DNA methyltransferase (DNMTs) expression in cervical cancer tissues and its relationship with HPV infection and tumor malignancy

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Objective: To study the DNA methyltransferase (DNMTs) expression in cervical cancer tissues and its relationship with human papilloma virus (HPV) infection and tumor malignancy.

Methods: The cervical cancer tissues and normal cervical tissues surgically removed in Department of Obstetrics and Gynecology of our hospital between July 2013 and March 2016 were collected, the fluorescent quantitative PCR method was used to determine the mRNA expression of DNMTs in the tissues, and enzyme-linked immunosorbent assay kits were used to determine the protein expression of DNMTs molecules and pro-apoptotic molecules in the tissues.

Results: DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l mRNA expression and protein expression in cervical cancer tissues were significantly higher than those in normal cervical tissues (\(P<0.05\)) while Fas, FasL, Bax and Caspase-3 protein expression were significantly lower than those in normal cervical tissues (\(P<0.05\)); DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l protein expression in cervical cancer tissues with high-risk HPV infection (+) were significantly higher than those in cervical cancer tissues with high-risk HPV infection (-) (\(P<0.05\)); Fas, FasL, Bax and Caspase-3 protein expression were negatively correlated with DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l protein expression.

Conclusions: Elevated DNMTs expression in cervical cancer tissues is associated with high-risk HPV infection, and highly expressed DNMTs can inhibit the expression of pro-apoptotic molecules.

1. Introduction

Cervical cancer is the malignant tumor of female reproductive system with the highest incidence worldwide, and high-risk human papilloma virus (HPV) infection is the currently known risk factor of cervical cancer\(^{[1,2]}\), but the molecular mechanism of HPV infection to cause cervical epithelial cancer is not clear. DNA methylation is the epigenetic regulating mechanism that has received wide attention in recent years, and the DNA methyltransferase (DNMT) catalyzes the methylation modification of 5' carbon atoms on the cytosine in DNA sequences into the 5'-methyl cytosine, which affects DNA transcription process\(^{[3]}\). In the occurrence and development of malignant tumors, DNA methylation modification can cause the decreased or deleted expression of a variety of tumor suppressor genes and pro-apoptotic molecules, and then promote the growth of cancer cells\(^{[4-6]}\). In order to define whether HPV infection inhibits the expression of tumor suppressor genes and pro-apoptotic molecules by DNA methylation in the development and change of cervical cancer, and then promote cancer cell proliferation, the DNA methyltransferase expression in cervical cancer tissues and its relationship with HPV infection and tumor malignancy were analyzed in the following study.

2. Materials and methods

2.1. Sample origin

All clinical tissue samples were patients with cervical cancer and...
patients with uterine fibroids who were treated in Department of Obstetrics and Gynecology of our hospital between July 2013 and March 2016. Patients with cervical cancer included 74 cases that were 43–72 years old and with BMI (22.7±3.1) kg/m²; patients with uterine fibroids included 65 cases that were 48–70 years old and with BMI (22.5±3.2) kg/m². Patients with cervical cancer and patients with uterine fibroids were not significantly different in general information (P>0.05).

2.2. Tissue sample collection and preservation methods

After surgical removal, suitable amount of tissue samples were collected, suitable amount of cervical cancer tissues were collected from patients with cervical cancer, suitable amount of normal cervical tissues were collected from patients with uterine fibroids, the cervical cancer tissues and normal cervical tissues were cleaned with saline for 3–5 times, then the moisture was absorbed with filter paper, and the tissues were put in cryopreserved tubes and quickly frozen in liquid nitrogen for 20–30 min; the cryopreserved tubes that contained tissue samples were taken out from the liquid nitrogen and placed in a -80 °C refrigerator.

2.3. mRNA expression detection methods

Proper amount of cervical cancer tissues and normal cervical tissues were collected, added in the RNAiso extract produced by Takara Company and fully grinded, the trichloromethane and isopropanol were added in turn for extraction and centrifuge, and then the obtained RNA precipitation was rinsed with 75% alcohol isopropanol were added in turn for extraction and centrifuge, and then the obtained RNA precipitation was rinsed with 75% alcohol twice, dissolved with DEPC water and reverse-transcribed into cDNA with cDNA first strand synthesis kits; cDNA samples were collected, the fluorescent quantitative PCR kits were used to amplify DNMT1, DNMT2, DNMT3a, DNMT3b, DNMT3l and GAPDH, and then the obtained RNA precipitation was rinsed with 75% alcohol twice, dissolved with DEPC water and reverse-transcribed into cDNA with cDNA first strand synthesis kits; cDNA samples were collected, the fluorescent quantitative PCR kits were used to amplify DNMT1, DNMT2, DNMT3a, DNMT3b, DNMT3l and GAPDH, and then the GAPDH was used as reference to calculate DNMT1, DNMT2, DNMT3a, DNMT3b, DNMT3l, Fas, FasL, Bax and Caspase-3 mRNA expression.

2.4. Protein expression detection methods

Proper amount of cervical cancer tissues and normal cervical tissues were collected, added in protein lysis buffer produced by Beyotime Company, fully grinded and then centrifuged to separate protein suspension, enzyme-linked immunosorbent assay kits were used to determine the DNMT1, DNMT2, DNMT3a, DNMT3b, DNMT3l, Fas, FasL, Bax and Caspase-3 protein levels in protein suspension, BCA kits were used to determine the total protein level in protein suspension, and the DNMT1, DNMT2, DNMT3a, DNMT3b, DNMT3l, Fas, FasL, Bax and Caspase-3 levels per each mg total protein were calculated.

2.5. Statistical analysis

SPSS16.0 software was used to input and analyze data, measurement data analysis between two groups was by t test, correlation analysis was by Pearson test and P<0.05 indicated statistical significance in differences.

3. Results

3.1. DNMTs expression in cervical cancer tissues and normal cervical tissues

Analysis of DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l mRNA and protein expression in cervical cancer tissues and normal cervical tissues is shown in Table 1. DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l mRNA expression in cervical cancer tissues were significantly higher than those in normal cervical tissues (P<0.05). DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l protein expression in cervical cancer tissues were significantly higher than those in normal cervical tissues (P<0.05).

3.2. Pro-apoptotic molecule expression in cervical cancer tissues and normal cervical tissues

Analysis of pro-apoptotic molecules Fas, FasL, Bax and Caspase-3 protein expression in cervical cancer tissues and normal cervical tissues is shown in Table 2: Fas, FasL, Bax and Caspase-3 protein expression in cervical cancer tissues were significantly lower than those in normal cervical tissues (P<0.05).

Table 1

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>mRNA</th>
<th>Protein (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical cancer</td>
<td>74</td>
<td>2.3±0.31</td>
<td>6.85±0.77</td>
</tr>
<tr>
<td>Normal cervix</td>
<td>65</td>
<td>1.03±0.16</td>
<td>3.15±0.45</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>13.572</td>
<td>11.598</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Tissue origin</th>
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<tr>
<td>P</td>
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<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 1

DNMTs mRNA and protein expression in cervical cancer tissues and normal cervical tissues (t=±s).

Table 2

Pro-apoptotic molecule protein expression in cervical cancer tissues and normal cervical tissues (ng/mg protein, t=±s).
3.3. Correlation between high-risk HPV infection and DNMTs expression in cervical cancer tissues

Analysis of DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l protein expression in cervical cancer tissues with high-risk HPV infection (+) and those with high-risk HPV infection (-) is shown in Table 3: DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l protein expression in cervical cancer tissues with high-risk HPV infection (+) were significantly higher than those in cervical cancer tissues with high-risk HPV infection (-) \((P<0.05)\).

Table 3

<table>
<thead>
<tr>
<th>High-risk HPV Infection</th>
<th>DNMT1</th>
<th>DNMT2</th>
<th>DNMT3a</th>
<th>DNMT3b</th>
<th>DNMT3l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection (+)</td>
<td>13.15±1.77</td>
<td>10.54±0.63</td>
<td>8.18±0.93</td>
<td>4.28±0.50</td>
<td>2.14±0.30</td>
</tr>
<tr>
<td>Infection (-)</td>
<td>7.16±0.87</td>
<td>5.24±0.63</td>
<td>8.58±0.92</td>
<td>6.97±0.75</td>
<td>6.26±0.75</td>
</tr>
<tr>
<td>t</td>
<td>8.928</td>
<td>7.681</td>
<td>8.287</td>
<td>8.414</td>
<td>6.973</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

3.5. Correlation between pro-apoptotic molecule expression and DNMTs expression in cervical cancer tissues

Pearson test analysis of the correlation of pro-apoptotic molecules Fas, FasL, Bax and Caspase-3 protein expression with DNMTs molecules DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l protein expression in cervical cancer tissues showed that Fas, FasL, Bax and Caspase-3 protein expression were negatively correlated with DNMTs expression in cervical cancer tissues.

4. Discussion

It has been confirmed that abnormal DNA methylation modification process is associated with the occurrence and development of a variety of malignant tumors. DNMTs are the key enzymes that catalyze DNA methylation modification, and include different subtypes such as DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l. DNMT1 can catalyze the methylation modification of the newly synthesized DNA single-strand, and can also act on the hemimethylated DNA and maintain the methylation state of DNA; DNMT3a and DNMT3b mainly catalyze the de novo methylation process of DNA; DNMT3l itself does not have the activity to catalyze methylation, and it is mainly responsible for regulating the catalytic activity of DNMT3a and DNMT3b in the methylation process; DNMT2 mainly catalyzes the methylation process of tRNA. In order to define the relationship between abnormal DNA methylation modification process and cervical cancer, the DNMTs expression in cervical cancer tissues and normal cervical tissues were analyzed in the study, and the results showed that DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l mRNA expression and protein expression in cervical cancer tissues were significantly higher than those in normal cervical tissues \((P<0.05)\). This means that the excessive DNA methylation modification mediated by highly expressed DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l is closely related to the occurrence of cervical cancer.

The currently known risk factor of cervical cancer is high-risk HPV infection, and about 90% of the women will only be mildly injured in the beginning after HPV infection and can remove the viruses within three years; about 10% of the women can't remove the viruses in time after HPV infection, and the persistent HPV infection may increase the risk of cervical intraepithelial neoplasia and cervical cancer. The most common type of HPV infection in patients with invasive cervical cancer is high-risk HPV, including HPV16 and HPV18. Although the relationship between high-risk HPV infection and cervical cancer is widely approved, the molecular mechanism of HPV infection to cause cervical epithelial cancer is not clear. As mentioned earlier, a variety of DNMTs molecules are highly expressed in cervical cancer tissues, and in order to further clarify whether high-risk HPV infection influences the expression of DNMTs to increase the occurrence risk of cervical cancer, the relationship between high-risk HPV infection and DNMTs expression in cervical cancer tissues was analyzed in the study, and the result showed that DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l protein expression in cervical cancer tissues with high-risk HPV infection (+) were significantly higher than those in cervical cancer tissues with high-risk HPV infection (-) \((P<0.05)\). This means that high-risk HPV infection can increase the expression of a variety of DNMTs in cervical cancer tissues, and then participate in the occurrence and development of cervical cancer through the DNA methylation modification process catalyzed by DNMTs.
of cancer cells. Fas/FasL and Bax are the pro-apoptotic molecules adjusting the Caspase cascade apoptosis pathways[16,17], and the analysis of the expression of the pro-apoptotic molecules in cervical cancer tissues in the study showed that Fas, FasL, Bax and Caspase-3 protein expression in cervical cancer tissues were significantly lower than those in normal cervical tissues (P<0.05). In order to further define whether the highly expressed DNMTs in cervical cancer tissues inhibited the expression of pro-apoptotic molecules by DNA methylation, the correlation between DNMTs expression and pro-apoptotic molecule expression was analyzed in the study, and the results showed that Fas, FasL, Bax and Caspase-3 protein expression were negatively correlated with DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l protein expression. This means that the DNA methylation process mediated by high DNMTs expression in cervical cancer tissues can inhibit the expression of pro-apoptotic molecule to promote the proliferation of cancer cells.

In conclusion, the DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3l expression significantly increase in cervical cancer tissues, high-risk HPV infection can increase the DNMTs expression, and highly expressed DNMTs can inhibit the expression of pro-apoptotic molecules.

References