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Diagnostic and prognostic significance of E-cadherin and Ki-67 expression in non-small cell lung cancer patients

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Abstract. – OBJECTIVE: To elucidate the relationship between E-cadherin (E-cad) and Ki-67, and to determine their clinical significance in non-small cell lung cancer (NSCLC).

PATIENTS AND METHODS: A total of 68 NSCLC paraffin embedded tissue specimens and tumor-adjacent normal tissue specimens were collected. The expression of E-cad and Ki-67 was examined by immunohistochemistry and the relationships between the expression of these two markers were evaluated. The clinicopathological features were correlated with the expression of E-cad and Ki-67 to check whether these proteins have any association and if exist an association whether E-cad and Ki-67 can be used in diagnosis and prognosis of NSCLSC.

RESULTS: E-cad was expressed in all the normal tissues but Ki-67 expressed in only 5.8% of normal tissues. Ki-67 and E-cad expression were observed in 61.8% and 25% of NSCLC tissues respectively. Correlation analysis revealed an inverse association between the expression of E-cad and Ki-67 ($r = 0.524$, $p = 0.000$). The clinicopathological features such as tumor differentiation, TNM stage, lymph node metastasis and pleural invasion were all significantly associated with E-cad and Ki-67 expression ($p < 0.05$).

CONCLUSIONS: E-cad and Ki-67 together play a key role in the development, invasion and metastasis of NSCLC and combined detection of them serve as a potential marker for clinical diagnosis in addition to exploiting them as a therapeutic target.

Key Words:
E-cadherin, Ki-67, Combined detection, Non-small cell lung cancer, Metastasis.

Introduction

The incidence and mortality of lung cancer are increasing year by year, and the overall 5-year survival rate remains poor around 15%[1], largely due to recurrence and metastasis. Non-small cell lung cancer (NSCLC) alone contributes to ~80% of all lung tumors. The epithelial-mesenchymal transition (EMT) is a complex reprogramming process of epithelial cells, plays an essential role in tumor invasion and metastasis[2] and is characterized by the loss of E-cadherin (E-cad) [3]. E-cad is expressed mainly on the epithelial cell membrane and is required for cell–cell adhesion, where it functions to maintain cell-cell junctions, thereby inhibiting aberrant cell proliferation and migration[4]. Ki-67 is a nuclear non-histone protein that is considered as a biomarker of tumor cell proliferation[5] and is found to be highly associated with tumor development, metastasis and prognosis. This study was carried out with aims of analyzing the expression of E-cad and Ki-67 in NSCLC and tumor-adjacent normal tissues and to identify association with clinicopathological features, thereby establishing diagnostic or prognostic markers for early diagnosis and therapeutic target for treatment.

Patients and Methods

Patients

The paraffin embedded NSCLC tissues were obtained from 68 patients who underwent pneumonectomy without any preoperative therapy in the Department of Thoracic Surgery, Hebei General Hospital from Jan. 1, 2009 to Dec. 31, 2011. Also, tumor adjacent normal lung tissues were collected from 17 of the above patients. Ethical approval for this study was obtained from the Institutional Review Board of Hebei General Hospital. Tumors were graded as well differentiated, moderately differentiated and poorly differentiated according to World Health Organization Classification of Tumors (WHO) criteria. Tumor histology, TNM staging, lymph node metastasis, and pleural invasion were all determined according to the International Association for the Study of Lung Cancer (IASLC) in 2009.

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Major Reagents

Rabbit anti-human E-cad and Ki-67 monoclonal antibodies were commercially availed (Epitomics, Burlingame, CA, USA). The Envision kit and diaminobenzidine stain (DAB) were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China.

Immunohistochemistry

Briefly, 4 m thick tissue sections were dewaxed in xylene and rehydrated in decreasing concentrations of ethanol. Then, endogenous peroxidase activity was blocked by incubating the sections with a peroxidase-blocking solution (Dakocytomation, Glostrup, Copenhagen, Denmark) for 5 min. Antigen retrieval consisted of autoclave treatment of sections for 30 min in Target Retrieval Solution (pH 6.0, DakoCytomation, Denmark). Then, the sections were incubated with primary antibodies such as E-cad (1:3000 dilution) and Ki-67 (1:3000) overnight. The next-morning, the sections were washed and incubated with horseradish peroxidase conjugated secondary antibody for 30 min. This was followed by incubation of sections with a substrate chromogen (diaminobenzidine) solution and light counterstaining with Mayer's hematoxylin. The known positive control was stained as described for the tumor specimens, whereas, in the negative control phosphate buffered saline was used instead of primary antibody.

Evaluation of Immunohistochemical Staining

The slides were reviewed and scored blindly by two expert pathologists. The results of E-cad and Ki-67 expression were analyzed by the semi-quantitative scoring method. A total of 10 fields were observed for each specimen and immunoreactivity in 1000 tumor cells was evaluated at 400 X magnification. We set the threshold > 10% to consider E-cad and > 5% to consider Ki-67 immunopositive. The specimens were assigned scores for immunoreactivity ranging from 0-4. For E-cad, 0 (≤ 10% stained cells), 1 (11-30% stained cells), 2 (31-60% stained cells), 3 (> 60% stained cells) and 4 (> 60% positive cells). For Ki-67, 0 (≤ 5% stained cells), 1 (6-25% stained cells), 2 (26-50% stained cells), 3 (51-75% stained cells) and 4 (≥ 76% stained cells). A separate score for the intensity of staining was given, "0" no staining, "1" for light yellow staining, "2" for brown-yellow staining and "3" for brown staining. The final score of each specimen was obtained by multiplication of the score of the stained cells (0-4) and a score of the color intensity (0-3). The final scores 0-4 was considered as negative, 5-12 considered as positive and used for further analysis.

Statistical Analysis

The Chi-square test and Fisher's exact test were used to analyze the expression of E-cad and Ki-67 in NSCLC and normal tissues and their association with clinicopathological features. Correlation analysis was performed using Spearman's correlation test to assess the relationship between E-cad and Ki-67. All statistical tests were two-sided, and statistical significance was set at $p < 0.05$. All analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Expression of E-cad and Ki-67 in Normal Lung Tissues and NSCLC Tissues

E-cad expression was observed in all of the normal and 25% of NSCLC tissues and this difference was statistically significant ($\chi^2 = 31.875$, $p = 0.000$). Ki-67 expression was seen in 5.9% of normal and 61.8% of NSCLC tissues with a statistically significant difference ($\chi^2 = 16.991$, $p = 0.000$, Table I, Figures 1 and 2).

Table I. Expression of E-cad and Ki-67 in normal and NSCLC tissues.

		E-cad				Ki-67			
	n	+	–	χ^2	p	+	–	χ^2	p
Normal lung tissues	17	17	0	31.875	0.000	1	16	16.991	0.000
NSCLC tissues	68	17	51			42	26		

$p < 0.05$ difference was statistically significant.

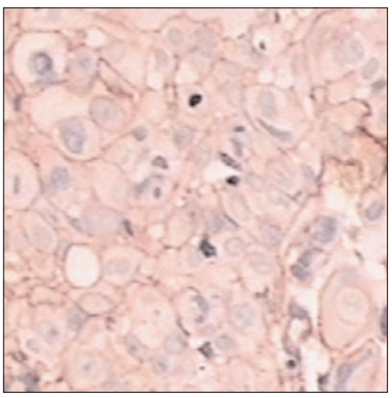

Figure 1. Expression of E-cad in NSCLC tissue (SP×100).

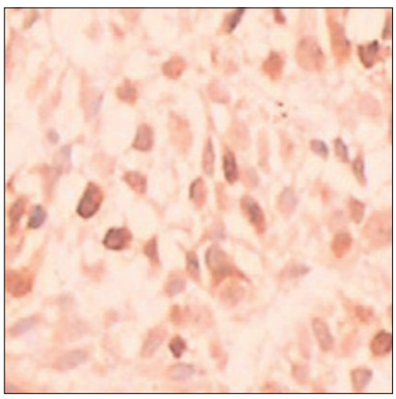

Figure 2. Expression of Ki-67 in NSCLC tissue (SP×100).

Relationship of Expression of E-cad and Ki-67 with Clinicopathological Features in NSCLC Tissues

No relationship was found between the expressions of E-cad and Ki-67 with sex, age and tumor histologic type ($p > 0.05$). E-cad expression was observed in 51.7% (15/29) of stage I + II NSCLC tissues. A significant reduction in E-cad expression was seen in NSCLC tissues comprising stage III and IV ($p < 0.05$). Ki-67 expression was found to be 44.8% (13/29) in stage I and II and 74.4% in stage III & IV NSCLC tissues ($p < 0.05$). It was found that E-cad expression had zdecreased ($p = 0.025$) and Ki-67 expression had increased ($p = 0.023$) as the disease progresses to advanced stages. Furthermore, both E-cad and Ki-67 were significantly associated with clinicopathological variables such as lymph node metastasis, tumor differentiation and pleural invasion ($p < 0.05$, Table II).

Table II. Association of expression of E-cad and Ki-67 with clinicopathological features of NSCLC tissues.

Clinicopathological variables	n	E-cad				Ki-67			
		+	–	χ^2	*p*	+	–	χ^2	*p*
Age (years)									
≤ 65	23	7	16	0.4548	0.459	12	11	1.354	0.245
> 65	45	10	35			30	15		
Gender									
Male	50	11	39	0.403	0.526	34	16	3.110	0.078
Female	18	6	12			8	10		
Histologic type									
Squamous carcinoma	32	8	24	0.185	0.912	23	9	2.661	0.264
Adenocarcinoma	26	7	19			14	12		
Other	10	2	8			5	5		
Differentiation									
Well	17	7	10	7.360	0.025	8	9	7.560	0.023
Moderately	30	9	21			16	14		
Poorly	21	1	20			18	3		
TNM stage									
I+II	29	15	14	19.260	0.000	13	16	6.142	0.013
III+IV	39	2	37			29	10		
Lymph node metastasis									
Yes	43	2	41	25.829	0.000	32	11	7.930	0.005
No	25	15	10			10	15		
Pleural invasion									
Yes	38	2	36	17.859	0.000	30	8	10.768	0.001
No	30	15	15			12	18		

$p < 0.05$ difference was statistically significant.

Correlation Between Expression of E-cad and Ki-67 in NSCLC Tissues

Of the 42 Ki-67 positive NSCLC tissues, only 7.1% was simultaneously positive for E-cad. But, of the 26 Ki-67 negative NSCLC tissues, 53.84% were positive for E-cad expression. It indicates that a negative correlation is existing between E-cad expression and the Ki-67 expression in NSCLC tissues (r = -0.524, $p = 0.000$, Table III).

Discussion

Lung cancer is the most common cancer and most frequent cause of cancer death worldwide. According to data from the World Health Organization, lung cancer accounts for 1.6 million deaths each year, or 19.5% of the total cancer mortality worldwide. Recurrence and metastasis are the major obstacles that cannot be solved by the surgery, radiotherapy, and chemotherapy[6]. Cancer metastasis is a multi-step cascade that starts with EMT, dissociation from the primary tumor, and distal invasion. Invasion into healthy tissue is thus a barrier to the progression of metastatic disease[7]. E-cad is an important adhesion molecule establishing the core of the epithelial adherens junction with neighboring cells. E-cad abnormal expression is the basis of molecular dispersion, and the reduction or loss of its expression can lead to the loss of adhesion of normal cells or the detachment from the primary tumor to other parts of the body[8]. The loss of E-cad is a hallmark of EMT, a process in which cells loose cell–cell contact, delaminate from the epithelium, and acquire a mesenchymal and migratory phenotype[9]. Indeed, some studies have confirmed reduced expression of E-cad in a variety of carcinomas[10-13]. A recent study[14] has shown that high expression of E-cad can reduce the capacity of invasion and metastasis of tumor cells, and low expression can lead to the increase of metastasis potential. Our study suggested the existence of a significant association between E-cad expression and tumor cell differentiation, TNM stage, lymph node metastasis and pleura invasion. The downregulation or absence of E-cad expression resulted in the invasion and metastasis in a previous study[15] and our study results are similar to that report.

Ki-67 is a nuclear non-histone protein expressed throughout the cell cycle except the G_0 state and is widely considered to be an ideal target antigen for detection of tumor cell proliferation. Several studies showed that higher Ki-67 expression indicates tumor cell proliferation, metastasis, and recurrence of cancer cells and thus being used as a marker to evaluate proliferation in NSCLC[16-17] and other tumors, such as lymphoma[18], breast cancer[19], and prostate cancer[20], basal cell carcinoma[21]. Takenaka et al[22] found that Ki-67 expression is closely related with the expression of vascular endothelial growth factor in NSCLC, and can be used as a reliable biological indicator of tumor progression and metastasis of lung cancer. IN our study, Ki-67 expression was associated with tumor cell differentiation, TNM stage, lymph node metastasis and pleural invasion as observed in a previous study by Ahn et al[23].

We also demonstrated in our study that, potential correlations existing between E-cad and Ki-67 and measuring their levels together may be useful for the determination of lung cancer aggressiveness. Statistical analysis revealed a negative correlation between these two biomarkers to define tumor proliferation (Ki-67) and invasiveness (E-cadherin), supporting the results of previous studies[24-25].

Conclusions

Our report showed that, the expression of E-cad and Ki-67 was closely related with clinicopathological features such as tumor cell differen-

Table III. Correlation between expression of Ki-67 and E-cad in NSCLC tissues.

Ki-67	E-cad		Total	r	*p*
	+	–			
+	3	39	42	-0.524	0.000
–	14	12	26		
Total	17	51	68		

$p < 0.05$ difference was statistically significant.

tiation, TNM stage, lymph node metastasis and pleural invasion. Furthermore, the expression of both E-cad and Ki-67 was inter-related. Hence, we suggest simultaneous detection of E-cad and Ki-67 may reflect the real-time situation of the tumor and can be exploited as targets for treating lung cancer.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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