Transfer RNA-derived small RNAs: A class of potential biomarkers in multiple cancers (Review)

CHUNYAN MAO[1,2], WENTAO YUAN[1,2], RONGHUA FANG[1,2], YI WU[1,2], ZHIHAN ZHANG[1,2] and HUI CONG[1,3]

[1]Department of Laboratory Medicine, Affiliated Hospital of Nantong University, Nantong, Jiangsu 226001, P.R. China;
[2]Department of Clinical Medicine, Medical School of Nantong University, Nantong, Jiangsu 226001, P.R. China;
[3]Department of Blood Transfusion, Affiliated Hospital of Nantong University, Nantong, Jiangsu 226001, P.R. China

Received November 10, 2023; Accepted April 11, 2024

DOI: 10.3892/ol.2024.14427

Abstract. Transfer (t)RNA-derived small RNAs (tsRNAs) are a class of novel non-coding small RNAs that are created via precise cleavage of tRNAs or tRNA precursors by different enzymes. tsRNAs are specific biological molecules that serve essential roles in cell proliferation, apoptosis, transcriptional regulation, post-transcriptional modification and translational regulation. Additionally, tsRNAs participate in the pathogenesis of several diseases, particularly in the development of malignant tumors. At present, the process of discovering and understanding the functions of tsRNAs is still in its early stages. The present review introduces the known biological functions and mechanisms of tsRNAs, and discusses the tsRNAs progression in several types of cancers as well as the possibility of tsRNAs becoming novel tumor biomarkers. Furthermore, tsRNAs may promote and hinder tumor formation according to different mechanisms and act as oncogenic or oncostatic molecules. Therefore, tsRNAs may be future potential tumor biomarkers or therapeutic targets.

Contents

1. Introduction
2. Biogenesis and classification of tsRNAs
3. Biological functions of tsRNAs
4. Roles of tsRNAs for diagnosis in several malignant tumors
5. Conclusion

Correspondence to: Professor Hui Cong, Department of Blood Transfusion, Affiliated Hospital of Nantong University, 20 Xisi Road, Nantong, Jiangsu 226001, P.R. China
E-mail: huicjs@163.com

Key words: non-coding RNA, transfer RNA-derived small RNAs, cancer, biological function, biomarker

1. Introduction

With the improvement of high-throughput sequencing technology, an increasing number of small non-coding (snc)RNAs have been elucidated. These are RNAs that do not code for proteins and include micro (mi)RNAs, circular RNAs, transfer (t)RNAs and P-element-induced wimpy testis (PIWI)-interacting (pi)RNAs (1). It has been reported that sncRNAs serve vital roles in the regulation of transcription and translation, as well as in the progression of multiple malignancies (2). In recent years, increasing evidence has demonstrated that tRNA-derived small (ts)RNAs are involved in several metabolic pathways and immunological processes (3), and are frequently dysregulated in malignant tumors (4). tsRNAs may also act as biomarkers and therapeutic targets for cancer prognosis and diagnosis, according to several studies (5,6).

tsRNAs are a group of sncRNAs produced from tRNAs, which are strongly associated with tRNA abundance and were initially thought to be degradation fragments (7). However, there is evidence that tsRNAs are byproducts of enzymatic digestion that specifically target mature or precursor tRNAs under particular circumstances , such as viral infection, stress induction (8,9). tsRNAs are clusters of functional molecules with great stability and inherently conserved features; they are not simply byproducts of tRNA breakdown (10). The present review describes the important characteristics and biological purposes of tsRNAs. The dysregulation and roles of tsRNAs in several malignancies are evaluated, and their potential as biomarkers and therapeutic targets for cancer detection and outlook is assessed.

2. Biogenesis and classification of tsRNAs

tsRNAs are classified into two groups based on length and cleavage site (Fig. 1 and Table I): One group comprises tRNA halves [tRNA-derived stress-induced (ti)RNAs], which have lengths of 28-36 nucleotides (nt) and are produced by angiogenin (ANG)-specific cleavage at the anticodon loop, including 3' and 5' tiRNAs (11); and the other group comprises tRNA-derived fragments (tRFs), which are 14-30 nt in length and are produced via cleavage of mature or precursor tRNAs at specific sites. A total of five subclasses of tRFs have been identified: tRF-1, tRF-2, tRF-3, tRF-5 and

internal (i)-tRF (12). Currently, it is unknown exactly how tRF-2 and i-tRF are produced (13); however, tRF-5 and tRF-3 are reported to be by-products of mature tRNA enzymatic digestion (14), whilst tRF-1 is a by-product of precursor tRNA enzymatic shearing (15). The mature tRNA's D-loop (tRF-5a, 14-16 nt) or the sequences between the D-loop and the anticodon loops (tRF-5b, 22-24 nt; and tRF-5c, 28-30 nt) are the sources of tRF-5 (16). tRF-5 is typically produced by Dicer and ANG cleavage to different termination sites between the 5' end and the anticodon loop. tRF-3, which is composed of tRF-3a and tRF-3b, is generated by Dicer cleavage on the TψC loop at the 3' end of mature tRNAs and the tail of each tRF-3 contains a specific CCA structure at the 3' end of the mature tRNA (17). tiRNAs are present in the cytoplasm, with tRF-5 common in the nucleus and tRF-3 and tRF-1 prominent in the cytoplasm (17). The 3' end of the tRNA precursor is where tRF-1 is produced, and it finishes in U bases. tRF-1 is the product of ribonuclease Z, shearing the 3' end of precursor tRNAs (15).

3. Biological functions of tsRNAs

The biological roles of tsRNAs have yet not been fully elucidated; however, they serve a wide range of significant biological functions, including in transcriptional regulation, post-transcriptional modification and translational regulation (18).

Transcriptional regulation. tsRNAs can regulate gene expression at the transcriptional level. tsRNAs are similar to piRNAs in physical structure and both are single-stranded RNAs. They are also similar to piRNAs, which can interact with PIWI proteins to form complexes and then interact with DNA methyltransferases to affect the methylation of genes, thereby exerting transcriptional repression (19,20). For example, Pekarsky *et al* (21) assessed whether ts-3676 and ts-4521 can interact with PIWI proteins as piRNAs by performing RNA immunoprecipitation experiments. They reported that ts-3676 and ts-4521 were notably enriched in complexes containing labeled PIWI-like protein (PIWIL)2 compared with controls, suggesting that ts-3676 and ts-4521 can interact with PIWIL2 as piRNAs. Zhang *et al* (22) reported that the tRNA-Glu-derived piRNA [td-piR(Glu)]/PIWIL4 complex recruits H3K9 methyltransferases (SETDB1, SUV39H1) and heterochromatin protein 1β to the CD1A promoter region and promotes H3K9 methylation, resulting in marked repression of CD1A transcription.

Post-transcriptional modification. tsRNAs serve important roles in the post-transcriptional regulation of several biological processes by regulating messenger (m)RNA stability. Similar to miRNAs, tsRNAs can take part in the formation of RNA-induced silencing complexes (RISCs) and regulate mRNA stability by RISC binding to the 3' untranslated region (3'-UTR) of target genes. They may also regulate gene expression through the post-transcriptional pathway (23). tsRNAs can also control gene expression by influencing the stability of targeted RNAs by competitively binding to RNA-binding proteins (RBPs) (24). Competitively binding to the 3'-UTR of RBP Y-box binding protein 1, tRFs displace oncogenic transcripts, reducing their stability and resulting in tumor suppressor and metastasis suppressor actions in breast cancer cells (Fig. 2A) (25).

Translational regulation. tsRNAs regulate gene expression at the translational level in different ways, including the Argonaute (AGO)-dependent/AGO-independent translational inhibition approaches, ribosome-related translation inhibition and ribosomal (r)RNA regulation. In the AGO-dependent translation inhibition mechanism, tsRNAs bind to AGO proteins and regulate the effectiveness of translation. tRFs preferentially bind to the AGO1, AGO3 and AGO4 proteins to silence mRNA translation (Fig. 2B) (13). The primary effector protein of miRNA-induced RISC is AGO2. In the AGO-independent translational regulation approach, tsRNAs can form RNA G-quadruplexes (RG4), whereas tiRNAs produced by ANG stress can form RG4 structures, target and displace eukaryotic initiation factors (eIF)4A/E/G, and can inhibit the initiation of mRNA translation (Fig. 2C) (26). Furthermore, it was reported that tRFs are associated with the Tetrahymena Piwi protein 12 (Twi12), which joins with 5'-3' exoribonuclease 2 (Xrn2) and Twi-associated novel 1 (Tan1) to form a complex that controls the regulation of rRNA translation (Fig. 2D) (27). In a study by Kim *et al* (28), it was reported that tsRNA binds to coding and noncoding 28'UTR sequences in ribosomal protein S28 (RPS28) mRNA, altering its secondary structure and enhancing its translation. Moreover, RPS28 mRNA translation was reduced by LeuCAG3'tsRNA inhibition (Fig. 2E). In conclusion, tsRNAs have several biological functions and important biological roles in cells.

4. Roles of tsRNAs for diagnosis in several malignant tumors

tsRNAs have a dual role in regulating cancers by acting both as promoters and inhibitors; thus, tsRNAs are both oncogenic and oncostatic molecules (24). This indicates that tsRNAs have clinical diagnostic value (29). The main cancers that have been investigated in detail include breast cancer (BC) (30), gastric cancer (GC) (31) and colorectal cancer (CRC) (32). Furthermore, other cancers have been studied, including urothelial bladder carcinoma (UBC) (33), lung cancer (34), gliomas (35), laryngeal squamous cell carcinoma (LSCC) (36,37), epithelial ovarian cancer (EOC) (38) and prostate cancer (PCa) (39). In these studies, the mechanisms by which tsRNAs regulate cancer are mainly characterized by cell apoptosis, invasion, proliferation and mediation of signaling pathways (40,41).

BC. tsRNAs serve crucial regulatory roles in BC expression, and they are anticipated to be developed as biomarkers for the diagnosis and monitoring of early-stage BC (42). Wang *et al* (43) reported that tRF-Glu-CTC-003, tRF-Gly-CCC007, tRF-Gly-CCC-008, tRF-Leu-CAA-003, tRF-Ser-TGA-001 and tRF-Ser-TGA-002 were notably downregulated in the plasma and tissues of a patient with early-stage BC. In addition, it was reported that these six tRFs may distinguish between patients with BC who had lymph node metastases and healthy individuals; however, further research revealed no statistically significant

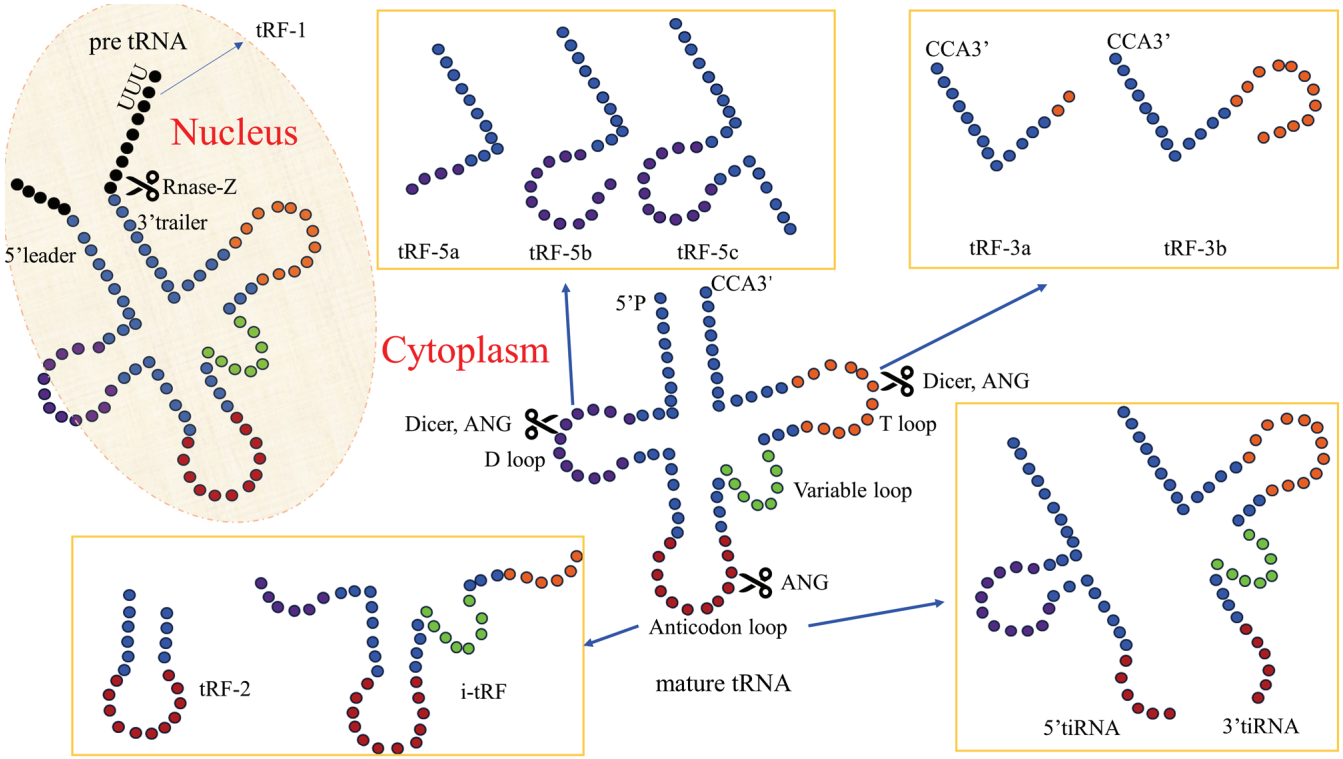

Figure 1. Classification of tsRNAs. tsRNAs can be divided into tRFs and tiRNAs. The tiRNAs are derived from mature tRNA in the cytoplasm and can be divided into 5'tiRNA and 3'tiRNA. The source of tRF-2 and i-tRF is not yet clear. tRF-1 is derived from pre-tRNA in the nucleus. tRF-5s and tRF-3s are generated from mature tRNA in the cytoplasm. tRNA, transfer RNA; tsRNA, tRNA-derived small RNA; tRF, tRNA-derived fragments; i-tRF, internal tRF; tiRNA, tRNA-derived stress-induced RNA; pre-tRNA, tRNA precursor; ANG, angiogenin; RnaseZ, ribonuclease Z.

results. Zhang *et al* (44) reported that tRF-Gly-CCC-046, tRF-Tyr-GTA-010 and tRF-Pro-TGG-001 were downregulated in both the sera and tissues of patients with BC, indicating that the three tRFs may function as circulating biomarkers for the identification of BC. When these three tRFs were combined with conventional biomarkers, the area under the curve (AUC) and sensitivity increased, which markedly enhanced the possibility of conventional biomarkers to diagnose early-stage BC. Moreover, according to a study by Sun *et al* (45), exosomal tRF-16-K8J7K1B targets tumor-necrosis factor related apoptosis-inducing ligand to induce tamoxifen resistance in BC. tRF-16-K8J7K1B was reported to be upregulated in the cells and sera of patients with tamoxifen-resistant BC, and its overexpression boosted BC cell proliferation, migration, invasion and apoptosis. To overcome tamoxifen resistance, exosomal tRF-16-K8J7K1B may be a useful forecasting marker and a treatment target. By targeting and suppressing ribosomal protein-L27A, tRF-19-W4PU732S was observed to increase the activity of BC cells in the study by Zhang *et al* (46) tRF-19-W4PU732S was notably highly expressed in BC tissues and cell lines (MCF-7 and MDA-MB-231) and was associated with a poor prognosis for survival. In a study by Mo *et al* (47) it was reported that tRF-17-79 MP9PP was downregulated in BC tissues and serum and inhibited BC cell invasion and migration via the thrombospondin 1/transforming growth factor β1/Mothers against decapentaplegic homolog 3 axis, whereas attenuated expression of THBS 1 reversed tRF-17-79MP9PP-mediated inhibition of BC cells. According to Ma and Liu (48), tRF-20-S998LO9D was upregulated in BC tissues and may be a cancer-promoting molecule in BC. Furthermore, the role of 5'-tRF-GlyGCC in the advancement of BC was assessed by Chen *et al* (49) who reported that 5'-tRF-GlyGCC was upregulated in BC tissues and 5'-tRF-GlyGCC restricted autophagy, enhanced fat mass and obesity-associated protein demethylase activity, lowered eIF4G1 methylation, and directly binded to proteins linked to adiposity and obesity. The results point to 5'-tRF-GlyGCC as a possible option for BC therapy. Additionally, research by Mo *et al* (50) assessed whether tiRNAs contribute to the progression of BC. The expression of 5'-tiRNA^{Val} was markedly downregulated in BC tissues, and stage and lymph node metastases were associated with serum 5'-tiRNA^{Val} downregulation. Moreover, 5'-tiRNA^{Val} prevented the Wnt/collagen signaling pathway that is regulated by frizzled class receptor 3, which could be a potential therapeutic target in BC. Wang *et al* (51) also reported that miRNA-34 directly targets the tRNA$_i^{Met}$ precursor via AGO-mediated cleavage to hinder the proliferation of BC cells. 6-phosphofructo-2-kinase/fructose 2, 6-bisphosphatase 3 (PFKFB 3) has been reported to be a possible target of tRiMetF31 by luciferase analysis. Whilst tRNA$_i^{Met}$ and PFKFB 3 were upregulated in BC and elevated PFKFB 3 was notably associated with metastasis, miR-34a was downregulated in BC. Given the research, tRiMetF31 offers an innovative target for therapeutic intervention in BC by suppressing PFKFB 3 and inhibiting angiogenesis. In conclusion, tsRNAs serve a vital regulatory role in the biogenesis of BC, and investigations into their dysregulation roles in BC and the possibility for them to serve as novel diagnostic biomarkers are required.

Table I. tsRNA types.

tsRNA	Length, nt	Subclass of tsRNA	Generation mechanism	Location
Trf	14-30	tRF-1	Produced by shearing the 3' end of pre-tRNA by Rnase Z	Cytoplasm
		tRF-3	Generated by Dicer cleavage on the TψC loop at the 3' end of mature tRNAs	Cytoplasm
		tRF-5	Produced by Dicer and ANG cleavage in the D-loop or the region between the D-loop and the anticodon loop of mature tRNA	Nucleus
		tRF-2	Not clear	Not clear
		i-tRF	Not clear	Not clear
TiRNA	28-36	3'tiRNA	Produced by ANG-specific cleavage at the anticodon loop of mature tRNA	Cytoplasm
		5'tiRNA	Produced by ANG-specific cleavage at the anticodon loop of mature tRNA	Cytoplasm

tRNA, transfer RNA; tsRNA, tRNA-derived small RNA; tRF, tRNA-derived fragment; tiRNA, tRNA-derived stress-induced RNA; pre-tRNA, tRNA precursor; Rnase Z, ribonuclease Z; ANG, angiogenin; nt, nucleotide.

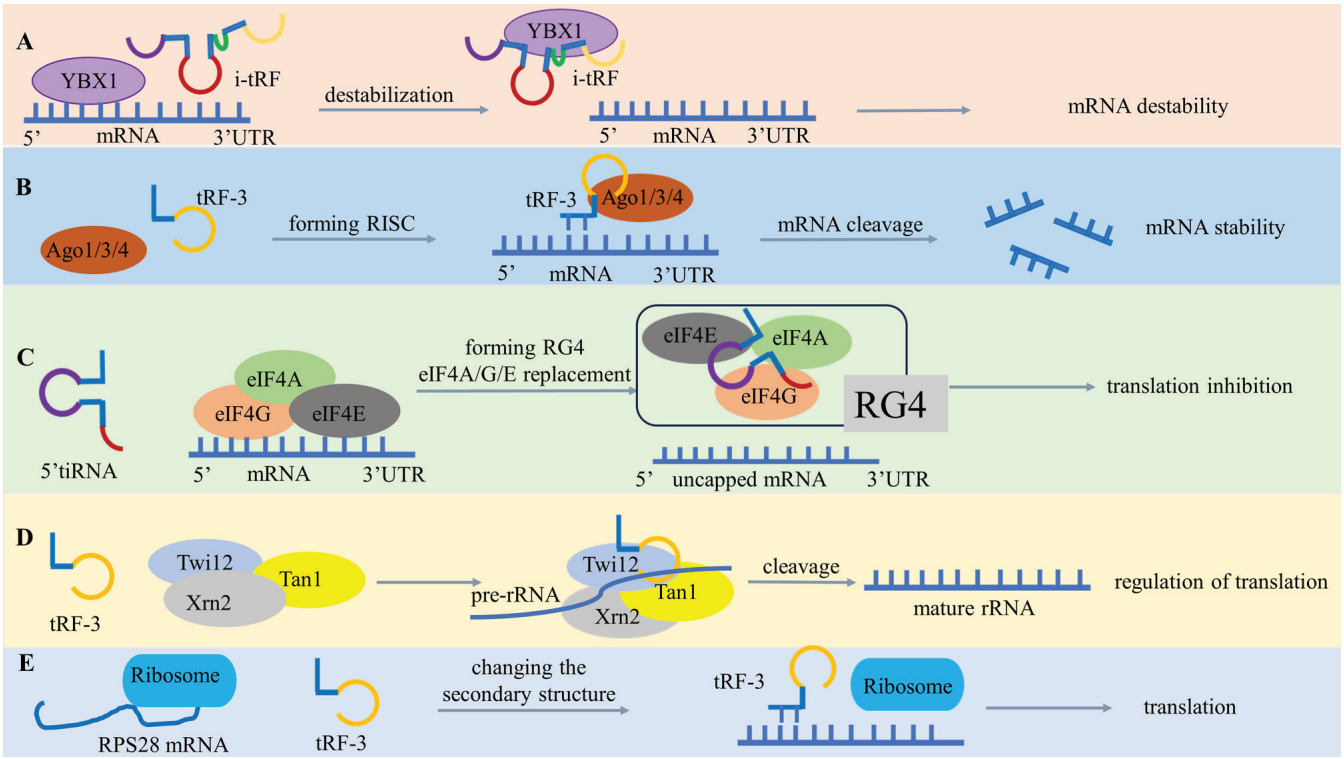

Figure 2. Biological functions of tsRNAs. (A) i-tRF bind to YBX1 to decrease oncogenic transcript stability. (B) tRF-3 forms RISC with Ago proteins to silence mRNA. (C) 5'tiRNA bind to the eIF4A/E/G complex, displacing mRNA without cap structure to cause translational inhibition. (D) tRF-3 binds to Twi12, Xrn2 and Tan1 to form a complex affecting the rRNA process. (E) Regulation of ribosome biogenesis by changing RPS28 mRNA secondary structure during translation. tRNA, transfer RNA; tsRNA, tRNA-derived small RNA; tRF, tRNA-derived fragment; i-tRF, internal tRF; YBX1, Y-box binding protein 1; RISC, RNA-induced silencing complex; Ago, argonaute; tiRNA, tRNA-derived stress-induced RNA; eIF4A/E/G, eukaryotic initiation factors 4A/E/G; Twi12, twinfilin actin binding protein 2; Xrn2, 5'-3' exoribonuclease 2; Tan-1, translocation-associated notch protein 1; rRNA, ribosomal RNA; RPS28, ribosomal protein S28; 3' UTR, 3' untranslated region; RG4, RNA G-quadruplexes.

GC. Studies have reported that tsRNAs can regulate GC development and modulate GC progression in several ways. An experimental investigation on the mechanism of tRF-Val-CAC-016 in GC was performed by Xu *et al* (52), which revealed that tRF-Val-CAC-016 was markedly down-regulated in GC tissues. tRF-Val-CAC-016 suppressed GC progression by controlling the calcium voltage-gated channel α1 D-mediated MAPK signaling pathway. This finding indicates that tRF-Val-CAC-016 may serve as a therapeutic target for the early detection of GC. Zhang *et al* (53) reported that a high expression of tRF-23-Q99P9P9NDD in GC serum could more effectively distinguish between patients with GC, gastritis and healthy donors. A high expression of tRF-23-Q99P9P9NDD was associated with a shorter lifespan, according to Kaplan-Meier survival curve analysis. Therefore, tRF-23-Q99P9P9NDD may be used for the potential

monitoring of patients with GC. According to Gu *et al* (54), serum tRF-17-WS7K092 expression in patients with GC notably decreased compared with healthy donors, and its high expression was associated with a poor prognosis. After being combined with common biomarkers, the sensitivity and AUC values of tRF-17-WS7K092 markedly improved, indicating that it may be a GC diagnostic and prognostic biomarker. Zheng *et al* (55) reported that tRNA-Val-CAC-001 was downregulated in GC tissues and cells, and could exert its effects by targeting LDL receptor-related protein 6 through the Wnt/β-collagen signaling pathway in GC. Xu *et al* (56) reported that tRF-Glu-TTC-027 had a low expression of GC tissues compared with normal tissues. In addition, tRF-Glu-TTC-027 could regulate the progression of GC both *in vivo* and *in vitro* through the MAPK signaling pathway. The aforementioned study reported that tRF-Glu-TTC-027 could be exploited as a target for molecularly targeted treatment in GC. Shen *et al* (57) revealed that tRF-33P4R8YP9LON4VDP was downregulated in the preoperative serum of patients with GC, which tended to be lower than that of healthy donors. The finding predicted that tRF-33P4R8YP9LON4VDP may act as a tumor suppressor. In the study by Li *et al* (58), the tRF-29-R9J8909NF5JP expression levels in GC serum were associated with the degree of differentiation, tumor stage, lymph node metastasis, tumor lymph node metastasis stage and nerve/vascular infiltration. A high expression of serum tRF-29-R9J8909NF5JP was associated with a decreased rate of survival, based on the results of Kaplan-Meier survival curve analysis. Wang *et al* (59) observed that tRF-41-YDLBRY73W0K5KKOVD expression was decreased in GC cells and tissues. Functionally, overexpression of tRF-41-YDLBRY-73W0K5KKOVD diminished the cell cycle, induced apoptosis and hindered cell proliferation and migration. This implies that the protein tRF-41-YDLBRY73W0K5KKOVD is a tumor suppressor and may potentially be used in the future to treat GC. Cui *et al* (60) reported that tRF-Val is highly expressed in GC tissues and cell lines. Mechanistically, tRF-Val directly binds to the chaperone molecule eukaryotic translation elongation factor 1 α1 specifically and then translocates to the nucleus, increasing GC cell proliferation and inhibiting GC cell apoptosis. These findings indicate new molecular mechanisms for the development of GC. Furthermore, according to Shen *et al* (61), there was low-level expression of tRF-19-3L7L73JD in the preoperative plasma group compared to post-operative plasma group and healthy donors. Moreover, tRF-19-3L7L73JD had low expression in GC cell lines (BGC-823/AGS/SGC-7901) compared with human epithelial cells (GES-1). Functionally, tRF-19-3L7L73JD overexpression prevented GC cells from proliferating and migrating, encouraged apoptosis and preventing the cells from entering the G0/G1 phase. In a study by Huang *et al* (62), tRF-31-U5YKFN8DYDZDD was upregulated in the serum of patients with GC compared with that of healthy donors. High expression of serum tRF-31-U5YKFN8DYDZDD is associated with the tumor-node-metastasis stage, tumor infiltration depth, lymph node metastasis and vascular infiltration in GC. Receiver operating characteristic curve analysis demonstrated that the detection efficiency of tRF-31-U5YKFN8DYDZDD was greatly improved after combining it with a conventional marker. Therefore, tRF-31-U5YKFN8DYDZDD may be a therapeutic target in GC. Moreover, previous research by Wang *et al* (63) revealed low expression of tRF-24-V29K9UV3IU in GC tissues compared with that of adjacent tissues. Knockdown of tRF-24-V29K9UV3IU expression increased the expression of vimentin and N-cadherin, whilst decreasing the expression of E-cadherin in mouse tumors. As a downstream target gene of tRF-24-V29K9UV3IU, G protein-coupled receptor 78 attenuated the inhibitory effects of overexpressed tRF-24-V29K9UV3IU on GC cell proliferation, migration and invasion. Additionally, Tong *et al* (64) reported the high expression of tRF-3017 A in GC tissues and cell lines. tRF-3017 A regulated the tumor suppressor gene neural EGFL like 2 (NELL2) by creating an RISC with the AGO protein. The authors reported that tRF-3017 A promoted GC cell invasion and migration by cutting off the tumor inhibitor NELL2 and promoting GC cell migration and invasion. Zhu *et al* (65) revealed that tRF-5026a was upregulated in GC tissues, serum and cell lines, and high expression of tRF-5026a was positively associated with decreased survival time. Western blotting analyses demonstrated that tRF-5026a diminished the proliferation, migration and cell cycle progression of GC cells by controlling the PTEN/PI3K/AKT signaling pathway. Finally, the impact of tRF-5026a on tumor growth was assessed using a subcutaneous tumor model in nude mice and animal tests revealed that upregulating tRF-5026a had a major inhibitory impact on tumor growth. In summary, tsRNAs are crucial to the development of GC and further studies are needed to explore the molecular processes of tsRNAs in GC and discover more potential therapeutic targets and biomarkers.

CRC. Wu *et al* (66) assessed the diagnostic value of 5'-tRF-GlyGCC in CRC and reported that the expression of 5'-tRF-GlyGCC was markedly upregulated in CRC tissues and plasma. The sensitivity and AUC of 5'-tRF-GlyGCC were notably increased when it was combined with the conventional biomarkers carcinoembryonic antigen (CEA), carbohydrate antigen (CA)199 and CA724. This suggests that 5'-tRF-GlyGCC has the potential to be a novel biomarker for CRC treatment. Lu *et al* (67) identified a novel tRNA-derived fragment, tRF-3022b, which was upregulated in CRC tissues and plasma exosomes. The study revealed that tRF-3022b regulated migration inhibitory factor (MIFs) in M2 macrophages by binding to Galectin 1 and MIFs in CRC cells to reduce polarization. Tsiakanikas *et al* (68) reported that 5'-tiRNA-ProTGG was markedly upregulated in CRC tissues. They assessed the value of 5'-tiRNA-ProTGG in CRC prognosis. Kaplan-Meier survival curve analysis demonstrated that high levels of 5'-tiRNA-ProTGG were associated with an unfavorable prognostic value in patients with rectal cancer and/or moderately differentiated CRC (grade II). As the underlying mechanisms of epithelial-mesenchymal transition (EMT) in CRC are still unclear, Chen *et al* (69) evaluated the participation of tRF in EMT and its role in CRC progression. tRF-phe-GAA-031 and tRF-VAL-TCA-002 were screened using high-throughput sequencing and quantitative PCR, and it was reported that their expression levels in CRC tissues were markedly higher than that in paraneoplastic non-tumor tissues, and the expression was associated with tumor metastasis and clinical stage. tRF-phe-GAA-031 and tRF-VAL-TCA-002 may serve vital roles in the metastasis of CRC. In conclusion, they

could serve as probable markers in the therapy of CRC. The study by Luan *et al* (70) assessed the roles of key tRFs in CRC progression and their associated mechanisms, and reported that there was a low expression of tRF-20-M0NK5Y93 in CRC cell lines (RKO/SW480). Functionally, tRF-20-M0NK5Y93 inhibited CRC cell migration and invasion by targeting the EMT-associated molecule Claudin-1. In a follow-up study, tRF-20-M0NK5Y93-induced metastasis associated lung adenocarcinoma transcript 1 was reported to promote CRC metastasis through selective splicing of structural maintenance of chromosomes 1A. The two aforementioned findings suggest that tRF-20-M0NK5Y93 may be a new potential therapeutic target (71). In a study by Tao *et al* (72), 5'tiRNA-His-GTG was reported to be upregulated in CRC tissues. It was revealed to be produced in response to the tumor hypoxic microenvironment and was regulated through the hypoxia-inducible factor-1α/angiopoietin axis. Large tumor suppressor kinase 2 (LATS2) was identified as an important target of 5'tiRNA-His-GTG and it was reported that LATS2 'switches off' the 5'tiRNA-His-GTG through the regulation of the Hippo signaling pathway and promotes anti-apoptosis-associated gene expression. In summary, the potential mechanism by which tsRNAs affect CRC development is not yet clear and needs to be further explored to discover more potential tumor biomarkers and therapeutic targets.

UBC. Qin *et al* (33) observed that tiRNA-Gly-GCC-1 was markedly upregulated in UBC tissues and tiRNA-Gly-GCC-1 inhibited toll-like receptor 4 (TLR4) expression by directly targeting the 3'UTR of TLR4.

Lung cancer. Hu *et al* (73) reported that tsRNA-5001A was markedly elevated in lung adenocarcinoma tissues and high expression of tsRNA-5001A was associated with decreased survival time. Cell function assays indicated that tsRNA-5001A overexpression promoted the proliferation of lung cancer cell lines (A549/PC9).

Glioma. Ren *et al* (74) proposed that tRFdb-3003a and tRFdb-3003b serve key roles in glioma development. tRFdb-3003a and tRFdb-3003b may bind directly to vav guanine nucleotide exchange factor 2 to inhibit glioma progression. tRFdb-3003a/b expression is notably reduced in glioma tissues. Xu *et al* (75) reported that in tsRNA derived from tRNA-Leu-CAA, ts-26, tRFdb-3012 a/b expression was downregulated in diffuse glioma tissues. The expression of tRFdb-3012 a/b in gliomas was associated with isocitrate dehydrogenase mutation status and O6-methylguanine-DNA methyltransferase promoter mutations. This suggests that ts-26, tRFdb-3012 a/b may be used as diagnostic and prognostic biomarkers for diffuse gliomas and that tRFdb-3012 a/b and ts-26 may affect glioma progression by binding RNA-binding motif protein 43 and homeobox A13, respectively.

LSCC. Deng *et al* (36) reported that tRF-33-Q1Q89P9L842205 expression was markedly downregulated in LSCC tissues. In addition, tRF-33-Q1Q89P9L842205 expression was associated with lymph node metastasis in LSCC. The results revealed that tRF-33-Q1Q89P9L842205 inhibits the proliferation, migration, invasion and induction of apoptosis of LSCC cells through the direct silencing of the catalytic subunit of phosphatidylinositol 3-kinase. It is hypothesized that tRF-33-Q1Q89P9L842205 acts as a potential tumor suppressor by directly targeting phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit Δ (PIK3D). In a study by Zhao *et al* (37), tRF^{Tyr} was reported to be markedly elevated in LSCC tissues and cell lines (AMC-HN8/TU212/TU686). Mechanistic studies showed that tRF^{Tyr} interacted with lactate dehydrogenase A to increase phosphorylation levels and activate lactate dehydrogenase A to induce lactate accumulation in LSCC cells.

EOC. The clinical utility of tRF in EOC was first assessed by Panoutsopoulou *et al* (76) who reported that serum i-tRF-GlyGCC expression was upregulated in patients with EOC, indicating an unfavorable prognostic value of highly expressed i-tRF-GlyGCC for the therapy and survival of patients with EOC.

PCa. Wang *et al* (77) reported that the expression of tRF-Glu-TTC-2 in PCa tissues and cell lines (PC3) was upregulated, and overexpressed tRF-Glu-TTC-2 promoted the proliferation of PCa cells. These results suggest that tRF-Glu-TTC-2 may be a novel oncogene. Furthermore, according to Yang *et al* (78), tRF-315 expression was increased in PCa cells (LNCaP, DU145 and PC3) compared with that in normal prostate cells. In LNCaP and DU145 cells, tRF-315 not only alleviated cisplatin induced apoptosis, but also regulated the cisplatin-altered cell cycle by targeting the tumor suppressor gene, growth arrest and DNA damage inducible α.

In summary, tsRNAs reflect the characteristics and changes of tumor tissues and have potential tumor diagnostic value. Analyzing tsRNAs in tumor tissues or body fluids could help predict the treatment response and prognosis of patients.

5. Conclusion

The roles and mechanisms of tsRNAs in several malignant tumors are summarized in Table II. The occurrence, progression and prognosis of diseases have all been reported to be strongly associated with anomalies in tsRNA expression in malignancies.

With the continuous innovation and development of technical means, tsRNAs have received attention (79). Several studies (80,81) have reported that dysregulated tsRNAs serve a regulatory role in several cancers, which is of great significance to the diagnosis, treatment and prognosis of cancers, and thus tsRNAs have been regarded as potential tumor biomarkers. However, there are still limitations to the investigation of tsRNA as a potential biomarker. The majority of existing studies are single longitudinal studies, and research methods are not standardized. Furthermore, tsRNA research is still in its infancy, the understanding of the distribution and biological functions of tsRNAs is still incomplete, and the mechanism by which tsRNAs regulate cancer has not yet been fully elucidated. Therefore, tsRNAs currently cannot be used for clinical diagnosis, and more in-depth studies are required to improve and optimize them.

Finally, several studies have reported that the properties of tsRNAs, such as high sensitivity, have a broad application prospect in the early diagnosis of cancer. Currently, there are

Table II. Roles and mechanisms of tsRNAs in malignant tumors.

First author/s, year	Cancer type	tsRNA	Expression of tsRNA compared with normal tissue/healthy samples/normal cells	Effect	(Refs.)
Wang *et al*, 2020	BC	tRF-Glu-CTC-003, tRF-Gly-CCC007, tRF-Gly-CCC-008, tRF-Leu-CAA-003, tRF-Ser-TGA-001 and tRF-Ser-TGA-002	Downregulated in BC tissue/plasma	Inhibited BC cell proliferation	(43)
Zhang *et al*, 2021		tRF-Gly-CCC-046, tRF-Tyr-GTA-010 and tRF-Pro-TGG-001	Downregulated in BC tissue/sera	Functioned as circulating biomarkers for the identification of early-stage BC	(44)
Sun et al, 2023		tRF-16-K8J7K1B	Upregulated in BC sera	Targeted TRAIL to induce tamoxifen resistance in BC	(45)
Zhang *et al*, 2022		tRF-19-W4PU732S	Upregulated in BC tissue	Targeted RPL27A to increase the activity of BC cells	(46)
Mo *et al*, 2021		tRF-17-79 MP9PP	Downregulated in BC tissue/sera	Suppressed the invasion and migration of BC cells through the THBS 1/TGF-β1/Smad 3 axis	(47)
Ma and Liu, 2022		tRF-20-S998LO9D	Upregulated in BC tissue	Maybe an oncogene in BC	(48)
Chen *et al*, 2023		5'-tRF-GlyGCC	Upregulated in BC tissue	Promoted BC metastasis by increasing fat mass and obesity-associated protein demethylase activity	(49)
Mo et al, 2019		5'-tiRNA^{Val}	Downregulated in BC tissue/sera	Suppressed the Wnt/β-catenin signaling pathway by targeting FZD3 in BC	(50)
Wang *et al*, 2022		tRiMetF31	Downregulated in BC cell lines (MCF7, HCC1806, HCC1419 and ZR75-1)	Suppressed migration and angiogenesis of BC cells via targeting PFKFB3	(51)
Xu *et al*, 2022	GC	tRF-Val-CAC-016	Downregulated in BC tissue	Modulated the transduction of the CACNA1d-mediated MAPK signaling pathway to suppress GC cell proliferation	(52)
Zhang *et al*, 2022		tRF-23-Q99P9P9NDD	Upregulated in BC tissue/sera	Promoted GC cell proliferation, migration and invasion	(53)
Gu 0*et al*, 2022		tRF-17-WS7K092	Upregulated in BC serum	Improved diagnostic sensitivity and AUC when combined with CEA, CA19-9 and CA72-4	(54)
Zheng *et al*, 2022		tRNA-Val-CAC-001	Downregulated in BC tissue	Prevented cell proliferation by targeting LRP 6 through the Wnt/β-collagen signaling pathway	(55)
Xu *et al*, 2021		tRF-GluTTC-027	Downregulated in BC tissue	Regulated the progression of GC through the MAPK signaling pathway	(56)
Shen *et al*, 2021		tRF-33P4R8YP9LON4VDP	Downregulated in BC serum	Maybe a tumor suppressor	(57)
Li *et al*, 2023		tRF-29-R9J8909NF5JP	Upregulated in BC serum	High expression was associated with a decreased rate of survival	(58)

Table II. Continued.

First author/s, year	Cancer type	tsRNA	Expression of tsRNA compared with normal tissue/healthy samples/normal cells	Effect	(Refs.)
Wang *et al*, 2023		tRF-41-YDLBRY73W0K5KKOVD	Downregulated in BC tissue	Suppresses cell cycle progression, induced apoptosis and hindered cell proliferation and migration	(59)
Cui *et al*, 2022		tRF-Val	Upregulated in BC tissue	Promotes proliferation and inhibited apoptosis by targeting EEF1A1	(60)
Shen *et al*, 2021		tRF-19-3L7L73JD	Downregulated in BC plasma	Inhibited cell proliferation, migration and invasion; promoted apoptosis; and inhibited the cells from entering the G0/G1 phase	(61)
Huang *et al*, 2021		tRF-31-U5YKFN8DYDZDD	Upregulated in BC serum	Improved detection efficiency after combining with conventional biomarkers	(62)
Wang *et al*, 2022		tRF-24-V29K9UV3IU	Downregulated in BC tissue	Prevents GC progression by inhibiting GPR78 expression	(63)
Tong *et al*, 2020		tRF-3017 A	Upregulated in BC tissue	Promoted metastasis by inhibiting NELL2	(64)
Zhu *et al*, 2021		tRF-5026 a	Downregulated in BC tissue/serum	Diminished cell proliferation, migration and cell cycle progression by controlling the PTEN/PI3K/AKT signaling pathway	(65)
Wu *et al*, 2021	CRC	5'-tRF-GlyGCC	Upregulated in CRC tissue/plasma	May be a new biomarker for CRC diagnosis	(66)
Lu *et al*, 2022		tRF-3022b	Upregulated in CRC tissue/plasma	Modulated cell apoptosis and M2 macrophage polarization via binding to cytokines	(67)
Tsiakanikas *et al*, 2022		5'-tiRNA-Pro^{TGG}	Upregulated in CRC tissue	High expression levels were associated with poor prognosis	(68)
Chen *et al*, 2022		tRF-phe-GAA-031 and tRF-VAL-TCA-002	Upregulated in CRC tissue	Served as probable markers in the therapy of CRC	(69)
Luan *et al*, 2021 and Luan *et al*, 2023		tRF-20-M0NK5Y93	Downregulated in CRC cell lines (RKO and SW480) under hypoxic conditions	Inhibited CRC cell migration and invasion in part by targeting the EMT-associated molecule Claudin-1	(70, 71)
Tao *et al*, 2021		5'ti RNA-His-GTG	Upregulated in CRC tissue	Responded to hypoxia via the HIF-1α/ANG axis and promoted CRC progression by regulating LATS2	(72)
Qin *et al*, 2022	UBC	tiRNA-Gly-GCC-1	Upregulated in UBC tissue	Target TLR4 to promote progression of UBC	(33)
Hu *et al*, 2021	Lung Cancer	tsRNA-5001a	Upregulated in lung cancer tissue	Promoted lung cancer cell proliferation	(73)
Ren *et al*, 2022	Glioma	tRFdb-3003a/b	Downregulated in glioma tissue	Attached to VAV2 to inhibit glioma progression	(74)

Table II. Continued.

First author/s, year	Cancer type	tsRNA	Expression of tsRNA compared with normal tissue/healthy samples/normal cells	Effect	(Refs.)
Xu *et al*, 2022		ts-26, tRFdb-3012 a/b	Downregulated in glioma tissue	Affected glioma progression by binding RBM 43 and HOXA 15	(76)
Deng *et al*, 2022	LSCC	tRF-33-Q1Q89P9L842205	Downregulated in LSCC tissue	A potential diagnostic biomarker for LSCC. Acted as a tumor suppressor by directly targeting PIK3CD	(36)
Zhao *et al*, 2023		tRF^{Tyr}	Upregulated in LSCC tissue	Induced oncogenesis and lactated accumulation in LSCC by interacting with LDHA	(37)
Panoutsopoulou *et al*, 2021	EOC	i-tRF-GlyGCC	Upregulated in EOC serum	High levels of i-tRF-GlyGCC expression associated with a lower survival	(76)
Wang *et al*, 2022	PCa	tRF-Glu-TTC-3	Upregulated in PCa tissue	May be a novel oncogene	(77)
Yang *et al*, 2021		tRF-315	Upregulated in PCa cell lines (LNCaP, DU145 and PC3)	Prevented cisplatin-induced apoptosis and alleviated cisplatin-induced mitochondrial dysfunction in PCa cells	(78)

tRNA, transfer RNA; tsRNA, tRNA-derived small RNA; tRF, tRNA-derived fragment; tiRNA, tRNA-derived stress-induced RNA; i-tRF, internal tRF; BC, breast cancer; GC, gastric cancer; CRC, colorectal cancer; UBC, urothelial bladder cancer; NSCLC, non-small cell lung cancer; LSCC, laryngeal squamous cell carcinoma; EOC, epithelial ovarian cancer; PCa, prostate cancer; TRAIL, tumor-necrosis factor related apoptosis-inducing ligand; RPL27A, ribosomal protein L27a; THBS 1, thrombospondin 1; TGF-β1, transforming growth factor β1; Smad 3, Mothers against decapentaplegic homolog 3; FZD3, frizzled receptor 3; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; CACNA1d, calcium voltage-gated channel α1 D; AUC, area under the curve; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; CA72-4, cancer antigen 72-4; LRP 6, LDL receptor-related protein 6; EEF1A1, eukaryotic translation elongation factor 1 α1; GPR78, G Protein-coupled receptor 78; NELL2, neural EGFL like 2; EMT, epithelial-mesenchymal transition; HIF-1α, hypoxia-inducible factor-1α; ANG, angiogenin; LATS2, large tumor suppressor kinase 2; TLR4, toll-like receptor 4; VAV2, vav guanine nucleotide exchange factor 2; RBM 43, RNA-binding motif protein 43; HOXA 13, homeobox A13; PIK3CD, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit Δ.

fewer studies on tsRNA, and the potential of tsRNAs as tumor biomarkers and therapeutic targets are being explored. In the future, multi-longitudinal studies need to be performed to combine multiple tsRNAs with traditional biomarkers such as CEA and CA199 to improve the diagnostic efficiency of cancer. Moreover, it is necessary to expand the sample size to study whether tsRNAs can be utilized for clinical analysis. Therefore, extensive further research is still required to use tsRNA as a biomarker in clinical practice.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Key Medical Research Projects of Jiangsu Provincial Health Commission (grant no. ZD2022008).

Availability of data and materials

Not applicable.

Authors' contributions

CM contributed to writing the original manuscript. CM, WY and RF provided the direction and guidance for design of this manuscript. ZZ, YW and HC contributed to the conceptualization. HC contributed to revising the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Xiong Q, Zhang Y, Li J and Zhu Q: Small non-coding RNAs in human cancer. Genes (Basel) 13: 2072, 2022.
2. Romano G, Veneziano D, Acunzo M and Croce CM: Small non-coding RNA and cancer. Carcinogenesis 38: 485-491, 2017.
3. Gornalusse G, Spengler RM, Sandford E, Kim Y, Levy C, Tewari M, Hladik F and Vojtech L: Men who inject opioids exhibit altered tRNA-Gly-GCC isoforms in semen. Mol Hum Reprod 29: gaad003, 2023.
4. Wang Y, Weng Q, Ge J, Zhang X, Guo J and Ye G: tRNA-derived small RNAs: Mechanisms and potential roles in cancers. Genes Dis 9: 1431-1442, 2022.
5. Jia Y, Tan W and Zhou Y: Transfer RNA-derived small RNAs: Potential applications as novel biomarkers for disease diagnosis and prognosis. Ann Transl Med 8: 1092, 2020.
6. Kim HK, Yeom JH and Kay MA: Transfer RNA-derived small RNAs: Another layer of gene regulation and novel targets for disease therapeutics. Mol Ther 28: 2340-2357, 2020.
7. Qin C, Xu PP, Zhang X, Zhang C, Liu CB, Yang DG, Gao F, Yang ML, Du LJ and Li JJ: Pathological significance of tRNA-derived small RNAs in neurological disorders. Neural Regen Res 15: 212-221, 2020.
8. Taxis TM, Bauermann FV, Ridpath JF and Casas E: Analysis of tRNA halves (tsRNAs) in serum from cattle challenged with bovine viral diarrhea virus. Genet Mol Biol 42: 374-379, 2019.
9. Drino A, Oberbauer V, Troger C, Janisiw E, Anrather D, Hartl M, Kaiser S, Kellner S and Schaefer MR: Production and purification of endogenously modified tRNA-derived small RNAs. RNA Biol 17: 1104-1115, 2020.
10. Chen Q, Zhang X, Shi J, Yan M and Zhou T: Origins and evolving functionalities of tRNA-derived small RNAs. Trends Biochem Sci 46: 790-804, 2021.
11. Chu X, He C, Sang B, Yang C, Yin C, Ji M, Qian A and Tian Y: Transfer RNAs-derived small RNAs and their application potential in multiple diseases. Front Cell Dev Biol 10: 954431, 2022.
12. Shen Y, Yu X, Zhu L, Li T, Yan Z and Guo J: Transfer RNA-derived fragments and tRNA halves: Biogenesis, biological functions and their roles in diseases. J Mol Med (Berl) 96: 1167-1176, 2018.
13. Yu X, Xie Y, Zhang S, Song X, Xiao B and Yan Z: tRNA-derived fragments: Mechanisms underlying their regulation of gene expression and potential applications as therapeutic targets in cancers and virus infections. Theranostics 11: 461-469, 2021.
14. Yuan Y, Li J, He Z, Fan X, Mao X, Yang M and Yang D: tRNA-derived fragments as new hallmarks of aging and age-related diseases. Aging Dis 12: 1304-1322, 2021.
15. Lee YS, Shibata Y, Malhotra A and Dutta A: A novel class of small RNAs: TRNA-derived RNA fragments (tRFs). Genes Dev 23: 2639-2649, 2009.
16. Kumar P, Anaya J, Mudunuri SB and Dutta A: Meta-analysis of tRNA derived RNA fragments reveals that they are evolutionarily conserved and associate with AGO proteins to recognize specific RNA targets. BMC Biol 12: 78, 2014.
17. Kumar P, Mudunuri SB, Anaya J and Dutta A: tRFdb: A database for transfer RNA fragments. Nucleic Acids Res 43 (Database Issue): D141-145, 2015.
18. Zhao Y, Li X, Ye C, Huang C, Lv X and Li J: The biogenesis, mechanism and function of the tRNA-derived small RNA (tsRNA): A review compared with microRNA. Am J Cancer Res 13: 1656-1666, 2023.
19. Siddiqi S and Matushansky I: Piwis and piwi-interacting RNAs in the epigenetics of cancer. J Cell Biochem 113: 373-380, 2012.
20. Siddiqi S, Terry M and Matushansky I: Hiwi mediated tumorigenesis is associated with DNA hypermethylation. PLoS One 7: e33711, 2012.
21. Pekarsky Y, Balatti V, Palamarchuk A, Rizzotto L, Veneziano D, Nigita G, Rassenti LZ, Pass HI, Kipps TJ, Liu CG, *et al*: Dysregulation of a family of short noncoding RNAs, tsRNAs, in human cancer. Proc Natl Acad Sci USA 113: 5071-5076, 2016.
22. Zhang X, He X, Liu C, Liu J, Hu Q, Pan T, Duan X, Liu B, Zhang Y, Chen J, *et al*: IL-4 Inhibits the biogenesis of an epigenetically suppressive pIWI-interacting RNA to upregulate CD1a molecules on monocytes/dendritic cells. J Immunol 196: 1591-1603, 2016.
23. He L and Hannon GJ: MicroRNAs: Small RNAs with a big role in gene regulation. Nat Rev Genet 5: 522-531, 2004.
24. Li J, Zhu L, Cheng J and Peng Y: Transfer RNA-derived small RNA: A rising star in oncology. Semin Cancer Biol 75: 29-37, 2021.
25. Goodarzi H, Liu X, Nguyen HC, Zhang S, Fish L and Tavazoie SF: Endogenous tRNA-derived fragments suppress breast cancer progression via YBX1 displacement. Cell 161: 790-802, 2015.
26. Akiyama Y, Kharel P, Abe T, Anderson P and Ivanov P: Isolation and initial structure-functional characterization of endogenous tRNA-derived stress-induced RNAs. RNA Biol 17: 1116-1124, 2020.
27. Couvillion MT, Bounova G, Purdom E, Speed TP and Collins K: A Tetrahymena Piwi bound to mature tRNA 3' fragments activates the exonuclease Xrn2 for RNA processing in the nucleus. Mol Cell 48: 509-520, 2012.
28. Kim HK, Xu J, Chu K, Park H, Jang H, Li P, Valdmanis PN, Zhang QC and Kay MA: A tRNA-derived small RNA regulates ribosomal protein S28 protein levels after translation initiation in humans and mice. Cell Rep 29: 3816-3824, 2019.
29. Fu BF and Xu CY: Transfer RNA-derived small RNAs: Novel regulators and biomarkers of cancers. Front Oncol 12: 843598, 2022.

30. Wang J, Ma G, Ge H, Han X, Mao X, Wang X, Veeramootoo JS, Xia T, Liu X and Wang S: Circulating tRNA-derived small RNAs (tsRNAs) signature for the diagnosis and prognosis of breast cancer. NPJ Breast Cancer 7: 4, 2021.
31. Xu J, Wang Y, Li X, Zheng M, Li Y and Zhang W: Clinical value assessment for serum hsa_tsr013526 in the diagnosis of gastric carcinoma. Environ Toxicol 39: 2753-2767, 2024.
32. Huang T, Chen C, Du J, Zheng Z, Ye S, Fang S and Liu K: A tRF-5a fragment that regulates radiation resistance of colorectal cancer cells by targeting MKNK1. J Cell Mol Med 27: 4021-4033, 2023.
33. Qin C, Chen ZH, Cao R, Shi MJ and Tian Y: A novel tiRNA-Gly-GCC-1 promotes progression of urothelial bladder carcinoma and directly targets TLR4. Cancers (Basel) 14: 4555, 2022.
34. Liang Y, Zhang X, Peng J, Liu J, Chen H and Guo S: Vitamin D-mediated tsRNA-07804 triggers mitochondrial dysfunction and suppresses non-small cell lung cancer progression by targeting CRKL. J Cancer Res Clin Oncol 150: 51, 2024.
35. Tu M, Zuo Z, Chen C, Zhang X, Wang S, Chen C and Sun Y: Transfer RNA-derived small RNAs (tsRNAs) sequencing revealed a differential expression landscape of tsRNAs between glioblastoma and low-grade glioma. Gene 855: 147114, 2023.
36. Deng H, Wang J, Ye D, Chen J, Qiu S, Tang M, Zhou C, Shen Y, Fang S, Shen Z, *et al*: A 5'-tiRNA fragment that inhibits proliferation and migration of laryngeal squamous cell carcinoma by targeting PIK3CD. Genomics 114: 110392, 2022.
37. Zhao R, Yang Z, Zhao B, Li W, Liu Y, Chen X, Cao J, Zhang J, Guo Y, Xu L, *et al*: A novel tyrosine tRNA-derived fragment, tRF^{Tyr}, induces oncogenesis and lactate accumulation in LSCC by interacting with LDHA. Cell Mol Biol Lett 28: 49, 2023.
38. Panoutsopoulou K, Magkou P, Dreyer T, Dorn J, Obermayr E, Mahner S, van Gorp T, Braicu I, Magdolen V, Zeillinger R, *et al*: tRNA-derived small RNA 3'U-tRF^{ValCAC} promotes tumour migration and early progression in ovarian cancer. Eur J Cancer 180: 134-145, 2023.
39. Zhao C, Tolkach Y, Schmidt D, Muders M, Kristiansen G, Müller SC and Ellinger J: tRNA-halves are prognostic biomarkers for patients with prostate cancer. Urol Oncol 36: 503.e1-503.e7, 2018.
40. Di Fazio A and Gullerova M: An old friend with a new face: TRNA-derived small RNAs with big regulatory potential in cancer biology. Br J Cancer 128: 1625-1635, 2023.
41. Gu X, Zhang Y, Qin X, Ma S, Huang Y and Ju S: Transfer RNA-derived small RNA: An emerging small non-coding RNA with key roles in cancer. Exp Hematol Oncol 11: 35, 2022.
42. Pekarsky Y, Balatti V and Croce CM: tRNA-derived fragments (tRFs) in cancer. J Cell Commun Signal 17: 47-54, 2023.
43. Wang J, Ma G, Li M, Han X, Xu J, Liang M, Mao X, Chen X, Xia T, Liu X, *et al*: Plasma tRNA fragments derived from 5' ends as novel diagnostic biomarkers for early-stage breast cancer. Mol Ther Nucleic Acids 21: 954-964, 2020.
44. Zhang Y, Bi Z, Dong X, Yu M, Wang K, Song X, Xie L and Song X: 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 12: 2314-2323, 2021.
45. Sun C, Huang X, Li J, Fu Z, Hua Y, Zeng T, He Y, Duan N, Yang F, Liang Y, *et al*: Exosome-transmitted tRF-16-K8J7K1B promotes tamoxifen resistance by reducing drug-induced cell apoptosis in breast cancer. Cancers (Basel) 15: 899, 2023.
46. Zhang Z, Liu Z, Zhao W, Zhao X and Tao Y: tRF-19-W4PU732S promotes breast cancer cell malignant activity by targeting inhibition of RPL27A (ribosomal protein-L27A). Bioengineered 13: 2087-2098, 2022.
47. Mo D, He F, Zheng J, Chen H, Tang L and Yan F: tRNA-derived fragment tRF-17-79MP9PP attenuates cell invasion and migration via THBS1/TGF-β1/Smad3 axis in breast cancer. Front Oncol 11: 656078, 2021.
48. Ma J and Liu F: Study of tRNA-derived fragment tRF-20-S998LO9D in pan-cancer. Dis Markers 2022: 8799319, 2022.
49. Chen F, Song C, Meng F, Zhu Y, Chen X, Fang X, Ma D, Wang Y and Zhang C: 5'-tRF-GlyGCC promotes breast cancer metastasis by increasing fat mass and obesity-associated protein demethylase activity. Int J Biol Macromol 226: 397-409, 2023.
50. Mo D, Jiang P, Yang Y, Mao X, Tan X, Tang X, Wei D, Li B, Wang X, Tang L, *et al*: A tRNA fragment, 5'-$tiRN^{AVal}$, suppresses the Wnt/β-catenin signaling pathway by targeting FZD3 in breast cancer. Cancer Lett 457: 60-73, 2019.
51. Wang B, Li D, Ilnytskyy Y, Kovalchuk I and Kovalchuk O: A miR-34a-guided, tRNAiMet-derived, piR_019752-like fragment (tRiMetF31) suppresses migration and angiogenesis of breast cancer cells via targeting PFKFB3. Cell Death Discov 8: 355, 2022.
52. Xu W, Zheng J, Wang X, Zhou B, Chen H, Li G and Yan F: tRF-Val-CAC-016 modulates the transduction of CACNA1d-mediated MAPK signaling pathways to suppress the proliferation of gastric carcinoma. Cell Commun Signal 20: 68, 2022.
53. Zhang Y, Gu X, Qin X, Huang Y and Ju S: Evaluation of serum tRF-23-Q99P9P9NDD as a potential biomarker for the clinical diagnosis of gastric cancer. Mol Med 28: 63, 2022.
54. Gu X, Zhang Y, Huang Y and Ju S: Comprehensive evaluation of serum tRF-17-WS7K092 as a promising biomarker for the diagnosis of gastric cancer. J Oncol 2022: 8438726, 2022.
55. Zheng J, Li C, Zhu Z, Yang F, Wang X, Jiang P and Yan F: A 5'-tRNA derived fragment namedtiRNA-Val-CAC-001 works as a suppressor in gastric cancer. Cancer Manag Res 14: 2323-2337, 2022.
56. Xu W, Zhou B, Wang J, Tang L, Hu Q, Wang J, Chen H, Zheng J, Yan F and Chen H: tRNA-derived fragment tRF-Glu-TTC-027 regulates the progression of gastric carcinoma via MAPK signaling pathway. Front Oncol 11: 733763, 2021.
57. Shen Y, Yu X, Ruan Y, Li Z, Xie Y, Yan Z and Guo J: Global profile of tRNA-derived small RNAs in gastric cancer patient plasma and identification of tRF-33-P4R8YP9LON4VDP as a new tumor suppressor. Int J Med Sci 18: 1570-1579, 2021.
58. Li X, Zhang Y, Li Y, Gu X and Ju S: A comprehensive evaluation of serum tRF-29-R9J8909NF5JP as a novel diagnostic and prognostic biomarker for gastric cancer. Mol Carcinog 62: 1504-1517, 2023.
59. Wang Y, Li Z, Weng Q, Zheng Y, Lin Y, Guo J and Ye G: Clinical diagnostic values of transfer RNA-derived fragment tRF-41-YDLBRY73W0K5KKOVD and its effects on the growth of gastric cancer cells. DNA Cell Biol 42: 176-187, 2023.
60. Cui H, Li H, Wu H, Du F, Xie X, Zeng S, Zhang Z, Dong K, Shang L, Jing C, *et al*: A novel 3'tRNA-derived fragment tRF-Val promotes proliferation and inhibits apoptosis by targeting EEF1A1 in gastric cancer. Cell Death Dis 13: 471, 2022.
61. Shen Y, Xie Y, Yu X, Zhang S, Wen Q, Ye G and Guo J: Clinical diagnostic values of transfer RNA-derived fragment tRF-19-3L7L73JD and its effects on the growth of gastric cancer cells. J Cancer 12: 3230-3238, 2021.
62. Huang Y, Zhang H, Gu X, Qin S, Zheng M, Shi X, Peng C and Ju S: Elucidating the role of serum tRF-31-U5YKFN8DYDZDD as a novel diagnostic biomarker in gastric cancer (GC). Front Oncol 11: 723753, 2021.
63. Wang H, Huang W, Fan X, He X, Chen S, Yu S and Zhang Y: The tRNA-derived fragment tRF-24-V29K9UV3IU functions as a miRNA-like RNA to prevent gastric cancer progression by inhibiting GPR78 expression. J Oncol 2022: 8777697, 2022.
64. Tong L, Zhang W, Qu B, Zhang F, Wu Z, Shi J, Chen X, Song Y and Wang Z: The tRNA-derived fragment-3017A promotes metastasis by inhibiting NELL2 in human gastric cancer. Front Oncol 10: 570916, 2020.
65. Zhu L, Li Z, Yu X, Ruan Y, Shen Y, Shao Y, Zhang X, Ye G and Guo J: The tRNA-derived fragment 5026a inhibits the proliferation of gastric cancer cells by regulating the PTEN/PI3K/AKT signaling pathway. Stem Cell Res Ther 12: 418, 2021.
66. Wu Y, Yang X, Jiang G, Zhang H, Ge L, Chen F, Li J, Liu H and Wang H: 5'-tRF-GlyGCC: A tRNA-derived small RNA as a novel biomarker for colorectal cancer diagnosis. Genome Med 13: 20, 2021.
67. Lu S, Wei X, Tao L, Dong D, Hu W, Zhang Q, Tao Y, Yu C, Sun D and Cheng H: A novel tRNA-derived fragment tRF-3022b modulates cell apoptosis and M2 macrophage polarization via binding to cytokines in colorectal cancer. J Hematol Oncol 15: 176, 2022.
68. Tsiakanikas P, Adamopoulos PG, Tsirba D, Artemaki PI, Papadopoulos IN, Kontos CK and Scorilas A: High expression of a $tRNA^{Pro}$ derivative associates with poor survival and independently predicts colorectal cancer recurrence. Biomedicines 10: 1120, 2022.
69. Chen H, Xu Z, Cai H, Peng Y, Yang L and Wang Z: Identifying differentially expressed tRNA-derived small fragments as a biomarker for the progression and metastasis of colorectal cancer. Dis Markers 2022: 2646173, 2022.

70. Luan N, Chen Y, Li Q, Mu Y, Zhou Q, Ye X, Deng Q, Ling L, Wang J and Wang J: TRF-20-M0NK5Y93 suppresses the metastasis of colon cancer cells by impairing the epithelial-to-mesenchymal transition through targeting Claudin-1. Am J Transl Res 13: 124-142, 2021.
71. Luan N, Wang J, Sheng B, Zhou Q, Ye X, Zhu X, Sun J, Tang Z and Wang J: tRF-20-M0NK5Y93-induced MALAT1 promotes colon cancer metastasis through alternative splicing of SMC1A. Am J Cancer Res 13: 852-871, 2023.
72. Tao EW, Wang HL, Cheng WY, Liu QQ, Chen YX and Gao QY: A specific tRNA half, 5'tiRNA-His-GTG, responds to hypoxia via the HIF1α/ANG axis and promotes colorectal cancer progression by regulating LATS2. J Exp Clin Cancer Res 40: 67, 2021.
73. Hu F, Niu Y, Mao X, Cui J, Wu X, Simone CB II, Kang HS, Qin W and Jiang L: tsRNA-5001a promotes proliferation of lung adenocarcinoma cells and is associated with postoperative recurrence in lung adenocarcinoma patients. Transl Lung Cancer Res 10: 3957-3972, 2021.
74. Ren J, Wu X, Shang FF, Qi Y, Tang Z, Wen C, Cao W, Cheng Q, Tan L, Chen H, *et al*: The tRNA-Cys-GCA derived tsRNAs suppress tumor progression of gliomas via regulating VAV2. Dis Markers 2022: 8708312, 2022.
75. Xu B, Liang J, Zou H, Wang J, Xiong Y and Pei J: Identification of Novel tRNA-Leu-CAA-Derived tsRNAs for the diagnosis and prognosis of diffuse gliomas. Cancer Manag Res 14: 2609-2623, 2022.
76. Panoutsopoulou K, Dreyer T, Dorn J, Obermayr E, Mahner S, Gorp TV, Braicu I, Zeillinger R, Magdolen V, Avgeris M, *et al*: tRNA(GlyGCC)-derived internal fragment (i-tRF-GlyGCC) in ovarian cancer treatment outcome and progression. Cancers (Basel) 14: 24, 2021.
77. Wang L, Liu Y, Yan W, Huang C, Ding Z, Yang J, Jiang S and Sun L: Clinical significance of high expression of tRF-Glu-TTC-2 in prostate carcinoma and its effect on growth. Am J Mens Health 16: 15579883221135970, 2022.
78. Yang C, Lee M, Song G and Lim W: $tRNA^{Lys}$-derived fragment alleviates cisplatin-induced apoptosis in prostate cancer cells. Pharmaceutics 13: 55, 2021.
79. Yang N, Li R, Liu R, Yang S, Zhao Y, Xiong W and Qiu L: The emerging function and promise of tRNA-Derived small RNAs in cancer. J Cancer 15: 1642-1656, 2024.
80. Chen Q, Li D, Jiang L, Wu Y, Yuan H, Shi G, Liu F, Wu P and Jiang K: Biological functions and clinical significance of tRNA-derived small fragment (tsRNA) in tumors: Current state and future perspectives. Cancer Lett 587: 216701, 2024.
81. Salehi M, Kamali MJ, Rajabzadeh A, Minoo S, Mosharafi H, Saeedi F and Daraei A: tRNA-derived fragments: Key determinants of cancer metastasis with emerging therapeutic and diagnostic potentials. Arch Biochem Biophys 753: 109930, 2024.