Relationship between high risk HPV infection and potential malignant biological behavior of patients with cervical intraepithelial neoplasia

Cai–Yun Bai*, Yan–Ming Wang

Department of Gynecology, Yanan University Affiliated Hospital, 716000

Objective: To study the relationship between high risk HPV infection and potential malignant biological behavior of patients with cervical intraepithelial neoplasia.

Methods: Patients with cervical intraepithelial neoplasia treated in our hospital from May 2012 to May 2014 were chosen for study; 40 cases of patients each in CIN I stage, CIN II stage and CIN III stage were screened; HPV subtypes as well as mRNA contents of proliferation related genes and chromosome related genes were detected.

Results: (1) HPV subtypes: there were no differences in positive rates and contents of HPV-31, 33, 51, 52 and 58 of patients in different CIN stages; there were differences in positive rates and contents of HPV-16 and 18, and the higher the CIN stage is, the higher the positive rates and contents of HPV-16 and 18 are; (2) Proliferation related genes: mRNA contents of proliferation genes IMP3, MUC1 and TS in cervical tissue of patients with high risk HPV infection displayed higher expressions; mRNA contents of tumor suppressor genes p16ink4a and p53 displayed lower expressions; (3) Chromosome related genes: mRNA contents of MCM2, MCM7, hTERC, hTERT, hTP1 and CA-IX in cervical tissue of patients with high risk HPV infection displayed higher expressions.

Conclusion: HPV-16 and 18 are high risk HPV of patients with cervical intraepithelial neoplasia and will cause increased expressions of proliferation genes and inhibited expressions of tumor suppressor genes, and affect chromosome replication and extension process.

1. Introduction

Cervical carcinoma is the second largest malignant tumor of women. Its incidence rises year by year. Patients experience the pathological processes of cervical intraepithelial neoplasia (CIN), cervical carcinoma in situ and invasive carcinoma of cervix uteri in turn. Human papillomavirus (HPV) infection is the most important cause of the occurrence of CIN. HPV-16, 18, 31, 33, 51, 52, 58, and so on can all infect cervical tissue and cause the occurrence of malignant proliferation of cells[1]. HPV-16 and 18 are currently known high risk HPV and are most closely related to the occurrence of CIN and cervical carcinoma[2]. In the following research, the relationship between high risk HPV infection and potential malignant biological behavior of patients with CIN was explored.

2. Materials and methods

2.1. Clinical data

Patients with CIN treated in our hospital from May 2012 to May 2014 were chosen for study. Cervical tissue was generally collected for pathologic biopsy. Patients were clearly diagnosed of CIN, notified of research matters and signed informed consent. 40 cases of patients each in CIN I stage, CIN II stage and CIN III stage
were screened according to different CIN stages. Patients in CIN I stage are (45.56±6.62) years old with disease course of 4.95±0.64 months; patients in CIN II stage are (44.95±6.88) years old with disease course of (5.01±0.58) months; patients in CIN III stage are (45.29±6.52) years old with disease course of (5.13±0.67) months. There were no differences among three groups’ general data (P>0.05).

2.2. HPV detecting methods

Primers that could specifically identify HPV-16, 18, 31, 33, 51, 52 and 58 DNA were designed. DNA of cervical tissue was extracted with medical kits for rapid hybridization of nucleic acid molecules, HPV-DNA was amplified and then gel electrophoresis was conducted to distinguish that different HPV types were positive or negative. HC- II (hybrid capture II ) kits from Digane Company were used to detect HPV-16, 18, 31, 33, 51, 52 and 58 contents.

2.3. Gene mRNA contents detecting methods

Cervical tissue for biopsy was taken. Trizol lysate was added to it to extract RNA for RT into cDNA with RT kits from Promega Company. Then PCR amplification was conducted, target genes including IMP3, MUC1, TS, P16ink4a, p53, MCM2, MCM7, hTERC, hTERT, hTP1 and CA-IX. Ct values were read from software and then mRNA contents were calculated.

2.3. Statistical methods

Detected data was input by SPSS18.0 software, analysis of measurement data between two groups for t test, comparison of measurement data among three groups for variance analysis and pairwise comparison for LSD-t test; percentage data among three groups for variance analysis and pair wise comparison for chi-square test. Differences were considered to be significant at a level of P<0.05.

3. Result

3.1. HPV infection conditions of patients in different CIN stages

HPV infection is an important cause of the occurrence of CIN.

Table 1

Relationship between high risk HPV infection and expressions of chromosone related genes.

<table>
<thead>
<tr>
<th></th>
<th>MCM2</th>
<th>MCM7</th>
<th>hTERC</th>
<th>hTERT</th>
<th>hTP1</th>
<th>CA-IX</th>
</tr>
</thead>
<tbody>
<tr>
<td>High risk</td>
<td>234.20±36.20</td>
<td>314.80±44.10</td>
<td>278.90±34.80</td>
<td>309.40±40.80</td>
<td>259.10±38.60</td>
<td>288.40±41.50</td>
</tr>
<tr>
<td>Non–high risk</td>
<td>100.00±16.62</td>
<td>100.00±17.92</td>
<td>100.00±18.02</td>
<td>100.00±16.77</td>
<td>100.00±15.93</td>
<td>100.00±17.27</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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</tbody>
</table>

HPV-16, 18, 31, 33, 51, 52, 58, and so on can all infect cervical tissue. In order to clear the relationship between the occurrence of CIN and different types of HPV infection, types of HPV infection in cervical tissue of patients in different CIN stages were detected and analysis results showed that: (1) there were no differences in positive rates and contents of HPV-31, 33, 51, 52 and 58 of patients in different CIN stages (P>0.05); (2) there were differences in positive rates and contents of HPV-16 and 18, and the higher the CIN stage is, the higher the positive rates and contents of HPV-16 and 18 are.

Figure 1. HPV infection conditions of patients in different CIN stages. There were no differences in positive rates and contents of HPV-31, 33, 51, 52 and 58 of patients in different CIN stages; there were differences in positive rates and contents of HPV-16 and 18. a: compared with patients in CIN I stage, there were differences; b: compared with patients in CIN II stage, there were no differences.

3.2. Relationship between high risk HPV infection and expressions of proliferation related genes

HPV infection will cause HPV-DNA integration of genome DNA that enters cervical epithelial cells, thus causing abnormal expressions of a variety of proliferation related genes. By the results of previous section, HPV-16 and 18 are high risk HPV. In order to clear the relationship between high risk HPV infection and expressions of proliferation related genes, expressions of proliferation related genes in cervical tissue of patients with high risk HPV infection (HPV-16 and 18 infection) and non-high risk HPV infection (HPV-31, 33, 51, 52 and 58) were compared. Results showed that mRNA contents of proliferation genes IMP3, MUC1 and TS in cervical tissues of patients with high risk HPV infection displayed higher expressions; mRNA contents of tumor suppressor genes p16ink4a and p53 displayed lower expressions.
about 50% of cervical carcinoma is caused by HPV16 infection. HPV-18 are known high risk HPV. Epidemiological data shows that epithelial cells and interfere with expressions of proliferation and apoptosis. When HPV infects cervical tissue, viruses and according to different genotypes, it can be divided into and development into cervical carcinoma. HPV infection has already emerged in early CIN and lasts until late CIN and cancer period. Human papillomavirus cervical carcinoma. HPV infection is an important reason that causes CIN occurrence and development process of CIN. Integration between chromosomes related genes in cervical tissue of patients with high risk HPV infection, which indicated that high risk HPV infection would cause increased expressions of proliferation genes and inhibited expressions of tumor suppressor genes.

3.3. Chromosome related genes

Abnormal amplification of the long arm of chromosome 3 is an important pathological feature of CIN. Maintenance of chromosome structure and function relies on proteins encoded by MCM2, MCM7, hTERC, hTERT, hTP1, CA-IX and many other genes. Abnormally high expressions of the above proteins will directly cause chromosome replication enhancement, length increase and structure change. In order to clear the relationship between high risk HPV infection and expressions of chromosome related genes, expressions of chromosome related genes in cervical tissue of patients with high risk HPV infection and non-high risk HPV infection were compared. Results showed that mRNA contents of MCM2, MCM7, hTERC, hTERT, hTP1 and CA-IX in cervical tissue of patients with high risk HPV infection displayed higher expressions, expression levels rising by 2.34, 3.15, 2.79, 3.09, 2.59 and 2.88 times respectively.

4. Discussion

Cervical intraepithelial neoplasia is a clear precancerous lesion of cervical carcinoma. HPV infection has already emerged in early CIN and lasts until late CIN and cancer period. Human papillomavirus (HPV) infection is an important reason that causes CIN occurrence and development into cervical carcinoma. HPV is a type of DNA viruses and according to different genotypes, it can be divided into more than 100 types, among which more than 40 types can infect human genital and anal area. When HPV infects cervical tissue, HPV-DNA can integrate genome DNA that enters cervical epithelial cells and interfere with expressions of proliferation and apoptosis related genes, thus leading to malignant transformation of cervical epithelial cells[3]. Current studies on HPV have shown that HPV that can infect cervical tissue and cause cervical lesions include HPV-16, 18, 31, 33, 51, 52, 58 and so on[4], among which HPV-16 and HPV-18 are known high risk HPV. Epidemiological data shows that about 50% of cervical carcinoma is caused by HPV16 infection and about 10-20% of cervical carcinoma is caused by HPV18[5].

In order to identify high risk HPV infection conditions in patients with CIN, different types of HPV in cervical tissue were detected and HPV infection conditions of patients in different CIN stages were analyzed. Results showed that there were no differences in positive rates and contents of HPV-31, 33, 51, 52 and 58 of patients in different CIN stages; there were differences in positive rates and contents of HPV-16 and 18, and the higher the CIN stage is, the higher the positive rates and contents of HPV-16 and 18 are, which indicated that HPV-16 and HPV-18 were high risk HPV that caused the occurrence of CIN and they were closely related to the development of the disease.

The most direct influence of high risk HPV infection on cervical epithelial tissue is HPV-DNA integration of genome DNA that enters cervical epithelial cells, thus affecting expressions of a variety of proliferation related genes and leading to malignant proliferation of epithelial cells. Insulin-like growth factor II mRNA binding protein 3 (IMP3) is a binding protein that can identify messenger RNA, containing RNA identifying domain of N-terminal and HK identifying domain of C-terminal[6]. On the one hand, IMP3 can raise IGF-II and promote cell proliferation, and on the other hand, it can increase expressions of adhesion molecules and proteases and promote cell migration and invasion[7]. MUC1 is a high molecular weight protein that specifically exists in epithelial cells. Normally expressed MUC1 has protective effect; abnormally high expression and non-polar distribution of MUC1 will cause deformed or incomplete glycosylation of a variety of proteins in epithelial cells, thus leading to malignant cell proliferation phenotype[8]. Thymidylate synthetase (TS) is the key enzyme that catalyzes de novo synthesis of thymidylate. Its high expression can directly result in uncontrolled cell proliferation and can also inhibit the function of tumor suppressor gene p53 to induce cell proliferation[9]. P16ink4a is a newly discovered tumor suppressor gene, also known as the multiple tumor suppressor gene p53 to induce cell proliferation[9]. P16ink4a is a newly discovered tumor suppressor gene, proteins encoded by which can compete with cell cycle protein CyclinD to combine with CDK4, accelerate CyclinD degradation and block the starting phase of cell cycle; P16ink4a can also directly combine with CDK6 and inhibit pRb phosphorylation, thus stagnating cell cycle in G1 phase[10].

Proliferation related genes in cervical tissue of patients with high risk HPV infection and non-high risk HPV infection were compared and analyzed. Results showed that mRNA contents of proliferation genes IMP3, MUC1 and TS in cervical tissue of patients with high risk HPV infection were higher than those of patients with non-high risk HPV infection and contents of tumor suppressor genes p16ink4a and p53 were lower than those of patients with non-high risk HPV infection, which indicated that high risk HPV infection would cause increased expressions of proliferation genes and inhibited expressions of tumor suppressor genes.

Studies on genomics in recent years have shown that there is abnormal amplification of the long arm of chromosome 3 in the occurrence and development process of CIN. Integration between high risk HPV-DNA and host DNA is an important reason that
causes structure change and abnormal replication of chromosome and is also one of the ways that high risk HPV-DNA is involved in the occurrence and development of CIN. Chromosome replication and extension process is regulated by many genes. DNA chain elongation minichromosome maintenance protein (MCM) is a highly conserved family of proteins. It has the function of helicase and plays a regulatory role in chromosome replication initiation and extension.[11] MCM2 and MCM7 of MCM family participate in replication of helicase complex composition. MCM2 and MCM7 are largely expressed in malignant proliferation phenotype of cells while their expressions are barely detectable in resting cells.[12] Telomere is a substance at the end of chromosome of eukaryotic cells. Its length and function are regulated by telomerase. Compositions of telomerases include telomere RNA (hTERC), human telomerase reverse transcriptase (hTERT) and telomerase binding protein (hTP1).[13] Nature of telomere lengthening process is the reverse transcription of the template, hTERC into DNA single strand under the catalysis of hTERT.[14] Under physiological conditions, telomerase activity is mostly negative, which can avoid abnormal increase of telomere length; abnormally activated telomerase will lead to lengthened telomere, thus preventing cell apoptosis and enabling cells with immortalized ability.[15] The function of CA-IX is to catalyze the reaction between carbon dioxide and water molecules and generate bicarbonate ions and hydrogen ions. It can maintain steady state of the environment within cells. In malignant proliferation phenotype of cells, increased cell metabolic rate will cause large generation of hydrogen ions; increased CA-IX expression can catalyze and generate HCO3- and neutralize H+, keeping chromosome in suitable micro-environments. Chromosome related genes in cervical tissue of patients with high risk HPV infection and non-high risk HPV infection were compared and analyzed. Results showed that mRNA contents of MCM2, MCM7, hTERC, hTERT, hTP1 and CA-IX in cervical tissue of patients with high risk HPV infection displayed higher expressions, which indicated that high risk HPV infection would affect chromosome replication and extension process.

Based on above discussions and it can be concluded that HPV-16 and 18 are high risk HPV of patients with cervical intraepithelial neoplasia and will cause increased expressions of proliferation genes and inhibited expressions of tumor suppressor genes, and affect chromosome replication and extension process.

References


