RESEARCH ARTICLE

Expression of Connexin 43 and E-cadherin Protein and mRNA in Non-small Cell Lung Cancers in Chinese Patients

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Abstract

Aim: Connexin 43 (Cx43) and E-cadherin are important biomarkers related with cancer. Their expression at protein and mRNA levels was here investigated in 50 primary lung carcinoma tissues and 20 samples of adjacent normal tissue of Chinese patients with non-small cell lung cancer (NSCLC). Methods: Protein and mRNA expression were evaluated by ABC immunohistochemistry and RT-PCR. Results: (1) The positive expression rates of Cx43 and E-cadherin protein were higher in the adjacent normal tissues than those in the primary lung carcinoma tissues; (2) the positive expression rates of Cx43 and E-cadherin protein decreased with NSCLC progression; (3) the expression of E-cadherin protein was not related with the pathological type of NSCLC; and (4) the relative quantity of the Cx43 or E-cadherin mRNA expression was correlated with the the histological type, clinical stage, cancer cell differentiation and the lymph node metastasis. Conclusion: The data suggested that the Cx43 and E-cadherin are reduced with NSCLC progression, and might be important biomarkers for judging the metastasis and prognosis.

Keywords: Connexin 43 - E-cadherin - non-small cell lung cancer - Chinese patients

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Introduction

Lung cancer is the most common cancer in the world. Non-small cell lung cancer (NSCLC) is the most common bronchial tumor, which can be classified in two major histological subtypes, the adenocarcinoma (AC) and the squamous cell carcinoma (SCC). In 2008, among males, the highest lung cancer incidence rates are in Eastern and Southern Europe, North America, Micronesia and Polynesia, and Eastern Asia, and Chinese females have higher lung cancer rates than those in certain European countries such as Germany and Italy. Lung cancer rates are also increasing in China (Parkin, 2001; Jemal et al., 2011).

Connexins was a group of homologous proteins which from the inter membrane channels of gap junctions (Proksch et al., 2008). The connexins are the products of an identified gene family which has both highly conserved and highly divergent regions. The variety contributes to the wide range of functional properties of gap junction (Sohl and Willecke, 2004; Laird, 2006; Solan and Lampe, 2009).

The abnormal connexin expression and distribution are closely related to the tumor formation (Laird, 2006; Cronier et al, 2008).The wild-type connexin genes, which were shifted into tumor cells, could inhibit the tumor growth and up-regulate the gap junction intercellular communication so as to recover the normal growth of tumor cells (Tomai et al., 1999; Yamasaki et al., 1999; McLachlan et al., 2006; Hattori et al., 2007; Langlois et al., 2010). These indicated that connexin genes were a family of tumor suppressor genes (Plante et al., 2011; Ogawa et al., 2012). Previous studies have demonstrated that connexin 43 (Cx43) in different tumor tissues act different expressions that were correlated with the tumor differentiation and prognosis (Laird et al., 1999; Huang et al., 1999; Murray et al., 2000; King and Bertram, 2005; Mesnil et al., 2005; Langlois et al., 2010).

Adhesion molecules play an important role in tumor metastasis by controlling the gap junction function (Fujimoto et al., 1997; Pertz et al., 1999; Kobayashi et al., 2007; Dittmar et al., 2007; Jeanes et al., 2008; Wheelock et al., 2008; Makrilia et al., 2009). Cell surface adhesion molecules, especially Ca^{2+}-dependent adhesion molecule E-cadherin, can provide a stable structure that induces cell membrane move closer to another cell membrane and promote two connected bodies relative to each other, so as to form a gap junction (Jongen et al., 1991; Fujimoto et al., 1997; Wheelock et al., 2008).These calcium-dependent adhesion molecules on the regulation of gap junction

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Table 1.

	Forward primer	Reverse primer	
Cx43	5'CTCAGTCACCGAACTTTGAAAATTTTCGAGAC	5'TATGTTTATACTAAATTAAAACTTTATTGAAT	14.13kb
E-cadherin	5'CCAGACCCAACGCGCGACCCGGACCTCCC	5'TTTTTTTTTTTTTTTTTTTTTGGCCAGAAAGCAA	4.02kb

function are affected by calcium ion concentration changes (Pertz et al., 1999).

Although so many studies have proven that connexin and E-cadherin are related with the tumor progress, it is not clear that how of connexin and E-cadherin protein and mRNA expression in Chinese patients with lung cancer. The present study tried to investigate Cx43 and E-cadherin protein and mRNA expression in Chinese patients with NSCLC, so as to understand possible Cx43 and E-cadherin protein and mRNA expression as biomarkers for judging the metastasis and prognosis of NSCLC.

Materials and Methods

Case information

Fifty Chinese patients with non-small cell lung cancer including 39 male and 11 female, aged 26-77 years, average aged 46.5 years, were studied. The experiments were approved by the Ethics Committee of 117 hospital of People's Liberation Army and all of the patients were signed the informed consents. The patients did not accept the treatments of chemotherapy and radiotherapy before the study. The diseases were diagnosed by the pathological methods after the surgery and biopsy, in which 32 cases had lymph node metastasis and 18 not, 25 cases suffered with adenocarcinoma, 19 with squamous carcinoma and 7 with large cell undifferentiated carcinoma. Tumor-node-metastasis (TNM) stage designations were according to UICC standard, and all the tumors were classified as stage I, II, III and IV respectively (n=12, 15, 20 and 3). For the pathological grade, 10 cases were in well-differentiation, 20 in moderate-differentiation and 20 in poor- differentiation. For 20 control samples, the lung tissues, which were diagnosed as normal by the pathological methods, were taken from the tumor tissue 3 cm or more away in the patients with NSCLC.

Materials

Rabbit anti-human monoclonal Cx43 antibody, Rabbit anti-human monoclonal E-cadherin antibody and ABC kit were purchased from Zymed Co., Ltd., USA. The specific steps of ABC immunohistochemical method were operated according to the manual instruction.

Immunohistochemical evaluation

Cx43 expression evaluation: After ABC immunohistochemical test, Cx43 protein showed brown stains that distributed on the adjacent membranes between lung cancer cells. The Cx43 expression evaluation standard was depended on the brown stain intensity and extent. It was considered as positive if over 10% lung cancer had brown stains, otherwise negative.

E-cadherin expression evaluation: After ABC immunohistochemical test, E-cadherin protein showed brown stains that distributed on the membrane of lung cancer cells. The E-cadherin expression evaluation standard was depended on the brown stain intensity and extent. It was considered as positive if over 60% lung cancer had brown stains, otherwise negative.

RT-PCR

During surgery, the lung tissues isolated were quickly semi-frozen. Using TRIzol reagent isolated the total RNA from each tissue sample. TRIzol reagent 0.3 ml maintains the integrity of the RNA during the sample lysis. Addition of 0.3 ml chloroform was followed by centrifugation and the aqueous phase was transferred. The RNA is recovered by precipitation with isopropyl alcohol. The extracted RNA was treated with DNase according to the manufacturer's protocol. Thereafter, to 1 μg of RNA, the sample was mixed with 1 μl of DNase (1 U/μl, Invitrogen), 10× DNase reaction buffer, and adjusted to 10 μl with DEPC-water. The mixed solutions were incubated at 25 °C for 15 min and stopped by adding 1 μl of 25mM EDTA at 65 °C for 10 min.

A review of GenBank using BLAST program showed that these primers were specific for Cx43 and (Gene Bank accession No. NC000006) and E-cadherin mRNA (Gene Bank accession No. NM001670.2) as shown in Table 1.

The cDNA solution (RT-product, 2 μl) was mixed with 5 μl 10 × PCR buffer, 2 μl 50 mM MgCl2, 1 μl 10 mM dNTP, 1 μl 10 mM forward and reverse primers, 1 μl Platinum Taq DNA polymerase and 37 μl DEPC water. Each cycle at 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min for 30 cycles. The PCR products of Cx43 and E-cadherin were subjected to 2% NuSieve agarose gel electrophoresia, stained with ethidium bromide, and then detected with a Gel-Doc (Bio-Rad, USA).

Statistical analysis

All values were expressed as the positive expression rate (%) and mean ± standard error of the mean (SEM). The data were analyzed by SPSS 17.0, which χ^2 test and two-way analysis of variance (ANOVA), followed by the Bonferroni test and one-way ANOVA followed by Dunnett test and Newmann–Keuls test. $P < 0.05$ was considered statistically significant.

Results

The relationship between Cx43 and E-cadherin protein expression

In 50 Chinese patients with NSCLC, there were 21 cases (42%) showing the positive expression of both Cx43 protein and E-cadherin protein; there were 2 cases (4%) showing the negative expression of Cx43 protein and the positive expression of E-cadherin protein; there were 6 cases (12%) showing the positive expression of Cx43 protein and the negative expression of E-cadherin protein; and there were 21 cases (42%) showing the negative

Table 2. Cx43 or E-cadherin Protein Expression in 50 Chinese Patients with NSCLC

Case		E-cadherin protein expression	
		positive	negative
Cx43 protein expression	positive	21	2
	negative	6	21

χ^2=23.86, P<0.01, γ=0.96

Table 3. Correlation of Cx43 or E-cadherin Protein Expression and Clinical Pathology

Group	Total cases	Cx43 protein expression		E-cadhein protein expression	
		Positive (Case)	Positive rate (%)	Positive (Case)	Positive rate (%)
Stage					
I~II	27	18	66.67	17	62.96
III~IV	23	9	39.14*	6	26.08**
Differentiation					
Well- or moderate-	30	20	66.67	18	50.00
Poor-	20	7	35.00*	5	35.00*
Histological type					
Adenocarcinoma	25	18	76.00	12	48.00
Squamous cell carcinoma	19	7	31.58*	9	46.37
Large cell carcinoma	6	2	33.33*	2	33.33
Lymph node metastasis					
No	18	10	55.56	13	72.22
Yes	32	17	53.13	10	31.25**

*P<0.05, **P<0.01 compared with the other group in the same clinical pathology

expression of both Cx43 protein and E-cadherin protein. There was very significant (χ^2=23.86, P<0.01, γ=0.96) (Table 2).

Correlation of Cx43 or E-cadherin protein expression and clinical pathology

In 50 NSCLC patients, the Cx43 positive expression was correlated with the histological type, clinical stage and cancer cell differentiation, but without the lymph node metastasis; E-cadherin protein expression was associated with clinical stage, cancer cell differentiation and lymph node metastasis, but without histological type.

The positive expression rates of Cx43 and E-cadherin protein in NSCLC with stage I and II were higher than those with stage III and IV (Cx43: 66.67% vs. 39.14%, P<0.05; E-cadherin: 62.96% vs. 26.08%, P<0.01); the positive expression rates of Cx43 and E-cadherin protein in NSCLC with moderate- and well-differentiated carcinoma higher than those with poor-differentiated (Cx43: 66.67% vs. 35.00%, P<0.05; E-cadherin: 50.00% vs. 35.00%, P<0.05); the positive expression rate of Cx43 protein in NSCLC with adenocarcinom (76.00%) was higher than those with the squamous cell carcinoma (31.58%) or large cell un-differentiated (33.33%) (P<0.05); and the positive expression rate of E-cadherin protein in NSCLC without lymph node metastasis (72.22%) was higher than those with lymph node metastasis (31.25%) (P<0.01) (Table 3).

Correlation of Cx43 or E-cadherin mRNA expression and clinical pathology

In 50 NSCLC patients, the relative quantity of the Cx43 or E-cadherin mRNA expression was calculated by comparing with the neighboring health lung tissue of each lung cancer sample. As the results of the protein expressin above, the relative quantity of the Cx43 or E-cadherin mRNA expression was correlated with the the histological type, clinical stage, cancer cell differentiation and the lymph node metastasis (Table 4).

Table 4. Correlation of Cx43 or E-cadherin mRNA Expression and Clinical Pathology

Group	Total cases	Cx43 mRNA expression Mean ± SEM	E-cadhein mRNA expression Mean ± SEM
Stage			
Health tissue	50	0	0
I~II	27	8.9 ± 1.6***	5.7 ±2.3**
III~IV	23	23.4 ± 7.2***	17.2 ± 6.1***
Differentiation			
Health tissue	50	0	0
Well- or moderate-	30	4.2 ± 1.8*	3.1 ± 2.0
Poor-	20	31.3 ± 11.4***	23.5 ± 16.2***
Histological type			
Health tissue	50	0	0
Adenocarcinoma	25	20.5 ± 9.6**	21.2 ± 14.3*
Squamous cell carcinoma	19	14.4 ± 8.9*	11.3 ± 6.7*
Large cell carcinoma	6	7.5 ± 4.2*	4.3 ± 3.1
Lymph node metastasis			
Health tissue	50	0	0
No	18	7.8 ± 4.2*	7.5 ± 3.2**
Yes	32	27.8 ± 12.5***	19.9 ± 5.6***

For each lung cancer sample, the value was compared with the value of neighboring health lung tissue; * P<0.05, ** P<0.01, *** P<0.001 compared with the group in the different clinical pathology

Discussion

Reduced connexin (Cx) 43 gene expression has been shown in most of lung tumors and cancer cell line (Chen et al., 2003; Kato et al., 2005; Brehm et al., 2006; Xu et al., 2008; Jinn et al., 2010; Losa et al., 2011). Jinn et al. detected connexin 32 (Cx32) and Cx43 expression levels by immunohistochemistry in human lung cancer tissue (Chen et al., 2005). They found that Cx43 expression level reduced, but Cx32 expression was not, and Cx43 expression level was correlation with the degree of differentiation of lung cancer. With Cx43 expression decreasing, the degree of differentiation was lower, and the prognosis was worse. In our research, 50 cases of primary lung cancer were studied by using Immunohistochemistry. The date showed that Cx43 expression positive rate was 54% (27/50), whose number and distribution were different and showed a significant heterogeneity. Alveolar cells, macrophages, epithelial cells, lymphocytes had a strong expression of Cx43, but have no significant relationship with Cx43 expression in lung cancer cells.

In the study of Cx43 expression and clinical stage, histological grade of lung cancer, we found that with tumor progression, Cx43 expression gradually decreased, and the difference of the positive rate between stageI~II and stage III~IV was statistically significant (P <0.05), and Cx43 positive expression rate of moderately, well differentiated lung cancer cases was significantly higher than that in poorly differentiated cancer (P <0.05). These results suggested that, Cx43 expression was corrected with histological grade and clinical stage of lung cancer. The tumor cells are easy to escape normal growth control

and host immune surveillance because of decreased Cx43 expression. Therefore, decreased Cx43 expression may be one of the reasons that lung cancer cells in the middle, late stage and poorly differentiated are easy to spread and distant metastasis. These show that Cx43 plays an important role in judging the prognosis of patients with lung cancer. In Lung cancer with or without lymph node metastasis, the difference of Cx43 positive expression rate was not statistically significant ($P>0.05$). In addition, adenocarcinoma was prone to invasiveness and blood transfer. In this group, Cx43-positive expression rate in lung adenocarcinoma was significantly higher than squamous cell carcinoma and large cell undifferentiated carcinoma. It is not yet clear whether Cx43 positive expression of lung cancer cells are prone to hematogenous shift and the expression have no significant relationship with lymph node metastasis, the exact mechanism awaits further studies.

The study of 54 cases with NSCLC conducted by Smyth et al. showed that E-acdherin and its associated proteins were associated with the differentiation of lung cancer (Smythe et al., 1999), but not with its clinical stage and histological type. However, Chen XF et al. believed that E-cadherin and nm23 functions asputative metastasis-suppressor genes in the progression of malignancies in NSCLC (Jinn et al., 1998). Aberrationsin mRNA expression were observed and associated with degrees of histological differentiation and increasing stage as well as lymph node metastases, although no change of genetic structure was detected in the E-cadherin and nm23 genes. Our study showed that (1) E-cadherin expression level of lung cancer without lymph node metastasis was higher than those with lymph node metastasis; (2) E-cadherin positive expression in stage I~II was significantly higher than that in stage III~IV; (3) E-cadherin positive expression rate of moderately, well differentiated lung cancer was significantly higher than the poorly differentiated level of lung cancer; (4) E-cadherin expression had no correction with the tissue type of NSCLC. These demonstrated that E-cadherin played a key role in lung cancer occurrence, development and metastasis, which could be used as an important prognostic indicator.

Musil et al. reported that E-cadherin fens were transected into cell communication dysfunction of tumor cell lines, whose gap junctional intercellular communication increased (Musil et al., 1990). The E-cadherin expression showed high in the cell while connexin protein increased. The data indicated that E-cadherin might have control function for transcription and translation of connexin protein mRNA (Naus and Laird, 2010).

In our study, the results also showed that the relationship of E-cadherin expression lever with Cx43 protein expression was positive; indicating that in the development and metastasis of lung cancer, Cx43 and E-cadherin played a common control function.

In summary, the investigation demonstrated that dysfunction of Cx43 and E-cadherin had a role in progression of NSCLC, and that the examination of Cx43 and E-cadherin expression could provide experimental evidence for clinical treatment. In clinical work, detection of the expression of these two indicators had significance in the metastasis and prognosis of lung cancer.

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References

Brehm R, Rüttinger C, Fischer P, et al (2006). Transition from preinvasive carcinoma in situ to seminoma is accompanied by a reduction of connexin 43 expression in Sertoli cells and germ cells. *Neoplasia*, **8**, 499-509.

Chen JT, Cheng YW, Chou MC, et al (2003). The correlation between aberrant connexin 43 mRNA expression induced by promoter methylation and nodal micrometastasis in non-small cell lung cancer. *Clin Cancer Res*, **9**, 4200-4.

Chen XF, Zhang HT, Qi QY, et al (2005). Expression of E-cadherin and nm23 is associated with the clinicopathological factors of human non-small cell lung cancer in China. *Lung Cancer*, **48**, 69-76.

Cronier L, Crespin S, Strale PO, et al (2009). Gap Junctions and Cancer: New Functions for an Old Story. *Antioxid Redox Signal*, **11**, 323-38.

Dittmar T, Heyder C, Gloria-Maercker E, et al (2007). Adhesion molecules and chemokines: the navigation system for circulating tumor (stem) cells to metastasize in an organ-specific manner. *Clin Exper Metastasis*, **25**, 11-32.

Fujimoto K, Nagafuchi A, Tsukita S, et al (1997). Dynamics of connexins, E-cadherin and alpha-catenin on cell membranes during gap junction formation. *J Cell Sci,* **110**, 311-22.

Hattori Y, Fukushima M, Maitani Y (2007). Non-viral delivery of the connexin43 gene with histone deacetylase inhibitor to human nasopharyngeal tumor c ells enhances gene expressi on and inhibits in vivo tumor growth. *Int J Oncol*, **30**, 1427-39.

Huang RP, Hossain MZ, Sehgal A, et al (1999). Reduced connexin43 expression in hig- grade human brain glioma cells. *Surg Oncol*, **70**, 21-4.

Jeanes A, Gottardi CJ, Yap AS (2008). Cadherins and cancer: how does cadherin dysfunction promote tumor progression? *Oncogene*, **27**, 6920-9.

Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.

Jinn Y, Ichioka M, Marumo F (1998). Expression of connexin32 and connexin43 gap junction proteins and E- cadherin in human lung cancer. *Cancer Lett*, **127**, 161- 9.

Jinn Y, Inase N (2010). Connexin43, E-Cadherin, β-Catenin and ZO-1 Expression, and Aberrant Methylation of the Connexin43 Gene in NSCLC. *Anticancer Res*, **30**, 2271-8.

Jongen WM, Fitzgerald DJ, Asamoto M, et al (1991). Regulation of connexin 43-mediated gap junctional intercellular communication by Ca^{2+} in mouse epidermal cells is controlled by E-cadherin. *J Cell Biol*, **114**, 545-55.

Kato Y, Hirano T, Yoshida K, et al (2005). Frequent loss of E-cadherin and/or catenins in intrabronchial lesions during carcinogenesis of the bronchial epithelium. *Lung Cancer*, **48**, 323-30.

King TJ, Bertram JS (2005). Connexins as targets for cancer chemoprevention and chemotherapy. *Biochim Biophys Acta*, **1719**, 146-60.

Kobayashi H, Boelte KC, Lin PC (2007). Endothelial Cell Adhesion Molecules and Cancer Progression. *Curr Med Chem*, **14**, 377-86.

Laird DW (2006). Life cycle of connexins in health and disease. *J Biochem*, **394**, 527-43.

Laird DW, Fistouris P, Batist G, et al (1999). Deficiency of connexin43 gap junction is an independent marker for breast tumors. *Cancer Res*, **59**, 4101-10.

Langlois S, Cowan KN, Shao Q, et al (2010). The tumor-suppressive function of connexin43 in keratinocytes is mediated in part via interaction with caveolin-1. *Cancer Res*, **70**, 4222-32.

Losa D, Chanson M, Crespin S (2011). Connexins as therapeutic targets in lung disease. *Expert Opin Ther Targets*, **15**, 989-1002.

Makrilia N, Kollias A, Manolopoulos L, et al (2009). Cell adhesion molecules: role and clinical significance in cancer. *Cancer Invest*, **27**, 1023-37.

McLachlan E, Shao Q, Wang HL, et al (2006). Connexins act as tumor suppressors in three-dimensional mammary cell organoids by regulating differentiation and angiogenesis. *Cancer Res*, **66**, 9886-94.

Mesnil M, Crespin S, Avanzo JL, et al (2005). Defective gap junctional intercellular communication in the carcinogenic process. *Biochim Biophys Acta*, **1719**, 125-45.

Murray SA, Davis K, Fishman LM, et al (2000). Alpha connexin43 gapjunction are decreased in human adrenocortical tumors. *J Clin Endocrinol Metab*, **85**, 890-5.

Musil B, Linda S, Gerald M, et al (1990). Differential phosphorylation of gap junction protein connexin43 in junctional communication- competent and different cell lines. *J Cell Biol*, **111**, 20777-9.

Naus CC, Laird DW (2010). Implications and challenges of connexin connections to cancer. *Nat Rev Cancer*, **10**, 435-41.

Ogawa K, Pitchakarn P, Suzuki S, et al (2012). Silencing of connexin 43 suppresses invasion, migration and lung metastasis of rat hepatocellular carcinoma cells. *Cancer Sci*, **103**, 860-7.

Parkin DM (2001). Global cancer statistics in the year 2000. *Lancet Oncol*, **2**, 533-43.

Pertz O, Bozic D, Koch AW, et al (1999). A new crystal structure, Ca^{2+}dependence and mutational analysis reveal molecular details of E-cadherin homoassociation. *EMBO J,* **18**, 1738-47.

Plante I, Stewart MK, Barr K, et al (2011). Cx43 suppresses mammary tumor metastasis to the lung in a Cx43 mutant mouse model of human disease. *Oncogene*, **30**, 1681-92.

Proksch E, Brandner JM, Jensen JM (2008). The skin: an indispensable barrier. *Exper Dermatol*, **17**, 1063-72.

Smythe W, Williams J, Wheelock M, et al (1999). Cadherin and catenin expression in normal bronchial epithelium and non-small cell lung cancer. *Lung Cancer*, **24**, 157-68.

Sohl G, Willecke K (2004). Gap junctions and the connexin protein family. *Cardiovasc Res*, **62**, 228-32.

Solan JL, Lampe PD (2009). Connexin43 phosphorylation-structural changes and biological effects. *J Biochem*, **419**, 261-72.

Tomai E, Brownell HL, Tufescu TV, et al (1999). Gap junctional intercellular communication in cultured human lung carcinoma cells. *Lung Cancer,* **23**, 223-31.

Wheelock MJ, Shintani Y, Maeda M, et al (2008). Cadherin switching. *J Cell Sci*, **121**, 727-735.

Xu HT, Li QC, Zhang YX, et al (2008). Connexin 43 recruits E-cadherin expression and inhibits the malignant behaviour of lung cancer cells. *Folia Histochem Cytobiol*, **46**, 315-21.

Yamasaki H, Krutovskikh V, Mesnil M, et al (1999). Role of connexin (gap junction) genes in cell growth control and carcinogenesis. *CR Acad Sci III*, **322**, 151-9.