Research Article

LncRNA LINC01116 Regulates the Proliferation, Migration, and Invasion of Cervical Cancer Cells by Targeting miR-744-5p

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Objective. To investigate the effects and potential molecular mechanisms of LncRNA LINC01116 on proliferation, migration, and invasion of cervical cancer cells. Method(s). The content of miR-744-5p and LINC01116 in cervical cancer cells HeLa, SiHa, and C33a was detected by RT-PCR, the proliferative activity and clone number of SiHa cells were determined by MTT and clone formation assay, the number of invaded and migrated cells was determined by Transwell assay, the expressions of Cyclin D1 and MMP-2 in cells were detected by Western blot, and the activity of luciferase detected by dual-luciferase reporting system verified the regulatory relationship between LINC01116 and miR-744-5p. Result(s). Compared with human normal cervical epithelial cells Ect1/E6E7, the content of LINC01116 in cervical cancer cells HeLa, SiHa, and C33a was increased significantly [(1.04 ± 0.12) vs. (3.34 ± 0.38)/(4.48 ± 0.45)/(3.59 ± 0.45)] (P < 0.05), the content of miR-744-5p was decreased significantly [(0.98 ± 0.09) vs. (0.39 ± 0.04)/(0.34 ± 0.05)/(0.41 ± 0.05)] (P < 0.05). Silencing LINC01116 could inhibit the protein expression of Cyclin D1 [(0.68 ± 0.06) vs. (0.31 ± 0.04)] and MMP-2 [(0.74 ± 0.08) vs. (0.34 ± 0.05)] in SiHa cells, and inhibition of cell proliferation [(100.98 ± 7.89)% vs. (67.26 ± 6.14)%], clone formation ability [(180.31 ± 11.34) vs. (53.56 ± 6.18)], migration [(234.67 ± 15.31) vs. (98.46 ± 7.46)], and invasion [(134.83 ± 9.12) vs. (62.44 ± 5.34)]. LINC01116 targeted and negatively regulates the expression of miR-744-5p. Overexpression of miR-744-5p could inhibit the proliferation of cervical cancer SiHa cells [(101.64 ± 4.89)% vs. (62.34 ± 5.73%)], clone formation ability [(179.24 ± 12.34) vs. (47.67 ± 5.18)], migration [(241.06 ± 16.64) vs. (84.52 ± 7.57)], and invasion [(127.92 ± 8.16) vs. (65.24 ± 7.36)]. Inhibition of miR-744-5p reversed the effects of silencing LINC01116 on SiHa cell proliferation [(102.46 ± 7.64)% vs. (146.72 ± 12.23%)], clone formation ability [(47.64 ± 6.20) vs. (104.26 ± 8.64)], migration [(81.52 ± 7.42) vs. (153.64 ± 12.57)], and invasion [(69.12 ± 5.56) vs. (94.31 ± 7.44)]. Conclusion(s). LncRNA LINC01116 inhibits proliferation, migration, and invasion of SiHa cells by targeting miR-744-5p, and LINC01116 is a potential molecular target for cervical cancer.

1. Introduction

Cervical cancer is a common gynecological malignancy tumor, and there were about 100,000 new cases in 2014, with the incidence rate of about 15.3/100,000 [1]. Despite continuous progress in the treatment and method of cervical cancer, the median survival of patients with metastatic cervical cancer is only 8–13 months [2]. Studies have shown that a large number of IncRNA (long noncoding RNA) and miRNA (microRNA) are abnormally expressed in cervical cancer and are involved in the progression, migration, and invasion and prognosis and drug resistance of cancer cells [3–9]. Long-chain noncoding RNA LINC01116 is highly expressed in nasopharyngeal carcinoma cell line [10], gastric cancer tissue [11], and oral squamous cell carcinoma [12] and is closely related to the proliferation and migration of cancer cells. In this study, starBase prediction showed that there was a binding site for miR-744-5p and LINC01116. miR-744-5p is abnormally expressed in a variety of tumor tissues or cells, such as ovarian cancer and non-small-cell lung cancer [13, 14], targeting different genes involved in tumor proliferation and invasion. This study was aimed at exploring the effects and possible molecular mechanisms of LncRNA LINC01116 on the proliferation, invasion, and migration of SiHa cells and observing the role of miR-
744-5p in this mechanism, in order to provide new targets for cervical cancer.

2. Materials and Methods

2.1. Material. The following materials are used: human cervical cancer cell lines HeLa, SiHa, C33a, and the human normal cervical cancer epithelial cell line Ect1/E6E7 (purchased from ATCC); fetal calf serum (fetal bovine serum, FBS) and DMEM (Dulbecco’s modified eagle medium) medium (purchased from Gibco); RNA extraction reagent TRizol, Trypsin, and tetrazolium blue (Thiazolyl Blue Tetrazolium Bromide, MT) (purchased from Sigma-Aldrich Company); Transwell board (purchased from Corning Company, USA); Lipofectamine 2000, Real-time PCR kit, and reverse transcription kit (RT-PCR) (purchased from Invitrogen Company, USA); primers and vectors (purchased from the Shanghai Gemma gene); real-time PCR instrument (purchased from Bio-Rad, USA).

2.2. Method

2.2.1. Cell Transfection. si-LINC01116 and mutant (MUT-LINC01116) double luciferase vectors were transfected into si-LINC01116 and using si-con as the control); the miR-744-5p group (transfected with miR-744-5p and using miR-con as the control); si-LINC01116 + anti-miR-744-5p group (transfected with si-LINC01116 + anti-miR-744-5p and using si-LINC01116 + anti-miR-con as the control); and dual-luciferase reporting experimental group (cotransfected with miR-con + WT-LINC01116, miR-744-5p + WT-LINC01116, miR-con + MUT-LINC01116, miR-744-5p + MUT-LINC01116, respectively).

2.2.2. The Expression of RNA Was Determined by Real-Time PCR. The cells were collected, and the total RNA of cervical epithelial cells Ect1/E6E7 and cervical cancer cell lines HeLa, SiHa, and C33a was extracted with TRizol reagent, and then, cDNA was synthesized according to the instructions of the reverse transcription kit. The cDNA was extracted and real-time PCR was performed as follows: 94°C for 5 min; 94°C 30s, 58°C 45s, 72°C 40s, 35 cycles; extension at 72°C for 10 min. Primers are as follows: LINC01116 upstream: 5′-TCAAGTCATCCTCCACCTC-3′, downstream: 5′-GGGCCTTTGGGTCAAGT-3′; miR-744-5p upstream: 5′-TGCGGGCTAGGGCTAACAGCA-3′, downstream: 5′-GGTGCTAGGGCTAGGGCTAACAGCA-3′, U6 upstream: 5′-GCTTACGAGCCACATAGAGA-3′, downstream: 5′-GGTGCTAGGGCTAGGGCTAACAGCA-3′.

2.2.3. Cell Activity Was Determined by the MTT Assay. SiHa cells (1 × 10^5 cells/mL) were inoculated into 96 microplates (200 μL/well). After the cells were cultured for 72 h, 20 μL MTT (5 mg/mL) was added, and the supernatant was discarded after culture for 4 h. After the supernatant was added with 150 μL DMSO, the cells were mixed evenly at room temperature for 5 min. The absorbance (A) at 490 nm was measured by the SpectraMax i3x (Meigu Molecular Instruments (Shanghai) Co., Ltd).

2.2.4. Cell Migration and Invasion Were Examined by the Transwell Assay. Migration experiments: SiHa cells (2 × 10^5 cells/mL) were collected and added into 100 μL of the upper chamber of Transwell, and 500 μL 10% FBS medium was added into the culture wells of the lower layer of Transwell as the migration chemotaxis agent. After culture for 48 h, the cells were fixed with formaldehyde, stained, and observed and counted under the microscope.

 Invasion experiment: After diluting Matrigel, the medium was added to the upper Transwell chamber and dried. The following procedures were the same as those for migration experiment.

2.2.5. Clone Formation Experiments Determine That Ability of Cell to Form Clones. The transfected cells of SiHa in each group were collected, digested with trypsin and counted, and the cells were diluted and inoculated in 200 cells/dish in a cell culture dish, mixed with 10 mL of culture solution, and cultured for 10–14 days until visible cell clones appeared, discard culture medium, wash cells twice, fix cells with formaldehyde, stain with crystal violet, and count the number of cell clones, the number of cell clones > 50 is effective.

2.2.6. Western Blot Analysis. The cells were collected and the total protein was extracted with RIPA reagent. After the protein content was detected by BCA method, 12% SDS-PAGE electrophoresis was performed. The protein isolates were transferred to PVDF membrane and sealed in 5% skim milk powder for 1 h. The primary antibody was incubated at 4°C overnight, and the membrane was washed 3 times, 10 min each. The second antibody was incubated at room temperature for 1 h, and the membrane was washed for 3 times, 10 min each. The electrochemiluminescence substrate was added and the image was taken by gel imager.

2.2.7. Dual-Luciferase Reporter Experiments. Cells were cultured and transfected according to Section 2.2.1, and SiHa cells were inoculated into a 24-well plate with 2 × 10^4 cells/well for culture for 24 h and then transfected. The constructed wild-type (WT-LINC01116) and mutant (MUT-LINC01116) double luciferase reporter vectors of LINC01116 were cotransfected with SiHa cells with miR-con or miR-744-5p, respectively. After

<table>
<thead>
<tr>
<th>Group</th>
<th>LINC01116 (expression level)</th>
<th>miR-744-5p (expression level)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ect1/E6E7</td>
<td>1.04 ± 0.12</td>
<td>0.98 ± 0.09</td>
</tr>
<tr>
<td>HeLa</td>
<td>3.34 ± 0.38*</td>
<td>0.39 ± 0.04*</td>
</tr>
<tr>
<td>SiHa</td>
<td>4.48 ± 0.45*</td>
<td>0.34 ± 0.05*</td>
</tr>
<tr>
<td>C33a</td>
<td>3.59 ± 0.45*</td>
<td>0.41 ± 0.05*</td>
</tr>
</tbody>
</table>

Note: Comparison with the Ect1/E6E7 group. *P < 0.05.
transfection for 48 h, the luciferase activity was detected by the Modulus Single Tube Luminometer (TurnerBioSystems, USA).

2.3. Statistical Treatment. The results of this experiment were statistically analyzed by SPSS 20.0 (SPSS Co., Ltd., Chicago, USA). Measurement data were expressed by mean ± standard deviation, and t-test was used for their comparison between groups. $P < 0.05$ indicates that the difference is statistically significant.

### 3. Results

#### 3.1. Expression of LINC01116 and miR-744-5p in Normal Cervical and Cervical Cancer Cells. The results of qRT-PCR showed that compared with human normal cervical epithelial cells Ect1/E6E7, the content of LINC01116 in cervical cancer cells HeLa, SiHa, and C33a increased significantly...
(\(P < 0.05\)), while the content of miR-744-5p decreased significantly \((P < 0.05)\), as shown in Table 1.

3.2. Silencing of LINC01116 Inhibits SiHa Proliferation, Migration, and Invasion of Cervical Cancer Cells. Compared with the si-con group, the content of LINC01116 in si-LINC01116 group decreased significantly \((P < 0.05)\), the expression of Cyclin D1 protein decreased significantly, the cell activity and the number of clones decreased significantly \((P < 0.05)\), which indicated that silencing LINC01116 could inhibit the proliferation of SiHa cells. Compared with the si-con group, the number of SiHa migration and invasion cells in si-LINC01116 group decreased significantly \((P < 0.05)\), and the content of migration and invasion protein MMP-2 decreased significantly \((P < 0.05)\), indicating that knocking down LINC01116 can inhibit the migration and invasion of SiHa cells, as shown in Figure 1 and Table 2.

3.3. LINC01116 Targeting miR-744-5p to Regulate Its Expression. The starBase prediction revealed that the sequence of LINC01116 contains sites complementary to miR-744-5p, as shown in Figure 2. Overexpression of miR-744-5p reduced the luciferase activity of WT-LINC01116 \((P < 0.05)\), with no significant effect on the luciferase activity of MUT-LINC01116, as shown in Table 3. Compared with the si-con group, the expression of miR-744-5p in the si-LINC01116 group was significantly increased \((P < 0.05)\). Compared with the pcDNA group, the expression of miR-744-5p in the pcDNA-LINC01116 group was significantly decreased \((P < 0.05)\), as shown in Table 4.

3.4. Transfection of miR-744-5p Inhibited the Proliferation, Migration, and Invasion of SiHa Cells in Cervical Cancer. Compared with the control miR-con group, the MIRI-744-5P content in the miR-744-5p group was significantly increased \((P < 0.05)\), the protein contents of Cyclin D1 and MMP-2 in SiHa cells were decreased, and the number of migrated and invaded cells, cell activity, and clone formation was all significantly decreased \((P < 0.05)\), suggesting that overexpression of miR-744-5p could inhibit the proliferation, migration, and invasion of cervical cancer SiHa cells, as shown in Figure 3 and Table 5.

3.5. Transfection of Anti-miR-744-5p Partially Reversed the Inhibitory Effect of Silencing of LINC01116 on SiHa in Cervical Cancer Cells. Silencing LINC01116 also inhibited miR-744-5p

<table>
<thead>
<tr>
<th>Groups</th>
<th>miR-744-5p</th>
<th>CyclinD1</th>
<th>MMP-2</th>
<th>Cell migration number</th>
<th>Cell invasion number</th>
<th>Cell viability (%)</th>
<th>Number of colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-con</td>
<td>1.04 ± 0.15</td>
<td>0.65 ± 0.09</td>
<td>0.71 ± 0.08</td>
<td>241.06 ± 16.64</td>
<td>127.92 ± 8.16</td>
<td>101.64 ± 4.89</td>
<td>179.24 ± 12.34</td>
</tr>
<tr>
<td>miR-744-5p</td>
<td>5.14 ± 0.58*</td>
<td>0.28 ± 0.04*</td>
<td>0.42 ± 0.06*</td>
<td>84.52 ± 7.57*</td>
<td>65.24 ± 7.36*</td>
<td>62.34 ± 5.73*</td>
<td>47.67 ± 5.18*</td>
</tr>
</tbody>
</table>

Note: Compared with the miR-con group, *\(P < 0.05\).

Figure 3: Effects of miR-744-5p transfection on proliferation, migration, invasion, and cell cloning of SiHa cells. (a) Western blot was used to detect the expression of MMP-2 and CyclinD1 proteins in SiHa cells. (b) Transwell assay was used to detect the migration and invasion of cells. (c) The clone formation experiment assays the ability of cell clone formation.

Table 5: miR-744-5p transfection inhibited proliferation, migration, and invasion of cervical cancer SiHa cells.

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and found that compared with the si-LINC01116+anti-miR-con group, the content of miR-744-5p in SiHa cells of the si-LINC01116+anti-miR-744-5p group was decreased ($P < 0.05$), and the protein content of Cyclin D1 and MMP-2 was increased ($P < 0.05$). The number of migrated and invaded cells, cell activity, and clone formation was significantly increased (all $P < 0.05$), indicating that inhibition of miR-744-5p reversed the inhibition of proliferation, migration, and invasion of SiHa cells by silencing lncRNA LINC01116, as shown in Figures 4 and Table 6.

### Table 6: Effect of transfection of anti-miR-744-5p and silencing LINC01116 on proliferation, migration, and invasion of SiHa cells.

<table>
<thead>
<tr>
<th>Groups</th>
<th>miR-744-5p (copies/μg)</th>
<th>CyclinD1 (μg)</th>
<th>MMP-2 (μg)</th>
<th>Cell migration number</th>
<th>Cell invasion number</th>
<th>Cell viability (%)</th>
<th>Number of colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>si-LINC01116+anti-miR-con</td>
<td>1.08 ± 0.12</td>
<td>0.36 ± 0.03</td>
<td>0.41 ± 0.05</td>
<td>81.52 ± 7.42</td>
<td>69.12 ± 5.56</td>
<td>102.46 ± 7.64</td>
<td>47.64 ± 6.20</td>
</tr>
<tr>
<td>si-LINC01116+anti-miR-744-5p</td>
<td>0.73 ± 0.08*</td>
<td>0.45 ± 0.04*</td>
<td>0.61 ± 0.08*</td>
<td>153.64 ± 12.57*</td>
<td>94.31 ± 7.44*</td>
<td>146.72 ± 12.23*</td>
<td>104.26 ± 8.64*</td>
</tr>
</tbody>
</table>

Note: Compared with the siLINC01116+anti-miR-con group, $*P < 0.05$.

#### 4. Discussion

Numerous studies have shown that lncRNA LINC01116 located on chromosome 2 is abnormally expressed in multiple malignancies and is involved in tumor development. LINC01116 is upregulated in breast cancer, epithelial ovarian cancer, head and neck squamous cell carcinoma, and glioma and functions as an oncogene in promoting cancer progression by targeting miR-145, EMT pathway, or targeting VEGFA [15–18]. However, its expression and role in cervical cancer are still unclear. In this study, the normal cervical epithelial cells were used as a control to detect LINC01116 content in different cervical cancer cell lines. The results showed that the content of LINC01116 in cervical cancer cells HeLa, SiHa, and C33a was increased significantly, while the expression of silent LINC01116 inhibited the activity of SiHa cells as well as clonal formation, migration, and invasion, indicating that LINC01116 could promote the development of cervical cancer. However, the molecular mechanism of LINC01116 in the development of cervical cancer is unknown.

Studies have shown that the expression of Mir-744-5p is downregulated in epithelial ovarian cancer tissues and cells, and overexpression of Mir-744-5p can reduce the proliferation, migration, and invasion of epithelial ovarian cancer cells, which may become a new therapeutic target for ovarian cancer [19]. Downregulation of Mir-744-5p expression can enhance the proliferation of thyroid papillary carcinoma cells and block cell apoptosis [20]. This study is the first to confirm that IncRNA LINC01116 targeting negatively regulates the expression of miR-744-5p. In lung adenocarcinomas, IncRNA MAFG-AS1 promotes lung adenocarcinoma cell proliferation by regulating the miR-744-5p/MAFG axis [21]. The expression of miR-744-5p is upregulated in the serum of oesophageal squamous cell carcinoma (ESCC) patients and may be an early detection marker of ESCC [22]. This study found that miR-
744-5p was downregulated in HeLa, SiHa, and C33a of cervical cancer cells, and overexpression of miR-744-5p inhibited proliferation, migration, and invasion of cervical cancer SiHa cells, and inhibition of miR-744-5p reversed the inhibitory effect of silencing LINC01116 on proliferation, migration, and invasion of SiHa cell, confirming the regulatory relationship between IncRNA LINC01116 and miR-744-5p in cervical cancer SiHa cells. This study shows that there are indeed binding sites for miR-744-5p on the LINC01116 gene sequence, which can competitively bind to miR-744-5p, that is, LINC01116 can participate in the occurrence and development of cervical cancer by targeting the expression of miR-744-5p, but the LINC01116 gene sequence contains multiple binding sites for the miRNA. Further studies are needed to determine which miRNA LINC01116 can target and exert effects. In addition, any downstream target genes regulated by miR-744-5p during the formation of cervical cancer need to be further verified.

5. Conclusion
This study illustrates that IncRNA LINC01116 is upregulated, and miR-744-5p is downregulated in the cervical cancer cell lines HeLa, SiHa, and C33a. LINC01116 targets to negatively regulate the expression of miR-744-5p and suppresses the proliferation, migration, and invasion of SiHa cells. LncRNA LINC01116 is expected to be a molecular target for cervical cancer. The next step will be in vivo animal experiments to verify the mechanism of LINC01116/miR-744-5p molecular axis in the occurrence and development of cervical cancer, so as to lay the experimental foundation for further revealing the pathogenesis of cervical cancer.

Data Availability
The data can be obtained from the authors upon reasonable request.

Conflicts of Interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors’ Contributions
Jing Wang and Lei Yue are co-first authors.

References


