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REVIEW ARTICLE

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]

tsRNAs, 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).

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—11] tiRNAs are divided into 5′-tiRNAs and 3′-tiRNAs.[12,13] There is another type of tiRNA—sex hormone-dependent tRNA 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′U-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.[19].

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 RNA stability regulation (Fig. 2).

RNA silencing

Many studies have shown that tsRNAs may mediate RNA silencing by binding with Argonauts (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 A 1 (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.[26] 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].

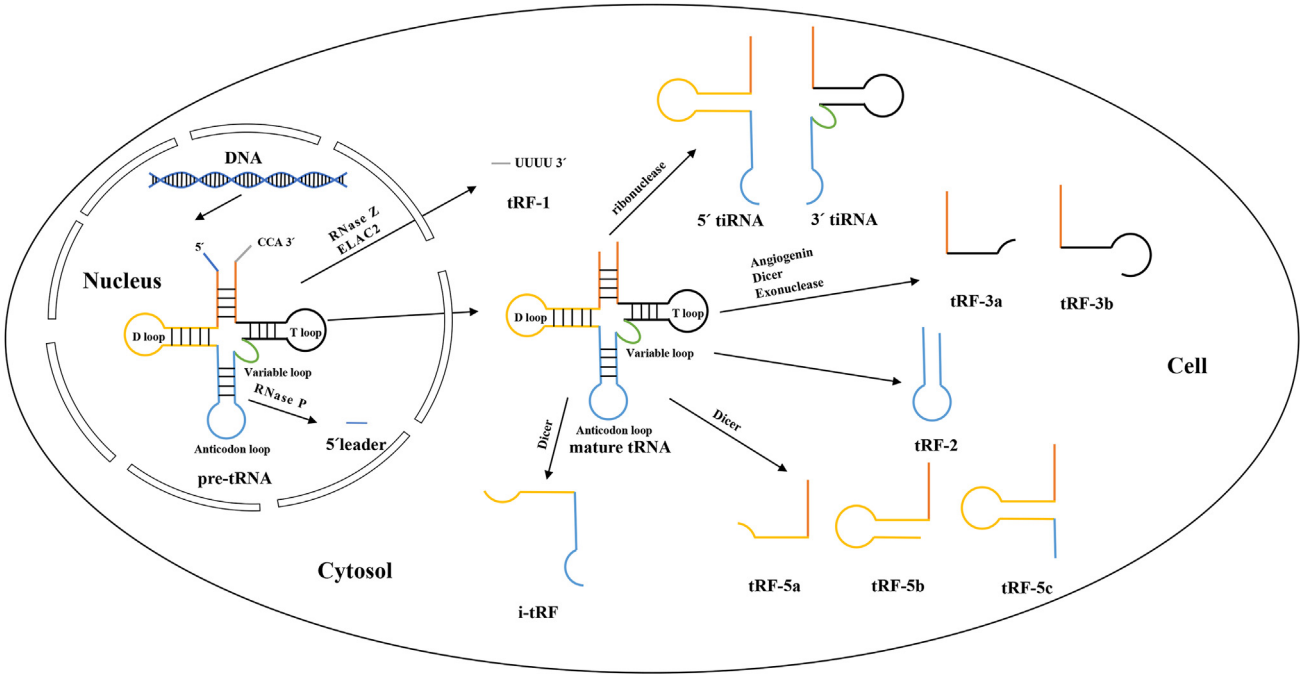

Figure 1 Two main subtypes of tsRNAs.

Translational regulation

Some tsRNAs can regulate translation in a sequence-specific way.[31] 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.[32] Some tsRNAs have been found to play a role in global translation inhibition in plant and animal cells.[33,34] 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.[35] Ivanov et al found that 5′-tiRNAs inhibited protein synthesis and triggered phosphor-eIF2α-independent assembly of stress granules (SGs).[35] 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.[36] 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.[35] 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.[37] In 2014, Ivanov et al found that 5′-tiRNAs can be assembled into a unique G-quadruplex (G4) structure.[36] G4 plays an important role in 5′-tiDNA^{Ala} and 5′-tiDNA^{Cys} (DNA analogues of 5′-tiRNA^{Ala} and 5′-tiRNA^{Cys}) inhibition of protein synthesis, promoting SG formation and protecting motor neurons exposed to stress.[36] 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*.[38].

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.[39] Additionally, in yeast, tsRNA was found to affect the aminoacylation of tRNA by interacting with aminoacyl-tRNA synthetase, thereby inhibiting translation *in vitro*.[40].

Moreover, other tsRNAs can regulate translation by interacting with ribosomes (Fig. 2B). In *Haloferax volcanii*, tRF from tRNA^{Val} 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*.[41,42] LeuCAG3' tsRNA derived from the tRNA$^{Leu-CAG}$ 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.[43,44] Fricker et al found that the 3′-tiRNA^{Thr} in *Trypanosoma brucei* was significantly induced under starvation.[45] After nutritional recovery, 3′-tiRNA^{Thr} binds to ribosomes and polymers to promote the loading of mRNA into these ribosomes, thus enhancing translation.[45] Keam et al found that the 19 nt 5′-tRF from tRNA^{Gln} interacts with human multisynthetase complex (MSC) to inhibit translational elongation.[46].

Furthermore, studies have shown that the regulation of translation by tsRNAs is affected by post-transcriptional modifications.[47,48] 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.[47] 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.[48].

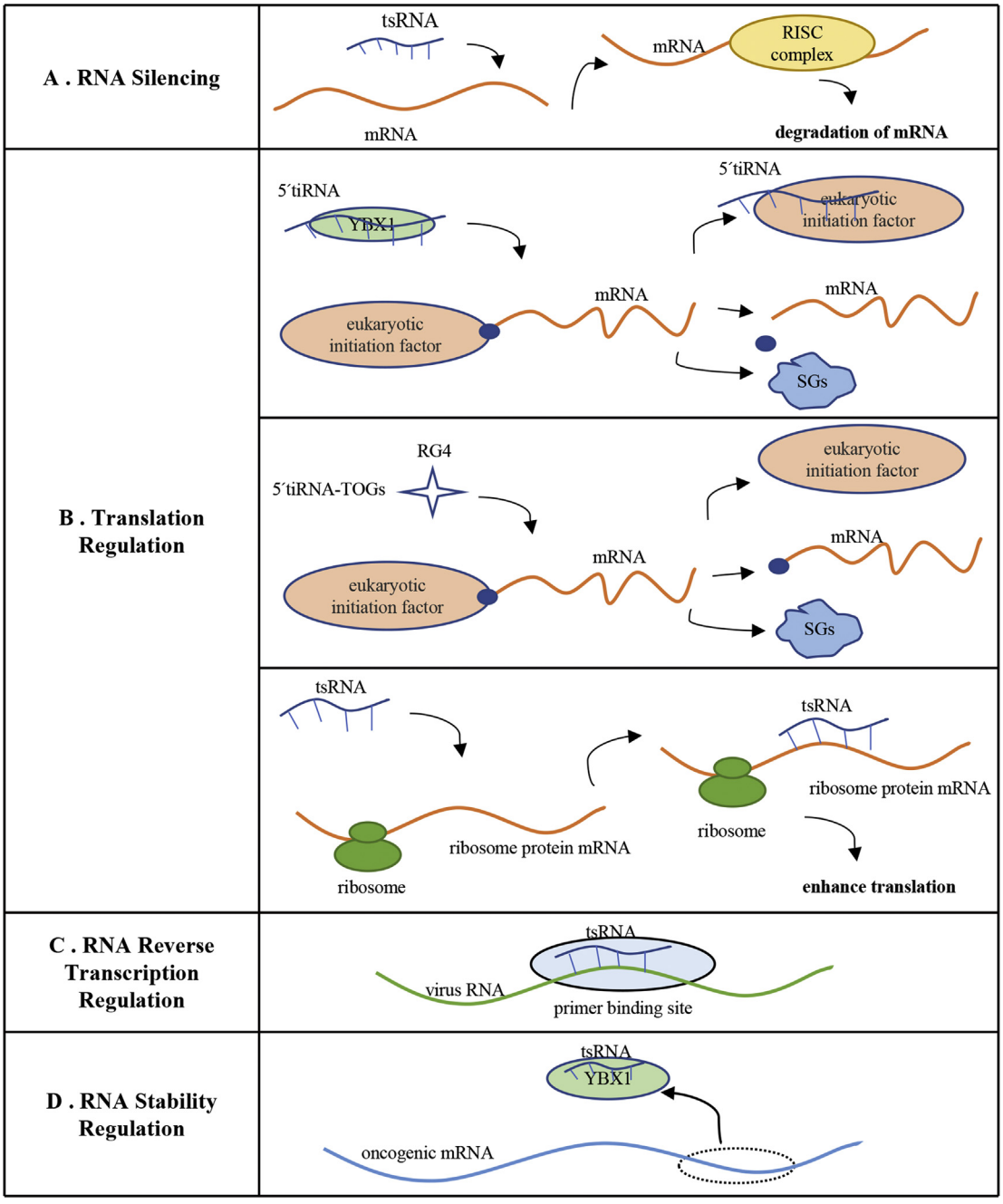

Figure 2 The four function categories of tsRNAs.

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′-tRFs are key regulators of TEs in cells.[49] In mouse stem cells, many 18 nt 3′CCA tRFs target tRNA PBSs in a sequence-dependent manner. 18 nt 3′CCA tRFs specifically inhibit the reverse transcription of TEs without affecting the expression of TEs. 22 nt 3′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 $tRNA^{Lys-UUU}$ 3′ 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].

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).[53] 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.[53] Goodarzi et al found that when exposed to hypoxia, breast cancer cells could induce tRFs derived from $tRNA^{Asp}$, $tRNA^{Glu}$, $tRNA^{Gly}$, and $tRNA^{Tyr}$. 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.[53].

In addition, the stability of mRNA mediated by tsRNA is related to the assembly of SGs.[54] 5′-tiRNA can induce the phosphorylation of eukaryotic initiation factor 2α (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.[54]

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.[55] 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.[55,56] 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.[57].

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 tsRNA-26576 in breast cancer cells promoted cell proliferation and migration, and inhibited cell apoptosis.[58] In breast cancer cells, overexpression of 5′-tiRNA regulates cell proliferation and migration by inhibiting the FZD3/Wnt/β-Catenin signaling pathway.[59] Saikia et al suggested that ANG-induced mouse embryonic fibroblasts (MEF) to produce tiRNAs by interacting with cytochrome c (Cyt c).[60] 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.[12,60].

In addition, other studies have found that tsRNAs play a role through the formation of tsRNA-RNP complex.[61,62] 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.[61] After the tRF-1001 derived from the 3′ end of pre-$tRNA^{Ser}$ was knocked out, the cells were blocked in G_2 stage, which led to the inhibition of DNA biosynthesis and cell proliferation.[8,62] 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.[63] 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 induces pluripotency.[63]

tsRNAs also play an important role in immune responses, intercellular communication, and intergenerational inheritance. Chiou 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.[64] Shen et al found that TdR-001292 exists in the endometrium of patients with endometriosis and participates in the signal transmission between cells.[65] 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.[66].

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 clinicopathological characteristics.[67–71] 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.[67,68] Our group found that the expression level of tiRNA-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.[67] The expression level of tRF-29-RRJ89O9NF5JP in the serum of gastric cancer patients was significantly increased and was positively correlated with lymph node metastasis and tumor grade.[68] 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 and migration and promoted cell apoptosis.[69–71] Dong et al found similar effects of tRF-24-V29K9UV3IU on gastric cancer cells.[70] Zhang et al found that tRF-18-8R1546D2 derived from $tRNA^{Ala-AGC-1-1}$ regulated the proliferation, migration, and invasion of gastric cancer cells by targeting the tumor suppressor gene F-box protein 47 (FBXO47).[72] tRF-19-FRJ4O1E2 from $tRNA^{Val-TAC}$ might form RISC with Ago protein to regulate tumor suppressor gene nerve epidermal growth factor-like like protein 2 (NELL2).[73].

In colorectal cancer cells, the expression of tRF-24-NMEH623K25, tRF-30-XSXMSL73VL4Y, tRF-29-QU7BPN6ISBJO, and tRF-27-Q99P9P9NH5N increased significantly and was related to tumor differentiation.[74] These tRFs 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

Table 1 Functional tsRNAs in different types of cancer.

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-RRJ89O9NF5JP	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
	tRF-19-FRJ4O1E2	Tissue	Up	Promote invasion and migration	72
Colorectal cancer	tRF-24-NMEH623K25, tRF-30-XSXMSL73VL4Y, tRF-29-QU7BPN6ISBJO, tRF-27-Q99P9P9NH5N	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
	tRF-31-P4R8YP9LON4VD	Plasma	Up	Diagnostic biomarker	77
Hepatocellular carcinomas	tRF-40-EFOK8YR951K36D26, tRF-34-QNR8VP94FQFY1Q, tRF-32-79MP9P9NH57SJ, tRF-31-87R8WP9N1EWJ0	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 and prognosis 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-31-87R8WP9I1EWJ0	Serum	Down	Diagnostic and prognosis biomarker	82
Non-triple negative breast cancer	tRF-18-18VBY9DV, tRF-23-NB57BK87DZ	Serum	Down	Diagnostic biomarker	83

Trastuzumab-resistant breast cancer	tRF-30-JZOYJE22RR33, tRF-27-ZDXPHO53KSN	Serum	Up	Biomarker and treatment target	86
Doxorubicin-resistance triple-negative breast cancer	tRF-31-P4R8YP9LON4VD, tDR-7336	Cell	Up	Biomarker and treatment Target	87
Leukemia	tRF-21-ZPEK45H5D	Peripheral blood mononuclear cell	Down	Diagnostic and prognosis biomarker	89
	tRF-18-HR0VX6D2	Peripheral blood mononuclear cell	Up	Diagnostic and prognosis biomarker	90
	ts-101, ts-43, ts-44	Peripheral blood mononuclear cell	Down	Diagnostic biomarker	91,92
	miR-3676	Peripheral Blood Mononuclear Cell	Down	Tumor suppressor	93
Prostate cancer	tRF-315	Cell	Up	Protect apoptosis induced by cisplatin treatment	95
Renal cell carcinoma	5′ tiRNA-Arg-CCT, 5′ tiRNA-Leu-CAG, 5′ tiRNA-Glu-CTC, 5′ tiRNA-Lys-TTT	Tissue and serum	Down	Prognosis biomarker	96
Ovarian cancer	tRF-03358	Serum	Up	Diagnostic biomarker	98
	tRF-03357	Serum	Up	Promote proliferation, migration and invasion	98
Non-small cell lung cancer	tRF-Leu-CAG	Tissue and serum	Up	Promote proliferation and cell cycle progression	99
Lung cancer	tRF-30-RK9P4P9L5HMV, tRF-31-RK9P4P9L5HMVE, tRF-26-MI7O3B1NR8E, tRF-27-WJ9X0UD394N, tRF-26-SP5830MMUKD, tRF-29-MIF91SS2P4IR, tRF-30-3JVIJMRPFQRD, tRF-31-ROD8N0X0JYOYE, tRF-32-ROD8N0X0JYOYO	Tissue	Up	Diagnostic biomarker	100
Bladder cancer	5′-tRF-Lys-CTT	Tissue	Up	Diagnostic and prognosis biomarker	101
Papillary thyroid cancer	tRF-39-0VL8K87SIRMM12E2, tRF-38-0VL8K87SIRMM12V	Tissue	Up	Diagnostic biomarker	102
	tRF-34-YSV4V47Q2WW1J1, tRF-27-PIR8YP9LON3	Tissue	Down	Diagnostic biomarker	102
Oral squamous cell carcinoma	tRF-20-S998LO9	Tissue	Up	Prognosis biomarker	103

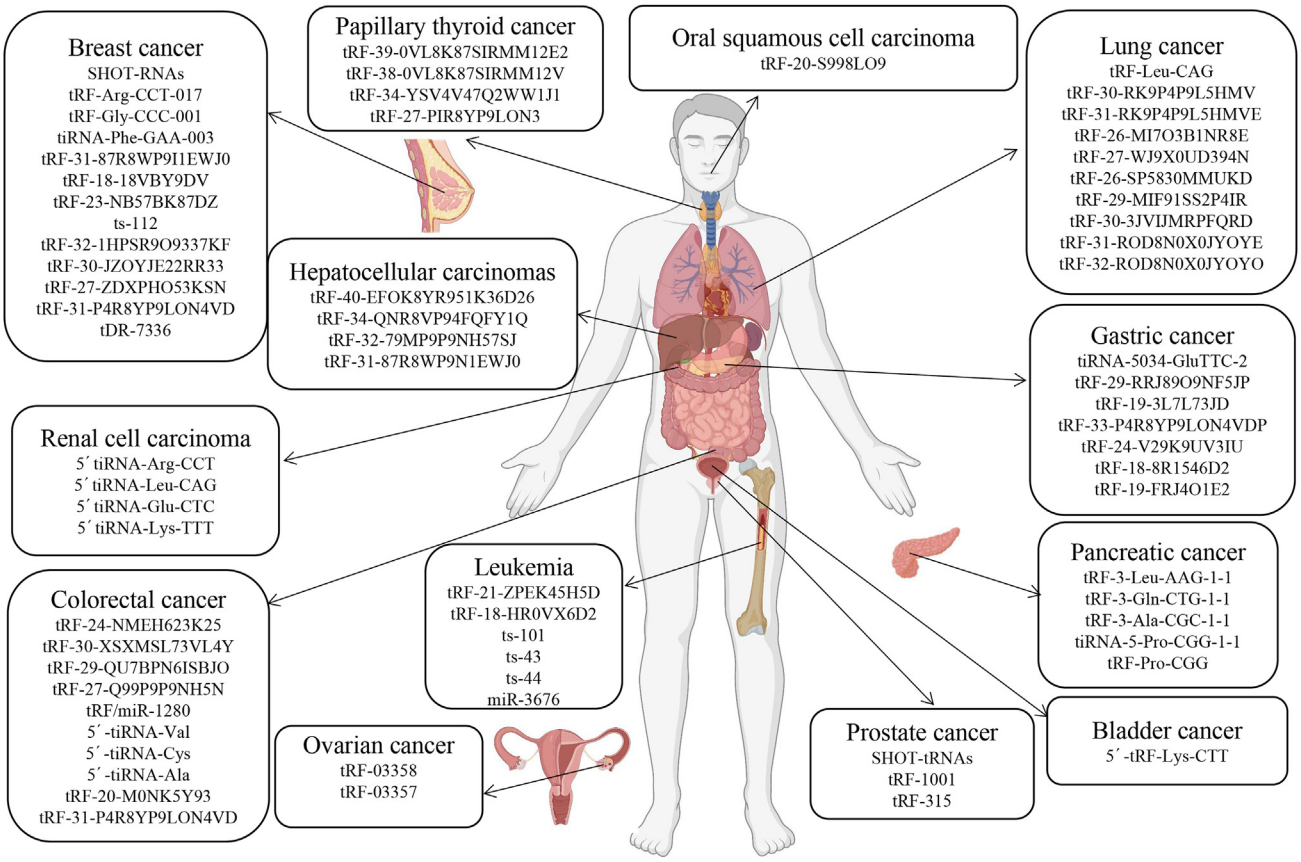

Figure 3 The roles of tsRNAs in cancers.

expressed in colorectal cancer tissues.[75] 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.[75] In colorectal cancer tissues, elevated ANG level 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.[76] A mechanistic study showed that tRF-20-M0NK5Y93 inhibited the migration and invasion of colorectal cancer cells by targeting Claudin-1.[77] 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.[78].

The expression of tRF-40-EFOK8YR951K36D26, tRF-34-QNR8VP94FQFY1Q, tRF-32-79MP9P9NH57SJ, and tRF-31-87R8WP9N1EWJ0 in the plasma exosomes of patients with hepatocellular carcinoma (HCC) was up-regulated, suggesting that these tsRNAs may be novel diagnostic biomarkers for HCC.[79] 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.[43] 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-000102], and tiRNA-5-Pro-CGG-1-1[AS-tDR-001391] were abnormally expressed in pancreatic cancer cells.[80] 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.[80] 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.[81] Furthermore, a low level of tRF-Pro-CGG predicted poor prognosis and short overall survival (OS).[81].

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.[14] The expression levels of tRF-Arg-CCT-017, tRF-Gly-CCC-001, and tiRNA-Phe-GAA-003 in the plasma of patients with BC were significantly up-regulated and were related to disease-free survival (DFS) and OS.[82] In triple-negative BC (TNBC) patients, the expression of tRF-31-87R8WP9I1EWJ0 was decreased.[83] 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.[84]

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 $tRNA^{Asp}$, $tRNA^{Gly}$, and $tRNA^{Tyr}$ 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-32-1HPSR9O9337KF) from mature $tRNA^{Glu}$ 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-ZDXPHO53KSN were significantly up-regulated in trastuzumab-resistant patients.[87] The expression of tRF-31-P4R8YP9LON4VD from $tRNA^{Gly-GCC-1-1}$ and tDR-7336 from $tRNA^{Gly-GCC-1-2}$ were significantly up-regulated after hypoxia stimulation of TNBC cells and promoted doxorubicin resistance in TNBC.[88] 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-ZPEK45H5D and increased expression of tRF-18-HR0VX6D2 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.[92]

tsRNAs are also potential therapeutic targets for CLL. Veneziano et al found that ts-43 and ts-44 from pre-$tRNA^{His}$ 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.[93] 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.[14] tRF-1001 derived from $tRNA^{Ser}$ 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 $tRNA^{Lys}$ 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′-tiRNA-Arg-CCT, 5′-tiRNA-Leu-CAG, 5′-tiRNA-Glu-CTC, and 5′-tiRNA-Lys-TTT decreased in renal cell carcinoma (RCC) patients.[97] The expression level of tsRNA from $tRNA^{Gly}$ 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-RK9P4P9L5HMV, tRF-31-RK9P4P9L5HMVE, tRF-26-MI7O3B1NR8E, tRF-27-WJ9X0UD394N, tRF-26-SP5830MMUKD, tRF-29-MIF91SS2P4IR, tRF-30-3JVIJMRPFQRD, tRF-31-ROD8N0X0JYOYE, and tRF-32-ROD8N0X0JYOYO were significantly up-regulated in lung cancer.[101] The expression of 5′-tRF-Lys-CTT from $tRNA^{Lys-CTT}$ increased in bladder cancer.[102] In papillary thyroid cancer, the expression of tRF-39-0VL8K87SIRMM12E2 and tRF-38-0VL8K87SIRMM12V were up-regulated, while tRF-34-YSV4V47Q2WW1J1 and tRF-27-PIR8YP9LON3 were down-regulated.[103] 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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