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Plasma SNORD42B and SNORD111 as potential biomarkers for early diagnosis of non-small cell lung cancer

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Abstract

Background: Non-small-cell lung cancer (NSCLC) still occupied the leading reason of cancer death due to lack of availability of early detection. This study aimed to identify the effective biomarkers for the early-stage NSCLC diagnostics based on plasma snoRNAs.

Materials and Methods: The differential snoRNAs between lung cancer patients and healthy donors were analyzed using the SNORic and TCGA databases. SNORD42B and SNORD111 were screened out and further verified in 48 FFPE NSCLC and adjacent normal tissues, as well as in plasma from 165 NSCLC patients and 118 health donors using qRT-PCR. Next, their diagnostic efficiency, as well as combined with carcinoembryonic antigen (CEA), was obtained by the analysis of receiver operating characteristic (ROC).

Results: We first screened out 47 top differential snoRNAs, among which the top 10 upregulated snoRNAs in LUAD were U44, U75, U78, U77, SNORD72, SNORD13, SNORD12B, SCARNA5, U80, SNORD41, and in LUSC were U44, U75, U78, SNORD41, SNORD111, SNORA56, U17a, SNORD35A, SNORD32A, SNORA71D. SNORD42B and SNORD111 was significantly increased not only in tumor tissues but also in plasma from NSCLC and early-stage NSCLC patients. They were capable to act as promising biomarkers for NSCLC and early-stage NSCLC diagnosis. Moreover, CEA diagnostic efficiency for early-stage NSCLC was significantly improved when combined with these two plasma snoRNAs.

Conclusion: SNORD42B and SNORD111 could act as the potential and non-invasive diagnostic biomarkers for NSCLC and early-stage NSCLC.

KEYWORDS
biomarker, diagnosis, NSCLC, SNORD111, SNORD42B, SnoRNAs

1 | INTRODUCTION

Non-small-cell lung cancer (NSCLC), the most common type of lung cancer accounting for about 80%–85% of all lung cancer, remains the leading cause of cancer death among men and women worldwide largely due to the delayed diagnosis.[1-4] 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 tumor when diagnosed.[5] Therefore, there is an urgent need to develop novel and reliable biomarkers such as circulating biomarkers for early-stage NSCLC diagnosis.

Small nucleolar RNAs (snoRNAs), medium length non-coding RNAs, mainly locate within introns of protein-coding genes and long non-coding RNA[6–8] and fall into two categories according their structural basis: Box C/D snoRNAs (SNORDs) and H/ACA snoRNAs (SNORAs). At first, the researches on snoRNAs mainly focused on the post-transcriptional modification of rRNAs and some spliceosomal RNA[9,10]; With the deepening of research, accumulating evidence have revealed that snoRNAs play important roles in the tumorigenesis and tumor progression,[11] and aberration of their expression has been observed in multiple many cancers, some of which are cancer type-specific. For example, SNORD113-1 expression in hepatocellular carcinoma (HCC) was significantly lower than that in paracancerous tissues and its downregulation was significantly associated with poor survival of patients.

Notably, snoRNAs in circulation are usually assembled by the set of core proteins named ribonucleoproteins (RNPs) into mature SNORNP, which are required for stability and making them resistant to digestion by ribonuclease.[12] Therefore, they can be stably expressed in plasma, serum, urine, and other body fluids and accurately detected,[13] empowering snoRNAs with the potential as fluid-based diagnostic biomarkers for malignancies. For example, SNORD44, stably expressed and measurable in serum, has been reported to participate in the diagnosis of breast cancer with U6[14]; Besides,. SNORD33, SNORD66, SNORD76, upregulated not only in tumor tissues but also in plasma, could be the potential biomarkers for NSCLC[11]; Recently, our previous studies also had demonstrated that SNORD63 and SNORD96A were not only stable in plasma and urinary sediment, but also acted as reliable and promising biomarkers for the diagnosis of renal clear cell carcinoma,[15] further illustrating the potential of snoRNAs as the circulating biomarker for cancer diagnosis.

In present study, SNORD42B and SNORD111 were screened out based on databases and then further verified in the expanded cohorts. SNORD42B, located at the intronic region of RPL23A in chromosome 17q11.2 with a 67-nt length, played a part in complementally directing 2′-O-methylation of U116 nucleotide in 18S rRNA[16,17]; At the same time, SNORD111, transcribed by the intronic region of SF3B3 in chromosome 16q22.1 with a 94-nt length, functioned by directing 2′-O-methylation of G3929 nucleotide in 28S rRNA.[17,18] These two snoRNAs were significantly increased not only in tissue but also in plasma from NSCLC as well as from early-stage NSCLC compared with those from healthy donors, processing with the favorable diagnostic efficiency. Moreover, the diagnostic efficiency of CEA was significantly elevated for early-stage NSCLC when combined with the two plasma snoRNAs.

2 | MATERIALS AND METHODS

2.1 | Data set source

The snoRNAs expression data of lung adenocarcinoma (LUAD) and lung squamous carcinoma (LUSC) tissues and adjacent normal tissues were downloaded from the SNORic database (http: //bioinfo.life.hust.edu.cn/ SNORic)[19] and their corresponding clinical information including a total of 989 NSCLC cases (Table S1) was obtained from TCGA (http://cancergenome.nih.gov).

2.2 | Patients and healthy donors

Formalin-fixed paraffin embedded (FFPE) specimens of NSCLC tissues and paired para-cancerous tissues from 48 NSCLC patients , as well as plasma from 165 NSCLC patients prior to any anticancer treatment and 118 healthy volunteers from Shandong Cancer Hospital and Institute were enrolled between September, 2018 and July, 2019. Cancer diagnosis was determined through a histological examination of tumor specimens, and tumor staging was determined according to the 8th edition of the lung cancer TNM staging standards formulated by IASLC. Healthy volunteers were excluded from any malignant, metabolism, immune diseases after examination.

2.3 | Isolation of exosomes and microvesicles (MVs)

The microvesicles and exosomes were isolated as described previously.[20] First, peripheral blood was centrifugated at 3000 g at 4°C for 10 min to collect plasma, followed by twice centrifugations at 14,000 g at 4°C for 35 minutes to gain MV pellets. Subsequently, the above MV-poor plasma underwent ultracentrifugation (Class H, R, and S Preparative Ultracentrifuges, Type 50.4 Ti Rotor; Beckman Coulter) at 100,000 g for 120 min at 4°C to isolate exosomes.

2.4 | RNA extraction and qRT-PCR

RNAs from FFPE specimens were isolated using FFPE RNA purification Kit (Norgen), while RNAs from plasma were extracted by TRIzol LS reagent (Thermo Fisher Scientific) according to the manufacturer's instruction.

The extracted RNAs were reverse-transcribed into cDNA using the Mix-X miRNA First Strand Synthesis Kit (TaKaRa Bio, Nojihigashi). QRT-PCR was performed using TB-Green Premix Ex Taq II Reagent (TaKaRa) according to the manufacturer's instruction. U6 was used

as an endogenous control. The qRT-PCR reaction was evaluated by melting curve analysis. The relative expression of snoRNAs was calculated using ΔCT ($Ct^{snoRNA}-Ct^{U6}$).[11,21] Each sample was analyzed in duplicate. The qRT-PCR primers are listed in Table 1.

2.5 | Statistical analysis

Statistical analysis was performed using SPSS 21.0 software (IBM, Ehningen) and GraphPad Prism version 8.0. The normality of the

TABLE 1 Sequence information of the primers for qRT-PCR

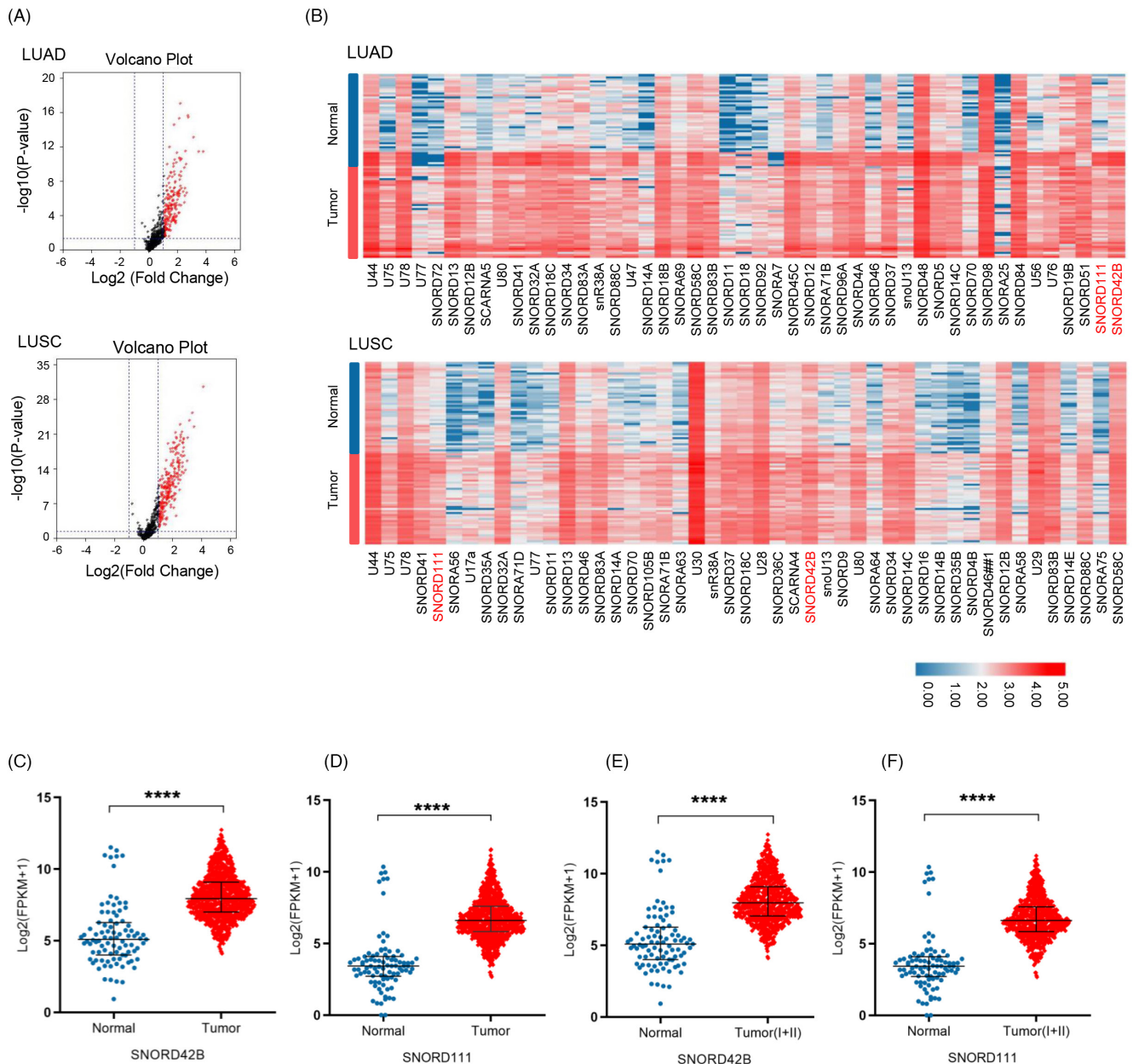| snoRNA	Forward primer	Reverse primer
SNORD42B	TGGAAAAGTTTTAATCTCCTGACACT	GTGCATCAGTGGTTCCTTTGA
SNORD111	CTTCTCTGACATTTTTCTCTGGACA	AGCCTGATCAGATTCATAAGGCA
U6	TGGAACGCTTCACGAATTTGCG	GGAACGATACAGAGAAGATTAGC

FIGURE 1 Differential snoRNAs identified in NSCLC from database. (A) Two volcano plots respectively showed the differential expressions of snoRNAs in tumor tissues compared with paired adjacent tissues in LUAD and LUSC database; (B) The two heatmaps showed the top 47 snoRNAs with the most significant difference in LUAD and LUSC databases, respectively; (C–F) Analysis of SNORD42B and SNORD111 differential expression using SNORic database, including normal vs NSCLC and normal vs early-stage NSCLC. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma

distribution was checked using the Kolmogorov–Smirnov test. If the data followed normal analysis, unpaired t-test would be used, if not, Mann–Whitney test would be used. Chi-square test was used for analyzing categorical variables. Multi-group analysis was tested by one-way analysis of variance (ANOVA) or Kruskal–Wallis test. In paired data, the normally distributed numeric variables were evaluated by paired t-test, whereas non-normally distributed variables were analyzed by Wilcoxon rank-test. Receiving operating characteristic (ROC) curve was used to evaluate diagnostic efficiency. The data were presented as the median with interquartile range. All tests were two-sided and $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Differential snoRNAs identified in NSCLC from database

We downloaded the normalized snoRNAs expression data from 989 NSCLC patients, of which paired data (paired tumor and its adjacent tissue) were derived from 46 LUAD and 45 LUSC patients. We used paired data and drew volcano plots and the heatmaps through log2(FC) and p value. As shown in Figure 1A, the cutoff values of the volcano plots were set at $p < 0.05$ and log2(FC) > 1 or < −1. Most of the differential genes were up-regulated. However, no down-regulated genes were in the interval. As shown in Figure 1B, the top 47 snoRNAs with the most significance (all log2(FC) ≥1.5) from these differential snoRNAs were listed in the heatmaps, among which only 26 were present in both LUAD and LUSC and treated as the candidates for next validation. Firstly, we used 24 paired FFPE samples to analyze their expressions by qRT-PCR. Unexpectedly, most were ruled out due to low primer specificity and expression level, as well as no difference in expression between cancer and adjacent tissues, finally SNORD42B and SNORD111 were identified with the log2 (FC) = 1.84,1.84 and p

value = 0.0001, 8.1E-05 in LUAD and the log2 (FC) = 2.4, 2.9 and p value = 4.4E-16, 3.3E-21 in LUSC.

Next, we carried out a large sample verification of the differential expression of SNORD42B and SNORD111 using TCGA. As shown in Figure 1C–F, these two snoRNAs were significantly increased in tumor tissues ($n = 989$) as well as in early-stage tumor tissues ($n = 784$) compared with those in normal tissues ($n = 91$), thus were selected for further research. Moreover, the correlation between the levels of SNORD42B and SNORD111 and clinicopathological characteristics was also analyzed. As given in Table S1, SNORD42B was related with lymph node metastasis and histology, whereas SNORD111 was related with gender and histology.

3.2 | SNORD42B and SNORD111 were upregulated in NSCLC and early NSCLC tissues

To further identify the differential expression of SNORD42B and SNORD111 in NSCLC, SNORD42B and SNORD111 were subjected to validation in FFPE tissues including 48 NSCLC and paired paracancerous tissues. Consistently, SNORD42B and SNORD111 were significantly increased in NSCLC ($p < 0.0001$, $p = 0.0008$, respectively. Figure 2A,B) as well as in 34 early-stage NSCLC ($p = 0.0019$, $p = 0.0463$, respectively. Figure 2C,D) compared with those in healthy donors. In addition, we also analyzed the correlation between the levels of SNORD42B, SNORD111, and clinicopathological paraments. However, no significant relationship was observed, which might attribute to small sample size (Table 2).

3.3 | SNORD42B and SNORD111 were upregulated in NSCLC and early-stage NSCLC in plasma stably

To identify the differential expression of SNORD42B and SNORD111 in plasma of NSCLC patients, we first assessed the stability of the
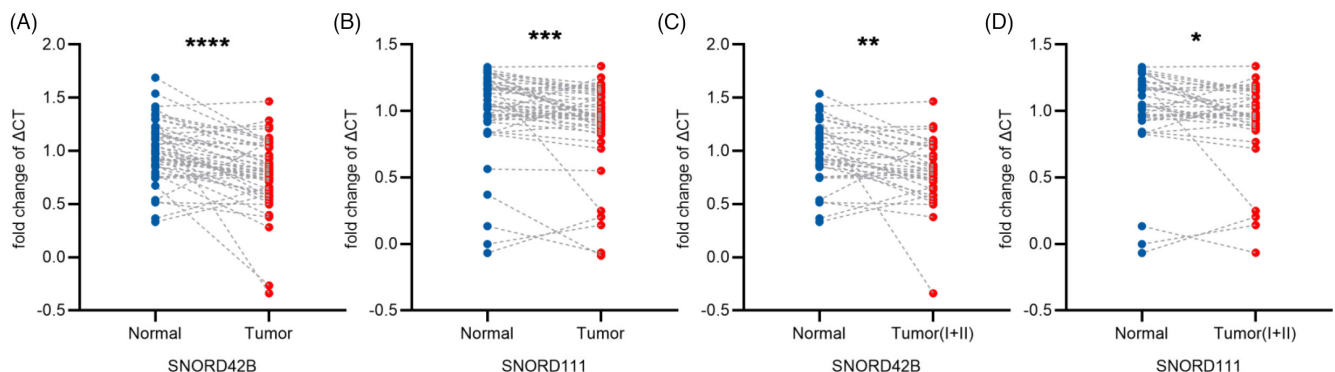

FIGURE 2 SNORD42B and SNORD111 were upregulated in NSCLC and early NSCLC tissues. (A,B) Analysis of SNORD42B and SNORD111 expressions in 48 paired NSCLC FFPE samples and adjacent samples; (C,D) Analysis of SNORD42B and SNORD111 expressions in 34 paired early-stage NSCLC FFPE samples and adjacent samples. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$

TABLE 2 Characteristics of NSCLC patients for SNORD42B and SNORD111 in FFPE specimens

Parameters	Samples, n	SNORD42B expression Δ Ct Median (interquartile range)	p-value	SNORD111 expression Δ Ct Median (interquartile range)	p-value
Age (years)					
≤62	24	3.325 (2.665–4.423)	0.5985	6.710 (6.183–7.990)	0.3382
>62	24	3.298 (2.415–4.175)		6.630 (5.205–7.373)	
Gender					
Male	32	3.415 (2.483–4.295)	0.5627	6.615 (6.085–7.638)	0.3047
Female	16	3.078 (2.594–4.305)		6.790 (5.905–8.108)	
Smoking					
NO	18	3.230 (2.476–4.368)	0.6839	6.790 (5.888–8.103)	0.4729
YES	27	3.450 (2.430–4.300)		6.650 (6.250–7.640)	
Not Available	3				
Drinking					
N0	23	3.225 (2.545–4.450)	0.6879	6.770 (5.820–8.050)	0.6881
YES	21	3.520 (2.420–4.320)		6.650 (6.080–7.635)	
Not Available	4				
Histology					
AC	24	3.145 (2.293–4.325)	0.2983	6.790 (5.843–7.990)	0.6642
SCC	24	3.415 (3.034–4.223)		6.615 (6.320–7.570)	
Tumor size					
V > 6 cm^3	22	3.775 (3.169–4.490)	0.0631	7.110 (6.280–7.953)	0.4206
V ≤ 6 cm^3	23	3.065 (2.410–3.580)		6.650 (5.820–7.680)	
Not Available	3				
T stage					
T1	26	3.153 (2.425–4.295)	0.2742	6.710 (5.888–7.953)	0.7329
T2	14	3.360 (1.961–4.490)		6.720 (5.235–7.743)	
T3	3	3.870 (3.860–4.300)		6.620 (6.500–8.100)	
T4	2	4.515 (3.690–5.340)		7.630 (7.390–7.870)	
TX	3				
LN meta.					
N0	32	3.428 (2.665–4.423)	0.5061	6.740 (6.063–7.930)	0.8531
N1	4	3.608 (3.113–4.208)		7.060 (6.340–7.668)	
N2	4	2.185 (−0.5325–3.703)		6.335 (0.9700–7.815)	
N3	4	3.425 (1.828–4.618)		7.350 (4.520–8.260)	
NX	4				
Distant meta.					
M0	44	3.388 (2.569–4.330)		6.740 (6.063–7.930)	
M1	1	1.650		3.810	
MX	3				
TNM stage					
I	25	3.270 (2.535–4.460)		6.770 (5.970–7.980)	
II	9	3.610 (3.150–4.135)		6.500 (6.270–7.390)	
III	9	3.690 (1.765–4.575)		7.390 (6.180–8.075)	
IV	1	1.650		3.810	
Not Available	4				

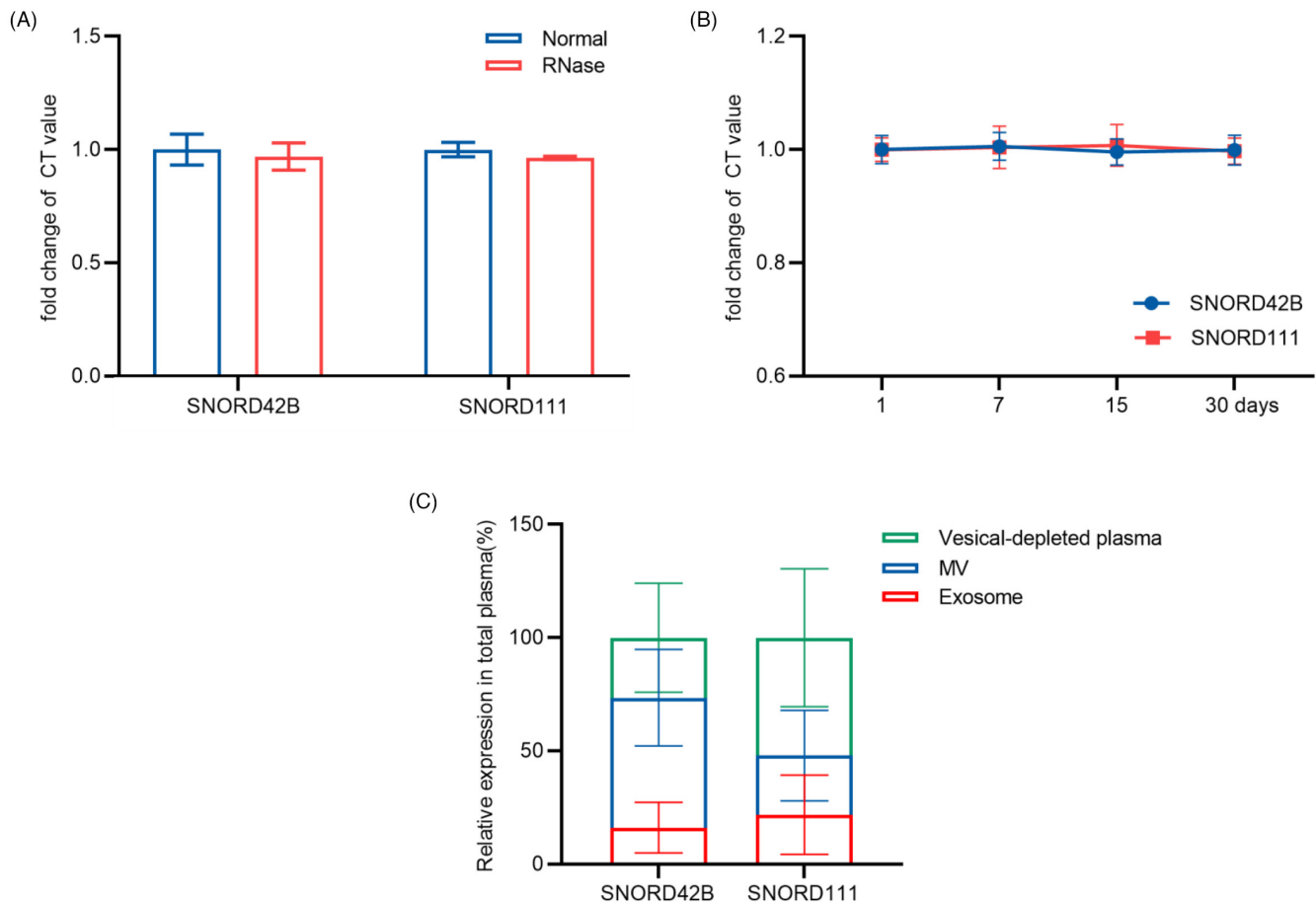Abbreviations: AC, adenocarcinoma; SCC, squamous cell carcinoma.

FIGURE 3 SNORD42B and SNORD111 existed in plasma stably. (A) Plasma SNORD42B and SNORD111 expressions treated with RNase A, (B) or stored at −80°C at time-points of 1, 7, 15, 30days; (C) The distribution of SNORD42B and SNORD111 in plasma. MV, microvesicles

snoRNAs in plasma as described previously.[11] As shown in Figure 3A,B, the expression of SNORD42B and SNORD111 in the plasma seemed no obvious changes after treatment by Ribonuclease A (RNase A), or storage for 30days, indicating they were stable and consistently measurable in the plasma. Besides, we explored the position of SNORD42B and SNORD111 in the plasma. As shown in Figure 3C, SNORD42B and SNORD111 were existed in microvesicles (MV), exosome and vesicle-depleted plasma, indicating their stability in plasma might not attribute to the protection by bilayer membrane. Unexpectedly, no difference of their position in plasma was observed.

Next, two snoRNAs were detected in a large validation in plasma with 165 NSCLC patients and 118 healthy donors. As shown in Figure 4A,B,D,E, SNORD42B and SNORD111 were significantly upregulated in NSCLC patients ($p < 0.0001$ and $p < 0.0001$, respectively), as well as in 72 early-stage NSCLC ($p < 0.0001$ and $p < 0.0001$, respectively) compared with healthy donors.

In addition, when compared with the healthy donors, these two snoRNAs levels were significantly upregulated in various TNM stage (Figure 4C,F). The association between SNORD42B, SNORD111, and clinicopathological characteristics of NSCLC patients was summarized in Table 3, but no significant relationship was observed.

3.4 | Plasma SNORD42B and SNORD111 as effective biomarkers of NSCLC and early-stage NSCLC diagnosis

To explore the potential of SNORD42B and SNORD111 as circulating diagnostic markers for NSCLC, ROC curves were employed. As shown in Figure 5A,B, their areas under the curve (AUCs) were 0.7191 with 61.8% sensitivity and 77.1%specificity, and 0.6993 with 55.2% sensitivity and 73.7% specificity, respectively. Moreover, the diagnostic efficiency of their combination was also calculated, possessing AUC of 0.7303 with a relative sensitivity of 73.3% and a relative specificity of 63.6% (Figure 5C).

Subsequently, when comparing the patients with early-stage NSCLC to healthy controls, ROC curves demonstrated favorable diagnostic efficiencies of SNORD42B, SNORD111, possessing AUCs of 0.7235 with 61.1% sensitivity and 77.1% specificity, and 0.7306 with 44.4% sensitivity and 90.7% specificity, respectively (Figure 5D,E), as well as 0.7556 with a relative sensitivity of 83.3% and a relative specificity of 57.6% for their combination (Figure 5F), indicating SNORD42Band SNORD111 act as the promising non-invasive diagnostic biomarkers for NSCLC.
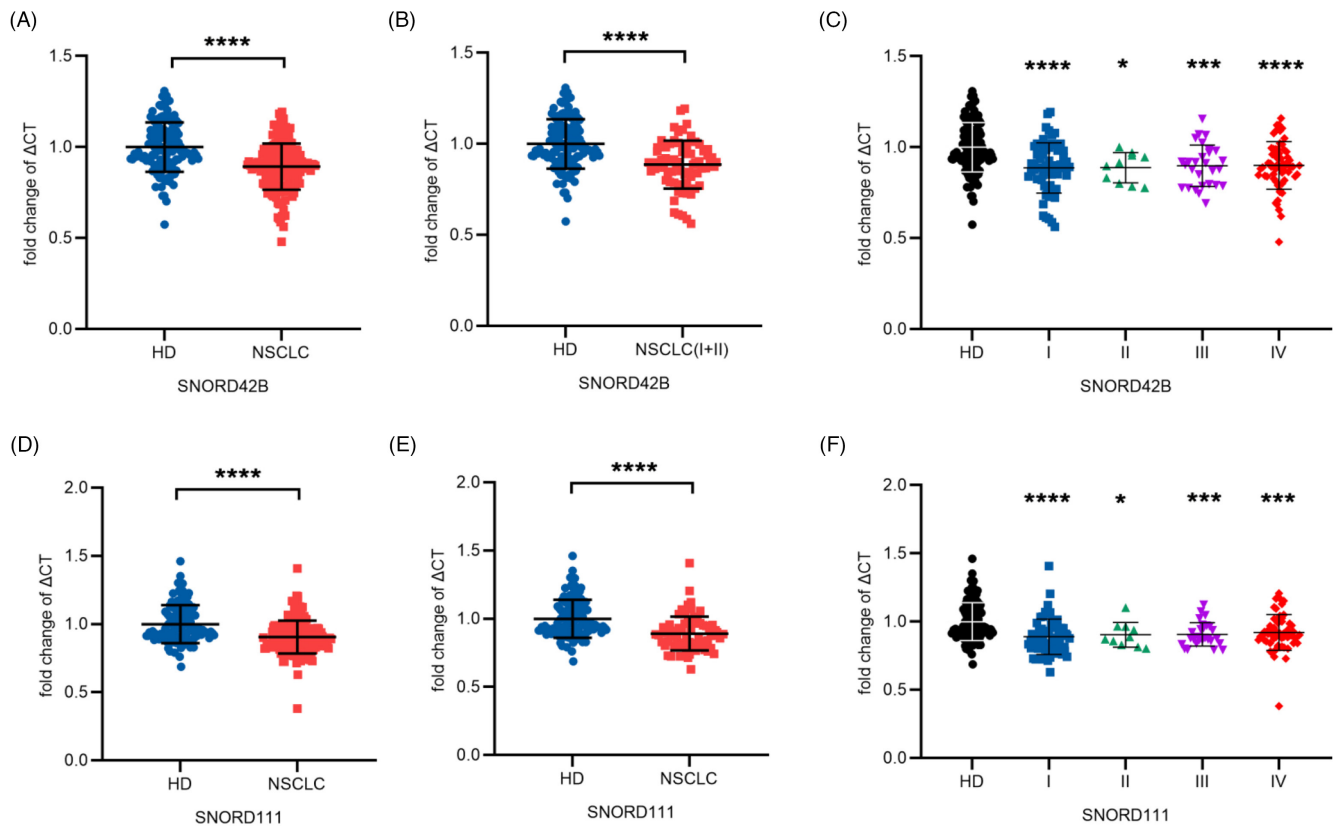
FIGURE 4 Plasma SNORD42B and SNORD111 were upregulated in NSCLC and early NSCLC. (A,D) Differential expressions of SNORD42B and SNORD111 in plasma of 118 healthy individuals and 165 patients with NSCLC; (B,E) Differential expressions of SNORD42B and SNORD111 in 118 healthy individuals and 72 patients with early-stage NSCLC; (C,F) SNORD42B and SNORD111 expressions in various TNM stage. ns, no significance; $*p < 0.05$; $***p < 0.001$; $****p < 0.0001$

3.5 | Plasma SNORD42B and SNORD111 facilitate diagnostic performance of CEA for early-stage NSCLC

Carcinoembryonic antigen (CEA), a traditional biomarker, is now generally accepted clinically for NSCLC diagnosis,[22] but possesses the poor clinical diagnostic efficiency at the early stage of cancer development.[23] In present study, we detected the expression levels of CEA in 158 NSCLC patients including 68 early NSCLC patients and 96 healthy donors, and calculated the diagnostic efficiency combined with plasma snoRNAs expressions. The combination of CEA with SNORD42B or SNORD111 significantly improved the diagnostic efficiency of early-stage NSCLC, although the significance of combined diagnosis of NSCLC was not obvious (data not shown). As shown in Figure 6A–C, the AUCs of CEA were significantly elevated from 0.6787 to 0.8056 or 0.7986. As expected, combining all three markers increased the AUC value to 0.8220, suggesting plasma SNORD42B and SNORD111 facilitate diagnostic performance of CEA (Figure 6D).

4 | DISCUSSION

Despite of significant advances in the treatment of NSCLC, it is still the most pervasive factor of malignancy-related death globally,

mainly because the majority of patients with NSCLC are diagnosed with local progression or metastatic disease.[24] Therefore, there is an urgent need for effective and novel biomarkers to identify with NSCLC patients, especially those with early-stage NSCLC.

Recently, more and more evidence has demonstrated that snoRNAs are dysregulated in tumor tissue, closely related to carcinogenesis and fairly encouraging as biomarkers for cancer diagnosis. However, the relevant studies of SNORD42B and SNORD111 are few, especially in tumors. In our study, we demonstrated SNORD42B and SNORD111 were significantly increased in NSCLC, as evidence from the results from databases, FFPE and plasma samples, indicating their potential roles in tumorigenesis of NSCLC. More importantly, in our study we validated SNORD42B and SNORD111 were capable to act as the promising biomarkers for NSCLC and early-stage NSCLC. First, the diagnostic performance of these two snoRNAs was assessed, they acted as the non-invasive diagnostic biomarkers for NSCLC and early-stage NSCLC, possessing considerable diagnostic efficiency; Second, CEA, as a traditional biomarker, has been the most extensively used for NSCLC diagnosis, but possesses lower sensitivity and clinic diagnostic efficiency for early-stage NSCLC than for advanced NSCLC.[23,25] Our data demonstrated plasma SNORD42B and SNORD111 facilitated diagnostic performance of CEA for early-stage NSCLC significantly. Taken together,

TABLE 3 Characteristics of NSCLC patients for SNORD42B and SNORD111 in plasma

Parameters	Samples , *n*	SNORD42B expression Δ Ct Median (interquartile range)	*p*-value	SNORD111 expression Δ Ct Median (interquartile range)	*p*-value
Age (years)					
≤62	88	5.355 (4.889–5.746)	0.0545	6.185 (5.799–6.686)	0.8355
>62	77	5.445 (4.560–5.978)		6.220 (5.718–6.715)	
Gender					
Male	98	5.468 (5.025–6.001)	0.7140	6.220 (5.711–6.898)	0.5475
Female	67	5.460 (4.945–5.905)		6.160 (5.840–6.635)	
Smoking					
No	87	5.500 (5.110–5.970)	0.4732	6.160 (5.730–6.660)	0.5345
Yes	78	5.413 (4.798–5.944)		6.230 (5.781–6.769)	
Drinking					
No	126	5.468 (5.018–5.985)	0.4383	6.175 (5.718–6.700)	0.4417
Yes	39	5.465 (4.775–5.760)		6.245 (6.050–6.715)	
Histology					
AC	126	5.478 (5.033–6.000)	0.1709	6.175 (5.756–6.641)	0.8462
SCC	27	5.325 (4.775–5.595)		6.175 (5.585–6.715)	
Others	12				
Tumor size					
<5.8cm^3	65	5.400 (5.033–5.990)	0.7594	6.315 (5.718–6.738)	0.3117
≥5.8cm^3	64	5.465 (4.870–5.900)		6.123 (5.733–6.551)	
Not Available	36				
T stage					
Tis	7	5.505 (4.615–6.205)	0.5644	5.575 (5.450–6.160)	0.4178
T1	55	5.430 (5.110–5.980)		6.260 (5.705–6.700)	
T2	54	5.488 (4.953–6.021)		6.343 (5.758–7.034)	
T3	11	5.555 (4.805–6.035)		6.165 (5.850–6.440)	
T4	28	5.423 (4.803–5.606)		6.140 (5.838–6.769)	
TX	10				
LN meta.					
N0	79	5.480 (4.995–5.975)	0.7476	6.120 5.575–6.660)	0.4387
N1	12	5.548 (5.083–6.051)		6.335 (5.900–6.684)	
N2	35	5.300 (4.825–5.905)		6.175 (5.840–6.745)	
N3	31	5.440 (4.850–5.925)		6.245 (5.995–6.760)	
NX	8				
Distant meta.					
M0	101	5.465 (4.838–5.938)	0.7171	6.120 (5.685–6.648)	0.1071
M1	59	5.440 (5.110–6.000)		6.270 (5.860–6.880)	
MX	5				
TNM stage					
0	6	5.453 (4.318–5.973)	0.9752	5.563 (5.418–6.003)	0.0770
I	56	5.448 (5.003–5.999)		6.203 (5.613–6.713)	
II	10	5.513 (4.846–5.838)		6.115 (5.744–6.704)	
III	28	5.528 (4.806–5.954)		6.115 (5.873–6.695)	
IV	62	5.488 (5.110–6.005)		6.288 (5.924–6.861)	
Not Available	3				

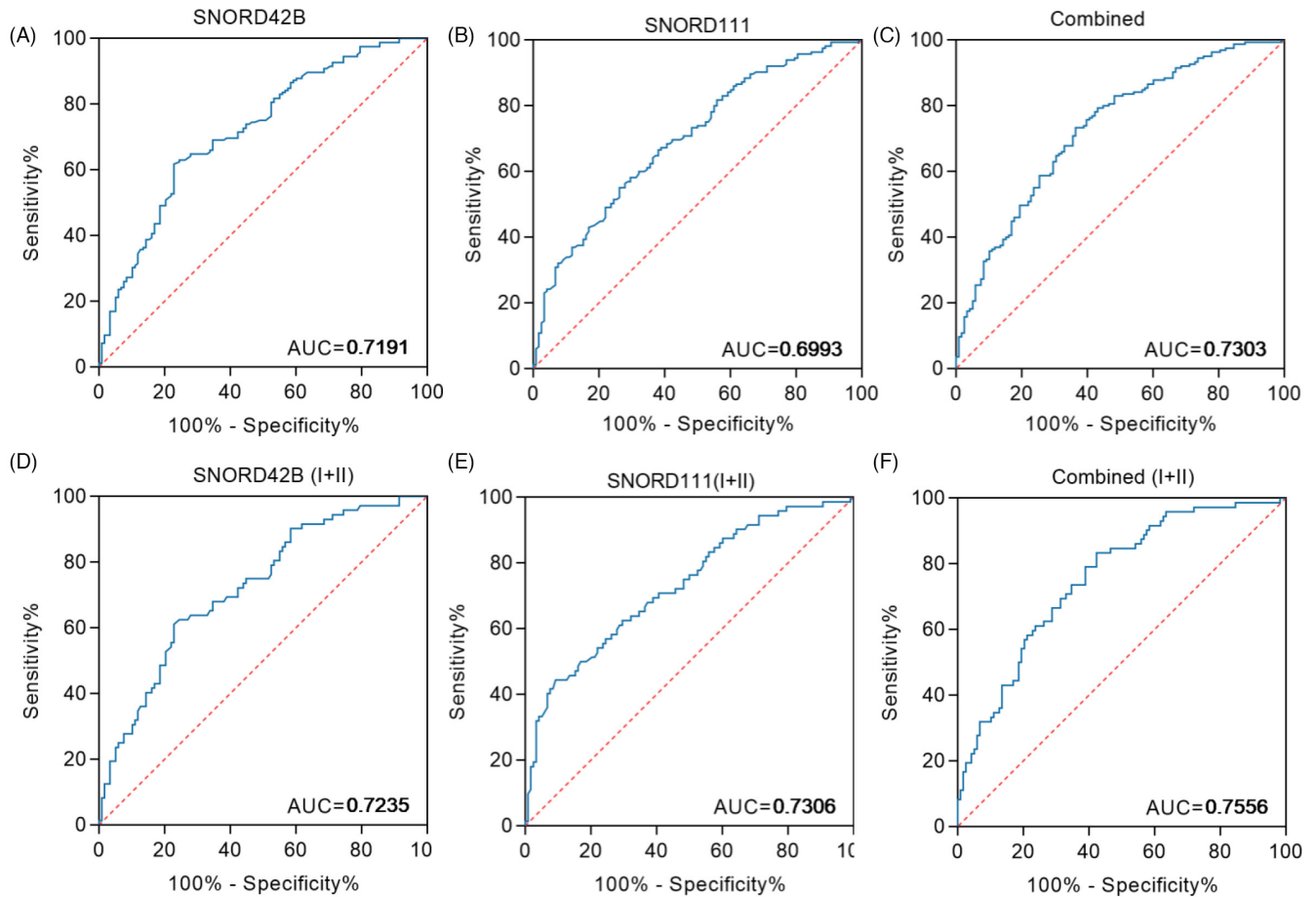Abbreviations: AC, adenocarcinoma; SCC, squamous cell carcinoma.

FIGURE 5 Plasma SNORD42B and SNORD111 as biomarkers for diagnosis and early diagnosis of NSCLC. (A–C) The AUC analysis of SNORD42B, SNORD111, and their combination for NSCLC; (D–F) The AUC analysis of SNORD42B, SNORD111, and their combination for early-stage NSCLC
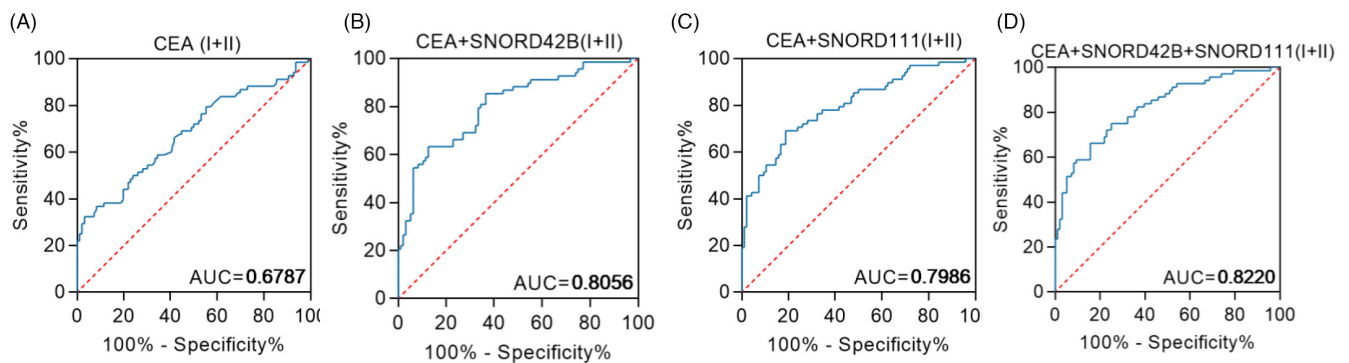

FIGURE 6 Plasma SNORD42B and SNORD111 facilitate diagnostic performance of CEA for early-stage NSCLC. The ROC curve analysis of CEA (A) and the combination of CEA and SNORD42B (B), SNORD111 (C), and both (D) for early-stage NSCLC. AUC, area under curve

the current data indicated that plasma SNORD42B and SNORD111 could act as potential diagnostic biomarkers for NSCLC and early-stage NSCLC. However, the small sample size limits us to discover more cancer-related properties of SNORD42B and SNORD111. This problem is caused by our research criteria on the selection of appropriate research subjects and the insufficient time span of the study.

In conclusion, the current data suggests that the levels of SNORD42B and SNORD111 are significantly upregulated in NSCLC patients and can serve as promising and non-invasive diagnostic markers for NSCLC and early-stage NSCLC.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICAL APPROVAL

The study involving human participants (201806004) was reviewed and approved by Ethics Committee of Shandong Cancer Hospital and Institute.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.