REVIEW

tRNA cleavage: a new insight

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Abstract

Over the past decades, tRNA was found to be a rich hub of RNA modifications such as 1-methyladenosine and 5-methycytosine modifications and others, holding more than half of all modifications occurring in RNA molecules. Moreover, tRNA was discovered to be a source of various small noncoding RNA species, such as the stress induced angiogenin cleaved tRNA halves (tiRNA) or the miRNA like tRNA derived fragments. tRNA cleavage under stress was fist discovered in bacteria and later was found to be conserved across different species, including mammals. Under cellular stress conditions, tRNA undergoes conformational changes and angiogenin cleaves it into 3′ and 5′ halves. 5′tiRNA halves were shown to repress protein translations. tRNA cleavage is thought of to be a cytoprotective mechanism by which cells evade apoptosis, however some data hints to the opposite; that tiRNA are cytotoxic or at least related to apoptosis initiation. tRNA cleavage also was shown to be affected by tRNA modifications via different enzymes in the cytosol and mitochondria. In this review, we will highlight the biology of tRNA cleavage, show the evidence of it being cytoprotective or a marker of cell death and shed a light on its role in disease models and human diseases as well as possible future directions in this field of RNA research.

Key Words: *angiogenin; apoptosis; cell stress; RNA modification; stress granules; stroke; tiRNA; translation repression; tRNA; tRNA cleavage*

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Introduction

tRNA is a ubiquitous RNA present across all forms of life. Traditionally, its function is to transfer amino acids to the ribosome-mRNA complex for protein synthesis (Anderson and Ivanov, 2014). With the advent of modern RNA sequencing and new analysis tools, tRNAs were found to be a hub for RNA modifications, with more than half of the known RNA modifications to occur in tRNA (Boccaletto et al., 2018). Moreover, tRNA was discovered to be a rich source of small RNA species, produced via different mechanisms under different stress and resting conditions, and yet some of these mechanisms are still not fully understood (Lee et al., 2009; Yamasaki et al., 2009; Lyons et al., 2018). While several small tRNA-derived RNA species' functions were identified in health and in diseases (Yamasaki et al., 2009; Anderson and Ivanov, 2014; Goodarzi et al., 2015; Lyons et al., 2018), we are still scratching the surface of their function, and everyday new evidence to their diverse roles come to light, adding layers of complexity to epigenetic control of the cell biology.

Indeed, several tRNA-derived small RNA species were identified; tiRNAs, which are the product of angiogenin (Ribonuclease 5) cleavage of tRNA at the anti-codon site during stress (Yamasaki et al., 2009; Elkordy et al., 2018, 2019) and tRFs, which are miRNA like fragments, acting in a similar way by Argonaut protein interaction and involving DICER in their generation (Cole et al., 2009; Lee et al., 2009; Martinez et al., 2017; Lyons et al., 2018) are the most well studied small tRNA-derived RNA species.

Here, we will focus this review on the recent advances in stress induced-tRNA cleavage derived fragments, highlighting their production pathways, biological function and prospective translational research applications.

Search Strategy and Selection Criteria

A literature search in PubMed and Scopus database using the keywords: tRNA cleavage, tiRNA, tRNA derived RNA and tRNA derived fragments was performed. Articles and reviews that specifically reported findings on tiRNAs were analyzed and their references also analyzed for any articles not appearing in the master search. The article search was performed between the first and March 10, 2019. Articles included in this review were limited to articles covering tiRNAs and tRNA cleavage under stress.

tRNA Cleavage and Angiogenin

The first evidence of tRNA cleavage was observed in E. coli as a response to bacteriophage infection (Levitz et al., 1990). It was not until 2009 that angiogenin was identified to mediate such a cleavage by two independent groups (Fu et al., 2009; Yamasaki et al., 2009). Angiogenin-mediated tRNA cleavage characteristically occurs at the anti-codon loop of mature tRNA, leading to accumulation of 5′ and 3′ halves in response to stresses (Thompson and Parker, 2009; Yamasaki et al., 2009; Lyons et al., 2018). Other mechanisms for tRNA cleavage were also reported and reviewed in Lyons et al. (2018). However, we will focus in this review on the stress induced angiogenin mediated tRNA cleavage process.

Angiogenin itself is known to promote angiogenesis in tumors (Fett et al., 1985), as well as being a neuroprotective and inflammatory modulating agent (Thomas et al., 2018). And while it promotes tRNA cleavage, leading to translation

repression (Yamasaki et al., 2009; Emara et al., 2010; Ivanov et al., 2011, 2014), it was shown to transcribe ribosomal RNA, promoting protein synthesis and cell growth (Pizzo et al., 2013; Sheng and Xu, 2016). These seemingly contradictory functions of angiogenin are in fact regulated by its subcellular localization (Pizzo et al., 2013). This localization is controlled by the ribonuclease inhibitor 1 (RNH1) (Pizzo et al., 2013). Under growth conditions, angiogenin is localized in the nucleus, promoting rRNA transcription and cell growth, however, under stress conditions it is mobilized to the cytosol to promote stress granule formation and tRNA cleavage leading to translation repression (Yamasaki et al., 2009; Emara et al., 2010; Ivanov et al., 2011; Saikia et al., 2012; Pizzo et al., 2013). Indeed, when RNH1 was knocked down or angiogenin induced tRNA cleavage increased under oxidative stress conditions (Yamasaki et al., 2009; Saikia et al., 2012; Elkordy et al., 2018) indicating a tight control of the cleavage process by their interaction.

Regulation of Cleavage through tRNA Modifications

While still there is no evidence that modifications in the tiRNA halves themselves have an impact on their biological function, given the fact that synthetic unmodified oligo-tiRNAs had the same effect as natural tiRNAs (Ivanov et al., 2011), different mature tRNA modifications were shown to impact tRNA cleavage under different conditions (Tuorto et al., 2012; Martinez-Zamora et al., 2015; Blanco et al., 2016; Liu et al., 2016).

tRNA was shown to be a hub for post-transcriptional modifications, with more than half of the RNA modifications discovered occurring in tRNA species (Machnicka et al., 2014; Oerum et al., 2017; Boccaletto et al., 2018; Lyons et al., 2018). These modifications impact the structure, stability and function of tRNA, leading to wide ranging cellular effects (Liu et al., 2016; Oerum et al., 2017). Of these modifications, methylation modifications are the most common (Oerum et al., 2017; Zhang and Jia, 2018).

One of the most studied methylation modifications of tRNA is the 1-methyladenosine (m^1A) modification. m^1A modifications occur at positions 9, 14, 22, 57, and 58 in cytosolic tRNA, and at positions 9 and 58 in mitochondrial tRNA (Oerum et al., 2017). While m^1A modifications are common in tRNA, they are comparatively rare in mRNA (Clark et al., 2016; Dominissini et al., 2016). The m^1A9 and m1A58 modifications, occurring in both cytosolic and mitochondrial tRNA, were shown to impact the structural stability of tRNA (Voigts-Hoffmann et al., 2007; Liu et al., 2016) as well as tRNA's thermostability (Tomikawa et al., 2010; Oerum et al., 2017). Interestingly, ALKBH1 induction, which is a m1A demethylase, was shown to repress protein translation by reducing the levels of cellular $tRNA^{imet}$ (Liu et al., 2016). Moreover, when ALKBH1 was knocked down, the levels of $tRNA^{imet}$ increased by almost 2 folds compared to control cells (Liu et al., 2016). It is predicted that the demethylation of m^1A58 by ALKBH1 leads to rapid degradation of $tRNA^{imet}$ (Liu et al., 2016), yet the exact nature of this degradation, as well as whether it is angiogenin mediated or not is still unknown.

Another well characterized post-transcriptional methylation modification of the tRNA is the cytosine-5 methylation (m^5C) (Blanco et al., 2014; Machnicka et al., 2014; Boccaletto et al., 2018). Two methyltransferases were shown to impact tRNA function via m^5C methylation regulation; Dnmt2 and Nsun2 (Schaefer et al., 2010; Tuorto et al., 2012; Blanco et al., 2016). Both enzymes, via their m^5C methyltransferase activity, were shown to protect tRNA from stress induced cleavage (Schaefer et al., 2010; Tuorto et al., 2012). Dnmt2, upon heat shock stress, was shown to colocalize with cytoplasmic stress granule (Schaefer et al., 2010), and its deletion indeed increased stress induced tRNA cleavage as well as reduced drosophila flies' resistance to heat stress (Schaefer et al., 2010). On the contrary, Dnmt2 over expression protected tRNA from angiogenin mediated cleavage via m^5C methylation (Schaefer et al., 2010). Nsun2 deletion from tumor cells reduced protein translation and induced an undifferentiated phenotype of the tumors produced (Blanco et al., 2016). Indeed, reduced Nsun2-mediated m^5C methylation leads to increased angiogenin-mediated tRNA cleavage and accumulation of 5′tiRNAs leading to translation repression (Blanco et al., 2014). Although the accumulation of 5′tiRNAs was shown to be protective, Nsun2 deletion from cells rendered them more sensitive to external stressors and to the cytotoxic agent 5-fluorouracil (Okamoto et al., 2014; Blanco et al., 2016). Nsun2, as with Dnmt2, localized to the cytosolic stress granules after cell stress (Blanco et al., 2014) and its deletion rendered cells more sensitive to ultraviolet stress (Blanco et al., 2014). Moreover, Dnmt2 and Nsun2 were shown to be complementary in their m^5C methylation activity (Tuorto et al., 2012). Dnmt2 knockout mice lost cytosine-38 methylation in $tRNA^{Asp}$-GTC, $tRNA^{Val}$-AAC and $tRNA^{Gly}$-GCC (Tuorto et al., 2012). Nsun2 knockout mice, on the other hand, showed no loss of cytosine-38 methylation while methylation at cytosine 34 ($tRNA^{Leu}$-CAA), cytosine 40 ($tRNA^{Gly}$-GCC), cytosines 48 and 49 ($tRNA^{Asp}$-GTC, $tRNA^{Val}$-AAC and $tRNA^{Gly}$-GCC) and cytosine 50 ($tRNA^{Gly}$-GCC) was completely lost in a complementary pattern to Dnmt2 deletion (Tuorto et al., 2012). While disrupting tRNA methylation by deletion either enzymes did not have apparent effects on mice in terms of viability, double knockout mice were smaller at birth compared to their littermates and subsequently died (Tuorto et al., 2012). It's important to note though that Nsun2 deletion causes reduced brain size in mice embryos (Blanco et al., 2014). While Nsun2 deletion did not affect viability, as with double Dnmt2/Nsun2 deletion, it caused cognitive dysfunction in adult mice (Blanco et al., 2014). Analysis of tRNA cleavage from double knockout mice showed increased tRNA cleavage in the common tRNA substrates of the two enzymes, while tRNAs which are methylated by only one enzyme did not show cleavage (Tuorto et al., 2012). Loss of tRNA methylation affected its protein translation ability, and cells deficient in both enzymes were significantly affect-

ed compared to single enzymatic loss (Tuorto et al., 2012).

Another interesting tRNA modifier which was linked to angiogenin mediated tRNA cleavage is GTPBP3 (Martinez-Zamora et al., 2015). GTPBP3 is an evolutionarily conserved mitochondrial GTP-binding protein involved in the tRNA modifications (Li and Guan, 2003). GTPBP3 is involved in modifying the wobble uridine in mitochondrial tRNA (Villarroya et al., 2008; Boutoual et al., 2018). GTPBP3 deletion altered the development and mitochondrial function in zebrafish (Chen et al., 2016) and induced AMP-activated protein kinase activation, increased levels of uncoupling protein 2 and peroxisome proliferator-activated receptor γ, and inactivation of hypoxia-inducible factor-1α in siRNA knocked down cells (Boutoual et al., 2018). Mitochondrial tRNA isolated from GTPBP3 knockdown cells showed increased sensitivity to angiogenin cleavage (Martinez-Zamora et al., 2015). Moreover, GTPBP3 knockdown cells showed deficiency in mitochondrial translation, though this translation repression was mild, and alteration in mitochondrial metabolism (Villarroya et al., 2008; Martinez-Zamora et al., 2015).

Cytoprotective or Cell Death Marker?

Intrinsic and extrinsic apoptosis, while their initiating factors are different, both end up with the activation of effector caspases, leading to the elimination of damaged cells (Hou and Yang, 2013). The initial step of intrinsic apoptosis entails mitochondrial outer membrane depolarization and release of cytochrome C to the cytosol, where it bind to Apaf-1 forming the apoptosome and activating pro-caspase 9 and initiating the downstream apoptotic cascade (Sugawara et al., 1999; Hou and Yang, 2013). Interestingly, both mature and cleaved tRNA were observed to interact with cytochrome C (Mei et al., 2010; Saikia et al., 2014). Saikia et al. (2014) in their report, showed that cytochrome C interacts with tiRNAs *in vitro* in cell extracts and *in vivo* in stressed cells, blocking its oligomerization to Apaf-1. They also showed that Angiogenin was protective to the cells, and that it exerts its protective function via tRNA cleavage (Saikia et al., 2014). Contrary to that, Mei et al. (2010) demonstrated that non-cleaved mature tRNA binds to cytochrome C to block cytochrome C-Apaf-1 oligomerization. The contradictory results came from the fact that when they induced RNA hydrolysis, this oligomerization blocking effect was abolished (Mei et al., 2010). However, it is important to note that they induced tRNA hydrolysis by adding onconase to the cells, and not angiogenin. Thus, differences may arise from different tRNA processing mechanisms, which may explain the different results between both reports. It is important also to note that the abundance of tiRNA fragments is 10 folds less than tRNA (Ivanov et al., 2011), a fact that should be taken into account when dissecting the above mentioned data.

Another mechanism by which tiRNA can protect the cells is via translation repression (Yamasaki et al., 2009; Emara et al., 2010; Ivanov et al., 2011, 2014; Saikia et al., 2012). Reprogramming of protein translation is essential for cells to survive stress and adverse environment (Yamasaki et al., 2009). During stress, endogenous angiogenin-cleaved tRNA was shown to repress translation independent of eukaryotic initiation factor (eIF) 2α (Yamasaki et al., 2009; Emara et al., 2010). Moreover, adding angiogenin or 5′tiRNA fragments (and not 3′tiRNAs) to cell cultures enhanced stress granules formation (Emara et al., 2010; Ivanov et al., 2011). This effect on translation is indeed specific to some tiRNA species, as 5′tiRNA^{Ala} and 5′tiRNA^{Cys} were shown to be more potent translation repressors in comparison to some other species, such as 5′tiRNA^{Val}, which did not have a significant translational repressive activity (Ivanov et al., 2011). Synthetic 5′tiRNA^{Ala} targeted the eIF4F complex and displaced eIF4G from capped and uncapped mRNA (Ivanov et al., 2011). Structurally, both 5′tiRNA^{Ala} and 5′tiRNA^{Cys} have terminal oligoguanine (TOG) motifs, which are very rare in other tRNA species. This TOG motif appears to be crucial for the translation repression activity (Ivanov et al., 2011, 2014; Lyons et al., 2016). Interestingly, while the protein YB-1, a translation repressor itself (Nekrasov et al., 2003), was essential for the ability of 5′tiRNA^{Ala} to stimulate stress granules formation, it did not affects its translation repressive activity, suggesting a separate mechanism by which YB-1 and tiRNAs suppress translation (Ivanov et al., 2011; Lyons et al., 2016). Interestingly, Schwenzer et al. (2019) recently reported that under conditions of oxidative stress, induced by hydrogen peroxide, tRNA was re-imported to the nucleus in a RIDD1-mTor depended mechanism. This nuclear import on selective tRNA species was linked to translation regulation under stress conditions, and to the unfolded protein response (Schwenzer et al., 2019). Whether tRNA cleavage is complementary to tRNA nuclear import or not remains to be determined, but it appears that these two mechanisms may be working in tandem to regulated protein translation under stress.

Elkordy et al. (2018, 2019), however, showed in their work that while angiogenin induced tRNA cleavage and generation of tRNA halves, there was a consistent trend of upregulation of tiRNAs with increasing stress and cell death, that is; the more cell death observed, the more tiRNAs were observed. Their observations hinted to a role of tiRNAs as death signaling molecules, or to be involved in cell death pathways, which they capitalized on in a later report and showed the utility of tiRNAs as biomarkers for cell death assessment (Elkordy et al., 2019).

Interestingly, tRNA-derived small RNA fragment (tRFs) were shown to be onco-suppressors in breast cancer via interacting with YB-1 protein, the same protein reported to interact with tiRNAs (Ivanov et al., 2011; Lyons et al., 2016), and inducing transcription repression (Goodarzi et al., 2015). These implicated tRFs were produced under hypoxic conditions, and by blunting their production, cancer cells were able to evade onco-suppression (Goodarzi et al., 2015). Angiogenin itself can be toxic in certain situations (Thomas et al., 2018). When RNH1 was knocked-out, angiogenin accumulated the cytosol, inducing tRNA cleavage and accumulation. Some of the accumulated tiRNA species were shown to be cytotoxic and potent cell death inducers (Thomas et

al., 2018). Moreover, hypomethylation of tRNA by NSUN2 or Dnmt2 deletion leads to increased vulnerability to angiogenin mediated cleavage (Schaefer et al., 2010; Tuorto et al., 2012; Blanco et al., 2014). This results in accumulation of 5′tiRNA fragments, translation repression, activation of cell stress pathways and increase neuronal apoptosis (Blanco et al., 2014). NSUN2 mutations are known to induce microcephaly and neurologic abnormalities in mice and humans (Martinez et al., 2012; Blanco et al., 2014). In addition, NSUN2 deletion in stem cells renders them hypersensitive to cytotoxic stress (Blanco et al., 2016). As mentioned above, both Nsun2 and Dnmt2 colocalize to the cytosolic stress granules under stress conditions (Schaefer et al., 2010; Blanco et al., 2014). Since both enzymes confer an increased stability to tRNA, the exact regulation of tRNA cleavage and 5′tiRNA accumulation in stress granules should be explored in more details to unveil this seemingly complex regulation of tRNA cleavage.

All this together shows how complex the process of interpreting the exact role of tiRNAs in cell biology. It seems that cell specific/stress specific differences do exist, and these discrepancies cannot be settled unless more research is done using different disease models, techniques and approaches. A summary of these mechanisms can be found in **Figure 1**.

Role of Angiogenin and tiRNA in Stem Cells' Biology

Recently two reports have shown that tiRNAs play an important role in regulating stem cell function (Blanco et al., 2016; Goncalves et al., 2016). Blanco et al. (2016) reported that the regulation of protein synthesis via Nsun2-mediated tRNA methylation modifications maintain stem cells and tumor stem cells, and that deletion of Nsun2 resulted in more undifferentiated tumors in mice. Moreover, this translation repression induced by Nsun2 deletion disrupted tumor stem cells' response to cytotoxic stress (Blanco et al., 2016). Goncalves et al. (2016) reported a non-cell autonomous role of angiogenin for the maintenance of hematopoiesis. Angiogenin reduces the proliferative capacity of hematopoietic stem/progenitor cells by increasing tiRNAs and reducing protein synthesis thus maintaining quiescence. On the other hand, Angiogenin when added to myeloproliferative cells enhanced rRNA transcription and protein synthesis. Indeed, at baseline hematopoietic stem/progenitor cells had higher tiRNA levels in comparison to myeloproliferative cells (Goncalves et al., 2016). This confirms that angiogenin and tiRNA generation have a degree of cell-type specificity that is still largely unexplored, and also show a physiologic role for tiRNAs, extending their functions beyond cell stress situations.

tiRNAs in Diseases

Recently, tiRNAs were shown to associate with different diseases. Mishima et al. (2014) showed that tRNA undergoes conformational changes and cleavage under oxidative stress in renal and hepatic rodent injury models. Using an antibody against the m^1A modification in tRNA, which detects m^1A signal after the tRNA loses its tertiary structure under oxidative stress (Mishima et al., 2014, 2015), conformational changes in tRNA were detected much faster that apoptosis and DNA damage in hepatic ischemic injury and in Cisplatin kidney injury models in mice (Mishima et al., 2014). Mishima et al. (2014) used the fact that m^1A is detectable using ELISA and liquid chromatography-mass spectrometry to detect its level in patients undergoing aortic surgery and chronic kidney disease patients and found a correlation between peripheral m1A levels and the degree of tissue damage and mortality. 5′tiRNA halves were also abundant in livers of patients with hepatitis B and C infections (Selitsky et al., 2015), however their levels were reduced in cancerous hepatic lesions (Selitsky et al., 2015). This resonates with the similar observation of reduced tRFs in aggressive breast cancer lesion (Goodarzi et al., 2015), suggesting a common or complementary functional relationship between tiRNAs and tRFs at least in malignancies.

tRNA cleavage was also shown to play a role in neurodegenerative diseases. Nsun2, which when mutated is associated with Dubowitz-like syndrome in humans (Martinez et al., 2012), was shown to regulated tRNA cleavage via methylation modification as we discussed above (Blanco et al., 2014). Angiogenin was also shown to be a risk factor for Parkinson's disease and amyotrophic lateral sclerosis (van Es et al., 2011). Progranulin mutations, in particular progran-

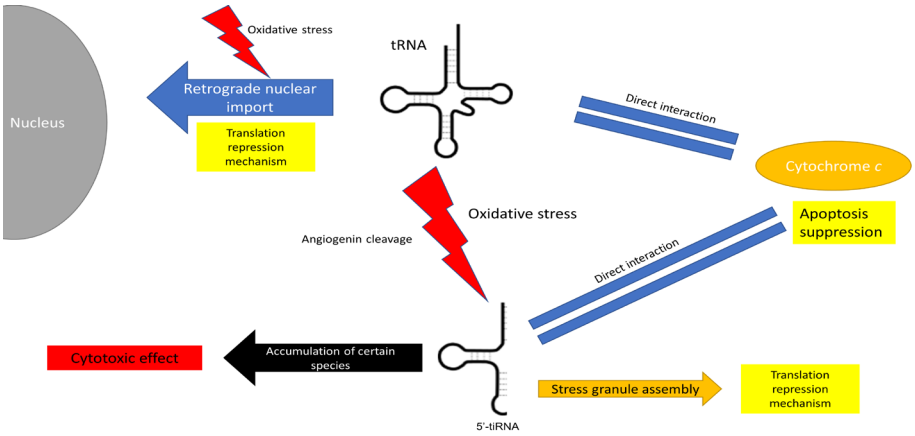

Figure 1 A schematic simplified summary of the effects of tRNA and tiRNA on cell survival under stress.
Under stress condition tRNA can be retrogradely transported to the nucleus to suppress translation. tRNA itself can retard apoptosis by binding with cytochrome C. Under cellular stress conditions, Angiogenin cleaves tRNA leading to 3′ and 5′tiRNA fragments formation. So far only 5′tiRNA fragments were shown to have biological function. 5′tiRNA fragments can bind to cytochrome C, retarding apoptosis. 5′tiRNA fragments also induce translational repression, leading to cellular protection. Accumulation of certain 5′tiRNA fragments can also be cytotoxic, indicating an underlying complex regulatory mechanism that is not fully explored yet.

ulin A9D, were shown to be linked to amyotrophic lateral sclerosis and dementia as well (Baker et al., 2006; Schymick et al., 2007; Li et al., 2018). Progranulin A9D was shown to retard angiogenin's localization to the stress granules and induce apoptosis (Li et al., 2018), while 5'tiRNAAla was able to rescue the cells from this effect (Li et al., 2018).

Recently Elkordy et al. (2019) developed an *in vitro* ischemia/reperfusion (I/R) injury model, in order to study the mechanism of I/R injuring in cell cultures. Using this model, they were able to show that tiRNAs were generated in proportion to the level of injury from I/R (Elkordy et al., 2019). They also utilized the same antibody used by (Mishima et al., 2014) to evaluate the levels of tiRNAs harboring the m^1A modification (Mishima et al., 2015). Using the anti-m^1A antibody they were able to detect m^1A containing tiRNAs after I/R injury, again proportionate to the degree of cell death (Elkordy et al., 2019). In the same work, they tested the possibility of utilizing tiRNAs as a biomarker for stroke therapy. Elkordy et al. (2019) used Minocycline, a tetracycline antibiotic that was shown to be neuroprotective in hyperperfusion syndrome (Rashad et al., 2016), to test whether cellular protective therapies would impact the level of tiRNAs. Indeed, they observed a difference between treatment and no treatment in cell cultures exposed to I/R insult, providing first evidence of the utility of tiRNAs and m^1A as biomarkers for stroke (Elkordy et al., 2019).

tiRNAs were also detected in animal model of I/R as well as hindlimb ischemia (Li et al., 2016). In the same report, when oligo-tiRNA particles (synthetic tiRNA particles) were transfected to endothelial cells they induced angiogenesis repression (Li et al., 2016). This angiogenesis repression might be a sequel of translation repression as adding synthetic tiRNA particles in other reports induced stress granule formation and translation repression (Yamasaki et al., 2009; Emara et al., 2010; Ivanov et al., 2011). However, the exact mechanism was not highlighted in that report.

tRNA fragments were detected in the urine of cancer patients almost 4 decades ago (Borek et al., 1977; Speer et al., 1979). 5′tiRNA halves were also detected in the plasma using next generation sequencing techniques (Dhahbi et al., 2013). 5′tiRNA halves circulate in the blood in stable 100–300 kDa particles, and not in exosomes or extracellular vesicles (Dhahbi et al., 2013). This indicates a great value as biomarkers for diseases, although this notion is not fully exploited yet.

Future Directions

Several open questions still exist regarding tiRNAs biology and function. Does the regulation of tRNA cleavage extend beyond Angiogenin, RNH1 and the tRNA modifiers we reviewed herein? Is the effect on cell survival a cell-specific one or a non-specific one? Why adding 5′tiRNA particles protected cells while inducing tRNA cleavage via enhancing demethylation had the opposite effect? What is the effect of modifications on the function of tiRNA fragments? Moreover, is the cleavage specific to specific pathologies (in terms of the species of tRNA cleaved) or is it a non-specific cleavage of all tRNA species?

All these questions need to be addressed in future research, in parallel with studies exploring the value of tiRNAs as biomarkers for diseases and/or therapy. So far it seems that tRNA cleavage is a very sensitive biomarker for cell stress. How sensitive tiRNA detection is in the clinical setting, however, is yet to be explored.

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