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Circulating long non-coding RNA AFAP1-AS1 is a potential diagnostic biomarker for non-small cell lung cancer

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ABSTRACT

Background: Recent studies have indicated that long non-coding RNA actin filament–associated protein 1 antisense RNA 1 (lncRNA AFAP1-AS1) was increased in non–small cell lung cancer and associated with unfavorable patient prognosis. AFAP1-AS1 also participates in promoting invasion and metastasis in non–small cell lung cancer cells. However, the diagnosis value of serum AFAP1-AS1 in non–small cell lung cancer was unclear. In this study, we aimed to explore whether circulating AFAP1-AS1 can be used as a diagnostic biomarker for non–small cell lung cancer.

Method: The serum AFAP1-AS1 expression level in 126 non–small cell lung cancer patients and 60 healthy controls was detected by quantitative real-time polymerase chain reaction (qRT-PCR). The concentrations of serum cyfra21-1 were detected through chemiluminescence method using the Roche Cobas e601. Receiver operating characteristic curve analysis was applied to assess the diagnostic value of serum AFAP1-AS1 and cyfra21-1 in non–small cell lung cancer.

Result: The results demonstrated that AFAP1-AS1 expression level was significantly elevated in non–small cell lung cancer patients compared with that in normal controls ($p = 0.000$). Serum AFAP1-AS1 could be used as molecular marker for distinguishing non–small cell lung cancer patients from healthy people with an area under the curve of 0.759 (95% confidence interval = 0.692–0.826; $p = 0.000$). The combination of FAP1-AS1 and cyfra21-1 showed that the area under the curve was 0.860 (95% confidence interval = 0.808–0.912; $p = 0.000$). Further analysis found that high serum AFAP1-AS1 expression levels correlated with distant metastasis ($p = 0.03$), lymph node metastasis ($p = 0.017$), poor clinical stage ($p = 0.019$), and larger tumor size ($p = 0.015$). Furthermore, AFAP1-AS1 was significantly upregulated in positive distant metastasis group ($p = 0.003$), positive lymph node metastasis ($p = 0.017$), poor clinical stage group ($p = 0.019$), and larger tumor size group ($p = 0.015$).

Conclusion: Serum AFAP1-AS1 could serve as an ideal combined biomarker for the diagnosis of non–small cell lung cancer.

1. Introduction

Lung cancer is one of the common malignant neoplasms, also the number one cause of death in cancer-related deaths all over the world [1,2]. In China, lung cancer is a serious health problem, which was estimated as 730,000 new cases and almost > 610,000 deaths in 2015 [3]. The most common type of lung cancer is non–small cell lung cancer (NSCLC); it occupies > 80% of all lung cancer [4]. Recently, although the development of diagnosis and treatment modalities has become more rapid, the average 5-year survival rate of NSCLC cancer patients is still very low, at 16%. It may be due to the reason that most of the NSCLC patients did not show apparent clinical symptoms until disease progression and lack of efficient detection methods for NSCLC at early stage [5]. Therefore, the study to explore and identify new diagnostic markers with high sensitivity and specificity in early NSCLC patients has important clinical significance.

In recent years, growing evidences indicated that long non-coding RNAs (lncRNAs) played a vital role in tumorigenesis, progression, and prognosis of cancers [6–9]. LncRNAs are commonly considered as RNA molecules with a length > 200 nucleotides and with no protein coding ability [10,11]. Many studies have demonstrated that lncRNAs were dysregulated in various cancers and serve as tumor suppressors or oncogenes through a variety of mechanisms to regulate gene expression [12–16]. In addition, accumulating studies suggested that lncRNAs

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stably exist in blood or even urine, which displayed promising capacity for use as biomarkers in various tumors, including NSCLC [16–20]. However, the classical serum tumor biomarkers such as carcinoembryonic antigen (CEA), cancer antigen 125 (CA125), neuron-specific enolase (NSE), and cyfra21-1 have limited sensitivity and specificity for NSCLC diagnosis, which restricts the clinical application of these tumor markers [21–23]. Therefore, it becomes necessary to develop new alternative biomarkers for the diagnosis of NSCLC.

The actin filament–associated protein 1 antisense RNA 1 (AFAP1-AS1) has been reported to be significantly increased in NSCLC, which was related to unfavorable patient prognosis [24,25]. Besides, AFAP1-AS1 could promote invasion and metastasis in NSCLC cells [25]. These findings imply that AFAP1-AS1 may serve as a new predictive marker for prognosis and diagnosis of NSCLC patients. Thus, in this study, we detected the serum AFAP1-AS1 levels to analyze its diagnostic value and the association with clinicopathological features in NSCLC patients.

2. Materials and methods

2.1. Patients and specimens

Before treatment, all serum samples of 126 patients (69 men and 57 women, mean age: 63.51 ± 12.72 years) with NSCLC were collected based on histopathological examination. In addition, 60 healthy people (33 men and 27 women, mean age: 54.23 ± 10.61 years) were randomly recruited from healthy volunteers without cancer during the same period. The research protocol was approved by the ethics board of Xiangya Hospital of Central South University. Approximately 3 mL of blood sample was gathered from the venous blood of each participant. All sera were isolated using a two-step centrifugation protocol to completely remove the blood cells (2000 g for 5 min at 4 °C; 12,000 g for 5 min at 4 °C). All supernatant plasma was stored in nuclease-free tubes at − 80 °C until use to extract total RNA.

2.2. Total RNA isolation

Total RNA was isolated from serum using the plasma RNA extraction kit (BioTeke, Beijing, China) according to the product manual, and each sample was eluted in 30 μL of nuclease-free water. The absorbance of 260/280 (RNA/DNA) and 260/230 (RNA/protein) was measured using NanoDrop 2000 spectrophotometer to evaluate the quality of RNA. All RNA samples were conserved at − 80 °C until analysis.

2.3. Quantitative real-time polymerase chain reaction

The RNAs were reverse transcribed to complementary DNAs (cDNAs) using PrimeScript RT Reagent Kit (TaKaRa, Dalian, China). The 20 μL RT reaction system comprising 8 μL of total RNA, 4 μL of 5 × PrimeScript Buffer, 0.5 μL of RT Primer Mix, 0.5 μL of PrimeScript RT Enzyme Mix I, and 7 μL of RNase-free water was used for the analysis. The reaction was carried out using the following protocol: 37 °C for 15 min, then 85 °C for 5 s and 4 °C. Subsequently, the cDNA samples were detected by quantitative real-time polymerase chain reaction (qRT-PCR) using SYBR Premix Ex Taq (TaKaRa) in 20 μL reaction mixture at once, which consisted of 10 μL of SYBR® Premix Ex Taq II, 2 μL of RT reaction products, 0.8 μL of forward primers, 0.8 μL of reverse primers, and 6.4 μL of nuclease-free water. The conditions were as set as follows: primary denaturation at 95 °C for 30s, 40 cycles of 95 °C for 5 s, and 60 °C for 30s. Considering the expression of β-actin messenger RNA (mRNA) was proven to be relatively stable in serum, we used β-actin as the standard for normalizing the relative levels of AFAP1-AS1 [26,27]. The relative expression levels of AFAP1-AS1 was computed using the Δcycle threshold (CT) value method, and ΔCt = Ct (AFAP1-AS1) − Ct (β-actin) [18]. Samples with CT values > 40 were treated as negative results. The primers used in this study were as follows: AFAP1-AS1: 5′-AATGGTGGTAGGAGGGAGGA-3′(forward) and AFAP1-AS1: 5′-CACACAGGGGAATGAA GAGG-3′ (reverse); β-actin: 5′-AAGCCACCCCACTTCTCTCTAA-3′ (forward) and β-actin: 5′-AATGCTATCACCT CCCCTGTGT-3′(reverse). The concentrations of serum cyfra21-1 were detected through chemiluminescence method using the Roche Cobas e601 (Roche, Switzerland), and the cutoff value was 3.32 ng/mL.

2.4. Statistical analysis

The SPSS 18.0 software package and GraphPad Prism 5.0 software were used to perform all the statistical analyses and generate graphs. The analyses between NSCLC patients and healthy individuals were evaluated using Mann–Whitney *U* test. The connection between the expression of AFAP1-AS1 in serum and clinicopathological features was assessed using Pearson's chi-square test and Mann–Whitney *U* test. The diagnostic values were evaluated using receiver operating characteristic (ROC) curves. All p values < 0.05 were regarded as statistical significance.

3. Results

3.1. The expression level of AFAP1-AS1 in serum of NSCLC patients

To investigate whether serum AFAP1-AS1 levels can be used as potential circulating biomarkers, we first detected the relative expression levels of AFAP1-AS1 in 126 NSCLC patients and 60 normal controls using RT-PCR. The result showed plasma AFAP1-AS1 levels were distinctly increased in patients compared with those in normal controls (p = 0.000; Fig. 1).

3.2. Potential diagnostic value of AFAP1-AS1 in the diagnosis of NSCLC

To examine the diagnostic potential of AFAP1-AS1, 126 NSCLC patient serum samples and 60 healthy volunteer serum samples were analyzed. The ROC curve analysis was utilized to evaluate the predictive capability of AFAP1-AS1. The result showed that serum AFAP1-AS1 levels are promising to be a biomarker for the diagnosis of NSCLC. The area under the ROC curve (AUC) was 0.759 (95% confidence interval (CI) = 0.692–0.826; Fig. 2). It indicates that serum AFAP1-AS1 level shows a moderate diagnostic accuracy. Generally, serum cyfra21-1 level is commonly used to screen NSCLC [22,28,29]. Thence, we compared the predictive value for the AFAP1-AS1 with cyfra21–1. The concentrations of cyfra21-1 were detected in the same plasma specimens and found that the AUC value of cyfra21-1 is 0.777 (95% CI = 0.705–0.850; Fig. 2). Subsequently, we also explored the diagnostic value of combination of serum AFAP1-AS1 and cyfra21-1. The result showed that predictive ability of the combination (AFAP1-

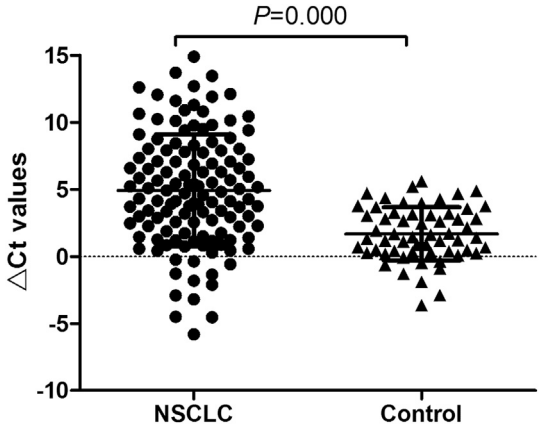

Fig. 1. The expression levels of lncRNA AFAP1-AS1 in serum. AFAP1-AS1 was detected by QRT-PCR in NSCLC patients (n = 126) and healthy controls (n = 60).