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REVIEW



# Novel insights into the roles of tRNA-derived small RNAs

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**ABSTRACT**

tRNA-derived small RNA (tsRNA) is a novel class of non-coding RNA that is usually produced from tRNA following endonuclease cleavage which occurs under stress conditions. There are two types of tsRNAs: tRNA-derived fragments (tRFs) and stress-induced tRNA halves (tiRNAs), which differ in their cleavage position. Many studies have demonstrated that tsRNAs are involved in various physiological and pathological processes apart from cancer and gene expression. In this review, we briefly described the biogenesis, classification, and characteristics of tsRNAs and summarized the current research progress of tsRNAs in metabolic diseases, senescence, reproduction, stress, and organ injury, and finally put forward some problems to be solved.

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## Introduction

tRNAs are important non-coding RNAs in organisms that primarily drive protein synthesis. In the past, tRNAs were thought to only function in transporting the corresponding amino acids to the ribosome for peptide chain elongation, while the small RNAs derived from tRNAs, named tRNA-derived small RNAs (tsRNAs), were considered as by-products of random tRNA cleavage [1]. However, further studies have proven that tsRNAs are not by-products but also played a significant role in various physiological and pathological processes, including suppressing breast cancer development via YBX1 displacement [2], regulating ribosome biogenesis by binding mRNA and ribosomal proteins [3], inhibiting translation by interfering with peptide bond formation *in vitro* [4] and so on. These discoveries illustrate that tsRNAs have complex functions and suggest that tsRNAs are vital molecules in gene expression and protein synthesis. Thus, an important question was asked: what roles do tsRNAs play in the development of other complex diseases and processes outside of regulating gene expression and cancer development? In this review, we briefly describe the biogenesis, classification, and characteristics of tsRNAs, summarize the research progress of tsRNAs in metabolic diseases, senescence, reproduction, stress, and organ injury, and discuss some unknown aspects of tsRNA biology to be addressed in the future.

### *tsRNAs species and biogenesis*

tsRNAs are derived from tRNAs. Mature tRNAs come from tRNA precursors (pre-tRNAs) through a series of processing events in the following order: RNA polymerase III transcribes the DNA sequence into RNA, ribonucleases P (RNase P) and Z (RNase Z), respectively, cut the transcript-specific sequences at the 5′ end and the 3′ end, the intron sequences are removed by tRNA endonucleases, and final post-transcriptional modifications are made, such as pseudouridylation, methylation, and the addition of the trinucleotide sequence CCA to the 3′ end of the transcript by tRNA nucleic acid transferases. The tertiary structure of a mature tRNA is L-shaped, including a D loop, a TψC loop, an anticodon loop, and a variable loop. When different enzymes cut the specific position of tRNA precursors or mature tRNAs, tsRNAs are produced. According to their cutting site, tsRNAs can be divided into two categories [5,6]. The first category is a tRNA-derived fragment (tRF), usually produced by Dicer or angiogenin, which can be divided into five subtypes described in the following table [7,8]. Under normal conditions, tRFs are located ubiquitously in various biofluids and their abundance is affected by diet, diseases, or external stimulation [2,9–11]. The second category of tsRNAs is stress-induced tRNA halves (tiRNA), which possesses a double-stranded structure and is 30–40 bases in length. Under stress conditions such as heat shock, ultraviolet radiation, starvation, hypoxia, and viral infection [12–14], angiogenin cuts the mature tRNA into two halves from the anticodon loop to obtain 3′-tiRNA and 5′-tiRNA [15] (Table 1 and Figure 1). In addition, different RNA modification has different effect on tsRNAs biogenesis. Pseudouridylation (Ψ) is an abundant and widespread nucleoside modification, which isomerize uridine to pseudouridine. Ψ is catalysed by evolutionarily conserved pseudouridine synthases (PUSs). When knocking out PUS7 in human

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**Table 1.** tsRNAs species.

| Classification | Subtype | Biogenesis | Characteristics |
|---|---|---|---|
| tRFs | 1-tRFs [20] | Cut the 3′ end of pre-RNA in the nucleus by RNase Z | Single-stranded, 16–48nt, with poly-U at the end, without redundant sequences of tRNA, distributed in the nucleus and cytoplasm |
| | 2-tRFs [2] | Produced under hypoxic conditions from $tRNA^{Glu}$, $tRNA^{Asp}$, $tRNA^{Gly}$, and pre-$tRNA^{Tyr}$ | Only four types, containing complete anticodon stem and anticodon loop |
| | 3-tRFs [21] | From mature tRNA 3′ end. Part of 3-tRFs are produced by angiogenin cleaving the TψC loop of tRNA | Containing RNA polymerase III promoter B box and the CCA sequence, including tRF-3a (18nt) and tRF-3b (22nt) |
| | 5-tRFs [21] | From mature tRNA 5′ end | Containing RNA polymerase III promoter A box, including tRF-5a (14–16nt), tRF-5b (22–24nt), and tRF-5 c (28–30nt) |
| | i-tRFs [22] | From mature tRNA inside | Starting from the second or more downstream nucleotide at mature tRNA 5′ end, spaning the anticodon, and extending to the T-loop farthest |
| tiRNA [12,23] | 3′-tiRNA | Produced by angioge-nin in mammalian cell (RNY1 in yeast) cutting the mature tRNA anticodon loop | From tRNA 3′ end to the cutting position of anticodon loop |
| | 5′-tiRNA | | From tRNA 5′ end to the cutting position of anticodon loop |

embryonic stem cells (hESCs), tRNAs lost Ψ, leading to change in biogenesis of 5-tRFs and 3-tRFs. And a special class of 5-tRFs derived from tRNA-Ala, tRNA-Lys, tRNA-Val which contain five consecutive guanine residues at 5′ end decreased significantly [16]. Another frequent RNA modification is methylation. Previous study has proved Dnmt2, a tRNA methyltransferase, protected tRNA from being cleaved by ribonuclease under stress [17]. Dnmt2 methylate the C38 position ($m^5C$) of tRNA-Gly whose loss promoted tRNA fragmentation [18]. In $Dnmt2^{-/-}$ mice sperm, $tsRNA^{Gly}$ were up-regulated [19]. However, we need further investigations of how tRNA modifications influence tsRNAs biogenesis.

## The latest progress of tsRNAs

### tsRNAs involved in the occurrence and development of metabolic diseases (Figure 2)

Obesity is a risk factor for diabetes, non-alcoholic fatty acid diseases, and cardiovascular disease [24,25]. All the time, scientists turn to seek robust methods to reduce weight, but there is no completely reliable one up to now. Fat accumulation directly results in obesity, which is associated with pre-adipocytes proliferation and differentiation, and mature adipocyte hypertrophy. Emerging evidence indicates that tsRNAs are involved in adipocyte fate and function. Zhu Li group found that a tRF from $tRNA^{Glu\text{-}TTC}$, referred to as $tRF^{Glu\text{-}TTC}$, promoted the proliferation of preadipocytes while inhibiting differentiation in the prerenal adipose tissue of rats fed a high-fat diet. This tRF not only promoted 3T3-L1 preadipocyte proliferation via increasing the expression of cell cycle regulatory factors (CDK4, CyclinD1, and Cyclin E), but also targeted the key pro-adipogenic transcription factors *KLF9, KLF11*, and *KLF12* directly, which led to decreases in aP2, PPARγ, and C/EBPα expression. $tRF^{Glu\text{-}TTC}$ reduced and increased the expression of fatty acid oxidation-related and synthesis-related genes, respectively, and the expression patterns of these genes were consistent with studies focused on fatty acid phenotypes in other systems [26–28]. What's more, a new study demonstrated that a lack of tsRNAs led to a significant decrease in the expression levels of PPARγ, FABP4, and C/EBPα [29]. According to the importance of PPARγ and C/EBPα in the regulation of adipogenesis, we inferred that tsRNAs might facilitate adipogenesis in adipocytes to support lipid accumulation and meanwhile promote pre-adipocytes proliferation and differentiation, so that influence obesity. However, this hypothesis requires further study. Besides this, a tsRNA named tsRNA-06018, promoted adipogenic differentiation by targeting the 3′UTR of *Stanniocalcin-2* (STC2) mRNA via the extracellular signal-regulated kinase 1/2 (ERK1/2) [29].

Alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) are serious global health issues whose incidences are rising in recent decades [30]. Although the pathogenesis is different, ALD and NAFLD share a similar developmental course beginning at steatosis and leading to hepatitis, cirrhosis, and hepatocellular carcinoma [30]. So, there might be something common participating in disease progression. In patients with ALD, the expression level of $tRF^{GLy}$ increased and the expression of *Sirt1* decreased, and the ALD mice models exhibited the same phenotype [11]. He Songqing group further found that $tRF^{GLy}$ can downregulate *Sirt1* expression through binding to Ago3 and acting on the 3′ UTR of *Sirt1* mRNA to promote lipogenesis and inhibit β-oxidation of fatty acids, thus promoting the development of liver steatosis and liver damage in ALD mice [11]. This outcome suggests that tsRNAs function in ALD progression. On the other hand, in mice with NAFLD, elevated tRF-300b can target and inhibit the expression of the autophagy-related gene *Prkaa1* and promote lipid formation, which aggravates the development of NAFLD [31]. However, the existing research on tsRNAs and liver diseases is relatively simple and we need to continue to conduct in-depth research on their mechanisms of development.

Epidemiology and basic research have shown that the health and nutritional status of parents is one of the risk factors for chronic diseases in the offspring [32–34]. Studies have shown that tsRNAs are the most abundant small RNAs in mature mouse sperm [35]. The role of tsRNAs in the intergenerational or transgenerational inheritance of metabolic diseases has also drawn much attention. Cropley J. E. et al. used Avy/a male mice crossed to a/a female mice to set up a congenic rodent model of obesity and pre-diabetes. Pre-diabetic obese fathers made the F1 generation potentially

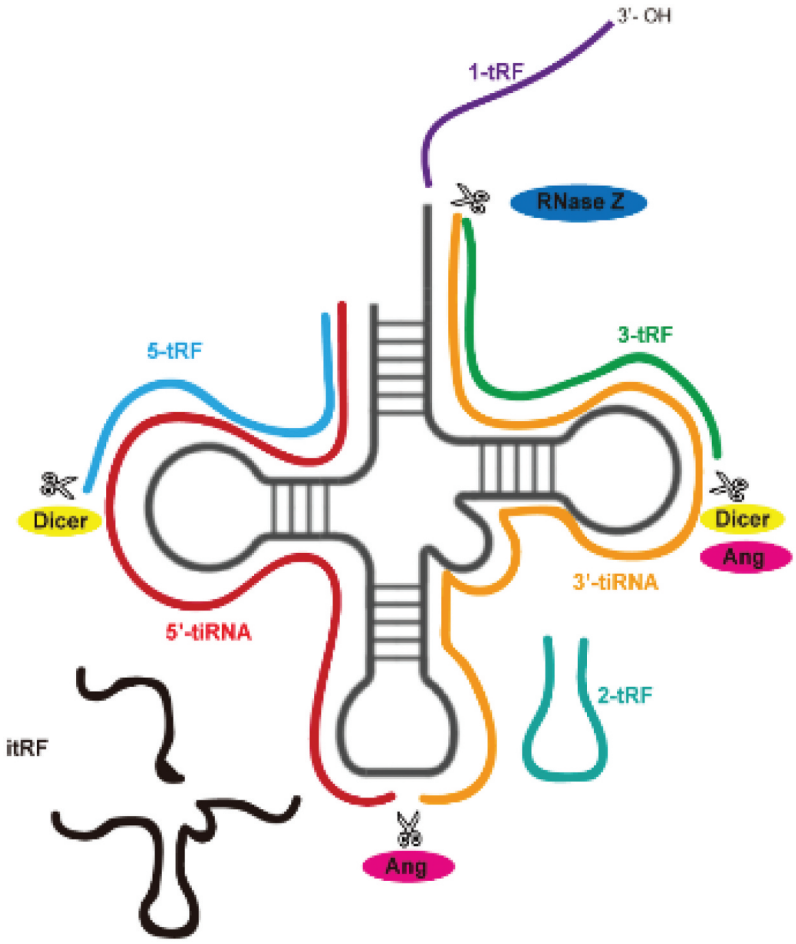


**Figure 1.** tsRNAs species and biogenesis.

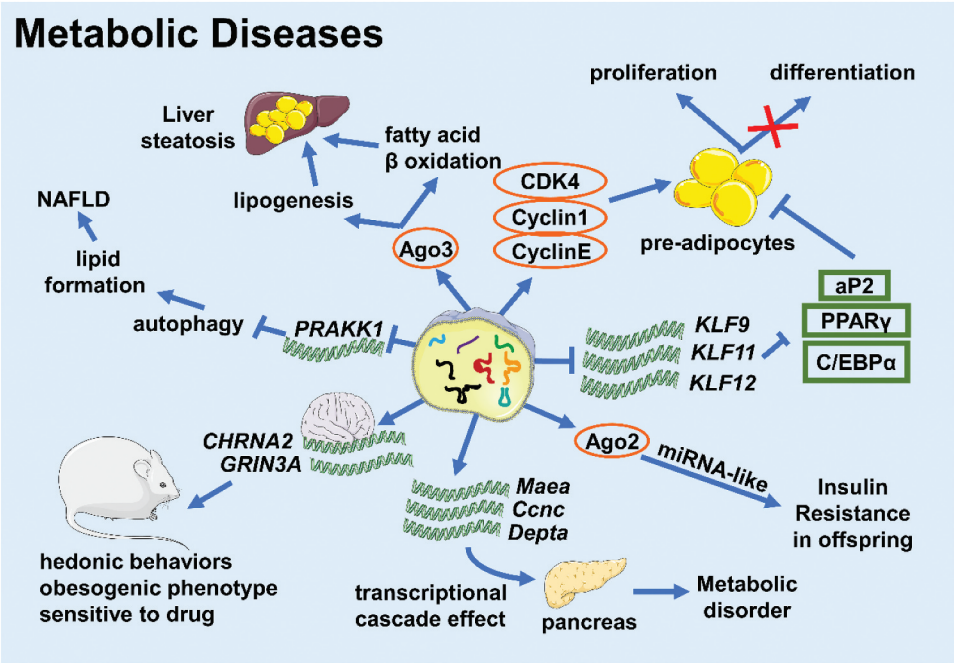


**Figure 2.** tsRNAs take part in various metabolic diseases (Created with Servier Medical Art, https://smart.servier.com).

susceptible to hepatic insulin resistance, and the sperm of F1 males could still transmit the induced metabolic phenotype to the F2 generation without obesity, changes in diet, or any significant metabolic damage. Matching with this, it was

found that approximately a quarter of the small RNAs in F1 male sperm are 5-tRF$^{Glu-CTC}$ and 5-tRF$^{Gly-GCC}$, and the content of 5-tRF$^{Glu-CTC}$ was reduced about 30% in the sperm of the F1 of obese paternal mice compared to the control group. It was suspected that 5-tRF$^{Glu-CTC}$ combined with Ago2 and might act in a miRNA-like manner in the zygote. This example suggests that tsRNAs might influence the intergenerational inheritance of metabolic phenotypes without extrinsic factors, which means that tsRNAs might act as a novel epigenetic factor [36]. Chinese scientists further verified this conjecture, as they produced a study showing that the expression profile of 5′-tiRNAs changed in the sperm of the parents fed on a high-fat diet. After the injection of purified tsRNAs into the zygote, multiple metabolic regulation-related genes were downregulated in the early embryonic stage. Such changes would affect the expression of metabolic genes through the transcriptional cascade effect into adulthood, and influence the reprogramming of the pancreatic islets of the F1 generation, leading to metabolic disorders [19]. Studies have also demonstrated that the metabolic profile of the maternal line could be passed to offspring through sperm tsRNAs. Sarker G. et al. observed that compared to male offspring of female mice fed a chow diet, sperm tsRNA expression increased in those fed a high-fat diet. After microinjecting all or part of specific sperm tsRNAs of these F1 males into normally fertilized eggs, scientists found that the F2 generation who received 30–34 nt sperm tsRNAs had similar obese bodies and hedonistic behaviours as the father, with a preference for delicious food and alcohol with increased sensitivity to central stimulant drugs. This result indicated that sperm tsRNAs were indeed carriers of genetic information, which helped to spread maternal high-fat diet-induced hedonistic behaviour and obesogenic phenotypes across generations via targeting *CHRNA2* and *GRIN3A* in the brain [37].

However, in a sparrow study, by comparing the small RNA expression profiles of mature sperm of adult and old sparrows, there was no statistical difference in tRFs between the two, suggesting that the carrier of epigenetic information might also be related to RNA modification [38]. Chen Qi group injected endogenous tsRNAs from high-fat diet-fed mice and artificially synthesized tsRNAs into fertilized eggs, respectively, and found that endogenous tsRNAs can produce offspring with impaired glucose tolerance and insulin resistance but synthetized tsRNA cannot, indicating that the modification of RNA affected the function of tsRNAs [19]. The scientists further found that compared to the tsRNAs of mice fed a normal diet, the $m^5C$ and $m^2G$ modifications of tsRNAs of mice fed with a high-fat diet were significantly up-regulated [19]. Although the function of $m^2G$ was unknown, early studies had shown that $m^5C$ could improve RNA stability and was related to RNA-mediated cross-generational inheritance [18,39]. Another study found that tsRNAs$^{Gly}$ levels rose in Dnmt2$^{-/-}$ sperm. Dnmt2 deletion caused tRNA $m^5C$ hypomethylation, changed tRNA secondary structure significantly, and promoted tRNA fragmentation, thereby eliminating the ability of sperm tsRNAs to induce the metabolic changes of the offspring. In addition, three artificially synthesized tsRNAs$^{Gly}$ with varying degrees of $m^5C$ modification induced different transcriptome responses in specific gene categories [40]. Therefore, we believe that tsRNAs are possible carriers of epigenetic information that relate to RNA modification, but how and where tsRNAs obtain this epigenetic information needs further study.

### *tsRNAs have multiple roles in the ageing process (Figure 3)*

Recent studies have shown that the abundance of tsRNAs changed in ageing insects and animals. The expression of *Drosophila* tsRNAs increased in an age-dependent manner [41]. *C. elegans* tsRNA levels generally rose during ageing [42]. American scientists found that the level of 5′-tiRNA in mice serum changed significantly with age [43]. The

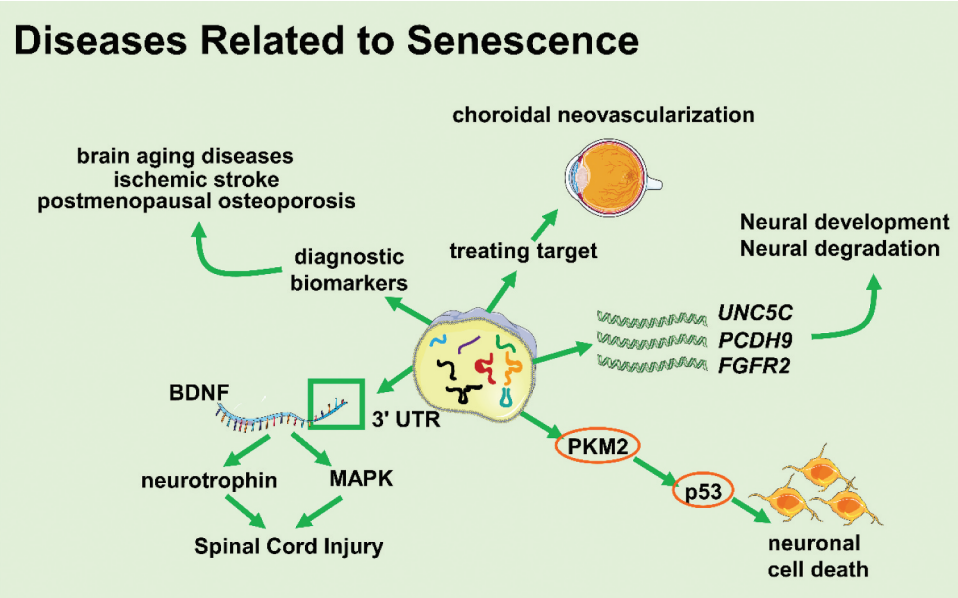


**Figure 3.** tsRNAs participate in diseases related to senescence (Created with Servier Medical Art, https://smart.servier.com).

abundance of 3-tRF in mouse brain cells increased with age, and the abundance of 3-tRF in older rats was also higher than that in younger rats [44]. These data above show that tsRNAs might participate in, or are at least associated with, the ageing process.

The degeneration of the nervous system is coupled with ageing. Many studies have shown that tsRNAs were involved in the development or degeneration of the nervous system and the occurrence and development of related diseases. Early studies demonstrated that compared to miRNAs, the levels of age-related mRNAs targeted by tRFs decreased more significantly. The targets of tRFs included *UNC5C, PCDH9, FGFR2*, etc. *UNC5C* mutations are associated with the susceptibility of Alzheimer's disease and *PCDH9* is related to the development and maintenance of the central nervous system structure. *FGFR2* is a powerful regulator during the development of the nervous system [44–47]. A study has also demonstrated that 5′-tRF$^{Tyr}$ derived from tyrosine pre-tRNA was more likely to cause p53-dependent neuronal death than other types of tRFs. 5′-tRF$^{Tyr}$ contained the 5′ leader sequence and 5′ exon of tRNA$^{Try}$, directly bound to PKM2, enhanced p53 activation, and led to neuronal cell death. Injecting 5′-tRF$^{Tyr}$ into zebrafish single-cell embryos caused embryonic development defects and induced zebrafish larvae microcephaly [48]. All these data suggest that tsRNAs are closely related to the development and degeneration of the nervous system. A study has also proved that tiRNA$^{Gly\text{-}GCC\text{-}001}$ might participate in the MAPK and neurotrophic factor pathways by targeting brain-derived neurotrophic factor (BDNF) and regulating the pathophysiological processes following spinal cord injury [49], prompting that tsRNAs might play an important role in injury and recovery of the nervous system.

In addition, it has also been reported that patients with Parkinson's disease and healthy controls had a set of tRFs that could clearly distinguish the two groups in the brain prefrontal cortex, cerebrospinal fluid, and serum [50]. Changes in the expression profiles of tRFs have been detected in the brains of SAMP8 mice (a model of neurodegenerative disorders) and SAMR1 mice (a control model) [51]. These pieces of evidence indicate that tsRNAs are related to brain ageing-related diseases. Angiogenin is one of the key enzymes in the production of tRFs, and at the same time, angiogenin mutations have been reported in patients with amyotrophic lateral sclerosis and Parkinson's disease [52]. Angiogenin directly participated in the pathways leading to motor neuron degeneration or dopaminergic neuron degeneration. Therefore, angiogenin might be involved in the occurrence and development of neurodegenerative diseases by protecting central neurons or making changes in tRFs levels [50,52]. Zhang Shuai group found that the differential expression of target genes of tRFs in the brains of SAMP8 mice and SAMR1 mice were involved in various brain functions, including synapse formation and synaptic vesicle circulation pathways. They speculated that tRFs might participate in the pathogenesis of Alzheimer's disease and Parkinson's disease through miRNA-like patterns, so the expression pattern of tRFs could be used as a potential biomarker for the diagnosis of brain ageing and related diseases [51].

In addition to brain ageing, stroke is also one of the diseases that seriously affects the quality of life of the elderly. Elkordy et al. found that various oxidative stresses induced the production of tRNA fragmentation in neuronal PC12 cells of rats and that there was tiRNA production during ischaemia-reperfusion injury. Interestingly, tiRNAs were produced before severe cell damage and death, and their production was related to the severity of the cellular injury. When the cells had recovered from stresses after a few hours, the content of tiRNAs decreased significantly, showing that tiRNAs could be a potential early biomarker of ischaemic stroke [53,54].

The WHO survey showed that osteoporosis was an age-related disease, with a very high prevalence among the elderly [55]. Zhang Yan et al. found that tRFs played an important role in osteoporosis. They collected and tested 40 plasma samples of osteoporosis patients and healthy controls, and found that tRF-25, tRF-38, and tRF-18 from plasma exosomes can be used as a diagnostic biomarker of osteoporosis to prognosticate osteoporosis [56]. Aside from osteoporosis, tsRNAs were found to be associated with choroidal neovascularization, which is one of the major clinical characteristics of neovascular age-related macular degeneration and a major cause of elderly blindness. Zhang Liwei group found that 72 tsRNAs were differentially expressed in choroidal neovascularization mice models and the target genes of these tsRNAs were most enriched in the NOD-like receptor signalling pathway, which plays a crucial role in angiogenesis, suggesting that tsRNAs might be a novel potential target in treating choroidal neovascularization [57,58].

In a study on longevity, it was found that inhibiting RNA polymerase III in the gut of adult worms or flies could prolong lifespan by reducing protein synthesis and lowering the level of pre-tRNAs. Pre-tRNAs can be processed into tsRNAs by RNase Z. This study further supported that changes in tsRNAs levels might contribute to lifespan and health [59]. In summary, tsRNAs are differentially expressed in young and ageing individuals in multiple species and have special targets, which suggests that tsRNAs might not only be potential biomarkers but might also play other pivotal roles in ageing and ageing-related diseases.

### *Sperm gets the payload of tsRNAs in the epididymis*

As mentioned above, the mature sperm cells have an abundance of tsRNAs, which promotes the intergenerational transmission of specific metabolic traits in mice. Godoy, P. M. et al. used RNA-sequencing technology to compare small RNAs systematically in 12 types of human biological fluids and found that tsRNAs are mainly found in bile, urine, seminal plasma, amniotic fluid, follicular fluid, and semen [9]. Many researches regarded that extracellular tsRNAs work as potential biomarkers of diseases for tsRNAs were easy to obtain without damage to body and their abundance showed obvious differences between normal physiological body and pathological body [50,53,56,60,61].

tsRNAs expression varied across different parts of the male reproductive system. The level of tsRNAs increased in the distal epididymis, and the specific tRFs expression profile was diverse in the testis, epididymal head (proximal), and

epididymal tail (distal). Besides, scientists have also detected differences in the expression of tsRNAs within a cell. The sperm head contained moderate levels of tRFs. A few specific tRFs (especially $tRF^{Gly\text{-}TCC}$ and $tRF^{Val\text{-}Val}$) were relatively abundant in the sperm tail [62–64]. These observations strengthened the opinion that tsRNAs are enriched in mature germ cells. Also, low levels of tRFs were detected in immature sperm cells purified from mouse testes, but studies have found that tRNAs had a large amount of cleavage in the epididymis – the place where sperm mature. Conine C.C. et al. found that epididymal epithelial cells could secrete epididymosomes containing rich 5-tRF. While sperm moving from the epididymal head to the epididymal tail, the sperm fused with 5-tRF-rich epididymosomes, suggesting that sperm might obtain tRNA fragments when passing through the epididymis [63–66]. This shows that tRF pools can be transferred between different types of cells to achieve the transfer of biological information from somatic cells to germ cells and tRFs may work as information carriers and exchangers as previously described.

But where does this biological information come from? Many researchers demonstrated that diet might affect tsRNAs profiles in germaria. A human study showed that the levels of i-tRFs and 3-tRFs in sperm cells from healthy donors were upregulated and positively correlated with an increase in sperm motility, while donors accepted a two-step diet intervention-healthy diet for the first week and a high-sugar diet for the second week[10]. And in mice fed by a low-protein diet, $tRF^{Gly\text{-}GCC}$ in sperm was upregulated and it could inhibit the active endogenous reverse transcription element-related genes in the preimplantation embryo, thereby affecting the biogenesis in mammalian reproduction [65]. But a newer study had different results. Colin et al. separated caput sperm and cauda sperm. The embryos produced with caput sperm showed significant overexpression of various regulatory factors and even caused embryonic lethality. Microinjection of purified small RNAs from cauda sperm into such embryos could rescue molecular defects and lethality. However, these small RNAs were miRNAs rather than tRFs, which indicated that the role of tsRNAs in embryonic development needs further studies [64].

In conclusion, in reproductive and metabolic diseases mouse models, sperm tsRNAs might be a driving factor of sperm epigenetics or at least play an indispensable role [19,67]. Sperm tsRNAs might also interfere with mRNA synthesis of proximal gene regulation region directly, and inhibit genes driven by transposable elements [19,65], and this process is required for early embryonic development (Figure 5).

### *tsRNAs participate in stress in different forms (Figure 4)*

Previous studies at the cellular level have shown that tsRNAs can be upregulated under various stresses (physical, chemical, and viral) [68,69]. Furthermore, there are abundant tsRNAs in haematopoietic and lymphatic tissues, as well as in the blood circulatory system [43] . All of these observations have inspired researchers to explore whether tsRNAs are related to pathological conditions.

The level of tsRNAs existing in the circulatory system increases rapidly during inflammation, indicating that tsRNAs might play a vital role in the immune response [70]. On the one hand, during the maturation of monocytes to dendritic cells, $5\text{-}tRF^{Glu}$ formed a complex with the Ago-like proteins piwil4 and piwil1 and then recruited SETDB1, SUV39H1, and HP1β to the *CD1A* promoter to methylate histone H3K9, thereby inhibiting the expression of *CD1A* [71]. On the other hand, tsRNAs from $tRNA^{Ala\text{-}UGC}$ with a CCACCA sequence could directly interact with Toll-like receptors to activate the immune response of Th1 and toxic T lymphocytes. Research by Chiou et al. showed that immune

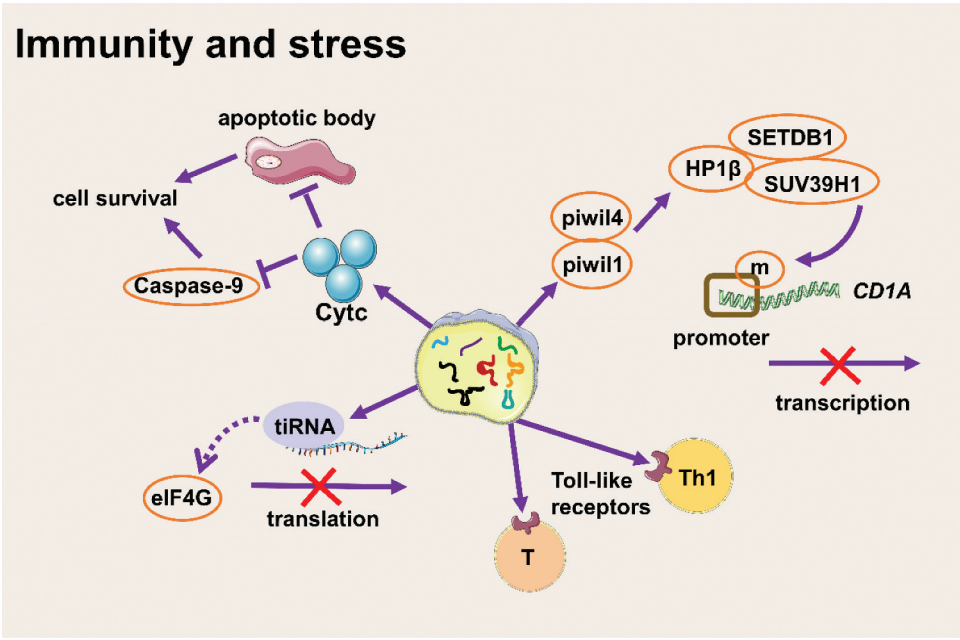


**Figure 4.** tsRNAs participate in stress in different forms (Created with Servier Medical Art, https://smart.servier.com).

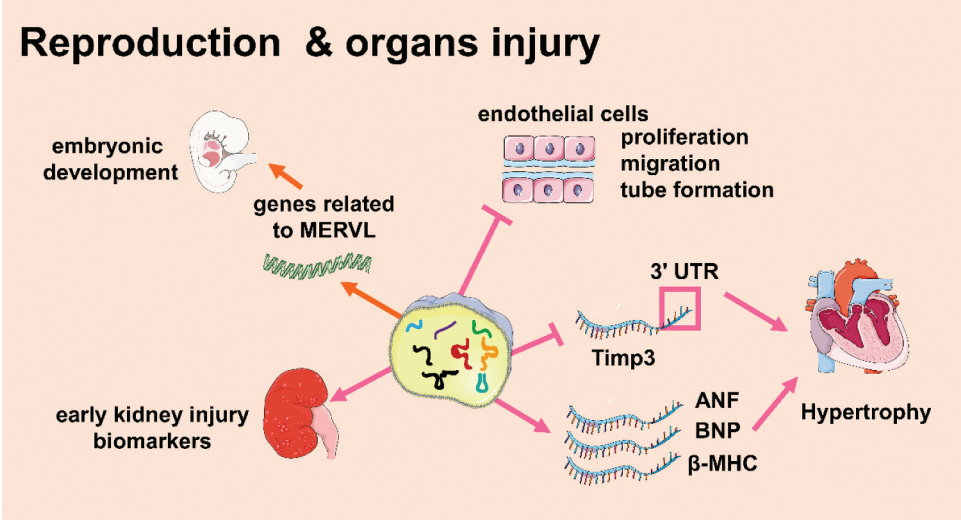


**Figure 5.** tsRNAs are associated with multiple organs injuries (Created with Servier Medical Art, https://smart.servier.com).

activation signals promoted the secretion of vesicles containing specific tRFs that inhibited T cell activation and cytokine production [72]. These results indicated that tsRNAs might work as new immune signalling molecules to participate in an immune response.

Apart from participating in stress as immune signalling molecules, tsRNAs also participate in stress response by inhibiting translation [73]. When cells are exposed to unfavourable environmental conditions, they will automatically trigger the stress response to conserve energy resources for surviving. Generally, eIF2α is phosphorylated and eIF2α phosphorylation inhibits translation initiation. Then, some proteins containing domains that have low sequence complexity and a high content of glycines bind to messenger ribonucleoprotein (mRNP) rapidly and form stress granules [74–76]. But tiRNAs inhibit translation in another way. Pavel Ivanov et al. found that tiRNA (mainly 5′-tiRNA) was a component of stress response after stimulating stress conditions, and tiRNA could promote translation stagnation and reduce the global translation speed by 10%-15% in a phospho-eIF2α–independent way [13]. Stress conditions induced the production of 5′-tiRNA$^{Ala}$ and 5′-tiRNA$^{Cys}$. The two tiRNAs contained terminal oligoguanine motif (TOG) and had ability to assemble G-quadruplexes structure (a stable guanine-rich nucleic acid structure [77]), then disrupted the scanning step of translation initiation by directly binding the HEAT1 domain of eIF4G (a large scaffold protein in eIF4F) on the 5′ end of mRNA [74,78]. What's more, a study on *Trypanosoma brucei* showed that a large amount of 3′-tiRNA$^{Thr}$ was produced in malnourished trypanosomes. This tiRNA was related to ribosomes and polymers. Once starvation stopped, 3′-tiRNA$^{Thr}$ promoted mRNA translation during stress recovery [79]. This evidence showed that tsRNAs, especially tiRNAs, are important translation regulators during stress.

Early studies have shown that mature tRNA molecules can bind to cytochrome C and inhibit the formation of apoptotic bodies and inhibit the activity of caspase-9, thus promoting cell survival [80]. Mridusmita Saikia. et al. found that when cells were exposed to a high osmotic pressure environment, angiogenin cut tRNAs to produce tiRNAs rapidly, and tiRNAs accumulated in the cytoplasm and preferentially formed a complex with cytochrome C released from mitochondria, thereby blocking autophagy [81]. Thus, we can consider that tiRNAs 'inherit' part of the characteristics of tRNA and they play a role in stabilizing the cell state under stress.

### *tsRNAs are associated with multiple organ injuries (Figure 5)*

Several lines of research showed that tsRNAs were involved in regulating cardiomyocyte activity [82]. Cardiomyocytes are prone to ischaemia. Studies have shown that tRFs were involved in tissue ischaemia [83,84]. In ischaemic rat brains, a mouse hindlimb ischaemia model, and a cellular hypoxia model, tRF$^{Val}$ and tRF$^{Gly}$ levels increased, and they could inhibit the proliferation, migration, and tubular formation of endothelial cells, resulting in ischaemia[84]. It seems like tRFs participate in myocardial ischaemia, but the relationship is not fully understood. Besides, tsRNAs were also involved in regulating cardiac hypertrophy. Zhu Li et al. used isoproterenol to induce cardiac hypertrophy in mice. In this model, tRF$^{Gly\text{-}GCC}$ and tRF$^{Glu\text{-}TTC}$ were overexpressed in the heart, which both increased the surface area of H9c2 cells (rat cardiomyocyte cell line) and increased the expression of the cardiomyocyte hypertrophy markers ANF, BNP, and β-MHC. Among them, tRF$^{Gly\text{-}GCC}$ inhibited the 3′ UTR of Timp3 mRNA directly, proving that tRFs were involved in the regulation of cardiac hypertrophy. [85]This study also found that a variety of tRFs were highly expressed in the sperm of F0 generation mice with cardiac hypertrophy and in the heart of F1 generation mice. Compared to the control, the expression of *β-MHC* and *ANP* in the F1 generation of cardiac hypertrophic mice was higher, and fibrosis and autophagy in the heart increased. This indicated that tRFs might act as a new type of epigenetic factor to participate in the intergenerational inheritance of myocardial hypertrophy [85]

Many studies have shown that tsRNAs are associated with kidney damage. For example, the concentration of tiRNAs

increased in the blood of patients with chronic kidney disease [86]. The plasma level of tiRNAs increased in animals with kidney injury and renal ischaemia-reperfusion [60]. Studies have shown the relationship between tsRNAs and kidney damage, when the kidney was damaged, the expression of angiogenin in renal epithelial cells significantly increased. While angiogenin participated in cell protection, it also interfered with the initiation of protein translation by producing tiRNAs. Then, tiRNAs reduced protein synthesis and promoted the adaptability of cells upon acute kidney injury [87]. The levels of plasma tiRNAs and angiogenin in patients with acute or chronic kidney injury could reflect the severity of the prognosis [60]. In addition, the two could also be detected in the urine, suggesting that tiRNAs and angiogenin are potential biomarkers that reflect the degree of kidney injury [60].

## Summary

As a new class of non-coding RNAs, tsRNAs encompass various types of tRNA-derived small molecules and have received extensive attention for their unique and diverse physiological and pathological functions. Recent studies on the role of tsRNAs have emphasized their important role in a variety of molecular processes, including mRNA stabilization [2], miRNA-mediated silencing [88,89], regulation of cap-dependent and cap-independent translation [79]. Also, tsRNAs are usually dysregulated in cancers,; thus,many current researches focused on the role of tsRNAs in cancer [90–94].

In this review, we summarized the progression of tsRNAs in disease processes other than cancer. For metabolic diseases, tsRNAs are not only involved in development but also work as paternal or maternal intergenerational and transgenerational genetic factors. In senescence, although there is no clear evidence of tsRNAs influencing the ageing process, tsRNAs seem to have a role in senescence-related diseases and might serve as potential biomarkers. In addition, tsRNAs are regulated quickly by diet and can be transferred from somatic cells to sperm during sperm passage through the epididymal head to the epididymal tail, working as a driving factor of sperm epigenetics. For immunity and stress, tsRNAs mostly act as a regulator to maintain cellular homoeostasis. What's more, tsRNAs play a significant role in suppressing or promoting organ injury to some extent. However, the extent of knowledge on tsRNAs in the fields of metabolic diseases, ageing, reproduction, and stress is still paltry. There are even some fields that are not yet discovered to be influenced by tsRNAs. More time is needed to explore the role and mechanism of tsRNAs during disease and physiology processes.

Although researches about tsRNAs have achieved some excellent results [73,77,95], there are still many questions to be answered. First, there is no consensus on the classification and nomenclature of tsRNAs. For this type of small RNA derived from tRNA, some people name tRFs and tiRNAs together as tRNA-derived fragments (tDFs), and some refer to tsRNAs as tRNA-derived microRNA [88]. Detailed naming is also more confusing given these numerous classifications and ppoorly definedconventions. Earlier, when we understood even less about tsRNAs, some studies mistook tsRNAs for micro RNAs [96], and some were named separately [88,97,98]. However, the current work on the classification of tsRNAs is also only to distinguish between tRFs and tiRNAs mostly, only a few with obvious characteristics such as 3-tRF are marked, which is not beneficial to the communication between researchers and the generalization of specific tsRNAs functions. So Jinghao Sheng proposed a feasible naming method -X-tsRNAs$^{AA\text{-}NNN}$ in 2018. Under these guidelines, tsRNAs are described with specific tRFs and tiRNAs, where X represents subclass, AA represents the amino acid corresponding to the tRNA, and NNN is the codon [83]. Thus, 3′-tiRNA and 5a-tRF are both derived from tRNA$^{Gly\text{-}GGG}$, so it can be written as 3′-tiRNA$^{Gly\text{-}GGG}$ and 5a-tRF$^{Gly\text{-}GGG}$.

Second, the general biogenesis process of tsRNAs is still largely undefined. Dicer, Ang, RNase, and ELAC2 are the key enzymes to produce tsRNAs, and most of tsRNAs are classified according to the cutting site and cutting enzyme. However, the length of various tsRNAs is not a certainty value, indicating that the recognition site of these endonucleases to tRNA is probably not unique and that the mechanism of these endonucleases to recognize and cleave tRNA still needs to be explored. In addition, there are more low-content tsRNAs such as 2-tRF, and it is not yet clear how they are produced. Are there other endonucleases? Does the three-dimensional structure of tRNA affect the production of tsRNAs? Except for pseudouridylation [16] and methylation [40], do other RNA modifications affect the production of tsRNAs? All these questions require further study and are necessary to understand the scope of tsRNA biological activities.

Thirdly, although in some studies, artificially synthesized tsRNAs can show similar activities to endogenous tsRNAs, more studies have confirmed that modifications on tsRNAs have a crucial impact on the production and activity of tsRNAs. The current research on tsRNAs is still limited by the development of technology. First of all, current sequencing methods cannot detect small RNAs with modifications. Therefore, it is often necessary to remove the modifications with a pretreatment kit before detecting the expression level of sample tsRNAs. Such processing may cause degradation of tsRNAs and affect the final test results. Secondly, compared with endogenous tsRNAs, artificially synthesized tsRNAs lose part of activity and are not as stable as endogenous tsRNAs. This is probably related to lack of RNA modification and changes in spatial structure. On the one hand, it is difficult to obtain sufficient and high-purity single specific tsRNA for mass spectrometry to verify the analysis. On the other hand, because the specific location and types of modifications on tsRNAs have not yet been fully clarified, it is difficult to modify tsRNAs precisely, leading to the endogenous tsRNAs unable to be fully reproduced. In order to obtain endogenous tsRNAs, scientists have made unremitting efforts. In 2016, Chen Qi et al. used polyacrylamide gel electrophoresis to separate tsRNAs. After gel cutting and recovery, tsRNAs with endogenous activity were obtained, but they could only be classified simply based on length, and specific individual tsRNAs could not be separated [19]. In

2020, Pavel Ivanov group captured and purified single endogenous tiRNAs successfully by using DNA oligo probes complementary to target tiRNAs [99]. This method is relatively efficient and can obtain high purity tsRNAs, which provides favourable conditions for the in-depth study of single tsRNAs. However, how to extract high-quality tsRNAs quickly and accurately from biological samples in large quantities and how to accurately modify artificially synthesized tsRNAs to obtain the correct spatial structure and activity requires more technological breakthroughs.

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