P53 and Murine Double Mimute 2 (MDM2) Expression Changes and Significance in Different Types of Endometrial Lesions

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Background:
Endometrial lesions are common in obstetrics and gynecology, including endometrial polyps, uterine adenomyosis, and malignant endometrial adenocarcinoma. Endometrial lesions seriously affect women's health, fertility, quality of life, and life safety. As a pro-apoptosis gene, p53 is considered to be closely related with human tumors. Murine double mimute 2 (MDM2) is an oncogene that can promote tumor occurrence and development. P53 and MDM2 expression and significance in different types of endometrial lesions have not been fully elucidated.

Material/Methods:
Normal endometrium, endometrial polyps, uterine adenomyosis, and endometrial adenocarcinoma tissue samples were collected. Real-time PCR was used to detect p53 and MDM2 mRNA expression. Immunohistochemical staining and Western blot analysis were applied to test p53 and MDM2 protein expression. Their correlation with clinical staging of endometrial adenocarcinoma was analyzed.

Results:
P53 and MDM2 mRNA and protein expression were significantly elevated in the endometrial polyps group and the endometrial adenocarcinoma group compared with the normal control group (P<0.05). Their levels increased more obviously in endometrial adenocarcinoma compared with endometrial polyps (P<0.05). P53 and MDM2 mRNA and protein expression were slightly enhanced in uterine adenomyosis compared with normal controls, but this difference lacked statistical significance (P>0.05). P53 and MDM2 mRNA and protein level showed a positive correlation. Significantly higher expression of p53 or MDM2 was observed in patients with stage III compared to those in patients with stage II. Higher expression was also observed in patients with stage II than in patients with stage I.

Conclusions:
P53 and MDM2 mRNA and protein were elevated in endometrial polyps and endometrial adenocarcinoma and their expressions were correlated with clinical staging of endometrial adenocarcinoma. They can promote cancer occurrence and development, and can be treated to assist diagnosis and provide a reference for treatment.

MeSH Keywords:
Adnexa Uteri • Endometrial Ablation Techniques • Endometrial Hyperplasia • Uterine Cervical Neoplasms

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Background

As society progresses, work rhythm speeds up, and living and eating habits change, younger women are at increased risk of different types of endometrial lesions [1,2]. The most common types of endometrial lesions are endometrial polyps, uterine adenomyosis, and malignant endometrial adenocarcinoma [3,4]. The incidence of endometrial polyps in women reached 25%, with the main clinical symptoms being large menstrual flow, intermenstruum bleeding, menstrual abnormalities, and infertility. Endometrial polyps in the perimenopausal or postmenopausal period easily induce cancer [5]. As a common type of endometrial disease, endometrial polyps frequently occur in women, with easy relapse that seriously affects life quality and fertility [6]. In uterine adenomyosis, uterine glands or uterine stromal endometrial tissue enters the myometrium, inducing pathological changes such as myometrium nodular changes, especially uterine adenomyoma, which is common in young women [7]. As uterine adenomyosis is often accompanied with compensatory hyperplasia and hypertrophy, and occurs in fertile women, it often causes severe symptoms, mainly increased menstrual volume, abnormal menstrual period, severe abdominal pain, and infertility [8]. Endometrial cancer is among the 3 most common malignant tumors in the female reproductive system. Endometrial adenocarcinoma is the most common type, with increased incidence and a trend toward affecting younger women [9,10].

Pro-apoptotic gene p53, which is a tumor suppressor gene, plays an important role in regulating cell apoptosis. P53 accumulated in the nucleus, leading to DNA damage by binding with the cell division control site [11,12]. Murine double mimute 2 (MDM2) was first found from the double mimute in transformation mice. It was further confirmed to be expressed in various tissues and organs. MDM2 overexpression in nude mice can lead to excessive cell proliferation and tumor formation. Thus, MDM2 may promote tumor occurrence and development by promoting tumor cell proliferation and growth. It is an oncogene that can promote tumor occurrence and development [13,14]. MDM2 can play a role in 2 ways, as its translation product can bind with p53 protein, thus inhibiting pP53 transcription activity. It further degrades wild-type p53 protein, and plays a stabilizing role in mutant P53 protein [15]. MDM2 also can play an oncogene role independent of p53 method, as a promotor gene. It can promote cell cycle from G1 to S phase, or suppress oncogenes through zinc finger structures. It also can stimulate cell proliferation by binding with Rb-related E2F [16,17]. Differences in P53 and MDM2 expression in various endometrial lesions have not been reported. This study aimed to analyze p53 and MDM2 mRNA and protein expression in normal endometrium, endometrial polyps, uterine adenomyosis, and endometrial adenocarcinoma tissues, to provide a reference for diagnosis and treatment of endometrial lesions in clinical practice.

Material and Methods

Study subject selection and specimen collection

A total of 71 patients with different types of endometrial lesions diagnosed between January 2014 and June 2015 in Chengdu Military General Hospital were selected and assigned to 4 groups. The control group consisted of 15 patients who underwent hysterectomy because of uterine prolapse, uterine fibroids, or ovarian benign tumor. The endometrial polyps group (polyps group) consisted of 20 patients diagnosed as having single or aggregated multiple endometrial polyps by pathology who underwent hysteroscopic operations. The uterine adenomyosis group (adenomyosis group) consisted of 17 uterine adenomyosis patients diagnosed by pathology who underwent hysterectomy. The endometrial adenocarcinoma group (adenocarcinoma group) consisted of 19 endometrial adenocarcinoma patients in stage I–III who underwent hysterectomy. The patients enrolled were ages 37–69 (58.6±10.2) years.

Table 1. General information comparison.

Index	Control	Polyps group	Adenomyosis group	Adenocarcinoma group
Cases (n)	15	20	17	19
Mean age (year)	58.1±11.8	59.6±10.3	58.7±12.7	57.9±13.1

Table 2. Primer sequence.

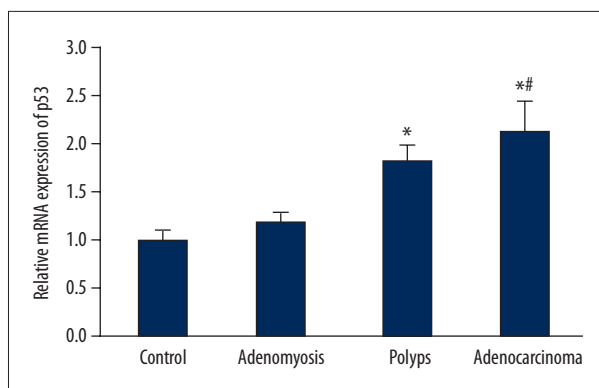| Gene	Forward 5'-3'	Reverse 5'-3'
GADPH	ACCAGGTATCTGCTGGTTG	TAACCATGATGTCAGCGTGGT
p53	GTGACCTCAGTGACCTTATG	GTTCTTCCGCTCAACTCTTATCT
MDM2	ATGCTTCATGATGCCGACT	TTGTCAACTCTTATCCTTC

Figure 1. P53 mRNA expression in different types of endometrial lesions. * *P*<0.05, compared with control; # *P*<0.05, compared with polyps group.
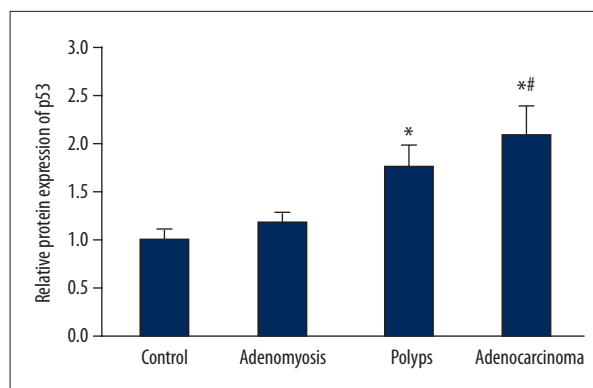

Figure 2. P53 protein expression in different types of endometrial lesions.

Figure 3. P53 protein expression in different types of endometrial lesions analysis. * *P*<0.05, compared with control; # *P*<0.05, compared with polyps group.
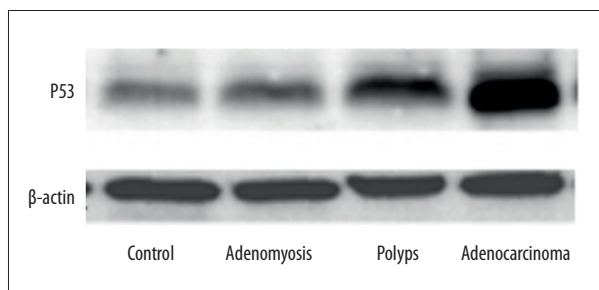
One-way ANOVA analysis revealed that general characteristics of patients in the 4 groups showed no statistically significant differences (Table 1). No patients received radiotherapy, chemotherapy, or hormone therapy before surgery. No patients received antibiotic therapy or intrauterine device. Patients whose cases were complicated with other reproductive system disease, severe organ failure, other malignant tumors, or severe complications were excluded. Intraoperative endometrial specimens were collected and preserved at –80°C until needed.

The study protocol was approved by the Research Ethics Committee of Chengdu Military General Hospital, and all patients gave their informed consent before study commencement.

Main materials and instruments

TRIzol reagent was from Invitrogen. Polyvinylidene difluoride (PVDF) membrane was from Pall Life Sciences. Western blot-related chemical reagents were bought from Beyotime. ECL reagent was from Amersham Biosciences. Mouse antihuman p53 primary antibody, mouse antihuman MDM2 primary antibody, and goat anti-mouse horseradish peroxidase (HRP)-tagged IgG secondary antibody were from Cell Signaling. The SABC immunohistochemical staining kit was purchased from Boster Bio-engineering, Ltd. (Wuhan, China). The RNA extraction kit and

reverse transcription kit were from Invitrogen. DNA amplifier was from PE Gene Amp PCR System2400. Other common reagents were purchased from Sangon.

Real-time polymerase chain reaction (PCR)

Total RNA was extracted from endometrial tissue using TRIzol after liquid nitrogen grinding, and reverse transcribed to cDNA according to the manual. The primers used in the experiment were designed by Primer 6.0 and synthesized by Invitrogen (Table 2). Real-time PCR consisted of 55°C for 1 min, followed by 35 cycles of 92°C for 30 s, 58°C for 45 s, and 72°C for 35 s. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was selected as the internal reference. Relative gene expression was semiquantitatively analyzed by $2^{-\Delta Ct}$ method.

Western blot

Endometrial tissues were treated by radioimmunoprecipitation assay (RIPA) solution for 15~30 min. Then the cells were ultra-sonicated 4 times at 5 s and centrifuged at 4 °C and 10 000×g for 15 min. The supernatant was stored at –20°C. The protein was separated by 10% SDS-PAGE and transferred to a PVDF membrane. After blocking by 5% skim milk for 2 h, the membrane was incubated in primary antibody (1:1000) at 4°C overnight. Next, the membrane was further incubated in secondary antibody (1: 2000) at room temperature for 30 min after washing with phosphate balanced saline with Tween 20 (PBST). Finally, the chemiluminescent agent was added to membrane for 1 min and developed. Protein image processing system software and Quantity One software were used for scanning and calculation. All experiments were repeated 4 times (n=4).

Immunohistochemical staining

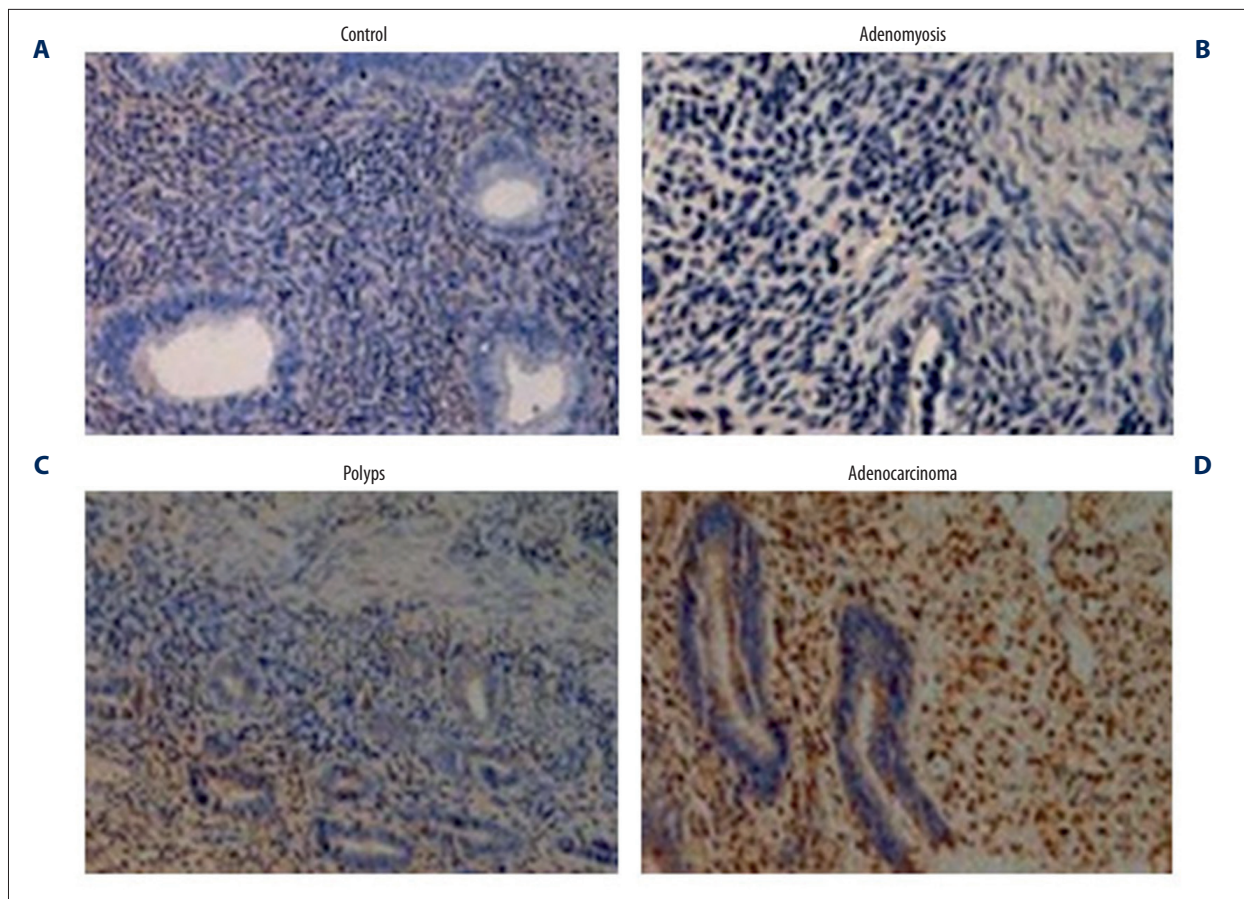Immunohistochemical staining was performed using a SABC kit according to manufacturer's instructions. Briefly, tissue

Figure 4. Immunohistochemical analysis of P53 expression.

Table 3. Expression of p53 and MDM2 measured by immmunohistochemical staining.

Group	N	p53			MDM2		
		(–)	(+ or ++)	%	(–)	(+ or ++)	%
Control	15	15	0	0	15	0	0
Adenomyosis	17	17	0	0	12	5	29.4
Polyps	20	12	8	40	10	10	50
Adenocarcinoma	19	7	12	63.15*	5	14	73.68*

* $P<0.05$.

slices were incubated with 3% H_2O_2 at room temperature and blocked with 10% goat serum. Then the slices were incubated with antibody against p53 (1 in 1000 dilution) or MDM2 (1 in 500 dilution) for 1 h at 37°C and then washed 3 times with PBS. After that, biotin-labelled goat anti-mouse secondary antibody was added. Color was developed with 3, 3'-diaminobenzidine. Positivity (+) was defined as less than 50% positive cells and Positivity (++) was defined as more than 50% positive cells.

Statistical analysis

SPSS19.0 was used for data analysis. Measurement data are presented as mean ± standard deviation ($\overline{\chi}$±S). One-way ANOVA was used to compare the differences of age and expression of p53 or MDM2 within different groups, and Spearman correlation analysis was used for to assess the correlation of p53 expression with MDM2. $P<0.05$ was considered as statistical significance.
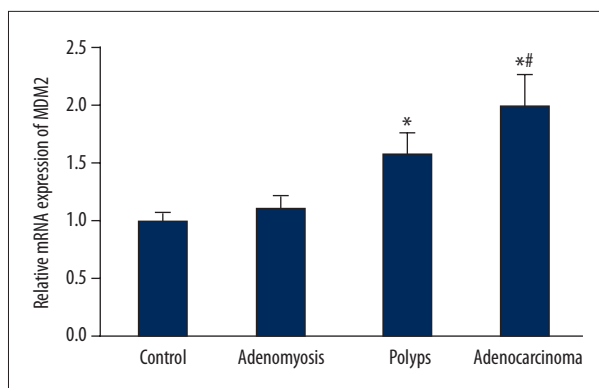
Figure 5. MDM2 mRNA expression in different types of endometrial lesions. * $P<0.05$, compared with control; # $P<0.05$, compared with polyps group.
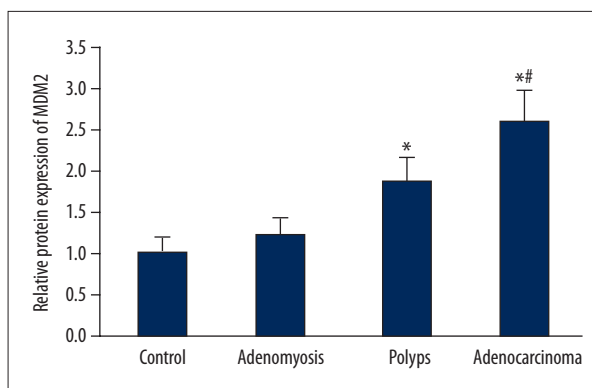

Figure 6. MDM2 protein expression in different types of endometrial lesions.

Results

P53 mRNA expression in different types of endometrial lesions

Real-time PCR was used to detect p53 mRNA expression in different types of endometrial lesions. The results showed that p53 mRNA was expressed in the control group and it increased in the adenomyosis group, but the difference lacked statistical significance (P>0.05). The polyps group and adenocarcinoma group both showed significantly overexpressed p53 mRNA compared with controls (P<0.05). Its level in the adenocarcinoma group was obviously higher compared with the polyps group (P<0.05) (Figure 1).

P53 protein expression in different types of endometrial lesions

Western blot was used to test p53 protein expression in different types of endometrial lesions. Similar to p53 mRNA expression, p53 protein was expressed in the control group and it was elevated in the adenomyosis group but the difference lacked statistical significance (P>0.05). The polyps group and adenocarcinoma group both presented markedly increased p53 protein expression compared with controls (P<0.05). Its level in the adenocarcinoma group was significantly higher compared

Figure 7. P53 protein expression in different types of endometrial lesions analysis. * $P<0.05$, compared with control; # $P<0.05$, compared with polyps group.

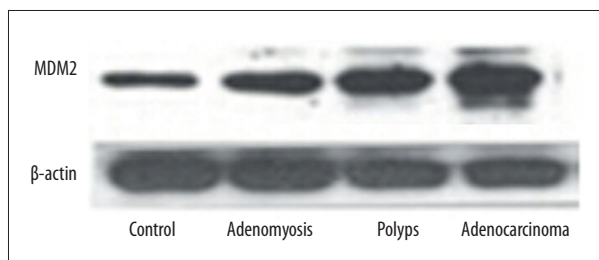with the polyps group (P<0.05) (Figures 2, 3). Consistent with these results, immunohistochemical staining analysis displayed the same patterns of p53 expression, with higher expression in the polyps group and adenocarcinoma group (Figure 4, Table 3).

MDM2 mRNA expression in different types of endometrial lesions

Real-time PCR was performed to detect MDM2 mRNA expression in different types of endometrial lesions. The results showed that MDM2 mRNA was expressed in the control group and was elevated in the adenomyosis group, but the difference lacked statistical significance (P>0.05). The polyps group and adenocarcinoma group both showed significantly enhanced MDM2 mRNA compared with controls (P<0.05). Its level in the adenocarcinoma group was obviously higher than that in the polyps group (P<0.05) (Figure 5).

MDM2 protein expression in different types of endometrial lesions

Western blot analysis was further used to test MDM2 protein expression in different types of endometrial lesions. Similar to MDM2 mRNA expression, MDM2 protein was expressed in the control group and it was elevated in the adenomyosis group, but the difference lacked statistical significance (P>0.05). The polyps group and adenocarcinoma group both presented markedly increased MDM2 protein expression compared with controls (P<0.05). Its level in the adenocarcinoma group was obviously higher compared with the polyps group (P<0.05) (Figures 6, 7). Consistent with these results, immunohistochemical staining analysis also displayed the same patterns of MDM2 expression, with higher expression in the polyps group and adenocarcinoma group (Figure 8, Table 3).
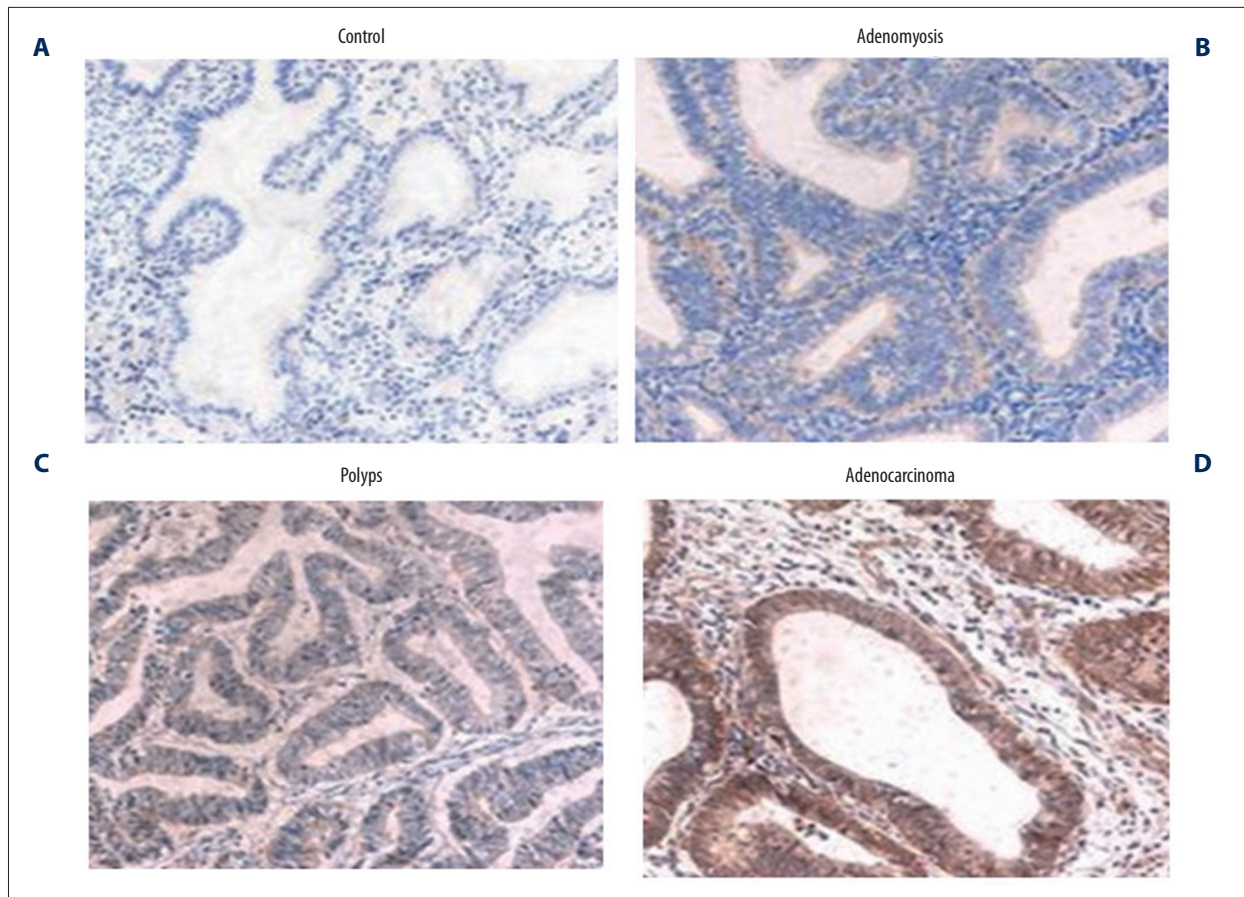
Figure 8. Immunohistochemical analysis of MDM2 expression.

Analysis of the correlation between MDM2 and p53

The relationships between MDM2 and p53 mRNA and protein level with different types of endometrial lesions were analyzed. The results showed that MDM2 and p53 mRNA and protein showed positive correlations in endometrial polyps, uterine adenomyosis, and endometrial adenocarcinoma (P<0.05) (Table 4).

Correlation analysis of expression of p53 or MDM2 with clinical staging of endometrial adenocarcinoma

Immunohistochemical staining was performed to measure the expression level of p53 and MDM2, and then we analyzed the correlation of expression of p53 and MDM2 with clinical staging of endometrial adenocarcinoma. As seen in Table 5, significantly higher expression of p53 or MDM2 was observed in patients with stage III compared to those in patients with stage II or I. Higher expression was also observed in patients with stage II than in patients with stage I, suggesting that expression of p53 or MDM2 might be associated with the clinical staging of endometrial adenocarcinoma.

Discussion

To date, the p53 gene shows the closest relationship with human tumors. It is involved in regulating cell cycle, cell growth, apoptosis, and cell division. Tumor suppressor gene p53 is a transcription factor that can directly or indirectly control the cell cycle genes or apoptosis-related gene expression, and is involved in the apoptosis-related death receptor pathway and mitochondrial pathway [18,19]. Wild-type p53 protein plays a negative role in regulating cell growth. P53 mutation or abnormal expression is an important mechanism of inducing tumors [20]. P53 gene mutation may lead to loss of negative regulation, resulting in abnormal cell proliferation and canceration. This study analyzed p53 mRNA and protein, which are expressed in several common types of endometrial lesions. The results confirmed that p53 mRNA and protein levels in uterine adenomyosis were similar to those in controls. Its expression was significantly elevated in the endometrial polyps group and the endometrial adenocarcinoma group, especially in endometrial adenocarcinoma. Research showed that p53 gene and protein have important roles in the process of endometrial carcinoma. P53 gene activation and increased protein expression can induce abnormal cell proliferation. Its elevation can

Table 4. Correlation analysis between MDM2 and p53.

Index	Normal control		Adenomyosis		Endometrial polyps		Endometrial adenocarcinoma	
	r	P	r	P	r	P	r	P
p53 mRNA MDM2 mRNA	0.874	0.005	0.751	0.032	0.727	0.041	0.727	0.041
p53 protein MDM2 protein	0.892	0.003	0.718	0.045	0.921	0.002	0.723	0.042

Table 5. Expression of p53 and MDM2 in samples with different stages measured by immmunohistochemical staining.

Staging	N	p53			MDM2		
		(–)	(+ or ++)	%	(–)	(+ or ++)	%
I	6	4	2	33.33	4	2	33.33
II	7	3	4	57.14*	1	6	95.71*
III	6	0	6	100*	0	6	100*

* $P<0.05$.

be seen in proliferative diseases, such as endometrial polyps and cancer. P53 gene mutation and abnormal protein expression often occur in the advanced stage of endometrial adenocarcinoma, and can assist diagnosing the degree of malignancy of endometrial cancer [15,21].

The zinc finger structure in MDM2 can mediate protein interaction and the combination of DNA and RNA. It participates in regulating cell cycle and promoting cell proliferation, thus facilitating tumor growth [22,23]. However, little is known about MDM2 expression in various endometrial lesions, including uterine adenomyosis, endometrial polyps, and endometrial carcinoma. This study confirmed that MDM2 mRNA and protein expression showed no statistically significant differences between uterine adenomyosis and normal endometrium. Its level obviously increased in endometrial polyps and endometrial adenocarcinoma, particularly in the latter. Furthermore, it had a positive correlation with p53 mRNA and protein expression.

The results revealed that p53 and MDM2 were closely related to occurrence and development of endometrial lesions.

Conclusions

p53 and MDM2 mRNA and protein expressions were clearly elevated in endometrial polyps and endometrial adenocarcinoma. In addition, expressions of p53 and MDM2 were correlated with clinical staging of endometrial adenocarcinoma. P53 and MDM2 can promote cancer occurrence and development, and can be used to assist in diagnosis and providing a reference for treatment.

Disclosure of conflict of interest

The authors declare no competing financial or commercial interests.

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