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Mini-review

tRNA-derived fragments and tRNA halves: The new players in cancers

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ABSTRACT

tRNA-derived fragments (tRFs) and tRNA halves (tiRNAs) are small non-coding RNAs derived from precursor tRNAs or mature tRNAs. Depending on the sources, tRFs can be divided into tRF-1, tRF-2, tRF-3, tRF-5, and i-tRF; tiRNAs can be divided into 5′tiRNA and 3′tiRNA. Both tRFs and tiRNAs play important roles in tumorigenesis. Some tRFs and tiRNAs promote cell proliferation and cell cycle progression by regulating the expression of oncogenes. Other tRFs and tiRNAs inhibit cancer progression. Mechanism studies have shown that tRFs and tiRNAs may bind to RNA binding proteins such as Y-box binding protein 1 (YBX1) and prevent transcription, inactivate initiation factor eIF4G/A, promote translation of ribosomal proteins, or activate aurora kinase A, the regulator of mitosis. Therefore, tRFs and tiRNAs regulate the occurrence and development of cancers, including lung cancer, colorectal cancer, prostate cancer, breast cancer, ovarian cancer, B cell lymphoma, chronic lymphocytic leukemia, etc. This article reviews the classification of tRFs and tiRNAs, their biological functions in the occurrence of cancers, and their relationships with some common cancers. It will provide new ideas for the diagnosis and treatment of cancers.

1. Introduction

Non-coding RNAs (ncRNAs) are RNAs that do not have the ability to translate into proteins. ncRNAs exist widely and have a wide variety of functions in organisms [1,2]. Transfer RNAs (tRNAs) are classical ncRNAs that are mainly responsible for converting genetic information on messenger RNA (mRNA) into amino acid sequence information on nascent proteins.

Under the effect of sex hormone, hypoxia, and other stress conditions, the anticodon loop of tRNAs may be specifically spliced into tRNA halves (tiRNAs) [3,4]. The importance of tRNA-derived fragments (tRFs) and tiRNAs has been ignored for quite some years. Recently, however, important functions of tRFs and tiRNAs have been gradually discovered. Acting as signal molecules and gene expression regulators, tRFs and tiRNAs have important regulatory functions in major diseases such as tumors, metabolic diseases, and neurological diseases [5–8]. Here, we introduced the classification of tRFs and tiRNAs, their biological functions, and especially focused on their roles in cancers.

2. Classification of tRFs and tiRNAs

According to the sites of cleavage, the tRNA-derived small RNAs (tsRNAs) can be divided into two main types (Fig. 1): (1) tRFs, approximately 14–30 nt in length and derived from mature or precursor tRNAs; (2) tiRNAs, 29–50 nt in length, induced by stress and produced by specific cleavage at the mature tRNA anticodon loop.

Based on whether 5′ or 3′ sequence is included, tiRNAs can be divided into two subclasses: 5′tiRNAs and 3′tiRNAs. 5′tiRNAs start from the 5′ end of the mature tRNA to the end of the anticodon loop; 3′tiRNAs start from the anticodon loop to the 3′ end of the mature tRNA [9]. Differing from microRNAs (miRNAs) and small interfering RNAs (siRNAs) produced by Dicer or type III RNase enzymatic cleavage, tiRNAs are cleaved by angiogenin (ANG, called RNY1 in yeast) and have a 5′ hydroxyl group instead of a 5′ phosphate [10,11]. Based on stress conditions, cancer cells produce a 5′tiRNA, while sex hormone-dependent tRNA-derived RNAs (SHOT-RNAs) are produced under sex hormone-induced conditions [4]. Additionally, 5′tiRNAs and 3′tiRNAs can be produced under hypoxic conditions [3,7,9,11].

Depending on the sources, tRFs can be divided into tRF-1, tRF-2, tRF-3, tRF-5, and i-tRF (Fig. 1). tRF-1 is derived from the 3′ end of precursor tRNA cleaved by RNase Z or its cytoplasmic homolog ribonuclease Z 2 (ELAC2) [12]. The 3′ end of tRF-1 contains a poly U sequence. tRF-2 is a newly discovered tRFs derived from $tRNA^{Glu}$, $tRNA^{Asp}$, $tRNA^{Gly}$, and $tRNA^{Tyr}$. tRF-2 is decomposed from the anticodon loop on tRNA, excluding the 5′ end and the 3′ end structures. It is induced under hypoxic condition [13]. tRF-3, derived from the 3′ end of mature tRNA, is produced by cleavage of ANG, Dicer, or members of ribonuclease A superfamily at the T-loop. Therefore, the tRF-3 tail

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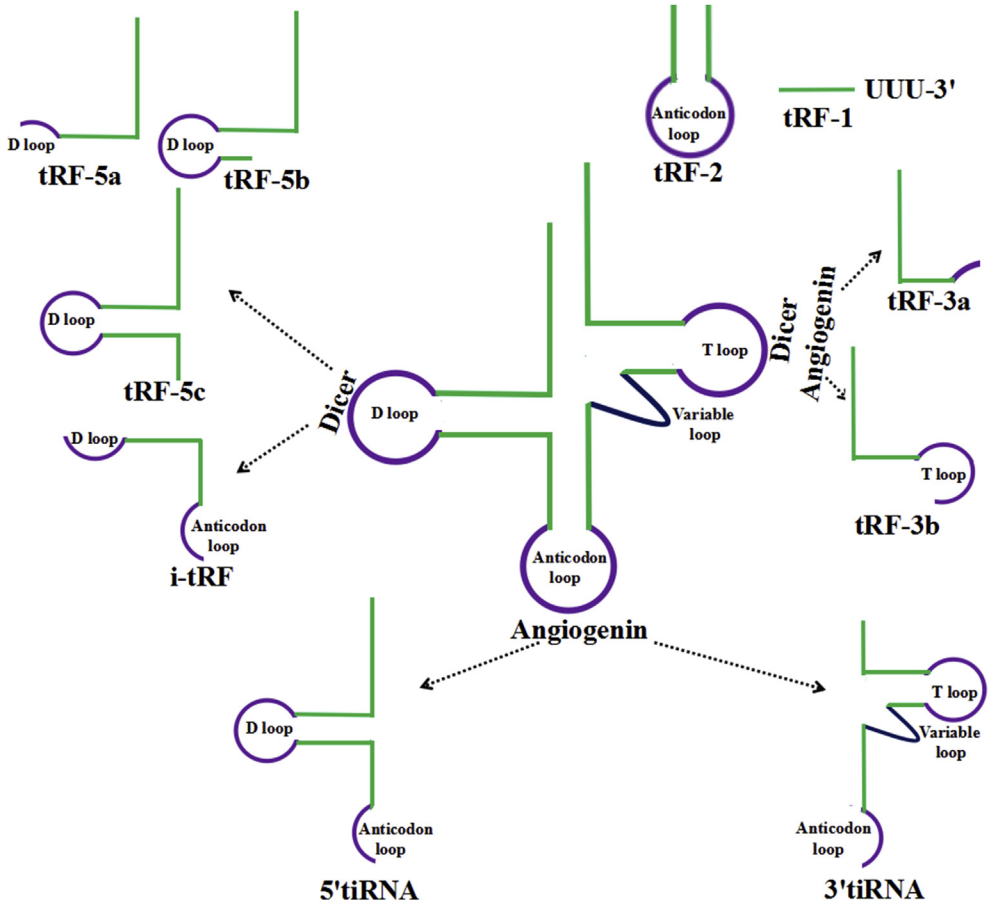

Fig. 1. Biogenesis and classification of tRFs and tiRNAs. tRF-1 is generated from precursor tRNA and cleaved by RNase Z or ELAC2 at 3′ trailer. tRF-2 is a tRNA fragment containing an anti-codon loop generated by an unknown cleavage method. tRF-3 and tRF-5 are generated from the 3′ and 5′ ends of the mature tRNAs, respectively. i-tRF is mainly from the internal region of mature tRNA. tiRNAs are generated by specific cleavage by angiogenin in the anticodon loops of mature tRNAs.

contains a CCA structure specific for the 3′ end of the mature tRNA. The major members of the tRF-3 are tRF-3a and tRF-3b, approximately 18–22 nt in length [10,14]. tRF-5, derived from the 5′-end of tRNA, is decomposed at D-ring. tRF-5 produced by Dicer cleavage at the D-loop of tRNA and is less than 30 nt in length. tRF-5 may further be divided into three sub-subcategories: tRF-5a, tRF-5b, and tRF-5c. i-tRF is mainly from the internal region of mature tRNA.

Besides the above-mentioned tRFs and tiRNAs, other tRNA fragments can also be seen by high-throughput sequencing. The diversity of tRFs and tiRNAs is found to be more diverse than existing classifications [15]. In addition, the rules of nomenclature for tRFs and tiRNAs are inconsistent. Sometimes, researchers name their tRFs and tiRNAs according to their own habits. Thus, the nomenclature of tRFs and tiRNAs is not yet unified.

In certain tissue cells, and even tumor cells, more and more mature tRNAs are found to be the sources of miRNA precursors [16]. tRFs and tiRNAs have different structures. Because tiRNAs are produced by the cleavage of ribonuclease ANG, they have no 5′ phosphate group [10,11]. tRFs have a 5′ phosphate and a 3′ hydroxyl group similar to miRNAs [13].

3. Biological roles and mechanisms of tRFs and tiRNAs in cancers

Studies have shown that the levels of tiRNAs are significantly elevated under stress conditions, such as hypoxia and sex hormone stimulation [10]. Therefore, in recent years, the mechanisms underlying tRFs and tiRNAs regulating cancer occurrence have received attention (Fig. 2).

First, tRFs and tiRNAs have been found to be important regulators of rRNA and protein biogenesis [17]. Kim et al. found that by binding two or more ribosomal proteins (RPS28 and RPS15), the specific $tsRNA^{Leu-CAG}$ 3′tsRNA promoted its target mRNA translation [18], thereby promoting cancer cell proliferation. Specifically, inhibition of $tsRNA^{Leu-CAG}$ 3′tsRNA induced the apoptosis of hepatocellular carcinoma cells. Inhibition of RPS28 mRNA translation halts processing of 18S pre-ribosomal RNA, resulting in a decrease in a number of the 40S ribosomal subunits [19]. In cancer cells, by binding with Argonaute (Ago), some tRFs exert their gene silencing roles by acting as miRNAs (Fig. 2A). These explain post-transcriptional mechanisms that can regulate gene expression during different life-activity states, providing new opportunities for cancer treatment. It is currently believed that tRFs and tiRNAs regulate translation at different levels depending on cell status or their subtypes [19].

Second, tRFs and tiRNAs regulate gene expression by combining with RNA binding proteins (RBPs). The first RBP atlas discovered by Castello et al. showed that there are at least 300 RBPs in the biological world, about half of which are encoded by known mutations in cancers or diseases [20]. It is speculated that the occurrence of some cancers or diseases is not mainly related to the loss of known-proteins' functions, but to the potential functions of these RBPs [20]. Goodarzi et al. found that tRFs derived from $tRNA^{Asp}$, $tRNA^{Glu}$, $tRNA^{Tyr}$, and $tRNA^{Gly}$ and some endogenous oncogene transcripts competed binding with Y-box binding protein 1 (YBX1) in breast cancer cells [21]. YBX1 is an RNA binding protein with a variety of biological functions. By binding with some endogenous oncogene transcripts, YBX1 maintains their stability and thus increases cell proliferation (Fig. 2B), whereas YBX1 inactivation leads to cell death. When the YBX1-oncogene transcript complex is dissociated, the stability of oncogene transcripts is disturbed. At this time, the expression of oncogenes is reduced, and then, the growth of cancer cells is inhibited [21]. YBX1 can bind several types of transcripts including tRFs and tiRNAs. As a result, under stress or other stress conditions, cancer cells produced more tRFs and tiRNAs that will

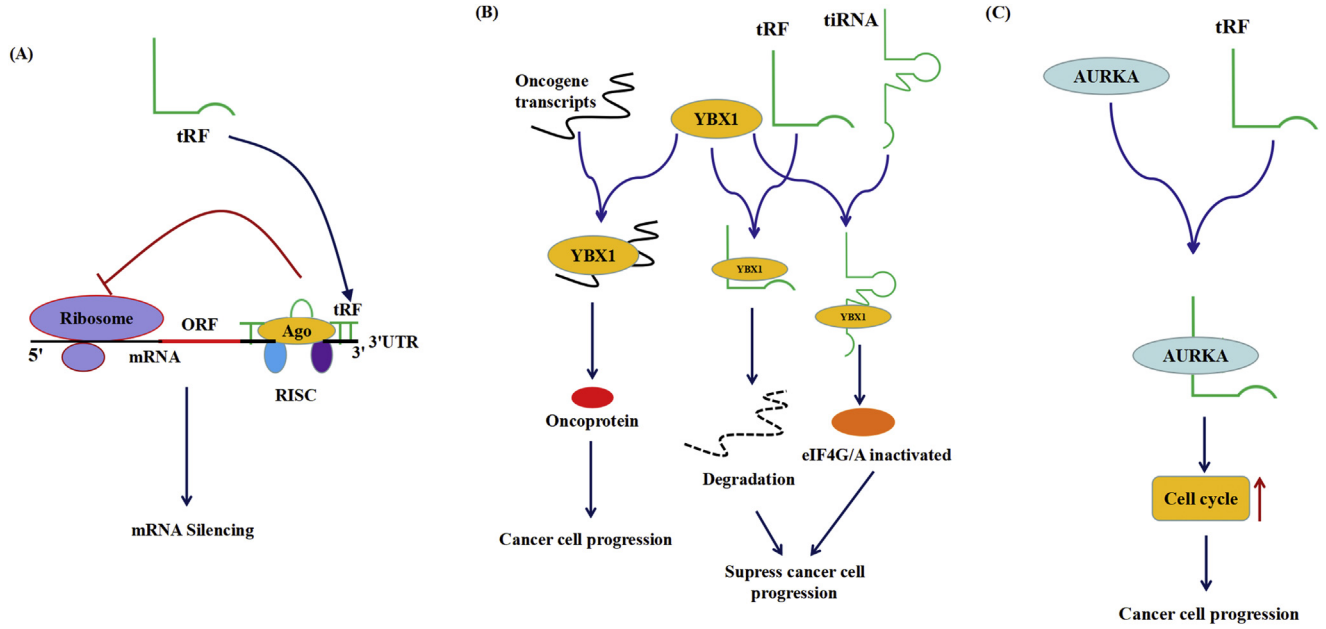

Fig. 2. Biological roles of tRFs and tiRNAs in cancers. (A) tRFs function like miRNAs to inhibit cancer-associated gene expression. Binding with 3′ untranslated region (3′UTR) of target mRNA, Argonaute (Ago) protein and other proteins form an RNA-induced silencing complex (RISC). Then, mRNA expression is suppressed. ORF, open reading frame. (B) tRFs or tiRNAs compete with endogenous oncogene transcripts for the binding of YBX1 that causes oncogene transcripts degradation or inactivates eIF4G/A, thereby inhibiting the oncogene expression in posttranscriptional or translational levels. (C) tRFs regulate aurora kinase A (AURKA) activity and then promote cell cycle progression and cell growth.

compete with oncogene transcripts and then bind with YBX1 (Fig. 2B). A study has shown that the binding of tRFs with YBX1 stimulated the proliferation of breast cancer cells [22]. In addition, the binding of tiRNA to YBX1 inactivates translation initiation factor eIF4G/A and thus inhibits the initiation of translation of several oncoproteins (Fig. 2B) [23]. Studies have shown that 5′tiRNAs from $tRNA^{Ala}$ and $tRNA^{Cys}$ assemble into a G-quadruplex-like structure (G4-motif) that competitively binds to eIF4G/A in translation initiation complexes [23,24]. They do not inhibit internal ribosome entry site (IRES)-mediated translation but inhibit the translation of cap-dependent mRNA, allowing cells to resist apoptosis and survival [23,24].

Third, tRFs and tiRNAs regulate kinase activity. Shao et al. found that $tRF^{Leu-CAG}$ regulated cell proliferation and cell cycle progression in non-small cell lung cancer (NSCLC) [25]. When the expression of $tRF^{Leu-CAG}$ was knocked down, the expression of aurora kinase A (AURKA) was inhibited [25]. AURKA, a serine-threonine kinase, plays important roles in mitosis. It is associated with centrosome maturation and separation and thereby regulates spindle assembly and stability. Previous studies have shown that miR-137 and miR-32 bind directly to AURKA and affect the progression of NSCLC [26,27]. Overexpression of $tRF^{Leu-CAG}$ increases the activity of AURKA and then promotes cell cycle progression at the G0/G1 phase in NSCLC [25]. This means that $tRF^{Leu-CAG}$ regulates cell cycle progression by regulating AURKA activity (Fig. 2C).

Besides above cancer-related mechanisms, tRFs and tiRNAs can also influence the development of cancers by affecting mRNA stability, regulating reverse transcription, inhibiting translation, and regulating ribosomal biogenesis [28]. tRFs and tiRNAs can influence the development of cancer by regulating cell proliferation, cell cycle, apoptosis, and migration [29]. They may regulate the expression of endogenous target genes and affect translation efficiency [13,30,31]. tRFs and tiRNAs also bind to Ago and Piwi complexes, suggesting that these tRFs and tiRNAs can function as miRNAs or Piwi-interacting RNAs (piRNAs), respectively [27,32,33]. Although this means that the mechanisms underlying tRFs and tiRNAs in cancers are more than the above-mentioned aspects, more detailed mechanisms regarding tRFs and tiRNAs in cancers need further study.

4. Dysregulation of tRFs and tiRNAs in cancers

How tRFs and tiRNAs are dysregulated in cancers? One of the causes is the dysregulation of precursor tRNAs in cancers. The dysregulation of tRNAs in cancers may result in the dysregulations of tRFs and tiRNAs in cancers. Transcription of tRNA by RNA Pol III is affected by proto-oncogenes and tumor suppressor genes, which mainly impacted the subunit Brf1 of TFIII B factor in Pol III, thus promoting or suppressing its function [19,34]. In estrogen receptor (ER)-positive breast cancer, the interaction of estrogen receptor α (ERα) with Brf1 mainly regulate the transcription of the Pol III genes—particularly $tRNA^{Leu}$ [34]. A significantly positive correlation between the expression of telomerase reverse transcriptase (TERT) and pretranscripts of $tRNA^{Tyr}$ and $tRNA^{Leu}$ has been found in some cancers [19]. Loss of TERT was related to reduced polyomavirus middle T oncogene-induced (PyMT-induced) mammary tumorigenesis and expression of pre-$tRNA^{Tyr}$ [35].

Sex hormone sensitivity and hormone receptor expression may be the main cause of tRFs and tiRNAs disorders in hormone-dependent cancers [29]. tRF-1 (ts-66) is dysregulated in prostate androgen receptor (AR) late-negative cell lines compared to AR-positive early and normal cells [36]. Alternatively, SHOT-RNA expression can be modulated by the changes in tRNA 5-methylcytidine (m5C) modification induced by the changes in DNA methyltransferase 2 (Dnmt2) and NOP2/Sun RNA methyltransferase 2 (NSun2) levels [4].

The proto-oncogenes AURKA and $tRF^{Leu-CAG}$ in cancer cells promotes the expression of each other [19,25]. When oncogene activation or tumor suppressor inactivation, the expression of tsRNAs may also be dysregulated [19,36].

Besides, the activation of ANG in cancers contributes the produce of tiRNAs. For example, in ER-positive breast cancer, sex hormones and their receptors promote the ANG cleavage of mature tRNA anticodon loops to produce a large number of tiRNAs [4].

5. tRFs and tiRNAs in common cancers

5.1. tRFs and tiRNAs in lung cancer

Lung cancer is the most common cancer in the world. Recent studies found that tRFs and tiRNAs are associated with the occurrence of lung cancer [25,27,36]. Pekarskya et al. found that ts-4521 and ts-3676, which are derived from $tRNA^{Ser}$ and $tRNA^{Thr}$, respectively, can not only interact with Ago1 and Ago2 proteins but also interact with Piwi-like protein 2 (Piwil2) as piRNA [27]. Moreover, the expression levels of these two tsRNAs are significantly downregulated in lung cancer tissue samples compared to matched normal lung tissue samples [27]. In addition, down-regulation of ts-4521 is associated with cell proliferation and apoptosis-related signaling pathways [36]. Balatti et al. also confirmed that the overexpression of ts-46 and ts-47 revealed a significant decrease in colony formation rate in lung cancer cells [36]. This means that tsRNAs are involved in the carcinogenesis of lung cancer.

In addition, as mentioned above, the overexpression of $tRNA^{Leu-CAG}$ derived tRF can promote cell proliferation and cell cycle progression and thus contributes to the progression of NSCLC [25].

5.2. tRFs and tiRNAs in colorectal cancer

Colorectal cancer is the third most common cancer in the Western world and the incidence increases with increasing age. Huang et al. observed that tRF/miR-1280, a 17 nt of $tRNA^{Leu}$ fragment and fragment of pre-miRNA, was involved in the Notch signaling pathway and promoted the function of cancer stem-like cells (CSCs) in the development of colorectal cancer [37]. As we know, the Notch signaling pathway is closely related to several cancer cell characteristics, such as proliferation, metastasis, and stem cell-like phenotype [38–40]. Low expression of tRF/miR-1280 inhibits colorectal cancer cell proliferation and colony formation [37]. Further mechanism studies found that the ligand jagged 2 (JAG2) of the Notch signaling pathway directly binds to tRF/miR-1280, thereby reducing the formation and metastasis of tumor cells [41]. These findings indicate the presence of a mutual activation of the Notch signaling pathway regulated by JAG2 or tRF/miR-1280. More importantly, acting as a bridge by tRF/miR-1280, the inactivation of Notch signaling inhibits the CSC phenotype through transcriptional repression of the miR-200b and Gata1/3 genes. Previous experimental results indicated the decreased levels of miR-200b and elevated levels of JAG2, Zeb1, Suz12, Gata1, and Gata3 in colorectal cancer tissue samples [37,42]. The results of these findings explain that the aberrant Notch signaling pathway can be driven by tRFs.

5.3. tRFs and tiRNAs in prostate cancer

Prostate cancer is the sixth most common type of neoplasm in the world and the second in prevalence among men (10% of all cases). Olvedy et al. first performed whole-genome sequencing of tRF expression levels in adjacent prostate cancer samples and samples at different stages of prostate cancer [5]. They found that the expression levels of many tRFs were different between adjacent tissues and cancer tissues [5]. Most tRFs are derived from the 5′ and 3′ ends of cytoplasmic mature tRNAs. A weak correlation between the use of codons in each tRNA and the expression of the corresponding tRF suggests that the levels of tRF expression are dependent on its precursor and that the expression levels of tRF in cells are most likely through special mechanisms [43]. The most abundant tRFs in prostate cancer cells are tRF-5s, and most of them are upregulated [5]. Most of tRF-3 are downregulated in prostate cancer [5]. The ratio of tRF from $tRNA^{Lys-CTT}$ and $tRNA^{Phe-GAA}$ was found to be a good indicator of biomarkers and progression-free survival [5].

tRF-1001 is related to prostate cancer cell proliferative capacity. A study has shown that silencing the expression of tRF-1001 arrested prostate cancer cells at G2 phase, inhibited DNA synthesis, and decreased cell viability and cell proliferation [35]. In addition, sequencing of whole-genome transcriptomes of small RNA in prostate cancer tissues and metastatic lymph nodes revealed that tRFs are enriched in all lymph nodes tested [35]. Martens-Uzunova et al. found that the length of tRFs in non-metastatic and metastatic prostate cancer samples were 18 and 27 nt, respectively [44]. This means that the cleavage of precursor tRNAs in prostate cancer is different from that in normal tissues.

Magee et al. analyzed the prostate cancer dataset (PRAD) from the Cancer Genome Atlas (TCGA) and found a large number of PRAD-associated tRFs [45]. Moreover, the authors found that the expression of tRFs was different in patients of different races [45].

As we know, prostate cancer is a sex hormone-associated cancer. Honda et al. observed that a hormone-dependent tsRNA was enriched in prostate cancer cells and enhanced the proliferative capacity of prostate cancer cells [4].

5.4. tRFs and tiRNAs in breast cancer

Breast cancer is another sex hormone-associated cancer. Honda et al. found that tiRNAs were induced by sex hormone in estrogen receptor (ER)-positive breast cancer cells and ER receptor promoting ANG cleavage of the anticodon loop of mature tRNAs [4]. Moreover, the expression of 5′-$tiRNA^{Asp}$ and 5′-$tiRNA^{His}$ was highly expressed in breast cancer tissues or cells compared with that in the normal control group. The experiments also showed that the expression of silencing 5′-tiRNA affected cell proliferation [4]. These results indicate that tiRNAs play an important role in the tumorigenesis of breast cancer.

In breast cancer, tsRNAs are found regulated in oncogene regulatory pathways. Balatti et al. performed a statistical analysis of the oncogenes of normal breast epithelial cells with oncogene activating mutations and at different developmental stage of breast cancer cells and found that oncogenes could regulate the expression of tsRNAs [36]. Besides, tsRNAs are expressed at certain stages of carcinogenesis. For example, ts-3 is significantly downregulated in advanced invasive breast cancer cells, but ts-6, ts-48, and ts-67 are upregulated in advanced cancer cell lines [36].

For the mechanism study, Goodarzi et al. observed that as competitive endogenous oncogene transcripts, tRFs derived from $tRNA^{Gly}$, $tRNA^{Asp}$, $tRNA^{Tyr}$, and $tRNA^{Glu}$ bound with YBX1 and destroyed the stability of oncogene transcripts, thereby inhibiting the development of breast cancer [21]. This means that some tRFs participate in post-transcriptional gene expression regulation.

Various epigenetic modifications of RNA may also play an important role in the function of tRFs. There are many epigenetic modifications in tRNAs and their breakdown products. These epigenetic modifications play an important role in regulating the function of endogenous tRFs [21]. The efficient actions of endogenous tRFs can be partially explained by the presence of RNA modifications that affect the structure, specificity, and stability of tRFs. In addition, the combination of tiRNA and YBX1 may stop the translation [23].

5.5. tRFs and tiRNAs in ovarian cancer

Besides breast cancer, the phenomenon that tRFs regulate gene expression is also observed in ovarian cancer. Zhou et al. demonstrated that $tRF5^{Glu}$ directly bound to the 3′ untranslated region (UTR) of breast cancer anti-estrogen resistance 3 (BCAR3) mRNA, thereby reducing the expression level of BCAR3 [46]. It is reported that BCAR3 is one of the players that have a role in ovarian cancer development [46]. Therefore, $tRF5^{Glu}$ affects the proliferative capacity of ovarian cancer cells by regulating the expression of BCAR3. These biological functions of tRFs are Ago-dependent. The direct binding of $tRF5^{Glu}$ to BCAR3 mRNA is confirmed by the method of improving miR-Catch and using the dual luciferase reporter gene assay [46]. Kumar et al. analyzed tRFs in AGO-CLIP dataset and found that tRFs, which were associated with AGO

proteins, recognized specific RNA targets [47]. Helwak et al. further found that tRF5^{Glu} and miR-2476 bound to mRNA in an AGO1-dependent manner [48]. These results suggest a novel mechanism for tRFs' regulating gene expression (Fig. 2A).

5.6. tRFs and tiRNAs in B-cell lymphoma

The B-cell lymphomas are types of lymphoma affecting B cells. They develop more frequently in older adults and in immunocompromised individuals. Maute et al. demonstrated a tRF called CU1276 with miRNA function [33]. The main biological functions of CU1276 include Dicer1-dependent biological production, binding to Ago protein, and inhibition of mRNA transcription by specific sequence [33]. In addition, CU1276 inhibits the endogenous essential gene replication protein A1 (RPA1) involved in DNA kinetics [33]. RPA1 is an essential gene for DNA kinetics, such as genomic replication [49]. Thus, stably expressed CU1276 inhibits cell proliferation in an RPA1-dependent manner in Burkitt lymphoma-derived cell lines. Lymphoma cell line and biopsy tissue examination reveal low expression of CU1276 [49]. Experimental results indicate that silencing of CU1276 and high expression of RPA1 protein levels can promote the growth of malignant cells [33]. Therefore, CU1276 inhibits the proliferation of lymphoma cells and regulates the response of molecules to DNA damage. CU1276 is a tRF-3 and has the biological function of miRNA. Interestingly, the authors observed that CU1276 was significantly down-expressed in germinal center (GC)-derived lymphoma relative to their source cells [33].

5.7. tRFs and tiRNAs in chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) affects white blood cells and tends to progress slowly over many years. It mostly affects people over the age of 60 and is rare in people under age 40 years. Pekarsky et al. found that ts-3676 and ts-4521 have mutations and significant down-regulation in CLL [27]. As we know, the Piwi/piRNA complex can regulate DNA methylation, and piRNAs and tsRNAs have some similarity. Pekarsky et al. investigated the interaction of ts-3676 and ts-4521 with Piwi proteins to form Piwi-complexes. They found that both ts-3676 and ts-4521 bound with Piwil2 [27]. To investigate whether more tsRNAs are involved in CLL, they performed a microarray analysis and found that 120 tsRNAs were associated with CLL [27].

Balatti et al. also found that ts-101, ts-53, ts-46, and ts-47 were underexpressed in CLL [36]. They also found that ts-101 and ts-53 bound to a silencing transposon protein Piwil2 [36]. Moreover, activated *Myc* proto-oncogene in lymphocytes inhibited ts-47 level [36]. In previous studies, ts-46 and ts-101 have been found to be involved in cancers [50]. It was found that ts-46 regulated cell proliferation by inhibiting the S1P/ceramide pathway [50]. In a ts-46 knockout (KO) experiment, integrin-linked kinase (ILK) signaling, which is involved in tumor proliferation and metastasis [51], was promoted. In addition, genes associated with chromatin morphology were silenced in ts-101 KO cells [36].

5.8. tRFs and tiRNAs in other cancers

As mentioned above, tRFs and tiRNAs are related to lung cancer, colorectal cancer, prostate cancer, breast cancer, ovarian cancer, B cell lymphoma, and CLL. Recent studies found that tRFs and tiRNAs are also found in other cancers (Table 1). Sobala et al. observed that a tRF from tRNA^{Gln} was highly expressed in cervical cancer cells [52]. Nientiedt et al. observed that compared with the control group, 5′tiRNA4$^{Val-AAC}$ was less expressed in clear cell renal cell carcinoma (ccRCC) tissues [53]. Martinez et al. revealed that three 5′tiRNA were significantly dysregulated in the serum of head and neck squamous cell carcinoma (HNSCC) patients compared to those of healthy controls [54]. Guo et al. performed high-throughput sequencing of some sncRNAs in patients with myelodysplastic syndrome and found that 6 tRFs were associated with treatment response [55]. Ruggero et al. isolated the virion of tRF-3019 from human T-cell leukemia virus type 1 (HTLV-1) infected cells, suggesting that tRF-3019 might play a role in HTLV-1 reverse transcription [56].

Table 1
tRFs and tiRNAs in cancers.

Cancer type	tRF/tiRNA	References
Lung cancer	ts-4521, ts-3676, ts-46, ts-47, tRF$^{Leu-CAG}$	[25,27,36]
Colorectal cancer	tRF/miR-1280	[37]
Prostate cancer	tRF$^{Lys-CTT}$, tRF$^{Phe-GAA}$, tRF-1001	[4,5,44,45]
Breast cancer	5′tiRNA^{Asp}, 5′tiRNA^{His}, ts-3,ts-6, ts-48, ts-67	[4,21,36]
Ovarian cancer	tRF5^{Glu}	[46]
B cell lymphoma	CU1276	[33]
Chronic lymphocytic leukemia	ts-3676, ts-4521, ts-46, ts-47, ts-53, ts-101	[27,36]
Lymphoma	tRF-3027	[13]
Cervical cancer	5′tRF, tRF(Gln)	[52]
Clear cell renal cell carcinoma	5′tRNA4$^{Val-AAC}$	[53]
Head and neck squamous cell carcinoma	5′tiRNA^{Ala}, 5′tiRNA^{Cys}, 5′tiRNA^{Tyr}	[54]
Myelodysplastic syndrome	chrM.tRNA10-TC, chr12.tRNA8-AlaTGC, chr16.tRNA4-ProAGG, chr1.tRNA58-LeuCAA, chr19.tRNA8-SeC(e)TCA(SeC(e)TCA, chr19.tRNA4-ThrA GT	[55]

6. tRFs and tiRNAs in body fluids and the applications

Through high-throughput sequencing technology, tRFs have been found in exosomes of semen [6]. Further studies found that tRFs and tiRNAs were highly enriched in body fluids, sometimes even higher than miRNAs did [7,8]. Dhahbi et al. found that tiRNAs were present as abundant complexes in serum [57]. Therefore, minimally invasive methods can be used in the detection of tRFs and tiRNAs from exosomes in body fluids from cancer patients [8]. tRFs and tiRNAs can also act as regulatory molecules to regulate signal transduction in body fluids from cancer patients [6]. Because tRFs and tiRNAs are widely involved in the pathogenesis of solid tumors and leukemia, the application of tRFs and tiRNAs-based non-invasive biomarkers in tumor diagnosis will have broad prospects.

7. Methods used for tRFs and tiRNAs detection

With the application and development of RNA high-throughput sequencing, more and more tiRNAs and tRFs have been discovered [13]. The high-throughput sequencing approaches enable the detection of more types of RNAs, such as tRNA, rRNA, snoRNA, and scaRNA. Because tRFs and tiRNAs are quite small (14–50 nt), how to distinguish bona fide tRFs from random degradation fragments should be first seriously considered. By designing specific amplification primers, tRFs and tiRNAs can be specifically detected by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) [5]. The expression of tRFs and tiRNAs can also be detected by Northern blot [28,29]. Recently, Mitochondrial and Nuclear TRF mapping (MINTmap; https://github.com/TJU-CMC-Org/MINTmap/), a method and a software package for the quick, deterministic, and exhaustive identification of tRFs in short RNA-seq datasets, have been developed [58]. In addition to identifying tRFs, MINTmap is able to unambiguously calculate and report both raw and normalized abundances for the discovered tRFs [58]. In the future, we believe that more tools and methods for the determination of tRFs and tiRNAs will be developed.

8. Conclusion and further perspectives

Animal models are promising tools for aiding in the discovery of novel non-coding RNAs and investigating the phenotypic significance of these RNAs. Kumar et al. found that tRFs and tiRNAs are precisely generated fragments in organisms from bacteria to humans [47]. Blanco et al. found that the consumption of NSUN2 resulted in the production of tRFs and tiRNAs being conserved between humans and other organisms [59]. Therefore, tRFs and tiRNAs are conserved between humans and other organisms.

In summary, with the development of high-throughput sequencing technology, more and more tRFs and tiRNAs have been identified. The roles and mechanisms of tRFs and tiRNAs in cancers will be elucidated.

Competing interests

None.

Acknowledgments

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