Molecular Therapy
Nucleic Acids
Review

The function of tRNA-derived small RNAs in cardiovascular diseases

Yan Zhao,[1,3] Kai Wang,[1,3] Chun Zhao,[2,3] Ning Liu,[1] Zhihong Wang,[1] Wenting Yang,[1] Zewei Cheng,[1] Luyu Zhou,[2] and Kun Wang[1]

[1]Institute for Translational Medicine, The Affiliated Hospital of Qingdao University, College of Medicine, Qingdao University, Qingdao 266021, P.R. China; [2]College of Biology, Hunan University, Changsha 410082, P.R. China

tRNA-derived small RNAs (tsRNAs) constitute a subgroup of small noncoding RNAs (ncRNAs) originating from tRNA molecules. Their rich content, evolutionary conservatism, high stability, and widespread existence makes them significant in disease research. These characteristics have positioned tsRNAs as key players in various physiological and pathological processes. tsRNA actively participates in regulating many cellular processes, such as cell death, proliferation, and metabolism. tsRNAs could be promising diagnostic markers for cardiovascular diseases (CVDs). tsRNAs have been identified in serums, suggesting their utility as early indicators for the diagnosis of CVDs. Moreover, the regulatory roles of tsRNAs in CVDs make them promising targets for therapeutic intervention. This review provides a succinct overview of the characteristics, classification, and regulatory functions of tsRNAs in the context of CVDs. By shedding light on the intricate roles of tsRNAs, this knowledge could pave the way for the development of innovative diagnostic tools and therapeutic strategies for CVDs.

INTRODUCTION

tRNA-derived small RNAs (tsRNAs) hold significant relevance in the field of cardiovascular molecular biology due to their potential implications in cardiovascular diseases (CVDs). tsRNAs are derived from the cleavage of mature tRNA or precursor tRNA by Dicer and ribonuclease 5, and exhibit evolutionary conservation, stability, and widespread presence. Recent studies have unveiled the diverse biological functions of tsRNAs, including their involvement in gene expression regulation, cell proliferation, apoptosis, metabolism, and epigenetic modifications.

tsRNAs can be categorized into two types: tRNA halves (tiRNAs) and tRNA-derived fragments (tRFs). tiRNAs are approximately 29–50 nt in length and usually are generated by the cleavage of tRNA anticodon loops by angiogenin (ANG).[1] ANG is a secreted ribonuclease, which functions in angiogenesis. ANG can hydrolyze RNA.[2] tiRNAs can be further divided into 5′ tiRNAs, which contain the 5′ end of tRNA and the anticodon loop, and 3′ tiRNAs, which encompass the 3′ end of tRNA.[3] tRFs are approximately 14–29 nt long and are derived from mature or precursor tRNAs through Dicer-dependent processes.

According to corresponding positions on tRNAs, tRFs can be further divided into tRF-5, tRF-3, tRF-1, tRF-2, and i-tRF. The production of tRFs may depend on the Dicer enzyme.[4] tRF-5 and tRF-3 are derived from the 5′ and 3′ ends of mature tRNA, respectively. tRF-1 is produced from the 3′ end of precursor tRNA and is also called 3′ U-tRF.[5] tRF-3 is divided into tRF-3a (T ring front cut, 18 nt) and tRF-3b (T ring inside cut, 22 nt). The “CCA” trinucleotide sequence in the tail of tRF-3 can be cleaved by the RNaseZ enzyme to form the 3′CCA tRF. tRF-5 can be divided into 3 categories according to different cleavage sites: tRF-5a (14–16 nt), tRF-5b (22–24 nt), and tRF-5c (28–30 nt). tRF-2 is derived from the anticodon loop of tRNA, such as $tRNA^{Glu}$, $tRNA^{Asp}$, $tRNA^{Gly}$, and $tRNA^{Tyr}$, during hypoxia.[6] i-tRF mainly originates from the tRNA internal region, such as the D loop to the T-loop of mature tRNA.[7] 3′i-tRF is enriched in extracellular vesicles and is derived from tRNA Leu-TAA and tRNA Ser-GCT (Figure 1; Table 1).[8]

tsRNA and microRNA (miRNA) are similar not only in size but also in function. Some miRNAs even originate from tRNAs. tsRNAs also behave like Piwi-interacting RNAs and may participate in epigenetic inheritance together with PIWI protein.[15] tsRNA and miRNA both could bind to Argonaute (AGO) proteins and regulate translation. They were both implicated in diseases.

MECHANISMS OF tsRNA FUNCTION

The function of tsRNA is extensive and complex. tsRNAs play a role in protein translation initiation, gene silencing, ribosome, and the cell cycle. tsRNAs regulate gene expression by binding to RNA-binding proteins or by directly interacting with mRNAs. Evidence suggests that tsRNA targets and regulates RNA function in a miRNA-like manner. Similar to miRNA, some tsRNAs bind to AGO proteins and targets to mRNA using seed sequences.[16] tRF-5s and tRF-3s

https://doi.org/10.1016/j.omtn.2024.102114.

[3]These authors contributed equally

Correspondence: Luyu Zhou, College of Biology, Hunan University, Changsha 410082, P.R. China.
E-mail: lyzhoucas@163.com
Correspondence: Kun Wang, Institute for Translational Medicine, The Affiliated Hospital of Qingdao University, College of Medicine, Qingdao University, Qingdao 266021, P.R. China.
E-mail: wangk696@163.com

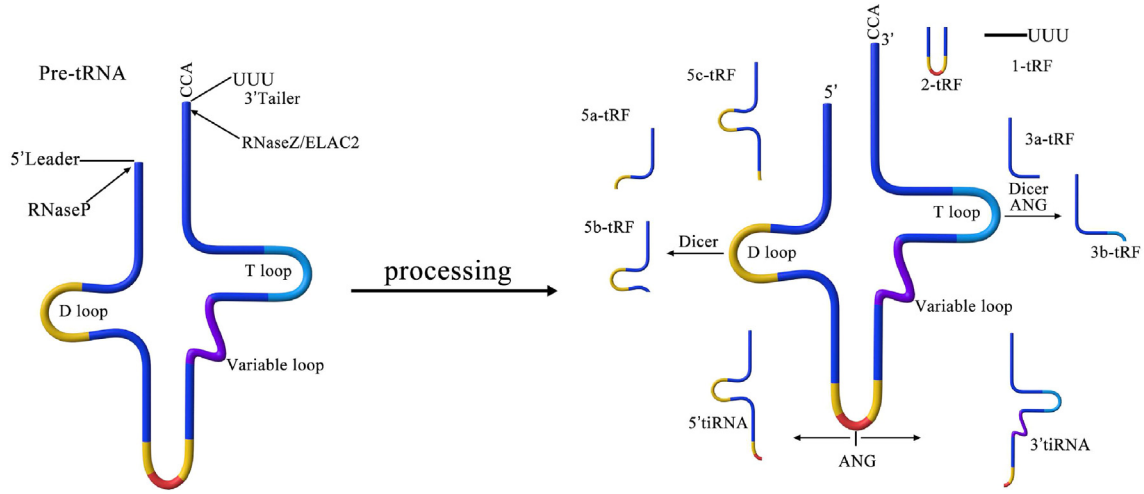

Figure 1. Structure and classification of tsRNAs

tsRNA was cleaved from tRNA and regulates gene expression at the transcriptional and translational levels. tsRNAs are mainly divided into tRNA-derived fragments (tRFs) and tRNA halves (tiRNAs). tRFs are approximately 16–28 nt in length, derived from mature tRNA or precursor tRNA, and can be further classified into tRF-5, tRF-3, tRF-1, and i-tRF.[4] tiRNAs are 5′ and 3′ tRNA half-molecules, approximately 29–50 nt in length, produced by ANG-specific cleavage at the anticodon loops of mature tRNAs under various stress conditions.

contain abundant seed sequences that match the central region of RNA cross-linking.[16] In adipose tissues, the seed sequence of tsRNA, the base at positions 2–7 of tsRNAs, had sequence priority.[17]

The functions and mechanisms of tsRNAs can be summarized as follows:

Regulating mRNA translation

tiRNAs can competitively bind to mRNA, inhibiting the function of the eukaryotic initiation factor 4G(eIF4G) and eukaryotic initiation factor 4A(eIF4A), and finally inhibit protein translation. Terminal oligo guanine (TOG) sequences can enhance the ability of 5′ tiRNA to inhibit translation initiation.[18] 5′-tiRNAs modulate the expression of its target genes and influence the development of CVDs by binding to Y-box binding protein 1 (YBX1) and block eukaryotic initiation factor 4F(eIF4F) complex initiated translation (Figure 2A).[18,19] tsRNAs mediate gene silencing through the RNA silencing complex. tsRNAs induce the AGO2-containing silencing complex targets to mRNA or long noncoding RNAs in the nucleus in a sequence-specific manner.[20] Many studies have shown that tRFs are associated with AGO complexes and inhibit gene expression as miRNAs.[4,10,21] Overexpression of tRFs, such as tRF-3001A, tRF-3003a, and tRF-3009A, downregulates the target mRNA level (Figure 2B). Under stress conditions, interleukin (IL)-1β-stimulated osteoarthritis chondrocytes increase the abundance of tRFs. tRNA overexpression increased the expression of the tRF-t003a fragment. In response to IL-1β stimulation, tRF-t003a interacts with the AGO-GW182 complex to silence gene expression.[22] 5′ tiRNAs impair 40s ribosome scanning on the mRNA and ultimately inhibit translation initiation in angiogenesis and inflammation.[19] 5′ tRF interacts with multiple synthase complexes to inhibit the translation process.[23] The 3′ end of the 5′ tRF found in HeLa cells has a conserved "GG" nucleic acid sequence that gives it translational inhibitory activity.[24]

Regulating RNA-binding proteins

LeuCAG3′ tsRNA is a small noncoding RNA derived from 3′ LeuCAG tRNA. The 22-nt LeuCAG3′ tsRNA enhances cell viability.[25] LeuCAG3′ tsRNA binds to two sites in the *ribosomal S28 (RPS28)* mRNA (Figure 2C).[25] It is also important for maintaining *RPS28* mRNA translation and the stabilization of ribosomal number.[26] The decreased expression level of LeuCAG3′ tsRNA inhibits the translation level of *RPS28* mRNA, reduces the biosynthesis of the first 18S rRNA, and reduces the generation of the 40S ribosomal subunit.[27] 5′ tRF^{Cys} has two G-rich motifs and may act as a nucleator for oligomerization from monomeric mRNA-nucleolin complexes. 5′ tRF^{Cys} promotes the oligomerization of an RNA-binding protein, thereby stabilizing its target transcripts.[28]

Inducing epigenetic modification

tsRNAs also induce epigenetic modifications. tsRNAs carry epigenetic information and transmit metabolic disorder phenotypes to offspring.[29] 5′ tiRNAs induce histone modifications that regulate the expression of genes involved in angiogenesis and inflammation.[19] Complexes of PIWI-tsRNA play important roles in intracellular RNA processing and histone modification.[30]

Regulating miRNA activity

tsRNAs regulate the activity of other small noncoding RNAs, such as miRNAs. By modulating the activity of miRNAs, tsRNAs influence gene expression and contribute to the development of CVDs.[19] 3′ tRF-IleAAT and 3′ tRF-LysTTT may participate in the regulation of the miRNA profile.[31] Some studies have also suggested that tsRNAs

Table 1. Classification and characterization of tsRNAs

Name	Classification		Source	Characteristic	Cell line	Tissue sample	Animal model
tsRNA	tRF	tRF-1[9]	Derived from the 3′ UTR of tRNA before RNase Z dissection	Contains poly-U sequence	Prostate cancer cell lines, HCT116 cells	–	–
		tRF-2[6]	Produced in anoxic conditions by anticodon ring	Typical 5′ ended and 3′ ended sequences are not included	HEK293T, MDA-MB-231, and CN34 cells	–	Mouse
		tRF-3[10]	TψC ring can be digested by ANG, Dicer, or exonuclease	Generally contains 18-nt CCA tail sequence; has tRF-3a and tRF-3b subtypes	B lymphoma BCP1 cell line	–	–
		tRF-5[11,12]	Dicer cuts D rings in tRNAs	The 3′ terminal is usually adenine, which can be further divided into tRF-5a, tRF-5b, and tRF-5c	H9 human embryonic stem cells, HEK293T cells	Healthy donor cord blood	NOD/SCID/$_{\gamma}$ mice
		i-tRF[7,8]	Originates from internal region of mature tRNA	Includes parts of D-loop to the T-loop of mature tRNA	–	Colorectal cancer and adjacent normal colon tissues	–
	tiRNA	5′ tiRNA[11,13]	Produced by angiogenesis-specific cleavage of tRNA in or near the region of the anticodon ring	Starts at the tRNA 5′ end and ends at the anticodon ring	Mouse embryo fibroblasts	–	–
		3′ tiRNA[11,14]		Includes anticodon rings and 3′ ends	U2OS cells, mouse embryo fibroblasts	–	–

do not change miRNAs abundance greatly, but affect the silencing function of miRNAs.[32] tsRNA-23678 targets to miR-29b-1-5p and negatively regulates scar formation.[33] Although tsRNAs and miRNAs are both important regulators, tsRNAs are considered to be more complex than miRNAs.

tsRNAs FUNCTION IN PHYSIOLOGICAL AND PATHOLOGICAL PROCESSES

tsRNA, beyond being a mere degradation fragment of tRNA, has been acknowledged to possess regulatory functions in various pathological processes. tsRNAs play a crucial role in regulating essential biological processes, including cell proliferation, necrosis, and apoptosis. tsRNAs are involved in the biological process of embryonic skeletal muscle development.[34]

Cell death

tiRNAs can interact with cytochrome *c* and block the apoptotic response induced by the binding of cytochrome *c* to APAF-1. ANG mutations reduce the generation of ANG-derived tiRNAs and inhibited cytochrome *c*-mediated apoptosis.[35] Oxidative stress reactive oxygen species (ROS) are also inducers of tiRNAs.[36] LeuCAG3′ tsRNA can induce apoptosis in rapidly dividing cells *in vitro* and *in vivo*.[25] tsRNA-26576 inhibits cell apoptosis *in vitro*.[37] Depletion of tRNA methyltransferase causes accumulation of tsRNAs and alleviates necrosis.[38] tsRNAs also exist in extracellular vesicle and exosomes. tsRNA-5006c exists in the extracellular vesicle and could regulate the differentiation of aortic valve interstitial cells through mitophagy, based on the study of the mouse mononuclear macrophage leukemia cell line and human aortic valve interstitial cells.[39]

Proliferation

Numerous tRNA-derived fragments actively participate in the regulation of cell proliferation. Abnormal expression of TRF-1001 has been observed in many cancer cell lines, and its knockdown has been shown to reduce cell viability and impede cell proliferation.[9] High levels of tRFs ts-46 and ts-47 have inhibited cell proliferation.[15] tRF-26-P4R8YP9lond and tRF-25-P940KK5Y93 inhibit the proliferation of colon cancer cells.[4] tRF-Leu-CAG promotes the proliferation of non-small cell lung cancer (NSCLC) cells and causes progression of the G0/G1 cell cycle.[40] Inhibition of ts-112 expression reduces the proliferation of MCF10CA1a breast cancer cells, and inhibition of ts-112 prevents excessive proliferation of breast epithelial cells.[41]

Cardiomyocyte hypertrophy

Expression of tsRNA is associated with myocardial hypertrophy.[42] ANG, which is the main inducer of tsRNA, is closely related to myocardial hypertrophy and has been shown to be a biomarker of heart failure.[43] The content of tRFs in the hypertrophy group was significantly higher than that in the control group. tRFs1 targets to the 3′ terminal noncoding region of the downstream molecule tissue inhibitors of metalloproteinase 3 (TIMP3).[44] This evidence suggests that tRFs may play a role in myocardial hypertrophy.

Metabolism

tsRNAs play an important role in metabolism and are involved in many metabolic diseases. tRF-3009 regulates mitochondrial oxidative phosphorylation in systemic lupus erythematosus (SLE) $CD4^+$ T cells.[45] tRF-3009 participated in respiratory metabolism and increased mitochondrial membrane potential, ATP, and ROS in $CD4^+$ T cells. Knockdown of DNA methyltransferase 2 in human

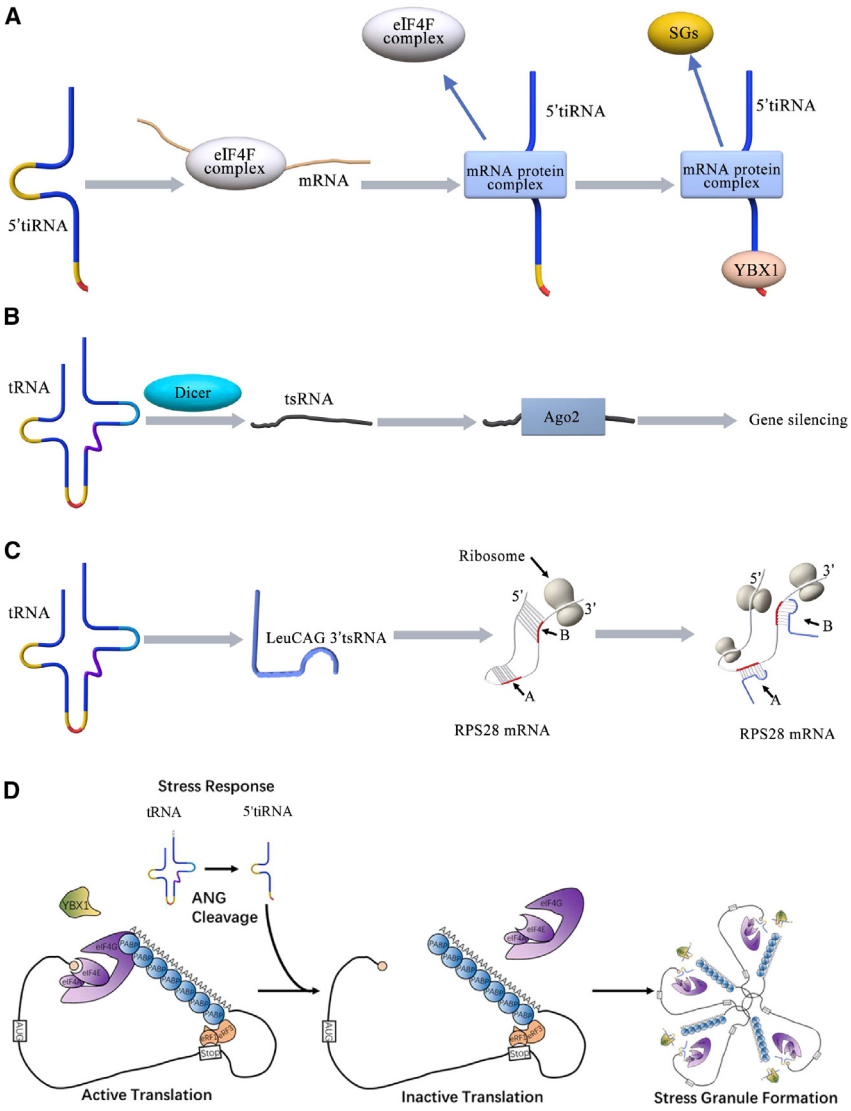

Figure 2. Mechanisms of tsRNA

(A) The 5′ tiRNA contains the TOG sequence, displaces the eIF4F complex from the mRNA, and binds to YBX1, leading to the formation of stress granules (SGs) and inhibition of translation.[18,19] (B) Dicer-dependent tsRNA induces gene silencing by interacting with AGO2, resulting in gene silencing.[4,10,20,21] (C) The LeuCAG 3′ tsRNA interacts with RPS28 mRNA, breaks its inner hairpin structure, and enhances the translation of *RPS28* mRNA.[25] (D) eIF4F(purple), PABP(blue), and the translation termination factor eRF1/eRF3(orange) participate in the active translation of mRNA. Stress induced ANG mediated tRNA cleavage produces tiRNA that induces the eIF4F complex removing and promotes translation inhibition. The stagnant translation complex is then condensed into stress granules (SGs) by combining 5 'tiRNAs with the cold-shock domain (CSD) of YBX1.

mature sperm can increase the 5′ tiRNA level, which plays an important role in the establishment of an intergenerational metabolic response in zygotes. After a period of high-sugar diet in healthy men, the levels of tsRNA from nuclear DNA organisms were upregulated.[46] In obese men, tsRNA levels were reduced.[47] This suggests that tsRNA may be closely related to human metabolism. tsRNAs from spermatozoa also affect human metabolism.[29,48] Sperm tsRNAs function in the intergenerational inheritance of diet-induced metabolic disorders.[29] Mouse protein restriction increased the number of fragments of glycine 5′-terminal tRNA.[48]

tsRNAs FUNCTIONS IN CVDs

Numerous studies have indicated the participation of tsRNAs in CVDs (Figure 3; Table 2). The role of tsRNA has been investigated in several cardiovascular conditions, including atherosclerosis (AS), aortic dissection, and myocarditis.

Role of tsRNA in AS

AS is a chronic inflammatory disease with a complex mechanism and is also the main cause of CVDs.[60] Its typical pathological features involve lipid deposition on vascular walls, which leads to proliferative cascade reactions and inflammation. The formation of atherosclerotic plaque is associated with vascular endothelial cells, white blood cells, and vascular smooth muscle cells (VSMCs).[61]

The expression levels of specific tsRNAs were altered in AS. The expression level of tRF-5a was increased in AS, whereas tRF-3b and tRF-2 levels were significantly decreased. Notably, tRF-Gly-GCC showed significantly higher expression in the AS group compared to the control group, suggesting its potential as a biomarker in AS.[51] tRF-Gly-GCC negatively regulates major histocompatibility complex (MHC) protein levels, promoting the proliferation and migration of endothelial cells and VSMCs.[51] These findings indicate that tRF-Gly-GCC may be a potential therapeutic target in AS. In addition, other tsRNAs, such as tRF-Gly-GCC-009 and tRF-Pro-AGG-006, were found to be significantly different between the AS and control groups, indicating their potential roles in the disease.[51] These tsRNAs suggest that they have important roles, and their functions need to be studied further.

Role of tsRNA in aortic dissection (AD)

AD is a very serious CVD with low morbidity but high mortality. The aortic wall consists of three layers: intima, media, and peripheral adventitia. AD refers to the rupture of the intima of the aorta, from which the blood in the aortic cavity passes into the media of the aorta, forming the true lumen and the false lumen.[62] The onset is usually accompanied by tearing pain. The pathogenesis of AD is mainly

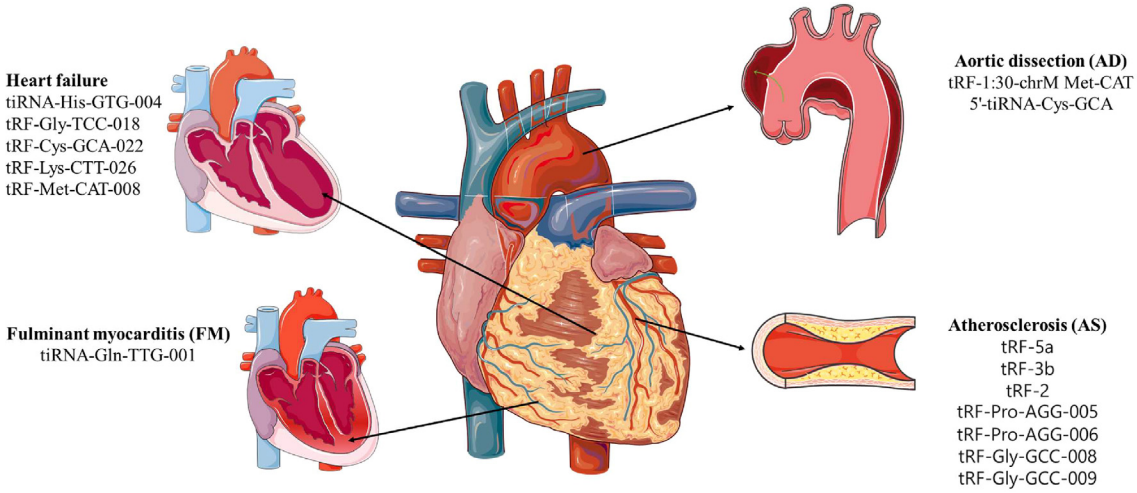

Figure 3. Functions of tsRNAs
The role of tsRNAs in heart failure, FM, AD, and AS.

related to inflammatory cells such as lymphocytes and macrophages. The expression of protease and calmodulin increased, and ROS are released, which ultimately leads to apoptosis of VSMCs and the rupture of AD.[62,63]

Recent studies have shown that tsRNA is associated with multiple pathogeneses of AD and may also be a new target for AD therapy. Differentially expressed tRFs and tiRNAs were detected in AD and control groups. RNA sequencing and qRT-PCR showed that tRF-1:30-chrM.Met-CAT was upregulated in VSMCs during AD.[55] In addition, it could enhance the migration and proliferation of VSMCs.[55] Under pathological conditions, 5′ tiRNA-Cys-GCA expression was significantly reduced in AD compared with the normal aorta.[56] It directly binds to its downstream target signal transducer and activator of transcription 4 (STAT4), resulting in the downregulation of STAT4 expression and increased expression of α-smooth muscle actin (α-SMA). These effects ultimately regulate the proliferation, phenotypic transformation, and migration of VSMCs.[55] These findings suggest that tsRNAs play important roles in the regulation of signal transduction pathways associated with AD.

Role of tsRNA in myocarditis

Myocarditis is an inflammatory disease of the myocardium[64] that can cause edema and dysfunction of the myocardium tissue, resulting in arrhythmia and shock. It has high mortality and sudden death in children and adolescents. Myocarditis usually involves cardiomyocyte damage directly caused by viruses and tissue damage mediated by immune reactions.[64] At present, there is no marker or better treatment for myocarditis.

In myocarditis, tsRNAs also show promise as potential targets for therapeutic intervention.[45] The overexpression of tiRNA-Gln-TTG-001 has been detected in juvenile myocarditis, indicating its potential as an early-stage marker for myocarditis. The expression level of tiRNA-Gln-TTG-001 could be used as a criterion for assessing the stage of fulminant myocarditis (FM) disease.[45] Furthermore, tiRNA-Gln-TTG-001 has been implicated in the modulation of Ras, mitogen-activated protein kinase MAPK, and other signaling pathways, suggesting its involvement in the pathogenesis of myocarditis.

Role of tsRNA in heart failure

Heart failure is a rapidly growing public health problem worldwide. Heart failure has a high incidence and mortality in CVDs, and the quality of life of patients with heart failure will be seriously affected, such as fatigue, poor breathing, and other life problems.[65] The main population of heart failure is older than 60 years of age, and the prevalence increases with age. Heart failure is characterized by the inability of the heart to pump enough blood to meet the body's metabolic needs, resulting in a variety of severe syndromes.[66]

The role of tsRNA in heart failure has been investigated, particularly in the context of cardiac preconditioning. Liu conducted a study to explore the association between tsRNA and cardiac preconditioning induced by caloric restriction (CR).[58] Through high-throughput analysis, five tsRNA targets (tiRNA-His-GTG-004, tRF-Gly-TCC-018, tRF-Cys-GCA-022, tRF-Lys-CTT-026, and tRF-Met-CAT-008) associated with CR preconditioning were predicted using an miRNA algorithm. Subsequent studies confirmed that the functions of these tsRNA targets, except for tRF-Gly-TCC-018, were related to the improvement of metabolic disorders and the signaling pathways involved in free radical accumulation.[58] These findings suggest that tsRNAs may play a therapeutic role in myocardial ischemia–related pathways and have potential implications in heart failure.

CONCLUSION AND PROSPECTS

tsRNA has emerged as a novel class of noncoding RNA with important biological functions. Initially considered to be by-products of

Table 2. The mechanism of tsRNA in disease

Disease	Cell line	Tissue sample	Animal model	Mechanism
Ischemic injury	Human umbilical vein endothelial cell	Adult male Sprague-Dawley rat brain	Middle cerebral artery occlusion model, the murine model of hindlimb ischemia	The upregulation of $tRNA^{Val}$ and $tRNA^{Gly}$ expression hinders the proliferation, migration, and angiogenesis of endothelial cells[49]
Myocardial hypertrophy	Rat H9c2 cells		Myocardial hypertrophy rat model (male Sprague-Dawley rats)	tRFs1 and tRFs2 both enlarged the surface area of cardiac cells and increased the expression of hypertrophic markers (Atrial natriuretic peptide (ANP), Brain natriuretic peptide (BNP) and β-MHC)[44]
				tRF-5 inhibits the expression of Timp3 mRNA's 3′ UTR, a regulator of hypertrophy[50]
AS	Humanumbilical veinendothelial cells and VSMCs	Atherosclerotic arterial tissues and arteries from healthy subjects		tRF-Gly-GCC regulates cell migration, adhesion, and proliferation of humanumbilical veinendothelial cells and VSMCs, contributing to the pathogenesis of AS[51]
		Carotid arteries of AS patients and three organ donors		tRF-Gly-GCC-009 plays a role in AS by regulating Apelin signaling, Notch signaling, and calcium signaling[52]
	Aortic segment digestion isolated cells	Aortas, the serum of healthy donors	$ApoE^{-/-}$ mice on a C57BL/6 background	Interferon-γ upregulates mini-tryptophanyl-tRNA synthetase (mini-TrpRS), affecting phenotypic conversion of VSMCs[53]
Varicose veins		Vascular tissue		Differentially expressed tRFs in varicose veins regulate Wnt and MAPK signaling pathways, influencing varicose veins[54]
Myocarditis	Human cardiomyocyte	Peripheral blood samples		Elevated expression of tiRNA-Gln-TTG-001 is observed in myocarditis[45]
Aortic dissection	VSMC, a human aortic smooth muscle cell line	AD tissues		tRFs and tiRNA can promote phenotypic transformation, proliferation, and migration of VSMCs[55]
	Human aortic VSMCs	The mouse thoracic aortas, human aortic dissection tissue and normal human aorta.	AD model (3-week-old male FVB mice)	5′ tiRNA-Cys-GCA downregulates STAT4 and increases α-SMA expression, regulating the proliferation, phenotypic transformation, and migration of VSMCs[56]
Moyamoya disease		Peripheral blood		Differentially expressed tRFs in moyamoya disease are involved in regulating pathways related to the disease[57]
Myocardial ischemia		Blood samples from the inferior vena cava of rats, heart tissues	Myocardial ischemic injury (adult male Sprague-Dawley rats)	Prediction of tsRNA associated with CR preconditioning showed their function is related to the improvement of metabolic disorders and signaling pathways related to free radical accumulation[58]
SLE	$CD4^+$ T cells from SLE patients and healthy control people (HCs)	Peripheral blood mononuclear cells		tRF-3009 participates in metabolic modulation in interferon-α-induced $CD4^+$ T cell oxidative phosphorylation in SLE patients[59]
Obesity		Semen samples, venous blood		tsRNAs from spermatozoa also affect human metabolism[47]
Lung cancer	The human bronchial epithelial cell line 16HBE, the human NSCLC cell lines A549, H1650, PC-9, 95-D, SPCA-1, H1299, and H23 cell	Tissue and serum samples		tRF-Leu-CAG promotes proliferation of NSCLC cells and causes progression of the G0/G1 cell cycle[40]

(*Continued on next page*)

Table 2. *Continued*

Disease	Cell line	Tissue sample	Animal model	Mechanism
Breast cancer	MCF10A cells			Inhibition of ts-112 expression reduces the proliferation of MCF10CA1a breast cancer cells[41]
Neuroinflammation		The thalamus and the top 10 from the cortex of rats	Traumatic brain injury rat	3′ tRF-IleAAT and 3′ tRF-LysTTT were associated with increased expression of miR-146a profile and a worse behavioral outcome[31]

tRNA degradation, tsRNAs have been shown to interact with proteins and mRNA, regulate gene expression, and participate in the regulatory mechanisms of signaling pathways involved in various diseases, including CVDs. The abundance of tsRNAs in different diseases is cell specific and tissue specific, and their functions span a wide range of biological processes.

Currently, many tsRNA databases are also reported and will facilitate the study. tsRBase is a comprehensive and systematic tsRNA library that contains information on tsRNAs from different data sources from multiple species.[67] tsRFun evaluated the expression profile and prognostic value of tsRNAs in 32 types of cancer, identified tsRNA target molecules using high-throughput sequencing, and constructed an interaction network between tsRNA, miRNA, and mRNA. At the same time, tsRFun also provides various real-time online tools for tsRNA recognition, target prediction, and functional enrichment analysis.[68]

tsRNA exists in the patient plasma are promising biomarker for many diseases.[69,70] Although the clinical value of tsRNAs requires further investigation, more and more studies have suggested that tsRNAs could be used as potential biomarkers for disease diagnosis and prognosis. Due to specific changes in the expression level of tsRNA in many CVDs, it is possible that tsRNAs can be biomarkers of CVDs.

The therapeutic value of tsRNAs is an area of active research. tsRNAs have been associated with many diseases, as summarized earlier in this article. There are several potential therapeutic applications and implications associated with tsRNA. Modulating tsRNA expression or activity could be explored as a therapeutic approach. tsRNAs are potential functional targets. It is worth noting that the abundance of tsRNAs in different types of diseases is cell specific and tissue specific, and they function in a wide range of biological processes. Regulating tsRNAs may become a key factor in controlling disease progression. Overall, tsRNAs offer new insights into disease mechanisms and potential therapeutic targets. Further studies are needed to elucidate the precise molecular mechanisms and therapeutic potential of tsRNAs in these conditions. tsRNA is expected to be a promising treatment for CVDs.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (81870236) and the Qingdao University Core Curriculum Construction Project, Innovation and entrepreneurship project for college students (2016-478, 201911065031X, 202011065318). Ethical approval was obtained from the Ethics Committee of Qingdao University. We thank Prof. Kun Wang and Prof. Peifeng Li for their helpful suggestions and discussions.

AUTHOR CONTRIBUTIONS

Conceptualization, Y.Z., K.W., and L.Z.; information collection and validation, Y.Z., K.W., C.Z., N.L., Z.W., W.Y., Z.C., and L.Z.; figure design and drawing, K.W., Y.Z., C.Z., and L.Z.; resources and data curation, K.W., N.L., Z.W., and L.Z.; writing – original draft, Y.Z., K.W., C.Z., and L.Z.; supervision, L.Z. and K.W. All of the authors have read and agreed to the published version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Rashad, S., Niizuma, K., and Tominaga, T. (2020). tRNA cleavage: a new insight. Neural Regen. Res. *15*, 47–52.
2. Tello-Montoliu, A., Patel, J.V., and Lip, G.Y.H. (2006). Angiogenin: a review of the pathophysiology and potential clinical applications. J. Thromb. Haemost. *4*, 1864–1874.
3. Li, S., and Hu, G.F. (2012). Emerging role of angiogenin in stress response and cell survival under adverse conditions. J. Cell. Physiol. *227*, 2822–2826.
4. Kuscu, C., Kumar, P., Kiran, M., Su, Z., Malik, A., and Dutta, A. (2018). tRNA fragments (tRFs) guide Ago to regulate gene expression post-transcriptionally in a Dicer-independent manner. RNA *24*, 1093–1105.
5. Veneziano, D., Di Bella, S., Nigita, G., Laganà, A., Ferro, A., and Croce, C.M. (2016). Noncoding RNA: Current Deep Sequencing Data Analysis Approaches and Challenges. Hum. Mutat. *37*, 1283–1298.
6. Goodarzi, H., Liu, X., Nguyen, H.C.B., Zhang, S., Fish, L., and Tavazoie, S.F. (2015). Endogenous tRNA-Derived Fragments Suppress Breast Cancer Progression via YBX1 Displacement. Cell *161*, 790–802.
7. Wang, X., Zhang, Y., Ghareeb, W.M., Lin, S., Lu, X., Huang, Y., Huang, S., Xu, Z., and Chi, P. (2020). A Comprehensive Repertoire of Transfer RNA-Derived Fragments and Their Regulatory Networks in Colorectal Cancer. J. Comput. Biol. *27*, 1644–1655.
8. Wang, S., Luo, Z., Yuan, L., Lin, X., Tang, Y., Yin, L., Liang, P., and Jiang, B. (2023). tRNA-Derived Small RNAs: Novel Insights into the Pathogenesis and Treatment of Cardiovascular Diseases. J. Cardiovasc. Transl. Res. *16*, 300–309.
9. Lee, Y.S., Shibata, Y., Malhotra, A., and Dutta, A. (2009). A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). Genes Dev. *23*, 2639–2649.
10. Li, Z., Ender, C., Meister, G., Moore, P.S., Chang, Y., and John, B. (2012). Extensive terminal and asymmetric processing of small RNAs from rRNAs, snoRNAs, snRNAs, and tRNAs. Nucleic Acids Res. *40*, 6787–6799.

11. Xie, Y., Yao, L., Yu, X., Ruan, Y., Li, Z., and Guo, J. (2020). Action mechanisms and research methods of tRNA-derived small RNAs. Signal Transduct. Target. Ther. *5*, 109.

12. Guzzi, N., Cieśla, M., Ngoc, P.C.T., Lang, S., Arora, S., Dimitriou, M., Pimková, K., Sommarin, M.N.E., Munita, R., Lubas, M., et al. (2018). Pseudouridylation of tRNA-Derived Fragments Steers Translational Control in Stem Cells. Cell *173*, 1204–1216.e26.

13. Saikia, M., Krokowski, D., Guan, B.J., Ivanov, P., Parisien, M., Hu, G.F., Anderson, P., Pan, T., and Hatzoglou, M. (2012). Genome-wide identification and quantitative analysis of cleaved tRNA fragments induced by cellular stress. J. Biol. Chem. *287*, 42708–42725.

14. Yamasaki, S., Ivanov, P., Hu, G.F., and Anderson, P. (2009). Angiogenin cleaves tRNA and promotes stress-induced translational repression. J. Cell Biol. *185*, 35–42.

15. Balatti, V., Nigita, G., Veneziano, D., Drusco, A., Stein, G.S., Messier, T.L., Farina, N.H., Lian, J.B., Tomasello, L., Liu, C.G., et al. (2017). tsRNA signatures in cancer. Proc. Natl. Acad. Sci. USA *114*, 8071–8076.

16. Kumar, P., Anaya, J., Mudunuri, S.B., and Dutta, A. (2014). Meta-analysis of tRNA derived RNA fragments reveals that they are evolutionarily conserved and associate with AGO proteins to recognize specific RNA targets. BMC Biol. *12*, 78.

17. Wang, L., Gu, H., Liao, T., Lei, Y., Qiu, Y., Chen, Q., Chen, L., Zhang, S., Wang, J., Hao, X., et al. (2023). tsRNA Landscape and Potential Function Network in Subcutaneous and Visceral Pig Adipose Tissue. Genes *14*, 782.

18. Ivanov, P., Emara, M.M., Villen, J., Gygi, S.P., and Anderson, P. (2011). Angiogenin-induced tRNA fragments inhibit translation initiation. Mol. Cell *43*, 613–623.

19. Wang, Y., Weng, Q., Ge, J., Zhang, X., Guo, J., and Ye, G. (2022). tRNA-Derived Small RNAs: Mechanisms and Potential Roles in Cancers. Genes Dis. *9*, 1431–1442.

20. Di Fazio, A., Schlackow, M., Pong, S.K., Alagia, A., and Gullerova, M. (2022). Dicer dependent tRNA derived small RNAs promote nascent RNA silencing. Nucleic Acids Res. *50*, 1734–1752.

21. Burroughs, A.M., Ando, Y., de Hoon, M.J.L., Tomaru, Y., Suzuki, H., Hayashizaki, Y., and Daub, C.O. (2011). Deep-sequencing of human Argonaute-associated small RNAs provides insight into miRNA sorting and reveals Argonaute association with RNA fragments of diverse origin. RNA Biol. *8*, 158–177.

22. Green, J.A., Ansari, M.Y., Ball, H.C., and Haqqi, T.M. (2020). tRNA-derived fragments (tRFs) regulate post-transcriptional gene expression via AGO-dependent mechanism in IL-1beta stimulated chondrocytes. Osteoarthritis Cartilage *28*, 1102–1110.

23. Keam, S.P., Sobala, A., Ten Have, S., and Hutvagner, G. (2017). tRNA-Derived RNA Fragments Associate with Human Multisynthetase Complex (MSC) and Modulate Ribosomal Protein Translation. J. Proteome Res. *16*, 413–420.

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

25. Kim, H.K., Fuchs, G., Wang, S., Wei, W., Zhang, Y., Park, H., Roy-Chaudhuri, B., Li, P., Xu, J., Chu, K., et al. (2017). A transfer-RNA-derived small RNA regulates ribosome biogenesis. Nature *552*, 57–62.

26. Kim, H.K., Xu, J., Chu, K., Park, H., Jang, H., Li, P., Valdmanis, P.N., Zhang, Q.C., and Kay, M.A. (2019). A tRNA-Derived Small RNA Regulates Ribosomal Protein S28 Protein Levels after Translation Initiation in Humans and Mice. Cell Rep. *29*, 3816–3824.e4.

27. Robledo, S., Idol, R.A., Crimmins, D.L., Ladenson, J.H., Mason, P.J., and Bessler, M. (2008). The role of human ribosomal proteins in the maturation of rRNA and ribosome production. RNA *14*, 1918–1929.

28. Liu, X., Mei, W., Padmanaban, V., Alwaseem, H., Molina, H., Passarelli, M.C., Tavora, B., and Tavazoie, S.F. (2022). A pro-metastatic tRNA fragment drives Nucleolin oligomerization and stabilization of its bound metabolic mRNAs. Mol. Cell *82*, 2604–2617.e8.

29. Chen, Q., Yan, M., Cao, Z., Li, X., Zhang, Y., Shi, J., Feng, G.H., Peng, H., Zhang, X., Zhang, Y., et al. (2016). Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. Science (New York, NY) *351*, 397–400.

30. Pekarsky, Y., Balatti, V., Palamarchuk, A., Rizzotto, L., Veneziano, D., Nigita, G., Rassenti, L.Z., Pass, H.I., Kipps, T.J., Liu, C.G., and Croce, C.M. (2016). Dysregulation of a family of short noncoding RNAs, tsRNAs, in human cancer. Proc. Natl. Acad. Sci. USA *113*, 5071–5076.

31. Puhakka, N., Das Gupta, S., Vuokila, N., and Pitkänen, A. (2022). Transfer RNA-Derived Fragments and isomiRs Are Novel Components of Chronic TBI-Induced Neuropathology. Biomedicines *10*, 136.

32. Haussecker, D., Huang, Y., Lau, A., Parameswaran, P., Fire, A.Z., and Kay, M.A. (2010). Human tRNA-derived small RNAs in the global regulation of RNA silencing. RNA *16*, 673–695.

33. Zhang, Y., Deng, Q., Tu, L., Lv, D., and Liu, D. (2020). tRNA-derived small RNAs: A novel class of small RNAs in human hypertrophic scar fibroblasts. Int. J. Mol. Med. *45*, 115–130.

34. Gan, M., Ma, J., Chen, L., Zhang, S., Niu, L., Zhao, Y., Li, X., Pan, H., Zhu, L., and Shen, L. (2022). Identification of tRNA-derived small RNAs and their potential roles in porcine skeletal muscle with intrauterine growth restriction. Front. Physiol. *13*, 962278.

35. Saikia, M., Jobava, R., Parisien, M., Putnam, A., Krokowski, D., Gao, X.H., Guan, B.J., Yuan, Y., Jankowsky, E., Feng, Z., et al. (2014). Angiogenin-cleaved tRNA halves interact with cytochrome c, protecting cells from apoptosis during osmotic stress. Mol. Cell Biol. *34*, 2450–2463.

36. Chen, Y., Zhou, Z., and Min, W. (2018). Mitochondria, Oxidative Stress and Innate Immunity. Front. Physiol. *9*, 1487.

37. Zhou, J., Wan, F., Wang, Y., Long, J., and Zhu, X. (2019). Small RNA sequencing reveals a novel tsRNA-26576 mediating tumorigenesis of breast cancer. Cancer Manag. Res. *11*, 3945–3956.

38. Ying, S., Li, P., Wang, J., Chen, K., Zou, Y., Dai, M., Xu, K., Feng, G., Zhang, C., Jiang, H., et al. (2023). tRF-Gln-CTG-026 ameliorates liver injury by alleviating global protein synthesis. Signal Transduct. Target. Ther. *8*, 144.

39. Xia, H., Gao, M., Chen, J., Huang, G., Xiang, X., Wang, Y., Huang, Z., Li, Y., Su, S., Zhao, Z., et al. (2022). M1 macrophage-derived extracellular vesicle containing tsRNA-5006c promotes osteogenic differentiation of aortic valve interstitial cells through regulating mitophagy. PeerJ *10*, e14307.

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

41. Farina, N.H., Scalia, S., Adams, C.E., Hong, D., Fritz, A.J., Messier, T.L., Balatti, V., Veneziano, D., Lian, J.B., Croce, C.M., et al. (2020). Identification of tRNA-derived small RNA (tsRNA) responsive to the tumor suppressor, RUNX1, in breast cancer. J. Cell. Physiol. *235*, 5318–5327.

42. Cao, J., Cowan, D.B., and Wang, D.Z. (2020). tRNA-Derived Small RNAs and Their Potential Roles in Cardiac Hypertrophy. Front. Pharmacol. *11*, 572941.

43. Jiang, H., Zhang, L., Yu, Y., Liu, M., Jin, X., Zhang, P., Yu, P., Zhang, S., Zhu, H., Chen, R., et al. (2014). A pilot study of angiogenin in heart failure with preserved ejection fraction: a novel potential biomarker for diagnosis and prognosis? J. Cell Mol. Med. *18*, 2189–2197.

44. Shen, L., Gan, M., Tan, Z., Jiang, D., Jiang, Y., Li, M., Wang, J., Li, X., Zhang, S., and Zhu, L. (2018). A Novel Class of tRNA-Derived Small Non-Coding RNAs Respond to Myocardial Hypertrophy and Contribute to Intergenerational Inheritance. Biomolecules *8*, 54.

45. Wang, J., Han, B., Yi, Y., Wang, Y., Zhang, L., Jia, H., Lv, J., Yang, X., Jiang, D., and Zhang, J. (2021). Expression profiles and functional analysis of plasma tRNA-derived small RNAs in children with fulminant myocarditis. Epigenomics *13*, 1057–1075.

46. Nätt, D., Kugelberg, U., Casas, E., Nedstrand, E., Zalavary, S., Henriksson, P., Nijm, C., Jäderquist, J., Sandborg, J., Flinke, E., et al. (2019). Human sperm displays rapid responses to diet. PLoS Biol. *17*, e3000559.

47. Donkin, I., Versteyhe, S., Ingerslev, L.R., Qian, K., Mechta, M., Nordkap, L., Mortensen, B., Appel, E.V.R., Jørgensen, N., Kristiansen, V.B., et al. (2016). Obesity and Bariatric Surgery Drive Epigenetic Variation of Spermatozoa in Humans. Cell Metab. *23*, 369–378.

48. Sharma, U., Conine, C.C., Shea, J.M., Boskovic, A., Derr, A.G., Bing, X.Y., Belleannee, C., Kucukural, A., Serra, R.W., Sun, F., et al. (2016). Biogenesis and Function of tRNA Fragments during Sperm Maturation and Fertilization in Mammals. Science (New York, NY) *351*, 391–396.

49. Li, Q., Hu, B., Hu, G.W., Chen, C.Y., Niu, X., Liu, J., Zhou, S.M., Zhang, C.Q., Wang, Y., and Deng, Z.F. (2016). tRNA-Derived Small Non-Coding RNAs in Response to Ischemia Inhibit Angiogenesis. Sci. Rep. *6*, 20850.

50. Shen, Y., Yu, X., Zhu, L., Li, T., Yan, Z., and Guo, J. (2018). Transfer RNA-derived fragments and tRNA halves: biogenesis, biological functions and their roles in diseases. J. Mol. Med. *96*, 1167–1176.

51. He, X., Yang, Y., Wang, Q., Wang, J., Li, S., Li, C., Zong, T., Li, X., Zhang, Y., Zou, Y., and Yu, T. (2021). Expression profiles and potential roles of transfer RNA-derived small RNAs in atherosclerosis. J. Cell Mol. Med. *25*, 7052–7065.

52. Wang, J., Dong, P.K., Xu, X.F., Huang, T., Mao, S., Wang, Q.G., Hao, J., Liu, X.H., Sun, X.D., Kang, K., et al. (2021). Identification of tRNA-derived Fragments and Their Potential Roles in Atherosclerosis. Curr. Med. Sci. *41*, 712–721.

53. Biros, E., Reznik, J.E., and Moran, C.S. (2022). Role of inflammatory cytokines in genesis and treatment of atherosclerosis. Trends Cardiovasc. Med. *32*, 138–142.

54. Yu, C., Wang, X., Hong, Y., Chen, G., Ge, J., Cao, H., and Zhou, B. (2019). Expression profile of tRNAderived fragments and their potential roles in human varicose veins. Mol. Med. Rep. *20*, 3191–3201.

55. Fu, X., He, X., Yang, Y., Jiang, S., Wang, S., Peng, X., Tang, G., Zong, T., Li, X., Zhang, Y., et al. (2021). Identification of transfer RNA-derived fragments and their potential roles in aortic dissection. Genomics *113*, 3039–3049.

56. Zong, T., Yang, Y., Lin, X., Jiang, S., Zhao, H., Liu, M., Meng, Y., Li, Y., Zhao, L., Tang, G., et al. (2021). 5'-tiRNA-Cys-GCA regulates VSMC proliferation and phenotypic transition by targeting STAT4 in aortic dissection. Mol. Ther. Nucleic Acids *26*, 295–306.

57. Wang, C., Zhao, M., Wang, J., Zhang, D., Wang, S., and Zhao, J. (2019). Expression analysis of transfer RNAderived fragments in the blood of patients with moyamoya disease: A preliminary study. Mol. Med. Rep. *19*, 3564–3574.

58. Liu, W., Liu, Y., Pan, Z., Zhang, X., Qin, Y., Chen, X., Li, M., Chen, X., Zheng, Q., Liu, X., and Li, D. (2020). Systematic Analysis of tRNA-Derived Small RNAs Discloses New Therapeutic Targets of Caloric Restriction in Myocardial Ischemic Rats. Front. Cell Dev. Biol. *8*, 568116.

59. Geng, G., Wang, H., Xin, W., Liu, Z., Chen, J., Danting, Z., Han, F., and Ye, S. (2021). tRNA derived fragment (tRF)-3009 participates in modulation of IFN-α-induced CD4(+) T cell oxidative phosphorylation in lupus patients. J. Transl. Med. *19*, 305.

60. Frostegård, J. (2013). Immunity, atherosclerosis and cardiovascular disease. BMC Med. *11*, 117.

61. Falk, E. (2006). Pathogenesis of atherosclerosis. J. Am. Coll. Cardiol. *47*, C7–C12.

62. Wen, D., Zhou, X.L., Li, J.J., and Hui, R.T. (2011). Biomarkers in aortic dissection. Clin. Chim. Acta *412*, 688–695.

63. Luo, F., Zhou, X.L., Li, J.J., and Hui, R.T. (2009). Inflammatory response is associated with aortic dissection. Ageing Res. Rev. *8*, 31–35.

64. Veronese, G., Ammirati, E., Cipriani, M., and Frigerio, M. (2018). Fulminant myocarditis: Characteristics, treatment, and outcomes. Anatol. J. Cardiol. *19*, 279–286.

65. Ziaeian, B., and Fonarow, G.C. (2016). Epidemiology and aetiology of heart failure. Nat. Rev. Cardiol. *13*, 368–378.

66. Dassanayaka, S., and Jones, S.P. (2015). Recent Developments in Heart Failure. Circ. Res. *117*, e58–e63.

67. Zuo, Y., Zhu, L., Guo, Z., Liu, W., Zhang, J., Zeng, Z., Wu, Q., Cheng, J., Fu, X., Jin, Y., et al. (2021). tsRBase: a comprehensive database for expression and function of tsRNAs in multiple species. Nucleic Acids Res. *49*, D1038-d1045.

68. Wang, J.-H., Chen, W.-X., Mei, S.-Q., Yang, Y.-D., Yang, J.-H., Qu, L.-H., and Zheng, L.-L. (2022). tsRFun: a comprehensive platform for decoding human tsRNA expression, functions and prognostic value by high-throughput small RNA-Seq and CLIP-Seq data. Nucleic Acids Res. *50*, D421–D431.

69. Zhu, L., Li, J., Gong, Y., Wu, Q., Tan, S., Sun, D., Xu, X., Zuo, Y., Zhao, Y., Wei, Y.-Q., et al. (2019). Exosomal tRNA-derived small RNA as a promising biomarker for cancer diagnosis. Mol. Cancer *18*, 74.

70. Wang, J., Ma, G., Ge, H., Han, X., Mao, X., Wang, X., Veeramootoo, J.S., Xia, T., Liu, X., and Wang, S. (2021). Circulating tRNA-derived small RNAs (tsRNAs) signature for the diagnosis and prognosis of breast cancer. NPJ Breast Cancer *7*, 4.