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A research on the protein expression of p53, p16, and MDM2 in endometriosis

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Abstract

This study aims to examine the expression of p53, p16, and murine double minute 2 (MDM2) protein in normal endometrium and endometriosis, in order to discuss the role of p53, p16, and MDM2 protein and apoptosis in the pathogenesis and development of endometriosis, and provide a theoretical basis for clinical diagnosis and treatment.

The immunohistochemical streptavidin-biotin peroxidase method was used to detect the expression of p53, p16, and MDM2 in tissue samples obtained from 30 women with pathologically confirmed ovarian endometriosis and 29 women with pathologically confirmed normal endometrium. The relationship between p53, p16, and MDM2 expression and apoptosis was analyzed.

In normal endometrium, the positive rate of p53 in the secretory phase was higher than that in the proliferative phase ($P < .05$). Furthermore, the positive rate of p53 in normal endometrium was higher than that in ovarian endometriosis ($P < .05$). There was a significant difference between normal endometrium and ovarian endometriosis.

The positive rate of p16 in normal endometrium was higher than that in ovarian endometriosis ($P < .05$). Furthermore, there was a significant difference between normal endometrium and ovarian endometriosis. The positive rate of MDM2 in normal endometrium was lower than that in ovarian endometriosis ($P < .05$).

In ovarian endometriosis, the expression of p53 and p16 was positively correlated with each other ($r = 0.611$, $P < .01$). However, the expression of p53 and MDM2 was negatively correlated with each other ($r = -0.541$, $P < .01$). Furthermore, the expression of p16 and MDM2 might not be relevant in the endometriosis ($r = 0.404$, $P > .05$).

As important apoptosis regulatory genes, p53, p16, and MDM2 might be involved in the pathogenesis and development of endometriosis.

Abbreviations: EMs = endometriosis, MDM2 = murine double minute 2, SP = streptavidin-biotin peroxidase.

Keywords: endometriosis, endometrium, MDM2 protein, p16 protein, p53 protein

1. Introduction

Endometriosis (EMs) is a disease, in which tissue that normally grows inside of your uterus (endometrium) grows outside the uterus (not including the myometrium). EMs is a disease affected by multiple environmental factors and genetic factors, and presents with family aggregation. Regardless of drug treatment, surgical treatment, or surgery combined with drug treatment, the recurrence rate is high. There is a lack of ideal treatment for it. Although EMs is a benign disease, it presents with biological behaviors similar to malignant tumors, including invasiveness, distant metastasis, and dissemination.[1] Therefore, to explore the pathogenesis of EMs from the perspective of occurrence of malignant tumors has become a hot and difficult concern in the academic community.[2] As everyone knows, the occurrence and development of malignant tumors are closely correlated to the apoptosis regulation of tumor cells.[3] Therefore, research with the apoptosis regulation of EMs cells as the target may provide clues and evidence for the treatment of EMs.

Tumor suppressor factor p53 protein in tumor cells is a key apoptosis regulatory factor in human body. The role of its accumulation in the nucleus is to destroy DNA. Murine double minute 2 (MDM2) is an important regulatory gene for p53, its function is closely correlated top53.[4] p16 is a CDK inhibitory protein, competitively binds to CDK4 against CyelinDl, and plays a role in negative regulation of cell cycle. Therefore, p53, p16, and MDM2 proteins can regulate cell apoptosis, to control the growth of tumor cells. We assumed that since p53, p16, and MDM2 proteins could control the apoptosis of tumor cells, these proteins may play the roles in the regulation of cycle of EMs cells that have the biological behaviors similar to malignant tumors. Based on this assumption, immunohistochemistry was adopted.[5] The present experiment used immunohistochemistry to detect the expression of p53, p16, and MDM2 protein in normal endometrium and EMs. This would provide new insights and a theoretical basis for the early diagnosis, treatment and prognosis improvement, and prevention of EMs.[6]

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2. Data and methods

This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with approval from the Ethics Committee of our Hospital. Written informed consent was obtained from the participants.

2.1. Specimen source and grouping

The tissue samples of 30 patients who underwent operation and EMs was confirmed by pathological findings from August 2014 to May 2015 at the Hefei Hospital Affiliated to Medical University of Anhui were randomly selected.

Inclusion criteria:

(1) patients with EMs confirmed by pathology after operation;
(2) patients underwent surgery;
(3) patients with an age of 20 to 49 years old;
(4) patients who were not treated with hormone therapy in the past 6 months before the operation.

According to the menstrual cycle combined with H&E staining analysis of tissue sections, 19 patients were in the hyperplastic stage, and 11 patients were in the progestational stage.

Exclusion criteria:

(1) patients with medical complications, such as hypertension, diabetes, and thyroid dysfunction; the tissue samples of normal endometria from 29 patients with uterine fibroids who underwent operation in the same period, were randomly selected.

Inclusion criteria:

(1) patients in whom uterine leiomyoma was confirmed by pathology after the operation;
(2) patients underwent surgery;
(3) patients with an age of 20 to 49 years old;
(4) patients who were not treated with hormone therapy in the past 6 months before the operation.

Among these patients, 18 patients were in the hyperplastic stage, and 11 patients were in the progestational stage.

Exclusion criteria:

(1) patients with medical complications, such as hypertension, diabetes and thyroid dysfunction.

2.2. Main reagents

Streptavidin-biotin peroxidase (SP) kit (Catalog: SP-9000), mouse anti-human p53 monoclonal antibody (Catalog: ZM-0405), mouse anti-human p16 protein monoclonal antibody (Catalog: ZM-0205), and mouse anti-human MDM2 protein monoclonal antibody (Catalog: ZM-0405) were provided by Zhongshan Golden Bridge Biotechnology (Beijing, China).

2.3. Experiment method

All the tissue samples of patients included in the present study were fixed with 10% formaldehyde, paraffin-embedded and cut into 4-μm serial sections. Then, H&E and immunohistochemical staining were performed on the sections. Immunohistochemistry was performed according to kit instructions, and each specimen was confirmed by 2 professional pathologists after staining.

2.4. Positive results determination

Image-pro plus, developed by Media Cybernetics Co in America, was applied to do the imaging analysis. The positive staining of p53 was defined as a nucleus with yellow or clay bank particles. The positive staining of p16 and MDM2 protein was defined as a nucleus and (or) cytoplasm with yellow or clay bank particles. Under high magnification (200 unit), 10 visual fields were randomly selected, and 1000 cells were counted. According to the percentage of positive cells (0% = 0; <10% = 1; 10–50% = 2; 51–80% = 3; 80% = 4) and staining intensity (colorless = 0; pale yellow = 1; clay bank = 2; tan = 3), a comprehensive analysis and judgment was made. If both produced more than 3, this was regarded as a positive standard.

2.5. Statistical process

All data were obtained from the Excel database, and analyzed by SPSS 11.5. The distribution of p53, p16, and MDM2 protein in each group was calculated. The rate of these 2 groups was compared by the chi-square test, and the correlation of the distribution of p53, p16, and MDM2 protein was analyzed by Spearman nonparametric correlation analysis. $P < .05$ was considered statistically significant.

3. Results

3.1. Protein expression of p53

In normal endometrium tissues, the rate of p53 protein in 18 patients in proliferative phase was 16.67% and the rate of p53 protein in 11 patients in the secretory phase was 72.73%. The rate of p53 in the secretory phase was higher than that in the proliferative phase ($X^2 = 9.114$, $P = .003$). In EMs tissues, the rate of p53 protein in 19 patients in the proliferative phase was 10.53% and the rate of p53 protein in 11 patients in the secretory phase was 18.18%. The difference was not statistically significant ($X^2 = 0.353$, $P = .552$). As shown in Table 1, the positive rates of p53 protein in normal endometrium and EMs tissues were 37.93% and 13.33%, respectively. The rate of p53 protein in normal endometrium was higher than in EMs ($X^2 = 4.706$, $P = .03$). The rate of p53 protein for EMs is shown in Table 1 and Figure 1.

Table 1

Expression of p53, p16, and MDM2 in 2 groups.

Group	Number	P53			P16			MDM2		
		−	+	Positive rate (%)	−	+	Positive rate (%)	−	+	Positive rate (%)
Normal endometrium	29	18	11	37.93	20	9	31.03	28	1	0.03
Endometriosis	30	26	4	13.33	27	3	10.00	14	16	53.33

MDM2 = murine double minute 2.

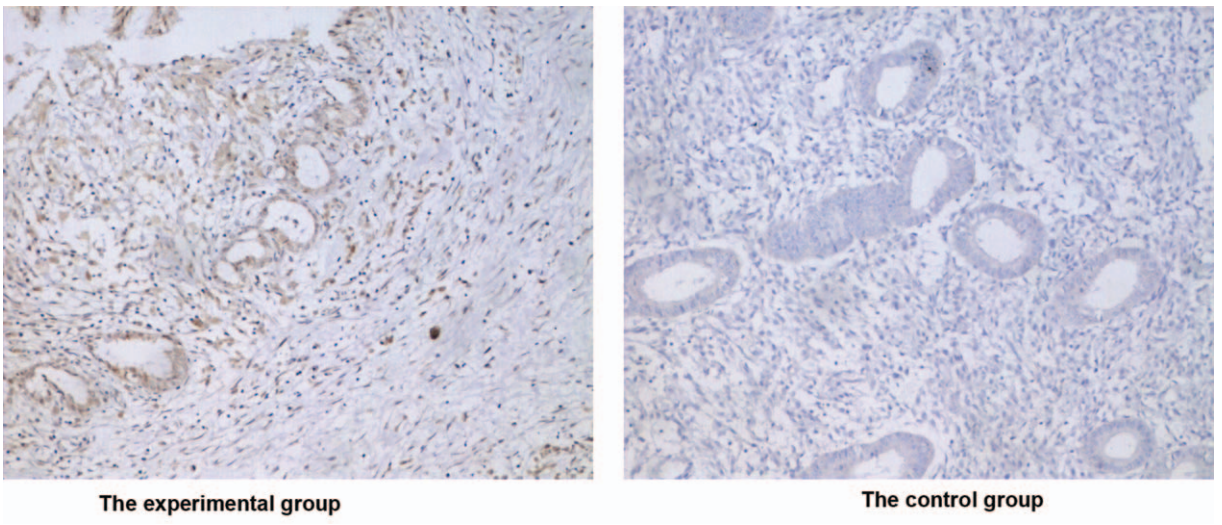

Figure 1. Expression of p53 protein in endometriosis (SP ×100). SP = streptavidin-biotin peroxidase.

3.2. Protein expression of p16

As shown in Table 1, the rates of p16 protein in normal endometrium and EMs tissues were 31.03% and 10%, respectively. The rate of p16 protein was higher in normal endometrium than in EMs ($X^2=4.027$, $P=.045$) (Fig. 2).

3.3. Protein expression of MDM2

As shown in Table 1, the rates of MDM2 protein in normal endometrium and EMs tissues were 0.03% and 53.33%, respectively. The rate of MDM2 protein was lower in normal endometrium than in EMs ($X^2=17.89$, $P=.000$) (Fig. 3).

3.4. The correlation analysis of p53, p16, and MDM2 in EMs

As shown in Tables 2–4, in EMs tissues obtained from 30 patients, when the rate of p53 increased, the rate of p16 decreased, and the rate of MDM2 increased. Hence, there was a positive correlation between p53 and p16 ($r=0.611$, $P<.01$) and a negative correlation between p53 and MDM2 ($r=-0.541$, $P<.01$). However, there was no correlation between p16 and MDM2 ($r=0.404$, $P>.05$).

4. Discussion

4.1. p53 and EMs

Among the genes that have been found to date, the p53 gene is the most prominent tumor suppressor gene in human cancers.[7] The p53 gene encodes nucleophosmin, which comprises of 393 amino acids. The molecular weight of p53, which is also known as the p53 protein located in nuclei 53×10^3 D, acts as a transcription factor by binding to defined DNA targets. The p53 gene and protein are regulators of cell division in terms of cell cycle regulation, cell growth, and apoptosis.[8] The stable expression of p53 is vital for the performance of its function, and p53 activity is

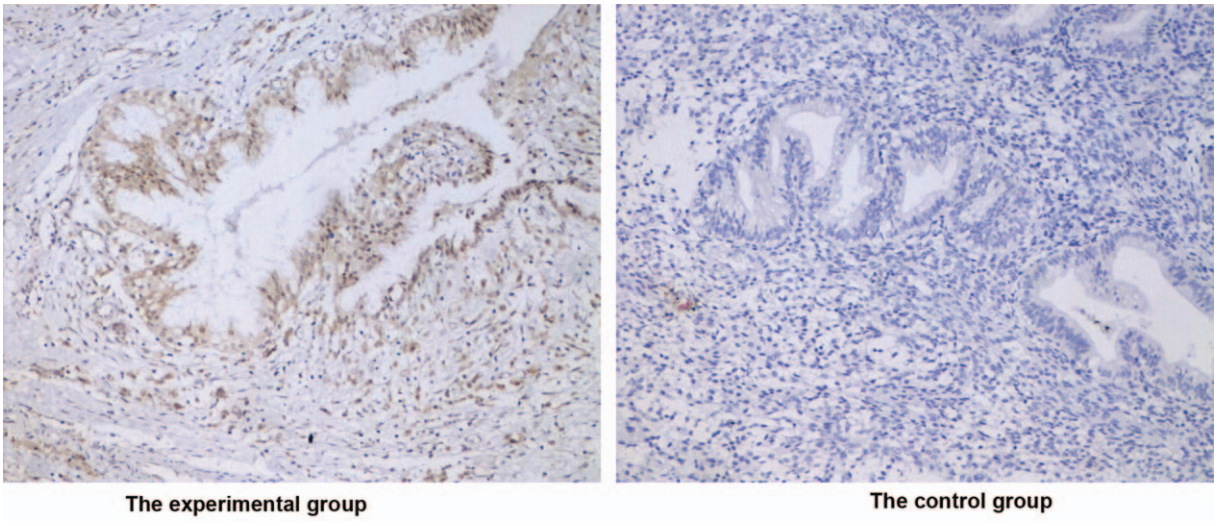

Figure 2. Expression of p16 protein in endometriosis (SP ×100). SP = streptavidin-biotin peroxidase.

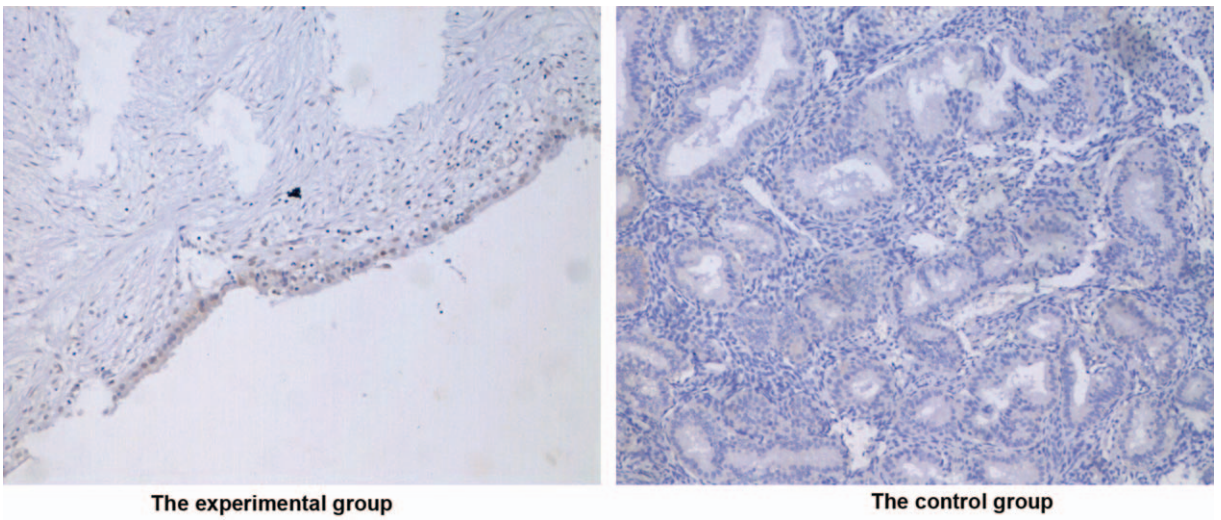

Figure 3. Expression of MDM2 protein in endometriosis (SP ×100). MDM2 = murine double minute 2, SP = streptavidin-biotin peroxidase.

regulated by posttranslational modification and its interaction with other proteins.[9] The deletion or mutation of p53 is an important step in malignant lesions.[6] The occurrence of a tumor is induced by p53 gene rs1042522 polymorphisms, through the involvement of the regulation of cell cycle arrest, cell proliferation, and the apoptosis process.[8,10] Apoptosis is a physiological process that eliminates redundant, aging and damaged cells to maintain the metabolism of the body. More and more studies have confirmed that cell apoptosis is a key factor in the stability of endometrial cells, and that it is correlated to endometrial cyclical growth and reconstruction during the fertile period. A recent study found that there was a significant difference between the apoptosis rate of EMs cells and normal endometrial cells.[11] Abnormal apoptosis is one of the most important reasons in the pathogenesis and development of EMs.[12,13] The study conducted by Dmowski et al[14] revealed that apoptosis rate has a periodical change in the endometrium. In particular, apoptosis rate in the late secretory phase and menstrual period is higher than that in the early secretory phase and proliferative phase, and there is a significant difference. It has been speculated that it gives priority to cell proliferation and reduces apoptosis, which is beneficial to the repair and functional recovery of endometrium in the proliferation phase. It also gives priority to apoptosis to clear senescent cells from the function layer, which leads to periodic endometrium denudation and maintains its function in the late secretory phase and menstruation. Therefore, periodic spontaneous apoptosis is present in normal endometrium cells, which mainly occurs in the function layer of the endometrium and plays a key role in maintaining the normal structure and function of the endometrium.

Table 2

Correlation analysis of expression of p53 and p16 in endometriosis shows that there is a positive correlation ($r=0.611$, $P<.01$).

	P16 expression		
p53 expression	**–**	**+**	**Sum**
–	26	0	26
+	1	3	4
Sum	27	3	30

Table 3

Correlation analysis of expression of p53 and MDM2 in endometriosis shows that there is a negative correlation ($r=-0.541$, $P<.01$).

	MDM2 expression		
p53 expression	**–**	**+**	**Sum**
–	11	15	26
+	3	1	4
Sum	14	16	30

MDM2 = murine double minute 2.

Table 4

Correlation analysis of expression of p16 and MDM2 in endometriosis shows that there is no correlation ($r=0.404$, $P>.05$).

	MDM2 expression		
p16 expression	**–**	**+**	**Sum**
–	12	15	27
+	2	1	3
Sum	14	16	30

MDM2 = murine double minute 2.

The experimental results show that in normal endometrium, the p53 expression rate was higher in the secretory phase than in the proliferation phase ($P<.05$). This result is the same as that reported by Agui et al.[15,16] This indicates that apoptosis in normal endometrial tissues changes with the menstrual cycle, and apoptosis in the proliferation phase is low, causing endometrial cell proliferation and endometrium proliferation. Apoptosis in the secretory phase is high, which reduces endometrial proliferation until it breaks down in the menstrual phase. The expression rate of p53 is higher in normal endometrial tissues than in EMs ($P<.05$). This indicates that the decrease in expression rate of p53 results in the decrease in apoptosis in the EMs and the obvious increase in proliferation, which affects other synergistic factors in the pathogenesis and development of EMs.

4.2. p16 and EMs

The p16 gene is the first antioncogene directly involved in cell cycle regulation, and is located on chromosome 9P21. p16 protein competes with CDK4/CDK6 to inhibit its activity, inducing cells to stop in the G1 phase, rather than in the S phase, thereby inhibiting cell proliferation. This research result indicates that the protein expression of p16 is significantly lower in EMs than in the control group. It was hypothesized that the sustained low expression of p16 may decrease the inhibition of DNA synthesis and cell proliferation, thereby reducing the role of negative cell cycle regulation.[17]

4.3. MDM2 and EMs

Oncogene MDM2 is a gene found in the double minute chromosome of the transformed murine cell line reported by Cahilly et al in 1987. It is located in segment 13-14 of the long arm of chromosome 12 (12q13-14). As oncogenes, MDM2 can enhance the vitality of cells, and promote cell proliferation and tumor growth.[18] Furthermore, MDM2 is a downstream gene of the regulation network of the p53 gene, and it is an important regulatory factor of p53, which participates in cell growth inhibition, apoptosis, and cell cycle regulation, among others. These experimental results show that the protein expression rate of MDM2 in normal endometrium tissues is lower than that in EMs ($P < .05$). These results suggest that the increased expression of MDM2 in EMs leads to the disordered function of normal endometrium and the cell cycle, and promotes the development of EMs. The exact mechanism of action remains to be further confirmed.

4.4. The correlation of the expression of p53, p16, and MDM2 in EMs tissues

These experimental results show that in EMs tissues when the expression rates of p53 and p16 decrease, the expression rate of MDM2 increases. Hence, there was a positive correlation between p53 and p16 ($P < .01$), a negative correlation between p53 and MDM2 ($P < .01$), and no correlation between p16 and MDM2 in the ovarian EMs tissues obtained from 30 patients. The occurrence and development of EMs is complex, and has a multistage and multistep process participated by various genes. The role of abnormal apoptosis in this has been taken more and more seriously. Cell apoptosis is the outcome of the combined action of many kinds of gene regulation. Excessive apoptosis promotes genes, while the absence of apoptosis inhibits genes that promote the pathogenesis and development of EMs. Abnormal cell cycle regulation is closely associated with EMs. The G1-S phase control is an important control point. There are 3 negative regulating approaches in the G1-S phase cell cycle regulation mode, which may be mediated by p16, p53. This experiment result shows that the inactivation of p16 and p53 jointly promotes the increase in ectopic endometrium cells. MDM2 protein is the p53 negative regulating protein that has been most studied. MDM2 is a kind of E3 ubiquitin ligase that promotes p53 hydrolysis by combining with p53.[19] Moreover, there is an overlap between the DNA binding domain of MDM2 and activating domain of p53, thereby allowing it to inhibit p53 transcriptional activation function.[16,20] The experimental result shows that MDM2 and p53 restrain and influence each other in the pathogenesis and development of EMs. Although p16 and MDM2 are correlated to the pathogenesis and development of EMs, the correlation between p16 and MDM2 expression has not been found. It has been speculated that p16 and MDM2 plays a role through a different pathway.[21]

The research on the endometrial cell apoptosis mechanism of EMs patients can promote the clinical pathway for drugs and help in determining different kinds of treatments to induce the apoptosis of ectopic endometrium cells, in order to result in its spontaneous regression and achieve the treatment aim. It is a promising clinical application that is worth studying in the future. The experiment results on the research of 3 kinds of apoptosis regulatory proteins (p53, p16, and MDM2) may provide a new clue to the pathogenesis and treatment method of EMs.

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Author contributions

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Software: Qian-Jin Fang.
Writing – original draft: Lin Sang.
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