# The Correlation Between Non-small Cell Lung Cancer Lymph Node Metastasis and E-CD mRNA Expression

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**Abstract** **Objective** To examine the mRNA expression of the E-CD gene and to analyse the relation between metastasis of non-small cell lung carcinomas and the gene expression abnormalities. **Method** By using semiquantitative RT-PCR, The E-CD mRNA expression were studied in 24 cases of non-small cell lung cancer. The normal lung tissue were used as control. **Results** Of 24 cases of NSCLC, 10 cases(41.7%) had abnormal expression of E-CD, low expression of E-CD mRNA were found in 9 of 15 lung cancer cases with lymph node metastasis, The rate (9/15, 60.0%) of was significantly higher than that in the non-metastasis cancers (1/9, 11.1%) ($P$<0.05). **Conclusion** These data indicate that E-CD may be a putative metastasis suppressor gene which may have reverse regulating activation in the metastasis progression of lung cancer, and the leval of E-CD mRNA expression may be considered as one of the pathological indications in predicting metastasis potential of lung cancers.

**Key Words** non-small cell lung cancer; E-cadherin gene; mRNA expression.

Lung cancer is one of the malignant diseases that severely threatens human´s health. As the improvement of industrialization, the morbidity of lung cancer appears an ascending tendency in many countries of the world [1]. Tumor metastasis is a complex multiple-stage procedure, and cell cohesion plays an important role in these procedures[2].

Through many years researching, four sorts of cell adhesion molecule related to tumor metastasis have been isolated and identified. They are integrin protein, immunoglobulin superfamily molecule, calcium adhesion protein and selectin[3]. Calcium adhesion protein is a kind of transmembrane glycoprotein that can mediate intercellular homogenous adhesion. It has the significant function in maintaining cell morphologic state, movement and adhesion. Expression of E-CD has close relation to the differentiation degree, invasion, metastasis and prognosis of malignant tumors, the low and unstable expression of E-CD can promote the occurrence of metastasis.

In order to clarify the relation between E-CD gene quantitative expression and lymph node metastasis, we examined E-CD exon 8, 9 mRNA in 24 cases of primary non-small cell lung cancer with gene fragments including eight introns aimed at extron 8, 9 using RT-PCR method.

## *MATERIAL AND METHOD*

### Specimen

24 cases of lung cancer specimen were taken from patients who hospitalized in the thoracic surgery department of our hospital from Jan. 8, 2000 to Nov 18, 2001, which include 16 male and 8 females, aged from 26 to 77 (averaging 60) years old. There were 10 cases of squamous carcinoma, 10 cases of adenocarcinoma, 1 case of adenosquamous carcinoma, and 3 cases of large cell cancer, with 15 cases of lymph nodes metastasis. All the patients didn´t receive preoperative radiotherapy and chemotherapy. Fresh specimens were placed in −70℃ liquid nitrogen.

### Oligonuelceotide primer

Using DNAStar software, we have found mRNA sequence of E-CD in GenBank storeroom, designing gene fragments including eight introns aimed at Exon 8, 9 whose length is 250bp and the sequence of its primer is: 5´>CCAGGAACCTCTGTGAT<3´, Anti-Sence primer: 5´>TTGTTACGTGGTGGGATT<3´. The length of β-action fragment is 152bp, and

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the sequence of its primer is: Sense primer: 5′> CTACAATGAGCTGCGTGTGG <3, Anti-Sense primer: 5′>ATAGCAACGTACATGGCTGG<3′. The primers are synthesized by Shanghai Sheng Gong Bioengineering Company.

**Extraction of total RNA**

The total RNA of carcinoma tissue was extracted by using Tripure reagent ( ROCH Company of America). The optical density(OD) value of specimens were measured by ultraviolet spectrophotometer in 260nm/280nm wavelength, all optical density (OD) ratio of the specimens >1.8.

**Reverse transcription of NSCLC specimen**

4μg total RNA was taken, and conducted predenaturation in 70℃ for 5 min, then suddenly refrigerated on ice for 2 min, added RNA enzyme inhibitor 0.5 μl, 4 × dNTP 2 μl, oligo($dT_{18}$) 2 ul, 5 × buffer 4 μl, AMV reverse transcription enzyme 1 μl, complementing water to the total volume 25 μl. cDNA synthesis reaction was performed in 37℃ for 1 h, 70℃ for 10 min, and then reserved in −20℃.

**Semiquantitative RT-PCR**

We conducted semiquantitative analysis to the Exon 8, 9 mRNA of E-CD gene using tumor sample and the cDNA of the normal tissue as the template, β-action as the inside control: cDNA production 2 μl, 4 × dNTP 0.5 ul, 25 mmol/L $MgCl_2$ 3 μl, Taq DNA polymerase 0.5 μl, 10×PCR buffer 2.5 μl, primer of Exon8, 9 3 μl, were added in 25 μl reaction system, double distillated water being complemented to 25 μl. this circle as follow: 95℃ pre-denaturation for 5min, 95℃ denaturalization 30 s → 60℃ annealing 30s → elongation in 72℃ for 1 min, 30 circles, elongation in 72℃ for 10min, reserved in 4℃. After RT-PCR production 5 μl through 1.5% agarose gel electrophoresis for 1 h, image was processed through the gel analyzation image system (Bio Rad America) and saved. We scaned signal intensity of E-CD gene, β-action gene RT-PCR spreading band, the relative expression intensity of specimen Exon 8, 9 mRNA, and intensity ratio of Exon 8,9/β-action by using GDS-5000 image analyzing machine. We definited confidence interval based on average Exon 8, 9 expression intensity of normal lung control group as well as on significant deviation ($P$>0.05). The expression intensity lied in confidence interval was normal, surpassed over high limit was divided into enhancement, on the contrary, was regarded as attenuation.

**Statistics dealing**

Accounting data were examined by $X^2$.

## *RESULTS*

Of 24 cases of NSCLC, 41.7% of them had abnormal expression of E-CD, and 15 cases accompanied by lymph node metastasis., whereas 9 cases with lymph node metastasis had abnormal E-CD Exon 8, 9 expression (60.0%, 9/15), the other 6 cases showed normal expression (40%, 6/15). In 9 cases without lymph node metastasis, the expression of E-CD Exon 8, 9 decreased in one case (11.1%, 1/9), and the other 8 cases were normal (table 1). Graph 1 showed RT-PCR electrophoresis band of specimen marks from 15 to 24 of 24 cases with NSCLC, attenuation occurred in 15, 20, 21, 22 specimen mark, and the expressions weren´t observed in specimens marked 23, 24.

Table 2 shows the relation of E-CD mRNA abnormal expression and clinical pathology. In this experiment, 6 cases with high and moderate differentiation represented normal expression of E-CD; while among 18 cases with low differentiation, the reduced expression of E-CD was 10 cases account for 55.6% ($P$<0.05). In terms of histological classification, 10 cases were squamous carcinoma, in which 6 cases had abnormal expression (60.0%); while 10 adenocarcinoma cases all expressed normal E-CD. One adenosquamocarcinoma case had low level expression (100%), and 3 large cell cancer cases as well (100%).

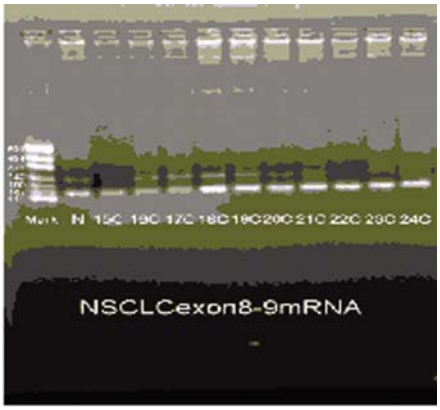


Fig. 1 weakness in 15C, 20C, 21C, 22C; non-expression in 23C, 24C
N: normal specimen of lung; C: specimen of cancer

**Table 1** Expression abnormalities of E-CD in NSCLC Cases

| Group | Cases | relative expressing intensity of Exon8-9 mRNA | | *P* value |
|---|---|---|---|---|
| | | Abnormality | normal | |
| Lymph metastasis group | 15 | 9(60.0%)* | 6(40.0%) | |
| Lymph non-metastasis Group | 9 | 1(11.1%) | 8(88.9%) | <0.05 |
| Summation | 24 | 10(47.7%) | 14(58.3%) | |

* There is the massive difference of the expression abnormalities of mRNA between lymph node metastasis group and non-metastasis group.

**Table 2** The relationship between expression abnormalities of E-CD mRNA in NSCLC and clinical Pathology

| Classification | case | relative expressing intensity of Exon8-9 mRNA | | *P* value |
|---|---|---|---|---|
| | | weaken | normal | |
| Types of tissue | | | | |
| Squamous carcinoma | 10 | 6(60.6%)* | 4(40.0%) | <0.05 |
| Adenocarcinoma | 10 | 0 | 10(100%) | |
| Adenosquamous carcinoma | 1 | 1(100%) | – | – |
| Large cell carcinoma | 3 | 3(100%) | – | – |
| The degree of differentiation | | | | |
| High, moderate differentiation | 6 | 0△ | 6(100%) | <0.05 |
| Low differentiation | 18 | 10(55.6%) | 8(44.4%) | |
| Lymph node metastasis | | | | |
| Yes | 15 | 9(60.0%)▲ | 6(40.0%) | <0.05 |
| No | 9 | 1(11.1%) | 8(88.9%) | |

* $P<0.05$, significant squamous carcinoma VS adenocarcinoma; △ $P<0.05$, low differentiation VS high, moderate differentiation; ▲ $P<0.05$, Lymph node metastasis group VS non-metastasis group.

## *DISCUSSION*

E-CD is a kind of homogenous calcium-dependent transmembrane glycoprotein[4] that can mediate adhesion between cells. The main function of E-CD is mediating homogenous specific adhesion among cells, also it plays a marked role in shaping and maintaining epidermis tissue. Human E-CD gene is located at 16q22.1, the length is 4.8 kb, open reading frame 2.65 kb, with coding transmembrane glycoprotein whose length is 882 amino acid. The expression degree of E-CD has negative relation to metastasis potential of cancer cells. The invasion disappeared when high invasive cancer cell, was transfected with mRNA plasmid of E-CD, but the invasion would be acquired by dealing with E-CD mono-antibody or using antisense mRNA of E-CD to transfect non-invasive cells. The cells that are absent of E-CD can´t form epithelioid tissue. E-CD is the definite symbol of differentiation of cephalo-cevical squamous carcinoma, carcinoma of stomach, breast cancer and thyroid cancer. The inordinate expression of E-CD is founded in infiltrative tumor. The expression in infiltrative growing tumors is much lower than that in endogenous growing tumors [6]. E-CD may have some affinities with processes of the differentiation and invasion of tumors as well as the losing epithelia antetypes and acquiring invasion of cells.

In researching the cancer of breast, esophaguse, stomach, rectum and prostate, the scholars found that the frequency of extending lymph node metastasis was much higher in the patients with low expressions of E-CD protein than that in the patients with normal expression of E-CD protein[7]. The breast cancer patients with low E-CD expression had more possibility of blood metastasis to bone and lung than those with normal E-CD expression. In the immune-histochemisty study of cephalo-cervial cancer it was also found that the depressed E-CD protein expression could promote cancer metastasis to lymph node. Bahrawy et al.[8] in the research of large intestine carcinoma found that the low expression of mRNA had the positive

relation to lymph node metastasis. The same conclusion was got in the research of testicle germ cell tumor by Wang et al[9].

Using immune-histochemical method, we found that there was 88% abnormal expression (including heterotopic expression and non-expression) of E-CD in the specimen of lung cancer with lymph node metastasis. There is relation between the abnormal of expression E-CD and non-small cell lung cancer lymph node metastasis. According to the research about E-CD gene of lung cancer before, we found that lung cancer mainly represented exon 8, 9 abnormality of E-CD.

The mechanism of depression or absence of E-CD gene expression isn´t identified yet. It is generally thought as the aspects below: ① the transcription and translation abnormality of E-CD gene; ② methylate of E-CD promoting gene; ③ the cleavage of E-CD polypeptidase.

The results reveal that there are E-CD mRNA expression abnormalities in 41.7% of NSCLC patients. There is close correlation between expression abnormalities of E-CD mRNA and NSCLC lymph node metastasis as well as histological types and differentiation degree ($P<0.05$). Examining the expression of E-CD exon 8, 9 mRNA can be conductive to the pathological diagnosis of lung cancer.

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