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Estrogen receptor ER-α36: A diagnostic biomarker for endometrial cancer

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ABSTRACT

Background: In this experiment, we intend to investigate the relationship between ER-α36 expression and endometrial cancer. ***Materials and Method:*** A total of 172 healthy control and endometriosis patients were selected from our hospital. These included 43 cases of normal endometrium (NE group), 43 cases of endometrium without atypical hyperplasia (EH group), 43 cases of atypical hyperplasia (AH group) and 43 cases of endometrial cancer (EC group). The expression of ER-α36 in these tissues was detected by immunohistochemical methods. ***Results:*** The positive rate of estrogen receptor (ER-α36) in each group was 4.24%±5.02%, 5.74%±6.34%, 9.69%±9.42%, and 11.78%±10.39%, respectively. The expression of ER-α36 demonstrated a notably higher level in the AH and EC groups compared to the EH and NE groups. A statistically significant difference was observed between the NE group and the AH group (P=0.0112). The NE group was statistically different from the EC group (P=0.0001).There was a statistical difference between the EH group and the EC group (P=0.0040). Among endometrial cancers, the mean positive rate of ER-α36 expression was 11.67%±6.74% in highly differentiated endometrial cancers, 9.45%±11.38% in moderately differentiated, and 14.82%±11.35% in poorly differentiated. Comparison between the two groups showed that there was no statistically significant difference in the expression positivity rate of ER-α36 in endometrial cancer of different degrees of differentiation (P>0.05). ***Conclusion:*** ER-α36 has a certain diagnostic efficacy for endometrial cancer and can be used as an auxiliary judgment tool for pathological examination.

INTRODUCTION

Endometrial cancer (EC) is one of the three major malignant tumors in gynecology that seriously endanger women's physical and mental health and life safety, and its incidence has been the first gynecological malignant tumor in some developed countries such as the United States [1-3]. With the changes in lifestyle, such as poor diet and living habits, work pressure, rest and relaxation, exogenous estrogen intake and other EC high-risk exposure factors, the incidence of EC is continuously rising. Although traditional surgery and radiotherapy are currently the first-line EC treatment modalities, they are traumatic and affect patients' quality of life after treatment [4-5]. Although highly effective progestin can reverse endometrial lesions and are currently effective conservative treatments that may provide hope for preserving the reproductive needs of patients with fertility needs, they may increase the risk of breast cancer and thrombosis [6]. There is a lack of effective assessment indexes for predicting the prognosis of conservative treatments [7]. Mining the correlates of EC can provide a theoretical basis for subsequent research and exploration of the pathogenesis of EC, searching for therapeutic targets, prognostic factors, and diagnosis, which is an urgent issue for gynecologic oncology researchers [8-9].

Endometrial cancer is classified into type I and type II. Type I, also known as endometrioid adenocarcinoma, is associated with prolonged estrogen stimulation, and this type of adenocarcinoma is the predominant pathological type, accounting for nearly 90% of all endometrial cancers. In instances characterized by a high positive rate [10-11] of estrogen receptor (ER) and progesterone receptor (PR) expression, estrogen binds to ER, initiating downstream responses. Classical estrogen receptors encompass estrogen receptor α (ERα) and estrogen receptor β (ERβ), which regulate the biological effects of estrogen in the nucleus.ERα is a member of the nuclear receptor superfamily, and ER-α is subdivided into three isoforms, ER-α66, ER-α46, and ER-α36 which mainly exert their biological functions.ERα plays a role in the development of the female reproductive system, affecting the proliferation and differentiation of the endometrium [12].Studies have confirmed that The transcription of genes mediated by ERα plays a pivotal role in the process of type I endometrial

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carcinogenesis [13].

An increasing number of studies have elaborated the relationship that exists between ER-α36 and the development of malignant tumors, which has potential application in the diagnosis of endometrial cancer. Firstly, estrogen passes through ER- α 36 can activate PKC δ Signal pathway. This suggests that estrogen dependent endometrial cancer proliferation may be associated with ER-α. By regulating ER-α the expression of 36 can affect PKC δ The activity of. This in turn affects the proliferation and apoptosis of tumor cells. Secondly, PKC α The increase in activity is related to the migration and proliferation of tumor cells. Therefore, by regulating ER- α. The expression of 36 may also affect PKC α The activity of. This affects the migration and proliferation of tumor cells. Currently, the diagnosis of endometrial cancer mainly relies on pathologic examination and imaging, but these methods have certain limitations and shortcomings. Therefore, the search for new biomarkers is important to improve the diagnostic accuracy and early detection of endometrial cancer [14]. Within the scope of this investigation, we intend to detect the positive level of ER-α36 expression in normal endometrium, endometrium without atypical hyperplasia, endometrium with atypical hyperplasia, endometrial carcinoma and to fully analyze the correlation between ER-α36 and endometrial carcinomas, so as to provide a basis for the future exploration of endometrial carcinomagenesis. Explore new therapeutic targets and provide new drug mechanisms for the treatment of endometrial cancer.

MATERIALS AND METHODS

Study subjects and sample collection

Patients who visited our hospital and underwent segmental diagnostic scraping from 2021 to June 2023 were collected and divided into four groups: 43 instances characterized by normal endometrium (NE), 43 cases of endometrium without atypical hyperplasia (EH), A total of 43 cases involving atypical hyperplasia (AH) and an additional 43 cases associated with endometrial carcinoma (EC), a total of 172 patients were enrolled (table 1), and endometrial cancer was classified according to the histological grading into highly differentiated, moderately differentiated and poorly differentiated endometrial cancer. In this study, we will use immunohistochemical staining to compare the expression of ER-α36 in the NE, EH, AH and EC groups. Having obtained approval from the Medical Ethics Committee of the Fourth Hospital of Shijiazhuang on January 3, 2021 (Ethics No: 20210030), the study proceeded with patients duly signing an informed consent form.

Table 1. General information of patients.

		NE	EH	AH	EC	*P* values
Age (ys)		44.58±7.29	44.84±6.45	47.49±9.73	51.19±8.34	<0.001
Height (cm)		158.49±4.92	158.75±5.42	156.24±4.29	158.14±5.37	0.089
Weight (kg)		59.27±8.61	62.40±7.35	62.21±8.05	60.59±8.39	0.238
body mass index BMI (kg/m^2)		23.77±3.09	24.87±2.30	25.46±3.25	24.27±3.28	0.057
CA125 (U/ml)		36.29±57.81	32.61±57.06	36.78±62.54	29.37±27.05	0.907
CA199 (U/ml)		15.21±29.24	17.75±45.31	27.59±40.25	40.35±64.28	0.055
preoperative endothelial thickness (mm)		10.07±58.39	15.03±12.37	11.26±5.24	17.01±10.34	0.002
ER-α36 positivity rate (%)		4.24±5.02	5.74±6.34	9.69±9.42	11.78±10.39	<0.001
Pregnancy frequency						0.778
0		2 (4.65%)	4 (9.30%)	3 (6.98%)	2 (4.65%)	
1		9 (20.93%)	8 (18.60%)	7 (16.27%)	4 (9.30%)	
2		11 (25.59%)	12 (27.91%)	14 (32.56%)	16 (37.20%)	
3		6 (13.95%)	6 (13.95%)	9 (20.93%)	11 (25.58%)	
4		6 (13.95%)	5 (11.63%)	8 (18.60%)	3 (6.98%)	
5		4 (9.30%)	2 (4.65%)	1 (2.33%)	3 (6.98%)	
6		3 (6.98%)	3 (6.98%)	1 (2.33%)	3 (6.98%)	
7		2 (4.65%)	2 (4.65%)	0 (0.00%)	0 (0.00%)	
8		0 (0.00%)	1 (2.33%)	0 (0.00%)	1 (2.33%)	
Production frequency						0.198
0		2 (4.87%)	4 (10.26%)	3 (7.32%)	1 (2.44%)	
1		9 (21.94%)	8 (20.51%)	7 (17.07%)	4 (9.76%)	
2		11 (26.83%)	12 (30.77%)	14 (34.15%)	16 (39.01%)	
3		6 (14.63%)	5 (12.82%)	8 (19.51%)	11 (26.83%)	
4		6 (14.63%)	5 (12.82%)	7 (17.07%)	3 (7.32%)	
5		4 (9.76%)	2 (5.13%)	1 (2.44%)	3 (7.32%)	
6		3 (7.32%)	3 (7.69%)	1 (2.44%)	3 (7.32%)	
high blood pressure	no	41 (95.35%)	39 (90.70%)	35(81.40%)	37 (86.05%)	0.046
	yes	2 (4.65%)	4 (9.30%)	8(18.60%)	6 (13.95%)	
diabetes	no	43 (100%)	43 (100%)	42 (97.67%)	40(93.02%)	0.108
	yes	0 (0.00%)	0 (0.00%)	1 (2.33%)	3(6.98%)	
menopause	no	36 (83.72%)	41 (95.35%)	36(8372%)	16(37.21%)	<0.001
	yes	7 (16.28%)	2 (4.65%)	7 (16.28%)	27(62.79%)	

NE: normal endometrium, EH: Denoting atypical hyperplasia as AH and endometrial carcinoma as EC, the "statistical characteristics" were computed using a Student t-test, facilitating a comparison between the control group and the treatment group.

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Inclusion criteria: 1) women aged 18 years or older; 2) patients who underwent diagnostic curettage or hysteroscopic diagnostic curettage in the Department of Gynecology of our hospital at this time, and whose postoperative pathological diagnosis was normal endometrium, endometrium without atypical hyperplasia, endometrial atypical hyperplasia, endometrial carcinoma; and 3) patients who do not have moderate-to-severe uterine adhesion, and who have not been previously operated on for endometrial debridement, and who can successfully complete the diagnostic curettage.

Collection and processing of specimens

All specimens were taken from the Department of Pathology of our hospital, Subjected to fixation in 10% formalin (Nanjing Fomax Biotechnology Co, China) and subsequent embedding in paraffin (Nanjing Fomax Biotechnology Co, China), followed by prepared tissue sections for pathological diagnosis and immunohistochemical detection. All specimens were processed by paraffin sectioning, and five consecutive sections were taken, each section was first stained with HE staining to clarify the pathological diagnosis, and the sections confirmed by pathological diagnosis were then stained by immunohistochemistry. All sections were selected under the same conditions after sectioning was completed by placing them in a 60°C oven and baking the slices for 2 hours, so that the adhesive was tightly attached to the slices to prevent the tissues from being delaminated during the test. Afterwards, they were placed in a section box and stored in a 4°C refrigerator. Use a microscope (Leica, Germany) to observe [15].

① Baking: Tissue paraffin sections were preheated on a 60□ baking machine (Hubei Yaguang Medical Electronic Technology Co, China) for 45 minutes.

②Dewaxing: The preheated sections were immersed in xylene A and B cylinders for 15 minutes each.

Remove the sections from the xylene cylinders and place them in different gradients of alcohol (Nanjing Fomax Biotechnology Co, China) (100%, 95%, 90%, 80%, 70%) for 10 minutes in each cylinder to fully dewax the sections.

At the end of dewaxing, the sections were immersed into the PBS vat for 3 times, each time for 2 minutes.

③ Permeabilization: 200 μl of TritonX-100 working solution (Beijing Solabao Biotechnology Co, China) was added dropwise to each section. After incubating for 20 minutes at room temperature, the samples were rinsed with PBS buffer (Biological Industries, Israel) three times, each session lasting 3 minutes.

④ Blocking endogenous peroxidase: 200 μl of endogenous peroxidase blocker (Dalian Meilun Biotechnology Co, China) was aspirated and placed dropwise on the sections and incubated at room temperature for 12 minutes. Subsequently, the sections were washed 3 times with PBS buffer for 3 minutes each time.

⑤ Closure: 200 μl of closure was aspirated with normal goat serum closure solution (Nanjing Fomax Biotechnology Co, China), dropped on the sections and placed at room temperature for 12 minutes of incubation, goat serum closure solution was discarded and not washed.

⑥ Incubation of primary antibody: aspirate 200 μl drops of freshly prepared ER-α36 antibody (GeneTex Corporation, China) on the sections. Overnight in a thermostatic incubator (ThermoFisher Scientific, USA) at 4°C. Wash 3 times with PBS buffer for 3 minutes each time.

⑦ Manipulation of biotin-tagged goat-derived anti-mouse/rabbit IgG polymer: A 200 μl droplet of aspirated biotin-tagged goat-derived anti-mouse/ rabbit IgG polymer (Beijing Zhongsui Jinqiao Biotechnology Co, China) was administered onto the specimens and subjected to a 12-minute treatment at ambient temperature. Subsequently, rinsing was conducted thrice using PBS buffer, with each wash lasting 3 minutes.

⑧ Treatment of horseradish enzyme labeled streptavidin ovalbumin: 200 μl of aspirated horseradish enzyme labeled streptavidin ovalbumin (Nanjing Fomax Biotechnology Co, China) was used as the working fluid and treated for 12 minutes at room temperature. Wash with PBS buffer 3 times for 3 minutes each time.

⑨ Color development: 200 μl of freshly prepared DAB color development solution (Nanjing Fomax Biotechnology Co, China) was aspirated and dropped on the section, incubated at room temperature for 1-2 minutes, and the excess staining solution was rinsed off with tap water.

⑩ Restaining: immerse the section in hematoxylin staining solution (Shanghai Biyuntian Biotechnology Co, China) for 30-60 seconds to stain the nuclei. Differentiate for a few seconds to remove excess staining and place in tap water to return to blue for 5-7 minutes.

⑪ Tissue dehydration and sealing: Sections were automatically dehydrated according to the following procedure: distilled water (5min) → 50% alcohol (5min) → 75% alcohol (5min) → 85% alcohol (5min) → 95% alcohol (5min) → anhydrous ethanol (10min) → xylene A (10min) → xylene B (10min). The slices were removed and dried, one drop of neutral resin (Wuhan Doctor Bio-engineering Co, China) was added to each slice, covered with a coverslip and placed in a ventilated area to dry, observed under a light microscope and photographed (figure.1).

ER-α36 positivity rate detection

In this study, Image 1.8.0 image analysis software will be applied for quantitative analysis, and the proportion of ER-α36-positive cells to all cells (the total number of cells was obtained by calculating the nuclear staining of the cells) was analyzed by ImageJ

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1.8.0 software and was recorded as N% as a quantitative result of the overall ER-α36 expression positivity rate.

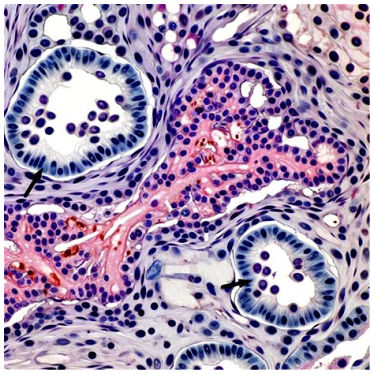

Figure 1. Immunohistochemical image. Note: Here is an immunohistochemical image showing the expression of estrogen receptor ER-α36 in uterine endometrial cancer tissue. The image displays a microscopic view of tissue cells with ER-α36 expression, highlighted by specific staining, and regions of interest are indicated with arrows. The magnification is 50x.

Statistical methods

ER 2.0 software was used for statistical analysis as follows: representation of measurement data was conveyed as mean ± standard deviation. Comparative analyses among various groups were conducted using ANOVA. Meanwhile, count data were presented as N%, and the chi-square test was employed to assess inter-group differences in unordered count data within multiple groups, and $P<0.05$ was recognized as the difference was statistically significant. Correlation analysis was performed using logistic regression correlation analysis. Expression of ER-α36 in different endometrial lesions and endometrial cancers of different degrees of differentiation was analyzed by ANOVA. Statistical significance was deemed established when the P-value fell below 0.05.

RESULTS

Impact of different factors

As can be seen in table 1, the higher the ER-α36 positivity rate, the higher the risk of endometrial lesions ($P<0.001$); when the ER-α36 expression positivity rate was sorted and then grouped into three equal groups, it was found that compared with the Q1 group (0.03%-2.29%), the patients in the Q2 group (2.41%-8.55%) and the Q3 group (8.60%-49.22%) had an increased were at increased risk of lesions ($P=0.7005$, 0.0001). The risk of endometriosis was positively correlated with age in the patients included in this study, and the risk of endometriosis was significantly lower in the younger age group (Q1, 26-45 ys) than in the older age group (Q3, 51-85 ys) when the age was sorted and grouped into three equal categories ($P=0.0003$), as shown in table 2.

Table 2. Correlation analysis of different associated factors with the occurrence of endometriosis in the enrolled cases.

		Sample Size	α/OR	95%CI	*P* values
Age (ys)		47.03±9.35	1.10	(1.05,1.14)	<0.0001
Age group (ys)	Q1 (26-45)	55 (31.98%)	ref		
	Q2 (46-50)	57 (33.14%)	1.02	(0.48,2.18)	0.9640
	Q3 (51-85)	60 (34.88%)	4.35	(1.97,9.60)	0.0003
ER-α36 positivity rate (%)		7.88±8.57	1.11	(1.05,1.16)	<0.0001
Grouping of ER-α36 positivity rates (%)	Q1 (0.03-2.29)	57 (33.14%)	ref		
	Q2 (2.41-8.55)	57 (33.14%)	1.16	(0.55,2.47)	0.7005
	Q3 (8.60-49.22)	58 (33.72%)	4.80	(2.16,10.66)	0.0001
CA125 (U/ml)		33.76±50.27	1.00	(0.99,1.01)	0.7944
CA199 (U/ml)		25.23±60.88	1.02	(0.97,1.05)	0.0724
preoperative endothelial thickness (mm)		13.34±8.37	1.01	(0.97,1.05)	0.7770

Note: Statistical properties were evaluated using the student t-test, which involved a comparison between the control group and the treatment group. Effects of confounding factors.

Table 3 shows that in the unadjusted model, the risk of developing endometriosis increased by 11% for each unit increase in ER-α36 (OR: 1.11, 95% CI 1.05, 1.16, $P<0.0001$); after adjusting for the age factor, the OR value of developing endometriosis was 1.09 (OR: 1.09, 95% CI 1.04,1.15, and $P=0.0005$). After sorting the ER-α36 expression positivity rate and then grouping into three equal groups, the risk value of developing endometriosis in groups Q2 and Q3 increased with increasing ER-α36 expression positivity rate compared with group Q1, and the trends were statistically different. The OR of age for developing endometriosis in the unadjusted model was 1.10 (OR: 1.10, 95% CI 1.05, 1.14, $P<0.0001$). After adjusting for factors related to body mass index, hypertension, diabetes mellitus, and menopause, the OR was 1.07 (OR: 1.07, 95% CI 1.01, 1.13, $P=0.0237$).

Table 3. Multiple regression analysis of the correlation between ER-α36 and the occurrence of endometrial lesions.

		Unadjusted model (OR, 95%CI, *P*)	Adjusted model I (OR, 95%CI, *P*)	Adjusted model II(OR, 95%CI, *P*)
ER-α36 positivity rate		1.11 (1.05,1.16) <0.0001	1.09 (1.04,1.15) 0.0005[a]	1.09 (1.03,1.15) 0.0014[b]
grouping of ER-α36 positivity rates	Q1	1.0	1.0	1.0
	Q2	1.16 (0.55,2.47) 0.7005	1.03 (0.46,2.28) 0.9498[a]	1.07 (0.45,2.60) 0.8862[b]
	Q3	4.80 (2.16,10.66) 0.0001	3.66 (1.58,8.49) 0.0025[a]	3.74 (1.47,9.45) 0.0053[b]
trend testing		<0.0001	0.0008	0.0019
group (ys)		1.10 (1.05,1.14) <0.0001	1.11 (1.06,1.16) <0.0001[c]	1.07 (1.01,1.13) 0.0237[d]

Note: a: Controlled for age; b: Controlled for age and body mass index, hypertension, diabetes, and menopause; c: Controlled for body mass index; d: Controlled for body mass index, hypertension, diabetes, and menopause. Statistical features were determined through a Student t-test, involving a comparison between the control group and the treatment group. Comparison of ER-α36 in different style of EC.

ER-α36 was expressed in different endometrial

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tissues, and the results showed that the average ER-α36 positivity rate in NE group was 4.23% ± 5.17%, the average ER-α36 positivity rate in EH group was 5.76% ± 6.50%, the average ER-α36 positivity rate in AH group was 9.69% ± 9.57%, and the average ER-α36 positivity rate in EC group was 11.78% ± 10.00% (Table 4). Upon comparing each group with the other, notable statistical differences emerged: there was a significant distinction between the NE group and the AH group (P=0.0111), a marked difference between the NE group and the EC group (P=0.0001), and a statistically significant variance between the EH group and the EC group (P=0.0040)., and no statistical difference in the average positive rate of ER-α36 expression between the rest of the groups of the NE and the EH group, the EH and the AH group, and the AH and the EC group (P>0.05) (table 5).

Table 4. Expression of ER-α36 positivity in different endometrial lesion types.

pathology	number of samples	average	standard deviation	minimum value	median	maximum values
NE	43	4.23	5.17	0.07	2.45	24.47
EH	43	5.76	6.50	0.04	3.42	27.53
AH	43	9.69	9.57	0.03	5.50	30.60
EC	43	11.78	10.00	1.77	9.76	49.22

Note: NE: normal endometrium, EH: atypical hyperplasia is denoted as AH, and endometrial carcinoma is represented by EC.

Table 5. Comparison of ER-α36 positive expression in different endometrial lesions.

pathology	pathology	mean difference	lower limit of the 95% range	upper limit of the 95% band	*P* value
EH	NE	1.50	-3.06	6.05	0.8288
AH	NE	5.46	0.93	9.98	0.0112
EC	NE	7.55	3.01	12.05	0.0001
AH	EH	3.94	-0.60	8.51	0.1133
EC	EH	6.04	1.49	10.59	0.0040
EC	AH	2.09	-2.44	6.61	0.6297

Note: NE: normal endometrium, EH: atypical hyperplasia is designated as AH, and endometrial carcinoma is denoted as EC. The determination of "statistical characteristics" was conducted through a Student t-test, involving a comparison between the control group and the treatment group.

Comparison of ER-α36 in different degrees of EC

The enrolled endometrial cancer cases were divided into three groups according to the pathological results: low-differentiated, middle-differentiated, and highly-differentiated endometrial cancer groups, and ANOVA was performed. The mean positive rate of ER-α36 expression in the highly-differentiated, middle-differentiated, and low-differentiated endometrial cancer groups was 11.67%±6.74%, 9.45%±11.38%, and 14.82% ±11.35%, respectively (table 6).Comparisons between the two groups Comparison revealed that there was no statistically significant difference in the positive rate of ER-α36 expression between endometrial cancer groups of different differentiation degrees (*P*>0.05) (table 7).

Table 6. Expression of ER-α36 in patients with endometrial cancer of different degrees of differentiation.

pathology	number of samples	average	standard deviation	minimum value	median	maximum values
high differentiation	14	11.67	6.74	2.64	11.09	21.46
middle differentiation	18	9.45	11.38	2.06	6.06	49.22
low differentiation	11	14.82	11.35	1.77	12.79	41.97

Table 7. Comparison of ER-α36 expression in patients with endometrial cancer of different degrees of differentiation.

pathology	pathology	mean difference	lower limit of the 95% range	upper limit of the 95% band	*P* value
high differentiation	middle differentiation	-2.23	-10.95	6.51	0.8089
high differentiation	low differentiation	3.15	-7.31	13.61	0.7477
middle differentiation	low differentiation	5.37	-4.62	15.38	0.3974

Note: Statistical properties were assessed using a Student t-test, comparing the control group to the treatment group.

DISCUSSION

ER-α36 has the capability to activate the mitogen-activated protein kinase/extracellular signal -regulated kinase (MAPK/ERK) signaling pathway. Causing related estrogen- and anti-estrogen-dependent activation and stimulating cell growth [16]. ER-α36 can work with epidermal growth factor receptor (EGFR) to regulate tumor biological behaviors [17]. ER-α36 has the capacity to collaborate with the epidermal growth factor receptor (EGFR) in order to regulate the biological behavior of tumors, such as promoting the proliferation of hepatocellular carcinoma cells, and participating in the resistance of breast cancer to platinum and tamoxifen, etc. ER-α36 also participates in the resistance of tumors to drugs by mediating the PI3K/Akt pathway, which is related to the cell growth and survival, as well as exerting neuroprotective effects using rapid hormone signaling. Numerous studies have demonstrated the involvement of ER in a variety of tumor development processes. Bonkhoff [18] showed that up-regulation of ER-α, in an animal model of high-grade prostatic intraepithelial neoplasia (HGPIN), mediated the oncogenic effects of estradiol. Partial loss of ER-β in HGPIN suggests an oncostatic role for ER-β. Cheng [19] *et al.* in a large lung cancer sample study confirmed that both ER-α (β = 45.0, *P*<0.001) and ER-β (β = 25.9, *P*<0.001) were higher in the cytosol of tumor tissues of patients with a history of smoking than in patients without a history of smoking, and elevated levels of ER-α and ER-β expression were linked to diminished survival outcomes. Ge [20] *et al.* also summarized in the meta-analysis of gastric cancers of the TCGA the relationship between ER- α and ER-β

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with clinicopathologic features and overall survival time of gastric cancer, ER-α could be a correlate of poor prognosis in patients with helminthic cancer, whereas the lower the expression of ER-β, the higher the lymph node metastasis. The above studies suggest that the estrogen receptor family plays an extremely important role in tumor development.

In this study, we proposed to examine the expression of ER-α36 in different endometrial lesions to reveal whether the abnormal expression of ER-α36 is associated with the severity of endometrial lesions. Commonly used detection methods for protein expression include protein blotting (western blot, WB) and immunohistochemistry (IHC), which both have high specificity in the binding of antibodies and antigens, and are commonly used for protein determination and quantitative analysis. Therefore, immunohistochemistry was used in this study. The results are shown by table 5. From this, we hypothesized that the high expression of ER-α36 altered the signaling pathway of the receptor, which promoted the associated tumorigenesis. However, the accuracy of the statistical efficacy of this study is not fully guaranteed due to the limited sample size when subdividing the subgroups, and further studies to expand the sample size will be carried out in the future, in addition to the need to further clarify the mechanism of action from the perspective of basic research.

Although the exact etiology of endometrial cancer is still unclear, most experts believe that it is associated with prolonged endogenous or exogenous estrogen stimulation without progesterone antagonism. Domestic scholars Wu [21] and others applied RT-PCR to detect the expression of ER-α36 in normal endometrial tissues and endometrial cancer tissues. The results showed that ERα mRNA expression was lower in normal endometrial tissues than in endometrial cancer tissues. It was also confirmed that ERα expression was not related to the degree of pathological differentiation and prognosis of endometrial cancer. The results of this study showed that the expression of ER-α36 was different in endometrial cancers with different degrees of differentiation, and there was no statistically significant difference in the analysis of the expression positivity rate of each group ($P>0.05$). The results of this paper are consistent with most reports in the literature, suggesting that ER-α36 has no significant effect on the progression of endometrial cancer progression [22-23]. Qiu *et al.* [24] and Li *et al.* [25] found that age is a risk factor for most malignant tumors. As age increases, the risk of developing malignant tumors increases. At the same time, tumor mortality rates likewise increase with age. From table 2, it can be seen that age is associated with AH and EC, and the risk of endometriosis increases with age after adjusting for hypertension, diabetes mellitus, BMI and other related factors. Therefore, postmenopausal women should also adhere to regular physical examinations for early detection and treatment, especially in patients with combined endometrial cancer risk factors (e.g., obesity, hypertension, diabetes mellitus, late menopause, etc.).

CONCLUSION

The expression of ER- α36 is associated with the occurrence of endometrial lesions. The higher the positive rate of ER- α36 expression, the higher the risk of developing endometrial lesions. Mean ER-α36 expression was progressively higher in NE, EH, AH, and EC. As age increases, the risk of developing endometrial lesions also increases.

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