Original Paper

CircRNA8924 Promotes Cervical Cancer Cell Proliferation, Migration and Invasion by Competitively Binding to MiR-518d-5p /519-5p Family and Modulating the Expression of CBX8

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Key Words

Cervical cancer • CircRNA • CeRNA • MiRNA family • CircRNA8924 • MiR-518d-5p/519-5p • CBX8

Abstract

Background/Aims: Circular RNAs (circRNAs) play a significant role in the development and progression of various human cancers. However, the expression and function of circRNAs in cervical cancer (CC) have rarely been explored. The aim of this study was to investigate the biological function of circRNA8924 in CC and elucidate the possible molecular mechanism involved. **Methods:** Quantitative polymerase chain reaction was used to determine mRNA expression of circRNA8924, miR-518d-5p/519-5p and CBX8 in CC tissues and cells. CBX8 protein expression was measured by Western blotting. The CCK-8 assay was used to evaluate cell proliferation, and the transwell assay to determine cell migration and invasion. The luciferase reporter assay was used to determine the direct regulation of miR-518d-5p/519-5p and circRNA8924 or CBX8. **Results:** The study demonstrated that the expression level of circRNA8924 in CC was significantly higher than that in the adjacent normal tissues ($P < 0.001$), and that it was also associated with tumor size, FIGO staging and myometrial invasion. The knockdown of circRNA8924 significantly inhibited the proliferation, migration and invasion of CC cells SiHa and HeLa. The expression level of miR-518d-5p/519-5p was negatively correlated with circRNA8924, and circRNA8924 regulated CBX8 by competitively binding to miR-518d-5p/519-5p. **Conclusions:** CircRNA8924 is highly expressed in CC tissue and can be considered a competitive endogenous RNA of the miR-518d-5p/519-5p family to promote the malignant biological behavior of CC cells. It is suggested that it may serve as a new biomarker for CC diagnosis and disease progression and provide potential targets for targeted therapy.

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Published by S. Karger AG, Basel

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Introduction

Cervical cancer (CC) is one of the most common gynecological cancers worldwide [1], and it has been proved that persistent infection with high-risk type human papillomavirus (HR-HPV) is an important pathogenic factor in its histogenesis and progression [2]. With the widespread application of HPV vaccine in recent years, primary prevention of CC has been achieved to a certain degree, with its morbidity and mortality effectively controlled in many developed countries [3]. Nevertheless, higher HPV infection rate is always accompanied by relatively lower incidence of CC, which not only makes CC screening and primary prevention a heavy economic burden of hygienic economics but also hints that there are individual genetic differences and other important factors in the progression of CC [4, 5]. Therefore, it is essential to explore the exact pathogenesis of CC and find brand new diagnostic markers as potential therapeutic targets.

The human genome has just less than 2% of nucleotide sequence encoding proteins, while most genes are translated into non-coding RNAs (ncRNA) [6]. In recent years, circRNAs have become a research hotspot in diseases following the appearance of the microRNA and long non-coding RNA [7]. Different from miRNA and lncRNA with traditional linear structure, circRNAs are a covalently closed continuous loop [8]. Due to its absence of 3', 5' end and poly A tail, circRNAs can avoid the degradative effect of exonuclease and RNase R. Compared with linear RNA, circRNAs are more conservative and stable and show cell-specific, tissue-specific and stage-specific [9, 10]. Chen et al [11]. confirmed that hsa_circ_0000190, as a marker for gastric cancer, had a higher sensitivity and specificity than CEA and CA19-9. Wang et al [12]. reported that hsa_circ_001988 expression was significantly reduced in patients with colorectal cancer and closely related to tumor differentiation and neuro-invasion. Yao et al [13]. found that circRNA_100876 was tightly correlated with the survival cycle of patients with non-small cell lung cancer. Liu et al [14]. indicated that hsa_circRNA_104980 and hsa_circRNA_103801 might play important roles in the initiation of osteosarcoma.

At present, research on the biological function of circRNAs is still at the exploration stage. It has been demonstrated that circRNAs act as competitive endogenous RNAs (ceRNAs), namely microRNA sponges, regulating target gene expression [15]. They could also serve as a transcriptional regulator or protein-binding RNA [16, 17], or even be translated directly into proteins under certain circumstances [18]. Many potential circRNA molecular markers and their pathways as miRNA sponges have been discovered in many tumors, including esophageal squamous carcinoma, gastric cancer, pancreatic ductal adenocarcinoma, liver cancer, colorectal cancer, early-stage lung adenocarcinoma, glioma, and ovarian cancer [19-21]. However, the role of circRNA in the progression of cervical cancer has not been reported yet.

In this study, human circRNA Microarray V2.0 was used to screen CC tissues and pair-matched adjacent normal tissues, showing that circRNA8924 was the circRNA that was significantly increased. CircRNA8924 (DeepBase) is also called hsa_circ_0141539 (circBase), with designated gene name Clorf116. It is an exonic circRNA and located in chr1:2072008388-207201024. By using bioinformatics analysis, we found the combinations of miR-519a-5p, miR-519c-5p and miR-518e-5p with circRNA8924 were highly rated. All these miRNAs belong to the miR-518d-5p/519-5p family, and most of the family members have the same nucleotide sequence, while the others differ in only 1 or 2 nucleotides. Some of these members have been reported in tumors. Wang et al [22]. demonstrated that patients with prostate cancer would benefit from preoperative evaluation and monitoring of disease prognosis by using miR-519c-5p serum level combined with their age, PSA, stage and biopsy. Recent studies have revealed that low expression of miR-526a in liver cancer tissues can target the regulation of PAK7. Overexpression of miR-526a significantly inhibits proliferation, migration, invasion and heterotopic planting of HCC cells Huh7 and HepG2 [23]. However, the expression and mechanism of the miR-518d-5p/519-5p family in CC remain unclear.

Our research was the first to prove the vital role of circRNA8924 in the tumorigenesis of CC and reveal its function as a ceRNA to regulate the expression of CBX8 by sponging miR-518-d-5p/519-5p, establishing a new theoretical basis for CG development and creating a new direction for molecular targeted therapy.

Materials and Methods

Clinical specimens

CC tissues and adjacent normal tissues were collected from 33 cases of patients who underwent surgery at Cancer Hospital of China Medical University from February 2016 to June 2016. None of them had received preoperative chemotherapy or radiotherapy. All collected specimens were dipped in liquid nitrogen immediately after resection and then stored in freezer at -80°C for further analysis. Postoperative diagnoses were pathologically confirmed by three independent pathologists. Meanwhile, corresponding patient clinicopathological data were recorded. This study has received approval from the Ethics Committee of Cancer Hospital of China Medical University.

Cell culture

Human cervical cancer cell lines (SiHa and HeLa) and 293T cells were all obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in DMEM high glucose medium (Hyclone) supplemented with 10% fetal bovine serum (FBS; PAN-Biotech, Adenbach, Germany) and maintained in a humidified incubator with 5% CO₂ at 37°C.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from specimens and cells using TRIzol reagent (Takara, Tokyo, Japan) according to the instructions of manufacturer. The quality and concentration of RNA were assessed by a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The reverse transcription reactions of circRNA and mRNA were performed using the PrimeScript RT reagent kit (RR037A, Takara, Tokyo, Japan), and the reverse transcription reaction of miRNA was carried out with the miRNA First Strand Synthesis Kit (638315, Takara, Tokyo, Japan). The qRT-PCR was done with the SYBR Premix Ex Taq Kit (RR420A, Takara, Tokyo) and ABI 7500 fast real-time PCR system (Applied Biosystems, Darmstadt, Germany). GAPDH was regarded as the endogenous control for circRNA and mRNA, U6 was employed as control for miRNA normalization. The amplification efficiencies of primers were in a range of 100% to 105%. Therefore, fold changes in expression of RNAs were recorded using the 2^{-ΔΔCt} method. All qRT-PCR assays were performed in triplicate. Divergent primers were designed to amplify circRNA8924 transcripts by Geneseech (Guangzhou, China), while other primers were designed by PrimerPremier5.0 software (Premier, Canada). All sequences are summarized in Table 1.

Lentivirus constructs and cell transfection

For *in vivo* studies, circRNA8924-overexpressing cells (circRNA8924 OE), circRNA8924- knockdown cells (sh-circRNA8924) and negative control cells (NC) were used. Lentivirus particles were purchased from Hanbio Biotechnology (Shanghai, China). At 48 h after transfection, stable-transfection cells were established by puromycin selection (2 μg/ml) applied for 10 days.

The miR-519a-5p mimic, miR-519a-5p inhibitor and negative control were obtained from GenePharma (Shanghai, China). SiHa and HeLa cells were seeded in 6-well plates and then transfected the next day using Lipofectamine3000 (Invitrogen, CA, USA) according to the manufacturer's instructions. Cells were harvested at 48 h after transfection followed by confirmation of transfection efficiency using qRT-PCR.

Table 1. Primers used for qRT-PCR

Name	Primer Sequence(Forward)	Primer Sequence(Reverse)
circRNA8924	CAGCTGTGACAGCATGATGA	TCGGGCATCACCAGAAACAA
miR-519a-5p	CTCTAGAGGGAAGCGCTTTC	—
CBX8	GGCCTCGGAGAGTACCTCAA	ACACCACTGACACAGGTC
GAPDH	GCACCGTCAAGGCTGAGAAC	TGGTGAAGACGCCAGTGGA
U6	GGAACGATACAGAGAAGATTAGC	TGGAACGCTTCACGAATTTCGG

Cell proliferation assay

The cell proliferation assay was performed using Cell Counting Kit-8 (CCK-8, Dojindo, Osaka, Japan). Siha and HeLa cells in different groups were seeded into 96-well plates with serum-free DMEM medium. Each group was replicated with three independent wells. According to the experimental requirements, cell proliferation was observed at 24, 48 and 72 h. Before observation, each well was treated with 10 μ l CCK-8 reagent and then cells were incubated at 37°C for another 3 h before measuring absorbance at 450 nm using a spectrophotometer. All experiments were performed in triplicate.

Transwell migration assay and invasion assay

Siha and HeLa cells (5×10^4) in different groups were resuspended in 200 μ l serum-free medium and seeded into the upper chamber of 24-well plates (Corning, New York, NY, USA) with (invasion) or without (migration) Matrigel (BD Biosciences, New York, NY, USA), while 600 μ l medium containing 20% FBS were supplied at the lower chamber as chemoattractant. After incubation at 5% CO₂ /37°C for 20 h, cells were fixed with 4% paraformaldehyde for 30 min and then stained with 0.1% crystal violet for 30 min. The number of cells that migrated or invaded was determined in five randomly selected fields using an inverted microscope.

Cell cycle assay

Cells (2×10^6) were collected and fixed with 70% ethanol at 4°C overnight, and 100 μ l RNase A (Keygen Biotech, Nanjing, China) were then added, followed by incubation at 37°C for 30 min. Cells were then stained with 400 μ l propidium iodide (Keygen Biotech) for 30 min. A FACSCalibur flow cytometer (Becton Dickinson) was applied to evaluate cells at 488 nm, and ModFit LT software (Verity Software House) for analysis. All assays were repeated at least three times.

Luciferase reporter assay

We constructed the plasmid psiCHECK-circRNA8924-WT/-Mut and psiCHECK-CBX8-WT/-Mut (Promega) on the basis of the bioinformatics-predicted results for further investigation and the sequence of wild-type circRNA8924 and CBX8 mRNA was obtained by PCR. Meanwhile, a mutant circRNA8924 without miR-519a-5p binding sites and mutant CBX8 3'UTR fragments were obtained by overlap extension PCR with mutant primers. Briefly, 293T cells were co-transfected with psiCHECK-circRNA8924-WT or psiCHECK-circRNA8924-Mut (psiCHECK-CBX8-WT or psiCHECK-CBX8-Mut) and miR-519a-5p mimics or miR-NC using Lipofectamine3000 (Invitrogen). Luciferase activity was measured at 48 h after transfection by a Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Western blotting

Total protein was extracted from cells using RIPA lysis buffer (Keygen Biotech). The lysate protein was separated by 10% SDS-PAGE and electrophoretically transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA). After blocking with 5% non-fat milk in TBST for 2 h, proteins were then incubated with primary and secondary antibodies. Signal detection was visualized by using enhanced chemiluminescence. Finally, relative concentration was calculated using Quantity One software (Bio-Rad). Primary antibodies included those against CBX8 (dilution 1:1000, 14696) from Cell Signaling Technology, (CA, USA), and Cyclin D1 (dilution 1:200, 60186-1-Ig), Bax (dilution 1:2000, 50599-2-Ig), and GAPDH (dilution 1:5000, 10494-1-AP) from Proteintech (Chicago, USA).

Statistical analysis

Data were reported as means $\pm$ standard deviation (SD) and analyzed using SPSS 23.0 software (SPSS Inc., Chicago, USA). Chi-squared test was used to compare clinicopathological features and circRNA8924 expression. Between-group differences were tested for significance using one-way analysis of variance and Student's t-test. Pearson correlation coefficients were calculated to determine the correlation. P-value<0.05 was considered significant.

Results

CircRNA8924 expression is up-regulated in cervical cancer tissues and correlated with clinicopathological variables

The level of circRNA8924 expression was examined in CC tissues and matched adjacent normal tissue samples for the first time. The expression of circRNA8924 was significantly upregulated in 96.97% (32/33) CC tissues compared with their adjacent normal tissues (Fig. 1A,1B). The patient cohort was then divided into high-expression group and low-expression group using the median expression level (10.93) as cutoff value. The level of circRNA8924 expression was significantly correlated with tumor size ($P=0.008$), FIGO (International Federation of Gynecology and Obstetrics) stage ($P=0.041$) and myometrial invasion ($P=0.022$), but it has no correlation with patient's age or tumor differentiation or lymph node metastasis ($P>0.05$ in all cases) (Table. 2).

CircRNA8924 affects cell proliferation, cell cycle, migration and invasion in cervical cancer cells in vivo

Since circRNA8924 was up-regulated in CC tissues, we performed gain and loss of function analyses *in vitro* to investigate its effects on cells by transfection with overexpression and

Table 2. Correlation between circRNA8924 expression and clinicopathological features of cervical cancer patients

Clinicopathological features	Number (n=33)	CircRNA8924 expression		P value
		Low(%)	High(%)	
Age(years)				
<45	14	6(42.86)	8(57.14)	0.579
≥45	19	10(52.63)	9(47.37)	
Tumor size(cm)				
<4	26	14(80.77)	12(19.23)	0.008 **
≥4	7	2(28.57)	5(71.43)	
Tumor differentiation				
Well and mediate	27	11(40.74)	16(59.26)	0.059
Poor	6	5(83.33)	1(16.67)	
FIGO stage				
Ib	21	13(61.9)	8(38.1)	0.041 *
IIa-IIb	12	3(25.00)	9(75.00)	
Myometrial invasion				
<1/2	18	12(66.67)	6(33.33)	0.022 *
≥1/2	15	4(26.67)	11(73.33)	
Lymph node metastasis				
Negative	23	12(52.17)	11(47.83)	0.520
Positive	10	4(40.00)	6(60.00)	

Fig. 1. CircRNA8924 expression in cervical cancer tissues. (A) The expression level of circRNA8924 was up-regulated in CC tissues (Fold change≈6). (B) CircRNA8924 expression was assayed in 33 pairs of CC tissues and matched noncancerous tissues by qRT-PCR. The expression was upregulated in 96.97% (32/33) CC tissues. Data were reported as means ± standard deviation. ** P-value<0.001.

Fig. 2. Effect of circRNA8924 on cervical cancer cell proliferation and cell cycle in vitro. (A, B) Compared with NC, the expression of circRNA8924 could be effectively increased by circRNA8924 OE and inhibited by sh-circRNA8924-1 and sh-circRNA8924-2. The sh-circRNA8924-2 was selected for the follow-up study. (C) The CCK-8 assay showed that the proliferation rate of SiHa and HeLa cells in sh-circRNA8924 was significantly reduced. (D) After transfection of sh-circRNA8924, the flow cytometry assay verified a higher percentage of cell in Go/G1 phase and a lower percentage of cell in S phase. CircRNA8924 OE showed an opposite trend. Data were reported as means ± standard deviation. *P-value<0.05, **P-value<0.001.

knockdown lentivirus. The expression of circRNA8924 could be effectively increased by circRNA8924 OE (Fig. 2A) and inhibited by sh-circRNA8924-1 and sh-circRNA8924-2 (Fig. 2B) compared with NC. We chose sh-circRNA8924-2 for the follow-up study with superior interference effect. The CCK-8 assay showed that the proliferation rate of SiHa and HeLa cells in sh-circRNA8924 was significantly reduced compared with the NC group (Fig. 2C). To explore the effect of circRNA8924 on CC cell cycle, the cells were analyzed by flow cytometry. Results showed that compared with the NC group, the proportion of sh-circRNA8924 cells blocked in G0/G1 phase was increased, and cells in S phase decreased (Fig. 2D). At the same time, the migration rate and invasion rate of the SiHa and HeLa cells of the sh-circRNA8924 group also decreased significantly (Fig. 3A,3B). However, cell proliferation, migration and invasion of the circRNA8924 OE group were significantly increased, with up-regulated cell proportion in S phase and down-regulated percentage of G0/G1 phase (Fig. 2, 3). The functional study indicated that proliferation, migration and invasion of CC cells were significantly inhibited by circRNA8924 knockdown.

CircRNA8924 acts as a molecular sponge of miR-518d-5p/519-5p

To determine whether circRNA8924 could act as a ceRNA, we used TargetScan and miRanda to predict potential circRNA-miRNA interactions, and miR-518d-5p/519-5p family had higher scores. We used miR-519a-5p as the representative of the miR-518d-5p/519-5p family. The expression of miR-519a-5p was decreased in CC tissues compared with matched adjacent normal tissue samples (Fig. 4A), and had a significant negative correlation with circRNA8924 level (Fig. 4B). Furthermore, the activity of luciferase reporters containing the binding sequence of circRNA8924 in miR-519a-5p was significantly decreased in

Fig. 3. The role of circRNA8924 in CC cell invasion and migration. (A, B) The transwell migration and invasion assay showed the inhibition in the sh-circRNA8924 group and enhancement in the circRNA8924 OE group. Data were reported as means $\pm$ standard deviation. **P-value<0.001.

Fig. 4. MiR-519a-5p expression in cervical cancer tissues and correlation with circRNA8924. (A) The expression level of miR-519a-5p was down-regulated in CC tissues (n=33), which was accomplished by qRT-PCR. (B) There was a remarkable negative correlation between the expression level of circRNA8924 and miR-519a-5p in the pair-matched CC tissues. (C) The predicted binding site and sequence of circRNA8924-Mut were shown. (D) The relative luciferase activity was reduced after co-transfection of circRNA8924-WT and miR-519a-5p mimics. (E) Transfection of circRNA8924 OE and sh-circRNA8924 caused corresponding change of miR-519a-5p in CC cell. Data were reported as means $\pm$ standard deviation. *P-value<0.05, **P-value<0.001.

circRNA8924-WT constructs; nevertheless, the luciferase activity of circRNA8924-Mut constructs was not affected (Fig. 4C, 4D). Additionally, miR-519a-5p was markedly decreased in SiHa and HeLa cells. After transfection of circRNA8924 OE, the expression of miR-519a-5p was declined, and oppositely the miRNA expression was raised by sh-circRNA8924 transfection (Fig. 4E). All these observations suggested that miR-518d-5p/519-5p could be targeted by circRNA8924.

MiR-518d-5p/519-5p reverses the tumorigenic effects of circRNA8924 on CC cells

We transfected SiHa and HeLa cells with miR-519a-5p mimics and NC. The CCK-8 assays showed that miR-519a-5p blocked the promotion of cell proliferation (Fig. 5A, 5B), and cell migration and invasion were also inhibited (Fig. 5C, 5D). These results showed that circRNA8924 promoted tumor malignancy by competitively binding to miR-518d-5p/519-5p.

CircRNA8924 modulated the target of miR-518d-5p/519-5p, CBX8

Combining bioinformatics analysis, CBX8 was found to be one of the downstream target genes of great significance. CBX8 expression level was high (Fig. 6A), with positive correlation with circRNA8924 expression and negative correlation with miR-519a-5p (Fig. 6B). Luciferase reporter assays showed a clear difference in co-transfection of CBX8-WT or CBX8-Mut with miR-519a-5p mimics/NC (Fig. 6C, 6D). The expression of CBX8 mRNA was significantly decreased in cells transfected with sh-circRNA8924 or miR-519a-5p mimics (Fig. 6E). After we transfected SiHa and HeLa cells with sh-circRNA8924, CBX8 protein was significantly reduced, and could be reversed by miR-519a-5p inhibitor, which resembled

Fig. 5. MiR-519a-5p reverses the effects of circRNA8924 in cervical cancer cells. (A) The CCK-8 assay was performed to determine the proliferation of CC cells after transfection of miR-519a-5p mimics, and co-transfection with circRNA8924 OE. (B) The flow cytometry was used to test the proportion of cell cycle phases. (C, D) The cell migration capability and invasion capability were detected by the transwell assay. Data were reported as means $\pm$ standard deviation. *P-value<0.05, **P-value<0.001.

Fig. 6. CBX8 expression in cervical cancer tissues and correlation with circRNA8924/miR-519a-5p. (A, B) The expression level and correlation of CBX8 in CC cell were analyzed by qRT-PCR and Pearson correlation coefficients respectively. (C, D) The combination with miR-519a-5p was determined by bioinformatics prediction and luciferase reporter assay. (E) The expressions of CBX8 were analyzed using qRT-PCR in CC cell followed by circRNA8924 knockdown and miR-519a-5p overexpression. Data were reported as means $\pm$ standard deviation. *P-value<0.05, **P-value<0.001.

Fig. 7. Transfection leads to the alteration of CBX8 and other protein expression. (A, B) Relative protein expression of CBX8, Bax and Cyclin D1 in CC cell following treatment with sh-circRNA8924 or miR-519a-5p mimics were measured by Western blotting (GAPDH as control). Co-transfection could reverse the transformation of protein level. Data were reported as means $\pm$ standard deviation. *P-value<0.05, **P-value<0.001.

miR-519a-5p mimics and its changeover by circRNA8924 OE. In addition, Bax was used as a biomarker of cell apoptosis, and the expression of Cyclin D1 was measured to explore the influence of circRNA8924 and miR-519a-5p in CC cell cycle. Bax and Cyclin D1 were increased and decreased, respectively, with the knockdown of circRNA8924 or overexpression of miR-519a-5p (Fig. 7). These results showed that circRNA8924 abrogated the repression of CBX8 induced by miR-518d-5p/519-5p by sequestering endogenous miRNA, and exerted oncogenic functions by modulating miR-518d-5p/519a-5p and CBX8.

Discussion

The etiology of cervical cancer and HPV infection is widely recognized. HPV vaccine has made a significant contribution to the primary prevention of cervical cancer, but there are still some new challenges ahead. Prevention of cervical cancer in some developing countries is still difficult to achieve in the short term [24, 25]. Therefore, seeking a molecular biomarker of cervical cancer is still a hotspot issue.

CircRNAs were first found in RNA viruses in the 1970s, and were not paid much attention as an abnormal RNA formed by transcription disorder [26]. With the rapid development of high-throughput sequencing and bioinformatics technology, circRNA has recently emerged as a new class of endogenous RNAs that form a covalently closed continuous loop and that exist extensively in mammalian cells [27, 28], suggesting its potency as a tumor biomarker. In this study, a circRNA microarray was conducted for cervical cancer tissues and matched adjacent normal tissues. Among differentially expressed circRNAs, circRNA8924 showed significantly higher expression in 96.97% (32/33) cervical cancer tissue than the pair-matched adjacent normal tissue. The results of qRT-PCR were consistent with microarray results. Further analysis, combined with parameters of clinical cases, indicated that circRNA8924 expression level was closely correlated with patients' tumor size, FIGO stage, and myometrial invasion, illustrating that high expression of circRNA8924 might prompt progression of cervical cancer. The blocking of circRNA8924 might be a meaningful gene therapy for keeping the tumor development within limits especially to the CC patients who have a high expression in cancer tissue. However, to minority patients with a low expression of circRNA8924, it could be another question entirely. Studies on circRNAs of cervical cancer have not been reported. Further studies *in vitro* showed that: knockdown of circRNA9024 could inhibit cervical cancer cell proliferation, migration and invasion, with the expression of cell cycle protein Cyclin D1 significantly reduced, cells in G0/G1 phase increased, S phase cell proportion reduced and the expression of apoptosis related proteins such as Bax increased. On the contrary, overexpression of circRNA8924 led to opposite consequences. The features described above indicated that circRNA8924 served as an oncogene in CC cells and was closely related to the malignant biological behavior of CC cells.

The ceRNA hypothesis presumes that mRNA, gene transcription, lncRNA and circRNA can be combined by miRNAs following the principle of complementary base pairing by competitive microRNA response elements (MREs), functionally liberating mRNA transcripts targeted by miRNAs [29, 30]. Similar to lncRNA, circRNAs were mainly used as a miRNA sponge to compete with miRNAs, reversing the inhibitory effect of miRNA downstream genes, thus increasing the expression of target genes [31]. Memczak found that CDR1as had 63 conservative binding sites to miR-7 in nervous tissue. And CDR1as was also involved in several biological process, such as insulin secretion, liver cancer cell proliferation and invasion. Furthermore, it was able to regulate gastric cancer cells through the PTEN/PI3K/AKT pathway by binding to miR-7 [32-34]. circ_0015756 was reported to be involved in the progression of hepatoblastoma by binding to miR-1250-3p [35]. Can circRNA8924 also regulate the cellular biological behavior of cervical cancer by acting as a miRNA sponge? To support this hypothesis, we determined microRNAs that interacted with circRNA8924 by bioinformatics prediction, and found circRNA8924 had the MRE of the miR-518-d-5p/518-5p family. Dual luciferase reporter gene assays further demonstrated the combination of the circRNA and microRNAs. Tissue experiments confirmed that miR-518-d-5p/518-5p had low expression in cervical cancer tissue and was significantly negatively correlated with circRNA8924 expression level. We used miR-519a-5p as the representative of the miR-518d-5p/519-5p family. Cancer cell proliferation, migration and invasion were suppressed after miR-519a-5p mimics were transfected into cervical cancer cells, and the effect was similar to that of knockdown of circRNA8924. The overexpression of both circRNA8924 and miR-519a-5p could reverse the above changes in the biological function of the cell, with no significant difference compared to the negative control group. Above all, circRNA8924 affected the malignant biological behavior of cervical cancer cells by competing with the miR-518d-5p/519-5p family.

MiRNA was repressed by the target gene in combination with 3' UTR of target gene [36]. CBX8 was defined as an important target gene of the miR-518d-5p/519-5p family, which was determined by bioinformatics and the dual-luciferase reporter gene assays. PcG family proteins play a vital role in epigenetic regulation during the process of transcription, and they are divided into two kinds of protein complexes, PRC1 and PRC2, participating in tumorigenesis, regulating stem cell differentiation, and X chromosome inactivation [37-39].

CBX8, as the core component of the PRC1 complex, is closely related to the histogenesis, invasion, metastasis and prognosis of the tumor, and has been shown to be an initial factor in the progression of multifarious cancers. A previous study showed, a significant difference of CBX8 expression in esophageal squamous cell carcinoma by deep sequencing. Proliferation, invasion and cloning ability of ESCC cells (KYSE2 and KYSE510) were suppressed by silencing CBX8[40]. Tang et al [41]. found that CBX8 was highly expressed in colorectal cancer tissue, suggesting a poor prognosis and that knockdown of CBX8 would increase the expression of p53 and its downstream effect factors, which would restrain the proliferation of colorectal cancer cells. CBX8 was also found by Lee et al [42].to be able to interact with STRT1 to reverse premature senility and retarded growth of MCF cells induced by stress. The expression level and effects of CBX8 in CC is currently unknown. This study found for the first time that the expression level of CBX8 is high in cervical cancer tissues and significantly correlated with the expression level of circRNA8924. The knockdown of circRNA8924 clearly reduced CBX8 expression level. With co-transfection of miR-519a-5p inhibitor, CBX8 protein levels recovered, accompanied by some changes in cell proliferation, cell cycle, cell invasion and migration ability of cervical cancer cells. We concluded that by binding to miR-518-d-5p/519-5p family, circRNA8924 regulated the miRNA family target gene CBX8 indirectly, influencing the cell cycle and proliferation, migration and invasion ability of CC cells.

Taken together, our study demonstrated that circRNA8924 was highly expressed in cervical cancer tissues and was closely associated with tumor size, FIGO stage and myometrial invasion. In a further study *in vitro*, circRNA8924 competitively combined with the miR-518d-5p/519-5p family, upregulated the expression level of CBX8, leading to the enhancement of the malignant biological behavior of CC cells. The circRNA8924-miR-518d-5p/519-5p-CBX8 axis provided a brand new insight into molecular treatment of CC, and revealed that the circRNA8924 might serve as a new biomarker and potential therapeutic target for CC.

Acknowledgements

This study was supported by the National Key Research and Development Program of China sub-project (No. 2017YFC0114204), and the Scientific Research Foundation for Introduced Talents, Cancer Hospital of China Medical University.

Disclosure Statement

No conflict of interests exists.

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