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The expression and significance of p-STAT3, Twist, and E-cadherin in ovarian epithelial carcinoma

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Summary

Objective: To observe the expression of p-STAT3, Twist, and E-cadherin in ovarian epithelial carcinoma and normal ovarian tissue and to investigate the correlation and significance among them and clinicopathological parameters of ovarian cancer. *Materials and Methods:* The expression of p-STAT3, Twist, and E-cadherin in 60 cases of ovarian cancer were detected by immunohistochemistry. Forty normal ovaries were used as controls. *Results:* The positive rates of p-STAT3, Twist, and E-cadherin in ovarian cancer were 68.3%, 65%, 28.3%, and those of normal ovarian tissue rates were 20%, 27.5%, and 87.5%. The differences were statistically significant and the expressions of p-STAT3 and Twist were positively correlated, but negatively correlated with E-cadherin expression. The expressions of P-STAT3, Twist, and E-cadherin were correlated with the ovarian cancer clinical stage, tumor differentiation, and lymph node metastasis ($p < 0.05$). *Conclusion:* In ovarian epithelial carcinoma, the expressions of p-STAT3 and Twist are related to the biological characteristics of tumor to a certain extent, and both of them may be involved in epithelial and mesenchymal transition of epithelial ovarian cancer.

Key words: STAT3; Twist; E-cadherin; Ovarian cancer.

Introduction

Epithelial mesenchymal transition (EMT) refers to the epithelial cells losing the polarity and normal morphology, transfering into mesenchymal cells with the ability to migrate the form, which is more common in the early embryonic development. Research shows [1, 2] that EMT is closely related to the occurrence and metastasis of malignant tumors. In recent years, the study found, signal transducers and activators of transcription 3 (STAT3) and twist protein (Twist) are newly discovered oncogene, and both of them are closely related to EMT. STAT3 can affect cell proliferation, differentiation, apoptosis, invasion and metastasis, and many molecules that regulate EMT are upstream or downstream of STAT3. Many studies have shown that STAT3 is the upstream gene of Twist, STAT3 activation can promote EMT by up-regulating the expression of Twist. However the researchs of both ovarian cancers are scant at home and abroad. In this study, the expressions of activated form (phosphorylated) STAT3 (p-STAT3), Twist, and epithelial marker E-cadherin in epithelial ovarian cancer tissue were detected by immunohistochemistry, and the correlation between the expressions among them, and clinical pathological parameters of ovarian cancer were analyzed, preliminarily demonstrating the relationship among p-STAT3, Twist, and EMT in ovarian cancer.

Materials and Methods

Sixty ovarian cancer resection specimens were selected by the Pathology Department in Yantai Yuhuangding Hospital between 2013 and 2014, and all cases were diagnosed as epithelial ovarian cancer, and neoadjuvant chemotherapy was not performed before surgery in all patients. The ages of the patients were from 34 to 68 (mean 52) years old. Normal ovarian tissues were selected from 40 cases in the same period, from total hysterectomy plus double accessory resection patients due to uterine fibroids or adenomyosis hysterectomy, aged from 44 to 62 (mean 53) years. According to the surgical pathological staging criteria of the International Federation of Gynecology and Obstetrics (FIGO) in 2000, cases of Stage I and II were 18, and the cases of Stage III and IV were 32; pathological grading: G1 in 14 cases, G2 in 18 cases, and G3 in 28 cases. According to WHO histological classification: serous carcinoma were 43 cases, mucous carcinoma were ten cases, and Moulting cancer in the uterus were seven cases. After collection, the specimens were immediately frozen in liquid nitrogen, and then they were placed in a 70% refrigerator. All diagnoses were confirmed by pathology. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Yantai Yuhuangding Hospital. Written informed consent was also obtained from all participants.

Rabbit anti-human Twist, STAT3, E-cadherin polyclonal antibody Streptavidin-biotin complex SABC Reagent kit, and DAB color reagent kits were used. Immunohistochemistry included the SABC method, conventional paraffin sectioning, dewaxing to water, PBS washing two times each for five minutes, and sealed with 3% H_2O_2 at room temperature for ten minutes. Washed three times with distilled water, antigen repaired in ten minutes, PBS

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wash three times, each for five minutes. Drop serum sealing liquid of normal goat occurred at room temperature for 20 minutes, shaken off the excess liquid, and added the primary antibody four times for a an entire night. PBS was three times every minute. Biotinylated secondary antibody was placed at 37°C for 20 minutes, and PBS washed for three times, every two minutes. Drop reagent SABC, 20-37°C for 20 minutes, PBS was washed four times every five minutes. DAB stained, washed with distilled water, and redyed with Hematoxylin for one minute. Hydrochloric acid ethal differentiation, dehydration, and transparent seal microscopic examination followed.

The judgment standard of results: the immunohistochemical results were evaluated by two physicians who did not know any of the clinical and pathological data. The judgement of positive results: positive results were judged by semi-quantitative integral method. Staining intensity score: no staining 0, light yellow 1, brown 2, tan 3. Ten fields of view were randomly selected for each slice, and were counted by the percentage of positive cells at the view of high magnification. The positive rate of cells was 0, < 25%, 25-50%, and > 50%, respectively, 0 points, 1 point, 2 points, and 3 points. The product of two integral < 2 denoted negative expression, while > 2 a positive expression. The X^2 test and rank correlation analysis were also used.

Results

The positive rate of p-STAT3 protein in ovarian cancer tissue was 68.3%, and the positive rate in normal ovarian tissue was 20%%. The positive rate of Twist protein in ovarian cancer tissue was 65%%, and that in normal ovarian tissue was 27.5%%. The positive rates of Twist and STAT3 protein in ovarian cancer tissues were significantly higher than that in normal ovarian tissues ($p < 0.01$). The positive rate of E-cadherin in ovarian cancer tissue was 28.3%%, which was significantly lower than that of normal ovarian tissue 87.5%% ($p < 0.01$, Table 1).

The results showed that the expressions of p-STAT3 and Twist in ovarian cancer tissues, which were in advanced stage, poorly differentiated and lymph node metastasis increased, while the expression of E-cadherin decreased. The expressions of p-STAT3, Twist, and E-cadherin in the stage of ovarian cancer, differentiation degree, and lymph node metastasis were statistically significant ($p < 0.05$, Tables 2-4). The results showed that Twist and STAT3 expression and E-cadherin expression were negatively correlated ($p < 0.01$, Table 5).

Discussion

Recent studies have indicated that EMT is an important event in the process of tumor invasion, which is closely related to the occurrence and metastasis of tumor [3, 4]. EMT means that the epithelial cells acquire the original fibroblast-like phenotype, and expresses downregulation of expression of intercellular adhesion, overexpression of stromal cell markers, cytoskeletal rearrangement, the increasing of cell invasion and migration ability, and the formation of new cell matrix adhesion, *etc* [5-7]. EMT is helpful to the invasion and metastasis of cancer cells, and plays a key role in the invasion and metastasis of epithelial tumors.

Ovarian cancer mortality is the highest in gynecological malignancies. Invasion and metastasis are the main factors influencing the survival rate of ovarian cancer patients, but the mechanism of its occurrence, development, and infiltration diffusion is not clear. EMT is also found in ovarian cancer, and its role in the invasion and metastasis of the ovarian cancer has been verified [8-15]. Twist is a newly discovered oncogene, and is one of the key factors in the process of EMT [16, 17]. Ru *et al.* [18] found that the high expression of Twist in gastric cancer was significantly related to the tumor invasion and the prognosis of the patients. It is suggested that Twist overexpression is significantly linked to cervical cancer tumorigenesis and progression, likely related to EMT through (YB-1)-Twist-(E-cadherin) pathway [19]. Twist promotes tumor metastasis in basal-like breast cancer by transcriptionally upregulating ROR1 [20]. TWIST1 as a target protein to enhance the sensitivity of colorectal cancer cells to 5FU [21]. Studies of Yoshida *et al.* [10] found that the expression of Twist in epithelial ovarian cancer was significantly higher, and it was significantly correlated with its FIGO Stage, suggesting that Twist related to the metastasis of ovarian cancer. Gao *et al.* [15] study showed that NAC1 and FOXQ1 were upregulated in ovarian carcinoma. The overexpression of NAC1 promoted invasion and metastasis of the ovarian cancer cell, and induced NF-kappa B phosphorylation by downregulating the metastasis suppressor gene MKK4, promoting Twist expression, thereby inhibiting the expression of E-cadherin, leading to ovarian cancer the occurrence of EMT, and increasing the ability of invasion and metastasis. The results of this study showed that the high expression of Twist was closely related to the low expression of E-cadherin, and the positive rate was higher in the advanced stage and lymph node metastasis group than in the early stage and no lymph node metastasis group. Therefore, the present authors deduced that the expression of oncogene Twist in ovarian cancer increased and inhibited the expression of E-cadherin. The decrease of E-cadherin expression decreased the adhesive strength of ovarian cancer cells, and it was simple to fall off from the primary tumor. Ovarian cancer tissues lack of natural barrier, so the shedding cancer cells spread easily in the abdominal cavity and plant transfer occurred.

STAT3 is a key molecule of the downstream of JAK kinase/signal transduction activator transcription. STAT3 phosphorylation is closely related to the proliferation, differentiation, and apoptosis of tumor cells. STAT3 could have context-dependent molecular roles of in breast cancer, results which warrant further prospective verification in clinical trials [22]. Interleukin-12 inhibits the hepatocellular carcinoma growth by inducing macrophage polarization to the M1-like phenotype through downregulation of STAT3

Table 1. — *Expressions of p-STAT3, Twist, and E-cadherin in ovarian carcinoma and normal ovarian tissues (n).*

Index	Ovarian cancer tissue (n=60)		Normal ovarian tissue (n=40)		X^2	*p*
	Positive	Positive rate (%)	Positive	Positive rate (%)		
p-STAT3	41	68.33	8	20.00	22.44	< 0.01*
Twist	39	65.00	11	27.50	13.50	< 0.01*
E-cadherin	17	28.33	35	87.50	33.66	< 0.01*

**The differences were statistically significant.*

Table 2. — *Relationship between expressions of p-STAT3 and clinical pathological parameters of ovarian cancer (n).*

Pathological parameters	n	Positive	Positive rate (%)	X^2	*p*
Stage				19.55	< 0.01*
I-II	18	5	27.78		
III-IV	42	36	85.71		
Grade				11.79	< 0.01*
G1-2	31	15	48.39		
G3	29	26	89.66		
Lymph node metastasis				7.29	< 0.01*
Negative	43	25	58.14		
Positive	17	16	94.12		

**The differences were statistically significant.*

Table 3. — *Relationship between expressions of Twist clinical pathological parameters of ovarian cancer (n).*

Pathological parameters	n	Positive	Positive rate (%)	X^2	*p*
Stage				11.33	< 0.01*
I-II	18	6	33.33		
III-IV	42	33	78.57		
Grade				19.49	< 0.01*
G1-2	31	12	38.71		
G3	29	27	93.10		
Lymph node metastasis				8.84	< 0.01*
Negative	43	23	53.49		
Positive	17	16	94.12		

**The differences were statistically significant.*

Table 4. — *Relationship between expressions of E-cadherin and clinical pathological parameters of ovarian cancer (n).*

Pathological parameters	n	Positive	Positive rate (%)	X^2	*p*
Stage				18.61	< 0.01*
I-II	18	12	66.67		
III-IV	42	5	11.90		
Grade				5.84	0.016*
G1-2	31	13	41.94		
G3	29	4	13.79		
Lymph node metastasis				4.45*	0.035*
Negative	43	16	37.21		
Positive	17	1	5.88		

**The differences were statistically significant.*

Table 5. — *Relationship between expressions of Twist and p-STAT3 in ovarian carcinoma and E-cadherin expression (n).*

E-cadherin	p-STAT3 expression			Twist expression		
expression	Negative	Positive	Total	Negative	Positive	Total
Netative	5	36	43	9	34	43
Positive	14	3	17	12	5	17
total	19	41	60	21	39	60
r	-0.68	-0.47				
p	< 0.01	< 0.01				

[23]. At present, many studies have confirmed that STAT3 and EMT are closely related, and many molecules that regulate EMT are target molecules of upstream or downstream of STAT3. STAT3 signal pathway activation induced by IL-6 is related with the invasion, metastasis, and drug resistance of head and neck cancers, ovarian cancer, hepatocellular carcinoma, and breast cancer [24-27]. Sullivan *et al.* [28] found that activation of STAT3 and abnormal IL-6 production in breast cancer MCF-7 cells can promote the expression of Twist and inhibit the expression of E-cadherin at the transcriptional level to induce EMT and promote the metastasis of breast cancer. p-STAT3 can also promote the development of gastric cancer by regulating Twist [27] and mediate the invasion and metastasis of HCC [29, 30]. Therefore, p-STAT3 can promote EMT by upregulating the expression of Twist. The results showed that p-STAT3 in ovarian cancer tissue expression increased, and with Twist expression was positively correlated, while with the expression of E-cad was negatively correlated. The positive rate in the advanced stage and lymph node metastasis was higher than that in the early stage and no lymph node metastasis group. Therefore, it can be considered that there are STAT3 activation and EMT in human ovarian cancer, and it is related to the invasion and metastasis of ovarian cancer. p-STAT3, Twist, and E-cadherin may be predictive index of invasion and metastasis of ovarian cancer. However the mechanism and relationship between STAT3 activation and Twist in ovarian cancer EMT still need to be further studied.

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