RESEARCH ARTICLE

Expression of ER, PR, C-erbB-2 and Ki-67 in Endometrial Carcinoma and their Relationships with the Clinicopathological Features

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Abstract

Background: To analyze the expression of estrogen receptors (ER), progesterone receptors (PR), C-erbB-2 and Ki-67 in endometrial carcinoma (EC) and their relationships with the clinicopathological features. Materials and Methods: Sixty-seven EC samples, 53 normal endometrial samples and 53 atypical hyperplasia endometrial samples were all selected in Shaanxi Provincial People's Hospital from Jun., 2012 to Jun., 2014. The expression of ER, PR, C-erbB-2 and Ki-67 in EC tissue, normal endometrial tissue and atypical hyperplasia endometrial tissue was respectively detected using immunohistochemical SP method. The relationships between the expression of ER, PR, C-erbB-2 and Ki-67 and the patients' clinicopathological features as well as their correlations in EC tissue were also analyzed. Results: The positive expression rates of ER and PR in EC tissue were 44.8% and 41.8%, respectively, dramatically lower than in atypical hyperplasia endometrial tissue and normal endometrial tissue (P<0.01). The positive expression rates of C-erbB-2 and Ki-67 in EC tissue were 80.6% and 64.2%, respectively, significantly higher than in atypical hyperplasia endometrial tissue and normal endometrial tissue (P<0.01). In EC tissue, the expression of ER and PR was closely associated with the differentiated degrees and depth of myometrial invasion (P<0.05), while that of C-erbB-2 and Ki-67 with the clinical staging, differentiated degrees, depth of myometrial invasion and presence or absence of lymph node metastasis (P<0.05). Spearman correlation analysis further displayed that the expression of ER was positively correlated with PR (r=0.393, P=0.001), but negatively with C-erbB-2 and Ki-67 (r=-0.469, P=0.000; r=-0.329, P=0.007); The expression of PR was negatively correlated with C-erbB-2 and Ki-67 (r=-0.273, P=0.025; r=-0.251, P=0.041), but that of C-erbB-2 positively with Ki-67 (r=0.342, P=0.005). Conclusions: Abnormal expression of ER, PR, C-erbB2 and Ki-67 might play an important role in endometrial malignant transformation and cell differentiation, so their joint detection is likely to be a comprehensive combination of immune factors, which is of great importance for EC prognosis.

Keywords: Estrogen receptors - progesterone receptors - C-erbB-2 - Ki-67 - endometrial carcinoma

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Introduction

Endometrial carcinoma (EC), originating from malignant epithelial tumors, is the most common malignancy of the female genital tract in developed countries (Kajo et al., 2015). It can cause about 74 000 deaths annually around the world and is classified into several histological subtypes including endometrioid and serous histologies (Hong et al., 2015). In recent years, the incidence of EC has been on a progressive rise due to extensive application of hormone-replacement therapy in clinic, especially for the young people. The occurrence of EC is not only related to the levels of estrogen receptors (ER) and progesterone receptors (PR), but also to the abnormal activation of proto-oncogenes and abnormal proliferation of cells (Srijaipracharoen et al., 2010; Binder et al., 2014).

Tumorigenesis is the lesion caused by activation of oncogenes or inactivation of tumor suppressor genes. EC pertains to hormone-dependent tumors, and both ER and PR in its tumor tissue are of great importance for the prognosis and clinical selection of endocrine therapy. C-erbB-2 that can inhibit tyrosinase activity plays a pivotal role in the cell growth, proliferation and differentiation, and its overexpression is positively correlated with the malignant degrees of the tumor (Uharcek, 2008). As one of the extensively-applied cell proliferation markers, Ki-67 can reflect the proliferation degree of malignant cells and is associated with the progression, metastasis and prognosis of various malignancies. A lot of studies have shown that that the prognosis of patients with EC is closely related to the age, histological grade, depth of myometrial invasion and/or cervical invasion, and the presence of lymph node metastases (Faria et al., 2015; Takahashi et al., 2015), and some biological indicators including hormone receptors, oncogenes and tumor suppressor genes are also

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involved (Ito et al., 1997), but these indicators are short of comprehensive verification of clinical trials with large sample sizes. Hence, in this study, the expression of ER, PR, C-erbB-2 and Ki-67 in EC tissue was detected using immunohistochemical SP method and their relationships with the clinicopathological features of EC were also investigated so as to provide valuable indicators for the clinical treatment and prognostic evaluation of EC.

Materials and Methods

Sample source

Sixty-seven EC samples archived in Department of Pathology and confirmed by two pathologists in Shaanxi Provincial People's Hospital were selected from Jun., 2012 to Jun., 2014. They were at the age of 29-68, with the mean age of 49.6. Among 67 patients with EC, there were 22 cases with high differentiation, 19 with moderate differentiation and 26 with poor differentiation; According to the depth of myometrial invasion, the invasive depth was less than 1/2 in 39 cases, equal to or more than 1/2 in 28 cases; 29 cases suffered from lymph node metastasis, while 38 not; Based on the clinical staging, there were 22 cases in phase I, 16 in phase II and 29 in phase III-IV. Besides, the endometrial samples with other gynecological diseases that underwent surgical resection or uterine curettage were also selected at the same term, in which normal endometrial samples and atypical hyperplasia endometrial samples were both 53 cases. All the enrolled samples had no history of radiotherapy, chemotherapy, ER and PR administration, and the clinicopathological files were complete. This research was approved by Ethics Committee of Shaanxi Provincial People's Hospital, and the patients or their relatives signed the informed consent form.

Major reagents

Mouse anti-human ER, PR, C-erbB-2 and Ki-67 monoclonal antibodies were all purchased from Fujian Maixin Biotech Co., Ltd. Enzyme-labeled goat anti-mouse IgG polymer as the secondary antibody and ready-to-use DAB coloration kit were provided by Beijing Zhongshan Golden Bridge Biotech Co., Ltd.

Detection method

All the samples were fixed by paraformaldehyde, embedded by paraffin and continuously cut into slices with the thickness of 4 μm. The expression of ER, PR, C-erbB-2 and Ki-67 in EC tissue, atypical hyperplasia endometrial tissue and normal endometrial tissue was all detected using immunohistochemical SP method. Specific steps were as follows: (1) bake the sections at 68°C for 20 min; (2) carry out dewaxing and dehydration respectively with xylene and gradient alcohol, and then apply 3% H_2O_2 to incubate 10 min at 37°C and PBS to swash 3 times, 5 min per time; (3) conduct antigen repair in citrate for 15-20 min, and then apply PBS to swash 3 times, 5 min per time after cooling to room temperature; (4) use the normal goat serum working solution for sealing 10 min at 37°C, dropwise add primary antibody, stay overnight for incubation at 4°C, and then conduct secondary antibody incubation after applying PBS to swash 3 times; (5) apply DAB for coloration, hematoxylin for counterstaining, xylene for transparency and neural resin for sealing sections. All the operations were carried out strictly according to the kit instructions. Known positive sections of EC were regarded as positive controls and PBS as negative controls instead of primary antibodies.

Judging criteria

All the sections were observed and judged independently by two pathologists under the condition of unknown clinical data. The positive expression of ER and PR was mainly located in cell nuclei, manifesting different sizes of brown-yellow granules; The positive expression of C-erbB-2 was primarily located in cytomembrane; The positive expression of Ki-67 was defined as presence of brown-yellow granules in cell nuclei or in both cell nuclei and cytomembrane.

The results were evaluated using semi-quantitative scoring method: (1) Five high-power visual fields (×400) in each section were randomly selected to calculate the total number of tumor cells and number of positive cells. The proportions of positive cells ≤5%, being 6%-25% 26%-50%, 51%-75% and >75% were respectively scored 0, 1, 2, 3 and 4 points; (2) The scores were made according to staining intensity, namely colorless, light yellow, brown yellow and sepia were represented by 0, 1, 2, and 3 points, respectively. The product of two scores above was as the final score, namely 0 point being negative (-), 1-4 points being weakly positive (+), 5-8 points being positive (++) and 9-12 points being strongly positive (+++).

Statistical data analysis

SPSS 15.0 software package was used for statistical analysis. The enumeration data were compared using x^2 test and expressed by percentages. Relationships between the expression of ER, PR, C-erbB-2 and Ki-67 in EC tissue and the clinicopathological features were analyzed by x^2 test of the four-fold table, and their correlations in EC tissue were analyzed by Spearman correlation analysis. All statistical tests were performed in a two-sided way. $P<0.05$ was considered to be statistically significant.

Results

Expression of ER, PR, C-erbB-2 and Ki-67 in different endometrial tissues

The positive expression of ER and PR was mainly located in cell nuclei, manifesting different sizes of brown-yellow granules (Figure 1; Figure 2). The positive expression of C-erbB-2 was primarily located in cytomembrane (Figure 3). The positive expression of Ki-67 was defined as presence of brown-yellow granules in cell nuclei or in both cell nuclei and cytomembrane (Figure 4).

As shown in Table 1 and Table 2, the positive expression rates of ER and PR in EC tissue were 44.8% and 41.8%, respectively, dramatically lower than in atypical hyperplasia endometrial tissue (79.2%, $P_{ER}=0.000$; 77.4%, $P_{PR}=0.000$) and normal endometrial tissue (90.6%, $P_{ER}=0.000$; 92.5%, $P_{PR}=0.000$). Significant difference was

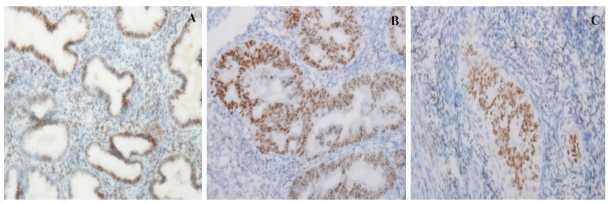

Figure 1. A. Positive Expression of ER in Normal Endometrial Tissue (×400); B. Positive Expression of ER in Atypical Hyperplasia Endometrial Tissue (×400); C: Positive Expression of ER in EC tissue (×400)

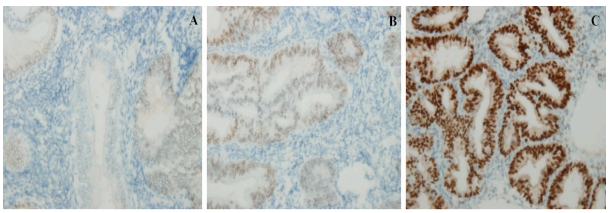

Figure 2. A. Positive Expression of PR in Normal Endometrial Tissue (×400); B. Positive Expression of PR in Atypical Hyperplasia Endometrial Tissue (×400); C. Positive Expression of PR in EC tissue (×400)

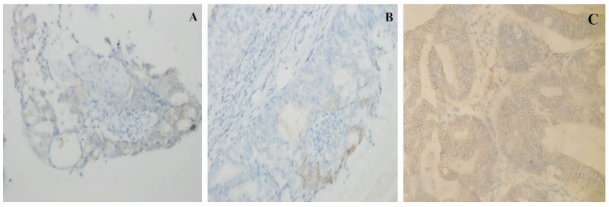

Figure 3. A. Positive Expression of C-erbB-2 in Normal Endometrial Tissue (×400); B. Positive Expression of C-erbB-2 in Atypical Hyperplasia Endometrial Tissue (×400); C. Positive Expression of C-erbB-2 in EC tissue (×400)

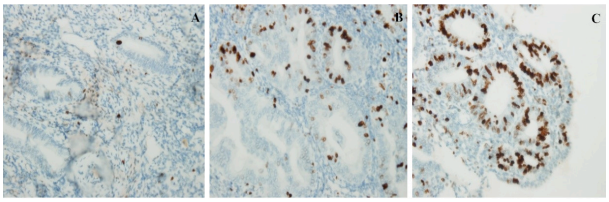

Figure 4. A. Positive Expression of Ki-67 in Normal Endometrial Tissue (×400); B. Positive Expression of Ki-67 in Atypical Hyperplasia Endometrial Tissue (×400); C. Positive Expression of PR in Ki-67 tissue (×400)

Table 1. Comparison on ER Expression in Different Endometrial Tissues [n(%)]

Histological types	n	ER			
		Negative expression	Positive expression	x^2	P
EC tissue	67	37(55.2)	30(44.8)	32.708	
Atypical hyperplasia endometrial tissue	53	11(20.8)	42(79.2)		
Normal endometrial tissue	53	5(9.4)	48(90.6)		

Table 2. Comparison on PR Expression in Different Endometrial Tissues [n(%)]

Histological types	n	PR			
		Negative expression	Positive expression	x^2	P
EC tissue	67	39(58.2)	28(41.8)	37.975	0.000
Atypical hyperplasia endometrial tissue	53	12(22.6)	41(77.4)		
Normal endometrial tissue	53	4(7.5)	49(92.5)		

Table 3. Comparison on C-erbB-2 Expression in Different Endometrial Tissues[n(%)]

Histological types	n	C-erbB-2			
		Negative expression	Positive expression	x^2	P
EC tissue	67	13(19.4)	54(80.6)	78.002	0.000
Atypical hyperplasia endometrial tissue	53	25(47.2)	28(52.8)		
Normal endometrial tissue	53	53(100.0)	0(0.0)		

Table 4. Comparison on Ki-67 Expression in Different Endometrial Tissues[n(%)]

Histological types	n	Ki-67			
		Negative expression	Positive expression	x^2	P
EC tissue	67	24(35.8)	43(64.2)	43.777	0.000
Atypical hyperplasia endometrial tissue	53	35(66.0)	18(34.0)		
Normal endometrial tissue	53	50(94.3)	3(5.7)		

also presented by comparison to the positive expression rates of ER and PR in atypical hyperplasia endometrial tissue and normal endometrial tissue (x^2=2.650, P=0.104; x^2=4.711, P=0.030).

As shown in Table 3 and Table 4, the positive expression rates of C-erbB-2 and Ki-67 in EC tissue were 80.6% and 64.2%, respectively, significantly higher than in atypical hyperplasia endometrial tissue (52.8%, $P_{C\text{-}erbB\text{-}2}$=0.001; 34.0%, $P_{Ki\text{-}67}$=0.001) and normal endometrial tissue (0.0%, $P_{C\text{-}erbB\text{-}2}$=0.000; 5.7%, $P_{Ki\text{-}67}$=0.000). There

Table 6. Relationships Between C-erbB-2, Ki-67 Expression and the Patients Clinicopathological Features[n(%)]

Clinicopathological Features	n	C-erbB-2 Expression		x^2	P	Ki-67 Expression		x^2	P
		Negative	Positive			Negative	Positive		
Clinical staging									
I-II	38	12(31.6)	26(68.4)	8.323	0.004	19(50.0)	19(50.0)	7.678	0.006
III-IV	29	1(3.4)	28(96.6)			5(17.2)	24(82.8)		
Differentiated degrees									
High differentiation	22	8(36.4)	14(63.6)	6.486	0.039	13(59.1)	9(40.9)	7.765	0.021
Moderate differentiation	19	3(15.8)	16(84.2)			5(26.3)	14(73.7)		
Poor differentiation	26	2(7.7)	24(92.3)			6(23.1)	20(76.9)		
Depth of myometrial invasion									
<1/2	39	12(30.8)	27(69.2)	7.710	0.005	18(46.2)	21(53.8)	4.334	0.037
≥1/2	28	1(3.6)	27(96.4)			6(21.4)	22(78.6)		
Lymph node metastasis									
Yes	29	2(6.7)	28(93.3)	6.364	0.012	6(20.7)	23(79.3)	5.092	0.024
No	38	12(31.6)	26(68.4)			18(47.4)	20(52.6)		

Table 5. Relationships Between ER, PR Expression and the Patients' Clinicopathological Features[n(%)]

Clinicopathological Features	n	ER Expression		x^2	P	PR Expression		x^2	P
		Negative	Positive			Negative	Positive		
Clinical staging									
I-II	38	18(47.4)	20(52.6)	2.191	0.139	19(50.0)	19(50.0)	0.969	0.325
III-IV	29	19(65.5)	10(34.5)			18(62.1)	11(37.9)		
Differentiated degrees									
High differentiation	22	9(40.9)	13(59.1)	8.096	0.017	8(36.4)	14(63.6)	11.349	0.003
Moderate differentiation	19	8(42.1)	11(57.9)			8(42.1)	11(57.9)		
Poor differentiation	26	20(76.9)	6(23.1)			21(80.8)	5(19.2)		
Depth of myometrial invasion									
<1/2	39	16(41.0)	23(59.0)	7.608	0.006	15(38.5)	24(61.5)	10.604	0.001
≥1/2	28	21(75.0)	7(25.0)			22(78.6)	6(21.4)		
Lymph node metastasis									
Yes	29	18(62.1)	11(37.9)	0.969	0.325	19(65.5)	10(34.5)	2.191	0.139
No	38	19(50.0)	19(50.0)			18(47.4)	20(52.6)		

was statistical significance by comparison to the positive expression rates of C-erbB-2 and Ki-67 in atypical hyperplasia endometrial tissue and normal endometrial tissue (x^2=38.051, P=0.000; x^2=13.361, P=0.000).

Relationships between the expression of ER, PR, C-erbB-2 and Ki-67 and the patients' clinicopathological features

In EC tissue, the expression of ER and PR was closely associated with the differentiated degrees and depth of myometrial invasion (P_{ER}=0.017, 0.006; P_{PR}=0.003, 0.001) instead of the clinical staging and presence or absence of lymph node metastasis (P>0.05) (Table 5). The expression of both C-erbB-2 and Ki-67 was intimately related to the clinical staging, differentiated degrees, depth of myometrial invasion and presence or absence of lymph node metastasis ($P_{C\text{-}erbB\text{-}2}$=0.004, 0.039, 0.005, 0.012; $P_{Ki\text{-}67}$=0.006, 0.021, 0.037, 0.024) (Table 6).

Correlation among the expression of ER, PR, C-erbB-2 and Ki-67 in EC

In EC tissue, the expression of ER was positively correlated with PR (r=0.393, P=0.001), but negatively with C-erbB-2 and Ki-67 (r=-0.469, P=0.000; r=-0.329, P=0.007); The expression of PR was negatively correlation with C-erbB-2 and Ki-67 (r=-0.273, P=0.025; r=-0.251, P=0.041); The expression of C-erbB-2 had a significantly positive correlation with Ki-67 (r=0.342, P=0.005).

Discussion

EC, a primary epithelial tumor, is usually accompanied by gland differentiation and has the potency of infiltration into uterine walls and distant dissemination. In recent years, the incidence of EC is on the rise due to the extension of average life in the population and application of hormone-replacement therapy during menopause. The uterus in female genital system is a target organ of sexual hormone, where the tumors are regarded as the sexual hormone-dependant tumor. Extensively distributed in the histocytes of female reproductive organs, both ER and PR are essential in the systemic regulation of estrogen and progestogen in female reproductive organs. Their production and functions can be affected when normal histocytes suffer from variation. Nevertheless, the therapeutic response of EC to hormone depends on the level of hormone receptors in the tumor tissue. High level of receptors has better response to endocrinotherapy (Kuramoto et al., 1989). This study applied immunohistochemical SP method to detect the expression of ER and PR in three different endometrial tissues, and the results showed that the positive expression of ER and PR in EC tissue was markedly lower than

in atypical hyperplasia endometrial tissue and normal endometrial tissue, and that in normal endometrial tissue was the lowest. Additionally, with the increase of tumor differentiated grades and myometrial invasive depth, the positive expression rates of ER and PR went down, which might be related to increased malignant degrees of tumor cells, elevated DNA content in cancer cell nuclei and change of normal enzyme activity that all impact the synthesis of ER and PR.

C-erbB-2 is a chief proto-oncogene of EC, which plays a pivotal role in its biological behavior (Zhao et al., 2009). Its gene amplification and protein overexpression have been associated with poor prognosis in several solid tumors, including breast and gastric cancer (Thompson et al., 2011; Sharifah et al., 2008). C-erbB-2 is not expressed or lowly expressed in normal endometrial tissue, but its expression rate goes up gradually from atypical endometrial hyperplasia to EC (Ioachin 2005). In this study, the positive expression rate of C-erbB-2 in EC tissue was 80.6%, significantly higher than in normal endometrial tissue (0.0%) and atypical hyperplasia endometrial tissue (2.8%), conforming to the above reports. Amplification and overexpression of C-erbB-2 gene can cause the malignant transformation of normal cells. The invasion of highly-expressed tumors is higher, and prognosis is poorer. There are a lot of research results regarding the relationships between amplification or overexpression of C-erbB-2 gene and EC biological behaviors. Morrison et al. analyzed 483 patients with EC and found that C-erbB-2 overexpression was related to histological grades (Morrison et al., 2006). However, Seki et al. believed that the positive expression of C-erbB-2 was not associated with histological grades or FIGO staging (Seki et al., 1998). The research results in this study displayed that the positive expression of C-erbB-2 in phase III-IV patients was higher than in phase I-II, showing that C-erbB-2 expression increases with the clinical staging elevating; As the tumor differentiated grades increased, C-erbB-2 expression went up obviously, indicating that C-erbB-2 expression is related to histological differentiation. The higher the positive expression of C-erbB-2 was, the poorer the histological differentiation was; The positive expression of C-erbB-2 in patients with lymph node metastasis or depth of myometrial invasion ≥1/2 was also dramatically higher than those without lymph node metastasis or depth of myometrial invasion <1/2, showing C-erbB-2 expression increases with aggravation of lymph node metastasis and myometrial invasion. All above results suggest that the tumors with high C-erbB-2 expression are stronger in invasion, rapid in disease progression and poor in prognosis.

The proliferation of tumor cells depends on their biological behaviors. The occurrence and progression of EC are related to various factors, but abnormal cell proliferation exerts a certain effect (Gurda et al., 2014). Ki-67 pertains to non-histone proteins and is an affirmative proliferation marker at present. In cell cycle, its expression begins to appear in phase G_1, increases in phases S and G_2, reaches to the peak in phase M, and disappears rapidly in the advanced stage of cell division, but it is not expressed in phase G_0. Ki-67 becomes the most reliable index in the detection of tumor cell proliferation activity due to short half-life period. In this study, the positive expression of Ki-67 was 64.2%, dramatically higher than in normal endometrial tissue (5.7%) and atypical hyperplasia endometrial tissue (34.0%), indicating that Ki-67 expression goes up successively in normal endometrial tissue, atypical hyperplasia endometrial tissue and EC tissue, which may be related to the enhanced cell proliferation. The expression of Ki-67 in EC tissue was intimately related to the clinical staging, differentiated degrees, depth of myometrial invasion and presence or absence of lymph node metastasis, illustrating that Ki-67 expression increases with the elevation of tumor malignant degrees; The stronger cell proliferation is, the higher malignant degrees are. Hence, Ki-67 can be an important index for evaluating EC proliferation and differentiation and has key biological and clinical significance in EC occurrence, progression and diagnosis.

Correlation analysis further revealed that the expression of ER in EC tissue was positively correlated with PR, and negatively with C-erbB2 and Ki-67, illustrating that C-erbB2 is expressed weakly in hormone-dependant EC tissue, while strongly in non-hormone-dependant EC tissue; The lower the expression rates of ER and PR are, the more significant cell proliferation is. Besides, the expression of C-erbB-2 had a significantly positive correlation with Ki-67. All these results suggest that abnormal expression of ER, PR, C-erbB2 and Ki-67 might play an important role in endometrial malignant transformation and cell differentiation, so their joint detection is likely to be a comprehensive combination of immune factors, which is of great importance for EC prognosis.

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