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Expression of HPV 16-E6 in Esophageal Carcinoma and its Clinical Significance

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ABSTRACT

Background and Objective: The role of (Human Papilloma Virus) HPV in cancer of certain anatomical location, such as cervix, has been widely recognized. The present study was conducted to explore the association between HPV 16-E6 protein and esophageal squamous cell carcinoma. **Methods:** SP immunohistochemical method was used to examine the expression of HPV 16-E6 in 50 cases of esophageal squamous cell carcinoma, 10 cases of normal esophageal squamous cell and 10 cases of adjacent tissue. **Results:** The expression of HPV 16-E6 was significantly higher in esophageal carcinoma than in normal esophageal mucosa and in adjacent tissue. The expressions of HPV 16-E6 had significant correlation with invasive depth ($P<0.05$), but not with patient age, lymph node metastasis, tumor size ($P>0.05$). **Conclusion:** HPV 16-E6 can promote the growth and metastasis of esophageal squamous cell carcinoma and can be a prognostic factor of esophageal squamous cell carcinoma.

Key words: Esophageal carcinoma; HPV 16-E6; Immunohistochemistry

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INTRODUCTION

Gastrointestinal cancer is the most common malignant tumor in China. In the recent years, it has become one of the hot-spots in modern medical research. The role of high-risk human papilloma virus (HPV) has been discussed in a plethora of cancer researches. Studies have shown that HPV has coding regions in genes E1, E2, E4, E5, E6 and E7, among which, E6 can activate the transcription of genes. Meanwhile, it can promote the proliferation of cells.[1,2] The role of HPV in cervix cancer has been widely recognized. There are many studies showing the relationship between cervical diseases and HPV-16-E6 mRNA.[3,4] HPV18 and HPV16 may be pathogenic factors of mammary invasive ductal carcinomas, and the former may also be related to benign breast lesions.[5,6]

The present study was designed to explore the association between HPV 16-E6 protein and esophageal squamous cell carcinoma. Immuno histochemical technique was used to study the expression of HPV-16-E6 in normal esophageal mucosa, esophageal carcinoma and adjacent tissue, to explore its effect in esophageal epithelial cells infected with HPV. Furthermore, the prognostic factors of esophageal squamous cell carcinoma were also analyzed.

MATERIALS AND METHODS

A total of 50 esophageal squamous cell carcinoma specimens, 10 normal esophageal mucosa specimens (negative resection margin) and 10 adjacent tissue specimens (a field that contains genetically abnormal cells, which can be as large as three cm in diameter around the carcinoma) which were procured from the individuals who underwent

esophagectomy with lymph node dissection, at Affiliated Hospital of Chengde Medical College between the period from 2008 to 2011 were included in this study. All specimens were formalin-fixed and paraffin embedded. Neither of them underwent radiotherapy, chemotherapy and immunotherapy. Patients' gender, age, lymph node metastases, tumor sizes and invasive depths were included in the study. The histologic subtypes of esophageal carcinoma were sub classified by experienced pathologists.

All the esophageal tissue were fixed in 40g/L formaldehyde fixative and embedded in paraffin. Sections were dewaxed and rehydrated according to a standard procedure, incubated with 3ml/L hydrogen peroxide in methanol for 15 minutes at room temperature. After washing twice with phosphate-buffered saline (PBS) for five minutes, tissue sections were incubated at 37^0C for 20 minutes with blocking solution. Sections were incubated at 37^0C for two hours with primary antibody HPV 16-E6 (SC-584, Santa Cruz Biotechnology, Inc). After washing twice with PBS (0.01mol/L, PH,7.4) for 10 min, tissue sections were incubated at 37^0C for 30 min with biotin-anti-rabbit IgG. After washing twice with PBS for 5 min, tissue sections were incubated with streptavidin-HRP for 30min.Then the sections were washed twice in PBS, and they were incubated with metal-enhanced 3,3-diaminobenzidene solution for 15 min, then they were washed two times with distilled water and counterstained with hematoxylin. Negative control sections were incubated with PBS instead of primary antibody. The positive staining for HPV 16-E6–synthesizing cells was expressed as red brown granules, which were mainly located in cell nucleus under microscopy. At least 5 high-power (×400 field) were chosen randomly for cell counting. The ratio of the positive staining for HPV 16-E6–synthesizing cells was calculated by dividing the number of positive cells over the total number of cells. Tumors were then classified according to their expression of HPV 16-E6 upon overview of the section. The percentage of positive cells was divided into five grades (percentage cores): ≤ 5% = score 0; 6%-20% = score 1; 21%-50% = score 2; 51%-75% = score 3; and > 75% = score 5. HPV 16-E6 staining positivity was determined by the scores, the scores ≤ 1 was defined as negative, and > 1 as positive.

The chi-square (χ^2) test was used to compare the relationship in frequency distributions between the expressions of HPV 16-E6 and clinical indicators. Statistical significance was defined as $p<0.05$. All the calculations were performed using SPSS18.0.

RESULT

Expression of HPV 16-E6 protein in esophageal carcinoma, adjacent tissue and normal esophageal squamous cell

The positive stainings for HPV 16-E6 synthesizing cells were mainly located in cell nucleus under microscopy. Immunohistochemistry of different groups showed that, of the 50 cases of esophageal squamous cell carcinoma, the rate of positive expression was 56.00%. The rate of HPV 16-E6 positive expression was lower in adjacent tissue (50%), and normal esophageal squamous cell (30%) respectively than in esophageal squamous cell carcinoma. (Table 1).

Relationship of HPV 16-E6 protein expression to clinicopathologic features

The expressions of HPV-16-E6 had significant correlation with invasive depth ($P<0.05$), but not with patient age, lymph node metastasis, tumor size ($P>0.05$). (Table 2, Figure 1, Figure 2).

Table 1: Expression of HPV 16-E6 protein in esophageal carcinoma, adjacent tissue and normal esophageal squamous cell

Variables	No. of Cases	+Ve	-Ve	χ^2	P
Normal Mucosa	10	3	7	2.26[a]	0.17 [a]
Adjacent Tissue	10	5	5	0.83[b]	0.65 [b]
Esophageal Carcinoma	50	28	22	0.73[c]	0.74 [c]
No. of Cases	440	200	240		

[a]esophageal carcinoma versus normal mucosa
[b]normal mucosa versus adjacent tissue
[c]esophageal carcinoma versus adjacent tissue

Table 2. Relationship of HPV 16-E6 protein expression to clinicopathologic features in the cases of esophageal squamous cell carcinoma

Parameters	N	HPV 16-E6 Expression		χ^2	P
		Positive	Negative		
Age (years)					
≤60	23	14	9	0.41	0.58
>60	27	14	13		
Tumor Size (cm)					
<3	18	7	11	4.09	0.13
3-5	23	14	9		
>5	9	7	2		
Invasion Depth					
Mucosa/ Submucosa	5	0	5	65.71	0.02
Muscularis	10	5	5		
Outer Membrane	35	23	12		
Lymph Node Metastasis					
Positive	22	14	8	0.93	0.39
Negative	28	14	14		

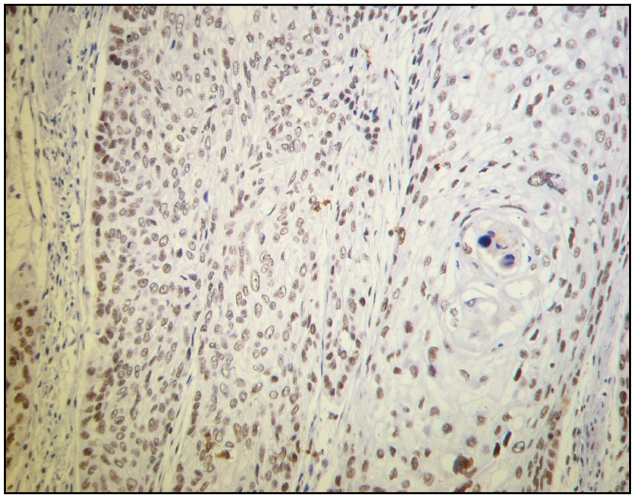

Figure 1. HPV16-E6 expression is more in cases with lymphatic metastasis SCC SP×200

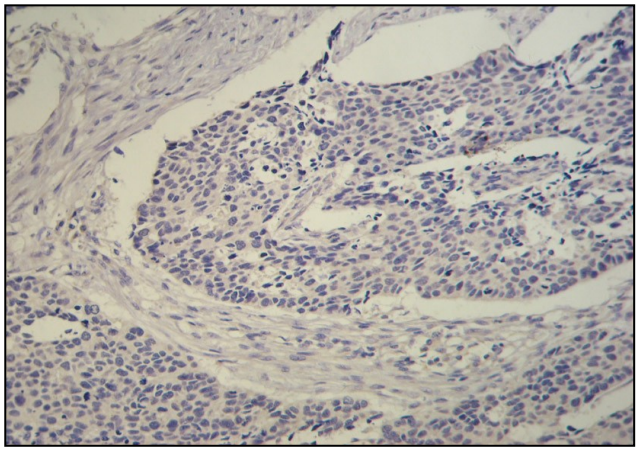

Figure 2. HPV16-E6 expression is less in cases without lymphatic metastasis SCC SP×200

DISCUSSION

Human papilloma virus is a group of DNA-containing viruses, notable for the double-stranded structures. Currently identified more than 100 types of HPV are described in detail. More than 70 types of HPV can infect a strictly defined type of epithelium and cause specific changes. There are many methods to diagnose papillomavirus: for example, histological morphological examination, Immunohistochemistry and PCR. The most sensitive method currently recognized is the polymerase chain reaction (PCR). The pathological criterion for diagnosis of HPV is the presence of koilocytic changes. Immunohistochemistry allows the identification of capsid protein expression, which exists in a period of virus replication. This can result in false negatives. So immunohistochemistry is also not ideal.[7]

Many reports suggest association of HPV with lesions of various anatomical locations such as cervix, urethra, pharynx, nasal cavity, oral cavity, bronchus and oesophagus[8-10] Recently, there has been much focus on the relationship of HPV and esophageal carcinoma.[11,12] It was reported that HPV -E6, E7 protein played a part in the occurrence and development of esophageal cancer. According to the carcinogenicity, HPV can be divided into low and high carcinogenicity groups. HPV-16 is considered to be a high carcinogenicity group. Studies showed that HPV had a coding region in

genes E1, E2, E4, E5, E6 and E7. Among them, E6 can activate the transcription of genes and promote cell multiplication leading to runaway cell division and eventually tumor formation. Open reading frame encodes the protein of E6 and E7. The increased expression of E6 and E7 in cervical carcinoma are indispensable for maintaining the transition states of tumour cells.[13,14]

Immunohistochemical method was used in this study to examine the expression of HPV 16-E6 protein. HPV 16-E6 protein contained 151 amino acids and 2 zinc fingers motifs. Its carcinogenesis is reflected as follow: E6 protein expressed by high-carcinogenicity-HPV-16, 18 inhibits the action of P53 by accelerating its degradation and inhibiting its entry into the nucleus. Its carcinogenesis is reflected as follow: E6 protein expressed by high-carcinogenicity-HPV-16, 18 inhibits the action of P53 by accelerating its degradation and inhibiting its entry into the nucleus. On the other hand, HPV 16-E6 plays an important role in the immortalization of normal cells induced by HPV16. The essential attribute of immortalization is the loss of control in the cell cycle regulation.

Li T et al[7] have reported that loss of HPV 16-E6 protein expression is significantly associated with esophageal squamous cell carcinoma. Using PCR and ISH protocols, they reported that the prevalence of expression of HPV 16-E6 protein gene in the high incidence area was higher than that in the low incidence area. In addition, an association of HPV with esophageal carcinoma has been reported in China.[15,16] Using SP immunohistochemical method, we examined the expression of HPV 16-E6 in 50 cases of esophageal squamous cell carcinoma, 10 cases of normal esophageal squamous cell and 10 cases of adjacent tissue. Our analysis found an association of HPV with esophageal cancer.

CONCLUSION

Esophageal cancer is the most common malignant tumor in China, human papilloma virus (HPV) plays an important role in the development of the esophageal cancer. More experimental methods are needed to search the other factors related to the disease.

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