Expressions of NF-κBp50, p53 and Bcl-2 in cervical cancer and their relationship with human papillomavirus infection*

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Abstract  Objective: To explore the relationship between expressions of NF-κBp50, p53 and Bcl-2 in tissue of cervical cancer and human papillomavirus (HPV) infection. Methods: The expressions of NF-κBp50, p53 and Bcl-2 were detected using immunohistochemical staining in 46 specimens of cervical cancer and 26 specimens of normal cervical tissue. The infection of HPV DNA were determined by PCR. Results: The expressions of NF-κBp50, p53 and Bcl-2 in tissue of cervical cancer were significantly higher than that in normal cervical tissue (P<0.01), and the expressions of NF-κBp50 and p53 or Bcl-2 were closely related (P<0.05). The expression of NF-κBp50 in HPV DNA positive group was significantly higher than that in HPV negative group (P<0.05), but there were no significantly differences in the expressions of p53 and Bcl-2 between HPV DNA positive group and HPV negative group (P>0.05). Conclusion: The expressions of NF-κBp50, p53 and Bcl-2 were significantly correlated with cervical carcinogenesis. NF-κBp50 may be activated by HPV infection.

Key words cervical cancer; NF-κBp50; p53; Bcl-2; HPV

NF-κB is composed of homo- and heterodimers of five members of the Rel family including NF-κB 1(p50), NF-κB 2 (p52), RelA (p65), RelB, and c-Rel (Rel), which could regulate several vital proteins’ expression and control apoptosis, cell proliferation, differentiation, etc. NF-κB is sequestered inactively in the cytoplasm of most cell types by inhibitory proteins. It was reported that the NF-κB transcription factor could be activated by some virus infection and involved in the development or progression of human cancers1-3. The development of human cervical cancer was strongly associated with human papillomavirus infections, especially types 16 and 18, together with the abnormal expression of apoptotic associated genes4. However, the expression of major apoptotic associated genes, including NF-κBp50, p53 and Bcl-2, in tissue of cervical cancer and their relationship with the infection of HPV are still unclear. In this research we adopted the immunohistochemistry technique to study the expression of NF-κBp50, p53 and Bcl-2 in tissue of cervical cancer and their relationship with the infection of HPV.

Materials and methods

Patients

Forty-six patients with primary cervical cancer were included in the study. The median age was 51 years (range 34–70 years). Twenty-six hysteromyoma patients were included as the control group with the median age of 49 years (range 35–70 years). All the patients were evaluated at the Department of Obstetrics and Gynecology of the 1st and 2nd affiliated Hospital of Wenzhou Medical College. The diagnosis of the specimens were confirmed by pathological examination. The histopathologic features of the surgical specimens were classified according to the World Health Organization criteria. There were 10, 7, 25 and 4 samples of the carcinoma in situ, grade I, grade II and grade III respectively.

Immunohistochemistry

The tissue specimens were fixed with 10% buffered formalin, embedded in paraffin, and sectioned serially to 4 μm slices. The immunohistochemistry kit was purchased from Beijing Zhongshan Biotechnology Co., Ltd (China). Immunohistochemistry (IHC) SP techniques were adopted to detect the expressions of NF-κBp50 (polyclone 1:50, Santa Cruze, USA), p53 (monoclonal 1:50, Santa Cruze, USA) and Bcl-2 (monoclonal 1:50, Santa Cruze, USA) according to the manufactory’s guide.

The staining evaluation was assessed through estimating the fraction of positive cells out of 200 tumour cells. All the slides were assessed twice by computer. The expressions were graded as following: −, 0%–5%; +, 6%–20%; ++, 21%–50%; ++++, >50%.

PCR detection

The presence of HPV DNA was determined by PCR. The general PCR kit was purchased from the Molecular Patho-
logical Laboratory, School of Medicine, Beijing University, China. The HPV DNA typing kit was purchased from Shanghai Jingmei Biotech Company, China. The primer pairs were kindly donated by Dr. Jian Zhou, Medical Research Center, Loyola University, USA. For PCR, 35 cycles were used at 95 °C for 30 s, 55 °C for 45 s, and 72 °C for 1 min. Each amplification mixture was subjected to electrophoresis on 1.5% agarose gel and visualized by ethidium bromide staining.

Statistical analysis
Using SPSS 10.0 software, the statistical data were analyzed by Chi-square test, Fisher exact probabilities and Spearman rank correlation.

Results
The expressions of NF-κBp50, p53 and Bcl-2 in tissue of cervical cancer and normal cervical tissue
The results were shown in Table 1.

The correlations of the expressions of NF-κBp50 and p53, Bcl-2 in cervical cancer tissue
After grading the expression levels of NF-κBp50, p53, and Bcl-2 in 46 cervical cancer tissue, their correlations were assessed with Spearman Analysis Method. It was shown that NF-κBp50 and p53, NF-κBp50 and Bcl-2 had significantly correlations with each other ($P<0.05$) while p53 and Bcl-2 had no significantly correlation ($P>0.05$, Table 2, 3).

The relationship between pathological grade and expression levels of NF-κBp50, p53 and Bcl-2 in cervical cancer tissue
The expression of NF-κBp50 was significantly different between the carcinoma in situ (CIS) and squamous carcinoma ($P<0.05$), while not the case with the expressions of p53 and Bcl-2 (Table 4).

Detection of HPV DNA in the cervical cancer tissue and normal cervical tissue
30 samples were randomly selected from the cervical cancer group for HPV DNA detection. 16/30 (53.3%) were HPV positive, 13 (43.3%) samples were HPV 16/18 positive and none (0%) was HPV 6/11 positive. Among 26 normal cervical samples, there were 5 samples (19.2%) with HPV positive. One sample (3.8%) was HPV 16/18 positive and none (0%) was HPV 6/11 positive (Fig. 1). The detection rate of HPV 16/18 was significantly different between the cervical cancer group and the normal group ($P<0.01$).

The correlations between the expressions of NF-κBp50, p53, and Bcl-2 and HPV infection
The HPV infection had correlation with the expression of NF-κBp50 ($P<0.05$), but not correlations with the expressions of p53 and Bcl-2 (Table 5).