Expression and clinical significance of HER2/neu, aromatase P450 and adhesion molecule CD24 in endometrial cancer

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ABSTRACT

This study aimed at exploring the expression and clinical significance of aromatase P450, adhesion molecule CD24 and HER2/neu in endometrial cancer. The expression of aromatase P450, adhesion molecule CD24 and HER2/neu was detected by immunohistochemistry in 15 cases of endometrial hyperplasia group, 50 cases of endometrial adenocarcinoma and 3 cases of uterine papillary adenocarcinoma, with 15 cases of normal endometrium as control group. We detected no expression of aromatase P450, adhesion molecule CD24 or HER2/neu in control group. Aromatase P450 positive expression rate was 66.7% in endometrial hyperplasia group and 70.3% in endometrial carcinoma group, without significant difference ($p>0.05$). There was no significant difference ($p>0.05$) in the positive expression rate of aromatase P450 between different myometrial invasion groups of endometrial adenocarcinomas. CD24 positive expression rate was 40.0% in endometrial hyperplasia group and 79.6% in endometrial carcinoma group, with significant difference ($p<0.05$). HER2/neu positive expression rate was 26.7% in the endometrial hyperplasia group and 57% in endometrial carcinoma group, with significant difference ($p<0.05$). In conclusion, aromatase P450 may be one factor associated with endometrial cancer cell proliferation, while CD24 and HER2/neu may be important factors associated with the invasion and metastasis of endometrial cancer.

Key words: aromatase P450; adhesion molecule CD24; HER2/neu; endometrial cancer.

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Introduction

Endometrial cancer is one common malignant tumor of the female genital tract, accounting for 20-30% of female genital tract malignancies. The incidence of endometrial cancer has increased significantly in recent years.[1] The etiology of endometrial cancer remains unclear. Endometrial cancer can be divided into two types: estrogen-dependent type, which is related to the long-term stimulation of estrogen without progesterone antagonism, and most of the pathological types are adenocarcinoma; non-estrogen dependent type, which has unclear pathogenesis.[2] For example, although uterine serous papillary carcinoma accounts for only 10% of endometrial carcinoma, it has the characteristics of high malignancy, early metastasis and poor prognosis.

Currently, it is proposed that the occurrence and development of endometrial cancer, especially endometrial adenocarcinoma, is related to the elevated level and continuous estrogen stimulation.[3] Cytochrome P450 aromatase is the key enzyme that catalyzes the conversion of androstenedione and testosterone into estrone and estradiol (E2).[3] It has been reported that aromatase P450 can promote the conversion of local estrogen in the endometrium, and the locally produced estrogen may play a role in the occurrence of endometrial cancer.[4] In addition, estrogen can regulate the expression of adhesion molecule CD24.[5] The adhesion molecule CD24 is a double-chain glycosyl-phosphatidylinositol located on the surface of the cell membrane. The O-linked glycosylation site of CD24 could mediate the adhesion between cells as well as the adhesion between cells and matrix. CD24 can regulate the development and maturation of lymphocytes, participate in the formation of neural networks, and maintain immune homeostasis. In addition, increased expression of CD24 can promote the proliferation of tumor cells.[6] However, the role of CD24 in endometrial cancer is still unclear. HER2/neu gene is located on the 17p21 band of the human chromosome and encodes the cell membrane glycoprotein with tyrosine kinase activity.[7] Recent evidence suggests that HER2/neu is a potential therapeutic target in endometrial cancer.[8] However, the correlation of HER2/neu expression with the expression of P450 and CD24 in endometrial cancer is still unclear.

Therefore, in this study we aimed at investigating the clinical significance of aromatase P450, adhesion molecule CD24 and HER2/neu in endometrial cancer. We employed immunohistochemical method to detect the expression of aromatase P450, adhesion molecule CD24 and HER2/neu in clinical samples of endometrial cancer and control tissues.

Materials and Methods

Samples

This study was conducted in accordance with the Declaration of Helsinki and was approved by Ethics Board of Hebei Medical University (approval No. 202871), and written informed consent was provided by all the patients. Paraffin tissues from the patients who were admitted to the Fourth Hospital of Hebei Medical University during 2000-2007 and underwent hysterectomy were collected. Histological classification was based on the World Health Organization (WHO) standard in 2000, there were 50 cases of endometrial adenocarcinoma, 3 cases of papillary adenocarcinoma and 15 cases of endometrial hyperplasia, and 15 cases of normal endometrium tissues were set as the control group. All cases had not received hormone replacement therapy.

Main reagents

Immunohistochemical method

Tissue sections were fixed in formalin and embedded in paraffin, then cut into 5 μm thin sections. Antigen retrieval was performed by boiling the sections in 10 mM citrate buffer (pH 6.0) for 10 min. The sections were incubated in 0.3% H_2O_2 for 30 min to block endogenous peroxidases, and then stained with primary antibodies overnight at 4°C and with secondary antibodies for 1 h. Phosphate-buffered saline instead of the primary antibody was used as the negative control. Finally, the sections were detected using DAB chromogenic kit, and sealed for observation under microscope (Olympus IX71, objective 100×; Olympus, Tokyo, Japan). For each slide, 5 fields of view were evaluated by an experienced pathologist in a blind manner.

Anti-aromatase mouse polyclonal antibody was purchased from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China) with a working concentration of 1:100; rabbit anti-C-erB-2 antibody was purchased from Beijing Biosynthesis Biotechnology Co., Ltd., with a working concentration of 1:100; anti-CD24 sheep polyclonal antibody was purchased from Santa Cruz Biotechnology (Dallas, TX, USA), with a working concentration of 1:100. The appropriate secondary antibodies and the DAB chromogenic kit were purchased from Beijing Zhong Shan Golden Bridge Biological Technology Co., Ltd. (Beijing, China).

Evaluation of immunohistochemical staining

Aromatase P450 was mainly distributed in the cytoplasm of endometrium; brown-yellow granules in the cytoplasm were judged as positive, the number of positively stained cells <10% was judged as negative, 10%–25% as (+), 25%–50% as (++), and >50% as (+++). Adhesion molecule CD24 was mainly distributed in the cell membrane and cytoplasm of endometrium: the brown-yellow granules in the cell membrane and cytoplasm were judged as positive, the number of positively stained cells <10% was judged as negative, 10%–25% as (+), 25%–50% as (++), and >50% as (+++). HER2/neu was mainly located in the cell membrane and cytoplasm of endometrium; the brown-yellow granules in the cell membrane and cytoplasm were judged as positive, the number of positively stained cells <10% was judged as negative, 10%–25% as (+), 25%–50% as (++), and >50% as (+++).

Statistical analysis

SPSS12.0 statistical software was used for statistical analysis. The two-way χ^2 test was used to compare multiple samples. The Spearman's correlation analysis was used to analyze the correlation between aromatase P450, adhesion molecule CD24 and HER2/neu, and $p<0.05$ was considered statistically significant.

Results

Expression of aromatase P450 in endometrial cancer

Aromatase P450 was not detected in normal endometrium, but was positively expressed in endometrial hyperplasia group and endometrial carcinoma group, with positive expression rate of 66.8% and 70.4%, respectively (Figure 1 A-C). Pairwise comparison showed that there was no significant difference in positive expression rate of aromatase P450 between endometrial hyperplasia group and endometrial carcinoma group ($\chi^2=0$, $p>0.05$). Due to the differences in the differentiation of endometrial adenocarcinoma and the depth of myometrial invasion, endometrial adenocarcinoma with deep myometrial invasion and low differentiation was

compared with papillary adenocarcinoma, and 50 cases of endometrial adenocarcinoma were compared pairwisely for aromatase P450 expression according to the depth of myometrial invasion (Table 1). The results showed that the p-values were 0.286, 0.091 and 0.699, respectively, (all p>0.05), indicating that aromatase P450 was not associated with the depth of myometrial invasion.

Expression of adhesion mold CD24 in endome-trial cancer

CD24 was not detected in normal endometrium, but was positively expressed in endometrial hyperplasia group and endometrial carcinoma group, with positive expression rates of 40.0% and 79.6%, respectively (Figure 1 D-F). Pairwise comparison showed that positive expression rate of CD24 was significantly higher in endometrial carcinoma group than in endometrial hyperplasia group (χ^2=7.135, p=0.008). As shown in Table 2, positive expression rate of CD24 in stage I endometrial adenocarcinoma was 68.7% and that in stage II-IV endometrial adenocarcinoma was 95.4%, and the difference between the two groups was statistically significant (p<0.05), indicating that CD24 was related to surgical staging of endometrial adenocarcinoma. The positive expression rate of CD24 in the non-lymph node metastasis group was 80% and that in the lymph node metastasis group was 80%, showing no

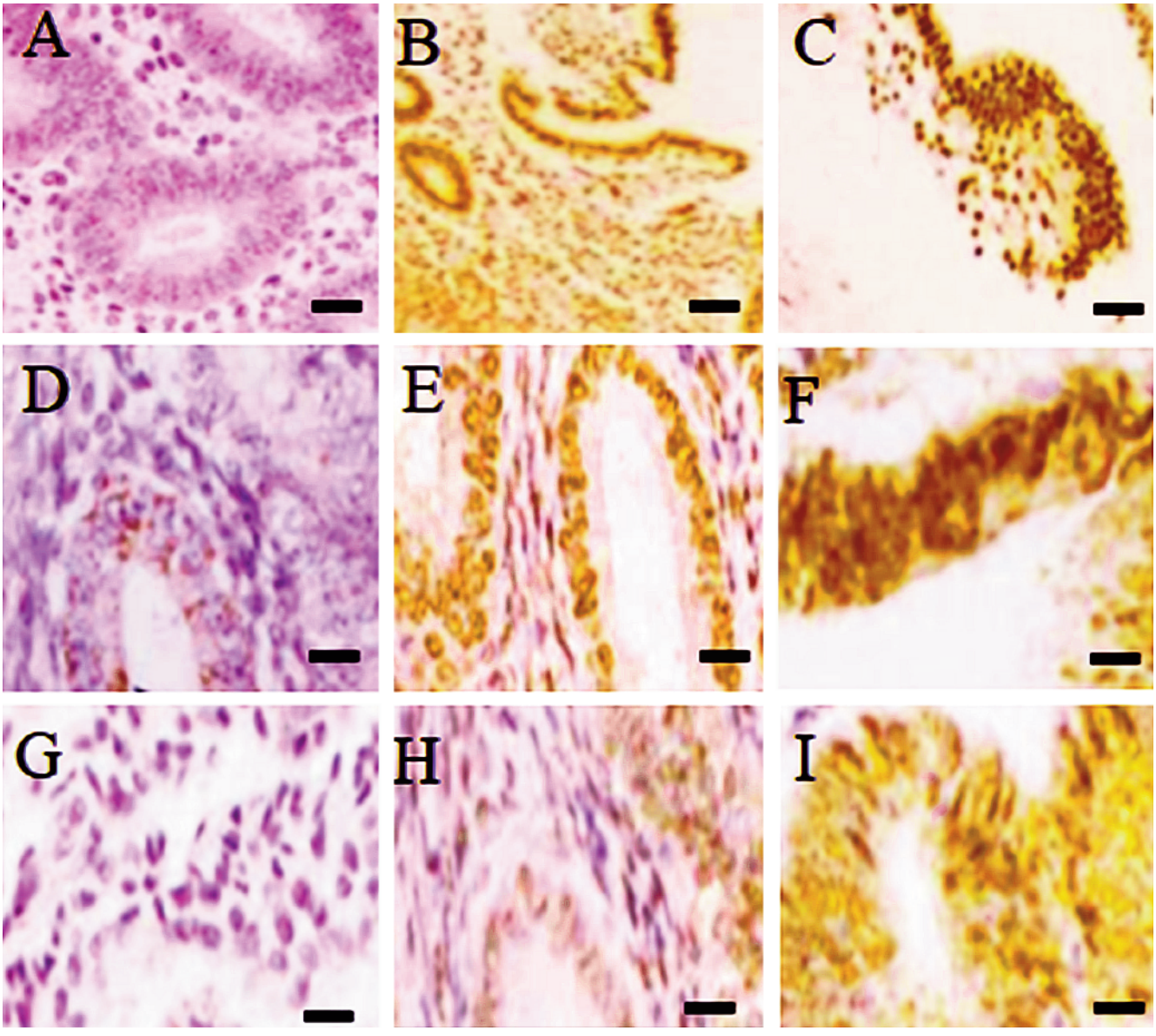

Figure 1. Immunohistochemical staining of aromatase P450, CD40 and HER2/neu in different endometrium tissues. **A**) No positive staining of P450 was detected in normal endometrium tissues in control group. **B**) Positive staining of P450 was detected in endometrium tissues in endometrial hyperplasia group. **C**) Strong positive staining of P450 was detected in endometrium tissues in endometrial carcinoma group. **D**) No positive staining of CD40 was detected in normal endometrium tissues in control group. **E**) Positive staining of CD40 was detected in endometrium tissues in endometrial hyperplasia group. **F**) Strong positive staining of CD40 was detected in endometrium tissues in endometrial carcinoma group. **G**) No positive staining of HER2/neu was detected in normal endometrium tissues in control group. **H**) Positive staining of HER2/neu was detected in endometrium tissues in endometrial hyperplasia group. I) Strong positive staining of HER2/neu was detected in endometrium tissues in endometrial carcinoma group. Scale bars: 20 μm.

significant difference between the two groups. The positive expression rate of CD24 in no myometrial invasion group was 40% and that in myometrial invasion group was 88.9%, and the difference between the two groups was statistically significant, indicating that CD24 was related to myometrial invasion of endometrial adenocarcinoma.

Expression of HER2/neu in endometrial cancer

HER2/neu was not detected in normal endometrium, but was positively expressed in endometrial hyperplasia group and endometrial carcinoma group, with positive expression rates of 26.7% and 57%, respectively (Figure 1 G- I). Pairwise comparison showed that positive expression rate of HER2/neu was significantly higher in endometrial carcinoma group than in endometrial hyperplasia group (χ^2=3.92, p=0.048). As shown in Table 3, positive expression rate of HER2/neu in stage I endometrial adenocarcinoma was 39.2% and that in stage II-IV endometrial adenocarcinoma was 72.7%, and the difference between the two groups was statistically significant (p<0.05), indicating that HER2/neu was related to surgical staging of endometrial adenocarcinoma. The positive expression rate of HER2/neu in the non-lymph node metastasis group was 60% and that in the lymph node metastasis group was 53.3%, showing no significant difference between the two groups. The positive expression rate of HER2/neu in no myometrial invasion group was 30% and that in myometrial invasion group was 60%, and the difference between the two groups was statistically significant, indicating that HER2/neu was related to myometrial invasion of endometrial adenocarcinoma.

Correlation of the expression of aromatase P450, CD24 and HER2/neu in endometrial cancer

Spearman analysis showed that the correlation coefficient between the expression of CD24 and aromatase P450 in endometrial adenocarcinoma was r=−0.127, p=0.361 >0.05, indicating that aromatase P450 had no correlation with the expression of CD24 in endometrial adenocarcinoma (Table 4). The correlation coefficient between CD24 and HER2/neu in endometrial adenocarcinoma was r=0.103, p =0.459 >0.05, indicating that HER2/neu had no correlation with the expression of CD24 in endometrial adenocarcinoma (Table 5). The correlation coefficient between HER2/neu and aromatase P450 in endometrial adenocarcinoma was r=0.073, p=0.602 >0.05, indicating that HER2/neu had no correlation with the expression of aromatase P450 in endometrial adenocarcinoma (Table 6).

Table 1. Pairwise comparison of aromatase P450 expression in endometrial carcinoma according to invasion depth.

Invasion depth	Positive cases	Negative cases	Positive rate (%)
Deep myometrial invasion	15	3	83.3
Shallow myometrial invasion	14	8	66.7
No invasion	5	5	50.0

Table 2. Relationship between CD24 and surgical-pathological staging, metastasis and myometrial invasion of endometrial carcinoma.

CD24	−	+ / +++	Positive rate (%)	p
Stage				
I	8	20	71.4	<0.05
II	2	13	86.7	
III–IV	0	7	100	
Metastasis				
Yes	1	4	80	
No	9	36	80	
Myometrial invasion				
No	6	4	40	
Shallow	3	19	86.3	<0.05
Deep	1	17	94.4	

Table 3. Relationship between HER2/neu and surgical-pathological staging, metastasis and myometrial invasion of endometrial carcinoma.

HER2/neu	−	+ / +++	Positive rate (%)	p-value
Stage				
I	17	11	39.2	
II - IV	6	16	72.7	<0.05
Metastasis				
Yes	2	3	60	
No	21	24	53.3	
Myometrial invasion				
No	7	3	30	
Shallow	12	10	45.5	<0.05
Deep	4	14	66.7	

Discussion

Aromatase P450 is a rate-limiting enzyme in the last step of estrogen biosynthesis, and aromatase inhibitor anastrozole has been used to treat endometrial cancer.[9] It is postulated that the occurrence and development of endometrial carcinoma, especially endometrial adenocarcinoma, is related to the elevated level and continuous stimulation of estrogen.[10] Most endometrial carcinomas are estrogen-dependent, and estrogen concentration in endometrial carcinoma tissues is higher than that i mal endometrium, indicating that locally produced estrogen y a role in the occurrence of endometrial carcinoma. In this pizza we showed that there was no expression of aromatase P450 mal endometrium, consistent with previous [11] Aroma 450 was expressed in endometrial hyperpla p and endometrial group, but the difference in negative expression rate of 450 in different myometri n groups was not significant ndicating that aromatase P4 promote the occurdometrial carcinoma, but has no significant effect on of endometrial cancer.

Cancer invasion and metastasis is a complex process of interaction between host cells and tumor cells, which is related to the adhesion and movement ability of tumor cells, and cell adhesion molecules that mediate cell adhesion play an important role in tumor invasion and metastasis.[12] A recent study reported that high expression of CD24 promoted endometrial cancer progression.[13] Consistently, this study showed that there was no expression of adhesion molecule CD24 in normal endometrium, and adhesion molecule CD24 was expressed at higher level in endometrial carcinoma group than in endometrial hyperplasia group. Moreover, CD24 expression was related to surgical staging and myometrial invasion of endometrial adenocarcinoma. Therefore, CD24 may be closely related to the metastasis of endometrial cancer.

HER2/neu is a proto-oncogene, and the amplification and overexpression of HER2/neu could promote cell proliferation and cell cycle, leading to malignant manifestations.[14,15] The gene amplification and protein overexpression of HER2/neu have not been detected in normal endometrium. However, in endometrial carcinoma HER2/neu was overexpressed, indicating that HER2/neu plays an important role in endometrial carcinoma. Relevant studies have shown that the overexpression of HER2/neu is related to many factors such as the depth of invasion and the differentiation of tumor.[16,17] In this study, we found no expression of HER2/neu in normal endometrium. However, HER2/neu was positively expressed in endometrial hyperplasia group and endometrial carcinoma group, and positive expression rate of HER2/neu was significantly higher in endometrial carcinoma group than in endometrial hyperplasia group. Moreover, HER2/neu was related to surgical staging and myometrial invasion of endometrial adenocarcinoma. These results are consistent with recent study that amplification of HER-2/neu gene and high expression of HER-2/neu were associated with low differentiation grade and deep myometrial invasion of endometrial carcinoma.[18] Afify *et al.* analyzed the expression of HER-2/neu in patients with stage I and III serous ovarian cancer by fluorescence *in situ* hybridization, and the results showed that the expression of HER-2/neu in patients of stage III was significantly higher than that in patients of stage I.[19] However, prognostic value of HER-2/neu in ovarian cancer has been controversial.[20,21] The different results may be due to the differences in detection methods, the disparity in the number of sample cases and other factors. Further studies are needed to confirm prognostic value of HER-2/neu in endometrial cancer.

Furthermore, we investigated the correlation of the expression of aromatase P450, CD24 and HER2/neu in endometrial cancer. While aromatase P450 has been reported to regulate the synthesis of estrogen, and estrogen could regulate the expression of CD24, we found no significant correlation between the expression of aromatase P450 and CD24 in our samples. In addition, we found no significant correlation between the expression of aromatase P450

Table 4. No correlation between the expression of aromatase P450 and CD24 in endometrial adenocarcinoma.

Adhesion molecule CD24	Aromatase P450		Total
	Negative cases	Positive cases	
Negative cases	2	9	11
Positive cases	14	29	43
Total	16	38	54

Table 5. No correlation between the expression of HER2/neu and CD24 in endometrial adenocarcinoma.

Adhesion molecule CD24	HER2/neu		Total
	Negative cases	Positive cases	
Negative cases	6	5	11
Positive cases	18	25	43
Total	24	30	54

Table 6. No correlation between the expression of HER2/neu and aromatase P450 in endometrial adenocarcinoma.

HER2/neu gene	Aromatase P450		Total
	Negative cases	Positive cases	
Negative cases	8	16	24
Positive	8	22	30
Total	16	38	54

and HER-2/neu, and between the expression of HER-2/neu and CD24 in our samples. The reason remains unclear and needs further investigations. Epigenetics such as histone modification could regulate the expression of oncogenes and tummor suppressors.[22] Therefore, it is important to investigate epigenetic mechanisms that regulate P450, CD24 and HER-2/neu expression in endometri-al cancer.

This study has several limitations. First, the sample size is relatively small. Second, this is a single-center study. These may partially explain why we could not find the correlation between the expression of aromatase P450, CD24 and HER-2/neu in endometrial cancer. Further multiple center study with large sample size will help validate clinical significance of aromatase P450, CD24 and HER-2/neu in endometrial cancer.

In summary, our results suggest that abnormal high expression of aromatase P450 is related to the occurrence and development of endometrial cancer, while high expression of CD24 and HER2/neu may be important factors for the metastasis of endometrial carcinoma.

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