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Clinicopathology of EpCAM and EGFR in human epithelial ovarian carcinoma

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Abstract: The objective of this study was to explore the expression of EpCAM and EGFR in human epithelial ovarian cancer (EOC) and their correlation with clinicopathological parameters.

The protein expression levels of epithelial cell adhesion molecule (EpCAM) and epidermal growth factor receptor (EGFR) were evaluated by immunohistochemistry in formalin-fixed paraffin-embedded specimens from 30 patients with epithelial ovarian carcinoma and 15 normal ovary tissues. Clinicopathological characteristics were gathered by retrospective review of the patients' files. The correlation between EpCAM and EGFR expression, as well as their association with clinical pathological parameters were investigated. The SPSS 17.0 package was used to perform statistical analyses.

The positive expression rates of EpCAM and EGFR were significantly elevated in epithelial ovarian cancer tissues than in normal ovary tissues. The positive expressions of EpCAM and EGFR in EOC were associated with International Federation of Gynecology and Obstetrics (FIGO) stage and tumor differentiation, lymph node metastasis. Spearman correlation analysis demonstrated a significant positive association between EpCAM and EGFR expression in EOC.

The co-expression of EpCAM and EGFR may play an important role in the carcinogenesis of EOC and might provide a promising molecular therapeutic target.

Keywords: Epithelial ovarian cancer; EpCAM; EGFR; Immunohistochemistry

1 Introduction

Epithelial ovarian cancer (EOC) is the leading cause of mortality from gynecological malignancies and account for 85%~90% of ovarian cancer. Based on GLOBOCAN estimates, there were an estimated 238,700 new ovarian cancer cases and 151,900 deaths worldwide in 2012 [1]. The relatively asymptomatic early stages and the absence of accurate screening methods and biomarkers result in the majority ovarian cancers being diagnosed at advanced stages when metastasis has occurred, followed by migration, implantation and invasion throughout the peritoneal cavity. Despite the use of cytoreduction surgery and subsequent platinum-paclitaxel combination chemotherapy during advanced stages, 25% of patients show resistance to chemotherapy, and the majority ultimately relapse without response to treatment. Accordingly, ovarian cancer has a high mortality rate, with a five year survival near 45% [2]. Given the complex biological behavior of ovarian cancer, the identification of a novel potential therapeutic target is the present clinical challenge.

The epithelial cell adhesion molecule (EpCAM, also called CD326) is a 39~42kDa calcium-independent transmembrane glycoprotein initially discovered as an antigen on human colon carcinoma, and functions as a homophilic, epithelial-specific intercellular adhesion molecule [3]. EpCAM protein is consisted of an extracellular domain with epidermal growth factor-like domain and presumed thyroglobulin domain, a single transmembrane region, and a small 26-amino acid intracellular domain [4]. EpCAM expression is found in epithelium of healthy individuals, except epidermal keratinocytes, gastric pari-

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etal cells, hepatocytes, and squamous epithelium. It has been demonstrated that EpCAM is also expressed in the majority of human epithelial-derived carcinomas at levels greater than in human normal epithelium [5]. The role of EpCAM is not only limited to mediating epithelial-specific intercellular adhesion, but it is also involved in cellular signaling, cell proliferation, migration, invasion and differentiation, suggesting it plays an important role in cancer progression and metastasis [6].

Epidermal growth factor receptor (EGFR) is a member of the human epidermal receptor (HER) family, comprisingfour type I transmembrane tyrosine kinase receptors: EGFR (HER1, erbB-1), erbB-2 (HER2 or Neu), HER3 and HER4. EGFR is the expression product of oncogene c-erbB1 and is structurally subdivided into an extracellular ligand-binding domain, a single lipophilic transmembrane domain, and an intracellular tyrosine kinase domain [7]. Upon binding of the ligand to the extracellular domain, EGFR is activated and undergoes homodimerization or heterodimerization, which in turn induces the activation of intrinsic tyrosine kinase and ultimately triggers multiple signaling pathways [8]. The signaling pathways activated by EGFR dimerization play a key role in variety cancer cell behaviors, including regulation of cell proliferation, adhesion, migration, invasion, angiogenesis and resistance to apoptosis [9]. It has been well-documented that overexpression of EGFR is detected in epithelial ovarian cancer and is associated with poor prognosis and chemoresistance [10].

As it is known that overexpression of EpCAM and EGFR are associated with the development and progression of human carcinomasthe present study was conducted to investigate whether there is a correlation between EpCAM and EGFR in EOC progression. Our study utilized immunohistochemistry to detect the expression of EpCAM and EGFR in EOC tissues, and identified the relationship between protein expression and clinical pathological parameters. Further objective were to elucidate the potential associations with EpCAM and EGFR, and determine if EpCAM and EGFR could be a joint molecular therapeutic target in epithelial ovarian cancer.

2 Methods

2.1 Tissue specimens

In this study, 30 Formalin-fixed paraffin-embedded (FFPE) specimens from epithelial ovarian cancer (EOC) patients and 15 FFPE normal ovarian epithelial tissue specimens obtained from normal ovaries during surgery for other gynecological diseases were collected between April 2013 and December 2015 in the Department of Pathology of the Second Hospital of Jilin University. All hematoxylin-eosin slides were reviewed by two pathologists to establish a histological gradiant and type using World Health Organization (WHO) criteria to achieve a consensus diagnosis. None of the EOC patients included in this study had received chemotherapy or radiotherapy before surgical operation. This study was approved by the Medical Ethics Committee of the Second Hospital of Jilin University and written informed consent was obtained from each participant.

2.2 Immunohistochemical Staining

4-micrometre sections were cut from FFPE tissue specimens. The sections were mounted and baked at 60°C for 1 hour. Then the sections were deparaffinized in xylene and rehydrated in a series of graded alcohol. Endogenous peroxidase activity was removed by incubation the sections with 3% H_2O_2 for 5 minutes. The sections were heated in an autoclave in retrieval buffer (10mM citric acid buffer, pH 6.0) at 100°C for 15 minutes for antigen retrieval. After washing three 5-minute with PBS (0.1M Tris-HCl, pH 7.6), non-specific binding was blocked by immersing the sections in PBS containing 10% goat serum at room temperature for 30 minutes. Then the sections were incubated with the primary antibody against EGFR (rabbit anti-human monoclonal antibody, 1:250, Abcam, MA, USA) and EpCAM (mouse anti-human monoclonal antibody, 1:200, Abcam, MA, USA) overnight at 4°C. After rinsing with PBS three times for 5-minutes, sections were incubated with horseradish-peroxidase-labeled goat anti-mouse/rabbit IgG/HRP conjugated polymer (PV-6000, ZSGB-BIO) for 20 minutes at 37°C. 3,3-diaminobenzidine tetrahydrochloride with hydrogen peroxidase (DAB-H_2O_2 substrate) was used as a chromogen. Next, the sections were washed with distilled water and counterstained with hematoxylin, dehydrated with ascending grades of ethanol, cleared with xylene and mounted with resinous mounting medium. The procedure was repeated in the negative control slides, using phosphate buffered saline solution instead of the primary antibody.

2.3 Histology scoring

EpCAM and EGFR positive staining were observed in the membrane of epithelial ovarian cancer cells. All sections

were evaluated using a double-blind method. For each tissue section, immunostaining results were scored quantitatively in 5 high-power fields (200×magnification) and 100 tumor cells were counted in each field. The staining intensity was classified according to the following criteria: 0 = no staining; 1 = weak staining, 2 = moderate staining, 3 = strong staining. The percentage of positive staining tumor cells was scored as follows: 0 for ≤ 5%, 1 for 6 -25%, 2 for 26-50%, 3 for 51-75% and 4 for ≥76%. Values of staining intensity score were multiplied by the values of positive cells score to define the expression level: a score of 0-2 was considered negative, 3-4 was weak positive, 6-8 was moderate positive and 9-12 was strong positive.

2.4 Statistical methods

The Statistical Package for Social Sciences version 17.0 was used for all statistical analyses (SPSS 17.0, IBM, Chicago, IL, USA). Association between protein expression level of EpCAM or EGFR and clinicopathologic characteristics was identified using the Chi-square test or Fisher's exact test. The Spearman correlation analysis was used to evaluate the correlations between EpCAM expression and EGFR expression. In all analyses, a *P* value <0.05 was considered as statistically significant.

3 Results

3.1 Clinical pathology information

The clinical and pathological parameters of patients were collected by retrospective review of the patients' files and are described in Table 1. The diagnosis of histological type and histological grade were assigned according to the Classification of Ovarian Cancer (WHO 2004), among which 22 cases were serous cystadenocarcinoma, 5 mucinous cystadenocarcinoma, and 3 endometrioid carcinoma. Regarding tumor histological grading (tumor differentiated), 6 samples were well differentiated (Grade 1), 6 samples were moderately differentiated (Grade 2) and 18 samples were poorly differentiated (Grade 3). The epithelial ovarian carcinoma staged following the standards of International Federation of Gynecology and Obstetrics (FIGO, 2006), 8 patients had stage I, 2 patients had stage II, 13 patients had stage III, 7 patients had stage IV. 19 of

Table 1: Correlation of EpCAM and EGFR expression with clinical pathological parameters in epithelial ovarian carcinoma

Parameters	n	EpCAM Positive (%)	P	EGFR Positive (%)	P
Age					
≥55	14	10(71.4)	0.378	8(57.1)	0.442
<55	16	14(87.5)		12(75.0)	
Histological type					
Serous	22	19(86.4)	0.343	16(72.7)	0.375
Mucinous	5	3(60.0)		2(40.0)	
Endometrioid	3	2(66.7)		2(66.7)	
Differentiation					
Well - moderately	12	7(58.3)	0.026	4(33.3)	0.004
Poorly	18	17(94.4)		16(88.9)	
FIGO stage					
I - II	10	5(50.0)	0.009	3(30.0)	0.005
III - IV	20	19(95.0)		17(85.0)	
Lymphatic metastasis					
No	11	6(54.5)	0.016	4(36.4)	0.015
Yes	19	18(94.7)		16(84.2)	

Notes: *P<0.05* statistically significant

the 30 patients had lymph node metastasis. The ages of patients ranged from 44 to 78 years (median 55.2 years).

3.2 Expression of EpCAM and its correlation with clinicopathological parameter

EpCAM immunostaining pattern was diffuse throughout the tumor cell membrane. The proportion of immunoreactive cells among epithelial ovarian carcinoma specimens was 80% (Figure 1). In contrast, EpCAM expression in normal ovarian epithelial tissue was weak in 4 of 15 samples (26.7%, Figure 1). These result suggest that the level of EpCAM expression is increased in epithelial ovarian carcinoma compared to levels found in normal ovaries (X^2=12.101, P<0.05, Table 2).

As shown in Table 1, a statistically significant association was observed between EpCAM expression and FIGO stage and the degree of differentiation (histological grade), lymph node metastasis (*P<0.05*). No statistically significant correlation was found between EpCAM expression and age or histological type (*P>0.05*). The highest EpCAM expression was found in patients with lymph node metastasis. The level of EpCAM protein expression was found to be significantly elevated in patients with FIGO advanced stages (III-IV) versus FIGO early stages (I-II). The expression level of EpCAM ranged from moderately differentiated and well-differentiated (G1-G2) to poorly differentiated (G3).

3.3 Expression of EGFR and its correlation with clinicopathological parameter

The expression of EGFR was seen in the cell membrane of epithelial ovarian carcinoma (Figure 2). However EGFR immunostaining was negative in most normal ovarian epithelial tissue (Figure 2). Among 30 epithelial ovarian carcinoma patients, 24 patients were positive for EGFR staining (66.7%). In contrast, EGFR immunostaining was weakly positive in normal ovary (20%). Expression of EGFR was significantly greater in ovarian carcinoma samples than in the non-cancerous controls (*X^2=8.715, P<0.05*, Table 3).

Correlations between the incidence of immunoreactivity for EGFR and the clinicopathological parameter of

Table 2: Chi-square test analyses of EpCAM expression in EOCs and normal ovaries

		EpCAM expression						
	n	-	+	++	+++	Positive rate (%)	X^2	P
EOC	30	6	10	12	12	80	12.101	0.001
Normal	15	11	2	2	0	26.7		

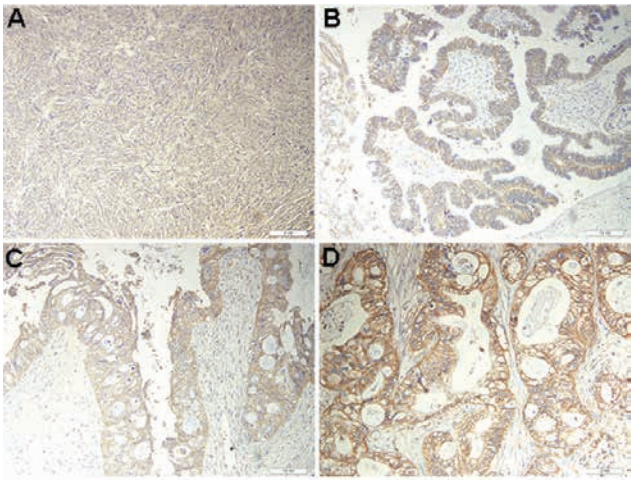

Figure 1: Representative immunohistochemical results of EpCAM in human normal ovary and epithelial ovarian carcinoma tissue sections. (A) Mild staining of EpCMA in normal ovary tissue. (B) Epithelia ovarian carcinoma tissue with weak positive (+) of EpCAM. (C) Epithelia ovarian carcinoma tissue with moderate positive (++) of EpCAM. (D) Epithelia ovarian carcinoma tissue with strong positive (+++) of EpCAM

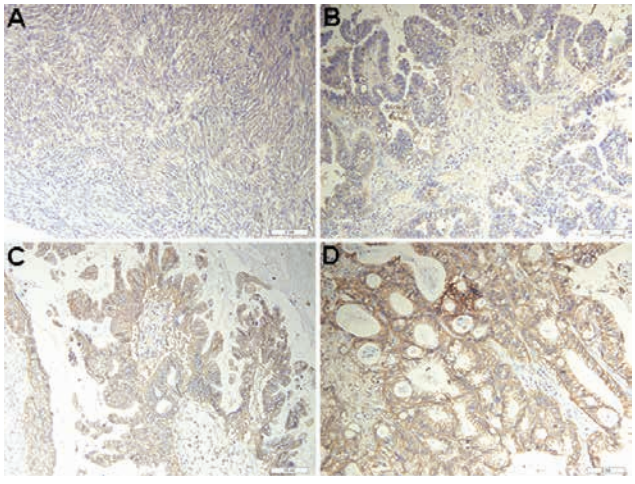

Figure 2: Representative immunohistochemical results of EGFR in human normal ovary and epithelial ovarian carcinoma tissue sections. (A) Mild staining of EGFR in normal ovary tissue. (B) Epithelia ovarian carcinoma tissue with weak positive (+) of EGFR. (C) Epithelia ovarian carcinoma tissue with moderate positive (++) of EGFR. (D) Epithelia ovarian carcinoma tissue with strong positive (+++) of EGFR

epithelial ovarian carcinoma are summarized in Table 1. The data show that a positive EGFR status is positively correlated with FIGO stages, the degree of differentiation (histological grade), and lymph node metastasis ($P<0.05$). The correlation between EpCAM expression and age orhistological type is non-significant ($P>0.05$). The percentage of EGFR positive tumor cells increase with increasing FIGO stages. Positive EpCAM expression rates were higher in poorly differentiated samples (G3) than those which were moderately differentiated and well-differentiated (G1-G2). The increased expression of EpCAM protein was more prevalent in lymph node metastasis of epithelial ovarian carcinoma compared to non-metastatic samples.

3.4 EpCAM and EGFR expression association in epithelial ovarian carcinoma

Spearsman was used to investigate the potential association between EpCAM and EGFR expression in epithelial ovarian carcinoma. 30 epithelial ovarian carcinoma specimens were used in this study. Among these, 19 epithelial ovarian carcinoma specimens exhibited positive immunostaining for both EpCAM and EGFR, 5 specimens exhibited negative for both EpCAM and EGFR, 5 specimens were only positive for EpCAM, and only 1 specimen was positive for EGFR. We found a significant positive correlation between EpCAM and EGFR expression in epithelial ovarian carcinoma tissues (Table 4, $r=0.530$, $P=0.003$).

4 Discussion

Acting as a mitogenic signal transducer, EpCAM, enhances cell proliferation with a direct influence on the cell cycle control, upregulates the proto-oncogene c-myc and the cell cycle regulating genes cyclin A and E, and induces signal transduction into the cell nucleus via the Wnt signaling pathway, which finally lead to tumorigenesis and development [4]. Previous studies have confirmed that EpCAM promotes invasion and metastasis of tumor cells, and EpCAM positive cells have a stronger capacity of cell proliferation compared with EpCAM negative cells [11]. The percentage of positive EpCAM expression in metastatic gastric carcinoma has also been found to be higher than that of nonmetastatic gastric carcinoma [12]. The intensity of EpCAM expression is associated with tumor differentiation, stage of disease, and metastasis [13-15]. Our study revealed that epithelial ovarian carcinoma tissues exhibit higher expression of EpCAM when compared with normal ovarian tissues, and demonstrated that upregulation of EpCAM was associated with FIGO stage and the degree of differentiation, lymph node metastasis.

EGFR, as a transmembrane tyrosine kinase receptor, specifically binds to epidermal growth factor (EGF) or transforming growth factor-α (TGF-α), causing tyrosine kinase auto-phosphorylation, which in turn activates the phosphatidylinositol 3-kinase pathway (PI3K/Akt pathway) and ras- raf-mitogen-activated protein kinase pathway (Ras/Raf/MAPK pathway) [16]. In physiological processes, EGFR regulates cell differentiation and the cell cycle, and also promotes repair of wound tissue. In pathological processes, overexpression of EGFR has been observed in a variety of human cancers, such as breast, gastric, and cervical cancers [17-19]. The overexpression of EGFR continuously transmits signals to cells, which interfere with the normal regulation of cell differentiation and cycle, which in turn contribute to cells' continued proliferation and malignant transformation [20]. One study reported that the percentage of EGFR expression in ovarian serous carcinomas was 64%, and the strong positive EGFR expression was detected in high grade tumors [21]. The present study revealed that there was more frequent expression of EGFR in epithelial ovarian cancer than normal ovary, and also found a positive correlation between EGFR expression and FIGO stage, tumor differentiation, and lymph node metastasis. Our results further suggest that the overexpression of EGFR enhances the metastasis and invasion of tumor cells.

Multiple genes co-regulate the proliferation of ovarian cancer cells. Our study revealed that EpCAM and EGFR

Table 3: Chi-square test analyses of EGFR expression in EOCs and normal ovaries

	n	EGFR expression				Positive rate (%)	X^2	P
		-	+	++	+++			
EOC	30	10	7	7	6	66.7	8.715	0.003
Normal	15	12	2	1	0	20		

Table 4: Correlation between EpCAM and EGFR expression in EOC

EGFR expression	EpCAM expression		Total	r	P
	Positive	Negative			
Positive	19	1	20	0.530	0.003
Negative	5	5	10		
Total	24	6	30		

are associated with the malignant potential of EOC, and detected a positive correlation between EpCAM and EGFR protein expression in EOC. These results further suggest that the co-expression of EpCAM and EGFR may serve as a potential biomarker for evaluating the progression of epithelial ovarian cancer and may provide a promising molecular therapeutic target for advanced stage epithelial ovarian cancer. However larger scale investigations with more specimens at different histological types and stages are required.

Conflict of interests: No authors report any conflict of interest.

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