A META-ANALYSIS OF THE RELATIONSHIP BETWEEN PROTEIN EXPRESSION OF HEDGEHOG SIGNALING PATHWAY AND INFECTION OF HUMAN PAPILLOMAVIRUS TYPE 16 IN CERVICAL CANCER

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ABSTRACT

Objective: To systematically estimate the relationship between protein expression of Hedgehog (Hh) signaling pathway and human papillomavirus type 16 (HPV16) infection in cervical cancer.

Methods: Literature published from 2001 to date on the relationship between protein expression of Hh signaling pathway and HPV16 infection in cervical cancer was retrieved from databases such as PubMed, Embase, Medline, CNKI and Wanfang. The data were screened and collated independently by two evaluators. Thereafter, the data were subjected to meta-analysis using RevMan5.2 analysis software after the evaluation of risk of bias.

Results: Ten published works were included in this study. These consisted of 520 cases of cervical intra-epithelial neoplasia (CIN) and 642 cases of cervical carcinoma (CC). The meta-analysis showed that in the Hh signaling pathway, the expression of sonic hedgehog (Shh) protein in CC group was significantly higher than that in CIN group (OR = 0.22; 95 % CI = 0.16, 0.31; P < 0.00001); the positive expression of HPV16 in CC group was higher than that in CIN group (OR = 0.26; 95 % CI: 0.16, 0.43; P < 0.00001), and the positive expression of Shh protein in HPV16 positive group was higher than that in HPV16 negative group (OR = 4.95; 95 % CI: 2.34, 10.43; P < 0.00001).

Conclusion: The protein expression of Hh signaling pathway is enhanced in cervical cancer and is positively correlated with HPV16 infection.

Keywords: Hedgehog, Human papillomavirus type 16, Cervical cancer, Meta-analysis.

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Introduction

Cervical cancer is a common malignant tumor which seriously endangers the health of women, and ranks second in mortality amongst malignant tumors of women. Cervical cancer has several predisposing factors, amongst which human papillomavirus (HPV) is considered very important. There are more than 100 different HPVs, but the common genotypes for cervical cancer involve HPV 16, 18, 45, 33 and 52. It has been established that HPV16 has the highest-risk of carcinogenicity to the reproductive system.

Studies have shown that the HPV infection rate increases with the aggravation of cervical lesions.

Hedgehog (Hh) protein is present in the tumor of epithelial organic tissues such as basal cell carcinoma, lung cancer, gastrointestinal tumor, ovarian cancer, oral cancer and breast cancer. The Hh signaling pathway is an important signal pathway that regulates cell proliferation and tissue differentiation during human embryonic development which is often in the resting state after maturation of embryo, and is activated abnormally in a variety of malignant tumors. It is actively expressed in cervical cancer, suggesting that the Hh signaling pathway is closely related to the development of this malignancy.

Studies on the association of Hh signaling pathway, HPV and cervical cancer have important clinical significance for cervical cancer treatment.
However, not much is known about the association between Hh signaling pathway and HPV16 in cervical cancer. In this study, the key protein expressions in Hh signaling pathway, and the expression of HPV16 were compared and analyzed in order to investigate the relationship between them.

**Subjects and Methods**

**Inclusion and exclusion criteria**

*Type of studies included:* Literature on the relationship between protein expression of Hh signaling pathway and HPV16 infection in cervical cancer was searched. This study involved only literature written in Chinese and English, and the number of cases included in each selected study was not less than 20.

*Study subjects:* Only patients clinically diagnosed with cervical cancer (CC) or cervical intraepithelial neoplasia (CIN) were included.

*Exposure factors:* The exposure factors were protein expression of Hh signaling pathway and positive expression of HPV16.

*Main outcome indices:* These were the amounts of protein expressed in Hh signaling pathway and positive expression of HPV16.

*Exclusion criteria:* (1) literature without data integrity or invasive clinical features; (2) literature in languages other than Chinese or English; (3) case reports, review lectures and conferences, and (4) literature involving duplicated publication.

**Search strategy**

Literature was retrieved from databases such as PubMed, Embase, Medline, CNKI and Wanfang. The keywords in English for retrieval included “Hedgehog”, “Shh”, “Ihh”, “Dhh”, “Ptc”, “SMO”, “HPV16”, “cervical cancer”. The keywords in Chinese (translated) for retrieval included “signaling pathway”, “expression”, “cervical cancer” and “human papillomavirus”. The retrieval was performed by the means of free combination of keywords.

**Literature screening, data extraction and bias risk assessment**

The screening of literature, extraction of data, and assessment of bias risk in the included studies were independently performed by two evaluators. Any differences in their opinions were resolved through mutual discussion, or by a third party.

The data were extracted using self-made data extraction tables, and the main extracted contents included: (1) general data such as titles, authors, year of publication and source of original references; (2) baseline characteristics and outcomes of research subjects, including cases, sample sizes, operation time, intraoperative blood loss, postoperative drainage volume for the first day, extubation time and hospitalization time in control group, and (3) key factors in the evaluation of bias risk. Risk of bias was evaluated by the Newcastle Ottawa Scale (NOS scale).

**Statistical Analysis**

The data were processed and analyzed using the analysis software Review Manager 5.2. The odds ratio (OR) was used as the effect index of numeration data, and the mean deviation (MD) was regarded as the effect index of measurement data. The estimated value and 95% CI of each effect index were established. Heterogeneity between the research results was analyzed by chi-square test (size of test: $\alpha = 0.1$), and the degree of heterogeneity was determined in combination with the I2 quantitative method. An I2 value less than 50 % showed no presence of heterogeneity between studies. At first, a meta-analysis was performed by the fixed effect model, and the source of heterogeneity was further analyzed when there was statistically significant heterogeneity between the results. Then, after the significant influence of clinically heterogeneity was excluded, meta-analysis was performed by the random effect model. The size of test in meta-analysis was $\alpha = 0.05$.

**Results**

**Results of literature retrieval**

A total of 648 studies were retrieved from PubMed (n = 136), EMBASE (n = 84), Medline (n = 124), Wanfang (n = 64) and VIP (n = 237). Ten case-control studies were eventually included in this study after successive screening. The process and results of literature screening are summarized in Figure 1.

**Basic characteristics of included studies and evaluation of bias risk**

The basic characteristics of the included studies and results of the bias risk assessment are shown in Table 1. The 10 studies included control studies between CIN group and CC group, 5 of which
involved the comparison of Shh protein expressions in Hh signaling pathway. Another set of 5 studies involved comparison of HPV16 expressions, while 2 studies involved the comparison of Shh expressions under the negative and positive states of HPV16 in CC group.

### Results of meta-analysis

#### Comparison of Shh protein expressions

Five of the 10 studies involved comparison of Shh protein expressions in Hh signaling pathway, and included 319 cases of CIN and 440 cases of CC. Meta-analysis with fixed effect model showed that the Shh protein expressions in CC group were significantly higher than those in the CIN group (OR = 0.22; 95% CI: 0.16, 0.31; P < 0.00001). These results are shown on Table 2.

#### Comparison of HPV16 positive expressions

Five of the 10 studies involved comparison of HPV16 positive rates, including 201 cases in CIN group and 202 cases in CC group. The meta-analysis with fixed effect model showed that the HPV16 positive rates in CC group were higher than those in CIN group, and the differences were statistically significant (OR = 0.26; 95% CI: 0.16, 0.43; P < 0.00001, Table 3).

#### Comparison of Shh expressions under different states of HPV16

Two of the 10 studies involved comparison of Shh protein expressions under different states of HPV16 in cervical cancer tissues. They comprised 111 cases of HPV16-positive group and 78 cases of HPV16-negative group. Meta-analysis with fixed effect model showed that the Shh protein expressions in HPV16 positive group were significantly higher than those in HPV16-negative group (OR = 4.95; 95% CI: 2.34, 10.43; P < 0.00001, Table 4).

### Determination of publication bias

Funnel plot of Shh protein expressions in Hh signaling pathways in CIN and CC groups showed that the included literature with asymmetric distribution were basically within 95% confidence interval (95% CI).

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Table 1: The basic features of included literature.

<table>
<thead>
<tr>
<th>Name of author and year</th>
<th>CIN</th>
<th>CC</th>
<th>HPV16</th>
<th>HPV16 (NC)</th>
<th>Total score of NOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ang, Nan, 2018 [6]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Ding-sheng Lin, 2016 [7]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Ming-yue Zhu, 2015 [10]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Nan, Yu, 2016 [11]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Yan, Nan, 2009 [12]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Nan, Cheng, 2015 [14]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Xu, Xiang, 2013 [15]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Wei-xi Tu, 2014 [17]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Yan, Li, 2015 [18]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 2: Meta-analysis of Shh expressions in CIN group and CC group.

Table 3: Meta-analysis of HVP16 positive expressssions in CIN group and CC group.

Table 4: Meta-analysis of Shh expressions under different states of HPV16.
This indicates that this study was affected by publication bias (Figure 2).

Table 4: Meta-analysis of Shh expressions under different states of HPV16 in CC group.

<table>
<thead>
<tr>
<th>Author name and year</th>
<th>HPV16+</th>
<th>HPV16-</th>
<th>Weight</th>
<th>Random 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na, Ming 2016</td>
<td>44</td>
<td>30</td>
<td>0.05</td>
<td>0.04 (0.01-0.04)</td>
</tr>
<tr>
<td>Yang, Xiao 2014</td>
<td>35</td>
<td>20</td>
<td>0.06</td>
<td>0.05 (0.03-0.07)</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>50</td>
<td>0.05</td>
<td>0.04 (0.03-0.06)</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>85</td>
<td>70</td>
<td>0.05</td>
<td>0.04 (0.03-0.06)</td>
</tr>
</tbody>
</table>

Figure 2: Funnel plots of Shh expressions.

Discussion

Hedgehog gene was discovered in 1980. The Hedgehog signaling pathway proposed by Forbes in 1993 is mainly composed of receptor complexes (including three Hh ligands: Shh, Ihh and Dhh); transmembrane protein receptor Patched, transmembrane protein Smo with signal transduction, and downstream proteins in three Gli transcription factors. Disorders in Hh signals lead to a large number of developmental anomalies which are very important in the pathogenesis of human tumors. Over-expressions of Hh genes in multiple tissues and mutations in their target genes can lead to cell-specific tissue proliferations and tumor progression.

Studies have shown that Shh protein expression is increased in normal cervical tissues, CIN tissues and cervical cancer tissues, even in the staging of cervical cancer. This implies that compounds involved in Hh signaling pathway may be related to the etiology of cervical cancer. Recombinant Shh protein promotes the proliferation of cervical cancer cells, while Hh signaling pathway inhibitor blocks the proliferation and migration of cervical cancer cells, and also reduces Smo levels, indicating that the Hh signaling pathway is closely related to cervical cancer cell proliferation and migration.

Human papillomavirus (HPV) is a double-stranded closed circular DNA virus from the virus family of Papovaviridae, which can infect human skin and mucosumembranous epithelial cells, and even cause many malignant pathologic lesions. The hypothesis raised by HauselIs on the correlation between HPV and cervical cancer has now been verified. The HPV can be divided into two types: low-risk and high-risk types. The most common high-risk types in cervical cancer are HPV16 and HPV18. Different types of HPV are associated with pathological grouping and clinical staging of cervical cancer, in which HPV16 is more common in cervical squamous carcinoma, while HPV-18 is more frequent in cervical adenocarcinoma. Statistically, more than 90% of cervical cancers are associated with HPV infection.

The detection rates of HPV infections in normal populations, and in patients with CIN I, CIN II and CIN III, and cervical cancer are 4, 30, 55, 65, and 99.8%, respectively. Thus, HPV is closely related to the formation and development of cervical cancer, and its infection rate increases with aggravation of cervical lesions.

In the present study, comparison of Shh expressions in Hh signaling pathway showed that Shh protein expression in cervical cancer tissues was significantly higher than that in cervical intraepithelial neoplasia tissues, which is consistent with the cell proliferation caused by the overexpression of Hh genes. The expression of Shh as a key ligand of Hh signaling pathway was increased accordingly when Hh genes were overexpressed. Results from comparison showed that the HPV16 positive expression in cervical cancer tissues was higher than that in cervical intraepithelial neoplasia tissues, which indicates that HPV16 is closely related to the etiology of cervical cancer. This is in agreement with existing research results. In addition, Shh expression in HPV16-positive group was higher than that in HPV16-negative group, which indicates that both of them are interrelated. Previous studies showed that the expressions of other protein factors (SMO, Ihh, and Ptch) in HPV16-positive group were significantly higher than those in HPV16-negative group in Hh signaling pathway under the different states of HPV16.

This suggests that HPV16 has an impact on each link in the Hh signaling pathway. It can be speculated that each major factor in Hh signaling pathway is positively correlated with the positive expression of HPV16, but the interaction between...
Hh signaling pathway and HPV16 in cervical cancer need to be further explored and validated, in view of the relatively small sample size used in the present study.

**Study Limitations**

There were some bias in this study as was evident from the funnel plot, due probably to the small size of included literature. This implies that this study still needs to be further verified using larger sample sizes. In addition, the collected data were not analyzed with respect to the expressions of other factors in Hh signaling pathway, again because of relatively small number of studies used.

**Conclusion**

Cervical cancer is associated with a high expression of Shh protein in the Hh signaling pathway, and a high HPV16-positive expression. The increased Hh protein expression in Hh signaling pathway is positively correlated with HPV16 infection in cervical cancer.

**References**


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