Expression of E-cadherin, β-catenin and uPA proteins in endometrial carcinoma and its clinical significance

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Abstract Objective The aim of this study was to investigate the expression of E-cadherin, β-catenin, and uPA proteins in endometrial carcinoma and their clinical significance. **Methods** The expression of E-cadherin, β-catenin, and uPA in the specimens of 89 cases with endometrial carcinoma, 65 cases with atypical hyperplasia and 30 cases with normal endometrium were determined by immunohistochemistry. **Results** ①The aberrant expression of E-cadherin and β-catenin in 89 cases of endometrial carcinoma were 51.1% and 62.2% respectively; The postitive expression of uPA were 68.9%. There was a significant different between atypical hyperplasia endometrium, normal endometrium and carcinoma tissue (*P<0.05*). ②The aberrant expression of E-cadherin in endometrial carcinoma had some relationship with its clinical FIGO staging and lymph node metastasis (*P<0.05*), but had no relationship with age, the histological classification and myomterial invasion (*P>0.05*); β-catenin and uPA had relationship with its clinical FIGO staging, myomterial invasion and lymph node metastasis (*P<0.05*), but had no relationship with the histological classification and age (*P>0.05*). ③ There was a negative correlation between the decreased expression of E-cadherin and the high expression of uPA in endometrial carcinoma (*P<0.05*); There also was a positive correlation between the aberrant expression of β-catenin and the high expression of uPA (*P<0.05*). **Conclusions** Aberrant expression of E-cadherin and β-catenin could induce activation or facilitation of uPA overexpression, and affect the pathogenesis and progression of endometrial carcinoma.

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1. Introduction

Endometrial carcinoma (EC) is a common malignant tumor in the female reproductive system. There is an obviously increase of the incidence of EC in recent year [1-2]. Tumor invasion and metastasis is the leading cause of death in patients, which is also an important indicator to determine the prognosis of patients. Many studies have confirmed that E-cadherin, β-catenin related with tumor cell adhesion. The uPA promote the invasion and metastasis of tumor by increasing extracellular matrix degradation. Foreign research suggests uPA may be one of the downstream target genes of the Wnt signaling pathway and its expression levels can be changed through the regulation of β-catenin. There was no similarly report in domestic at present. This study is to detect expression of three kinds of factors in EC and its correlation by immunohistochemistry. Which to provide basis for revealing mechanism of endometrial cancer invasion and metastasis , predicting tumor invasion and metastasis, making individual, predictable treatment options.

2. Material and Methods

2.1 Subjects

89 cases of EC resection specimens were collected in Kaifeng City Maternity Hospital from January 2005 to December 2007. The patients ranged in age from 38 to 64 years old, with a median age of 44 years old. These cases are classified according to the 2009 FIGO surgical stage standard: Pathological stage Ⅰ 56 cases (62.9%), Phase II 18 cases (20.2%), Ⅲ ~ Ⅳ 15 cases (16.9%). All of those patients hadn't received radiotherapy, chemotherapy and hormone drugs treatment before surgery. No myometrial invasion 15 cases (16.9%), superficial myometrial infiltration 56 cases (62.9%), deep myometrial invasion 18 cases (20.2%); there are 23 patients (26.7%) cases with lymph node metastasis and 66 patients cases(73.3%) without lymph node metastasis. 65 cases of Atypical hyperplasia endometrial samples and 30 cases of normal proliferative endometrium specimens are randomly selected from cases archived at the same time. All of those cases are diagnosed by pathological examination after surgery and having complete clinical and pathological data.

2.2 Reagents

Mouse anti-human monoclonal antibody E-cadherin, mouse anti-human monoclonal antibody β-catenin and mouse anti-human monoclonal antibody uPA were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., SP kit and DAB reagent were provided by the Fujian Maixin Biotechnology Co., Ltd.. The best working concentration of β-catenin antibody, E-cadherin antibody and uPA antibody were 1:100, 1:50, and 1:100, respectively.

2.3 Methods

All Paraffin-embedded tissues were cut into 4ul serial sections, the conventional dewaxing to the water, and retored by microwave antigen repair technique for 20min. Then 4ul serial sections were returned to room temperature, washed with PBS to eliminate endogenous peroxidase and closed by rabbit serum. Then add the primary antibody overnight at 4°C, the next day add secondary antibody 37°C for 30 min, horseradish peroxidase-labeled chain avidin incubated for 20 min at room temperature. Observe and control color under microscope when dropping DAB, and mounted after hematoxylin counterstaining. In this experiment, 0.01mol / L PBS solution instead of primary antibody is as a negative control, E-cadherin, β-catenin, uPA protein-positive breast cancer organization used as a positive control.

2.4 Assessment criteria

Each case was randomly observed under the microscope 10 high power field (× 400), 100 cells per visual field. E-cadherin and β-catenin-positive signal is in the cell membrane, showing up continuous stained cytomembrane; uPA is positive signals of in the cytoplasm, which is Brown yellow granules, scoring standard use a semi-quantitative integration of the three parameters [3]: the stained intensity (A), positive cell number (B) and the product of the two scoring. Completely stained light brown yellow, brown, dark brown and yellow are evaluated as 0, 1, 2, 3, respectively; Percentage of positive cells from 0% to 24%, from 25% to 49%, from 50% to 74%, 75% to 100%, respectively, are classified as points 1, 2, 3, 4; multiplying the two positive score is equal to Positive score. The product of two points is divided into negative (-); weakly positive (+), (point 1 to 4); moderately positive (+ +), (point 5 to 8); strongly positive (+ + +), (point 9 to 12). "(+ + ~ + + +)" Count as positive rate or normal expression rates; "(~ +)" count as negative rate and abnormal expression rate.

2.5 Statistical analysis

All data were analyzed using SPSS 16.0 software. X^2 test was applied in the intergroup analysis. The correlation analysis uses Spearman rank correlation analysis. A value of *P<0.05* was considered statistically significant.

3. Results

3.1 Expression of E-cadherin, β-catenin and uPA protein in different endometrial tissue

There is an obviously upward trend of abnormal expression rate of E-cadherin, β-catenin protein from proliferative endometrium group, EIN group, to EC group in 45 cases of EC. There was significant difference between any two groups *(P <0.05)*. There is an obviously increase of positive expression rate of uPA protein. Compared with the proliferative endometrium group, there was higher in between EIN group, and EC group, any two groups *(P <0.05)*. Which were shown in Table 1.

Table 1. E-cadherin, β-catenin and uPA protein expression of different endometrial tissues

Group	n	E-cadherin Anomaly expression number(%)	β-catenin Anomaly expression number(%)	uPA Positive expression number (%)
Proliferative Endometrium group	30	0(0%)	2(6.7%)	0(0)
EIN group	65	22(34.4%)[a]	28(43.8%)[b]	16(25.0%)[c]
EC group	89	45(51.1%)	55(62.2%)	61(68.9%)

Note: Compared with normal EC group, **a:** x^2=7.470, p<*0.05*;b:x^2=8.341,p<0.05;c:x^2=11.356,p <0.05

3.2 The relationship between expression of E-cadherin, β-catenin and uPA with endometrial cancer clinical pathology parameters

The results showed that abnormal expression of E-cadherin and β-catenin, and positive expression of uPA in endometrial carcinoma were correlated with tumor stage (*P <0.05*). There is no significant difference in expression of these three tissue factors in various differentiation degrees of endometrial carcinoma (*P> 0.05*). Nucleus / perinuclear abnormal expression rate of β-catenin and positive expression rate of uPA in tumor deep myometrial invasion and lymph node metastasis groups are higher than that in the control group, and the difference is significant *(P*

< *0.05)*. The decline and lack of E-cadherin expression in membrane is only associated with lymphatic metastasis (*P <0.05*), and has no significant correlation with the depth of myometrial invasion in these groups *P> 0.05*). Abnormal expression of the three factors in these tissues has nothing to do with gender. Which were all shown in Table 2.

3.3 Relationship of E-cadherin, β-catenin and uPA expression in endometrial carcinoma

The results of spearman correlation test showed that there are positively correlation not only between low expression of E-cadherin and abnormal expression of β-catenin (r=0.430, p=0.043), but also between the low expression of E-cadherin and overexpression of uPA (r=0.495, p=0.031). And there are also positively correlation between ectopic expression of β-catenin and overexpression of uPA (r =0.763, p=0.018) in 45 cases of endometrial cancer tissues.

Table 2: The relationship between expression of E-cadherin, β-catenin and uPA with endometrial cancer clinical pathology parameters

Pathological parameters	n	E-cadherin		*β*-catenin		uPA	
		Anomaly expression number(%)	*P*value (x2)	Anomaly expression number(%)	*P*value (x2)	Positive expression number(%)	*P*value (x2)
Age							
≤44	41	21(51.2%)	0.873 (0.025)	31(75.6%)	0.071 (5.632)	29(70.7%)	0.731 (0.118)
＞44	48	24(50.0%)		24(50.0%)		43(89.6%)	
Surgical Stages							
I	56	27(48.2%)	0.036 (6.666)	27(48.2%)	0.007 (9.909)	33(58.9%)	0.031 (6.928)
II	18	12(66.7%)		14(77.8%)		9(50.0%)	
III-IV	15	13(86.7%)		15(100.0%)		15(100.0%)	
Pathology classification							
G1	69	37(53.6%)	0.375 (1.961)	43(62.3%)	0.715 (0.671)	45(65.2%)	0.072 (5.250)
G2	8	2(25.0%)		6(75.0%)		4(50.0%)	
G3	12	4(33.3%)		6(50.0%)		12(100.0%)	
muscular layer infiltrates							
no	18	10(55.6%)	0.138 (3.962)	10(55.6%)	0.007 (9.909)	12(66.7%)	0.020 (7.843)
<1/2	53	22(41.5%)		29(54.7%)		31(58.5%)	
≥1/2	18	14(77.8%)		18(100.0%)		18(100.0%)	
lymphatic metastasis							
No	66	26 (39.4%)	0.023 (5.155)	32(48.5%)	0.002 (9.935)	40(60.6%)	0.047 (3.961)
Yes	23	19(82.6%)		23(100.0%)		21(91.3%)	

4. Discussions

Endometrial cancer is one of the common malignant tumors in the female reproductive system. Despite the advances that have been made in other cancers, both the annual incidence of and the death rate associated with endometrial cancer appear to be rising, both in China and around the world [2,4,5]. Currently, it is still a serious threat to women's health [6,7]. Especially, local tumor recurrence and metastasis is the most important cause of death for patients. Endometrial cancer metastasis has become a research focus in diagnosis and treatment of endometrial cancer.

E-cadherin is well known as a transmembrane glycoprotein which has the tumor suppressor function. In recently years, some studies have confirmed that the lack of E-cadherin expression in breast cancer has correlation with axillary lymph node metastasis, and there is the E-cadherin re-expression of in the metastasis in the distance [8,9]. From above study they concluded that cancer local invasion and distant metastasis is due to the lack of E-cadherin. Research of E-cadherin expression missing promoting tumor metastasis suggested that intracellular domain of E-cadherin has interaction with the key molecule β-catenin in the Wnt signaling pathway. In tumor progression, the lack of E-cadherin expression can result in β-catenin decomposition block. Then it can lead to the cytosolic free β-catenin accumulation which actives Wnt signal transduction pathway mediated by β-catenin. In the Wnt signal transduction pathway, β-catenin interacts with the transcription factor Tef / lef, and it enters into the nucleus which can affect gene transcription. Finally, β-catenin makes the downstream target genes activation and promotes tumor metastasis. In other studies it is also confirmed that tyrosine phosphorylation of β-catenin can promote the separation of β-catenin and E-

cadherin in cell junction, and lead to abnormal aggregation of intracytoplasmic free β-catenin and upregulation of target genes in the Wnt pathway at the same time in the process of invasion and metastasis of colorectal cancer [10,11]. Therefore, it suggests that the role of E-cadherin abnormal expression in tumor metastasis was related with the activation of Wnt signal pathway. In this study, we detected E-cadherin protein in 89 cases of endometrial cancer tissue by immunohistochemistry. Which show that the expression of E-cadherin and was significantly decreased in the 44 cases (48.9%) of cancerous tissue, compared with in atypical hyperplasia membrane and normal endometrium. Low expression of E-cadherin has correlation with lymph node metastasis and loss of E-cadherin expression may have certain reference value in early metastasis of endometrial cancer which is consistent with previous conclusions. Meanwhile, our study suggested that abnormal expression of β-catenin in endometrial cancer was significantly higher than in normal and atypical endometrial hyperplasia. There is a gradually increasing trend of abnormal expression of β-cateninthe with increasing of FIGO stage, lymph node metastasis and myometrial invasion ($P < 0.05$), and the results were consistent with research of Wang [3], and Shaco-L[12], which suggested that the abnormal accumulation of β-catenin in patients has stronger function of invasion and metastasis. In our study, the correlation analysis of E-cadherin and β-catenin show that with the expression of E-cadherin in cytomembrane reduced or lacking in endometrial carcinoma, the abnormal expression of β-catenin is significantly increased and both were significantly correlated ($r = 0.430$, $p = 0.003$). Therefore, it is speculated that the lack of E-cadherin expression may active the Wnt pathway by inducing abnormal accumulation of β-catenin in cytoplasmic, then promoting the tumor metastasis in the development of endometrial cancer.

Plasminogen activator (PA) is a special kind of serine proteases, which can be divided into the tissue-type (tPA) and urokinase-type (uPA). Among of above, uPA can promote tumor cell invasion and metastasis through activation of plasminogen, degradation of fibrin in matrix and fiber junction protein, regulation of tumor angiogenesis, and so on. In recent years, some foreign study put forward that uPA may be one of the downstream target genes in the Wnt signaling pathway [13]. At the same time, some research found that the missing of E-cadherin function was related with the upregulation expression of uPA [14], which suggests that it maybe has a signal pathway between in tumor cell adhesion and in protein degradation. But the underlying mechanism is not yet clear. There are not the relevant reports about whether the Wnt signal pathway is actived by abnormal accumulation of β -catenin and cause upregulation of uPA gene transcription and separation of E-cadherin/β-catenin complex, And then Wnt signal pathway participate in decreased of cell adhesion strength and degradation of tumor stroma -the two invasion and metastasis stages. In this study show that the expression of uPA in endometrial cancer was significantly higher than that in normal endometrium. The expression of uPA in endometrial cancer was significantly related with lymph node metastasis, depth of myometrial invasion and clinical stage, but which is not related with age or histopathological grading. It suggests that the high expression of uPA is also a malignant behavior of endometrial cancer which has a catalytic role in promoting tumor invasion and metastasis. Our study is consistent with the result of Gerstein ES [15] and Fredstorp L [16]. In addition, the result of correlation analysis of E-cadherin, β-catenin and uPA in endometrial cancer showed there is a significant correlation of expression of the three factors in endometrial cancer tissue. With the increased abnormal expression of β-catenin, E-cadherin membrane expression was significantly reduced or absent, while uPA positive expression showed a significant upward trend. It suggests that of E-cadherin, β-catenin and uPA may interact with each other in the development of endometrial cancer. So we speculated that the related factors in Wnt signal pathway may participate in the decline in cell adhesion and degradation of tumor stroma.

In conclusion, we consider that E-cadherin, β-catenin and uPA are interrelated. Abnormal expression of E-cadherin and β-catenin may promote decrease of adhesion, stromal degradation and metastasis in endometrial cancer cell by activated the overexpression of uPA in downstream signal. However, the exact mechanism needs to be further explored. Meanwhile, The results suggest that joint detection of the factors'expression levels of E-cadherin, β-catenin and uPA have important significance on the evaluation of metastatic potentiality, forecast of clinical prognosis and options therapeutic schedule for endometrial cancer.

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