Original Article

Expression profiles of tRNA-derived fragments and their potential roles in lung adenocarcinoma

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Background: Transfer RNA-derived fragments (tRFs) and transfer RNA halves (tiRNAs) have been shown to play crucial roles in gene regulation. This study aims to reveal the expression profiles of tRFs and tiRNAs and their possible biological roles in lung adenocarcinoma (LUAD).

Methods: Five paired clinical lung adenocarcinoma tissues (LAT) and adjacent normal lung tissues (ANLT) were selected to analyze the expression of tRFs and tiRNAs. Six significantly expressed tRFs and tiRNAs were selected and validated by Quantitative Real-time PCR (qPCR). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed.

Results: The sequencing results showed that 109 tRFs and tiRNAs were differentially expressed between LAT and ANLT, out of which 60 were upregulated and 49 were downregulated. Compared with ANLT, lower expression levels of 3 tRF-1s (tRF-Ser-TGA-010, tRF-Arg-CCT-018, and tRF-Val-CAC-017) in LAT were verified by qPCR. Subsequently, the putative target genes of tRF-1s were analyzed by computational prediction and the top 10 significant results of GO and KEGG pathway enrichment analysis were presented.

Conclusions: This study has revealed the landscape of tRF and tiRNA expression profiles in LUAD. Three newly found differentially expressed downregulated tRF-1s may be involved in the pathogenesis of LUAD and may serve as potential diagnostic biomarkers, or otherwise reconcile target genes for drug development.

Keywords: Lung adenocarcinoma (LUAD); regulatory networks; transfer RNA-derived fragments (tRFs); tRF and tiRNA sequencing; tRF-1s

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Introduction

Lung cancer ranks first in terms of cancer morbidity and mortality worldwide. In 2018, the estimated number of fatalities was 1.8 million, close to one fifth (18.4%) of the total cases (1). Lung adenocarcinoma (LUAD), which accounts for about 40% of all lung cancer cases, is the most prevalent subtype (2). However, since the majority of LUAD patients are diagnosed at the advanced stages, there

is a very low 5-year survival rate among these patients (3).

Small non-coding RNAs (sRNAs) have been found to play important roles in the regulation of diverse biological processes (4). LUAD is associated with dysregulated expression of sRNAs such as long non-coding RNAs (lncRNAs) and microRNAs (miRNAs). Significantly expressed sRNAs serve as diagnostic biomarkers and potential therapeutic targets of LUAD (5). With the rapid development of high-throughput sequencing technologies, new classes of sRNAs, namely transfer RNA-derived fragments (tRFs) and transfer RNA halves (tiRNAs), have been identified by several independent studies (6-8). Produced by endonucleolytic cleavage of precursor and mature transfer RNAs (tRNAs), these tRNA derivatives are classified into 30–40 nt long tiRNAs (tiRNA-3 and tiRNA-5) and 18–22 nt long tRFs (tRF-1, tRF-3, tRF-5, i-tRF) (9,10). Although dysregulated expression of miRNAs has been found to be associated with the development of LUAD by many studies previously, fewer studies have focused on the biological function of tRFs and tiRNAs in LUAD, which need to explore.

It has been demonstrated that tRFs and tiRNAs are aberrantly expressed in many diseases, especially cancers (11). In 2009, Lee *et al.* first reported upregulated tRF-1001 in prostate cancer cells and revealed that the specific tRF expression was related to cell proliferation (7). Subsequently, more and more tRFs and tiRNAs with aberrant expression patterns have been discovered in various kinds of cancers, including breast cancer, lung cancer, colorectal cancer, chronic lymphocytic leukemia, and others (12-15). These tRNA derivatives were also shown to be related to many biological functions, such as cancer cell proliferation, progression, metastasis, and survival, by regulating protein translation, affecting gene expression, and so on (16-18).

Downregulated tRF-1s have been found in lung cancer cells, such as ts-46, ts-47, ts-53, and ts-101, which may regulate cancer progression (19,20). Shao *et al.* showed that higher expression of tRF-Leu-CAG (a type of tiRNA) was detected in non-small cell lung cancer tissues, cell lines, and sera, and that the inhibition of tRF-Leu-CAG suppressed cancer cell proliferation and impeded the cell cycle (14). It was also found that AURKA was repressed with the knockdown of tRF-Leu-CAG (14). These studies suggest that tRFs and tiRNAs could be novel diagnostic markers and potential therapeutic targets in lung cancer.

With the rapid development of RNA sequencing and the abundant data and information, we explored tRF and tiRNA expression profiles and their potential functions in LUAD in greater detail. In our study, we screened the tRF and tiRNA profiles of LUAD patients by high-throughput sequencing. Then, we identified and verified the differentially expressed tRFs and tiRNAs. Subsequently, their possible biological functions in the oncogenesis of LUAD were analyzed through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) biological pathway analysis.

We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-119/rc).

Methods

Study samples

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved and supervised by the Clinical Research Ethics Committee of Shengjing Hospital (Approval No. 2019PS700K). Written informed consent was obtained from all patients. In this study, five patients with early-stage lung adenocarcinoma were selected at the Department of Thoracic Surgery, Shengjing Hospital, in March 2020. Five pairs of lung adenocarcinoma tissues (LAT) and adjacent normal lung tissues (ANLT) were collected. The samples were fresh frozen in liquid nitrogen and stored at −80 ℃ until the specimens were used. No treatment was administered to patients prior to diagnosis.

RNA extraction and quality control

The quantification and quality assurance of each RNA sample were checked using the NanoDrop ND-1000 instrument (Thermo Fisher Scientific, Waltham, MA, USA). RNA integrity and DNA contamination tests were performed by denaturing agarose gel electrophoresis.

Pretreatment of tRFs and tiRNAs and library preparation

The rtStar™ tRF&tiRNA Pretreatment Kit (Catalog NO. AS-FS-005, Arraystar, MD, USA) was utilized to treat total RNA samples. According to the manufacturer's instructions, some RNA modifications that interfere with small RNA-seq library construction were removed. The instructions were as follows: (I) charged 3'-aminoacyl deacylation to 3'-hydroxyl group (3'-OH) for 3'-adaptor ligation; (II) 3'-cyclic phosphate (3'-cP) removal to 3'-OH for 3'-adaptor ligation, (III) 5'-OH phosphorylation to 5'-phosphate for

5'-adaptor ligation; (IV) demethylation of m1A and m3C for efficient reverse transcription.

Total RNA of each sample was ligated to 3' and 5' small RNA adapters sequentially. With Illumina's proprietary and amplification primers, complementary DNA (cDNA) was synthesized and then amplified. The polymerase chain reaction (PCR) amplified fragments (134–160 bp) were extracted and purified from the PAGE gel. Subsequently, the Agilent 2100 Bioanalyzer (Agilent, CA, USA) was used for accurate assessment of the quality and concentration of the sequencing library by the Agilent DNA 1000 chip kit (Agilent, part # 5067-1504).

Sequencing of tRFs and tiRNAs

The denatured and diluted libraries were loaded onto the reagent cartridge and forwarded to the sequencing run on the Illumina NextSeq 500 system for 50 cycles, according to the manufacturer's instructions.

We got the cytoplasm tRNA sequences from GtRNAdb database (21), and the mitochondria tRNA sequences from tRNAscan-SE software (22). The predicted intronic sequences (if present) were removed and an additional 3'-terminal "CCA" to each tRNA was added to generate the mature tRNA libraries. 40 nucleotides of flanking genomic sequence on either side of the original tRNA sequence were included to generate the precursor tRNA libraries (23).

Base calling and image analysis were performed with Solexa pipeline v1.8 software. Sequencing quality was examined by FastQC, and trimmed reads [pass Illumina quality filter, trimmed 5,3-adaptor bases by cutadapt (24)] were aligned allowing for 1 mismatch only to the mature tRNA sequences, then reads that did not map were aligned allowing for 1 mismatch only to precursor tRNA sequences with bowtie software (25). The abundance of tRFs and tiRNAs was assessed with sequencing counts and was standardized as counts per million (CPM) of total aligned reads.

Differential expression of tRFs and tiRNAs

Whether the results could be used for subsequent data analysis was based on alignment statistical analysis including read length, mapping ratio, and fragment sequence bias. If so, we determined not only the expression profiling of tRFs and tiRNAs, but also the differentially expressed tRFs and tiRNAs. The scatter plots, volcano plots, and hierarchical clustering analyses were used to show the expression signatures of tRFs and tiRNAs. If CPM values in all samples were less than 20, the tRFs and tiRNAs were filtered. Based on the count value using R package edgeR, the differentially expressed tRFs and tiRNAs were screened (26). For statistical computing and graphics, principal component analysis (PCA), pie charts, Venn diagrams, scatter plots, volcano plots, and hierarchical clustering were generated for the expressed tRFs and tiRNAs using R and perl environment. Fold change (cutoff 1.5) and P value (cutoff 0.05 performed only when there were replicates) were used for screening differentially expressed tRFs and tiRNAs.

Primer design and quantitative real-time PCR

Total RNA was isolated from clinical samples with TRIZOL (Invitrogen Life Technologies), then cDNA was synthesized with the rtStar™ First-Strand cDNA Synthesis Kit (3' and 5' adaptor) (catalog no. AS-FS-003, Arraystar, MD, USA). The primers were designed for the tRFs and tiRNAs using Primer 5.0 and synthesized using 2X PCR master mix (Arraystar, MD, USA). U6 was used for internal control. Quantitative real-time PCR (qPCR) was performed on the QuantStudio5 Real-time PCR System (Applied Biosystems, MA, USA). Then, the relative expression level of each tRF and tiRNA was calculated by 2-ΔCt (or $2^{-\Delta\Delta Ct}$) method.

Target prediction and functional enrichment analysis of tRFs and tiRNAs

The computational prediction of regulatory relationships between selected tRFs and tiRNAs and targets was performed using TargetScan and miRanda (27,28).

To determine the specific biological functions of the differentially expressed tRFs and tiRNAs, functional enrichment analysis was performed for the target genes based on GO and KEGG pathways. GO consists of 3 domains: biological process, cellular component, and molecular function (http://www.geneontology.org). Fisher's exact test was used to determine whether there was greater similarity among the list of differentially expressed genes and GO annotation lists than expected by chance. Significant GO term enrichment for differentially expressed genes was denoted by $P \leq 0.05$. Pathway enrichment analysis included functional analysis of mapped genes using KEGG (http://www.genome.jp/kegg/). The P value cutoff was 0.05, which indicated the significance of the pathway correlated with the conditions. The lower the P value, the more significant the pathway.

Statistical analysis

Statistical analyses were performed with SPSS v.19.0 software (SPSS Inc., Chicago, IL, USA) and presented graphs were generated using R and perl environment. A P value <0.05 was considered as the level of significance.

Results

Overview of tRF and tiRNA profiles

A total of 581 tRFs and tiRNAs were identified in this study (Table S1). Among them, 499 tRFs and tiRNAs have never been annotated in the tRFdb database (29,30) (*Figure 1A*), and 330 of them have never been annotated in the MINTbase (9). There were 228 tRFs and tiRNAs identified as novel that have never been found. A total of 375 tRFs and tiRNAs were selected for further analysis. The raw sequencing data has been uploaded to NCBI GEO, and the accession number is GSE165918 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE165918).

The stacked plot suggested that the tRF and tiRNA subtype distribution came from the same anticodon tRNA (*Figure 1B,1C*). The frequency of subtypes relative to tRFs and tiRNAs lengths is shown in *Figure 1D,1E*. The length of tRF-1s varied between 14 and 33 nt both in LAT and ANLT. The tRF and tiRNA subtypes distribution in LAT and ANLT is shown in *Figure 1F,1G*. It was found that tRF-5c and tRF-1 were the most abundant isoforms in LAT and ANLT, respectively. PCA showed differentially expressed tRF and tiRNA profile among the samples (*Figure 1H*).

Expression profiles of tRFs and tiRNAs

The scatter plots revealed that tRFs and tiRNAs were differentially expressed between LAT and ANLT (*Figure 2A*). The volcano plot showed 2-fold changes in differentially expressed tRFs and tiRNAs with statistical significance (*Figure 2B*). The result from hierarchical clustering shows distinguishable tRF and tiRNA expression profiling between LAT and ANLT (*Figure 2C*). We finally found 109 significantly differentially expressed genes, 60 of which were upregulated and 49 of which were downregulated (Table S2).

Validation using qPCR

The qPCR method was utilized to prevent false positive results in sequencing. Three upregulated tRFs, namely tRF-Val-CAC-005, tRF-Val-CAC-008, and tiRNA-Val-CAC-001, and 3 downregulated tRFs, namely tRF-Ser-TGA-010, tRF-Arg-CCT-018, and tRF-Val-CAC-017, were selected, as shown in *Table 1*. U6 was used as an internal control for tRF and tiRNA.

The results demonstrated that 3 tRF-1s (tRF-Ser-TGA-010, tRF-Arg-CCT-018, and tRF-Val-CAC-017) were significantly lower in the 5 LAT compared with ANLT (P<0.05), and they were selected for further analysis (*Figure 3*). tRF-Val-CAC-005, tRF-Val-CAC-008, and tiRNA-Val-CAC-001 were higher in LAT, but the difference was not significant (*Figure 3*). The differentially expressed tRFs are listed in *Table 2* according to fold change.

Predicted target genes of tRFs and functional enrichment analysis

We found that tRF-Ser-TGA-010 was predicted to have 79 target genes by miRanda and TargetScan, tRF-Arg-CCT-018 was predicted to have 202 target genes, and tRF-Val-CAC-017 was predicted to have 177 target genes. In addition, the tRF-1 gene regulatory module network was constructed (*Figure 4*).

We predicted the target genes of the 3 downregulated tRFs through GO and KEGG pathway analysis. GO analysis revealed that the target genes were mostly enriched in 'nervous system development' (biological process), 'golgi transport complex' (cellular component), and 'endodeoxyribonuclease activity' (molecular function) (*Figure 5A*). In the KEGG pathway analysis, 31 significantly enriched pathways related to target genes were found (P<0.05), and the top one was the 'MAPK signaling pathway' (*Figure 5B*). A number of genes involved in the MAPK signaling pathway were targets of tRFs (*Figure 6*) (31).

Discussion

At the time of introduction of new classes of sRNAs, tRFs and tiRNAs, they were formerly regarded as other types of miRNAs. Subsequently, they were verified to be the cleavage products from tRNAs, with specific sequence structure, exact expression patterns, and biological functions (7). It has been demonstrated that tRFs and tiRNAs act like miRNAs in a range of cellular processes, including oncogenic transformation (32). Furthermore, tRFs and tiRNAs are more abundant and stable than miRNAs. Therefore, the distinct expression and function of tRNA-derived fragments may involve in the development of

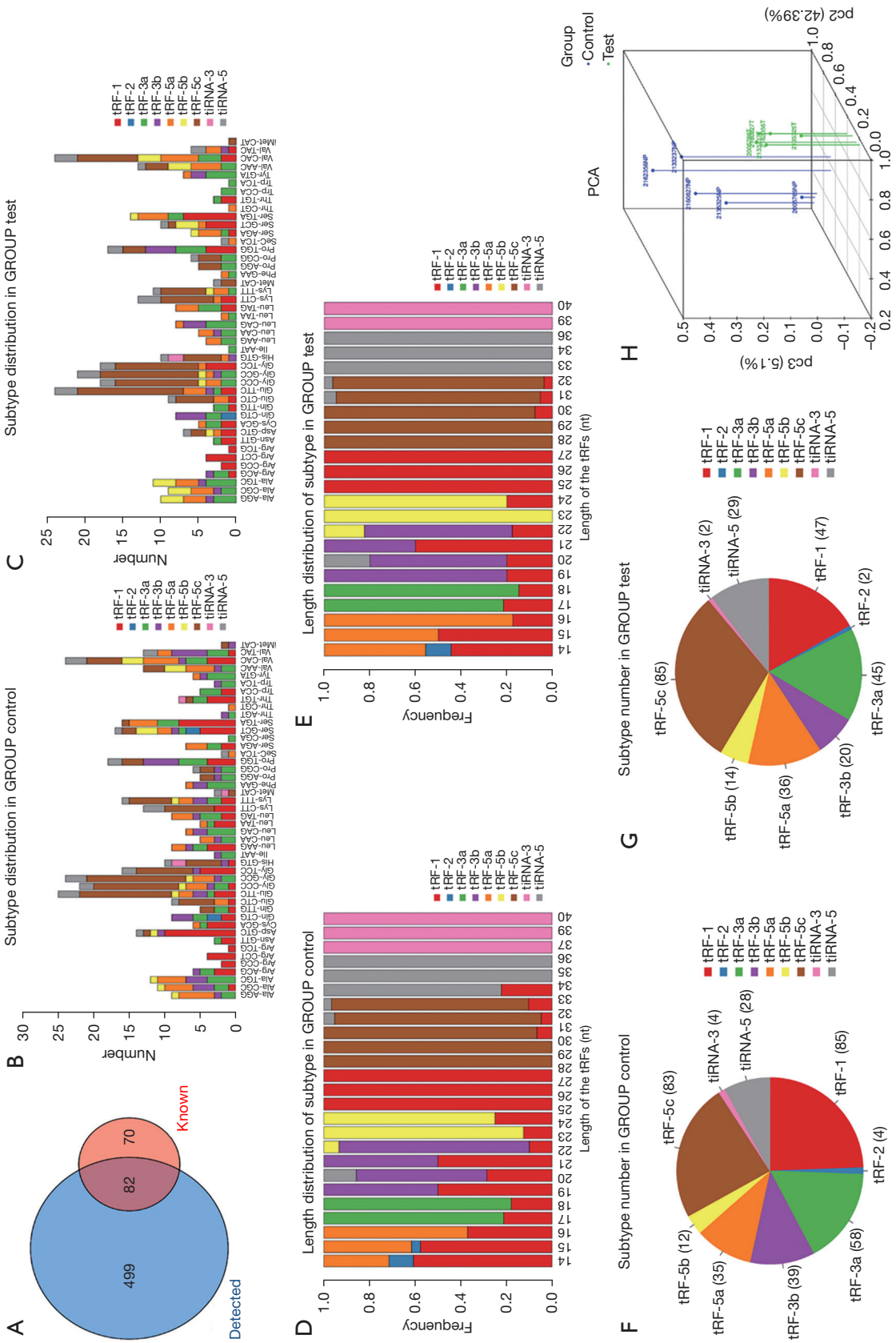

Figure 1 Overview of the expression profiles of tRFs and tiRNAs. (A) A total of 581 tRFs and tiRNAs were identified in this study, and 499 of them were identified as new tRFs and tiRNAs that had never been annotated in the tRFdb database. (B,C) The stacked plots show the distribution of tRF and tiRNA subtypes derived from the same anticodon tRNA in ANLT (B) and LAT (C). (D,E) The frequency of subtypes against the length of the tRFs and tiRNAs is shown in ANLT (D) and LAT (E). (F,G) The distribution of tRF and tiRNA subtypes in ANLT (F) and LAT (G). (H) PCA showed distinguishable tRF and tiRNA expression profiling among the samples. ANLT, adjacent normal lung tissues; LAT, lung adenocarcinoma tissues; PCA, principal component analysis; tRFs, transfer RNA-derived fragments; tiRNAs, transfer RNA halves.

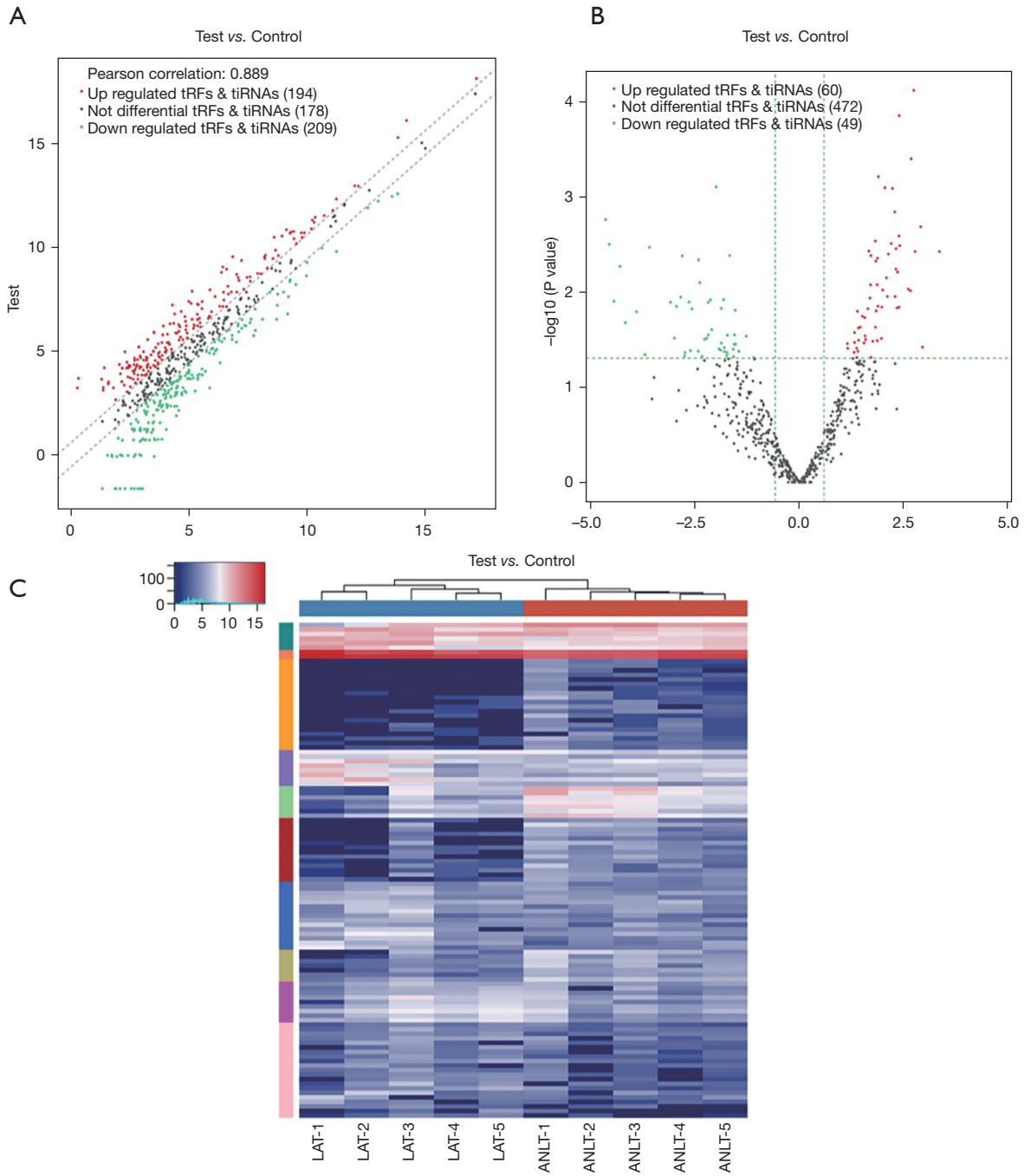

Figure 2 Sequencing analysis of tRNA-derived fragments. (A) Scatter plots of tRFs and tiRNAs show genes above the top line (red: upregulated) or below the bottom line (green: downregulated) between the 2 groups. Gray dots indicate tRFs and tiRNAs without differential expression. (B) Volcano plot shows a 2-fold change in differentially expressed tRFs and tiRNAs with statistical significance (red: upregulated; green: downregulated). (C) Hierarchical clustering indicated the expression levels of various tRFs and tiRNAs. Each square represents a gene, and its color represents the amount of expression of the gene. The darker the color, the higher the amount of expression (red is upregulated, blue is downregulated). The colored bar at the left side of the panel indicates the divisions which were performed using K-means. The group information of the tRFs and tiRNAs in each comparison can be found in Table S3. ANLT, adjacent normal lung tissues; LAT, lung adenocarcinoma tissues; tRFs, transfer RNA-derived fragments; tiRNAs, transfer RNA halves.

Table 1 The primer sequences and the optimal annealing temperatures in qPCR

Genes	Two-way primer sequence	Annealing temperature (℃)	Length (bp)
U6	F: 5'-GCTTCGGCAGCACATATACTAAAAT-3'; R: 5'-CGCTTCACGAATTTGCGTGTCAT-3'	60	89
tRF-Val-CAC-005	F: 5'-TTCTACAGTCCGACGATCGCT-3'; R: 5'-TGCTCTTCCGATCTGATAACCA-3'	60	55
tRF-Val-CAC-008	F: 5'-GACGATCGCTTCTGTAGTGTAG-3'; R: 5'-TTCCGATCTGAACGTGATAA-3'	60	45
tiRNA-Val-CAC-001	F: 5'-TGTAGTGTAGTGGTTATCACGTTCG-3'; R: 5'-CGTGTGCTCTTCCGATCTGA-3'	60	48
tRF-Ser-TGA-010	F: 5'-ACAGTCCGACGATCGAAGC-3'; R: 5'-CCGATCTAAAAAATAAGAGCACC-3'	60	43
tRF-Arg-CCT-018	F: 5'-GGCTGTGCTCGCAAGGTT-3'; R: 5'-CGTGTGCTCTTCCGATCTAAA-3'	60	42
tRF-Val-CAC-017	F: 5'-TCTACAGTCCGACGATCAAGTG-3'; R: 5'-CTCTTCCGATCTAAAACGGG-3'	60	45

qPCR, quantitative real-time polymerase chain reaction; tRF, transfer RNA-derived fragments; tiRNAs, transfer RNA halves.

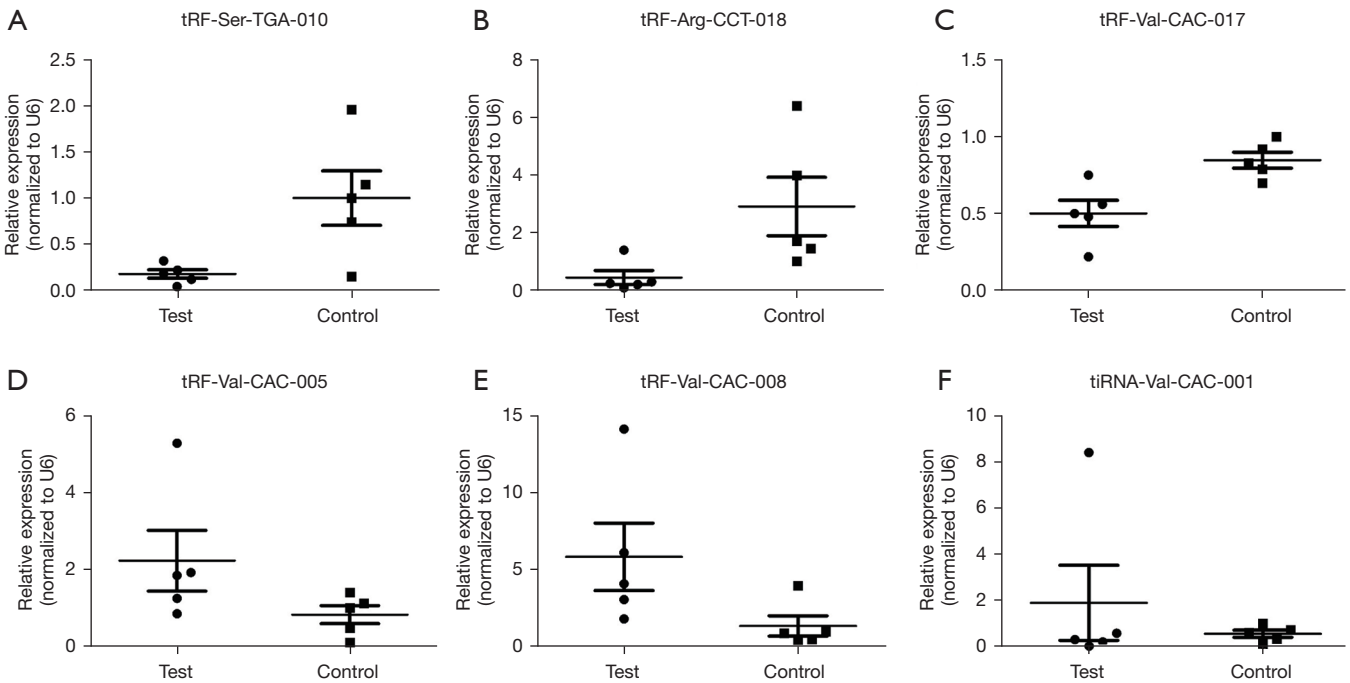

Figure 3 Validation of the expression of tRF-1s by qPCR. U6 was used for template normalization of tRF-1s. The results showed that tRF-Ser-TGA-010 (A), tRF-Arg-CCT-018 (B), and tRF-Val-CAC-017 (C) were significantly lower in the 5 LAT compared with ANLT ($P<0.05$), while tRF-Val-CAC-005 (D), tRF-Val-CAC-008 (E), and tiRNA-Val-CAC-001 (F) were higher in LAT, but the difference was not significant. ANLT, adjacent normal lung tissues; LAT, lung adenocarcinoma tissues; qPCR, quantitative real-time polymerase chain reaction; tRFs, transfer RNA-derived fragments; tiRNAs, transfer RNA halves.

cancers. They have been reported to decrease mRNA stability and to suppress mRNA translation of ribosomal proteins (33,34). At present, the research on tRFs and tiRNAs is still limited and their expression profiles and roles in cancer are still largely undefined.

We identified a total of 581 tRFs and tiRNAs were identified in this study, and 228 of them were discovered for the first time in both the tRFdb and MINTbase databases (30). This means that we are entering uncharted territory, where these new tRNA derivatives may contain some surprising answers to the pathogenesis of LUAD.

By comparing the subtype distribution in LAT and

Table 2 Three significantly differentially expressed tRFs

tRF_ID	tRF_Seq	Type	Length	Fold change	P value	q value	Regulation
tRF-Ser-TGA-010	GAAGCGGGTGCTCTTA TTTTTT	tRF-1	22	0.2824363	0.0120602	0.158925	Down
tRF-Arg-CCT-018	TCGAGAGGGGCTGTGC TCGCAAGGTTTCTTTT	tRF-1	32	0.3319403	0.0399906	0.2500561	Down
tRF-Val-CAC-017	AAGTGGTTCCCGTTTT	tRF-1	16	0.3343091	0.0478761	0.2604676	Down

tRFs, transfer RNA-derived fragments.

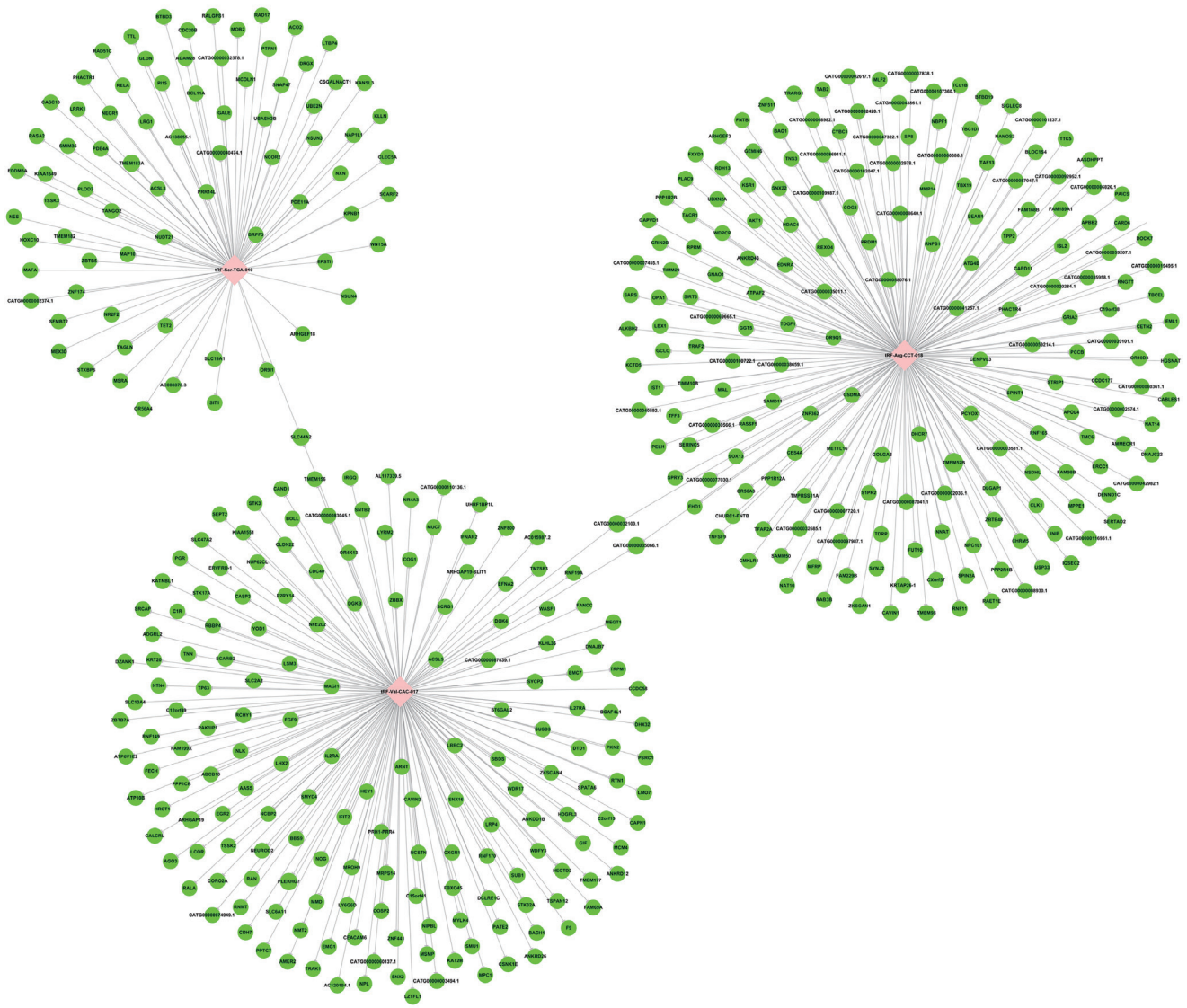

Figure 4 tRF-1-related gene regulatory module network construction including putative genes of tRF-1s (tRF-Ser-TGA-010, tRF-Arg-CCT-018, and tRF-Val-CAC-017). tRFs, transfer RNA-derived fragments.

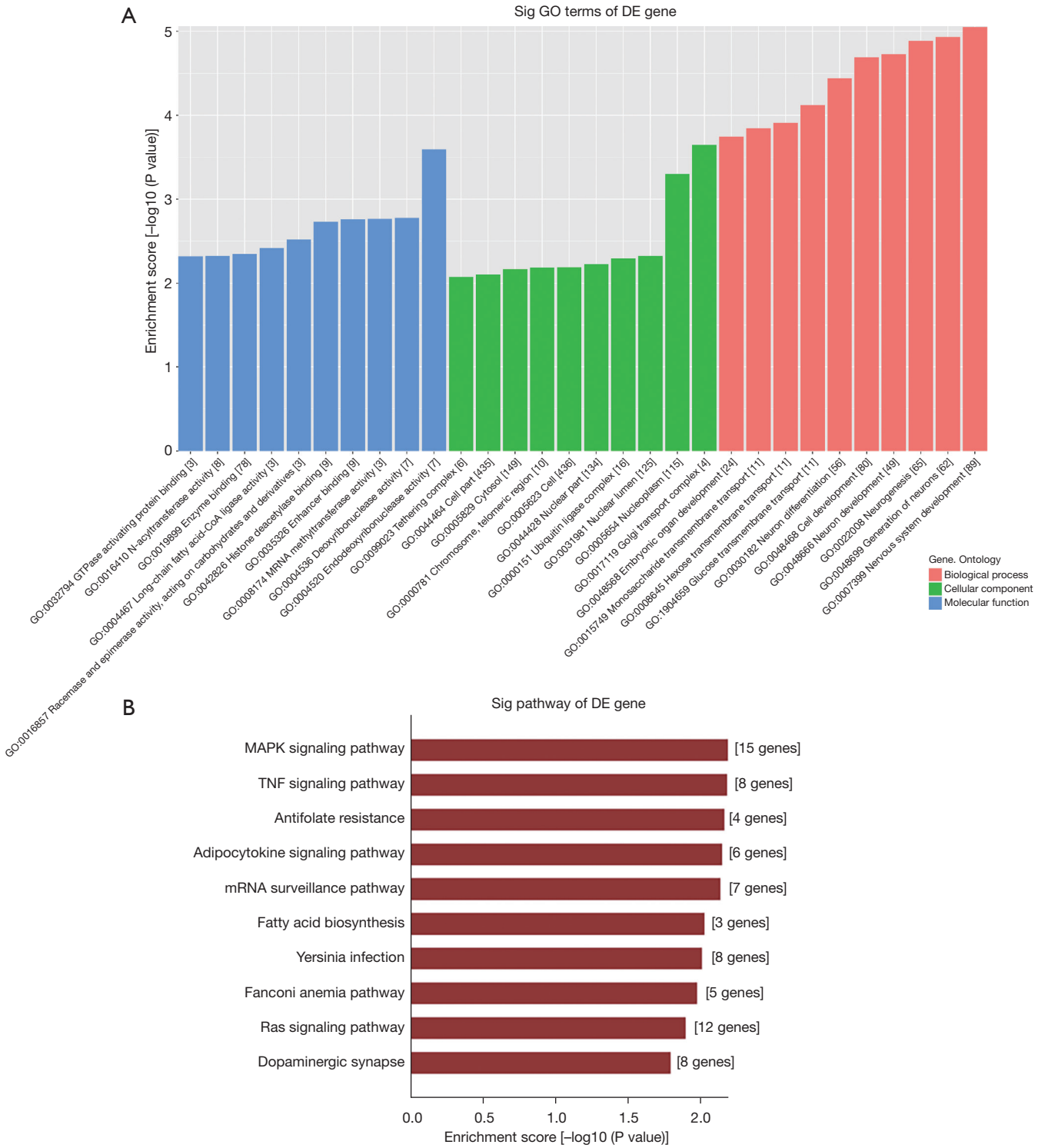

Figure 5 tRF-1-related functional enrichment analysis. (A) GO analysis showed that the target genes of tRF-1s mainly participated in 'nervous system development' (biological process), 'golgi transport complex' (cellular component), and 'endodeoxyribonuclease activity' (molecular function). (B) In the KEGG pathways, there were 31 significantly enriched pathways that corresponded to the tRF-1 target genes (P<0.05), and the top one was 'MAPK signaling pathway'. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; tRFs, transfer RNA-derived fragments.

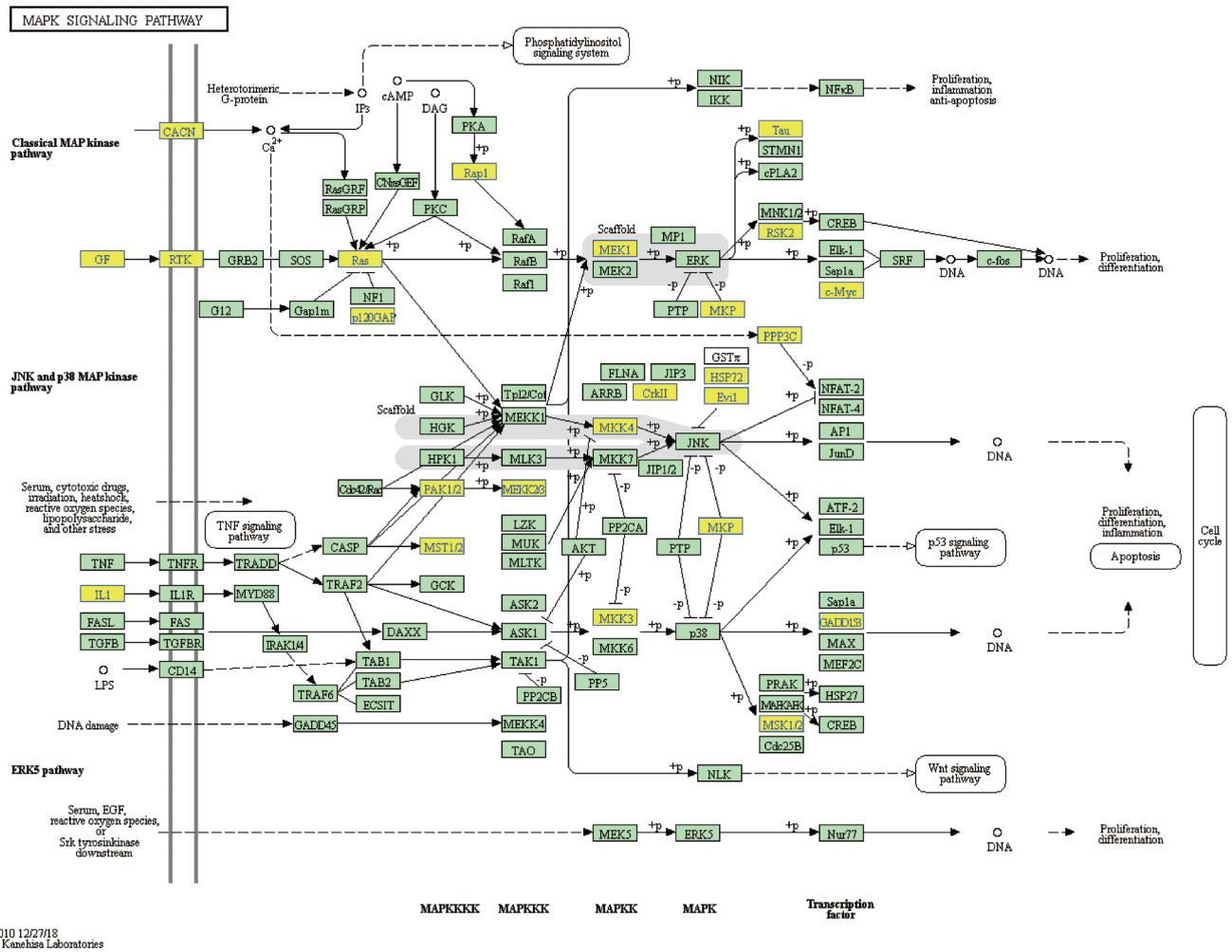

Figure 6 Mapping of the MAPK signaling pathway. Yellow marked nodes are putative molecules associated with the downregulated tRF-1s in lung adenocarcinoma. This figure was reused with permission from the KEGG website (https://www.kegg.jp/pathway/map04010). tRFs, transfer RNA-derived fragments.

ANLT, we found that tRF-5a and tRF-5c were more frequent in LAT, and tRF-3a, tRF-3b, and tRF-1 were more frequent in ANLT. In the hierarchical clustering analyses, most of the differentially expressed tRF-5s were upregulated, whereas most of the differentially expressed tRF-1s and tRF-3s were downregulated in LAT.

Strongly expressed tRFs and tiRNAs in PCA were completely distinct between LAT and ANLT. However, considering the small sample size in this study, we selected 6 differentially expressed tRNA derivatives by using qPCR in 5 paired LUAD tissues and confirmed that the 3 newly found tRF-1s (tRF-Ser-TGA-010, tRF-Arg-CCT-018, and tRF-Val-CAC-017) were all significantly downregulated in LAT. The positive results suggest that these tRFs may serve as new candidate diagnostic biomarkers for LUAD. Tissue or blood expression level in larger samples of LUAD patients with different stages and healthy people could further help determine the diagnostic and prognostic value of tRF-1s.

tRF-1s are derived from the cleavage of 3'-ends of precursor tRNAs by RNase Z or its cytoplasmic homologue ribonuclease Z 2 (35). Although there were less tRF-1s in LUAD compared with the control in this study, we still found significantly expressed downregulated tRF-1s. Previous studies showed that tRF-1s (ts-46, ts-47, ts-53, and ts-101) were downregulated in lung cancer, and that

they bound to Ago and PiwiL2 proteins and regulated pre-transcriptional gene expression (20). In our study, we found that ts-46 (tRF-His-GTG-039), ts-47 (tRF-Arg-TCG-003), and ts-101 (tRF-Ser-GCT-033) were downregulated in LAT, which was consistent with previous research (20). In a recent study, three tRF-5s (tiRNA-Lys-CTT-002, tRF-Val-CAC-011, and tRF-Val-CAC-010) were upregulated, and one tRF-1 (tRF-Ser-TGA-005) was upregulated in LUAD, which were all consistent with our research (36). However, despite breakthroughs in the roles of tRNA derivatives, the current understanding of the mechanisms of tRNA derivatives in LUAD remains limited. Our results confirmed previous studies and revealed that 3 newly found tRF-1s are significantly downregulated in LUAD. Thus, we strongly recommend the tRF-1 subtype in LUAD as the next research direction. The tRF-1 subtype can therefore be utilized as a candidate therapeutic marker in LUAD. The target genes and molecular signal pathways of tRF-1s need to be explored, which will help to discover whether tRF-1s are candidate therapeutic markers for LUAD.

The current understanding of the biological function of tRFs and tiRNAs in cancers remains limited. Previous preliminary studies have suggested that its functions may include cancer cell progression, metastasis, proliferation, and survival, by affecting gene expression, regulating protein translation, and so on (16-18). We conducted GO and KEGG pathway analysis to explore the potential biological functions and pathways of the 3 downregulated tRF-1s. Based on the results of GO analysis, nervous system development was found to be regulated by the downregulated tRFs. Several studies have reported that CNTN-1, a neural cell adhesion molecule, functions not only in nervous system development but also in the metastasis and invasion of LUAD (37,38). Whether the low expression of tRF-1 is related to the role of CNTN-1 in LUAD remains to be further studied.

By using KEGG pathway analysis, it was identified that these downregulated tRF-1s were primarily involved in signaling pathways. Among them, the MAPK signaling pathway was the top one. MAPK is a group of serine-threonine protein kinases that are associated with progression to malignant adenocarcinoma (39,40). Cicchini *et al.* demonstrated that MAPK signaling in the lungs played a key role not only in malignant progression but also in tumor initiation (41). Furthermore, Telonis and Rigoutsos demonstrated that tRFs were associated with the regulation of the MAPK and Wnt/β-catenin signaling pathways (42). In addition, it has been found that the association of specific mRNAs with tRFs depends on and differs from cancer to cancer, and the tRF-mRNA wiring is involved in the MAPK signaling pathway in LUAD as demonstrated through integrative analysis (43). In the present study, the target genes of tRF-Ser-TGA-010, tRF-Arg-CCT-018, and tRF-Val-CAC-017 may regulate MAPK signaling and contribute to the development and progression of LUAD. The results need to be supported by protein functional experiments.

This study may have some limitations. First, the number of samples is limited. With newly found down-regulated tRF-1s that may be involved in the development of LUAD, it suggests that it makes sense to use a larger sample size for further research. Second, there is still no clear evidence that tRF-1s may work like miRNAs. tRF-1s can be detected in Ago complexes, but much less than tRF-3s and tRF-5s (29). We will perform q-PCR for tRF-1 targets mRNA to examine whether these mRNAs are indeed changing expression in these samples in future studies.

Conclusions

In summary, we reported a more detailed landscape of tRF and tiRNA expression profiles in LUAD. We identified 3 newly-found downregulated tRF-1s that may be involved in the development and progression of LUAD via nervous system development and the MAPK signaling pathway. The specific roles of tRFs and tiRNAs in LUAD should be explored further.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-119/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-119/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-119/coif).

The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved and supervised by the Clinical Research Ethics Committee of Shengjing Hospital (Approval No. 2019PS700K). Written informed consent was obtained from all study patients.

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