European Review for Medical and Pharmacological Sciences 2019; 23: 8771-8778

Circular RNA circRNA_0000285 promotes cervical cancer development by regulating FUS

R.-X. CHEN, H.-L. LIU, L.-L. YANG, F.-H. KANG, L.-P. XIN, L.-R. HUANG, Q.-F. GUO, Y.-L. WANG

Department of Gynecology, Women and Children's Hospital, School of Medicine, Xiamen University, Xiamen, China

Abstract. – OBJECTIVE: Recently, the vital role of circular RNAs (circRNAs) in human diseases has attracted much attention. The aim of this research was to verify the potential role of circRNA_0000285 in the development of cervical cancer (CC).

PATIENTS AND METHODS: CircRNA_0000285 expression in both CC cells and tissue samples was detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Functional experiments were performed, including cell counting kit-8 (CCK-8) assay, cell cycle assay and transwell assay. Meanwhile, the underlying mechanism was explored through qRT-PCR and Western blot assay, respectively. In addition, the function of circRNA_0000285 was identified *in vivo*.

RESULTS: CircRNA_0000285 expression level was significantly higher in CC samples than that of corresponding normal tissues. Moreover, the growth and migration abilities of CC cells were significantly inhibited after circRNA_0000285 was knocked down *in vitro*. Furthermore, the expression of FUS was remarkably down-regulated after knockdown of circRNA_0000285. Subsequent results indicated that the expression level of FUS was positively correlated with the expression of circRNA_0000285 in CC tissues. In addition, the knockdown of circRNA_0000285 significantly inhibited the formation and metastasis of CC in nude mice.

CONCLUSIONS: CircRNA_0000285 could enhance the proliferation and metastasis of CC by up-regulating FUS, which might be a potential therapeutic target for CC treatment.

Key Words:
Circular RNA, CircRNA_0000285, Cervical cancer (CC), FUS.

Introduction

Cervical cancer (CC) is one of the top ten malignant cancers in both incidence and mortality in the world[1]. According to the World health organization (WHO), 570,000 new cases were diagnosed of CC, with about 311,000 deaths worldwide in 2018[2]. For the past decade, there has been a common trend that patients with CC are suffering at a relatively young age. Though the cure rate of localized CC accounts for 91.5%[3], the prognosis of patients with metastatic CC remains dismal. Statistics have shown that the median survival time of CC patients is only 8 to 13 months[4]. Moreover, the intervention for advanced CC patients is limited due to the heterogeneous mechanism of CC. Thus, there is an urgent need to formulate a deep understanding of CC progression and to find out new therapeutic strategies for patients.

Circular RNAs (circRNAs), formed by a covalently closed loop, have been emerged as a new hot topic in the noncoding RNA network. Recently, circRNAs have been indicated to be involved in multiple physiological and pathological processes of tumorigenesis *via* serving as microRNA sponges. Meanwhile, they can modulate the expression of genes and proteins. For example, circ_LARP4 is significantly down-regulated in ovarian cancer, serving as a potential biomarker for the prognosis of ovarian cancer patients[5]. Regulating the expression of miR-29a, circ MYLK functions as an oncogene and promotes the progression of prostate cancer[6]. Mediated by miR-503/LARP1 signaling, silence of circ-BANP suppresses the proliferation and migration of lung cancer cells[7]. Knockdown of circRNA-CER restrains the proliferation and migration of breast cancer *via* modulating the activity of miR136/MMP13 signaling[8]. CircRNA_0000285 has been demonstrated a novel oncogene in many tumors. However, the exact role of circRNA_0000285 in CC and the potential molecular mechanism have not been fully elucidated.

Corresponding Author: Yanlong Wang, MM; e-mail: xmfy_wyl@126.com

In this work, circRNA_0000285 expression was remarkably up-regulated in CC tissues. Knockdown of circRNA_0000285 suppressed the proliferation, migration and invasion of CC cells. Meanwhile, the underlying mechanism of circRNA_0000285 function in CC proliferation and metastasis was explored. Furthermore, the role of circRNA_0000285 in CC progression was detected in nude mice.

Patients and Methods

Clinical Samples

Before the study, human CC tissues were collected from 55 CC cases. Tumor tissues and paired adjacent normal tissues were preserved in liquid nitrogen for use. The clinicopathological characters were analyzed by two pathologists. This investigation was approved by the Ethics Committee of Women and Children's Hospital, School of Medicine, Xiamen University. Signed written informed consents were obtained from all participants before the study.

Cell Culture

Human CC cell lines (HeLa, SiHa, C4-1 and C-33a) and normal cervical epithelium cell line (NC104) were offered by the Institute of Cell Research, Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and penicillin. Meanwhile, the cells were maintained in an incubator with 5% CO_2 at 37°C.

Cell Transfection

Lentivirus expressing short-hairpin RNA (shRNA) directly against circRNA_0000285 was compounded by GenePharma (Shanghai, China). Negative control shRNA (NC) was synthesized as well. Moreover, pLenti-EF1a-EGFP-F2A-Puro vector (Biosettia Inc., San Diego, CA, USA) was used for cloning the shRNA and negative control, which was transfected into C-33a cells.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from CC cells and tissues using the TRIzol kit (TaKaRa Bio, Inc., Otsu, Shiga, Japan). RNA concentration was measured using an ultraviolet spectrophotometer (Hitachi, Tokyo, Japan). Subsequently, extracted RNA was reverse transcribed into complementary Deoxyribose Nucleic Acid (cDNA) according to the instructions of the PrimeScript™ RT MasterMix kit (Invitrogen, Carlsbad, CA, USA). Primers used for qRT-PCR were as follows: circRNA_0000285 primers forward 5′-TATGTTGGTGGATCCTGTTCGGCA-3′, reverse 5′-TGGTGGGTAGACCAAGACTTGTGA-3′; Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers forward 5′-CCAAAACCAGATGGGGCAATGCTGG-3′ and reverse 5′-TGATGGCATGGACTGTGGCCATCCA-3′. Thermal cycle was as follows: 30 sec at 95°C, 5 sec for 40 cycles at 95°C, and 35 sec at 60°C.

Cell Proliferation Assay

The proliferation of transfected CC cells was monitored by Cell Counting Kit-8 (CCK-8; Beyotime Institute of Biotechnology, Shanghai, China) assay. Briefly, 5 mg/ml CCK-8 was added to each well at 0, 24, 48, and 72 h after transfection, respectively, followed by incubation for 1 h. OD 450 was measured by a spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Cell Cycle Assay

2×10^5/mL cells were diluted by RNase A in 75% ice-cold ethanol overnight. Subsequently, these cells were stained with propidium iodide (PI; 50 mg/mL; MultiSciences Biotech Co., Ltd, Hangzhou, China) in the dark for 30 min at 4°C. Cell cycle was finally determined by flow cytometer (FACScan, BD Bioscience, Franklin Lakes, NJ, USA).

Transwell Assay

Transwell chambers with 8 μm pores were provided by Corning (Corning, NY, USA). The membrane was pre-coated with or without 50 μL Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). Cells were first seeded into the upper chamber of a 24-well plate. Meanwhile, 20% FBS-DMEM was added to the lower chamber of culture inserts. After incubation for 24 h, these inserts were fixed with methanol for 30 min and stained with hematoxylin for 20 min. Finally, the number of invaded cells was counted by a light microscope (Olympus, Tokyo, Japan).

Western Blot Analysis

First, the cells were washed with precooled phosphate-buffered saline (PBS) and lysed with cell radioimmunoprecipitation lysis solution (RIPA; Beyotime, Shanghai, China). Protein concentration was detected using the bicinchonin-

ic acid (BCA; Thermo Fisher Scientific Inc., Waltham, MA, USA) method. Subsequently, extracted proteins were separated and transferred on to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking with Tris-Buffered Saline and Tween-20 (TBST) (25 mM Tris, 140 mM NaCl, and 0.1% Tween 20, pH 7.5) containing 5% skimmed milk for 2 h, the membranes were incubated with primary antibodies of FUS and GAPDH (Abcam Inc., Cambridge, MA, USA) at 4°C overnight. After washing with TBST (3×10 min), the membranes were incubated with corresponding secondary antibody at room temperature for 1 h. Immuno-reactive bands were analyzed by Image J software (NIH, Bethesda, MD, USA).

Xenograft Model

After transfection, C-33a cells (6×10^5/mL) were replaced into NOD/SCID mice (6-week-old) subcutaneously. Tumor diameters were detected every 5 days. Tumor volume was calculated as the formula: volume= length × width^2 × 1/2. Tumors were extracted after 4 weeks. Transfected C-33a cells were injected into tail vein of NOD/SCID mice (4-5 weeks old). After 4 weeks, the mice were sacrificed, and lung tissues were extracted. The number of metastatic nodules in the lungs was counted. Animal experiments were approved by the Animal Ethics Committee of School of Medicine, Xiamen University.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (SPSS, Chicago, IL, USA) was used for all statistical analysis. The student's *t*-test was used to compare the differences between the two groups. $p<0.05$ was considered statistically significant.

Results

CircRNA_0000285 Expression Level Was Higher in CC Tissues and Cells

QRT-PCR was first conducted to detect circRNA_0000285 expression in 55 CC patients' tissues. CircRNA_0000285 expression was significantly higher in CC tissue samples than that of adjacent normal tissues (Figure 1A). Moreover, qRT-PCR was performed to detect circRNA_0000285 expression in CC cells as well. Results indicated that circRNA_0000285 was significantly up-regulated in CC cells when compared with NC104 cells (Figure 1B).

Knockdown of CircRNA_0000285 Inhibited Proliferation and Invasion of CC Cells

In our work, C-33a cell line was chosen for the knockdown of circRNA_0000285. QRT-PCR was utilized to verify the transfection efficiency (Figure 2A). To explore the exact role of circRNA_0000285 in the proliferation of CC cells, CCK8 assay and cell cycle assay were performed. As shown in Figure 2B, the proliferation of C-33a cells was remarkably suppressed after knockdown of circRNA_0000285. As shown in Figure 2C, the percentage of G0/G1 cells increased markedly after knockdown of circRNA_0000285 in C-33a cells, while the percentage of S cells was reduced. As shown in Figure 2D, the number of

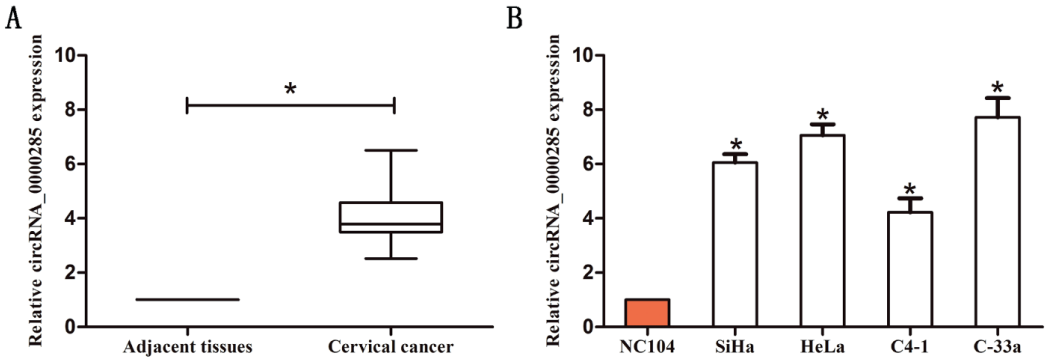

Figure 1. Expression level of circRNA_0000285 was up-regulated in CC tissues and cells. **A,** CircRNA_0000285 expression was significantly up-regulated in CC tissues compared with adjacent tissues. **B,** Expression levels of circRNA_0000285 relative to GAPDH in human CC cell lines and NC104 cells were determined by qRT-PCR. GAPDH was used as an internal control. Data were presented as mean ± standard error of the mean. *$p<0.05$.

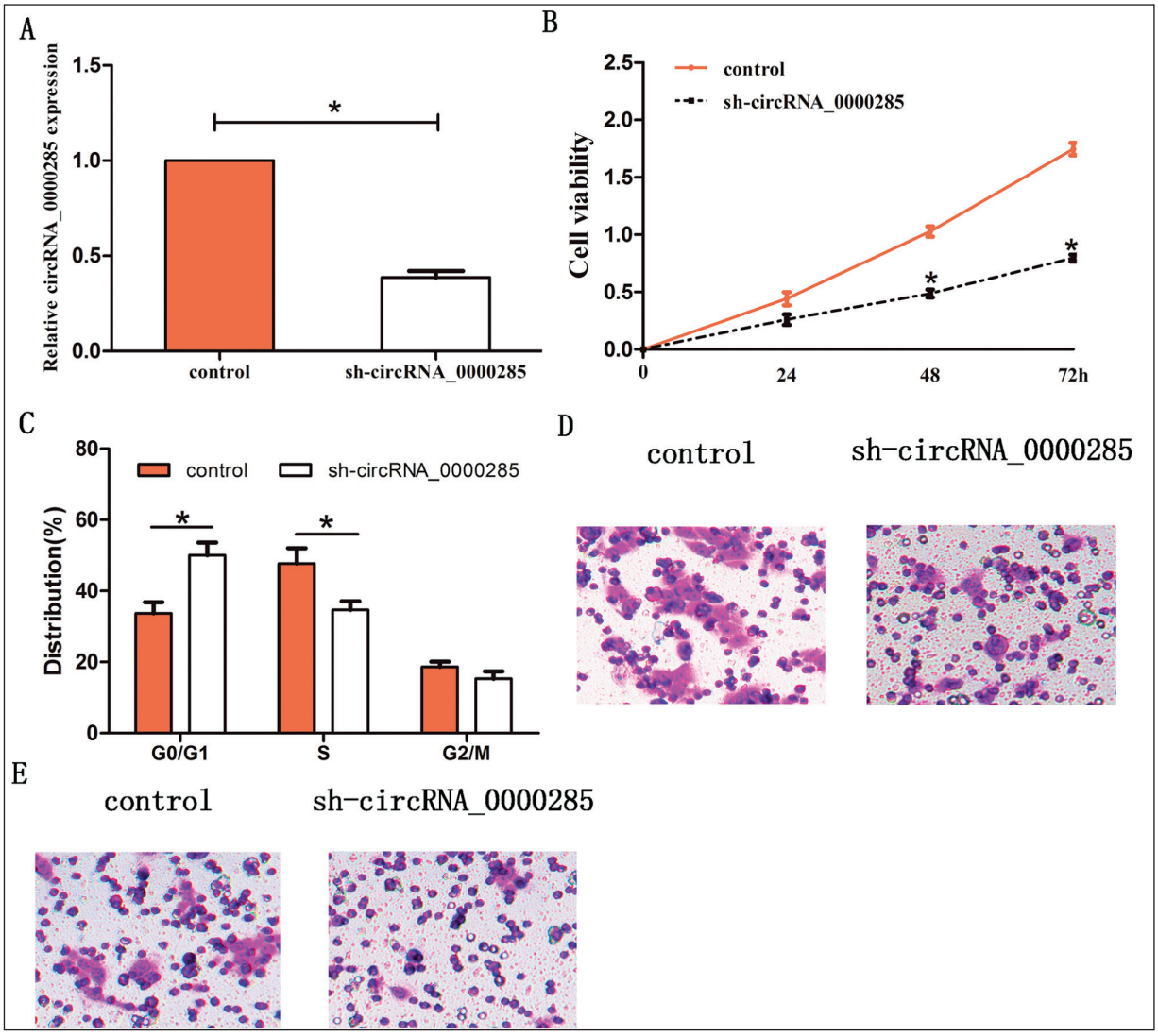

Figure 2. Knockdown of circRNA_0000285 inhibited CC cell proliferation and metastasis. **A,** CircRNA_0000285 expression in C-33a cells transduced with sh-circRNA_0000285 and control was detected by qRT-PCR. GAPDH was used as an internal control. **B,** CCK8 assay showed that the proliferation of C-33a cells was remarkably suppressed *via* knockdown of circRNA_0000285. **C,** Cell cycle assay showed that the the percentage of G0/G1 cells increased and the percentage of S cells decreased after knockdown of circRNA_0000285 in C-33a cells. **D,** Transwell assay showed that the number of migrated cells was reduced *via* knockdown of circRNA_0000285 in C-33a CC cells (magnification: 40×). **E,** Transwell assay showed that the number of invaded cells was reduced *via* knockdown of circRNA_0000285 in C-33a CC cells (magnification: 40×). The results represented the average of three independent experiments (mean ± standard error of the mean). *$p<0.05$, as compared with control cells.

migrated C-33a cells was remarkably reduced after knockdown of circRNA_0000285. As shown in Figure 2E, the number of invaded C-33a cells was remarkably reduced after knockdown of circRNA_0000285 *in vitro*.

The Interaction Between FUS and CircRNA_0000285 in CC

Previous studies have reported that the key regulator, FUS, promotes the development of many cancers including CC. FUS has also been predicted as the potential target protein of circRNA_0000285. In our study, the interaction between FUS and circRNA_0000285 was investigated. QRT-PCR results demonstrated that compared with control group, FUS expression was significantly lower in sh-circRNA_0000285 group (Figure 3A). Western blot assay indicated that after circRNA_0000285 was knocked down, FUS was significantly down-regulated at

the protein level (Figure 3B). Furthermore, we verified the interaction between FUS and circRNA_0000285 in CC tissues. FUS expression was significantly higher in CC tissues compared with that of adjacent normal tissues (Figure 3C). In addition, correlation analysis demonstrated that FUS expression level positively correlated with circRNA_0000285 expression in CC tissues (Figure 3D).

Knockdown of CircRNA_0000285 Inhibited Tumor Formation and Metastasis in Nude Mice

The ability of circRNA_0000285 in CC formation and metastasis was detected in nude mice. Tumor size in sh-circRNA_0000285 group was significantly smaller when compared with control group (Figure 4A). The number of metastatic nodules in lung tissues of sh-circRNA_0000285 group was markedly less than that of control group (Figure 4B). Moreover, the expression level of circRNA_0000285 and FUS in dissected tumor tissues was detected by qRT-PCR. Results demonstrated that circRNA_0000285 expression was down-regulated in sh-circRNA_0000285 group compared with control group (Figure 4C). Furthermore, FUS expression was reduced in sh-circRNA_0000285 group than that of control group (Figure 4D).

Discussion

Increasing evidence have suggested that circRNAs are crucial regulators in the carcinogenesis of CC. For instance, through restraining miR-8075, hsa_circRNA_101996 promotes the proliferation and invasion of CC cells *via* regulat-

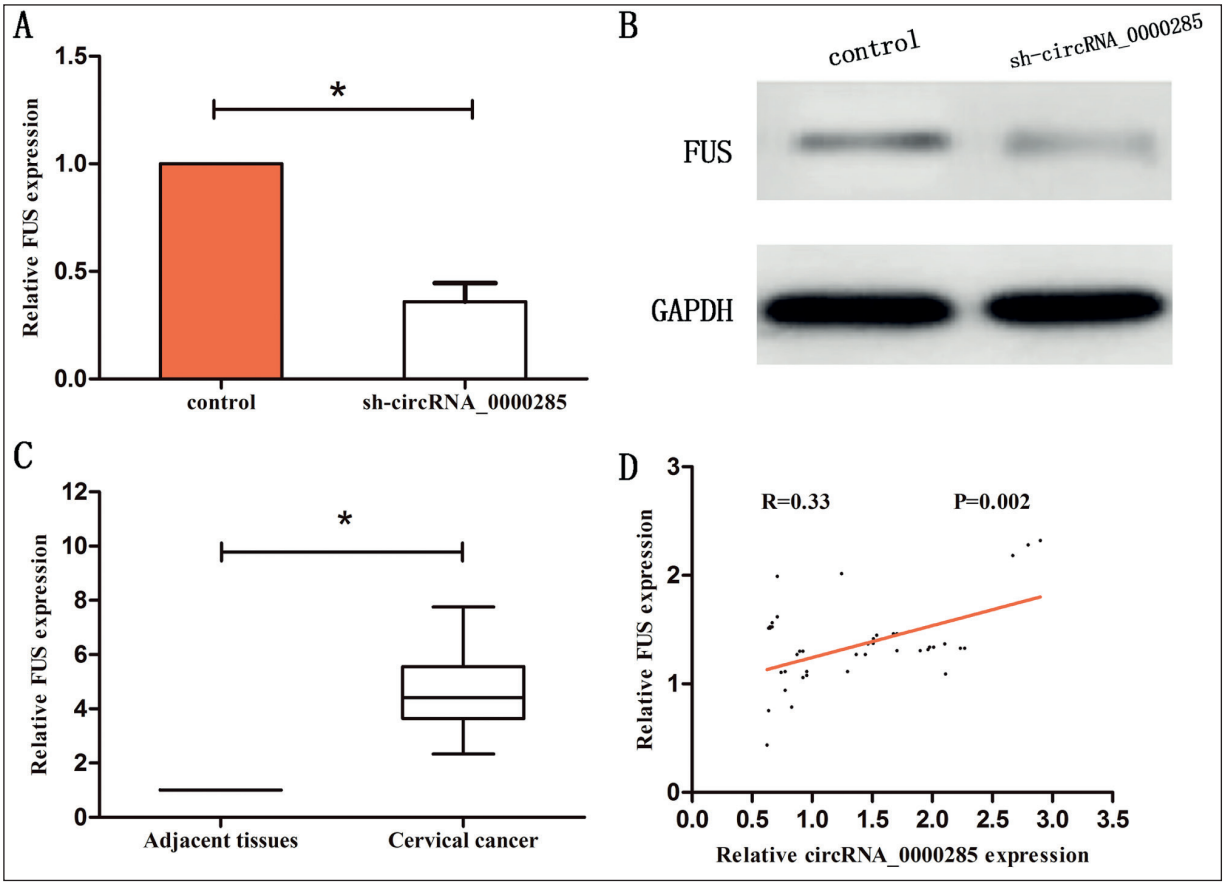

Figure 3. The association between circRNA_0000285 and FUS. **A,** QRT-PCR showed that FUS expression was significantly lower in sh-circRNA_0000285 group compared with control group. **B,** Western blot assay revealed that FUS protein expression decreased obviously in sh-circRNA_0000285 group compared with control group. **C,** FUS was significantly up-regulated in CC tissues compared with corresponding normal tissues. **D,** Linear correlation between the expression levels of FUS and circRNA_0000285 in CC tissues. Data were presented as mean ± standard error of the mean. *p<0.05.

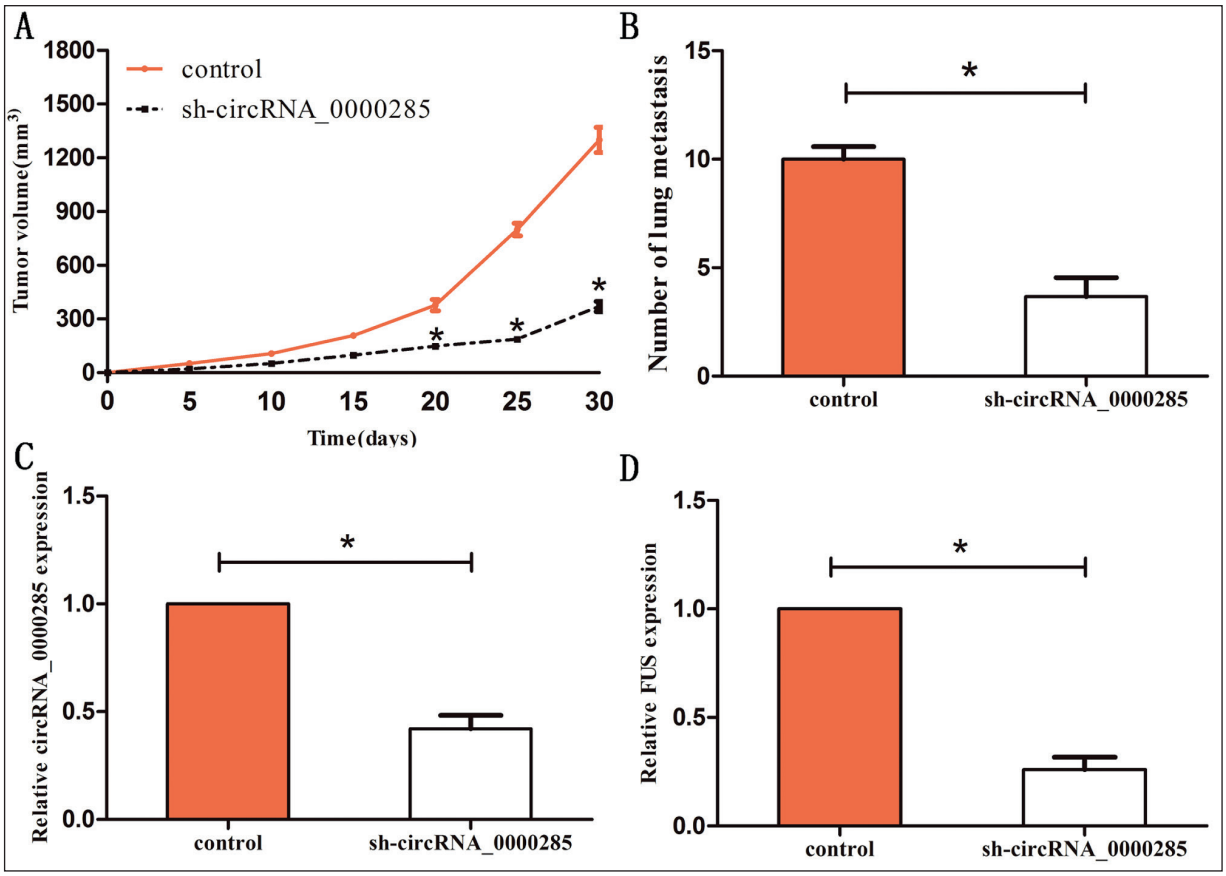

Figure 4. Knockdown of circRNA_0000285 inhibited tumor formation and metastasis of CC in nude mice. **A,** Tumor size in sh-circRNA_0000285group was smaller compared with control group. **B,** The number of metastatic nodules in lung tissues of sh-circRNA_0000285 group was significantly reduced compared with control group. **C,** CircRNA_0000285 expression in dissected tumors of sh-circRNA_0000285 group was lower than control group. **D,** FUS expression in dissected tumors of sh-circRNA_0000285 group was lower than control group. The results represented the average of three independent experiments (mean ± standard error of the mean). *p<0.05, as compared with control cells.

ing the expression of TPX2[9]. Hsa_circ_0023404 functions as an oncogene in CC by regulating miR-136/TFCP2/YAP pathway[10]. Through sponging miR-506, silence of circRNA-000284 inhibits the proliferation and invasion of CC cells[11]. Furthermore, overexpression of circ_0067934 serves as an oncogene and facilitates the progression of CC through modulating miR-545/EIF3C axis[12].

Recently, circRNA_0000285, initially discovered in nasopharyngeal carcinoma, has been served as a prognostic biomarker in radiosensitivity[13]. It has been found abnormally expressed in other cancers, which promotes tumor progression. For example, circRNA_0000285 induces cell invasion by regulating miR-181b in pancreatic cancer[14]. CircRNA_0000285 accelerates the proliferation and invasion of pancreatic cancer through depressing the endogenous function of miR-181b[14]. CircRNA_0000285 promotes the proliferation and metastasis of non-small cell lung cancer through regulating miR-144[15]. In this report, circRNA_0000285 was found significantly up-regulated in both CC tissues and cells. The proliferation of CC cells was significantly inhibited and cell cycle was regulated after knockdown of circRNA_0000285. Meanwhile, the migration and invasion of CC cells were found markedly inhibited through the knockdown of circRNA_0000285. The above results indicated that circRNA_0000285 might act as an oncogene in CC development and metastasis.

FUS (Fused in sarcoma) is a protein encoded by the FET gene family. Previous studies have confirmed that it plays a crucial role in a variety of biological behaviors, including DNA recombination and repair and pre-mRNA splicing. More-

over, FUS has been reported to be a fusion gene with DNA damage inducible transcript 3 (*CHOP*) in human myxoid liposarcoma[16]. Recently, FUS has been found to participate in the development of several cancers. For instance, by interacting with NEAT1, the low-expression of FUS/TLS induces cell apoptosis and inhibits cell growth in breast cancer[17]. FUS/TLS participates in the mediation of cell-cycle progression androgen-dependent and cancer growth in prostate cancer[18].

Our findings indicated that FUS expression was significantly inhibited after knockdown of circRNA_0000285. Moreover, FUS expression was positively correlated with circRNA_0000285 expression in CC tissues. Further investigations demonstrated that the knockdown of circRNA_0000285 inhibited tumor formation and metastasis in nude mice.

Conclusions

In summary, circRNA_0000285 could enhance CC cell development and metastasis by up-regulating FUS. All these findings implied that circRNA_0000285 could be used as a prospective therapeutic target for CC.

Conflicts of interest

The authors declare no conflicts of interest.

References

1) Global Burden of Disease Cancer Collaboration. Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, Allen C, Hansen G, Woodbrook R, Wolfe C, Hamadeh RR, Moore A, Werdecker A, Gessner BD, Te AB, McMahon B, Karimkhani C, Yu C, Cooke GS, Schwebel DC, Carpenter DO, Pereira DM, Nash D, Kazi DS, De Leo D, Plass D, Ukwaja KN, Thurston GD, Yun JK, Simard EP, Mills E, Park EK, Catala-Lopez F, deVeber G, Gotay C, Khan G, Hosgood HR, Santos IS, Leasher JL, Singh J, Leigh J, Jonas JB, Sanabria J, Beardsley J, Jacobsen KH, Takahashi K, Franklin RC, Ronfani L, Montico M, Naldi L, Tonelli M, Geleijnse J, Petzold M, Shrime MG, Younis M, Yonemoto N, Breitborde N, Yip P, Pourmalek F, Lotufo PA, Esteghamati A, Hankey GJ, Ali R, Lunevicius R, Malekzadeh R, Dellavalle R, Weintraub R, Lucas R, Hay R, Rojas-Rueda D, Westerman R, Sepanlou SG, Nolte S, Patten S, Weichenthal S, Abera SF, Fereshtehnejad SM, Shiue I, Driscoll T, Vasankari T, Alsharif U, Rahimi-Movaghar V, Vlassov VV, Marcenes WS, Mekonnen W, Melaku YA, Yano Y, Artaman A, Campos I, MacLachlan J, Mueller U, Kim D, Trillini M, Eshrati B, Williams HC, Shibuya K, Dandona R, Murthy K, Cowie B, Amare AT, Antonio CA, Castaneda-Orjuela C, van Gool CH, Violante F, Oh IH, Deribe K, Soreide K, Knibbs L, Kereselidze M, Green M, Cardenas R, Roy N, Tillmann T, Li Y, Krueger H, Monasta L, Dey S, Sheikhbahaei S, Hafezi-Nejad N, Kumar GA, Sreeramareddy CT, Dandona L, Wang H, Vollset SE, Mokdad A, Salomon JA, Lozano R, Vos T, Forouzanfar M, Lopez A, Murray C, Naghavi M. The global burden of cancer 2013. JAMA Oncol 2015; 1: 505-527.
2) Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.
3) Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D, Bray F. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer 2013; 49: 1374-1403.
4) van Meir H, Kenter GG, Burggraaf J, Kroep JR, Welters MJ, Melief CJ, van der Burg SH, van Poelgeest MI. The need for improvement of the treatment of advanced and metastatic cervical cancer, the rationale for combined chemo-immunotherapy. Anticancer Agents Med Chem 2014; 14: 190-203.
5) Zou T, Wang PL, Gao Y, Liang WT. Circular RNA_LARP4 is lower expressed and serves as a potential biomarker of ovarian cancer prognosis. Eur Rev Med Pharmacol Sci 2018; 22: 7178-7182.
6) Dai Y, Li D, Chen X, Tan X, Gu J, Chen M, Zhang X. Circular RNA myosin light chain kinase (MYLK) promotes prostate cancer progression through modulating mir-29a expression. Med Sci Monit 2018; 24: 3462-3471.
7) Han J, Zhao G, Ma X, Dong Q, Zhang H, Wang Y, Cui J. CircRNA circ-BANP-mediated miR-503/LARP1 signaling contributes to lung cancer progression. Biochem Biophys Res Commun 2018; 503: 2429-2435.
8) Qu Y, Dou P, Hu M, Xu J, Xia W, Sun H. CircRNACER mediates malignant progression of breast cancer through targeting the miR136/MMP13 axis. Mol Med Rep 2019; 19: 3314-3320.
9) Song T, Xu A, Zhang Z, Gao F, Zhao L, Chen X, Gao J, Kong X. CircRNA hsa_circRNA_101996 increases cervical cancer proliferation and invasion through activating TPX2 expression by restraining miR-8075. J Cell Physiol 2019; 234: 14296-14305.
10) Zhang J, Zhao X, Zhang J, Zheng X, Li F. Circular RNA hsa_circ_0023404 exerts an oncogenic role in cervical cancer through regulating miR-136/TFCP2/YAP pathway. Biochem Biophys Res Commun 2018; 501: 428-433.
11) Ma HB, Yao YN, Yu JJ, Chen XX, Li HF. Extensive profiling of circular RNAs and the potential regulatory role of circRNA-000284 in cell proliferation and invasion of cervical cancer via sponging miR-506. Am J Transl Res 2018; 10: 592-604.
12) Hu C, Wang Y, Li A, Zhang J, Xue F, Zhu L. Overexpressed circ_0067934 acts as an oncogene to facilitate cervical cancer progression via the miR-

545/EIF3C axis. J Cell Physiol 2019; 234: 9225-9232.

13) Shuai M, Hong J, Huang D, Zhang X, Tian Y. Upregulation of circRNA_0000285 serves as a prognostic biomarker for nasopharyngeal carcinoma and is involved in radiosensitivity. Oncol Lett 2018; 16: 6495-6501.

14) An Y, Chen XM, Yang Y, Mo F, Jiang Y, Sun DL, Cai HH. LncRNA DLX6-AS1 promoted cancer cell proliferation and invasion by attenuating the endogenous function of miR-181b in pancreatic cancer. Cancer Cell Int 2018; 18: 143.

15) Huang Y, Ni R, Wang J, Liu Y. Knockdown of lncRNA DLX6-AS1 inhibits cell proliferation, migration and invasion while promotes apoptosis by downregulating PRR11 expression and upregulating miR-144 in non-small cell lung cancer. Biomed Pharmacother 2019; 109: 1851-1859.

16) Crozat A, Aman P, Mandahl N, Ron D. Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature 1993; 363: 640-644.

17) Ke H, Zhao L, Feng X, Xu H, Zou L, Yang Q, Su X, Peng L, Jiao B. NEAT1 is required for survival of breast cancer cells through FUS and miR-548. Gene Regul Syst Bio 2016; 10: 11-17.

18) Brooke GN, Culley RL, Dart DA, Mann DJ, Gaughan L, McCracken SR, Robson CN, Spencer-Dene B, Gamble SC, Powell SM, Wait R, Waxman J, Walker MM, Bevan CL. FUS/TLS is a novel mediator of androgen-dependent cell-cycle progression and prostate cancer growth. Cancer Res 2011; 71: 914-924.