Study on the Expression and Significance of P16 Gene Methylation Based on Information Technology Statistics in Female Cervical Cancer in Hunan Province

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Abstract. To explore the significance of p16 gene methylation and abnormal expression in cervical cancer, Methylation specific PCR (MSP) was used to detect 5’ CpG island methylation of p16 gene promoter in 60 cervical cancer tissues of different pathological types and clinical stages, who was chosen in Human province. Detection of homozygous deletion of exon 1 (E1) and exon 2 (E2) of p16 gene by PCR. Immunohistochemical analysis of p16 protein expression. The results showed that there was no methylation of p16 gene in normal control tissues and paracancerous tissues and no E1 and E2 deletions and abnormal p16 protein expression were found. The methylation rate of 60 cervical cancer samples was 21.67% (13 / 60), the deletion rate of p16 gene was 15.00% (9 / 60), and the expression rate of p16 protein was 51.67% (31 / 60). It can be seen that the positive expression rate of p16 gene protein decreased with the increase of clinical stage. The results suggest that p16 gene inactivation is common in cervical cancer and closely related to the pathological grade. p16 gene methylation plays a role in the development of cervical cancer.

Keywords: P16 Gene Methylation, Cervical Cancer, Hunan Province

1. Introduction
P16, as a tumor suppressor gene, frequently occurs heterozygous deletion, homozygous deletion, mutation and methylation in a variety of tumors and cell lines [1]. Previously, it was thought that there were two ways to inactivate tumor suppressor genes: gene mutation and chromosomal material loss. It has been proved that DNA methylation is the third mechanism of tumor suppressor gene inactivation . DNA methylation can occur before cell malignant transformation. Therefore, the detection of gene promoter methylation is helpful for the early detection of cancer prone cells, as well as for the prediction of response to chemotherapy and prognosis [2]. The study of p16 gene has become one of
the hot spots in tumor molecular biology and molecular genetics. This study shows that p16 inactivation exists in a variety of human malignant tumors [3]. However, there are few studies on the abnormal expression of p16 gene and the methylation status of 5′ CpG island in the process of cervical cancer, especially the comprehensive analysis of the relationship between p16 gene abnormal expression and pathological types, clinical stages, and the understanding of these changes and interactions is still incomplete.

2. Materials and methods

2.1. Research object

60 cases of cervical cancer and its adjacent tissue samples were from hospital in Hunan province. According to the standards proposed by FIGO in 1988, pathological grades are divided into G1, G2 and G3 grades. The clinical stages were divided into stages I, II, III and IV according to the new stage method published by FIGO in 1989[4]. Among them, 30 were paraffin embedded tissue specimens, 30 were fresh surgically excised specimens (fresh tissue specimens were quickly preserved in -70 °C refrigerator for DNA extraction) [5]. 10 normal cervical tissues of corresponding age were selected as negative control.

2.2. Extract DNA

Using DNA extraction kit, the integrity of the extracted genomic DNA was checked by 1.5% agarose electrophoresis, and by UV spectrophotometer, a260 / A280 (a is absorbance, formerly known as optical density OD) were all between 1.5 and 1.9.

2.3. Methylation of p16 gene in cervical cancer

(1) DNA bisulfite modification

Take 2 μg of distilled water of template DNA and dilute to 50 μL, add 5.5 μL NaOH solution with a new configuration of 2 mol / L (make the final concentration of 0.3mol / L), incubate it at 37 °C for 20 min. Add 30 μL 10mmol / L hydroquinone, and see the solution is yellow. Add 520 μl newly prepared 3 mol / L sodium bisulfite (sigma s-8890). After fully mixing, centrifuge and cover with mineral oil. Incubate at 50 °C for 16 h. The next day, the modified DNA was purified with wizard DNA clean up system (Promega a7280) [6]. The purified DNA was stored at 4 °C or -20 °C. 5.5 μl of 3 mol / L NaOH was added into each centrifuge tube and incubated at room temperature for 15 min. Add 33 μl of 10 mol / L ammonia acetate for neutralization, add 3 volumes of ethanol to precipitate DNA (-20 °C overnight, shake for 30 min), wash with 70% ethanol, dry, and suspend with 20 μL water. -20 °C frozen storage (avoid repeated freezing and thawing).

(2) PCR amplification of specific methylated and unmethylated fragments

Reaction mixture 50 μL system: 10 × PCR buffer, 5 μL; 25 mmol / L dNTP, 2.5 μL; forward primer P1 (p16-mf or p16-uf), 1 μL; reverse primer P2 (p16-mr or p16-ur), 1 μL; sterilized distilled water, 28.5 μL; modified DNA, 2 μL; mineral oil, 25-50 μL; 2.5 u Taq enzyme, 10 μL water, added to each sample after hot start. The amplification conditions were 95 °C, 5 min; 95 °C, 30 s; 55 °C, 30 s; 72 °C, 30 s, 35 cycles; 72 °C, 4 min. 4 °C for analysis.1.5
2.4. Expression of p16 protein in cervical cancer

(1) Experimental method

In this experiment, p16 protein was determined by S-P method [7-8]. In the experiment, p16 gene monoclonal antibody, S-P hypersensitive kit was purchased from Fuzhou Maixin biotechnology development company, and other reagents and tools were obtained from the pathology department of our hospital.

(2) Decision result

A: Score according to the color of cytoplasm or nucleus in the section. The nucleus or cytoplasm showed no color, 0 point; the cytoplasm or nucleus showed light yellow, 1 point; the cytoplasm or nucleus showed brown yellow, 2 points. B: according to the percentage of color development in sections, no color development is found, 0 point; color development ≤ 30%, 1 point; color development in 30% - 60%, 2 points; color development > 60%, 3 points [9]. The slice integral of each case was a × B. According to the high and low scores, the scores are "–" negative; the scores < 2 are "+" (weak positive); the scores 2-4 are "++" (positive); the scores > 4 are "+++" (high expression). The percentage of "++", "+++", and "++", the percentage of "+", or "–", the total number of cases were decreased or no expression.

3. Result

In this paper, chi square test (χ²) was used for statistical analysis, the results are as follows.

3.1. Detection of methylation of p16 gene in cervical cancer

Methylation of p16 promoter region was not detected in 10 normal cervical tissues and 60 paracancerous tissues (Fig. 1). Methylation was detected in 13 of 60 cases of cervical cancer, accounting for 21.67% (13 / 60), which was not related to clinical pathological grade and pathological type. Among them, there were 6 cases in stage I-Ⅱ, accounting for 25.00% (6 / 24), 7 cases in stage III-Ⅳ, accounting for 19.44% (7 / 36). There was no significant difference between the two (P > 0.05) (Table 2).

![Figure 1](image_url)

**Figure 1** MSP of p16 gene in cervical carcinoma

| Clinical stages | Number | Number of methylation | Percentage/% |
|-----------------|--------|-----------------------|--------------|
| I ~ II          | 24     | 6                     | 25.00        |
| III ~ IV        | 36     | 7                     | 19.44        |

**Table 1. Significance of methylation of p16 gene in cervical carcinoma**
3.2. Expression of p16 protein in cervical cancer

The positive rate of p16 protein expression in 60 cases of cervical cancer was 48.33% (29 / 60) (+ + - + +). The decreased or no expression of p16 protein (+ or -) was 51.67% (31 / 60), of which 79.17% (19 / 24) was positive in stage I-II cases, 20.83% (5 / 24) was negative in stage III-IV cases, 27.78% (10 / 36) was positive in stage III-IV cases, 72.22% (26 / 36) was positive in stage III-IV cases, 72.22% (26 / 36).

4. Discussion

Cervical cancer is a serious threat to women's lives. At present, some molecular biological methods lack specific indicators for its occurrence, development, prognosis and metastasis. P16 gene deletion, mutation and protein expression in cervical cancer. However, there are few reports on the methylation of CpG island in the 5' promoter region of p16 gene, especially in the study of cervical cancer by methylation specific PCR (MSP). From the basic research and clinical observation two aspects of comprehensive analysis of its incidence of the report is also rare.

The methylation of p16 gene and the expression of p16 protein were studied in 60 cases of cervical cancer of different stages and pathological types, who was choses in Hunan province. The results showed that p16 gene inactivation was common in cervical cancer and closely related to pathological grade. P16 gene methylation plays a role in cervical cancer. This research conclusion can be applied to the screening of cervical cancer in women. It is of great significance for early diagnosis of cervical cancer to detect the occurrence and high incidence factors of cervical cancer through abnormal methylation state detection.

In this study, MSP method was first used in cervical cancer research in China. Paraffin section and fresh operation were selected to study the CpG island methylation of p16 gene 5' promoter region. It can be seen that no p16 gene promoter region methylation was detected in 10 normal cervical tissues and 60 adjacent tissues. In 60 cases of cervical cancer, there were 13 cases of methylation, accounting for 21.67% (13 / 60), 6 cases of stage I-II, accounting for 25.00% (6 / 24), 7 cases of stage III-IV, accounting for 19.44% (7 / 36). There was no significant difference (P > 0.05). It also suggested that p16 gene methylation might be an early change of cervical cancer, which had no significant relationship with clinical stage. The results are consistent with those detected by the method of Southern blot hybridization after digestion with methylation sensitive restriction enzyme. In this study, the expression of p16 protein was detected by immunohistochemistry in 60 cases of cervical cancer. The results showed that the expression rate of p16 protein was significantly correlated with the clinical stage of cervical cancer, and the expression rate of p16 protein in stage III - IV was significantly lower than that in stage I - II.

The results showed that the methylation rate of p16 gene was 21.67% (13 / 60) in 60 cases of cervical cancer in Hunan Province. Because of the specificity of p16 gene acting on CDK4, the gene was small and easy to operate, so it gradually became one of the target genes for gene therapy. Therefore, it is of great significance for further gene therapy in Hunan Province and even the whole country to explore the changes of p16 gene in clinical tumors.
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