Experimental study of peripheral-blood pro-surfactant protein B for screening non-small cell lung cancer[1]

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Abstract

Purpose: To evaluate the possibility of using peripheral-blood presurfactant protein B (Pro-SFTPB) for screening non-small cell lung cancer (NSCLC).

Methods: A total of 873 healthy volunteers and 165 lung cancer patients hospitalized in the Fifth People's Hospital of Dalian were tested Pro-SFTPB once every half year from January 2014 to September 2015. The healthy volunteers were also conducted spiral computed tomography (CT) examination once every year. The data were then com-pared and statistically analyzed.

Results: The positive expression rate of Pro-SFTPB in NSCLC was significantly higher than that in healthy volunteers, and significantly higher in lung adenocarcinoma than in squamous cell carcinoma; additionally, the expression rate was increased with the in-crease of smoking index, and the intergroup differences showed statistical signifi-cance ($p \leq 0.05$). The positive rate of newly diagnosed lung cancer was 29.55%, higher than healthy volunteers (22.34%), but there was no significant difference ($p > 0.05$).

Conclusion: Pro-SFTPB is over expressed in non-small cell lung cancer, especially in lung adeno-carcinoma, but it can't be used as a clinical screening tool for lung cancer.

Key words: Non-Small Cell Lung Cancer. Lung Neoplasms. Mass Screening.

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■ Introduction

In recent years, the incidence of lung cancer increases year by year, and it's still the primary cancer that causes death in China. The survival outcome of lung cancer patients depends more on their stages when diagnosed. Early detection and diagnosis is the key of improving the survival rate of lung cancer. Scholars have been various exploring means over years, aiming to improve the five-year survival rate of lung cancer through early diagnosis and treatment. The National Lung Screening Trial (NLST) data show that low-dose chest computed tomography (LDCT) scan for high-risk groups can reduce the total mortality by 20% and 7% in current and pre-smokers, re-spectively[1]. Japanese scholars have further improved the detection rate of pulmonary nodules by combining positron emission tomography (PET) and LDCT[2,3]. But in re-cent years, controversies about the application of LDCT for early lung cancer screen-ing have also emerged. European Journal of Radiology reported that chest CT scans can cause osteoporosis and even spinal fractures[4]. Jubal also proposed CT scan for lung cancer screening may increase cardiovascular diseases, especially can accelerate coronary artery calcification[5]. It has also been reported that LDCT used in lung cancer screening exhibited too many false-positive, overdiagnosed, and treated cases[6,7]; therefore, exploring new effective lung cancer screening method has become a hotspot in medical fields. Some scholars have tried to achieve the purpose of early diagnosis by chromatographically analyzing the exhaled gases and secretions[8]. Studies about detecting blood tumor markers for screening lung cancer have been carried out for many years, but no target markers with high specificity and high sensitivity have been found ever[9-12]. At the same time, if LDCT-lung cancer screening was to applied to Chinese populations, it will bring great economic burden to the country, so exploring a simple and economical blood test screening method is particularly important.

Our previous studies have shown that Pro-SFTPB often presents in the peripheral blood of immaturely developed children and is associated with many chronic lung diseases, such as respiratory distress syndrome (RDS)[13-16], which may cause chronic lung dysfunction in chronic obstructive pulmonary disease (COPD) patients[17]. Mean-while, the abnormal regulation of surfactant protein B in non-small cell lung cancer (NSCLC) may cause its overexpression of Pro-SFTPB. Dr. Ayumu Taguchi, MD, MD Anderson Cancer Center, University of Texas, USA (2013), showed that there is ab-normal SFTPB synthesis and regulation in breast tumors, especially in the adenocarci-noma cells, so Pro-SFTPB is overexpressed, and the modification ability of its pro-somes after being translated toward mature hydrophobicity will also occur mutation. Ayumu found that compared with the matched control, the level of mature SFTPB in the peripheral blood of surgery-treatable lung cancer patients is increased. The results of mass spectrometry screening has shown that the N-terminal of the front peptide of SFTPB can be used as a potential biomarker for NSCLC. Ayumu established and veri-fied the enzyme-linked immunosorbent assay (ELISA) of blood Pro-SFTPB between surgery-treatable NSCLC patients and healthy controls. Preliminary studies have sug-gested that serum Pro-SFTPB levels may be associated with certain lung cancer-independent known clinical risk factors, and high levels of blood Pro-SFTPB may be used to evaluate the lung cancer predictions in high-risk lung cancer patients. Wikoff *et al.*[18] also confirmed that there indeed exists the overexpression of Pro-SFTPB in the peripheral blood of NSCLC patients. This study examined the peripheral blood Pro-SFTPB in healthy volunteers and lung cancer patients, together with chest CT exami-nation, aiming to investigate whether this test indicator can be used as a specific tu-mor marker for the early

screening of lung cancer.

■ Methods

This study was conducted in accordance with the declaration of Helsinki, and approval from the Ethics Committee of 5th Hospital of Dalian. Written informed con-sent was obtained from all participants.

Healthy volunteers and patients

A total of 1000 volunteers were selected from the physical examination center of the Fifth People's Hospital of Dalian from January 2014 to September 2015. Addi-tionally, another 189 pathologically-diagnosed NSCLC patients (pathologically diag-nosed but not performed surgery, radiotherapy, or chemotherapy). A total of 127 healthy volunteers and 24 lung cancer patients were excluded due to not being regu-larly tested or lost. So, the cases that finally completed the experiment included a total of 873 healthy volunteers.

Data of healthy volunteers and patients

532 males and 341 females; 294 cases >65 years old and 579 cases ≤65 years old; 657 cases with smoking index >30 packs/year and 294 cases with smoking index ≤30 packs/year) and a total of 165 patients with lung cancer (including 102 males and 63 females; 97 cases >65 years old and 68 cases ≤65 years old; 116 cases with smoking index >30 packs/year and 216 cases with smoking index ≤30 packs/year (or non-smoking); 128 cases with adenocarcinoma and 37 cases with squamous cell carcinoma) (Table 1).

Table 1 - Data analysis of healthy volunteers and lung cancer patients.

Item	Classification	Healthy volun-teers	Control group
Gender	M	532	102
	F	341	63
Age	> 65	294	97
	≤65	579	68
Smoking index (including passive smoking)	>30 packs/year	657	116
	≤30 packs/year	216	49 (or non-smoking)
Pathology	Adenocarcinoma	-	128
	Squamous cell carcinoma	-	37

All of they were tested Pro-SFTPB in the peripheral blood (once every six months) by ELISA (the Pro-SFTPB ELISA kit is from Shanghai Jingbang Compony, China) and chest spiral CT (once a year) (the CT machine is produced by Siemens Compony of Germany) and followed up for two years.

Inclusion criteria

(1) Smoking, or quit smoking for less than 15 years, smoking index >30 packs/year, including passive smoking (long-term common life partner of a smoker, and the smoking amount was the same as the above); (2) Without a history of lung cancer or other cancers; (3) Volunteered to carry out the test and can cooperate with regular follow-up.

Statistical analysis

The χ^2 test and multivariate correlation analysis were used, and the data pro-cessing was done using SPSS19.0.

■ Results

Positive rate of Pro-SFTPB of healthy volunteers and expression in lung cancer patients

The comparison between the positive rate of Pro-SFTPB in the healthy volun-teers and lung cancer patients showed that the positive rate in lung cancer patients was higher than 50%, which was significantly higher than that in the healthy volun-teers, and the intergroup difference was statistically significant ($p \leq 0.05$). The positive rate in the healthy volunteers did not change, while that in the lung cancer patients showed an increasing trend with the development of lung cancer (Table 2).

Table 2 - Comparative analysis of positive rate of Pro-SFTPB in healthy volunteers and lung cancer patients.

Detection time	Healthy volunteers (%)	Control group (%)	*P*
2014-3	201 (23.02)	108 (65.45)	≤0.05
2014-9	172 (19.70)	97 (58.79)	≤0.05
2015-3	226 (25.89)	122 (73.94)	≤0.05
2015-9	182 (20.85)	116 (70.30)	≤0.05

Relationship of positive rate of Pro-SFTPB with patient features

Through analyzing the detection results of Pro-SFTPB of the two groups, it can be seen that the positive rate of Pro-SFTPB was not related to the age and sex ($p > 0.05$). At the same time, the expression of Pro-SFTPB increased with the increase of smoking index, and the difference was statistically significant ($p \leq 0.05$). The expressions of Pro-SFTPB in lung adenocarcinoma was significantly overexpressed than in SqCa, and the intergroup difference was statistically significant ($p < 0.05$) (Table 3).

Table 3 - Relationship of positive rate of Pro-SFTPB with patient features

Item	Category	Positive rate of Pro-SFTPB (%)	X^2	*P*
Age	>65	120 (30.69)	0.4423	>0.05
	≤65	186 (28.75)		
Sex	M	196 (30.91)	0.8569	>0.05
	F	114 (27.23)		
Smoking index	>30 packs/year	241 (31.18)	4.1966	≤0.05
	≤30 packs/year	65 (24.62)		
Pathology	Adenocarcinoma	20 (53.38)	3.7853	≤0.05
	Squamous cell carcinoma	91 (71.09)		

Positive rate of Pro-SFTPB in healthy volunteers and newly diagnosed lung cancer patients during follow-up

The CT follow-up revealed 6 cases of lung cancer in the first follow-up examina-tion and 5 cases of lung cancer in the second follow-up, and all the patients were pathologically diagnosed by surgery, biopsy, or bronchoscopy.

The 11 newly diag-nosed lung cancer patients were performed a total of 44-time Pro-SFTPB follow-up detection, and the comparison with the healthy volunteers revealed that the positive rate of Pro-SFTPB in these newly diagnosed lung cancer patients was 29.55%, which was higher than the healthy volunteers (22.34%), but no statistical significance was found (p>0.05) (Table 4).

Table 4 - Comparison of positive rates of Pro-SFTPB between newly diagnosed lung cancer patients and healthy volunteers.

Pro-SFTPB	Newly diagnosed lung cancer patients (%)	Healthy volunteers (%)	*P*
Positive rate	13 (29.55)	195 (22.34)	>0.05

■ Discussion

The total five-year survival rate of lung cancer is only 18.7% because of its hid-den symptoms and non-easy early detection[13]. So how to screen and early detect lung cancer has become the focus of medical fields over years. There exists an abnormal expression of SFTPB in lung cancer, especially in the adenocarcinoma cells, so Pro-SFTPB may be overexpressed. The level of blood Pro-SFTPB may be associated with certain lung cancer-independent known clinical risk factors. Therefore, the blood Pro-SFTPB level in individuals with high-risk lung can-cer may be used as a specific marker for the early diagnosis of lung cancer[19].

Other studies[20,21] have suggested that Napsin A is an important pre-gene of Pro-SFTPB. Brasch *et al.*[22] observed by immunoelectron microscopy that Napsin A, SP-B, and Pro-SFTPB are localized on the same position (lamellar body) of type II alveolar epithelial cells. Napsin A is a more specific marker of primary lung adenocarcinoma. Napsin A and free lamellar bodies can lead to the cleavage of three identical Pro-SFTPBs, suggesting

that the expression deletion of Napsin A may increase the aggres-siveness of lung cancer, thereby affecting the prognosis of the disease. Furthermore, the expression deletion of Napsin A-caused the aggressiveness increase of lung cancer is due to its reducing the cleavage of Pro-SFTPB into mature SP-B, so its roles are reduced. Therefore, the increase of Pro-SFTPB in the peripheral blood is associated with the expression decrease of Napsin A. One report in the USA in 2013 pointed out that the incidence rate of high-risk population developing into lung cancer was 3%[18]. The correlations of the mutation and expression of Pro-SFTPB gene with races are also reported[23,24]. Blood Pro-SFTPB tests have achieved initial results in North America, but whether it is applicable to Asian populations, especially for screening lung can-cer in Chinese populations, is our concern. In this study, the 873 healthy volunteers were also the people with high-risk lung cancer, and 2-year follow-up revealed 11 newly diagnosed lung cancer patients, and the incidence rate was 1.26%, which was lower than that reported in the USA, but combined with the mean incidence of lung cancer in Dalian, this incidence is still

higher than general populations by about 15 times and meets the epidemic law of lung cancer in Dalian area.

The results of this study showed that the level of Pro-SFTPB in the peripheral blood of lung cancer patients was significantly higher than the healthy volunteers, and the expression in lung adenocarcinoma was significantly higher than that in squamous cell carcinoma, thus further confirming that there exists abnormal SFTPB synthesis in NSCLC, especially in the adenocarcinoma cells, so Pro-SFTPB will be overexpressed. Matsui *et al.*[25] examined the expressions of surfactant proteins and vascular endotheli-al growth factors in lung cancer patients and healthy people, and found that the above two indicators are clearly overexpressed in lung cancer, and higher in adenocarcinoma than in squamous cell carcinoma. There also exists a positive correlation with lung cancer stages, consistent with the findings of this study.

This study found that the Pro-SFTPB expression was significantly increased with the development of tumor progression, indicating that the overexpression of Pro-SFTPB may be an indirect manifestation of tumor invasion and development, which may provide a new idea for the follow-up observation and efficacy assessment of NSCLC.

Other studies have shown that the occurrence and development of tumors is inseparable from the local hypoxic environment, and too high CO_2 concentration-caused local hypoxic environment can inhibit the synthesis of SFTPC, reduce the cell activity, and increase the apoptotic rate[26], indicating that SFTPC may also be involved in the occurrence and development of tumors. However, the regulation of Pro-SFTPB is affected by cyclooxygenase-2 inhibitors. Cyclooxygenase-2 inhibitors may alleviate the inflammatory response by inhibiting prostaglandins, increase the cleavage of Pro-SFTPB, and increase the amount of

mature SFTPB, thereby relieving lung injury under hyperoxic conditions, as well as reducing the possibility of tissue cell proliferation, which may also be an important mechanism for the occurrence and development of lung cancer, especially adenocarcinoma[27].

In this study, the detection of Pro-SFTPB in newly discovered lung cancer pa-tients revealed that the positive rate was higher than the healthy volunteers, but no statistical difference can be found, indicating that Pro-SFTPB is not the only mecha-nism of lung cancer, and its occurrence and development is also interfered by many other tumor regulatory factors. Meanwhile, due to the small number of the samples enrolled in this study, the number of patients with lung cancer found in follow-up was also less and limited to one medical center of the Fifth People's Hospital in Dalian, which may be likely to cause the error and offset of the results. Therefore, although Pro-SFTPB in the peripheral blood can't be used as an alternative for screening and detecting NSCLC, as an important specific tumor marker of NSCLC, especially lung adenocarcinoma, it can provide more evidence-based medical evidence for the early diagnosis of lung cancer. Our next step is to confirm the possible roles of Pro-SFTPB in screening NSCLC by further enlarging large-sample multi-center prospective studies.

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