The Expression of NF-kB and Slug in NSCLC and the Effect on EMT

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ABSTRACT Objective: To investigate the expression of NF-kB and Slug in NSCLC and their relationship with EMT, to provide theoretical basis to the treatment of NSCLC. Methods: (1) PV9000 two-step immunohistochemical method was used to detect the expression of NF-κBP65, Slug, E-cadherin and Vimentin in 50 NSCLC tissues and 20 adjacent normal lung tissues. (2) RT-PCR was performed to determine the expression of NF-κBP65mRNA and SlugmRNA in 25 NSCLC tissues and 10 adjacent normal lung tissues. Results: In NSCLC tissues, NF-κBP65 expression was higher than that in adjacent normal lung tissues ($Z=-2.370$, $P<0.05$); mRNA expression of NF-kBP65 was significantly higher than that in adjacent normal lung tissues ($t=4.967$, $P<0.01$); Slug expression was significantly higher than that in adjacent normal lung tissues ($Z=-4.443$, $P<0.01$); The expression of Slug was significantly higher than that in adjacent normal lung tissues ($t=6.483$, $P<0.01$). In NF-kBP65 positive NSCLC tissues, E-cadherin expression decreased ($X^2=5.024$, $P<0.05$) while vimentin expression increased ($X^2=4.723$, $P<0.05$). In Slug positive NSCLC tissues, E-cadherin expression decreased ($X^2=5.984$, $P<0.05$) and Vimentin expression increased ($X^2=5.028$, $P<0.05$). In addition, NF-kBP65 had very significantly positively correlated with Slug ($r=0.443$, $P<0.01$). NF-kB was related to differentiation degree ($X^2=5.024$, $P<0.05$), lymph node metastasis ($X^2=7.933$, $P<0.01$) and neoplasm staging ($X^2=7.317$, $P<0.01$), but not related to gender, age and tissue type ($P>0.05$). Slug was related to lymph node metastasis ($X^2=6.174$, $P<0.05$) and neoplasm staging ($X^2=7.317$, $P<0.01$), but not related to gender, age, tissue type and differentiation degree ($P>0.05$). Conclusion: The increase of NF-kB expression and Slug expression in NSCLC may be related to the generation, development and metastasis of NSCLC; in addition, NF-kB and Slug may synergistically inhibit E-cadherin expression, promote Vimentin expression and induce EMT of NSCLC, which further provides theoretical basis to the researches on EMT of NSCLC.

Key words: NF-κB; Slug; EMT; RT-PCR; Immunohistochemistry

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Introduction

Lung cancer is one of the common malignant tumors and has become the leading cause of death in China, of which non-small cell lung cancer (NSCLC) accounts for more than 75% of lung cancer[1]. Otherwise, some researches show that epithelial-mesenchymal transition (EMT) of malignant tumors is the committed step for tumor progression and metastasis [2]. Thus, EMT of NSCLC has become the focus of recent researches. However, the genesis mechanism of EMT of malignant tumors remains unclear, it may be caused by multiple genes and multiple steps. In recent years, numerous researches show that NF-kB and Slug play an important role in EMT of Pancreatic carcer[3,4]and other malignant tumors, however, the action of NF-kB and Slug in EMT of NSCLC has not been reported. NF-kB was found in B cells by Bltimore [5], which widely exists in eukaryotes and is the protein family formed by RELA\P65, P50, RELB, C-Rel, P52 [6]. Slug belongs to Snail zinc lipoprotein superfamily and can encode zinc lipoprotein [7]. Through the determining of the expression of NF-kBP65 and Slug in NSCLC and the expression of Vimentin and E-cadherin, which were landmark factors of EMT[8], this study investigated the function of NF-kB and Slug in NSCLC, the effect on EMT of NSCLC and the correlation with EMT. It also discussed the correlation of NF-kB and Slug with clinical pathology.

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1 Materials and methods

1.1 Specimen source

Patients treated by operation and without chemoradiotherapy before operation in Affiliated Hospital of Qingdao University Medical College between 2010 and 2011 were selected as subjects. 50 NSCLC tissues and 20 adjacent normal lung tissues (5cm adjacent to cancer) were collected from these subjects and then divided into two parts. One part was fixed in neutral formalin for IHC and the other was quickly placed into a refrigerator at -80℃ for storage. All collected specimens were confirmed by pathological section as NSCLC. The clinical and pathological data were integral. Of these subjects, 31 were males and 19 were females; 26 were ≤60 ages and 24 were>60 ages, with the average age of 59; 15 were squamous carcinoma and 35 were adenocarcinoma; 26 were highly or moderately differentiated and 24 were poorly differentiated; 15

were with lymph node metastasis and 35 were without lymph node metastasis. According to the TNM Classification of Malignant Tumors: 30 were at I~II stage and 20 were at III~IV stage.

1.2 Laboratory reagents and methods

1.2.1 Reagents Mouse Anti-human NF-κBp65 sc-8008 Monoclonal Antibody was purchased from Santa Cruz Biotechnology Inc., Rabbit Anti-human Slug Monoclonal Antibody from Beijing Biosynthesis Biotechnology Co., Ltd.,and Mouse Anti-human Vimentin Monoclonal Antibody, Mouse Anti-human E-cadherin Mnoclonal Antibody, PV-9000 Immunohistochemistry Kit and DAB Kit all from Zhongshan GoldenBridge Biotechnology Co, Ltd. All antibodies were diluted at the concentration of 1:100. RNAiso Reagent was purchased from TAKARA Bio Inc. and Super One Step RT-PCR Kit from Beijing BioTeke Corporation. NF-kBP65 (RELA), Slug and GAPDH primers were synthesized by Shanghai Sangon Biotech Co., Ltd.

1.2.2 Immunohistochemical methods All tissues were fixed in neutral formalin, embedded in paraffin and serially sectioned with a thickness of 4μm. Then, the tissues were dewaxed and hydrated, and endogenous enzymes were blocked by 3% hydrogen peroxide for 20min. NF-κBp65,Slug and Vimentin were prepared with citrate (pH=6.0) at high pressure for 4min and E-cadherin was prepared with EDTA (pH=9.0) at high pressure for 4min. Then, antibodies and PV-9000 reagent were dripped. The tissues were developed with DAB and stained with hematoxylin. The primary antibody was substituted by the citrate solution to be used as the negative control. The known positive tissues were used as the positive control and treated according to the aforementioned steps. The appearance of yellow-brown fine particles indicated immunohistochemical positive. The immunohistochemical scoring method of this trial was as follows [9]: 5 high power fields (× 400) were selected for each section and 100 cancer cells were counted under each field. Base on the staining intensity of positive cells: 0 was for unstained, 1 for light yellow, 2 for yellow and 3 for yellow brown. Based on the percentage of positive cells: 0 was for <5%, 1 for 5%-10%, 2 for 11%-50% and 3 for 51%-100%. Analysis was conducted by adding the staining intensity of positive cells and the percentage of positive cells: 0 was negative (-), 1-2 was weak positive (+), 3-5 was positive (++) and 5-6 was strong positive, of which (-) indicated negative and (+), (++) and (+++) indicated positive.

1.2.3 RT-PCR methods The collected 50 fresh NSCLC tissues and 20 adjacent normal lung tissues were placed into the refrigerator at -80℃ for storage. 25 NSCLC tissues and 10 adjacent normal lung tissues were selected randomly to conduct the trial.Total RNA was extracted with RNAiso Reagent. One-step method was performed to reverse transcription and PCR reaction. The obtained product was electrophoresed in 2% agarose. NF-κB, Slug and reference GAPDH target bands were analyzed by Quentity One software and values were obtained. The primers were as follows:

Table 1 The primers of NF-kBP65, Slug and GAPDH

Grouping	Primers	Product length
NF-kBP65(RELA) forward	5'-CGAGAGGAGCACAGATACCAC-3'	228bp
NF-kBP65(RELA) reverse	5'-CGCTTCTTCACACACTGGATT-3'	
Slug forward	5'-AGATTTGACCTGTCTGCAAATGCTC-3'	158bp
Slug reverse	5'-ATGCATATTCGGACCCACACATTAC-3'	
GAPDH forward	5'-CGGGAAACTGTGGCGTGAT-3'	299bp
GAPDH reverse	5' AGTGGGTGTCGCTGTTGAAGT-3'	

1.2.4 Statistical method Data were analyzed using SPSS17.0 statistics software. Mann-Whitney Test, X^2Test and Spearman Correlation analysis were conducted for the immunohistochemical results. Independent-Samples T test and Pearson Correlation were conducted for the RT-PCR results. P<0.05 was considered as there was difference with statistical significance.

2 Results

2.1 Immunohistochemical results

2.1.1 Protein expression of NF-κBP65 and Slug in NSCLC and adjacent normal lung tissues NF-κBP65 expression in NSCLC was significantly higher than that in adjacent normal lung tissues (Z=-2.370，P＜0.05); Slug expression in NSCLC was significantly higher than that in adjacent normal lung tissues (Z=-4.443，P＜0.01)（Table 1）.

2.1.2 Relation of NF-κBP65 expression and Slug expression with clinical pathology of NSCLC NF-kBP65 was related to differentiation degree, lymph node metastasis and neoplasm staging (P<0.05), but not related to gender, age and tissue type (P＞0.05). Slug was related to lymph node metastasis and neoplasm staging (P<0.05), but not related to gender, age, tissue type and differentiation degree (P＞0.05).（Table 2）

2.1.3 Correlation of NF-κBP65 expression and Slug expression in NSCLC NF-kBP65 expression was very significantly positively correlated with Slug expression (r=0.443 P＜0.01)（Table 3）.

2.1.4 Expression of NF-kBP65 and Slug in NSCLC and the

Table 1 Protein expression of NF-kBP65 and Slug in NSCLC and adjacent normal lung tissues

Classificatio	NF-kBP65		Slug	
n	Tumor	Normal	Tumor	Normal
-	11	16	9	14
+	14	3	14	5
++	16	1	21	1
+++	9	0	6	0
n	50	20	50	20
	Z=-2.370		Z=-4.443	
	P=0.018＜0.05		P=0.00＜0.01	

Table 2 Relation of NF-kBP65 expression and Slug expression with clinical pathology of NSCLC

Clinicopathologic							
Parameters	n	-	+		-	+	
Sex							
Male	31	5	26	P=0.201＞0.05	7	24	P=0.282＞0.05
Female	19	6	13	x^2=1.639	2	17	x^2=1.160
Age							
≤60	26	4	22	P=0.240＞0.05	5	21	P=0.814＞0.05
＞60	24	7	17	x^2=1.381	4	20	x^2=0.056
Histotype							
Squamous cell carcinoma	15	2	13	P=0.316＞0.05	3	12	P=0.810＞0.05
Adenocarcinoma	35	7	28	x^2=0.574	6	29	x^2=0.058
Differentiation							
High or moderate	26	9	17	P=0.025＜0.05	7	19	P=0.087＞0.05
Poor	24	2	22	x^2=5.024	2	22	x^2=2.922
LNM							
Negative	32	11	21	P=0.005＜0.01	9	23	P=0.013＜0.05
Positive	18	0	18	x^2=7.933	0	18	x^2=6.174
TNM staging							
I-II stage	30	10	20	P=0.018＜0.05	9	21	P=0.007＜0.01
III-IV stage	20	1	19	x^2=5.614	0	20	x^2=7.317

Table 3 Correlation of NF-kBP65 expression and Slug expression in NSCLC

NF-kBP65 \ Slug	-	+	++	+++
-	6	2	2	1
+	2	7	4	1
++	1	3	9	3
+++	0	2	6	1
		r=0.443 P=0.001＜0.01		

relationship with E−cadherin and Vimentin E-cadherin expression in NF-κBP65 positive group was significantly lower than that in NF-κBP65 negative group (X^2=5.024，P＜0.05), and Vimentin expression in NF-κBP65 positive group was significantly higher than that in NF-κBP65 negative group (X^2=4.723，P＜0.05). E-cadherin expression in Slug positive group was significantly

lower than that in Slug negative group (X^2=5.984，P＜0.05) and Vimentin expression was significantly higher than that in Slug negative group (X^2=5.028，P＜0.05)，(Table 4) .

Table 4 Expression of NF-κBP65 and Slug in NSCLC and the relationship with E-cadherin and Vimentin

Grouping		NF-κBP65			Slug		
		-	+		-	+	
E-cadherin	-	2	22	x^2=5.024	1	23	x^2=5.984
	+	9	17	P=0.025	8	18	P=0.014
Vimentin	-	8	14	x^2=4.723	7	15	x^2=5.028
	+	3	25	P=0.030	2	26	P=0.024

2.2 RT-PCR results

Correlation of NF-κBP65 mRNA expression and Slug mRNA expression in NSCLC tissues and adjacent normal lung tissues The expression of NF-κBP65mRNA in NSCLC was higher than that in adjacent normal lung tissues and the difference had statistical significance (t=4.967, P＜0.01); the expression of Slug mRNA in NSCLC was higher than that in adjacent normal lung tissues and the difference had statistical significance (t=6.483,P＜0.01). In addition, the expression of NF-κBP65 mRNA was significantly positively correlated with the expression of SlugmRNA in NSCLC (r=0.439,P＜0.05)，(Table 5) .

Table 5 Expression of NF-kBP65 mRNA and Slug mRNA in tissues and correlation thereof

Classification	n	NF-kBP65	Slug	r	P
NSCLC	25	0.622± 0.212	0.751± 0.188	0.439	0.028
Normal lung tissues	10	0.263± 0.131	0.297± 0.183		
		t=4.967 P＜0.01	t=6.483 P＜0.01		

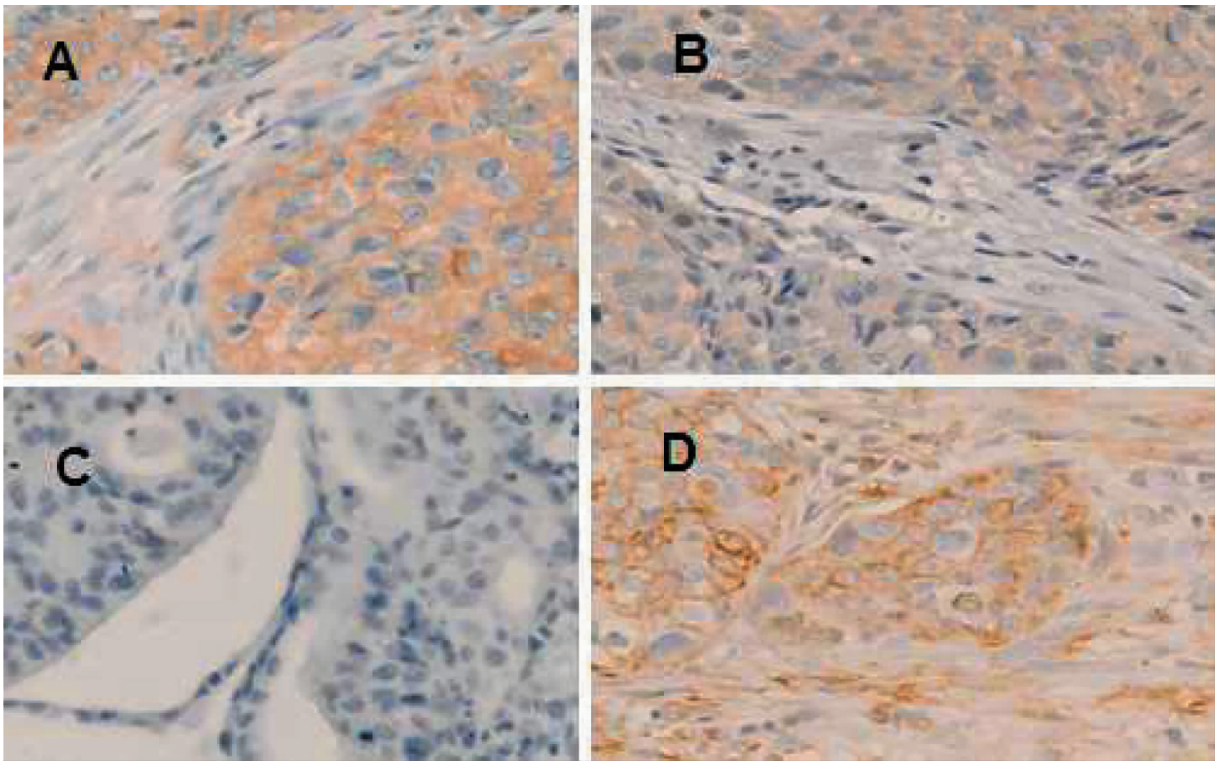

Fig.1 The Expression of NF-kBP65,Slug,E-cadherin and Vimentin protein in NSCLC

A is strong positive expression of NF-kBP65 in NSCLC; B is strong positive expression of Slug in NSCLC; C is negative expression of E-cadherin in NSCLC; D is strong positive expression of Vimentin in NSCLC(× 400)

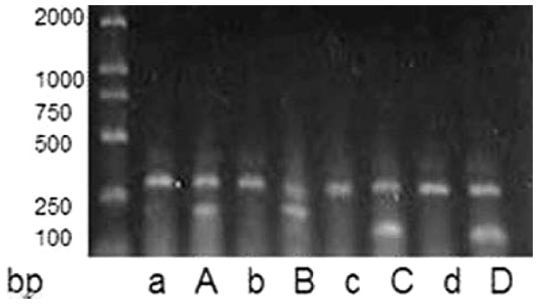

Fig.2 The Expression NF-kBP65, Slug and GAPDH mRNA in NSCIC
A and B are the expression of NF-kBP65 mRNA in NSCLC; C and D are the expression of Slug mRNA in NSCLC; a, b, c and d are the expression of NF-kBP65mRNA and Slug mRNA in adjacent normal lung tissues

3 Discussion

The generation, development, metastasis[10-12] and EMT[13,14] of NSCLC were related with multiple factors. The results of this paper showed that NF-κB and Slug played an important role in the generation, development, metastasis and EMT of NSCLC.

Li reported that the increase of NF-κBP65 expression would induced NSCLC cell proliferation, inhibited cell apoptosis and promoted the generation and development of NSCLC[15]. Li R reported that Slug played an important role in the lymph node metastasis and staging of lung carcinoma [16] .It was showed in the present study that the protein and mRNA expression of NF-κBP65 and Slug in NSCLC were significantly higher than that in adjacent normal lung tissues. The expression of NF-κBP65 correlated with TNM staging, differentiated group and lymph node metastasis. The expression of Slug correlated with differentiated group and lymph node metastasis. The results of the present study indicated that NF-κB and Slug played an important role in the generation, development and metastasis of NSCLC, and lung carcinoma could be induced by the increase of NF-κB and Slug expression in lung tissues. Otherwise, Maybe malignancy degree and metastasis rate increased and the prognosis worsened with the increasing of NF-κB and Slug expression. The results of this study were consistent with the aforementioned studies. However, it was reported by Li that there was no correlation between NF-κBP65 and metastasis of NSCLC; Shih indicated that there was no significant correlation between Slug and lymph node metastasis and staging. [17] The contradictions might result from the different research methods or different case constitutions. It needs further research. The positive correlation of NF-κ BP65 and Slug in protein level and mRNA level indicated that NF-κB and Slug synergistically promoted the generation and development of NSCLC. However, the detail mechanism remains unclear and needs further studies.

IkB protein family in cytoplasm could be combined with NF-κB and kept in resting state. When NF-κB was stimulated by external factors, IkB dissociation would be induced by NF-κB to dissociate P50\P65 dimer in order to regulate the expression of target genes, of which, Ras gene was recognized as one of the genes related to EMT. In this trial, E-cadherin expression in NF-κB positive NSCLC tissues was lower than that in NF-κB negative NSCLC tissues, and Vimentin expression in NF-κB positive NSCLC tissues was higher than that in NF-κB negative NSCLC tissues. It was purposed that when NF-κB dissociated with IkB under stimulation, NF-κB inhibited E-cadherin expression, increased Vimentin expre-ssion and promoted EMT of NSCLS by regulating Ras pathway. Huber reported that EM T of Ras transfecte d epithelial cell was stopped by inhibiting NF-κB activities, which indicated that NF-κB played an important role in inducing EMT of breast cancer [18]. The results of this study were consistent with the results of Huber. In Slug positive NSCLC tissues, E-cadherin expression decreased and Vimentin expression increased, which indicated that Slug could also inhibit E-cadherin expression, increase Vimentin expression and induce EMT of NSCLC. Thiery rep orted that Slug cou ld competitively combine with E-box of E-cadherin during the formation of chicken primary germ layer to inhibit E-cad herin expression, increase V imentin expression and induce EMT [2]. Come reported that EMT of breast cancer could be promoted by Snail\Slug [19]. The results of this study were consistent with Thiery and Come. Otherwise, the results showed that NF-κB was significantly correlated with Slug, which indicated that NF-κB may a ct on Slug in mu ltiple ways to synergistically regulate E-cadherin and Vimentin expression and induce EMT of NSCLC. It was showed in researches on mice malignant tumor by Wu that NF-κB activation played an important role in the enhancement of the expression of Snail\Slug and the stability of Snail\Slug [20]. The detailed mechanis m of NF-κB and Slug in synergistically promoting EMT of NSCLC remains unclear and needs further studies.

In conclusion, the generation, development and metastasis of NSCLC were promoted synergistically by the increasing of NF-κB and Slug expression in lung tissues. During to the effects of NF-κB and Slug, the E-cadherin expression was inhibited, the Vimentin expression was increased and the EMT of NSCLC was promoted. The results of this study suggest that the generation, development, metastasis of NSCLC and the generation of EMT in NSCLC could be suppressed by the inhibition of activities of NF-κB and Slug.

References

[1] Ministry of Healthy of the People's Republic of China. Major situation of the third survey of national death causes [J]. China Cancer, 2008,17 (5):344-345

[2] Thiery JP. Epithelial-mesenchymal transitions in tumour progression [J]. Nat Rev Cancer, 2002, 2(6):442-454

[3] Shi Lei, Wang Shi-ming, He Jie-feng. Expression of NF-kB and its relation with epithelial-mesenchymal transition in pancreatic cancer [J]. Cancer Research and Clinic,2011,23(3):191-193

[4] Hotz B, Arndt M, Dullat S, et al. Eepithelial to mesenchymal

transition:expression of regulators snail, slug, and twist in pancreatic cancer[J]. Clin Cancer Res, 2007,13(16):4769-4776

[5] Singh H, Sen R, Baltimore D. A nuclear factor that binds to a conserved sequence motif in transcriptional control elements of immunoglobulin genes[J]. Nature, 1986, 319(6049):154-158

[6] Zuo Jing, Liu Wei. The research on development of NF-kb in chemoresistance in cancer[J]. Chinese Journal of Clinical Oncology. 2004,31(20):1195-1197

[7] Bolos V, Peinado H, Perez-MORENO MA, et al. The transcription factor Slug represses E-cadherin expression and induces epithelial to mesenchymal transitions: a comparison with Snail and E47 repressor [J]. J Cell Sci, 2003, 116:499-511

[8] Welis A, Yates C, Shepard CR. E-cadherin as an indicator of mesenchymal to epithelial reverting transitions during the melastatic seeding of disseminsted carcinomas[J]. Clin Exp Metastasis, 2008, 25(6):621-628

[9] Pacifico F, Mauro C, Barone C, et al. Oncogenic and anti-apoptotic activity of NF-kappa B in human thyroid carcinomas [J]. J Biol Chem, 2004, 279(52):54610-54619

[10] Zhang Yang, Li Cai, Zhang Lian-zhu, et al. Expression and clinical significance of MMP-26 and MMP-7 in non-small cell lung cancer [J]. Progress in Modern Biomedicine, 2009,9(18):3471-3473

[11] Dang Shou-qin, Xiang Feng-gang, Zhao Yong-gang, et al. Expression of Bmi-1 in NSCLC and its relationship to clinicopathologic characters[J]. Progress in Modern Biomedicine, 2011, 11(7):1301-1304

[12] Yan Kun, Ji Zong-zheng, Cheng Wei, et al. Study on expression of nm23, EGFR and Bcl-2 in lung neoplasms and their clinical significance[J]. Progress in Modern Biomedicine, 2007, 7(11):1677-1680

[13] Deng Qin-fang, Zhou Cai-cun, Su Chun-xia. Clinicopathologic features and epidermal growth factor receptor mutations associated[J]. Cancer Research on Prevention and Treatment, 2008, 35(4):258-262

[14] Ye Ting, Jiang Wei, Pang Xu-guang, et al. Research progress in epithelial-mesenchymal transition phenomenon in lung cancer [J]. Journal of Fudan University(Medical Sciences) , 2009, 36(3):372-375

[15] Li Qi-zhi, Jiang Yang, Zhang Sheng-ming, et al. Expression implications of P65, c-myc and P73 proteins in non-small cell lung cancer[J]. Chinese Journal of Cancer Prevention and Treatment, 2007, 14(5): 324-327

[16] Li Rui, Zhang Dao-rong, Cai Cun-wei, et al. The clinical signifcance of claudin-7 and slug expression in lung squamous cell carcinoma and adenocarcinoma[J]. Chinese Journal of Lung Cancer,2011,14(6): 492-496

[17] Shi JY, Tsai MF, Chug TH, et al. Transcription repressor slug promotes carcinoma invasion and predicts outcome of patients with lung adenocarcinoma[J]. Clin Cancer Res, 2005, 11(22):8070-8078

[18] Huber MA, Azoitein N, Baumann B, et al. NF-kappa B is essential for epithelial-mesenchymal transition and metastasis in a modal of breast cancer progression[J]. J Clin Invest, 2004, 114(4):569-581

[19] Come C, Magnino F, Bibeau F, et al. Snail and Slug play distinct roles during breast carcinoma progression[J]. Clin Cancer Res, 2006, 12 (18):5395-5402

[20] Wu Y, Deng J, Rvchahou PG, et al. Stabilization of snail by NF-kB is required for inflammation-induced cell migration and invasion[J]. Cancer Cell, 2009, 15(5):416-428

NF-kB 与 Slug 在非小细胞肺癌及其上皮间质转化中的作用

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摘要 目的：研究核因子 NF-kB 与 slug 在非小细胞肺癌（NSCLC）中的表达情况、及二者与非小细胞肺癌上皮间质转化（EMT）的关系，为非小细胞肺癌的诊断治疗提供理论依据。方法：(1) 采用免疫组化 PV9000 二步法测定 50 例 NSCLC 组织及 20 例相应正常肺组织中 NF-kBP65、slug、E-cadherin 及 Vimentin 蛋白表达情况。(2) 采用 RT-PCR 测定其中 25 例 NSCLC 组织及 10 例相应正常肺组织中 NF-kBP65、slug 的 mRNA 表达情况。结果：NSCLC 中 NF-kBP65 蛋白表达量高于癌旁正常肺组织（$Z=-2.370$, $P<0.05$），NF-kBP65mRNA 表达量明显高于癌旁正常肺组织（$t=4.967$, $P<0.01$）；Slug 蛋白表达量明显高于癌旁正常肺组织（$Z=-4.443$, $P<0.01$），SlugmRNA 表达量明显高于癌旁正常肺组织（$t=6.483$, $P<0.01$）。在 NF-kBP65 阳性癌组织中，E-cadherin 蛋白表达下降（$\chi^2=5.024$, $P<0.05$），Vimentin 蛋白表达上升（$\chi^2=4.723$, $P<0.05$）；Slug 阳性癌组织中，E-cadherin 蛋白表达下调（$\chi^2=5.984$, $P<0.05$），Vimentin 表达上调（$\chi^2=5.028$, $P<0.05$）。另外，NF-kBP65 与 Slug 在蛋白水平呈极显著正相关（$r=0.443$, $P<0.01$），在 mRNA 水平呈显著正相关（$r=0.439$, $P<0.05$）。NF-kB 与分化程度（$\chi^2=5.024$, $P<0.05$）、有无淋巴结转移（$\chi^2=7.933$, $P<0.01$）及肿瘤的分期（$\chi^2=5.614$, $P<0.05$）有关，与性别、年龄、组织类型无明显相关性（$P>0.05$）；Slug 与淋巴结转移（$\chi^2=6.174$, $P<0.05$）及肿瘤的分期（$\chi^2=7.317$, $P<0.01$）有关，与性别、年龄、组织类型、分化程度无明显相关性（$P>0.05$）。结论：NF-kB、Slug 在 NSCLC 中表达增强，可能与 NSCLC 的发生、发展、转移有关；并且 NF-kB 与 Slug 可能协同抑制 E-cadherin 表达，促进 Vimentin 表达，诱使 NSCLC 的 EMT 发生，从而为进一步研究 NSCLC 的 EMT 提供理论依据。

关键词：NF-kB；Slug；EMT；RT-PCR；免疫组织化学；NSCLC

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