Correlation of high-risk HPV infection in cervical lesions with Th cell differentiation and abnormal cell proliferation

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ABSTRACT

Objective: To investigate the correlation of high-risk HPV infection in cervical lesions with Th cell differentiation and abnormal cell proliferation. Methods: A total of 80 patients who underwent gynecological examination and were diagnosed with high-risk HPV infection and 64 patients who were diagnosed with low-risk HPV infection in our hospital between August 2016 and December 2017 were enrolled in high-risk HPV infection group and low-risk HPV infection group respectively, and 50 healthy women who underwent examination in our hospital during the same period were enrolled in normal control group. The differences in the expression levels of Th1/Th2 cytokines, Th17/Treg cytokines and proliferation-related genes in cervical tissue were compared among the three groups. Results: IL-2 and IL-3 levels in cervical tissue of high-risk HPV infection group were lower than those of low-risk HPV infection group and normal control group whereas IL-4 and IL-10 contents were higher than those of low-risk HPV infection group and normal control group; IL-17, IL-23, IL-6 and TGF-β contents were higher than those of low-risk HPV infection group and normal control group; Prdx4, Furin and STAT3 mRNA expression were higher than those of low-risk HPV infection group and normal control group whereas FOXF2 mRNA expression was lower than that of low-risk HPV infection group and normal control group. Conclusion: High-risk HPV infection can directly affect the balance of Th1/Th2 and Th17/Treg cells in cervical tissues, and promote the abnormal proliferation of cervical cells in the lesion area.

1. Introduction

Human papillomavirus (HPV) infection is a high-risk factor for the occurrence of cervical cancer in women, and the specific harm varies according to the different risk degree of HPV. Now, it is basically clear that high-risk HPV infection is one of the causes of cervical cancer, but it generally takes ten years for high-risk HPV infection to develop into invasive cervical cancer, and the changes in both cervical tissue microenvironment and cervical cell proliferation activity caused by virus during this period are the direct causes of cervical cancer[1-3]. At present, it is pointed out that high-risk HPV can change the immune environment of the cervical tissue to activate the cellular infinite proliferation ability, but the specific pathway has not yet been reported. In this study, the differences in Th1/Th2 and Th17/Treg cell function as well as proliferation gene expression in cervical tissue were compared among women with different types of HPV infection and healthy women, and the specific harm and pathway of high-risk HPV were discussed in order to lay the foundation for disease intervention for women with high-risk type HPV infection in the future.

2. Materials and methods

2.1. Case information

A total of 80 patients who underwent gynecological examination and were diagnosed with high-risk HPV infection and 64 patients who were diagnosed with low-risk HPV infection in our hospital between August 2016 and December 2017 were enrolled in high-risk HPV infection group and low-risk HPV infection group respectively, and 50 healthy women who underwent examination in
our hospital during the same period were enrolled in normal control group. High-risk HPV infection group were 36-68 years old; low-risk HPV infection group were 35-70 years old; normal control group were 38-67 years old. The research plan was reviewed and approved by the hospital ethics committee.

2.2. Inclusion and exclusion criteria

Inclusion criteria: (1) the above clinical diagnosis was confirmed by cervical biopsy combined with high-risk HPV mRNA detection and immunohistochemistry; HPV mRNA detection was by QuantiVirusTM HPV mRNA detection kit; immunohistochemistry kit was purchased from Maxim Company; (2) patients were without history of cervical surgery; (3) they were 80 years old. Exclusion criteria: (1) combined with primary malignant tumor disease in other tissue viscera; (2) combined with severe autoimmune diseases; (3) combined with serious infectious diseases; (4) pregnant or breast-feeding women

2.3. Th cytokine content detection

Cervical tissue samples were obtained from the three groups of research subjects immediately after they were included, ground, placed in sterile PBS liquid and centrifuged to get the supernatant, and enzyme-linked immunosorbent assay kit from Shanghai Westang Biotechnology Company was adopted to detect the contents of Th1 cytokines interleukin-2 (IL-2) and interleukin-3 (IL-3), Th2 cytokines interleukin-4 (IL-4) and interleukin-10 (IL-10), Th17 cytokines interleukin-17 (IL-17) and interleukin-23 (IL-23) as well as Treg cytokines interleukin-6 (IL-6) and transforming growth factor-β (TGF-β).

2.4. Proliferation gene expression detection

During the examination, the cervical tissue specimens were obtained from the suspected lesion area, and the Prdx4, Furin, STAT3 and FOXF2 mRNA expression were detected by fluorescence quantitative PCR. The reverse transcription kits and fluorescence quantitative PCR kits involved in the above processes were purchased from the American Sigma Company, the corresponding PCR amplification curve was obtained from computer software and the target gene expression was calculated.

2.5. Statistical methods

Th1/Th2 cytokines, Th17/Treg cytokines and proliferation-related genes were input in SPSS 25.0 as measurement data, the P value was calculated and \( p<0.05 \) was set as the standard of statistical significance in differences.

3. Results

3.1. Th1/Th2 cytokines

Comparison of the contents of Th1 cytokines IL-2 and IL-3 as well as Th2 cytokines IL-4 and IL-10 in cervical tissue among three groups of research subjects was as follows: IL-2 and IL-3 levels in cervical tissue of high-risk HPV infection group were lower than those of low-risk HPV infection group and normal control group whereas IL-4 and IL-10 contents were higher than those of low-risk HPV infection group and normal control group; IL-2 and IL-3 levels in cervical tissue of low-risk HPV infection group were lower than those of normal control group whereas IL-4 and IL-10 contents were higher than those of normal control group (\( P<0.05 \)), shown in Table 1.

3.2. Th17/Treg cytokines

Comparison of the contents of Th17 cytokines IL-17 and IL-23 as well as Treg cytokines IL-6 and TGF-β in cervical tissue among three groups of research subjects was as follows: IL-17, IL-23, IL-6 and TGF-β.
and TGF-β contents in cervical tissue of high-risk HPV infection group were higher than those of low-risk HPV infection group and normal control group; IL-17, IL-23, IL-6 and TGF-β contents in cervical tissue of low-risk HPV infection group were higher than those of normal control group ($P<0.05$), shown in Table 2.

### 3.3. Proliferation-related genes

Comparison of proliferation-related genes Prdx4, Furin, STAT3 and FOXF2 mRNA expression in cervical tissue among three groups of research subjects was as follows: Prdx4, Furin and STAT3 mRNA expression in cervical tissue of high-risk HPV infection group were higher than those of low-risk HPV infection group and normal control group whereas FOXF2 mRNA expression was lower than that of low-risk HPV infection group and normal control group; Prdx4, Furin and STAT3 mRNA expression in cervical tissue of low-risk HPV infection group were higher than those of normal control group whereas FOXF2 mRNA expression was lower than that of normal control group ($P<0.05$), shown in Table 3.

### 4. Discussion

The local immune response of cervical cancer tissue plays an extremely important role in both host anti-HPV infection and inhibition of cervical lesion progress, and Th1/Th2, as the two kinds of main immune cells from Th cell differentiation, are directly involved in above processes\[4,5\]. At present, it has been found that Th2 cytokines predominate in many tumors, and the Th1/Th2 immune balance in cervical tissue also shifts to the Th2 cells after HPV infection, which leads to the decreased synthesis of Th1 cytokines such as IL-2 and IL-3 as well as the increased synthesis of Th2 cytokines IL-4 and IL-10\[6,7\]. The Th cellular immune response mediated by IL-2 and IL-3 has the effects such as anti-HPV infection, and the continuous HPV infection is directly related to the weakening of Th1 cell function\[8,9\]. IL-4 and IL-10 play an important role in immunosuppression, which can inhibit T cell activation and Th0 cell differentiation to Th1 cell\[10\]. In the study, the differences in the Th1/Th2 cytokine expression in cervical tissue were compared among the three groups, and the results showed that compared with those of low-risk HPV infection group and normal control group, Th1 cytokine levels in lesion tissue of high-risk HPV infection group were lower whereas Th2 cytokine contents were higher, which has to do with the immune state change in cervical tissue after HPV infection, also shows that high-risk type HPV infection can further lead to Th1/Th2 immune imbalance, and is one of the important immune mechanisms leading to subsequent lesions.

Th17 cells mainly secrete cytokines such as IL-17 and IL-23, which play an important role in the development of various infectious diseases and tumors\[11,12\]. Studies have shown that the proportion of Th17 cell subset in total CD4+T lymphocytes increases with the aggravation of cervical lesions, and the expression level is the highest in cervical cancer tissue\[13,14\]. It is also found that Treg cells participate in the immune escape of HPV, they mainly exert immunosuppressive effect, and the percentage of Treg cells in peripheral blood is positively correlated with the tumor staging and lymph node metastasis\[15,16\]. In the study, the differences in Th17 cytokins and Treg cytokine contents in cervical tissue were compared among the three groups, and the results showed that compared with those of low-risk HPV infection group and normal control group, the contents of Th17 cytokines IL-17 and IL-23 as well as Treg cytokines IL-6 and TGF-β in lesion tissue of high-risk HPV infection group were higher, it indicates that the enhancement of Th17 and Treg cell function participates in cervical lesions, and high-risk type HPV infection can further worsen the change and promote the malignant transformation of cervical tissue.

The changes of immune environment may influence the proliferation activity of cervical cells, tumor progression occurs when the normal apoptosis of cells is blocked and the cells show abnormal proliferation activity, the change in proliferation-related gene expression is directly involved in this process, and testing their specific expression is an important means to measure the risk of cellular malignant transformation. Prdx4 is a typical ethylenedicysteine Prdx, it plays an important role in maintaining the hydrogen peroxide concentration under physiological condition, and the increased Prdx4 expression induced by oxidative stress can protect cells from apoptosis and lead to tumorigenesis\[17\]. It is also found that the proliferation activity of tumor cells decreases and the apoptosis index increases after the specific down-regulation of Prdx4 expression. Furin is involved in cell proliferation and adhesion, and cell research has found that it can increase the expression of matrix metalloproteinases, promote the proliferation activation of tumor cells to increase and promote them to invade and transfer\[18,19\]. STAT3 is a bifunctional protein coupled with tyrosine phosphorylation signal channel, it regulates cell proliferation, differentiation, apoptosis and other key gene expression to participate in the occurrence and development of malignant tumor, and its high expression can promote cell proliferation and prevent apoptosis\[20,21\]. FOXF2 is a mesenchymal transcription factor specifically expressed in the epithelium and mesenchyme, and study has shown that it is highly expressed in normal prostate tissues and lowly expressed in prostate cancer; another study indicates that the low expression of

Table 3.

Comparison of proliferation-related gene expression in cervical tissue among three groups of research subjects.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Prdx4</th>
<th>Furin</th>
<th>STAT3</th>
<th>FOXF2</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-risk HPV infection group</td>
<td>80</td>
<td>183.04±22.18</td>
<td>153.28±16.19</td>
<td>161.48±17.09</td>
<td>50.88±6.21</td>
</tr>
<tr>
<td>Low-risk HPV infection group</td>
<td>64</td>
<td>132.64±15.93</td>
<td>117.45±13.59</td>
<td>130.27±14.58</td>
<td>75.91±8.46</td>
</tr>
<tr>
<td>Normal control group</td>
<td>50</td>
<td>94.27±10.74</td>
<td>85.63±9.21</td>
<td>101.28±13.47</td>
<td>99.74±10.51</td>
</tr>
</tbody>
</table>

$p<0.05$; compared with normal control group, $p<0.05$.

Note: compared with low-risk HPV infection group, $p<0.05$; compared with normal control group, $p<0.05$.
FOXF2 is related to the early metastasis of breast cancer[22,23]. The study results showed that compared with those of low-risk HPV infection group and normal control group, Prdx4, Furin and STAT3 mRNA expression in lesion tissue of high-risk HPV infection group were higher whereas FOXF2 mRNA expression was lower, confirming that high-risk HPV infection can significantly increase the cervical cell proliferation activity and make it become the tumor. To sum up, it is concluded that the high-risk HPV infection in cervical lesions can lead to the imbalance of Th1/Th2 cellular immunity and Th17/Treg cellular immunity and eventually increase the cervical cell proliferation activity, and it is one of the direct causes of cervical cancer.

References


