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Clinical significances of PTEN and HER-2 protein expression in endometriosis associated ovarian carcinomas and their correlations.

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Abstract

This study aimed to investigate the clinical significances of phosphatase and tension homology deleted on chromosome ten (PTEN) and human epidermal growth factor receptor 2 (HER-2) protein expression in endometriosis associated ovarian carcinomas (EAOC) and their correlations. Seventy-eight cases of EAOC specimen and 60 cases of ovarian endometrial cyst (OEC) specimen were collected. The expressions of PTEN and HER-2 protein were determined using immunohistochemical analysis. Their relationships with clinicopathological features of EAOC and their correlations were analyzed. Results showed that, in 78 cases of EAOC, the expression level of PTEN protein in EAOC tissues was significantly lower than that in OEC tissues (P<0.01), and that of HER-2 protein in EAOC tissues was significantly higher than that in OEC tissues (P<0.01). The positive rates of PTEN and HER-2 protein had significant difference between EAOC with FIGO stage I-II and III-IV (P<0.01) and between EAOC with G1-G2 and G3 differentiation (P<0.01), respectively. In EAOC the expressions of PTEN and HER-2 protein were negatively correlated (r=-0.714, P<0.001). In conclusion, the expressions PTEN and HER-2 protein are significantly related to the FIGO stage and differentiation grade of EAOC, and they are negatively correlated.

Keywords: Endometriosis associated ovarian carcinomas, PTEN, HER-2, Relation.

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Introduction

Endometriosis is a benign disease in women of child-bearing age, which has the malignant transformation potential [1]. Gene mutation and apoptosis are closely related to the occurrence and development of malignant tumors [2]. The modern molecular pathology shows that, endometriosis is a monoclonal malignant disease. A part of endometriosis will develop into ovarian cancer, especially the ovarian endometrioid carcinoma (OEC), clear cell carcinoma (CCC) and mucinous ovarian carcinoma (MOC), which are also called endometriosis associated ovarian carcinomas (EAOC) [3]. The prognosis of EAOC is poor, and there is no effective targeted therapy scheme [4]. Phosphatase and tension homology deleted on chromosome ten (PTEN) is a tumor suppressor gene, only found so far, which has dual phosphatase activity. When the PTEN gene mutation, deletion or inactivation occurs, the expression of PTEN protein is down-regulated, and its activity is decreased. Therefore, the ability of PTEN protein in inhibiting tumor cell proliferation, adhesion, migration and angiogenesis is weakened, which thus promotes the development of tumor [5]. Human epidermal growth factor receptor 2 (HER-2) is a transmembrane glycoprotein with tyrosine protein kinase activity, and is one of the members of epidermal growth factor receptor (EGFR) family [6]. It is found that, the over-expression of HER-2 can potentially activate the EGFR signaling pathway, and promote EGFR-mediated transformation and tumor occurrence [7]. The clinical significance of PTEN and HER-2 protein expression in EAOC, especially their correlation, is relatively less reported, and the results are not consistent. This study investigated the clinical significances of PTEN and HER-2 protein expression in EAOC and their correlations. The objective was to provide a new basis for the diagnosis and treatment and prognosis evaluation of different types of EAOC.

Materials and Methods

Materials

Seventy-eight cases of EAOC specimen obtained by surgical resection in Heze Municipal Hospital from May 2010 to May 2016 were enrolled in this study. All samples were confirmed by pathology. In addition, 60 cases of ovarian endometrial cyst (OEC) specimen were collected during the same period. Before surgery, all patients had not been treated with radiation, chemotherapy, or hormone therapy. The pathological staging was performed according to the International Federation of Obstetrics and gynecology (FIGO) standards in 2009. This study was approved by the ethics committee of Heze Municipal Hospital. Written informed consent was obtained from all participants.

Immunohistochemical analysis

The paraffin sections of EAOC tissues and OEC tissues were prepared, and then were normally de-waxed. The enzyme closure was performed using 3% hydrogen peroxide and antigen retrieval with citrate buffer (Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, China). After closing non-specific sites using non-immune goat serum, primary antibody (rabbit anti-human PTEN monoclonal antibody, rat anti- human HER-2 monoclonal antibody; Sigma-Aldrich Corp., MO, USA) with 1:200 dilution was added, followed by incubation at 4°C overnight. After washing with PBS, horseradish peroxidase-labelled secondary antibody (Abcam Company, MA, USA) was added to each section, followed by incubation at 37°C for 30 min and washing with PBS for 2 times. After coloration using DBA, washing with water, counterstain and mounting, the sections were observed under optical microscope (Olympus Corp., Tokyo, Japan).

Determination of staining results

The positive expressions of PTEN protein was presented by pale yellow particles in cytoplasm or nucleus [8]. The positive expression of HER-2 protein was presented by pale yellow particles in cell membrane [9]. Under the low magnification (100X), the most satisfactory staining sites were selected from each immunohistochemical section. Then, under the high magnification (400X), 10 fields of view were randomly selected. Each field of view contained 200 cells. The scoring was performed according to the staining intensity and percentage of positive cells. For staining intensity, no coloring, yellow, pale brown and brown presented 0, 1, 2 and 3 points, respectively. For percentage of positive cells, the percentage of positive cells ≤ 10%, 10%-40%, 41%-70% and >70% presented 0, 1, 2 and 3 points, respectively. Two types of score were multiplied to obtain the final score. The mean value of 10 fields of view was taken. The score with ≥ 2 points presented the positive expression, and that with 0-1 point presented the negative expression.

Statistical analysis

All statistical analysis was carried out using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). The enumeration data were presented as number and rate, and the comparison between two groups was performed using χ^2 test. The correlation between two indexes was analyzed by Spearman rank correlation analysis. P<0.05 presented the statistically significant difference of comparison.

Results

General information of EAOC patients

In 78 cases of EAOC, there were 34 cases with age ≤ 60 years and 44 cases with age>60 years, 42 cases with FIGO stage I-II and 36 cases with FIGO stage III-IV, 33 cases with OEC, 24 cases with CCC and 21 cases with MOC, 36 cases with G1-G2 differentiation and 42 cases with G3 differentiation.

Expressions of PTEN and HER-2 protein in EAOC and OEC tissues

The positive rates of PTEN and HER-2 protein expression in EAOC tissue were 28.2% (22/78) and 68.3%% (41/60), respectively. The positive rates of PTEN and HER-2 protein in OEC tissues were 60.3% (47/78) and 33.3% (20/60), respectively. The expression level of PTEN protein in EAOC tissues was significantly lower than that in OEC tissues (P<0.01), and the expression of HER-2 protein in EAOC tissues was significantly higher than that in OEC tissues (P<0.01) (Table 1).

Table 1. *Expressions of PTEN and HER-2 protein in EAOC tissues and OEC tissues (n(%)).*

Group	EAOC (n=78)		OEC (n=60)		χ^2	P
	-	+	-	+		
PTEN	56 (71.8%)	22 (28.2%)	19 (31.7%)	41 (68.3%)	22.010	<0.001
HER-2	31 (39.7%)	47 (60.3%)	40 (66.7%)	20 (33.3%)	9.841	<0.001

EAOC: Endometriosis Associated Ovarian Carcinomas; OEC: Ovarian Endometrioid Carcinoma; PTEN: Phosphatase and Tension Homology Deleted on Chromosome Ten; HER-2: Human Epidermal Growth Factor Receptor 2.

Relationships of PTEN protein expression with clinicopathological features of EAOC

In 78 cases of EAOC, the positive rates of PTEN protein expression in FIGO stage I-II and III-IV were 40.5% (17/42) and 13.9% (5/36), respectively, with significant difference between them (P<0.01). The positive rates of PTEN protein expression in EAOC with G1-G2 and G3 differentiation were 41.7% (15/36) and 16.7% (7/42), respectively, with significant difference between them (P<0.01). The later FIGO stage and poorer differentiation indicated the lower expression of PTEN protein. In addition, there was no significant difference of PTEN protein expression between EAOC patients with age ≤ 60 years and/or >60 years, or among EAOC with histological type of OEC, CCC and MOC, respectively (P>0.05) (Table 2).

Table 2. *Relationship of PTEN protein expression with clinicopathological features of EAOC.*

		-	+	Positive rate (%)	X~	P
Age	≤ 60 y	25	9	26.5	0.090	0.765
	>60 y	31	13	29.5		
FIGO stage	I-II	25	17	40.5	6.767	0.009
	III-IV	31	5	13.9		
	OEC	23	10	30.3	0.283	0.868
Histological type	CCC	17	7	29.2		
	MOC	16	5	23.8		
Differentiation	G1-G2	21	15	41.7	5.983	0.014

G3	35	7	16.7	

EAOC: Endometriosis Associated Ovarian Cancers; OEC: Ovarian Endometrioid Carcinoma; FIGO: International Federation of Obstetrics and Gynecology; CCC: Clear Cell Carcinoma; MOC: Mucinous Ovarian Carcinoma; PTEN: Phosphatase and Tension Homology Deleted on Chromosome Ten.

Relationship of HER-2 protein expression with clinicopathological features of EAOC

The positive rates of HER-2 protein expression in EAOC with FIGO stage I-II and III-IV were 45.2% (19/42) and 77.8% (28/36), respectively, with significant difference between them (P<0.01). The positive rates of PTEN protein expression in EAOC with G1-G2 and G3 differentiation were 41.7% (15/36) and 76.2% (32/42), respectively, with significant difference between them (P<0.01). The later FIGO stage and poorer differentiation indicated the higher expression of HER-2 protein. In addition, the HER-2 protein expression had no significant relation with the age and histological type, respectively (P>0.05) (Table 3).

Table 3. *Relationship of HER-2 protein expression with clinicopathological features of EAOC.*

		-	+	Positive rate (%)	X˜	P
Age	≤ 60 y	13	21	61.8	0.057	0.811
	>60 y	18	26	59.1		
FIGO stage	I-II	23	19	45.2	8.571	0.003
	III-IV	8	28	77.8		
	OEC	16	17	51.5	1.825	0.402
Histological type	CCC	8	16	66.7		
	MOC	7	14	66.7		
Differentiation	G1-G2	21	15	41.7	9.684	0.002
	G3	10	32	76.2		

EAOC: Endometriosis Associated Ovarian Cancers; OEC: Ovarian Endometrioid Carcinoma; FIGO: International Federation of Obstetrics and Gynecology; CCC: Clear Cell Carcinoma; MOC: Mucinous Ovarian Carcinoma; PTEN: Phosphatase and Tension Homology Deleted on Chromosome Ten.

Correlations of PTEN and HER-2 protein expression in EAOC

The Spearman rank correlation analysis showed that, in EAOC, the expression of PTEN protein was negatively correlated with HER-2 protein expression (r=-0.714, P<0.001).

Discussion

The incidence of EAOC accounts for 10%-15% of ovarian cancer [10]. The study on molecular biological mechanism of EAOC has gradually become the key point of the prevention and treatment of this disease. Studies [7,11] have shown that, the expression and mutation of various proto oncogenes in EAOC may play an important role in the process of adhesion, invasion and proliferation of endometriosis tissues. It is found that, the malignant transformation rate of endometriosis is about 0.7-1.6%. The 80% malignant transformation sites are in the ovary, which can increase the incidence of ovarian cancer [12]. Due to the high resistance to chemotherapy, the clinical prognosis of EAOC is poor [13]. Therefore, studying the molecular biological mechanism of EAOC, preventing the malignant transformation of endometriosis and finding new target drugs have gradually become the core of the prevention and treatment of ovarian cancer.

At present, the study on the relationship between PTEN and the malignant transformation of endometriosis is still at in the exploratory stage [14]. The loss of PTEN expression may be an early event in the process of malignant transformation of endometriosis, and may be one of the mechanisms of malignant change of endometriosis [15]. Previous study [16] has shown that, the occurrence and development of endometrial diseases are closely related to the functional inactivation of *PTEN* genes. Others scholars [17] believe that, *PTEN* gene is the housekeeping gene in the endometrium. Martini et al [18] have found that, the expression of PTEN protein is down-regulated in case of malignant endometriosis of ovary, but the mechanism is unknown. Therefore, in this study we investigated the expression of PTEN in EAOC tissues and OEC tissues, and analyzed its correlations with the clinicopathological features of EAOC. Results showed that, the positive rate of PTEN protein expression in EAOC tissues was significantly lower than that in OEC tissues (P<0.01). In 78 cases of EAOC, the positive rate of PTEN protein expression had significant difference between EAOC with FIGO stage I-II and III-IV (P<0.01) and between EAOC with G1-G2 and G3 differentiation (P<0.01), respectively. The later FIGO stage and poorer differentiation indicated the higher expression of PTEN protein. This suggests that, the inactivation of PTEN protein may play an important role in the malignant transformation of OEC to EAOC.

HER-2 is a transmembrane glycoprotein with tyrosine protein kinase activity, and is one of the members of EGFR family. It is confirmed that, the over-expression of HER-2 can potentially activate the signaling pathway of EGFR, and promote EGFR-mediated transformation and tumor progression [19]. HER-2 protein is usually expressed only during the fetal stage [20]. After adulthood, the expression level of HER-2 protein is relatively low, and only in a very small number of tissues [21]. However, the over-expression of HER-2 protein can be found in many human tumors such as breast cancer [22], gastric cancer [23], primary renal cell carcinoma [24], endometrial carcinoma [25], etc., which suggests the poor prognosis. As HER-2 is closely related to the occurrence, development and prognosis of cancer, it has now become one of the targets of immunotherapy. It is confirmed that, the expression rate of *HER-2* gene is 20-30% in epithelial ovarian cancer, and is correlated with the FIGO stage, histological grade, residual tumor size and pathological type of ovarian cancer [26]. The research on correlation of HER-2 protein expression with EAOC is relatively less reported, and the conclusions are controversial. This study also investigated the expression of HER-2 in EAOC tissues and OEC tissues,

and analyzed its correlations with the clinicopathological features of EAOC. Results showed that, the expression of HER-2 protein in EAOC tissues was significantly higher than that in OEC tissues ($P<0.01$). The positive rates of HER-2 protein had significant difference between EAOC with FIGO stage I-II and III-IV ($P<0.01$), and between EAOC with G1-G2 and G3 differentiation ($P<0.01$). The later FIGO stage and poorer differentiation indicated the higher expression of HER-2 protein. This suggests that, the high expression of HER-2 protein may play an important role in the malignant transformation of OEC to EAOC.

Both PTEN and HER-2 are involved in the regulation of cell cycle and apoptosis. Their action links are the same, but the biological effects are opposite [27,28]. PI3K/Akt signaling pathway can regulate the expression of *HER-2* gene, while PTEN can inhibit the PI3K/Akt pathway under normal physiological conditions, thus inhibiting the expression of HER-2 protein [29,30]. Results of this study showed that, PTEN protein was lowly expressed in EAOC, and HER-2 protein was highly expressed in EAOC. In addition, in EAOC the expressions of PTEN and HER-2 protein were negatively correlated ($r=-0.714$, $P<0.001$). Chen et al. [31] have found that, the expressions of PTEN and HER-2 protein were negatively correlated ($r=-0.9$, $P<0.05$) in endometrioid adenocarcinoma. This is basically the same with our study result.

In conclusion, the expressions PTEN and HER-2 protein are significantly related to the FIGO stage and differentiation grade of EAOC. In addition, they are negatively correlated. This study has provided a new basis for the diagnosis and treatment and prognosis evaluation of different types of EAOC. However, due to relatively small sample size, this should be further verified.

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