CircSEC24A promotes colorectal cancer progression by regulating miR-488-3p/TMEM106B axis

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Research

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Abstract

Background: Currently, more and more circular RNAs (circRNAs) have been identified to exert their functions in tumor progression, including colorectal cancer (CRC). However, the role of circSEC24A (circ_0003528) in CRC remains unknown.

Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was conducted to determine the levels of circSEC24A, SEC24A and microRNA-488-3p (miR-488-3p). The characterization of circSEC24A was investigated by Actinomycin D and RNase R digestion assays. 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) assay was used to assess cell proliferation. Flow cytometry analysis was adopted for cell apoptosis and cell cycle process. Transwell assay was employed to evaluate cell migration and invasion. Western blot assay was performed to determine protein levels. Dual-luciferase reporter assay was utilized to explore the relationship between miR-488-3p and circSEC24A or transmembrane protein 106B (TMEM106B). Murine xenograft model was constructed to explore the effect of circSEC24A in vivo .

Results: CircSEC24A level was increased in CRC tissues and cells. CircSEC24A deficiency impeded cell proliferation, cell cycle process, migration and invasion and induced apoptosis in CRC cells in vitro and blocked tumorigenesis in vivo . MiR-488-3p was a target of circSEC24A and miR-488-3p was downregulated in CRC tissues and cells. The inhibitory effect of circSEC24A silencing on CRC cell progression was restored by miR-488-3p inhibition. Moreover, TMEM106B could be negatively regulated by miR-488-3p via acting as a downstream gene of miR-488-3p. MiR-488-3p overexpression decelerated CRC cell progression by targeting TMEM106B.

Conclusion: CircSEC24A facilitated CRC progression by regulating miR-488-3p/TMEM106B axis, which might provide a promising treatment approach for CRC.

Introduction

With the improvement of people's living standards and the change of dietary structures, the incidence of colorectal cancer (CRC) has been on the rise and now ranks third among malignant tumors [1]. Although much progress has been made, the mortality of patients with CRC remains high [2]. Moreover, the overall survival rate of CRC patients is very poor [3]. Thus, there is a pressing need to elucidate the underlying mechanism of CRC and explore effect therapy targets for CRC.

As a group of novel identified non-coding RNAs (ncRNAs), circular RNAs harbor a covalently closed loop and have been revealed to function as essential regulators in human cancers, including CRC [4, 5]. For example, circ_0001178 played a promotional role in CRC metastasis [6]. CircIFT80 level was notably raised in CRC and contributed to CRC cell growth in vitro and tumorigenesis in vivo [7]. On the contrary, circ_0026344 overexpression hampered CRC cell metastasis and epithelial-mesenchymal transition (EMT) process [8]. Circ_0021977 repressed cell growth and motility in CRC cells [9]. All these reports indicated that circRNAs played different roles in CRC progression. Even so, the investigation on the functions of circRNAs in CRC remains largely unknown and the roles of circSEC24A (circ_0003528) in CRC have not been reported.

MicroRNAs (miRNAs) are a series of ncRNAs (~ 22 nucleotides), which can interact with the 3' untranslated region (3'UTR) of target mRNAs to modulate target gene expression [10]. It has been documented that miRNAs play a vital role in tumor progression [11]. For instance, miR-19a could facilitate CRC cell growth and migration by binding to TIA1 [12]. MiR-195 overexpression restrained CRC cell viability and induced cell cycle arrest by interacting with FGF2 [13]. MiR-495 repressed cell growth and motility and promoted apoptosis in CRC cells via targeting FAM84D [14]. MiR-488-3p acted as an anti-tumor regulator in some human cancers, such as glioma [15], retinoblastoma [16] and non-small cell lung cancer (NSCLC) [17]. Nevertheless, the effects of miR-488-3p in CRC are not fully understood. In CRC, Ren et al. have pointed out that transmembrane protein 106B (TMEM106B) can be targeted by miR-3163 to participate in the progression of CRC [18]. Nonetheless, whether miR-488-5p can target TMEM106B to take part in CRC development has not been documented yet.

In this article, we determined the expression patterns of circSEC24A, miR-488-3p and TMEM106B in CRC. Moreover, we investigated the roles and underlying mechanisms of circSEC24A in CRC progression.

Materials And Methods

Tissues collection

50 CRC tissues and adjacent normal tissues were collected from patients during operation at Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine. The patients have not received any treatment prior to the study. The specimens were preserved at -80°C before use.

Cell culture

Two CRC cell lines (SW480 and SW620) were bought from the American Type Culture Collection (ATCC, Manassas, VA, USA) and one normal human colon mucosal epithelial cell line (NCM460) was bought from Beina Biotechnology Co., Ltd. (Beijing, China). These cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS; Gibco) at 37°C in a humidified atmosphere consisting of 5% CO₂.

Quantitative real-time polymerase chain reaction (qRT-PCR)

The extraction of total RNA in tissues and cells was performed with RNAiso Plus kit (Takara, Dalian, China). After total RNA was examined on a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), M-MLV Reverse Transcriptase Kit (Promega, Madison, WI, USA) or miRNA 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China) was adopted for reverse transcription experiment. Next, qRT-PCR was conducted on an ABI 7900 PCR system (Applied Biosystems, Foster City, CA, USA) using AceQ Universal SYBR qPCR Master Mix (Vazyme). The relative expression was examined by the $2^{-\Delta\Delta Ct}$ method. U6 or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was chosen as the internal control. The primers sequences were: circSEC24A: (F: 5'-GCTCTCCTTAAACAGGATATACACA-3' and R: 5'-TGTCCTACTGAGAAGGAATAAGTCA-3'); SEC24A: (F: 5'-GGCCACAAGCCTTTACTCAG-3' and R: 5'-CTAGTCAGCGTGCATCGAAA-3'); miR-488-3p: (F: 5'-CGGGGCAGCUCAGUACAG-3' and R: 5'-CAGTGCGTGTCTGGAGT-3'); TMEM106B: (F: 5'-GGAGGAACCGGCAATTGATGGGAAAGTCTCTTTCTCACTTACC-3' and R: 5'-GGCGGGCCTCCTCGAGTTATTGTTGTGGCTGAAGGACATT-3'); GAPDH: (F: 5'-TGTTGCCATCAATGACCCCTT-3'

and R: 5'-CTCCACGACGTACTCAGCG-3'); U6: (F: 5'-CTCGCTTCGGCAGCACATATACTA-3' and R: 5'-ACGAATTTGCGTGTCATCCTTGCG-3').

Actinomycin D assay

To block transcription, 2 µg/mL Actinomycin D (Abcam, Cambridge, MA, USA) was added into the cell culture medium. After being treated for 0 h, 4 h, 8 h, 12 h and 24 h, total RNA in SW480 and SW620 cells was isolated and the abundance of circSEC24A and linear SEC24A was measured by qRT-PCR assay.

RNase R digestion assay

10 µg total RNA extracted from SW480 and SW620 cells was digested with or without RNase R (20 mg/mL; Solarbio, Beijing, China) at 37°C for 20 min. Thereafter, qRT-PCR was adopted to test the levels of circSEC24A and linear SEC24A.

Cell transfection

Small interfering RNA against circSEC24A (si-circSEC24A), miR-488-3p mimics (miR-488-3p), miR-488-3p inhibitors (anti-miR-488-3p), the overexpression vector of TMEM106B (TMEM106B), short hairpin RNA targeting circSEC24A (sh-circSEC24A) and their corresponding controls (si-NC, miR-NC, anti-miR-NC, vector and sh-NC) were synthesized by GeneCopoeia (Guangzhou, China). The transfection of SW480 and SW620 cells was conducted with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) assay

MTT assay was employed to assess the proliferation of SW480 and SW620 cells. In brief, transfected cells were plated into 96-well plates (1×10^3 cells/well). Next, 20 µL MTT (5 mg/mL; Solarbio) was added into each well after 0 h, 24 h, 48 h and 72 h of incubation. After being kept for another 4 h, 150 µL dimethyl sulfoxide (DMSO; Solarbio) was put into the well for the dissolution of formazan crystals. Finally, the optical density at 490 nm was monitored by a microplate reader (BioTek, Winooski, VT, USA).

Western blot assay

Total RNA was obtained from tissues and cells by using RIPA buffer (Beyotime, Shanghai, China) and measured using a BCA protein assay kit (Tiangen, Beijing, China). Then 20 µg proteins were resolved by 10% sodium dodecyl sulfonate-polyacrylamide gel (Solarbio) and then transferred onto polyvinylidene difluoride membranes (Pall Corporation, New York, NYC, USA). Next, the samples were immersed with 5% skim milk for 1 h and incubated with primary antibodies: cyclin D1 (ab16663; Abcam), proliferating cell nuclear antigen (PCNA; ab92552; Abcam), TMEM106B (ab264323; Abcam) or GAPDH (ab181602; Abcam) at 4°C overnight followed by incubation with relevant secondary antibody (ab205719; Abcam) for 2 h at room temperature. Finally, an enhanced chemiluminescence kit (Vazyme) was utilized to visualize the blots.

Flow cytometry analysis

For the analysis of cell apoptosis, Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) Apoptosis Detection Kit (Beyotime) was used. After 48 h of transfection, SW480 and SW620 cells were

harvested and stained with 5 μ L AnnexinV-FITC (Beyotime) and 10 μ L PI (Beyotime) for 15 min in the dark. Next, a flow cytometer (BD Biosciences, San Jose, CA, USA) was adopted to examine the percentage of apoptotic cells.

For the analysis of cell cycle, transfected SW480 and SW620 cells were collected, washed in PBS (Solarbio) and fixed in 70% ethanol overnight at 4°C. Then the ethanol was removed, cells were washed again and resuspended in PI (Beyotime) containing RNase (Solarbio) for 30 min at room temperature followed by detection with a flow cytometer (BD Biosciences).

Transwell assay

The transwell chambers (Corning, Corning, NY, USA) pre-coated with Matrigel (Corning) were used to determine cell invasion, while the chambers without Matrigel (Corning) were used to determine cell migration. Briefly, transfected SW480 and SW620 cells were harvested and fixed in serum-free medium and then seeded into the upper compartments. The bottom compartments were filled with 500 μ L DMEM (Gibco) including 10% FBS (Gibco). After 48 h, migrated or invaded cells were fixed with 4% formaldehyde (Sangon, Shanghai, China), dyed with 1% crystal violet (Sangon) and then counted under a microscope (Olympus, Tokyo, Japan).

Dual-luciferase reporter assay

The circSEC24A or TMEM106B 3'UTR sequences containing the wild-type or mutant binding sequences of miR-488-3p were cloned and inserted into pmirGLO vector (Promega) to create luciferase reporter vectors: circSEC24A WT, circSEC24A MUT, TMEM106B 3'UTR WT and TMEM106B 3'UTR MUT. Then miR-488-3p or miR-NC was transfected into SW480 and SW620 cells together with corresponding luciferase reporter vectors. 48 h later, the luciferase activity was measured by Dual-Luciferase Reporter Assay Kit (Promega).

Murine xenograft model

4-6 weeks old BALB/c nude mice were bought from Shanghai SLAC Laboratory Animal Co., Ltd, (Shanghai, China) and assigned to 3 groups (6 mice/group). Lentiviral vector for the silencing of circSEC24A (sh-circSEC24A) or its control sh-NC was transfected into SW480 and SW620 cells. Then transfected cells were subcutaneously injected into the right flank of the mice. Tumor volume was monitored every 5 days and calculated with the equation: $(\text{Length} \times \text{Width}^2)/2$. After 30 days of injection, the mice were euthanized and tumors were weighed and saved at -80°C for further experiments.

Statistical analysis

The data were collected from three independent experiments and analyzed using GraphPad Prism 7 (GraphPad Inc., La Jolla, CA, USA). The data were displayed as mean $\pm$ standard deviation. Student's *t*-test or one-way analysis of variance (ANOVA) was utilized to estimate the difference. If $P < 0.05$, the differences were defined as significant.

Results

High expression of circSEC24A in CRC tissues and cells

By searching GEO database (<https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE126094>), we found that circSEC24A was upregulated in 10 CRC tissues compared to corresponding normal tissues (Figure 1A). CircSEC24A derived from the Exons (14-18) of SEC24A gene, and its mature length after splicing is 741 bp (Figure 1B). Then to investigate the effect of circSEC24A in CRC development, we determined the expression level of circSEC24A in 50 CRC tissues and adjacent normal tissues by qRT-PCR assay. The data showed that circSEC24A was highly expressed in CRC tissues relative to normal tissues (Figure 1C). Similarly, the level of circSEC24A was notably elevated in SW480 and SW620 cells compared to NCM460 cells (Figure 1D). Subsequently, the stability of circSEC24A was evaluated by Actinomycin D assay. The data exhibited that the half-life of circSEC24A was longer than linear SEC24A in both SW480 and SW620 cells, indicating that circSEC24A was more stable than linear SEC24A (Figure 1E and F). RNase R digestion assay showed that circSEC24A was resistant to RNase R, while linear SEC24A was digested by RNase R in SW480 and SW620 cells, indicating that circSEC24A possessed a loop structure (Figure 1G and H). Collectively, we hypothesized that the abnormal expression of circSEC24A might be involved in the progression of CRC.

CircSEC24A knockdown suppressed cell proliferation, migration and invasion and promoted cell apoptosis and cell cycle arrest in CRC cells

In order to explore the biological function of circSEC24A in CRC, we established circSEC24A-knockdown SW480 and SW620 cells by transfecting si-circSEC24A into SW480 and SW620 cells. As presented in Figure 2A and B, the expression of circSEC24A, but not linear SEC24A, was effectively decreased in SW480 and SW620 cells after si-circSEC24A transfection compared to si-NC transfected cells. MTT assay indicated that the proliferation of SW480 and SW620 cells was markedly repressed after circSEC24A knockdown compared to control groups (Figure 2C and D). Then we determined the protein levels of cyclin D1 and PCNA in SW480 and SW620 cells. The results of western blot assay displayed that circSEC24A silencing apparently reduced the levels of cyclin D1 and PCNA in SW480 and SW620 cells (Figure 2E and F). Flow cytometry analysis showed that cell apoptosis was drastically induced in SW480 and SW620 cells transfected with si-circSEC24A in comparison with cells transfected with si-NC (Figure 2G and H). The data of flow cytometry analysis also showed that the percents of SW480 and SW620 cells in G1/G0 phase were increased and the percents of SW480 and SW620 cells in S phase were decreased after circSEC24A deficiency, indicating that cell cycle was arrest after circSEC24A silencing (Figure 2I and J). As demonstrated by transwell assay, silencing of circSEC24A obviously inhibited cell migration and invasion in SW480 and SW620 cells compared to si-NC control groups (Figure 2K and L). Taken together, circSEC24A knockdown could decelerate CRC cell progression.

CircSEC24A sponged miR-488-3p in CRC cells

To explore the potential molecular mechanism of circSEC24A in regulating CRC progression, bioinformatics software starBase was utilized to search the potential target of circSEC24A. MiR-488-3p was predicted to be a target of circSEC24A and their potential binding sites were shown in Figure 3A. Then we performed dual-luciferase reporter assay to verify this prediction. The results displayed that miR-488-3p markedly reduced the luciferase activity of circSEC24A WT vector in SW480 and SW620 cells, but did not affect the luciferase activity of circSEC24A MUT vector (Figure 3B and C). Next, to further investigate the regulatory relationship between miR-488-3p and circSEC24A, SW480 and SW620 cells were transfected with si-NC or si-circSEC24A.

The qRT-PCR data showed that circSEC24A knockdown led to a remarkable upregulation of miR-488-3p expression in SW480 and SW620 cells (Figure 3D). Thereafter, miR-488-3p expression in CRC tissues and cells was examined by qRT-PCR assay. The results showed that compared to normal tissues and cells, miR-488-3p level was strikingly diminished in CRC tissues and cells (Figure 3E and F). To sum up, circSEC24A negatively modulated miR-488-3p expression via acting as a sponge of miR-488-3p in CRC cells.

The effects of circSEC24A knockdown on cell proliferation, apoptosis, cell cycle process, migration and invasion were ameliorated by miR-488-3p inhibition in CRC cells

Based on the above results, we then explored whether circSEC24A promoted CRC cell progression by sponging miR-488-3p. SW480 and SW620 cells were transfected with si-NC, si-circSEC24A, si-circSEC24A+anti-miR-NC or si-circSEC24A+anti-miR-488-3p. The transfection efficiency was determined by qRT-PCR. The data showed that anti-miR-488-3p transfection effectively restored the upregulation of miR-488-3p caused by si-circSEC24A transfection in both SW480 and SW620 cells (Figure 4A and B). MTT assay indicated that the suppressive role of circSEC24A knockdown in cell proliferation was rescued by miR-488-3p inhibition in SW480 and SW620 cells (Figure 4C and D). Our results of western blot assay showed miR-488-3p deletion partly reversed the inhibitory effects of circSEC24A silencing on the levels of cyclin D1 and PCNA in SW480 and SW620 cells (Figure 4E and F). As illustrated by flow cytometry analysis, miR-488-3p inhibition partially overturned the promotional effect on cell apoptosis and the inhibitory effect on cell cycle process mediated by circSEC24A deficiency in SW480 and SW620 cells (Figure 4G-J). Besides, transwell assay revealed that circSEC24A knockdown conspicuously suppressed the migration and invasion of SW480 and SW620 cells, whereas miR-488-3p deletion weakened the impacts (Figure 4K-N). All these results suggested that circSEC24A facilitated CRC cell progression by binding to miR-488-3p.

TMEM106B was a downstream gene of miR-488-3p in CRC cells

By further searching software starBase, we found that TMEM106B might be a target gene of miR-488-3p (Figure 5A). Dual-luciferase reporter assay showed that the luciferase activity in SW480 and SW620 cells co-transfected with miR-488-3p and TMEM106B 3'UTR WT was distinctly suppressed compared to that in miR-NC and TMEM106B 3'UTR WT co-transfected cells, while no change was observed in TMEM106B 3'UTR MUT groups, confirming the combination between miR-488-3p and TMEM106B (Figure 5B and C). Then miR-NC or miR-488-3p was transfected into SW480 and SW620 cells and qRT-PCR results indicated that TMEM106B protein level was markedly decreased following miR-488-3p transfection (Figure 5D). Moreover, when circSEC24A was silenced, the protein level of TMEM106B was decreased in both SW480 and SW620 cells, whereas miR-488-3p inhibition attenuated this effect (Figure 5E). Besides, the protein level of TMEM106B was apparently raised in CRC tissues and cells when compared to normal tissues and cells (Figure 5F and G). These data demonstrated that circSEC24A positively regulated TMEM106B expression by sponging miR-488-3p.

MiR-488-3p overexpression suppressed CRC cell progression by targeting TMEM106B

In order to determine the effects of miR-488-3p and TMEM106B on the behaviors of CRC cells, we divided SW480 and SW620 cells into 4 groups: miR-NC, miR-488-3p, miR-488-3p+vector and miR-488-3p+TMEM106B. As we observed in Figure 6A and B, miR-488-3p transfection notably decreased the protein level of TMEM106B

in both SW480 and SW620 cells, whereas TMEM106B transfection significantly restored the decrease. As illustrated by MTT assay, the inhibitory effect on cell proliferation mediated by miR-488-3p was abolished by the elevation of TMEM106B in SW480 and SW620 cells (Figure 6C and D). The results of western blot assay showed that miR-488-3p overexpression drastically reduced the levels of cyclin D1 and PCNA in SW480 and SW620 cells, whereas the upregulation of TMEM106B partly restored the impacts (Figure 6E and F). Flow cytometry analysis indicated that miR-488-3p overexpression led to a marked promotion in cell apoptosis and a noteworthy suppression in cell cycle process in SW480 and SW620 cells, but the impacts were abrogated by TMEM106B elevation (Figure 6G-J). Transwell test data implied that the migration and invasion of SW480 and SW620 cells were repressed by miR-488-3p, while TMEM106B overexpression partly rescued the effects (Figure 6K and L). Taken together, miR-488-3p overexpression suppressed cell proliferation, migration and invasion and promoted cell apoptosis and cell cycle arrest in CRC cells by interacting with TMEM106B.

CircSEC24A knockdown hampered tumorigenesis of CRC *in vivo*

Subsequently, we explored the effect of circSEC24A on tumor growth *in vivo* by injecting sh-NC or sh-circSEC24A transfected SW480 and SW620 cells into the nude mice. As shown in Figure 7A and B, tumor volume and weight were all notably blocked by the knockdown of circSEC24A. Then the expression levels of circSEC24A, miR-488-3p and TMEM106B in the tissues collected from the mice were measured. The data indicated that the levels of circSEC24A and TMEM106B protein were reduced and the level of miR-488-3p was elevated in sh-circSEC24A groups compared to sh-NC groups (Figure 7C and D). Thus, we concluded that circSEC24A contributed to tumor growth of CRC *in vivo*.

Discussion

In this research, we proved the carcinogenic role of circSEC24A in CRC development. Our data indicated that circSEC24A was evidently increased in CRC tissues and cells in comparison with normal controls. Loss-of-function experiments showed that circSEC24A deficiency impeded CRC cell growth, cell cycle process and metastasis and facilitated apoptosis. Moreover, a novel regulatory network circSEC24A/miR-488-3p/TMEM106B axis was established in regulating CRC development.

Currently, some studies have verified that circRNAs exert their tumorigenic or tumor-suppressive role in CRC. For example, circ_0020397 accelerated CRC cell viability and invasion and hindered apoptosis by sponging miR-138 [19]. Circ_103809 was weakly expressed in CRC and its elevation suppressed CRC cell growth, cell cycle process and metastasis and facilitated apoptosis via targeting miR-532-3p [20]. As a novel identified circRNA, circ_0003528 has been demonstrated to be upregulated in tuberculosis patients [21, 22]. However, the effects of circSEC24A in CRC have not been addressed. Here, we observed that compared to corresponding controls, circSEC24A level was markedly raised in CRC tissues and cells. Moreover, we demonstrated the oncogenic properties of circSEC24A in CRC. Our results showed that circSEC24A silencing led to a noteworthy suppression in CRC cell growth, cell cycle process and metastasis and a remarkable promotion in apoptosis *in vitro*. Murine assay confirmed that circSEC24A could block the tumorigenesis of CRC *in vivo*. All these data suggested that circSEC24A played a carcinogenic role in CRC.

It's worth noting that circRNAs can exert their function via working as miRNA sponges [23]. Through bioinformatics analysis and dual-luciferase reporter assay, the direct combination between circSEC24A and miR-488-3p was proved. Yang et al. implicated that miR-488-3p was lowly expressed in esophageal squamous cell carcinoma (ESCC) and its overexpression suppressed ESCC cell growth and promoted apoptosis by targeting ZBTB2 [24]. Wan et al. manifested that circ_0020123 contributed to cell growth and motility and impeded apoptosis in NSCLC cells via miR-488-3p/ADAM9 axis [17]. Furthermore, in CRC, Yao et al. unraveled that miR-488 was reduced in CRC and its inhibition could target PHF8 to increase CRC cell growth and migration [25]. Consistently, we observed that miR-488-3p was declined in CRC, and its overexpression restrained cell growth, cell cycle process and motility and promoted apoptosis in CRC cells. Moreover, the inhibitory effect of circSEC24A knockdown on CRC progression was partly abolished by miR-488-3p inhibition, suggesting that circSEC24A promoted CRC progression by binding to miR-488-3p.

Subsequently, TMEM106B was verified to function as a target gene of miR-488-3p. Kundu et al. declared that TMEM106B played a positive role in lung cancer metastasis [26]. Ren et al. disclosed that TMEM106B could reverse the promotional role in cell apoptosis and the suppressive roles in cell proliferation and migration in CRC cells mediated by MAGI2-AS3 silencing via acting as a target of miR-3163, indicating that TEME106B might play a positive role in CRC progression [18]. Herein, TMEM106B expression was inversely modulated by miR-488-3p and TMEM106B upregulation restored the impacts of miR-488-3p overexpression on CRC cell growth, cell cycle, apoptosis and metastasis.

Conclusion

In summary, circSEC24A contributed to CRC cell progression by elevating TMEM106B expression through sponging miR-488-3p, which might be expected to be a valuable and promising strategy for CRC therapy.

Abbreviations

circRNAs: circular RNAs; CRC: colorectal cancer; qRT-PCR: Quantitative real-time polymerase chain reaction; miR-488-3p: microRNA-488-3p; TMEM106B: transmembrane protein 106B; ncRNAs: non-coding RNAs; EMT: epithelial-mesenchymal transition; miRNAs: MicroRNAs; NSCLC: non-small cell lung cancer; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; PCNA: proliferating cell nuclear antigen; ESCC: esophageal squamous cell carcinoma.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine and written informed consents were collected from all patients and hospitals.

The animal experiment was permitted by the Animal Research Committee of Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine. and performed in accordance with the guidelines of the National Animal Care and Ethics Institution.

Consent for publication

Not applicable

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no financial conflicts of interest

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None

Authors' contributions

Gaowu Hu participated in the conception and design of the study. Wei Peng performed the analysis and interpretation of data. Yongqing Cao contributed and approved the final submitted manuscript.

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Figures

Figure 1

CircSEC24A was upregulated in CRC tissues and cells. (A) GEO database showed circSEC24A was elevated in CRC patients. (B) The schematic illustration showed that circSEC24A was formed by the circularization of the Exons 14-18 of SEC24A. (C and D) The expression level of circSEC24A in CRC tissues, cells and corresponding normal tissues and cells was examined by qRT-PCR. (E and F) The levels of circSEC24A and linear SEC24A in SW480 and SW620 cells treated with Actinomycin D at indicated time points were measured by qRT-PCR. (G and H) The levels of circSEC24A and linear SEC24A in SW480 and SW620 cells treated with or without RNase R were measured by qRT-PCR. *P<0.05.

Figure 2

CircSEC24A deficiency repressed CRC cell progression. SW480 and SW620 cells were transfected with si-circSEC24A and si-NC. (A and B) The expression of circSEC24A and linear SEC24A in SW480 and SW620 cells was detected by qRT-PCR assay. (C and D) The proliferation of SW480 and SW620 cells was assessed by MTT assay. (E and F) The protein levels of cyclin D1 and PCNA in SW480 and SW620 cells were measured by western blot assay. (G and H) The apoptosis of SW480 and SW620 cells was determined by flow cytometry analysis. (I and J) Cell cycle process in SW480 and SW620 cells was analyzed by flow cytometry analysis. (K and L) The migration and invasion of SW480 and SW620 cells were tested by transwell assay. * $P < 0.05$.

Figure 3

CircSEC24A negatively regulated miR-488-3p expression by direct interaction in CRC cells. (A) The potential binding sites between circSEC24A and miR-488-3p were predicted by starBase. (B and C) The luciferase activity in SW480 and SW620 cells co-transfected with miR-488-3p/miR-NC and circSEC24A WT/circSEC24A MUT was determined by dual-luciferase reporter assay. (D) The level of miR-488-3p in SW480 and SW620 cells transfected with si-NC or si-circSEC24A was examined by qRT-PCR assay. (E and F) The expression level of miR-488-3p in CRC tissues, cells and matched normal tissues and cells was detected by qRT-PCR. *P<0.05.

Figure 4

MiR-488-3p inhibition reversed the inhibitory effect of circSEC24A silencing on CRC cell progression. Si-NC, si-circSEC24A, si-circSEC24A+anti-miR-NC or si-circSEC24A+anti-miR-488-3p was transfected into SW480 and SW620 cells. (A and B) The expression of miR-488-3p in SW480 and SW620 cells was determined by qRT-PCR. (C and D) SW480 and SW620 cell proliferation was evaluated by MTT assay. (E and F) The protein levels of cyclin D1 and PCNA in SW480 and SW620 cells were determined via western blot assay. (G-J) Cell apoptosis and cell cycle process were analyzed by flow cytometry analysis. (K-N) SW480 and SW620 cell migration and invasion were examined by transwell assay. *P<0.05.

Figure 5

TMEM106B was a direct target of miR-488-3p in CRC cells. (A) The complementary sequences between miR-488-3p and TMEM106B were shown. (B and C) MiR-488-3p or miR-NC and TMEM106B 3'UTR WT or TMEM106B 3'UTR MUT were co-transfected into SW480 and SW620 cells and then the luciferase activity was determined by dual-luciferase reporter assay. (D) The protein level of TMEM106B in SW480 and SW620 cells transfected with miR-NC or miR-488-3p was examined using western blot assay. (E) Si-NC, si-circSEC24A, si-circSEC24A+anti-miR-NC or si-circSEC24A+anti-miR-488-3p was transfected into SW480 and SW620 cells and then TMEM106B protein level was measured by western blot assay. (F and G) The protein level of TMEM106B in CRC tissues, cells and matched normal tissues and cells was determined by western blot assay. *P<0.05.

Figure 6

TMEM106B overexpression reversed the effects of miR-488-3p on cell proliferation, apoptosis, cell cycle, migration and invasion in CRC cells. MiR-NC, miR-488-3p, miR-488-3p+vector and miR-488-3p+TMEM106B was transfected into SW480 and SW620 cells. (A and B) The protein level of TMEM106B in SW480 and SW620 cells was tested by western blot assay. (C and D) Cell proliferation in SW480 and SW620 cells was evaluated by MTT assay. (E and F) The protein levels of cyclin D1 and PCNA in SW480 and SW620 cells were detected via western blot assay. (G-J) Cell apoptosis and cell cycle in SW480 and SW620 cells were analyzed by flow cytometry analysis. (K and L) The migration and invasion of SW480 and SW620 cells were assessed by transwell assay. *P<0.05.

Figure 7

CircSEC24A silencing suppressed tumor growth in vivo. SW480 and SW620 cells were stably transfected with sh-NC or sh-circSEC24A and then injected into the mice. (A) Tumor volume was determined every 5 days. (B) Tumor weight was measured after 30 days. (C) The levels of circSEC24A and miR-488-3p in the harvested tissues were measured by qRT-PCR. (D) The protein level of TMEM106B in the harvested tissues was examined by western blot assay. *P<0.05.