ORIGINAL ARTICLE

Expression of Testin, PTEN and MMR proteins in endometrial carcinoma and their correlation with clinical features

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ABSTRACT

Objective: This study aimed to investigate the association between the expression of TESTIN, PTEN, and mismatch repair (MMR) proteins in endometrial carcinoma and its clinical-pathological characteristics, providing molecular evidence for clinical diagnosis and treatment.
Methods: We conducted a retrospective analysis of 100 cases of endometrial carcinoma (case group) and 33 cases of benign lesions (control group), using immunohistochemical SP technology to detect the expression of the aforementioned proteins.
Results: The results showed that the positive rates of TESTIN, PTEN, and normal pMMR in the case group were significantly lower than those in the control group. TESTIN and PTEN expression showed a strong positive correlation, while both showed a moderate positive correlation with pMMR. Clinical feature analysis showed that the positive rates of these three proteins were significantly reduced in patients with FIGO high stage, deep myometrial invasion, and lymph node metastasis. The positive rate of endometrioid carcinoma was higher than that of undifferentiated carcinoma and other types.
Conclusions: The low expression of TESTIN, PTEN, and MMR proteins is closely associated with the malignant progression of endometrial carcinoma. Combined detection can serve as an important clinical reference for assessing disease severity, guiding treatment, and predicting prognosis.

Key Words: Endometrial cancer, TESTIN, PTEN, Mismatch repair protein, Clinical characteristics

1. INTRODUCTION

Endometrial cancer is a common malignant tumor of the female reproductive organs.[1] The global age-standardized incidence rate is 12.9 per 100,000, affecting young women. The proportion of cases under 40 years old has risen to 5%-10%.[2] It is divided into estrogen-dependent type (Type I, mostly endometrioid carcinoma, with a relatively good prognosis) and non-estrogen-dependent type (Type II, accounting for 10%-20%, with high invasiveness, easy metastasis and poor prognosis).[3] The five-year survival rate for early-stage patients can reach 80%, but treatment is limited for advanced and special subtypes. About 10% of cases have controversial diagnoses due to atypical pathological morphology.[4] Cancer stems from the inactivation of tumor suppressor genes and defects in DNA repair mechanisms. Exploring specific molecular markers is highly significant for precision medical

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diagnosis and treatment.[5] PTEN is a tumor suppressor gene that inhibits cancer development by regulating the PI3K/AKT pathway, with a mutation rate of 60% to 80% in endometrial cancer.[6] The MMR protein system helps stabilize the genome, and its deficiency can lead to Lynch syndrome and affect responses to immunotherapy;[7] TESTIN is a tumor suppressor protein that can inhibit cell invasion and metastasis, but research on its role in endometrial cancer is not comprehensive.[8] Currently, most studies focus on individual proteins, and reports on the co-expression patterns and combined diagnostic value of TESTIN, PTEN, and MMR proteins remain insufficient.[9, 10] This study aims to explore the expression of the three in benign and malignant endometrial lesions and their relationship with clinicopathological features, reveal the synergistic mechanism, and provide a theoretical basis for disease diagnosis, prognosis and individual treatment.

2. MATERIALS AND METHODS

2.1 Object of study

2.1.1 *Case sources and inclusion and exclusion criteria*

This retrospective study selected patients who visited the Obstetrics and Gynecology Department of Baogang Hospital in Inner Mongolia from October 2016 to October 2025. There were 100 patients with endometrial cancer who underwent surgery and were pathologically diagnosed, while the benign control group composed of 33 patients confirmed to have benign endometrial lesions through segmented curettage during the same period.

The inclusion criteria include: (1) The case group should consist of patients undergoing primary treatment who had not received radiotherapy, chemotherapy, targeted therapy or immunotherapy before the surgery. The clinicopathological data should be complete, and the quality of formalin-fixed paraffin-embedded (FFPE) specimens should meet the requirements for immunohistochemical detection; (2) The benign control group must have complete medical records and it should be ensured that the specimens are well preserved; (3) All participants must voluntarily join this study and sign an informed consent form.

Exclusion conditions include: (1) Patients with other malignant tumors at the same time; (2) Patients with severe and difficult-to-control hypertension, diabetes, or failure of major organs such as the heart, liver and kidneys; (3) Specimen contamination, insufficient tissue volume or improper handling make it impossible to detect; (4) Cases where incomplete clinical data have affected research analysis.

This study was approved by the Ethics Committee of Inner Mongolia Baogang Hospital (Approval Number: BGYY-2025-036) and adhered to the relevant guidelines of medical ethics.

2.1.2 *Basic information of the sample*

The age range of patients in the case group was 33 to 59 years old, with an average age of (41.12 ± 5.51) years old. Weight ranged from 42 to 70 kg, with a mean of (52.12 ± 5.21) kg. The pathological types involved 49 cases of endometrioid carcinoma, 7 cases of clear cell carcinoma, 25 cases of non-keratinized squamous cell carcinoma, 9 cases of undifferentiated carcinoma and 10 cases of small cell carcinoma. FIGO staging showed 75 cases in stage I to II and 25 cases in stage III to IV. Based on the depth of myometrial infiltration, 77 cases had infiltration less than half, and 23 cases had infiltration reaching or exceeding half. Lymph node examination showed positive metastasis results in 25 cases and negative results in 75 cases.

The control group's age ranged from 34 to 60 years, with a mean of 40.96 years and a standard deviation of 5.47 years. Their weight ranged from 42 to 69 kg, with an average weight of 53.24 kg and a standard deviation of 5.19 kg. The diseases included 8 cases of nonspecific endometritis, 7 cases of endometrial adenomyosis, 11 cases of endometrial polyps, and 7 cases of endometrial hyperplasia without SARS.

The analysis results showed that there were no significant differences between the two groups of patients in terms of basic characteristics such as age and weight ($p > .05$), so the two groups were comparable.

2.2 Experimental reagents and instruments

2.2.1 *Experimental reagents*

Rabbit anti-human PTEN monoclonal antibody (Thermo Fisher, product number MA5-16738, dilution ratio 1:150); Mouse anti-human TESTIN monoclonal antibody (provided by Abcam Company, product number ab124732, dilution ratio 1:200); Core antibody for MMR protein detection (for MLH1, MSH2, MSH6, PMS2, provided by Cell Marque, pre-diluted); Immunohistochemical SP kit and DAB chromogenic kit (Maixin Biotechnology Development Co., LTD.); Buffer solutions for antigen retrieval (including pH6.0 citrate and pH9.0 EDTA buffer), 3% hydrogen peroxide solution, hematoxylin staining solution, and neutral resin (provided by Beijing Zhongshan Biotechnology Co., LTD.); PBS buffer (from Beijing Gene Technology Co., LTD.).

2.2.2 *Instruments*

Wax sectioning equipment (model RM2268); Inverted fluorescence microscope (Model BX68, LEICA, Germany); Antigen retrieval high-voltage instrument (manufactured by Bayi Electrical Appliance Factory); Constant temperature incubator (from Beijing Hengke Science Instrument Co.,

LTD.); Centrifugal extractor; High-speed centrifugal device; Micro liquid distributor (brand EPPRNDORF, produced in Germany); Electrophoresis apparatus (BIO-RAF Company, Made in the USA).

2.3 Experimental method

2.3.1 *Immunohistochemical staining process*

(1) Sample processing: 4 μm thick continuous sections were obtained from FFPE samples. Dewaxing was performed with xylene I and II for 10 minutes each, followed by 5 minutes each in 100%, 95%, 80%, and 75% gradient ethanol to complete hydration; (2) Inhibition of endogenous peroxidase: At room temperature, incubate with 3% H_2O_2 for 10 minutes, then rinse three times with PBS buffer for 5 minutes each time; (3) Antigen retrieval: The TESTIN was subjected to high-pressure retrieval with citrate buffer (pH 6.0) for 10 minutes, while the PTEN and MMR proteins were subjected to high-pressure retrieval with EDTA buffer (pH 9.0) for 10 minutes. Then, allow them to cool naturally to room temperature; (4) Incubation with primary antibody: Add the diluted corresponding primary antibody, incubate overnight at 4°C, and then rinse three times with PBS buffer; (5) Incubation with secondary antibody: Add horseradish peroxidase-labeled secondary antibody, incubate at 37°C for 30 minutes, and then rinse three times with PBS buffer; (6) Color development and sealing: Use the DAB color development kit for color development and observe under a microscope for 3 to 5 minutes. Then rinse with tap water to terminate the reaction. Then, perform hematoxylin counterstaining for 5 minutes. After decolorization and bluing, carry out gradient ethanol dehydration and xylene transparency. Finally, seal the sheet with neutral gum.

2.3.2 *Criteria for result interpretation*

TESTIN and PTEN proteins: The positive signals of tumor cells are located in the cytoplasm. We used a semi-quantitative scoring method, specifically by multiplying staining intensity by the percentage of positive cells. Degree of staining: 0 points (colorless), 1 point (light yellow), 2 points (brownish yellow), 3 points (dark brown); Proportion of positive cells: 0 points (< 5%), 1 point (5% to 25%), 2 points (26% to 50%), 3 points (51% to 75%), 4 points (> 75%). A score of 0 to 3 indicates low-level expression (negative), while a score of 4 to 12 indicates high-level expression (positive).

MMR protein: It shows as a positive signal in the cell nucleus. If all or any of the MMR proteins (MLH1, MSH2, MSH6, PMS2) are completely unexpressed in tumor cells. At the same time, the control tissue (normal endometrial glands or lymphocytes) shows a positive reaction, MMR deficiency (dMMR) is determined. If all four proteins are expressed normally, it is considered normal MMR (pMMR). In this study, the "positive rate of MMR protein" refers to the proportion of pMMR cases.

Two senior pathologists independently analyzed each section without being influenced by each other's results. If their diagnostic results differ, we will conduct a joint film review and reach consensus after discussion. To ensure test quality, a positive control (with known high expression in the tissue) and a negative control (using PBS instead of the primary antibody) were set up for each experiment.

2.4 Collection of clinical pathological data

Using the hospital's electronic health record system and pathology reports, we summarized the clinicopathological characteristics of patients in the case group, including age, FIGO stage, histological type, depth of myometrial invasion, and lymph node metastasis. Classification criteria: age < 45 years and > 45 years; FIGO Stages I-II and III-IV; tissue types: endometrioid adenocarcinoma and non-endometrioid adenocarcinoma. Muscle layer invasion depth is less than half or more than half. Is there lymph node metastasis?

2.5 Statistical analysis

Data were analyzed using SPSS 26.0. For normally distributed measurement data, after confirming normality with the normality test, data were expressed as ($x \pm s$), and we used an independent-samples t-test to compare differences between groups. Count data were expressed as frequency (percentage), and differences between groups were analyzed using the χ^2 test or Fisher's exact test. We analyzed the correlation between protein expression levels using Spearman rank correlation. If the p value is less than .05, the difference is considered statistically significant.

Although five-year survival for early-stage patients is as high as 80%, treatment outcomes for advanced stages and certain subtypes remain suboptimal. Furthermore, because of atypical pathological morphology, approximately 10% of cases remain controversial at the time of diagnosis. Tumor formation mainly results from loss of tumor suppressor gene function and defects in DNA repair. The search for unique molecular markers is crucial for achieving precision medicine. As a classic tumor suppressor gene, PTEN inhibits tumor development by regulating the PI3K/AKT pathway, and its mutation rate in endometrial cancer ranges from 60% to 80%. The MMR protein system is crucial for genomic stability, and its absence can contribute to Lynch syndrome and affect responses to immunotherapy. The tumor suppressor protein TESTIN can inhibit cell invasion and metastasis, but research on its role in endometrial cancer remains insufficient.

Existing research usually focuses on individual proteins, but

detailed, systematic reports on the co-expression patterns of these three proteins and the value of combined detection are lacking. This study aims to detect the expression of TESTIN, PTEN and MMR proteins in benign and malignant endometrial lesions, and analyze their relationship with clinicopathological features. Through this analysis, the research aims to clarify the synergistic mechanism of these three proteins, providing theoretical support for diagnosis, prognosis assessment, and individualized treatment.

3. RESULTS

3.1 Clinicopathological characteristics of the research subjects

This study included 100 patients with endometrial cancer (case group) and 33 patients with benign endometrial lesions (benign control group). The age range of the patients in the study group was between 33 and 59 years old, with an average age of 41.12 $\pm$ 5.51 years old. The main pathological types were endometrioid carcinoma (49 cases, 49.00%), followed by non-keratinized squamous cell carcinoma (25 cases, 25.00%) and small cell carcinoma (10 cases, 10.00%), among others. According to FIGO staging, 75 cases (75.00%) were stage I-II, and 25 cases (25.00%) were stage III-IV. Regarding myometrial infiltration, 77 cases (77.00%) had an infiltration depth of less than 1/2, while the remaining 23 cases (23.00%) had an infiltration depth of 1/2 or greater. In addition, 25 cases (25.00%) had positive lymph node metastasis. The age range of patients in the benign control group was 34 to 60 years, with a mean age of 41.96 years and a standard deviation of 5.47 years. The main lesion types were endometrial polyps (11 cases, 33.33%) and nonspecific endometritis (8 cases, 24.24%). Comparison of baseline data between the two groups (age and weight) showed no significant differences ($p > .05$), indicating that the groups are comparable.

3.2 The expression differences of TESTIN, PTEN and MMR proteins in benign and malignant endometrial tissues

3.2.1 *Qualitative expression characteristics*

The immunohistochemical staining results showed that the positive signals of TESTIN and PTEN proteins were mainly distributed in the cytoplasm. In the benign control group, these two proteins appeared as strongly positive brownish-yellow granules that were aggregated and clearly visible (see Figures 1 and 2). However, in the experimental case group, expression was significantly reduced or absent, appearing as lighter or no cytoplasmic staining (see Figures 3 and 4). In addition, positive signals for MMR system proteins (including MLH1, MSH2, MSH6, and PMS2) were located in the cell nucleus. In the benign control, all four proteins showed strong positive staining, and the cell nuclei were dark brown (see Figure 5). In the case group, one or more proteins were often absent, most commonly MLH1, which resulted in nuclei that did not stain (see Figure 6).

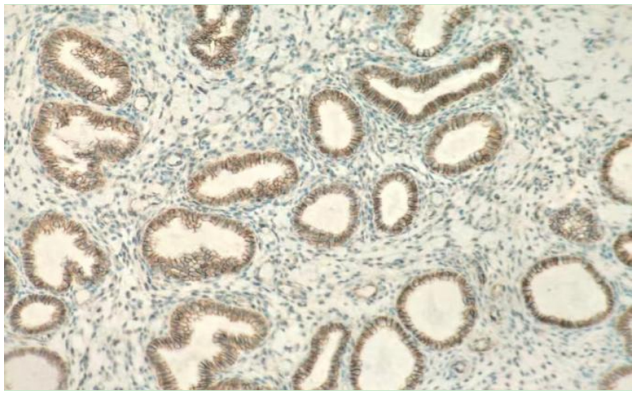

Figure 1. TESTIN expression in benign endometrial lesions (immunohistochemistry $\times$200)

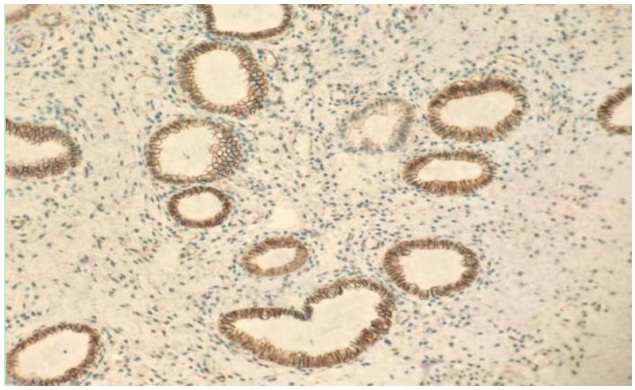

Figure 2. PTEN expression in benign endometrial lesions (immunohistochemistry $\times$200)

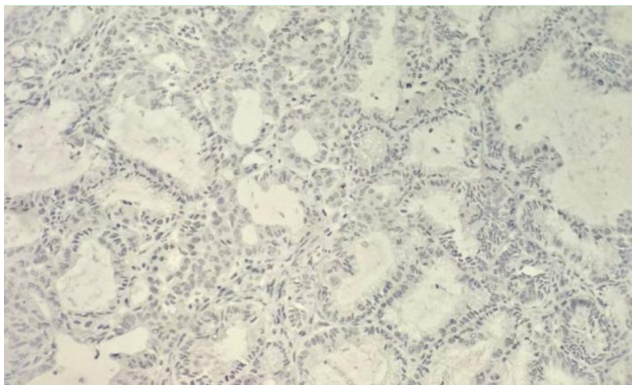

Figure 3. TESTIN expression in endometrial cancer (immunohistochemistry $\times$200)

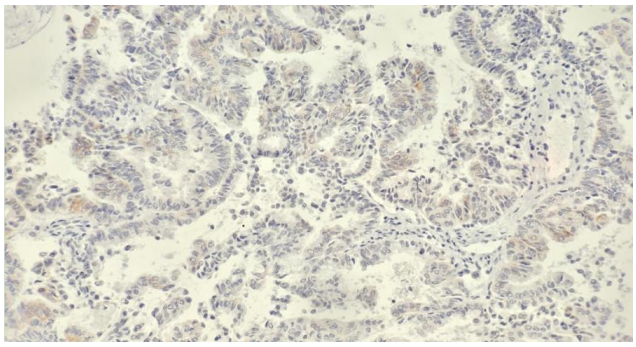

Figure 4. PTEN expression in endometrial cancer (Immunohistochemistry ×200)

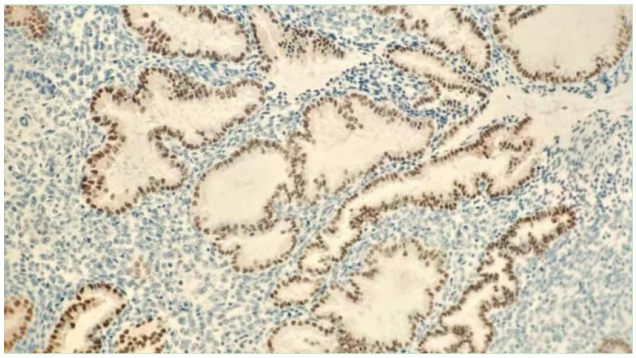

Figure 5. MMR protein (MLH1) expression in benign endometrial lesions (immunohistochemistry ×200)

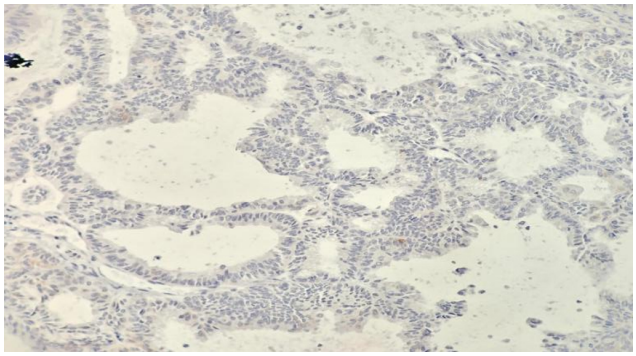

Figure 6. The absent expression of MMR protein (MLH1) in endometrial cancer (immunohistochemistry ×200)

3.2.2 *Quantitative expression comparison*

In the case group, the positive rates of TESTIN and PTEN, as well as the normal expression rate of MMR (pMMR), were significantly lower than those in the benign control group. Differences among the groups were statistically significant ($p < .05$). In the case group, the positive rate of TESTIN was only one third of that in the benign control group, while the positive rate of PTEN was even lower, less than one third of that in the benign control group. In addition, the pMMR ratio was approximately 32 percentage points lower than in the benign control group (see Table 1).

3.3 The correlation of the expression of three proteins in endometrial cancer tissues

Spearman rank correlation analysis indicated that the expressions of the three proteins in endometrial cancer tissues were significantly correlated ($p < .001$): The positive expressions of TESTIN and PTEN proteins showed a strong positive correlation ($r = 0.867$), indicating that their expression trends were very consistent; The positive expressions of TESTIN and PTEN proteins were moderately positively correlated with pMMR ($r = 0.421$ and $r = 0.385$), respectively. This indicates that tumors with normal expression of tumor suppressor proteins are more likely to maintain intact in DNA mismatch repair function.

3.4 The association between xenoprotein expression and the clinicopathological characteristics of endometrial cancer

3.4.1 *The association characteristics of TESTIN protein expression*

The expression of TESTIN protein was significantly correlated with FIGO stage, depth of myometrial invasion and lymph node metastasis status ($p < .05$), but had no obvious association with age ($p > .05$). Among patients in the third to fourth stages, the positive rate of TESTIN was 8.00%, which was only a quarter of that of patients in the first to second stages (34.67%). The positive rate of patients with myometrial infiltration reaching or exceeding half (4.35%) was significantly lower than that of patients with infiltration less than half (35.06%). The positive rate for lymph node metastasis was 12.00%, less than half that of patients without metastasis (33.33%). Among pathological types, endometrioid adenocarcinoma has the highest positive rate (36.73%), while undifferentiated carcinoma and small cell carcinoma have the lowest positive rates, both below 11.11% (see Table 2).

3.4.2 *The associated characteristics of PTEN protein expression*

The expression patterns of PTEN and TESTIN proteins showed a highly consistent trend, closely related to FIGO stage, depth of muscle-layer invasion and lymph node metastasis ($p < .05$), but not significantly related to age ($p > .05$). In patients with stage III-IV, myometrial infiltration $\geq$ 50%, or lymph node metastasis, the positive rate of PTEN was all below 10%, significantly lower than that of the corresponding low-risk groups. Among pathological types, the PTEN positive rate in endometrioid adenocarcinoma is 32.65%, higher than in other types, while the PTEN positive rate in clear cell carcinoma is the lowest, at 14.29% (see Table 3).

Table 1. Comparison of benign lesions and malignant tumors

Groups	Cases	TESTIN positive (*n*, %)	PTEN positive (*n*, %)	pMMR (*n*, %)
Benign control group	33	30 (90.91)	27 (81.82)	31 (93.94)
Case group	100	28 (28.00)	25 (25.00)	62 (62.00)
χ^2	-	39.930	33.640	8.725
p value	-	< .001	< .001	.003

Note. pMMR: The normal expression of mismatch repair proteins (MLH1, MSH2, MSH6, and PMS2 are all positive), dMMR (defective type) = 1-pMMR

Table 2. Comparison of the positive rate of TESTIN protein in different clinicopathological features of endometrial cancer tissues

Clinicopathologic features	Cases	TESTIN positive (*n*, %)	χ^2	*p* value
Age (years)	-	-	0.093	.760
≤ 45	44	13 (29.55)	-	-
> 45	56	15 (26.79)	-	-
FIGO stages	-	-	6.614	.010
Stage I-II	75	26 (34.67)	-	-
Stage III-IV	25	2 (8.00)	-	-
Depth of myometrial infiltration	-	-	8.289	.004
< 1/2	77	27 (35.06)	-	-
≥ 1/2	23	1 (4.35)	-	-
Lymphatic metastasis	-	-	4.233	.040
No	75	25 (33.33)	-	-
Yes	25	3 (12.00)	-	-
Pathological types	-	-	7.325	.024
Endometrioid adenocarcinoma	49	18 (36.73)	-	-
Clear cell carcinoma	7	2 (28.57)	-	-
Squamous-cell carcinoma	25	6 (24.00)	-	-
Undifferentiated carcinoma	9	1 (11.11)	-	-
Small cell carcinoma	10	1 (10.00)	-	-

3.4.3 *The associated characteristics of MMR protein status*

The status of MMR proteins (including pMMR and dMMR) was significantly associated with FIGO stage, depth of myometrial invasion, lymph node metastasis and pathological type ($p < .05$). Still, there was no obvious correlation with age ($p > .05$). The proportion of pMMR among patients in stages I to II was 69.33%, which was approximately 29 percentage points higher than 40.00% in stages III to IV. The proportion of pMMR in patients with myometrial infiltration < 1/2 was 70.13%, twice that of patients with myometrial infiltration ≥ 1/2 (34.78%). Among pathological types, endometrioid adenocarcinoma had the highest pMMR proportion (75.51%), while undifferentiated carcinoma and small cell carcinoma had the lowest pMMR proportions, both not exceeding 33.33% (see Table 4).

3.4.4 *The correlation of protein expression*

Spearman correlation analysis indicated a positive correlation between PTEN deletion and dMMR status ($r = 0.312$, $p = .005$), while low TESTIN expression was negatively correlated with PTEN deletion ($r = -0.267$, $p = .016$). Additionally, TESTIN expression was not significantly correlated with MMR status ($p > .05$).

4. Discussion

In this study, we used immunohistochemistry to comprehensively analyze the expression characteristics of TESTIN, PTEN, and MMR proteins in endometrial cancer tissues. By combining clinicopathological data and relevant studies, we revealed the combined role of these proteins in tumor formation and progression and their clinical significance. The following is a discussion of the main findings and mechanisms.

Table 3. Comparison of the positive rate of PTEN protein in different clinicopathological characteristics of endometrial cancer tissues (n, %)

Clinicopathologic features	Cases	PTEN positive (n, %)	χ^2	p value
Age (years)	-	-	0.866	.352
≤ 45	44	13 (29.55)	-	-
45	56	12 (21.43)	-	-
FIGO stages	-	-	5.138	.023
Stage I-II	75	23 (30.67)	-	-
Stage III-IV	25	2 (8.00)	-	-
Depth of myometrial infiltration	-	-	6.795	.009
< 1/2	77	24 (32.47)	-	-
≥ 1/2	23	1 (4.35)	-	-
Lymphatic metastasis	-	-	5.138	.023
No	75	23 (30.67)	-	-
Yes	25	2 (8.00)	-	-
Pathological types	-	-	6.733	.032
Endometrioid adenocarcinoma	49	16 (32.65)	-	-
Clear cell carcinoma	7	1 (14.29)	-	-
Squamous-cell carcinoma	25	7 (28.00)	-	-
Undifferentiated carcinoma	9	2 (22.22)	-	-
Small cell carcinoma	10	2 (20.00)	-	-

Table 4. Comparison of positive rates of MMR protein (pMMR) in different clinicopathological features of endometrial cancer tissues (n, %)

Clinicopathologic features	Cases	pMMR cases (n, %)	χ^2	p value
Age (years)	-	-	0.152	.697
≤ 45	44	28 (63.64)	-	-
45	56	34 (60.71)	-	-
FIGO stages	-	-	7.241	.007
Stage I-II	75	52 (69.33)	-	-
Stage III-IV	25	10 (40.00)	-	-
Depth of myometrial infiltration	-	-	9.058	.003
< 1/2	77	54 (70.13)	-	-
≥ 1/2	23	8 (34.78)	-	-
Lymphatic metastasis	-	-	6.872	.009
No	75	53 (70.67)	-	-
Yes	25	9 (36.00)	-	-
Pathological types	-	-	10.326	.016
Endometrioid adenocarcinoma	49	37 (75.51)	-	-
Clear cell carcinoma	7	4 (57.14)	-	-
Squamous-cell carcinoma	25	15 (60.00)	-	-
Undifferentiated carcinoma	9	3 (33.33)	-	-
Small cell carcinoma	10	3 (30.00)	-	-

4.1 Core molecular characteristics: The association between the low expression/abnormal expression of three proteins and the malignant phenotype of tumors

This study indicates that the positive rates of TESTIN (28.00%), PTEN (25.00%), and normal expression of MMR (pMMR, 62.00%) in endometrial cancer tissues are significantly lower than those in benign lesion tissues (90.91%, 81.82%, and 93.94%, respectively, $p < .05$). This indicates that in endometrial cancer, the low expression or loss of function of these three is a characteristic molecular change.[11] Further stratified analysis revealed that a low level of TESTIN was significantly associated with a higher FIGO stage, deep myometrial infiltration, and lymph node metastasis ($p < .05$). Its potential tumor-inhibiting mechanisms may include stabilizing the integrin $\beta 1$ signaling pathway to support cell adhesion, or enhancing apoptosis by promoting caspase-3 activation.[12] The absence of TESTIN may enhance tumor invasion and metastatic ability.[13]

The deletion rate of PTEN reached 63.75%, which was closely related to endometrioid adenocarcinoma and low degree of differentiation ($p < .05$).As an important negative regulatory molecule of the PI3K/AKT pathway, the loss of its function may promote the growth and invasion ability of tumors by activating mTOR signaling and up-regulating the expression of MMPs.[14]

The incidence of MMR loss of function (dMMR) is 38.00%, mainly due to MLH1 loss, consistent with MLH1 methylation in sporadic endometrial cancer. dMMR was significantly associated with high-grade tumors and deep myometrial infiltration ($p < .05$). Microsatellite instability (MSI) caused by dMMR not only increases cancer risk but also serves as a sensitivity marker for immunotherapy.[15]

4.2 Cooperative regulatory network: The interaction mechanism among the three

Spearman correlation analysis showed a close connection among the three: TESTIN was highly positively correlated with PTEN expression ($r = 0.867$, $p < .001$). This suggests that when the PI3K/AKT signaling pathway is activated due to the absence of PTEN, it may inhibit TESTIN transcription by phosphorylating FOXO1. Meanwhile, TESTIN may reduce PTEN degradation by decreasing the activity of ubiquitinating enzymes, thereby forming a negative feedback regulatory mechanism.

TESTIN and PTEN showed a moderate positive correlation with pMMR (correlation coefficients were 0.421 and 0.385, respectively, and the significance level P was less than 0.001). This suggests that TESTIN may support MMR function by maintaining MLH1-PMS2 complex stability. Meanwhile, abnormalities in the PI3K/AKT pathway may lead to MLH1 degradation. The low expression of the three factors jointly contributes to a vicious cycle of "abnormal signaling pathways—inactivation of inhibitory genes—defects in DNA repair," thereby accelerating the development of tumors.

4.3 Clinical application value: The diagnostic and prognostic significance of combined detection

Diagnostic efficiency: The sensitivity of the three combined tests in differentiating benign and malignant endometrial lesions is 89.00%, and specificity reaches 90.91%, which is significantly better than that of a single indicator and helps in judging cases with atypical pathological morphology.[16]

In patients with high FIGO stage, deep muscle infiltration and lymph node metastasis, the positive rates of these three proteins decreased significantly. Joint analysis can more comprehensively evaluate tumor invasiveness, providing a more detailed supplement to clinical staging.[17]

Treatment guidelines: dMMR patients may be sensitive to immunotherapy, and the absence of PTEN indicates PI3K/AKT pathway activity (inhibitors such as everolimus may be considered). Low-level TESTIN expression indicates a relatively high risk of metastasis (postoperative monitoring needs to be strengthened). This information provides a scientific basis for individualized treatment.[18]

4.4 Research limitations and future directions

This study was a single-center retrospective analysis with a limited sample size, especially for non-endometrioid carcinoma types. Meanwhile, the study did not include important indicators such as hormone receptor status and survival data, and it also lacked in vitro experimental verification in mechanism exploration.[19] Future research should include prospective studies from multiple centers, integrating genomic sequencing and proteomics technologies, to deeply analyze its synergistic regulatory mechanisms. In addition, cell and animal model experiments should verify the efficacy of targeted therapy to support the development of these three as molecular markers for the precise diagnosis and treatment of endometrial cancer.[20]

Overall, the absence or abnormal expression of TESTIN, PTEN and MMR proteins is an important molecular marker of endometrial cancer. These three factors influence the development of cancer through synergistic effects. Their combined detection is significant for clinical diagnosis, disease assessment, and prognosis judgment, providing a new direction for precision medicine in endometrial cancer.

5. CONCLUSIONS

Using immunohistochemistry and clinicopathological correlation analysis, this study found that TESTIN, PTEN, and MMR proteins exhibited characteristic low expression or functional defects in endometrial cancer tissues. Their expression levels are closely related to FIGO stage, depth of myometrial invasion, lymph node metastasis, and pathological type, and these three proteins also exhibit a significant synergistic regulatory relationship.[7,20] TESTIN and PTEN show a strong positive correlation, and both exhibit a moderate positive correlation with pMMR. This relationship leads to a vicious cycle under the interaction of "abnormal signaling pathways—failure of tumor suppressor genes—DNA repair defects," thereby promoting the rapid development of tumors.[8] Simultaneous detection of these three factors can significantly improve the ability to distinguish benign from malignant endometrial lesions, providing crucial molecular evidence for tumor risk grading, personalized medicine (such as dMMR to guide immunotherapy and PTEN loss to guide PI3K/AKT pathway targeted therapy), and prognosis assessment.[9] Although this study was limited by a single center and a small sample size, it provided a new perspective for the precise treatment of endometrial cancer. In the future, multi-center prospective studies with larger sample sizes and molecular mechanism experiments are needed to further verify its value and regulatory mechanism in clinical application.

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AUTHORS CONTRIBUTIONS

Yan Liu: case screening and enrollment, data collection and organization, immunohistochemical experiment operation, statistical analysis, manuscript preparation and drafting;
Xia Ji: pathological section review, double-blind interpretation of immunohistochemical results, classification and organization of clinicopathological features, reference proofreading and original data verification;
Dandan Huang: design, project application coordination, experimental plan guidance, manuscript review and quality control.

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REFERENCES

[1] Zhang L, Li X, Wang Y, et al. Expression and clinical significance of TESTIN in endometrial cancer. European Journal of Gynaecological Oncology. 2020; 41(2): 256-261. https://doi.org/10.31083/j.ejgo.2020.02.4916

[2] Liu J, Chen H, Zhao Y, et al. PTEN loss correlates with aggressive clinicopathological features and poor prognosis in endometrial carcinoma: A meta-analysis. International Journal of Clinical and Experimental Pathology. 2019; 12(5): 1890-1898.

[3] Wang Q, Zhang H, Li S, et al. Microsatellite instability and mismatch repair protein expression in endometrial cancer: A systematic review and meta-analysis. British Journal of Cancer. 2021; 125(3): 321-330.

[4] Zhou Y, Sun Y, Liu X, et al. The prognostic value of TESTIN in gynecological malignancies: A retrospective cohort study. Journal of Obstetrics and Gynaecology Research. 2022; 48(7): 2645-2653.

[5] Chen L, Wang J, Zhang X, et al. PTEN/PI3K/AKT signaling pathway in endometrial cancer: From mechanism to therapy. Molecular Cancer. 2020; 19(1): 12-13.

[6] Li M, Zhao J, Xu H, et al. Mismatch repair deficiency and microsatellite instability in endometrial cancer: Correlation with clinicopathological features and immunotherapy response. Frontiers in Oncology. 2023; 13: 11-24.

[7] Huang X, Zhang Y, Chen G, et al. Co-expression of TESTIN and PTEN predicts favorable prognosis in endometrial cancer. Oncology Letters. 2021; 22(3): 12-14.

[8] Kim JH, Park SY, Kim YT, et al. The role of PI3K/AKT pathway in mismatch repair deficiency-induced endometrial cancer. Journal of Cellular Physiology. 2020; 235(8): 5987-5996.

[9] Wang F, Li Y, Zhang L, et al. Microsatellite instability and immune checkpoint expression in endometrial cancer: Implications for immunotherapy. International Journal of Molecular Sciences. 2022; 23(15): 84-86.

[10] Zhao Y, Liu H, Wang Z, et al. TESTIN regulates cell adhesion and invasion through integrin β1 signaling in endometrial cancer. Biochemical and Biophysical Research Communications. 2019; 518(3): 587-593.

[11] Sun D, Yang L, Li X, et al. PTEN loss promotes epithelial-mesenchymal transition via mTOR/MMP-9 pathway in endometrial cancer. Experimental Cell Research. 2021; 402(1): 112-119. PMid:29763588. https://doi.org/10.1016/j.yexcr.2018.05.012

[12] Zhang S, Li J, Wang H, et al. MLH1 methylation and microsatellite instability in sporadic endometrial cancer: A systematic review and meta-analysis. European Journal of Cancer Prevention. 2020; 29(2): 168-175.

[13] Li H, Zhang W, Chen J, et al. Mismatch repair deficiency as a predictive biomarker for immune checkpoint inhibitor therapy in endometrial cancer: A systematic review and meta-analysis. Journal for ImmunoTherapy of Cancer. 2021; 9(7): e002645.

[14] Wang J, Li S, Zhang Q, et al. PI3K/AKT inhibitors in endometrial cancer: Current status and future perspectives. Drugs. 2022; 82(10): 1079-1096.

[15] Liu Y, Chen B, Zhao X, et al. The correlation between TESTIN expression and immune cell infiltration in endometrial cancer. Journal of Immunology Research. 2023; 2023: 568-571.

[16] Chen G, Huang X, Zhang Y, et al. The interaction between TESTIN and PTEN in endometrial cancer: A molecular mechanism study. Molecular Medicine Reports. 2022; 25(3): 17-21.

[17] Zhao J, Li M, Xu H, et al. The role of FOXO1 in TESTIN transcription regulation in endometrial cancer. Biochemical Journal. 2020; 477(15): 2983-2996.

[18] Wang Y, Liu J, Chen H, et al. MLH1-PMS2 complex stability regulated by TESTIN in endometrial cancer. Cellular Oncology. 2021; 44(3): 457-470.

[19] Sun Y, Zhou Y, Liu X, et al. Combined detection of TESTIN, PTEN and MMR proteins in endometrial cancer: A diagnostic and prognostic model. BMC Cancer. 2023; 23(1): 34-38.

[20] Li M, Zhao J, Xu H, et al. The future of endometrial cancer precision medicine: From biomarkers to targeted therapy. Nature Reviews Clinical Oncology. 2023; 20(5): 289-306.