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Circrna ABCB10 promotes cell proliferation and invasion by affecting emt signaling pathway in cervical cancer

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Abstract

Recently, many emerging circular RNAs (circRNAs) have been studied in human malignancies including cervical cancer and are involved in tumor development and oncogenesis. Here, we found that circABCB10 was higher in 58 cervical cancer tissue sample than that in the adjacent normal tissues. Higher circABCB10 expression significantly associated with advanced the International Federation of Gynecology and Obstetrics (FIGO) stage and greater lymphatic invasion, and worse survival time in cervical cancer patients. Function assays showed that circABCB10 silencing inhibited cell proliferation, migration and invasion capacities. Moreover, we demonstrated that inhibition of circABCB10 upregulated epithelial-mesenchymal transition (EMT)-related proteins E-cadherin expression and downregulated the Vimentin expression, which suggested that affected EMT signaling pathway in cervical cancer. Thus, the circABCB10 may contribute to the development of cervical cancer by affecting epithelial-mesenchymal transition signaling pathways.

Keywords: cervical cancer; circular RNA; circABCB10; cell proliferation; cell invasion.

Practical Application: In the study, we found that circABCB10 was higher in cervical cancer tissue and high circABCB10 expression significantly associated with advanced T category and greater lymphatic invasion, and poor prognosis in patients.

1 Introduction

Cervical cancer (CC) is the 4th most common malignancy with about 450,000 new cases diagnosed each year (Song et al., 2019). Although some methods including surgery combined with chemotherapy or radiotherapy has advancement for patients with locally-advanced tumors, the 5-year overall survival is under 40%, due to tumor metastasis and recurrence (Petignat & Roy, 2007; Sanches et al., 2018). Thus, it is urgent to explore the molecular analysis for novel therapeutic targets for cervical cancer.

Circular RNAs (circRNAs) are a novel class of endogenous RNA that has a covalent closed loop structure (Kristensen et al., 2018). Increasing evidence has demonstrated that circRNAs regulate tumor gene expression in some tumors to affects tumor expression (Meng et al., 2017; Yi et al., 2019). For example, 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 (Liu et al., 2018). Overexpressed circ_0067934 acts as an oncogene to facilitate cervical cancer progression via the miR-545/EIF3C axis (Hu et al., 2019). CircRNA hsa_circRNA_101996 increases cervical cancer proliferation and invasion through activating TPX2 expression by restraining miR-8075 (Song et al., 2019). Circ-ATP8A2 promotes cell proliferation and invasion as a ceRNA to target EGFR by sponging miR-433 in cervical cancer (Ding & Zhang, 2019).

Circular RNA ABCB10 (CircABCB10) has been found to play important role in some tumors. Such as, CircABCB10 could promote hepatocellular carcinoma progression by increasing HMG20A expression by sponging miR-670-3p (Yang et al., 2020). However, the roles and underlying mechanisms of Circular RNA ABCB10 remain unclear in CC progression.

In the study, we found that circABCB10 was higher in cervical cancer tissue and high circABCB10 expression significantly associated with advanced T category and greater lymphatic invasion, and poor prognosis in patients. Function assays showed that circABCB10 silencing inhibited tumor growth and invasion. Moreover, we demonstrated that circABCB10 affected cell invasion by regulating EMT signaling pathway in cervical cancer. Thus, our data identified that CircABCB10 may be a potential prognostic marker and therapeutic target in Cervical cancer.

2 Materials and methods

2.1 Patients tissue samples

Human primary CC tissues and adjacent normal tissues (ANT; at least 3 cm away from the edge of the tumor and no tumor cells were observed) from 58 cases of patients were obtained in Gynaecological Department, Cancer Hospital of China Medical University from 2009 to 2014. The fresh samples were immediately frozen in liquid nitrogen and stored until

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total RNA extraction. All patients signed the informed consent forms. The study was approved by the Ethic Committee of Gynaecological Department, Cancer Hospital of China Medical University. No patient received chemotherapy or radiotherapy before surgery.

2.2 Cell culture and cell transfection

The CC cell lines (HeLa, SiHa C-33Aand CaSki) and normal cervical epithelium cell line (HCvEpC) were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), all cells were maintained in DMEM (Gibco, USA), supplemented with 10% FBS (Invitrogen, USA) in a humidified incubator containing 5% CO_2 at 37 °C (Gaspar-Pintiliescu et al., 2020). Si-NC and si-circABCB10 (GenePharma, Shanghai, China) was transfected into cells using Lipofectamine 2000 (Invitrogen, Waltham, MA, USA). The sequence of si-circABCB10 was as follows: 5'-TGGTGAAATAAATATGCGCAA-3'.

2.3 CCK-8 cell proliferation assays

Transfected cells were seeded into 96-well plates (5000 cells/well) and cell were cultured at 37 °C, 5% CO_2. At 0, 24, 48 and 72 hours, 10 μL of CCK-8 solution was added to each well. Then, cell was detected in a microplate reader and the optical density (OD) value of each well at 450 nM according to the manufacturer's instructions and has been reported according previous study (Balthazar et al., 2021; Rafiq et al., 2020).

2.4 RNA extraction and quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from cells and tissues using TRIzol (TaKaRa, Shiga, Japan) according to manufacturer's instructions. RNA was reversely transcribed into cDNA using PrimeScript RT Reagent Kit (TaKaRa, Shiga, Japan). RNA expression was quantified by qRT-PCR with SYBR Premix Ex Taq™ (TaKaRa, Shiga, Japan). GAPDH was adapted as internal controls for circRNAs. The ΔΔCt method was employed to assay relative expression levels. The primer sequences were as follows:

CircABCB10 (divergent primer): Forward: 5'-CTTATCCACTTGGCCGGAG-3'; CircABCB10 (divergent primer): Reverse 5'-CGCGTAGATCTCAGGGG-3'. GAPDH: Forward: 5'-CGAGAGAATCCGCGGACAT-3'GAPDH: Reverse: 5'-TTGTGCAATACAGCGTGGAC-3'.

2.5 Transwell migration and invasion assays

Cell migration and invasion was examined by transwell invasion assay using transwell chamber (Corning Costar, Cambridge, MA, USA). After 48 h of transfection, cells were placed in the upper compartment pre-coated with Matrigel (Sigma-Aldrich, St. Louis, MO, USA) in non-serum medium and cells were cultured using 10% FBS in the lower. Invasing cells in the bottom of the membrane were fixed and stained using 0.1% crystal violet. The pictures were captured utilizing the microscope. The number of cells was evaluated by ImageJ software.

2.6 Western blot analysis

Total protein was extracted from transfected HeLa or SiHa cells lysed using the Pierce cell lysis buffer (Pierce; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Bicinchoninic acid protein assay kit (Takara Biotechnology Co., Ltd., Dalian, China) was used to quantify protein concentration. Equal amounts of protein (40 μg) were separated by 10% SDS-PAGE and then were subsequently transferred onto polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). The membranes were blocked in 5% skim milk for 1 h, and then incubated with primary antibodies overnight at 4°C. The primary antibodies used were anti-E-cadheirn (1:1,500, Abcam, Cambridge, UK), Vimentin (1:1,500, Abcam, Cambridge, UK) and anti-β-actin (1:3,000 Abcam, Cambridge, UK). Subsequently, the membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated secondary antibody (1:1,000; Abcam, Cambridge, UK). Bands were visualized using an enhanced chemiluminescence detection kit (EMD Millipore).

2.7 Statistical analysis

Statistical analysis was performed using the SPSS 20.0 (Chicago, USA) and GraphPad Prism 5 software (San Diego, CA). Student's *t* test (two-tailed) and one-way ANOVA were employed for comparing the differences between two groups or the differences among multiple groups. The statistical significance of differences was determined by $P < 0.05$.

3 Results

3.1 Upregulation of circABCB10 was found in CC patients

To explore the clinical importance of circABCB10 expression in CC patients, we collected 45 pairs of CC and adjacent normal tissues from the CC patients. As the expression of circABCB10 in CC patients has not been reported, we detected the expression of circABCB10 in CC patients by qRT-PCR. The results showed that circABCB10 expression was drastically upregulated in CC tissues than that in the adjacent normal tissues (Figure 1A, $P < 0.05$). We also detected the expression of circABCB10 between the CC cells and the normal human cervical epithelial cells, we further proved the circABCB10 expression was significantly higher in all tested CC cells (HeLa, CaSki, SiHa) than that in the normal human cervical epithelial E6E7 cells (Figure 1B, $P < 0.01$). To examine the clinical significance of circABCB10 in CC patients. Our results showed that high circABCB10 expression significantly associated with advanced T category and greater lymphatic invasion compared to low circABCB10 expression in CC patients (Table 1, $P < 0.05$). To detected the association between circABCB10 expression and prognosis, Kaplan-Meier curve analysis was performed. The results suggested that increased expression levels of circABCB10 were associated with poor prognosis in patients with CC (Figure 1C).

3.2 CircABCB10 enhances CC cell growth and cell invasion ability

To explore the biological functions of circABCB10 in CC, the two siRNAs targeting circABCB10 was transfected into SiHa

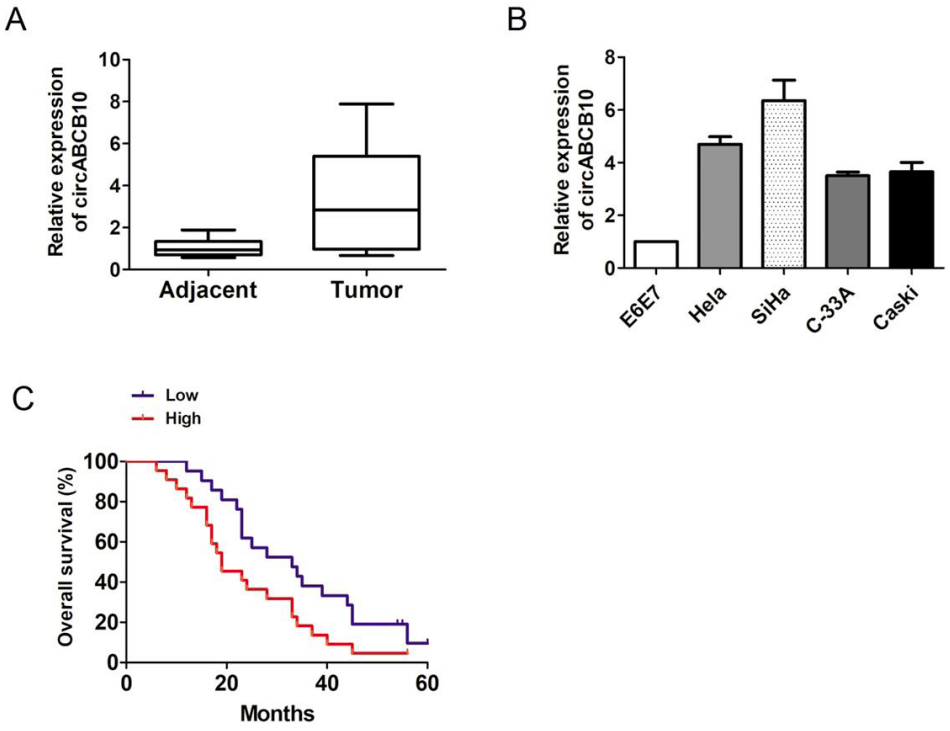

Figure 1. Expression of CircABCB10 is upregulated in human cervical cancer tissues and cells. (A) QRT-PCR was used to detect the expression of CircABCB10 in 49 pairs of human cervical cancer tissues and adjacent normal tissues; (B) The expression of CircABCB10 in cervical cancer cell lines and normal cervical cell line E6E7 were explored using qRT-PCR. (C)High expression of CircABCB10 predicted a poor prognosis compared with low CircABCB10 expression in in 49 pairs of human cervical cancer patients. * $P < 0.05$.

Table 1. The association between circABCB10 and clinicopathological feathers was shown.

Clinicopathological feathers	Patients (n = 58)	Low (n = 30)	High (n = 28)	P-value
Age				0.599
≤ 45	29	14	15	
> 45	29	16	13	
Tumor size				0.192
≤ 4 cm	28	12	16	
> 4 cm	30	18	12	
HPV 16/18 infection				0.786
Positive	30	15	15	
Negative	28	15	13	
Differentiation				0.444
Well and moderately	32	18	14	
Poor	26	12	14	
Lymph node metastasis				0.018*
Negative	30	20	10	
Positive	28	10	18	
FIGO stage				0.009*
I	35	23	12	
II	23	7	16	

* $P < 0.05$.

and CaSki cells (Figure 2A). Next, we found that the expression of circABCB10 is mainly in cytoplasm in SiHa and CaSki cells (Figure 2B). Compared with the negative control (si-NC), the CCK-8 assay showed that cell viability of CaSki cells at 48 and 72hour after transfection was statistically significantly reduced ($P < 0.05$ at 48hrs, $P < 0.05$ at 72hrs). The number of cell migration and invasion was significantly reduced in the si-circABCB10 group compared with that in the control group (Figure 3A, B, $P < 0.05$). The above results indicated that circABCB10 exerted a carcinogenic effect and promoted the growth, migration and invasion of CC cells.

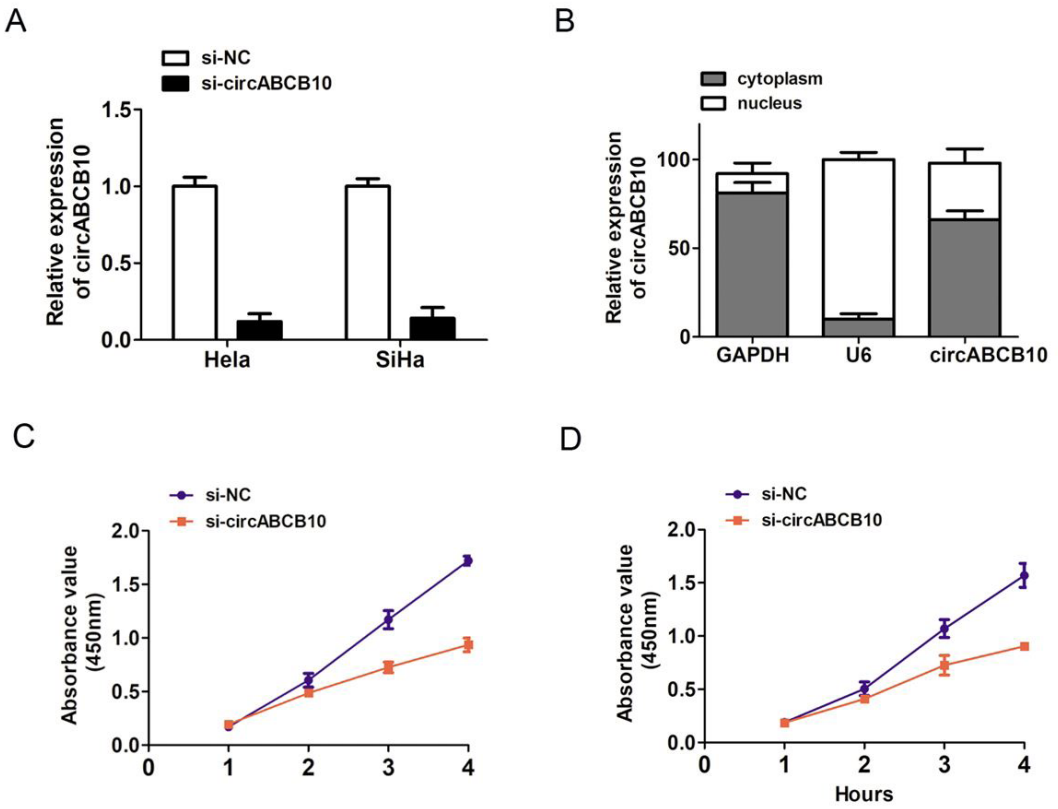

Figure 2. CircABCB10 expression promoted tumor growth in cervical cancer cells. (A) miR-584 expression in HeLa and SiHa cells transfected with si-NC or si-CircABCB10 was detected by quantitative reverse transcription PCR; (B) miR-584 expression in HeLa and SiHa cells was detected by quantitative reverse transcription PCR in cytoplasm or nucleus; (C) The cell viability of HeLa cells was tested transfected with si-NC or si-CircABCB10 with a CCK-8 assay; (D) The cell viability of SiHa cells was tested transfected with si-NC or si-CircABCB10 with a CCK-8 assay. * $P < 0.05$.

3.3 CircABCB10 promotes cell proliferation by affecting EMT signaling pathway in Cervical cancer

To determine whether circABCB10 could affect EMT signaling pathway, we first observed that circABCB10 silencing reduced the protein level of Vimentin, whereas silencing circABCB10 increased the E-cadherin levels, which indicated circABCB10 could affect EMT signaling (Figures 4A, B). Taken together, our results demonstrated that circABCB10 could inhibit the carcinogenesis of CC through repression of EMT signaling.

4 Discussion

An increasing number of studies have showed the crucial role of circRNAs in many human diseases including cancer. Such as, circRNA-AKT1 sequesters miR-942-5p to Upregulate AKT1 and Promote Cervical Cancer Progression[(Ou et al., 2020). Circular RNA HIPK3 Promotes EMT of Cervical Cancer Through Sponging miR-338-3p to Up-Regulate HIF-1α (Qian et al., 2020) circ-MYBL2 Serves As A Sponge For miR-361-3p Promoting Cervical Cancer Cells Proliferation And Invasion (Wang et al., 2019) Overexpression of circular RNA hsa_circ_0001038 promotes cervical cancer cell progression by acting as a ceRNA for miR-337-3p to regulate cyclin-M3 and metastasis-associated in colon cancer 1 expression (Wang et al., 2020).

Circular RNA ABCB10 has been found to regulate some tumor progression. Such as, Circular RNA ABCB10 promotes hepatocellular carcinoma progression by increasing HMG20A expression by sponging miR-670-3p (Fu et al., 2019). CircABCB10 promotes non small cell lung cancer cell proliferation and migration by regulating the miR-1252/FOXR2 axis (Tian et al., 2019). However, the roles and underlying mechanisms of Circular RNA ABCB10 remain unclear in CC progression. In the study, we found that circABCB10 was higher in cervical cancer tissue and high circABCB10 expression significantly associated with advanced T category and greater lymphatic invasion, and poor prognosis in patients.

Next, function assays showed that circABCB10 silencing inhibited tumor growth and invasion. EMT is closely associated with tumor progression and invasion. The previous studies have shown that circRNAs could induce EMT to promote tumor progression (Xu et al., 2020). To determine whether circABCB10 could affect EMT signaling pathway, we first observed that circABCB10 silencing reduced the protein level of Vimentin, whereas silencing circABCB10 increased the E-cadherin levels, which indicated circABCB10 could affect EMT signaling. Taken together, our results demonstrated that circABCB10 could inhibit the carcinogenesis of CC through repression of EMT signaling.

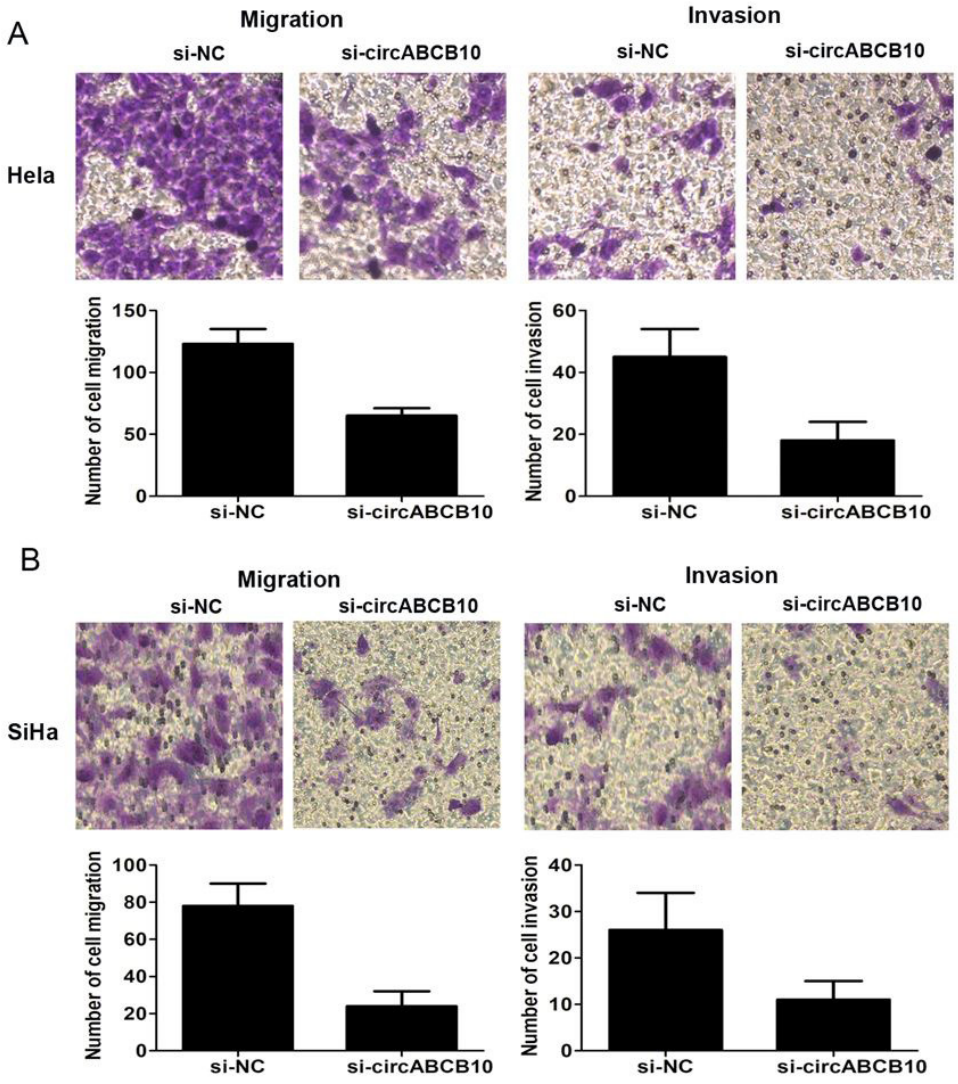

Figure 3. CircABCB10 expression promoted tumor migration and invasion in cervical cancer cells. (A)Transwell assay was used to analyze the migration and invasion capability of HeLa cells. (Scale bar, 100 μM; magnification, ×100); (B) A Transwell assay was used to analyze the migration and invasion capability of SiHa cells (Scale bar, 100 μM; magnification, ×100). *P < 0.05.

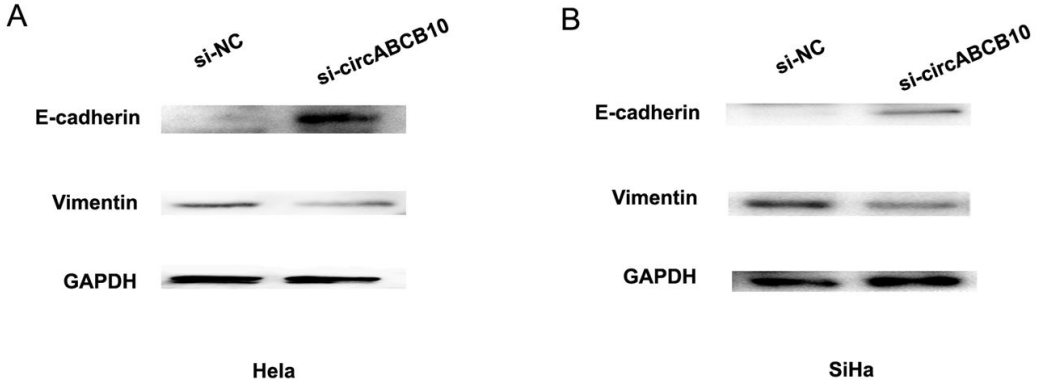

Figure 4. Downregulation of CircABCB10 expression inhibited PI3K/AKT signaling pathway in cervical cancer cells. (A) HeLa cells were co-transfected with si-NC and si-CircABCB10. The protein expression levels of PI3K and AKT were then analyzed by western blotting; (B) SiHa cells were co-transfected with si-NC and si-CircABCB10. The protein expression levels of E-cadherin and Vimentin were then analyzed by western blotting.

In conclusion, we found that circABCB10 was higher in cervical cancer and circABCB10 promote cell proliferation and invasion capacities. Moreover, we demonstrated that circABCB10 affected cell invasion by regulating EMT signaling pathway in cervical cancer. Thus, our data identified that circABCB10 may be a potential prognostic marker and therapeutic target in cervical cancer.

Author contributions

Wei Ben and Guangmei Zhang designed the study. Lu Zhao and Tian Liang performed the experiments. Wei Ben analyzed the data. Guangmei Zhang drafted the manuscript.

References

Balthazar, C. F., Moura, N. A., Romualdo, G. R., Rocha, R. S., Pimentel, T. C., Esmerino, E. A., Freitas, M. Q., Santillo, A., Silva, M. C., Barbisan, L. F., Cruz, A. G., & Albenzio, M. (2021). Synbiotic sheep milk ice cream reduces chemically induced mouse colon carcinogenesis. *Journal of Dairy Science*, 104(7), 7406-7414.

Ding, L., & Zhang, H. (2019). Circ-ATP8A2 promotes cell proliferation and invasion as a ceRNA to target EGFR by sponging miR-433 in cervical cancer. *Gene*, 705, 103-108. http://dx.doi.org/10.1016/j.gene.2019.04.068. PMid:31029604.

Fu, Y., Cai, L., Lei, X., & Wang, D. (2019). Circular RNA ABCB10 promotes hepatocellular carcinoma progression by increasing HMG20A expression by sponging miR-670-3p. *Cancer Cell International*, 19(1), 338. http://dx.doi.org/10.1186/s12935-019-1055-z. PMid:31889891.

Gaspar-Pintiliescu, A., Oancea, A., Cotarlet, M., Vasile, A. M., Bahrim, G. E., Shaposhnikov, S., Craciunescu, O., & Oprita, E. I. (2020). Angiotensin-converting enzyme inhibition, antioxidant activity and cytotoxicity of bioactive peptides from fermented bovine colostrum. *International Journal of Dairy Technology*, 73(1), 108-116.

Hu, C., Wang, Y., Li, A., Zhang, J., Xue, F., & Zhu, L. (2019). Overexpressed circ_0067934 acts as an oncogene to facilitate cervical cancer progression via the miR-545/EIF3C axis. *Journal of Cellular Physiology*, 234(6), 9225-9232. http://dx.doi.org/10.1002/jcp.27601. PMid:30362562.

Kristensen, L. S., Hansen, T. B., Venø, M. T., & Kjems, J. (2018). Circular RNAs in cancer: opportunities and challenges in the field. *Oncogene*, 37(5), 555-565. http://dx.doi.org/10.1038/onc.2017.361. PMid:28991235.

Liu, J., Wang, D., Long, Z., Liu, J., & Li, W. (2018). 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. *Cellular Physiology and Biochemistry*, 48(1), 173-184. http://dx.doi.org/10.1159/000491716. PMid:30007986.

Meng, S., Zhou, H., Feng, Z., Xu, Z., Tang, Y., Li, P., & Wu, M. (2017). CircRNA: functions and properties of a novel potential biomarker for cancer. *Molecular Cancer*, 16(1), 94. http://dx.doi.org/10.1186/s12943-017-0663-2. PMid:28535767.

Ou, R., Mo, L., Tang, H., Leng, S., Zhu, H., Zhao, L., Ren, Y., & Xu, Y. (2020). circRNA-AKT1 Sequesters miR-942-5p to Upregulate AKT1 and promote cervical cancer progression. *Molecular Therapy. Nucleic Acids*, 20, 308-322. http://dx.doi.org/10.1016/j.omtn.2020.01.003. PMid:32193155.

Petignat, P., & Roy, M. (2007). Diagnosis and management of cervical cancer. *BMJ*, 335(7623), 765-768. http://dx.doi.org/10.1136/bmj.39337.615197.80. PMid:17932207.

Qian, W., Huang, T., & Feng, W. (2020). Circular RNA HIPK3 promotes EMT of cervical cancer through sponging miR-338-3p to up-regulate HIF-1alpha. *Cancer Management and Research*, 12, 177-187. PMid:32021434.

Rafiq, S., Gulzar, N., Huma, N., Hussain, I., & Murtaza, M. S. (2020). Evaluation of anti-proliferative activity of Cheddar cheeses using colon adenocarcinoma (HCT-116) cell line. *International Journal of Dairy Technology*, 73(1), 255-260. http://dx.doi.org/10.1111/1471-0307.12665.

Sanches, J. G. P., Xu, Y., Yabasin, I. B., Li, M., Lu, Y., Xiu, X., Wang, L., Mao, L., Shen, J., Wang, B., Hou, L., Ju, J., Zhao, J., & Song, B. (2018). miR-501 is upregulated in cervical cancer and promotes cell proliferation, migration and invasion by targeting CYLD. *Chemico-Biological Interactions*, 285, 85-95. http://dx.doi.org/10.1016/j.cbi.2018.02.024. PMid:29477382.

Song, T., Xu, A., Zhang, Z., Gao, F., Zhao, L., Chen, X., Gao, J., & Kong, X. (2019). CircRNA hsa_circRNA_101996 increases cervical cancer proliferation and invasion through activating TPX2 expression by restraining miR-8075. *Journal of Cellular Physiology*, 234(8), 14296-14305. http://dx.doi.org/10.1002/jcp.28128. PMid:30633364.

Tian, X., Zhang, L., Jiao, Y., Chen, J., Shan, Y., & Yang, W. (2019). CircABCB10 promotes nonsmall cell lung cancer cell proliferation and migration by regulating the miR-1252/FOXR2 axis. *Journal of Cellular Biochemistry*, 120(3), 3765-3772. http://dx.doi.org/10.1002/jcb.27657. PMid:30417418.

Wang, J., Li, H., & Liang, Z. (2019). circ-MYBL2 Serves As A Sponge For miR-361-3p promoting cervical cancer cells proliferation and invasion. *OncoTargets and Therapy*, 12, 9957-9964. http://dx.doi.org/10.2147/OTT.S218976. PMid:31819492.

Wang, Y., Wang, L., Wang, W., & Guo, X. (2020). Overexpression of circular RNA hsa_circ_0001038 promotes cervical cancer cell progression by acting as a ceRNA for miR-337-3p to regulate cyclin-M3 and metastasis-associated in colon cancer 1 expression. *Gene*, 733, 144273. http://dx.doi.org/10.1016/j.gene.2019.144273. PMid:31809842.

Xu, G., Chen, Y., Fu, M., Zang, X., Cang, M., Niu, Y., Zhang, W., Zhang, Y., Mao, Z., Shao, M., Qian, H., Xu, W., Cai, H., Jiang, P., & Zhang, X. (2020). Circular RNA CCDC66 promotes gastric cancer progression by regulating c-Myc and TGF-beta signaling pathways. *Journal of Cancer*, 11(10), 2759-2768. http://dx.doi.org/10.7150/jca.37718. PMid:32226494.

Yang, W., Ju, H. Y., & Tian, X. F. (2020). Circular RNA-ABCB10 suppresses hepatocellular carcinoma progression through upregulating NRP1/ABL2 via sponging miR-340-5p/miR-452-5p. *European Review for Medical and Pharmacological Sciences*, 24(5), 2347-2357.

Yi, Y., Liu, Y., Wu, W., Wu, K., & Zhang, W. (2019). Reconstruction and analysis of circRNAmiRNAmRNA network in the pathology of cervical cancer. *Oncology Reports*, 41(4), 2209-2225. PMid:30816541.