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Review Article

Role of tRNA-derived fragments in cancer: novel diagnostic and therapeutic targets tRFs in cancer

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Abstract: Recent studies have revealed that tRNAs are not always the terminal molecules and small RNA fragments can be mapped to precursor tRNA sequences or mature tRNA sequences. tRNA-derived fragments (tRFs) are a novel class of small RNAs in miRNA-size found in a diverse range of organisms and can be the source of small regulatory RNAs, a previously unanticipated concept. tRFs have a diverse range of effects on cells involving in cell differentiation and homeostasis. They play a critical role in pathological processes, particularly in cancer, and therefore can modulate complicated regulatory networks. Recent studies on the role of tRFs in tumorigenesis suggest that they are promising targets for diagnosis and therapeutics. Improvement in experimental and computational approaches permit a greater understanding of the regulatory networks and will have a significant impact on both basic and clinical research.

Keywords: tRNA-derived fragments, cancer, transfer RNA, microRNA, piRNA, diagnosis, treatment

Introduction

Only about 1% of the human genome is composed of protein-coding genes. Our understanding of the rest of the genome, namely noncoding genes, is still limited. The discovery of tens of thousands of noncoding RNAs (ncRNAs), possessing either infrastructural or regulatory functions, has changed our way of thinking about genetics, physiology, pathophysiology and treatment of diseases including cancer [1]. In recent years, it becomes clear that some ncRNAs are relatively stable and can serve as biomarkers for cancer diagnosis, treatment and prognosis. Complementary oligonucleotides can be effective treatments in some animal models and human clinical trials [2].

Infrastructural ncRNAs are composed of transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), while regulatory ncRNAs comprises long ncRNAs (lncRNAs, >200 nucleotides) and small ncRNAs (sRNAs, <200 nucleotides). Small RNAs consist of microRNAs (miRNAs), Piwi-interacting RNAs (piRNAs), tRNA-derived fragments (tRFs), small interfering RNAs (siRNAs), small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs) and exosomal RNAs (exRNAs). A large variety of sRNA fragments are derived from the processing and fragmentation of longer transcripts, such as tRNAs. They have been identified and classified by high throughput sequencing methods and bioinformatic analysis.

The tRNA, one of the most abundant cellular ncRNAs, plays a crucial role in protein translation. Recent experimental and computational data have revealed that tRNAs are not always the terminal molecules. Furthermore, fragments derived from tRNAs (tRFs) can be a source of small regulatory RNAs. tRFs are a novel class of small RNAs in miRNA-size with precise ends found in a diverse range of organisms, from bacteria to humans. This suggests that tRFs may be associated with diverse cellular biological processes, besides protein translation. Thus, they may serve as functional molecules in human disease. Accumulating evidence has demonstrated that tRFs play a critical role in human cancer [3-12] and are promis-

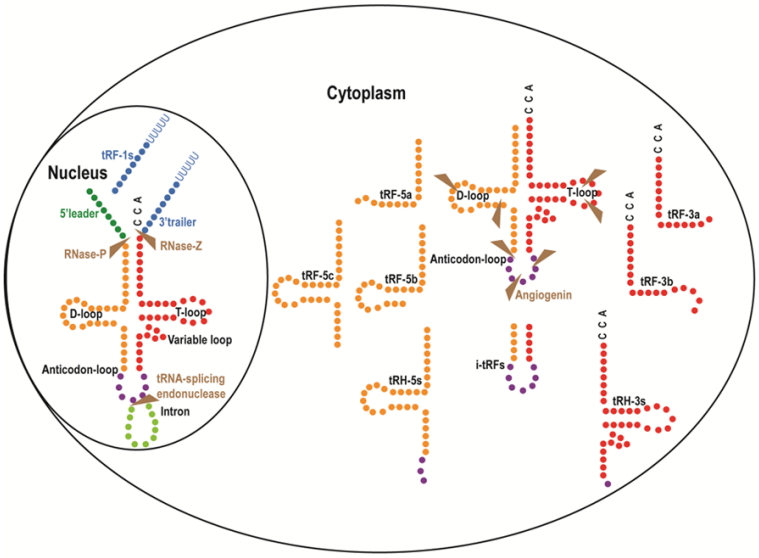

Figure 1. Biogenesis of tRNA-derived fragments (tRFs). tRFs have different lengths. The colors denote different origin. Green circles indicate an intron present in some tRNAs.

ing as diagnostic biomarkers and therapeutic targets in the management of cancer. tRFs can modulate protein translation and interact with ribosomes and aminoacyl tRNA synthetases [13, 14]. In addition, tRFs can associate with Ago and Piwi proteins in a cell-type specific manner, potentially affecting gene expression. Moreover, the interaction between RNA-binding proteins and tRFs [4, 15] may be involved in cancer development and metastasis. Taken together, these findings strongly suggest the involvement of tRFs in tumorigenesis. This article will overview tRFs and their association with cancer, focusing on their biogenesis and biological functions as regulatory ncRNAs, as well as their diagnostic and therapeutic potential.

Biogenesis

Beyond their role in translation, tRNAs further serve as precursors for tRFs. Initially, researchers misclassified tRFs as miRNAs or piRNAs without realizing they were actually derived from tRNAs. tRFs can be classified into three distinct categories based on their origin and length (**Figure 1**): 1) tRF-1s; 2) tRF-3s, tRF-5s and internal tRFs (i-tRFs or tRF-2s); and 3) tRNA halves (tRHs or tiRNAs), namely tRH-3s and tRH-5s. Each class of tRFs is generated by specific ribonucleases and regulated by specific pathways. The main characteristics and biogenesis of each type of tRFs are described below.

tRF-1s

tRF-1s, also called tsRNAs, are derived from the 3' trailer of primary tRNA and formed during the tRNA precursor sequence maturation process. They span from the 3' end of the tRNA to the stretch of four Ts which is the stop signal for RNA pol III (**Figure 1**). Generally, tRF-1s vary in length depending on the location of termination signal in each precursor tRNA. Usually, tRF-1s are characterized as single-stranded small ncRNAs with 16-48 nucleotides that are generated in the nucleus by RNase Z. As tRF-1s do not arise from redundant consensus sequences in precursor tRNA, they are distinct in the sequence. Although both tRF-1s and tRF-3s arise from the 3' end of tRNA (poly 'U' in tRF-1s and CCA in tRF-3s), they target distinct 3' UTRs due to their different 5' ends.

tRF-3s, tRF-5s and i-tRFs

tRF-3s and tRF-5s originate from the 3' and 5' ends of mature tRNAs respectively and can be produced in a Dicer-dependent or Dicer-independent manner [5, 7, 16-18]. The enzymes responsible for their production are currently under investigation. tRF-3s are subclassified into two types: 1) tRF-3a (18 bases) single stranded and cleaved right before the T-loop; and 2) tRF-3b (22 bases) single stranded and cleaved within the T-loop. tRF-5s are subclassified into three types (**Figure 1**): 1) tRF-5a (14-16 bases) mostly single stranded and cleaved right before the D-loop; 2) tRF-5b (22-24 bases) partially double stranded and cleaved right after the D-loop; and 3) tRF-5c (28-30 bases) double stranded and cleaved right before the anticodon loop. As tRF-3s and tRF-5s are derived from functionally conserved mature tRNAs, they may be present across species. i-tRFs [4, 19] have been discovered and characterized recently. They are derived from internal region of mature tRNAs (**Figure 1**) and

are highly abundant and may be involved in many pathological processes.

tRNA halves

tRNA halves (tRHs) are the earliest discovered tRFs. They are distinctly cleaved from anticodon loops of mature tRNA containing 31-40 nucleotides. Previously, tRHs were known as stress-induced fragments. However, they can be detected under non-stressed conditions too. tRHs are grouped into two subclasses (**Figure 1**): 1) tRH-5s (from the 5' end of mature tRNAs to the anticodon loop), 2) tRH-3s (from the anti-codon loop to the 3' end of mature tRNAs). tRHs are produced by the ribonuclease angiogenin (ANG) and thus possess a 5' hydroxyl instead of a 5' phosphate, in contrast to Dicer or Rnase III enzymes to produce miRNAs and siRNAs [20].

Distribution

Some 3' trailers derived from precursor tRNA are generated in the nucleus. They accumulate and are exported by yet unidentified mechanisms. For example, this was found for tRF-1001 (one of tRF-1s) [12]. Usually, tRF-5s are found in the nucleus of HeLa cells, while tRF-3s and tRF-1s are in the cytoplasm [18, 21]. tRFs are also found in the cytoplasm and they may serve as mediators for information exchange between the mitochondria and the nucleus [13].

Role in cancer

tRF-1s

In 2010, it was demonstrated that 3' trailer-derived tRNA are capable of regulating small RNA silencing by differential Argonaute association [6]. miR-3676 (recently removed from miRbase) is a tRF-1 generated during tRNA processing. In B-cell chronic lymphocytic leukemia (CLL), miR-3676 can inhibit the expression of TCL1 by targeting the 3' UTR of TCL1 [39]. Low levels of miR-3676 are significantly associated with four forms of CLL (carrying 11q deletions, 13q deletions, 17p deletions or a normal karyotype) compared with normal CD19+ cord blood and peripheral blood B cells [39]. Similarly, miR-4521/3676, part of the tRF-1s group downregulates and mutates in CLL and lung cancers [40]. Like miRNAs and piRNAs, these two tRFs can associate with the Piwi-like protein 2 complexes and serve as oncogenes or tumor suppressors in hematopoietic malignancies and solid tumors [40]. Veronica et al. investigated tRF signatures in colon, breast and ovarian cancer patients and corresponding cell lines. The expression level of tRFs changed with oncogene activation and cancer progression. In addition, expression of ts-101 and ts-46 (tRF-1s) is associated with chromatin structure, cell survival, cell proliferation and apoptosis [41]. Overexpression of these tRF-1s can strongly inhibit colony formation [41]. Furthermore, tRF-1001 depletion impaired cell proliferation with decreased DNA synthesis and increased G2-phase cells [42].

tRF-3s

tRF-3s play a fundamental role in tumorigenesis and development. Some tRF-3s have similar functions to known miRNAs and piRNAs, binding to Ago or Piwi proteins and playing a regulatory role in tumor, while others affect proliferation, apoptosis and metastasis of tumor cells through regulation of ribosomal proteins and oncogenes.

Two related miRNAs (miR-1274a and miR-1274b) share an 18-nucleotide sequence with tRNA-Lys3 and tRNA-Lys5, respectively. This indicates that they are not bona fide miRNAs but tRFs. Moreover, the percentage of tRNA-Lys3 and tRNA-Lys5 is similar to the known tRNA ratio [16]. In the same study, the miR-720, which has an identical 3' CCA motif to tRNA-Thr, was also reported to be a tRF rather than a miRNA [16]. Li et al. found that in 105 breast cancer patient samples, the elevated level of 3' tRNA-Thr was associated with increase in overall survival [27]. Additionally, 3' tRNA-Thr could suppress epithelial-mesenchymal transition and metastasis with a downregulation of E-cadherin and upregulation of N-cadherin and MMP-2 [28-30] in breast cancer cells by targeting TWIST1. The downregulation was positively associated with HER2 expression and negatively associated with VEGF [27]. Similarly, CU1276 (22nt-tRF-3) in mature B cells shared structural and regulatory traits with miRNAs, including DICER1-dependent biogenesis and a physical association with four Ago proteins. Furthermore, CU1276 was capable of binding the endogenous 3' UTRs of RPA1 and

suppressing mRNA transcription in a miRNA-like fashion in lymphoma cells. Consequently, CU1276 was confirmed to inhibit proliferation and regulate the molecular response to DNA damage [17]. A recent meta-analysis demonstrated that tRF-3s and tRF-5s, rather than tRF-1s, are enriched in Ago1, 3 and 4 PAR-CLIP (photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation) data and associate with mRNAs. Analysis of Ago1 CLASH (crosslinking, ligation and sequencing of hybrids) data further revealed that tRF-5s and tRF-3s tend to interact with a large number of human cellular RNAs [18]. Additionally, ~18- to 22-nucleotide tRFs can interact with Twi12, a tetrahymena Piwi protein essential for growth, in mouse embryonic stem cells and human cancer cells [31].

Additionally, tRFs may regulate the occurrence and development of tumor through other novel pathways with distinct mechanisms [32]. For example, repression of a specific tRF-3 (LeuCAG 3' tsRNA) induced apoptosis *in vitro* and in a xenotransplanted mouse model of patient-derived orthotopic hepatocellular carcinoma through binding to the mRNA of two ribosomal proteins (RPS28 and RPS15) to promote protein synthesis [32]. Anti-Leu 3' tsRNA locked nucleic acids (LNAs) treatment for 4 weeks can significantly reduce tumor growth and final tumor xenograft volume [32], which suggests that LeuCAG 3' tsRNA is a novel target for cancer treatment [32]. Moreover, in human colorectal cancer, miR-1280 (a novel tRF-3 previously described as miRNA) is significantly associated with cancer growth and metastasis. The upregulation of tRF/miR-1280 *in vitro* represses cell proliferation and colony formation. tRF/miR-1280 may target the Notch ligand JAG2 and consequently inhibit cancer stem cell phenotypes through repressing the expression of Gata1/3 and miR-200b transcriptionally [33]. In 2018, Ezequiel et al. demonstrated that 275 tRFs were differentially expressed in MDA-MB-231 (147 downregulated tRFs and 128 upregulated tRFs). The high level of some tRFs after GPAT2 silencing was validated to be related to phospholipid biosynthesis and cell growth, two significant biological processes previously associated with GPAT2 [34]. Interestingly, tRF-3019 was detected abundantly in HTLV-1 virus particles (a crucial gene in adult T-cell leukemia/lymphoma (ATLL)) and capable of enhancing the reverse transcription of HTLV-1 and therefore inhibiting the overall replication of reverse transcription [35].

tRF-5s

tRFs are expressed in a tissue-state-specific and race-specific manner [9, 15, 22]. tRFs affected energy metabolism, cell signaling and immune response in a cohort of patients with triple-negative breast cancer [15]. In a study of prostate cancer with different clinicopathological stages [23] tRFs were significantly downregulated in cancer. The percentage of tRFs produced from tRNA-LysCTT and tRNA-PheGAA was correlated with progression-free survival. Thus, they might be a promising prognostic marker [23]. In testicular germ cell tumors, tRFs are mainly derived from the 5' end of mature tRNAs and the sequences generated from tRNA-GluGAG and tRNA-AspGAY were highly expressed in tumor state [10]. Furthermore, tRF-5s can interact with Piwi protein, similar to piRNAs. For example, immunoprecipitation of Hiwi2 (the human Piwi ortholog protein ubiquitously expressed in different cell types) from MDA-MB-231 cells enriches piRNAs mostly generated from processed tRNAs, indicating that tRF-5s have an uncharacterized function in a Piwi-piRNA-like pathway [11].

tRFs also exist in various biofluids as well as cell culture supernatants. tRF-5s and tRH-5s were the most abundant tRFs in exosomes found in plasma or in cell culture medium of liver cancer [24]. Importantly, significantly higher level of four tRFs (tRNA-ValTAC-3, tRNA-GlyTCC-5, tRNA-ValAAC-5 and tRNA-GluCTC-5) was found in plasma-derived exosomes from hepatocarcinoma patients [24]. Furthermore, tRFs were elevated in MCF7 extracellular vesicles in breast cancer [25]. Thus, tRFs are potential diagnostic biomarkers. Treatment with IL-4 significantly decreased the biogenesis of tRNA-Glu and subsequently, tRNA-Glu-derived tRFs. Furthermore, these tRFs can interact with the PIWIL4 complex and recruit SETDB1, SUV39H1 and heterochromatin protein 1β to the CD1A promoter region and facilitate H3K9 methylation. tRFs are also involved in the differentiation of immune cells, indicating a potential role in tumor immunity [26].

i-tRFs

In 2015, i-tRFs were discovered and characterized by analyzing breast cancer data from The Cancer Genome Atlas (TCGA) [9]. The characteristics of i-tRFs were associated with gender, population, race, amino acid identity, genomic loci, tissue and disease subtype [9]. The expression level of i-tRFs in tumor tissue was significantly altered [9]. Recently, 20,722 tRFs from TCGA datasets representing 32 human cancer types were analyzed, and i-tRFs were the most abundant tRFs [13]. They regulate cellular processes post-transcriptionally [4, 13, 36, 37]. i-tRFs originating from tRNA-Glu, tRNA-Asp, tRNA-Gly, and tRNA-Tyr were found to suppress breast cancer [4, 19]. This set of i-tRFs competitively bind (via their 3' UTRs) with YBX1-binding motifs, which are generally found in multiple oncogenic transcripts, acting as tumor suppressors [4]. In prostate cancer, i-tRFs are the most abundant tRFs (66%) and 42% are derived from mitochrondrial tRNA. Recently, in ovarian cancer circulating tRFs are ubiquitous in serum [38]. Interestingly, the majority of the tRFs were tRF-5s and tRH-5s followed by i-tRFs, while tRF-3s, tRH-3s and tRF-1s were relatively rare [38]. Furthermore, i-tRFs were increased significantly, while no statistical difference among benign, borderline, and cancerous cases. Thus, circulating i-tRFs may play an essential role in cell proliferation and tumorigenesis [38].

tRNA halves

tRNA halves (tRHs) were originally defined as stress-induced RNAs, because they can be induced by numerous stress stimuli, such as oxidative stress, heat/cold shock and irradiation. However, they can also be observed under non-stressed conditions. tRHs can be generated by angiogenin and affect cell proliferation, apoptosis, global translation and epigenetic inheritance. tRHs may associate with Ago and other proteins related to silencing pathways, therefore may be involved in cellular RNAi pathways. Sex hormone-dependent tRNA-derived RNAs (SHOT-RNAs) in exosomes may be used as biomarkers [43]. They accumulate specifically and abundantly in estrogen receptor-positive breast cancer, androgen receptor-positive prostate cancer cell lines and luminal-type breast cancer patient tissues [44]. Moreover, their expression increases after addition of sex hormone and their receptors [44]. Their production and augmented signaling pathways promote cell proliferation, therefore may contribute to tumorigenesis [45].

tRH-5s may preferentially suppress translation both *in vitro* and *in vivo* [46]. However, the tRH-5s used for transfection were gel-purified sRNAs in this study, thus might contain other non-tRNA-derived sRNAs. Therefore, verification is needed. Human RNASET2 is a T2-RNase glycoprotein that is associated with malignancies. Angiogenin treatment can enhance tRNA cleavage and RNASET2 production, consequently affecting tumor growth [47-49]. In other studies, natural and synthetic 5' but not 3' tRNA halves (5'-tiRNAAla and 5'-tiRNACys) collected from angiogenin-treated U2OS (osteosarcoma) cells significantly inhibit translation and trigger a phospho-eIF2α-independent assembly of stress granules (components of the stress response program) in rabbit reticulocyte lysates [50]. Additionally, some tRHs repress translation through displacement of eIF4G/eIF4A from uncapped > capped RNAs and replacing eIF4F, rather eIF4E:4EBP1, from isolated m7G caps. Furthermore, YB-1 (a tRH-associated translational silencer) contributes to angiogenin-, tRH-, and oxidative stress-induced translational inhibition [50].

A tRH-5 generated from tRNA-LeuCAG is found to be elevated in non-small cell lung cancer tissues and cell lines [51]. Depletion of it inhibits cell proliferation and halts the cell cycle. The tRH-5 level in patients' sera is positively correlated with tumor progression, indicating a potential for diagnostic biomarker and therapeutic target [51]. In contrast, tRH-5 produced from tRNA-ValAAC is significantly downregulated in clear-cell renal cell carcinoma (ccRCC). It is inversely associated with tumor staging and grading, which. were confirmed in 118 ccRCC and 74 normal controls [52]. Hence, tRH-5-ValAAC may serve as a diagnostic biomarker. In patients with head and neck squamous cell carcinoma circulating level of tRH-5s from isoacceptors of tRNA-Ala, -Cys, and -Tyr increased, but from tRNA-Arg, -Glu, -Gly, -Lys, -Trp, and -Val decreased [53]. Moreover, in MCF-7 and MCF-10A cells, tRH-5s were significantly higher in extracellular fractions

Table 1. Dysregulation of tRNA-derived framents (tRFs) in cancer

tRF type	Cancer type	Clinical correlation	Refs	tRNA name
tRF-1s	CLL	TCL1 expression and CLL progression	[39]	tRNA-Thr
	CLL & lung	Cancer progression	[40]	
	Multiple	Cell proliferation and DNA synthesis	[42]	tRNA-Ser-TGA-3
tRF-3s	Breast	Aggressive clinicopathological features, EMT and metastasis	[27]	tRNA-Thr-3
	Lymphoma	Proliferation and DNA damage	[17]	
	Liver	Apoptosis and therapeautic target	[32]	tRNA-Leu-CAG-3
	Colorectal	Cancer stem cell	[33]	
tRF-5s	Prostate	Clinicopathological stages and progression-free survival	[23]	tRNA-Lys-CTT, tRNA-Phe-GAA
	TGCT	Clinicopathological features	[10]	tRNA-Glu-GAG, tRNA-Asp-GAY
	Breast	Cancer progression	[11]	
	Liver	Diagnostic biomarker	[24]	tRNA-Val-TAC-3, tRNA-Gly-TCC-5, tRNA-Val-AAC-5, tRNA-Glu-CTC-5
i-tRFs	Breast	Tumor suppressors	[4]	tRNA-Glu, tRNA-Asp, tRNA-Gly, tRNA-Tyr
tRNA halves	Breast & Prostate	Cell proliferation and tumorigenesis	[44, 45]	
	NSCLC	Cell proliferation and cell cycle	[51]	tRNA-Leu-CAG-5
	ccRCC	Tumor staging and grading.	[52]	tRNA-Val-AAC-5

Abbreviations: tRF: tRNA-derived fragments; TGCT: testicular germ cell tumor; CLL: B-cell chronic lymphocytic leukemia; SHOT-RNAs: Sex HOrmone-dependent TRNA-derived RNAs; NSCLC: non-small cell lung cancer; ccRCC: clear cell renal cell carcinoma; Refs: references.

(microvesicles, exosomes and ribonucleoprotein complexes) than the intracellular content. Thus, these molecules may exert their action from outside of the cell [54]. Interestingly, tRFs are positively correlated with shorter genes with a higher repeat density, but negatively correlated with longer genes with a lower repeat density, implying a possible dichotomy between cell proliferation and differentiation [13] (**Table 1**).

Other essential functions

Parental sperm tRFs can affect metabolism of offspring [55-57]. tRH-5s and tRF-5s are significantly associated with epigenetic inheritance [55, 56]. Sperm of low-protein-diet mice contained a high level of tRH-5s and tRF-5s (tRF-Gly-CCC, -TCC, and -GCC; tRF-Lys-CTT; and tRF-His-GTG). Interestingly, tRF-GlyGCC, namely tRF-5002c in tRFdb, inhibited the expression of almost 70 genes related to the endogenous retroelement MERVL in embryonic stem cells and embryos. Thus, tRFs may modulate gene expression [55]. Offsprings of high-fat-diet mice have impaired glucose tolerance. tRH-5s from several tRNAs increased in spermatozoa and passed through the epididymis. Injection of tRH-5s into an early embryo downregulated several genes. Many of them have promoter regions base-paired to the tRH-5s found in the spermatozoa [56]. This indicates that tRH-5s may regulate gene expression, by altering the epigenetic states of genes and their promoters.

Challenges and prospects

The advancement of high-throughput sequencing technology permits investigation of sRNAs in unprecedented detail. However, many challenges remain [58, 59]. One of the major problems is associated with isolating bona fide tRFs from a large random pool of sRNA degradation fragments. In addition, the analysis of RNA-seq at tRNA loci imposes some limits. Accurate multi-mapping either across the whole genome or on the tRNA loci alone may lead to ambiguous or erroneous results due to mismatches and tRNA-lookalikes found in the human genome. Furthermore, precursors and mature tRNAs undergo many modifications before and after transportation to the cytoplasm, respectively. Base modification to tRFs

needs consideration, although a recent report indicates a rather limited impact on exploring tRFs through TCGA-wide analysis [13].

Although patients with cancer encounter diverse therapies, acquired drug resistance require targeted and more aggressive treatments, such as chemotherapy. Moreover, the advent of RNA therapies, including those targeting tRFs, bodes well for the future development of therapies against sRNAs that are biologically relevant to tumor initiation and progression. What initially seemed like a complexity in our genome has emerged as an unprecedented opportunity for novel cancer treatments.

Conclusions

In conclusion, we now know that tRFs regulate many cellular biological processes, exert critical functions and possess potential as biomarkers for early diagnosis and treatment of cancer. Although much has been learned about tRFs, our current knowledge is likely to be just the tip of the iceberg. We still need to shed light on multiple aspects of their biogenesis and functions. It is necessary to address the need for the appropriate therapeutic delivery of tRFs.

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Disclosure of conflict of interest

None.

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