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Prognostic value of circ_0000043/miR-590-5p in cervical cancer and regulation of tumor progression

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Received: 18 December 2024 / Accepted: 12 May 2025
Published online: 23 May 2025

Abstract

Objective To investigate the prognostic value of circ_0000043 in cervical cancer (CC) and its involvement in the modulation of patients' prognostic progression.

Methods Clinical data of 136 CC patients were included, postoperative CC and adjacent normal tissues were stored at −80 °C, and patients were followed up for 5 years for prognosis. RT-qPCR detected circ_ 0000043 and miR-590-5p expression, Kaplan–Meier curves recorded for prognostic survival, multivariate cox analysis of patients' prognostic risk factors. Moreover, CCK8 assessed cell proliferation, Transwell detected migration and invasion. Dual luciferase reporter assay and RIP examined the interactions between circ_ 0000043 and miR-590-5p.

Results circ_ 0000043 was down-regulated in CC patients, and patients with low expression of circ_ 0000043 had worse survival. Low expression of circ_0000043, lymph node metastasis, and FIGO were all unfavorable factors threatening the prognosis of CC. circ_0000043 overexpression markedly inhibited the proliferation, migration, and invasion of cancer cells. miR-590-5p is a direct target miRNA of circ_0000043. miR-590-5p mimic prominently resisted the altered cellular functions induced by circ_0000043.

Conclusion Patients with low circ_0000043 expression have poor prognosis as it fuels CC progression by boosting cancer cell proliferation, migration, and invasion via miR-590-5p. Our study provides new ideas for the prognosis and treatment of CC.

Keywords Circ_ 00000431 · Cervical cancer · MiR-590-5p · Prognosis

1 Introduction

Cervical cancer (CC) is a major threat to women's health worldwide, and it is a common malignancy in gynecological care [1, 2]. Currently, although the treatment of CC has been accelerated in our country [3], for example, free vaccination and cervical screening for women of the right age [4], the female population of deaths due to CC is still

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Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12672-025-02670-5.

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increasing every year [5, 6]. As screening has become more widespread it has been found that the CC population is getting younger [7, 8], and although hysterectomy is a safe and reliable method for young patients with early-stage cervical cancer [9, 10], the high cost and uncertainty about the prognosis of the patients after the procedure remain problematic. Earlier studies have shown that some cervical cancer cells are severely infiltrative, which may be an important reason for the poor prognosis of patients [11].

Circular RNA (circRNAs) is a competing endogenous non-coding RNA [12], which does not encode proteins but is rich in miRNA-binding sites, forming a unique closed-loop structure that exists stably in cells [13]. In recent years, more and more studies have focused on the role of circRNAs in the pathogenesis of cancer [14, 15], especially CC [16, 17]. It has been shown that dysregulated circRNAs expression on the one hand causes proliferation of cervical epithelial cells [18], leading to carcinogenesis [19]; on the other hand, it can target miRNAs cooperating with it to participate in the progression of CC [20, 21]. For example, circ_YPEL2 expression was found to be involved in cervical cancer progression [22], and circ_0000228 was able to bind miR-195-5p to promote the progression of cervical cancer [23], and all of these studies can prove that circRNAs play a very important function in the disease progression of cervical cancer. In the pre-experimental stage, we found that circ_0000043 was down-regulated in GSE113696 (Cervical Cancer Database) and aberrantly expressed in other diseases by microarray analysis [24, 25]. In addition, retrospective analysis found that miR-590-5p was up-regulated in CC patients [26], which can combine with other genes to regulate endocervical inflammation [27]. circ_0000043 and miR-590-5p are both involved in the pathogenesis of CC as a kind of non-coding RNA, and it is expected to be a potential marker for predicting CC progression. However, we are not fully aware of the mechanism of action by which circ_0000043 regulation predicts CC progression.

Based on the above background, the present study collected clinical data from 136 patients undergoing cervical cancer surgery, and further explored the involvement of circ_0000043 in assessing the predictive value of CC by transfection of mimics overexpressing circ_0000043 and the use of miR-590-5p inhibitor, to reveal the mechanism of action of circ_0000043/miR-590-5p in regulating CC.

2 Materials and methods

2.1 Source of data

Differentially expressed circRNAs involved in CC progression (circ_0000043): retrieved from the Gene Expression Omnibus (GEO). In addition, the original dataset of cervical cancer (GSE113696) was also extracted, including human normal cervical epithelial cells HcerEpic (GSM3112199) and cervical cancer cell lines HeLa (GSM3112200), CaSki (GSM3112201), SiHa (GSM3112202), C-33 A (GSM3112203), and SW756 (GSM3112204). The data were normalized by the R package and default parameters, where differentially expressed genes (DEGs) were used to output the results in logarithmic base, and DEGs corrected for $P < 0.05$ were selected for visualization.

2.2 Study population

This study included 136 CC patients who came to First People's Hospital of Linping District from May 2017 to February 2018. Intraoperative cervical cancer and adjacent normal tissues were preserved at −80 °C, and patients' lives were followed up for 5 years after surgery. The criteria for patient inclusion were [28, 29]: (1) adult female patients who were not in pregnancy; (2) having complete medical data and follow-up records; (3) having been diagnosed by two or more specialists from the department of pathology of the hospital; (4) patients who had not received radiotherapy or cervical surgery; (5) patients who suffered from a history of serious diseases (renal failure, stroke, cardiac infarction, etc.) or autoimmune diseases, patients with combination of other malignancies were excluded from the study. CC staging was classified according to the International Society of Gynecology and Obstetrics (FIGO) criteria [30].

The study was approved by the Ethics Committee of First People's Hospital of Linping District and participants signed a written informed consent.

2.3 Gene extraction and RT-qPCR

Total RNA from cancer tissues and cells was extracted by the Trizol (Sigma, U.S.). RNA concentration was tested using a NanoDrop Ultra Micro Nucleic Acid Analyzer. Reverse transcription and quantitative of circ_0000043 were carried out using circRNA RT-qPCR Kit (IVDSHOW, Zhangjiakou, China). miR-590-5p sample cDNA was synthesized by TaqMan™ MicroRNA Reverse Transcription Kit (Thermo, U.S.), and quantitative reaction was performed by miRNA qPCR Assay Kit (BioLabs, Beijing, China). Refer to the corresponding instructions for reaction conditions. Raw data were recorded by CFX Connect Real-Time Fluorescent Quantitative PCR System (Bio-Rad, U.S.). circRNA and miRNA data were normalized by using GAPDH and U6 as internal reference genes, respectively, and the statistical results were calculated by $2^{-\Delta\Delta Ct}$. Relevant sequence information is provided in Supplementary Table 1.

2.4 Cell culture and transfection

Human cervical immortalized squamous cells (Ect1/E6E7) and cervical cancer cells (MS751, Hela, CaSki, SiHa) were purchased from the American Cell Culture Collection (ATCC). Among them, Ect1/E6E7 were grown in high-saccharide DMEM (Gibco, U.S.) medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). MS751, Hela, SiHa were cultured in DMEM (Gibco, U.S.) complete medium, and CaSki were cultured in RPMI- 1640 (Gibco, U.S.) for growth. All cells were grown in an incubator at 37 °C with 5% CO_2. Logarithmic growth phase cells with more than 70% confluence were taken and inoculated in 6-well plates, and the cells were transfected for 2 days under suitable conditions using Lipofectamine™ 3000 (Lipo 3000, invitrogen, U.S.) in the amount of 5 µl transfection reagent per well.

2.5 Cell proliferation assay

Cell proliferation assay was performed by Cell Counting Kit-8 (CCK8, beyotime, Shanghai, China). CC cells [31] with different transfection treatments were inoculated in 96-well plates at a density of 2×10^3, and after the cells were attached to the wall, 10 µl of CCK8 solution was added proportionally and incubated in a cellular thermostat incubator for 2 h. The absorbance (OD) of the solution was detected by the Synergy LX multifunctional enzyme marker at a 450 nm filter, and the proliferative capacity of the cells was plotted according to the standard curve.

2.6 Transwell test for cell migration and invasion

Cells were starved using serum-free Opti-MEM (Gibco, U.S.) medium for 24 h prior to the experiment, and matrix gel was spread flat on the basement membrane of the upper chamber of the infiltrated transwell microtiter plates [32] (Becton Dickinson, U.S.) for invasion assay, whereas uncoated matrix gel was used for migration experiments. Cells were inoculated at a density of 1×10^4 in microtiter wells of the upper chamber [33], while the lower chamber was supplemented with DMEM complete medium containing 20% FBS, and incubate for 24 h. The cells were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet dye, the cell status was observed under a microscope (Nikon, Tokyo, Japan).

2.7 Dual luciferase reporter assay (DLR)

Wild-type (WT- circ_0000043) and mutant (MUT- circ_0000043) cervical cancer cell lines were first constructed by pGL3-Basic Vector. The target genes (mimic NC, miR-590-5p mimic, inhibitor NC, miR-590-5p inhibitor) were co-transfected into the cells by Lipo™ 3000 Transfection Reagent. After 24 h, the interactions between circ_0000043 and miR-590-5p were examined using Dual Luciferase Reporter Assay Kit (Vazyme, Nanjing, China) under light-avoidance conditions. The fluorescence intensity of the reporter genes after different treatments was observed by a fluorescence detector, and the experimental results were normalized by the luminescence intensity of sea kidney fluorescein.

2.8 RNA immunoprecipitation (RIP)

The interactions between circRNA and miRNA were verified using the RNA Binding Protein Immunoprecipitation (RIP) Kit (Sangon, Shanghai, China) according to the manufacturer's instructions. Later, reprecipitate the target RNA bound by the magnetic beads by Trizol reagent, and verify by RT-qPCR.

2.9 Data analysis

All statistical data of the experiment were processed by SPSS 23.0 and GraphPad 9.0. All samples had three biological replicates and two technical replicates, and statistical results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used for between-group comparisons, Kaplan–Meier curves recorded the prognostic survival rate of patients operated on for CC, and multivariate cox regression analyzed the factors affecting the prognosis of patients with CC. Pearson correlation analysis was performed to verify the relationship between the expression of circ_0000043 and miR-590-5p. $P < 0.05$ represents a statistically noticeable difference in the dataset results.

3 Results

3.1 Expression of circ_0000043 in cervical cancer patients

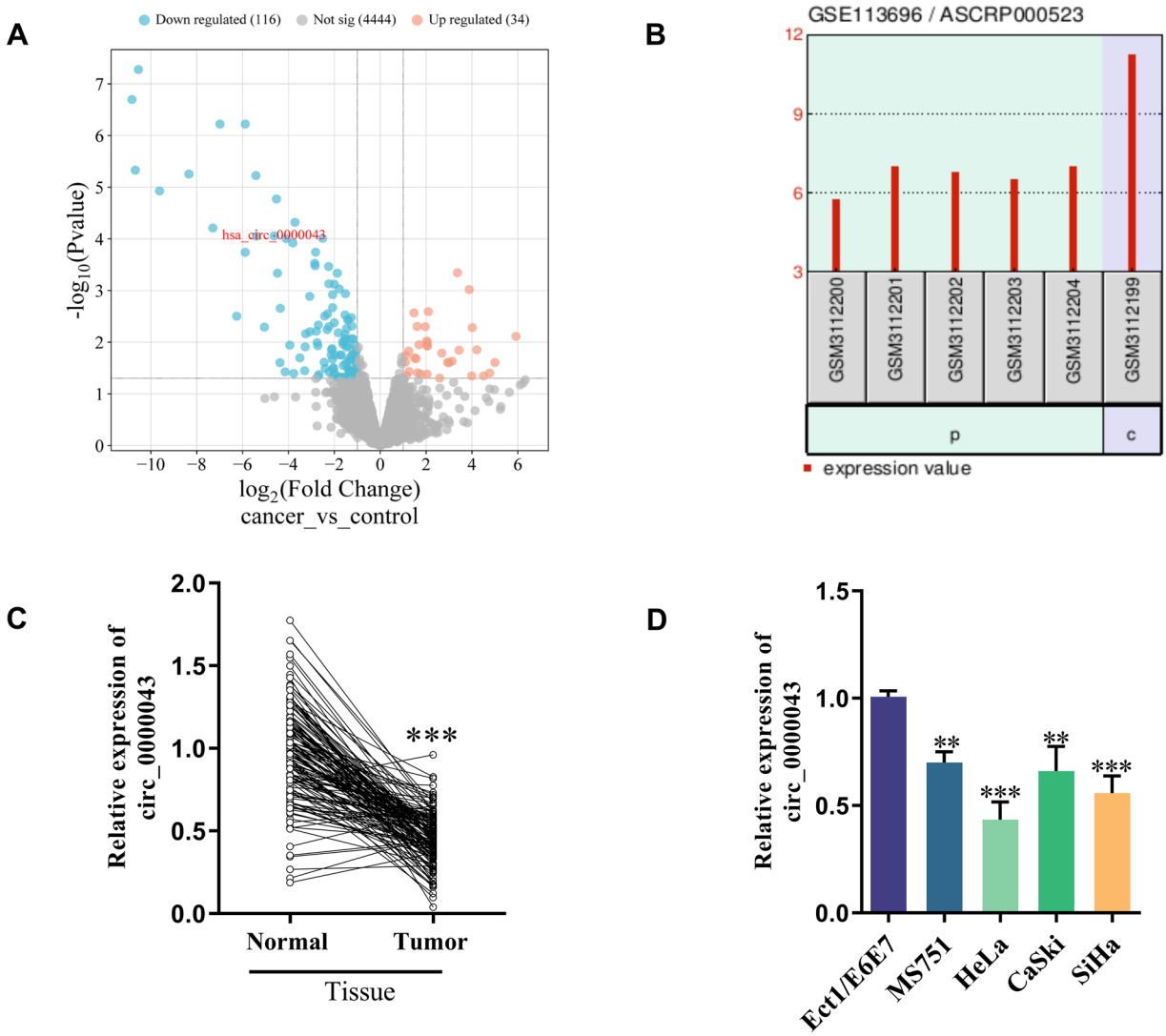
Enrichment analysis of differentially expressed genes (DEGs) revealed that circ_0000043 was down-regulated in CC patients (Fig. 1A). Database analysis showed that circ_0000043 (ASCRP000523) was obviously downregulated in each sub-data of the CC compared to controls (Fig. 1B). According to the results of the pathology department, postoperative excised cervical cancer tissues were used as the experimental group, and normal tissues adjacent to the cervical cancer tissues were used as the experimental control. The results of gene quantification showed that the expression level of circ_0000043 was persistently lower than that of normal tissues or cells in cervical cancer tissues (Fig. 1C) and cells (Fig. 1D, $P < 0.01$). The basic information of the patients showed that the average age of the patients was 37.26 ± 4.39, the size of the tumour was 4.39 ± 2.41, 43.4% of the patients had severe differentiation, 46.3% of the patients had lymph node metastasis, and 47.1% of the patients were in stage III, IV (Table 1). In addition, analysis of the clinical data of the two groups of patients revealed that CC patients with low expression of circ_0000043 had a higher rate of lymph node metastasis and worse FIGO stage ($P < 0.05$, Table 2). This result suggests that low expression of circ_0000043 may be an unfavorable factor for CC progression.

3.2 Lower expression of circ_0000043 affects the prognosis of CC patients

Follow-up of the prognosis of all CC patients at 5 years after surgery revealed that patients with lower expression of circ_0000043 had the potential to develop a fatal outcome after surgery (Fig. 2A). Kaplan–Meier curve results indicated that patients with down-regulated circ_0000043 had worse survival (Fig. 2B). Multivariate cox analysis revealed that circ_0000043 (HR: 0.277, 95% CI 0.122–0.628, $P < 0.01$), Lymph node metastasis (HR: 2.216, 95% CI 1.110–4.427, $P < 0.05$), and FIGO (HR: 3.968, 95% CI 1.479–10.644, $P < 0.01$) were all risk factors for CC patients towards death (Table 3). This result suggests that reduced circ_0000043 expression also has an impact on the prognosis of CC patients.

3.3 circ_0000043 affects the function of cervical cancer cells

Based on the above background, this study continued to investigate the effect of circ_0000043 on cervical cancer cells by transfection circ_0000043. Quantitative results showed that the expression of circ_0000043 was obviously increased in HeLa (Fig. 3A) and SiHa (Fig. 3B) cervical cancer cells after transfection with circ_0000043. CCK8 results suggested that overexpression of circ_0000043 notably inhibited the proliferation ability of the cancer cells ($P < 0.001$, Figs. 3C, D). Transwell results indicated that circ_0000043 also significantly prevented the migration ($P < 0.001$, Figs. 3E, F) and invasion



Cervical cancer differentially expressed genes (A), sub-database (B), tissue samples (C), and cells (D) show downregulation of circ_0000043.

Fig. 1 circ_0000043 expression in cervical cancer. **A** cervical cancer differentially expressed gene volcano map, **B** circ_0000043 expression levels in control and cervical cancer sub-datasets, **C** circ_0000043 quantitative results in cervical cancer tissues and adjacent normal tissues next to cancer, the quantitative results for circ_0000043 in cerebral cancer tissues were decreased compared to those in adjacent normal tissues. **D** circ_0000043 expression in normal cervical cells and cervical cancer cells, circ_0000043 expression in cervical cancer cells was downregulated compared to that in normal cervical cells. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ vs. Normal tissue or Ect1/E6E7

($P < 0.001$, Figs. 3G, H) of cancer cells. The above results indicate that circ_0000043 expression does affect cell function, and overexpression of circ_0000043 notably inhibited the abnormal proliferation and migration of cervical cancer cells.

3.4 miR-590-5p is a possible target gene of circ_0000043

The target site map of the predicted results showed the possibility of binding interactions between miR-590-5p and circ_0000043 (Fig. 4A). The results of luciferase reporter assay showed that the fluorescence activity of supernatant of WT- circ_0000043 cells after transfection with miR-590-5p mimic were substantially decreased ($P < 0.001$), whereas the wild-type cervical cancer cells were obviously enhanced ($P < 0.001$), but there was no such significant difference in fluorescence activity changes in MUT- circ_0000043 cells (Figs. 4B, C). In addition, RIP results showed that both circ_0000043 and miR-590-5p were distinctly enriched on the antibody to the target protein (Ago2, $P < 0.01$, Figs. 4D, E), and the expression of miR-590-5p was significantly elevated in CC tissues compared to normal tissues ($P < 0.01$, Fig. 4F). Correlation analysis revealed that circ_0000043 was notably negatively correlated with miR-590-5p expression in CC tissues (r = 0.7081,

Table 1 Baseline characteristics of cervical cancer patients

Parameters	Cases (n = 136)
Age (years)	37.26 ± 4.39
Tumor size (cm)	4.39 ± 2.41
Differentiation (n/%)	
Well, moderate	77/56.6
Poor	59/43.4
Lymph node metastasis (n/%)	
Yes	63/46.3
No	73/53.7
FIGO stage (n/%)	
I, II	72/52.9
III, IV	64/47.1

Data are presented as mean ± standard deviation or n/%

Table 2 Correlation of the has_circ_0000043expression with clinical characteristics in cervical cancer

Parameters	Cases (n = 136)	has_circ_0000043expression		*P*
		High (n = 65)	Low (n = 71)	
Age				0.880
≤ 40	95/69.9	45/69.2	50/70.4	
> 40	41/30.1	20/30.08	21/29.6	
Tumor size				0.061
≤ 4 cm	66/48.5	37/56.9	29/40.8	
> 4 cm	70/51.5	28/43.1	42/59.2	
Differentiation				0.146
Well, Moderate	77/56.6	41/63.1	36/50.7	
Poor	59/43.4	24/36.9	35/49.3	
Lymph node metastasis				**0.014**
Yes	63/46.3	23/35.4	40/56.3	
No	73/53.7	42/64.6	31/43.7	
FIGO stage				**0.009**
I, II	72/52.9	42/64.6	30/42.3	
III, IV	64/47.1	23/35.4	41/57.7	

Bold value indicates that the indicator corresponds to a $P \leq 0.05$

The count data is expressed as n/%. *P < 0.05* represents a statistically significant difference

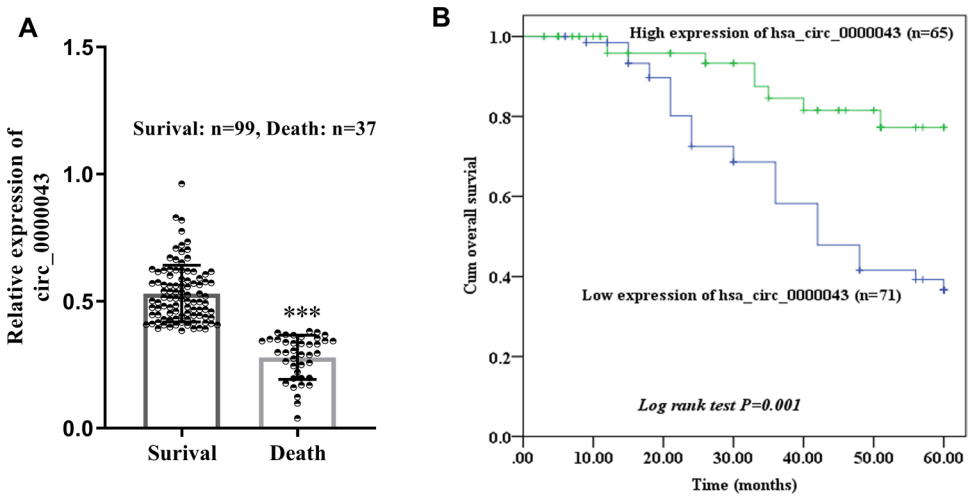


Fig. 2 circ_0000043 expression and prognostic outcome of CC patients. **A** Prognostic outcome as the change of circ_0000043 expression in the survivor (n = 99) or death (n = 37) population, and the CC patients who died expressed low levels of circ_0000043. **B** Kaplan–Meier curves for survival analysis of patients with cervical cancer over 5 years. * *P < 0.05*, ** *P < 0.01*, *** *P < 0.001*, **** *P < 0.0001* vs. Survival group

Table 3 Multivariate Cox analysis of clinical characteristics in relation to overall survival

Items	Multivariate analysis		
	P	HR	95% CI
Has_circ_0000043	**0.002**	0.277	0.122–0.628
Age	0.501	1.325	0.584–3.009
Tumor size	0.090	2.443	0.869–6.873
Differentiation	0.471	1.445	0.531–3.934
Lymph node metastasis	**0.024**	2.216	1.110–4.427
FIGO	**0.006**	3.968	1.479–10.644

Bold value indicates that theindicator corresponds to a $P \leq 0.05$

P < 0.05 represents a statistically significant difference

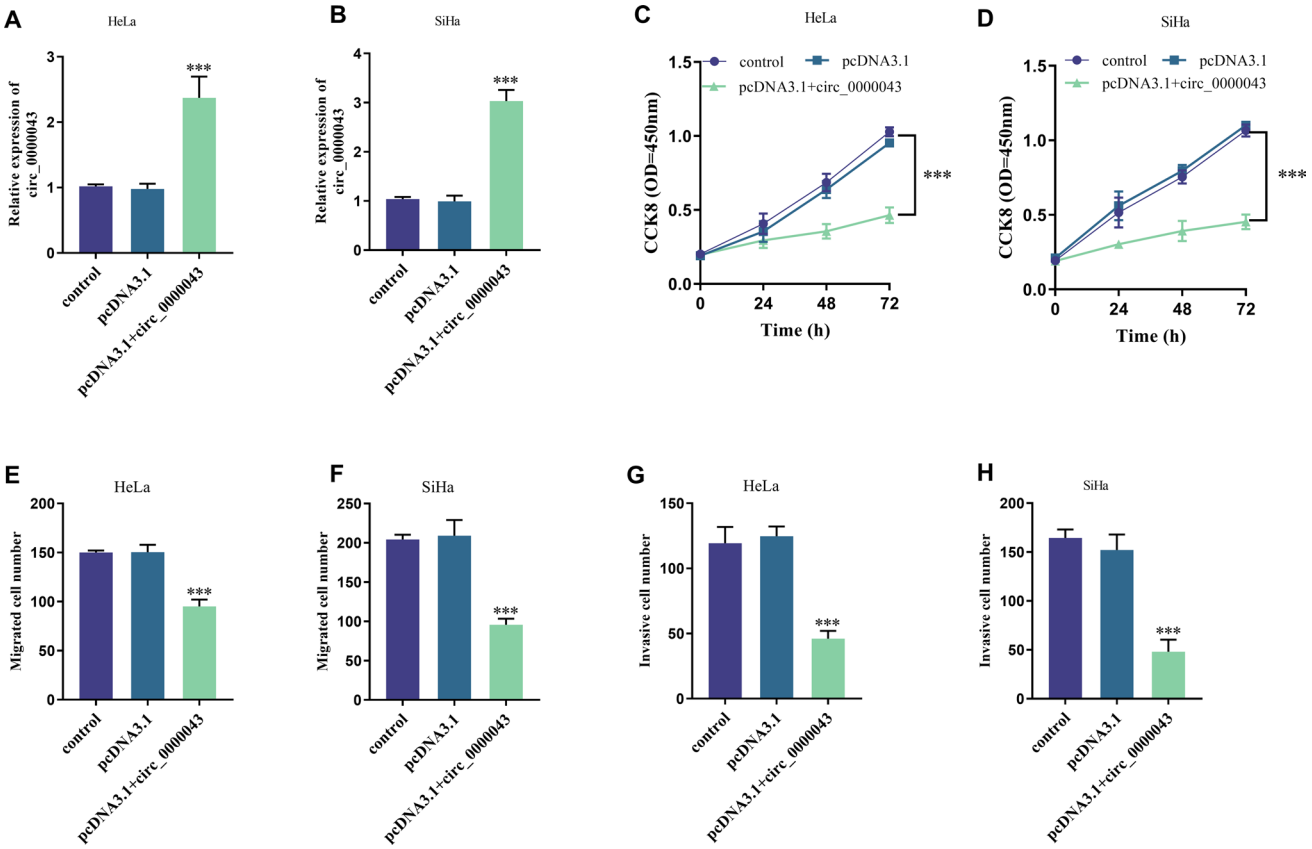


Fig. 3 Effect of circ_0000043 expression on the function of cervical cancer cells. circ_0000043 expression in 2 types of cervical cancer cells HeLa **(A)** and SiHa **(B)** after transfection, **C**, **D**. Changes in proliferative ability of cervical cancer cells after transfection with circ_0000043, and the effect of transfection with circ_0000043 on the function of cervical cancer cell migration **(E**, **F)** and invasion **(G**, **H)**. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ vs. pcDNA 3.1

P < 0.0001, Figs. 4G, H). The above results suggest that circ_0000043 may participate in cervical cancer cell function and regulate CC progression through direct binding to miR-590-5p and exerting antagonistic effects.

3.5 circ_0000043 regulates cervical cancer cell function through miR-590-5p

Overexpression of circ_0000043 substantially inhibited the expression of miR-590-5p in cervical cancer cells HeLa (*P < 0.01*, Fig. 5A) and SiHa (*P < 0.01*, Fig. 5B), but this inhibitory effect was significantly attenuated by transfection of miR-590-5p mimic. The results of cellular experiments showed that simultaneous transfection of miR-590-5p mimic notably enhanced

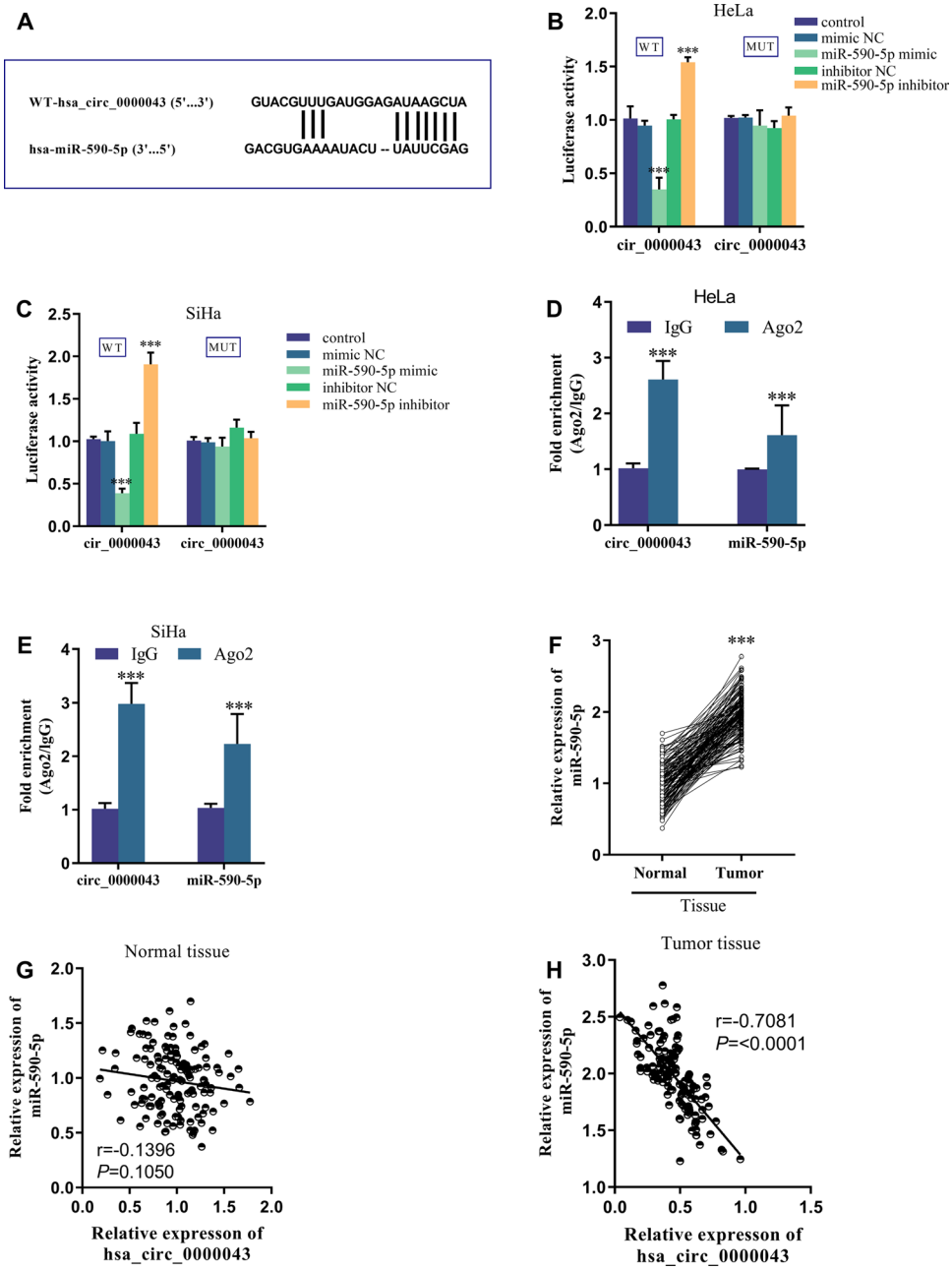


Fig. 4 Validation of miR-590-5p interactions with circ_0000043. **A** Binding target site map of miR-590-5p and circ_0000043. Luciferase reporter assay to detect interactions between miR-590-5p and circ_0000043 in HeLa (**B**) and SiHa (**C**), the results showed a targeting relationship between circ_0000043 and miR-590-5p. **D**, **E**. RIP validation of binding relationship between miR-590-5p and circ_0000043 in cervical cancer cells, and this occurs in direct interaction. **F** miR-590-5p expression levels in normal tissues and cervical cancer, up-regulation of miR-590-5p levels in tissues from cervical cancer patients. **G**, **H** Pearson correlation analysis of miR-590-5p and circ_0000043 expression relationship in cervical cancer cells. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ vs. control, IgG, or normal tissues

the proliferation ($P < 0.001$, Figs. 5C, D), migration ($P < 0.001$, Figs. 5E, F), and invasion ($P < 0.001$, Figs. 5G, H) abilities of the cervical cancer cells inhibited by the overexpression of circ_0000043.

4 Discussion

Cervical cancer mainly harms the female population, and its infiltrative and invasive characteristics have brought deep disaster to the majority of female patients [6, 34]. Although the development of CC treatment technology is fast [35, 36], the problems of expensive surgery [37] and poor prognosis of advanced patients still exist [38]. The previous analyses revealed that circRNAs can participate in the pathogenic progression of CC, and circ_0000043 expression was dysregulated in CC patients, which was speculated to be possibly involved in the mechanism of action of CC. In this study, enrichment of differentially expressed genes in CC patients revealed that circ_0000043 was down-regulated in all patients, and quantitative results also showed that patients' tissues and cells also contained low levels of circ_0000043. Follow-up of

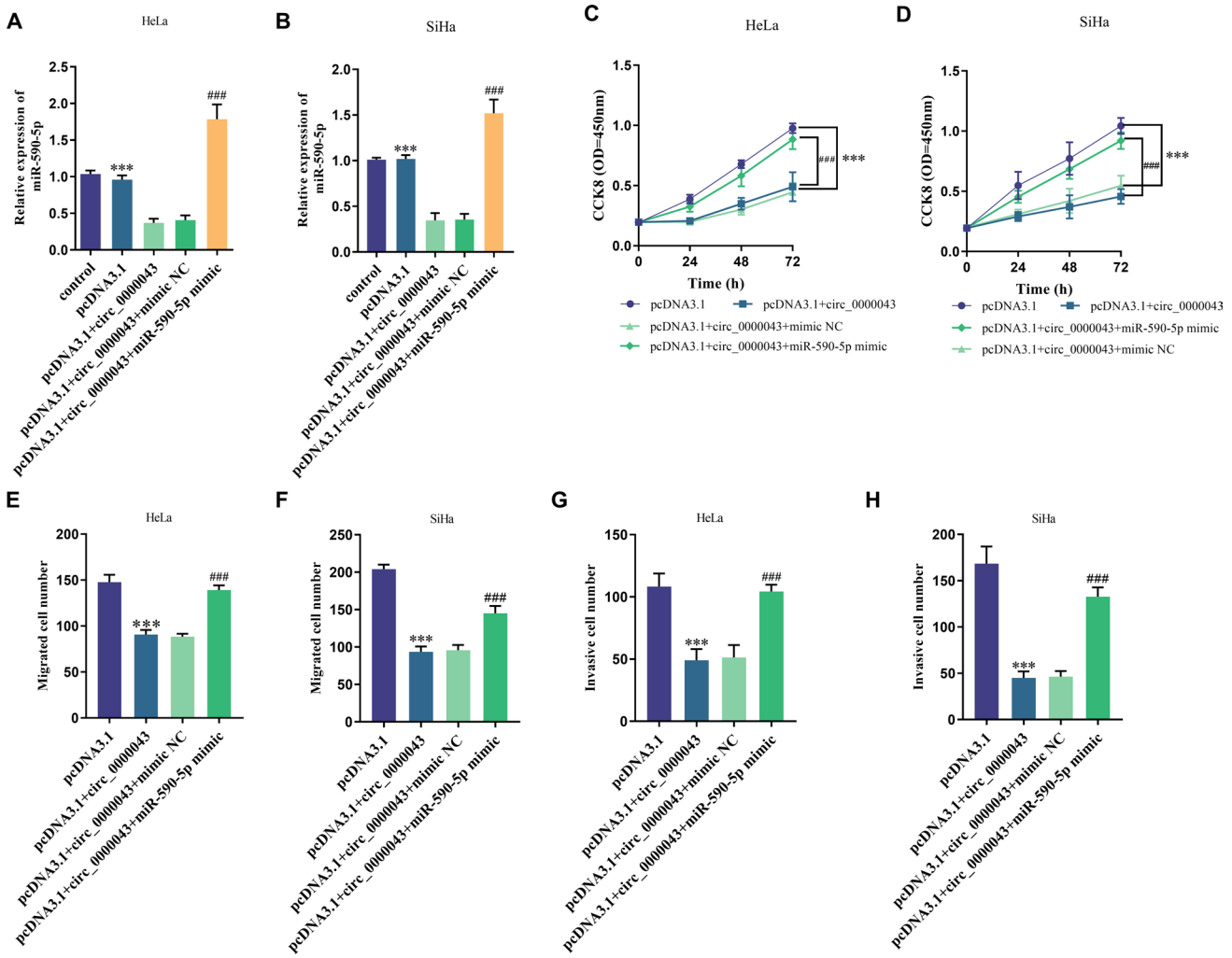


Fig. 5 circ_0000043/miR-590-5p affects cervical cancer cell function rescue assay. The expression of miR-590-5p in cervical cancer cells HeLa (**A**) and SiHa (**B**) after transfection with circ_0000043, miR-590-5p mimic, reduced miR-590-5p levels in cervical cancer cells transfected with circ_0000043. The effect of transfection with circ_0000043, miR-590-5p mimic on the proliferation (**C**, **D**), migration (**E**, **F**), and invasion (**G**, **H**). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ *vs. pcDNA3.1;* ###$P < 0.001$ vs. pcDNA3.1 + circ_0000043 + mimic NC

the patients' prognosis over a 5 years period revealed that patients with low expression of circ_0000043 had a worse survival rate, and the prognostic outcome was more likely to progress to death. In addition, analysis of the patient data showed that there were no significant differences in age, tumor size and degree of differentiation in the low-circ_0000043 group compared to the high-circ_0000043 group. However, multivariate cox analysis of CC patient data showed that circ_0000043, lymph node metastasis, and FIGO were all risk factors affecting prognosis. The above findings suggest that circ_0000043 may be involved in the prognostic progression of CC patients. Then in this study, we overexpressed circ_0000043 in cervical cancer cells by transfection, and found that the proliferation ability of cervical cancer cells was prominently reduced, and cell migration and invasion were also inhibited. Similar results were also reflected in other studies, for example, Circ-HMGCS1 was found to be one of the indicators of prognosis in cervical cancer patients [39], and the up-regulation of circ_TMCO3 was able to distinctly inhibit the viability and invasion of cervical cancer cells [40]. This suggests that low levels of circ_0000043 are involved in the mechanism of action of prognostic progression in CC patients.

circRNAs usually act in conjunction with miRNAs, e.g., circ_0039787 promotes CC occurrence through miR-877-5p [41], and circ_0001589/miR-1248 mediates drug resistance in CC [42]. Retrospective analysis revealed that circ_0000043 could participate in CC progression through miR-1271-5p [43]. The prediction results of this study showed that the miR-590-5p target site map had multiple binding sites with circ_0000043. The dual luciferase reporter assay demonstrated that overexpression of miR-590-5p inhibited circ_0000043 expression, and the RIP results showed that there was a direct reciprocal relationship between the two. Quantitative results showed that miR-590-5p was noticeably up-regulated in

CC tissues, and Pearson correlation analysis demonstrated that circ_0000043 was significantly negatively correlated with miR-590-5p expression in cancer tissues. When transfected with circ_0000043, miR-590-5p expression was markedly reduced in cervical cancer cells, while the inhibited proliferation, invasion and migration of both cervical cancer cells were substantially restored by concurrent miR-590-5p mimic. Thus, the present study initially identified the mechanism of action of circ_0000043/miR-590-5p involved in the prognostic progression of CC patients, i.e., the inhibited circ_0000043 could promote miR-590-5p-induced accelerated cervical cancer cell proliferation and enhanced migration and invasion, thus adding to the poor prognostic progression of the patients.CC is still a threat to the health of women as a risk factor, in this study, we found that the circ_0000043/miR-590-5p axis is involved in the prognostic mechanism of CC patients, which can provide a theoretical basis for the treatment of malignant CC.

circRNAs are miRNA precursor mRNA reverse splicing products that often act as regulators of cellular function and disease occurrence. Abnormal expression of circ_0000043 (circPUM1) has been found to be associated with a variety of diseases through an extensive literature review [44, 45]. For example, circ_0000043 promotes follicular growth and oocyte maturation by regulating the oncogene PTEN [46]. circPUM1 targets miR-1208/MAP3 K2 to promote epithelial mesenchymal metastasis (EMT) in hepatocellular carcinoma [47]. circPUM1 promotes esophageal squamous cell carcinoma by regulating oxidative phosphorylation and inhibiting cellular proptosis via UQCRC2 in mitochondrial complex III [48]. In this study, we found that down-regulated circ_0000043 was unable to inhibit miR-590-5p expression, resulting in enhanced cancer cell proliferation. This phenomenon promotes cervical carcinogenesis and is detrimental to the prognostic recovery of patients. It has been reported that circ_0000043 is down-regulated in patients with recurrent spontaneous abortion, with impaired proliferation of trophoblast cells and elevated levels of apoptosis and inflammation [49]. In addition, Li et al. [50] also found that circ_0000043 targeted miR-590-5p to upregulate METTL3 and promote non-small cell carcinoma (NSCLC) progression and glycolytic processes. These reports are similar to our findings. In clinical practice, routine serum marker testing is an important criterion for determining cervical cancer [51], and more and more serum markers (e.g., miR-4534) have been found to be involved in cervical cancer progression [52]. From the results of the study, downregulation of circ_0000043 can predict cancer progression and patient prognosis to some extent. That is, patients with cancer tissues containing lower circ_0000043 suffer from malignant transformation and poor prognosis. However, the degree of pathogenicity and prognostic effect of cervical cancer of different subtypes are different [53], so it is important to follow up the attention and differentiate the effect of circ_0000043 down-regulation on cancer subtypes.

There are many reasons affecting tumor genesis, and in addition to abnormal expression of the gene itself, HPV infection, smoking, and alterations in the tumor microenvironment (TME) are an indispensable factor [54, 55]. The tumor microenvironment contains immune cells, blood vessels, extracellular matrix, etc., which is one of the conditions for tumor metastasis [55]. circ_0000043 in exosomes promotes ovarian cancer metastasis by targeting miR-6753-5p to upregulate MMP2 expression [56]. The MMP family are matrix metalloproteinases involved in the inflammatory response, and TME alterations are bound to alert immune cells. In addition, circPUM1 in oral squamous cell carcinoma increases natural killer cytotoxicity and promotes chemoresistance [57]. The development of tumor immunotherapy has also provided new approaches to cervical cancer treatment [58]. Therefore, it is a very interesting aspect to focus on the changes in the tumor microenvironment and immune cell responses during cervical cancer treatment.

miR-590-5p is a double-edged sword regulating carcinogenesis [59], and miR-590-5p plays different roles in different cancers. In cervical cancer studies, miR-590-5p acts as an oncogene, i.e. miR-590-5p can target CHL1 to promote the proliferation of cervical cancer cells [26]; or degrade the mRNA of downstream target genes (e.g., tumorigenic inhibitory factor 15 protein RECK, TGFBR2, FOXO1, Fas ligand) to promote EMT in tumor cells and inhibit cell apoptosis [59]. It has been shown that miR-590-5p is exponentially increased in patients with cervical cancer [59], suggesting that miR-590-5p has the potential to develop into a progression and prognostic marker for cervical cancer. In this study, we found that down-regulated circ_0000043 and aberrant expression of miR-590-5p in patients were significantly correlated with survival and disease risk, which is expected to be a potential marker for cervical cancer prediction and prognosis. However, our discovery of circ_0000043 or miR-590-5p was determined by collecting patient tissues, and the experimental results still lacked further serological evidence and in vivo animal experiments compared with the identified classical cervical cancer markers. Therefore, we will subsequently collect sera from relevant patients and add in vivo animal experiments to validate the results of this paper.

The results of this paper suggest that circ_0000043 is down-regulated in patients with cervical cancer and that it causes poor prognosis in patients as a result of targeting miR-590-5p and promoting the proliferation of cervical cancer cells. Thus, we have identified another molecular mechanism that affects the progression and prognosis of cervical cancer. This result may provide some theoretical basis for the development of targeted drugs for the treatment of cervical cancer, and likewise provide new ideas for clinical prediction and assessment of cervical cancer

progression. However, our initial exploration only provided cytological evidence that circ_0000043 is involved in regulating cervical cancer progression. Statistically, more down-regulation of circ_0000043 seems to be more willing to appear in patients with severe staging, but this trend was not significantly different. Later on, we will collect more samples to carefully study the effect of circ_0000043 expression level and cervical cancer stage or subtype. Thus, it seems that there is still a long way to go to find a cure for cervical cancer.

From the current clinical results, patients with early-stage cervical cancer have a low risk of treatment and a better prognosis [60], but patients with advanced cervical cancer have unsatisfactory radiotherapy [61] and recovery. In addition to surgical treatment, targeted drug combination therapy [62] is widely used in the treatment of advanced patients. The results of this study suggest that circ_0000043 downregulation is involved in cervical cancer progression and has the potential to predict cancer progression and patient prognosis. Therefore, it is a good research direction to use circ_0000043 as an entry point to develop targeted drugs for cervical cancer. On the one hand, the development of gene editing technology and targeted drug development has made it possible to develop targeted drugs for disease treatment. The development of exosome-detected circ_0000043 [55] and nanotechnology [63] has provided the possibility of RNA delivery [64]. The development of gene editing also holds promise for the development of circ_0000043 targeted drugs for cervical cancer. On the other hand, combination therapy is increasingly being used for targeted drug development and disease research. whether circ_0000043 expression affects the sensitivity of cervical cancer cells to chemotherapy or targeted therapy is not clear to us, but we believe that this is a very interesting topic. In addition, although this preliminary study confirmed that circ_0000043 may be involved in CC progression by targeting miR-590-5p, the transcription factors and epigenetic modifications involved were not investigated. This represents a potential limitation of the current study, and we will analyze these issues in depth in future research. The subsequent in depth studies have also examined circ_0000043's impact on CC apoptosis, metabolic reprogramming, tumor growth and metastasis in vivo, as well as radiotherapy sensitivity. Additionally, they evaluated its predictive accuracy when combined with other prognostic biomarkers.

In summary, this study found that circ_0000043 was lowly expressed in CC patients, and low level of circ_0000043 was a risk factor for the prognosis of shadow patients, which was more likely to lead to the prognosis of patients to develop into a fatal outcome. circ_0000043 was involved in the prognosis and progression of CC patients by binding to miR-590-5p, which was mainly manifested by the fact that the low expression of circ_0000043 promotes miR-590-5p-induced accelerated proliferation and enhanced migration and invasion of cervical cancer cells.

Acknowledgements Not applicable.

Author contributions Conceptualization, Y.Z., X.S., S.Z., H.C., B.W.; Data curation, Y.Z., X.S., S.Z., H.C.; Formal analysis, Y.Z., X.S., S.Z., H.C.; Funding acquisition, H.C.; Investigation, H.C.; Methodology, Y.Z., X.S., S.Z., B.W., H.C.; Project administration, H.C.; Resources, S.Z.; Software, Y.Z., X.S., S.Z.; Supervision, H.C., B.W.; Validation, S.Z.; Visualization, S.Z.; Roles/Writing—original draft, S.Z.; Writing—review & editing,, Y.Z., X.S., H.C., B.W.

Funding The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

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

Declarations

Ethics approval and consent to participate The study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of First People's Hospital of Linping District before the study began.

Informed consent Informed consent was obtained from all individual participants included in the study.

Consent for publication Not applicable.

Competing Interests The authors declare no competing interests.

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