Expression and clinical significance of Oct-4 and E-cad in non-small-cell lung cancer

LI LI[1], YANLING LV[1], YING ZHANG[2], LIYA HE[3] and HONGZHEN ZHANG[3]

Departments of [1]Prevention and Health Care, [2]Personnel and [3]5th Oncology, Hebei
People's Hospital, Shijiazhuang, Hebei 050051, P.R. China

Abstract. Non-small-lung cancer (NSCLC) is a common malignant tumor and is a leading cause of cancer mortality. Tumor stem cells are associated with tumor pathogenesis and development as well as invastion and metastasis. In the present study, the expression and correlation of tumor stem cell markers, octamer-binding transcription factor 4 (Oct-4) and E-cadherin (E-cad) in NSCLC and normal lung tissue were investigated. Additionally, the molecular mechanisms of invasion and metastasis of NSCLC were assessed. The expression of Oct-4 and E-cad was examined in 65 pathologically diagnosed cases of NSCLC using immunohistochemistry. The correlation between Oct-4 and E-cad, as well as the association with pathological grade and clinical staging were also analyzed. Fifteen cases of normal lung tissues served as the control. The positive expression of Oct-4 and abnormal expression of E-cad was higher in the NSCLC tissue compared to the normal lung tissue, and increased as NSCLC malignancy increased. The differences in each grade each stage were statistically significant ($P<0.05$). A correlation was identified between the abnormal expression of Oct-4 and E-cad ($P<0.05$, coefficient of contingency C=0.439). In conclusion, the expression of Oct-4 promoted the epithelial-mesenchymal transition in lung cancer.

Introduction

Non-small-lung cancer (NSCLC) is a common malignant tumor, and a leading cause of mortality (1). Invasion and metastasis of NSCLC are the most dominant reasons for cancer mortality and treatment failure. Findings of previous studies have suggested that tumor pathogenesis was induced by tumor stem cells (2). Tumor stem cells were identified in many solid tumors, such as glioma, as well as breast, prostate, pancreatic and colon cancer (2). These stem cells have been associated with tumor pathogenesis and development, as well as invasion and metastasis of tumor cells (2). Octamer-binding transcription factor 4 (Oct-4) is a marker of tumor stem cells. Epithelial-mesenchymal transition (EMT) indicates the phenomenon that epithelial cells transit to fibroblast or mesenchymal cells and acquire migration ability (3). The most important marker of EMT is the downregulation or silencing of E-cadherin (E-cad) expression. The occurrence of EMT causes a loose cellular arrangement and increased cell mobility, leading to further invasion and metastasis of tumor cells (3).

The present study analyzed the expression of Oct-4 and E-cad in NSCLC and normal lung tissue, and examined the association between tumor stem cells in NSCLC and EMT.

Materials and methods

Specimen source. Specimens were obtained from 65 NSCLC patients at the Department of Thoracic Surgery, Hebei People's Hospital (Shijiazhuang, China) between January 2013 and June 2014. The patients included 38 males and 27 females, with an age range of 26-78 years and a median age of 53 years. The pathogenic grade of the patients was: high differentiation, 22 cases; medium differentiation, 30 cases; and low differentiation, 13 cases. The clinical stage of the patients was: stage 0-I, 9 cases; stage II-III, 31 cases; and stage IV, 25 cases. The control group included 15 cases of normal lung tissue following lobectomy for bronchiectasis and pulmonary bullae resection, which were confirmed pathologically. The age range for the control group was 18-48 years, with a median age of 35 years. These specimens were fixed in 10% formaldehyde, embedded with paraffin, and cut into serial tissue sections (5 μm).

Reagents. Mouse anti-human E-cad mAb (Beckman Coulter, Brea, CA, USA), and rabbit anti-Oct-4 polyclonal antibodies (Abcam, Cambridge, UK) were used. The dilutions for the antibodies was 1:500. Diaminobenzidine (DAB) chromogenic agent and ELISA kit were purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd. (Beijing, China). The protocols followed were as specified in the kit instructions.

Correspondence to: Dr Hongzhen Zhang, Department of 5th Oncology, Hebei People's Hospital, 348 Heping West Street, Shijiazhuang, Hebei 050051, P.R. China
E-mail: 1256596529@qq.com

Table I. Correlation between the expression of Oct-4 and E-cad in NSCLC tissue and between pathological grade and clinical stage.

Group	No. of cases	Oct-4		E-cad	
		No. of positive cases (%)	P-value	No. of abnormal expression cases (%)	P-value
Normal lung tissue	15	0 (0.00)		2 (13.3)	
Pathological grade					
High differentiation	13	4 (30.77)		3 (23.08)	
Medium differentiation	30	16 (53.33)	0.009[a]	19 (63.33)	0.002[a]
Low differentiation	22	18 (81.82)		18 (81.82)	
Clinical stage					
0-I	9	2 (22.22)		1 (11.11)	
II-III	31	18 (58.06)	0.034[a]	18 (58.06)	0.001[a]
IV	25	18 (72.00)		21 (84.00)	

[a]$P<0.05$. Oct-4, octamer-binding transcription factor 4; E-cad, E-cadherin; NSCLC, non-small-cell lung cancer.

Table II. Correlation between Oct-4 and E-cad expression.

Characteristics	E-cad Negative	Oct-4 Positive	Total	P-value
Normal expression	18	7	25	
Abnormal expression	9	31	40	0.000[a]
In total	27	38	65	

[a]$P<0.05$. Oct-4, octamer-binding transcription factor 4; E-cad, E-cadherin.
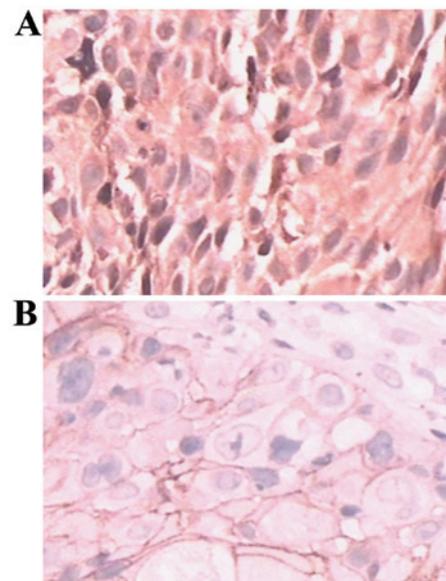

Figure 1. (A) Expression of E-cadherin in non-small-cell lung cancer (NSCLC) tissue [hematoxylin and eosin (H&E); maginification, x100]. (B) Expression of octamer-binding transcription factor 4 in NSCLC tissue (H&E; maginification, x100).

Interpretation of results. Brown granules in the cell nucleus indicated a positive expression of Oct-4. The score was calculated by combining the staining intensity and the percentage of positive cells in the tumor cells. Values for the staining intensity were: colorless, 0; light yellow, 1; tan, 2; and brown, 3. The score for positive cell percentage was: <5%, 0; 5-25%, 1; 26-50%, 2; and >50%, 3. The final score was calculated using the score of staining intensity multiplied by the score of positive cell percentage, as follows: 0-1, negative (-); 2-6, weak positive (+); and 7-12, strongly positive (++). Both (+) and (++) were classified as a positive expression (4). Light-yellow to brown granules were observed in the cells with a positive expression of E-cad, and the staining was located on the cell membrane with a few granules in the cytoplasm. The scoring criteria used were as previously described by Mahler-Araujo *et al* (5): <10% positive cells, 0: 10-25% positive cells, 1; 25-50% positive cells, 2; 50-75% positive cells, 3; ≥75% positive cells, 4. Scores of 0-3 indicated a negative expression, i.e., abnormal expression; and ≥3 indicated a positive expression, i.e., normal expression.

Statistical analysis. SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. The χ^2 test was used for enumeration data, with an inspection level of $\alpha=0.05$. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Relationship between grade, stage of Oct-4 and E-cad. Abnormal expression of E-cad was observed in NSCLC tissue and the staining demonstrated light-yellow to brown granules. E-cad expression was located on the cell membrane, with a few granules in the cytoplasm (Fig. 1A). A positive expression of Oct-4 was observed in the NSCLC tissue, and the staining demonstrated brown granules. Oct-4 expression was located on the cell membrane (Fig. 1B). The abnormal expression

of E-cad and positive expression of Oct-4 in the NSCLC specimens was higher than that of the normal lung tissue, and statistically significant (P<0.05). The expression of Oct-4 and E-cad were associated with the pathological grade and clinical stage of the patient, and were increased as the NSCLC malignancy increased. The differences in each grade and each stage were statistically significant (P<0.05) (Table I).

Correlation between Oct-4 and E-cad expression. Of the 65 NSCLC specimens, the abnormal expression rate of E-cad was 61.54% and the positive expression rate of Oct-4 was 58.46% (Table II). The difference between E-cad and Oct-4 was statistically significant (P=0.000, coefficient of contingency=0.439), and demonstrated that E-cad expression was correlated with Oct-4 expression.

Discussion

Invasion and metastasis are basic characteristics of malignant tumor. Tumor invasion and metastasis includes three key features: decreased adhesion, degraded matrix and enhanced migration, and EMT attributes to these features. E-cad is distributed in various epithelial cells and may mediate cell adhesion. The most important EMT tumor marker is the downregulation or silencing of E-cad, which is considered as the prerequisite for the ability of invasion and metastasis of epithelial cells (6). The findings of the present study have demonstrated that the increase in abnormal expression of E-cad in the NSCLC specimens was higher than that of the normal lung tissue, and the result was statistically significant. The decreased NSCLC differentiation level and increased clinical stage led to the abnormal expression of E-cad being increased, and the result was statistically significant.

Tumor stem cells are the cells that are characteristic of self-renewal with differentiation potential, and contribute to tumor relapse, metastasis and drug resistance (7). The expression of Oct-4 is a marker of tumor stem cells. Tumor cells with a positive Oct-4 expression have increased tumorigenic capacity *in vitro* and the characteristics of tumor stem cells. Akunuru *et al* (8) reported that although tumor stem cells have various phenotypes, they can express the genes of pluripotent stem cells, particularly Oct-4. The overexpression of Oct-4 protein may enhance tumor malignancy and promote tumor growth (9). The results of the present study have demonstrated that Oct-4 was not expressed in normal lung tissue. This indicates that the normal lung tissue achieved developmental maturation and lost the differentiation ability. The expression of Oct-4 was higher in NSCLC tissue compared to the normal lung tissue. This finding suggested that the expression of Oct-4 was associated with NSCLC pathogenesis, as well as pathological grade and clinical stage. Thus, the positive expression of Oct-4 is associated with NSCLC pathogenesis.

Tumor stem cells have the characteristics of mesenchymal cells. The tumor microenvironment theory suggests that the tumor epithelial cells may have the characteristics of mesenchymal cells after EMT. This finding indicates that the formation of tumor stem cells is associated with the EMT of tumor cells. It was previously reported that the EMT promoted mammary epithelial cells and breast cancer cells to acquire the properties of stem cells, leading to limitless proliferation and cell growth (3). The tumorigenicity of tumor cells was significantly increased after EMT, indicating that EMT is associated with the formation of tumor stem cells (10). Our results have demonstrated that the positive expression of Oct-4 was associated with the abnormal expression of E-cad, i.e., the expression of Oct-4 may be associated with the EMT of NSCLC.

In summary, Oct-4 is highly expressed in NSCLC tissue, and is a potential new biomarker of NSCLC pathogenesis, development and differentiation. Currently, E-cadherin is considered as a tumor suppressor. The downregulation or deficiency of E-cadherin can induce EMT, leading to invasion and metastasis of tumor cells. Specific therapy against tumor stem cells may block EMT, which is a promising target of NSCLC therapy, and can assist in the clinical treatment of NSCLC.

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