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Research Article

Research Progress of HPV L1 Capsid Protein in Prediction of Cervical Lesions

Jing Cheng[1], Lin Xu[2*], Beibei Liu[1], Biao Wang[1], Xicui Long[1], Zhihong Li[1], Ruiting Wu[1], Ruili Chen[1]

[1]The First Affiliated Hospital of Kunming Medical University, China;

[2]Kunming Tongren Hospital, Kunming 650000, Yunnan, China

Abstract: Cervical cancer is one of the most common malignant gynecological tumors and has the second highest incidence of all malignancies in females. Chronic and persistent infection with High Risk Human Papillomavirus (HR-HPV) is the main cause of cervical cancer. There is a distinct lack of methodology by which to determine whether cervical epithelial dysplasia is cancerous following HPV infection. HPV L1 capsid protein is a major structural protein of human papillomavirus (HPV), and it is the main target of the local cellular immune response aiming to combat human papillomavirus after HPV infection within cervical cells. Greater understanding of HPV L1 capsid protein and its association with cervical cytology, histopathology, patient age and human papillomavirus viral load has the potential to contribute toward improved the diagnosis and management of cervical cancer, providing useful information for gynecological clinicians in the hope of improving patient treatment and quality of life. This article reviews the predictive utility of HPV L1 capsid protein for cervical lesions.

Keywords: HPV L1 capsid protein; Cervical lesions; Prognosis

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[*]***Corresponding author:*** Lin Xu,xulin64@163.com

Cervical cancer is a major cause of morbidity and mortality in women worldwide and is one of the four most common malignant tumors in women. Globally, more than 560,000 new cases of cervical cancer are diagnosed each year, 80% of which occur in developing countries. Around 310,000 deaths from cervical cancer each year are reported worldwide. In China alone, over 130,000 new cases and 80,000 deaths are reported per annum[1]. 99.7% of women with cervical cancer are caused by HR-HPV infection[2]. HPV L1 protein is the main capsid protein of HPV, and is understood to be a major protein recognized by the immune system in combating HPV infection in humans, making it a principal cervical cancer-associated antigen. The local cellular immune response generated after HPV infection is mainly directly against the HPV L1 protein. Loss of HPV L1 expression may be related to integration of the HPV DNA into the host genome, leading to persistent infection. Therefore, determination of HPV L1 protein expression may have value for predicting progression of cervical lesions.

1 The structural basis of HPV and HPV L1 capsid protein

Human papillomavirus (HPV) belongs to the genus Papillomavirus, comprising a DNA genome and protein capsid. HPV is a spherical, uncoated double-stranded DNA virus with a diameter of 50-55nm. The human papillomavirus genome is relatively small with a super helical double-stranded closed loop of approximately 8000 base pairs. This DNA genome is divided into three regions: firstly, the non-coding regulatory region (about 400-1000bp), including promoter, enhancer and silencing subsequence, whose function is to regulate virus replication and gene transcription; secondly, the early-expression gene-encoding region, which encodes 6 early-expressed viral proteins (E1, E2, E4, E5, E6 and E7), whose functions are related to viral replication of HPV and

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tumorigenesis. E1 and E2 proteins are the basis of viral replication. Lastly, the late expressed gene-coding region, which expresses the L1 and L2 capsid proteins. L1 is highly conserved and is the primary capsid protein, comprising around 80% of the capsid; conversely, the L2 secondary capsid protein is highly mutated. L1 and L2 form a viral capsid with a spherical 20-plane three-dimensional structure to enclose the viral DNA, which protects the DNA genome from nucleases.

2 The biological activity of HPV and HPV L1 capsid protein

The naked-core HPV enters the existing damaged human cells after passively contacting the superficial cells, and then uses the cytoplasmic proteins of the human cells to synthesize the capsid protein of the HPV virus. Subsequently, the HPV virus transforms from a dormant immature virus into an aggressive mature virus. After HPV virus invades the nucleus of cervical basal cells, the HPV L1 capsid protein is removed, and then the viral DNA is loaded into the DNA of human cells. At this time, the human immune system can produce the phagocytosis effects of the CD3 and CD4 systems through the nucleus and nuclear membrane channels to eliminate HPV-infected cells. During infection, HPV E6 and E7 proteins interfere with multiple regulatory processes within the host cell, including processes regulated by pRB and p53. Overexpression of HPV E6 and E7 and associated host cell genomic instability may occur in patients with persistent HPV infection[3]. There are two different ways of virus replication: synthetic "coating" HPV directly enters the superficial cell nucleus and removes the "coating". The HPV DNA after removing the "coating" and the host DNA of superficial cells co-exist in the same cell nucleus. Because the viral DNA is not integrated into the DNA of human cells and is limited to the superficial cell layer, it can only cause rapid and excessive proliferation of infected cells in the superficial cell layer, but will not become cancerous. After removing the "coating", the DNA of the HPV virus is integrated into the host cell DNA; basal cells with integrated HPV DNA are driven to undergo rapid excessive mitosis, promoting transformation toward a malignant cell type. Integration of HPV DNA into the host genome is necessary for HPV-driven transformation and is a marker of cancer[4].

3 HPV L1 capsid protein and cervical lesions

3.1 Correlation between HPV L1 capsid protein and cervical cytology of patients

HPV L1 protein is only expressed in the cervical surface cells; cervical brushes are routinely obtainable in clinical practice, making correlation between HPV L1 protein expression and degree of abnormal cervical cytology easily assessable. A study by Su Guang et al[5] using samples from 101 patients identified a positive HPV L1 expression rate of 60.00% (12/20), 44.12% (15/34), 11.90% (5/42) and 0% (0/5) within the control, LSIL, HSIL and SCC groups, respectively. The differences between the control group and HSIL and SCC groups were statistically significant (P<0.05). Previous studies[6-7] have identified correlations between HPV L1 capsid protein expression and the degree of cervical cytological lesions. With progression of cervical lesion from LSIL to HSIL and SCC, the positive rate of HPV L1 capsid protein is lower and lower. Conversely, the negative rate increases gradually, highly indicating the probability of HPV DNA integration into the host genome.And the HPV genome is likely to be fully integrated into the host genome within cervical cancer patients [8-9].

Sarmadi S et al.[10] followed 65 female cases for 24 months. 28 cases were HPV L1 capsid protein positive LSIL patients, and the spontaneous regression rate was 60.7% (17/28); in 15 patients with HPV L1 capsid protein negative LSIL, the fading rate was 33.3% (5/15). Within the 22 cases of HPV L1 protein capsid negative HSIL cases, the fading rate was 13.6% (3/22) patients. Spontaneous subsistence is therefore more likely in HPV L1 protein capsid positive patients. HPV L1 protein negative patients are more prone to progression of the cervical lesion. therefore, HPV L1 protein immunocytochemistry detection can provide information on the prognosis of early proliferative cervical lesions, and can be used to predict the malignant potential of these lesion.

3.2 Correlation between HPV L1 capsid protein and histopathology

Cervical intraepithelial neoplasia (CIN) is a precancerous lesion of cervical cancer and is therefore of great importance with regard to cervical

cancer prevention and treatment. Wei X et al et al.[11] used the above methods to detect the expression of HPV L1 capsid protein in cervical smears from 274 patients with a pathological diagnosis of inflammation, CIN I, CIN II, CIN III or SCCs respectively. HPV L1 capsid protein accounted for 69.79% in cervicitis, 83.53% in CIN I and 41.81% in CIN II and III. It has also been shown that the decreased expression of HPV L1 may be related to the progress of cytopathology. Yu L et al.[12] used the same method to detect HPV L1 capsid protein using 63 tissue specimens. In the samples diagnosed as CIN I, CIN II/III and SCC, detection rates of HPV L1 were 40%, 19.4% and 0% respectively, suggesting an inverse correlation between HPV L1 positivity and severity of the cervical lesion. Ki EY et al.[13] analyzed 70 (ASCUS) and 215 LSIL Pap smears for HPV L1 expression by immunohistochemistry: CIN II+ samples were commonly HPV L1 negative, while HPV L1 expression was significantly higher in the CIN I/cervicitis samples (P<0.05). This indicates that the expression rate of HPV L1 capsid protein decreased with the increase of the severity of cervical lesions. It is widely acknowledged that precancerous cervical lesions may spontaneously regress. Choi YS et al.[14] examined 101 cases of histologically confirmed CIN I women with at least 12 months follow-up: 60.4% of women spontaneous regressed. The fading rate of HPV L1 capsid protein positive was 72.7% (48/66), and the rate of CIN I maintaining or progressing to higher-grade lesions was 27.3% (18/66). The fading rate of HPV L1 capsid protein-negative patients was 37.1% (13/35), and the lesions continued or progressed accounted for 62.9% (22/35; P<0.001). Mehlhorn G et al.[15] studied 908 patients with abnormal hyperproliferative lesions (LSIL/HSIL) in the early stage of HR-HPV infection, including the follow-up period of 54 months, and the results showed that the clinical outcome of HPV L1 negative and HPV L1 positive cases was statistically significant (P<0.0001). It can be seen that the positive expression of HPV L1 capsid protein is mostly associated with the natural regression of CIN I lesions, while for cervical precancerous lesions of CIN II and above, clinically active treatment measures are often taken, few follow-up observations are made, and few relevant studies have been reported.

3.3 Age correlation between HPV L1 capsid protein and patients

Rauber D et al.[16] followed 279 cases of CIN I or CIN II; the HPV L1 fading rate was 49.1%, the lesion persistence rate was 41.5%, and the lesion progression rate was 9.4%. The rate of progression in the HPV L1 negative group was 25.9% with a fading rate of 33.3% and persistence rate of 40.7% (P = 0.001). It was found that negative HPV L1 status was associated with disease progression in women with CIN I and CIN II, especially in women under 30 years old, which was of great significance for prognosis. Similarly, Stemberger-Papić S et al.[17] found that the difference in clinical outcome of HPV L1 positive staining was statistically significant in the age groups under 30 years old and above 30 years old (P=0.04). Lee H et al.[18] demonstraed that the expression rate of L1 capsids decreased in the age group above 40 years old (49.2% and 50.8% respectively). Together, these data suggest that expression rate of HPV L1 capsid protein may decrease with age; however, further studies are required to confirm this association.

3.4 Correlation between HPV L1 capsid protein and viral load

Huang Bin et al.[19] studied 309 patients, including 33 cases of normal or chronic cervicitis, 168 cases of CIN I, 84 cases of CIN II/III and 24 cases of SCC. The detection rate of HPV L1 capsid protein in the HR-HPV DNA load ≥1000 RLU/PC group was 73.1%. By comparison, in the <1000 RLU/PC group, its expression rate was significantly higher (P<0.05). Qian Min et al.[20] tested HPV L1 capsid protein in 409 patients, and found that the positive expression rates of HPV L1 capsid protein in patient groups with HR-HPV DNA loads of 0-9.9, 10-99.9, 100-999.9 and ≥1000 pg/mL were 6.67%, 17.24%, 36.72%, and 51.77%, respectively. Comparison between 0-9.9 and 10-99.9 pg/mL groups versus higher load patient groups demonstrated a significantly difference in HPV L1 positivity (P<0.05). Yan li et al.[21] studied 248 patients and found that the expression rate of HPV L1 capsid protein in the HPV load > 100 RLU/CO group (27/82,32.93%) was higher than that in the HPV load ≤100 RLU/CO group (13/163, 7.83%), with a statistically significant difference (P=0.000). Therefore, the expression rate of HPV L1 capsid protein in cervical exfoliated cells showed an increasing trend with the increase of viral load.

4 Conclusion

With the increasing standard of screening for cervical cancer and implementation of further cervical cancer prevention measures, improved understanding of the role and impact of HPV L1 capsid protein in cervical lesion progression is of great clinical interest. Robust correlation of HPV L1 expression with cytology and histopathology has the potential to improve the management of patients with both cancerous and precancerous cervical lesions in order to improve the fidelity of problematic lesion diagnosis and to prevent excessive treatment of lesions unlikely to develop into malignancy. Current studies demonstrate that HPV L1 protein capsid expression is useful for predicting the progression of cervical lesions; however, further research is required to robustly validate existing associations, and to dissect the complex mechanisms by which HPV L1 may influence progression or remission of precancerous lesions.

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