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Review

The roles of tRNA-derived fragments and tRNA modifications in cancer: Diagnostic and therapeutic perspectives

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ABSTRACT

Transfer ribonucleic acid (tRNA)-derived small RNAs (tsRNAs), including tRNA-derived fragments (tRFs) and tRNA halves, are factors that significantly contribute to the initiation, progression, and resistance to treatment processes of cancer. It is shown that tRNA processing, cleavage, and modification patterns are significantly altered in many types of malignancies. These alterations influence codon-dependent translation, messenger RNA stability, cellular stress responses, and the accuracy of protein synthesis, thereby promoting tumor growth and metastatic potential. Irregular tRNA modifications can reprogram translation to support oncogenic signaling and create therapeutic vulnerabilities. Therefore, targeting tRNA-modifying enzymes and reprogramming translation has been proposed as a promising therapeutic strategy. The presence and stability of tsRNAs in diverse biofluids, including plasma, serum, urine, and cerebrospinal fluid, underscore their potential as non-invasive diagnostic biomarkers. Studies show that circulating tsRNAs correlate with tumor stage, metastasis, recurrence risk, and overall survival, and may provide higher diagnostic accuracy than traditional microRNAs. This study highlights the role of tRNA modifications and tRFs in cancer biology, translational regulation, and precision diagnostics.

Keywords: Cancer biomarkers, tiRNAs, tRFs, tRNA modifications. tRNA-derived fragments.

Transfer ribonucleic acids (tRNAs) have long been characterized as fixed and passive components of the protein synthesis mechanism in the field of molecular oncology. However, recent developments involving transcriptomic analysis have shown that these molecules are not solely responsible for transporting amino acids. Their functions involve dynamic regulation in gene expression and cellular stress response processes.[1-3] It has been concluded that disruptions in tRNA processing stages and abnormal chemical modifications are key factors that initiate or accelerate tumor development in cancer biology.[4-8]

There are two main mechanisms by which tumor cells manipulate tRNA biology to meet their metabolic needs and gain survival advantages. First, they alter tRNA modification profiles (e.g., by rearranging methylation patterns), thereby enabling more efficient translation of oncogenic proteins by ribosomes.[2,7,9] In the second mechanism, tumor cells cleave tRNAs at specific sites using specialized ribonucleases, producing small non-coding RNAs known as tRNA-derived fragments (tRFs) and tRNA halves (tiRNAs).[3,5-8,10] These fragments are not random degradation byproducts; they have highly functional roles, including silencing tumor suppressor genes, suppressing protein synthesis, and promoting metastatic processes.[6,8,10-13]

Owing to their remarkable stability in physiological fluids, tRFs and tiRNAs are increasingly recognized as clinically significant molecules.[3,8,14-16] As they exhibit high

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stability in plasma and serum, they constitute reliable candidates for non-invasive liquid biopsy applications.[8,14-16] Some studies have demonstrated that these RNA types provide higher diagnostic sensitivity than traditional biomarkers and are directly related to tumor stage.[3,8,16-19]

In this review, the term tRNA-derived small RNAs (tsRNAs) is used as a general definition encompassing both tRFs and tiRNAs. tRFs are typically 14-32 nucleotides in length and originate from specific cleavage sites of precursor or mature tRNAs, whereas tiRNAs (30-50 nucleotides) are generated primarily under stress conditions through angiogenin-mediated cleavage at the anticodon loop.[12]

Building upon these definitions, this review comprehensively examines the molecular basis of abnormal tRNA modifications in cancer cells and tRF/tiRNA biogenesis processes. Rather than focusing solely on the biological mechanisms, their regulatory roles in tumor development are discussed, particularly regarding their potential as novel therapeutic targets and diagnostic markers.[2-4,8,16]

Classification and biogenesis of transfer ribonucleic acid-derived fragments

Transfer RNA-derived fragments are regulatory sequences generally 14-32 nucleotides long and are classified according to the tRNA form from which they derive. Under stress conditions, longer fragments known as tiRNAs (30-50 nucleotides) are generated. Transfer RNA processing mechanisms are complex and tightly coordinated, contributing to the structural heterogeneity of the resulting fragments. In eukaryotic cells, precursor tRNAs (pre-tRNAs) are transcribed by RNA polymerase III and undergo a multistep maturation process involving RNase P-mediated 5′ leader removal, RNase Z (ELAC2)-mediated 3′ trailer cleavage, CCA tail addition, and extensive post-transcriptional chemical modifications. Dysregulation of these processes can directly influence fragment generation

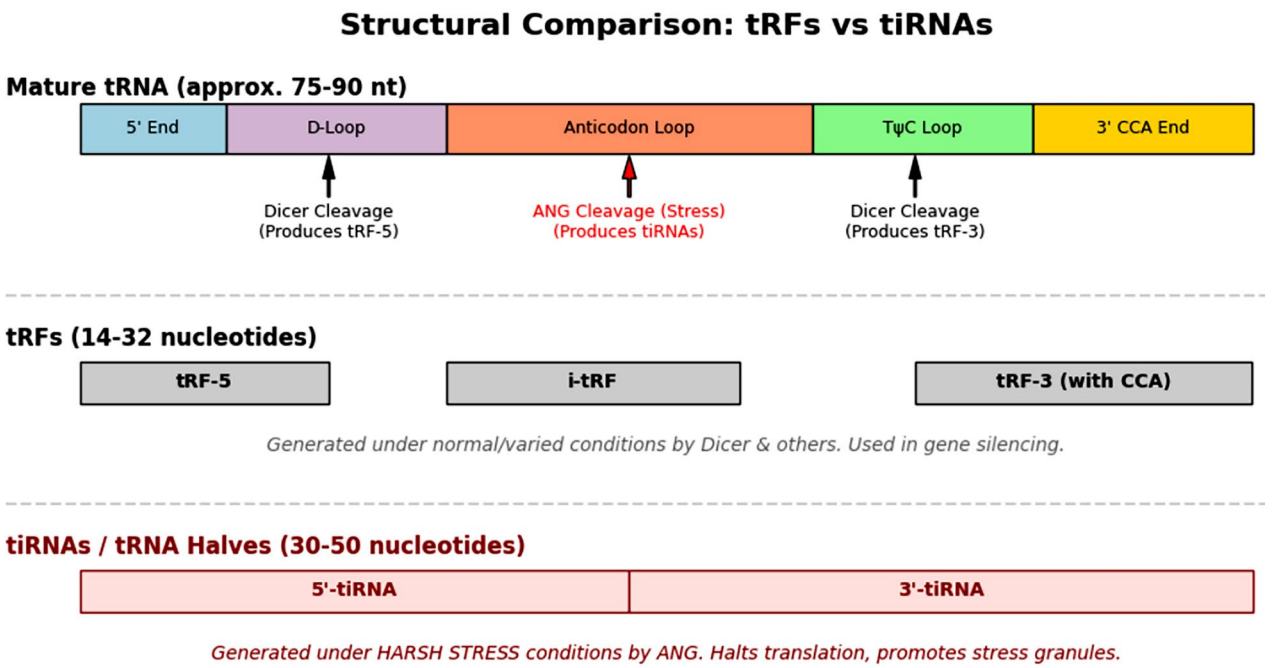


Figure 1. Structural comparison of tRFs and tiRNAs and their cleavage sites: The first illustration shows the basic regions (5' end, D-loop, Anticodon, TψC, and 3' CCA end) of mature tRNA, approximately 75-90 nucleotides in length, used as a reference. The second illustration represents shorter tRF forms (tRF-5, i-tRF, tRF-3) that are 14-32 nucleotides long and are generated by Dicer and similar enzymes cutting from the ends or internal regions. The third illustration shows tiRNA halves (5'-tiRNA and 3'-tiRNA) 30-50 nucleotides in length, which arise from a single central cleavage made by the ANG enzyme at the anticodon loop under cellular stress.

tRFs, transfer ribonucleic acids-derived fragments; tiRNAs, transfer ribonucleic acids halves; tRNAs, transfer ribonucleic acids; ANG, angiogenin.

patterns.[3,6,7,9,19] A structural comparison of tRFs and tiRNAs, including their respective cleavage sites, is illustrated in Figure 1.

Importantly, tRFs and tiRNAs are not passive byproducts of RNA turnover. Accumulating evidence demonstrates that these fragments can directly interact with messenger RNA (mRNA) transcripts, ribosomes, and RNA-binding proteins, thereby fine-tuning gene expression at post-transcriptional and translational levels.[1,3,5-7,10,20] Such multilayered regulatory capacity contributes to tumor cell plasticity and adaptive stress responses.[1,3,5-7,10]

Under stress conditions, the ribonuclease angiogenin (ANG) cleaves mature tRNAs at the anticodon loop, producing 5′- and 3′-tiRNAs.[12] Notably, 5′-tiRNAs are frequently more abundant and have been shown to suppress global translation initiation and promote stress granule assembly, enabling cancer cells to survive hostile microenvironments.[3,5-7,12,18]

Transfer RNA processing mechanisms are complex, so that these fragments acquire a wide structural diversity (heterogeneity).[1,3,6,7,10] tRFs and tiRNAs are far from being simple byproducts or 'cellular trash' of the biogenesis process; on the contrary, they are assigned as active participants in gene regulatory networks.[3,5,6,11,14,21] They can directly interact with mRNA transcripts, ribosomes, and specific RNA-binding proteins.[1,3,5-7,10,19,20] In this way, they fine-tune gene expression at both post-transcriptional and translational levels. The multilayered structure of these control mechanisms ultimately enables cancer

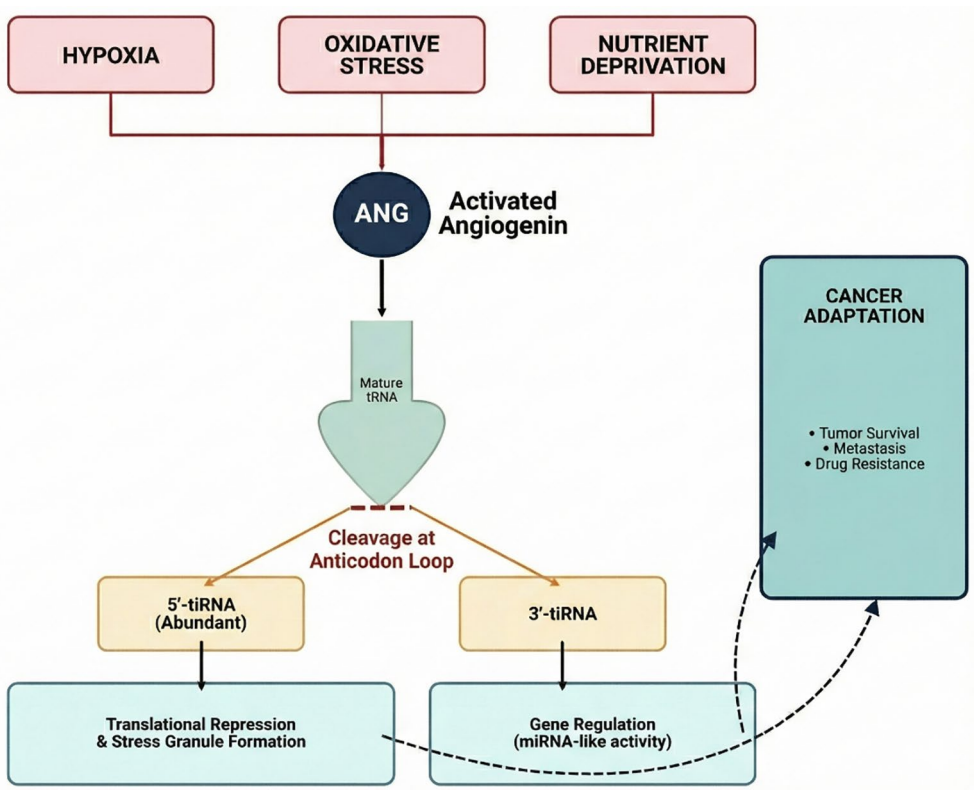


Figure 2. Mechanism of stress-induced tiRNA biogenesis and its functional impact on cancer adaptation: Ribonuclease ANG activation is triggered by cellular stress factors such as hypoxia, oxidative stress, and nutrient deprivation. Activated ANG specifically cleaves mature tRNAs within the anticodon loop, resulting in 5′-tiRNA and 3′-tiRNA fragments. 5′-tiRNA fragments are more abundant and promote translation suppression and stress granule formation. As a result of these molecular events, cancer cells can adapt to hostile microenvironments, and tumor survival, metastatic potential, and resistance to treatment are likely to increase.

tiRNA, transfer ribonucleic acids halve; ANG, angiogenin.

cells to shape their adaptive capabilities (plasticity).[1,3,6,7,10,13] The specific mechanism of stress-induced tiRNA biogenesis and its downstream functional impact on cancer adaptation are illustrated in Figure 2.

The production of tRFs is a process that undergoes intensive regulation, integrated into the stages of transcription, maturation, chemical modification, and transport out of the nucleus, extending from the nucleus to the cytoplasm. In eukaryotic cells, pre-tRNAs are transcribed by RNA polymerase III. These structures must undergo a multi-step enzymatic process to become functional. In this process, the RNase P enzyme cleaves the 5′ leader sequence; the RNase Z enzyme cleaves the 3′ trailer sequence.[3,6,7,14,19,21] Following these steps, the CCA tail is added, and specific post-transcriptional chemical modifications are completed. Once all steps are completed, the molecule reaches its final form.[3,6,7,14,19] The specific tRNA cleavage sites and the biogenesis of resulting fragments are schematically represented in Figure 3.

The subclass known as tRF-1 arises exclusively during the maturation of precursor tRNAs, distinguishing it from fragments derived from mature tRNA forms. During the nuclear maturation phase, the RNase Z enzyme cuts and removes the 3′ trailer sequence, resulting in the formation of these fragments.[21] These fragments have characteristic poly(U) tails, which give them a clear distinction from mature tRNA products.[3,7,11,14,21] On the other hand, the main architect of fragments originating from mature tRNAs is the cytoplasmic Dicer enzyme.[21] The Dicer enzyme targets the D-loop or stem region, thereby generating the tRF-5 series from the 5′ end. Additionally, it has cleavage activity in the TψC loop, resulting in tRF-3 forms that carry the CCA end.[3,5-7,14,21] Apart from all these structures formed, there is another type of fragment that can be generated by a different pathway: these are internal fragments and are named 'i-tRF.' They are formed as a result of cleavage within the anticodon loop by different enzymes.[3,5-7,11]

For tiRNAs, at the center of this defense mechanism is the previously mentioned ribonuclease ANG. Angiogenin makes a specific cut in the anticodon loop of mature tRNAs; as a result of this process, it generates 5′-tiRNA and 3′-tiRNA halves.[1,3,5-7,12] This process typically exhibits an asymmetric character in cancer cells, favoring 5′-tiRNA.[3,5,6,10,12] Consequently, the numerically dominant 5′-tiRNAs undertake critical roles. These roles are known to include displacing translation initiation factors from the ribosome and promoting the assembly of stress granules.[12] Thus, the cell halts global protein synthesis and, on the other hand, prioritizes the production of survival proteins.[3,5,6,8,10,12] Essentially, this pathway functions as a sensitive molecular tuning knob that adjusts translational output according to metabolic needs.[1,3,6,12] The comprehensive stages of this process, spanning from nuclear maturation to cytoplasmic cleavage under normal and stress conditions, are illustrated in Figure 4.

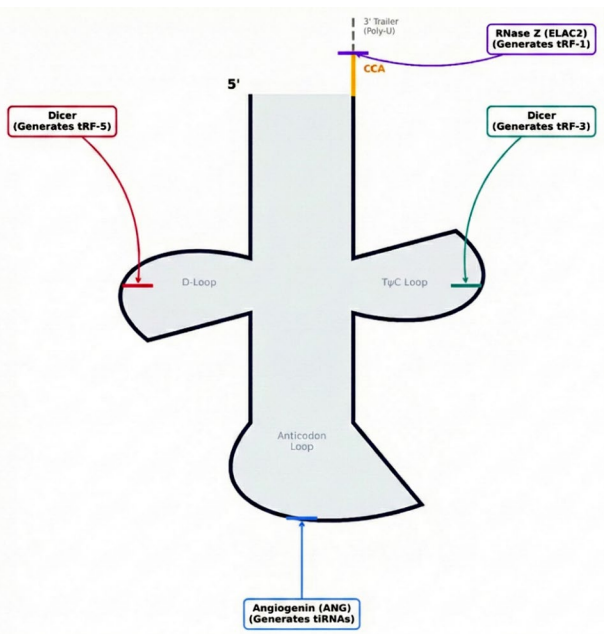


Figure 3. Schematic representation of tRNA cleavage sites and fragment biogenesis: This visual material schematically depicts specific regions of the tRNA molecule targeted by different ribonucleases. For tRF-1 to be produced, the 3′ trailer sequence is processed by RNase Z (ELAC2). Subsequently, Dicer targets the D-loop and TψC loop for the production of tRF-5 and tRF-3 fragments, respectively. Under stress conditions, the anticodon loop is cleaved by ANG, resulting in the production of tRNA halves.

tRNA, transfer ribonucleic acids; tRF, transfer ribonucleic acids-derived fragment; ANG, angiogenin.

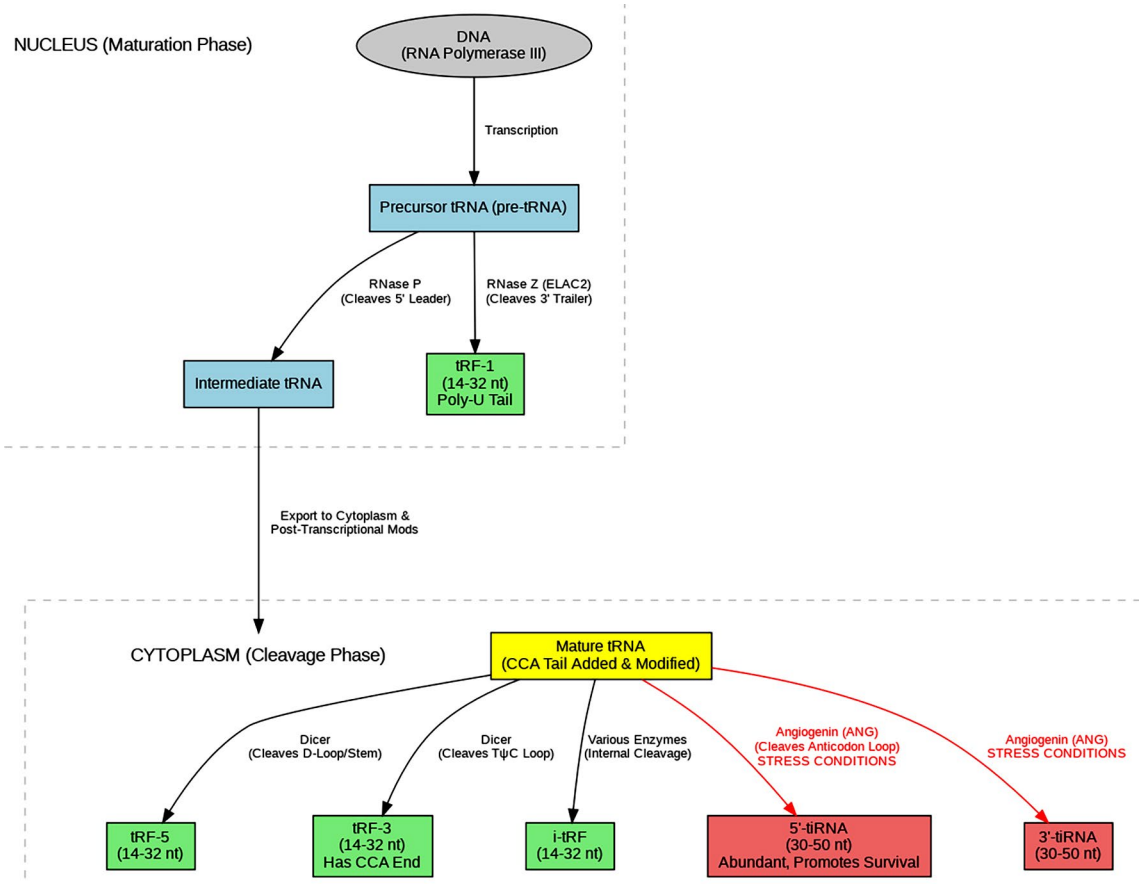


Figure 4. Biogenesis pathways of tRNA-derived small RNAs (tRF and tiRNA): The process consists of two main stages: Maturation in the nucleus and enzymatic cleavage in the cytoplasm. In the stage occurring in the nucleus, the pre-tRNA is processed by RNase Z (ELAC2), resulting in the formation of the poly(U)-tailed tRF-1 fragment. The mature tRNA transferred to the cytoplasm becomes the target of different ribonucleases depending on cellular conditions. In the normal cellular cycle, enzymes such as Dicer produce short fragments (tRF-5, tRF-3, i-tRF); under severe stress conditions (red arrows), the ANG enzyme is activated, cleaving the mature tRNA at the anticodon region and forming tiRNA halves (5'- and 3'-tiRNA).

tRNA, transfer ribonucleic acid; RNA, ribonucleic acids; tRF, transfer ribonucleic acids-derived fragment; tiRNA, transfer ribonucleic acids halve; ANG, angiogenin.

The impact of transfer ribonucleic acid dysregulation on cancer biology

Cancer cells reprogram protein synthesis to sustain proliferation, survive metabolic stress, and adapt to therapy. This reprogramming is not determined only by mRNA abundance. A growing body of evidence indicates that altered tRNA modification pathways and regulated tsRNA production reshape the translational landscape in parallel, thereby creating a more adaptable malignant state.[6,7,9,15,20] The dual impact of abnormal tRNA modifications on cancer progression is illustrated in Figure 5a and b.

Transfer ribonucleic acid modifications and translational bias

Chemical modifications are essential for correct tRNA folding, ribosome interaction, and decoding fidelity. In cancer cells, dysregulation of modification enzymes can generate a translational bias rather than a nonspecific increase in protein synthesis. In practical terms, this means that mRNAs enriched in particular codons may be translated more efficiently when the corresponding tRNAs are hypermodified or more abundant. Such preferential decoding has been linked to oncogenic and proliferation-associated transcripts, including MYC-, CCND1,

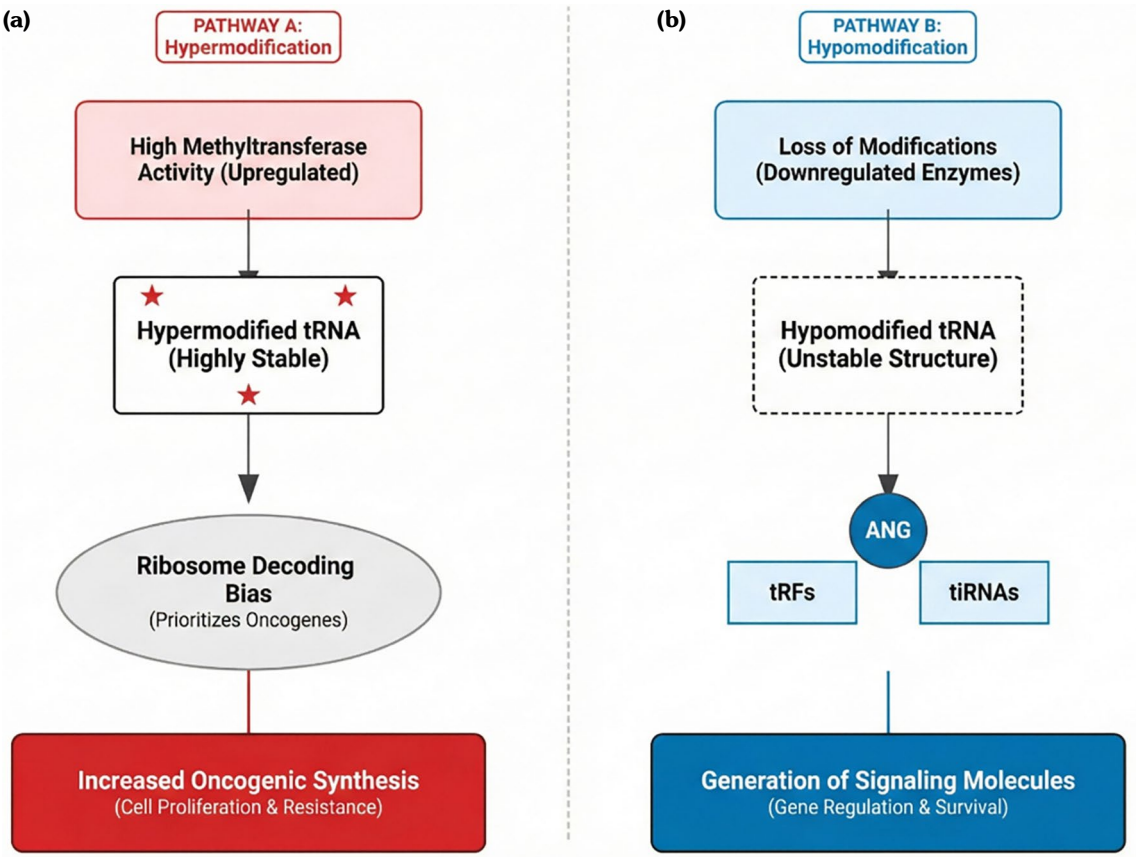


Figure 5. Dual impact of abnormal tRNA modifications on cancer progression: The diagram compares two different mechanisms caused by tRNA irregularities. **(a)** Hypermodified tRNAs are formed as a result of the upregulation of specific methyltransferases, and this situation promotes increased ribosomal decoding efficiency and the selective translation of oncogenic proteins that trigger cell proliferation. **(b)** Loss of critical modifications (hypomodification) destabilizes tRNA structure. This fragility promotes degradation by ribonucleases such as angiogenin, resulting in the production of tRFs and tiRNAs that function as regulatory signaling molecules.

tRNA, transfer ribonucleic acid; tRFs, transfer ribonucleic acids-derived fragments; tiRNAs, transfer ribonucleic acids halves.

and KRAS-related programs in selected tumor contexts.[2,6,7,9,15]

This concept is important in explaining how tumor cells selectively enhance the synthesis of proteins involved in cell-cycle progression, metabolic rewiring, and stress adaptation, rather than uniformly increasing the translation of all transcripts.[20]

Hypomodification and fragment generation

Not all changes favor increased decoding efficiency. Loss of critical tRNA modifications can destabilize tRNA structure and render the molecule more susceptible to cleavage by stress-responsive ribonucleases such as ANG. Under hypoxia, oxidative stress, or treatment pressure, hypomodified tRNAs are therefore more likely to generate tRFs and tiRNAs.[12,13] These fragments can then participate in downstream regulatory programs that help tumor cells tolerate hostile microenvironments.[3,5,6,9,13]

Integrated implications for tumor biology

Taken together, hypermodification and hypomodification should not be viewed as unrelated events. Hypermodification can enhance selective translation of oncogenic transcripts, whereas hypomodification

can expand pools of regulatory tsRNAs. This dual axis allows cancer cells to calibrate protein synthesis dynamically in response to hypoxia, nutrient limitation, or therapeutic stress.[13,20] Enzymes such as methyltransferase-like 1 (METTL1) and NOP2/Sun RNA methyltransferase 2 (NSUN2) illustrate this principle: altered activity of these methyltransferases can influence both translational output and stress adaptation, depending on tumor type and cellular context.[2,3,6,7,9]

Clinically, these converging mechanisms create measurable molecular signatures. Altered expression of tRNA-modifying enzymes and cancer-associated tsRNA profiles have therefore been proposed as candidate biomarkers and as potential intervention points in precision oncology. However, the strength of evidence varies by cancer type, and mechanistic validation remains essential before such signatures can be adopted routinely.[17]

Functional roles of tRFs and tiRNAs in tumorigenesis

Transfer RNA-derived fragments govern gene regulatory networks and regulate carcinogenesis. These molecules directly integrate into signaling pathways that control proliferation, apoptosis, and metastatic invasion.[3,6,7,13,20] They exert their tumorigenic potential through post-transcriptional gene silencing, manipulating translational output, and establishing strategic partnerships with RNA-binding proteins.[3,6,8,13,21]

Post-transcriptional gene silencing

A large portion of the tRF population has the ability to mimic microRNA (miRNA) function at the molecular level. These fragments form a physical complex with the Argonaute (AGO) protein family, thereby constituting the core of the RNA-induced silencing complex.[1,3,5,6,10,21] The role of tRFs after being loaded onto AGO is to act as a guide directing the complex to homologous sequences in regions of target mRNAs that are not located at the 3′ end (3′ UTR).[3,5,7,19,21] This sequence-specific recognition results in the degradation of the target transcript or the blockage of translation. tRFs use this miRNA-like silencing mechanism to deactivate tumor suppressor genes, thereby directly promoting uncontrolled proliferation and survival signals.[3,6,7,10,11]

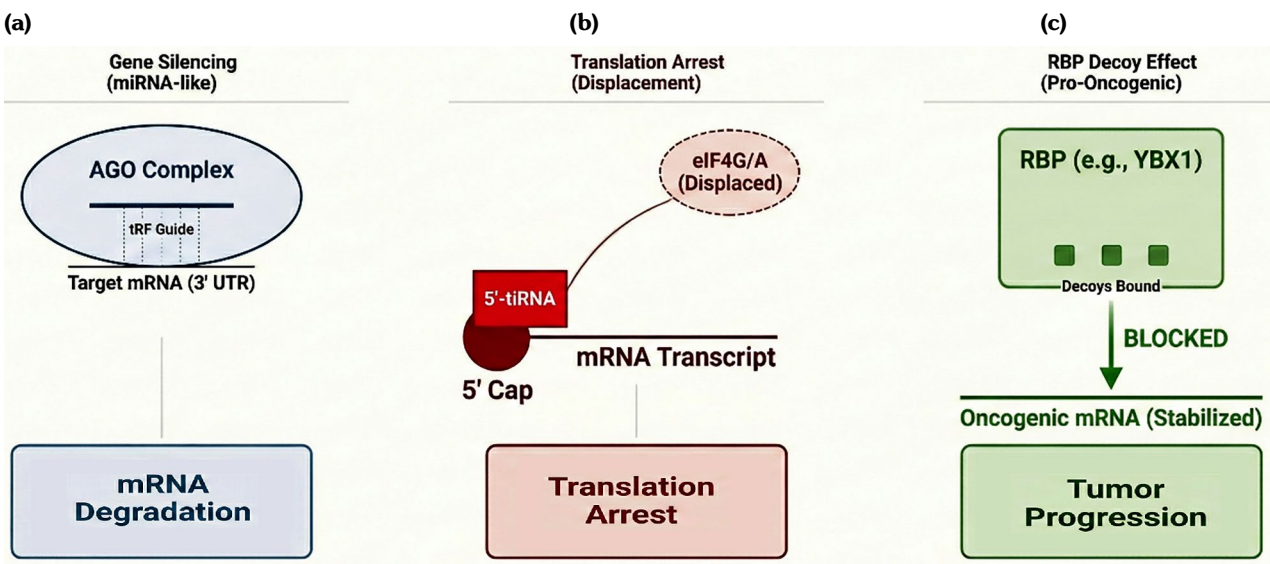


Figure 6. Molecular mechanisms of tRF and tiRNA function in tumorigenesis: The figure illustrates three primary mechanisms of action: **(a)** Small tRFs form a silencing complex that targets and degrades tumor-suppressor mRNAs by binding to AGO proteins. **(b)** Stress-induced 5′-tiRNAs bind to the mRNA cap structure, displacing translation initiation factors (eIFs) and inhibiting overall protein synthesis. **(c)** It is shown that tRFs interact with RNA-binding proteins (RBPs) and act as traps, preventing RBPs from destabilizing oncogenic transcripts.

tRF, transfer ribonucleic acids-derived fragment; tiRNA, transfer ribonucleic acids halve; mRNAs, messenger ribonucleic acids; AGO, Argonaute; RNA, ribonucleic acids; RBPs, RNA-binding proteins.

Modulation of protein synthesis

Transfer RNA fragments that go beyond gene silencing functions physically disrupt the translational machinery. Stress conditions cause the accumulation of ANG-derived tiRNA; these accumulated structures bind to the 5' cap structure of mRNA and inhibit the recruitment of critical translation initiation factors such as eIF4G and eIF4A to the complex.[3,6,7,12,14,15] This molecular displacement disrupts global protein synthesis, and as a result, the cell enters a 'protective hibernation' mode in response to hypoxia or nutrient deprivation.[12,13] However, this blockade is highly selective. General production is paused, while the synthesis of proteins vital for tumor stress adaptation and oncogenic signaling is preferentially preserved.[1,3,6,8,15,20]

Interaction with RNA-binding proteins

The functional repertoire of tRFs also includes competitively binding to RNA-binding proteins (RBPs) or serving as a structural platform. These fragments bind specific RBPs and reassign the stability of oncogenic mRNAs. For example, when tRFs sequester proteins that normally carry out mRNA degradation, they prevent the breakdown of transcripts that trigger metastasis and extend their lifespan.[3,5,6,7,13,19] On the other hand, some fragments that contend with target mRNAs for binding to RBPs disrupt vital cellular processes. These dynamic interactions in the tRF-RBP axis establish a pathogenic cellular environment that fuels tumor invasion and drug resistance.[3,6,9,10,13] The primary molecular mechanisms of tRFs and tiRNAs in tumorigenesis are illustrated in Figure 6a, b, and c.

tRNA-derived fragments as biomarkers in liquid biopsy

Maximizing survival rates in cancer treatment depends on detecting malignancies at early stages. In this context, liquid biopsy overcomes the limitations of surgical tissue sampling and allows the search for tumor-derived molecular traces in body fluids; therefore, it can be defined as a revolutionary alternative.[4,8,11,16,18] Current literature highlights the extraordinary biochemical stability exhibited by tRFs in many biological fluids (from plasma to urine); this feature makes them diagnostically much more reliable compared to unstable traditional markers.[6,8,11,16,18] In discussions on the origin of this molecular robustness, Wang et al.[18] emphasized the critical role of exosomal packaging, while other contemporary studies pointed to the protective shield mechanism formed by RNA-binding proteins.[3,6,9,14]

The workflow underlying tRF-based liquid biopsy applications begins with the release of tRFs from tumor cells into the extracellular environment.[11,16-18] These fragments, either encapsulated within exosomes or bound to carrier proteins, enter the circulation where they exhibit high resistance to enzymatic degradation and maintain their stability.[6,8,10,14,17,18] Due to these properties, tRFs can be efficiently isolated from biological fluids such as blood or urine and quantitatively analyzed using standard molecular techniques, including polymerase chain reaction (PCR) and next-generation sequencing (NGS). The resulting expression profiles provide reliable biomarkers for a wide range of clinical applications, including early diagnosis, prognosis prediction, patient stratification, and real-time monitoring of treatment response.[1,4,8,10,16-18,20]

The circulating profiles of tRNA fragments create disease-specific, consistent molecular signatures rather than random fluctuations.[17] These fragments can specifically distinguish oncology patients from healthy individuals.[8,9,15-17] These patterns generally reflect tissue origin and are directly related to the histological type and clinical stage of tumor. Studies on breast, prostate, and liver cancers have found that specific tRF changes observed outperform conventional protein biomarkers in terms of diagnostic sensitivity.[8,15-18,20] This superior identification power offers the opportunity to capture malignancies at early, still treatable stages.[8,16-18]

Recent profiling studies have identified tRNA fragments that function as suitable biomarkers in certain types of malignancies to explain their diagnostic potential.[4,8,16,18] For example, fragments such as tRF-Arg-CCT-017, 5′-tiRNA-Glu-CTC, and tRF-Leu-CAG have been found to be significantly dysregulated in breast, hepatocellular, and prostate cancers.[1,14,16-,20] The different expression patterns of these specific

molecules help confirm the tissue origin of the tumor. They also carry significant clinical value in non-invasive cancer screening and patient classification.[4,8,10,16-18]

The clinical value of tRFs is not limited to the detection of the disease; it also extends to prognostic evaluation and therapeutic monitoring. Especially when the amount of stress-induced tiRNAs is high, it has been found to show a strong correlation with tumor aggressiveness, disease progression, and low survival rates.[1,3,6,8,9,17] Dynamic changes observed in the levels of these fragments throughout treatment serve as a real-time indicator to measure the effectiveness of the applied therapy or to detect emerging drug resistance.[6,8,9,16,18,20]

Therapeutic potential of targeting tRNA modifications and tRF pathways

Interest in therapeutically targeting tRNA biology stems from the observation that dysregulated tRNA modification and tsRNA production are functionally linked to malignant growth, stress adaptation, and metastasis. At present, most therapeutic evidence remains preclinical and comes from cell culture and animal studies. For that reason, therapeutic statements in this field should be framed as promising but still exploratory. Current approaches can be grouped into three main categories: inhibition of dysregulated tRNA-modifying enzymes, direct neutralization of oncogenic tsRNAs, and interference with stress-induced tsRNA biogenesis.[1,3-6,20] Current strategies highlighted in the literature are grouped into three main categories: silencing modification enzymes, silencing (neutralizing) tRFs, and blocking biogenesis pathways. The overview of therapeutic strategies targeting tRNA modifications and tRF pathways is illustrated in Figure 7a, b, and c.

Targeting tRNA-modifying enzymes

Enzymes such as METTL1 and NSUN2 are attractive targets due to the dependency of certain tumors on hypermodified tRNA pools to sustain selective translation of growth-promoting transcripts. In hepatocellular carcinoma, for example, METTL1-mediated m7G modification has been linked to enhanced translation of proliferation-associated programs, and experimental METTL1 suppression reduced tumor growth in preclinical models. Likewise, NSUN2 overexpression has been reported in breast and squamous cell carcinomas, where its depletion impaired migration or metastatic traits *in vitro* and in xenografts.[2,6,7,9,15]

These findings support a rationale for pharmacological inhibition, but they do not yet establish clinical efficacy. Given that many tRNA-modifying enzymes serve essential functions in normal tissues, the challenge lies in defining therapeutic windows and identifying specific tumor contexts where dependency is strongest.

Direct silencing of oncogenic tsRNAs

A second strategy uses antisense oligonucleotides (ASOs) or related sequence-directed approaches to neutralize individual oncogenic tRFs.[20] These agents are designed to base-pair with a specific target tsRNA, thereby preventing its interaction with AGO complexes, ribosomes, or RNA-binding proteins; in some settings, they may also promote target degradation. Preclinical studies in chronic lymphocytic leukemia and breast cancer models suggest that tsRNA-directed silencing can restore tumor-suppressive pathways and increase apoptosis.[3,6,8,10,11] These molecules bind to the target fragments, creating a barrier; thus, they physically block the infiltration of tRFs into the translational machinery or the occupation of gene silencing complexes (Figure 7b).

Again, the field is at an early stage. The most compelling future applications will likely depend on identifying recurrent, cancer-type-specific tsRNAs with clearly validated driver functions.[17,20]

Blocking stress-induced biogenesis pathways

A third approach seeks to reduce production of pro-survival tiRNAs by targeting upstream biogenesis enzymes, particularly ANG. In principle, ANG inhibition may prevent stress-induced tRNA cleavage, reduce tiRNA accumulation, and sensitize cancer cells to chemotherapy or other cellular stressors.[12] This idea is biologically plausible and supported by preclinical observations; however, the translational path remains uncertain given

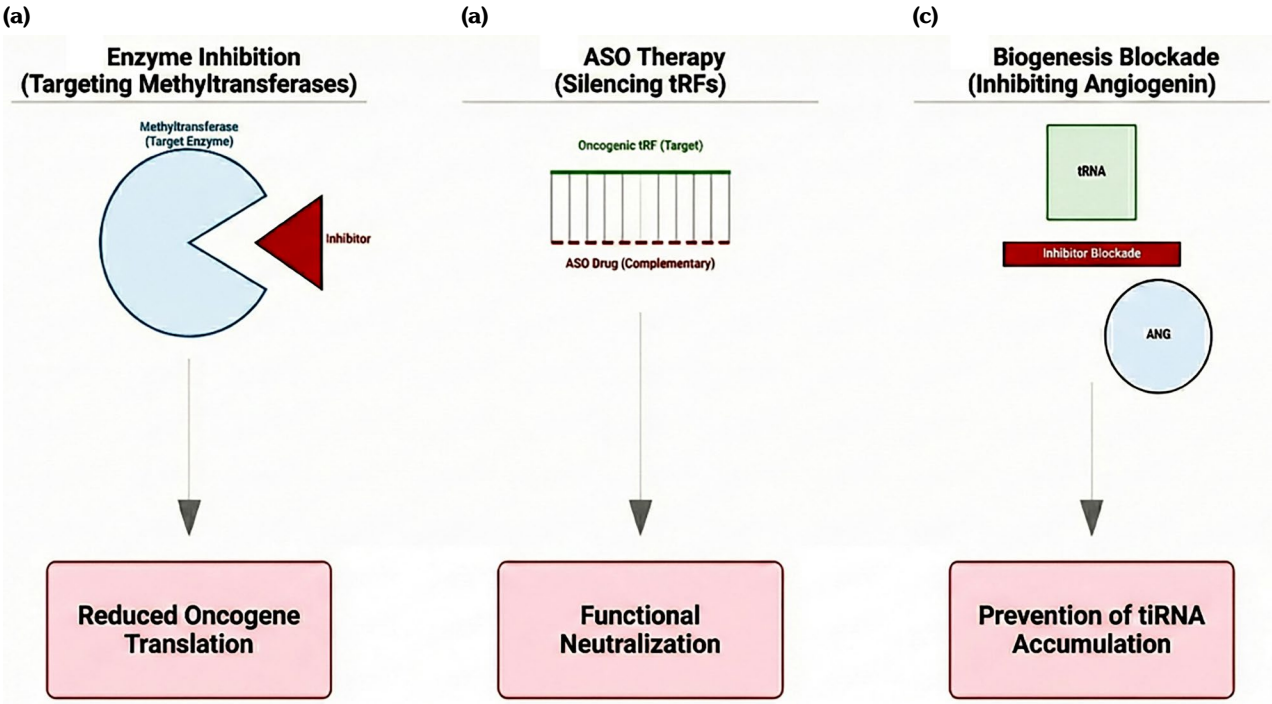


Figure 7. Overview of therapeutic strategies targeting tRNA modifications and tRF pathways: The figure shows three main intervention points in cancer treatment: **(a)** Pharmacological inhibition of up-regulated methyltransferases prevents the formation of hypermodified tRNAs and reduces oncogenic protein translation. **(b)** By binding ASOs to specific oncogenic tRFs, their activity is suppressed and their interactions with cellular molecular mechanisms are prevented. **(c)** Angiogenin inhibitors prevent stress-induced tRNA cleavage and limit the accumulation of tiRNAs that support cancer cell survival.

tRNA, transfer ribonucleic acid; tRF, transfer ribonucleic acids-derived fragment; ASOs, antisense oligonucleotides; tiRNA, transfer ribonucleic acids halve.

ANG's involvement in broader physiological processes.[3,5,7,9] As summarized in Figure 7c, the combination of this approach with standard chemotherapeutics creates a synergistic effect in breaking drug resistance and achieving maximum treatment success.

Overall, the therapeutic relevance of tRNA biology is best described as a developing preclinical opportunity. Promising results justify continued investigation, but robust translational progress will require stronger cancer-type-specific evidence, clearer distinction between preclinical and clinically validated findings, and improved delivery strategies.[20]

Technological advances enabling tRNA and tRF research

The biggest obstacle to unraveling the biology of tRNA and tRF has long been the rigid secondary structures and dense chemical modifications of these molecules. Standard RNA sequencing protocols have failed in these regions, which can be described as 'hard to read. Widespread methylations have blocked the reverse transcriptase enzyme, causing premature termination of complementary DNA synthesis.[6,7,9,11] However, innovations in NGS technologies have made these regions of the transcriptome visible.[17] Demethylation pre-treatments with the AlkB enzyme have been performed, and special enzymes resistant to modification have been developed. Due to these steps, a critical turning point has been reached in obtaining full-length tRNA and tRF profiles.[3,7,11,17]

This advancement in hardware has been accompanied by strategic improvements in bioinformatics analyses. The multiple copy numbers of tRNA genes in the genome and the short structure of tRFs create significant confusion in aligning data to the reference genome.[3,4,6] To overcome this problem, computational tools

and databases specifically designed for tRF analysis, going beyond standard algorithms, have been developed.[17] Through these new tools, researchers can distinguish biologically regulated true signals from random degradation noise.[3,11,16-18] This distinction is vital to reveal the true roles of tRFs in cancer pathogenesis.[20]

With molecular characterization studies, the adaptation of these technologies to the clinical field has gained momentum. Especially in liquid biopsy applications, optimized qRT-PCR protocols and digital PCR platforms offer critical sensitivity in capturing tRFs present in trace amounts in biological fluids (blood, urine, etc.).[8,14,16,17,19,20] However, capturing only the presence of tRFs is not sufficient to complete the clinical picture; mapping specific modification patterns is also essential. At this point, liquid chromatography-tandem mass spectrometry (LC-MS/MS) plays an important role, analyzing small changes in cancer-associated modifications such as m1A or m5C with very sensitive resolution.[2,6,7,9] These technological leaps have established the necessary infrastructure to effectively translate tRF research from the laboratory bench to the patient's bedside.[3,6,8,16,17] The overview of technologies supporting tRNA and tRF research is illustrated in Figure 8.

Current challenges and future directions

Despite substantial progress, several barriers continue to limit the routine clinical implementation of tsRNA-based approaches. First, methodological standardization remains insufficient. Variation in RNA isolation kits, library preparation methods, sequencing platforms, and normalization strategies complicates cross-study comparison and weakens reproducibility. This issue is particularly important for liquid biopsy,

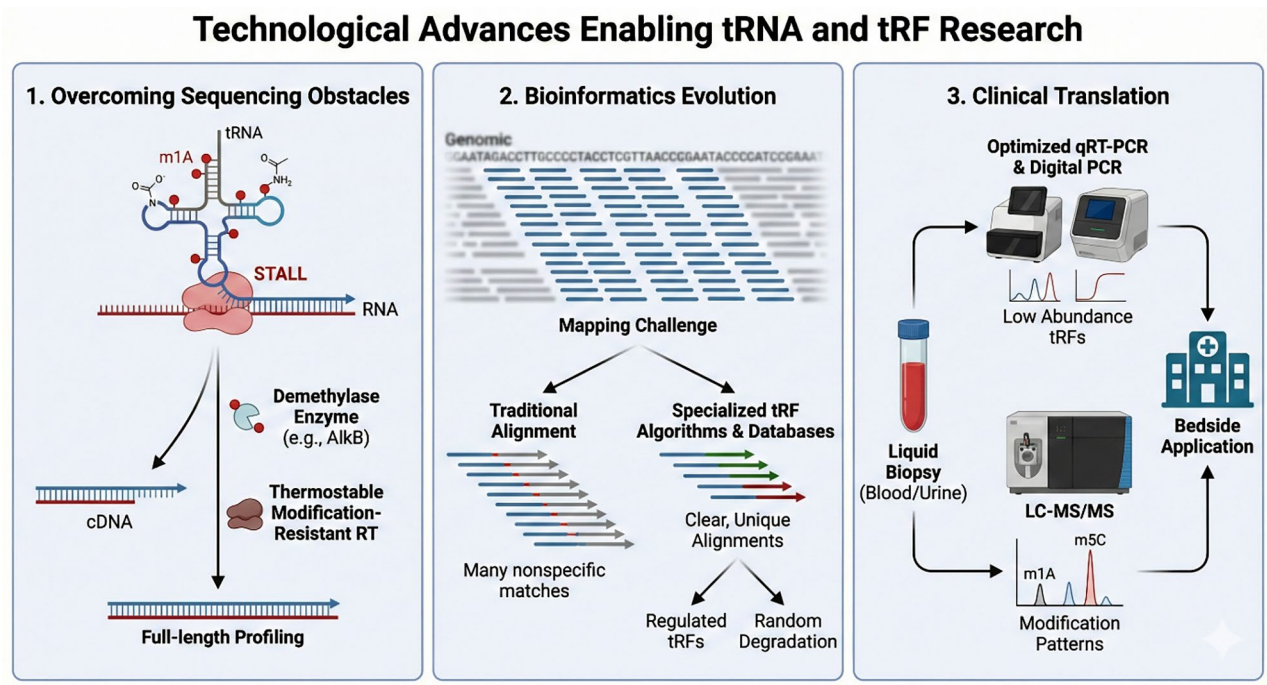


Figure 8. Overview of technologies supporting tRNA and tRF research: This diagram shows the workflow from molecular profiling to clinical application. **(1)** Overcoming sequencing challenges: Full-length complementary DNA (cDNA) is generated by removing modifications using enzymes (such as AlkB) and employing thermostable reverse transcriptases. **(2)** Bioinformatics improvements: Specialized computational tools help distinguish real tRFs from random degradation fragments and make genome mapping more reliable. **(3)** Clinical applications: Precise platforms such as digital PCR and LC-MS/MS enable researchers to accurately measure tRFs and modification patterns in liquid biopsy samples, aiding in diagnosis.

tRNA, transfer ribonucleic acid; tRF, transfer ribonucleic acids-derived fragment; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; LC-MS/MS, liquid chromatography-tandem mass spectrometry.

where preanalytical variables such as sample collection, storage, hemolysis, and extraction efficiency can strongly affect small RNA measurements.[1,7,10,14,16-18]

Second, not all detected tsRNAs are likely to be functional drivers. Much of the current literature is correlation-based, making it difficult to distinguish causal tsRNAs from fragments that simply reflect cellular stress or RNA turnover.[12] Mechanistic studies must therefore move beyond expression profiling and establish tissue-specific target networks, upstream regulators, and loss-of-function phenotypes.[20] The same tsRNA may have oncogenic effects in one cancer type and tumor-suppressive effects in another, which further underscores the need for context-specific interpretation.[3,4,6,8,11,13,18]

Third, therapeutic translation faces safety and delivery constraints. Inhibiting tRNA-modifying enzymes may disrupt malignant translation programs, but these enzymes also contribute to normal cellular homeostasis, raising concerns about off-target toxicity. Likewise, ASO-based strategies require delivery systems that can maintain molecular stability, improve tumor selectivity, and minimize systemic exposure.[20] Nanoparticle-based and other targeted delivery platforms may be especially important for future development.[2,3,6,10]

From a translational perspective, future work should also address practical implementation issues that influence clinical feasibility. These include inter-platform reproducibility, assay harmonization across laboratories, definition of robust endogenous or external controls for normalization, and assessment of cost, turnaround time, and scalability for routine diagnostics. Large multicenter validation studies will be needed before tsRNA panels can be incorporated into clinical workflows.[17]

Looking ahead, the most productive direction for the field will likely combine mechanistic validation with cancer-type-specific biomarker studies and carefully designed translational pipelines.[17,20] Integrating tRNA modification profiles, tsRNA abundance, transcriptomics, and proteomics may ultimately provide a more precise framework for patient stratification and therapeutic targeting in oncology.[17]

In conclusion, tsRNAs and abnormal tRNA modifications play critical roles in cancer development by regulating translation, stress adaptation, and oncogenic signaling pathways. Their high stability and detectability in biological fluids make them promising candidates for non invasive cancer diagnosis, prognosis, and treatment monitoring. Although current findings highlight significant therapeutic and diagnostic potential, further mechanistic investigations and large-scale clinical validation studies are required for their successful integration into precision oncology.

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