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Review

Regulatory Mechanisms of Transfer RNA-Derived Small Non-Coding RNAs in Tumor Immunity

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Abstract

Transfer RNA-derived small RNAs (tsRNAs) are small non-coding RNAs originating from transfer RNAs (tRNAs). Recent studies have revealed that tsRNAs mediate intercellular communication in the tumor microenvironment (TME) via extracellular vesicles such as exosomes and migrasomes, and are extensively involved in modulating the immunogenicity of tumor cells, affecting key immune processes including macrophage polarization, dendritic cell differentiation, natural killer cell cytotoxicity, and T cell activation. This review systematically summarizes the major types of tsRNAs (tRFs and tiRNAs) and their biogenesis pathways, with an emphasis on their regulatory mechanisms at the levels of transcription, translation, epigenetic modification, and intercellular signaling. On this basis, the functional evidence of tsRNAs in tumor immune response and immune evasion, including their association with the efficacy of immune checkpoint inhibitors, their potential as liquid biopsy biomarkers for early tumor diagnosis and prognosis assessment, and their feasibility as novel targets for immunotherapy are further delineated. Current findings suggest that tsRNAs not only reveal a new paradigm of non-coding RNA-mediated regulation of immune responses within the TME at the fundamental level, but also provide a theoretical foundation for the development of tsRNAs-based diagnostic tools and therapeutic strategies in tumor immunotherapy.

Keywords: tsRNAs; tumor immunity; tumor microenvironment; biomarker; tumor immunotherapy

1. Introduction

Tumors remain a leading cause of mortality worldwide. Despite considerable advancements in treatment modalities such as surgery, radiation therapy, and chemotherapy, challenges such as drug resistance and tumor recurrence persist, particularly in advanced cases, necessitating the development of novel therapeutic approaches [1, 2]. In recent years, tumor immunotherapy has emerged as a promising strategy, aiming to enhance the body's immune response to eliminate tumor cells, which is heavily influenced by the tumor microenvironment (TME) [3, 4]. The TME encompasses the microenvironment surrounding tumor cells, including blood vessels, immune cells, fibroblasts, inflammatory cells, various signaling molecules, and extracellular matrix components. Collectively, these elements can mediate both anti-tumor and pro-tumor effects by secreting diverse cytokines, chemokines, and other factors, thereby playing a decisive role in tumor initiation, progression, and response to immunotherapy [5, 6].

Transfer RNA (tRNA)-derived small RNAs (tsRNAs) are small non-coding RNAs (sncRNAs) originating from tRNAs. These fragments, ranging from 15 to 40 nucleotides in length, are produced through specific nuclease cleavage of mature or precursor tRNA under stress conditions such as hypoxia, nutrient deprivation, or DNA damage. tsRNAs include tRNA-derived fragments (tRFs) and

tRNA halves (tiRNAs) [7-9]. Recent research has highlighted the wide-ranging biological functions of tsRNAs and their close association with tumor development [10]. Evidence indicates that tsRNAs can be encapsulated within extracellular vesicles (EVs), such as exosomes and microvesicles, facilitating their transport through the microenvironment to the cytoplasm and extracellular space of recipient cells, where they play crucial roles in intercellular signaling [11, 12]. tsRNAs finely regulate gene expression and cellular processes, participating in cell proliferation, differentiation, cell cycle regulation, epigenetic modulation, and apoptosis, thus rendering them key participants in various physiological and pathological conditions, including tumors [13-17]. Owing to their origin from highly modified tRNAs, tsRNAs exhibit greater stability than other sncRNAs in cells, tissues, and peripheral blood. This positions them as promising candidates for novel, stable, and easily detectable tumor markers, particularly in non-invasive diagnostics, efficacy evaluations, and therapeutic targets for tumor treatment, exhibiting broad application prospects [18, 19].

2. Classification and biogenesis of tsRNAs

Transfer RNA, as a vital class of non-coding RNAs (ncRNAs), possesses a characteristic cloverleaf-shaped secondary structure and an L-shaped tertiary structure. It is responsible for the specific recognition of codons and the delivery of corresponding amino acids to ribosomes during protein synthesis [20]. The secondary structure of tRNA comprises three loops (D, anticodon, and TΨC loops), four stems (D, anticodon, TΨC, and amino acid acceptor stems), and one variable arm [21]. Precursor and mature tRNAs can undergo enzymatic cleavage to yield tRFs ranging from 14 to 30 nucleotides in length. Based on their origin and position on the tRNA molecule, these fragments are categorized into five subtypes: tRF-1, tRF-2, tRF-3, tRF-5, and i-tRF. tRF-1 is derived from the 3′ trailer sequence of precursor tRNAs (pre-tRNAs) and is generated by RNase Z cleavage, typically ranging from 16 to 30 nucleotides in length [22]. Notably, tRF-5 is further subdivided into three subcategories based on cleavage sites: tRF-5a (14–16 nt), tRF-5b (22–24 nt), and tRF-5c (28–30 nt) [23]. Among these, tRF-5a and tRF-5b are generally generated by the endonuclease Dicer, which cleaves the D loop and D stem, respectively, while tRF-5c is formed by cleavage of the anticodon stem [13, 24]. In addition, tRF-3, which arises from T-loop cleavage by enzymes such as Dicer, Angiogenin (ANG), and RNase A, can be further classified into tRF-3a (18 nt) and tRF-3b (22 nt), with cleavage sites located within the TΨC loop [22]. The atypical tsRNAs tRF-2 and i-tRF, discovered in recent years, are produced from nuclear- and mitochondrial-encoded tRNAs. tRF-2 results from cleavage in the anticodon region, while i-tRF predominantly originates from the internal region of mature tRNA, typically between the D and T loops [25]. Furthermore, i-tRF can be further categorized into A-tRF, D-tRF, and V-tRF based on distinct cleavage initiation sites, corresponding to fragments originating from the anticodon loop, D-loop, and variable loop, respectively [26]. However, the specific biological mechanisms and functions of tRF-2 and i-tRF remain to be elucidated [11].

Transfer RNA halves are generated by ANG cleavage of the anticodon loop of mature tRNAs in response to stressors such as hypoxia, oxidative stress, ultraviolet radiation, heat shock, viral infections, and amino acid deficiency, and are therefore referred to as tRNA-derived stress RNAs [11, 13]. Based on the location of the anti-codon cleavage site, tiRNAs are classified into two subtypes: 5′ tiRNAs and 3′ tiRNAs, with lengths ranging from 31 to 40 nucleotides [22, 27]. Notably, 5′ tiRNAs initiate from the 5′ end of mature tRNA and terminate at the anticodon loop, whereas 3′ tiRNAs begin at the anticodon loop and extend to the 3′ end of mature tRNA (Fig. 1) [22, 28, 29]. These tiRNAs play critical roles in stress responses and are implicated in the development of various conditions, including colorectal and gastric tumors, diabetes, renal ischemia-reperfusion injury, and other diseases. Nevertheless, the specific mechanisms underlying their generation and the associated functional pathways have yet to be elucidated [30-34].

3. Regulatory mechanism of tsRNAs: from nucleus to extracellular signaling

3.1. Intracellular regulatory functions

3.1.1. Transcription and post-transcriptional regulation

As a pivotal class of ncRNA regulatory molecules, tsRNAs modulate gene expression through diverse molecular mechanisms and participate in numerous physiological and pathological processes, including tumorigenesis and viral infections [11, 35, 36]. Canonically, microRNAs (miRNAs) recognize target mRNAs through sequence complementarity and, together with Argonaute (AGO) proteins, mediate post-transcriptional gene silencing through translational repression, mRNA deadenylation, and transcript degradation [37]. Similarly, a subset of cytoplasmic tsRNAs can associate with AGO proteins and regulate target RNA expression through sequence-dependent base pairing, leading to

translational repression and/or target RNA destabilization (Fig. 2A) [38]. Notably, unlike canonical miRNAs, the biogenesis and AGO-dependent regulatory functions of many tRFs can occur independently of Dicer [38].

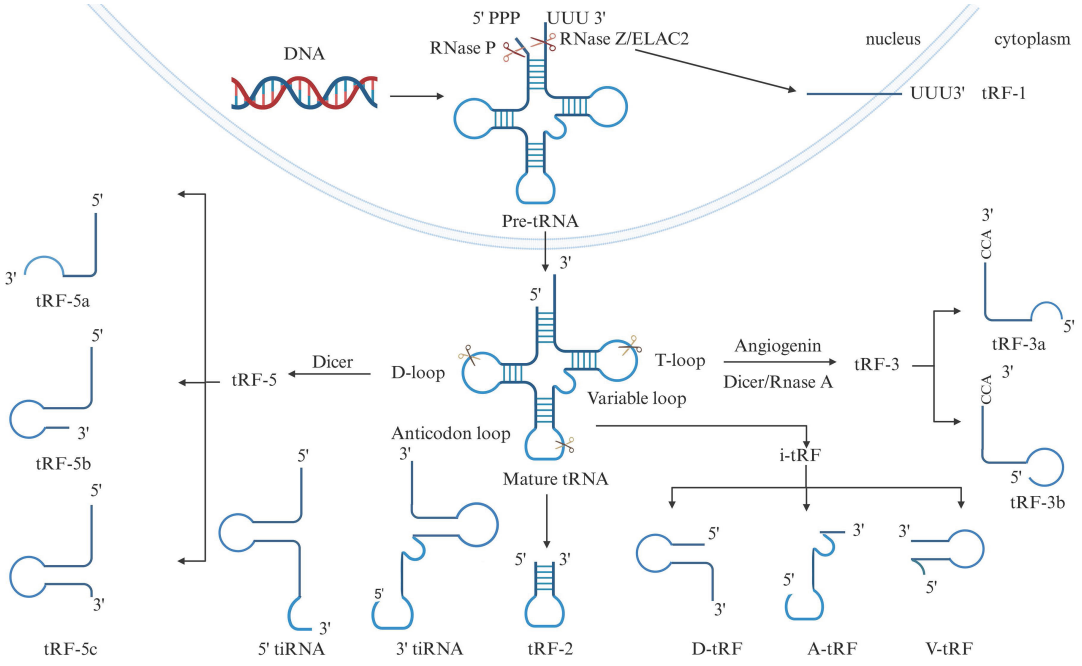

Figure 1. Biogenesis and classification scheme of tsRNAs. Pre-tRNAs are processed by specific enzymes, including RNase P for 5'leader cleavage, RNase Z/ELAC2 for 3' trailer cleavage, Dicer, and Angiogenin, in the nucleus and cytoplasm, yielding several main types: tRF-5, tRF-3, tRF-1, tRF-2, i-tRF, and tiRNA.

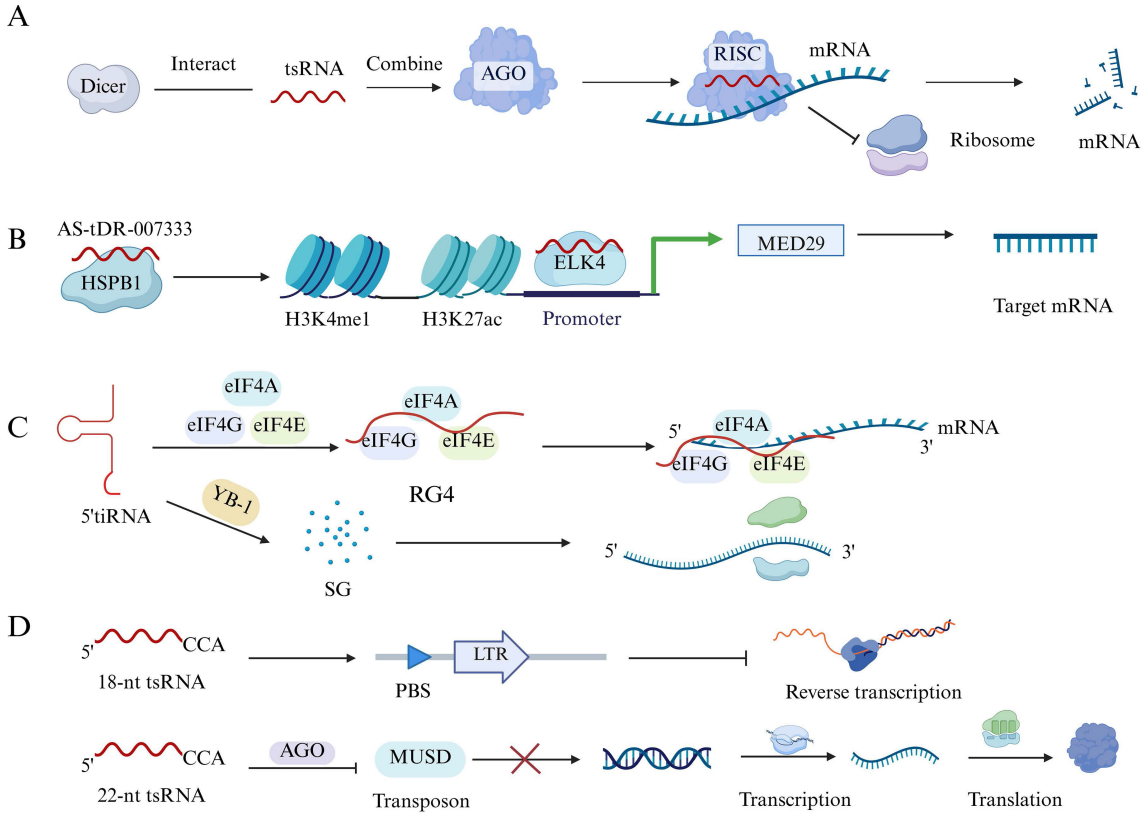

Figure 2. Regulation of transcription and translation by tsRNAs. (A) A representative Dicer-dependent route of tsRNAs-mediated post-transcriptional regulation. tsRNAs facilitate mRNA degradation and translational inhibition by interacting with Dicer, integrating into AGO-based RNA-induced silencing complex (RISC), and base-pairing with target mRNAs. Dicer is depicted here as one representative processing factor for Dicer-dependent tsRNAs and is not an obligate requirement for all tRF–AGO interactions. (B) AS-tDR-007333 binds to the HSPB1 protein, enhancing histone activation marks (H3K4me1/H3K27ac) at the MED29 promoter and upregulating the transcription factor ELK4, which subsequently activates the transcription of the MED29 oncogene. (C) 5'-tiRNAs inhibit translation initiation by binding to the eIF4F complex, forming RG4 structures that displace eIF4F from the m7G cap and partially replace eIF4G. (D) 18-nt tsRNAs inhibit reverse transcription by targeting the primer binding site, while 22-nt tsRNAs suppress retrotransposons (e.g., MusD) via AGO-mediated post-transcriptional silencing, collectively contributing to the maintenance of genomic stability.

Research indicates that distinct types of tsRNAs preferentially bind to different members of the AGO protein family. For instance, tRF-1 typically interacts with AGO3 and AGO4, whereas tRF-5 and tRF-3 can interact with AGO1, AGO3, and AGO4 [25]. Recent evidence further indicates that tsRNA–AGO interactions are context-dependent rather than static. For example, tRF3008A binds to AGO proteins and promotes the destabilization of the oncogenic transcript Forkhead box K1 (FOXK1) in colorectal cancer cells, thereby inhibiting cell proliferation and migration [39]. In another study, tRF-22 was shown to interact with the 3′ untranslated (3′ UTR) region of methyltransferase-like protein 3 (METTL3) mRNA in an AGO2-dependent manner, thereby reducing METTL3 expression, inhibiting m6A methylation of Axin1 and Arid1b transcripts, and consequently increasing their expression [40]. These findings illustrate that distinct tsRNAs can engage different AGO paralogs depending on the cellular context and target transcripts, underscoring the diversity and context specificity of tsRNA–AGO interactions.

Beyond this canonical cytoplasmic post-transcriptional mode of regulation, a distinct Dicer-dependent mechanism has been reported in the nucleus. RNA sequencing, Northern blotting, qRT-PCR, cell transfection, *in vitro* cleavage assays, and photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation analyses have shown that Dicer-dependent tsRNAs can guide AGO2-mediated silencing of nascent RNAs by targeting intronic regions. This non-canonical nuclear mechanism is distinct from conventional cytoplasmic post-transcriptional silencing and may contribute to the regulation of gene expression at the nascent RNA level [41]. Additionally, a foundational *in vitro* and *in vivo* study demonstrated that a novel category of CU-box-containing tRFs competitively binds YBX1 and disrupts YBX1-mediated stabilization of oncogenic transcripts at the post-transcriptional level, thereby altering oncogene mRNA stability and inhibiting the invasion, metastasis, and proliferation of breast tumor cells [42]. Another *in vitro* experiment confirmed that respiratory syncytial virus (RSV)-induced tRF5-GluCTCs recognize target mRNA in a partially complementary manner by directly binding to AGO4 protein and utilizing AGO1's auxiliary mechanism, thus achieving post-transcriptional gene silencing. The absence of AGO4 significantly impairs RSV replication, suggesting new tRF5-GluCTC-based antiviral strategies [43]. Recent findings from tRF sequencing, *in vitro* functional experiments, and animal models indicate that AS-tDR-007333 is highly expressed in non-small cell lung cancer (NSCLC). By directly binding to heat shock protein B-1 (HSPB1), AS-tDR-007333 increases the levels of the activating histone marks H3K4me1 and H3K27ac at the mediator complex subunit 29 (MED29) promoter. In parallel, it upregulates the expression of ETS-domain-containing protein 4 (ELK4), thereby activating MED29 transcription through coordinated epigenetic and transcriptional mechanisms and promoting NSCLC progression (Fig. 2B) [44].

3.1.2. Translation regulation

Under stressful conditions, tsRNAs regulate ribosome biogenesis to control protein translation, promote stress granule (SG) assembly, and mediate apoptosis [45]. In an NSun2-knockout (NS-KO) mouse model, loss of NSun2 led to the accumulation of tRF-1s, among which tRF-Gln-CTG-026 (tG026) was identified as a functional fragment. Administration of tG026 ameliorated liver injury by repressing global protein synthesis [46]. Furthermore, other researchers have employed locked nucleic acid/DNA mixed antisense oligonucleotides (LNAs/ASOs) to inhibit specific tsRNAs (LeuCAG3′ tsRNA) in both *in vitro* and *in vivo* functional experiments, including cell apoptosis assays and mouse liver malignant tumor models, multi-ribosome analyses, and *in vitro* translation systems. These studies, combined with bioinformatics predictions and structural analyses, demonstrate that LeuCAG3′ tsRNA binds to specific structural domains (e.g., hairpin structures) of ribosomal protein mRNAs such as RPS28 and RPS15 through base pairing. This process disrupts their secondary structures, thus facilitating ribosome binding and initiating translation, which enhances translation efficiency and promotes ribosome generation. tsRNAs inhibition can block 18S rRNAs processing, reduce 40S subunits, lead to defects in ribosome assembly, induce tumor cell apoptosis, and consequently inhibit tumor growth, revealing a novel mechanism by which tsRNAs regulate forward translation [47]. Additionally, tsRNAs may not only positively influence translation efficiency but can also inhibit it. For instance, studies utilizing *in vitro* translation assays, protein interaction analyses, and gene knockout techniques have demonstrated that tiRNAs produced via angiogenin cleavage (particularly fragments with a 5′ terminal oligoguanine motif) can displace mRNA by binding to eukaryotic translation initiation factor 4F (eIF4F) complexes (eIF4E, eIF4G, and eIF4A) [48]. Specifically, tiRNAs can almost entirely displace eIF4G/A complexes that bind to cap-free mRNA. For mRNAs with a 5′ cap structure, 5′ tiRNA-Ala contains a 5′ TOG motif at its end, which can further assemble into an RNA G-quadruplex (RG4) structure. This structure can bind to an eIF4F complex, causing it to dissociate

from the m^7GTP cap at the 5′ end of mRNA and partially replace eIF4G [49, 50]. This competitive binding to eIF4G/A diminishes the affinity of eIF4E for the 5′ cap structure of mRNA, effectively hindering cap-dependent translation initiation. Furthermore, tiRNAs can synergize with YB-1 protein to enhance translation inhibition and promote SG assembly, ultimately regulating gene expression reprogramming under cellular stress conditions (Fig. 2C) [48, 51].

3.1.3. Epigenetic regulation

Numerous studies suggest that tsRNAs are involved in epigenetic regulatory processes and may function as novel epigenetic regulators [52]. The primary regulatory mechanisms of epigenetics encompass multiple levels, including DNA methylation, histone covalent modifications, chromatin structural remodeling, and ncRNA-mediated gene expression regulation. These mechanisms collaboratively fine-tune gene expression without altering DNA sequences, playing a critical role in the development of diseases, including tumors [53]. Epigenome dysregulation can promote the occurrence and progression of tumors through aberrant transcriptional programs while also influencing anti-tumor immune responses by altering tumor immunogenicity and immune cell function. Understanding these mechanisms is crucial for advancing tumor immunotherapy [53, 54].

tsRNAs reportedly inhibit transposon activity. *In vitro* and *in vivo* functional experiments reveal that tsRNAs, including the two main types (18- and 22-nt tsRNAs) derived from the 3′ end of mature tRNA and carrying a cytosine–cytosine–adenine (CCA) end, can target the primer binding site (PBS) of long terminal repeat retrotransposons in mouse embryonic stem cells. This targeting achieves epigenetic regulation of transcription and transposition activity through a dual mechanism. Specifically, 18-nt tsRNAs directly inhibit the reverse transcription process and interfere with the reverse transcription of viral RNA without affecting its transcript levels. Conversely, 22-nt tsRNAs downregulate the RNA and protein expression of coding transposons, such as MusD, through an AGO protein-mediated post-transcriptional silencing mechanism, thereby collectively inhibiting transposon activity and maintaining genomic stability (Fig. 2D) [55]. This suggests that tsRNAs may represent a novel class of epigenetic regulators [56].

Moreover, a recent study successfully simulated a mitochondrial dysfunction model by subjecting male mice to a high-fat diet 2 weeks prior to conception. This intervention significantly increased the concentrations of mitochondrial tRNAs and 5′ mt-tsRNAs in the sperm of these male mice, which were subsequently transmitted to the oocyte during fertilization. This transmission triggered transgenerational genetic effects, resulting in decreased glucose tolerance in 30% of offspring mice [57]. The findings indicate that mitochondrial tRNAs and their fragments (mt-tsRNAs) carried by sperm can serve as vehicles of paternal dietary information. A high-fat diet induces the upregulation of sperm mt-tsRNAs expression, which is subsequently conveyed to the egg through fertilization, thereby regulating the transcriptional program of early embryos and ultimately affecting phenotypes such as offspring glucose metabolism [57]. This discovery underscores the role of mt-tsRNAs as novel epigenetic mediators in the transgenerational transmission of paternal metabolic information.

In addition, tsRNAs can regulate gene expression by modulating histone modifications and chromatin accessibility [11, 58]. For instance, 5′ tRF-Gly-GCC promotes the production of ncRNAs, such as U7 snRNA, in Cajal bodies by binding to the hnRNPF/H protein, thereby maintaining normal processing of histone mRNA and protein expression levels. This interaction can influence the expression of genes related to mouse embryonic stem cell-associated virus-like elements (MERVL) and alter chromatin accessibility within heterochromatin, ultimately inhibiting the transcriptional expression of reverse transcription elements such as MERVL [59]. Current research on the regulation of epigenetics by tsRNAs remains in its early stages, necessitating further studies to elucidate the underlying mechanisms [52].

3.2. Intercellular communication messengers

EVs are membrane-bound vesicles actively released by cells into the extracellular microenvironment. They possess a lipid bilayer structure and can carry various molecules, including proteins, nucleic acids, and lipids [60, 61]. Based on their formation in organisms, EVs can be classified into three main categories: exosomes, microvesicles, and apoptotic bodies [62-64]. tsRNAs are highly expressed in EVs derived from cells and plasma, and tsRNAs from EVs can participate in intercellular communication [65].

Research demonstrates that following T cell activation, specific tRNA fragments—particularly tRFs lacking hairpin structures at both the 5′ and 3′ ends—are selectively packaged and secreted into EVs via the multivesicular body pathway, resulting in reduced levels of these tRFs within the cell. Further functional experiments indicate that inhibiting these tRFs can enhance T cell activation and cytokine production, suggesting that the active secretion of tRFs through EVs promotes T cell immune responses [66].

Furthermore, a newly identified type of EV, the migrasome, has emerged as a mode of communication between cells [67]. A recent *in vitro* and *in vivo* study discovered that migrasomes derived from hepatocellular carcinoma (HCC) can promote M2 polarization of macrophages, enhance the immunosuppressive microenvironment, and subsequently facilitate HCC progression. Through small RNA sequencing and PCR validation, the study explored the specific mechanisms underlying migrasome-mediated M2 polarization in macrophages. They observed an increase in the expression of tsRNA-10105 in migrasomes from the serum of patients with HCC. Inhibiting tsRNA-10105 significantly negated the induction of M2 macrophage polarization by tumor cell migrasomes. Therefore, migrasomes from HCC cells drive macrophage M2 polarization by delivering tsRNA-10105 to macrophages, influencing the TME of HCC and promoting the survival and metastasis of tumor cells [68]. Collectively, these findings indicate that EVs, including exosomes and migrasomes, serve as key vehicles for tsRNA-mediated intercellular communication. Beyond EVs, lipid carriers such as high-density lipoprotein (HDL) have also been reported to transport tRNA-derived fragments to immune cells and modulate inflammatory signaling in atherosclerosis, suggesting that tsRNAs may exploit lipid-based transport mechanisms for intercellular communication independent of EVs [69]. Whether similar lipid-associated transport operates within the TME and contributes to tsRNA-mediated immune regulation warrants further investigation.

4. Primary role of tsRNAs in tumor immunity

4.1. Regulating the immunogenicity of tumor cells

The immunogenicity of tumor cells significantly influences the immune system's ability to recognize and eliminate these cells. As a class of sncRNAs with vital regulatory functions, tsRNAs are instrumental in modulating the immunogenicity of tumor cells. Recent studies indicate that Notch homolog 1 (NOTCH1) signaling impacts the heterogeneity of tumor immune infiltration by regulating angiogenic functions. The NOTCH1 gene is notably enriched in the processes of angiogenesis and tRNA metabolism in immunocompetent "hot tumors." As a key regulator of angiogenesis, angiogenin can cleave tRNAs to produce tsRNAs. This study specifically identified a tsRNA, tsRNA-Ala-5-0084, associated with NOTCH1 and its ligand Delta-like ligand 4, and performed functional enrichment analysis, suggesting that this tsRNA may play a role in maintaining tumor stem cell characteristics and reshaping the TME by regulating immune-related processes such as oxidative phosphorylation, protein ubiquitination, and natural killer (NK) cell signaling pathways, thereby indirectly influencing the immunogenicity of tumor cells [70].

In addition, another study suggests that tsRNAs can regulate tumor-associated fibroblasts to promote the formation of an immunosuppressive TME. Tumor-associated fibroblasts secrete exosomes that deliver tsRNA-10522 to CD8+ T cells activated by programmed cell death protein 1 (PD-1) antibodies. This tsRNA-10522 inhibits key functions of CD8+ T cells, including Granzyme B expression and interferon-γ secretion, thereby attenuating the ability of CD8+ T cells to recognize and eliminate tumor cells. This process impairs the capacity of the immune system to recognize tumor cells and to activate anti-tumor immune response [71]. Recently, researchers conducted *in vitro* experiments revealing that tumor cells from patients with lung adenocarcinoma undergoing neoadjuvant therapy secrete exosomes rich in tsRNA 3′ tiRNA-AlaCGC. These exosomes are taken up by fibroblasts in the TME, where tsRNA induces fibroblast aging and triggers a senescence-associated secretory phenotype by inhibiting the longevity gene Forkhead box O3 (FOXO3) and activating the transforming growth factor-β (TGF-β)/Smad3 signaling pathway [72]. This results in the increased secretion of Galectin-9, a ligand for the immune checkpoint TIM-3. Similar to the PD-1/ programmed death ligand 1 (PD-L1) mechanism, Galectin-9 induces the functional impairment of cytotoxic CD8+ T cells [72, 73]. Further *in vivo* experiments have demonstrated that the elevated expression of 3′ tiRNA-AlaCGC correlates with the reduced infiltration of CD8+ T cells and decreased cytotoxicity in mouse tumors, resulting in resistance to anti-PD-L1 therapy [72]. Bioinformatic and expression profiling analyses have associated that tsRNAs with the PD-L1 immune checkpoint and PD-L1 signaling pathway-related genes in lung tumors. tsRNAs such as 5a-TRF-Cys-GCA, 3P-tRNA-Ser-GCT-6-1, 3P-tRNA-Thr-CGT-4-1, 3P-tRNA-Arg-TCT-4-1, and 5P-tRNA-Trp-CCA-3 have been identified in this context [74]. These findings support an indirect role for tsRNAs in modulating tumor immunogenicity through immune checkpoint pathways. In contrast, direct regulation of major histocompatibility complex (MHC) class I molecules or the transporter associated with antigen processing (TAP) by tsRNAs has not yet been experimentally demonstrated. Because tsRNAs can guide Argonaute-mediated silencing of target mRNAs through seed-sequence pairing, a mechanism established for other target transcripts, we speculate

that tsRNAs might directly modulate MHC class I molecules or components of the antigen-processing machinery, thereby altering tumor cell immunogenicity. However, this remains a hypothesis that requires experimental validation. Future studies should determine whether tsRNAs directly bind key mRNAs in the antigen presentation pathway.

In summary, current experimental evidence indicates that tsRNAs primarily act as intercellular messengers within the TME. They are selectively packaged into EVs and other carriers and indirectly modulate tumor cell immunogenicity by regulating immune checkpoint molecules such as PD-L1. To our knowledge, no study has yet directly demonstrated that a specific tsRNA binds and functionally regulates MHC class I or TAP-associated transcripts in a tumor model. This possibility therefore remains speculative. Nevertheless, this field is still in its infancy, and the mechanisms underlying tsRNA sorting, secretion, and intercellular action are not fully understood. Whether these functions are tumor-type specific also requires further investigation. Future studies should focus on defining the functional networks of tsRNAs within the TME, identifying additional mechanisms that regulate tumor cell immunogenicity, and evaluating the clinical potential of tsRNAs as predictive biomarkers or therapeutic targets for tumor immunotherapy.

4.2. Regulating innate immunity

Innate immunity constitutes the body's first line of defense against pathogens, initiating a broad immune response by rapidly identifying conserved pathogen-associated molecular patterns (PAMPs) [75]. Innate immune cells, which include macrophages, dendritic cells (DCs), NK cells, and mast cells, can perform immune functions without prior exposure to specific pathogens. They swiftly recognize pathogen-related molecular patterns through pattern recognition receptors and eliminate pathogens via phagocytosis, the release of inflammatory factors, and the activation of complement, among other mechanisms [76]. Tumor-derived exosomes can modulate immune activity, influencing macrophage polarization and inhibiting NK cell activity [77, 78]. Recent studies have revealed that tsRNAs can regulate tumor immune responses by affecting macrophages, NK cells, and other factors.

Macrophages exhibit two distinct phenotypes: the classically activated pro-inflammatory (M1) phenotype and the anti-inflammatory (M2) phenotype. These phenotypes can dynamically transition based on environmental signals, primarily regulated by specific signaling molecules within the TME [79]. Tumor-associated macrophages initially exhibit an M1 phenotype in the early stages of tumor development. However, as the tumor progresses, growth factors and anti-inflammatory mediators such as interleukin (IL)-4, IL-10, and TGF-β are expressed in the TME, prompting macrophage polarization from the anti-tumor M1 phenotype to the pro-tumor M2 phenotype, which can inhibit immune activity [79, 80]. For instance, migrasomes derived from HCC cells enhance the migration and survival of liver tumor cells by inducing macrophage polarization towards the M2 phenotype through tsRNA-10105 enrichment [68]. Another *in vitro* experiment demonstrated that tRF-3022b binds to Galectin-1 and macrophage migration inhibitory factor (MIF) in colorectal tumor cells, promoting M2 polarization of macrophages through TME interactions with MIF in M2 macrophages, thereby advancing colorectal tumor progression (Fig. 3A) [81].

DCs serve as a crucial link between innate and adaptive immunity and are the most efficient antigen-presenting cells, capable of recognizing, capturing, processing, and presenting antigens. They can also produce cytokines in response to antigens and PAMPs or damage-associated molecular patterns, thereby inducing T-cell immunity [82-84]. tsRNAs regulate the differentiation of monocytes into DCs under the stimulation of cytokines such as IL-4 by methylating histones in monocytes. Specifically, the 5′-half body td-piR (Glu) derived from tRNA-Glu binds to the PIWIL4 protein, recruiting histone H3K9 methyltransferase (SETDB1/SUV39H1) and heterochromatin protein 1β to the promoter region of CD1A and other genes. This recruitment promotes H3K9 methylation, thereby inhibiting CD1A transcription and hindering the normal differentiation of DCs at the epigenetic level. IL-4 reduces td-piR(Glu) production by downregulating tRNA-Glu expression, thereby relieving its inhibitory effect on chromatin and promoting DC differentiation and maturation. These findings indicate that td-piR(Glu) can epigenetically regulate CD1A expression and monocyte-to-DC differentiation in vitro [85]. Given that DCs are key bridges between innate and adaptive immunity, this mechanism may have implications for anti-tumor immune responses. Currently, research on the regulation of DCs by tsRNAs is limited. Elucidating how different tsRNAs shape the functional state of DCs not only uncovers new mechanisms of tumor immune escape but also may provide innovative strategies and biomarkers for reshaping the TME and enhancing the efficacy of tumor immunotherapy by targeting tsRNAs.

NK cells are integral members of the innate lymphocyte family and play an indispensable role in innate immune responses. They are significant immune cells within the TME, exhibiting cytotoxic

capabilities and the capacity to secrete various cytokines. NK cells are pivotal in mediating both innate and adaptive immune responses to tumors and viral infections [86-88]. An *in vitro* study revealed an abnormal elevation in the expression level of a tsRNA known as tRF-3021a (3′ tRF-AlaCGC) in colorectal tumors. This tsRNA upregulates the expression of the metalloprotease ADAM10, which facilitates the cleavage of membrane-bound MHC class I polypeptide-related sequence A (mMICA) on the surface of tumor cells, resulting in the generation of soluble MICA (sMICA). sMICA is released into the TME, where it competitively binds to the activation receptor NKG2D on NK cells, functioning analogously to bait. Consequently, NK cells are unable to recognize mMICA on tumor cells, leading to a paralysis of their cytotoxic function and allowing tumor cells to evade immune detection [89].

4.3. Regulating adaptive immunity

Adaptive immunity is characterized by precise antigen recognition and the formation of immunological memory, enabling rapid responses upon re-exposure to the same pathogen [90-92]. The adaptive immune system comprises mature B cells in the bone marrow and mature T cells in the thymus [91, 93]. Research has demonstrated that tsRNAs can modulate the functional status of adaptive immune cells. Notably, tRFs were evidently enriched in T cell-derived EVs, accounting for 45%, while miRNAs constituted less than 1%. T cell activation triggers the EV-mediated release of a specific set of tRFs, derived from the 5′ terminal and 3′internal regions of tRNA, excluding variable loops. Functional assays have confirmed that these tRFs act as a "brake" on T cell activation. Inhibition of these tRFs using antisense oligonucleotides significantly enhances T cell activation levels and IL-2 production (Fig. 3B). This indicates that T cells actively eliminate inhibitory tRFs via EVs, thereby ensuring effective immune activation [66]. Furthermore, another study demonstrated a positive correlation between ts-49 and overall survival rates in breast cancer, while ts-34 was negatively correlated with overall survival. Significant interactions were detected between T cell activation and ts-34 and ts-49. In patients with T cell depletion, low levels of ts-34 or high levels of ts-49 were associated with improved survival rates, whereas no significant differences were observed in the activated group [94]. These findings suggest that tsRNAs can

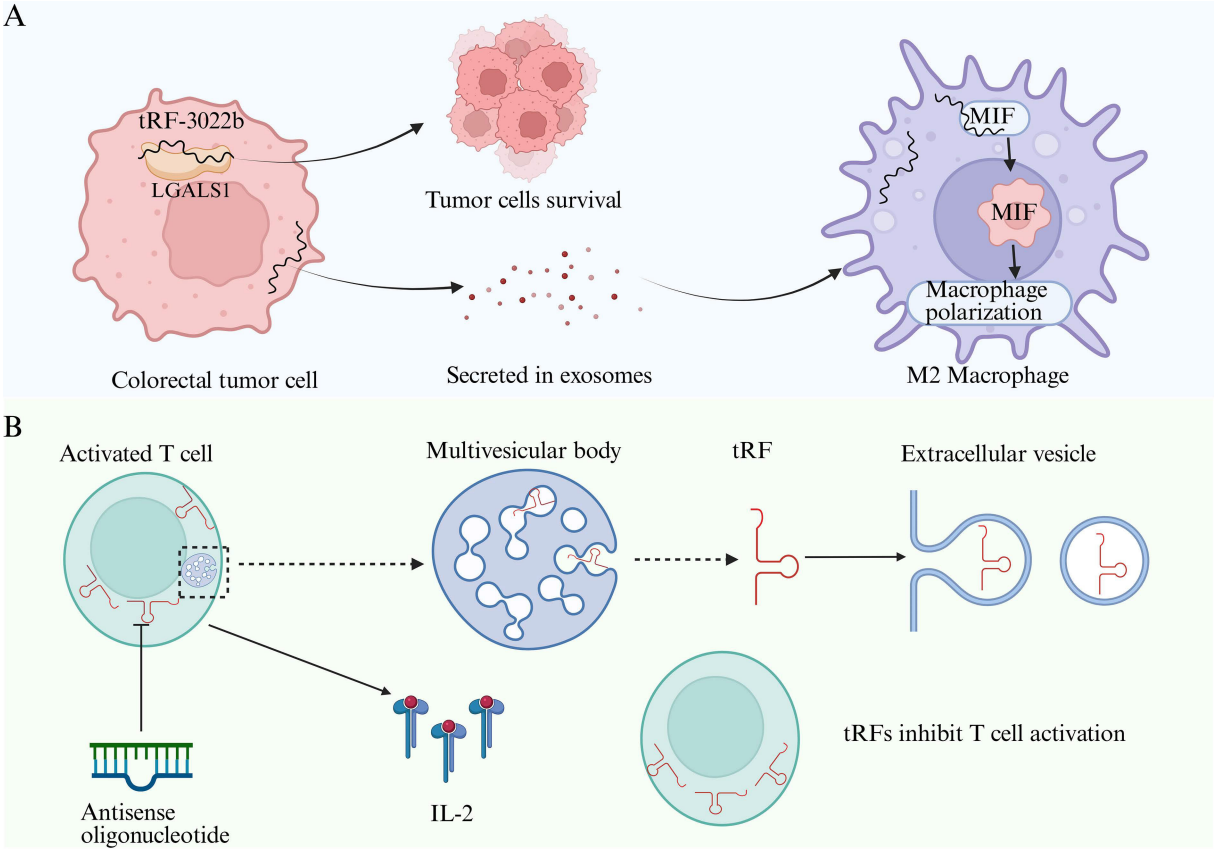

Figure 3. tsRNAs modulate innate and adaptive immunity. (A) In colorectal cancer, tRF-3022b binds to galectin-1 (LGALS1) and macrophage migration inhibitory factor within tumor cells. By interacting with MIF in M2 macrophages, it promotes M2 polarization, thereby accelerating tumor progression. (B) In adaptive immunity, T cell activation induces the release of a specific set of tRFs (derived from the 5' end and internal regions of tRNAs, which lack variable loops) via extracellular vesicles. Suppression of these tRFs using antisense oligonucleotides enhances T cell activation and IL-2 production, indicating their role in inhibiting T cell responses.

influence tumor progression by regulating T cells. Although direct evidence for tsRNAs regulation of B cell biology remains limited, studies on miRNA biology provide a conceptual framework for hypothesizing how tsRNAs might function in humoral immunity. miRNAs are well-established regulators of B cell differentiation, germinal center (GC) formation, and antibody class switching [95]. For instance, miR-125b is preferentially expressed by GC centroblasts and inhibits premature plasma cell differentiation by targeting the B lymphocyte-induced maturation protein-1 (BLIMP-1) and IFN regulatory protein-4 (IRF-4) [96]. miR-28 regulates the GC reaction in primary B cells by impairing class switch recombination and memory B/plasma cell differentiation [97]. Furthermore, EV-mediated transfer of T cell-derived miRNAs to B cells promotes B cell survival, proliferation, and antibody class switching [98]. Given that tsRNAs share key features with miRNAs, including AGO loading and EV-mediated transfer, they may participate in similar regulatory circuits. Notably, tRF-3 CU1276 is highly expressed in mature B cells but absent in germinal center-derived lymphomas, where it functions in an AGO-dependent, miRNA-like manner [99]. Nevertheless, relative to advancements in T cell research, the regulatory role of tsRNAs in B cell-mediated humoral immunity, particularly concerning antibody production and class switching, remains an area requiring further exploration.

5. Clinical translational potential of tsRNAs in tumor immunity

5.1. Predictive biomarkers for tumor immunotherapy

The rapid advancement of tumor immunotherapy has established it as a critical strategy for tumor treatment following surgery, radiotherapy, and chemotherapy. Multiple immunotherapy modalities have demonstrated significant clinical potential, including immune checkpoint inhibitors (ICIs), oncolytic virus therapy, and tumor vaccines [100]. Among these, immune checkpoint blockade (ICB) therapy targets T cell function regulation, alleviating the immunosuppressive state within the TME and restoring and enhancing the body's anti-tumor immune response [100]. Unlike traditional therapies that directly attack tumor cells, ICB represents a transformative approach capable of eliciting durable clinical responses and improving long-term patient survival across diverse malignancies [101, 102]. Currently, several ICIs have received clinical approval, including the PD-L1 inhibitor Atezolizumab, PD-1 inhibitor Nivolumab, cytotoxic T-lymphocyte-associated protein 4 inhibitor Ipilimumab, and lymphocyte-activation gene 3 inhibitor Relatlimab [103, 104]. Notwithstanding, owing to the complex mechanisms of immune evasion employed by tumors—such as low expression of immune checkpoints, limited tumor mutational burden, and intrinsic drug resistance—some patients exhibit poor responses to ICI therapy [103, 105, 106].

Recent studies have highlighted the essential role of tsRNAs in regulating tumor immunity and their potential as predictive biomarkers for immunotherapy efficacy. For example, in a study of lung adenocarcinoma, high levels of 3′ tiRNA-AlaCGC were associated with reduced CD8+ T-cell infiltration and diminished cytotoxicity in C57BL/6 mouse lung tumors, leading to resistance to anti-PD-L1 therapy. Consistent with this immunosuppressive mechanism, high 3′ tiRNA-AlaCGC expression was significantly associated with poor response to anti-PD-L1 therapy and poor prognosis. This study supports the potential of 3′ tiRNA-AlaCGC as a predictive biomarker for anti-PD-L1 resistance in lung adenocarcinoma [72]. In gastric malignancies, N6 methyl adenosine-modified 3′ tRF-AlaAGC was revealed to promote glycolysis and lactic acid accumulation within the gastric TME, altering the balance between regulatory T cells (Tregs) and CD8+ T cells in the TME. This enhancement of PD-1+ Treg immunosuppressive activity undermined the effectiveness of PD-1 blockade, indicating that this tsRNA may serve as a reliable marker for predicting immunotherapy responses [107]. Conversely, in prostate malignancies, methyltransferase-like protein 1 (METTL1) deficiency was found to induce the production of 5′ tsRNA (5′ TOG), which enhanced the efficacy of ICB therapy by activating the interferon signaling pathway, promoting M1 macrophage polarization, and facilitating CD8+ T cell infiltration, thereby reshaping the TME [108]. In esophageal squamous cell carcinoma (ESCC), researchers identified tRF-3024b to be highly expressed in ESCC cells co-cultured with cytotoxic T lymphocytes. Further investigation confirmed that tRF-3024b functions by sequestering miR-192-5p, which increases the expression of the anti-apoptotic protein B-cell lymphoma 2 (BCL-2), thus inhibiting the therapeutic efficacy of ICI. This relationship is indicative of ESCC's resistance to ICI, suggesting that this tRF could serve as a promising biomarker for ESCC immunotherapy [109].

5.2. Diagnostic and prognostic liquid biopsy biomarkers in malignancies

Beyond their predictive value for immunotherapy efficacy, tsRNAs can be detected in various body fluids, including blood, urine, saliva, and

exosomes, making them valuable non-invasive diagnostic and prognostic biomarkers in malignancies [110, 111]. In the context of pancreatic malignancies, serum tRF-Pro-AGG-004 and tRF-Leu-CAG-002 have emerged as promising biomarkers for pancreatic cancer diagnosis, demonstrating superior diagnostic potential compared to CA19-9 and CEA. The combination of these two tsRNAs significantly enhances the diagnostic accuracy for early-stage pancreatic malignant tumors (phases I and II), achieving an area under the curve (AUC) of 0.840, with a sensitivity of 75.0% and specificity of 83.0%. This performance surpasses that of CA19-9 (sensitivity of 63.5% and specificity of 59.6%) and CEA (sensitivity of 63.1% and specificity of 58.5%) [112]. In HCC, tsRNA-Thr-5-0015 is associated with tumor–node–metastasis (TNM) stage and lymph node metastasis in patients with liver cancer, exhibiting excellent stability, accuracy, precision, and linear range. Additionally, ROC curve analysis indicated that serum tsRNA-Thr-5-0015 can distinguish patients with HCC from healthy individuals with an AUC of 0.731 (95% confidence interval, 0.652–0.811), sensitivity of 71.6%, and specificity of 72.4%. Although this AUC is not as high as that of alpha-fetoprotein and des-γ-carboxy prothrombin, its sensitivity is superior [113]. In renal tumors, tRF-19-DRMD5112 in plasma has been identified as a diagnostic biomarker, with an AUC of 0.855, sensitivity of 80.9%, and specificity of 77.5% [114]. In breast cancer, the upregulation of tRF-tDR-7816 expression in serum positively correlates with tumor size, lymph node metastasis, TNM stage, and Ki-67 index. ROC analysis revealed that tDR-7816 possesses high diagnostic efficiency, with an AUC of 0.892, sensitivity of 0.867, and specificity of 0.834 [115]. Additionally, the downregulation of tRF-Gly-CCC-046, tRF-Tyr-GTA-010, and tRF-Pro-TGG-001 in breast cancer presented AUC values of 0.722, 0.781, and 0.709, respectively, indicating their potential as biomarkers for early breast cancer diagnosis [116]. Recent experimental investigations have demonstrated that i-tRF-LeuCAA promotes the migration, invasion, and proliferation of glioma cells while inhibiting their apoptosis, positioning it as a non-invasive biomarker for glioma diagnosis and prognosis [117].

5.3. Potential targets for tumor immunotherapy

In addition to their roles as predictive, diagnostic, and prognostic biomarkers, tsRNAs also represent potential targets for tumor immunotherapy [118]. A recent study on bladder cancer identified a METTL1-catalyzed, m^7G-modified tsRNA, namely, m^7G-3′-tiRNA-LysTTT, which drives malignant progression by binding to the tumor protein Annexin A2 and enhancing its phosphorylation at Tyr24, mediated by the Yes1 proto-oncogene [119]. In colorectal cancer, tsRNA-Gly-5-0007 expression is downregulated in tumor tissues, particularly among patients with early-stage disease. The diagnostic efficiency of exosomal tsRNA-Gly-5-0007 for colorectal cancer is 0.7812, with a value of 0.7726 for early-stage tumors. Research indicates that tsRNA-Gly-5-0007 may affect the adhesion and motility of colorectal tumor cells through involvement in Wnt and mechanistic target of rapamycin signaling pathways, suggesting its potential as both a diagnostic marker and a therapeutic target [120]. In non-small cell lung cancer (NSCLC), vitamin D upregulates the expression of tsRNA-07804, which directly targets and binds to the 3′ UTR region of the *CRKL* gene, inhibiting its expression. When downregulated, CRK-like protein (CRKL)—an adaptor protein that promotes tumor progression—leads to mitochondrial dysfunction, subsequently inhibiting the proliferation, migration, and invasion of NSCLC cells while promoting apoptosis. Consequently, tsRNA-07804 plays a crucial role in regulating CRKL within vitamin D-mediated anti-tumor mechanisms, presenting it as a potential therapeutic target for NSCLC [121]. Furthermore, recent studies have demonstrated that tsr-019759 is highly expressed in liver tumors. By directly targeting and inhibiting the expression and secretion of TNFSF15, it disrupts the TNFSF15/DR3/NF-κB signaling pathway, inhibiting macrophage polarization towards the M1 phenotype and promoting their transformation towards the M2 phenotype. M2 macrophages subsequently activate the epidermal growth factor receptor on tumor cell surfaces by secreting epidermal growth factor, further activating the JAK2/STAT3 signaling pathway. This process creates a positive feedback loop that promotes tumor progression and immunosuppressive TME formation. Additionally, researchers have developed nanoparticles (in-tsr/PEI/PLGA@CCM) that specifically inhibit tsr-019759, effectively blocking this signaling axis in both *in vitro* and *in vivo* models, suppressing tumor growth, and remodeling the immunosuppressive microenvironment. This highlights the potential of tsRNA-targeted interventions in tumor immunotherapy [122]. In addition, therapeutic inhibition or targeted silencing of the previously mentioned tsRNA-10522 and 3′ tRF-AlaAGC has been proposed to improve PD-1 blockade efficacy in esophageal and gastric tumors, respectively (Fig. 4) [71, 107].

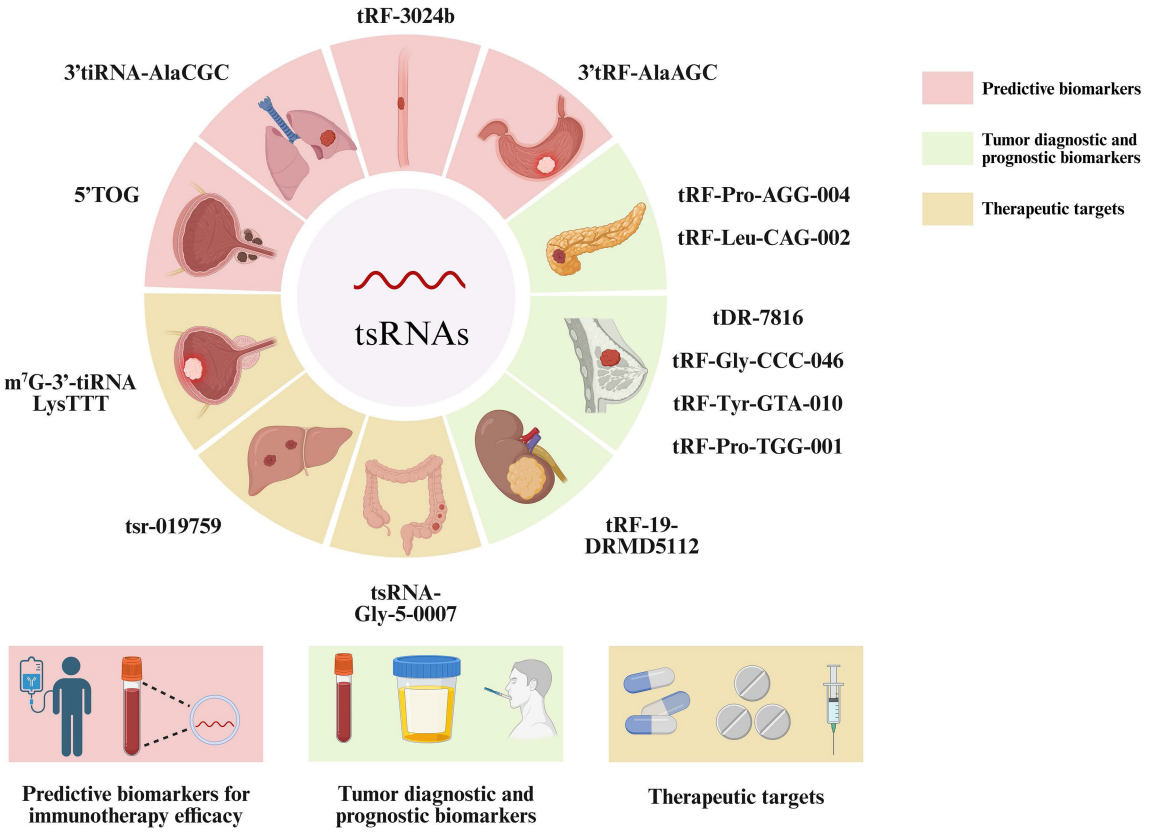

Figure 4. Clinical translational potential of tsRNAs in tumor immunotherapy and oncology diagnostics. tsRNAs serve as predictive biomarkers for tumor immunotherapy efficacy, diagnostic and prognostic biomarkers in malignancies, and therapeutic targets.

6. Technical and Translational Bottlenecks in tsRNAs Research

6.1. Sequencing challenges and methodological considerations

A notable feature of tRNAs and tsRNAs is that they inherit the dense post-transcriptional modifications present on parental tRNAs. Although AlkB-assisted sequencing strategies improve the detection of tRNAs and tsRNAs carrying reverse transcription-blocking modifications, the extent of modification removal can differ among RNA substrates and modification types [123,124]. Therefore, some modified tRNA-derived molecules may remain underrepresented after enzymatic pretreatment. Moreover, AlkB-dependent reactions are performed in the presence of Fe^{2+} and alpha-ketoglutarate (alpha-KG), and the reaction conditions may affect RNA stability if not carefully optimized [125]. Such technical factors could alter the relative recovery of tsRNAs species, particularly short fragments, and should therefore be considered when comparing tsRNAs profiles across studies. Continued optimization of reaction conditions, together with standardized quality-control procedures, will be important for improving the reproducibility of tsRNA sequencing analyses [126]. In order to overcome these obstacles, a variety of specialized library preparation and enzymatic sequencing protocols have been developed in recent years [127].

6.1.1. Demethylation strategy

AlkB-facilitated RNA de-methylation sequencing (ARM-seq), which is based on the Escherichia coli dealkylating enzyme AlkB, can remove reverse transcription–blocking modifications such as m^1A, m^3C, and m^1G, thereby significantly improving the detection of tRNA-derived fragments [128].

6.1.2. Panoramic RNA Display by Overcoming RNA modification Aborted Sequencing (PANDORA-seq)

Building on demethylation, this approach incorporates an end-repair step in which T4 polynucleotide kinase converts non-canonical 2′,3′-cyclic phosphate, 3′-phosphate, and 5′-hydroxyl termini into ligatable 5′-phosphate/3′-hydroxyl ends, allowing AlkB-cleared and end-repaired fragments (especially tRFs and rRNA-derived small RNAs) to be recovered with markedly reduced bias [129].

6.1.3. Thermostable Group II Intron Reverse Transcriptase (TGIRT) Sequencing

This approach employs a thermostable group II

intron reverse transcriptase (TGIRT) enzyme to read through residual modifications by incorporating mismatches, thereby enabling precise mapping of modification sites at single-nucleotide resolution [123].

6.1.4. Adapter-Ligated Libraries of tRNA-derived Sequences (ALL-tRNA-seq)

Combining demethylation, the high-processivity MarathonRT reverse transcriptase, and random adapter ligation reduces ligation bias and preserves fragment information, making this approach particularly robust for partially degraded tissue-derived samples and enabling simultaneous quantification of mature tRNAs and their fragments [130].

These methodological advances have made systematic studies of tsRNAs increasingly feasible. However, it should be noted that the efficiency of demethylation remains uncertain and may introduce random RNA backbone cleavage via Fenton chemistry mediated by α-ketoglutarate and ferrous iron, potentially enriching shorter fragments independently of their biological origin. Therefore, methodological standardization remains an urgent challenge in this field [126].

6.2. Pharmacological barriers to tsRNA-based therapeutics

Efficient *in vivo* delivery of tsRNAs mimics or inhibitors remains a critical bottleneck for their clinical translation. Similar to siRNAs and miRNAs, tsRNAs face multiple delivery barriers, including serum nuclease degradation, renal clearance, nonspecific tissue accumulation, and inefficient target cell uptake [131]. Among these, endosomal escape is one of the most critical rate-limiting steps: after internalization, nucleic acid drugs are retained in endosomes, and if they fail to escape into the cytoplasm in a timely manner, they are trafficked to lysosomes for degradation [132]. Histidine-rich peptides can promote endosomal escape through the proton sponge effect of their imidazole groups at the acidic pH of endosomes, whereas disulfide bonds allow intracellular dissociation [133, 134].

In terms of biodistribution, lipid nanoparticles, polymeric carriers, and tissue-specific targeting ligands currently represent the main delivery strategies [135]. Off-target hybridization is another important concern: tsRNAs rely on a "seed sequence" to recognize target mRNAs, and the short nature of this sequence may lead to off-target repression of numerous non-target transcripts [39,136]. Furthermore, exogenous tsRNAs mimics may competitively interfere with the endogenous tsRNAs network. Chemical modifications of tsRNAs profoundly influence their function. Therefore, in the design of therapeutic tsRNAs, it is necessary to balance the trade-off between the functional enhancement conferred by modifications and the increased difficulty of synthesis.

7. Conclusion

Overall, tsRNAs represent an emerging class of key regulatory molecules that play a vital role in shaping the TME by regulating gene transcription and translation, influencing epigenetic states, and facilitating intercellular communication. They not only modulate the immunogenicity of tumor cells but also directly impact the functional status of both innate and adaptive immune cells, thereby influencing immune recognition and tumor clearance. Importantly, specific tsRNAs have demonstrated significant correlations with the efficacy of ICIs, serving as potential biomarkers for predicting responses to tumor immunotherapy and innovative targets for reversing immune suppression and enhancing treatment outcomes. Although the precise mechanisms of action and clinical applications of tsRNAs remain in the exploratory stage, existing evidence underscores their profound potential in translational medicine. Future research should comprehensively analyze the functional networks of tsRNAs within specific TMEs, focusing on the development of targeted delivery technologies, such as nanomedicine, and advancing their validation in large clinical cohorts, thereby paving the way for novel tumor immunotherapy strategies [137].

Abbreviations

tsRNAs: transfer RNA-derived small RNAs; tRNAs: transfer RNAs; TME: tumor microenvironment; tRFs: tRNA-derived fragments; tiRNAs: tRNA halves; sncRNAs: small non-coding RNAs; EVs: extracellular vesicles; ncRNAs: non-coding RNAs; pre-tRNAs: precursor tRNAs; ANG: Angiogenin; miRNAs: microRNAs; AGO: Argonaute; FOXK1: Forkhead box K1; 3′ UTR: 3′ untranslated region; RSV: respiratory syncytial virus; HSPB1: heat shock protein B-1; MED29: mediator complex subunit 29; ELK4: ETS-domain-containing protein 4; NSCLC: non-small cell lung cancer; SG: stress granule; LNAs/ASOs: locked nucleic acid/DNA mixed antisense oligonucleotides; eIF4F: eukaryotic translation initiation factor 4F; eIF4E: eukaryotic translation initiation factor 4E; eIF4G: eukaryotic translation initiation factor 4G; eIF4A: eukaryotic translation initiation factor 4A; RG4: RNA G-quadruplex; CCA: cytosine-cytosine-adenine; PBS: primer binding site; MERVL: mouse embryonic stem cell-associated virus-like elements; HCC:

hepatocellular carcinoma; HDL: high-density lipoprotein; NOTCH1: Notch homolog 1; NK: natural killer; PD-1: programmed cell death protein 1; PD-L1: programmed death-ligand 1; TGF-β: transforming growth factor-β; FOXO3: Forkhead box O3; MHC: major histocompatibility complex; TAP: transporter associated with antigen processing; PAMPs: pathogen-associated molecular patterns; DCs: dendritic cells; IL: interleukin; MIF: macrophage migration inhibitory factor; mMICA: membrane-bound MHC class I polypeptide-related sequence A; sMICA: soluble MICA; GC: germinal center; BLIMP-1: B lymphocyte-induced maturation protein-1; IRF-4: IFN regulatory protein-4; ICIs: immune checkpoint inhibitors; ICB: immune checkpoint blockade; Tregs: regulatory T cells; METTL3: methyltransferase-like protein 3; METTL1: methyltransferase-like protein 1; ESCC: esophageal squamous cell carcinoma; BCL-2: B-cell lymphoma 2; AUC: area under the curve; TNM: tumor-node-metastasis; CRKL: CRK-like protein; ARM-seq: AlkB-facilitated RNA de-methylation sequencing; PANDORA-seq: Panoramic RNA Display by Overcoming RNA modification Aborted Sequencing; TGIRT: Thermostable Group II Intron Reverse Transcriptase; ALL-tRNA-seq: Adapter-Ligated Libraries of tRNA-derived Sequences; RISC: RNA-induced silencing complex; LGALS1: galectin-1.

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Authorship contribution statement

Zihan Liu: Writing-original draft, Investigation; **Jingzhi Wang**: Writing-original draft; **Xinyi Miao**: Writing-original draft, Visualization; **Tuo Zheng**: Funding acquisition, Writing-reviewing & editing; **Min Miao**: Conceptualization, Methodology, Supervision; **Yongfu Shao**: Conceptualization, Writing-reviewing & editing.

Competing Interests

The authors have declared that no competing interest exists.

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