tRNA-derived small RNAs: Mechanisms and potential roles in cancers

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Abstract  Transfer RNAs (tRNAs) are essential for protein synthesis. Mature or pre-tRNAs may be cleaved to produce tRNA-derived small RNAs (tsRNAs). tsRNAs, divided into tRNA-derived stress-induced RNA (tiRNAs) and tRNA-derived fragments (tRFs), play versatile roles in a number of fundamental biological processes. tsRNAs not only play regulatory roles in gene silencing, RNA stability, reverse transcription, and translation, but are also closely related to cell proliferation, migration, cell cycle, and apoptosis. Their abnormal expression is associated with the occurrence and development of various human diseases, especially cancer. This paper reviews the classification, biogenesis, and mechanism of action of tsRNAs, and the research progress to date on tsRNAs in cancers. These findings provide new opportunities for diagnostic biomarkers and treatment targets of several types of cancers including gastric cancer, colorectal cancer, hepatocellular carcinomas, pancreatic cancer, breast cancer, prostate cancer, renal cell carcinoma, ovarian cancer, lung cancer, bladder cancer, thyroid cancer, oral cancer, and leukemia.

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Introduction

In recent years, with the development of high-throughput sequencing technology and bioinformatics, transfer RNA (tRNA)-derived small RNAs (tsRNAs) were discovered. A mature tRNA is about 70–90 nucleotides (nt) in length and has a secondary structure composed of four stems and four loops, including an acceptor stem, an anticodon stem, a dihydrouracil stem, a pseudouracil stem, an anticodon loop, a dihydrouracil loop (D loop), a pseudouracil loop (TψC loop or T loop), and a variable loop. tRNAs play an important role in protein translation. In eukaryotes, tRNA genes are transcribed into pre-tRNA by RNA polymerase III. These pre-tRNAs include a 5' leader sequence and a 3' trailer sequence.1–3

tRNAs, processed from the specific cleavage sites of mature tRNAs or pre-tRNAs, is a type of RNA with a precise sequence structure and a specific biological function.4,5 tsRNAs are involved in gene silencing, the regulation of translation, RNA reverse transcription, and mRNA stability. Therefore, tsRNAs participate in many biological activities, such as cell proliferation, differentiation, migration, cell cycle, and apoptosis. Many studies suggest that tsRNAs play an important role in various human diseases, including cancers, neurodegenerative diseases, viral infectious diseases, and metabolic diseases.6,7

In this review, we summarize the classification and biogenesis of tsRNAs, describe the regulatory mechanisms mediated by tsRNAs, and provide an overview of recent reports on the roles of tsRNAs in cancers.

Classification and biogenesis of tsRNAs

According to their cleavage site and length, tsRNAs can be divided into two main subtypes (Fig. 1): tRNA-derived stress-induced RNA (tiRNAs) and tRNA-derived fragments (tRFs).14,15

tiRNAs are 30–50 nt in length and have a 5′-hydroxyl rather than a 5′-phosphate. tiRNAs are produced by ribonuclease specifically cleaving the anticodon loop of mature tRNAs under hypoxia, phosphorus deficiency, amino acid deficiency, ultraviolet irradiation, starvation, viral infection, heat shock, or heavy metal stress.8,9 tiRNAs are divided into 5′-tiRNAs and 3′-tiRNAs.12,13 There is another type of tiRNA—sex hormone-dependent tiRNA derived RNA (SHOT-RNA)—which is highly expressed in hormone-dependent cancers.14

The length of tRFs is about 14–30 nt. The structure and size of tRFs are similar to those of microRNAs (miRNAs), which contain 5′-phosphate and 3′-hydroxy groups.15,16

According to different sources, tRFs can be roughly divided into five categories: tRF-5, tRF-3, tRF-2, tRF-1, and i-tRF. tRF-5, 14–30 nt in length, is produced by Dicer cutting the D-loop or the stem between the D-loop and the anticodon loop of the mature tRNA transcript. tRF-5 can be divided into three subtypes with different specific lengths: tRF-5a (14–16 nt), tRF-5b (22–24 nt), and tRF-5c (28–30 nt). tRF-5a, tRF-5b, and tRF-5c are produced by cutting the D-loop, D-stem, and anticodon stem, respectively. tRF-3, 18–22 nt in length, is produced by ANG, Dicer, or exonuclease cleaving the TψC loop of mature tRNAs. The subtypes of tRF-3 include tRF-3a (18 nt) and tRF-3b (22 nt). tRF-1, also known as 3′-tRF, is produced by the RNase Z enzyme with a PolyU sequence or Elac domain protein 2 (ELAC2) cleaving the 3′ tail sequence of the pre-tRNA. tRF-1 ends with a polyuridine sequence (UUUUU, UUCUU, GUCUU, or AUCUU), which is an RNA polymerase III termination signal.5,17–19 tRF-2 is produced by the anticodon loop of tRNA under hypoxic conditions and contains only the stem loop sequence of the anticodon.20 i-tRF mainly comes from the internal region of mature tRNA (between the D loop and T loop), rather than the 5′ and 3′ terminal regions.15

There is currently no standardized nomenclature for tsRNAs. Recently, there is a universal consensus to establish such a standardized nomenclature.21 To better understand tsRNAs, the names based on MINTBase (http://cm.jefferson.edu/MINTbase/) are used in this paper. Other tsRNAs whose names are not included in MINTBase are referred to as what they were named in each respective study.

Functions of tsRNAs

The functions of tsRNAs can be divided into four categories: RNA silencing, translation regulation, RNA reverse transcription regulation, and mRNA stability regulation (Fig. 2).

RNA silencing

Many studies have shown that tsRNAs may mediate RNA silencing by binding with Argonautes (Ago)/Piwi protein.1,5,22 Ago/Piwi protein is a known component of the RNA-induced silencing complex (RISC).23–25 A study found that a tRNA fragment tRF-22-WE8SPOX52 from tRNA-Gly- GCC could bind to Ago.26 tRF-22-WE8SPOX52 is highly expressed in normal germinal center B cells, but not in germinal center-derived lymphomas. Overexpression of tRF-22-WE8SPOX52 reduces the proliferation of lymphoma cells by inhibiting the expression of replication protein A1 (RPA1) and regulates the molecular response to DNA damage.26 The function of RAP1 is to stabilize single-stranded DNA intermediates during DNA replication or stress.27 Some tRFs can down-regulate the expression of target genes in a sequence-dependent and Ago-dependent manner, similar to miRNAs (Fig. 2A).27

Another way that RNA silences tRFs is through the competitive binding of target proteins and mRNAs. tRF_U3_1, derived from Chr10.tRNA2-Ser (TGA), is not involved in Ago2-mediated gene silencing, but it can interact with the RNA chaperone La/SSB. By isolating cytoplasmic La/SSB to inhibit its binding to hepatitis virus C internal ribosome entry sites (IRES), tRF_U3_1 negatively regulates the expression of viral genes.28

In addition, some studies have shown that tiRNAs may inhibit RNA expression by pairing with bases in the target mRNA. For example, human respiratory syncytial virus (RSV)-infected human airway epithelial cells are enriched with a large number of 30 nt tRNA derived fragments.29 The tsRNAs from the 5′ termini of GluCTC, GlyCCC, and LysCTT mature tRNAs showed the ability to trans-silence the target gene, inhibiting the target mRNA in the cytoplasm and promoting the replication of RSV.29,30
Translational regulation

Some tsRNAs can regulate translation in a sequence-specific way. The process of translation is usually divided into three basic steps: initiation, elongation, and termination. In eukaryotes, the eukaryotic initiation factor 4F (eIF4F) initiation complex is composed of the cap binding protein eIF4E, the scaffold protein eIF4G, and the helicase eIF4A. Some tsRNAs have been found to play a role in global translation inhibition in plant and animal cells. tsRNA plays a role by interacting with ribonucleoprotein (RNP) to form a tsRNA-RNP complex. It has been shown that some tsRNAs interact with the initiation complex to inhibit translation. Ivanov et al found that 5’-tiRNAs inhibited protein synthesis and triggered phosphor-elF2α-independent assembly of stress granules (SGs). The stem-loop structure corresponding to the D-loop of the tRNA and 5’-terminal oligoguanine (TOG) motif are two structural features required for tiRNAs to inhibit translational initiation. tiRNAs inhibit translation by directly or indirectly binding to translation initiation complexes (Fig. 2B). Moreover, tiRNAs can cooperate with Y-box binding protein 1 (YBX1) to prevent eIF4G/A from initiating translation. YBX1 binds to tiRNAs directly through its cold shock domain, while the TOG motif is necessary for assembling the YBX1/tiRNA complex. However, YBX1 is not necessary for tiRNA-mediated translational inhibition. In 2014, Ivanov et al found that 5’-tiRNAs can be assembled into a unique G-quadruplex (G4) structure. G4 plays an important role in 5’-tiRNAAla and 5’-tiRNACys (DNA analogues of 5’-tiRNAAla and 5’-tiRNACys) inhibition of protein synthesis, promoting SG formation and protecting motor neurons exposed to stress. Later, Lyons’ team found that the destruction of the RNA G-quadruplex (RG4) made tRFs lose the ability to trigger the formation of SGs in vivo.

In addition to tiRNAs, the conserved GG dinucleotide in 5’tRFs can also inhibit protein synthesis. For example, 5’tRFs from tRNA^Gln interact with active polysomes to inhibit in vitro translation. Additionally, in yeast, tsRNA was found to affect the aminoclaylation of tRNA by interacting with aminocycl-tRNA synthetase, thereby inhibiting translation in vitro.

Moreover, other tsRNAs can regulate translation by interacting with ribosomes (Fig. 2B). In Haloferax volcanii, tRF from tRNAVal competed with the mRNA in the translation initiation complex to bind to the polymer and 30S subunit under high pH stress, resulting in a decrease in global translation in vivo and in vitro. LeuCAG3’ tsRNA derived from the tRNALeu/UCCG 3’ terminal selectively binds to the double stranded regions of the mRNA of the ribosomal proteins S28 and S15 (RPS28 and RPS15) to enhance their translation and ultimately increase the number of ribosomes. Fricker et al found that the 3’-tiRNAThr in Trypanosoma brucei was significantly induced under starvation. After nutritional recovery, 3’-tiRNA^Thr binds to ribosomes and polymers to promote the loading of mRNA into these ribosomes, thus enhancing translation. Keam et al found that the 19 nt 5’tRF from tRNAGln interacts with human multisynthetase complex (MSC) to inhibit translational elongation.

Furthermore, studies have shown that the regulation of translation by tsRNAs is affected by post-transcriptional modifications. The presence of pseudouracil can affect tsRNA-mediated translation regulation. In embryonic stem cells, inactivation of pseudouridine synthase 7 (PUS7) impairs translational regulation mediated by tRFs, resulting in increased protein synthesis. Gkatza et al found that the deletion of NSUN2, cytosine-5 RNA methyltransferase, leads to a decrease of methylation of specific tRNA sites, which affects the biogenesis of tRFs in stress responses and leads to the impairment of the regulation of protein synthesis.
RNA reverse transcription regulation

Some tsRNAs can regulate reverse transcription through a variety of mechanisms (Fig. 2C). Retrotransposon (TE), also known as endogenous retrovirus (ERV), has a good complementarity between the primer binding site (PBS) sequence of its long terminal repeat (LTR) and the sequence of tsRNA, so tsRNAs can interact with TEs to regulate reverse transcription.49,50 Schorn et al suggested that 3\textsuperscript{0}-tRFs are key regulators of TEs in cells.49 In mouse stem cells, many 18 nt 3\textsuperscript{0}CCA tRFs target tRNA PBSs in a sequence-dependent manner. 18 nt 3\textsuperscript{0}CCA tRFs specifically inhibit the reverse transcription of TEs without affecting the expression of TEs. 22 nt 3\textsuperscript{0}tRFs can affect the expression of TEs and reduce the RNA and protein levels of TEs through post-transcriptional silencing.49 Studies have demonstrated that tRF-18-HR6HFRD2 is perfectly complementary to human T-cell leukemia virus type 1 (HTLV-1) PBS, which can activate HTLV-1 reverse transcriptase and promote virus self-replication.51 Yeung et al reached a similar conclusion.52 In human immunodeficiency virus (HIV), the 18 nt tsRNA from tRNA\textsuperscript{Lys}\textsuperscript{UUG} 3\textsuperscript{0} terminus is complementary to HIV PBS and can bind to Ago2 protein. The 18 nt tsRNA can significantly reduce the copy number of HIV-1 RNA by interacting with Ago2 and Dicer.52

**Figure 2** The four function categories of tsRNAs.
RNA stability regulation

Some tsRNAs have been reported to affect the stability of mRNA (Fig. 2D). For example, a new class of tsRNA has been found to regulate RNA stability by competitively binding with Y box-binding protein 1 (YBX1). YBX1 is an RNA-binding protein (RBP) involved in a variety of cellular pathways. YBX1 binds to endogenous carcinogenic mRNA, maintains the stability of oncogene transcription, and promotes cell proliferation. Goodarzi et al found that when exposed to hypoxia, breast cancer cells could induce tRFs derived from tRNA^Glu, tRNA^Gly, and tRNA^Val. These tRFs compete with YBX1 in the carcinogenic transcription process. By replacing the 3′ untranslated region (UTR) of YBX1, the stability of endogenous oncogene transcripts is reduced, thus promoting the degradation of mRNA and ultimately inhibiting the proliferation of tumor cells. In addition, the stability of mRNA mediated by tsRNA is related to the assembly of SGs. 5′-tiRNA can induce the phosphorylation of eukaryotic initiation factor 2 (eIF2α) independent assembly. Stress induced phosphorylation of eIF2α promotes the formation of SGs. The assembly of SGs results in the temporary silencing of mRNA in cells.

tsRNAs may regulate RNA stability through other mechanisms. Elbarbary and his colleagues found that after interacting with miRNAs or tRFs, RNase Z (L), an endonuclease responsible for tRNA 3′ terminal maturation, can directly cleave sequence-matched RNA. The interaction between 5′ tiRNA from tRNA^Glu and PPM1F (protein phosphatase, Mg^2+/Mn^2+ dependent 1F) mRNA promoted the degradation of mRNA targets. In addition, it has been found that the 5′-tRF from tRNA^Gly^-GCC interacted with RNA binding proteins hnRNPF and hnRNPH to affect the stability of Cajal bodies and the activity of U7 snRNA.

Other potential regulatory mechanisms

tsRNAs may participate in a variety of biological activities, such as cell proliferation, migration, apoptosis, differentiation, and cell cycle. Zhou et al found that a high expression of tRNA-26576 in breast cancer cells promoted cell proliferation and migration, and inhibited cell apoptosis. In breast cancer cells, overexpression of 5′-tRNA regulates cell proliferation and migration by inhibiting the FZD3/Wnt/β-Catenin signaling pathway. Saikia et al suggested that ANG-induced mouse embryonic fibroblasts (MEF) to produce tiRNAs by interacting with cytochrome c (Cyt c). Cyt c is a component of apoptotic bodies. These tiRNAs inhibited the formation of apoptotic bodies and prevented the apoptosis of cortical neurons under hypertonic stress.

In addition, other studies have found that tsRNAs play a role through the formation of tsRNA-RNP complex. Some tsRNAs inhibit the binding of Apaf-1 and Cyt c by binding to Cyt c, thus preventing the activation of caspase-9 and inhibiting the formation of apoptotic bodies. After the tRF-1001 derived from the 3′ end of pre-tRNA^Ser was knocked out, the cells were blocked in G2 stage, which led to the inhibition of DNA biosynthesis and cell proliferation. Krishna et al showed that retinoic acid-induced differentiation of mouse embryonic stem cells (ESCs) resulted in increased expression of tsRNAs from GlnCTG, GlyGCC, GluTTC, LysTTT, and ValCAC/AAC tRNAs. These 5′-tsRNAs may interact with RBPs, such as IGF2BP1, to regulate the stability of c-Myc mRNA and the differentiation of stem cells. Overexpression of c-Myc pluripliotency.

tsRNAs also play an important role in immune responses, intercellular communication, and intergenerational inheritance. Chio et al found that activated T cells bind to multiple vesicles (MVB) through signal regulation, selectively secrete tRFs in extracellular vesicles (EVs), inhibit T cell activation, and play an important role in T cell-mediated immune responses. Shen et al found that TdR-001292 exists in the endometrium of patients with endometriosis and participates in the signal transmission between cells. Chen et al showed that diet-induced metabolic diseases might be transmitted from the paternal line to offspring through sperm, and this process was regulated by tsRNAs.

tsRNAs in cancers

In the past few years, the roles of tsRNAs in cancers have received increasing attention. Many studies have shown that the abnormal expression of tsRNAs in cancer cells contributes to tumor proliferation, metastasis, and clinical-pathological characteristics. tsRNAs may not only act as diagnostic and prognostic indicators but also as targets for cancer treatment (Table 1 and Fig. 3).

Gastrointestinal cancer

The expression of tsRNAs has been found abnormal in gastric cancer. Our group found that the expression level of tRNA-5034-GluTTC-2 in gastric cancer tissues was significantly decreased; its expression level was positively correlated with tumor size and negatively correlated with the survival rate of patients. The expression level of tsRF-29-RRJ890NF5JP in the serum of gastric cancer patients was significantly increased and was positively correlated with lymph node metastasis and tumor grade. Furthermore, tsRNAs are promising therapeutic targets for gastric cancer. For example, our group found that overexpression of tRF-19-3L7L73JD and tRF-33-P4R8YP9LON4VDP inhibited cell proliferation, migration, and invasion of gastric cancer. For example, our group found that overexpression of tRF-19-3L7L73JD and tRF-33-P4R8YP9LON4VDP inhibited cell proliferation, migration, and invasion of gastric cancer cells. Zhang et al found that tRF-18-8R1546D2 derived from tRNAAla correlated with tumor size and negatively correlated with the survival rate of patients. The expression level of tRF-18-8R1546D2 derived from tRNAAla was regulated by tsRNAs. These tRFs may participate in a variety of biological activities, such as cell proliferation, migration, and apoptosis.

In colorectal cancer cells, the expression of tRF-24-V29K9UV3IU on gastric cancer cells. Dong et al found similar effects of tRF-24-V29K9UV3IU on gastric cancer cells. Zhang et al found that tRF-18-8R1546D2 derived from tRNAAla regulated the proliferation, migration, and invasion of gastric cancer cells by targeting the tumor suppressor gene F-box protein 47 and tRF-19-3L7L73JD and tRF-33-P4R8YP9LON4VDP inhibited cell proliferation and migration and promoted cell apoptosis.

In colorectal cancer cells, the expression of tRF-24-NMEH623K25, tRF-30-XSXL73VL4Y, tRF-29-QU7BP61SBO and tRF-27-Q99PP99NH5N increased significantly and was related to tumor differentiation. These tsRFs are expected to be potential diagnostic biomarkers for colorectal cancer. A study by Huang et al showed that tRF/miR-1280 from tRNA^Leu and pre-miRNA was less
| Cancer                      | tsRNA          | Sample          | Dysregulation | Clinical value or biological function                                      | Reference |
|-----------------------------|----------------|-----------------|---------------|-----------------------------------------------------------------------------|-----------|
| Gastric cancer              | tiRNA-5034-GluTTC-2 | Tissue and plasma | Down          | Diagnostic biomarker                                                        | 66        |
|                             | tRF-29-RRJ8909NF5JP | Serum           | Up            | Diagnostic and prognosis biomarker                                          | 67        |
|                             | tRF-19-3L7L73JD | Plasma          | Down          | Inhibit proliferation and migration; Promote apoptosis; Affect cell cycle.  | 68        |
|                             | tRF-33-P4R8YP9LON4VDP | Plasma          | Down          | Inhibit proliferation, migration and apoptosis                             | 69        |
|                             | tRF-24-V29K9UV3IU | Tissue          | Down          | Inhibit proliferation, migration and invasion; Promote apoptosis            | 70        |
|                             | tRF-18-8R1546D2 | Tissue          | Up            | Promote proliferation, migration and invasion                              | 71        |
| Colorectal cancer           | tRF-19-FRJ4O1E2 | Tissue          | Up            | Promote invasion and migration                                               | 72        |
|                             | tRF-24-NMEH623K25, tRF-30-XSXM5L73VL4Y, tRF-29-QU7BP6ISBJO, tRF-27-Q99P9N5H5N | Tissue          | Up            | Diagnostic biomarker                                                        | 73        |
|                             | tRF/miR-1280    | Tissue          | Down          | Inhibit proliferation                                                        | 74        |
|                             | 5'-tiRNA-Val, 5'-tiRNA-Cys, 5'-tiRNA-Ala | Tissue          | Up            | Promote migration and invasion                                               | 75        |
|                             | tRF-20-M0NK5Y93 | Cell            | Down          | Inhibit invasion and metastasis                                              | 76        |
| Hepatocellular carcinomas   | tRF-31-P4R8YP9LON4VDP | Plasma          | Up            | Diagnostic biomarker                                                        | 77        |
|                             | tRF-40-EFOK8YR951K36D26, tRF-34-QNR8VP94QFY1Q, tRF-32-79MP99NH57SJ, tRF-31-87R8WP9N1EWOJ | Plasma exosome | Up            | Diagnostic biomarker                                                        | 78        |
| Pancreatic cancer           | tRF-3-Leu-AAG-1-1, tRF-3-Gln-CTG-1-1, tRF-3-Ala-CGC-1-1 | Tissue          | Up            | Diagnostic biomarker                                                        | 78        |
|                             | tiRNA-5-Pro-CGG-1-1 | Tissue          | Down          | Diagnostic biomarker                                                        | 79        |
|                             | tRF-Pro-CGG     | Tissue          | Down          | Diagnostic biomarker                                                        | 80        |
| Breast cancer               | tRF-Arg-CCT-017, tRF-Gly-CCC-001, tiRNA-Phe-GAA-003 | Plasma          | Up            | Diagnostic and prognosis biomarker                                          | 81        |
| Triple-negative breast cancer| tRF-31-87R8WP91EWOJ | Serum          | Down          | Diagnostic and prognosis biomarker                                          | 82        |
| Non-triple negative breast cancer | tRF-18-18VBY9DV, tRF-23-NB57BK87DZ | Serum          | Down          | Diagnostic biomarker                                                        | 83        |
| Disease Type                          | tRNA Sequences                                                                 | Sample Type      | Regulation | Function                                                                 |
|--------------------------------------|-------------------------------------------------------------------------------|------------------|------------|---------------------------------------------------------------------------|
| Trastuzumab-resistant breast cancer  | tRF-30-JZOYJE22RR33, tRF-27-ZDXPHO53KSN                                     | Serum            | Up         | Biomarker and treatment target                                            |
| Doxorubicin-resistant triple-negative breast cancer | tRF-31-P4R8YP9LON4VD, tDR-7336                                                | Cell             | Up         | Biomarker and treatment target                                            |
| Leukemia                            | tRF-21-2PEK45H5D, tRF-18-HROVX6D2                                             | Peripheral blood mononuclear cell | Down       | Diagnostic and prognosis biomarker                                        |
|                                     | ts-101, ts-43, ts-44, miR-3676                                                | Peripheral blood mononuclear cell | Up         | Diagnostic and prognosis biomarker                                        |
| Prostate cancer                     | tRF-315                                                                        | Peripheral Blood Mononuclear Cell | Down       | Tumor suppressor                                                          |
|                                     |                                                                                    | Cell             | Up         | Protect apoptosis induced by cisplatin treatment Prognosis biomarker       |
| Renal cell carcinoma                 | 5' tRNA-Arg-CCT, 5' tRNA-Leu-CAG, 5' tRNA-Glu-CTC, 5' tRNA-Lys-CTT             | Tissue and serum | Down       | Diagnostic biomarker                                                      |
|                                     |                                                                                    | Serum            | Up         | Promote proliferation, migration and invasion                             |
|                                     |                                                                                    | Serum            | Up         | Promote proliferation and cell cycle progression Diagnostic biomarker     |
| Ovarian cancer                      | tRF-03358, tRF-03357                                                           | Serum            | Up         | Diagnostic biomarker                                                      |
| Non-small cell lung cancer          | tRF-Leu-CAG                                                                    | Tissue and serum | Up         | Promote proliferation and cell cycle progression Diagnostic biomarker     |
| Lung cancer                         | tRF-30-RK9P4P9L5HMV, tRF-31-RK9P4P9L5HMVE, tRF-26-MI7O381NR8E, tRF-27-WJ9X0UD394N, tRF-26-SP5830MUKD, tRF-29-MIF91SS2P4IR, tRF-30-3JVLJMRPFQORD, tRF-31-RODB8NOXOJYOYE, tRF-32-ROD8NOXOJYOYO | Tissue           | Up         | Diagnostic biomarker                                                      |
| Bladder cancer                      | 5' tRF-Lys-CTT                                                                  | Tissue           | Up         | Diagnostic and prognosis biomarker                                        |
| Papillary thyroid cancer            | tRF-39-OVL8K87SIRMM12E2, tRF-38-OVL8K87SIRMM12V, tRF-34-YSV4V47Q2WJ1J1, tRF-27-PIR8YP9LON3 | Tissue           | Up         | Diagnostic biomarker                                                      |
| Oral squamous cell carcinoma        | tRF-20-S998LO9                                                                | Tissue           | Up         | Prognosis biomarker                                                       |

**tRNA-derived small RNAs**
expressed in colorectal cancer tissues. Overexpression of tRF/miR-1280 inhibited the expression of Notch1 and Notch2 receptors and inhibited cell proliferation and colony formation, thereby reducing tumor formation and metastasis. In colorectal cancer tissues, elevated ANG levels increased the expression of 5'-tiRNA-Val, 5'-tiRNA-Cys, and 5'-tiRNA-Ala, and promoted the invasion and metastasis of cancer cells without affecting cell proliferation. A mechanistic study showed that tRF-20-M0NK5Y93 inhibited the migration and invasion of colorectal cancer cells by targeting Claudin-1. The increased level of tRF-31-P4R8YP9LON4VD in the plasma of patients with colorectal cancer was dependent on the up-regulation of AlkB homolog 3 (ALKBH3), a tRNA demethylase, which promotes the cleavage of tRNA to produce tsRNAs. The expression of tRF-40-EFOK8YR951K36D26, tRF-34-QNR8VP94FQFY1Q, tRF-32-79MP9P9NH57SJ, and tRF-31-87R8WP9N1EW00 were abnormally expressed in pancreatic cancer cells. Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis and gene ontology (GO) analyses showed that these tsRNAs were mainly enriched in tumor-associated pathways, including the RAS signaling pathway, cancer pathways, axon guidance, and the PI3K/protein kinase-B (Akt) signaling pathway. Therefore, they may become biomarkers of pancreatic cancer and targets for its treatment. In addition, Li et al found that tRF-Pro-CGG was down-regulated in pancreatic ductal adenocarcinoma (PDAC) and was related to TNM stage and N stage. Furthermore, a low level of tRF-Pro-CGG predicted poor prognosis and short overall survival (OS).

Breast cancer

Several tsRNAs are abnormally expressed in breast cancer (BC) and may be a novel type of biomarkers for BC diagnosis and prognosis. Honda et al found that SHOT-RNA was specifically up-regulated in estrogen receptor (ER)-positive breast cancer and androgen receptor (AR)-positive prostate cancer cell lines. The expression levels of tRF-Arg-CCT-017, tRF-34-QNR8VP94FQFY1Q, tRF-32-79MP9P9NH57SJ, and tRF-31-87R8WP9N1EW00 in the plasma of patients with hepatocellular carcinoma (HCC) was up-regulated, suggesting that these tsRNAs may be novel diagnostic biomarkers for HCC. Kim et al found that in mouse HCC models, low expression of LeuCAG3-tsRNA levels could induce apoptosis in tumors but not in normal cells. This suggests that tsRNAs could be novel therapeutic targets of HCC.

Jin et al found that tRF-3-Leu-AAG-1-1[AS-tDR-000064], tRF-3-Gln-CTG-1-1[AS-tDR-000069], tRF-3-Ala-CGC-1-1[AS-tDR-00102], and tiRNA-Phe-GAA-1-1 were abnormally expressed in pancreatic cancer cells. In triple-negative BC (TNBC) patients, the expression of tRF-31-87R8WP9N1EW00 was decreased. However, in non-TNBC patients, the expression of tRF-18-18VBY9DV and tRF-23-NB57BK87DZ from tRNA Gly-CCC-5-1 and tRNA Phe-GAA-2-1 were significantly decreased.
tsRNAs have also been reported to be involved in many pathological processes of BC. Farina et al found that overexpression of runt-related transcription factor 1 (RUNX1), a tumor suppressor gene, inhibited proliferation by inhibiting ts-112.85 Hypoxia-induced tRFs from tRNA<sub>Asp</sub>, tRNA<sub>Gly</sub>, and tRNA<sub> Tyr</sub> inhibit the stability of multiple oncogenic transcripts by binding to the 3'-UTRs of YBX1 and replacing oncogenic transcripts of YBX1, thus inhibiting BC metastasis.53

Moreover, tsRNAs can act as potential therapeutic targets. tRF3E (Mintbase ID: tRF-32-1HPS909337KF) from mature tRNA<sub>Glu</sub> was specifically expressed in healthy breast tissues, but not in BC.86 tRF3E combines with nucleolin (NCL) to form the NCL-tRF3E complex, which can promote the translation of P53 and regulate the growth of cancer cells.86 NCL, an RBP that is overexpressed in BC, can inhibit the translation of P53.86

In addition, tsRNAs are related to BC chemoresistance. Sun et al discovered that tRF-30-JZOYJE22RR33 and tRF-27-ZDXPH05JKSN were significantly up-regulated in trastuzumab-resistant patients.73 The expression of tRF-31-P4R8YP9L0N4V and tRF-7336 from tRNA<sub>Gly</sub>-GCC-1-2 were significantly up-regulated after hypoxia stimulation of TNBC cells and promoted doxorubicin resistance in TNBC.86 This suggests that these tsRNAs may be potential biomarkers and intervention targets.

Leucocythemia
Guo et al found that tsRNA expression levels changed in the transformation of myelodysplastic syndrome (MDS) to acute myeloid leukemia (AML).89 This suggests that tsRNAs might be biomarkers to predict the progression of diseases. In chronic lymphoblastic leukemia (CLL), decreased expression of tRF-21-ZPEK45HSD and increased expression of tRF-18-HROVX6D2 are associated with OS.90,91 Some researchers found that in CLL, ts-101 was similar to Piwi-interacting RNAs (piRNAs) and bound to PiwiL2,92 which is a protein involved in transposon silencing.

tsRNAs are also potential therapeutic targets for CLL. Veneziano et al found that ts-43 and ts-44 from pre-tRNAs were down-regulated in CLL.93 To further study the expression level of tRFs in indolent and aggressive CLL, they found that mature tRF expression in CLL was seriously dysregulated, which may suppress cancer or carcinogenesis.94 miR-3676 (later found to be a tsRNA) was down-regulated in CLL.94 The deletion of miR-3676 led to a high expression level of the T-cell leukemia/lymphoma 1 (TCL1) gene, which promoted the progression of CLL.94

Prostate cancer
In AR-positive prostate cancer, SHOT-tRNA is highly expressed and plays an important role in cell proliferation.104 tRF-1001 derived from tRNA<sub>Glu</sub> is produced by the tRNA 3'-endonuclease ELAC2, a prostate cancer susceptibility gene. Inhibition of tRF-1001 can impair cell proliferation with the specific accumulation of cancer cells.95 tRF-315 from tRNA<sub>Glu</sub> is highly expressed in prostate cancer tissues. tRF-315 regulates the cell cycle by targeting the tumor suppressor gene GADD45A, thus inhibiting cisplatin-induced apoptosis and alleviating cisplatin-induced mitochondrial dysfunction.96

Other cancer types
In addition to the aforementioned cancers, tsRNAs can regulate other cancer types, but the molecular mechanisms are not completely known.

The expression of 5'-tRNA-Arg-CCT, 5'-tRNA-Leu-CAG, 5'-tRNA-Glu-CTC, and 5'-tRNA-Lys-CTT decreased in renal cell carcinoma (RCC) patients.97 The expression level of tRNA<sub>Glu</sub> was different in ovarian tumors.98 The expression levels of tRF-03357 and tRF-03358 were significantly increased in patients with high-grade serous ovarian cancer. tRF-03357 can promote the proliferation, migration, and invasion of ovarian cancer cells.99 tRF-Leu-CAG is highly expressed in non-small cell lung cancer (NSCLC), and promotes cell proliferation and the cell cycle.100 The expression levels of tRF-30-RK9P4P9L5HMW, tRF-31-RK9P4P9L5HMW, tRF-26-MI7O3B1NR8E, tRF-27-WJ9X0UD 394n, tRF-26-SP58OMU0KD, tRF-29-MIF91552P4IR, tRF-30-JVJMRFQQRD, tRF-31-ROD8N0X0JYOYE, and tRF-32-RD08 NOXOJYOYO were significantly up-regulated in lung cancer.101 The expression of 5'-tRF-Lys-CTT from tRNA<sub>Glu</sub>-Lys-CTT increased in bladder cancer.102 In papillary thyroid cancer, the expression of tRF-39-0VL8KB75SRMM12EZ and tRF-38-0VL8KB75SRMM12V were up-regulated, while tRF-34-Y5V4Q7Q2WW1J1 and tRF-27-PIR8YP9L0N3 were down-regulated.102 The expression level of tRF-20-S998LO9 in oral squamous cell carcinoma was significantly increased and was correlated with OS.104

Conclusion and prospects
In the late 1970s, tsRNAs were first discovered in the urine of cancer patients. However, at that time, tsRNAs were considered a non-specific degradation product of tRNAs.10,105 In recent years, studies have found that tsRNAs have precise sequence structures and specific biological functions.106 tsRNAs are involved in many cellular biological processes and are potential biomarkers for cancer diagnosis and treatment. With the development and application of sequencing technology, an increasing number of tsRNAs has been discovered, but our understanding is only beginning.

tsRNAs are known to be produced by cutting specific sites of tRNAs. However, the precise process of tsRNA production remains unclear. The distribution, expression level, and biological function of most tsRNAs remain unknown. Since tsRNAs are involved in the regulation of tumor proliferation, migration, apoptosis, and other biological processes, they can be used as potential biomarkers for cancer diagnosis and prognosis. Unfortunately, we do not fully understand the specific mechanism of tsRNAs in regulating the occurrence and development of tumors. Finally, many studies have shown that tsRNAs have high value in diagnosis, but whether they can be used in clinical treatment is still unclear. Further experiments are needed.
Conflict of interests

The authors declare that there is no conflict of interest.

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References

1. Phizicky EM, Hopper AK. tRNA biology charges to the front. Genes Dev. 2010;24(17):1832–1860.
2. Frank DN, Pace NR, Ribonuclease P. Unity and diversity in a tRNA processing ribozyme. Annu Rev Biochm. 1998;67:153–180.
3. Marai A, Lamichhane TN. 3’ processing of eukaryotic precursor tRNAs. Wiley Interdiscip Rev RNA. 2011;2(3):362–375.
4. Schimmel P. The emerging complexity of the tRNA world: mammalian tRNAs beyond protein synthesis. Nat Rev Mol Cell Biol. 2018;19(1):45–58.
5. Kumar P, Anaya J, Mudunuri SB, 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. 2014;12:78.
6. Xie Y, Yao L, Yu X, Ruan Y, Li Z, Guo J. Action mechanisms and research methods of tRNA-derived small RNAs. Signal Transduct Target Ther. 2020;5(1):109.
7. Zhu L, Ge J, Li T, Shen Y, Guo J. tRNA-derived fragments and tRNA halves: the new players in cancers. Cancer Lett. 2019;452:31–37.
8. Anderson P, Ivanov P. tRNA fragments in human health and disease. FEBS Lett. 2014;588(23):4297–4304.
9. Yu M, Lu B, Zhang J, Ding J, Liu P, Lu Y. tRNA-derived RNA fragments in cancer: current status and future perspectives. J Hematol Oncol. 2020;13(1):121.
10. Li S, Xu Z, Sheng J. tRNA-derived small RNA: a novel regulatory small non-coding RNA. Genes. 2018;9(5):246.
11. Xie Y, Xie Y, Zhang S, Song X, Xiao B, Yan Z. tRNA-derived mechanisms: underlining their regulation of gene expression and potential applications as therapeutic targets in cancers and virus infections. Theranostics. 2021;11(1):461–469.
12. Salkia M, Krokowski D, Guan BJ, et al. Genome-wide identification and quantitative analysis of cleaved tRNA fragments induced by cellular stress. J Biol Chem. 2012;287(51):42708–42725.
13. Shen Y, Yu X, Zhu L, Li T, Yan Z, Guo J. Transfer RNA-derived fragments and tRNA halves: biogenesis, biological functions and their roles in diseases. J Mol Med (Berl). 2018;96(11):1167–1176.
14. Honda S, Loher P, Shigematsu M, et al. Sex hormone-dependent tRNA halves enhance cell proliferation in breast and prostate cancers. Proc Natl Acad Sci U S A. 2015;112(29):E3816–E3825.
15. Couvillion MT, Sachidandaram R, Collins K. A growth-essential Tetrahymena Piwi protein carries tRNA fragment cargo. Genes Dev. 2010;24(24):2742–2747.
16. Kawai H, Nakamura M, Takahashi Y, et al. Hidden layers of human small RNAs. BMC Genom. 2008;9:157.
17. Kumar P, Mudunuri SB, Anaya J, Dutta A. tRFdb: a database for transfer RNA fragments. Nucleic Acids Res. 2015;43(Database issue):D141–D145.
18. Cole C, Sobala A, Lu C, et al. Filtering of deep sequencing data reveals the existence of abundant Dicer-dependent small RNAs derived from tRNAs. RNA. 2009;15(12):2147–2160.
19. Karoussi P, Katsaraki K, Papageorgiou SG, Pappa V, Scorilas A, Kontos CK. Identification of a novel tRNA-derived RNA fragment exhibiting high prognostic potential in chronic lymphocytic leukemia. Hematol Oncol. 2019;37(4):498–504.
20. Kumar P, Kuscu C, Dutta A. Biogenesis and function of transfer RNA-related fragments (tRFs). Trends Biochem Sci. 2016;41(8):679–689.
21. Thery C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles. 2018;7(1):153750.
22. Raina M, Ibma M. tRNAs as regulators of biological processes. Front Genet. 2014;5:171.
23. Gebetsberger J, Polacek N. Slicing tRNAs to boost functional ncRNA diversity. RNA Biol. 2013;10(12):1798–1806.
24. Garcia-Silva MR, Cabrera-Cabrera F, Guida MC, Cayota A. Hints of tRNA-derived small RNAs role in RNA silencing mechanisms. Genes. 2012;3(4):603–614.
25. Sobala A, Hutvagner G. Transfer RNA-derived fragments: origins, processing, and functions. Wiley Interdiscip Rev RNA. 2011;2(6):853–862.
26. Maute RL, Schneider C, Sumazin P, et al. tRNA-derived microRNA modulates proliferation and the DNA damage response and is down-regulated in B cell lymphoma. Proc Natl Acad Sci U S A. 2013;110(4):1404–1409.
27. Kuscu C, Kumar P, Kiran M, Su Z, Malik A, Dutta A. tRNA fragments (tRFs) guide Ago to regulate gene expression post-transcriptionally in a Dicer-independent manner. RNA. 2018;24(8):1093–1105.
28. Cho H, Lee W, Kim GW, et al. Regulation of La/SSB-dependent viral gene expression by pre-tRNA 3’ trailer-derived tRNA fragments. Nucleic Acids Res. 2019;47(18):9888–9901.
29. Wang Q, Lee I, Ren J, Ajay SS, Lee YS, Bao X. Identification and functional characterization of tRNA-derived RNA fragments (tRFs) in respiratory syncytial virus infection. Mol Ther. 2013;21(2):368–379.
30. Zhou J, Liu S, Chen Y, et al. Identification of two novel functional tRNA-derived fragments induced in response to respiratory syncytial virus infection. J Gen Virol. 2017;98(7):1600–1610.
31. Kim HK, Yeom JH, Kay MA. Transfer RNA-derived small RNAs: another layer of gene regulation and novel targets for disease therapeutics. Mol Ther. 2020;28(11):2340–2357.
32. Truitt ML, Ruggero D. New frontiers in translational control of the cancer genome. Nat Rev Cancer. 2016;16(5):288–304.
33. Yamasaki S, Ivanov P, Hu GF, Anderson P. Angelin cleaves tRNA and promotes stress-induced translational repression. J Cell Biol. 2009;185(1):35–42.
34. Zhang S, Sun L, Kragler F. The phloem-delivered RNA pool contains small noncoding RNAs and interferes with translation. Plant Physiol. 2009;150(1):379–387.
35. Ivanov P, Emara MM, Villen J, Gygi SP, Anderson P. Angelin-induced tRNA fragments inhibit translation initiation. Mol Cell. 2011;43(4):613–623.
36. Ivanov, P., O’Day, E., Emara, M.M., Wagner, G., Lieberman, J., Anderson, P. G-quadruplex structures contribute to the neuroprotective effects of angiogenin-induced tRNA fragments. *Proc Natl Acad Sci U S A*. 2014;111(51):18201–18206.

37. Lyons, S.M., Achorn, C., Kedersha, N.L., Anderson, P.J., Ivanov, Y.B. regulates tRNA-derived Stress Granule formation but not translational repression. *Nucleic Acids Res*. 2016;44(14):6949–6960.

38. Lyons, S.M., Gudanis, D., Coyne, S.M., Gdaniec, Z., Ivanov, P. Identification of functional tetramolecular RNA G-quadruplexes derived from transfer RNAs. *Nat Commun*. 2017;8(1):1127.

39. Sobala, A., Hutvagner, G. Small RNAs derived from the 5′ end of tRNA can inhibit protein translation in human cells. *RNA Biol*. 2013;10(4):553–563.

40. Mleczko, A.M., Celichowski, P., Bąkowska-Żywicka, K. Transfer RNA-derived fragments target and regulate ribosome-associated aminocyl-transfer RNA synthetases. *Biochim Biophys Acta BBA Gene Regul Mech*. 2018;1861(7):647–656.

41. Gebetsberger, J., Zywicki, M., Künzi, A., Polacek, N. tRNA-derived fragments target the ribosome and function as regulatory non-coding RNA in Haloferax volcanii. *Archaea*. 2012;2012:260909.

42. Gebetsberger, J., Wyss, L., Mleczko, A.M., Reuther, J., Polacek, N. A tRNA-derived fragment competes with mRNA for ribosome binding and regulates translation during stress. *RNA Biol*. 2017;14(10):1364–1373.

43. Kim, H.K., Fuchs, G., Wang, S., et al. A transfer-RNA-derived small RNA regulates ribosome biogenesis. *Nature*. 2017;552(7683):57–62.

44. Kim, H.K., Xu, J., Chu, K., et al. A tRNA-derived small RNA regulates ribosomal protein S28 protein levels after translation initiation in humans and mice. *Cell*. 2019;29(12):3816–3824.

45. Fricker, R., Brogli, R., Luidallepp, H., et al. A tRNA half modulates translation as stress response in Trypanosoma brucei. *Nat Commun*. 2019;10(1):118.

46. Keam, S.P., Sobala, A., Ten Have, S., Hutvagner, G. tRNA-derived RNA fragments associate with human multisynthetase complex (MSc) and modulate ribosomal protein translation. *J Proteome Res*. 2017;16(2):413–420.

47. Guzzi, N., Ciesla, M., Ngoc, P.C.T., et al. Pseudouridylation of tRNA-derived fragments steers translational control in stem cells. *Cell*. 2018;173(5):1204–1216.

48. Gkatza, N.A., Castro, C., Harvey, R.F., et al. Cytosine-5 RNA methylation links protein synthesis to cell metabolism. *PLoS Biol*. 2019;17(6):e3000297.

49. Schorn, A.J., Gubrod, M.J., LeBlanc, C., Martienssen, R. LTR-retrotransposon control by tRNA-derived small RNAs. *Cell*. 2017;170(1):61–71.

50. Slotkin, R.K., Martienssen, R. Transposable elements and the epigenetic regulation of the genome. *Nat Rev Genet*. 2007;8(4):272–285.

51. Ruggiero, K., Guffanti, A., Corradin, A., et al. Small noncoding RNAs in cells transformed by human T-cell leukemia virus type 1: a role for a tRNA fragment as a primer for reverse transcriptase. *J Virol*. 2014;88(7):3612–3622.

52. Yeung, M.L., Bennasser, Y., Watashi, K., Le, S.Y., Houzet, L., Jeang, K.T. Pyrosequencing of small non-coding RNAs in HIV-1 infected cells: evidence for the processing of a viral-cellular double-stranded RNA hybrid. *Nucleic Acids Res*. 2009;37(19):6575–6586.

53. Goodarzi, H., Liu, X., Nguyen, H.C.B., Zhang, S., Fish, L., Tavazoie, S.F. Endogenous tRNA-derived fragments suppress breast cancer progression via YBX1 displacement. *Cell*. 2015;164(1):738–902.

54. Emara, M.M., Ivanov, P., Hickman, T., et al. Angiogenin-induced tRNA-derived stress-induced RNAs promote stress-induced stress granule assembly. *J Biol Chem*. 2010;285(14):10959–10968.
86. Falconi M, Giangrossi M, Zabaleta ME, et al. A novel 3'-tRF/miR-1280 suppresses stem cell-like cells and metastasis in colorectal cancer. Cancer Res. 2017;77(12):3194–3206.

87. Sun C, Yang F, Zhang Y, et al. tRNA-derived fragments as novel biomarkers for colorectal cancer. Proc Natl Acad Sci U S A. 2019;116(48):24252–24258.

88. Balatti V, Rizzotto L, Miller C, et al. TCL1 targeting miR-3676 is codeleted with tumor protein p53 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A. 2015;112(7):2169–2174.

89. Lee YS, Shibata Y, Malhotra A, Dutta A. A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). Genes Dev. 2009;23(22):2639–2649.

90. Peng EY, Shu Y, Wu Y, et al. Presence and diagnostic value of circulating tscRNA for ovarian tumor. Mol Cancer. 2018;17(1):163.

91. Zhang M, Li F, Wang J, et al. tRNA-derived fragment tRF-03357 promotes cell proliferation, migration and invasion in high-grade serous ovarian cancer. Oncotargets Ther. 2019;12:6371–6383.

92. Shao Y, Sun Q, Liu X, Wang P, Wu R, Ma Z. tRF-Leu-CAG promotes cell proliferation and cell cycle in non-small cell lung cancer. Chem Biol Drug Des. 2017;90(5):730–738.

93. Gu W, Shi J, Liu H, et al. Peripheral blood non-canonical small non-coding RNAs as novel biomarkers in lung cancer. Mol Cancer. 2020;19(1):55.

94. Papadimitriou MA, Avgeris M, Levis P, et al. tRNA-derived fragments (tRFs) in bladder cancer: increased 5'-tRF-LysCTT and 3'-tRF-LysCGG are potential biomarkers in chronic lymphocytic leukemia. J Transl Oncol. 2021;14(1):431.

95. Wang F, Coates PJ, et al. tRNA-derived fragments (tRFs) in lung cancer. J Cell Physiol. 2021;236(6):8740–8751.

96. Borek E, Baliga BS, Gehrke CW, et al. High turnover rate of transfer RNA in tumor tissue. Cancer Res. 1977;37(9):3362–3366.

97. Zhu L, Li Z, Yu X, et al. The tRNA-derived fragment 5026a inhibits the proliferation of gastric cancer cells by regulating the PTEN/Pi3K/AKT signaling pathway. Stem Cell Res Ther. 2021;12(1):418.