



# Downregulation of hsa\_circ\_0007534 restricts the proliferation and invasion of cervical cancer through regulating miR-498/BMI-1 signaling

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## ABSTRACT

**Aims:** Hsa\_circ\_0007534 has been reported to be a novel cancer-related circRNA affecting multiple cancers. However, little is known about the role of hsa\_circ\_0007534 in cervical cancer specifically. In the current study, we aimed to explore the expression and function of hsa\_circ\_0007534 in cervical cancer.

**Main methods:** The expression of circRNA, miRNA and mRNA was measured using real-time quantitative polymerase chain reactions. Protein expression was assessed by Western blot. Knockdown of hsa\_circ\_0007534 was achieved by siRNA-mediated gene silencing. Cell proliferation was determined using a cell counting kit-8 and colony formation assays. Cell invasion was assessed using a Transwell invasion assay. RNA interactions were measured using an RNA pull-down and dual-luciferase reporter assays.

**Key findings:** The expression of hsa\_circ\_0007534 was upregulated in cervical cancer tissues and cell lines. Depletion of hsa\_circ\_0007534 decreased both the proliferation and invasion of cervical cancer cells. MicroRNA-498 (miR-498) was identified as a target of the miRNA encoded by hsa\_circ\_0007534. Levels of miR-498 were decreased in cervical cancer tissues, a finding that was inversely correlated with hsa\_circ\_0007534 expression. miR-498 overexpression repressed the proliferation and invasion of cervical cancer cells. B-cell-specific Moloney murine leukemia virus integration site 1 (BMI-1) was verified as a target gene of miR-498. BMI-1 overexpression reversed the effects of hsa\_circ\_0007534 depletion or miR-498 overexpression on cervical cancer cell proliferation and invasion.

**Significance:** Our study demonstrates that downregulation of hsa\_circ\_0007534 represses the proliferation and invasion of cervical cancer through regulating the miR-498/BMI-1 axis, suggesting the hsa\_circ\_0007534/miR-498/BMI-1 axis as a potential therapeutic target to treat cervical cancer.

## 1. Introduction

Cervical cancer is one of the most prevalent gynecological malignancies in females, exhibiting an increasing incidence and mortality rate in recent years [1]. Due to advances in cancer therapy, the survival rate of cervical cancer patients at all stages has been significantly increased. However, the survival rate for patients with distant metastatic cancer remains extremely low [1]. Persistent infection with high-risk human papillomavirus has been considered a critical pathogenic factor for cervical cancer pathogenesis [2]. However, the precise molecular mechanism underlying cervical cancer development and progression remains poorly understood. Therefore, it is essential to expand our knowledge regarding the molecular mechanisms underlying the pathology of cervical cancer. This enhanced knowledge base will likely

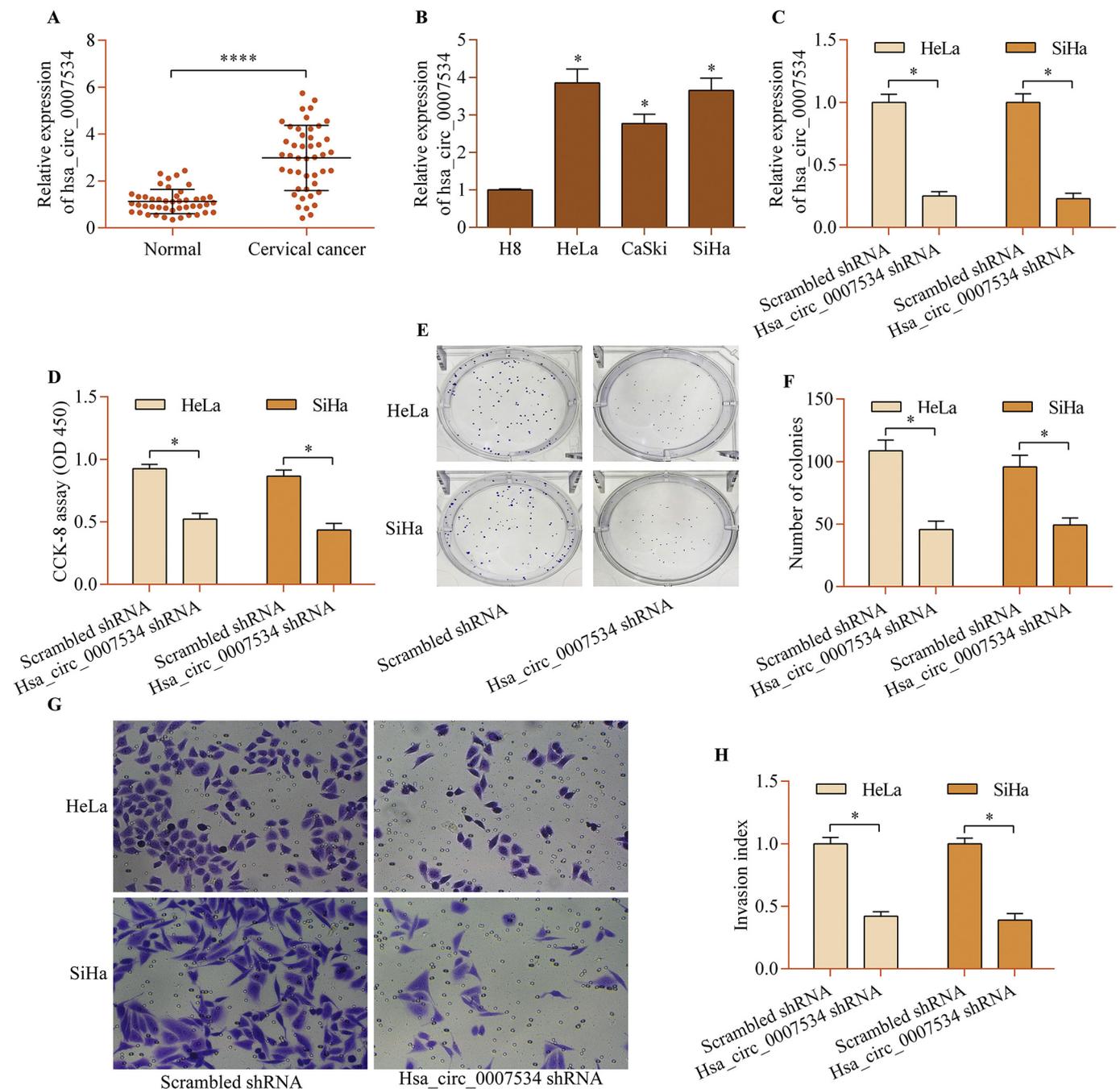
facilitate improvements in the treatment of cervical cancer.

Circular RNAs (circRNAs) are a novel type of endogenous noncoding RNAs, which have attracted a high level of attention in recent years due to their critical role in the regulation of gene expression [3,4]. CircRNAs are closed-loop RNAs without 5'-3' polarity or a polyadenylated tail [4]. CircRNAs are abundantly expressed in tissues and cells, are stable molecules and exhibit a high level of sequence conservation [5]. CircRNAs are involved in regulating gene expression through various methods, including through the use of microRNAs (miRNAs), a regulatory mechanism has been an especially hot topic in recent years [6,7]. circRNAs function through providing multiple binding sites for target miRNAs, thus acting like a molecular sponge. miRNA, when released, produce a miRNA-mediated inhibitory effect on the expression of their target genes [8]. A growing body of evidence has been

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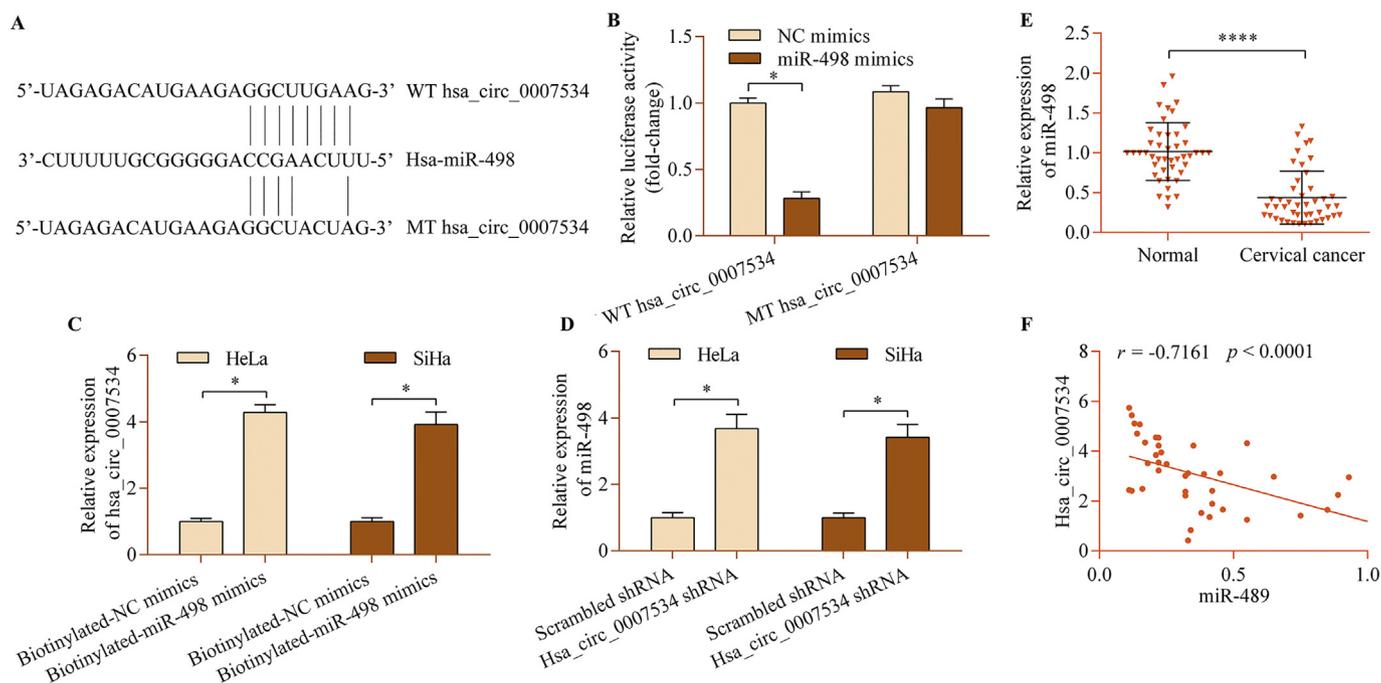
<sup>1</sup> These authors (Xuan Rong and Wei Gao) contributed equally to this work and shared the first authorship.



**Fig. 1.** Downregulation of hsa\_circ\_0007534 repressed the proliferation and invasion of cervical cancer cells. (A) Expression levels of hsa\_circ\_0007534 in cervical cancer tissues and adjacent normal tissues were examined by RT-qPCR. \*\*\*\* $p < 0.0001$ . (B) Relative expression of hsa\_circ\_0007534 in cervical cancer cell lines (HeLa, SiHa and CaSki) and the normal cervical epithelial cell line, H8, was evaluated by RT-qPCR, \* $p < 0.05$  vs. H8. (C) The knockdown efficiency of hsa\_circ\_0007534 shRNA was determined by RT-qPCR. HeLa and SiHa cells were transfected with either a pLKO.1-hsa\_circ\_0007534 shRNA expression vector or a pLKO.1-scrambled shRNA expression vector for 48 h. (D) Effects of hsa\_circ\_0007534 inhibition on cell proliferation was evaluated using a CCK-8 assay. (E, F) Effects of hsa\_circ\_0007534 inhibition on the colony-forming capacity of cervical cancer cells was assessed using a colony formation assay. (G, H) Effects of hsa\_circ\_0007534 inhibition on the invasive potential of cervical cancer cells was examined using a Transwell invasion assay, \* $p < 0.05$ .

accumulated that suggest circRNAs play key roles in various pathological processes [9,10]. Particularly, the role of circRNAs in cancers has been widely studied. Numerous circRNAs are dysregulated in cancer tissues, suggesting that the molecules may be useful as diagnostic and prognostic biomarkers as well as therapeutic targets [11,12]. Notably, recent studies have reported that multiple circRNAs are involved in regulating cell proliferation and invasion in cervical cancer, functioning as oncogenes or tumor suppressors [13,14]. Therefore, these circRNAs may potentially serve as therapeutic targets for cervical cancer.

miRNAs are small noncoding RNAs ~ 22 nucleotides in length, which suppress gene expression by binding to the 3'-untranslated regions (3'-UTRs) of target mRNAs. This set of interactions guide the formation of small RNA-induced silencing complexes resulting in either mRNA degradation or translational inhibition [15]. Through the regulation of the expression of target genes, miRNAs are involved in regulating various cellular processes, including proliferation, invasion, apoptosis, and differentiation [16]. The discovery of the contribution of miRNAs to the process of tumor progression has provided novel insights



**Fig. 2.** Hsa\_circ\_0007534 interacts with miR-498. (A) A graphical representation of the miR-498 binding site within hsa\_circ\_0007534. (B) A dual-luciferase reporter assay was performed to detect the interaction between miR-498 and hsa\_circ\_0007534 using 293T cells,  $*p < 0.05$ . (C) An RNA pull-down assay was performed using biotinylated miR-498 mimics. Biotinylated miR-498 mimics were transfected into HeLa and SiHa cells, and hsa\_circ\_0007534 expression in precipitates was measured using RT-qPCR,  $*p < 0.05$ . (D) The effect of hsa\_circ\_0007534 knockdown on miR-498 expression was measured using RT-qPCR,  $*p < 0.05$ . (E) Expression levels of miR-498 in cervical cancer tissues were assessed by RT-qPCR,  $****p < 0.0001$  vs. normal. (F) The correlation between hsa\_circ\_0007534 and miR-498 was evaluated by Spearman's rank correlation analysis ( $r = -0.7161$ ,  $p < 0.0001$ ).

into understanding cervical cancer pathogenesis [17,18]. miR-498 has been suggested as a cancer-related miRNA in diverse set of cancers, functioning as either oncogenic or tumor suppressive miRNA [19–21]. However, the precise role that the miRNA plays in the progression of cervical cancer remains unclear.

Recent studies have reported that hsa\_circ\_0007534 is a novel cancer-associated circRNA that regulates the progression of numerous cancers, including colorectal cancer, glioma, non-small cell lung cancer and breast cancer [22–25]. To date, little is known about the role of hsa\_circ\_0007534 in cervical cancer. The present study aimed to investigate the expression, function, and regulatory mechanism of hsa\_circ\_0007534 in cervical cancer.

## 2. Materials and methods

### 2.1. Tissue sample collection

Forty-five pairs of cervical cancer tissues and adjacent, normal tissues were obtained from Shaanxi Provincial People's Hospital with approval from the Ethics Committee of Shaanxi Provincial People's Hospital. Written informed consents were acquired from all enrolled patients. Tissues were collected during tumor resection and stored in liquid nitrogen at  $-80^{\circ}\text{C}$ . The experimental procedures were carried out following the guidelines and principles of the Declaration of Helsinki.

### 2.2. Cell culture

Human cervical cancer cell lines, including HeLa, SiHa and CaSki, and human cervical epithelial immortalized cell line H8, were purchased from BeNa Culture Collection (BNCC, Kunshan, China). HeLa and CaSki cells were cultured in RPMI 1640 Medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (FBS). SiHa cells were cultured in DMEM (Gibco) media

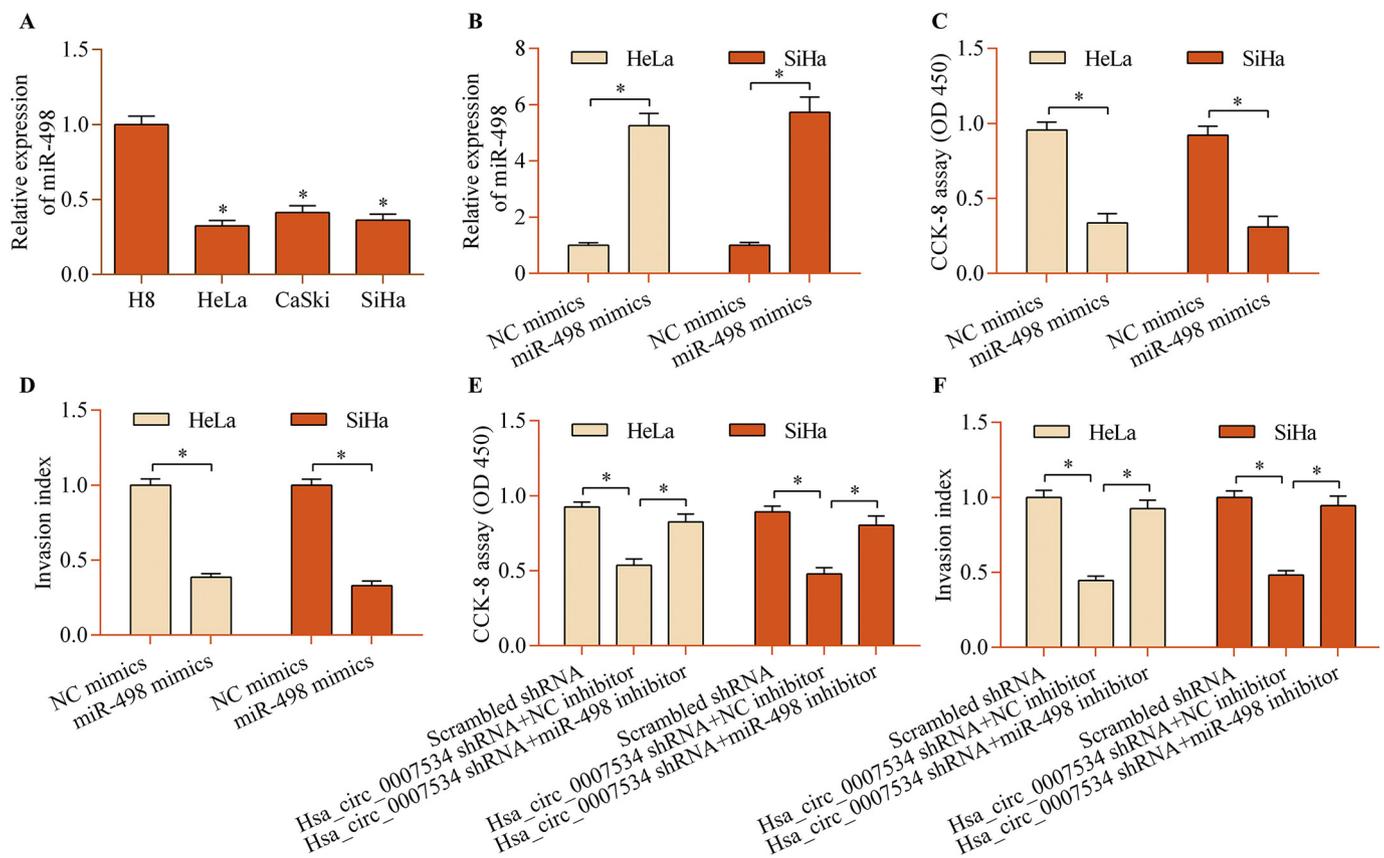
containing 10% FBS. The 293T cells were kindly provided by the Stem Cell Bank, Chinese Academy of Sciences (Shanghai, China) and grown in DMEM (Gibco) medium supplemented with 10% FBS.

### 2.3. Cell transfection

The shRNA sequences targeting hsa\_circ\_0007534 (5'-CACCGATCA TTCAGAGCTATTTGACGAATCAAAAUGCTCTGAATGATC-3') were inserted into a pLKO.1 vector. The miR-498 mimics, inhibitor, and negative controls (NC) were purchased from RiboBio (Guangzhou, China). The cDNA sequences of the BMI-1 open reading frame were subcloned into the pcDNA3.1 vector. Cell transfection was performed using Lipofectamine3000 Transfection Reagent (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions.

### 2.4. Real-time quantitative polymerase chain reaction (RT-qPCR) analysis

Total RNAs were extracted using TRIzol RNA Isolation Reagent (Invitrogen) according to standard protocols. To detect hsa\_circ\_0007534, cDNA was synthesized using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) and amplified using PowerUp SYBR Green Master Mix (Applied Biosystems). GAPDH was used as an internal reference for normalizing gene expression. To detect miR-498 expression, cDNA was generated using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) and amplified using TaqMan Fast Advanced Master Mix (Applied Biosystems). U6 was used as an internal control for normalizing miR-498 expression. The primer sequences used in this study were as follows: hsa\_circ\_0007534, forward: 5'-GTGACGGAAAT CCAATTGACC-3' and reverse: 5'-ATGGAATTGCTGGCGAGTTG-3'; GAPDH, forward: 5'-GAAGGTGAAGGTCGGAGTC-3' and reverse: 5'-GAAGATGGTGTGGATTTC-3'; miR-498, forward: 5'-GGTTTGAA GCCAGGCGTTTC-3' and reverse: 5'-CAGTGCAGGGTCCGAGGTAT-3'; U6, forward: 5'-CTCGCTTCGGCAGCAC-3' and reverse: 5'-AACGCTT



**Fig. 3.** miR-498 suppressed the proliferation and invasion of cervical cancer cells. (A) Relative expression of miR-498 in cervical cancer cell lines was measured by RT-qPCR,  $*p < 0.05$  vs. H8. (B) HeLa and SiHa cells were transfected with either miR-498 mimics or NC mimics for 48 h, and relative miR-498 expression was determined using RT-qPCR. (C) The effect of miR-498 overexpression on cell proliferation was assessed using the CCK-8 assay. (D) The effect of miR-498 overexpression on cell invasion was evaluated using a Transwell invasion assay. HeLa and SiHa cells were co-transfected with a miR-498 inhibitor and hsa\_circ\_0007534 shRNA for 48 h, (E) cell proliferation was monitored by CCK-8 assay, and (F) cell invasion was detected by Transwell invasion assay,  $*p < 0.05$ .

CACGAATTTGCGT-3'.

## 2.5. Cell counting kit-8 (CCK8) assay

Cells were seeded into a 96-well plate at a density of  $5 \times 10^3$  cells/well. After culturing overnight, cells were transfected with indicated vectors or miRNAs and incubated for 48 h. Then, 10  $\mu$ l of CCK-8 solution (Dojindo Laboratories, Shanghai, China) was added to each well and incubated for 1 h at 37 °C. Absorbance values of solutions at a wavelength of 450 nm were measured using a Microplate Reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

## 2.6. Colony formation assay

Colony-forming ability was evaluated using a 6-well tissue culture plates precoated with 0.7% agarose. Transfected cells were resuspended into media containing 0.3% agarose and seeded into the 6-well plates at a density of 500 cells/well. Cells were grown for 10 d at 37 °C. Thereafter, cells were fixed with methanol for 30 min and stained with 0.1% crystal violet. The colonies was observed and counted using a microscope.

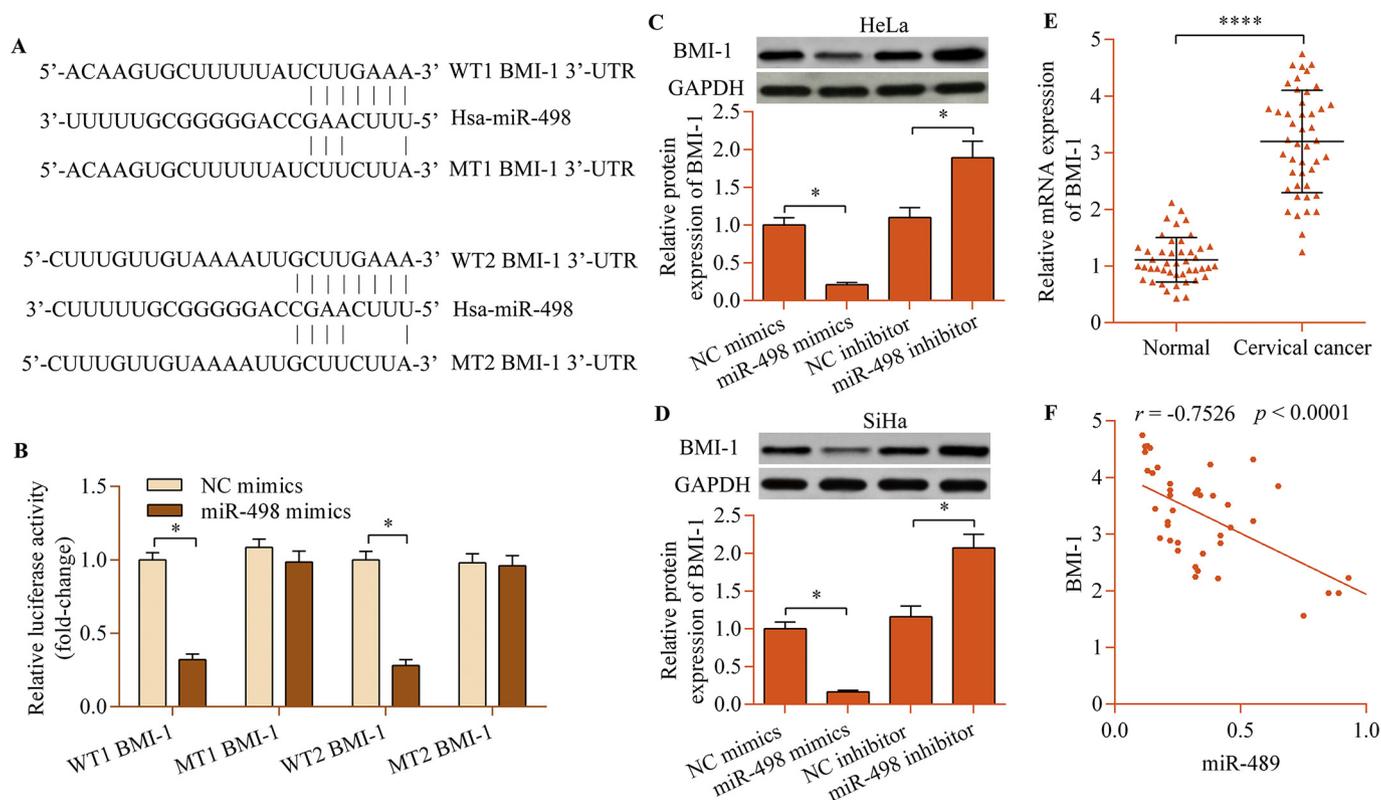
## 2.7. Cell invasion assays

Cell invasive potential was assessed using BD BioCoat Matrigel Invasion Chambers (BD Biosciences, San Jose, CA, USA) according to the manufacturer's protocols. Cells were suspended into serum-free medium and placed in upper chambers. The bottom chambers were filled with normal medium containing 20% FBS. After incubation for

24 h at 37 °C, residual cells on the upper surface of the membrane were removed with a cotton swab, while cells that had traversed through the membrane were fixed with methanol and stained with 0.1% crystal violet. Cells were counted using a microscope and invasion index values for each cell type were calculated.

## 2.8. Dual-luciferase reporter assay

The cDNA fragments of hsa\_circ\_0007534 or the BMI-1 3'-UTR that possessed a miR-498 binding site were inserted into the luciferase-reporter vector, pmirGLO, (Promega, Madison, WI, USA) downstream of the firefly luciferase gene. Renilla luciferase served as a reporter that controlled for differences in transfection efficiency. The constructed hsa\_circ\_0007534 or BMI-1 3'-UTR reporter vectors were cotransfected with miR-498 mimics or NC mimics into 293 T cells and incubated for 48 h. Thereafter, cells were harvested and relative luciferase activity was evaluated using the Dual-Luciferase Reporter Assay System (Promega) according to manufacturer protocols. Briefly, cells were collected, washed with PBS, and lysed using the provided lysis buffer. Lysate samples were cleared by centrifugation in a refrigerated micro-centrifuge and transferred to a fresh tube prior to reporter enzyme analyses. A total of 20  $\mu$ l of lysates were mixed with 100  $\mu$ l of Luciferase Assay Reagent II in a tube, which was then placed in the luminometer for measuring firefly luciferase activity. Then, 100  $\mu$ l of Stop & Glo Reagent was added to each of the tubes and the luminometer was used to measure Renilla luciferase activity. Relative luciferase activity was calculated by normalizing firefly luciferase activity with Renilla luciferase activity.



**Fig. 4.** miR-498 negatively regulates BMI-1 expression. (A) A graphical representation of the miR-498 binding site within BMI-1 3'-UTR. (B) The interaction between miR-498 and BMI-1 3'-UTR was detected by a dual-luciferase reporter assay using 293 T cells. HeLa and SiHa cells were transfected with miR-498 mimics or inhibitors for 48 h, and (C, D) protein expression of BMI-1 was determined by Western blot analysis.  $*p < 0.05$ . (E) BMI-1 mRNA expression levels in cervical cancer tissues were examined by RT-qPCR.  $****p < 0.0001$  vs. normal. (F) Correlation between BMI-1 mRNA expression and miR-498 expression was evaluated by Spearman's rank correlation analysis ( $r = -0.7526$ ,  $p < 0.0001$ ).

## 2.9. RNA pull-down assay

miR-498 and NC mimics were biotinylated using Biotin RNA Labeling Mix (Roche Diagnostics, Indianapolis, IN, USA) as per the manufacturer's instructions. Cells were transfected with biotinylated miR-498 or NC mimics for 48 h. Then, cells were harvested, lysed, and incubated with streptavidin-coated magnetic beads at 4 °C for 3 h. Bound RNA molecules were extracted using TRIzol RNA Isolation Reagents (Invitrogen).

## 2.10. Western blot analysis

Total proteins from cultured cells were extracted using Radio Immunoprecipitation Assay Lysis Buffer (Beyotime Biotechnology, Shanghai, China) with a protease inhibitor. The protein concentration of each sample was determined using an Enhanced BCA Protein Assay Kit (Beyotime Biotechnology). Equivalent amounts of proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane (Millipore, Billerica, MA, USA). The membrane was blocked with 5% non-fat milk for 1 h at room temperature and then incubated with primary antibodies against BMI-1 and GAPDH (Abcam, Cambridge, UK). After incubation at 4 °C overnight, the membrane was probed with HRP-conjugated secondary antibodies at 37 °C for 1 h. The immunoreactive bands were developed using Pierce ECL Plus Western Blotting Substrate.

## 2.11. Statistical analysis

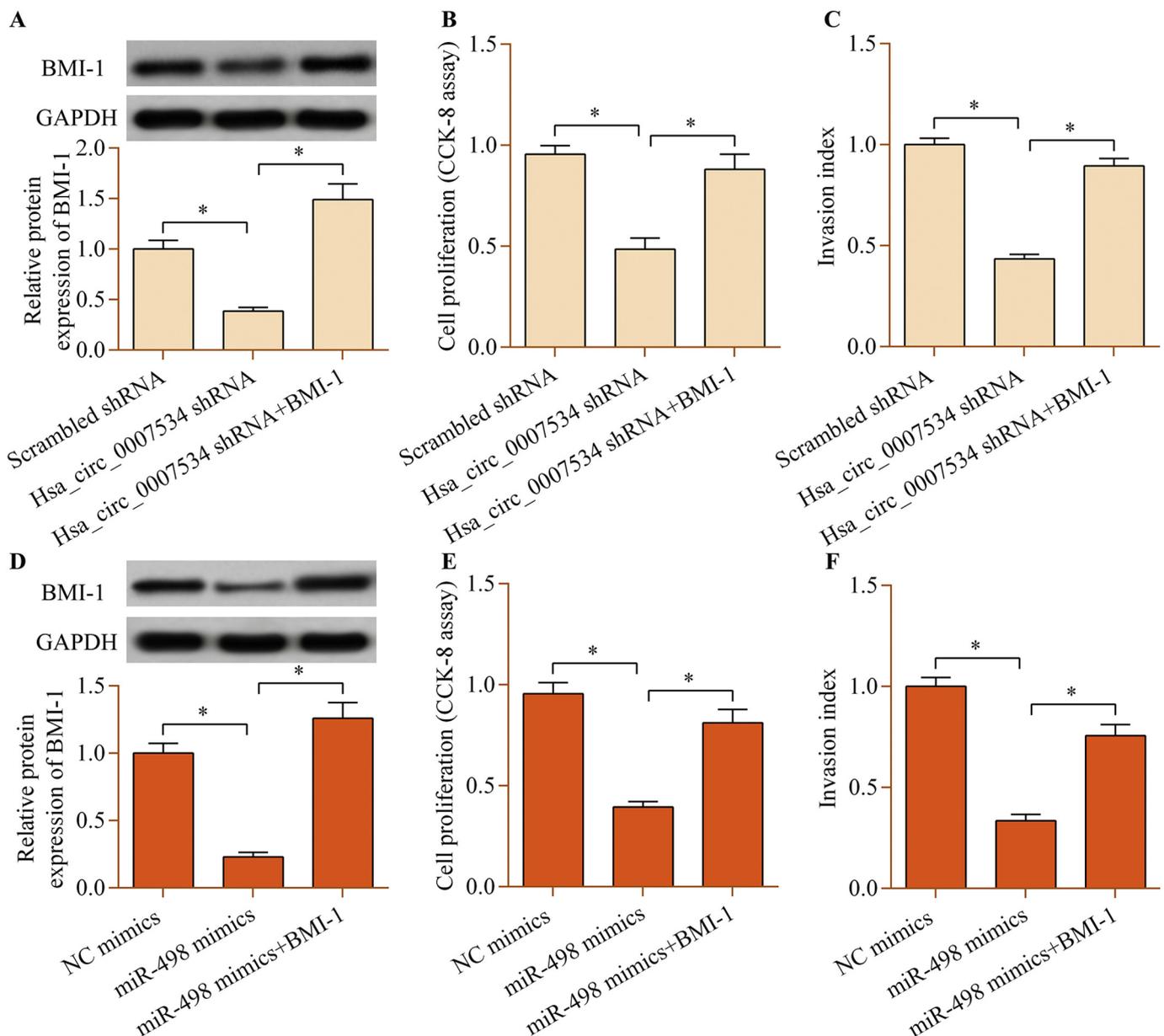
Data were expressed as mean  $\pm$  standard deviation. Statistical analyses were carried out using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS version 19.0 software (IBM, Armonk,

NY, USA). Differences were determined using Student's *t*-test and one-way analysis of variance. Correlation analysis was performed using Spearman's rank correlation coefficient. Differences in which  $p < 0.05$  were regarded as statistically significant.

## 3. Results

### 3.1. Inhibition of hsa\_circ\_0007534 impeded the proliferation and invasion of cervical cancer cells

To investigate whether expression of hsa\_circ\_0007534 is dysregulated in cervical cancer, we performed RT-qPCR analysis to quantify the expression of hsa\_circ\_0007534 in cervical cancer tissues and cell lines. The results showed that hsa\_circ\_0007534 expression levels were significantly upregulated in cervical cancer tissues compared to adjacent, normal tissues (Fig. 1A). Moreover, the expression of hsa\_circ\_0007534 was significantly higher in cervical cancer cell lines compared to the normal cervical epithelial cell line H8 (Fig. 1B). To investigate the biological function of hsa\_circ\_0007534 in cervical cancer, we performed loss-of-function experiments using siRNA-mediated gene silencing of hsa\_circ\_0007534. RT-qPCR revealed that transfection of hsa\_circ\_0007534 shRNA significantly depleted the expression of hsa\_circ\_0007534 in cervical cancer cells (Fig. 1C). Functional assays demonstrated that depletion of hsa\_circ\_0007534 significantly repressed the proliferation (Fig. 1D) and colony-forming capability (Fig. 1E and F) of cervical cancer cells. In addition, inhibition of hsa\_circ\_0007534 expression resulted in a significant reduction in the invasive potential of cervical cancer cells (Fig. 1G and H). Overall, these results suggest that hsa\_circ\_0007534 expression is upregulated in cervical cancer, and its inhibition restricts the capacity of cervical cancer cells to proliferate and invade neighboring tissues.

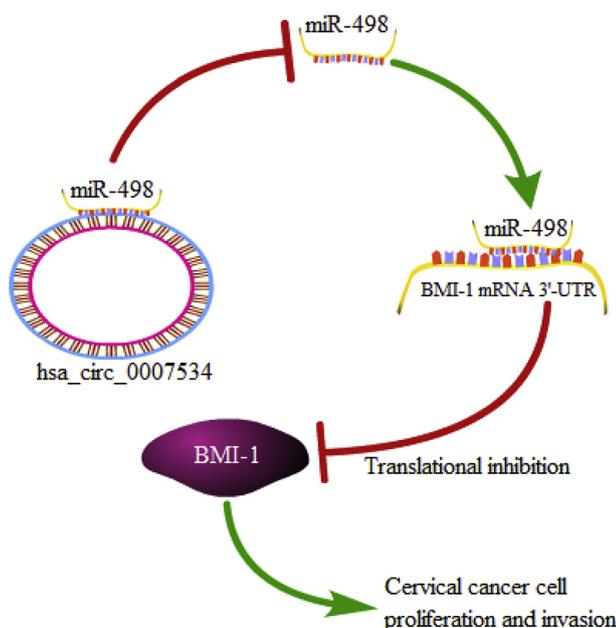


**Fig. 5.** The hsa\_circ\_0007534/miR-498 axis regulates cervical cancer cell proliferation and invasion through modulating levels of BMI-1. HeLa cells were co-transfected with either an hsa\_circ\_0007534 shRNA expression vector or a BMI-1 expression vector for 48 h, and (A) protein expression of BMI-1 was evaluated via Western blot. (B) The effect of BMI-1 overexpression on hsa\_circ\_0007534 knockdown-mediated cell proliferation was measured by CCK-8 assay. (C) The effect of BMI-1 overexpression on hsa\_circ\_0007534 knockdown-mediated cell invasion was evaluated using a Transwell invasion assay. HeLa cells were co-transfected with miR-498 mimics and a BMI-1 expression vector for 48 h, and (D) protein expression of BMI-1 was determined by Western blot. (E) Effects of BMI-1 overexpression on miR-498 overexpression-mediated cell proliferation was measured using a CCK-8 assay. (F) The effect of BMI-1 overexpression on miR-498 overexpression-mediated cell invasion was assessed using a Transwell invasion assay, \* $p < 0.05$ .

### 3.2. Hsa\_circ\_0007534 interacts with miR-498 and regulates miR-498 expression in cervical cancer cells

To uncover the underlying mechanism by which hsa\_circ\_0007534 modulates cervical cancer cell proliferation and invasion, we performed bioinformatics analyses to predict the potential miRNA target of hsa\_circ\_0007534 using the both starBase (<http://starbase.sysu.edu.cn/>) and circinteractome (<https://circinteractome.nia.nih.gov>) online databases. Analyses using both databases predicted that miR-498, a tumor suppressive miRNA in cervical cancer [26], was predicted to be a target of hsa\_circ\_0007534 (Fig. 2A). To verify whether hsa\_circ\_0007534 directly interacts with miR-498, we performed a dual-luciferase reporter assay. The results of the assay showed that overexpression of miR-498 significantly reduced the luciferase activity of the wild-type (WT)

hsa\_circ\_0007534 reporter vector (Fig. 2B). However, when the predicted binding site for MIR-498 was mutated, the molecule had no obvious effect on the luciferase activity of the mutant type (MT) hsa\_circ\_0007534 reporter vector (Fig. 2B). Moreover, we performed an RNA pull-down assays using biotinylated miR-498 mimics. The results of this assay revealed that levels of hsa\_circ\_0007534 were significantly higher in the precipitates of biotinylated miR-498 mimics compared with the precipitates of biotinylated NC mimics (Fig. 2C). Notably, knockdown of hsa\_circ\_0007534 significantly upregulated miR-498 expression in cervical cancer cells (Fig. 2D). In addition, we found that miR-498 expression was markedly decreased in cervical cancer tissues and had an inverse correlation with hsa\_circ\_0007534 expression (Fig. 2E and F). Taken together, these results suggest that hsa\_circ\_0007534 may interact with miR-498 in cervical cancer cells.



**Fig. 6.** A diagrammatic model of the hsa\_circ\_0007534/miR-498/BMI-1 axis for the regulation of cervical cancer cell proliferation and invasion. Hsa\_circ\_0007534 competitively binds to miR-498 and disrupts the interaction between miR-498 and BMI-1, leading to upregulation of BMI-1 expression, which contributes to the increased proliferation and invasion of cervical cancer.

### 3.3. Overexpression of miR-498 suppressed the proliferation and invasion of cervical cancer cells

To elucidate whether miR-498 exerts antitumor effects on cervical cancer cells, we detected the effects of miR-498 overexpression on the proliferative and invasive capacity of cervical cancer cells. We found that miR-498 expression was significantly decreased in cervical cancer cell lines (Fig. 3A). Transfecting miR-498 mimics resulted in a significant increase in miR-498 expression in cervical cancer cells (Fig. 3B). Functional experiments demonstrated that miR-498 overexpression significantly restricted the proliferation cervical cancer cells and limited their ability to invade (Fig. 3C and D). Notably, miR-498 inhibition significantly reversed the hsa\_circ\_0007534 knockdown-mediated phenotype, which produced inhibitory effects on cervical cancer cell proliferation and invasion (Fig. 3E and F). These data suggest that miR-498 exerts antitumor effects, at least with respect to cervical cancer cells.

### 3.4. BMI-1 was identified as a gene target of miR-498

To further elaborate on the mechanism by which the hsa\_circ\_0007534/miR-498 axis regulates cervical cancer cell proliferation and invasion, we next aimed to determine the identity of miR-498 gene targets. Among targets predicted using bioinformatic analyses, we were particularly interested in one gene that was identified, BMI-1, which had already been designated as an oncogene in cervical cancer (Fig. 4A). A luciferase reporter assay confirmed that miR-498 directly binds to the 3'-UTR of BMI-1 (Fig. 4B). Moreover, overexpression of miR-498 decreased BMI-1 expression, while miR-498 inhibition increased BMI-1 expression (Fig. 4C and D). Additionally, we were able to demonstrate that BMI-1 mRNA expression was significantly upregulated in cervical cancer tissues and was negatively correlated with miR-498 expression (Fig. 4E and F). Overall, these results confirm that BMI-1 is a target gene of miR-498.

### 3.5. BMI-1 overexpression reversed both hsa\_circ\_0007534 inhibition- and the miR-498 overexpression-mediated antitumor effects

To confirm whether BMI-1 affects cervical cancer cell proliferation and invasion via the hsa\_circ\_0007534/miR-498-mediated regulatory axis, we performed rescue experiments. BMI-1 protein expression was significantly decreased by expression of hsa\_circ\_0007534 shRNA, an effect which was significantly restored by transfecting cells with a BMI-1 expression vector (Fig. 5A). Restoration of BMI-1 levels partially reversed hsa\_circ\_0007534 knockdown-mediated inhibitory effects on cervical cancer cell proliferation and invasion (Fig. 5B and C). In addition, restoration of BMI-1 also reversed miR-498 overexpression-mediated antitumor effects observed in cervical cancer cells (Fig. 5D, E and F). These results suggest that BMI-1 is a functional target of the hsa\_circ\_0007534/miR-498 axis and that BMI-1 has a role in the regulation of cell proliferation and invasion in cervical cancer.

## 4. Discussion

Aberrant circRNA expression has been detected in cervical cancer [27–30]. In the present study, we identified hsa\_circ\_0007534 as a novel circRNA that was dysregulated in cervical cancer. Here, we found that hsa\_circ\_0007534 was highly upregulated in cervical cancer tissues and the inhibition of hsa\_circ\_0007534 expression repressed the proliferation and invasion of cervical cancer cells, suggesting an oncogenic role for hsa\_circ\_0007534. Our results further revealed that hsa\_circ\_0007534 inhibition reduced the expression of BMI-1 by upregulating the expression miR-498, suggesting an important role for the hsa\_circ\_0007534/miR-498/BMI-1 axis in cervical cancer progression.

The role of hsa\_circ\_0007534 in tumorigenesis has been studied in multiple cancers. High expression levels of hsa\_circ\_0007534 have been detected in colorectal cancer tissues, and have been correlated with tumor stage and lymph node metastasis [22]. Knockdown of hsa\_circ\_0007534 inhibits tumor growth in vitro and in vivo [22,31]. Overexpression of hsa\_circ\_0007534 promotes the proliferation, migration, and invasion of non-small cell lung cancer cells [24]. Moreover, the oncogenic role of hsa\_circ\_0007534 has also been reported in glioma, pancreatic cancers, and breast cancers [23,25,32]. Consistent with these findings, our results demonstrated that levels of hsa\_circ\_0007534 transcripts were increased in cervical cancer and contributed to the regulation of cervical cancer cell proliferation and invasion. Taken together, these data support the hypothesis that hsa\_circ\_0007534 has an oncogenic function in cervical cancer cells. Notably, hsa\_circ\_0007534 has been suggested to be a miRNA sponge that regulates various miRNAs, including miR-761, miR-593, miR-625, and miR-892b [23,25,32]. Herein, we identified miR-498 as a novel target of hsa\_circ\_0007534.

miR-498 plays an important role in various cancers. Low levels of miR-498 expression in ovarian cancer tissues are correlated with high tumor grade, lymph node metastases, and shorter overall and progression-free survival [33]. Overexpression of miR-498 inhibited the proliferation of ovarian cancer cells and was associated with the suppression of telomerase and Forkhead box O3 [21,34], suggesting a tumor-suppressive function for miR-498. Consistent with these findings, tumor-suppressive function of miR-498 has been reported in studies investigating in multiple cancer types, including colorectal, esophageal, gastric, and lung cancers [20,35–38]. Interestingly, miR-498 has been suggested to be one of the anti-cancer miRNAs induced by calcitriol in cervical cancer cells [26]. However, the precise role of miR-498 in cervical cancer remains unclear. In this study, our results demonstrated that miR-498 overexpression inhibited the proliferation and invasion of cervical cancer cells, supporting previous reports that suggested an antitumor function for miR-498 in cervical cancer. Moreover, our study revealed that the level of miR-498 expression was decreased in cervical cancer. Therefore, our study supports the hypothesis that miR-498 has tumor suppressive function in cervical cancer. However, an oncogenic

function for miR-498 has been reported in other types of cancer. It has been reported that miR-498 expression is upregulated in triple negative breast cancer and inhibition of miR-498 leads to reductions in the proliferation of triple negative breast cancer cells [19]. Therefore, the expression status and function of miR-498 may be critically dependent on both the type of cancer and cell-type involved.

Questions remain concerning the dysregulation of miR-498 in cervical cancer tissues. Interestingly, a recent study has reported that the expression of miR-498 is regulated by circRNAs, such as hsa\_circRNA\_100833 [39]. Herein, we identified miR-498 as a target miRNA of hsa\_circ\_0007534. We found that hsa\_circ\_0007534 expression was upregulated in cervical cancer, which was inversely correlated with miR-498 expression. Therefore, high levels of hsa\_circ\_0007534 expression may contribute to the decreased level of miR-498 expression observed in cervical cancer, indicating an important role for the hsa\_circ\_0007534/miR-498 axis in cervical cancer progression.

BMI-1 has emerged as an oncogene in cervical cancer. BMI-1 is highly expressed in cervical cancer and serves as a potential diagnostic and prognostic biomarker of the disease [40–42]. Knockdown of BMI-1 inhibits proliferation and invasion in cervical cancer cells [43,44]. In this study, we identified BMI-1 as a target gene of miR-498, which is consistent with the findings of a recent study [45]. Our study demonstrated that miR-498 repressed the proliferation and invasion of cervical cancer cells by targeting and inhibiting expression of BMI-1, highlighting the importance of miR-498/BMI-1 axis cervical cancer. Considering that hsa\_circ\_0007534 is highly expressed in cervical cancer, it may contribute to upregulation of BMI-1 expression by sponging miR-498, thus leading to the malignant proliferation and invasion of cervical cancer. Taken together, results of our study provide convincing evidence that the hsa\_circ\_0007534/miR-498/BMI-1 axis represents a novel mechanism for the regulation of cervical cancer progression.

## 5. Conclusions

In conclusion, our results demonstrate that knockdown of hsa\_circ\_0007534 downregulates the proliferation and invasion of cervical cancer through the inhibition of BMI-1 via miR-498 upregulation (Fig. 6). Our study highlights the importance of the hsa\_circ\_0007534/miR-498/BMI-1 axis in cervical cancer, providing novel insights into understanding the interaction of the circRNA-miRNA-mRNA network in the progression of cervical cancer. Our study suggests that hsa\_circ\_0007534 and miR-498 may serve as potential therapeutic targets for cervical cancer.

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## CRediT authorship contribution statement

**Xuan Rong:** Investigation, Writing - original draft. **Wei Gao:** Investigation, Writing - original draft. **Xiaomei Yang:** Methodology. **Junjun Guo:** Conceptualization, Writing - review & editing.

## Declaration of competing interest

The authors declare that they have no conflict of interest.

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