analysis of qRT-PCR was conducted to assess the expression level of miR-504 in four cervical cancer cell lines (SiHa, CaSki, HeLa, and C-33A) and one normal ectocervical cell line Ect1/E6E7, **$P<0.01$

normal ectocervical cell line Ect1/E6E7. Compared with Ect1/E6E7 cells, qRT-PCR results demonstrated that miR-504 expression was attenuated in all the above cervical cancer cell lines ($P<0.01$; Fig. 1c). These findings indicated that miR-504 might be implicated in the cervical cancer development.

## MiR-504 restricted proliferation, clonogenic potential, invasion and migration of cervical cancer cells

To examine the biological influence of abnormal miR-504 expressions on the cell behaviors, miR-504 mimic and miR-504 inhibitor were transfected into C-33A or HeLa cells, respectively. Result of CCK-8 analyses elucidated that the proliferation of C-33A cells transfected with miR-504 mimic was markedly inhibited compared with the control, especially at 48 h and 72 h ($P<0.01$; Fig. 2a). Colony formation analysis demonstrated that high regulation of miR-504 in C-33A cells inhibited the clonogenic ability and decreased the number of colonies ($P<0.01$; Fig. 2c). Transwell invasion and migration assays were performed in C-33A cells and disclosed that overexpression of miR-504 significantly restricted the invasive and migratory capabilities (Fig. 2e). The mean number of invasive and migratory cells showed the same trends ($P<0.01$; Fig. 2f). Conversely, the results of CCK-8, colony formation and transwell assays in HeLa cells transfected with miR-504 inhibitor exhibited the opposite tendency to C-33A cells with miR-504 mimic treatment. Compared with the control group, the transfection of miR-504 inhibitor significantly contributed to cell proliferation ($P<0.01$; Fig. 2b), colony formation ($P<0.01$; Fig. 2d), invasion and migration

($P<0.01$; Fig. 2g, h). All above observations disclosed that miR-504 might exert a suppressive effect on cell behaviors in cervical cancer.

## PAICS was a direct target gene of miR-504 in cervical cancer

Extensive studies have confirmed that the biological impacts of miRNAs to some extent depend on their direct target genes. Thus, we employed the miRWalk tool to predict the possible target genes of miR-504 in cervical cancer [19]. The putative target genes were crossed with the genes with unfavorable prognosis in cervical cancer obtained from an open source database TCGA (Fig. 3a). Combined with the context of literature and prognostic significance, PAICS was determined as a direct target gene of miR-504 in cervical cancer. Moreover, Pearson correlation analysis indicated that there was a negative correlation between miR-504 expression and PAICS expression (Fig. 3a; $r=-0.3085$, $P<0.0001$). Bioinformatics analysis showed the sequence of the binding site between miR-504 and PAICS (Fig. 3b). Dual-luciferase reporter analysis showed that the luciferase activity of cells co-transfected with miR-504 mimic and WT PAICS was markedly reduced while no effect of miR-504 mimic was found on the luciferase activity after co-transfection with the MUT PAICS ($P<0.01$; Fig. 3b). QRT-PCR analysis and western blot experiments revealed that PAICS expression was obviously attenuated in cervical cancer cells after miR-504 mimic transfection and significantly elevated after miR-504 inhibitor transfection relative to the control group ($P<0.01$; Fig. 3c–e). These findings uncovered that PAICS might serve as a direct target gene of miR-504 in cervical cancer.

**Fig. 2** MiR-504 restricted proliferation, clonogenic potential, invasive, and migratory abilities of cervical cancer cells. **a** and **b** CCK-8 assays were implemented to determine the role of miR-504 mimic and miR-504 inhibitor in C-33A or HeLa cells, **$P < 0.01$. **c** and **d** Clonogenic ability of cervical cancer cells was examined after miR-504 mimic or miR-504 inhibitor transfection. The number of colonies was quantified, **$P < 0.01$. **e** The invasion and migration of C-33A cells transfected with miR-504 mimic were investigated by transwell assays. **f** The invasive or migratory cells were counted artificially, **$P < 0.01$. **g** The invasion and migration of HeLa cells transfected with miR-504 inhibitor were explored by transwell experiment. **h** The invasive or migratory cells were counted artificially, **$P < 0.01$
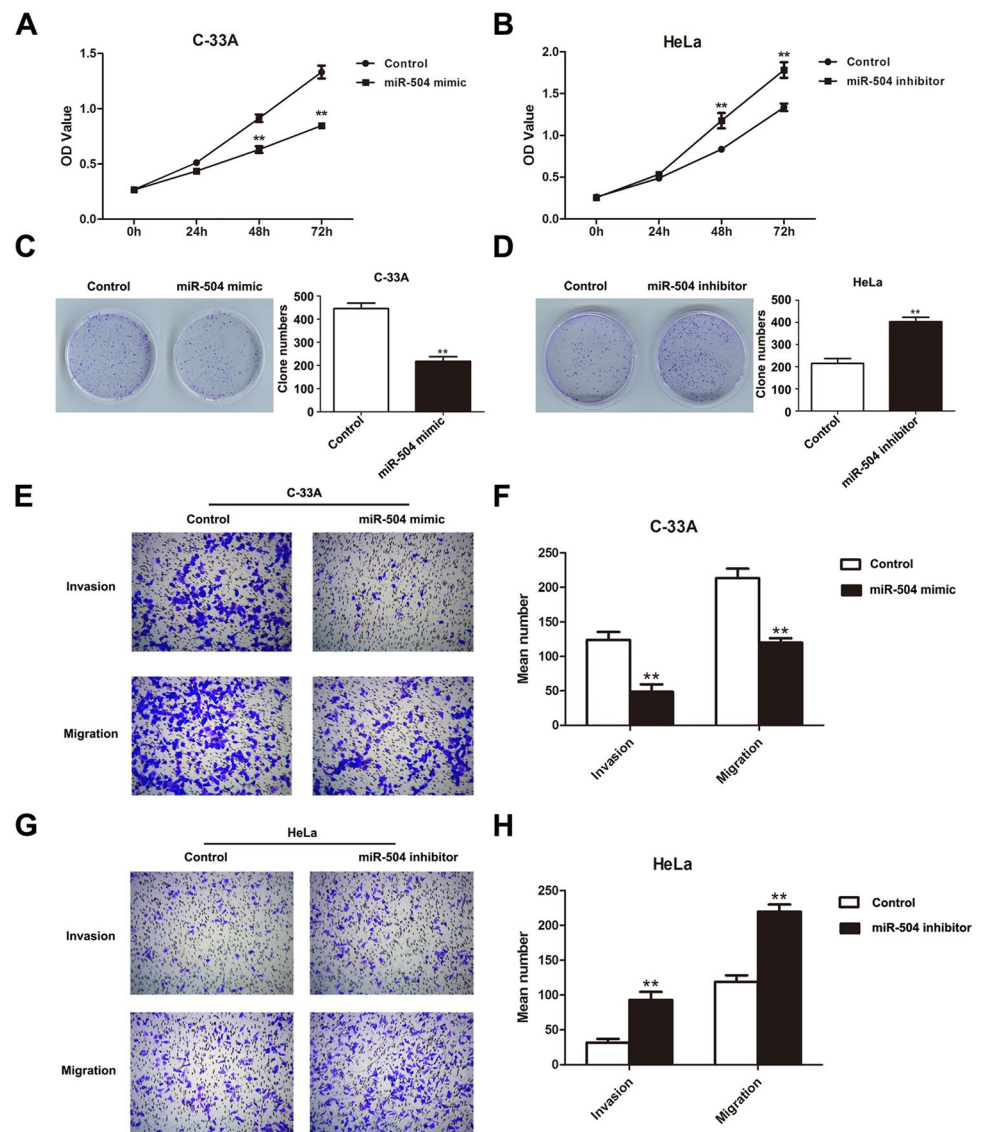


## PAICS expression was increased and associated with prognostic outcomes in cervical cancer

To validate the potential influence of PAICS in cervical cancer development, we also assessed PAICS expression in 13 normal specimens and 306 tumor specimens (derived from open source databases TCGA and GTEx). Results revealed that the PAICS expression was highly expressed in tumor samples compared to normal cases ($P < 0.0001$; Fig. 4a). Furthermore, in shorter period, the cervical cancer patients with high PAICS expression possessed worse outcomes than that of patients with low PAICS expression ($P = 0.01095$; Fig. 4b). All data elaborated that PAICS expression might be remarkably increased in cervical cancer and its high-regulation was associated with unfavorable outcomes of cervical cancer patients.

## PAICS can transpose the inhibitory effect of miR-504 on aggressiveness in cervical cancer cells

Given the correlation between miR-504 and PAICS, we therefore hypothesized that differential PAICS expression might mediate the phenotypes of cervical cancer cells regulated by miR-504. To verify our hypothesis, C-33A cells transfected with miR-504 mimic + pcDNA3.1-PAICS and HeLa cells transfected with miR-504 inhibitor + si-PAICS were prepared for the subsequent functional experiments. In C-33A cells, CCK-8 assays and colony formation analyses elucidated that the viability and colony formation of C-33A cells were inhibited upon the high-regulation of miR-504; the repressive impacts of miR-504 overexpression on proliferation and colony formation were restored by up-regulation of PAICS expression ($P < 0.01$; Fig. 5a, c). Transwell invasion and migration
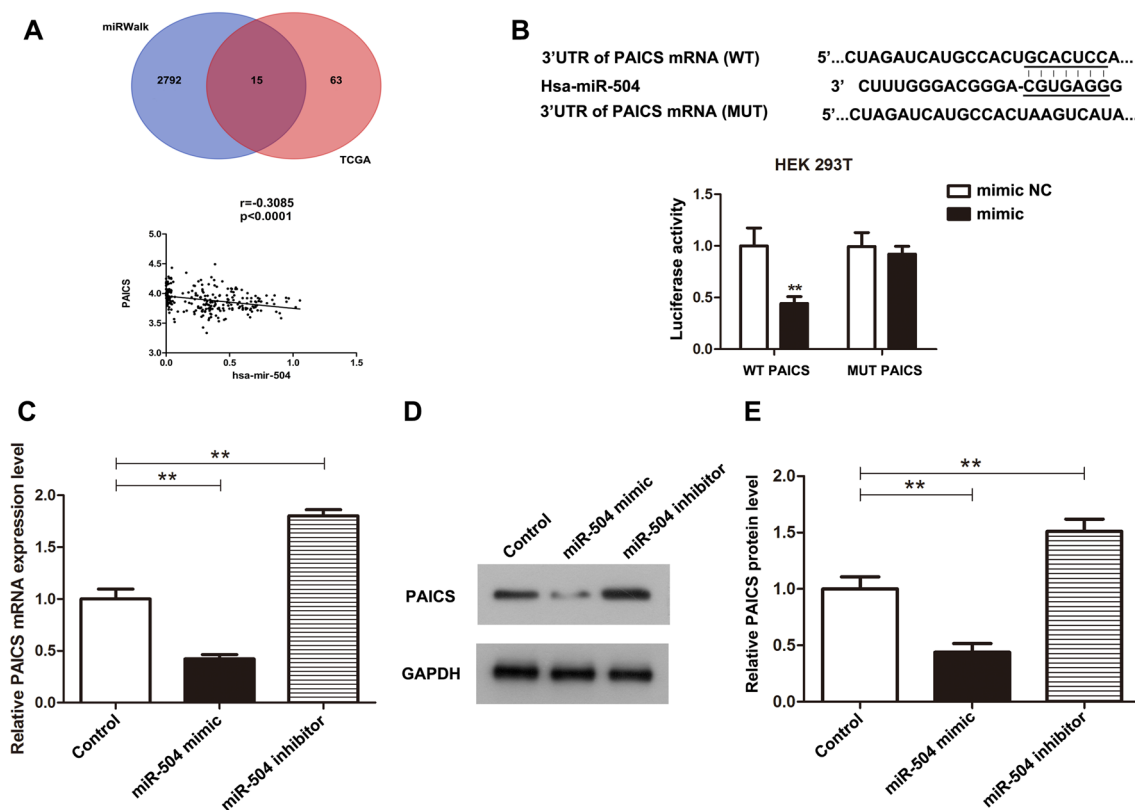
**Fig. 3** PAICS was a direct target gene of miR-504 in cervical cancer. **a** Intersection of target genes of miR-504 from miRWalk and up-regulated genes in cervical cancer from TCGA database. Pearson correlation analysis of miR-504 and PAICS. **b** The sequence of putative binding site between miR-504 and PAICS. Relative luciferase activity was examined in cervical cancer cells co-transfected with miR-504 mimic and WT PAICS or MUT PAICS, **$P < 0.01$. **c** The PAICS mRNA expression level was evaluated in cervical cancer cells transfected with miR-504 mimic or miR-504 inhibitor by qRT-PCR, **$P < 0.01$. **d** The protein expression level of PAICS was measured in cervical cancer cells after miR-504 mimic or miR-504 inhibitor transfection, **$P < 0.01$. **e** The gray value was scanned and quantified, **$P < 0.01$



**Fig. 4** PAICS was highly regulated and linked with prognosis in cervical cancer. **a** PAICS expression was high-regulated in cervical cancer tissue cases contrast to normal samples based on the integrated data of TCGA and GTEx databases, $P < 0.0001$. **b** The survival rate of cervical cancer patients illustrated that high-regulation of PAICS caused the poor outcome, $P = 0.01095$
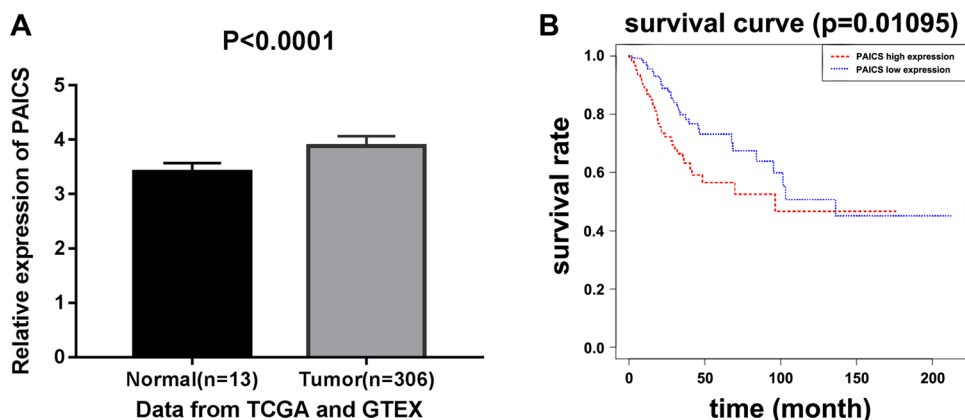
assays determined that the inhibition of invasive and migratory potentials induced by miR-504 mimic was also reversed by the overexpression of PAICS ($P < 0.01$; Fig. 5e, f). Consistently, the reversal effect of PAICS on miR-504-mediated cell behaviors was determined in HeLa cells. Promoting impacts

of miR-504 inhibitor on cell viability were significantly hindered by si-PAICS ($P < 0.01$; Fig. 5b, d). Results of transwell analysis determined that down-regulation of miR-504 facilitated the invasion and migration of HeLa cells, and this effect could be abrogated by si-PAICS ($P < 0.01$; Fig. 5g, h). All data
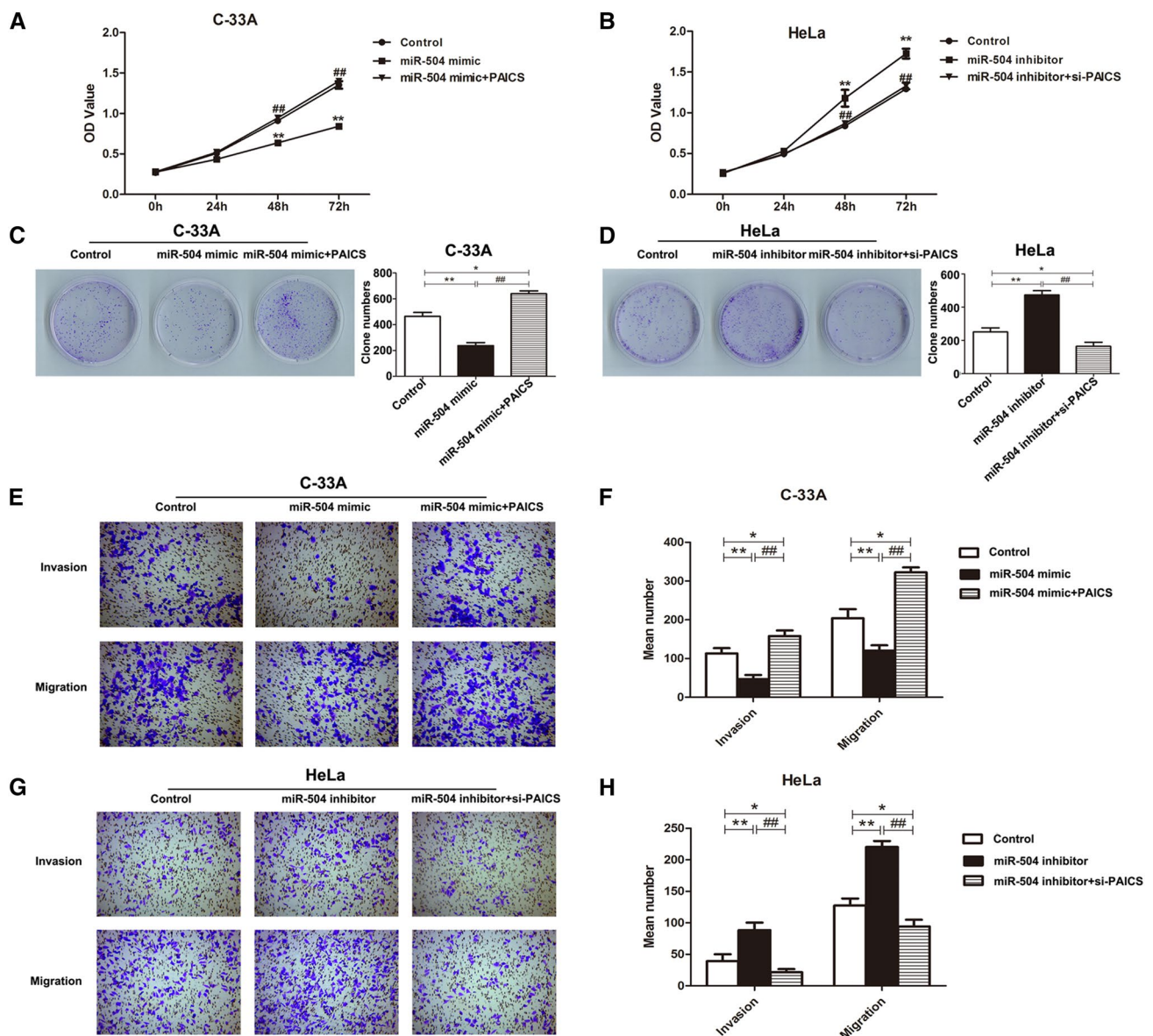
**Fig. 5** PAICS reversed the inhibitory influence of miR-504 on malignant aggressiveness in cervical cancer cells. **a** and **b** CCK-8 assays were conducted to assess the proliferative ability of C-33A and HeLa cells after indicated treatment, $**P < 0.01$, $^{##}P < 0.01$. **c** and **d** Colony formation analysis was carried out to determine the clonogenic activity in C-33A and HeLa cells with indicated transfection, $*P < 0.05$, $**P < 0.01$, $^{##}P < 0.01$. **e** C-33A cells transfected with miR-504 mimic or miR-504 mimic + PAICS were used to evaluate the invasion and migration by transwell assays. **f** Mean number of invasive and migratory cells was counted and quantified, $*P < 0.05$, $**P < 0.01$, $^{##}P < 0.01$. **g** HeLa cells transfected with miR-504 inhibitor or miR-504 inhibitor + si-PAICS were employed to investigate the invasion and migration using transwell assays. **h** Mean number of invasive and migratory cells was quantified, $*P < 0.05$, $**P < 0.01$, $^{##}P < 0.01$

suggested that PAICS can rescue the effect induced by miR-504 on aggressiveness in cervical cancer cells.

## MiR-504/PAICS axis mediated the expression of epithelial to mesenchymal transition (EMT)-related proteins

To further illuminate the potential mechanism of miR-504 and PAICS in cervical cancer, we evaluated the expression levels of some representatively EMT-related proteins using western blot assay. High-regulated miR-504 distinctly increased E-cadherin expression whilst attenuated the expression of N-cadherin, Vimentin and Snail in C-33A cells ($P < 0.01$; Fig. 6a, b). However, after transfection with miR-504 mimic + pcDNA3.1-PAICS, the expression changes of specific EMT-related proteins induced by miR-504 mimic were restored in C-33A cells ($P < 0.01$; Fig. 6a, b). Similarly, co-transfection of miR-504 inhibitor and si-PAICS

**Fig. 6** The EMT-related protein levels in cervical cancer cells were regulated by miR-504/PAICS axis. **a** The influence of miR-504 mimic and miR-504 mimic + PAICS on E-cadherin, N-cadherin, Vimentin and Snail expression levels in C-33A cells, and quantified in (**b**), *$P < 0.05$, **$P < 0.01$, ##$P < 0.01$. **c** The effect of miR-504 inhibitor and miR-504 inhibitor + si-PAICS on E-cadherin, N-cadherin, Vimentin and Snail expression levels in HeLa cells, and quantified in (**d**), *$P < 0.05$, **$P < 0.01$, ##$P < 0.01$
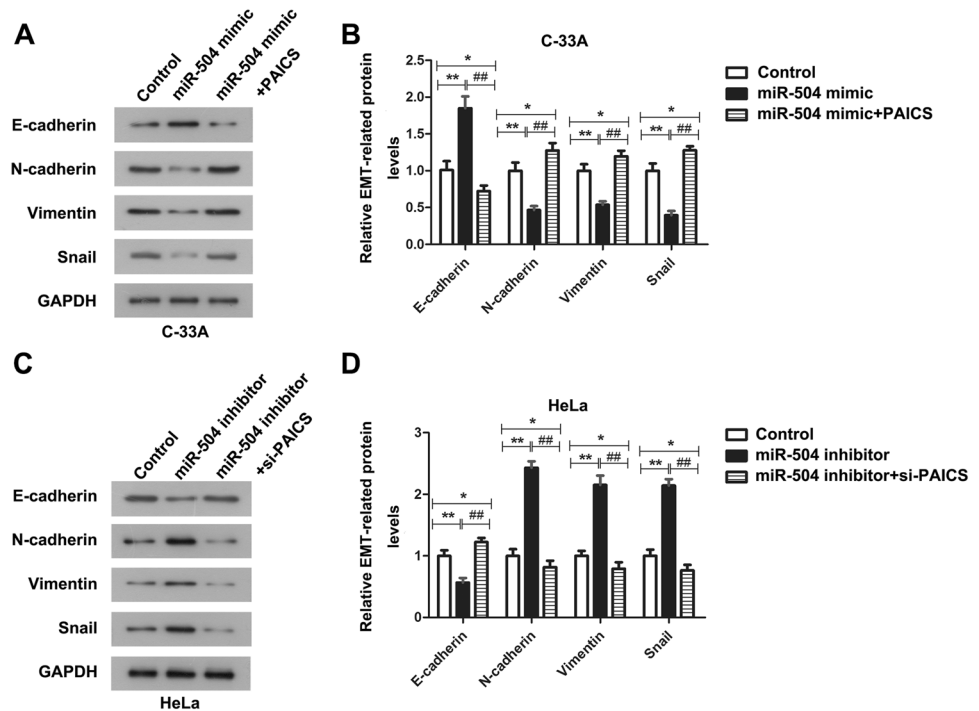


was proved to reverse the impacts of miR-504 inhibitor on the protein expression of specific EMT-related markers in HeLa cells ($P < 0.01$; Fig. 6c, d). These observations showed that miR-504/PAICS axis might affect cervical cancer cell behaviors via mediating the EMT process.

## Discussion

Over the past 5 years, a great deal of publications have indicated that miRNAs are expressed in cervical cancer with dysregulated expression levels, as cancer-promoting molecules or tumor suppressors. Thus, identifying the novel miRNAs involved in the progression of cervical cancer and deciphering their roles in cervical cancer may enhance our understanding of cervical cancer development, which provides important clues to improve cervical cancer treatments. This present study indicated that miR-504 may be decreased in cervical cancer. Low miR-504 expression was related with unfavorable outcome of cervical cancer patients. Abnormal miR-504 expression affected malignant aggressiveness of cervical cancer cells, including proliferation, colony formation, invasion, and migration. Furthermore, PAICS was identified as a target gene of miR-504. PAICS expression was increased in cervical cancer and its high-regulation-induced unfavorable prognosis of cervical cancer patients. Moreover, PAICS expression was negatively correlated with miR-504 expression. Further experiments in vitro demonstrated that PAICS restricted the effect of miR-504 on proliferation, migration, and invasion of cervical cancer cells. All data

illustrated that miR-504 may serve as a reliable therapeutic target for cervical cancer via directly targeting PAICS.

MiR-504 is differentially expressed in different types of human cancers. High-regulation of miR-504 in osteosarcoma can accelerate tumor growth and metastasis through modulating TP53INP1 [13]. Guan et al. summarized that miR-504 played an oncogenic role by negatively regulating p53, while impaired cancer cell growth in hypopharyngeal squamous cell carcinoma (HSCC). The poor prognosis of patients with high-grade glioma was also associated with miR-504 inhibition [20]. Interestingly, our findings were consistent with the above investigations: miR-504 was decreased in cervical cancer and its down-regulation was associated with poor prognosis. It has been well-known that miRNAs could regulate a variety of cellular processes by negatively modulating specific gene expression in tumorigenesis [21–23]. As above mentioned, we found that TP53INP1, p53, and LOXL2 were all identified as targeted genes of miR-504 in different cancers [12, 13, 20]. Therefore, we employed bioinformatics tool to predict the putative target gene of miR-504 in cervical cancer. Pearson correlation analysis showed that PAICS expression was negatively regulated by miR-504 and the luciferase reporter assay confirmed the correlation between PAICS and miR-504. In our study, PAICS expression might be increased in cervical cancer tissue samples and implicated in the prognosis of cervical cancer patients. More importantly, PAICS could counteract the inhibitory impacts of miR-504 in cervical cancer cell behaviors. These findings demonstrated that miR-504/PAICS pair plays crucial roles in cervical cancer development.

It has been previously reported that EMT is a hallmark of human cancers. Extensive evidence has determined that EMT can facilitate the invasion and metastasis of multiple cancers [24]. Many oncogenic molecules or tumor suppressors can affect cancer cell growth, migration, and invasion via regulating EMT process [25]. Interestingly, Liu et al. have discovered that miR-504 inhibited mesenchymal phenotype through directly regulating the FZD7 in glioblastoma [26]. Rehana et al. summarized the effect of EMT in cervical cancer, and determined the important significance of EMT in cervical cancer progression [27]. The rapid invasion characteristics of cancer cells are attributed to the loss of epithelial features and the acquisition of mesenchymal characteristics [28]. Therefore, we detected the EMT-related protein levels to identify whether the functions of miR-504/PAICS axis on cervical cancer cell aggressiveness are linked with EMT process. Results revealed that miR-504 can promote the expression level of E-cadherin, an epithelial marker, and attenuate mesenchymal marker expression levels, such as N-cadherin, Vimentin, and Snail [29]. Furthermore, up-regulation of PAICS can reverse the effects of miR-504 in EMT-related protein levels. The above observations demonstrated that miR-504 might inhibit the development of cervical cancer through mediating EMT process via targeting PAICS. Still, there are some concerns in the current study. Due to a lack of the sufficient number of normal samples, we might exaggerate the potential role of miR-504 and PAICS. Thus, more clinical specimens are needed for validation. In addition, implementation of in vivo experiments will further confirm the conclusions we obtained.

In summary, our investigation revealed that down-regulation of miR-504 might be revealed in cervical cancer and its low expression was linked with unfavorable prognosis. MiR-504 can inhibit cervical cancer cell proliferation, colony formation, invasion, and migration by mediating EMT process via directly targeting PAICS. Collectively, these data highlighted that miR-504/PAICS might act as an effective therapeutic target for cervical cancer treatments.

## Compliance with ethical standards

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