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Serological investigation of the clinical significance of fascin in non-small-cell lung cancer

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ABSTRACT

Objectives: Fascin, a conserved actin bundling protein family member, is frequently up-regulated in various cancer types, including non-small-cell lung cancer (NSCLC), and it plays increasingly important roles in the progression of tumor invasion and metastasis. However, variations in the serum fascin level in tumor patients are usually neglected.
Materials and Methods: In the present study, serum samples from 501 stage I-IV NSCLC patients and 109 healthy volunteers were investigated by an enzyme-linked immunosorbent assay.
Results: The serum fascin level was markedly increased in the NSCLC patients ($P<0.05$), particularly in advanced cases ($P<0.01$), compared with that in the healthy controls. We also found that the serum fascin level was significantly correlated with female sex ($P=0.02$), non-smoking status ($P=0.044$), and adenocarcinoma histology ($P<0.001$), with a higher positive rate relative to each counterpart. Furthermore, our results suggested that the serum fascin level reflects the aggressive progress of both lymphatic ($P<0.001$) and distant ($P<0.001$) metastases in NSCLC. A survival analysis of 283 eligible patients who underwent a follow-up examination after 3 years revealed that the patients in the serum fascin-positive group had a significantly lower overall survival rate compared with those in the negative group for 134 non-distant metastatic (stage M0) cases ($P=0.044$). A subsequent Cox regression analysis revealed that the serum fascin level was an independent prognostic factor for M0-stage NSCLC (univariate, $P=0.001$; multivariate, $P=0.038$).
Conclusion: Our study suggests that the serum fascin level is an effective indicator of tumor aggressiveness, and that it plays an important role in the prognosis of NSCLC, particularly for non-distant metastatic patients.

1. Introduction

Lung cancer (LC), one of the leading malignancies worldwide, causes millions of deaths annually. Non-small cell lung cancer (NSCLC), the most frequently occurring category of LC, accounts for approximately 80% of all cases. The incidence is closely related to multiple factors, including tobacco use, radon exposure, sex, and ethnicity. In addition, marked regional variation has been discovered, with most cases occurring in developing countries, including China. Regarding survival expectations, although the overall prognosis for LC remains poor, it varies considerably depending on the clinical stage. As described previously, the 10-year survival rate among surgically resected stage I LC patients is up to 92% [1], whereas the 5-year survival of LC patients ranges from 6 to 18% across different global regions [2].

Metastatic dissemination, one of the most complicated processes during carcinogenesis, is responsible for nearly 90% of cancer deaths. It involves a cascade of cellular events, including local invasion, intravasation, survival in the circulation, extravasation, proliferation, and colonization in distant tissues [3]. Although the molecular mechanism is not fully understood, enhanced cell motility is required. Accumulated data indicate that enhanced cell movement is correlated with greater metastatic potential and reduced overall survival (OS) [4,5]. Various molecules are involved in the regulation of cell motility by influencing the assembly of the actin cytoskeleton.

Fascin, an evolutionarily conserved actin bundling protein, localizes to diverse types of actin-based cortical cell protrusions, including filopodia, invadopodia, and microspikes, and it plays an

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important role in cell adhesion and motility [6–8]. To date, three isoforms of fascin have been identified in mammals: fascin-1, -2, and -3. Fascin-1, which is generally referred to as fascin, is widely distributed during embryonic development, but its expression is tissue- or cell-specific in adulthood, and is highly restricted to protrusive or migrating cells, including neurons, dendritic cells, endothelial cells, and macrophages [9,10]. Fascin is usually absent or present in small amounts in normal epithelial cells, but it is markedly up-regulated in a variety of human carcinomas [11–13], including NSCLC [14–16]. Increasing evidence indicates that, by stabilizing actin bundles, fascin overexpression enhances the potential for invasion and metastasis in transformed cells [7] and is correlated with more aggressive clinical behavior [17,18].

In this study, we first examined fascin expression in circulating serum, instead of in neoplastic tissues as reported previously [14–16], in a retrospective cohort of 501 stage I-IV NSCLC patients compared with 109 age-matched normal volunteers. Subsequent analyses revealed that serum fascin was correlated with advanced NSCLC, regional lymphatic invasion, distant metastasis, and overall prognosis, particularly for non-distant metastatic (M0-stage) NSCLC, implying its potential practical use as an effective indicator of the clinical prognosis of NSCLC.

2. Materials and methods

2.1. Patients and samples

According to the 7th edition of the tumor-node-metastasis (TNM) staging system for lung cancer [19], serum samples in the present study were consecutively collected from 501 cases of primary NSCLC (347 males and 154 females) and 109 healthy volunteers (71 males and 38 females), serving as age-matched normal controls, by the staff of Beijing Chest Hospital Sample Bank (Beijing, China) between January 2008 and December 2011. The median age of the patients was 59.5 years (range: 32–87 years). The NSCLC group included 306 smokers and 195 non-smokers and could be classified into 288 adenocarcinomas and 213 squamous cell carcinomas based on the pathological diagnosis complying with the World Health Organization histological classification system for lung tumors [20]. Detailed clinicopathological data were refined from the medical records and are listed in Table 1. Of the NSCLC cases, 283 eligible patients underwent a follow-up examination after 3 years, with a median survival of 14 months (95% confidence interval [CI]: 12.35–15.65 months); 14 patients were lost to follow-up at the time of analysis.

Serum samples were obtained from the patients at the time of diagnosis; those who had received preoperative adjuvant therapy such as radiotherapy or chemotherapy at enrollment were excluded from the study. Cases lost to follow-up or those who died of non-NSCLC causes were deemed censored data in the survival analysis. Normal volunteers recruited from individuals having an annual physical examination showed no evidence of malignancy. In detail, by centrifugation at 3000 rpm for 15 min at room temperature, clotted serum samples were separated and stored in aliquots at −80 °C until use. This project was approved by the Ethics Committee of Beijing Chest Hospital. Written informed consent was collected from each participant in advance.

2.1.1. Enzyme-linked immunosorbent assay (ELISA)

The serum fascin level was determined using a quantitative sandwich ELISA (USCN Life Science, Houston, TX, USA) according to the manufacturer's instructions. Briefly, 100-μl aliquots of sera diluted 100-fold were added to a pre-coated 96-well microplate at 37 °C for 2 h. After discarding the reaction solution, a subsequent incubation was performed with appropriately diluted detection antibodies at 37 °C for 1 h. Following three washes, secondary antibodies conjugated to horseradish peroxidase were added to each well followed by incubation at 37 °C for 30 min. After five additional washes, a 3,3′,5,5′-tetramethylbenzidine solution was pipetted into each well and incubated at 37 °C for 15 min under light-protected conditions. Finally, a 50-μl aliquot of 2 M sulfuric acid was added to stop the reaction, and the absorbance was measured at 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). Three independent experiments were performed, and each sample was detected in duplicate.

Table 1
Association between serum fascin level and clinicopathological characteristics in NSCLC patients ($n = 501$).

Variables Total cases	No. of cases 501	Fascin positive 96 (19.2%)	Fascin negative 405 (80.8%)	*P*
Age				
≤60	248	45 (18.1%)	203 (81.9%)	0.567
>60	253	51 (20.2%)	202 (79.8%)	
Gender				
Male	347	57 (16.4%)	290 (83.6%)	0.020*
Female	154	39 (25.3%)	115 (74.7%)	
Smoking history				
Smoker	306	50 (16.3%)	256 (83.7%)	0.044*
Non-smoker	195	46 (23.6%)	149 (76.4%)	
Histological subtype				
A	288	73 (25.3%)	215 (74.7%)	<0.001**
S	213	23 (10.8%)	190 (89.2%)	
pT status				
pT1	99	13 (13.1%)	86 (86.9%)	0.001**
pT2	222	32 (14.4%)	190 (85.6%)	
pT3	30	7 (23.3%)	23 (76.7%)	
pT4	150	44 (29.3%)	106 (70.7%)	
pN status				
pN0	200	23 (11.5%)	177 (88.5%)	<0.001**
pN1	30	4 (13.3%)	26 (86.7%)	
pN2	178	35 (19.7%)	143 (80.3%)	
pN3	93	34 (36.6%)	59 (63.4%)	
pM status				
pM0	318	37 (11.6%)	281 (88.4%)	<0.001**
pM1	183	59 (32.2%)	124 (67.8%)	
pTNM stage				
I	103	8 (7.8%)	95 (92.2%)	<0.001**
II	65	5 (7.7%)	60 (92.3%)	
III	149	24 (16.1%)	125 (83.9%)	
IV	184	59 (32.1%)	125 (67.9%)	

* Statistically significant at $P < 0.05$.
** Statistically significant at $P < 0.001$.

2.2. Statistical analysis

The normality of the distribution was assessed by the Kolmogorov–Smirnov test, and the Wilcoxon rank-sum test was used to evaluate differences between the NSCLC and healthy groups. The serum fascin concentrations of the two groups are presented as medians and ranges. The cut-off level of serum fascin was defined as the mean + 2 standard deviations of the healthy group. Associations of categorical variables were evaluated by means of a chi-square (χ^2) test. OS was defined as the time from diagnosis to death or the last follow-up. A survival analysis was performed using the Kaplan–Meier method, and differences in survival rates were calculated *via* the log-rank test. Cox's proportional hazards model was applied to perform univariate and multivariate analyses. The data were analyzed using SPSS version 16.0. $P < 0.05$ was deemed to be statistically significant.

3. Results

3.1. The serum fascin level is closely associated with advanced-stage NSCLC

Our ELISA results revealed that the serum fascin level was markedly increased in the NSCLC group compared with the healthy