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The role of epithelium-mesenchymal conversion in endometrial cancer patients with chemotherapy drug resistance

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Abstract: **To study the epithelial mesenchymal transition markers of E-cadherin and Vimentin expression in endometrial cancer patients with chemotherapy drug resistance. And then analyze relations with HER2 and epithelial mesenchymal transition. 75 cases of Endometrial carcinoma patients were collect in our hospital, which of 32 cases of patients were diagnosed by the clinical and pathological for adjuvant chemotherapy drug resistance. All patients were undergone surgical removal of the lesions and detected with pathologic examination. The epithelial mesenchymal transition markers of E-cadherin and Vimentin expression were detected by immunohistochemical. Level of Vimentin expression in resistant group were increased, and E-cadherin expression decreased (P<0.05). In 43 cases with HER2 positive, Vimentin expression level increased, E-cadherin decreased too, when compared with HER2 negative expression group, they had differences statistically significant (P<0.05). Spreamen correlation analysis showed that HER2 was negatively correlated with E-cadherin protein expression (R=0.336, P=0.336), and was positively related with Vimentin expression (R=0.587, P=0.587). In endometrial cancer patients with chemotherapy drug resistance, especially HER2 positive patients, it possible raise the expression of Vimentin and downgrade E-cadherin express to promote epithelial-mesenchymal transition, and received the occurrence of drug resistance. But the specific molecular biological mechanism still needs further research.**

Keywords: Ithelial-interstitial transition; Endometrial cancer; Adjuvant chemotherapy; Drug resistance

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1. Introduction

Endometrial carcinoma is a common tumor of women, and it is with more pathological subtypes and has different molecular phenotype, clinical phenotype and histological characteristics[1]. Study shows that the endometrial adenocarcinoma has highly sensitive to adjuvant chemotherapy, while spindle cell carcinoma and solid carcinoma have low sensitivity to chemotherapy drugs[2]. The reason may be relate to the characteristics of the tumor cells and immune phenotype. Therefore, this study will analysis the role of epithelium-mesenchymal conversion in endometrial carcinoma to explore its effect in endometrial cancer chemotherapy drug resistance, and provide ideas for clinical further treatment.

2. Data and methods

2.1. Clinical data

75 patients from January 2013 in January 2016 with endometrial carcinoma were randomly collected. All of them were diagnosed by clinical and pathological. Female, ages were (55.69-12.36) years old on average. Tumor lesion sizes were 1-4.3 cm, average (2.61 + 0.37) cm. There were 26 cases of pelvic lymph node metastasis and 49 cases without lymph node metastasis. All patients underwent surgery, and the postoperative pathologic diagnosis was endometrial carcinoma, which was diagnosed by clinical and pathological diagnosis in 32 patients with adjuvant chemotherapy. This study was approved by the hospital ethics committee and all patients signed informed consent.

2.2. Methods

The surgical excision of endometrial carcinoma was fixed and the paraffin package. Detection of epithelial mesenchymal transition markers such as E-cadherin and Vimentin were using immunohistochemical and the expression of HER2 in endometrial carcinoma used IHC too. Tissues were sliced into each concentration alcohol for 5min after high temperature baking for 2h to dewaxing. Endogenous peroxidase were treated by 3% hydrogen peroxide, using EDTA to repair antigen, PBS wash for 3 times respectively and add E-cadherin, Vimentin rabbit anti rat polyclonal antibody, while HER2 antibody were rabbit anti rat monoclonal antibody, incubation for 12 hours at low temperature and then flushing termination reaction. Plus Envision for 30min at 37℃, continue to PBS rinsed, DAB color, microscope observation and use of the suligin for redyeing.

2.3. Judgment standard

E-cadherin positive: tumor cell membranes are brown or yellow, Vimentin positive: tumor cell cytoplasm are brown or yellow, choose 10 horizon at high magnification, counting the number of positive cells in 100 cells, and calculate the percentage of positive cells, the percentage of positive cells <10%

is negative, 10% or higher is positive. The judgment was read by two or more attending pathologists. HER-2 criterion: negative: tumor cells have no tint or <10% cells have incomplete cell membrane staining. Positive: >10% tumor cell membrane with incomplete staining or <10% cell cell membrane is complete and strong staining.

2.4 Statistical methods

Using SPSS19.0 statistical software, test level a=0.05, P<0.05 on both sides to have statistical difference. Using chi-square test to compare two groups of qualitative data, using spreamen linear correlation analysis for the correlation.

3. Results

3.1. Comparison of the expression levels of epithelial mesenchymal transition markers in endometrial cancer

The expression level of Vimentin was increased in endometrial cancer of resistance group, and the expression of e-cadherin decreased (P < 0.05). Show in Table 1, Figure 1.

Table 1. the expression level of Vimentin and e-cadherin

.	cases	Vimentin		E-cadherin	
		Positive	negative	Positive	negative
Resistance group	32	21(65.64)	11(34.36)	13(40.62)	19(59.38)
Non resistance group	43	22(51.16)	21(48.84)	28(65.12)	15(34.88)
X^2 value		6.59		7.84	
P		<0.05		<0.05	

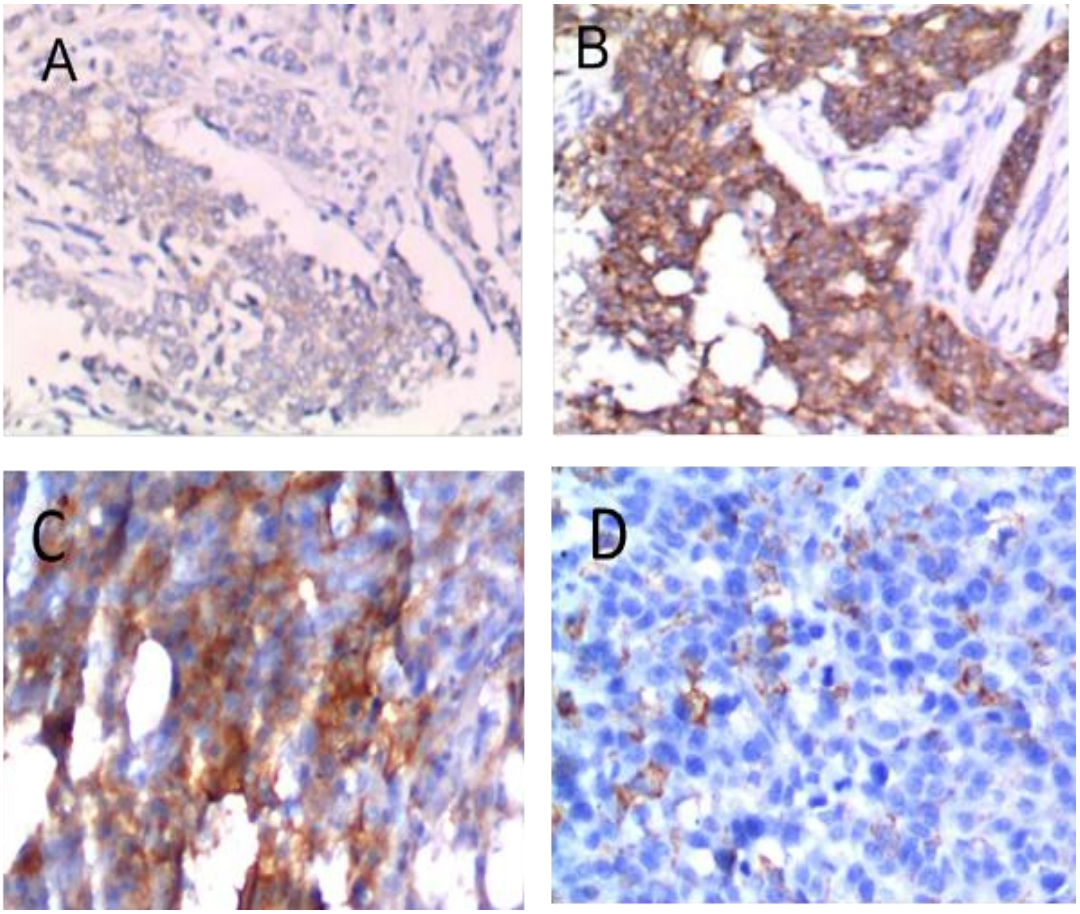

Figure 1. The expression of Vimentin and e-cadherin in the endometrial cancer resistance group A: e-cadherin weakly positive. C: e-cadherin strong positive expression B: Vimentin strongly positive expression D: Vimentin weak-positive expression.

Table 2. The relationship between HER-2 expression and epithelial - interstitial transition

		Vimentin		E-cadherin	
		+	-	+	-
HER2	+	29	14	16	27
	-	14	18	25	7

3.2. The relationship between HER-2 expression and epithelial - interstitial transition

In 43 cases of HER-2 positive tissues, Vimentin expression levels increased, e-cadherin decreased (P<0.05), shown in Table 2, Figure 2.

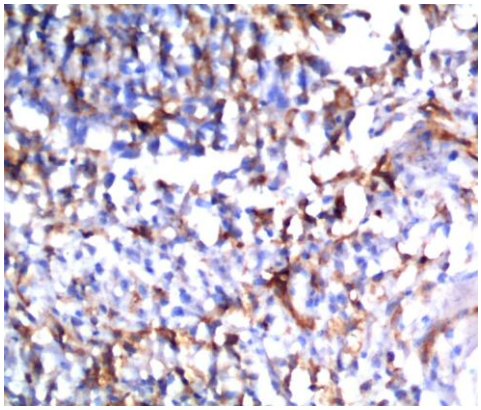

Figure 2. The Expression of HER-2 in endometrial cancer.

3.3. The relationship between the expression of her-2 and epithelial interstitial transition in spreamen

Spreamen analysis showed that there were negative correlation between her-2 and e-cadherin protein expression (R=-0.336, P=0.031), and positive correlation with Vimentin expression (R=0.587, P= 0.004), Figure 3.

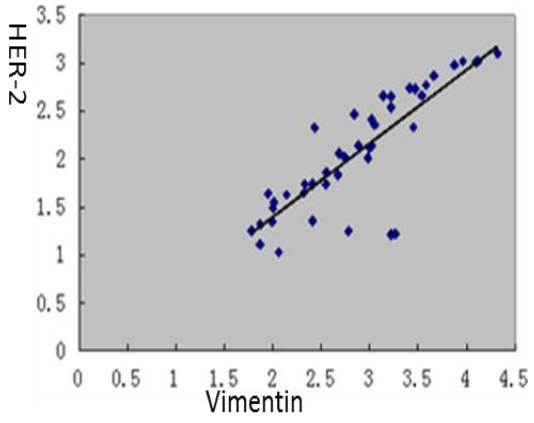

Figure 3. The relationship between the expression of her-2 and epithelial interstitial transition.

4. Discussion

A large number of studies have shown that endometrial cancer is highly heterogeneous, part of the same immune phenotype of endometrial cancer but tissue types were different, histologic. All of them have features characterized such as solid growth, with pleomorphic cells, and lack of gland structure[3]. The subtype of the tumor is not sensitive to endometrial cancer adjuvant chemotherapy or resistance, and it shows tumor epithelial mesenchymal transition[4]. Epithelium mesenchymal transition is one of the important transfer mechanism of tumor, which were reported in a variety of solid tumors[5,6]. The markers of Vimentin and E-cadherin have differences expression levels in endometrial carcinoma. Therefore, the discussion and analysis of epithelial-mesenchymal transition role in endometrial cancer chemotherapy drug resistance will have important clinical value.

E-cadherin is the epithelial calcium adhesion protein. It is an important marker of epithelial cells, and also has important value in maintaining the proliferation and biological function of epithelial cells[7]. In tumor, its positive expression increase appear significantly in epithelial mesenchymal transition, while the loss of expression showed that epithelial tumor cells adhesion disappear. Our study result shows that in 75 endometrial cancer patients, E-cadherin positive expression rate was 54.67%, and the rate was only 40.62% in the resistance group. It suggests that endometrial cancer cells present a loss of adhesion. Vimentin express in mesenchymal cells, and maintain the integrity of the cell with microtubule. Mesenchymal tumor tissues such as osteosarcoma and leiomyosarcoma had highly Vimentin expression[8,9]. But also in the nerve cells, gastrointestinal stromal tumor Vimentin has positive expression. Vimentin is an important marker of epithelial mesenchymal transition. It present the CK epithelial markers are alternative, by Vimentin, and cell morphological changes, and dynamic enhancement [10,11].

In our study, the positive rate of Vimentin in the endometrial cancer was 57.33%, and the positive rate in the resistance group was 65.64%. It indicates that the tumor showed obvious epithelial-mesenchymal transition. Our study preliminarily confirmed that it existed epithelium-mesenchymal transition in resistance groups, but how it mediated chemotherapy

drug resistance, and what it is possible mechanism. There is no clear research report. HER2 is a epidermal growth factor receptor. It is expressed in a wide variety of tumor. The HER2 is one of the important targets for clinical targeted therapy in endometrial carcinoma. Its has important value in mediating endometrial cancer biology behavior [12,13]. Therefore, our research will study expression of HER2 and analyze it whether mediated cells resistance by improving epithelial mesenchymal transition. Our results showed that the positive rate of HER2 in endometrial carcinoma was 57.33%, and in 43 cases HER2 positive organization. Vimentin expression level increased, while E-cadherin decrease. Chi-square proved HER2 have close relations to the expression of Vimentin and E-cadherin. Spreamen correlation analysis also showed that HER2 has negatively correlated with E-cadherin protein expression (R=0.336, P=0.336), and it also has positively related with Vimentin expression.

Through the establishment mice model of endometrial carcinoma, study found HER2 plays an important role in epithelial mesenchymal transition. By the inhibition of the expression of HER2, the transfer ability of endometrial cancer cells decreased obviously, and led to the decrease of the E-cadherin expression, decreased in intercellular adhesion ability, and activate the mesenchymal cells, enhance the vitality of cell movement[14,15]. Therefore, HER-2 may promote the metastasis and infiltration of tumor cells by regulating epithelial-interstitial transition.

Our study sample is less, the results may has certain limitations, but it confirmed that in endometrial cancer, it has epithelial-mesenchymal transition in patients with chemotherapy drug resistance, and the expression of HER2 has a close relationship to epithelial-mesenchymal transition.

5. Conclusion

The occurrence of chemotherapy drug resistance may be related to HER2 by raising the expression of Vimentin and decreasing E-cadherin expression, and then promote epithelial mesenchymal transition, but the specific molecular biological mechanism still needs further research.

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