Am J Cancer Res 2023;13(5):1656-1666
www.ajcr.us /ISSN:2156-6976/ajcr0144077

Review Article

The biogenesis, mechanism and function of the tRNA-derived small RNA (tsRNA): a review compared with microRNA

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Received May 11, 2022; Accepted July 30, 2022; Epub May 15, 2023; Published May 30, 2023

Abstract: Since the discovery of the first miRNA in 1993, numerous studies have focused on their biogenesis, their functions on regulating a diversity of cellular processes, and the molecular mechanisms underlying their regulatory activity. The critical roles they play during pathogenesis have also been explored. With the advancement on next-generation sequencing, new classes of small RNA with distinct functions have been discovered. Among them, tRNA derived fragment (tsRNAs) have become a center of studies due to their similarity to miRNA. In this review, we summarized the biogenesis of miRNA and tsRNAs, the molecular mechanisms of their functions, and their important roles during the development of diseases. The similarity and difference between miRNA and tsRNAs were also discussed.

Keywords: ncRNA, tsRNA, miRNA, gene silencing, cancer

Introduction

Although protein-coding DNA comprises only 2% of the total DNA content, the ENCODE project reveals that at least 80% of the human genome has biological activity [1, 2]. RNAs can be divided into coding RNAs (mRNAs) and non-coding RNAs (ncRNAs). ncRNAs are composed of several types of functional molecules (e.g, rRNA, tRNA, small nucleolar RNA [snoRNA] and microRNA [miRNA]) that is widely regarded as a key regulator of gene expression. miRNA is one of the most prominent and well-studied ncRNA, and what's more miRNA regulates up to two-thirds of human genes [3]. miRNAs are evolutionary conserved and characterized by a single-stranded RNA molecule of 21-23 nucleotides (nt). Currently, mounting data have provided evidence for their causal involvement in various diseases.

tsRNAs, a family of specific and regulable fragments derived from tRNA, are initially misunderstood as merely a byproduct of the random degradation of tRNA since the discovery of the first tsRNAs in the 1970s [4]. However, increasing evidence supports the notion that tsRNAs are also evolutionary conserved and play diverse roles across a variety of regulatory pathways [5]. Generally, tsRNAs can be classified into two species according to the length and the cleavage site of tRNAs. One species is the tRNA halves (also known as stress-induced tRNA fragments or tiRNAs) produced by cleaving at the anti-codon loop of the mature tRNA that are 28-36 nt long. The other species is the tRNA-derived fragments (also known as tRF) of 14-30 nt that result from a cleavage at any site from the mature or precursor tRNA loops [6]. Within tRFs, three main types of tRFs have been characterized according to their mapped positions: tRF-5, tRF-3, and tRF-1. tRF-5s are produced by cutting in the D-loop or the region between the D-loop and the anticodon loop; They can be further subdivided into tRF-5a (14-16 nt), tRF-5b (22-24 nt), and tRF-5c (28-30 nt). The cleavage sites of tRF-3s are in the TΨC loop, and the length of tRF-3s is ~18 or ~22

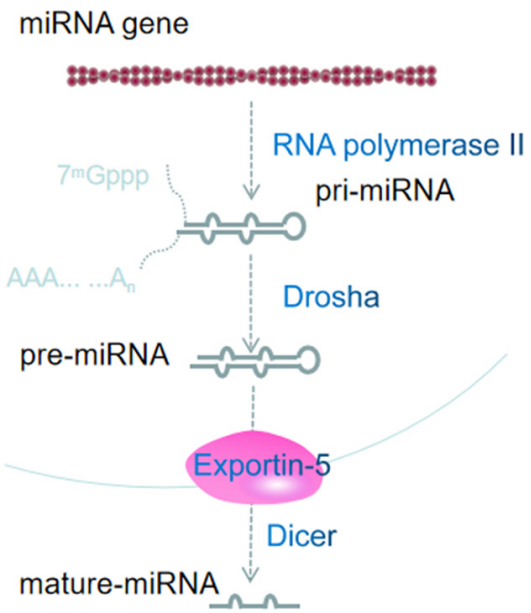

Figure 1. Biogenesis of the mature miRNA. The biogenesis of miRNAs begins with their transcription from miRNA genes in the nucleus, transcripted into pri-miRNAs by RNA polymerase II. The resulting pri-miRNAs is then processed into pre-miRNA by the RNase III enzyme Drosha and its dsRNA-binding domain (dsRBD), DGCR8. Next, the mature miRNAs can arise from the stem of the pre-miRNA by the Dicer after be exported to the cytoplasm by XPO5.

bases long. The biogenesis of tRF-1 is from the 3' end of the primary tRNAs which is cleaved by RNase Z or ribonuclease Z 2 (ELAC2) [7]. With the advancement on high-throughput sequencing, new types of tsRNA are continuing to be discovered, and the rules that guide their categorization are improving too.

Since miRNAs and tsRNAs share many common properties, some miRNAs are described as the derivatives of tRNAs, similar as tsRNA. However, in this review, we summarized the recent advances in the understanding of of miRNAs and tsRNAs, not only on their similarity but also on their unique features with a focus on their biogenesis, the mechanism of their functions, and their regulatory roles in disease.

Biogenesis

Dicer mediates cleavage

The biogenesis of miRNAs begins with their transcription from miRNA genes in the nucleus, the production of the nascent transcripts called primary miRNAs (pri-miRNAs). Most miRNA genes are present as a cluster of long transcriptional unit. which sometimes is considered as a family. It is quite common for a family of miRNAs to possess a similar seed sequence as short as 2-7 nt nucleotides, allowing them to have overlapping targets [8]. In addition, the alternative splicing of certain transcripts elicits the miRNA to be either an exon or an intron. More than 70% of miRNAs reside in introns in mammalian cells. If the pri-miRNAs are polycistronic, the expression of the clustered miRNA genes is under the control of a single promoter; otherwise each miRNA gene in the cluster has its own promoter but most likely shares the same enhancer [9]. Approximately one-third of intronic miRNAs and their host genes are expressed in an uncoupled manner [10].

The resulting pri-miRNAs receives 3' polyadenylated tails and 5' methylated caps to generate a stem-loop structure with some bulges. In some cases, the promoters may contain TATA boxes and some other features typical of Pol II promoters. The pri-miRNA is then processed into a 60- to 70-nucleotide precursor form (pre-miRNA) by the RNase III enzyme Drosha and its dsRNA-binding domain (dsRBD), DGCR8, in the nucleus [11]; Drosha and DGCR8 composed a microprocessor, which is necessary and sufficient for the processing of pri-miRNA into pre-miRNA. When either of these two proteins is depleted, the levels of pre-miRNA and mature miRNA are reduced [12]. Since the complex may only consist of Drosha and DGCR8, which suggests that more than one copy of these two components should exists [13].

Pre-miRNAs must be exported to the cytoplasm by exportin 5 (XPO5) where they are processed into a duplex strand by the RNase III enzyme Dicer (**Figure 1**). XPO5 binds to the transport substrates of pre-miRNAs in a Ran-GTP-dependent manner. A recent study has discovered the role of XPO5 in balancing pre-miRNA levels by its ability to prevent the degradation of pre-miRNA. In addition, XPO5 can regulate the step of the precursor to the mature form by transporting Dicer mRNA. Loss of XPO5 leads to a reduction in the Dicer protein and the accumulation of Dicer mRNA in a post-transcriptional regulation manner [14]. Dicer is a superfamily of enzymes that is responsible for recognizing and cleaving long dsRNA into 22 nt miRNAs, Structurally, dicer includes two RNase III domains as well as PAZ, helicase, and dsRNA

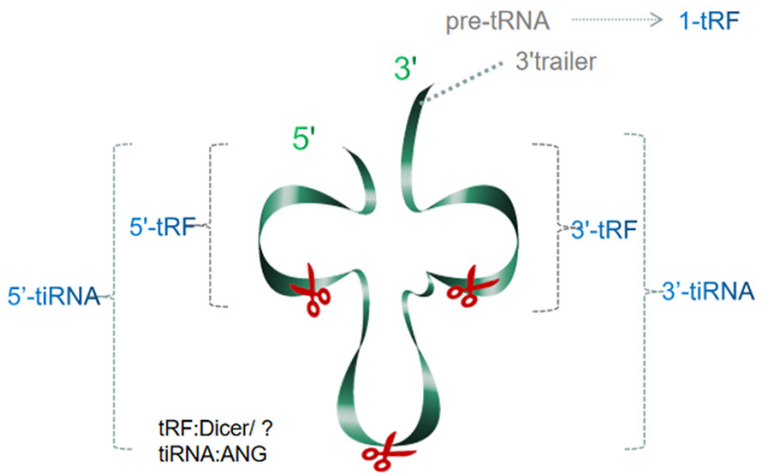

Figure 2. Biogenesis of the tsRNAs. tiRNAs also called tRNA halves, mainly produced by cleaving at the anti-codon loop of the mature tRNA by Ang protein. The produced tiRNA may directly lead to translation repression. The other species is the tRNA-derived frag-ments (also known as tRF) result from a cleavage at any site from the mature or precursor tRNA loops. It is found that Dicer, Ang and ELAC2 may involved in the production of tRFs.4.

binding domain. After the pre-miRNA is loaded into the Dicer protein, cleavage can occur to generate a two-stranded duplex. The cleavages can be influenced by the loop and the secondary structure of miRNA [15]. Next, miRNAs can arise from the stem of the pre-miRNA, or the mature guide strand can be deposited into an miRISC while the remaining, or passenger, strand (miRNA*) is discarded; the latter strand is rarely detected but, on occasions, can be used to form a functional RISC [16]. Argonaute (Ago) proteins, as one of the most important components of RISC, play vital roles in binding to miRNA and releasing Dicer. The structure of miRNA shows a specific affinity, among the different types of Ago proteins, to Ago2, among the different types of Ago proteins.

tRFs have become the center of some studies recently because of their similarities to miRNA in size and function. Since they may have 5' phosphates and 3' hydroxyls at the cleavage sites, similar as miRNA, tRFs may be processed in a Dicer-dependent manner as well [17]. tsRNAs have been found to be sensitive to AlkB and T4PNK, suggesting that some specific tsRNAs may have the 3'-P and 2'3'-cP termini [18]. tRF-3 starts by cleavage at the T-loop and ends at the 3' end of mature tRNAs; tRF-5 end before the anticodon loop but starts at the 5' ends of mature tRNAs (**Figure 2**). In contrast, other studies have shown that the tRF abundance is not decreased in Dicer knockout cells compared with wild-type cells, suggesting that Dicer is not essential for releasing tRFs from mature tRNA.

Another intriguing tRNA fragment, tRF-1001, is derived from a 3' trailer of tRNAser and is generated in the cytoplasm by ElaC ribonuclease Z 2 (ELAC2) during tRNA maturation [19]. Thus, some enzymes that may be involved in the biogenesis of tRFs remains to be identified.

Angiogenin (Ang) mediates cleavage

Ang is known to promote new blood vessel growth, making it a potential biomarker of cancer. It has also been characterized by its ability to promote protein synthesis and cell growth [20]. However, under stress situations, Ang can cleave tRNA into tiRNAs, which may directly lead to translation repression. As reported by Zhangli et al. [21] when the authors performed a global short RNA-seq, they found that ANG selectively cleaved tRNA to produce tiRNAs and long (26-30 nt) tRF-5. These contradictory findings could be explained by the differential subcellular localization of Ang.

Under growth conditions, Ang is trapped by ribonuclease inhibitor 1 (RNH1) in the nucleus; whereas, in the presence of stress (e.g., oxidative stress, heat shock, arsenite, ultraviolet irradiation), it mobilizes to the cytosol, where it promotes tRNA cleavage and stress granule (SG) formation [22]. Ribonuclease Rny1 has also been reported to be involved in this cleavage, and both molecules belong to the RNaz T2 family. In addition, careful studies have suggested the concept of cutting in the anticodon loop needs to be further validated as only 0.04% of tRNA cutting happens in the anticodon, while 55% of the tiRNA sequence ends just before the anticodon [23]. In human cells, the incidence of stress-induced tRNA cleavage is less than 1%, and certain tRNA sequences are more susceptible to this cleavage [24].

Data have suggested that Ang-associated tRNA cleavage can happen between any purine and

pyrimidine dinucleotides but with different efficiencies (CpA>CpG>UpA>UpG). Rny1, as another RNase, usually has no sequence specificity [25]. In addition, since more than half of the RNA modifications occur in tRNA species, these modifications can promote or restrict tRNA cleavage under different conditions. One of the best studied tRNA modifications is cytosine-5 methylation (m^5C). In eukaryotes, m^5C is primarily mediated by NSUN2 or DNMT2. NSUN2, as with DNMT2, are considered as tRNA cleavage protective markers through their methyl-transferase activity.

Under stress conditions, both enzymes localize to the cytosolic stress granules, and the m^5C deposition by either NSUN2 or DNMT2 may render cells more sensitive to stress. Indeed, tRNAs lacking NSUN2 or DNMT2 have decreased m^5C methylation and increased affinities for Ang, leading to more tRNA cleavage and the accumulation of tiRNA [2].

Mechanism of action

Transcriptional repression

miRNAs, as the most prominent and well-studied class of ncRNAs is miRNA, which have central roles in gene silencing and function post-transcriptionally. One mode of the function of miRNAs is to destabilize mRNA and inhibit translation by direct binding to the 3'UTRs of specific mRNAs [26]. This expressional repression can proceed at the initiation or elongation process of translation. miRNA exists in a ribonucleoprotein (RNP) complex called the RNA-induced silencing complex (RISC), which provides a sequence-specific binding component of approximately seven nucleotides in length to allow the AGO protein to cleave the bound mRNA or reduce the translation efficiency. In animals, miRNA interacts with target mRNA through partially complementary base pairing, unlike miRNA in plants, where miRNA needs perfectly complementary binding [27]. The canonical "seed" sequence is usually located at the 5' end of the miRNA and consists of an A at position 1 and complementary bases over the following seven nucleotides. Within a seed sequence, complementarity of 2-7 nt is not sufficient for target repression, however, adding another complementary base at position 8 can decrease target expression [28].

tRF is a class of tRNA fragments with a biogenesis pathway distinct from miRNA; however, the abundance of tRF is similar to miRNAs, and tFR exhibits some miRNA-like functions at least in some cases [29]. A recent study has suggested that tRF produced from Dicer-like 1 (DCL1) processing can specifically target mRNA derived from the transcriptionally active transposable elements in plants [30]. miRNA can bind to all four Ago proteins to exert an RNA silencing function. However, human photoactivatable-ribonucleoside-enhanced crosslink and immunoprecipitation data have shown that most tRFs preferentially associate with Ago1, Ago3, and Ago4 proteins rather than Ago2 (**Figure 3A**). Analysis of positional T to C mutational frequency has elucidated that the interaction method of Ago family is similar [6]. Interestingly, 1-tRFSer-TGA derived from pre-tRNASer-TGA can interact with Ago3 and Ago4 but does not exert a gene silencing function in an miRNA- or siRNA-like manner. miRNA physically obstructs translation by binding to complementary mRNA sequences. A recent study showed that tRF-5 could repress the expression of some reporter genes without the need of a complementary target site, suggesting that, in addition to binding to the Ago protein, tRF-5 could also bind to other components of RNAi machinery to suppress the activity of transcription factors or degrade the matrix. Another well-described study has shown that tRF regulates gene expression of genetic information at either the transcriptional or the translational level and which depends on the complementarity between the tRF and the transcript. In addition, tRFs can competitively bind to YB-1 and displace several oncogenic transcripts from YB-1 (**Figure 3B**), thereby suppressing their stability and expression and stability, leading to the inhibition of the metastatic progression of cancer cells [31]. Taken together, these data showed that tRFs could regulate gene expression through canonical or noncanonical miRNA pathways.

Buffer against stress conditions

Translational control of gene expression in eukaryotic cells is important for cell survival, which can happen at the initiation, elongation, termination, and ribosome recycling stage. This process is under tight regulatory control. Translation initiation is the most vulnerable

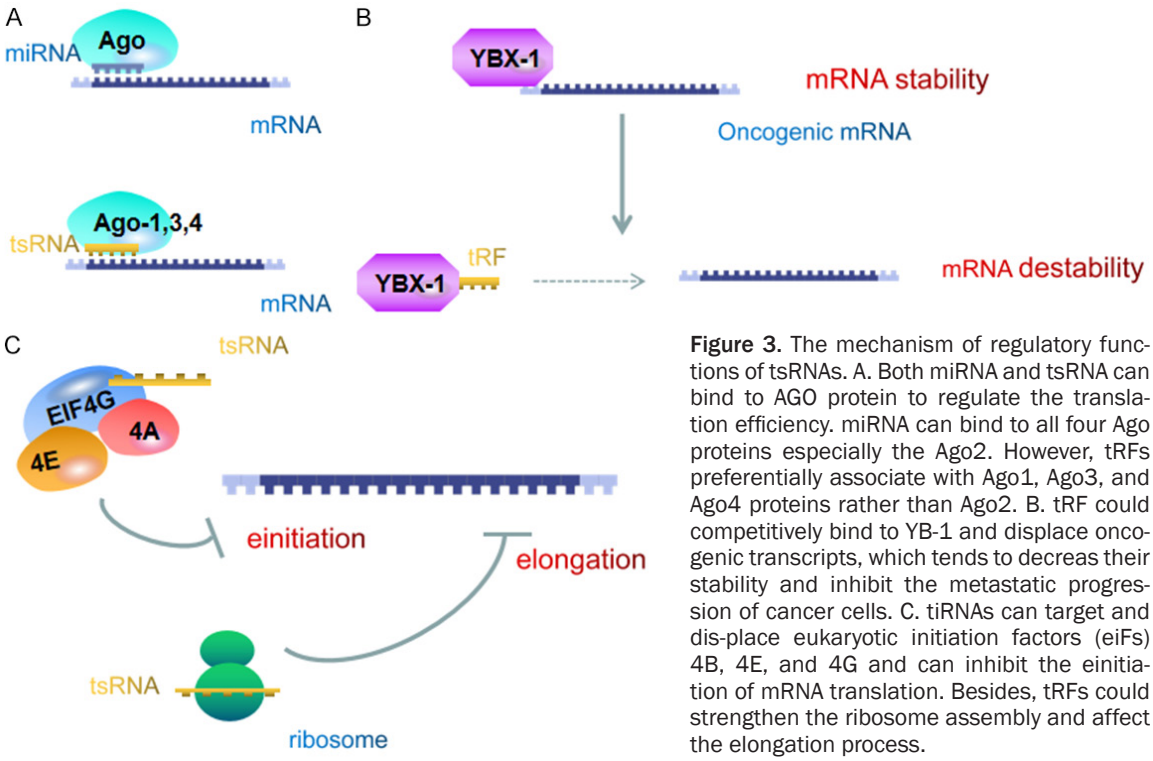

Figure 3. The mechanism of regulatory functions of tsRNAs. A. Both miRNA and tsRNA can bind to AGO protein to regulate the translation efficiency. miRNA can bind to all four Ago proteins especially the Ago2. However, tRFs preferentially associate with Ago1, Ago3, and Ago4 proteins rather than Ago2. B. tRF could competitively bind to YB-1 and displace oncogenic transcripts, which tends to decreas their stability and inhibit the metastatic progression of cancer cells. C. tiRNAs can target and dis-place eukaryotic initiation factors (eiFs) 4B, 4E, and 4G and can inhibit the einitiation of mRNA translation. Besides, tRFs could strengthen the ribosome assembly and affect the elongation process.

target under cellular stress [32]. The cap-dependent translation initiation is coordinated by interactions between initiation factors (eIFs) called eIF4F and mRNA at the 5' end and poly(A)-binding protein (PABP) at the 3' poly(A) tail. The recruitment of eIF4F to the 5' cap of mRNA is controlled by the mechanistic target of rapamycin (mTOR) activity. Under metabolic stress conditions, the inactivation of mTOR results in the underphosphorylation of eIF4E-binding protein (4E-BP), which can elicit more binding of eIF4E to 4E-BP thereby inhibiting translation. In eukaryotes, a ternary complex composed of eIF2, GTP, and $tRNA_i^{Met}$ will bind to the mRNA and scan along the 5'UTR for an AUG codon (the starting codon) [33]. However, mRNA must be activated by eIFs before the binding, so the activity of eIFs is downregulated under starvation or stress conditions. Also, under stress conditions, eIF2 can phosphorylate eIF2α as a stress-sensing serine/threonine kinase. Phosphorylated eIF2α impedes the GDP-GTP exchange, leading to a decrease in the assembly of the ternary complex, and dampens translation initiation.

The word "silencing" may cause misunderstanding about the function of miRNAs as miRNAs often act to fine-tune the expression rather than to turn on/off the expression [34]. Generally, most miRNAs can only decrease the target mRNA levels, and this repressive effect is cap dependent and mediated by eIF4F [35]. When cells undergo rapid changes, the role of miRNA can switch from gene regulators to molecular guards, which can protect the genome against the transcriptional fluctuations induced by extrinsic or intrinsic stresses. Numerous studies have validated the involvement of miRNA in stress responses [3].

Like miRNA, tiRNA can promote cellular adaption diverse adverse conditions. tiRNAs reportedly can directly inhibit protein translation speed by 10%-15%. To date, only 5' tiRNAs have been proven to possess the property of translation repression, which may in part relate to the stability of 5' tiRNAs. It has been well characterized that some specific tiRNAs, such as 5' halves from $tRNA^{Cys}$ and $tiRNA^{Ala}$, have a terminal oligoguanine (TOG) motif containing five guanosines at the 5' end, and this structure is critical for the translation arrest activity. 5' $tiRNA^{Ala}$ and 5' $tiRNA^{Cys}$ can target and displace eukaryotic initiation factors (eiFs) 4B, 4E, and 4G and can inhibit cap-dependent mRNA translation [36] (**Figure 3C**). Given that most proteins related to pro-survival and anti-apop-

tosis are translated via the IRES pathway rather than via cap-dependent translation, tiRNAs appear capable of exerting protective effects during unfavorable environmental conditions by selectively suppressing the general protein translation rates to decrease the cellular energy expenditure while leaving the synthesis of stress-response proteins unaffected [37]. In another mode of action, tiRNA indirectly interferes with the formation of translation initiation complex.

Interestingly, Ago proteins, miRNAs, and their target mRNAs are concentrated in SGs. SGs are a collection of nonmembranous cytoplasmic foci, and their formation is tightly connected to the status of mRNA translation. In addition, 5' tiRNAs can enhance the phospho-eiF2α-independent assembly of SGs [38].

Cell-to-cell communication

Accumulating evidence has indicated that miRNAs, as well as mRNA, DNA, and proteins, can be found in a variety of extracellular vesicles (EVs) that are released by many types of cells to mediate intercellular communication [39]. EVs are diverse and are generally classified as microvesicles (MVs) (100 nm-1 μm); apoptotic bodies, also known as microparticles (MPs, 0.5-2 μm), and exosomes (0.1-1 μm), depending on the size, shape, composition, and origin. MVs are formed from vesicles within the cell and ectosomes bud off from the plasma membrane. Although with different characteristics, all of them have the ability to transfer cargo to recipient cells, thereby contributing to a new mechanism of cell-to-cell communication [40]. Valadi et al. [41] have identified 120 miRNAs in the exosomes from mast cells and found that part of them were not expressed in the donor cell cytoplasm. In addition, miRNA can be packaged inside EVs but may also adhere to the membrane.

Different populations of cells and tissues can birth EVs containing different sets of miRNAs and deliver them to specific target cells. For example, the presence of miR-21 in the keratinocyte-derived MVs promotes the migration, differentiation, and contraction of fibroblasts [42]. Similarly, miR-142-3p can be transferred in microparticles from platelets to endothelial cells to exert its function [43]. Furthermore, different cells or tissues transport miRNAs by specific packaging mechanism for miRNAs by different cells or tissues. miRNAs delivered by EVs may also have the capability to suppress the target mRNA expression in recipient cells. Exosomes may be internalized into recipient cells by fusion with the target plasma cell membrane of the target cells or by endocytosis, through which miRNA is released in conjugation with either Ago or lipoproteins to evade degradation. Other functions of vesicle-delivered miRNAs have been reported too. For example, in a study on the renal ischemia-reperfusion injury process, miR-191 bearing platelet-derived MVs was transferred to HK-2 cells, and the experiment proved that miR-191 could inhibit CBS expression to promote HK-2 cell apoptosis [44].

Whether the concentration of miRNAs within EVs is always sufficient to instigate the variation in gene expression variation remains unknown. However, accumulating evidence has suggested that miRNAs within circulating exosomes could serve as biomarkers in numerous diseases. Studies have reported that miRNA and exosomal vesicles may serve as the prognostic indicators of acute and chronic liver diseases [45]. Moreover, circulating exosomal miRNA levels have been associated with cancer development and progression, and many attempts have been made to identify an exosomal miRNA that could become a specific diagnostic biomarker. For example, Zhang et al. have reported that exosomes derived from the bone marrow-derived mesenchymal stem cells (BMSCs) package specific microRNAs, including miR-193a-3p, miR-210-3p, and miR-5100, which can discriminate patients with lung cancer from control group patients without cancer. In addition, a panel of these three miRNAs showed higher diagnostic accuracy than individual exosomal miRNAs [46].

With the advancement on high-throughput sequencing, tiRNAs in EVs have become one of the research focuses. Dhahbi et al. [47] first reported in 2013 that tsRNAs circulated in the bloodstream, although only a small proportion (1.24%) of the reads were mapped to the tsRNA in EVs purified from human plasma. Sharma et al. detected tsRNAs in epididymis derived EVs. It is conceivable that extracellular RNAs (exRNAs) must associate with different carriers, including EVs, LPPs, and RNPs, to escape from degradation catalyzed by extracellular RNases. In particular, tsRNAs have

been found in epididymis-derived EVs by Sharma et al. [48]. Several lines of experimental evidence indicate that only a small proportion (1.24%) of the reads are mapped to the tsRNA in EVs purified from human plasma [47]. Although most circulating tsRNAs do not package into EVs, increasing evidence supports their roles as biomarkers in different diseases. A relevant experiment proved that plasma exosomal tRF-25, tRF-38, and tRF-18 were diagnostic biomarkers for osteoporosis detection [49]. Moreover, these EV-derived tRF fragments were also able to predict the diagnosis of gastric carcinoma.

Regulation of intergenerational inheritance

tsRNAs are the most abundant RNA species in particular, they count for ~80% of the 30-40 nt fraction of sperm RNA. The role of tsRNAs in epigenetic control was first identified in HFD-fed mice [50]. In that study, metabolic changes such as insulin resistance occurred in the male offspring of mice fed with a high-fat diet [50]. In another study, injection of tsRNA to the normal-zygote sperm of male mice on a high-fat diet showed the changes in the gene expression in the metabolic pathways in F1 offspring [51]. Other intergenerational experiments have revealed that protein restriction affects tsRNAs levels in mature sperm and induces glucose intolerance in offspring. Furthermore, tsRNAs are available by MVs from the somatic cells that line up the epididymis as sperm matures [52]. These tsRNAs could also repress the transcription of the endogenous retroelement MERVL [53]. The mechanism underlying the inherited tsRNA reprogramming of offspring phenotype remains to be elucidated. However, RNA modifications could play an important role here. A recent work has shown that the depletion of DNMT2 prevents the elevation of RNA modifications (m^5C, m^2G) and abolishes the intergenerational inheritance of high fat diet-induced metabolic disorders [54]. Collectively, these studies indicated the epigenetic information-carrier role of tsRNA in the mature sperm, but the precise mechanism requires further investigation.

Regulatory function in disease

Cancer

The uncontrolled proliferative nature of tumor cells grow very rapidly, which results in a microenvironment that outgrows the limits of oxygen and nutrients. Because tsRNAs derived from tRNAs can protect cell survival under a multitude of stress conditions, it is conceivable that tsRNA could be a powerful regulator during cancer initiation and progression. One of the mechanisms of tsRNA deregulation in cancers is through the alteration of precursor tRNAs. Oncogenic and tumor suppressive genes can affect the subunit of RNA Pol III, a transcriptional enzyme of tRNA, which may impact tRNA function [55]. Another mechanism of tsRNA deregulation in cancers is by the activation of Ang under stress conditions in several types of cancer [56]. Currently, some tsRNA signatures can distinguished between normal and cancer tissues, which could be used as cancer biomarkers [57]. One such example is tRF/miR-1280, derived from $tRNA^{Leu}$ and pre-miRNA in colorectal cancer (CRC), tRF/miR-1280 suppresses cancer stem cells by directly targeting Gata1/3 and miR-200b genes [6]. tsRNAs have also been found in several cancer types to regulate tumor progression by combining with RNA-binding proteins (RBPs), such as YBX1 (ref.), for which a functional role has been validated in various cancer types. Goodarzi et al. found that tRFs derived from $tRNA^{Asp}$, $tRNA^{Glu}$, $tRNA^{Tyr}$, and $tRNA^{Gly}$ led to destabilization of pro-oncogenic transcripts by displacing their 3'-UTRs from YBX1, thereby suppressing the development of breast cancer metastasis. Conversely, highly metastatic cells can evade this mechanism by blunting the induction of tsRNA [31]. A specific tsRNA, LeuCAG3' tsRNA, has been reported to inhibit the apoptosis of the rapidly dividing cells in a murine model of hepatocellular carcinoma. In addition, LeuCAG3' tsRNA can bind to the mRNA of two ribosomal proteins (RPS28 and RPS15) to attenuate their translation and thus interfere with the constitution of 40S ribosomal subunits [58].

Neurological diseases

All major neurodegenerative processes involve oxidative stress, so we speculate that oxidative stress and tsRNAs are intimately connected during the development and. progression of neurodegenerative diseases. In fact, the Ang-mediated production of tiRNAs may contribute to motor neuron survival. Ivanov et al. [59] found that the G-quadruplex (G4) structures allow 5' $tiRNA^{Ala}$ to enter motor neurons spontaneously and protect motor neurons from ad-

verse condition in a YB-1-dependent manner. Conversely, an RNA methyltransferase family member 2 (NSun2) mutation will increase the Ang-induced production of 5' tiRNAs, which directly induces the apoptosis of hippocampal, cortical, and striatal neurons. Furthermore, data from some other studies have shown that mutations of NSun2 have been shown to induce a syndromic form of intellectual disability and a Dubowitz-like syndrome.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the aberrant activity of the immune system and can affect almost any organ system, including renal, cardiovascular, skin, joints,lungs and central nervous system [60]. The current treatments for SLE can only delay the progression but cannot cure it completely, and the early diagnosis remains a challenge.

Xu et al. analyzed the tsRNA signatures in the peripheral blood mononuclear cells (PBMCs) of SLE patients. Gene Ontology (GO) analysis combined with Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses revealed that the altered target genes of tsRNA were most enriched in immune response and T cell receptor signaling pathway and primary immunodeficiency, which were related to the initiation of SLE [61]. Another study reported that tRF-His-GTG-1 and anti-dsDNA could serve as biomarkers for the diagnosis of SLE diagnosis, Moreover, the noninvasive serum tRF-His-GTG-1 could also be employed to distinguish between SLE with LN and SLE without LN but with AUC [62].

Other diseases

Human bone marrow mesenchymal stem cells (hMSCs) are self-renewing stem cells which can differentiate into a wide range of cell types, including adipocytes, osteoblasts and chondrocytes. Dysfunctional adipogenesis is associated with many metabolic disorders such as type II diabetes mellitus and cardiovascular disease. Wang et al. sequenced the total tsRNAs that were differentially expressed in hMSC during adipogenic differentiation and identified tsRNA-16902 as a novel regulator of adipogenesis, which by targeted RARγ via the Smad2/3 signaling pathway [63]. Additionally, Wang et al. found tsRNA-06018 as another regulator of this differentiation process. Decreased tsRNA-06018 led to the inhibition of adipogenesis by affecting the ERK1/2 signaling pathway-mediated STC2 activity [64].

A recent study showed that dexmedetomidine (DEX) treatment could ameliorate pulmonary injury, through inducing the abnormal expression of tsRNAs. The study by Fang et al. suggested that differential expression of tsRNA might be involved in the pathophysiology of steroid-induced osteonecrosis of the femoral head (SONFH). Furthermore, tsRNA-10277 enriched exosomes released by bone mesenchymal stem cells (BMSCs) could enhance osteogenic differentiation [65].

Conclusion

Both tsRNAs and miRNAs are important regulators in a diversity of normal and pathological processes. With respect to their regulatory roles in gene expression, tsRNA is more complex than miRNA. tsRNAs can participate in the Ago-mediated translation repression and interact with a wide range of molecules involved in the translational regulation and/or ribosomal biogenesis to exert their functions in translation regulation. tsRNA is also involved in other cellular activities, ranging from DNA damage response, cell proliferation, transposon silencing, to epigenetic inheritance.

Although tsRNAs are a newly discovered type of RNAs, a significant gap exists in our understanding of the molecular mechanisms how tsRNAs deliver their functions. Only 0.1%-5% of tRNAs isoacceptors will produce tsRNA under given stress conditions, so it is unclear how antisense oligonucleotides choose tsRNAs instead of other tRNA molecules. However, separating the functional tsRNA molecules from the other tRNA fragments is a challenge. Furthermore, both tsRNAs and miRNAs can be loaded into Ago to target their sequence-specific mRNAs, so it is conceivable that a crosstalk between them exists. Haussecker et al. [66] have reported that tsRNAs has a minor effect on miRNAs abundance but has a great influence on its silencing activity by competing in the interacttion with the Ago protein. Currently, connections between tsRNA and most human diseases remain mostly descriptive, which warrants future investigation.

Acknowledgements

This project was supported by the National Science Foundation of China (U19A2001), The University Synergy Innovation Program of Anhui Province (GXXT-2020-063, GXXT-2020-025).

Disclosure of conflict of interest

None.

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