

RESEARCH ARTICLE

Correlation Between Expression of Cell Adhesion Molecules CD₄₄v6 and E-cadherin and Lymphatic Metastasis in Non-small Cell Lung Cancer

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Abstract

Objective: To explore the relationship between expressions of cell adhesion molecules CD44 v6 and E-cadherin (E-cad) and lymphatic metastasis in non-small cell lung cancer (NSCLC). **Materials and Methods:** Eighty-seven tissue samples obtained from patients with primary NSCLC were collected in our hospital from Dec., 2007 to Dec., 2012, and the expressions of CD44 v6 and E-cad gene proteins in these samples were detected by immunohistochemical method. **Results:** In the tissue without lymphatic metastasis, the positive expression rate of CD44 v6 was significantly lower, whereas the normal expression rate of E-cad was notably higher than that with lymphatic metastasis (55.6% vs. 78.4%, 47.2% vs. 21.6%), and both differences had statistical significance ($P < 0.05$). Besides, CD44 v6 and E-cad expressions had a significant correlation in the NSCLC tissue with lymphatic metastasis ($P < 0.05$). **Conclusions:** The positive expression of CD44 v6 and abnormal expression of E-cad may play a very important role in promoting lymphatic metastasis of NSCLC, with synergistic effect. Hence, detection of CD44 v6 and E-cad expressions is conducive to judging the lymphatic metastasis in NSCLC.

Keywords: CD44 v6 - E-cad - non-small cell lung cancer - lymphatic metastasis

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Introduction

As the most essential biological characteristic in malignant tumors, infiltration and metastasis are the leading cause for the deaths of patients with tumors. At present, the morbidity and mortality of lung cancer are the highest among malignant tumors all over the world, in which most of them result from infiltration and metastasis (Li et al., 2013; Fei et al., 2013; Huang et al., 2013; Cui et al., 2014; Hou et al., 2014). In recent years, relevant studies have revealed that cell adhesion molecules (CAMs) CD₄₄v6 and E-cadherin (E-cad) are closely associated with infiltration and metastasis in a variety of tumors (Amirghofran et al., 2008; Wendt et al., 2011; Shiwu et al., 2012). Additionally, being the most commonly-encountered metastatic pathway in lung cancer, lymphatic metastasis not only affects the tumor staging, but also influences patients' prognosis (Cazes et al., 2014). Hence, in this study, the expressions of CD₄₄v6 and E-cad in non-small cell lung cancer (NSCLC) tissue were detected by immunohistochemical method, and their correlation with the lymphatic metastasis of NSCLC was investigated so as to provide evidences for NSCLC clinical treatment.

Materials and Methods

General data

Eighty-seven tissue samples obtained from patients with primary NSCLC were collected in Beijing Chest hospital from Dec., 2007 to Dec., 2012, all of which were resected by operation and wrapped by paraffin. Among 87 patients, there were 55 and 32 cases in males and females, respectively. They were at the age of 39~76, with the mean age of 57.6. Fifty-one suffered from lymphatic metastasis, but 36 didn't encounter. By comparison to the pathological grading and clinical staging, lymphatic metastasis and histological types in lung cancer, there were no significant differences ($P > 0.05$), with better compatibility.

Reagents

Rabbit anti-human CD₄₄v6 polyclonal antibody (BA0454), a series of E-cad immunohistochemical reagents (SA2029) and SABC kits purchased from Beijing Solarbio Technology Co., Ltd.

Methods

All samples were sliced up continuously, with

Table 1. Relationship Between CD₄₄ v6 and E-cad Expressions and Lymphatic Metastasis in NSCLC [n(%)]

Cell adhesion molecules	Expression	Metastasis (n=51)	No metastasis (n=36)
CD ₄₄ v6	Positive expression	40 (78.4)**	20 (55.6) [#]
	Negative expression	11 (21.6)	16 (44.4) [#]
E-cad	Abnormal expression	40 (78.4)**	19 (52.8) [#]
	Normal expression	11 (21.6)	17 (47.2) [#]

Compared with negative/normal expression, ** $P < 0.01$;
Compared with metastasis, [#] $P < 0.05$

the thickness of 4 μm . CD₄₄ v6 and E-cad were given immunohistochemical and HE staining, respectively, in which the steps of immunohistochemical staining were performed according to the instructions in the kit. The known section with positive staining tissue was considered as the positive, while phosphate buffer solution (PBS) as the negative control instead of primary antibodies.

Evaluation Criteria

Evaluation on the positive expression of CD₄₄ v6: take brown yellow granules in cell membrane and/or cytoplasm as positive cells, and the number of positive cells $>10\%$ being positive expression (Tran et al., 1997).

Evaluation on the normal expression of E-cad: take brown yellow granules with the same thickness in cell membrane as positive cells, and the number of positive cells $>50\%$ being normal expression, $\leq 50\%$ being abnormal expression (Sulzer et al., 1998).

The percent computational method of positive cell number: first, select five high power fields (10 \times 40 times) from each section, count 100 tumor cells randomly in each high power field, and then calculate the percentage of tumor cells with positive expression, finally calculate the percent mean value of tumor cells with positive expression in five high power fields, namely the percentage of tumor cells with positive expression in each section.

Statistical data analysis

SPSS 13.0 statistical software was used to analyze the data in this study. χ^2 test was applied to compare the positive rate, and Fisher's exact test to analyze the correlation between CD₄₄ v6 and E-cad expressions in NSCLC tissue with lymphatic metastasis. $P < 0.05$ was considered to be statistically significant.

Results

Relationship between CD₄₄ v6 and E-cad expressions and lymphatic metastasis in NSCLC

In the tissue with lymphatic metastasis, both CD₄₄ v6 positive expression rate and E-cad abnormal expression rate were obviously higher than their negative and normal expression rates, with statistical significance ($P < 0.01$); in the tissue without lymphatic metastasis, there were no significant differences by comparison to negative and positive expression rates of CD₄₄ v6 as well as abnormal and normal expression rates of E-cad ($P > 0.05$).

In the tissue without lymphatic metastasis, the

Table 2. Correlation of CD₄₄ v6 and E-cad Expressions in the NSCLC Tissue with Lymphatic Metastasis [n(%)]

CD ₄₄ v6	E-cad		Total cases
	+	-	
+	6 (11.8)	35 (68.6)	41 (80.4)
-	5 (9.8)	5 (9.8)	10 (19.6)
Total cases	11 (21.6)	40 (78.4)	51 (100.0)

positive expression rate of CD₄₄ v6 was significantly lower, whereas the normal expression rate of E-cad was notably higher than that with lymphatic metastasis (55.6% vs. 78.4%, 47.2% vs. 21.6%), and both differences had statistical significance ($P < 0.05$) (Table 1).

Correlation of CD₄₄ v6 and E-cad expressions in the NSCLC tissue with lymphatic metastasis

Among 51 NSCLC tissue samples with lymphatic metastasis, there were 35 ones (68.6%) with E-cad abnormal expression and CD₄₄ v6 positive expression, only 6 (11.8%) with E-cad normal expression and 5 (9.8%) with CD₄₄ v6 negative expression. It could be seen that CD₄₄ v6 and E-cad expressions had a significant correlation in the NSCLC tissue with lymphatic metastasis ($P < 0.05$).

Discussion

Lung cancer is one of the most commonly-encountered malignant tumors, with the highest mortality. In America, the mortality caused by lung cancer exceeds the summarization of breast cancer, colon cancer and prostate cancer. Although some therapeutic measures like chemotherapy, radiotherapy and surgery have been improved greatly, the 5-year survival rate of lung cancer is still very low, in which NSCLC is the most common, approximately accounting for 80%, and most patients are at the advanced stage of metastasis when diagnosed (Li et al., 2012; Liu et al., 2012; Liu et al., 2013; Lu et al., 2013; Lu et al., 2013; Yan et al., 2013; Yin et al., 2013; Yin et al., 2013; Yip et al., 2014). In 1991, Liotta first proposed three-step theory of tumor cells on infiltration and metastasis, namely adhesion, degradation and migration. He believes that tumor metastasis refers to tumor cells breaking away from primary lesions adhere to the extracellular matrix (ECM) structures, then degrade ECM and migrate by degrading matrix. During this process, the abnormal expression of cell adhesion molecules makes intracellular adhesion and that between tumor cells and other cells changed, consequently inhibiting or promoting the infiltration and metastasis of tumor cells (Liotta et al., 1991). Surgery is the optimal therapy for early NSCLC, whereas the major factor that affects surgery results is presence or absence of lymphatic metastasis. Additionally, as the primary metastatic pathway in lung cancer, lymphatic metastasis is also the principal factor that affects the radical resection and prognosis in NSCLC (Li et al., 2013; Nentwich et al., 2013).

In recent years, CD₄₄ v6 and E-cad are regarded as two subtypes of CAMs closely related to tumor infiltration

and metastasis. Human leukocyte differentiation antigen CD₄₄ gene is composed of 20 highly-conservative exons respectively consisting of 10 constitutive and variant exons. CD₄₄v6, a sort of transmembrane glycoprotein formed by the insertion of the 6th variant exon into mRNA in the process of transcription, has the function of binding various ligands including hyaluronic acid in extracellular region. Its intracellular region can be considered as the substrate of protein kinase C (PKC) to be phosphorylated so as to involve in the process of signal transmission. CD₄₄v6 exerts crucial effects in terms of regulating intracellular adhesion, mutual adhesion between cells and ECM and relevant cell migration. Günthert et al. first proposed CD₄₄v6 was associated with tumor metastasis (Günthert et al., 1991). Recently, studies have also demonstrated that CD₄₄v6 is abnormally expressed in a variety of human tumors, intimately related to tumor formation, progression and metastasis (Kawano et al., 2005; Chai et al., 2007). The results in this study revealed that the positive expression rate of CD₄₄v6 in the tissue without lymphatic metastasis (55.6%) was significantly lower than that with lymphatic metastasis (78.4%), suggesting that CD₄₄v6 may promote lymphatic metastasis in NSCLC. Besides, it could also be seen that CD₄₄v6 was positively associated with lymphatic metastasis in NSCLC, but not highly expressed in all metastatic tissues, indicating that CD₄₄v6 is not the only factor that can promote NSCLC metastasis.

The protein coded by E-cad gene, a transmembrane glycoprotein with 124 KD of relative molecular weight, is essential in the mutual connection of endothelial cells. The zippered structure can be formed between extracellular amino terminal of E-cad and the amino terminal of its adjacent cells under the presence of calcium ions, namely anti-parallel dimer, which can promote the intracellular connection more closely. However, the intracellular carboxyl terminal can cause E-cad to adhere to cytoskeleton indirectly, consequently stabilizing the cell morphology and location and exerting its effects of induced differentiation, cell adhesion and metastatic inhibition (Warrington et al., 2013). The research made by Frixen et al showed that E-cad was closely related to the infiltration and metastasis of tumor cells (Frixen et al., 1991). At present, a lot of researches have also revealed that loss of E-cad adhesion function is key for cells to achieve the high invasion (Dursun et al., 2007; Kolesnik et al., 2013). The results in this study demonstrated that the normal expression rate of E-cad in the tissue without lymphatic metastasis was notably higher than that with lymphatic metastasis, suggesting that E-cad abnormal expression is intimately associated with the lymphatic metastasis in NSCLC. And meanwhile, there were 35 tissue samples with E-cad abnormal expression and CD₄₄v6 positive expression, only 6 with E-cad normal expression and 5 with CD₄₄v6 negative expression, indicating that E-cad abnormal expression makes tumor cells break away from primary lesions and CD₄₄v6 positive expression endows them with stronger motor ability, they are correlated with each other instead of two independent processes. It can be concluded that CD₄₄v6 and E-cad have a synergistic effect in the process of NSCLC lymphatic metastasis.

To sum up, the positive expression of CD₄₄v6 and abnormal expression of E-cad may play a very important role in promoting lymphatic metastasis of NSCLC, with synergistic effect. Hence, detection of CD₄₄v6 and E-cad expressions is conducive to judging the lymphatic metastasis in NSCLC.

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