

RESEARCH PAPER

 OPEN ACCESS 

tRF-19-W4PU732S promotes breast cancer cell malignant activity by targeting inhibition of RPL27A (ribosomal protein-L27A)

Zhengxiang Zhang^a, Zhiping Liu^b, Weidong Zhao^a, Xiaohan Zhao^a, and Yunxiang Tao^{b,c}

^aDepartment of Oncology, Yijishan Hospital, First Affiliated Hospital of Wannan Medical College, Wuhu, China; ^bDepartment of Gastrointestinal Surgery, The Affiliated Hefei Hospital of Anhui Medical University, Hefei, China; ^cDepartment of Dermatology, Yijishan Hospital, First Affiliated Hospital of Wannan Medical College, Wuhu, China

ABSTRACT

Breast cancer (BC) is a serious threat to female health. tRNA-derived fragments (tRFs) are popular biomarkers for the diagnosis and treatment of cancer. The purpose of this study was to identify tRFs related to BC and to explore the function and regulatory mechanism of crucial tRFs in BC cells. Small RNA database was used to detect the tRF profiles from BC patients and controls. Differentially expressed tRFs were determined by quantitative reverse transcription PCR (RT-qPCR), and a crucial tRF was evaluated through silence and overexpression experiments, and the target gene was investigated by luciferase reporter gene assay, Western blot and rescue experiment. We screened tRF-19-W4PU732S, which was processed from the mature tRNA-Ser-AGA, and significantly highly expressed in BC tissues and cells. Inhibition of tRF-19-W4PU732S weakened MDA-MB-231 cell proliferation, migration and invasion, while enhanced apoptosis. On the contrary, overexpression of tRF-19-W4PU732S promoted MCF-7 cell proliferation, migration and invasion, whereas reduced apoptosis. Furthermore, tRF-19-W4PU732S induced BC cell epithelial-to-mesenchymal transition (EMT) and cancer stem-like cells (CSC) phenotypes, such as up-regulation of OCT-4A, SOX2 and Vimentin and down-regulation of E-cadherin. Ribosomal protein-L27A (RPL27A) was a downstream target of tRF-19-W4PU732S, which was lowly expressed in BC cells. The knockdown of RPL27A expression partially restored the promoting effects of tRF-19-W4PU732S on BC cell viability, invasion, migration, EMT and CSC phenotypes, and the suppression of apoptosis. In conclusion, our results manifested that tRF-19-W4PU732S promotes the malignant activity of BC cells by inhibiting RPL27A, which provides a new scientific basis for the treatment of BC.

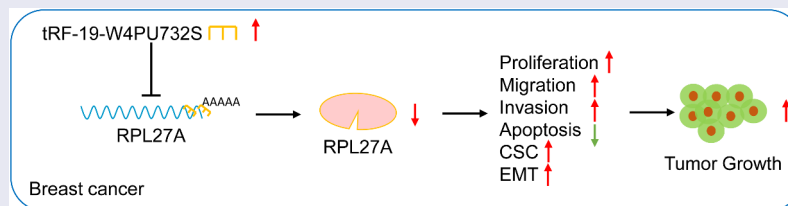
Abbreviations BC: breast cancer; tRNAs: transfer RNAs; tiRNAs: tRNA-derived stress-induced RNAs; tRFs: tRNA-derived fragments; CCK-8: Cell Counting Kit-8; PI: propidium iodide; EMT: epithelial-to-mesenchymal transition; CSC: cancer stem-like cells; RPL27A: ribosomal protein-L27A; RT-qPCR: quantitative reverse transcription PCR.

ARTICLE HISTORY

Received 23 September 2021
Revised 21 December 2021
Accepted 23 December 2021

KEYWORDS



Breast cancer; tRF-19-W4PU732S; RPL27A; biomarker; molecular mechanism




Introduction

Breast cancer (BC) is extremely harmful to female health [1]. As reported by World Health Organization, it is the second common account of cancer death among women [2]. Late diagnosis and fewer treatment options are the major reasons for the

high mortality rate in BC [3]. Effective treatment of BC depends on early diagnosis and therapy [4]. With early diagnosis and detection of BC, survival increases from 56% to more than 86% [5]. Nevertheless, due to the lack of sensitive and effective biomarkers for BC diagnosis, this goal is difficult to

CONTACT Yunxiang Tao  taoxyx@yeah.net  Department of Dermatology, Yijishan Hospital, First Affiliated Hospital of Wannan Medical College, Wuhu, Anhui241000, China

 Supplemental data for this article can be accessed [here](#)

© 2022 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

achieve [6]. Therefore, it is necessary to find biomarkers and effective molecular targets of BC.

Transfer RNAs (tRNAs) are non-coding RNAs that are responsible for converting genetic information on mRNAs into amino acid sequence information, which are acting as adapters in protein synthesis [7,8]. tRNA-derived fragments (tRFs) and tRNA-derived stress-induced RNAs (tiRNAs) were tRNA derivatives [9,10]. Nowadays, many studies have proved the important role of tRFs in cancer. For example, tRFs combined with miR-1280 suppressed the development of colorectal cancer [11]. The 5'-tRF-LysCTT level was positively correlated with the phenotype of invasive bladder cancer [12]. Moreover, some studies have manifested that, similar to miRNAs, tRFs can also bind to argonaute proteins, which may influence the development of cancer by adjusting target genes [10]. A growing body of researches indicated that tRNAs are closely related to human diseases and are good choice for tumor diagnosis and treatment [3,13,14].

Although many researches have claimed that tRNAs are involved in the cancer process, the related molecular mechanisms are still poorly understood [15,16]. In view of this, we speculated that tRFs play an important role in BC. Therefore, our aim is to screen BC-related tRFs and explore the potential roles of pivotal tRFs in the occurrence and development of BC, which provide scientific evidence for the future diagnosis and treatment of BC.

Materials and methods

Data mining analysis

The differentially expressed tRFs in BC from the deepbase3 database (<http://rna.sysu.edu.cn/deepbase3/index.html>). The differential expression analysis of target genes in BC was performed using the GEPIA database (<http://gepia.cancer-pku.cn/index.html>). tRF target genes were analyzed using RNA Hybrid (<https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid>). Cytoscape 3.7.1 (<https://cytoscape.org/>) was used for network graph analysis. The function of the target genes and signaling pathway were analyzed by DAVID 6.8 (<https://david.ncifcrf.gov/summary.jsp>). The bubble diagram adopts R package R i386 4.0.2.

Cell culture and cell transfection

The human embryonic kidney cell line (HEK293T), normal mammary epithelial cell line (MCF-10A), and human BC cell lines (MCF-7 and MDA-MB-231) were obtained from the Chinese Academy of Sciences (CAS). The cells were cultured in DMEM or RPMI-1640 medium supplemented with 1% antibiotics and 10% (v/v) fetal bovine serum, and placed in the incubator (5% CO₂, 37°C).

According to the reported literature, the tRF-19-W4PU732S single-strand mimics/inhibitors (tRF-19 mimics/inhibitor) and corresponding negative controls were transfected into MCF-7 and MDA-MB-231 cells by using LipofectamineTM 2000 (Invitrogen, USA) [3]. RNA interference (RNAi) targeting ribosomal protein-L27A (RPL27A), named si-RPL27A, was obtained from GenePharma Co., Ltd (Suzhou, China). Single si-RPL27A was transiently transfected into cells with Lipofectamine 2000, and then the cells were collected for subsequent detection after 48 h of incubation.

Clinical sample collection

The 15 female breast cancer patients' tumor tissues and paired tumor-adjacent tissues were obtained Yijishan Hospital, First Affiliated Hospital of Wannan Medical College. All patients provided written informed consent. This study was reviewed and approved by the Ethics Review Committee of Wannan Medical College and was performed in accordance with Declaration of Helsinki.

RNA isolation, cDNA synthesis and quantitative reverse transcription PCR (RT-qPCR)

According to manufacturer's instructions, total RNAs were extracted from cells and tissues using Trizol reagent (Invitrogen, USA) [17]. Some RNA modifications were then removed using rtStarTM tRF&tiRNA Pretreatment Kit & First-Strand cDNA Synthesis Kit (Aksomics, China). Then, the RT-qPCR was used for quantitative analysis of RNA by using a Bulge-Loop miRNA PCR Starter Kit (Ribobio, China) and reversed transcription into cDNA with Stem-loop RT primers for tRF-19-W4PU732S, tRF-21-80877S6VD and

tRF-18-IRM1JVV. RT reaction conditions of tRF-19-W4PU732S, tRF-21-80877S6VD and tRF-18-IRM1JVV were 5 min at 25°C, 60 min at 42°C and 5 min at 70°C. Then RT-qPCR was performed with the SYBR Green Mix containing dNTP mix, SYBR Green I, PCR buffer and Taq enzyme. RNU6B was used for tRF-19-W4PU732S, tRF-21-80877S6VD and tRF-18-IRM1JVV template normalization. Forward primers and reverse primers were added to form a mixture, and then incubated at 95°C for 10 min, then set 45 cycles, followed by 95°C for 15s, 60°C for 60s. RT-qPCR was followed the protocol described in previous study [18]. RT-qPCR was performed for RPL27A and other genes with SYBR® Premix Dimer-Eraser (TaKaRa), the housekeeping gene (GAPDH) was selected to analyze their transcriptional stability. The tRFs and mRNA levels were calculated by $2^{-\Delta\Delta Ct}$ and $2^{-\Delta Ct}$ methods for relative quantification of gene expression. The primer sequences of genes for RT-qPCR analysis were shown in Table S1.

Luciferase assay

Since there is a binding site of tRF-19-W4PU732S on the 3'UTR of RPL27A, we connected the 3'UTR to the pmirGLO-Reporter vector (GenePharma Co., Ltd). 3' of the luciferase gene: 5'-GAGCCA CTGCGCCCGGCCATGGC-3' (RPL27A 3'-UTR wild-type). While a negative control, the mutation sequence was: 5'-GGTAACGGGCGCCGA AGGCTTAC-3' (RPL27A 3'-UTR mutant). Referring to previous studies, we co-transfected tRF-19-W4PU732S mimics plasmid and corresponding negative control with wild-type plasmid or mutation plasmid into HEK293T cells by Lipofectamine 2000 [19]. After 48 h of transfection, we harvested cells to determine the luciferase activity by a Dual-luciferase reporter assay system (Promega).

Western blot analysis

After the total protein was isolated, the BCA assay kit (Invitrogen) was used to measure the protein concentration. Then the same amount of protein in each group was added to 10% SDS-PAGE gel. The proteins were transferred to PVDF membranes (Bio-Rad, USA) and then incubated with

RPL27A (Cat No. 16,002-1-AP; dilution 1:1,000; Proteintech); E-Cadherin (Cat No. 20,874-1-AP, dilution 1:50,000; Proteintech); Vimentin (Cat No. 10,366-1-AP; dilution 1:10,000; Proteintech); SOX2 (Cat No. 11,064-1-AP; dilution 1:1,000; Proteintech); Oct-4A (Cat No. 2890; dilution 1:1,000; Cell Signaling) or GAPDH antibody (Cat No. 10,494-1-AP; dilution 1:40,000, Proteintech) at 4°C overnight. After washing the membrane, it was incubated with goat anti-rabbit IgG-HRP for 1–2 h. The photos were taken using the Chemi Doc MP system (Bio-Rad, USA), and the quantitative pictures was obtained via the ImageJ software.

Cell proliferation assay

Cell Counting Kit-8 (CCK-8) was used to detect cell viability and proliferation. The cells were plated on 96-well plate with a density of 1×10^3 cells/well. After incubation for 24, 48, and 72 h, 10 μ L CCK-8 reagent and 100 μ L fresh DMEM or RPMI-1640 were added, then the cells were incubated for 1 h at 37°C. The absorbance at 450 nm was measured by a microplate reader.

Transwell assays

For migration assay, cells were plated in 0.8 μ m 24-well Transwell chambers (FALCON). For invasion assay, BioCoat™ Matrigel® (BioCoat) was used to detected cells. We aspirated the cell culture medium and washed the cells with an appropriate amount of 1x PBS containing 0.25% trypsin EDTA. The cells were then digested in an incubator for 3 minutes. After the cells were resected and rounded, digestion was stopped by adding an equal amount of complete medium. After centrifugation at $200 \times g$ for 3 min, the supernatant was removed. The cells were resuspended in serum-free McCoys 5A medium, and the cell concentration was adjusted to 8×10^4 cells/ml. After that, 500 μ L of cell suspension was added to the upper chamber, and 700 μ L medium with 10% serum was added to the lower chamber; then, the cells were incubated for 24 h. The lower cells were stained with 0.1% crystal violet after the upper cells were removed. Three visual fields were randomly selected for microscopic observation.

Annexin V/propidium iodide (PI) double staining assay

For cell apoptosis analysis, it was examined via PI and fluorescein isothiocyanate (FITC)-conjugated Annexin V staining [20]. Briefly, cells were washed with PBS and fixed with 70% ethanol. Fixed cells were then washed twice with PBS and stained in PI/FITC Annexin V, and then incubated at room temperature in the dark for 1 h. FACScan flow cytometry (Beckman Coulter, USA) was used to analyze apoptotic cells and necrotic cells. The data were analyzed using FlowJo software.

Statistical analysis

Data analysis was performed using the GraphPad Prism 8. The data were expressed as the mean \pm standard deviation (SD) and each experiment was repeated 3 times. The comparisons were performed using Student's t-test for two groups, and one-way ANOVA followed by Tukey's Post hoc test for three groups, and $p < 0.05$ was regarded as statistically significant.

Results

In our study, we speculated that the tRF-19-W4PU732S may play a dominant role in BC by silencing RPL27A. Therefore, we aimed to investigate the functional role of tRF-19-W4PU732S/RPL27A axis in BC. Our data revealed that tRF-19-W4PU732S was up-regulated in BC, and promoted malignant activity in BC. Furthermore, RPL27A could be suppressed by tRF-19-W4PU732S and RPL27A inhibition could partly reverse the effect of tRF-19-W4PU732S knock-down on BC cells. In general, our findings manifested that tRF-19-W4PU732S contributed to tumor progression in BC cells through silence RPL27A via directly targeting 3'-UTR, which may provide a novel mechanism and therapeutic target for BC.

Screening for tRFs associated with BC

To investigate the differential expression of tRFs in BC, we employed the deepbase3 dataset to screen tRFs in BC, and found 344 up-regulated

tRFs and 46 down-regulated tRFs in BC tissues compared to the normal tissues (Figure 1(a)). We obtained 12 tRFs significantly correlated with prognosis in BC patients (Table 1). We also analyzed the intersection between the prognostic tRFs and the differentially expressed tRFs (Figure 1(b)), and found that 5 differentially expressed tRFs expression was consistent with the prognostic trend. As shown in Figure 1(c), tRNA-Lys-TTT-3-1, tRNA-Gly-GCC-1-4, tRNA-Ser-AGA-2-5 and tRNA-Ser-AGA-3-1 (tRF-19-W4PU732S) were significantly up-regulated in BC compared to the control, and the survival time of BC patients with high expression was lower than that of BC patients with low expression, whereas tRNA-His-GTG-1-1 showed lower expression and was associated with good prognosis.

tRF-19-W4PU732S is up-regulated in BC

Through the deepbase tRF annotation file and the MINTbase database, we found that only tRNA-Ser-AGA-3-1 (MINTbase ID: tRF-19-W4PU732S), tRNA-Lys-TTT-3-1 (MINTbase ID: tRF-21-80877S6VD), and tRNA-Gly-GCC-1-4 (MINTbase ID: tRF-18-IRM1JVV) were uniquely mapped to the MINTbase database, so we detected the expression levels of these tRFs. RT-qPCR analysis showed that tRF-19-W4PU732S and tRF-21-80877S6VD were significantly increased in BC cells (MCF-7 and MDA-MB-231), compared with normal epithelial cells (MCF 10A). In addition, tRF-19-W4PU732S was the most significantly upregulated tRFs in highly transferable MDA-MB-231 cells, compared with lowly transferable MCF-7 cells (Figure 2(a)). In the MINTbase, the molecule of tRF-19-W4PU732S belonged to a class of 19nt small RNAs and the sequence was 5'-TCGTGGCCGAGTGGTTAAG-3' (Figure 2(b)). tRF-19-W4PU732S was derived from 5'-end of mature tRNA-Ser-AGA-3-1 with the carried anticodon AGA (Figure 2(c)). Finally, we detected BC and adjacent tissues and found that tRF-19-W4PU732S was highly expressed in cancer tissues compared with adjacent tissues (Figure 2(d)). Therefore, we further investigated the function of tRF-19-W4PU732S in BC.

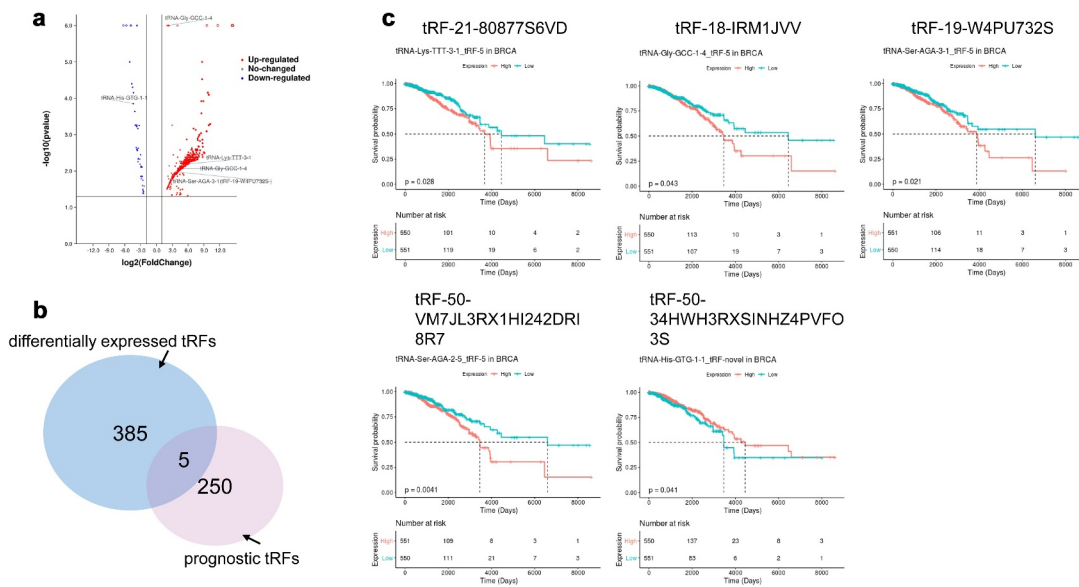


Figure 1. Screening for tRFs associated with BC. (a) The volcano plot showed the differentially expressed tRFs between different groups (the controls and BC patients). (b) Venn diagrams of the number of tRFs in prognostic analysis (pink) and differential expression analysis (blue) groups. (c) Prognostic effect of tRFs for BC was displayed in deepbase3 database, MINTbase ID (top) and deepbase3 ID (bottom).

Table 1. tRFs significantly correlated with prognosis in BC patients.

deepbase3 ID	Gene Symbol	FDR
tRNA-Arg-ACG-2-1	tRF-5	0.000718
nm-tRNA-Tyr-GTA-chr21-2	tRF-novel	0.003072
tRNA-Ser-AGA-2-5	tRF-5	0.004076
nmt-tRNA-Leu-TAA-4-1	tRF-5	0.011315
tRNA-Ser-AGA-2-2	tRF-5	0.019343
tRNA-Ser-AGA-3-1	tRF-5	0.021062
tRNA-Arg-TCT-5-1	tRF-5	0.024234
tRNA-Lys-TTT-3-1	tRF-5	0.028048
tRNA-Leu-AAG-1-2	tRF-5	0.037576
tRNA-His-GTG-1-1	tRF-novel	0.040731
tRNA-Gly-GCC-1-4	tRF-5	0.042585

tRF-19-W4PU732S enhances on BC cell proliferation, migration, invasion, apoptosis

To investigate the biological roles of tRF-19-W4PU732S in BC, we transfected the tRF-19-W4PU732S inhibitor and control RNA, tRF-19-W4PU732S mimics and control RNA into MDA-MB-231 and MCF-7 cells. The tRF-19-W4PU732S inhibitors and mimics efficiency were shown in the [Figure 3\(a\)](#). The CCK-8 assay demonstrated that silencing tRF-19-W4PU732S expression in MDA-MB-231 cells and MCF-7 cells decreased ability of cell proliferation compared to the control, and overexpression of tRF-19-W4PU732S in MCF-7 cells and MDA-MB-231 cells increased

ability of cell proliferation ([Figure 3\(b\)](#), [Figure S2A](#)). Meanwhile, we also found that tRF-19-W4PU732S inhibition markedly reduced the migration and invasion of BC cells, but tRF-19-W4PU732S mimics showed the opposite result ([Figure 3\(c\)](#), [Figure S2B](#)). Moreover, inhibition of tRF-19-W4PU732S significantly promoted cell apoptosis, while tRF-19-W4PU732S mimics had no significant effect on the apoptosis ([Figure 3\(d\)](#), [Figure S2C](#)). Because tRF-19-W4PU732S was highly expressed in transferable BC cells, we next examined the effect of tRF-19-W4PU732S on BC cell metastasis.

tRF-19-W4PU732S induces the EMT and CSC phenotypes of BC cells

As epithelial-to-mesenchymal transition (EMT) and cancer stem-like cells (CSC) phenotypes contributed to metastatic behaviors of cancer cells, we determined the expression of EMT (vimentin and E-cadherin) and CSC (OCT-4a and SOX2) biomarkers by the Western blot assay. As shown in [Figure 4\(a\)](#), tRF-19-W4PU732S inhibition reduced the OCT-4a, SOX2 and vimentin protein expression, and

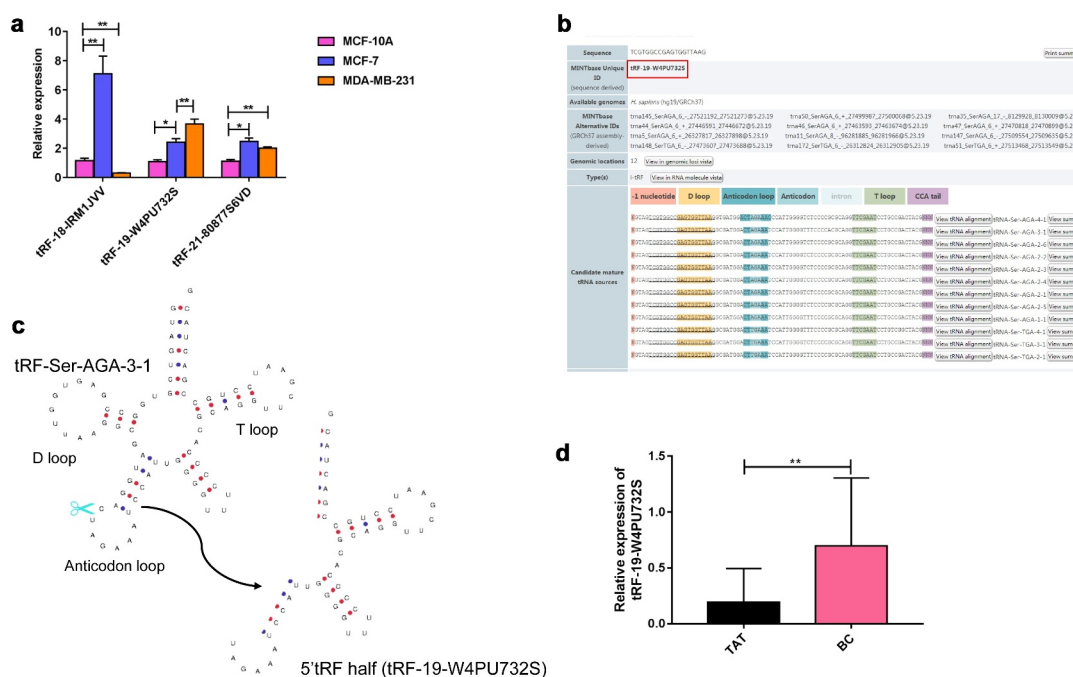


Figure 2. Validation of the differential expression tRFs. (a) Candidate tRF expression (tRF-19-W4PU732S, tRF-21-8087756VD, and tRF-18-IRM1JVJ) was measured by RT-qPCR ($n = 3$). (b) tRF-19-W4PU732S was derived from 5'-ends of mature tRNA-Ser-AGA-3-1 with the length of 19nt. (c) tRF-19-W4PU732S structure. (d) Detection of tRF-19-W4PU732S expression in BC tissues and adjacent tissues by RT-qPCR ($n = 15$). * $p < 0.05$; ** $p < 0.01$, statistically significant.

increased the E-cadherin protein expression in MDA-MB-231 cells. Additionally, tRF-19-W4PU732S mimics raised the OCT-4a, SOX2 and vimentin protein expression, and down-regulated the E-cadherin protein expression in MCF-7 cells (Figure 4(b)). The above results demonstrated that tRF-19-W4PU732S played an important role in the metastasis of BC cells.

tRF-19-W4PU732S reduces RPL27A expression by directly targeting its 3'-UTR

In order to study the molecular mechanism of tRF-19-W4PU732S in BC, we first used RNAhybrid software to predict target genes and obtained 941 potential target genes. Then, we analyzed the function of these potential target genes by GO and KEGG enrichment analysis. In GO analysis, we found that the targeted genes of the tRFs were mainly involved in signal transduction, positive regulation of GTPase activity, multicellular organism development and other processes (Figure 5(a)). For KEGG

analysis, we found that the tRFs primarily participated in the pathway in cancer, calcium signaling pathway, cAMP signaling pathway, and GnRh signaling pathway (Figure 5(b)). Next, we used the TCGA database to analyze the expression of these candidate target genes in BC and their relationship with prognosis. The results showed that 26 candidate target genes were lowly expressed and correlated with the prognosis of BC patients (Figure 5(c)). However, only 5 target genes were lowly expressed in BC with poor prognosis of patients (Figure S1 A and B, Figure 6(a,b)). We examined these 5 predicted target genes in BC cells by RT-qPCR. Compared with the negative controls, RPL27A and C7orf63 mRNA levels were significantly downregulated by tRF-19-W4PU732S mimics in MCF-7 cells (Figure 6(c)), whereas there was no significant difference in C16orf54, C2orf82 and ELMSAM expression. Since RPL27A was the most significant target gene, we chose the RPL27A to do the following experiment.

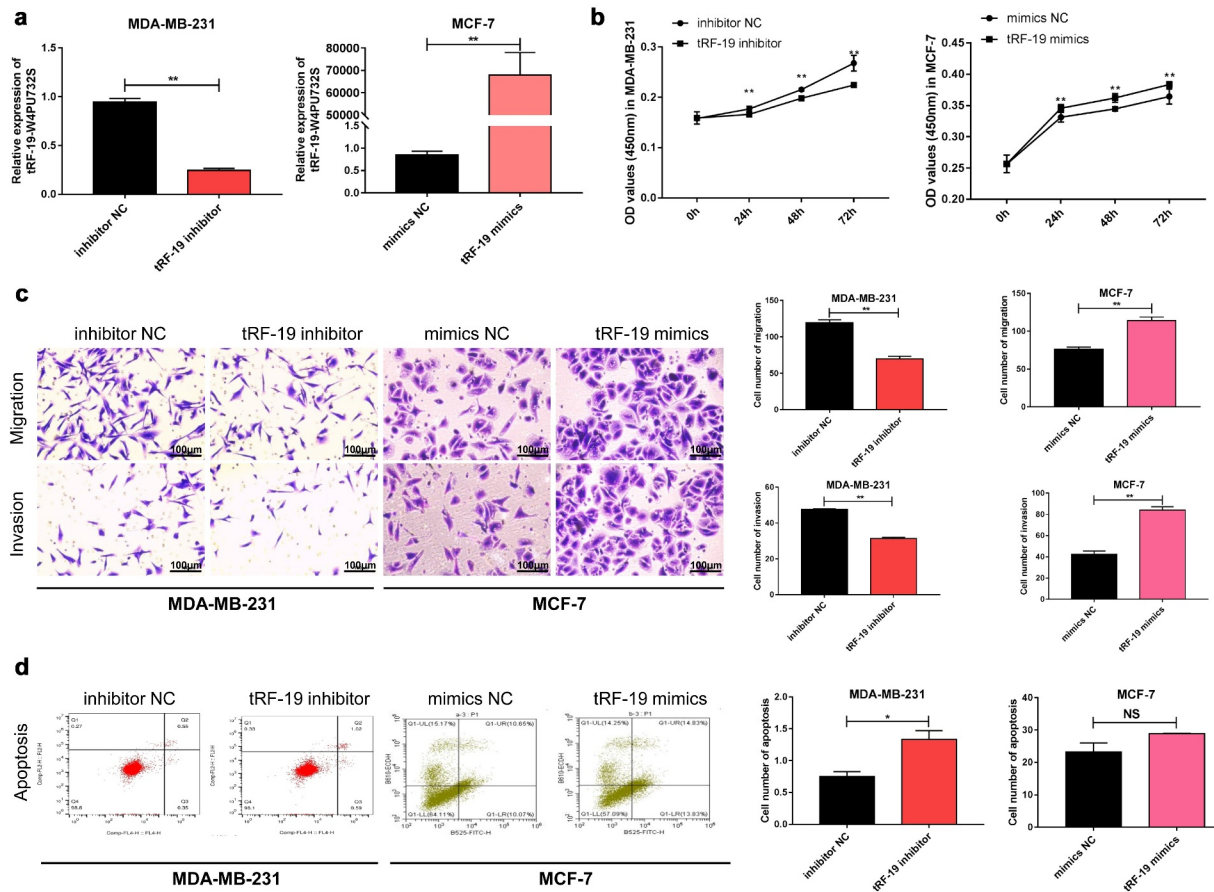


Figure 3. tRF-19-W4PU732S suppresses BC cell malignant activity. (a) tRF-19-W4PU732S inhibitors and overexpression efficiency ($n = 3$). (b) Cell viability of BC cell lines after transfection with tRF-19-W4PU732S inhibitor or inhibitor control, and tRF-19-W4PU732S mimics or mimics control were detected by a CCK-8 assay ($n = 6$). Cell migration assays, invasion assays (c) and apoptosis assays (d) were performed in treated cells ($n = 3$). NS: no significance. * $p < 0.05$, ** $p < 0.01$, statistically significant.

As expected, the tRF-19-W4PU732S mimics induced a significant decrease of RPL27A protein level in cells, whereas the tRF-19-W4PU732S inhibition showed an opposite result (Figure 6(d)). To further explore the regulatory effect of tRF-19-W4PU732S on RPL27A, we constructed a vector expressing of 3'-UTR of RPL27A (Figure 6(e)). The wild type or mutant constructs were co-transfected into HEK293T cells with tRF-19-W4PU732S mimics and negative control. After 48 h of transfection, compared with the control RNA, tRF-19-W4PU732S mimics significantly reduced the dual luciferase activity of wild type RPL27A. However, the mutant vector promoter activity was not affected (Figure 6(f)). In brief, these results indicated that tRF-19-W4PU732S regulated the expression of RPL27A by directly binding to its 3'-UTR binding site.

Attenuation of RPL27A expression enhances the tRF-19-W4PU732S-mediated improvement effects on BC cells

To determine whether the dysregulation of RPL27A is involved in the regulation of cell proliferation, migration, invasion and apoptosis by tRF-19-W4PU732S, we used specific siRNA against RPL27A to knock down RPL27A expression and verified that si-RPL27A has relatively high knockdown efficiency for MDA-MB-231 and MCF-7 cells (Figure 7(a,b), Figure S3A and B). Compared with the control group, the reduction of RPL27A expression partially enhanced the effect of tRF-19-W4PU732S in promoting the viability, invasion and migration of BC cells. The tRF-19-W4PU732S inhibitor + si-RPL27A group showed the lowest proliferative capacity, followed by tRF-19-W4PU732S inhibitor and inhibitor NC

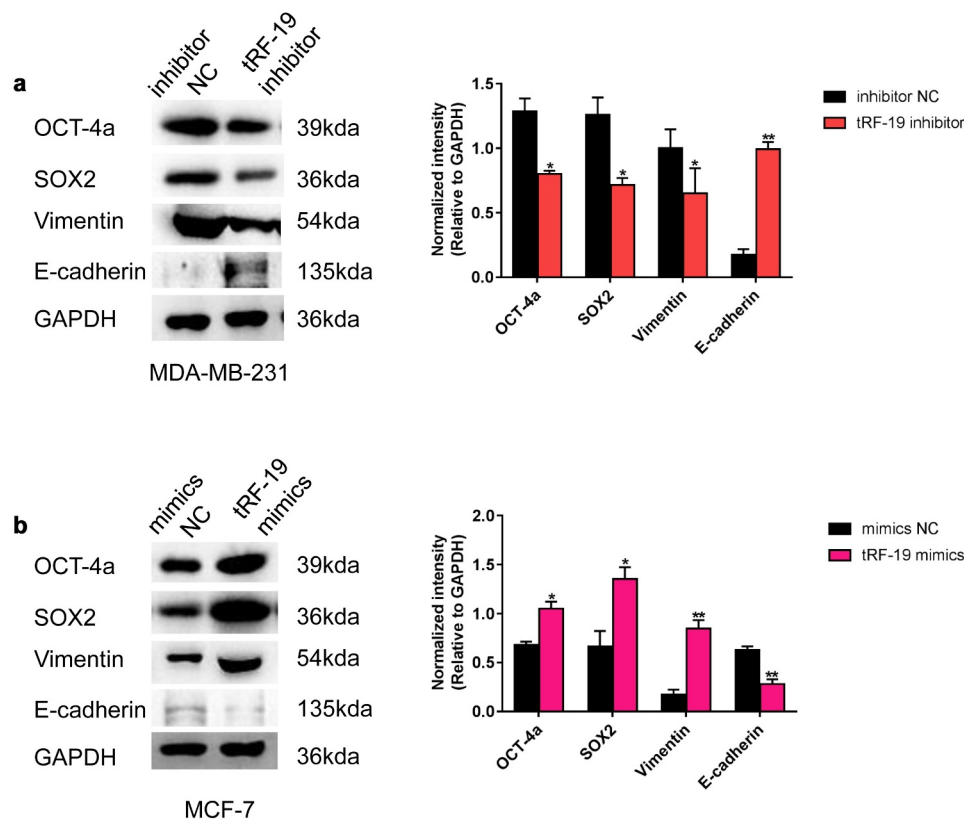


Figure 4. tRF-19-W4PU732S induces BC cell EMT and CSC phenotypes. (a) EMT and CSC biomarker proteins are shown in MDA-MB-231 (n = 3). (b) EMT and CSC biomarker proteins are shown in MCF-7 (n = 3). * $p < 0.05$; ** $p < 0.01$, statistically significant.

(Figure 7(c), Figure S3C). Compared with the tRF-19-W4PU732S inhibitor, the tRF-19-W4PU732S inhibitor + si-RPL27A group showed increased cell migration and invasion (Figure 7(d), Figure S3D). Besides, inhibition of RPL27A can partially weaken apoptosis of BC cells (Figure 7(e), Figure S3E). Compared with control, tRF-19-W4PU732S inhibitor reduced the OCT-4a, SOX2 and vimentin protein expression, and increased the E-cadherin protein expression, whereas knockdown of RPL27A reversed this phenomenon (Figure 7(f), Figure S3F). These data revealed that tRF-19-W4PU732S promoted BC cell malignant activities in a RPL27A-mediated manner.

Discussion

Due to late diagnosis, lack of treatment options, and cancer heterogeneity, BC is still the leading cause of cancer-related deaths in women worldwide. Although decades of researches have

provided considerable insights into BC transfer, the molecular basis involved in the development of BC is still unknown [21]. Generally, the 5 year survival rate of early-stage BC is obviously better than that of advanced stage, so early detection and early treatment [22]. Currently, the diagnosis of BC is mainly based on diagnostic imaging and pathological evaluation to determine the extent of the disease, such as pain or a palpable mass [23]. Mammography has been proven to be the only screening modality to reduce BC-specific mortality, and it is more beneficial for women in their 60s [24]. However, screening mammography has negative effects, such as pain, radiation exposure and other negative psychological effects [23]. Therefore, it is important to find a more effective and safer way of BC diagnosis. In our study, we used small RNA database to analyze the expression of tRFs in BC, and screened out tRF-19-W4PU732 as a new target for the treatment of BC. tRF-19-

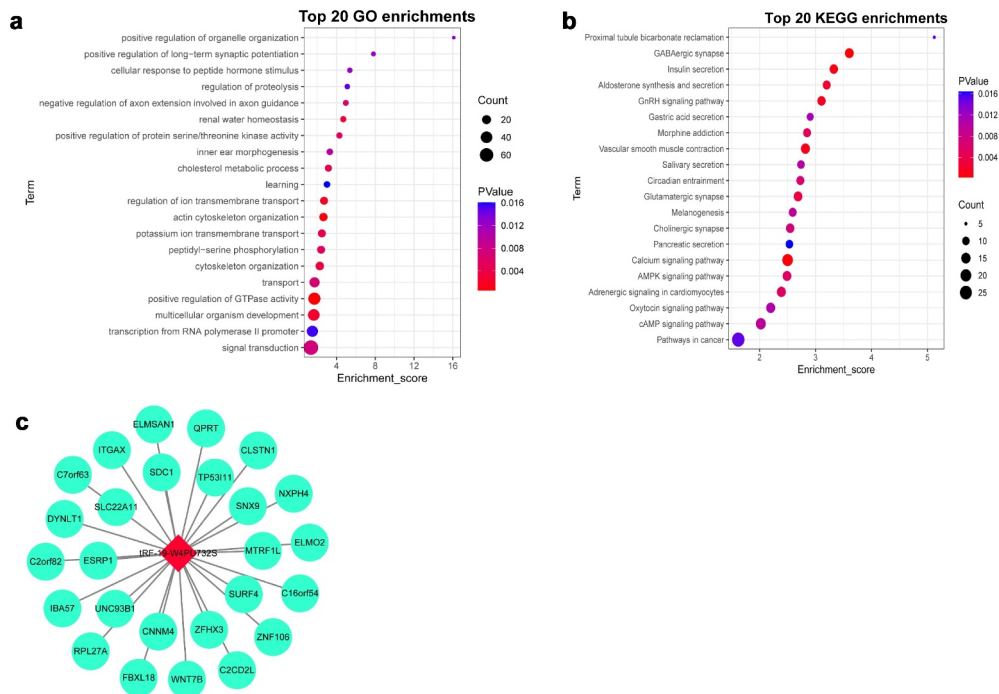


Figure 5. Target genes prediction and functional enrichment analyses of tRF-19-W4PU732S. (a) The gene ontology annotation analysis for predicted target genes of differentially expressed tRFs. (b) The KEGG pathways analysis for predicted target genes of differentially expressed tRFs. Rich factor included the gene numbers and *P*-values. (c) The interaction diagram of tRF-19-W4PU732S–mRNAs.

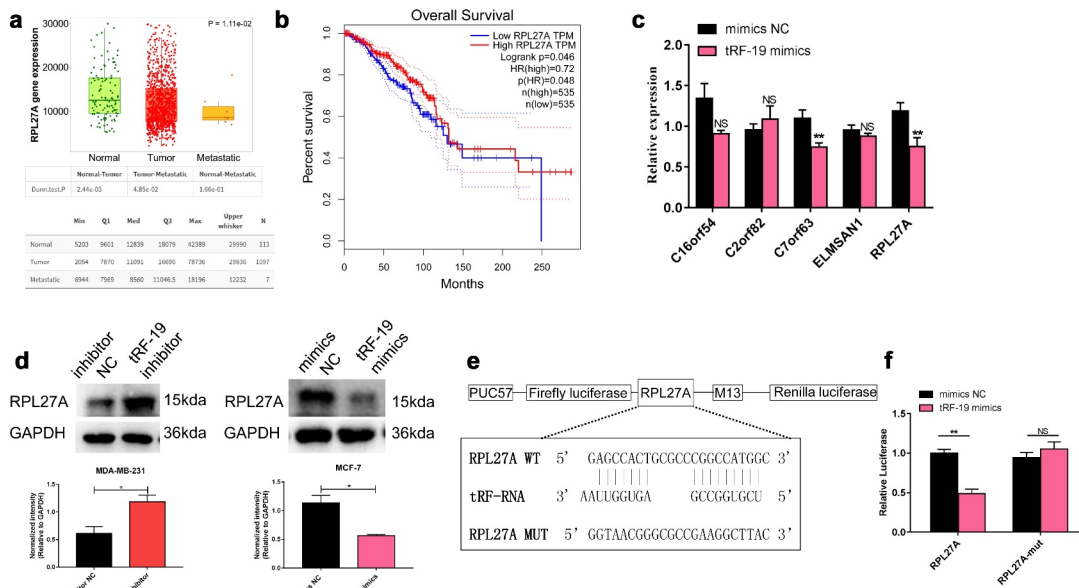


Figure 6. tRF-19-W4PU732S directly regulates RPL27A expression in BC cells. (a) The expression of RPL27A between cancer and normal samples in GEPIA database. (b) Prognostic effect of RPL27A for BC was displayed in deepbase3 database. (c) The mRNA levels of candidate target gene in treated MCF-7 cells (*n* = 3). (d) RPL27A protein levels expression in treated MDA-MB-231 and MCF-7 cells (*n* = 3). (e) The wide type (wt) and mutant of 3'-UTR fragment disrupted the interaction with tRF-19-W4PU732S. (f) Co-transfection of tRF-19-W4PU732S mimic plasmid and corresponding negative control with wild-type plasmid or mutant plasmid into HEK293T cells (*n* = 3). Luciferase activity of wt RPL27A 3'-UTR was significantly influenced by tRF-19-W4PU732S in BC cells. NS: no significance. **p* < 0.05, ** *p* < 0.01, statistically significant.

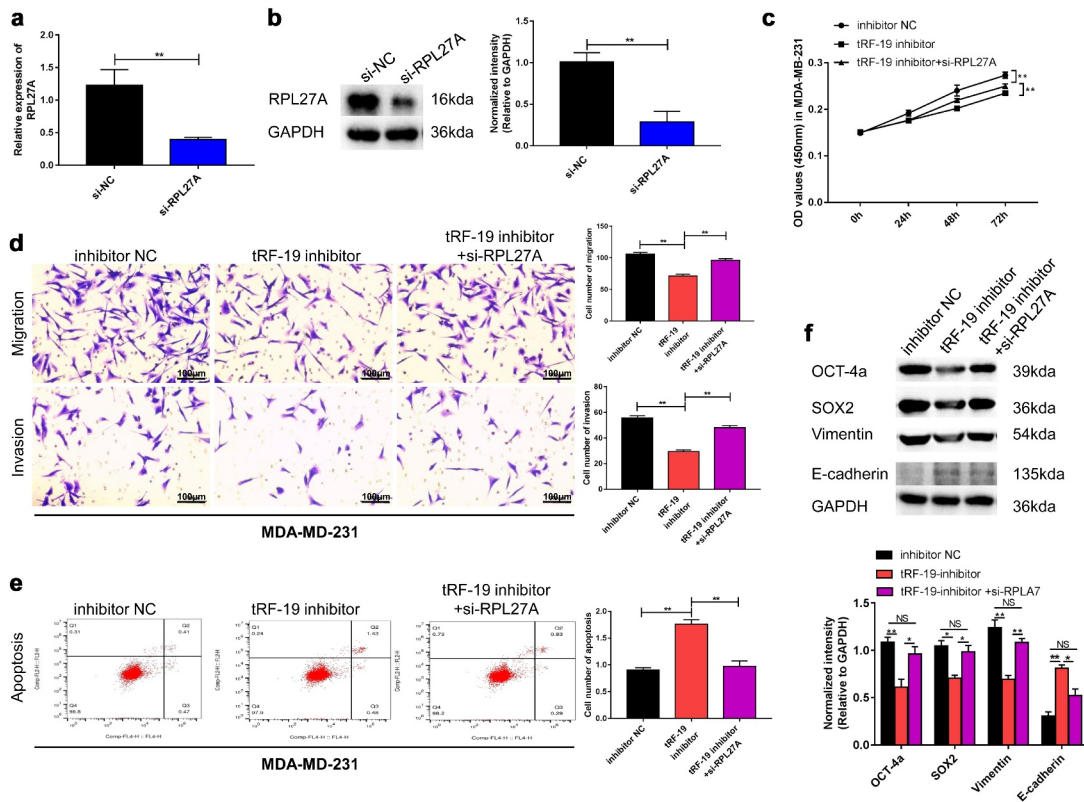


Figure 7. The decrease of RPL27A expression enhances the tRF-19-W4PU732S-mediated improvement influence on BC cells. The (a) mRNA and (b) Protein levels in MDA-MB-231 cells transfected with RPL27A siRNA or siRNA negative control ($n = 3$). (c) CCK-8 assay was used to detect the cell viability of MDA-MB-231 cells after transfection with tRF-19-W4PU732S inhibitor, si-RPL27A or negative control ($n = 6$). Cell migration assays, invasion assays (d) and apoptosis assays (e) were performed in treated BC cells ($n = 3$). (f) EMT and CSC phenotypes biomarker proteins were performed in treated BC cells. NS: no significance ($n = 3$). NS: no significance. * $p < 0.05$, ** $p < 0.01$, statistically significant.

W4PU732 belonged to the 5'-end of tRNA-Ser-AGA and promoted the malignant activity of BC cells. Through target gene prediction, tRF-19-W4PU732S reduced RPL27A expression by directly targeting its 3'-UTR. Inhibition of RPL27A expression enhanced tRF-19-W4PU732-mediated malignancy in BC cells. Our research provides new techniques for the diagnosis and treatment of BC.

With the development of genome sequencing technology, tRNA-derived fragments have been shown to be involved in the development of many diseases [8]. Half of the abnormally expressed tRFs and tRNAs in tumors are considered to be potential diagnostic biomarkers or therapeutic targets in tumor cells [10]. It has been reported that compared with the normal control group, 6 tRFs in the plasma samples of patients with BC stage were significantly down-regulated

[25]. Farina et al. demonstrated that RUNX1 repressed tsRNA to prevent overactive proliferation in breast epithelial cells [26]. In our study, we found that tRF-19-W4PU732S was the most significantly upregulated tRFs in BC cells compared with normal mammary epithelial cells, and tRF-19-W4PU732S was a 5'-end fragment of tRNA-Ser-AGA. Further study showed that overexpression of tRF-19-W4PU732S significantly promoted the ability of BC cell proliferation, migration, invasion, EMT and CSC phenotypes, and suppressed BC cell apoptosis, whereas inhibition of tRF-19-W4PU732S had the opposite effect. Collectively, the above results revealed that tRF-19-W4PU732S promoted the development of BC.

The mechanism of tRFs is similar to that of miRNAs, which can silence the expression of a series of target genes [27,28]. Some tRFs are known to bind mRNA targets directly similar to

miRNAs, such as the 3'-UTR prediction site of THBS1 that binds to tRF-17-79MP9PP, thereby regulating BC [3]. tRF-03357 might partly promote the proliferation, migration and invasion of ovarian cancer cells by regulating HMBOX1 [29]. Coincidentally, we found that tRF-19-W4PU732S downregulated its target gene RPL27A. The subsequent luciferase assay proved that tRF-19-W4PU732S could bind to the predicted site on 3'-UTR of RPL27A. RPL27A is typical genes encoding ribosomal proteins [30]. RPL27A was considered to result in myelodysplastic phenotype through ribosomal dysplasia [31]. Some studies suggested that RPL27A was a biomarker for monitoring a primary prognostic marker in renal cancer and BC [32]. Moreover, RPL27A regulated tumor immunity and tumor cell migration, and played an important role in tumor progression, but the molecular mechanism of RPL27A in BC have not been reported yet [33,34]. We reported for the first time that RPL27A played an important role in BC. Kaplan Meier survival analysis demonstrated that in the TCGA breast invasive carcinoma database, the overall survival of BC patients with low RPL27A expression was significantly reduced. Importantly, the suppression of RPL27A partially enhanced the promoting effect of tRF-19-W4PU732S on proliferation, migration, invasion, EMT and CSC phenotypes, and inhibition apoptosis in MDA-MB-231 cells. In sum, we confirmed that tRF-19-W4PU732S significantly promoted cell malignant activities as a new tumor-activator through RPL27A in BC.

Conclusion

In summary, tRF-19-W4PU732S significantly promoted the proliferation, migration, invasion, EMT and CSC phenotypes, and suppressed apoptosis of BC cells by targeting RPL27A inhibition. This study might provide a new target for BC diagnosis and treatment.

Highlights

- tRF-19-W4PU732S was up-regulated in BC with malignant survival rate.
- Overexpression of tRF-19-W4PU732S accelerated malignant activity of BC cells.

- tRF-19-W4PU732S regulated BC cells via targeting inhibition of RPL27A.

Authors' contributions

Yunxiang Tao designed the study. Zhengxiang Zhang designed the study and wrote the manuscript. Zhengxiang Zhang, Zhiping Liu, Weidong Zhao and Xiaohan Zhao performed the study and wrote the manuscript.

Consent to participate

All the authors listed have approved the manuscript.

Consent for publication

All the authors listed have approved the publication.

Ethics and consent statement

This study was performed with the approval of the Research Ethics Committee of Wannan Medical College. In addition, written informed consent forms were signed by all the patients who participated in this research.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

Data availability statement

All data are fully available without restriction.

ORCID

Yunxiang Tao  <http://orcid.org/0000-0001-8006-7341>

References

- [1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin.* 2019;69(1):7–34.
- [2] Marklund L, Hammarstedt L. Impact of HPV in oropharyngeal cancer. *J Oncol.* 2011;2011:509036.
- [3] Mo D, He F, Zheng J, et al. tRNA-derived fragment tRF-17-79MP9PP attenuates cell invasion and

- migration via THBS1/TGF- β 1/Smad3 axis in breast cancer. *Front Oncol.* **2021**;11:656078.
- [4] Wu X, Zeng R, Wu S, et al. Comprehensive expression analysis of miRNA in breast cancer at the miRNA and isomiR levels. *Gene.* **2015**;557(2):195–200.
- [5] Montazeri M, Montazeri M, Montazeri M, et al. Machine learning models in breast cancer survival prediction. *Technol Health Care.* **2016**;24:31–42.
- [6] Hamam R, Hamam D, Alsaleh KA, et al. Circulating microRNAs in breast cancer: novel diagnostic and prognostic biomarkers. *Cell Death Dis.* **2017**;8(9):e3045.
- [7] Zhu L, Ge J, Li T, et al. tRNA-derived fragments and tRNA halves: the new players in cancers. *Cancer Lett.* **2019**;452:31–37.
- [8] Sun C, Yang F, Zhang Y, et al. tRNA-derived fragments as novel predictive biomarkers for trastuzumab-resistant breast cancer. *Cell Physiol Biochem.* **2018**;49(2):419–431.
- [9] Shigematsu M, Kirino Y. 5'-Terminal nucleotide variations in human cytoplasmic tRNA HisGUG and its 5'-halves. *RNA.* **2017**;23(2):161–168.
- [10] Keam SP, Hutvagner G. tRNA-derived fragments (tRFs): emerging new roles for an ancient RNA in the regulation of gene expression. *Life (Basel).* **2015**;5(4):1638–1651.
- [11] Huang B, Yang H, Cheng X, et al. tRF/miR-1280 suppresses stem cell-like cells and metastasis in colorectal cancer. *Cancer Res.* **2017**;77(12):3194–3206.
- [12] Papadimitriou MA, Avgeris M, Levis P, et al. tRNA-derived fragments (tRFs) in bladder cancer: increased 5'-tRF-LysCTT results in disease early progression and patients' poor treatment outcome. *Cancers (Basel).* **2020**;12(12):3661.
- [13] Wang J, Ma G, Ge H, et al. Circulating tRNA-derived small RNAs (tsRNAs) signature for the diagnosis and prognosis of breast cancer. *NPJ Breast Cancer.* **2021**;7(1):4.
- [14] Zhang Y, Bi Z, Dong X, et al. tRNA-derived fragments: tRF-Gly-CCC-046, tRF-Tyr-GTA-010 and tRF-Pro-TGG-001 as novel diagnostic biomarkers for breast cancer. *Thorac Cancer.* **2021**;12(17):2314–2323.
- [15] Jia Y, Tan W, Zhou Y. Transfer RNA-derived small RNAs: potential applications as novel biomarkers for disease diagnosis and prognosis. *Ann Transl Med.* **2020**;8(17):1092.
- [16] Zhu P, Lu J, Zhi X, et al. tRNA-derived fragment tRF Lys-CTT-010 promotes triple-negative breast cancer progression by regulating glucose metabolism via G6PC. *Carcinogenesis.* **2021**;42(9):1196–1207. .
- [17] Rio DC, Ares M Jr, Hannon GJ, et al. Purification of RNA using TRIzol (TRI reagent). *Cold Spring Harb Protoc.* **2010**;2010(6):pdb.prot5439.
- [18] Mo D, Jiang P, Yang Y, et al. A tRNA fragment, 5'-tiRNAVal, suppresses the Wnt/ β -catenin signaling pathway by targeting FZD3 in breast cancer. *Cancer Lett.* **2019**;457:60–73.
- [19] Li SY, Zhu Y, Li RN, et al. LncRNA Lnc-APUE is repressed by HNF4 α and promotes G1/S phase transition and tumor growth by regulating miR-20b/E2F1 axis. *Adv Sci (Weinh).* **2021**;8(7):2003094.
- [20] Huang Q, Wang L, Ran Q, et al. Notopterol-induced apoptosis and differentiation in human acute myeloid leukemia HL-60 cells. *Drug Des Devel Ther.* **2019**;13:1927–1940.
- [21] Li P, Xu T, Zhou X, et al. Downregulation of miRNA-141 in breast cancer cells is associated with cell migration and invasion: involvement of ANP32E targeting. *Cancer Med.* **2017**;6(3):662–672.
- [22] DeSantis CE, Ma J, Gaudet MM, et al. Breast cancer statistics, 2019. *CA Cancer J Clin.* **2019**;69(6):438–451.
- [23] McDonald ES, Clark AS, Tchou J, et al. Clinical diagnosis and management of breast cancer. *J Nucl Med.* **2016**;57(1):9S–16S.
- [24] Berry DA, Cronin KA, Plevritis SK, et al. Cancer Intervention and Surveillance Modeling Network (CISNET) collaborators. Effect of screening and adjuvant therapy on mortality from breast cancer. *N Engl J Med.* **2005**;353(17):1784–1792.
- [25] Wang J, Ma G, Li M, et al. Plasma tRNA fragments derived from 5' ends as novel diagnostic biomarkers for early-stage breast cancer. *Mol Ther Nucleic Acids.* **2020**;21:954–964.
- [26] Farina NH, Scalia S, Adams CE, et al. Identification of tRNA-derived small RNA (tsRNA) responsive to the tumor suppressor, RUNX1, in breast cancer. *J Cell Physiol.* **2020**;235(6):5318–5327.
- [27] Huang SQ, Sun B, Xiong ZP, et al. The dysregulation of tRNAs and tRNA derivatives in cancer. *J Exp Clin Cancer Res.* **2018**;37:101.
- [28] Rashad S, Niizuma K, Tominaga T. tRNA cleavage: a new insight. *Neural Regen Res.* **2020**;15(1):47–52.
- [29] Zhang M, Li F, Wang J, et al. tRNA-derived fragment tRF-03357 promotes cell proliferation, migration and invasion in high-grade serous ovarian cancer. *Oncotargets Ther.* **2019**;12:6371–6383.
- [30] Frigerio JM, Dagorn JC, Iovanna JL. Cloning, sequencing and expression of the L5, L21, L27a, L28, S5, S9, S10 and S29 human ribosomal protein mRNAs. *Biochim Biophys Acta.* **1995**;1262(1):64–68.
- [31] Alkhatabi HA, McLornan DP, Kulasekararaj AG, et al. RPL27A is a target of miR-595 and may contribute to the myelodysplastic phenotype through ribosomal dysgenesis. *Oncotarget.* **2016**;7(30):47875–47890.
- [32] Zubor P, Hatok J, Moricova P, et al. Gene expression profiling of histologically normal breast tissue in females with human epidermal growth factor receptor 2-positive breast cancer. *Mol Med Rep.* **2015**;11(2):1421–1427.
- [33] Varis A, Wolf M, Monni O, et al. Targets of gene amplification and overexpression at 17q in gastric cancer. *Cancer Res.* **2002**;62(9):2625–2629.
- [34] Terzian T, Dumble M, Arbab F, et al. Rpl27a mutation in the sooty foot ataxia mouse phenocopies high p53 mouse models. *J Pathol.* **2011**;224(4):540–552.