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ORIGINAL ARTICLE

Expression and clinical significance of MCM5 and P16 in hyperplastic disease of the cervix

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Abstract

Objective The aim of the study was to study the expression and clinical significance of MCM5 and P16 in cervical intraepithelial neoplasia (CIN) and cervical cancer.

Methods The immunohistochemistry S-P method was used to detect the expression of MCM5 and P16 in 100 cases of cervical CIN and cancer.

Results The positive expression rates of MCM5 and P16 in normal cervical epithelium, CIN I, CIN II, CIN III, and cervical invasive cancer were 6.7%, 35.0%, 40.0%, 70.0%, and 97.5%, and 6.7%, 30.0%, 45.0%, 75.0%, and 100%, respectively. The positive expression rates of MCM5 and P16 significantly differed between CIN and infiltrating carcinoma ($P < 0.05$). The expression levels of MCM5 and P16 positively correlated in cervical cancer ($P < 0.01$). The positive expression of MCM5 and P16 closely correlated with the clinical stage and pathological grade of cervical cancer ($P < 0.05$).

Conclusion MCM5 and P16 might be used as markers for aggressive biological behavior in cervical cancer and might be useful for distinguishing CIN and cervical cancer.

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Key words: cervical cancer; P16; MCM5; immunohistochemistry

Cervical proliferative lesions, including cervical intraepithelial neoplasia (CIN) and cervical cancer, are common diseases that are harmful to women's health. CIN is a precancerous lesion of cervical cancer, and the basis of cervical cancer, and there have been many patients with cervical cancer cervical and intraepithelial neoplasia. Therefore, it is of considerable clinical significance to achieve a differential diagnosis of CIN and cervical cancer. Recently, MCM5 has been used as a new indicator of cell proliferation activity, and related studies have shown that the sensitivity of MCM5 was higher than that of Ki67 in the identification of benign and malignant lesions [1]. As a tumor suppressor gene, P16 directly participates in cell cycle regulation, and recently, P16 has frequently been used in the detection of cervical lesions. In this study, a immunohistochemical technique was used to detect the expression of MCM5 and P16 in normal cervical mucosa, CIN, and invasive carcinoma, to explore the clinical and pathological significance of these biomarkers.

Materials and methods

Materials and reagents

A total of 100 cases of cervical biopsies and surgical resected specimens were collected from the Sixth People's Hospital of Chongqing (China) and the First Affiliated Hospital of Medical University of Chongqing (China), during the past decade (2006–2016). Our study included 20 CIN I, 20 CIN II, 20 CIN III, and 40 invasive carcinoma cases, all of which were confirmed by postoperative pathological examinations. The ages of the 40 patients with invasive cancer ranged from 38 to 71 years, with an average age of 49.5 years. The pathological grades were as follows: grade G1, nine cases; grade G2, 24 cases; and G3 grade, seven cases. Clinical staging was carried out according to the staging criteria proposed by the International Union of Obstetrics and gynecology (FIGO): phase Ia, six cases; Ib, 15 cases; IIa, 10 cases; IIb, nine cases. In addition, 30 cases of normal cervical epithelial tissue were used as controls. The mouse anti-human MCM5 monoclonal antibody (CRCT5) was purchased from Abcam biology (Shanghai, China), and

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the mouse anti-human P16 monoclonal antibody (16P04/JC2) and the streptavidin-perosidase (SP) kit were purchased from Zhongshan biology (Beijing, China). The diaminobenzidine (DAB) kit was also purchased from Zhongshan biological.

Methods

Immunohistochemical staining was performed using the SP method. After paraffin section dewaxing, hydration, and microwave antigen repair, the peroxidase blocking agent was used to inactivate endogenous peroxidase activity. Sheep serum working fluid was used for closure and added four times, with a dose of anti-oxidant, overnight. We next added two biotin-labeled anti-streptavidin, followed streptavidin peroxidase solution. We then performed DAB coloration and hematoxylin re-staining. For a positive control, we used the tissue section from a tissue that was known to be positive, and phosphate buffer saline (PBS) was used instead as the negative control.

Microscopy

Under the microscope, the nuclei of MCM5- and P16-positive cells were found to be brown and yellow. The positive cases were quantified as follows: Five fields of vision were observed in each slice, and 100 tumor cells were counted in each field of vision. Negative (–): no brown staining or < 10% staining of positive cells. Weak positive (+): the number of positive cells ranged from 10% to 25%. Moderately positive (++): the number of positive cells ranged from 25% to 50%. Strong positive (+++): the number of positive cells was greater than 50%. The number of positive cells was greater than 10% in all positive cases.

Statistical analysis

The SPSS12.0 statistical software was used for χ^2 test and Spearman level correlation analysis.

Results

MCM5 expression in CIN and cervical cancer

The positive expression of MCM5 was brown in color and mainly located in the nucleus (Fig. 1). The positive rate of MCM5 expression gradually increased, from normal cervical epithelium, CIN, to cervical cancer (Table 1; $P < 0.05$). The expression rate of MCM5 in cervical cancer was significantly higher than in normal cervical epithelium and CIN, and the difference was statisticaly significant ($P < 0.05$). However, the expression of MCM5 was not significantly different between CIN I and CIN II ($P > 0.05$).

P16 expression in CIN and cervical cancer

The positive expression of P16 was brown and yellow in color, and was located predominantly in the nucleus and partly in the cytoplasm (Fig. 2). The positive rate of P16 expression gradually increased, from normal cervical epithelium, CIN, to cervical cancer, and the differences were statistically significant ($P < 0.05$). However, there was no difference in P16 expression between CIN I and CIN II ($P > 0.05$).

The clinicopathological relationship and correlation between MCM5 and p16 expression in cervical cancer

There was no significant correlation between the expression of MCM5 and P16 and the age of the patients with cervical carcinoma ($P > 0.05$), but MCM5 expression was related to cervical carcinoma clinical stage and pathological grade. The number of stage IIa–IIb cervical cancers was significantly higher than Ia to Ib ($P < 0.05$), and the number of pathological grades G2 and G3 were significantly higher than that of G1 ($P < 0.05$). However there were no significant differences in MCM5 expression between grades G2 and G3 (Table 2). The positive expression rates of MCM5 and P16 in cervical cancer tissues were 97.5% and 100%, respectively, and the ratios in the "++" cohort were 70% and 75%, respectively. With the development of cervical disease, the positive rates

Table 1 Expression of MCM5 and p16 in CIN and cervical invasive cancer

	MCM5								P16							
Type	*n*	–	+	++	+++	Positive (%)	> ++ (%)	*P*	*n*	–	+	++	+++	Positive (%)	> ++ (%)	*P*
Normal	30	28	2	0	0	6.7	0		30	28	2	0	0	6.7	0	
CINI	20	13	4	2	1	35.0	15.0	0.019[a]	20	14	4	1	1	30.0	10.0	0.018[a]
CINII	20	12	4	2	2	40.0	20.0	0.185[b]	20	11	5	2	2	45.0	20.0	0.197[b]
CINIII	20	6	4	6	4	70.0	50.0	0.028[c]	20	5	4	6	5	75.0	55.0	0.021[c]
Cancer	40	1	11	13	15	97.5	70.0	0.031[d]	40	0	10	14	16	100.0	75.0	0.033[d]

[a] $P < 0.05$, vs normal group; [b] $P > 0.05$, vs CINI group; [c] $P < 0.05$ vs CINII group; [d] $P < 0.05$ vs CINIII group

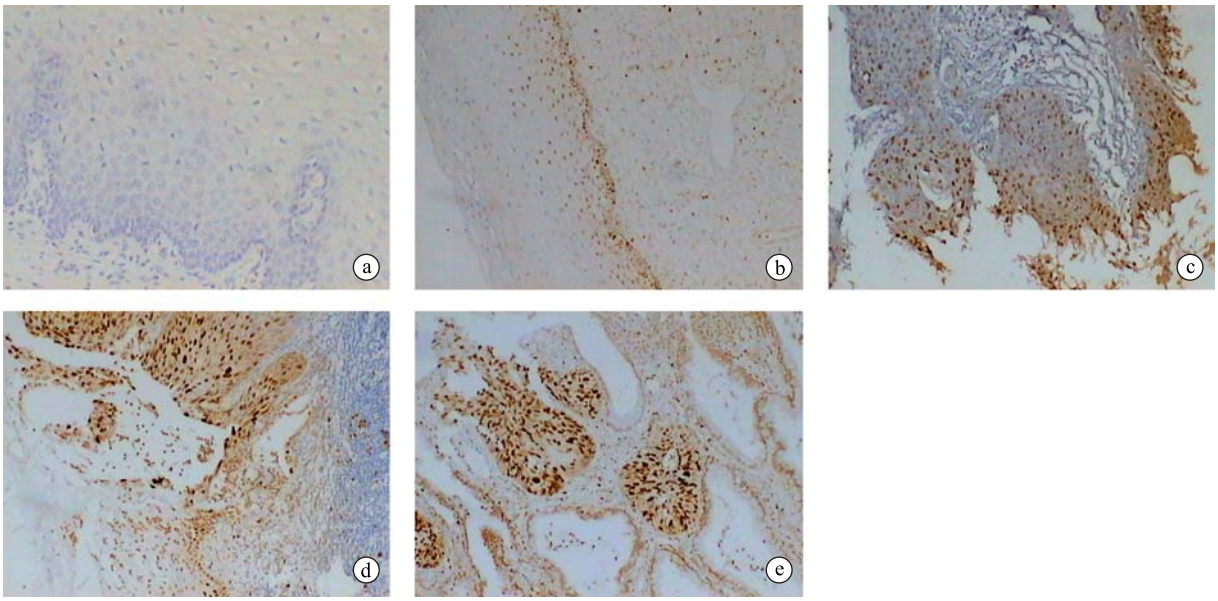

Fig. 1 MCM5 positive expression in CIN and cancer. (a) Negative expression in normal epithelium (SP x 200); (b) MCM5 positive expression in CIN I (SP x 200); (c) MCM5 positive expression in CIN II (SP x 200); (d) MCM5 positive expression in CIN III (SP x 200); (e) MCM5 positive expression in cervical cancer (SP x 200)

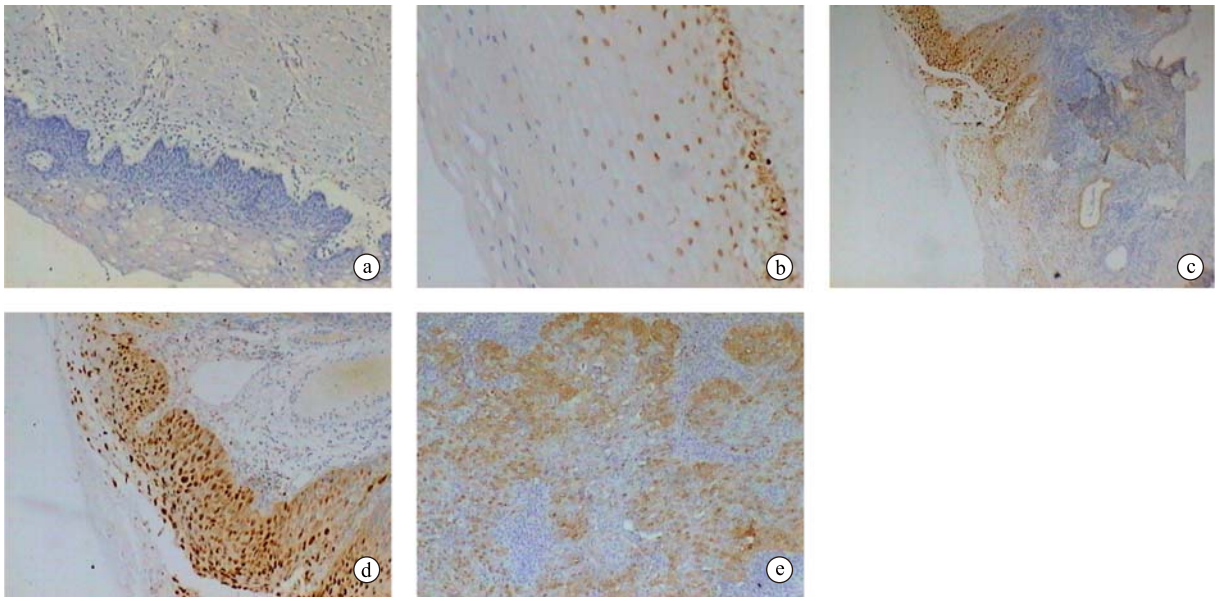

Fig. 2 P16 positive expression in CIN and cancer. (a) Negative expression in normal epithelium (SP x 200); (b) P16 positive expression in CIN I (SP x 200); (c) P16 positive expression in CINII (SP x 200); (d) P16 positive expression in CIN III (SP x 200); (e) P16 positive expression in cervical cancer (SP x 200)

of both MCM5 and P16 gradually increased, and the expression levels showed a positive correlation (Table 3; $r = 0.864$, $P < 0.01$).

Discussion

The abnormal regulation of cell proliferation is closely related to tumor occurrence, and the loss of control of any part of the cell cycle could lead to abnormal cell proliferation. MCM5, a member of the MCM protein

Table 2 The clinicpathology relation between MCM5 and P16 in cervical cancer

Type	MCM5						P16					
	n	–	+	++–+++	> ++ (%)	*P*	*n*	–	+	++–+++	> ++ (%)	*P*
Age (years)												
< 40	13	0	5	8	61.5		13	0	4	9	69.2	
> 40	27	1	6	20	74.1	0.316[a]	27	0	6	21	77.8	0.273[a]
Grade												
G1	8	1	4	3	37.5		8	0	5	3	37.5	
G2	25	0	6	19	76.0	0.022[b]	25	0	4	21	84.0	0.017[b]
G3	7	0	1	6	85.7		7	0	1	6	85.7	
Stage												
Ia–Ib	21	1	10	10	47.6		21	0	9	12	57.1	
IIa–IIb	19	0	1	18	94.7	0.029[c]	19	0	1	18	94.7	0.032[c]

[a] $P > 0.05$, vs age < 40 group; [b] $P < 0.05$ vs G1 group; [c] $P < 0.05$ vs Ia–Ib group

Table 3 The correlation of MCM5 and P16 in cervical cancer

MCM5	P16			
	–	+	++	+++
–	0	1	0	0
+	0	3	5	3
++	0	4	7	2
+++	0	2	2	11

family, is an essential for DNA replication in eukaryotes. MCM5 is rarely expressed in the rest period, but increases in the G1 phase, and reaches its peak in the S phase of the cell cycle. It has been shown to be an important factor that is closely related to cell proliferation [2]. Some scholars have discovered that MCM5 is strongly positive in esophageal cancer and negative in normal esophageal mucosa [3]. Investigations of genitourinary tumors have also found that MCM5 expression was very weak in normal epithelium, yet strong in tumor tissues [4]. All of these studies suggest that MCM5 could reflect the proliferation of tumor cells. In this study, the expression of MCM5 was investigated in 130 cases of normal cervical mucosa epithelium, CIN, and cervical cancer. Our results showed that MCM5 expression was barely detectable in the normal epithelium of the cervix, and weakly positive expression was observed in the first and second basal cell layers. From CIN to cancer, the number of MCM5-positive cells increased, and the MCM5-positive cell distributions also increased significantly. The clinical stage II cases had significantly higher numbers of MCM5-positive cells than clinical stage I cases, and pathological grades G2 and G3 had significantly higher rates of MCM5-positive cells than the G1 grade. The expression patterns of MCM5 suggest that cells entering the proliferative cycle increased gradually, from normal cervical epithelial to atypical hyperplasia and invasive carcinoma. Compared with traditional cell proliferation markers, PCNA and Ki67, MCM5 participates in chromosome DNA replication earlier and has a greater involvement in mitosis [5]. The detection of MCM5 could also reflect the activity of the MCM protein family. Therefore, the expression of MCM5 could reflect the proliferation activity of cervical cells in different proliferative stages and could be used as a specific biomarker for cervical cancer cell proliferation [6].

P16 is another important tumor suppressor gene that has recently been discovered, after P53, and its tumor suppressor effect might be more direct than that of P53 [7]. The P16 gene is located on the short arm of human chromosome 9 (9q21), with a total length of 8.5 kb, is divided into three exons and two introns, and encodes a cell cycle-dependent kinase 4 (INK4A). The P16 gene negatively regulates mitosis and prevents the G1 to S phase transition, which subsequently inhibits cell division and proliferation [8]. If the P16 gene acquires a loss of function mutation, such a mutation could cause the cell to expand and grow indefinitely, and eventually acquire a tumor phenotype. Therefore, some tumors have shown a loss or inactivation of P16 expression [9].

In this experiment, P16 expression was barely or weakly detected in normal cervical epithelium, while the positive rates, from CIN I to CIN III to invasive carcinoma, were 30%, 45%, 75%, and 100%, respectively. While P16 is a tumor suppressor gene, it was found to be overexpressed in our study. This finding is similar to that of another tumor suppressor gene, p53, which is often mutated and overexpressed in various tumors. We speculate that the reason for this might be due to the clinical association of cervical tumors with HPV infection [10]. Currently, we know that high risk HPV is a major factor in determining cervical intraepithelial lesions, and even cancer [11]. The E6, and especially E7, HPV proteins might compete with the cell cycle regulator protein, PRb [12–13]. Simultaneously, the transcription factor E2F is activated and P16 is blocked by CDK4 / CDK6 [14]. The disrupted the feedback loop between PRb and the P16 tumor suppressor results in Rb gene inactivation and the

overexpression of P16 [15–16].

In summary, both MCM5 and P16 are good indicators of early cervical intraepithelial neoplasia. There was a significant difference in the expression of MCM5 and P16 between normal cervical tissue, CIN, and cervical cancer, but there was no significant difference between CIN I and CIN II. This might be because both CIN I and CIN II have middle and low levels of tumorigenicity, their cell proliferation activities are not very high, and their differences are not large. Therefore, the clinical significance of the differential diagnosis between CIN I and CIN II at middle and low levels is not obvious. Furthermore, the results of our study show that the expression of MCM5 and P16 in cervical cancer is highly correlated. The combined detection of these two genes might help uncover the malignant potential of CIN and determine the degree of cervical intraepithelial neoplasia, which could facilitate diagnosis, grading, and prognosis when using the CIN index. Therefore, the immunohistochemical detection of P16 and MCM5 could be used as an important marker for cervical cancer identification, in order to improve the accuracy of cervical lesion diagnosis and provide useful guidance and assistance for clinical staging and treatment.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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