

Role and clinical value of serum hsa_tsr011468 in lung adenocarcinoma

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Abstract. Transfer RNA-derived small RNAs (tsRNAs) are novel non-coding RNAs that are associated with the pathogenesis of various diseases. However, their association with lung adenocarcinoma (LUAD) has not been studied comprehensively. Therefore, the present study aimed to explore the diagnostic value of a tsRNA, hsa_tsr011468, in LUAD. The OncotRF database was used to screen tsRNAs and reverse transcription-quantitative PCR (RT-qPCR) was performed to detect the expression levels of hsa_tsr011468 in various samples. Subsequently, the diagnostic and prognostic values of hsa_tsr011468 for LUAD were determined via receiver operating characteristic (ROC) curve and survival curve analyses, and by assessing clinicopathological parameters. In addition, both nuclear and cytoplasmic RNA were extracted to assess the location of hsa_tsr011468. The OncotRF database identified high expression of hsa_tsr011468 in LUAD. In addition, the results of RT-qPCR showed that the relative expression levels of hsa_tsr011468 in the serum and tissues of patients with LUAD were higher than those in normal controls. Furthermore, its expression was lower in postoperative serum samples than in preoperative serum samples from patients with LUAD. ROC and survival curves indicated that hsa_tsr011468 had good diagnostic and prognostic value. Furthermore, the clinicopathological analysis revealed that hsa_tsr011468 was associated with tumor size. In addition, hsa_tsr011468 was mainly localized in the cytoplasm of LUAD cells. The present

study indicated that hsa_tsr011468 has good diagnostic value and, therefore, could be employed as a serum marker for LUAD.

Introduction

The estimated data for 2022 indicated that 12.5% of all new cancer cases and 18.7% of total cancer deaths worldwide were associated with lung cancer (1). Notably, research has indicated that the incidence of lung cancer in most countries will continue to rise until 2035, highlighting it as a significant global public health concern (2). Moreover, lung adenocarcinoma (LUAD) has been estimated to account for ~40% of all lung cancer cases (3). Therefore, it is crucial to identify new biomarkers that can accurately diagnose LUAD.

With technological advancements and the broad use of high-throughput RNA sequencing technology, the role of transfer RNA (tRNA)-derived small RNAs (tsRNAs), as a new type of non-coding small RNA, has been determined in the medical field (4,5). The length of tsRNAs is generally 18-40 nucleotides, which mainly includes tRNA-derived RNA fragments (tRFs) and tRNA halves. Depending on the cleavage site of the parent tRNA, tRFs can be categorized into four types: tRF-1, tRF-3, tRF-5 and i-tRF, while tRNA-derived stress-induced RNAs (tiRNAs) can be classified into 5' and 3' tiRNAs (6,7). Furthermore, it has been indicated that tiRNAs are crucially involved in gene expression modulation, protein translation inhibition and epigenetic regulation, all of which contribute to viral replication and intercellular communication (8). Moreover, tsRNAs, as untranslated RNA molecules, serve various roles in different types of cancer. For example, tsRNA-42 has been reported to be inactivated in chronic lymphocytic leukemia primarily through promoter methylation (9). Additionally, epigallocatechin gallate can promote ferroptosis in non-small cell lung cancer by downregulating tsRNA-13502 (10). Furthermore, the downregulation of tRFdb-3013a/b has been shown to be associated with a poor prognosis for survival in patients with colon and rectal adenocarcinoma (11). In addition, tsRNAs have been reported to be associated with cancer development, and may have oncogenic or oncostatic functions (12).

Several studies on tsRNAs have indicated that they can serve as novel biomarkers for the diagnosis of various

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diseases (13). For example, tRF-Pro-AGG-004 and tRF-Leu-CAG-002 in the serum have been identified as novel biomarkers for diagnosing pancreatic cancer (14). Furthermore, tRNA-26576 has been shown to enhance the proliferation of breast cancer cells, indicating its potential as a diagnostic marker and therapeutic target for breast cancer (15). However, there is limited research on the role of small molecule RNAs in LUAD. At present, the studies on small molecule RNAs and screening mechanisms have mainly focused on microRNAs (miRNAs) (16,17). tsRNAs are highly stable and abundant in various body fluids, and are extensively involved in pathological processes (18), sometimes more so than miRNAs; therefore, the identification of abnormally expressed tsRNAs in LUAD may improve clinical diagnosis and prognosis.

The present study aimed to investigate LUAD-related tsRNAs using the OncotRF database. Reverse transcription-quantitative PCR (RT-qPCR) analysis of the differential expression of tsRNAs in serum and tissue samples from patients with LUAD and normal subjects was conducted to identify hsa_tsr011468. Moreover, receiver operating characteristic (ROC) curves and survival analyses were performed to evaluate the diagnostic and prognostic value of hsa_tsr011468 in LUAD. The relative expression levels of hsa_tsr011468 in the preoperative and postoperative serum samples from patients with LUAD were also compared to assess its dynamic monitoring ability. Additionally, functional enrichment analyses of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) predicted target genes and signaling pathways, providing new research directions for exploring effective biomarkers and specific mechanisms of action in LUAD.

Materials and methods.

Collection of serum and tissue specimens. Serum samples were collected from a total of 84 patients with LUAD (mean patient age: 64±9 years, male-to-female ratio: 45%) and 88 healthy individuals (mean patient age: 59±9 years, male-to-female ratio: 63%) between April 2020 and April 2022 from Nantong First People's Hospital (Nantong, China). A total of 70 of the 84 patients diagnosed with LUAD underwent surgery, and postoperative serum samples were also collected for analysis. Furthermore, 20 serum samples were collected from patients with squamous cell carcinoma (SCC) (mean patient age: 66±8 years, male-to-female ratio: 54%) and 20 serum samples were collected from patients with large cell carcinoma (LCC) (mean patient age: 62±9 years, male-to-female ratio: 67%) at The Affiliated Hospital of Nantong University (Nantong, China) between September 2023 and May 2024. A total of 42 LUAD tissue samples (mean patient age: 66±9 years, male-to-female ratio: 57%) and their corresponding paired paracancerous tissue samples (2-3 cm away from the tumor) were collected between April 2020 and April 2022 from the Affiliated Hospital of Nantong University. None of the patients from whom tissues were collected underwent prior radiotherapy or chemotherapy. The present study was approved by the First People's Hospital of Nantong City (approval no. 2023KT197) and the Affiliated Hospital of Nantong University (approval no. 2019-L071), and was performed in accordance with The Declaration of Helsinki.

Cell culture. The human LUAD cell lines (A549, NCI-H1299 and PC9) and the human normal lung epithelial cell line BEAS-2B used in the present study were obtained from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. The cells were cultured in RPMI 1640 medium (Corning, Inc.) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin. Cells were passaged by trypsin digestion when they entered the logarithmic growth phase and reached a maximum of 70-80% confluence. Passage was performed 1-2 times per experiment. The medium was changed when the cells reached 80% density, approximately once every 2 days. The cells were observed microscopically to be of good condition with a translucent appearance and intact cell membranes. The cells were cultured in an incubator at 37°C and 5% CO₂ until they reached the logarithmic growth phase for subsequent experiments.

LUAD tissue, cell and serum RNA extraction. Total RNA was extracted from cells (A549, NCI-H1299 and PC9), LUAD serum and milled tissue using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). The concentration and purity of acquired RNA were assessed using a UV spectrophotometer. The RNA was then subjected to RT or was stored at -80°C.

Database screening. The OncotRF database (<http://bioinformatics.zju.edu.cn/OncotRF/>) was utilized to screen meaningful tsRNAs in LUAD. Basic information on tsRNAs was obtained and defined using the tsRBase database (https://ngdc.cnca.ac.cn/databasecommons/database/id/7266#:~:text=Taken%20together,%20tsRBase%20is%20the%20most%20comprehensive%20and%20systematic%20tsRNA,)), the tsRFun database (<https://rna.sysu.edu.cn/tsRFun/>), and the UCSC database (<https://genome.ucsc.edu/>). TargetScan (https://www.targetscan.org/vert_80/), miRanda (<http://mirtoolsgallery.tech/mirtoolsgallery/node/1055>), RNAhybrid (<https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/submission.html/>) and tRFTar (<http://www.rnanut.net/tRFTar/>) databases were used to predict target gene of selected tsRNA. KOBAS software (<http://bioinfo.org/kobas>) was used to perform GO and KEGG pathway analyses.

Evaluation of hsa_tsr011468 assay. The linearity of hsa_tsr011468 was evaluated using continuous 10-fold dilutions of hsa_tsr011468 cDNA extracted from A549 cells. Mixed serum samples, prepared by combining multiple normal human sera, were maintained at room temperature for 0, 4, 8, 12 and 24 h, and were subjected to repeated freeze-thaw cycles for 1, 3, 5, 7 and 9 times. Subsequently, the expression levels of these samples were measured using RT-qPCR to analyze their stability. The specificity of the hsa_tsr011468 mRNA assay was evaluated by unimodal dissolution curve. RNA integrity was assessed by electrophoresis on 1% agarose gels containing ethidium bromide. The PCR product and the loading buffer were mixed in proportion and loaded into the agarose gel for electrophoresis. After 43 min of separation at 110 V, the results were observed in comparison to DNA markers.

RT-qPCR. RNA concentration and purity were evaluated using UV spectrophotometry, while RNA integrity was determined

through agarose gel electrophoresis (2% concentration). RNA was then used to synthesize cDNA using the Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The amplification conditions were as follows: Incubation at 42°C for 60 min and 70°C for 5 min, followed by maintenance at 4°C. The cDNA was then utilized as a template for qPCR amplification to detect the expression of target genes. The reaction mix comprised SYBR Green I Mix (ABclonal Biotech Co., Ltd.), forward primer, reverse primer, cDNA and DEPC water in a ratio of 10:1:1:3:5. The qPCR reaction was performed using the QuantStudioQ5 system (Thermo Fisher Scientific, Inc.), and the cycle conditions were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 30 sec. The primers used for qPCR analysis were: 5'-tRNA-Ala-AGC-11-1_L25, forward 5'-GGG GGAATTAGCTCAAATGG', reverse 5'-AGTGCAGGGTCC GAGGTATT-3', based on its sequence 5'-GGGGAATTAGCT CAAATGGTAGAGC-3'; 5'-M-tRNA-Gln-CTG-5-1_L24, forward 5'-CGGGTTCCATGGTGTAAATGG-3', reverse 5'-AGTGCAGGGTCCGAGGTATT-3', based on its sequence 5'-GGTTCATGGTGTAAATGGTTAGCA-3'; 3'-mito-tRNA-Arg-TCG_L25, forward 5'-CGCGCGTTATGA TAATCATATTTAC-3', reverse 5'-AGTGCAGGGTCCGAG GTATT-3', based on its sequence 5'-TTATGATAATCATAT TTACCAACCA-3'; 3'-M-tRNA-Glu-TTC-4-2_L28, forward 5'-GTTTCGATTCCCGGTCAGG-3', reverse 5'-AGTGCA GGGTCCGAGGTATT-3', based on its sequence 5'-CCG GGTTCGATTCCCGGTCAGGGAACCA-3'; 3'-M-tRNA-iMet-CAT-1-8_L24, forward 5'-CGGATCGAAACCATC CTCTG-3', reverse 5'-AGTGCAGGGTCCGAGGTATT-3', based on its sequence 5'-GATCGAAACCATCCTCTGCTA CCA-3'; hsa_tsr011468, forward 5'-CCTCGTGGCGCA ATGG-3' and reverse 5'-AGTGCAGGGTCCGAGGTATT-3', based on its sequence 5'-GACCTCGTGGCGCAATGGTAG CGC-3' (all from GeneAdv Co., Ltd.). The sequences of the above molecules were obtained from the tsRBase. U6 was employed as a standard control and its primer sequence was: Forward 5'-CGCTTCGGCAGCCACATATAC-3' and reverse 5'-TTCACGAATTTGCGTGTATC-3'. 18S rRNA was also employed as a standard control and its primer sequence was: Forward 5'-CGGCTACCACATCCAAGGAA-3' and reverse 5'-GCTGGAATTACCGCGGCT-3' (Guangzhou RiboBio Co., Ltd.). The expression levels of the product were determined using the $2^{-\Delta\Delta C_q}$ method (19).

Nuclear and cytoplasmic RNA extraction assay. Trypsin digestion of adherent cells (A549, NCI-H1299, PC9 and BEAS-2B) was performed and the cell precipitate was collected. Nuclear and cytoplasmic RNA were extracted using a Nucleus and Cytoplasmic Protein Extraction Kit (Beyotime Institute of Biotechnology), where the protease inhibitor was substituted with an RNase inhibitor. The extracted RNA was reverse transcribed and stored at -80°C for qPCR analysis.

Statistical analysis. All data were statistically analyzed using GraphPad 8.0 (Dotmatics). The data are presented as the mean $\pm$ SD. Unpaired Student's t-tests were performed to compare the results between patients with LUAD and healthy controls, as well as between lung cancer cells and normal lung epithelial cells. Paired Student's t-tests were performed to

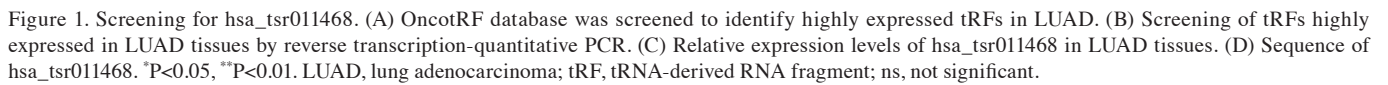
compare pre-operative and post-operative serum samples, as well as tumor and paracancerous tissue samples from patients with LUAD. A one-way ANOVA followed by Bonferroni post hoc test was used to compare the differences in the levels of hsa_tsr011468 in mixed sera at different times and the number of freeze-thaw cycles. Associations between hsa_tsr011468 expression and clinicopathological features were analyzed using the χ^2 test or Fisher's exact test. The log-rank test was employed to compare Kaplan-Meier survival curves. The diagnostic and combined diagnostic values of hsa_tsr011468 and other serum diagnostic markers for lung cancer were evaluated via receiver ROC analysis, with the area under the curve (AUC) values assessed. To determine the use of hsa_tsr011468, Cyfra21-1, CEA and SCC antigen to distinguish patients with LUAD from healthy controls, sensitivity, specificity, overall accuracy, positive predictive value and negative predictive value were calculated as follows: Sensitivity=number of true positives/total number of patients with LUAD in the population; Specificity=number of true negatives/total number of healthy controls in the population; Overall accuracy=(true positive + true negative)/total number of samples; Positive predictive value=true positive/(true positive + false positive); Negative predictive value=true negative/(true negative + false negative). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Database screening and validation of hsa_tsr011468 by qPCR. The OncotRF database was searched to investigate the significance of tsRNAs in LUAD. The search results were ranked based on the criterion of log2 fold-change and the top three ranked 3'tRF and 5'tRF were selected for validation (Fig. 1A). To further screen for tsRNAs significantly associated with LUAD, the expression of the top three ranked 3'tRF and 5'tRF were analyzed in 12 pairs of LUAD tissues. The results showed that 5'-tRNA-Trp-CCA-2-1_L24 was differentially expressed in LUAD tissues compared with in paracancerous tissues (Fig. 1B). This finding was later confirmed by the addition of 30 pairs of tissues to assess their relative expression levels; the results indicated that the expression of 5'-tRNA-Trp-CCA-2-1_L24 was higher in LUAD tissues than that in paracancerous tissues (Fig. 1C). Furthermore, to simplify the nomenclature of 5'-tRNA-Trp-CCA-2-1_L24, the tsRBase database was searched using molecular data, which revealed that 5'-tRNA-Trp-CCA-2-1_L24 could be named hsa_tsr011468 (Fig. 1D).

Methodological evaluation of hsa_tsr011468. Detailed information was obtained and the molecular structure of hsa_tsr011468 was determined using the tsRFun database (Fig. 2A). Furthermore, the UCSC database was utilized to identify the chromosomal location of hsa_tsr011468, which was chr 17:19,508,181-19,508,204 (Fig. 2B). The length of the hsa_tsr011468 PCR product was confirmed to be ~75 bp through agarose gel electrophoresis (Fig. 2C).

To ensure the reliability of the experimental results, a methodological evaluation of hsa_tsr011468 was conducted. The regression equation for hsa_tsr011468 was $Y = -1.357 \times X + 24.37$ ($R^2 = 0.960$) and for U6 was $Y = -1.429 \times X + 22.72$



Clinical advantages of hsa_tsr011468 as a serum marker for LUAD. A total of 20 serum samples were collected from patients with LCC, SCC, and LUAD (mean patient age: 63±8 years, male-to-female ratio: 33%), and 20 samples were collected from normal controls (mean patient age: 58±8 years, male-to-female ratio: 54%). The expression levels of hsa_tsr011468 in these non-small cell lung cancer were detected by RT-qPCR. It was revealed that the expression of hsa_tsr011468 was not statistically different in the serum of patients with SCC and LCC compared with that from healthy controls; however, the expression of hsa_tsr011468 was significantly higher in serum samples

To validate that hsa_tsr011468 can serve as a serum diagnostic marker for LUAD, we continued to collect and expand the sample size, ultimately totaling 84 serum samples from patients with LUAD and 88 control samples from healthy subjects for qPCR analysis. The results indicated that the relative expression levels of hsa_tsr011468 were higher in samples from patients with LUAD compared with those from normal controls (Fig. 3B). Furthermore, the expression levels of hsa_tsr011468 were elevated in LUAD cell lines (Fig. 3C). Subsequently, the 84 patients with LUAD were categorized into high-expression (n=42) and low-expression (n=42) groups based on the median expression of hsa_tsr011468, in order to investigate the association between hsa_tsr011468 and clinicopathological features. Tumor-node-metastasis classification was performed according to the eighth edition of tumor-node-metastasis staging developed by the International Association for the Study of Lung Cancer (20). The χ^2 test and Fisher's exact test revealed that hsa_tsr011468 was associated with tumor size (Table I). Overall, these

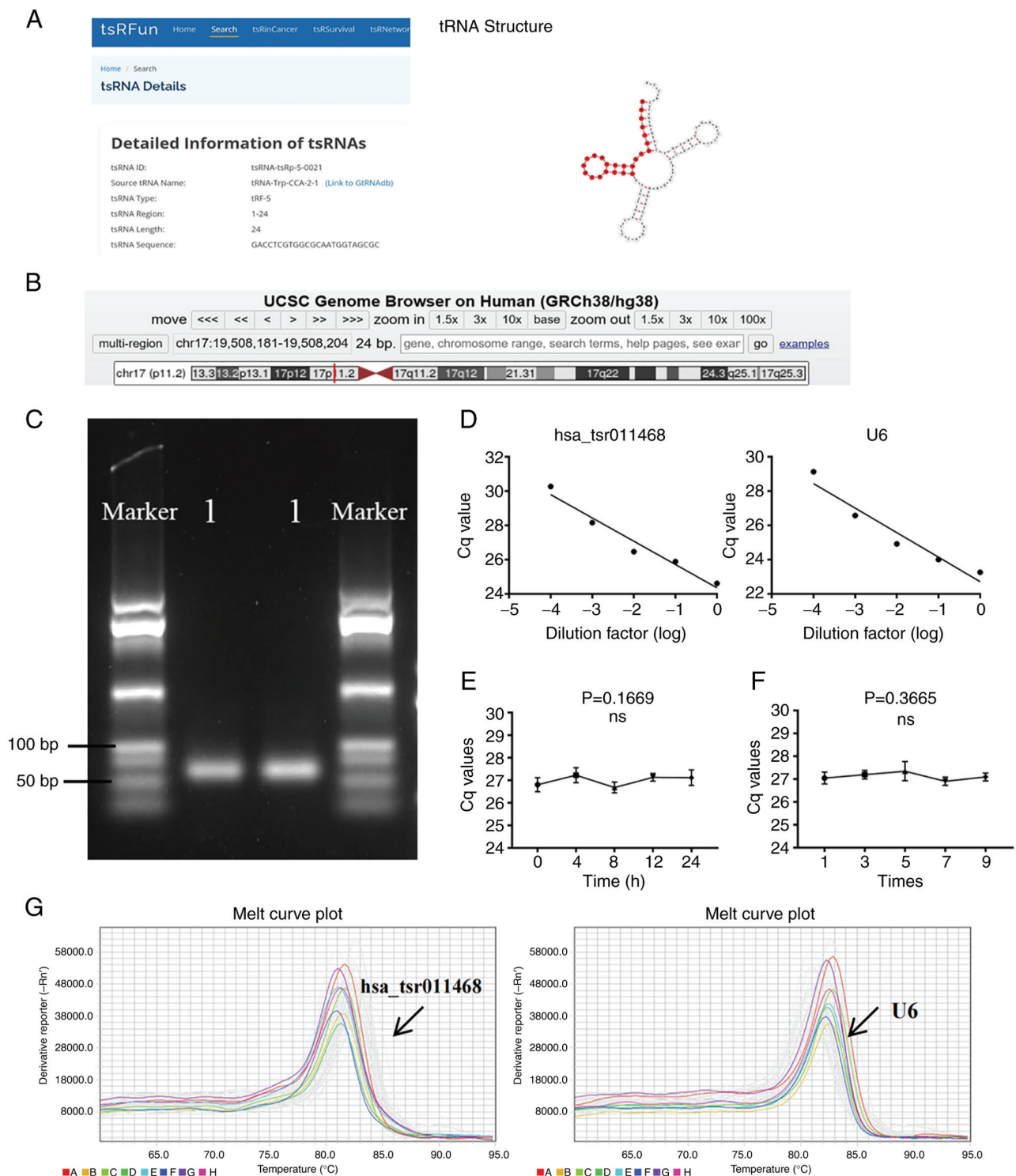


Figure 2. Identification of hsa_tsr011468. Database search results showed the (A) structure and (B) chromosomal localization of hsa_tsr011468. (C) Agarose gel electrophoresis of the PCR product of hsa_tsr011468. (D) Linearity of hsa_tsr011468 and U6. (E) Detection of hsa_tsr011468 expression in mixed sera after 0, 4, 8, 12 and 24 h of storage at room temperature. (F) Detection of the expression of hsa_tsr011468 in mixed sera after repeating freeze-thaw cycles 1, 3, 5, 7 and 9 times. (G) Smooth and single-peak-specific melting curves for hsa_tsr011468 and U6. tRNA, transfer RNA; tsRNA, tRNA-derived small RNA; ns, not significant.

data indicated that hsa_tsr011468 could serve as a specific biomarker for LUAD.

Diagnostic value of hsa_tsr011468 in LUAD. As aforementioned, hsa_tsr011468 is significantly associated with LUAD and is a highly expressed molecule in this type of cancer;

therefore, it may be considered a potential LUAD biomarker. However, its diagnostic value in LUAD requires further investigation. The results of ROC analysis showed that the AUC values of the common lung cancer diagnostic markers cytokeratin 19 fragment, carcinoembryonic antigen (CEA) and SCC antigen were 0.581, 0.686 and 0.641, respectively,

Table I. Relationship of hsa_tsr011468 expression with clinicopathological features in patients with lung adenocarcinoma.

Characteristic	Cases (n=84)	hsa_tsr011468 expression		P-value
		High (n=42)	Low (n=42)	
Age, years				0.498
≤60	31	14	17	
>60	53	28	25	
Sex				0.059
Male	26	17	9	
Female	58	25	33	
Tumor size, cm				0.023 ^a
<5	54	22	32	
≥5	30	20	10	
Tumor differentiation				0.078
Well + Moderate	48	20	28	
Poor	36	22	14	
Lymphatic metastasis				0.503
No	51	27	24	
Yes	33	15	18	
TNM stage				0.818
I-II	55	27	28	
III-IV	29	15	14	
Smoking status				0.287
Smoker	18	11	7	
Never-smoker	66	31	35	
Serum ProGRP, pg/ml				0.801
≤50	63	31	32	
>50	21	11	10	
Serum Cyfra21-1, ng/ml				0.763
≤3.3	71	36	35	
>3.3	13	6	7	
Serum NSE, ng/ml				0.533
≤16	72	35	37	
>16	12	7	5	
Serum CEA, ng/ml				0.067
≤5	71	32	39	
>5	13	10	3	
Serum SCC antigen, mg/l				0.313
<1.5	74	35	39	
≥1.5	10	7	3	

^aP<0.05. TNM, tumor-node-metastasis; ProGRP, progastrin-releasing peptide; Cyfra21-1, cytokeratin 19 fragment; NSE, neuron-specific enolase; CEA, carcinoembryonic antigen; SCC, squamous cell carcinoma.

whereas the AUC value of hsa_tsr011468 was 0.763, indicating a better diagnostic value (Fig. 4A). When combined with other common lung cancer diagnostic indicators, the AUC value for hsa_tsr011468 was notably increased (Fig. 4B and C). Furthermore, the AUC value increased to 0.840 after hsa_tsr011468 was combined with all common lung cancer indicators (Fig. 4D). In addition, the sensitivity of the diagnosis increased from 57.14 to 67.86% when

combined with all other common indicators of lung cancer (Table II).

Dynamic monitoring of hsa_tsr011468 in LUAD. To investigate the dynamic monitoring capability of hsa_tsr011468, preoperative and postoperative sera were collected from 70 patients with LUAD for qPCR analysis. The results revealed that the relative expression levels of hsa_tsr011468 in the

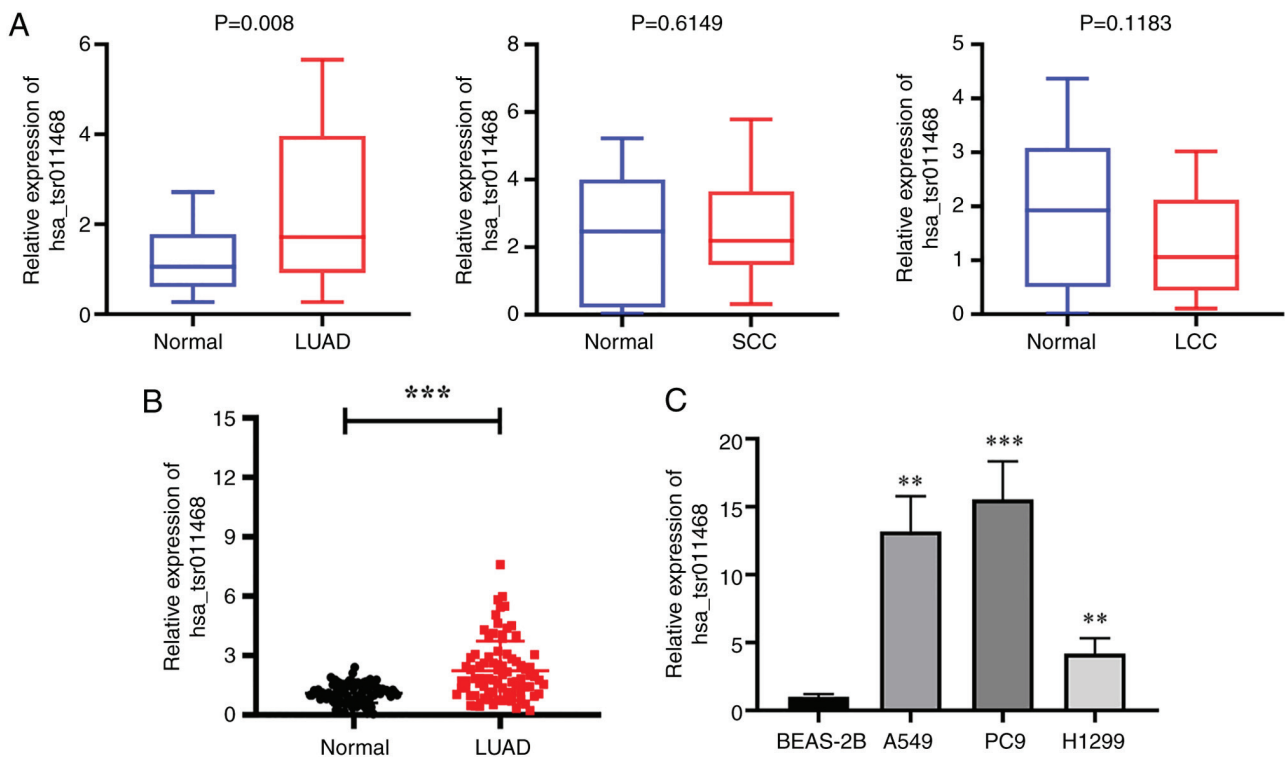


Figure 3. Diagnostic value of hsa_tsr011468. (A) Expression of hsa_tsr011468 in serum samples from patients with LUAD, SCC and LCC, and normal controls. (B) Relative expression levels of hsa_tsr011468 in LUAD serum samples. (C) Expression of hsa_tsr011468 in cell lines. ** $P < 0.01$, *** $P < 0.001$ vs. BEAS-2B or as indicated. LCC, large cell carcinoma; LUAD, lung adenocarcinoma; SCC, squamous cell carcinoma.

postoperative sera from patients was significantly lower than that in the preoperative sera (Fig. 5A). Furthermore, patients with LUAD were divided into high- and low-expression groups based on hsa_tsr011468 serum expression for Kaplan-Meier survival analysis and the survival curves were compared using the log-rank test. As shown in Fig. 5B, the disease-free survival time of the low-expression group was longer compared with that in the high-expression group. Moreover, the overall survival of the low-expression group of hsa_tsr011468 was higher than that of the high-expression group, providing additional evidence of the dynamic monitoring capability and prognostic significance of hsa_tsr011468.

Prediction of downstream regulatory mechanisms for hsa_tsr011468. The aforementioned experiments highlighted the significance of hsa_tsr011468 as a potential biomarker for LUAD. The present study also investigated its potential mechanism in LUAD and observed via cellular localization experiments that hsa_tsr011468, consistent with control 18S, was predominantly located in the cytoplasm (Fig. 6A). Subsequently, TargetScan, miRanda, RNAhybrid and tRFTar databases were used to predict the downstream target molecules of hsa_tsr011468, and a Venn diagram was generated. The analysis revealed an overlapping gene in these databases: DBF4 (Fig. 6B). In addition, KEGG bioinformatics analysis revealed that it was associated with the 'p53 signaling pathway', 'Cell cycle' and 'Protein processing in endoplasmic reticulum' (Fig. 6C). GO bioinformatics revealed that hsa_tsr011468 was associated with the 'regulation of cell development', 'cell projection membrane' and 'ctin cytoskeleton' (Fig. 6D). Therefore, hsa_tsr011468 may regulate LUAD progression at the cellular level via DBF4.

Discussion

Epidemiological data indicated that, in 2022, lung cancer was the most common type of cancer in China and accounted for the largest number of cancer-related deaths (21). LUAD is the most prevalent subtype of lung cancer, and researchers are increasingly investigating various functions of non-coding RNAs in LUAD. Specifically, long non-coding RNAs and circular RNAs have demonstrated potential as biomarkers for the detection, progression and drug resistance mechanisms of LUAD, thereby presenting new opportunities for scientific investigation (22-25). However, there are few studies on tsRNAs in LUAD. Therefore, the present study evaluated the effect of hsa_tsr011468 on LUAD pathogenesis and its potential as a LUAD marker.

tsRNAs are RNA molecules cut from mature tRNA or pre-tRNA that have a specific sequence structure and biological function (26). A growing number of studies have shown that aberrant expression of tsRNAs affects tumor development, and is indicative of early diagnosis and prognosis. For example, tRF-19-3L7L73JD expression in preoperative patients with gastric cancer was observed to be reduced compared with that in postoperative and healthy individuals, indicating its potential as a clinical diagnostic indicator for gastric cancer (27). Furthermore, the expression of 5'-tRF-GlyGCC in the plasma of patients with colorectal cancer was significantly higher than that in healthy individuals, and the AUC value of the working characteristics of the subjects verified that 5'-tRF-GlyGCC had a better diagnostic value than CEA or CA19-9 (28). Additionally, hsa_tsr016141 has been reported to have more

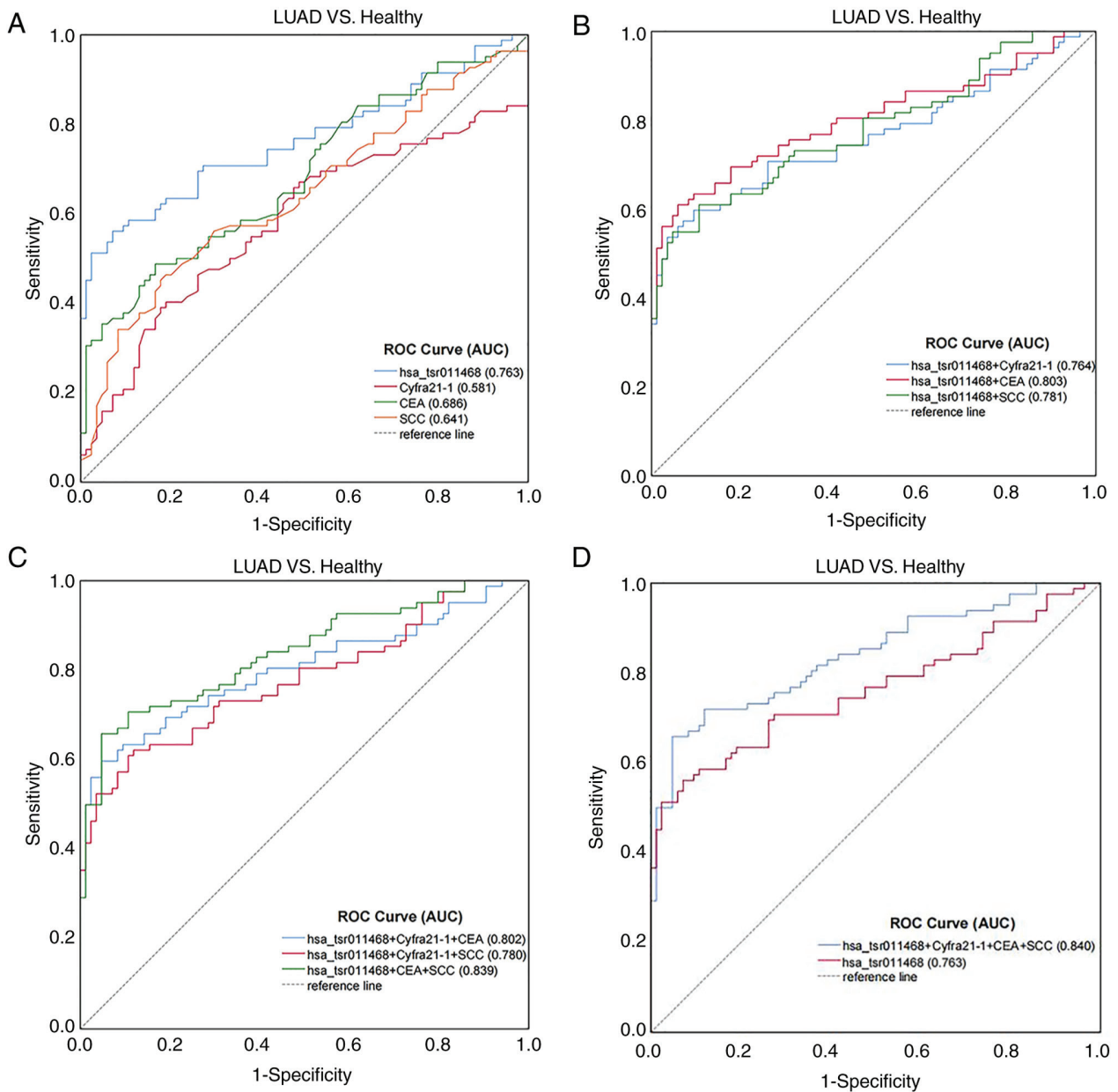


Figure 4. Evaluation of the diagnostic use of hsa_tsr011468 in LUAD. (A) ROC analysis of the independent use of plasma hsa_tsr011468, Cyfra21-1, CEA and SCC antigen in distinguishing patients with LUAD from healthy controls. (B) ROC curve analysis of the combined diagnostic value of hsa_tsr011468 + Cyfra21-1, CEA or SCC. (C) ROC curve analysis of the combined diagnostic value of hsa_tsr011468 + Cyfra21-1 + CEA or SCC, and of hsa_tsr011468 + CEA + SCC. (D) ROC curve analysis of the combined diagnostic value of hsa_tsr011468 + Cyfra21-1 + CEA + SCC. AUC, area under the curve; CEA, carcinoembryonic antigen; Cyfra21-1, cytokeratin 19 fragment; LUAD, lung adenocarcinoma; ROC, receiver operating characteristic; SCC, squamous cell carcinoma.

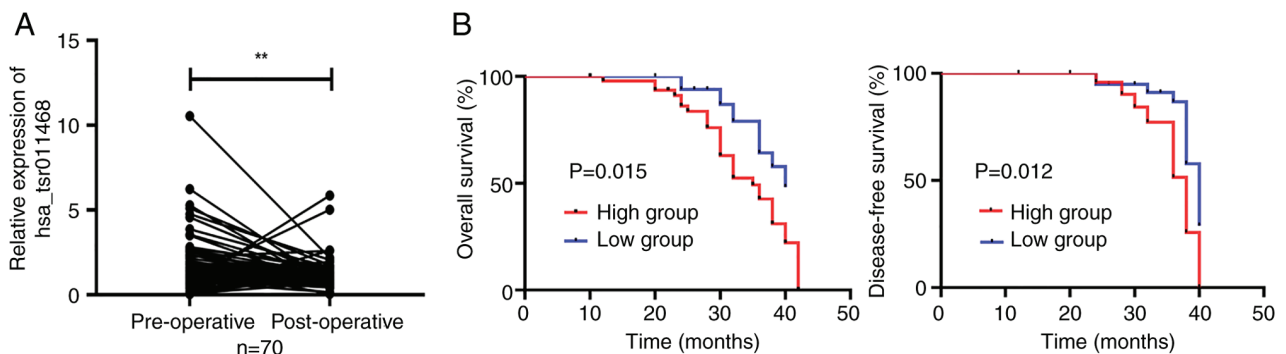


Figure 5. Dynamic monitoring and prognostic ability of hsa_tsr011468 in LUAD. (A) Serum expression levels of hsa_tsr011468 in preoperative and postoperative patients with LUAD. (B) Kaplan-Meier curve analysis of hsa_tsr011468 expression for disease-free survival and overall survival in patients with LUAD. **P<0.01. LUAD, lung adenocarcinoma.

Table II. Use of hsa_tsr011468, Cyfra21-1, CEA and SCC antigen to distinguish patients with LUAD from healthy controls.

Indicator	SEN (%)	SPE (%)	ACCU (%)	PPV (%)	NPV (%)
hsa_tsr011468	57.14	92.05	75.00	87.27	69.23
Cyfra21-1	15.48	93.18	55.23	68.42	53.59
CEA	15.48	94.32	55.81	72.22	53.90
SCC antigen	11.90	78.41	44.77	34.48	48.25
hsa_tsr011468 + Cyfra21-1	64.29	86.36	75.58	81.82	71.70
hsa_tsr011468 + CEA	59.52	87.50	73.84	81.97	69.37
hsa_tsr011468 + SCC antigen	59.52	73.86	66.86	68.49	65.66
hsa_tsr011468 + Cyfra21-1 + CEA	66.67	86.36	76.74	66.67	86.36
hsa_tsr011468 + Cyfra21-1 + SCC antigen	66.67	69.32	68.02	67.47	68.54
hsa_tsr011468 + CEA + SCC antigen	60.71	70.45	65.70	66.23	65.26
hsa_tsr011468 + Cyfra21-1 + CEA + SCC antigen	67.86	69.32	68.61	67.86	69.32

SEN, sensitivity; SPE, specificity; ACCU, overall accuracy; PPV, positive predictive value; NPV, negative predictive value; Cyfra21-1, cyto-keratin 19 fragment; CEA, carcinoembryonic antigen; SCC, squamous cell carcinoma.

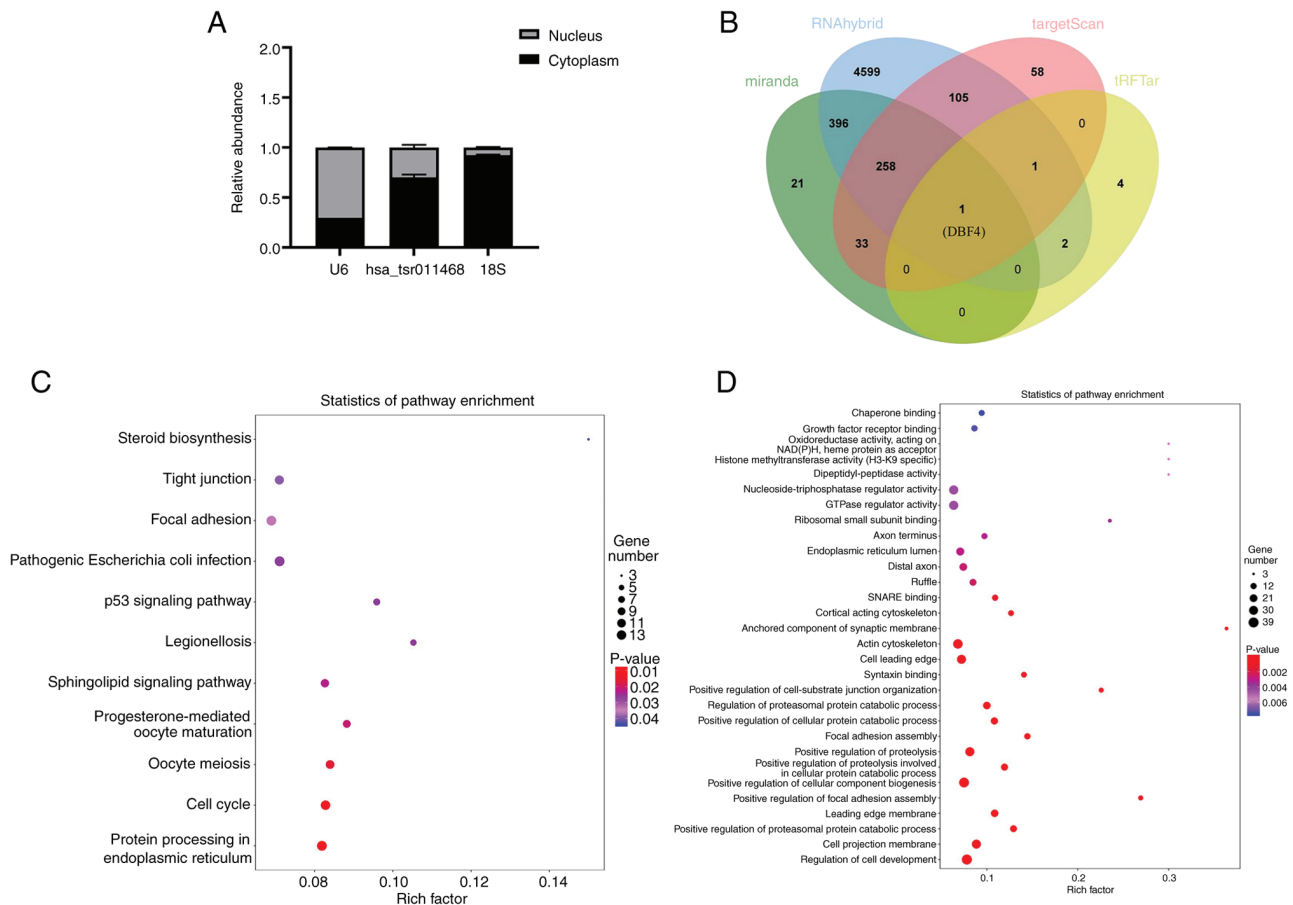


Figure 6. Mechanistic studies of hsa_tsr011468 in LUAD. (A) Localization of hsa_tsr011468 in LUAD. (B) Venn diagram plotting of hsa_tsr011468 database prediction results. (C) Kyoto Encyclopedia of Genes and Genomes and (D) Gene Ontology functional enrichment analyses of hsa_tsr011468. LUAD, lung adenocarcinoma.

diagnostic value than CA724 in gastric cancer (29). The aforementioned studies showed that tsRNAs have potential for cancer diagnosis, and provide new directions for cancer prognosis and targeted therapies.

The present study screened the OncotRF database and identified the highly expressed tsRNA, hsa_tsr011468, in LUAD. Statistical data on clinicopathological features showed that hsa_tsr011468 was associated with tumor size. ROC

curve analysis was conducted to assess the diagnostic value of hsa_tsr011468 compared with other lung cancer indicators in LUAD, and a survival curve was generated to evaluate the prognostic value of hsa_tsr011468. Furthermore, GO and KEGG analyses revealed that the downstream target molecule of hsa_tsr011468 may be DBF4. DBF4 acts as a crucial cell cycle regulator by binding to cell division cycle 7 to form a DBF4-dependent kinase, which serves a role in positively regulating DNA replication in the nuclear cell cycle and in regulating cell cycle phase transitions, and is significant in tumor development (30,31). In addition, the 'p53 signaling pathway' was identified as an enriched KEGG pathway. Notably, p53 is a classical signaling pathway that regulates cell survival and death, which has also been shown to be significant in a number of studies on the mechanism of RNA molecule-regulated cancer (32,33). A previous analysis of 201 microarray samples from various genetically engineered mouse breast cancer models indicated that p53-altered mammary tumors had elevated DBF4 mRNA expression (34). In addition, p53 as a transcription factor can regulate the expression of various genes including those associated with the cell cycle (35-37). However, further experiments are needed to verify the specific mechanisms regarding whether DBF4 can act as a target gene to affect the p53 pathway and hsa_tsr011468.

The present study has some limitations. Primarily, insufficient clinicopathological features related to LUAD were assessed, leading to an incomplete analysis of the results. Recently, EGFR mutation status, EML4-ALK and PD-L1 expression have been introduced as new targets and research directions for clinical lung cancer treatment (38-40); however, the present study lacks a discussion of these factors. Therefore, these factors will be explored in subsequent studies on the specific mechanism and targeted therapy of hsa_tsr011468 in LUAD to further determine the role of hsa_tsr011468 in LUAD.

In conclusion, the present study analyzed significant tsRNAs in LUAD. Firstly, RT-qPCR analysis of LUAD serum and tissue samples was performed, which indicated that patients with LUAD had increased expression of hsa_tsr011468. Subsequently, the ROC curve and survival analyses showed that hsa_tsr011468 had a good diagnostic and prognostic value. By comparing the relative expression levels of hsa_tsr011468 in the preoperative and postoperative serum of patients, it was revealed that its expression was decreased in the postoperative serum of patients. This finding suggested that hsa_tsr011468 may possess strong dynamic detection capabilities. Therefore, it could be proposed that hsa_tsr011468 may serve as a potential novel serological biomarker for LUAD. Finally, the target genes and signaling pathways of hsa_tsr011468 in LUAD were predicted, establishing a foundation for further research into its specific mechanism of action.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

PZ and KZ performed the experiments, analyzed and interpreted the data, and wrote the paper. CX contributed reagents, materials, analysis tools and collected data. SL and CX conceived and designed the experiments, and wrote the paper. PZ and KZ confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent for participation

The present study was approved by the Nantong First People's Hospital and Affiliated Hospital of Nantong University (approval nos. 2023KT197 and 2019-L071) and all participants provided written informed consent for this study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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