Effects of TSLC1 overexpression by adenovirus on proliferation, invasion and angiogenesis of cervical cancer cells

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Objective: To study the effect of TSLC1 overexpression by adenovirus on proliferation, invasion and angiogenesis of cervical cancer cells. Methods: Caski cervical cancer cells were cultured and divided into blank control group, Ad-RFP group and Ad-TSLC1 group that were treated with DMEM without adenovirus or serum, control adenovirus Ad-RFP and TSLC1-overexpressed recombinant adenovirus Ad-TSLC1 respectively. 12 h and 24 h later, the expression of proliferation, invasion, angiogenesis genes were determined respectively. Results: HERC4, CyclinD1, Bcl-2, S100A6, N-cadherin, Snail, Twist, EFEMP1, VEGF and Jagged1 mRNA expression in Ad-TSLC1 group were significantly lower than those in control group and Ad-RFP group; HERC4, CyclinD1, Bcl-2, S100A6, N-cadherin, Snail, Twist, EFEMP1, VEGF and Jagged1 mRNA expression in Ad-RFP group were not significantly different from those in control group. Conclusion: TSLC1 overexpression by adenovirus can inhibit the proliferation, invasion and angiogenesis of cervical cancer cells.

1. Introduction

Cervical cancer is a common malignant tumor of the female reproductive system, the cancer cells show abnormal proliferation, invasion and angiogenesis ability during the gradual progression from cervical intraepithelial neoplasia to cervical cancer, but the regulatory mechanism of cervical cancer cell proliferation, invasion and angiogenesis has not been elucidated. The tumor suppressor gene inactivation is a major cause of cervical intraepithelial neoplasia progression to cervical cancer. Tumor suppressor in lung cancer 1 (TSLC1) is the first tumor suppressor gene discovered in lung cancer tissues, and it has been found in recent years that TSLC1 expression deletion is associated with the occurrence of ovarian cancer¹, bladder cancer², esophageal cancer³ and other malignant tumors. The study of domestic ZHAO Xu-ye confirms that there is the pathological state of excessive TSLC1 gene promoter region methylation in cervical cancer lesions, and the corresponding gene expression is suppressed⁴. There is no clear relationship between the TSLC1 gene expression and the malignant biological behavior of cervical cancer cells at present. In the following study, adenovirus transfection was adopted to over-express the TSLC1 in cervical cancer cells, and then the effect of TSLC1 overexpression on cell proliferation, invasion and angiogenesis ability was further analyzed.

2. Experimental materials and methods

2.1 Experimental materials

Caski cervical cancer cell lines were bought from the ATCC cell center, the DMEM and fetal bovine serum for cell culture as well as the trypsin for cell subculture and amplification were bought from Gibco Company, control adenovirus Ad-RFP and TSLC1-overexpressed recombinant adenovirus Ad-TSLC1 were synthesized by Shanghai GenePharma Company, gene mRNA expression detection kits were bought from the TaKaRa Company, and the primers were synthesized by the Shanghai Sangon Company.
2.2 Experimental methods

2.2.1 Cell culture, grouping and treatment methods

Cells were cultured in culture medium containing 10% fetal bovine serum, digested and sub-cultured by trypsin and then inoculated in the cell plate, the culture medium in the cell plate was replaced once every 2 d, the cells were divided into blank control group, Ad-RFP group and Ad-TSLC1 group after the cell density reached 80%-90%, and the treatment method was as follows: blank control group were treated with DMEM without adenovirus or serum, Ad-RFP group were treated with control adenovirus Ad-RFP, and Ad-TSLC1 group were treated with TSLC1-overexpressed recombinant adenovirus Ad-TSLC1. At 12 h and 24 h of treatment, the cells were collected respectively for follow-up testing.

2.2.2 Gene mRNA expression detection methods

After 12 h and 24 h of treatment, RNA extraction kit was used to extract the RNA in the cells, and then the first-strand cDNA synthesis kit was used for reverse transcription from the RNA extracted in cells to cDNA. cDNA samples were taken, fluorescence quantitative PCR kit was used to amplify target genes HERC4, CyclinD1, Bcl-2, S100A6, N-cadherin, Snail, Twist, EFEMP1, VEGF, Jagged1 and reference gene β-actin respectively, and the amplification curve and β-actin standardization between groups were referred to calculate HERC4, CyclinD1, Bcl-2, S100A6, N-cadherin, Snail, Twist, EFEMP1, VEGF and Jagged1 mRNA expression.

2.3 Statistical methods

SPSS 20.0 statistical software was used for data processing, data comparison among three groups was by variance analysis and \( P<0.05 \) indicated statistical significance in differences.

### 3. Results

#### 3.1 Proliferation gene expression in three groups of cells

After 12 hours and 24 hours of treatment, analysis of proliferation genes HERC4, CyclinD1 and Bcl-2 mRNA expression in three groups of cells was as follows: HERC4, CyclinD1 and Bcl-2 mRNA expression in Ad-TSLC1 group were significantly lower than those in control group and Ad-RFP group, and HERC4, CyclinD1 and Bcl-2 mRNA expression in Ad-RFP group were not significantly different from those in control group. Differences in HERC4, CyclinD1 and Bcl-2 mRNA expression were statistically significant between Ad-TSLC1 group and Ad-RFP group as well as between Ad-TSLC1 group and control group after 12 hours and 24 hours of treatment (\( P<0.05 \)).

#### 3.2 Invasion gene expression in three groups of cells

After 12 h and 24 h of treatment, analysis of invasion genes S100A6, N-cadherin, Snail and Twist mRNA expression in three groups of cells was as follows: S100A6, N-cadherin, Snail and Twist mRNA expression in Ad-TSLC1 group were significantly lower than those in control group and Ad-RFP group, and S100A6, N-cadherin, Snail and Twist mRNA expression in Ad-RFP group were not significantly different from those in control group. Differences in S100A6, N-cadherin, Snail and Twist mRNA expression were statistically significant between Ad-TSLC1 group and Ad-RFP group as well as between Ad-TSLC1 group and control group after 12 h and 24 h of treatment (\( P<0.05 \)).

### Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>n</th>
<th>HERC4</th>
<th>CyclinD1</th>
<th>Bcl-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>After 12 h</td>
<td>5</td>
<td>1.03±0.15</td>
<td>1.02±0.17</td>
<td>1.05±0.12</td>
</tr>
<tr>
<td></td>
<td>of treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>After 24 h</td>
<td>5</td>
<td>1.35±0.19</td>
<td>1.20±0.18</td>
<td>1.29±0.22</td>
</tr>
<tr>
<td>Ad-RFP</td>
<td>After 12 h</td>
<td>5</td>
<td>1.05±0.13</td>
<td>1.01±0.15</td>
<td>1.03±0.17</td>
</tr>
<tr>
<td></td>
<td>of treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>After 24 h</td>
<td>5</td>
<td>1.30±0.22</td>
<td>1.28±0.12</td>
<td>1.31±0.18</td>
</tr>
<tr>
<td>Ad-TSLC1</td>
<td>After 12 h</td>
<td>5</td>
<td>0.65±0.08*</td>
<td>0.72±0.09*</td>
<td>0.76±0.10*</td>
</tr>
<tr>
<td></td>
<td>of treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>After 24 h</td>
<td>5</td>
<td>0.52±0.07*</td>
<td>0.59±0.07*</td>
<td>0.47±0.06*</td>
</tr>
</tbody>
</table>

* comparison between Ad-TSLC1 group and Ad-RFP group, \( P<0.05 \); \# comparison between Ad-TSLC1 group and control group, \( P<0.05 \).

### Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>n</th>
<th>S100A6</th>
<th>N-cadherin</th>
<th>Snail</th>
<th>Twist</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>After 12 h</td>
<td>5</td>
<td>1.06±0.13</td>
<td>1.02±0.15</td>
<td>0.98±0.11</td>
<td>1.03±0.17</td>
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<td>of treatment</td>
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<tr>
<td></td>
<td>After 24 h</td>
<td>5</td>
<td>1.36±0.20</td>
<td>1.22±0.15</td>
<td>1.33±0.19</td>
<td>1.28±0.23</td>
</tr>
<tr>
<td>Ad-RFP</td>
<td>After 12 h</td>
<td>5</td>
<td>1.04±0.16</td>
<td>0.99±0.10</td>
<td>1.02±0.14</td>
<td>1.01±0.18</td>
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<td>of treatment</td>
<td></td>
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<td></td>
<td>After 24 h</td>
<td>5</td>
<td>1.40±0.19</td>
<td>1.28±0.17</td>
<td>1.31±0.20</td>
<td>1.32±0.18</td>
</tr>
<tr>
<td>Ad-TSLC1</td>
<td>After 12 h</td>
<td>5</td>
<td>0.76±0.09*</td>
<td>0.68±0.08*</td>
<td>0.72±0.10*</td>
<td>0.65±0.08*</td>
</tr>
<tr>
<td></td>
<td>of treatment</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>After 24 h</td>
<td>5</td>
<td>0.52±0.08*</td>
<td>0.51±0.05*</td>
<td>0.42±0.07*</td>
<td>0.38±0.07*</td>
</tr>
</tbody>
</table>

* comparison between Ad-TSLC1 group and Ad-RFP group, \( P<0.05 \); \# comparison between Ad-TSLC1 group and control group, \( P<0.05 \).
3.3 Angiogenesis gene expression in three groups of cells

After 12 h and 24 h of treatment, analysis of angiogenesis genes EFEMP1, VEGF and Jagged1 mRNA expression in three groups of cells was as follows: EFEMP1, VEGF and Jagged1 mRNA expression in Ad-TSLC1 group were significantly lower than those in control group and Ad-RFP group, and EFEMP1, VEGF and Jagged1 mRNA expression in Ad-RFP group were not significantly different from those in control group. Differences in EFEMP1, VEGF and Jagged1 mRNA expression were statistically significant between Ad-TSLC1 group and Ad-RFP group as well as between Ad-TSLC1 group and control group after 12 hours and 24 hours of treatment (P<0.05).

4. Discussion

The product encoded by TSLC1 gene is a member of the immunoglobulin superfamily, it has the features of intercellular adhesion molecule and localizes in the cell membrane, and it can interact with each other to form homodimer and mediate the formation of intercellular junction. Within the squamous epithelium, TSLC1 can maintain the tight junction between the epithelium and inhibit cell migration and infiltration[14,15]. At the same time, the TSLC1 gene overexpression on the invasion gene expression in cervical cancer cells, the protein encoded by the gene is a type of E3 ubiquitin ligase, and there are HECT and RCC1 structure domain in the protein structure. The product encoded by HERC4 is involved in the composition of the ubiquitin-proteasome system, and can mediate the connection process between the substrate and the ubiquitin and cause the change in the substrate expression level[7]. In the proliferation of malignant tumor cells, HERC4 can regulate the expression of molecules such as CyclinD1 and bcl-2. CyclinD1 is a key molecule that regulates the cell cycle, and it forms compound with multiple CDK molecules to accelerate the cell cycle and thus facilitate cell proliferation[8,9]. The Bcl-2 is an important molecule that regulates mitochondrial apoptosis, and it can interfere with the cytochrome C release from mitochondria into cytoplasm, and then antagonize the apoptosis mediated by downstream caspase molecules and be conducive to cell proliferation[10]. In the study, analysis of the effect of TSLC1 gene overexpression on the proliferation gene expression in cervical cancer cells indicated that HERC4, CyclinD1 and Bcl-2 mRNA expression in Ad-TSLC1 group were significantly lower than those in control group and Ad-RFP group. This suggests that overexpressing TSLC1 gene can inhibit the cell proliferation mediated by HERC4 in cervical cancer cells.

In the invasive growth and distant metastasis of cervical cancer cells, the cell invasion mediated by S100A6 has played a critical role. The S100A6 is a member of the calcium-binding protein S100 family, which can promote cell invasion by inducing epithelial-mesenchymal transition[11]. Epithelial-mesenchymal transition is the process that epithelial phenotype transits to mesenchymal phenotype and obtains movement and migration ability, and the regulating effect of S100A6 on the process depends on the transcription factors Snail and Twist[12,13]. The increased expression of S100A6 will promote the generation of Snail and Twist and make them transfer into the nucleus, be combined with epithelial phenotype marker molecule E-cadherin and inhibit its expression, which causes the excessive transition from epithelial phenotype to mesenchymal phenotype and results in the increased expression of mesenchymal cell marker molecule N-cadherin[14,15]. In the study, analysis of the effect of TSLC1 gene overexpression on the invasion gene expression in cervical cancer cells are the important biological characteristics, and it is still not clear whether the lower expression of tumor suppressor gene TSLC1 in local lesions regulates the proliferation, invasion and angiogenesis ability of cancer cells. In the study, transfection of TSLC1 adenovirus was adopted to overexpress the TSLC1 gene in cervical cancer cells, and then the proliferation, invasion and angiogenesis ability of cancer cells were analyzed.
cervical cancer cells showed that S100A6, N-cadherin, Snail and Twist mRNA expression in Ad-TSLC1 group were significantly lower than those in control group and Ad-RFP group. This shows that overexpressing TSLC1 gene can inhibit the cell invasion and epithelial-mesenchymal transition mediated by the S100A6 in cervical cancer cells.

The proliferation, invasion and epithelial-mesenchymal transition of cancer cells in cervical cancer lesions all need new blood vessels to provide nutrients for them, and angiogenesis is also seen as an important malignant biological characteristic of cervical cancer. EFEMP1, also known as fibulin-3, belongs to the fibulin gene family, and the product encoded by it is a type of extracellular matrix protein. The MAPK signaling pathway is an important pathway of the EFEMP1 downstream, and EFEMP1 can up-regulate the expression of VEGF and Jagged1 by activating MAPK signaling pathway[16]. VEGF is the strongest cytokine that promotes endothelial neoplasm and angiogenesis, and it plays an important role in the process of tumor angiogenesis[17,18]. Jagged1 and Notch pathways are cross-activated, and the activation of the Notch signaling pathway in endothelial cells is beneficial to the formation of new blood vessels[19]. In the study, the analysis of effect of TSLC1 gene overexpression on above angiogenesis gene expression in cervical cancer cells showed that EFEMP1, VEGF and Jagged1 mRNA expression in Ad-TSLC1 group were significantly lower than those in control group and Ad-RFP group. This shows that overexpressing TSLC1 gene can inhibit the angiogenesis mediated by EFEMP1 in cervical cancer cells.

To sum up, it is believed that TSLC1 overexpression by adenovirus can inhibit the cell proliferation mediated by HERC4, the cell invasion and epithelial-mesenchymal transition mediated by S100A6, and the angiogenesis mediated by EFEMP1 in cervical cancer cells.

References


