Circ_0000228 Promotes Cervical Cancer Progression via Regulating miR-337-3p/TGFBR1 Axis

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Received: 24/December/2020, Accepted: 24/June/2021

Abstract
Objective: This study aims to investigate the biological function of circular RNA (circRNA) circ_0000228 in the cervical cancer (CC).

Materials and Methods: In this experimental study, the GSE113696 dataset was downloaded from the Gene Expression Omnibus (GEO). GEO2R was employed to obtain differentially expressed circRNA between CC tissues and matched paracancerous tissues. Quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot were employed to detect circ_0000228, microRNA-337-3p (miR-337-3p) and transforming growth factor, beta receptor 1 (TGFBR1) expression levels in the CC tissues and cells. Following gain-of-function and loss-of-function models establishment, CCK-8 and BrdU tests were conducted to examine cell proliferation. Tranwell experiment was executed to examine CC cells migration and invasion. A lung metastasis model was utilized to determine the ability of circ_0000228 on the lung metastasis. Bioinformatics analysis, dual-luciferase reporter experiment and RNA immunoprecipitation (RIP) assay were applied to verify the targeting relationship among miR-337-3p, circ_0000228, and TGFBR1.

Results: Circ_0000228 expression in the CC tissues and cells was up-modulated. Circ_0000228 overexpression markedly enhanced cell proliferation, migration, and invasion, while knocking down circ_0000228 remarkably repressed cell proliferation, migration, and invasion. MiR-337-3p could be adsorbed by circ_0000228. TGFBR1 was identified as a target gene of miR-337-3p that indirectly and positively modulated by circ_0000228 in the CC cells.

Conclusion: Circ_0000228 up-modulates TGFBR1 by targeting miR-337-3p to enhance CC cell proliferation, migration and invasion. Also, Circ_0000228 is a promising therapeutic target for the CC.

Keywords: Cervical Cancer, miR-337-3p, TGFBR1


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Introduction
Cervical cancer (CC), a common malignancy, seriously threatens women’s health (1) and also, surgery, chemotherapy and radiation therapy are considered as approaches for CC treatment (2). Unfortunately, the majority of patients have reached an advanced stage by the time of diagnosis, resulting in losing the opportunity for radical surgery (3). It is of great clinical value to decipher the CC pathogenesis to reach target therapy, particularly in the advanced stage.

Circular RNAs (circRNAs) as a member of endogenous non-coding RNAs, are closed-loop RNA molecules that are formed by reverse splicing. There are not 5'-end cap and 3'-end poly A tail in their structure. Also, they can be stably present in the diverse eukaryotic cells (4). Previously, circRNAs were considered as a "noise" of gene transcription (5, 6). However, recently, their biological function has been revealed (5, 7). Some studies have shown, abnormal expression of circRNAs in the human malignancies with unfavorable prognosis (8-15). For instance, in gastric cancer, circ-DONSON promotes the proliferation, migration, and invasion of cancer cells, and impedes apoptosis (13). In CC, circ_0000515 and circ_0007534 facilitate proliferation, migration and invasion of CC cells and repress apoptosis (14, 15). Also, circ_0000228 is generated from zinc finger E-box binding homeobox 1 (ZEB1) transcription. ZEB1 has been reported to be up-regulated in the CC and acts as an oncogene (16). Nonetheless, the biological function and mechanism of circ_0000228 in CC is undefined.

MicroRNA (miRNA, miR)-337-3p is down-modulated in the CC cells and suppresses proliferation, migration and invasion of these cells and induces apoptosis (17, 18). Also, TGFBR1 is reported to be overexpressed in the CC, and can enhance CC cell malignancy (19). Bioinformatics analysis predicts that miR-337-3p targets the 3'UTR of transforming growth factor, beta receptor 1 (TGFBR1). Moreover, circ_0000228 is predicted to be a potential molecular sponge for miR-337-3p. In this study, we probe function and mechanism of circ_0000228, miR-337-3p and TGFBR1 in the CC progression. We aim to offer clues to improve clinical diagnosis and therapy of CC.

Materials and Methods
Specimens collection
The work was approved by the Ethics Committee of Shengli Oilfield Central Hospital (2018-06). Totally, 57 CC specimens and corresponding cervical paracancerous specimens were surgically obtained of patients who
referred to the Shengli Oilfield Central Hospital. The patients with pathologically diagnosed CC who are willing to provide written informed consents participated in this study. The specimens were stored in the liquid nitrogen. All subjects did not undergo radiotherapy, chemotherapy or other anti-cancer treatments before surgery. All subjects signed an informed consent form before the surgery and tissue collection.

Cell culture

Human cervical epithelial cells (HUCECs) and CC cell lines (SiHa, HeLa, CaSkI, and C33A) were purchased from the Cell Center of Chinese Academy of Sciences (Shanghai, China). All cell lines were cultured in Dulbecco’s Modified Eagle Medium (DMEM) medium (Cat No. 11965-092, Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS, Cat No. 10270098, Gibco, Grand Island, NY, USA)+100 U/mL penicillin+100 μg/mL streptomycin (Cat No. 15140122, Hyclone, Logan, UT, USA). Then, all were incubated at 37°C, 5% CO₂, 95% humidity

Cell transfection

Small interfering RNA (siRNA) negative control (si-NC), siRNA against circ_0000228 (si-circ_0000228-1), siRNA against circ_0000228 (si-circ_0000228-2), pcDNA empty vector (NC), pcDNA-circ_0000228 (circ_0000228), mimics negative control (mimics NC), miRNA inhibitors negative control (inhibitors NC), miR-337-3p mimics, and miR-337-3p inhibitors were available from GenePharma Co., Ltd (Cat No. MIN0000578, Shanghai, China). All cell lines were cultured in Dulbecco’s Modified Eagle Medium (DMEM) medium (Cat No. 11965-092, Invitrogen, Carlsbad, CA, USA) and cultured at 37°C, 95% humidity.

Quantitative real-time polymerase chain reaction

Using TRIzol reagent (Cat No. 15596-018, Invitrogen, Carlsbad, CA, USA), total RNA was extracted from CC tissues and cells. Then, the PrimeScript™ RT Reagent kit (Cat No. RR037A, Takara Biotechnology Co., Ltd., Dalian, China) was utilized to reverse transcribe total RNA into cDNA. Next, qRT-PCR was implemented using SYBR Premix-Ex Taq™ (Cat No. 368706, Takara, Dalian, China) on the ABI7500 FAST Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). GAPDH was regarded as an internal reference to quantify circ_0000228 and TGFBR1 mRNA expression levels, and U6 was considered as an internal reference to detect miR-337-3p expression. Circ_0000228, miR-337-3p, and TGFBR1 mRNA relative expression was calculated using the 2^(-ΔΔCT) method. A PARIS™ Kit (Cat No. AM1556, Ambion, Austin, TX, USA) was employed for subcellular fractionation. After the cytoplasmic RNA and nuclear RNA were isolated respectively, qRT-PCR was executed to evaluate circ_0000228 expression in cytoplasm and nuclei. The primer sequences used for qRT-PCR were as follows:

\[
\begin{align*}
\text{circ}_0000228- n & = \text{GAGGTGTGGGGGTGGAGAAGGAGGAG}\text{GATGCGTCTATG} \quad \text{R: 5}^\prime\text{-GCCGTTGCAGCACATATACACAATAT} \quad \text{3}^\prime \\
\text{miR}_337-3p- \quad \text{F: 5}^\prime\text{-GCCGTTGCAGCACATATACACAATAT} \quad \text{R: 5}^\prime\text{-GCCGTTGCAGCACATATACACAATAT} \quad \text{3}^\prime \\
\end{align*}
\]

BrdU experiment

Cell proliferation was also assessed with the BrdU Cell Proliferation Assay kit (Cat No. 6813, Beyotime, Shanghai, China). The single-cell suspension was prepared with HeLa and C33A cells, and the cells were inoculated into 96-well plates (2×10^3 cells/well) and incubated. After 24 hours, 10 μL of cell counting kit8 (CCK-8) solution (Cat No. HY-K 0301, MedChemExpress, Monmouth Junction, NJ, USA) was supplemented to each well and then the cell culture was continued for 1 hour. The absorbance (OD_450nm value) of each well was recorded using a Bio-Tek Synergy HT Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA). Thereafter, with the same method, the absorbance of the cells was measured 48 hours and 72 hours later, respectively.
were stained with Hoechst staining solution. The total number of cells and the number of BrdU-positive cells in 10 high magnification fields were counted randomly under the microscope, and the percentage of BrdU-positive cells was calculated.

**Transwell experiment**

In the migration experiments, HeLa and C33A cells were resuspended in the serum-free medium, and the cell density was modulated (2×10^5 cells/mL), and then 100 μL of the cell suspension was supplemented to the upper compartment of the Transwell system (Cat No. 3422, Corning, Corning, NY, USA). Then, 500 μL of DMEM medium containing 10% FBS was supplemented to the lower compartment of the Transwell chamber. 24 hours’ incubation at room temperature, the upper compartment cells that did not migrate were gently wiped off with cotton swabs, and the attached cells on the lower surface of the membrane were fixed with 4% paraformaldehyde (Cat No. J61899, Alfa Aesar, averhill, MA, USA). After that, the cells were stained with 0.1% crystal violet (Cat No. C0121, Beyotime, Shanghai, China) for 10 minutes. After the membranes were washed, five randomly selected microscopic fields per membrane was selected and the numbers of stained cells were counted. To perform the cell invasion assay, the Transwell inserts were pre-covered with 50 μl of the Matrigel matrix. DMEM medium containing 10% FBS was placed in the lower chamber as a chemoattractant. Twenty-four hours later, 0.1% crystal violet was used to stain the cells that had invaded through the membranes. Then, the cells were observed by a microscope.

**Dual-luciferase reporter assay system**

The dual-luciferase reporter assay system (Cat No. 11752250, Promega, Madison, WI, USA) was used in this experiment. HeLa and C33A cells were trypsinized, counted, and planted in a 24-well plate (1×10^4 cells/well), and cultured for 24 hours. When cell confluence reached 80-90%, the transfected with mimics NC, miR-337-3p inhibitors, respectively. After the cells were cultured for 48 hours after transfection, HeLa and C33A cells were lysed with RIPA lysis buffer (Cat# P0013B, Beyotime Biotechnology, Shanghai, China) containing protease inhibitors (Cat No. 1183617001, Roche Applied Science, Penzberg, Germany), and the supernatants were collected after high-speed centrifugation, and protein concentrations were determined by a BCA kit (Cat No. P0012S, Beyotime, Shanghai, China). The supernatant was mixed with loading buffer, and then heated in a water bath at 100°C for 10 minutes to denature the protein. Next, the total proteins were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes by electrotransfer. The membranes were then incubated with the specific primary antibodies overnight at 4°C. After rinsing with Tween-20 (TBST, Cat No. AA177500K8, Fisher Scientific, Houston, TX, USA), the membranes were then incubated with corresponding secondary antibodies for 2 hours at room temperature. The protein bands were visualized by electrochemiluminescence automatic chemiluminescence imaging analysis system (Tanon 5500, Tanon Science & Technology, Shanghai, China), and β-actin was regarded as an internal reference. The antibodies used in this work were available from Abcam (Shanghai, China), including primary antibodies: anti-TGFBR1 antibody (ab31013, 1:1000), anti-β-actin antibody (ab179467, 1:1000), and a second antibody (ab205718, 1:2000).

**Western blot**

All data were analyzed using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA). Shapiro-Wilk
(SW) test was used to analyze the normal distribution of the data. All the measurement data were expressed as "mean ± standard deviation" (mean ± SD). Also, t test was adopted for comparison between two groups, and one-way ANOVA was used for comparison of the means among multiple groups. For skewed distributed data, the Wilcoxon signed-rank test was used. Counting data were expressed in contingency tables, and χ2 test was utilized to analyze differences between the two groups. Statistical significance was indicated by P<0.05.

Results

Circ_0000228 expression was up-modulated in cervical cancer

Detecting circRNA expression profile in the CC tissues, we observed that 122 circRNAs were down-modulated (P<0.05), while 34 circRNAs (including circ_0000228) were up-modulated (Fig.1A, B, P<0.05). Consistently, qRT-PCR indicated that circ_0000228 was up-modulated in the CC tissues (n=57) in comparison with the matched non-cancerous tissues (Fig.1C, P<0.001). Analyzing relationship between circ_0000228 expression in the CC tissues and clinical parameters, we observed that high circ_0000228 expression in the CC tissues was linked to lymph node metastasis and low differentiation of tumor tissues (Fig.1D, Table 1, P<0.05). Additionally, circ_0000228 expression was up-modulated in all of the 4 CC cell lines (SiHa, HeLa, CaSKi, C33A) relative to normal cervical epithelial cell line HUCEC cell (Fig.1E, P<0.01).

Circ_0000228 enhanced the proliferation, migration and invasion of cervical cancer cells

To examine the biological role of circ_0000228 in the CC, HeLa cells were transfected with circ_0000228 overexpression plasmid. Also, C33A cells were transfected with si-circ_0000228-1 and si-circ_0000228-2 (Fig.2A). CCK-8 colorimetric assay unveiled that circ_0000228 overexpression facilitates the proliferation of HeLa cells (P<0.001), while knock down circ_0000228 restrained C33A cell proliferation (Fig.2B, P<0.001). The data of BrdU experiments manifested, that the number of BrdU-positive cells was higher in the circ_0000228 overexpression group in comparison with the control group (P<0.001). The BrdU-positive cells number was lower in the si-circ_0000228-1 and si-circ_0000228-2 groups (Fig.2C, P<0.001). And, Transwell experiment was executed to examine the effects of circ_0000228 on the CC cell migration and invasion. The results demonstrated that circ_0000228 overexpression facilitates migration of HeLa cell and invasion (P<0.001), while circ_0000228 knockdown restrained C33A cells migration and invasion (Fig.2D, P<0.001). Finally, we used a lung metastasis model, in vivo model, to investigate the role of circ_0000228 in the CC cells metastasis regulation. The results indicated that circ_0000228 overexpression promoted lung metastasis in vivo (Fig.S1, See Supplementary Online Information at www.celljournal.org, P<0.001).

Table 1: Correlation between clinicopathological features and expression of circ_0000228 in the CC tissues

<table>
<thead>
<tr>
<th>Pathological parameters</th>
<th>Numbers</th>
<th>Circ_0000228 expression</th>
<th>χ²</th>
<th>P value</th>
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<tr>
<td></td>
<td>(n=57)</td>
<td>High (n=28) Low (n=29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (Y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>33</td>
<td>15</td>
<td>18</td>
<td>0.4220</td>
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<tr>
<td>≥45</td>
<td>24</td>
<td>13</td>
<td>11</td>
<td>0.5159</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4</td>
<td>29</td>
<td>12</td>
<td>17</td>
<td>1.4164</td>
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<tr>
<td>≥4</td>
<td>28</td>
<td>16</td>
<td>12</td>
<td>0.2340</td>
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<tr>
<td>FIGO stage</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>26</td>
<td>11</td>
<td>15</td>
<td>0.8884</td>
</tr>
<tr>
<td>II</td>
<td>31</td>
<td>17</td>
<td>14</td>
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<td>Lymph node metastasis</td>
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</tr>
<tr>
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<td>8</td>
<td>16</td>
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<td>20</td>
<td>13</td>
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<tr>
<td>Degree of differentiation</td>
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<td>21</td>
<td>10</td>
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<tr>
<td>Well</td>
<td>26</td>
<td>7</td>
<td>19</td>
<td>0.0021*</td>
</tr>
</tbody>
</table>

CC; Cervical cancer, FIGO; International federation of gynecology and obstetrics, and *; P<0.05.
Circ_0000228 directly targeted miR-337-3p

To probe the downstream targets of circ_0000228, the CircInteractome database (https://circinteractome.nia.nih.gov/) was searched. miR-337-3p was selected as a one of the predicted target miRNAs (Fig.3A, P<0.001). Subsequently, miR-337-3p expression in the CC cell lines was examined by qRT-PCR. miR-337-3p expression was demonstrated to be diminished in the CC cell lines (Fig.3B, P<0.05). Nucleocytoplasmic separation assay showed that circ_0000228 was expressed in the CC cells cytoplasm (Fig.3C, P<0.001). Dual-luciferase reporter experiment showed that miR-337-3p overexpression repressed the luciferase activity of WT circ_0000228, while miR-337-3p inhibition enhanced the luciferase activity of WT circ_0000228 (Fig.3D, P<0.001). However, neither miR-337-3p mimic nor miR-337-3p inhibitor affected the luciferase activity of MUT circ_0000228 (Fig.3D). Next, the results of RIP experiments showed that circ_0000228 and miR-337-3p were enriched in the Ago2-containing microribonucleoproteins relative to IgG group, suggesting a direct interaction between circ_0000228 and miR-337-3p (Fig.3E, P<0.001). Moreover, circ_0000228 overexpression suppressed miR-337-3p expression in the HeLa cells; while circ_0000228 knock down circ_0000228 increased miR-337-3p expression in the C33A cells (Fig.3F, P<0.001). Also, miR-337-3p was unveiled to be down-modulated in the CC tissues by qRT-PCR (Fig.3G, P<0.001). Pearson’s correlation analysis indicated that circ_0000228 was negatively correlated with miR-337-3p expression in the CC tissues (Fig.3H, P<0.001).

Fig.1: The expression characteristics of circ_0000228 in CC. A, B. Variations in the expression of circRNAs in the CC tissues were examined by analyzing dataset GSE113696. C. qRT-PCR was executed to examine circ_0000228 expression in the 57 cases of CC tissues and matched paracancerous tissues. D. qRT-PCR was implemented to examine circ_0000228 expression in the CC tissues of the patients with lymph node metastasis and without lymph node metastasis, different differentiation status, respectively. E. Circ_0000228 expression in the HUCECs and CC cell lines (SiHa, HeLa, CaSki and C33A) was measured by qRT-PCR. CC; cervical cancer, qRT-PCR; Quantitative real-time polymerase chain reaction, HUCECs; Human cervical epithelial cells, **; P<0.01, and ***; P<0.001.

Fig.2: Regulatory role of circ_0000228 in the CC cells phenotype. A. HeLa and C33A cells were transfected with circ_0000228 overexpression plasmid and circ_0000228 siRNAs, respectively, and also, the transfection efficiency was examined by qRT-PCR. B, C. The effects of circ_0000228 overexpression or knockdown on the proliferation of HeLa and C33A cells were detected using CCK-8 colorimetric assay and BrdU experiment. D. Transwell experiments were used to examine the effects of circ_0000228 overexpression and knockdown on the migration and invasion of HeLa and C33A cells. CC; Cervical cancer, qRT-PCR; Quantitative real-time polymerase chain reaction, **; P<0.01, and ***; P<0.001.

Fig.3: Circ_0000228 directly targeted miR-337-3p. A. Bioinformatics analysis projected the binding site between circ_0000228 and miR-337-3p. B. MiR-337-3p expression in the CC cell lines and HUCECs was examined by qRT-PCR. C. Nucleocytoplasmic separation experiment was conducted to verify the localization of circ_0000228 in the CC cells. D. Dual-luciferase reporter gene experiment was implemented to validate the bioinformatics predicted binding site. E, RIP assays were utilized to prove the interaction between circ_0000228 with miR-337-3p. F. The effect of circ_0000228 overexpression and knockdown on the miR-337-3p expression in the CC cells was detected by qRT-PCR. G. MiR-337-3p expression in the 57 CC tissues and 57 paracancerous tissues was modulated in the CC tissues by qRT-PCR (Fig.3G, P<0.001). Also, miR-337-3p was demonstrated to be diminished in the CC cell lines (Fig.3B, P<0.05). Nucleocytoplasmic separation assay showed that circ_0000228 was expressed in the CC cells cytoplasm (Fig.3C, P<0.001). Dual-luciferase reporter experiment showed that miR-337-3p overexpression repressed the luciferase activity of WT circ_0000228, while miR-337-3p inhibition enhanced the luciferase activity of WT circ_0000228 (Fig.3D, P<0.001). However, neither miR-337-3p mimic nor miR-337-3p inhibitor affected the luciferase activity of MUT circ_0000228 (Fig.3D). Next, the results of RIP experiments showed that circ_0000228 and miR-337-3p were enriched in the Ago2-containing microribonucleoproteins relative to IgG group, suggesting a direct interaction between circ_0000228 and miR-337-3p (Fig.3E, P<0.001). Moreover, circ_0000228 overexpression suppressed miR-337-3p expression in the HeLa cells; while circ_0000228 knock down circ_0000228 increased miR-337-3p expression in the C33A cells (Fig.3F, P<0.001). Also, miR-337-3p was unveiled to be down-modulated in the CC tissues by qRT-PCR (Fig.3G, P<0.001). Pearson’s correlation analysis indicated that circ_0000228 was negatively correlated with miR-337-3p expression in the CC tissues (Fig.3H, P<0.001).
**Circ_0000228 regulated the proliferation, migration and invasion of cervical cancer cells by adsorbing miR-337-3p**

Subsequently, circ_0000228 overexpression plasmid and miR-337-3p mimics were co-transfected into the HeLa cells. Also, si-circ_0000228-1 and miR-337-3p inhibitors were co-transfected into the C33A cells (Fig.4A). CCK-8 colorimetric assay, BrdU experiments and Transwell experiments showed that circ_0000228 overexpression facilitated CC cell proliferation, migration and invasion (P<0.05), while transfection with miR-337-3p mimics attenuated these effects (P<0.05). On the other hand, knocking down circ_0000228 repressed cell proliferation, migration, and invasion (P<0.05), while transfection of miR-337-3p inhibitors partially reversed these effects (Fig.4B-D, P<0.05).

**Circ_0000228 targeted miR-337-3p to up-modulate TGFBR1 expression**

The TargetScan database (http://www.targetscan.org/vert_72/) was used to predict the downstream targets of miR-337-3p, and TGFBR1 was predicted as one of the potential downstream targets of miR-337-3p (Fig.5A). Dual-luciferase reporter experiment showed that miR-337-3p overexpression repressed the luciferase activity of WT TGFBR1, while miR-337-3p inhibition enhanced the luciferase activity of WT TGFBR1 (Fig.5B, P<0.001). However, neither miR-337-3p mimic nor miR-337-3p inhibitor affected the luciferase activity of MUT TGFBR1 (Fig.5B).

Western blot showed that circ_0000228 overexpression enhanced TGFBR1 expression in the HeLa cells (P<0.001), whereas transfection of miR-337-3p mimics attenuated this effect (Fig.5C, P<0.001). Knocking down circ_0000228 impeded TGFBR1 expression in C33A cells (P<0.001), whereas inhibition of miR-337-3p counteracted this effect (Fig.5C, P<0.001). By qRT-PCR, TGFBR1 mRNA revealed overexpression in the CC tissues (Fig.5D, P<0.001). Notably, TGFBR1 mRNA expression in CC tissues was negatively correlated with miR-337-3p expression (P<0.001) and positively correlated with circ_0000228 expression (Fig.5E, F, P<0.001).

**Discussion**

In this study, we observed that circ_0000228 was up-regulated in the CC tissues and its overexpression was associated with several adverse clinical parameters in the CC patients. Our experiments demonstrated that circ_0000228 overexpression facilitates proliferation, migration and invasion of CC cells. We verified these findings with while knock down model of circ_0000228, and observed opposite effects. Several studies report that they are crucial regulators in cancer biology (20). For instance, circ-ITCH restrains the proliferation, migration and invasion of bladder cancer cells by sponging miR-17/miR-224 to up-regulate PTEN expression (8). Circ-SMARCAS5 represses the development of multiple myeloma by decoying miR-767-5p (21). Knocking down circ_0000228 suppresses the growth and migration of the CC cells (22). In the present work, our data indicated that circ_0000228 is a new oncogenic factor in the CC tissues and cells.

As mentioned above, miRNAs are often negatively regulated by circRNAs via a competitive endogenous RNA mechanism (8). In this work, it was found...
that circ_0000228 directly targets miR-337-3p and circ_0000228 enhances the CC cell proliferation, migration and invasion via adsorbing miR-337-3p. miRNAs are endogenous ncRNAs that are approximately 20 nucleotides in length that participate in the regulating diverse biological processes including epigenetic regulation, cell cycle, cell differentiation, proliferation, migration and so on (23, 24). miRNAs can function as either tumor-suppressive factors or oncogenic factors. For instance, miR-324-3p enhances the proliferation, migration, and invasion of colonic cancer cells, and impedes apoptosis (25). MiR-338-3p restrains CC progression by targeting MACC1 to regulate the MAPK signaling pathway (26). MiR-1284 represses the growth and metastasis of CC cells by targeting HMGBI1 and increases the sensitivity of CC cells to cisplatin (27). It is reported that miR-337-3p was a tumor suppressor in the CC cells and tissues (17, 18). Here, we reported that miR-337-3p counteracts with cancer-promoting effects of circ_0000228 in CC cells and tissues, which also validated the anti-cancer effects of miR-337-3p. Moreover, we demonstrated that miR-337-3p can be adsorbed by circ_0000228, which is a reasonable explanation for the aberrant expression of miR-337-3p in the CC cells and tissues.

Usually, miRNAs exert their biological functions through binding to the 3'UTR of mRNAs to induce translational repression or degradation of mRNAs (23). In this work, we found that miR-337-3p directly targets TGFBR1 mRNA 3'UTR and negatively regulates TGFBR1 mRNA expression, and circ_0000228 can promote TGFBR1 expression in the CC cells. TGFBR1 belongs to the TGF-β receptors family, which is involved in the TGF-β-mediated cell growth, differentiation and migration (28, 29). Accumulating studies have confirmed the regulatory role of TGFBR1 in the different cancers (30-32). For instance, TGFBR1 overexpression can enhance the proliferation, migration, invasion and the epithelial-mesenchymal transition process of gastric cancer cells (30).

In pancreatic cancer, LINC00462 overexpression enhances the expression of TGFBR1 and TGFBR2, thereby TGF-β/Smad pathway activating leads to facilitate proliferation, migration, and invasion of pancreatic cancer cells (31). In the non-small cell lung cancer (NSCLC), miR-3607-3p impedes tumor cell proliferation, invasion and migration by targeting TGFBR1 (32). Also, in the CC, TGFBR1 is reported to be a target of let-7a, and it mediates the activation of TGF-β/SMAD signaling in the CC cells (19). To our knowledge, this study is the first to identify miR-337-3p as an upstream miRNA of TGFBR1 in the CC cells.

Conclusion

This research reveals that circ_0000228 is highly expressed in the CC tissues and cells, and its highest expression is associated with adverse clinical parameters in the affected. Functionally and mechanistically, we confirm that circ_0000228 enhances proliferation, migration and invasion of CC cells via modulating the miR-337-3p/TGFBR1 axis. This work may provide novel ideas for the diagnosis, therapy, and prognosis of CC patients.

Acknowledgments

We thank the staff of the Department of Gynecology and Obstetrics and Department of Pathology of the Shengli Oilfield Central Hospital for their help and support in this study. We also thank Hubei Yican Health Industry Co., Ltd. for its linguistic assistance during the preparation of this manuscript. There is no financial support and conflict of interest in this study.

Authors’ Contributions

Y.X., X.D.: Contributed to conception and design. X.D., B.M., P.M.: Contributed to all experimental work, data, statistical analysis, and interpretation of data. Y.X., X.D.: Were responsible for overall supervision. Y.X., X.K., D.L.: Drafted the manuscript, which was revised by Y.X., X.D. All authors read and approved the final manuscript.

Reference