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Expression and clinical significance of high risk human papillomavirus and invasive gene in cervical carcinoma

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ABSTRACT

Objective: To study the expression of E6 and E7 mRNA in high-risk human papillomavirus (HPV) HPV-18 and the relationship between the expression of invasive gene and cervical carcinoma.

Methods: A total of 119 patients with cervical cancer, cervical erosion and cervical HPV infection who were diagnosed in our hospital were selected and randomly divided into two groups: cervical cancer group ($n = 58$) and non-cancerous group ($n = 61$). Another 60 patients with uterine leiomyoma were selected as normal control group. Detection of HPV18 E6, E7 mRNA expression and invasion, migration, proliferation inhibition genes, epithelial mesenchymal transition genes and proliferation related protein content.

Results: The relative expression of E6 and E7 HPV-18 in cervical cancer group was significant higher than that in non-cancerous group and control group (mRNA) ($P < 0.05$). The content of TRAF6 and c-FLIP in invasive cervical cancer group was significantly higher than that in non-cancerous group and control group ($P < 0.05$). The mRNA content of CD44v6 and MMP-9 in cervical cancer group was significantly higher than that in non-cancerous group and control group ($P < 0.05$). The content of DEC-1, IKK16, MBP-1 in cervical cancer group was significant lower than that in non-cancerous group and control group ($P < 0.05$). The mRNA content of beta -catenin and Vimentin in cervical cancer group was significantly lower than that in non cancerous group and control group ($P < 0.05$). The proliferation related protein E2F1 of cervical cancer group was significantly lower than that of non-cancerous group and control group, Bmi-1 content was significantly higher than non-cancerous group and control group ($P < 0.05$).

Conclusions: The expression of the detection of cervical cancer in high-risk human papilloma virus HPV-18 E6 and E7 mRNA, and the invasion, migration, proliferation inhibition gene, epithelial mesenchymal transition and proliferation related gene protein content, HPV expression rate of mRNA increased with the development of cervical cancer, the expression is also enhanced. The expression has a certain correlation between the level and development of cervical cancer. Through the above indicators, the development of cervical cancer monitoring and treatment to provide important clinical guidance.

1. Introduction

Cervical carcinoma is the second most common malignant tumor only after breast cancer in women, which is the highest degree of malignancy. Nearly ten thousand women were diagnosed with cervical cancer worldwide each year, especially in developing countries and shows younger trend, of which the mortality ranks first in female malignancies. The global average annual new cervical carcinoma is more than 50 million cases, with death of over 20 million cases. In China, there are 130000 new cases each year, accounting for 1/3 of the world. In 1970s, since German virologists has presented the hypothesis that human papillomavirus (HPV) is closely related to the incidence of cervical cancer, numerous studies have shown that HPV infection, in particular high-risk HPV persistent infection, is the main

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reason for vast majority of cervical precancerous lesions and cervical carcinoma [1,2]. HPV infection is the leading pathogenic factor of cervical carcinoma. The occurrence of 99.7% cervical carcinoma is related to HPV infection, and the same subtype of high-risk HPV persistent infection is the leading cause of cervical carcinoma [3–6]. There are about 193 kinds of HPV DNA or mRNA detection methods in the world, and it is very important to check HPV for the diagnosis, the evolution of cervical cancer, the follow-up and prognosis evaluation. To discuss the HPV18 E6, E7 mRNA expression in cervical carcinoma tissue and its relationship with cervical carcinoma, to analyze its interaction with cervical carcinoma and to study the role and mechanism of HPV18 E6, E7 mRNA in cervical carcinoma will provide biomolecular evidence for the formation of cervical carcinoma, thus guiding early diagnosis, prevention and treatment.

2. Materials and methods

2.1. General materials

A total of 119 patients with cervical cancer, cervical erosion and cervical HPV infection who were diagnosed in our hospital from 1st February to 30th June 2015 were selected and randomly divided into two groups: cervical cancer group (n = 58) and non-cancerous group (n = 61). In cervical cancer group (n = 58), the age range was 36–60 years, as for CIN type, there were 23 cases of type I, 19 cases was type II, and 16 cases was type III. While in non-cancerous group (n = 61), the average age range was 34–57 years, 34 cases were cervical erosion and 27 cases were cervical HPV infection. Another 60 patients who underwent resection of uterine leiomyoma were enrolled as normal control group. Inclusion criteria: (1) patients associated with cervical hyperemia, erosion and cervical hyperplasia; (2) patients are in line with "TBS classification" in the diagnostic criteria revised by the International Cancer Society; (3) patients without sex hormones treatment or undergoing cervical surgery before admission within 3 months. Clinical manifestations were mainly diagnosed with contact bleeding or abnormal vaginal bleeding, abnormal vaginal discharge, lower abdominal pain. All the patients had no history of CIN, cervical cancer, pelvis radiation therapy, total hysterectomy and without pregnancy. Three days before collecting samples, patients did not have vaginal irrigation and drug use. Normal cervical tissue was obtained from the uterine cervix for excision of uterine leiomyoma. All of the patients were not treated with radiotherapy and chemotherapy before undergoing operation. All patients were operated by a skilled clinician. Part of the tissue was used for pathological diagnosis, and the remaining part was frozen in liquid nitrogen for the extraction of mRNA. Consents were obtained from the patients before the experiment and informed consents were signed. General materials like gender and age in each group had no statistical difference and were comparable ($P > 0.05$).

2.2. Methods

2.2.1. Collection and preservation of the clinical samples

The appropriate amount of specimens of the lesion was taken and cleaned by saline for 3–5 times. After that, sample was transferred into the cryopreservation tube, frozen in liquid nitrogen for 30 min, and placed in refrigerator at −80 °C for further use.

2.2.2. Determination of HPV18 E6 and E7 mRNA

Reverse Transcription System (Promeg cat# A 3500) kits were used and strictly performed according to the Kit instructions for reverse transcription. RNA sample of 9.75 μL (1 μg) was added, and after sufficient mixing of reaction system (20 μL), centrifugation was used. The reaction condition of the sample was: 42 °C, 60 min, 99 °C, after 5 min the reaction was ended, and the outcome was cooling at 4 °C and placed at −20 °C for further use. Primer sequence of gene HPV18 E6 was U: GCGCTTTGAGGATCCAACAC, D: ACGAATGGCACT GGCCTCTA, with the amplified length of 415 bp; Primer sequence of gene HPV18 E7 was U: AAGAAAACGAT-GAAATAGATGGA, D: GGCTTCACACTTACAACACA, with the amplified length of 100 bp. The PCR reaction system was as follows: 2.5 μL 10 × PCR buffer, 0.5 μL dNTPs (10 mmol/L), 25 mmol/L $MgCl_2$ 0.8 μL, 0.1 μL Taq enzyme (5 U/μL), primer F (10 μg/L) 0.5 mL, 0.5 μL primer R (10 μg/L), probe (10 μg/L) 1 μL, 5 μL cDNA, adding ddH_2O to 25 μL system. After centrifugation for 10 s at low temperature, PCR was carried out, with the apparatus selected of Roche Light Cycler fluorescence PCR Thermal cyclers. The reaction temperature transmission rate of each step was set to 20 °C/s, and each cycle of fluorescence was detected at 62 °C of 40 s. A negative control was used to monitor the experimental contamination. Positive control was used to monitor if RNA was extracted successfully. The ratio of E6, E7 expression and internal reference β-actin expression was regarded to its relative expression.

2.2.3. Detection method of the target gene

Tissues of cervical cancer, cervical erosion and cervical HPV infection were collected and Trizol lysate was added to grind the tissues sufficiently. Then RNA was reverse transcribed into cDNA using a reverse transcription kit. cDNA sample was taken to perform fluorescent quantitation PCR and amplified the target gene TRAF6, c-FLIP, D44v6, MMP9, DEC-1, IKK16, MBP-1, β-catenin, Vimentin, E2F1, Bmi-1 and internal reference gene GAPDH, respectively. After obtaining amplification curve, the mRNA contents of the above-mentioned genes were calculated with GAPDH as the internal reference.

Table 1
Comparison of HPV18 E6, E7 mRNA and invasion gene expression in each group.

Group	n	HPV-18 E6 mRNA	HPV-18 E7 mRNA	TRAF6	c-FLIP
Cancerous group	58	3.76 ± 0.31	2.89 ± 0.21	2.32 ± 0.31	2.17 ± 0.24
Non-cancerous group	61	0.20 ± 0.04*	0.19 ± 0.03*	0.61 ± 0.07*	0.59 ± 0.16*
Control group	60	0.24 ± 0.04*	0.23 ± 0.02*	0.46 ± 0.04*	0.44 ± 0.06*

($\bar{x} \pm s$).
Note: Compared with cancerous group, *$P < 0.05$.