INTRODUCTION

The most frequent malignant tumor in humans is lung cancer. In China, mortality of lung cancer continually occupies the first place among all malignancies. Non-small cell lung cancer (NSCLC) is responsible for almost 80 percent of all cases related to lung cancer. Despite the development of molecular targeted therapies in lung cancers, patients’ 5-year survival estimates are still dismal, mainly because most patients reached an advanced stage when diagnosed. Traditional tumor markers, such as neuron-specific...
enolase (NSE) and cytokeratin fragment 21-1 (CYFRA21-1), were restricted in clinical utilization due to inferior sensitivity and specificity. Therefore, it is critical to explore novel and effective biomarkers in NSCLC.

Small non-coding RNAs (ncRNAs) formed from matured tRNAs or pre-tRNAs are known as transfer RNA (tRNA)-derived small RNAs (tsRNAs). Based on the cleavage site and length, they are divided into two categories: tRNA-derived fragments (tRFs) and tRNA halves (tiRNAs). tRFs are further split into tRF-1, tRF-3, and tRF-5 based on the tRNA splicing site, whereas tiRNAs are separated into two subtypes based on the anticodon cleavage site: 5’tiRNAs and 3’tiRNAs.

Growing evidence suggests that tsRNAs play important roles in multifarious human illnesses due to their part in cancer cells growth, translation regulation, and genetic silencing. Therefore, it is critical to explore novel and effective biomarkers in NSCLC.

To reverse transcribe total RNA, the ImProm-II Reverse Transcription System (Promega,) was utilized according to the directions provided by the manufacturer. Based on the reported literatures, stem-loop qRT-PCR method was used for quantification of mature tRFs, and miR-16 was selected as an internal control for the quantification of tRFs in serum. The 2−ΔΔCt technique was utilized in order to establish the related expression levels. RT and qPCR primers were used in this study: tRF-31-79MP9P9NH57SD stem-loop primer: 5’-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTG CATA CGACCGC GAA CGT-3’. The underlined nucleotides are complemented with tRF-31-79MP9P9NH57SD. miR-16 stem-loop primer: 5’-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTG CATA CGACCGC GAA CGT-3’, Forward qPCR primer for tRF-31-79MP9P9NH57SD: 5’-GACGACGGTTCGATG TAGTG-3’, Forward qPCR primer for miR-16: 5’-GTCGCCTGAGACCA GTAAAA-3’, Universal Reverse qPCR Primer: 5’-CCAGTGCAGGGTCC GAG GTA-3’.

**2.3 | qRT-PCR**

**2.4 | Statistical analysis**

GraphPad Prism 7.0 (GraphPad, Inc.,) was used for statistical analysis. For data comparison, statistical techniques such as Student’s t-test and chi-square test were utilized when needed. The ROC curve studies were performed using MedCalc 11.0 (MedCalc, Ostend, Belgium). In these studies, the significance was considered to be at p < 0.05.

**3 | RESULTS**

**3.1 | Characteristics of the tRF-31-79MP9P9NH57SD**

In MINTbase v2.0, tRF-31-79MP9P9NH57SD is a type of 5’-tRF with a length of 31 nt (5’-GGTTCCCGTAGTGATGTTATCACGTTGC GC-3’) (Figure 1A). tRF-31-79MP9P9NH57SD is derived from mature tRNAVal-CAC, and its secondary structure is shown in Figure 1B. Then, to amplify the tRF-31-79MP9P9NH57SD, we created one set of primers, and the amplified products were analyzed through melting curve and Sanger sequencing. Our findings revealed that the amplified product only produced a single peak. (Figure 1C), and the sequences were coincident with that in MINTbase v2.0 (Figure 1D). The data showed that tRF-31-79MP9P9NH57SD could be amplified by qRT-PCR availability.

**3.2 | Highly expressed tRF-31-79MP9P9NH57SD in serum of NSCLC patients**

qRT-PCR detected the tRF-31-79MP9P9NH57SD expression in serum samples of the 96 NSCLC patients and healthy controls. The
expression of tRF-31-79MP9P9NH57SD was substantially increased in NSCLC patients as to that of the healthy controls, according to our findings (Figure 2A). Next, tRF-31-79MP9P9NH57SD expression in another group including 20 NSCLC pre- and post-surgical patients and 20 healthy human subjects (termed as controls) was detected. The resulting outcome reveals that tRF-31-79MP9P9NH57SD was enriched in NSCLC patient’s serum before the surgical procedure (Figure 2B).

3.3 Clinical implications of serum tRF-31-79MP9P9NH57SD in NSCLC

We further explored the connection between the serum tRF-31-79MP9P9NH57SD expression and clinicopathological parameters in NSCLC. Based on qRT-PCR results, the patients were separated into two groups: those with elevated tRF-31-79MP9P9NH57SD expression and those with reduced tRF-31-79MP9P9NH57SD expression.
Table 1 shows a correlation between serum tRF-31-79MP9P9NH57SD expression and clinical stage (p = 0.002) and lymph node metastases (p = 0.012). However, no relationship was detected between tRF-31-79MP9P9NH57SD expression and other clinical features, such as age, gender, smoking status, and tumor differentiation stages. Then, the ROC curve was constructed to estimate the diagnostic value of tRF-31-79MP9P9NH57SD, NSE, CYFRA21-1, or a combination of these biomarkers (Panel). The area under the ROC curve (AUC) of tRF-31-79MP9P9NH57SD got to 0.733, with 48.96 percent sensitivity and 90.62 percent specificity, as shown in Figure 3. Moreover, we also found that the diagnostic accuracy of the Panel was higher than that of either single biomarker (Table S1). Collectively, these results suggested that serum tRF-31-79MP9P9NH57SD might have potential values for NSCLC.

### 3.4 Exploration of the downstream and function prediction of tRF-31-79MP9P9NH57SD

In the TargetScan, miRanda, and TargetRank databases, we predicted downstream targets of tRF-31-79MP9P9NH57SD in a Venny diagram (Figure 4A). Among the linked genes that demonstrated the
most overlap were ten genes (SACS, ERGIC2, KIF5C, IGF1R, ATP10D, MAP3K2, GALNT10, RMND5A, ZNF33B and OAS3). We then sought to look into the molecular mechanism of tRF-31-79MP9P9NH57SD. KEGG Enrichment analysis indicated that tRF-31-79MP9P9NH57SD was enriched in virus infection, a variety of tumors and mTOR signaling pathway (Figure 4B). tRF-31-79MP9P9NH57SD may have a role in RNA biosynthesis and transcription control, according to GO functional enrichment analysis of the target genes (Figure 4C). Therefore, the mechanisms of tRF-31-79MP9P9NH57SD in the regulation of NSCLC need to be further investigated.

4 | DISCUSSION

The primary function of tRNAs is to transport amino acids to the ribosome, allowing the synthesis of the appropriate protein to proceed more quickly under the supervision of the mRNA. tRFs and tRNAs have lately been discovered in a range of malignancies,\textsuperscript{20} according to some research. By regulating transcription, modifying mRNA stability, silencing target genes, and engaging in the cellular stress response, tRFs and tRNAs can influence cancer formation.\textsuperscript{21–23} Han et al. reported that tRF-3008a suppresses colorectal cancer (CRC) metastasis by repressing endogenous FOXK1, and it could be utilized as a possible prognostic biological marker for CRC.\textsuperscript{24} Parallel outcomes were observed that tRF-3008a could suppress cells malignant activity silencing THBS1 in breast cancer.\textsuperscript{25}

Recently, several tRFs have been reported to be diagnostic biomarkers in lung cancer and other malignancies.\textsuperscript{15,26,27} In this study, we screened tRFs from high-throughput sequencing databases and found tRF-31-79MP9P9NH57SD may be a lung cancer-associated tRF. Then, stem-loop qRT-PCR method was used for quantification of tRF-31-79MP9P9NH57SD. Our results showed that the NSCLC...
patients exhibited dramatically increased serum quantities of tRF-31-79MP9P9NH57SD compared with healthy donors. Statistical analysis demonstrated that increased tRF-31-79MP9P9NH57SD expression was directly linked to tumor size (p = 0.001) and malignant condition of the lymph node (p = 0.038). After measuring serum tRF-31-79MP9P9NH57SD expression pre- and post-surgical patients, it was discovered that levels of tRF-31-79MP9P9NH57SD declined dramatically after surgery, which could be a symptom of tumor recurrence. Zhu et al. reported that tRFs could be secreted from tumor cells by exosomes. We speculate that tRF-31-79MP9P9NH57SD might be secreted into serum by exosomes. However, this still needs further experiments to confirm.

At present, the mechanisms underlying tRFs on cancer occurrence are largely unknown. Bioinformatics analysis employing mRNA target-predicting techniques was used to investigate the downstream targets of tRF-31-79MP9P9NH57SD. Ten linked genes exhibited the most overlap between these approaches. Next, we need to carry out corresponding experiments to identify the targets and functions of tRF-31-79MP9P9NH57SD in NSCLC.

5 | CONCLUSION

To conclude, this research discovered that serum tRF-31-79MP9P9NH57SD expression is elevated in NSCLC, and has strong diagnostic importance, insinuating that tRF-31-79MP9P9NH57SD serum may be a novel diagnostic marker for NSCLC.

AUTHOR CONTRIBUTIONS

The study was conceived and carried out by LJ and YW. CC and FL were involved in obtaining ethical approval, collecting samples, performing qRT-PCR, and analyzing data. The manuscript’s first version was written by LJ. The final manuscript was reviewed and approved by all authors.

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CONFLICT OF INTERESTS

None.

DATA AVAILABILITY STATEMENT

On reasonable request, the corresponding author will provide the datasets used and/or analyzed during the current work.

CONSENT FOR PUBLICATION

Not applicable.

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**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of the article at the publisher’s website.

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