

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

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**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.

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## 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

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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 T $\psi$ C 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 1 $\beta$  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

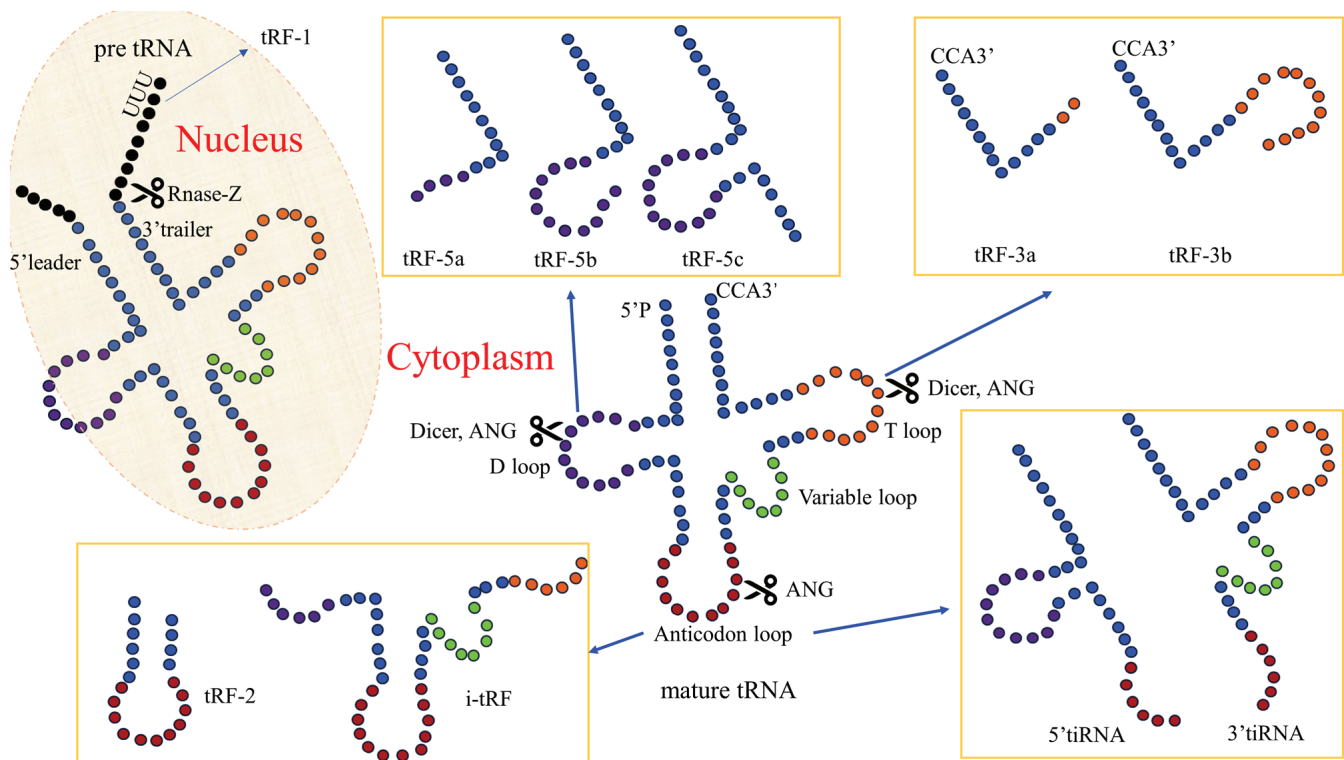


Figure 1. Classification of tsRNAs. tsRNAs can be divided into tRFs and tiRNAs. The tiRNAs are derived from mature tRNA in the cytoplasm and can be divided into 5'tiRNA and 3'tiRNA. The source of tRF-2 and i-tRF is not yet clear. tRF-1 is derived from pre-tRNA in the nucleus. tRF-5s and tRF-3s are generated 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 down-regulated 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  $\beta$ 1/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<sup>Val</sup> was markedly downregulated in BC tissues, and stage and lymph node metastases were associated with serum 5'-tiRNA<sup>Val</sup> downregulation. Moreover, 5'-tiRNA<sup>Val</sup> 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<sub>i</sub><sup>Met</sup> 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<sub>i</sub><sup>Met</sup> 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 T $\psi$ C 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.

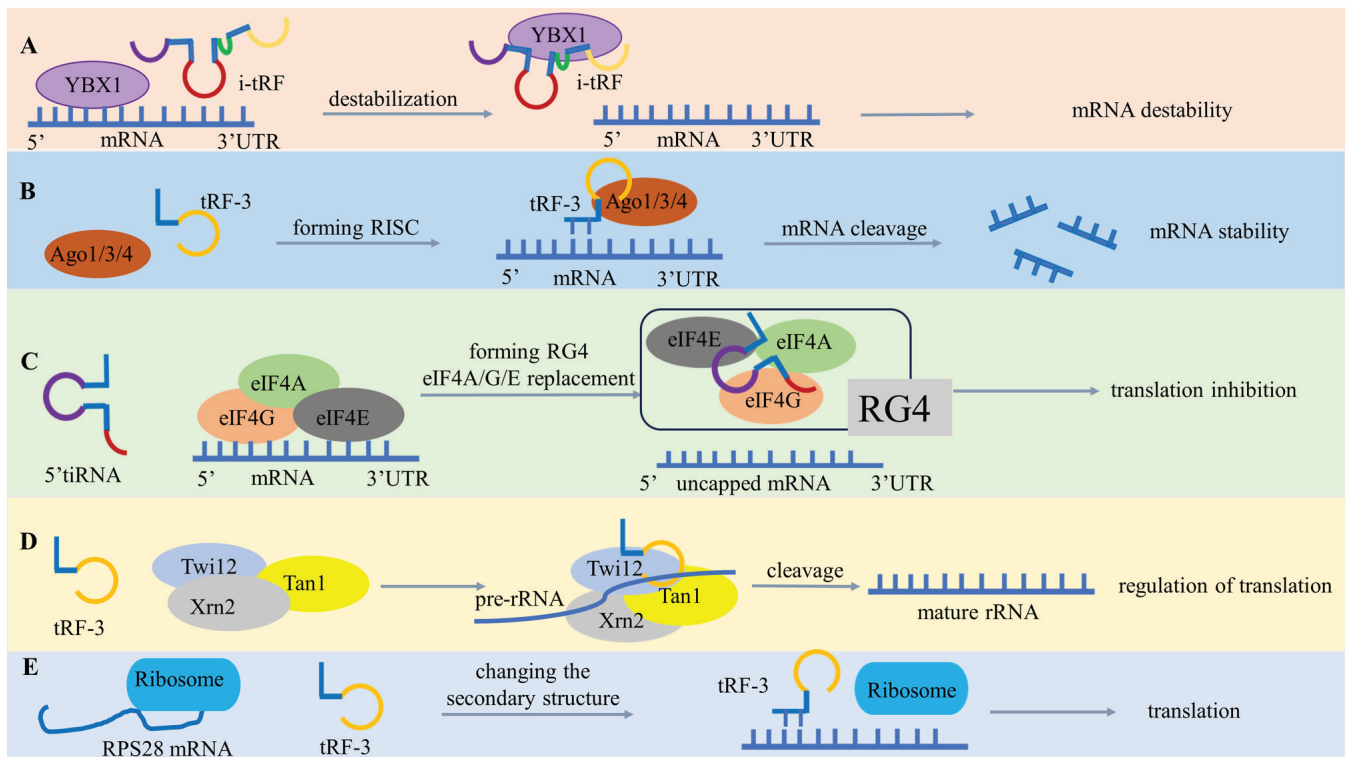


Figure 2. Biological functions of tsRNAs. (A) i-tRF bind to YBX1 to decrease oncogenic transcript stability. (B) tRF-3 forms RISC with Ago proteins to silence mRNA. (C) 5'tiRNA bind to the eIF4A/E/G complex, displacing mRNA without cap structure to cause translational inhibition. (D) tRF-3 binds to Twi12, 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  $\alpha 1$  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/ $\beta$ -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-YDLBRY73W0K5KKOVD 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  $\alpha$ 1 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-1 $\alpha$ /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  $\Delta$  (PIK3D). In a study by Zhao *et al* (37), tRF<sup>Tyr</sup> was reported to be markedly elevated in LSCC tissues and cell lines (AMC-HN8/TU212/TU686). Mechanistic studies showed that tRF<sup>Tyr</sup> 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  $\alpha$ .

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 <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 2023   |             | tRF-16-K8J7K1B                                                                                         | Upregulated in BC sera                                                       | Targeted TRAIL to induce tamoxifen resistance in BC                                                         | (45)    |
| Zhang <i>et al.</i> , 2022 |             | tRF-19-W4PU732S                                                                                        | Upregulated in BC tissue                                                     | Targeted RPL27A to increase the activity of BC cells                                                        | (46)    |
| Mo <i>et al.</i> , 2021    |             | tRF-17-79 MP9PP                                                                                        | Downregulated in BC tissue/sera                                              | Suppressed the invasion and migration of BC cells through the THBS 1/TGF- $\beta$ 1/Smad 3 axis             | (47)    |
| Ma and Liu, 2022           |             | tRF-20-S998LO9D                                                                                        | Upregulated in BC tissue                                                     | Maybe an oncogene in BC                                                                                     | (48)    |
| Chen <i>et al.</i> , 2023  |             | 5'-tRF-GlyGCC                                                                                          | Upregulated in BC tissue                                                     | Promoted BC metastasis by increasing fat mass and obesity-associated protein demethylase activity           | (49)    |
| Mo <i>et al.</i> , 2019    |             | 5'-tiRNA <sup>Val</sup>                                                                                | Downregulated in BC tissue/sera                                              | Suppressed the Wnt/ $\beta$ -catenin signaling pathway by targeting FZD3 in BC                              | (50)    |
| Wang <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 2022 |             | tRF-23-Q99P9P9NDD                                                                                      | Upregulated in BC tissue/sera                                                | Promoted GC cell proliferation and invasion                                                                 | (53)    |
| Gu <i>et al.</i> , 2022    |             | tRF-17-WS7K092                                                                                         | Upregulated in BC serum                                                      | Improved diagnostic sensitivity and AUC when combined with CEA, CA19-9 and CA72-4                           | (54)    |
| Zheng <i>et al.</i> , 2022 |             | tRNA-Val-CAC-001                                                                                       | Downregulated in BC tissue                                                   | Prevented cell proliferation by targeting LRP 6 through the Wnt/ $\beta$ -collagen signaling pathway        | (55)    |
| Xu <i>et al.</i> , 2021    |             | tRF-GluTTC-027                                                                                         | Downregulated in BC tissue                                                   | Regulated the progression of GC through the MAPK signaling pathway                                          | (56)    |
| Shen <i>et al.</i> , 2021  |             | tRF-33P4R8YP9LON4VDP                                                                                   | Downregulated in BC serum                                                    | Maybe a tumor suppressor                                                                                    | (57)    |
| Li <i>et al.</i> , 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 <i>et al.</i> , 2023                               |             | tRF-41-YDLBRY73W0K5KKOVD            | Downregulated in BC tissue                                                   | Suppresses cell cycle progression, induced apoptosis and hindered cell proliferation and migration                              | (59)     |
| Cui <i>et al.</i> , 2022                                |             | tRF-Val                             | Upregulated in BC tissue                                                     | Promotes proliferation and inhibited apoptosis by targeting EEF1A1                                                              | (60)     |
| Shen <i>et al.</i> , 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 <i>et al.</i> , 2021                              |             | tRF-31-U5YKFN8DYDZDD                | Upregulated in BC serum                                                      | Improved detection efficiency after combining with conventional biomarkers                                                      | (62)     |
| Wang <i>et al.</i> , 2022                               |             | tRF-24-V29K9UV3IU                   | Downregulated in BC tissue                                                   | Prevents GC progression by inhibiting GPR78 expression                                                                          | (63)     |
| Tong <i>et al.</i> , 2020                               |             | tRF-3017 A                          | Upregulated in BC tissue                                                     | Promoted metastasis by inhibiting NELL2                                                                                         | (64)     |
| Zhu <i>et al.</i> , 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 <i>et al.</i> , 2021                                 | CRC         | 5'-tRF-GlyGCC                       | Upregulated in CRC tissue/plasma                                             | May be a new biomarker for CRC diagnosis                                                                                        | (66)     |
| Lu <i>et al.</i> , 2022                                 |             | tRF-3022b                           | Upregulated in CRC tissue/plasma                                             | Modulated cell apoptosis and M2 macrophage polarization via binding to cytokines                                                | (67)     |
| Tsiakanikas <i>et al.</i> , 2022                        |             | 5'-tiRNA-Pro <sup>TGG</sup>         | Upregulated in CRC tissue                                                    | High expression levels were associated with poor prognosis                                                                      | (68)     |
| Chen <i>et al.</i> , 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 <i>et al.</i> , 2021 and Luan <i>et al.</i> , 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 <i>et al.</i> , 2021                                |             | 5'ti RNA-His-GTG                    | Upregulated in CRC tissue                                                    | Responded to hypoxia via the HIF-1 $\alpha$ /ANG axis and promoted CRC progression by regulating LAT52                          | (72)     |
| Qin <i>et al.</i> , 2022                                | UBC         | tiRNA-Gly-GCC-1                     | Upregulated in UBC tissue                                                    | Target TLR4 to promote progression of UBC                                                                                       | (33)     |
| Hu <i>et al.</i> , 2021                                 | Lung Cancer | tsRNA-5001a                         | Upregulated in lung cancer tissue                                            | Promoted lung cancer cell proliferation                                                                                         | (73)     |
| Ren <i>et al.</i> , 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 <i>et al</i> , 2022             |             | ts-26, tRFdb-3012 a/b | Downregulated in glioma tissue                                               | Affected glioma progression by binding RBM 43 and HOXA 15                                                     | (76)    |
| Deng <i>et al</i> , 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 <i>et al</i> , 2023           |             | tRF <sup>Tyr</sup>    | Upregulated in LSCC tissue                                                   | Induced oncogenesis and lactated accumulation in LSCC by interacting with LDHA                                | (37)    |
| Panoutsopoulou <i>et al</i> , 2021 | EOC         | i-tRF-GlyGCC          | Upregulated in EOC serum                                                     | High levels of i-tRF-GlyGCC expression associated with a lower survival                                       | (76)    |
| Wang <i>et al</i> , 2022           | PCa         | tRF-Glu-TTC-3         | Upregulated in PCa tissue                                                    | May be a novel oncogene                                                                                       | (77)    |
| Yang <i>et al</i> , 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- $\beta$ 1, transforming growth factor  $\beta$ 1; 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  $\alpha$ 1 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; EEFA1, eukaryotic translation elongation factor 1  $\alpha$ 1; GPR78, G Protein-coupled receptor 78; NELL2, neural EGFL like 2; EMT, epithelial-mesenchymal transition; HIF-1 $\alpha$ , hypoxia-inducible factor-1 $\alpha$ ; 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  $\Delta$ .

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.

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### 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.

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