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Tumor-derived exosomal proteins as diagnostic biomarkers in non-small cell lung cancer

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Accumulating evidence supports a role for exosomal protein in diagnosis. The purpose of this study was to identify the tumor-derived exosomal biomarkers in the serum that improve the diagnostic value in Chinese non-small cell lung cancer (NSCLC) patients. Serum exosomes were isolated from healthy donors (n = 46) and NSCLC patients (n = 125) by ultracentrifugation and were characterized using transmission electron microscopy, qNano, and immunoblotting. Proteomic profiles (by mass spectrometry) revealed multiple differentially expressed proteins in the healthy and NSCLC groups. The exosomal expression levels of alpha-2-HS-glycoprotein (AHSG) and extracellular matrix protein 1 (ECM1) increased significantly in the NSCLC patients compared to the healthy group. Alpha-2-HS-glycoprotein showed diagnostic values with a maximum area under the receiver operating characteristic curve (AUC) as 0.736 for NSCLC vs healthy individuals (P < .0001) and 0.682 for early stage NSCLC vs healthy individuals (P < .01). Extracellular matrix protein 1 showed the diagnostic capacity with AUC values of 0.683 (P < .001) and 0.656 (P < .05) in cancer and early stage NSCLC compared to healthy individuals. When AHSG was combined with ECM1, the AUCs were 0.795 and 0.739 in NSCLC and early stage patients, respectively. Taken together, the combination of AHSG, ECM1, and carcinoembryonic antigen improved the diagnostic potential of NSCLC. The diagnosis values were AUC of 0.938 for NSCLC and 0.911 for early stage NSCLC vs healthy individuals. Our results suggest that novel proteomic signatures found in serum exosomes of NSCLC patients show potential usefulness as diagnostic tools.

KEYWORDS
alpha-2-HS-glycoprotein, diagnostic marker, extracellular matrix protein 1, non-small cell lung cancer, tumor-derived exosomes

1 | INTRODUCTION

Non-small cell lung cancer (NSCLC) is the most common type that accounts for approximately 80% of all lung cancer[1] and is yet a global challenge due to the high morbidity and mortality. The major determinant of prognosis and treatment in patients with NSCLC is the disease stage, as defined by the International Association for the Study of Lung Cancer,[2] and detecting lung cancer at earlier stages could reduce the rate of mortality by 10- to 50-fold.[3] Despite continuous improvements in tissue biopsy and imageology examination, this disease is often diagnosed at an advanced stage, and approximately two-thirds of patients present with metastatic tumors at the time of diagnosis. The invasive tissue biopsy neither allows serial sampling, nor multiple biopsy attempts to assess the intratumor heterogeneity.

TABLE 1 Demographic and clinical features of healthy controls and patients with non-small cell cancer (NSCLC)

Categories	N	Age (years)	Male (n)	Female (n)
Healthy donors	46	54.5	5	41
NSCLC	125	60	80	45
I-IIA	35	59	18	17
IIB-IV	90	60	62	28

Median is indicated for age (years).

Imageology examination, such as low-dose computed tomography, yields conflicting results, although it is capable of detecting tumors at early stages.[4,5] Thus, developing new minimally invasive but reliable methods, such as circulating biomarkers, for the early detection of NSCLC is essential in order capture the molecular diversity of the disease, and the ease of serial testing facilitates the monitoring of its spatial and temporal progression.

Exosomes, 30-150 nm extracellular nanovesicles released by several different cell types, including cancer cells, and present in many bodily fluids such as blood, can be considered as potential circulating biomarkers for tumor diagnosis and prognosis.[6] The implications of these exosomes have been reported in different functionalities of the tumor such as growth,[7] metastasis,[8] immunomodulation,[9] and treatment.[10] Tumor-derived exosomes, released from the endosomal compartments of tumor cells, capture vital information from within the cell. Furthermore, the exosomes are found within circulation, allowing for minimally invasive isolation. They contain specific molecules that can provide information about the parent cell as well as the probable target cells, and can protect the information-carrying molecules from degradation, thereby rendering them advantageous for use as biomarkers. One of the most attractive aspects of exosome research is the discovery of novel biomarkers for the early and improved detection of lung cancer.[11]

The exosomal proteins possess unique features over conventional serological markers, have a higher sensitivity compared to the proteins directly detected in blood, as well as a higher specificity over secretory proteins.[12] In addition, exosomal proteins are highly stable and protected from external proteases and other enzymes by the lipid bilayer; also, the phosphorylation proteins can be separated from the frozen exosomal samples for up to 5 years.[13] A previous study provided evidence that serum exosomes contain specific proteins with potential diagnostic and prognostic value for cholangiocarcinomas.[14] Therefore, exosomal proteins could be considered as novel diagnostic and prognostic indicators for a variety of cancers as they carry the cargo that reflects the genetic or signaling alterations in cancer cells of origin.

In the present study, we hypothesize that exosomes from different sources contain different protein levels. Therefore, the evaluation of the differential expression of proteins in exosomes derived from NSCLC with different degrees of malignancy identified by microarray identified exosomal alpha-2-HS-glycoprotein (AHSG) and extracellular matrix protein 1 (ECM1) with significant differential expression in different groups. Alpha-2-HS-glycoprotein can promote breast cancer progression[15] and Lewis lung carcinoma

tumorigenesis[16]; it also positively associated with the risk of colorectal cancer.[17] Extracellular matrix protein 1 is found to promote progression and invasion in most cancers and identified as an indicator of increased metastasis and poor prognosis.[18,19] Thus, the detection of their expression levels in exosomes separated from the serum of NSCLC patients and healthy individuals prompted further exploration of the value of exosomal AHSG and ECM1 in cancer diagnosis.

2 | MATERIALS AND METHODS

2.1 | Patients and clinical samples

A total of 125 patients with NSCLC and 46 healthy donors were enrolled in this study at the Shandong Cancer Hospital Affiliated to Shandong University (Jinan, China) from February 2017 to October 2017. All patients signed written consent to use their samples for medical research. The characteristics of the samples are listed in Table 1. Non-small cell lung cancer was diagnosed by the combination of clinical, pathological, and radiological approaches, and the tumor stage was determined according to the 8th edition of the lung cancer TNM staging standards formulated by the International Association for the Study of Lung Cancer. None of the patients underwent any anticancer treatment or have any other endocrine, immune, or metabolic diseases; the healthy donors did not have any other tumor disease.
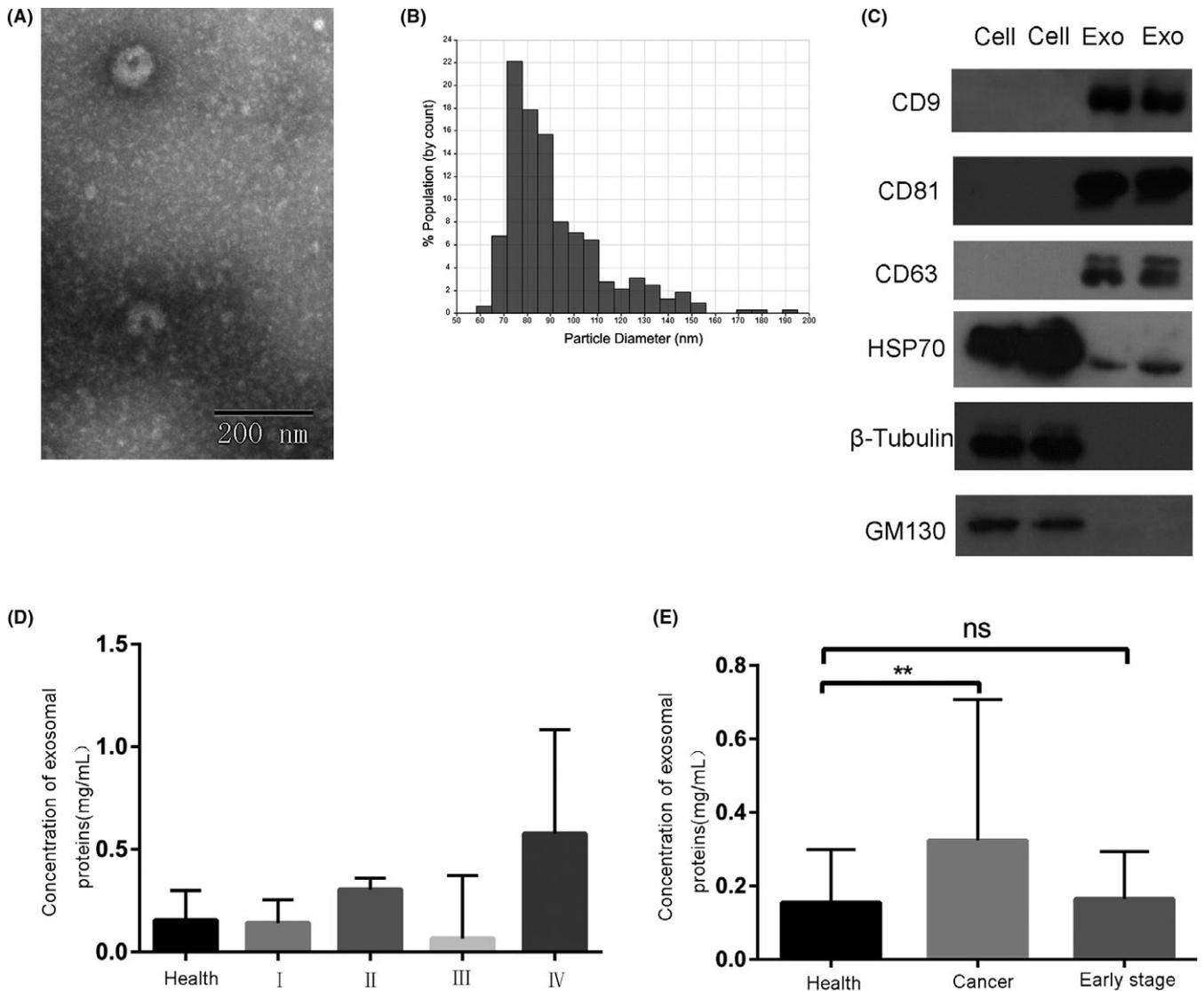
2.2 | Isolation of exosomes from serum

All eligible blood samples were coagulated at room temperature and centrifuged at 3000 g for 10 minutes. The serum was centrifuged at 3000 g for 10 minutes at 4°C, followed by 10 000 g for 30 minutes at 4°C to exclude the cell debris. Then 1 mL supernatant was ultracentrifuged (Class H, R, and S Preparative Ultracentrifuges, Type 50.4 Ti Rotor; Beckman Coulter, Brea, CA, USA) at 100 000 g for 2 hours at 4°C to pellet. The pellets were washed with PBS that was filtered through a 0.22-μm pore filter, followed by a second step ultracentrifugation at 100 000 g for 2 hours at 4°C to pellet the exosomes. The pellets of exosomes were resuspended in 100 μL PBS for the analysis of exosomes or 100 μL lysis buffer (Beyotime, Shanghai, China) for protein quantification.

2.3 | Transmission electron microscopy (TEM)

Exosomes (5 μL) were placed on 100 mesh formvar-coated copper grids (kaifeng, China) for 3 minutes. Phosphotungstic acid was prepared into a solution of approximately 1% with phosphate buffer, and a drop of the staining solution was dropped on the copper mesh of the sample with a dropper. After 1-2 minutes, the staining solution was removed by filter paper and the grid was air dried for 5-10 minutes. The TEM images were obtained under an electron microscope at 80 kV.

2.4 | Nanoparticle tracking analysis of exosomes

The measurement of size and distribution was based on tunable resistive pulse sensing and carried out using a qNano Gold system

FIGURE 1 Comparative analysis of serum exosomes from non-small cell lung cancer (NSCLC) and healthy individuals. The validation of the protocol for isolating serum exosomes was carried out in blood serum from NSCLC. A, Electronmicroscopic observation of exosomes purified from blood serum of NSCLC. Scale bar = 200 nm. B, qNano analysis of blood serum exosomes of NSCLC showing the typical exosomes-round size (~80 nm). C, Representative immunoblots indicated that the exosomes (Exo) isolated from blood serum of NSCLC present enrichment of the exosomes markers CD9, CD81, and CD63 but do not contain cellular marker protein β-tubulin and GM130 (negative control) compared to the whole cell extract (control). D, Concentration of serum exosomes in NSCLC patients and healthy donors. E, Concentration of serum exosomes in healthy donors (n = 46), NSCLC patients (n = 125), and patients with early stage NSCLC (n = 35). **$P < .01$

(Izon Science Ltd, Christchurch, New Zealand), which combined the tunable nanopores with proprietary data capture and analysis software (Izon Control Suite version 3.3.2.2001; Izon Science).

2.5 | Protein concentration assay

The protein concentration was determined using the Micro BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL, USA) according to the manual instructions. Consequently, the total proteins of exosomes were estimated using a fluorescent microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.6 | Serum biomarker measurements

The level of carcinoembryonic antigen (CEA) was determined by electrochemiluminescent immunoassay using the Roche Cobas E602 chemical luminescence immunity analyzer with CEA kits (Roche Diagnostics, Mannheim, Germany).

2.7 | Immunoblotting

The expression of the exosomal markers (CD9, CD81, CD63, and HSP70) and target proteins (ECM1 and AHSG) were analyzed by

immunoblotting. The protein extracts were separated using 10% SDS-PAGE and transferred onto a PVDF membrane (Millipore, Billerica, MA, USA). The membranes were blocked with 5% evaporated skimmed milk in TBS (50 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl) containing 0.1% Tween-20 for 1 hour, and probed overnight at 4°C with the appropriate primary Ab, followed by incubation with HRP-coupled secondary Ab for 1 hour at room temperature. Furthermore, the protein bands were visualized on photographic film using ECL blotting detection reagents (P0018; Beyotime).

2.8 | Enzyme-linked immunosorbent assay

Blood serum from patients with NSCLC and healthy individuals was analyzed using a Human AHSG ELISA Kit (KE00097; Proteintech, Rosemont, IL, USA) and Human ECM-1 ELISA Kit (ELH-ECM1; RayBiotech, Atlanta, GA, USA) according to the manufacturer's instructions.

2.9 | Statistical analysis

GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA) and SPSS 22.0 software (IBM, Ehningen, Germany) were used for statistical analyses. Data are shown as median with interquartile range. The differences between two groups were assessed by Mann–Whitney tests. The serum concentrations of the tested biomarkers with the best predictive value to discriminate between patients with NSCLC and healthy donors were determined using AUC with SPSS 22.0 software based on the sensitivity and specificity. All presented P values are two-sided and $P < 0.05$ was considered to be statistically significant.

3 | RESULTS

3.1 | Characterization of serum exosomes from NSCLC and healthy individuals

As the first step, the exosomes isolated from NSCLC patients and healthy donors were characterized using TEM, nanoparticle tracking analysis, and immunoblotting. These nanovesicles showed the typical exosome-like round morphology by TEM (Figure 1A). The qNano analysis of these exosomes indicated that the vesicles were approximately 80 nm in diameter (Figure 1B). In addition, a high level of CD9, CD81, and CD63, the exosomal protein markers, was detected in exosomes but not in the whole cell extract, whereas 50 kDa β-tubulin and 130 kDa GM130 (negative control) was only observed in the cell lysates but not in the isolated serum exosomes (Figure 1C).

The protein content was determined using the BCA protein assay kit, and the correlation with clinical data was analyzed statistically. The characteristics of the samples are listed in Table 2. As shown in Figure 1D, the median concentration of total protein in healthy individuals was 0.154 (range, 0-0.775) mg/mL, whereas that in patients with stage I, II, III, and IV NSCLC was 0.142 (range, 0-0.498), 0.304 (range, 0-0.514), 0.066 (range, 0.009-1.118), and 0.575 (range, 0-2.708) mg/mL, respectively. As shown in Figure 1E, the concentration of total protein from exosomes in patients with NSCLC was

TABLE 2 Characteristics of exosomal total protein expression in patients with non-small cell lung cancer

| Characteristic	No. of cases	Median (mg/mL)	P value
Age, years			
34-60	65	0.284	.229
61-84	60	0.381	
Sex			
Male	80	0.339	.673
Female	45	0.289	
Pathology diagnosis			
AC	83	0.321	.623
SCC	30	0.299	
Unknown	12		
Smoking history			
Smoker	64	0.339	.664
Non-smoker	61	0.304	
TNM staging			
I-IIA	35	0.164	<.0001
IIB-IV	90	0.435	

AC, adenocarcinoma; SCC, squamous cell carcinoma.

0.324 mg/mL, which was significantly higher than that of healthy individuals ($P = .0035$). However, no significant differences were observed between healthy donors and early stage (stage I-IIA) NSCLC.[20] The total protein concentration from exosomes in patients with metastatic NSCLC was significantly higher compared to those with nonmetastatic NSCLC ($P < .0001$), as described previously.

3.2 | Alpha-2-HS-glycoprotein and ECM1 in serum exosomes can serve as diagnostic markers for NSCLC

The results of proteomic profiling for NSCLC have been reported previously.[21] Compared to healthy donors, NSCLC induced 43 differentially expressed proteins: 34 upregulated and 9 downregulated. Based on the quantitative results, the number of proteins of interest was less, and functional studies were carried out for the target candidates. Thus, the proteins with remarkable changes in expression, such as AHSG and ECM1, were under intensive focus.

To further verify this result, the samples including NSCLC and healthy donors were analyzed using western blotting in an independent patient cohort, which confirmed the presence of exosomal markers CD81 and HSP70 (Figure 2A). The results revealed that patients showed high expression levels of AHSG and ECM1 compared to healthy donors (Figure 2A). Thus, samples from 168 individuals, consisting of 122 cancer patients and 46 healthy individuals, were subjected to ELISA to evaluate the expression of AHSG. The characteristics of the sample are listed in Table 3. As shown in Figure 2B, the median concentration of AHSG in healthy individuals was 0 (range, 0-97.497) ng/mL; the median concentrations of AHSG in patients with stage I, II, III, and IV NSCLC were 4.683 (range, 0-83.910), 4.224 (range, 0-77.369), 44.644 (range, 0-368.653), and 12.911 (range, 0-379.553) ng/mL,
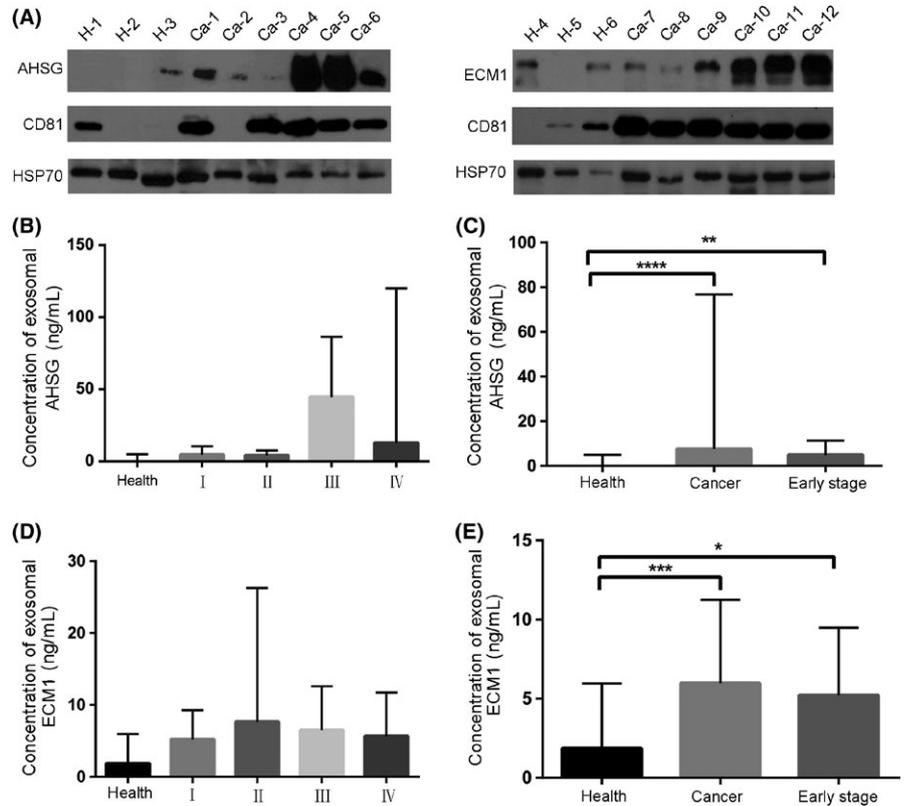
FIGURE 2 Alpha-2-HS-glycoprotein (AHSG) and extracellular matrix protein 1 (ECM1) as non-invasive biomarkers for non-small-cell lung cancer (NSCLC). A, The expression level of AHSG and ECM1 detected by Western blotting in NSCLC and healthy individuals. B, The concentration of exosomal AHSG in NSCLC patients and healthy donors. C, The concentration of exosomal AHSG in healthy donors (n = 46), NSCLC patients (n = 122), early stage of NSCLC (n = 31). **P < 0.01, ****P < 0.0001. D, The concentration of exosomal ECM1 in NSCLC patients and healthy donors. E, The concentration of exosomal ECM1 in healthy donors (n = 46), NSCLC patients (n = 109), early stage of NSCLC (n = 28). *P < 0.05, ***P < 0.001

respectively. As shown in Figure 2C, the level of exosomal AHSG was elevated significantly as detected in sera from patients with NSCLC (P < .0001) or early stage NSCLC (P = .0039) compared to that from healthy individuals. The content of ECM1 was also measured using ELISA in the symptomatic cohort comprising of 109 patients and 46 healthy individuals. The sample characteristics were listed in Table 4. As shown in Figure 2D, the median concentration of ECM1 in healthy individuals was 1.854 (range, 0-21.509) ng/mL, whereas in patients with stage I, II, III, and IV NSCLC the median concentrations were 5.217 (range, 0-23.998), 7.664 (range, 0-49.552), 6.511 (range, 0-37.371), and 5.654 (range, 0-47.738) ng/mL, respectively. Figure 2E shows significant differences in the level of ECM1 between NSCLC patients and healthy individuals (P = .0003), as well as between healthy donors and early stage NSCLC (P = .0239). The results provide evidence that ECM1 harbored the potential to diagnose NSCLC.

3.3 | Receiver operating characteristic curve analysis of biomarkers

To determine the diagnostic capacity of the selected biomarkers, AHSG and ECM1, we calculated the AUC. In serum exosomes from NSCLC, AHSG presented the diagnostic capacity with AUC 0.736 (95% confidence interval [CI], 0.659-0.812), sensitivity 54.9%, and specificity 84.8% compared to the healthy group (Figure 3A). When comparing the patients with early stage NSCLC to healthy donors, the ROC curves showed that AHSG revealed a classifier with an AUC of 0.682 (95% CI, 0.559-0.806) with 64.5% and 69.6% sensitivity and specificity, respectively (Figure 3B). The diagnostic ability of ECM1 protein was also evaluated. The results showed that the AUC was 0.683 (95% CI, 0.588-0.777) with 77.1% sensitivity and 58.7% specificity in cancer and healthy individuals (Figure 3C). When comparing the patients with early stage NSCLC to healthy donors, the ROC

TABLE 3 Characteristics of patients with non-small cell lung cancer for different expression of exosomal alpha-2-HS-glycoprotein proteins

Characteristic	No. of cases	Median (ng/mL)	P value
Age, years			
34-60	63	6.493	.136
61-84	59	15.782	
Sex			
Male	80	7.586	.503
Female	42	7.619	
Pathology diagnosis			
AC	79	7.40	.520
SCC	30	9.404	
Unknown	13		
Smoking history			
Smoker	63	7.771	.605
Non-smoker	59	7.618	
TNM staging			
I-IIA	31	4.952	.017
IIB-IV	91	14.707	

AC, adenocarcinoma; SCC, squamous cell carcinoma.

TABLE 4 Characteristics of non-small cell lung cancer patients for different expression of exosomal extracellular matrix protein 1 proteins

Characteristic	No. of cases	Median (ng/mL)	P value
Age, years			
34-60	56	5.741	.239
61-84	53	6.823	
Sex			
Male	69	6.022	.651
Female	40	5.932	
Pathology diagnosis			
AC	70	5.628	.415
SCC	29	6.823	
Unknown	10		
Smoking history			
Smoker	56	6.956	.111
Non-smoker	53	4.373	
TNM staging			
I-IIA	28	5.217	.390
IIB-IV	81	5.986	

AC, adenocarcinoma; SCC, squamous cell carcinoma.

curves indicated that exosomal ECM1 proteins had an AUC of 0.656 (95% CI, 0.528-0.785) with 75% sensitivity and 56.6% specificity (Figure 3D).

In order to present the better diagnostic capacity, we combined the two new biomarkers. The diagnostic capacity was elevated when comparing cancer and healthy individuals with AUC 0.795 (95% CI, 0.721-0.869), showing sensitivity and specificity of 53.8% and 89.1%, respectively (Figure 3E). When comparing the patients with early stage NSCLC to healthy donors, the ROC curves showed that the AUC was 0.739 (95% CI, 0.621-0.858) with a sensitivity of 87.5% and specificity of 54.3%, which improved the diagnostic capacity (Figure 3F).

3.4 | Improved diagnostic capacity using combined exosomal proteins and existing tumor markers in NSCLC patients

We combined AHSG with CEA (a non-specific serum tumor biomarker commonly used in the diagnostic process of NSCLC) for the diagnosis of NSCLC. The AUC was 0.925 (95% CI, 0.881-0.969) with a sensitivity of 79.2% and a specificity of 93.7%, higher than that for CEA alone (AUC = 0.854, with a sensitivity of 0.656 and a specificity of 0.906 (Figure 4A). Consecutively, we found that the combination of AHSG and CEA could greatly improve the diagnostic efficiency of early-stage NSCLC (AUC = 0.898, 95% CI: 0.810-0.986), and the optimal sensitivity and specificity were 81% and 87.5%, respectively (Figure 4B). However, CEA had an AUC of 0.776 with a sensitivity of 85.7% and a specificity of 59.4%. In addition, the diagnostic ability of ECM1 was observed

in combination with CEA. In NSCLC and healthy donors, the AUC value was 0.907 (95% CI, 0.853-0.961) with a sensitivity of 83.3% and a specificity of 87.5% (Figure 4C), higher than that for CEA alone. According to these data, the AUC of ECM1 in combination with CEA was 0.845 (95% CI, 0.744-0.946) when comparing early stage NSCLC vs healthy donors, and the sensitivity and specificity were 95.2% and 59.4%, respectively (Figure 4D).

In order to attain a better diagnostic capacity, we combined AHSG and ECM1 with CEA. The ability to diagnose NSCLC was improved, with an AUC value of 0.938 (95% CI, 0.899-0.978), higher than either alone, and the optimal sensitivity and specificity were 90.6% and 84.4%, respectively (Figure 4E). Furthermore, we evaluated the diagnostic value of the combination of the three biomarkers for early stage NSCLC patients. The AUC was 0.911 (95% CI, 0.836-0.986) with 85.7% sensitivity and 84.4% specificity (Figure 4F).

4 | DISCUSSION

Despite continuous improvements in treatment, NSCLC patients remain extremely vulnerable to relapse and mortality. Thus, detecting lung cancer at earlier stages could reduce the mortality rates significantly, and hence, new early diagnostic biomarkers are an urgent requisite for NSCLC. Recent studies have suggested that exosomes could serve as cancer biomarkers, as they carry several validated and surrogate noninvasive biomarkers with diagnostic, prognostic, and predictive value.[22] However, the use of exosomes as diagnostic markers has been evaluated in only a few studies focused on extracellular vesicles or exosomal microRNA; some other studies have previously referred to the diagnostic potential of exosomal proteins in NSCLC.[23]

Given the potential diagnostic role of exosomes in NSCLC, we prospectively isolated and characterized the exosomes from the serum of healthy donors and patients with different clinical stages of NSCLC. The size and number distribution of exosomes were quantified by qNano analysis and did not differ based on the clinical stage. Conversely, the exosomal protein concentrations were higher in NSCLC patients compared to healthy individuals. In addition, high levels of total protein expression in exosomes were associated with tumor metastasis. Thus, the exosomal proteins might reflect the pathological processes in NSCLC. Hence, the current data suggested that exosomal proteins could act as key contributors to biomarker diagnostic potential in NSCLC.

In previous studies, we screened the proteomic profiles in serum-derived exosomes coupled to tandem mass spectrometry analysis. The bioinformatic analysis revealed that the quantifiable proteins were involved in multiple biological functions and metastasis-related pathways. Furthermore, an in-depth analysis of the mass spectrometry data revealed the differential expression of proteins AHSG and ECM1; these were selected to determine the difference between NSCLC patients and healthy donors. Alpha-2-HS-glycoprotein
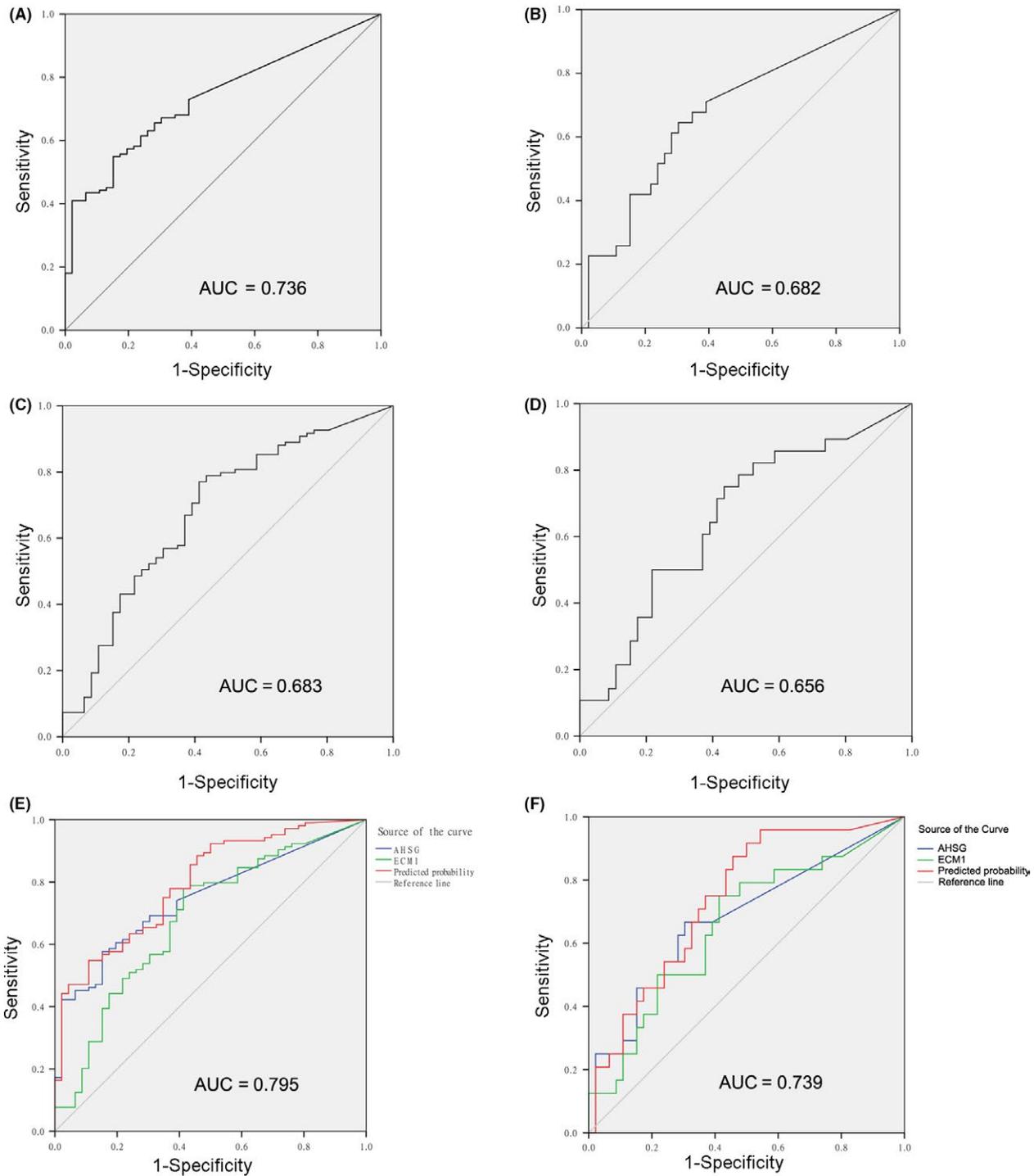
FIGURE 3 Diagnostic value of exosomal alpha-2-HS-glycoprotein (AHSG) and extracellular matrix protein 1 (ECM1) for non-small cell lung cancer (NSCLC). A,B, Diagnostic value of exosomal AHSG protein. Receiver operating characteristic (ROC) curves for distinguishing NSCLC patients from healthy donors (A) and early stage NSCLC patients from healthy donors (B). C,D, Diagnostic value of exosomal ECM1 protein. ROC curves for distinguishing NSCLC patients from healthy donors (C) and early stage NSCLC patients from healthy donors (D). E,F, Combination of AHSG and ECM1 to discriminate all NSCLC patients. ROC curves for distinguishing NSCLC patients from healthy donors (E) and early stage NSCLC patients from healthy donors (F)

shows a positive association with the risk of colorectal cancer,[17] whereas ECM1 is identified as an indicator of increased metastasis and poor prognosis.[18] In the current study, we proposed several lines of evidence to validate that AHSG and ECM1 served as

potential biomarkers for NSCLC. First, the level of AHSG and ECM1 in exosomes differed significantly between patients with early stage NSCLC and healthy donors. The analysis of the expression of exosomal AHSG and ECM1 showed that both could distinguish patients
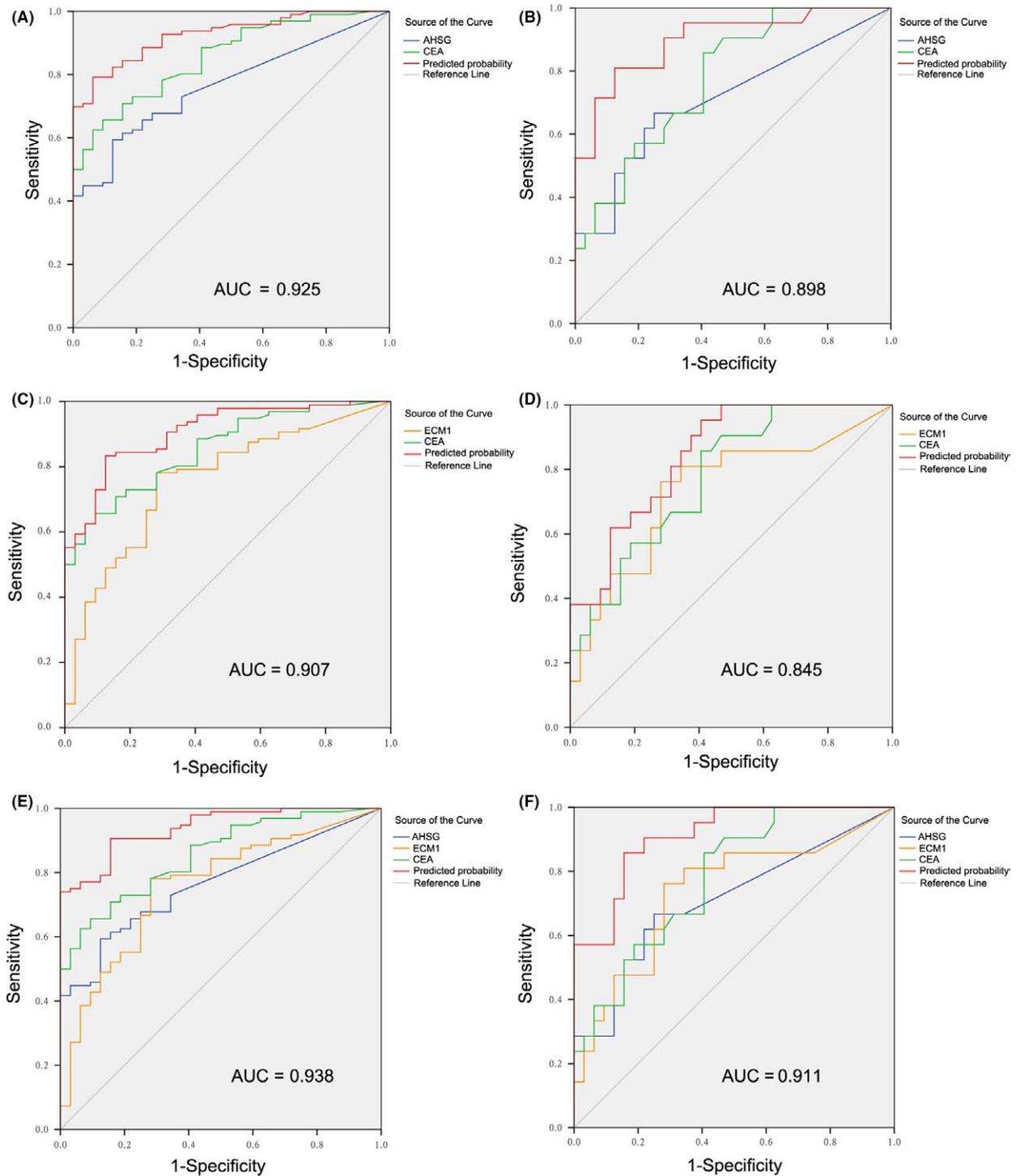
FIGURE 4 Diagnostic value of the combination of exosomal alpha-2-HS-glycoprotein (AHSG), extracellular matrix protein 1 (ECM1), and serum carcinoembryonic antigen (CEA) for non-small cell lung cancer (NSCLC). A,B, Combination of AHSG and CEA to diagnose NSCLC. Receiver operating characteristic (ROC) curves for distinguishing NSCLC patients from healthy donors (A) and early stage NSCLC patients from healthy donors (B). C,D, Combination of ECM1 and CEA to diagnose NSCLC. ROC curves for distinguishing NSCLC patients from healthy donors (C) and early stage NSCLC patients from healthy donors (D). E,F, Combination of AHSG, ECM1, and CEA to diagnose NSCLC. ROC curves for distinguishing NSCLC patients from healthy donors (E) and early stage NSCLC patients from healthy donors (F)

with NSCLC from healthy donors. Furthermore, the ROC curve analysis showed that AHSG with ECM1 improved the diagnostic potential, with AUC values of 0.795 when comparing cancer patients and healthy individuals. Finally, the expression of AHSG protein was also found to be associated with metastatic NSCLC, indicating its potential role in metastasis prediction.

The common molecular biomarkers for lung cancer diagnosis, such as CEA, are extensively applied in clinical settings. The level

of serum CEA was higher in patients with lung cancer than those with benign lung diseases; however, the specificity of CEA is low, with 61.9% of NSCLC patients detected with abnormal CEA serum levels.[24-26] As a biomarker, CEA is more accurate in late-stage disease than in early stage disease. In the present study, we propose that the combination of AHSG and CEA provides a more accurate diagnosis than CEA alone. Similarly, the combination of ECM1 with CEA also increased the diagnostic capabilities. The diagnostic ability of AHSG and ECM1 combined with CEA was higher than either of them alone. The AUC values were 0.938 or 0.911 for cancer and early stage NSCLC, respectively. These results suggest that AHSG and ECM1 can improve the diagnostic efficiency of CEA.

In conclusion, this study provides evidence that serum exosomes contain specific proteins with potential diagnostic and prognostic value for NSCLC, thereby opening new opportunities for their potential use as novel noninvasive tools. Non-small cell lung cancer-derived exosomes can be found in the serum of patients and carry oncogenic proteins that participate in the progression and dissemination of NSCLC. Thus, the manipulation of tumor-derived exosomes could represent a potential therapeutic strategy that deserves further investigation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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