Value of DNA ploid detection for the evaluation of cancer cell proliferation and invasion activity in cervical cancer

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ARTICLE INFO

Objective: To study the value of DNA ploid detection for the evaluation of cancer cell proliferation and invasion activity in cervical cancer. Methods: Patients with cervical cancer who underwent surgical resection in Yan’an University Affiliated Hospital between March 2015 and May 2017 were selected as cervical cancer group of the research, and patients with benign lesions who underwent hysterectomy in Yan’an University Affiliated Hospital during the same period were selected as the control group of the research. Before operation, cervical exfoliated cytology was taken for DNA ploid detection; before operation, the cervical tissue was taken to determine the expression of proliferation genes and invasion genes. Results: The number of exfoliated cells with abnormal DNA ploid in cervical cancer group was significantly higher than that in control group; Id-1, Ki-67, TET1, S6K1, CatL and ILK mRNA expression in cervical cancer tissue of cervical cancer group were significantly higher than those of control group whereas HSG, MCPH1, p16, TIMP1 and TIMP2 mRNA expression were significantly lower than those of control group; Id-1, Ki-67, TET1, S6K1, CatL and ILK mRNA expression in cervical cancer tissue with ≥3 exfoliated cells with abnormal DNA ploid were significantly higher than those in cervical cancer tissue with 1-2 exfoliated cells with abnormal DNA ploid whereas HSG, MCPH1, p16, TIMP1 and TIMP2 mRNA expression were significantly lower than those in cervical cancer tissue with 1-2 exfoliated cells with abnormal DNA ploid. Conclusion: DNA ploid detection can be used to evaluate the proliferation and invasion of cancer cells in cervical cancer.

1. Introduction

Cervical cancer is the most common malignant tumor in the female reproductive system, and its incidence is rising year by year[1]. Cervical exfoliative cytology is an auxiliary examination method for early clinical screening of cervical cancer and precancerous lesions, but the sensitivity of early diagnosis is poor. DNA ploid detection is an early screening method for cervical cancer in recent years, which measures the content of DNA in the nuclei to reflect the cell proliferation. In the occurrence and development of malignant tumor, abnormal cell proliferation can cause changes of DNA content and DNA ploid number in the nuclei, and DNA ploid detection is able to provide evidence for the diagnosis of malignant tumor[2]. Studies have reported that the cervical exfoliated cell DNA ploid detection combined with thinprep cytology test can improve the sensitivity and specificity in early diagnosis of cervical cancer[3], but it is unclear about the value of DNA ploid detection for evaluating the tumor cell malignancy within cervical cancer lesions. In the following study, we have specifically analyzed the number of cells with abnormal DNA ploid in cervical cancer lesions and its correlation with the expression of proliferation genes and invasion genes in order to reflect the value of DNA ploid detection for the evaluation of cancer cell proliferation and invasion activity in cervical cancer.
2. Information and methods

2.1 General case information

Patients with cervical cancer who underwent surgical resection in Yan’an University Affiliated Hospital between March 2015 and May 2017 were selected as the cervical cancer group of the research, the tissue properties of all patients were confirmed by pathological examination, and those who received preoperative chemoradiotherapy, targeted therapy or biological therapy were eliminated. Patients with benign lesions who underwent hysterectomy in Yan’an University Affiliated Hospital during the same period were selected as the control group of the research, and the cervical tissue was confirmed to be normal tissue by pathological examination. There were 58 cases in cervical cancer group, they were 42-64 years old, the gravidity was 2-4 and the parity was 1-3; there were 44 cases in the control group, they were 39-65 years old, the gravidity was 2-5 and the parity was 1-4. There was no significant difference in the general data between the two groups (P>0.05).

2.2 DNA ploid detection

Before surgery, two groups of patients underwent conventional gynecological examination, cervical exfoliated cells were collected, stained with thionine-Feulgen and made into cell smear, it was scanned and analyzed in DNA quantitative analysis system, DNA index was calculated, and the cells with DNA index > 2.5 were defined as those with abnormal DNA ploid.

2.3 Gene expression detection

After surgery, the cervical cancer tissue was collected from cervical cancer group, the normal cervical tissue was collected from control group, the kits were used to extract RNA and synthesize it into cDNA by reverse transcription; cDNA samples were taken for amplification with PCR kits, the adopted primers were the specific primers for Id-1, Ki-67, HSG, MCPH1, p16, TET1, S6K1, CatL, ILK, TIMP1 and TIMP2, and the PCR curve was referred to calculate the gene mRNA expression.

2.4 Statistical methods

Software SPSS 17.0 was adopted for data input and comparison of measurement data between two groups was by t test. P<0.05 meant that the difference was statistically significant.

3. Results

3.1 The number of cervical exfoliated cells with abnormal DNA ploid

The mean number of exfoliated cells with abnormal DNA ploid in cervical cancer group was (7.82±0.95), 38 cases had ≥3 cells with abnormal DNA ploid, 20 cases had 1-2 cells with abnormal DNA ploid and none was without cells with abnormal DNA ploid; the mean number of exfoliated cells with abnormal DNA ploid in control group was (0.42±0.07), 10 cases had 1-2 cells with abnormal DNA ploid, 34 cases were without cells with abnormal DNA ploid and none had ≥3 cells with abnormal DNA ploid. After t test, the number of exfoliated cells with abnormal DNA ploid in cervical cancer group was significantly higher than that in control group.

3.2 Proliferation gene expression

Analysis of proliferation genes Id-1, Ki-67, HSG, MCPH1 and p16 expression in cervical cancer tissue of cervical cancer group and in normal cervical tissue of control group was as follows: Id-1 and Ki-67 mRNA expression in cervical cancer tissue of cervical cancer group were significantly higher than those of control group whereas HSG, MCPH1 and p16 mRNA expression were significantly lower than those of control group.

Analysis of proliferation genes Id-1, Ki-67, HSG, MCPH1 and p16 expression in cervical cancer group of cervical cancer tissue with different number of cells with abnormal DNA ploid was as follows: Id-1 and Ki-67 mRNA expression in cervical cancer tissue with ≥3 cells with abnormal DNA ploid were significantly higher than those in cervical cancer tissue with 1-2 cells with abnormal DNA ploid whereas HSG, MCPH1 and p16 mRNA expression were significantly lower than those in cervical cancer tissue with 1-2 cells with abnormal DNA ploid.

Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Id-1</th>
<th>Ki-67</th>
<th>HSG</th>
<th>MCPH1</th>
<th>p16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical cancer group</td>
<td>58</td>
<td>2.89±0.42</td>
<td>3.29±0.51</td>
<td>0.31±0.06</td>
<td>0.28±0.05</td>
<td>0.48±0.08</td>
</tr>
<tr>
<td>Control group</td>
<td>44</td>
<td>1.04±0.15</td>
<td>1.01±0.13</td>
<td>0.98±0.14</td>
<td>1.05±0.12</td>
<td>1.03±0.18</td>
</tr>
<tr>
<td>t</td>
<td></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>

Table 2.

<table>
<thead>
<tr>
<th>No. of cells with abnormal DNA ploid</th>
<th>n</th>
<th>Id-1</th>
<th>Ki-67</th>
<th>HSG</th>
<th>MCPH1</th>
<th>p16</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥3</td>
<td>38</td>
<td>4.12±0.58</td>
<td>4.62±0.72</td>
<td>0.17±0.03</td>
<td>0.14±0.03</td>
<td>0.25±0.06</td>
</tr>
<tr>
<td>1-2</td>
<td>20</td>
<td>1.68±0.24</td>
<td>1.93±0.27</td>
<td>0.48±0.08</td>
<td>0.41±0.07</td>
<td>0.73±0.10</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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</tr>
</tbody>
</table>
Comparison of invasion genes in cervical cancer tissue with different number of cells with abnormal DNA ploid.

Table 4.

<table>
<thead>
<tr>
<th>No. of cells with abnormal DNA ploid</th>
<th>n</th>
<th>TET1</th>
<th>S6K1</th>
<th>CatL</th>
<th>ILK</th>
<th>TIMP1</th>
<th>TIMP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 cells with abnormal DNA ploid</td>
<td>20</td>
<td>1.05±0.12</td>
<td>1.02±0.17</td>
<td>0.97±0.14</td>
<td>1.01±0.15</td>
<td>1.04±0.16</td>
<td>1.05±0.14</td>
</tr>
<tr>
<td>1-2 cells with abnormal DNA ploid</td>
<td>38</td>
<td>23.218</td>
<td>21.383</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>1 cell with abnormal DNA ploid</td>
<td>10</td>
<td>19.958</td>
<td>16.579</td>
<td>10.038</td>
<td>0.24±0.04</td>
<td>0.23±0.07</td>
<td>0.24±0.07</td>
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</tbody>
</table>

3.3 Invasion gene expression

Analysis of invasion genes TET1, S6K1, CatL, ILK, TIMP1 and TIMP2 expression in cervical cancer tissue of cervical cancer group and in normal cervical tissue of control group was as follows: TET1, S6K1, CatL and ILK mRNA expression in cervical cancer tissue of cervical cancer group were significantly higher than those of control group whereas TIMP1 and TIMP2 mRNA expression were significantly lower than those of control group.

Analysis of invasion genes TET1, S6K1, CatL, ILK, TIMP1 and TIMP2 expression in cervical cancer tissue of cervical cancer group with different number of cells with abnormal DNA ploid was as follows: TET1, S6K1, CatL and ILK mRNA expression in cervical cancer tissue with ≥3 cells with abnormal DNA ploid were significantly higher than those in cervical cancer tissue with 1-2 cells with abnormal DNA ploid whereas TIMP1 and TIMP2 mRNA expression were significantly lower than those in cervical cancer tissue with 1-2 cells with abnormal DNA ploid.

4. Discussion

The early screening and diagnosis of cervical cancer is an effective method for improving the prognosis of diseases, thinprep cytology test is the most widely used clinical way of early cervical cancer screening, it is noninvasive, easy to operate and so on, and it has a certain value for early local screening. However, the detection method is greatly influenced by the operation level and subjective judgment level of the operator, and the sensitivity and specificity of the diagnosis are not ideal, which can easily lead to the missed diagnosis of early cervical cancer. DNA ploidy detection is a newly developed early screening method for malignant tumors in recent years, which can evaluate the illness by the determination of DNA ploid in exfoliated cells. The normal cells are the DNA diploid cells, the DNA index of quiescent cells is 1, and the DNA index of mitotic cells is 2, so the DNA index of normal cells is between 1 and 2; malignant tumor cells have extremely strong proliferative ability, and the DNA in the nucleus is also in abnormal replication state, and the DNA content increases abnormally, which is manifested as the change of DNA index and ploid. It has been reported that DNA ploid detection on the basis of thinprep cytology test can improve the sensitivity and specificity of early cervical cancer proliferation.[4,5]. In the above study, the DNA ploid detection results in exfoliated cells of patients with cervical cancer were analyzed at first, and the results showed that the number of exfoliated cells with abnormal DNA ploid in cervical cancer group was significantly higher than that in control group. This indicates that there is significant DNA ploid disorder in the occurrence and development of cervical cancer, and the DNA ploid detection can provide basis for the diagnosis of diseases.

In the development of cervical cancer, the abnormal proliferation of cancer cells mediated by abnormal expression of proliferation genes is an important biological link. Id-1 and Ki-67 are the genes that promote proliferation and promote the proliferation of cancer cells in the course of cervical cancer; the encoding product of Id-1 has inhibitory effect on cell differentiation, and can promote the cell proliferation and inhibit the cell senescence,[6], the encoding product of Ki-67 is a kind of proliferating cell nuclear antigen, and it is massively expressed in the process of mitosis and exerts positive regulatory effect.[7,8]. HSG, MCPH1 and p16 are the genes with anti-proliferation effect, and inhibit the proliferation of cancer cells in the course of cervical cancer; HSG is the fusion protein located in the mitochondrial membrane and can inhibit the cell proliferation process mediated by the Ras pathway, MCPH1 is the inhibitory molecule of telomerase function and can affect the function of telomerase to induce cell apoptosis and prevent cell proliferation[9], and p16 is the tumor suppressor gene involved in cell cycle regulation, and can make the cell cycle arrest and negatively regulate the cell proliferation.[10,11]. The analysis of the above proliferation gene expression in the cervical cancer lesions showed that Id-1 and Ki-67 mRNA expression in cervical cancer tissue of cervical cancer group were significantly higher than those of control group whereas HSG, MCPH1 and p16 mRNA expression were significantly lower than those of control group. This indicates that the high expression of pro-proliferation genes and the low expression of anti-proliferation genes in the lesion are related to the occurrence of cervical cancer. Further analysis of the correlation between DNA ploid expression and proliferation gene expression showed that Id-1 and Ki-67 mRNA expression increased significantly whereas HSG, MCPH1 and p16 mRNA expression significantly decreased in cervical cancer tissue with ≥3 cells with abnormal DNA ploid. This indicates that
the change of DNA ploid in cervical cancer is closely related to the change of proliferation gene expression, and the detection of DNA ploid can be used to evaluate the abnormal proliferation of cells in cervical cancer.

The cervical cancer lesion has the characteristic of invasive growth, and the cancer cells will continue to infiltrate towards the pelvic tissue and lymph node tissue and cause the progress of the disease. The extracellular matrix degradation mediated by invasion genes is a key biological link in the invasive growth of tumor lesion. TET1 and S6K1 are the upstream genes regulating cell invasion, the encoding product of the former main catalyzes DNA demethylation process and can increase the expression of a variety of downstream proteases[12,13], and the encoding product of the latter mainly increases the expression of a variety of downstream proteases through MAPK/mTOR and PI3K/AKT/mTOR pathway[14,15]; CatL and ILK are the important proteases in cervical cancer lesions, which can hydrolyze collagen, elastin and other components in extracellular matrix and promote cell invasion[16]. TIMP1 and TIMP2 are the negative regulatory elements of the cell invasion process, which can bind to the proteases and antagonize their hydrolysis activity so as to inhibit the hydrolysis of extracellular matrix and the invasion of cells[17,18]. The analysis of above invasion gene expression within cervical cancer lesions showed that TET1, S6K1, CatL and ILK mRNA expression in cervical cancer tissue of cervical cancer group were significantly higher than those of control group whereas TIMP1 and TIMP2 mRNA expression were significantly lower than those of control group. This indicates that the high expression of pro-invasion genes and the low expression of anti-invasion genes in the lesion are related to the occurrence of cervical cancer. Further analysis of the correlation between DNA ploid detection and invasion gene expression indicated that TET1, S6K1, CatL and ILK mRNA expression significantly increased in cervical cancer tissue with ≥3 cells with abnormal DNA ploid, it means that the change of DNA ploid in cervical cancer lesions is closely related to the change of invasion gene expression, and the detection of DNA ploid can be used to assess the abnormal invasion of cells in cervical cancer lesions.

Based on the above analysis of the correlation of DNA ploid detection with proliferation and invasion genes within cervical cancer lesions, it can be concluded that there is significant DNA ploid disorder in the occurrence and development of cervical cancer, and the DNA ploid detection can be adopted to evaluate the activity of cancer cell proliferation and invasion in cervical cancer lesions.

References


