Reproductive Sciences (2022) 29:2636–2646
https://doi.org/10.1007/s43032-022-00920-3

GYNECOLOGIC ONCOLOGY: ORIGINAL ARTICLE

Circ_0000263 facilitates the proliferation and inhibits the apoptosis of cervical cancer depending on the regulation of miR-1179/ABL2 axis

Yi Zhang[1] · Quanshu Di[2] · Jiao Chen[3] · Muyu Chang[1] · Yalin Ma[1] · Jianyun Yu[2]

Received: 30 November 2021 / Accepted: 12 March 2022 / Published online: 30 March 2022

Abstract

Circular RNA (circRNA) has been reported to participate in the progression of cervical cancer (CC). Studies on the role and mechanism of circ_0000263 in CC are limited, and more studies are needed. The expression of circ_0000263, microRNA (miR)-1179 and ABL proto-oncogene 2 (ABL2) mRNA in tissues and cells was analyzed by quantitative real-time PCR. The proliferation and apoptosis of CC cells were determined using cell counting kit 8 assay, Edu assay, colony formation assay and flow cytometry. The protein expression of proliferation markers, apoptosis markers and ABL2 was detected by western blot analysis. The interaction between RNAs was estimated via dual-luciferase reporter assay. Xenograft models were applied to explore the effect of circ_0000263 knockdown on CC tumorigenesis. Circ_0000263 was highly expressed in CC tumor tissues. Silencing of circ_0000263 suppressed CC cell proliferation and increased apoptosis. Circ_0000263 served as a sponge for miR-1179, and miR-1179 inhibitor reversed the regulation of si-circ_0000263 on CC cell proliferation and apoptosis. ABL2 could be targeted by miR-1179, and circ_0000263 could sponge miR-1179 to regulate ABL2. Overexpression of ABL2 reversed the anti-proliferation and pro-apoptosis roles of miR-1179 in CC cells. In addition, circ_0000263 knockdown reduced CC tumor growth by miR-1179/ABL2 axis. In brief, the results demonstrated that circ_0000263 exerted an oncogene role in CC, which suggested that circ_0000263 might be a promising therapeutic target for CC.

Keywords Cervical cancer · circ_0000263 · miR-1179 · ABL2

Introduction

Cervical cancer (CC) refers to malignant tumors that occur in the uterine vagina and cervical canal and is one of the common malignant tumors in women [19, 27]. CC is considered to be the major disease threatening women's health, and human papillomavirus infection is considered to be the main cause of CC [1, 20]. With the development of molecular biology and understanding of tumor development, molecular targeted therapy has gradually attracted attention in many cancers, including CC [11, 28]. Therefore, studying the mechanism of CC at the molecular level is of great significance for finding effective molecular targeted therapeutic targets for CC.

Circular RNA (circRNA) is a noncoding RNA with a circular structure, and its vital regulatory role in cancer development has been shown by many studies [10, 34]. Studies have suggested that cancer malignant progression is often accompanied by the abnormal expression of circRNA, confirming that circRNA is closely related to tumorigenesis [14, 29]. In addition, circRNA has been proven to act as the

Yi Zhang and Quanshu Di contributed equally to this work.

Highlights
1. Silencing of circ_0000263 inhibits CC cell progression and tumor growth.
2. Circ_0000263 serves as miR-1179 sponge.
3. ABL2 is a target of miR-1179.

✉ Jianyun Yu
yujianyun1999@163.com

[1] Department of Gynecology, Renmin Hospital, Hubei University of Medicine, Shiyan, Hubei, China

[2] Department of Oncology, Renmin Hospital, Hubei University of Medicine, No.39 Chaoyang Road, Maojian District, Shiyan 442000, Hubei, China

[3] Department of Cardiology, Renmin Hospital, Hubei University of Medicine, Shiyan, Hubei, China

sponge for small noncoding RNA, microRNA (miRNA), to mediate the expression of downstream genes, thereby affecting cell biological functions [6, 15]. In CC-related studies, many circRNAs have been confirmed to be involved in cancer progression and are expected to become targets for CC treatment [3, 33]. Hsa_circ_101996 was discovered to promote CC proliferation and invasion through miR-8075/TPX2 pathway, and it might be a potential therapeutic target for CC [24]. Besides, studies had reported that circ_0000515 could sponge miR-326 to upregulate ELK1 in CC, and targeted inhibition of circ_0000515 had been confirmed to restrain CC proliferation and promote apoptosis [25].

Circ_0000263 is located at chr10 with a length of 491 bp and is derived from TCONS_00017720 gene. Cai *et al.* analyzed the differentially expressed circRNA in the GEO database and found that circ_0000263 was significantly overexpressed in CC cells [2]. Also, further analysis revealed that circ_0000263 might promote the proliferation and metastasis of CC [2]. These results suggested that circ_0000263 might be a potential target for the treatment of CC. Of course, more evidence is needed to support this conclusion. Our study aims to further confirm the positive role of circ_0000263 in CC development and reveal its new molecular mechanism through looking for the targeted miRNA and downstream genes for circ_0000263. Our research hopes to provide new evidence for circ_0000263 as a therapeutic target of CC.

Materials and methods

Samples collection

CC tumor tissues and adjacent normal tissues were surgically retrieved from 55 CC patients in Renmin Hospital, Hubei University of Medicine. Tissues samples were stored at -80°C. For our study, each patient signed the written informed consent, and our research was approved by the Ethics Committee of Renmin Hospital, Hubei University of Medicine.

Cell culture and transfection

Human CC cells (HeLa and SiHa) were acquired from ATCC (Rockville, MD, USA) and were cultured in DMEM (Gibco, Waltham, MA, USA). Human normal cervical epithelial cells (HcerEpic) were bought from ScienCell Research Laboratories (Carlsbad, CA, USA) and cultured in Cervical Epithelial Cell Growth Supplement (ScienCell Research Laboratories). All media were additionally added with 10% FBS (Gibco) and 1% penicillin/streptomycin (Gibco). All cells were grown at 37°C with 5% CO_2. Cell transfection was carried out using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). The small interfering RNA and lentiviral short hairpin RNA of circ_0000263 (si-circ_0000263 and sh-circ_0000263), miR-1179 mimic and inhibitor (miR-1179 and anti-miR-1179), pcDNA ABL proto-oncogene 2 (ABL2) overexpression vector (pcDNA-ABL2), ABL2 siRNA (si-ABL2) and their controls were synthesized by RiboBio (Guangzhou, China).

Quantitative real-time PCR (qRT-PCR)

Trizol reagent (Invitrogen) was used to isolate total RNA. After obtaining cDNA using Reverse Transcription Kit (Invitrogen), qRT-PCR was performed by SYBR Green Real-Time PCR Kit (Toyobo, Osaka, Japan). Data were analyzed with $2^{-\Delta\Delta Ct}$ method. In this, β-actin (for circ_0000263 and ABL2) or U6 (for miRNA) was utilized as the internal control. The primer sequences are displayed in Table 1. In subcellular localization analysis, PARIS Kit (Invitrogen) was used for isolating the nuclear and cytoplasm RNA from HeLa and SiHa cells, and then, the distribution of circ_0000263, U6 (nuclear control) and GAPDH (cytoplasm control) in the nuclear and cytoplasm was measured using qRT-PCR.

Cell counting kit 8 (CCK8) assay

After transfection, HeLa and SiHa cells were harvested and then the cell suspensions were reseeded into 96-well plates (1×10^3 cells). After overnight, cells were incubated with CCK8 solution (Beyotime, Shanghai, China) for 4 h at the specific time points. The optical density (OD) value at 450 nm was determined to assess cell viability using a microplate reader.

Table 1 Primer sequences used for qRT-PCR

Name		Primers for PCR (5'-3')
Circ_0000263	Forward	CTGTGGGTACACGAGCCTCT
	Reverse	CTGACCAGGATGCAGAGACA
miR-1179	Forward	GCCGAGAAGCATTCTTTCATT
	Reverse	CCAGTGCAGGGTCCGAGGT
ABL2	Forward	GTTGAACCCCAGGCACTAAAT
	Reverse	CAACGAAGAGATTAGGGTCACTC
GAPDH	Forward	CTCTGCTCCTCCTGTTCGAC
	Reverse	CGACCAAATCCGTTGACTCC
β-actin	Forward	CTTCGCGGGCGACGAT
	Reverse	CCACATAGGAATCCTTCTGACC
U6	Forward	CTCGCTTCGGCAGCACATA
	Reverse	CGAATTTGCGTGTCATCCT

Edu assay

5×10^3 HeLa and SiHa cells were seeded into 96-well plates and cultured for 24 h. According to the instructions of Edu Kit (RibiBio), cells were incubated with Edu reagent and then stained with DAPI solution. Fluorescence images were captured by a fluorescence microscope, and the Edu^+ cells were counted as Edu- and DAPI-merged cells using ImageJ software.

Colony formation assay

HeLa and SiHa cells (250 per well) were seeded into six-well plates and cultured for 14 days. Cell colonies were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The colony pictures were acquired, and the number of colony cells was counted under a microscope.

Western blot (WB) analysis

RIPA lysis buffer (Beyotime) was used for isolating total protein. After quantification, the protein was electrophoresed on SDS–PAGE gel and transferred onto PVDF membrane. Following blocked with 5% nonfat milk, membranes were incubated with the primary antibodies against PCNA (1:1,000, ab18197, Abcam, Cambridge, MA, USA), Ki67 (1:1,000, ab16667, Abcam), Bax (1:2,000, ab32503, Abcam), Bcl-2 (1:1,000, ab32124, Abcam), ABL2 (1:1,000, ab126256, Abcam) or β-actin (1:1,000, ab8227, Abcam). Membranes were then hatched with secondary antibody (1:50,000, ab205718, Abcam) and treated with Super Enhanced Chemiluminescence Detection Reagents (Applygen, Beijing, China). Protein expression was analyzed by ImageJ software with β-actin as a reference gene.

Flow cytometry

Annexin V-FITC Apoptosis Detection Kit was bought from Beyotime. The transfected HeLa and SiHa cells were collected, and then, the cell suspensions suspended with binding buffer were stained with Annexin V/FITC and PI. Cell apoptosis rate was examined by a flow cytometer.

Dual-luciferase reporter assay

The binding sites between miR-1179 and circ_0000263 were predicted by circBank and circInteractome software (seed sequences: CUUACGA), and binding sites between miR-1179 and ABL2 3'UTR were predicted by Starbase3.0 software (seed sequences: CUUACGA). The predicted miR-1179 binding sequences (wild type, WT) or mutant sequences (mutant type, MUT) in circ_0000263 or ABL2 3'UTR were amplified and sub-cloned into psiCHECK-2 vectors by RiboBio. HeLa and SiHa cells were co-transfected with the circ_0000263-WT/MUT or ABL2-3'UTR-WT/MUT vectors and miR-1179 mimic or miR-NC. After 48 h, cells were harvested and luciferase activity was detected with Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Xenograft models

Male BALB/c nude mice (Vital River, Beijing, China) were subcutaneously injected with SiHa cells (2×10^6 /0.2 mL PBS) transfected with sh-NC or sh-circ_0000263 (n = 6). Tumor volume was calculated every 7 d by detecting tumor length and width. After 35 d, mice were killed, and the tumor was photographed and weighed. The circ_0000263, miR-1179 and ABL2 expression in the tumor tissues was detected by qRT-PCR and WB analysis. In addition, some tumor tissues were prepared for paraffin section and then immunohistochemical (IHC) staining was performed using Ki67 antibody (1:200, ab16667, Abcam). Animal experiments were approved by the Animal Ethics Committee of Renmin Hospital, Hubei University of Medicine.

Statistical analysis

Data were expressed as mean ± SD from three independent experiments. GraphPad Prism 7.0 software was used for statistical analysis. Linear correlation was analyzed by Pearson's correlation analysis. Student's *t*-test and ANOVA followed by Tukey post hoc test or Sidak's post hoc test were adopted for analysis of different groups. $P < 0.05$ was considered as significant difference.

Results

Circ_0000263 was highly expressed in CC tumor tissues

We measured circ_0000263 expression in 55 paired CC tumor tissues and adjacent normal tissues. Our data showed that circ_0000263 was significantly upregulated in CC tumor tissues compared to adjacent normal tissues (Fig. 1A). In the tumor tissues of CC patients with different clinical stages, we found that the higher the clinical stage of CC patients, the higher the expression of circ_0000263 (Fig. 1B).

Circ_0000263 knockdown inhibited proliferation and promoted apoptosis in CC cells

The circ_0000263 expression was found to be increased in both CC cells (HeLa and SiHa) compared to HcerEpic

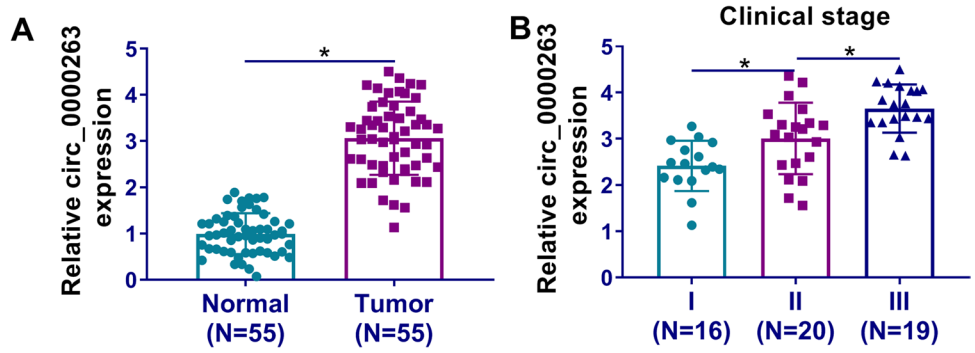

Fig. 1 The circ_0000263 expression in CC tumor tissues. (**A**) The circ_0000263 expression in CC tumor tissues and adjacent normal tissues was measured by qRT-PCR. (**B**) qRT-PCR was used to detect circ_0000263 expression in CC tumor tissues with different clinical stages. Paired t test: 1A; one-way ANOVA with Tukey's post hoc test: 1B. *$P < 0.05$.

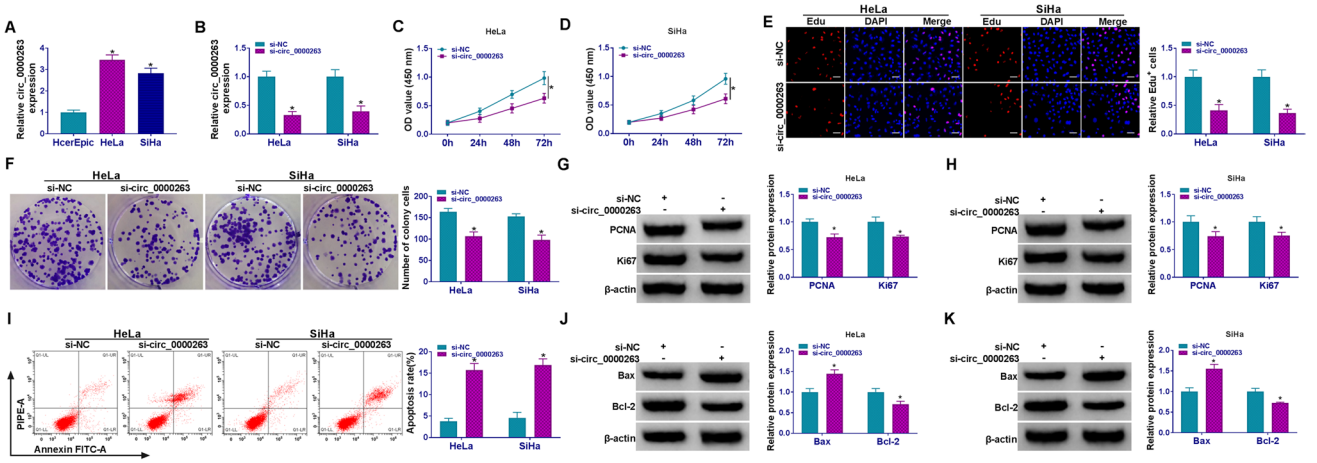

Fig. 2 Effects of si-circ_0000263 on CC cell proliferation and apoptosis. (**A**) The expression of circ_0000263 in CC cells (HeLa and SiHa) and HcerEpic cells was detected by qRT-PCR. (B-K) HeLa and SiHa cells were transfected with si-NC or si-circ_0000263. (**B**) The circ_0000263 expression was measured by qRT-PCR. CCK8 assay (**C**-**D**), Edu assay (**E**) and colony formation assay (**F**) were performed to measure cell proliferation. (**G**-**H**) The protein expression of PCNA and Ki67 was examined by WB analysis. (**I**) Cell apoptosis was determined by flow cytometry. (**J**-**K**) WB analysis was performed to measure the protein expression of Bax and Bcl-2. One-way ANOVA with Tukey's post hoc test: 2A; unpaired t test: 2B and 2E-K; two-way ANOVA with Sidak's post hoc test: 2C-D. *$P < 0.05$.

cells (Fig. 2A). To confirm the role of circ_0000263 in CC progression, we transfected with si-circ_0000263 into HeLa and SiHa cells. The detection of circ_0000263 expression showed that circ_0000263 expression was markedly reduced in HeLa and SiHa cells transfected with si-circ_0000263 (Fig. 2B). CCK8 assay, EdU assay and colony formation assay were performed to measure cell proliferation, and the results suggested that circ_0000263 knockdown significantly suppressed cell viability, Edu^+ cells and the number of colony cells (Fig. 2C-F). Not only that the protein expression of proliferation markers (PCNA and Ki67) also was decreased in HeLa and SiHa cells after circ_0000263 knockdown (Fig. 2G-H). Moreover, downregulated circ_0000263 enhanced the apoptosis rate of HeLa and SiHa cells (Fig. 2I), increased apoptosis marker Bax protein expression and reduced anti-apoptosis marker Bcl-2 protein expression (Fig. 2J-K). These data showed that circ_0000263 could promote CC proliferation and inhibit apoptosis.

MiR-1179 could be sponged by circ_0000263

Subcellular localization analysis showed that circ_0000263 was mainly distributed in the cytoplasm (Fig. 3A), which provided a necessary condition for circ_0000263 to become the sponge of miRNA. Then, the circBank and circInteractome software was used to joint predict the targeted miRNAs for circ_0000263 and a total of six miRNAs were identified as candidate miRNAs for circ_0000263 (Fig. 3B). After interfering with the expression of circ_0000263 in HeLa and SiHa cells, we found that the expression of miR-1179, miR-1265 and miR-338-3p was significantly increased, and the multiple of miR-1179 was the most obvious (Fig. 3C). So, miR-1179 was selected as the targeted miRNA for circ_0000263 in our research. According to the binding sites between circ_0000263 and miR-1179, we constructed the circ_0000263-WT/MUT vectors (Fig. 3D). MiR-1179 mimic was confirmed to increase miR-1179 expression in HeLa and SiHa cells

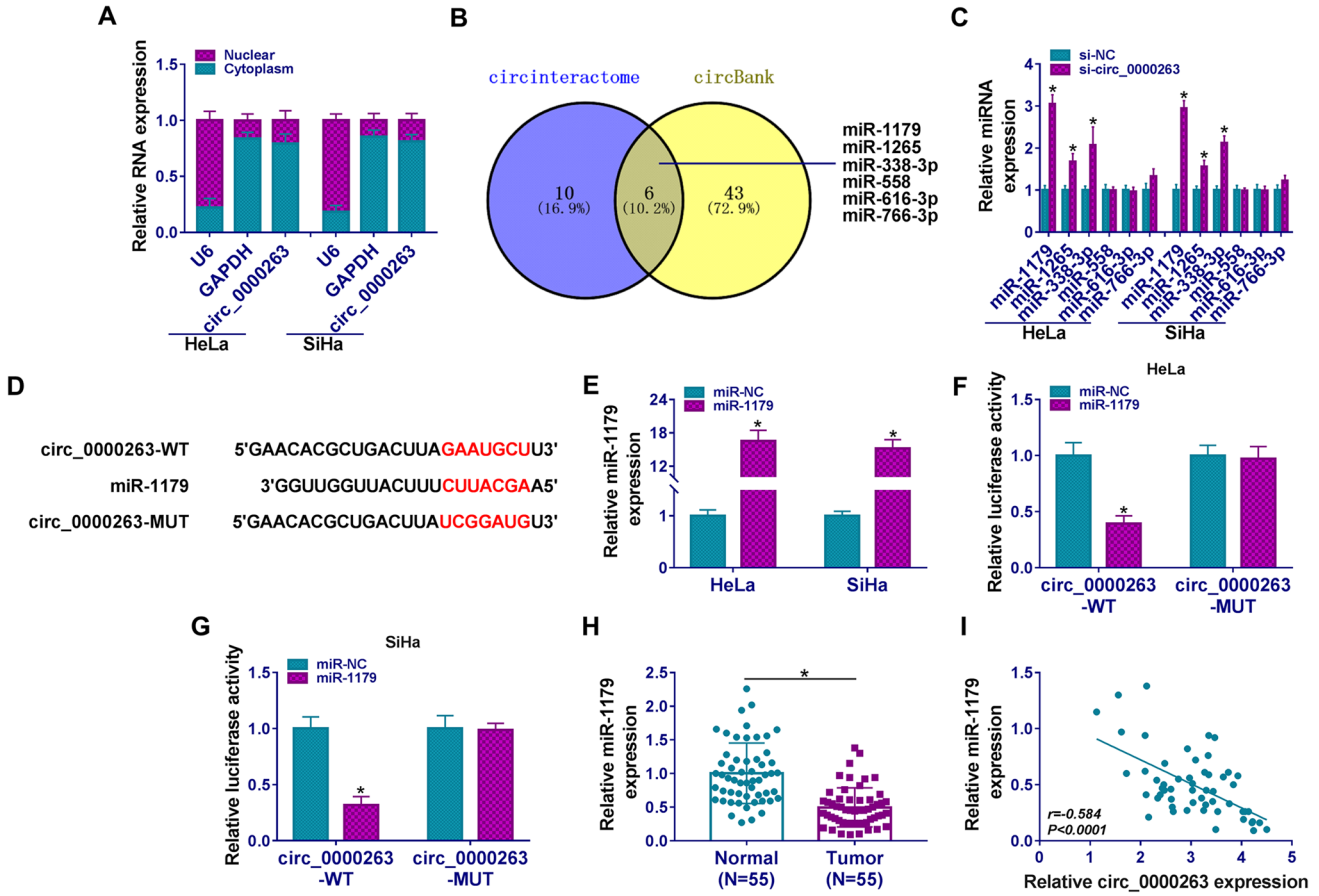

Fig. 3 Circ_0000263 sponged miR-1179. (**A**) Subcellular localization analysis was used to determine the distribution of circ_0000263 in nuclear and cytoplasm. (**B**) Venn diagram showed the targeted miRNAs of circ_0000263 predicted by circInteractome and circBank software. (**C**) The expression of candidate miRNAs was measured by qRT-PCR in HeLa and SiHa cells transfected with si-NC or si-circ_0000263. (**D**) The binding sites and mutant sites between circ_0000263 and miR-1179 were shown. (**E**) The transfection efficiency of miR-1179 mimic was confirmed by detecting miR-1179 expression using qRT-PCR. (**F-G**) Dual-luciferase reporter assay was utilized for measuring the interaction between circ_0000263 and miR-1179. (**H**) The expression of miR-1179 in CC tumor tissues and adjacent normal tissues was examined by qRT-PCR. (**I**) Pearson's correlation analysis was performed to assess the correlation between circ_0000263 expression and miR-1179 expression in CC tumor tissues. Unpaired t test: 3A and 3C; Welch's t test: 3E; two-way ANOVA with Sidak's post hoc test: 3F-G; paired t test: 3H; Pearson's correlation test: 3I. $*P < 0.05$.

(Fig. 3E). Then, the circ_0000263-WT/MUT vectors and miR-1179 mimic were co-transfected into HeLa and SiHa cells to perform dual-luciferase reporter assay. The results suggested that miR-1179 mimic inhibited the luciferase activity of circ_0000263-WT vector without affecting that of the circ_0000263-MUT vector (Fig. 3F-G). Besides, we confirmed that miR-1179 was lowly expressed in CC tumor tissues and its expression was negatively correlated with circ_0000263 expression (Fig. 3H-I). All data revealed that circ_0000263 acted as a sponge of miR-1179.

MiR-1179 inhibitor partially reversed the regulation of si-circ_0000263 on CC progression

In HeLa and SiHa cells, we found that miR-1179 also was lower than that in HcerEpic cells (Fig. 4A). To confirm whether circ_0000263 regulated CC progression by sponging miR-1179, the rescue experiments were performed. First, anti-miR-1179 indeed markedly reduced miR-1179 expression in HeLa and SiHa cells (Fig. 4B). Then, si-circ_0000263 and anti-miR-1179 were co-transfected into HeLa and SiHa cells. The results showed that miR-1179 inhibitor partially reversed the inhibition effects of circ_0000263 knockdown on cell viability, Edu^+ cells, the number of colony cells and the protein expression of PCNA

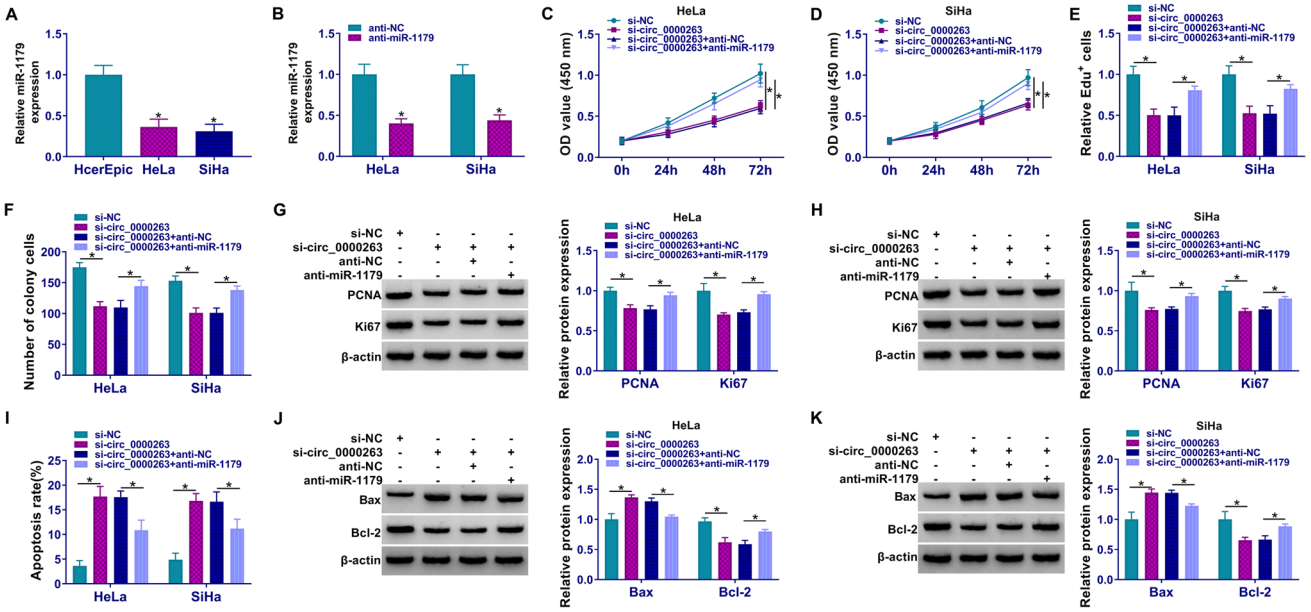

Fig. 4 Effects of si-circ_0000263 and anti-miR-1179 on CC progression. (**A**) The expression of miR-1179 in CC cells (HeLa and SiHa) and HcerEpic cells was analyzed by qRT-PCR. (**B**) The transfection efficiency of anti-miR-1179 was confirmed by measuring miR-1179 expression using qRT-PCR. (C-K) HeLa and SiHa cells were transfected with si-NC, si-circ_0000263, si-circ_0000263 + anti-NC or si-circ_0000263 + anti-miR-1179. CCK8 assay (**C**-**D**), Edu assay (**E**) and colony formation assay (**F**) were used for detecting cell proliferation. (**G**-**H**) WB analysis was performed to detect the protein expression of PCNA and Ki67. (**I**) Flow cytometry was used to measure cell apoptosis. (**J**-**K**) The protein expression of Bax and Bcl-2 was determined using WB analysis. One-way ANOVA with Tukey's post hoc test: 4A and 4E-K; unpaired t test: 4B; two-way ANOVA with Sidak's post hoc test: 4C-D.*$P < 0.05$.

and Ki67 (Fig. 4C-H). Also, the promotion of apoptosis induced by circ_0000263 downregulation also could be abolished by miR-1179 inhibitor in HeLa and SiHa cells (Fig. 4I). WB analysis results suggested that anti-miR-1179 also reversed the increasing effect of si-circ_0000263 on Bax protein expression and the decreasing effect on Bcl-2 protein expression in HeLa and SiHa cells (Fig. 4J-K). These data revealed that circ_0000263 promoted CC progression by sponging miR-1179.

ABL2 was a target of miR-1179

The Starbase3.0 software predicted that miR-1179 could bind with the 3'UTR of ABL2 (Fig. 5A). Also, dual-luciferase reporter assay was used to verify the interaction between miR-1179 and ABL2 and the results confirmed that miR-1179 mimic only could reduce the luciferase activity of ABL2 3'UTR-WT vector (Fig. 5B-C). To further assess the regulation of miR-1179 on ABL2 expression, we detected ABL2 expression in HeLa and SiHa cells transfected with miR-1179 mimic or inhibitor. Our data showed that ABL2 expression could be decreased by miR-1179 overexpression and promoted by miR-1179 inhibition at the mRNA level and protein level (Fig. 5D-E). Therefore, we confirmed that miR-1179 could target ABL2 in CC. In addition, the mRNA and protein expression of ABL2 was discovered to be upregulated in CC tumor tissues compared to adjacent normal tissues (Fig. 5F-G).

MiR-1179 inhibited CC proliferation and enhanced apoptosis by targeting ABL2

At the mRNA level and protein level, ABL2 expression was significantly increased in CC cells (HeLa and SiHa) compared with that in HcerEpic cells (Fig. 6A-B). To confirm the role of ABL2 in CC progression, si-ABL2 was constructed to silence ABL2 expression in CC cells (Supplementary Fig. 1A). We measured CC cell proliferation and apoptosis. The results indicated that ABL2 knockdown repressed cell viability, Edu^+ cells, colony numbers and the protein expression of PCNA and Ki67 (Supplementary Fig. 1B-G). Moreover, silencing of ABL2 accelerated cell apoptosis rate and Bax protein expression, but decreased Bcl-2 protein expression (Supplementary Fig. 1H-J). In this, we confirmed that ABL2 might contribute to CC progression. To reveal that miR-1179 targeted ABL2 to mediate CC progression, we performed the rescue experiments. The pcDNA-ABL2 could significantly promote ABL2 mRNA and protein expression in HeLa and SiHa cells (Fig. 6C-D). After that, miR-1179 mimic and pcDNA-ABL2 were co-transfected into HeLa and SiHa cells. Through evaluating cell proliferation, we found that miR-1179 overexpression could suppress cell viability, Edu^+ cells and the number

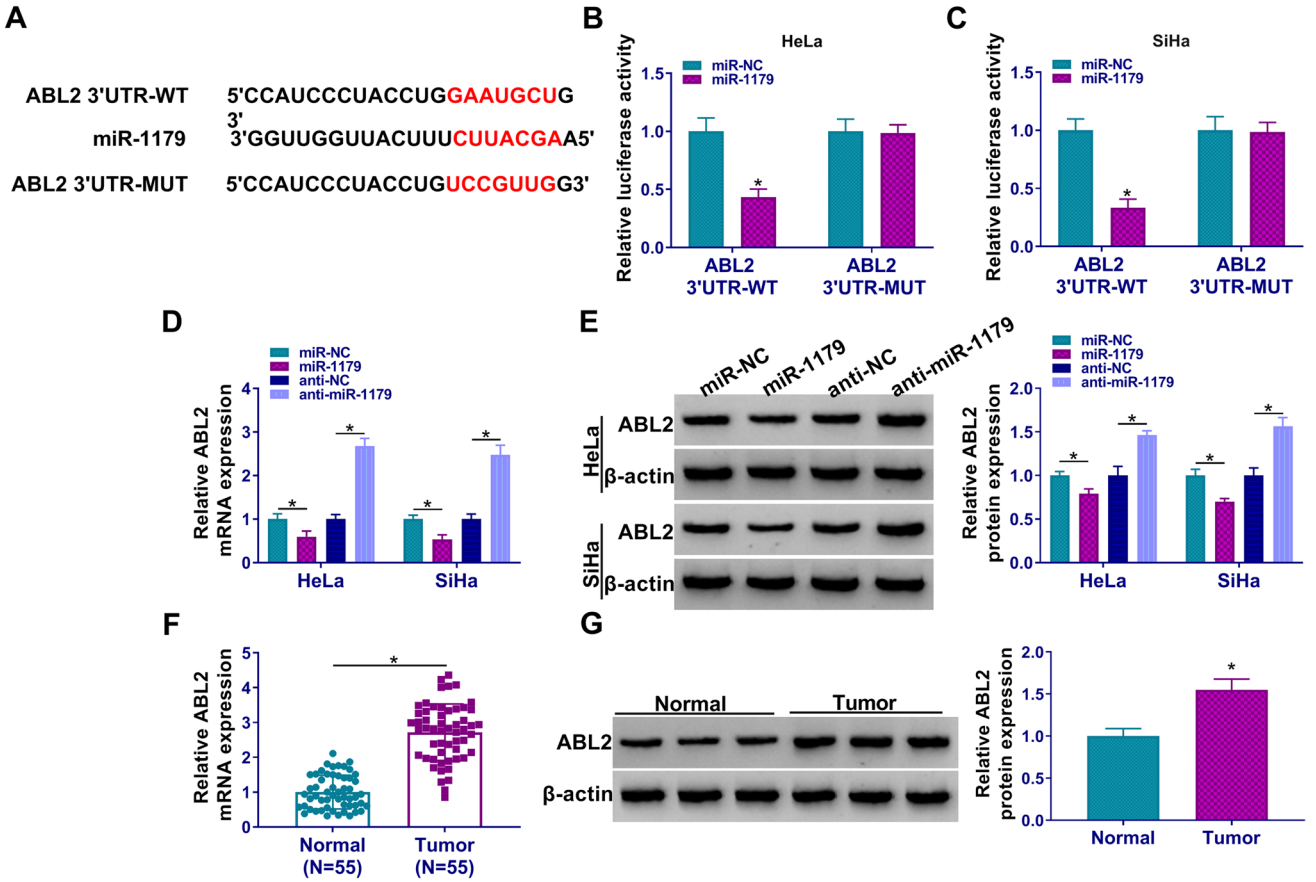

Fig. 5 ABL2 was a target of miR-1179. (**A**) The binding sites and mutant sites between ABL2 3'UTR and miR-1179 were shown. (**B-C**) Dual-luciferase reporter assay was used to evaluate the interaction between ABL2 and miR-1179. (**D-E**) qRT-PCR and WB analysis were used to measure the mRNA and protein expression of ABL2 in HeLa and SiHa cells transfected with miR-1179 mimic or inhibitor. (**F-G**) The mRNA and protein expression of ABL2 was examined by qRT-PCR and WB analysis in CC tumor tissues and adjacent normal tissues. Two-way ANOVA with Sidak's post hoc test: 5B-C; one-way ANOVA with Tukey's post hoc test: 5D-E; paired t test: 5F; unpaired t test: 5G.*$P < 0.05$.

of colony cells, as well as decrease the protein expression of PCNA and Ki67, while these effects could be abolished by overexpressing ABL2 (Fig. 6E-J). Meanwhile, miR-1179 mimic promoted cell apoptosis rate and Bax protein expression, but inhibited Bcl-2 protein expression. However, ABL2 overexpression also could reverse the pro-apoptosis effect of miR-1179 on CC cells (Fig. 6K-L). All data revealed that ABL2 participated in the regulation of miR-1179 on CC proliferation and apoptosis.

Circ_0000263 positively regulated ABL2 through sponging miR-1179

Through correlation analysis, we found that ABL2 mRNA expression was negatively correlated with miR-1179 expression and positively correlated with circ_0000263 expression in CC tumor tissues (Fig. 7A-B). To further illuminate that circ_0000263 could sponge to regulate ABL2, we detected ABL2 expression in HeLa and SiHa cells transfected with si-circ_0000263 and anti-miR-1179. Our results observed that circ_0000263 knockdown remarkably reduced the mRNA and protein expression of ABL2, while these effects were partially eliminated by miR-1179 inhibitor (Fig. 7C-D). The above data showed that circ_0000263 could regulate ABL2 by sponging miR-1179.

Interference of circ_0000263 inhibited CC tumor growth via miR-1179/ABL2 axis

Xenograft tumor was built to confirm the role of circ_0000263 in CC tumor growth *in vivo*. The results showed that the tumor volume and tumor weight in the sh-circ_0000263 group were markedly decreased compared to the sh-NC group (Fig. 8A-B). The downregulated circ_0000296 expression confirmed that circ_0000263 expression was indeed inhibited in the tumor tissues of sh-circ_0000263 group (Fig. 8C). Besides, we found that miR-1179 expression was increased, while ABL2 mRNA

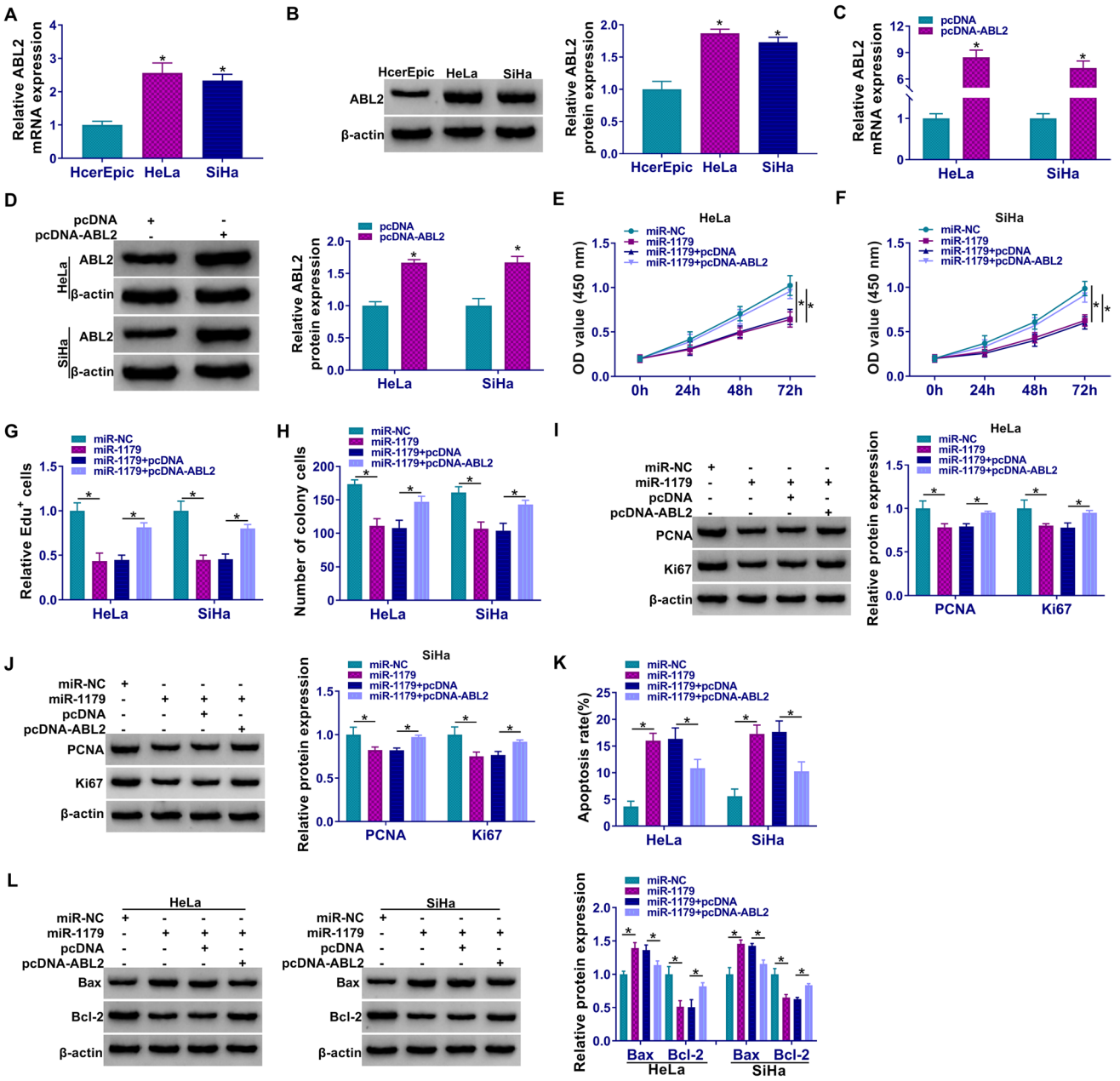

Fig. 6 Effects of miR-1179 and ABL2 on CC progression. (**A**-**B**) The mRNA and protein expression of ABL2 in CC cells (HeLa and SiHa) and HcerEpic cells was detected by qRT-PCR and WB analysis. (**C**-**D**) The transfection efficiency of pcDNA-ABL2 was confirmed by testing ABL2 mRNA and protein expression with qRT-PCR and WB analysis. (**E**-**L**) HeLa and SiHa cells were transfected with miR-NC, miR-1179, miR-1179 + pcDNA or miR-1179 + pcDNA-ABL2. Cell proliferation was determined using CCK8 assay (**E**-**F**), Edu assay (**G**) and colony formation assay (**H**). (**I**-**J**) WB analysis was used to examine the protein expression of PCNA and Ki67. (**K**) Cell apoptosis was analyzed using flow cytometry. (L) The protein expression of Bax and Bcl-2 was detected by WB analysis. One-way ANOVA with Tukey's post hoc test: 6A-B and 6G-L; Welch's t test: 6C; unpaired t test: 6D; two-way ANOVA with Sidak's post hoc test: 6E-F. *$P < 0.05$.

and protein expression was reduced in the tumor tissues of sh-circ_0000263 group (Fig. 8D-F). The results of Ki67 IHC staining revealed that the Ki67-positive cell rate in the tumor tissues of sh-circ_0000263 group was significantly lower than that in the control group (Fig. 8G). Above all, we confirmed that circ_0000263 played a tumor-promoting role in CC.

Discussion

Thanks to the rapid development of bioinformatics technology, the role of differentially expressed circRNAs in tumorigenesis has attracted great attention [4, 12]. At present, some circRNAs have been confirmed as the potential therapeutic

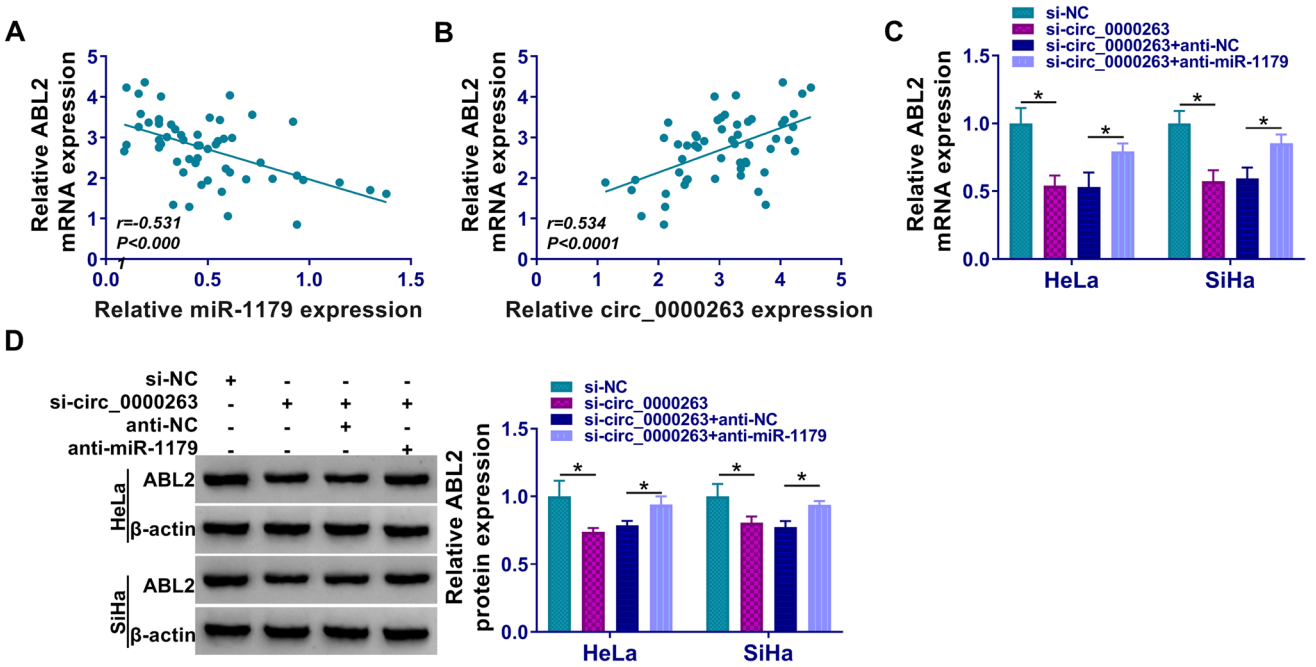

Fig. 7 Circ_0000263 regulated ABL2 by sponging miR-1179. (**A**-**B**) Pearson's correlation analysis was used to assess the correlation between ABL2 expression and miR-1179 expression or circ_0000263 expression in CC tumor tissues. (**C**-**D**) The mRNA and protein expression of ABL2 was determined by qRT-PCR and WB analysis in HeLa and SiHa cells transfected with si-circ_0000263 and anti-miR-1179. Pearson's correlation test: 7A-B; one-way ANOVA with Tukey's post hoc test: 7C-D. $^*P < 0.05$.

and prognostic targets for CC. CircRNAs that are highly expressed in CC often play a role as oncogenic genes in the progression of CC, such as circ_0000285 [5], circCLK3 [8] and circ_SLC26A4 [9]. Our research explored the role of circ_0000263, a circRNA reported to be upregulated in CC cells, in the progression of CC. Our results showed that circ_0000263 was overexpressed in CC tissues, and this high expression was related to clinical stage, tumor size and lymphoid node metastasis of CC patients. Consistent with the results of previous studies [2], we confirmed that circ_0000263 was an upregulated circRNA in CC cells, and its knockdown inhibited cell proliferation and promoted apoptosis. Moreover, silencing of circ_0000263 also showed an inhibitory effect on the growth of CC tumors. Here, the pro-cancer role of circ_0000263 had been further confirmed in CC, suggesting that circ_0000263 might be a potential target for CC treatment.

The mechanism by which circRNAs act as sponges for miRNAs has been widely demonstrated. Through subcellular localization analysis, we confirmed that circ_0000263 was mainly expressed in the cytoplasm, suggesting that circ_0000263 might affect the biological functions of CC cells at the post-transcriptional level. In combination with bioinformatics analysis and further detection, we determined that circ_0000263 could serve as a sponge for miR-1179. The anti-tumor role of miR-1179 had been confirmed in many cancers. For example, Li *et al.* showed that miR-1179 had a suppressive effect on gastric cancer proliferation [13], and Liu *et al.* reported that silenced miR-1179 could enhance the proliferation and metastasis of nasopharyngeal carcinoma [16]. Recently, the role of miR-1179 in CC had also been clarified, and it had a significant inhibitory effect on the proliferation, adhesion and migration of CC [22, 32]. According to a new study, miR-1179 was underexpressed in CC and could hinder CC invasion [18]. Here, the low expression of miR-1179 in CC tissues and cells was also confirmed in this study. MiR-1179 inhibitor reversed the anti-proliferation and pro-apoptosis effect of si-circ_0000263, indicating that circ_0000263 indeed targeted miR-1179 to mediate the malignant progression of CC. In addition, upregulated miR-1179 also showed a negatively regulation on CC proliferation, which was consistent with previous reports [18, 22, 32].

ABL2 is a cytoplasmic tyrosine kinase that regulates cell growth, metastasis and adhesion in the body [7, 26]. In general, ABL2 usually acts as a cancer-promoting gene in cancers, including gastric cancer [17], hepatocellular carcinomas [30] and renal cell carcinoma [31]. In previous

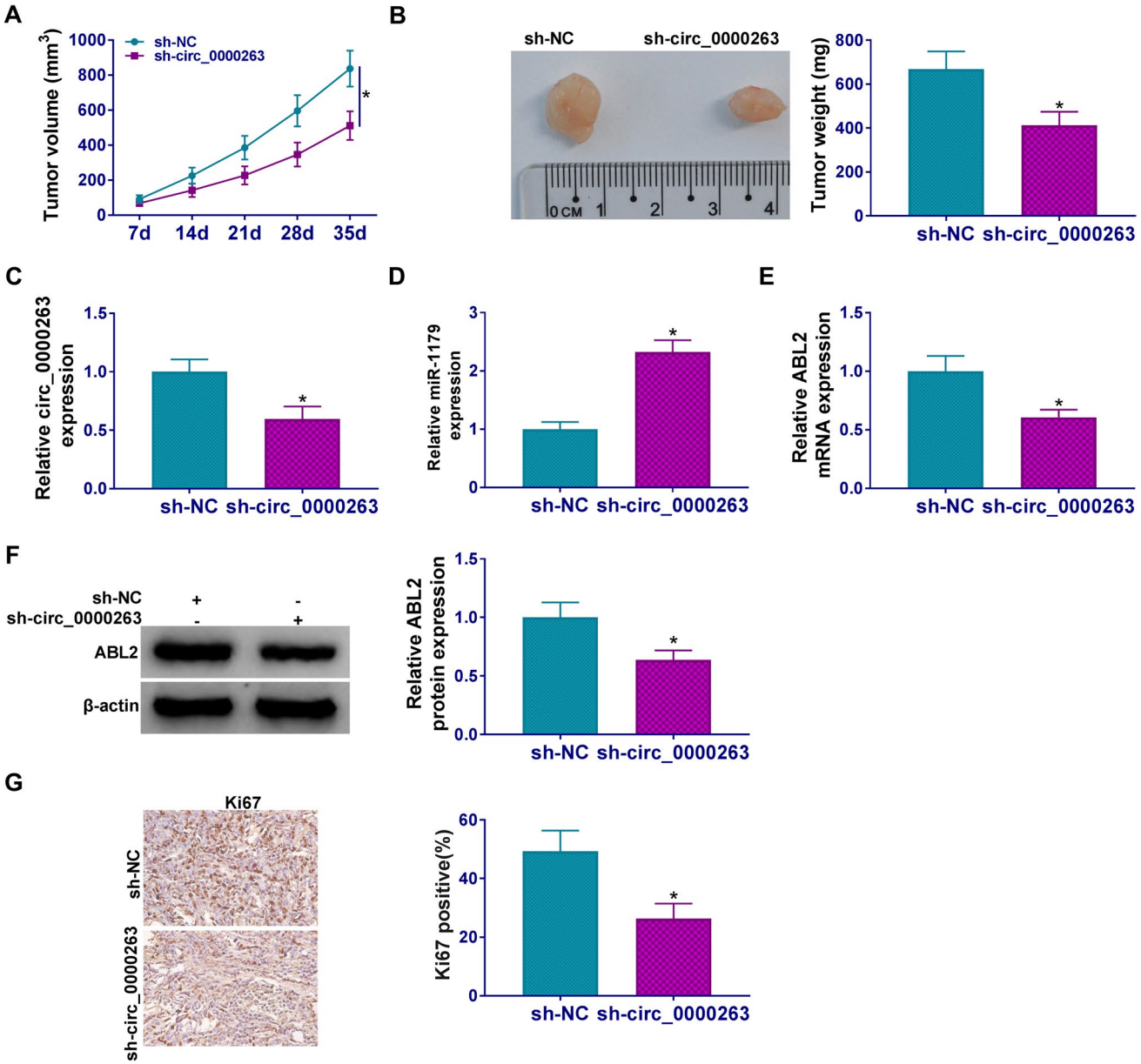

Fig. 8 Effects of sh-circ_0000263 on CC tumor growth. HeLa cells were transfected with sh-NC or sh-circ_0000263, followed by injection into nude mice. (**A**) Tumor volume was measured every 7 d. (**B**) After 35 d, tumor was photographed and weighted. (**C**-**E**) The expression of circ_0000263, miR-1179 and ABL2 in the tumor tissues was detected by qRT-PCR. (**F**) The protein expression of ABL2 in the tumor tissues was examined by WB analysis. (**G**) IHC staining was used to determine Ki67-positive cell rate in the tumor tissues. Two-way ANOVA with Sidak's post hoc test: 8A; unpaired t test: 8B-G. $^*P < 0.05$.

studies, ABL2 was discovered to be significantly overexpressed in CC, and its knockdown restrained the proliferation and migration of CC [21]. In addition, miR-214, as a tumor suppresser in CC, had been found to upregulate ABL2 expression in CC cells, confirming that ABL2 might act as oncogene role in CC [23]. In this, we put forward that ABL2 was overexpressed in CC tissues and cells, and miR-1179 could target ABL2. Knockdown of ABL2 hindered CC cell growth, confirming that ABL2 might play an pro-cancer role in CC. MiR-1179 contributed to the proliferation inhibition and apoptosis promotion in CC cells could be reversed by overexpressing ABL2, suggesting that miR-1179 inhibited CC progression through targeting ABL2. Moreover, we confirmed that circ_0000263 had a positively regulation on ABL2 expression *in vivo* and *in vitro*, which fully improved the conclusion that the circ_0000263/miR-1179/ABL2 axis mediated the progression of CC.

In conclusion, our findings elucidated that circ_0000263 promoted the malignant progression of CC through sponging miR-1179 to modulate ABL2, suggesting that circ_0000263 might be used as a target for CC therapy.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s43032-022-00920-3.

Declaration

Disclosure of interest The authors declare that they have no conflicts of interest.

Ethics approval and consent to participate Written informed consents were obtained from all participants, and this study was permitted by the Ethics Committee of Renmin Hospital, Hubei University of Medicine.

References

1. Burd EM. Human papillomavirus and cervical cancer. Clin Microbiol Rev. 2003;16(1):1–17.
2. Cai H, Zhang P, Xu M, Yan L, Liu N, Wu X. Circular RNA hsa_circ_0000263 participates in cervical cancer development by regulating target gene of miR-150-5p. J Cell Physiol. 2019;234(7):11391–400.
3. Chaichian S, Shafabakhsh R, Mirhashemi SM, Moazzami B, Asemi Z. Circular RNAs: A novel biomarker for cervical cancer. J Cell Physiol. 2020;235(2):718–24.
4. Chen L, Wang C, Sun H, Wang J, Liang Y, Wang Y, et al. The bioinformatics toolbox for circRNA discovery and analysis. Brief Bioinform. 2021;22(2):1706–28.
5. Chen RX, Liu HL, Yang LL, Kang FH, Xin LP, Huang LR, et al. Circular RNA circRNA_0000285 promotes cervical cancer development by regulating FUS. Eur Rev Med Pharmacol Sci. 2019;23(20):8771–8.
6. Dori M, Bicciato S. Integration of Bioinformatic Predictions and Experimental Data to Identify circRNA-miRNA Associations. Genes (Basel). 2019;10(9).
7. Gil-Henn H, Patsialou A, Wang Y, Warren MS, Condeelis JS, Koleske AJ. Arg/Abl2 promotes invasion and attenuates proliferation of breast cancer in vivo. Oncogene. 2013;32(21):2622–30.
8. Hong H, Zhu H, Zhao S, Wang K, Zhang N, Tian Y, et al. The novel circCLK3/miR-320a/FoxM1 axis promotes cervical cancer progression. Cell Death Dis. 2019;10(12):950.
9. Ji F, Du R, Chen T, Zhang M, Zhu Y, Luo X, et al. Circular RNA circSLC26A4 Accelerates Cervical Cancer Progression via miR-1287-5p/HOXA7 Axis. Mol Ther Nucleic Acids. 2020;19:413–20.
10. Kristensen LS, Hansen TB, Veno MT, Kjems J. Circular RNAs in cancer: opportunities and challenges in the field. Oncogene. 2018;37(5):555–65.
11. Kumar L, Harish P, Malik PS, Khurana S. Chemotherapy and targeted therapy in the management of cervical cancer. Curr Probl Cancer. 2018;42(2):120–8.
12. Li S, Teng S, Xu J, Su G, Zhang Y, Zhao J, et al. Microarray is an efficient tool for circRNA profiling. Brief Bioinform. 2019;20(4):1420–33.
13. Li Y, Qin C. MiR-1179 inhibits the proliferation of gastric cancer cells by targeting HMGB1. Hum Cell. 2019;32(3):352–9.
14. Liang HF, Zhang XZ, Liu BG, Jia GT, Li WL. Circular RNA circ-ABCB10 promotes breast cancer proliferation and progression through sponging miR-1271. Am J Cancer Res. 2017;7(7):1566–76.
15. Lin X, Chen Y. Identification of Potentially Functional CircRNA-miRNA-mRNA Regulatory Network in Hepatocellular Carcinoma by Integrated Microarray Analysis. Med Sci Monit Basic Res. 2018;24:70–8.
16. Liu D, Wang Y, Zhao Y, Gu X. LncRNA SNHG5 promotes nasopharyngeal carcinoma progression by regulating miR-1179/HMGB3 axis. BMC Cancer. 2020;20(1):178.
17. Liu Y, Shao C, Zhu L, Jiang S, Li G, Zhang W, et al. High Expression of ABL2 Suppresses Apoptosis in Gastric Cancer. Dig Dis Sci. 2018;63(9):2294–300.
18. Lv F, Zhong Y, Sang L, Wu X. MiR-1179 is downregulated in cervical cancer and its overexpression suppresses cancer cells invasion by targeting CHAF1A/ZEB1. Acta Biochim Pol. 2021;68(2):193–9.
19. Moore DH. Cervical cancer. Obstet Gynecol. 2006;107(5):1152–61.
20. Olusola P, Banerjee HN, Philley JV, Dasgupta S. Human Papilloma Virus-Associated Cervical Cancer and Health Disparities. Cells. 2019;8(6).
21. Qin X, Wan Y, Wang S, Xue M. MicroRNA-125a-5p modulates human cervical carcinoma proliferation and migration by targeting ABL2. Drug Des Dev Ther. 2016;10:71–9.
22. Qu X, Zhu L, Song L, Liu S. circ_0084927 promotes cervical carcinogenesis by sponging miR-1179 that suppresses CDK2, a cell cycle-related gene. Cancer Cell Int. 2020;20:333.
23. Sen P, Ghosal S, Hazra R, Arega S, Mohanty R, Kulkarni KK, et al. Transcriptomic analyses of gene expression by CRISPR knockout of miR-214 in cervical cancer cells. Genomics. 2020;112(2):1490–9.
24. Song T, Xu A, Zhang Z, Gao F, Zhao L, Chen X, et al. CircRNA hsa_circRNA_101996 increases cervical cancer proliferation and invasion through activating TPX2 expression by restraining miR-8075. J Cell Physiol. 2019;234(8):14296–305.
25. Tang Q, Chen Z, Zhao L, Xu H. Circular RNA hsa_circ_0000515 acts as a miR-326 sponge to promote cervical cancer progression through up-regulation of ELK1. Aging (Albany NY). 2019;11(22):9982–99.
26. Tripathi R, Liu Z, Plattner R. EnABLing Tumor Growth and Progression: Recent progress in unraveling the functions of ABL kinases in solid tumor cells. Curr Pharmacol Rep. 2018;4(5):367–79.
27. Tsikouras P, Zervoudis S, Manav B, Tomara E, Iatrakis G, Romanidis C, et al. Cervical cancer: screening, diagnosis and staging. J Buon. 2016;21(2):320–5.
28. Vora C, Gupta S. Targeted therapy in cervical cancer. ESMO Open. 2018;3(Suppl 1):e000462.
29. Wang C, Tan S, Liu WR, Lei Q, Qiao W, Wu Y, et al. RNA-Seq profiling of circular RNA in human lung adenocarcinoma and squamous cell carcinoma. Mol Cancer. 2019;18(1):134.
30. Xing QT, Qu CM, Wang G. Overexpression of Abl2 predicts poor prognosis in hepatocellular carcinomas and is associated with cancer cell migration and invasion. Onco Targets Ther. 2014;7:881–5.
31. Xu B, Wang C, Wang YL, Chen SQ, Wu JP, Zhu WD, et al. miR-143 inhibits renal cell carcinoma cells metastatic potential by suppressing ABL2. Kaohsiung J Med Sci. 2020;36(8):592–8.
32. Yang S, Jiang Y, Ren X, Feng D, Zhang L, He D, et al. FOXA1-induced circOSBPL10 potentiates cervical cancer cell proliferation and migration through miR-1179/UBE2Q1 axis. Cancer Cell Int. 2020;20:389.
33. Yi Y, Liu Y, Wu W, Wu K, Zhang W. Reconstruction and analysis of circRNAmiRNAmRNA network in the pathology of cervical cancer. Oncol Rep. 2019;41(4):2209–25.
34. Zhang HD, Jiang LH, Sun DW, Hou JC, Ji ZL. CircRNA: a novel type of biomarker for cancer. Breast Cancer. 2018;25(1):1–7.