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Review

Emerging Roles of tsRNAs: Novel Biomarkers and Therapeutic Targets in Gynecological Oncology

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Abstract

Transfer RNA-derived small RNA (tsRNAs) are a diverse group of non-coding RNAs that play key roles in controlling gene expression and the body's response to stress. Recent studies indicate that tsRNAs are not only byproducts of tRNA breakdown; rather, they actively participate in different biological processes, including tumor development. In gynecological cancers, including ovarian, cervical, and endometrial cancers, tsRNAs have been associated with the modulation of essential cancer-related pathways, affecting cell proliferation, migration, and apoptosis. This review examines the biosynthesis, categorization, and functional importance of tsRNAs, particularly regarding their role in gynecological cancers. The anomalous expression of tsRNAs in these malignancies underscores their potential as diagnostic tools and therapeutic targets. The increasing data supporting RNA-based methods for cancer diagnosis and therapy highlights the significance of tsRNAs as potential candidates for novel therapeutics in gynecological cancers.

1. Introduction

TsRNAs represent a remarkable category of non-coding short RNAs. They encompass tRFs and tiRNAs, which are RNAs produced from tRNA and generated by stress [1]. The many disorders linked to these molecules and their varied functions in controlling gene expression have brought them considerable attention [2]. Recent advancements in RNA sequencing have demonstrated that distinct cleavage patterns form these short RNAs, not merely resulting from tRNA degradation [3].

Only a few of the important physiological functions performed by tsRNAs, conserved from bacteria to humans, include translational repression, gene regulation, and stress responses [4]. Two of the most important enzymes in tsRNA synthesis are ANG and Dicer. They cleave mature or precursor tRNAs into their own tRF-1, tRF-3, tRF-5, 5'-tiRNA, and 3'-tiRNA forms [5,6]. Interestingly, while Dicer is essential for the synthesis of some tsRNAs, it does not play a role in the synthesis of other tsRNAs [7]. Current studies have underscored the pivotal function of tsRNAs not only in malignant conditions but also in non-neoplastic diseases [8,9].

Gynecological cancers (GCs), encompassing breast, cervical, ovarian, and uterine cancers, persist as a significant worldwide health concern, substantially affecting women's health [10]. Multiple variables, including genetic predisposition, lifestyle decisions, and hormone imbalances, enhance the vulnerability of females to these diseases. Recognized as significant regulatory molecules, tsRNAs have been linked to the genesis and development of various malignancies by regulating gene expression, influencing cell proliferation, and altering apoptosis [11,12]. Like with other types of cancer, molecular features, especially those found through genetic research, have become important for quickly identifying GCs and choosing the best treatment plans for every patient [13].

In the context of gynecological malignancies, tsRNAs have demonstrated the ability to affect critical cancer-related pathways, establishing them as prospective indicators for early detection and potential treatment targets [14]. Altered tsRNA expression patterns have been identified in breast cancer, the most common malignancy worldwide [15,16]. The advancing comprehension of tsRNA synthesis and their functional roles in cancer biology presents new prospects for diagnostic and therapeutic applications. Advancements in RNA-based cancer therapeutics suggest that tsRNAs may serve as vital components in the formulation of more effective and tailored treatment regimens [17,18].

This review examines the biogenesis, categorization, and biological functions of tsRNAs, emphasizing their relevance in gynecological malignancies. Furthermore, it underscores the specific roles of tsRNAs as molecular instruments for cancer diagnosis and treatment, facilitating future studies of RNA-based therapies.

2. Biogenesis Pathways and Categorization of tsRNAs: Mechanistic Insights

Either precursor or mature tRNAs can produce the non-coding RNAs recognized as transfer RNA-derived small RNAs. RNA Polymerase III starts the process of making tsRNAs by transcribing pre-tRNAs that have leader and trailer sequences at their 5′ and 3′ ends. The

ribonucleoprotein endonuclease RNase P cuts the leader sequence, and RNase Z cleaves the trailer sequence at the first unpaired nucleotide next to the 3′ end of the transfer RNA. After these cleavages, tRNA nucleotidyl transferase completes the maturation of processed tRNA by adding a non-templated "CCA" nucleotide to the 3′ end. Mature transfer RNAs usually lengthen between 75 and 93 nucleotides and keep their conserved structure, which includes four loops: the D-loop, the anticodon loop, the TψC loop, and the variable loop (Figure 1).

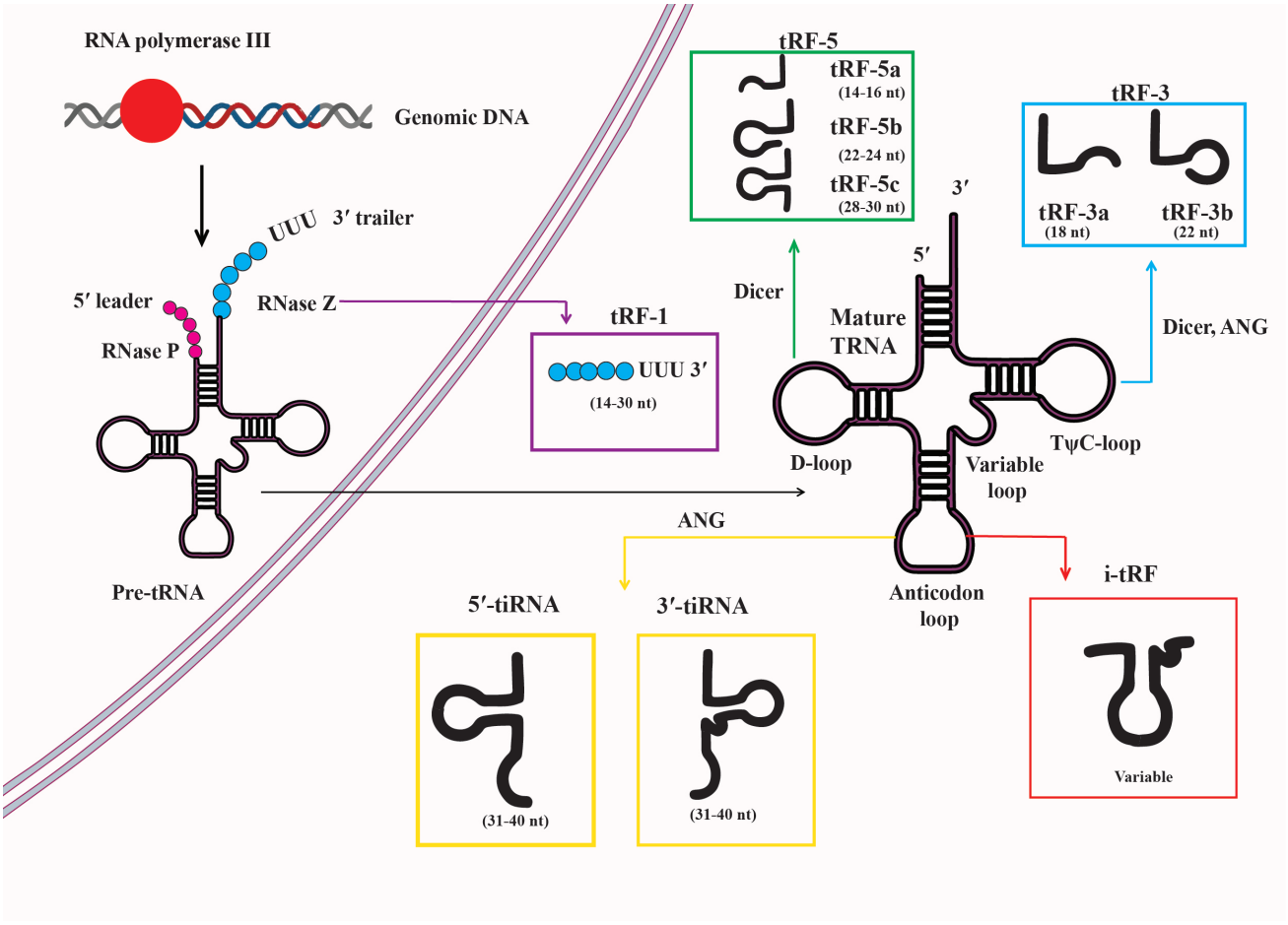

Figure 1. Transcribed by RNA polymerase III from tRNA genes in the nucleus. These pre-tRNAs undergo numerous processing and modification steps, including m5C, m7G, and Ψ, before maturing into functional tRNAs. Broadly, tsRNAs are categorized based on their cleavage sites into two major types: tRFs and tiRNAs. While tRFs can derive from either pre-tRNAs or mature tRNAs, they are further classified into subtypes such as tRF-1, tRF-3, tRF-5, and i-tRF, each defined by its cleavage location. For instance, tRF-1 is produced when RNase Z/ELAC2 cleaves pre-tRNAs. The TΨC loop of mature tRNAs, when cut by enzymes like Dicer or ANG, generates tRF-3, which is then divided into tRF-3a and tRF-3b. tRF-5, which results from cleavage at the D-loop, D stem, or the 5' segment of the anticodon stem in mature tRNAs, is further split into tRF-5a, tRF-5b, and tRF-5c. Meanwhile, under stress, tiRNAs emerge when ANG cuts the anticodon loops in mature tRNAs, yielding 5'-tiRNAs and 3'-tiRNAs. Additionally, i-tRFs arise from various internal regions of mature tRNAs.

2.1 Structural Classification of tsRNAs: Functional Subtypes and Their Origins

New high-throughput sequencing technology has shown that certain enzymes can cleave mature tRNAs and their precursors at precise locations, resulting in tsRNAs [19,20]. According to the location of their cleavage and their functional relevance, these tsRNAs, which are found across both prokaryotic and eukaryotic transcriptomes, are mainly regarded as two main groups: tRFs and tiRNAs [21].

2.1.1 tRFs

tRFs, which are produced from certain sections of either precursor or mature tRNAs, are a significant class of tsRNAs. These subtypes, classified according to the cleavage location of the tRNA molecule, are crucial for numerous physiological processes such as gene regulation and stress response [22].

2.1.1.1 tRF-1

The trailer sequence, located at the 3′ end of pre-tRNA, is the original source of these fragments. The production of tRF-1 fragments is the outcome of pre-tRNA cleavage by RNase Z or ELAC2. A poly-U tail, which is the RNA Polymerase III termination signal, is located in this area [1]. There is evidence that selective biological processes are involved in the biogenesis of tRF-1 fragments, which are involved in tRNA maturation. They may not be abundant, but they play an essential role in pathways that process RNA [23].

2.1.1.2 tRF-3

Mature tRNAs produce tRF-3 fragments. The length of the subtypes is used to further categorize them: tRF-3a is around 18 nucleotides long, and tRF-3b is about 22 nucleotides (nt) long [23]. During tRNA maturation, the CCA sequence is enzymatically added to the 3' end by tRNA nucleotidyl transferase. This sequence is crucial for the functionality of mature tRNAs and is retained in both tRF-3a and tRF-3b fragments. Enzymes such as ANG and Dicer cleave the TψC loop of tRNAs, generating these fragments while preserving the CCA sequence at their 3' termini. The conserved presence of the CCA sequence may influence the stability and functionality of tRF-3 fragments, contributing to their roles in gene regulation and cell survival, particularly during cellular stress [3].

2.1.1.3 tRF-5

Three subtypes of tRF-5 fragments are recognized according to their length: tRF-5a, which contains 14-16 nucleotides; tRF-5b, which contains 22–24 nucleotides; and tRF-5c, which contains 28–30 nucleotides [24]. The 5′ end of mature tRNAs generates these subtypes. These fragments are the product of a split in the D-loop or in the region where it joins the anticodon loop. The processing diversity of tRF-5s, in contrast to the homogeneity of tRF-3s, may be an indication of the wide variety of cellular operations that tRF-5s regulate, such as protein synthesis and stress response. Their appearance across a wide range of animals and environmental situations underscores their role in cellular homeostasis [25].

2.1.2 tiRNAs

TiRNAs, also known as tRNA sections, are fragments that are specifically generated in response to physiological stress. Endonucleolytic cleavage in the anticodon loop of mature tRNAs generates these fragments, which are associated with the suppression of protein synthesis [26]. This process facilitates the stress response and conserves energy during cellular stress. By regulating the production of stress granules, tiRNAs are known for their ability to inhibit translation and protect the cell from additional damage [27].

2.1.2.1 5′-tiRNAs

These fragments extend from the 5′ terminus of the mature tRNA to the cleavage site within the anticodon loop. Many stresses, including shock from heat, oxidative damage, viral infections, and hypoxia, frequently stimulate the synthesis of 5′-tiRNAs [26]. ANG is the principal enzyme that catalyzes the cleavage-producing tiRNAs during stress conditions [28]. The buildup of 5′-tiRNAs in cells inhibits global protein translation, enabling the cells to maintain energy and prioritizing stress response pathways [27].

2.1.2.2 3′-tiRNAs

Similar to 5′-tiRNAs, the cleavage of the anticodon loop produces 3′-tiRNAs, which extend from the cleavage site to the 3′ terminus of the mature tRNA. These fragments possess unique 5′-hydroxyl groups and are believed to serve different functions than their 5′ counterparts [29]. Alongside 5′-tiRNAs, these stress-induced fragments contribute to the production of stress granules, which are essential for sequestering mRNA and inhibiting superfluous translation during stress situations. Enzymes such as ANG and RNase L in vertebrates and Rny1 in yeast meticulously control the synthesis of tiRNAs [30]. Notably, stress does not exclusively create all tiRNAs; recent studies have discovered SHOT-RNAs (sex hormone-dependent tRNA-derived RNAs), a subset of tiRNAs synthesized in a hormone-dependent fashion. SHOT-RNAs are notably prevalent in hormone-dependent malignancies, including cancers of the breast and prostate, where they may facilitate tumor progression by modulating the response of cells to variations in hormones [31].

2.1.2.3 i-tRFs

Unlike other tRFs that originate from the terminals of tRNA molecules, cleavage within the middle region of mature tRNAs generates i-tRFs [2]. They do not reach the 5′ or 3′ termini and can arise from areas such as the anticodon arm. Generally, i-tRFs are longer than standard tRFs, with an average length of 36 nucleotides [32]. Despite their limited research, i-tRFs could have unique roles in gene regulation, potentially impacting specific cellular processes like translation and RNA stability [33].

2.1.3 Other tRNA-Derived RNAs (Specialized Subtypes)

In addition to the established categories of tRFs and tiRNAs, new research has uncovered several subtypes of tsRNAs with distinct functions. Sexual hormones modulate SHOT-RNAs, a distinct category of tsRNAs, and they contribute to hormone-dependent malignancies [34]. These fragments originate from completely aminoacylated mature tRNAs and participate in the hormonal regulation of gene expression, especially in tissues responsive to sex hormones such as estrogen and testosterone [35]. Another example is mitochondrial tRNA-derived RNAs, which originate from mitochondrial tRNAs rather than cytoplasmic tRNAs. These components play a crucial role in mitochondrial activity and energy metabolism, particularly in times of metabolic stress [36].

2.2 Functional Roles of tsRNAs

Originating from tRNA cleavage, tsRNAs serve numerous critical biological roles. These roles encompass gene expression regulation, translation control, cellular communication, and apoptosis suppression. Recent studies offer significant insights into the role of short RNA fragments in cellular stability and responses, establishing them as essential elements in gene regulation and intercellular functions [37].

2.2.1 Gene Expression Modulation

TSRNAs can modulate gene expression by silencing mRNA via either miRNA-like processes or competitive

suppression of oncogenic mRNA. By binding to specific sections of mRNA or RNA-binding proteins, tsRNAs exert substantial control over gene activity [38].

2.2.1.1 miRNA-Mimetic Silencing of mRNA

Similar to miRNAs, tsRNAs bind to the 3′ UTR of target mRNAs, thereby inhibiting their expression. Interactions with AGO proteins help make RISC, which stop the translation of mRNA in many species, including humans, mammals, and insects [39-41]. Research has demonstrated that tRF-3017A suppresses the expression of NELL2 genes in gastric cancer, thereby reducing tumor dissemination through the formation of RISC with AGO proteins (Figure 2A) [42].

2.2.1.2 Inhibition through YBX1 Binding

tsRNAs can decrease the expression of genes by binding competitively to YBX1, a protein that often stabilizes oncogenic mRNAs at the 3′ UTR. This relationship diminishes oncogenic mRNA stability and expression, hence restricting tumor proliferation. YBX1 identifies a CU-box motif that encompasses the silencing effect. Some tRFs, which are made by tRNA-Glu, tRNA-Asp, tRNA-Gly, and tRNA-Tyr, can lower the expression of oncogenes, which can stop cancer cells from spreading, especially breast cancer (Figure 2B) [43].

2.2.2 Translational Control

TSRNAs regulate translation by either inhibiting it during stress or boosting it through the change of ribosomal mRNA structures. The capacity to regulate protein synthesis in varying situations is crucial for cellular adaptability and responsiveness to environmental stresses [44].

2.2.2.1 Translation Inhibition via Stress Granule Formation

There is a process called cellular stress that causes eIF2 to be phosphorylated. This causes stress granules (SGs) to form, which temporarily stop translation. Some tiRNAs, like 5′ tiRNA-Ala, can help SG assembly on their own, which stops translation even when eIF2 is not activated. The 5′ tiRNA-Ala possesses a 5′ TOG motif that generates an RNA G-quadruplex (RG4), which interacts with eIF4F and displaces it from the m7GTP cap on mRNA, hence inhibiting translation. The growth of SGs that are caused by tiRNA depends on how it interacts with YBX1, which is important for stopping translation (Figure 2C) [45-47].

2.2.2.2 Ribosomal mRNA Modification Enhances Translation

Specific 3′ tsRNAs augment translation by interacting with ribosomal mRNAs and disrupting their secondary structures. The LeuC-AG 3′ tsRNA, a 22-nucleotide RNA, enhances cell survival by facilitating ribosome assembly. This tsRNA binds to the mRNAs of ribosomal proteins RPS28 and RPS15, disrupting secondary structures, hence enhancing translation and facilitating the production of 40S ribosomes. This specificity denotes a selective impact on ribosomal protein mRNAs since it does not influence other ribosomal proteins such as RPS9/RPS14 (Figure 2D) [48].

2.2.3 Epigenetic Regulation and Genome Stability

tsRNAs contribute to genome stability by inhibiting transposable elements, referred to as "genomic parasites," which have the potential to interfere with adjacent genes. By inhibiting transposon mobility, tsRNAs safeguard the genome from instability and avert unintentional gene activation. Transposable elements, or transposons, are DNA segments capable of autonomous or non-autonomous relocation within the genome, which may destabilize genetic expression. By attaching to the primer-binding sites of endogenous retroviruses, tsRNAs reinforce the genome by inhibiting their reverse transcription and mobility. Extreme conservation of the capacity to inhibit transposons supports the hypothesis that tsRNAs could serve as innovative epigenetic regulators (Figure 2E) [48-51].

2.2.4 Apoptosis Regulation

By their interaction with Cyt C, tsRNAs assist in inhibiting apoptosis, especially under stress situations. Cancer cells frequently detect the anti-apoptotic activity, which is essential for cell survival and facilitates continuous development by inhibiting apoptosis [52].

The interaction between mature tRNAs and Cyt C can inhibit apoptosis by obstructing apoptosome assembly and the activation of caspase-9 [53]. In response to cellular stress, angiogenin (ANG) cleaves tRNA to produce tiRNAs, which subsequently suppress apoptosis by binding to cytochrome c released from mitochondria, resulting in the formation of a cytochrome c-RNP complex. This combination inhibits apoptosis and promotes cell viability. Significantly, 5′ tiRNA-His-GTG inactivates the Hippo pathway by targeting big tumor suppressor kinase 2, promoting cell proliferation and anti-apoptotic functions. Colon cancer significantly increases this tiRNA, highlighting its role in facilitating tumor proliferation via anti-apoptotic pathways (Figure 2F) [54,55].

Apoptosis, a crucial mechanism in cellular regulation, is intricately linked to cancer progression, where its dysregulation enables tumor survival and growth. Transfer RNA-derived small RNAs (tsRNAs) have been identified as key regulators in modulating apoptosis, functioning to either promote or inhibit cell death based on the cellular context and environmental cues [52].

One of the primary ways tsRNAs influence apoptosis is through their interaction with cytochrome c. The interaction between mature tRNAs and Cyt C can inhibit apoptosis by obstructing apoptosome assembly and the activation of caspase-9 [53]. In response to cellular stress, angiogenin (ANG) cleaves tRNA to produce tiRNAs, which subsequently suppress apoptosis by binding to cytochrome c released from mitochondria, resulting in the formation of a cytochrome c-RNP complex. This combination inhibits apoptosis and promotes cell viability. Significantly, 5′ tiRNA-His-GTG inactivates

the Hippo pathway by targeting big tumor suppressor kinase 2, promoting cell proliferation and anti-apoptotic functions. Colon cancer significantly increases this tiRNA, highlighting its role in facilitating tumor proliferation via anti-apoptotic pathways (Figure 2F) [54,55]. An example is 5′ tiRNA-His-GTG, which not only blocks apoptotic processes but also inactivates the Hippo signaling pathway by targeting big tumor suppressor kinase 2. This specific tiRNA, highly expressed in colon cancer, has been implicated in facilitating tumor progression by its anti-apoptotic functions [55].

Further studies have demonstrated the role of tsRNAs in regulating apoptosis through interactions with ribosomal proteins. For instance, 3′-tsRNA-LeuCAG inhibits apoptosis by binding to ribosomal proteins RPS28 and RPS15, which stabilizes the processing of 18S preribosomal RNA. This stabilization promotes the production of 40S ribosomal subunits, thereby enhancing ribosome biogenesis and supporting the proliferation of cancer cells. However, when 3′-tsRNA-LeuCAG is inhibited, ribosome production decreases, leading to the induction of apoptosis [21]. This dual role was observed both in vitro and in murine models of patient-derived orthotopic hepatocellular carcinoma, highlighting the tsRNA's importance in apoptosis regulation and cancer cell survival [56].

Additionally, tsRNAs have been found to influence apoptotic pathways beyond cytochrome c binding. In their anti-apoptotic roles, tsRNAs may also regulate apoptosome assembly by promoting competitive interactions of cytochrome c with APAF1. Although these processes are well-documented in intrinsic pathways, the precise relationship between tiRNAs and Argonaute (AGO) proteins in apoptotic regulation remains poorly understood, requiring further research to elucidate these mechanisms fully [53].

To sum up, tsRNAs act as significant modulators of apoptosis, influencing intrinsic apoptotic pathways through mitochondrial interactions and ribosome biogenesis. Their ability to prevent apoptosome formation and suppress caspase activation underscores their relevance in cancer progression and therapeutic resistance. By targeting these small RNAs, novel therapeutic strategies may be developed to restore apoptotic signaling and improve outcomes in apoptosis-resistant cancers.

2.2.5 Intercellular Communication

TsRNAs play a pivotal role in intercellular communication through their incorporation into extracellular vesicles (EVs), such as exosomes and microvesicles [57]. These tsRNAs regulate immune system responses by modulating the activity of immune cells and influencing immunological signaling pathways. After exosome uptake by recipient cells, tsRNAsengage in a wide range of regulatory activities, including modulating gene expression, immune responses, and protein synthesis. For example, 5′ tRFs packaged in exosomes from T cells have been shown to alter T-cell activation and immune signaling cascades, directly impacting the immune response. Specifically, these tsRNAs can modulate cytokine release, macrophage polarization, and T-cell receptor signaling, which are critical for orchestrating immune responses. These effects are mediated by their interactions with key signaling proteins and RNA-binding complexes, ultimately influencing cellular signaling pathways [57].

Once internalized, tsRNAs associate with pivotal proteins and ribonucleoprotein complexes to alter cellular functions. For instance, Garcia-Silva et al. highlighted that exosomal 5′-tRFs and 3′-tRFs from *Trypanosoma cruzi* can bind Argonaute (AGO) proteins in recipient cells, subsequently affecting mRNA stability and translation in HeLa cells [58]. This illustrates the potential of exosome-delivered tsRNAs to significantly modify gene expression patterns in target cells.

Furthermore, tsRNAs derived from EVs can contribute to immune evasion mechanisms by tumor cells, suppressing anti-tumor immune responses and promoting immune tolerance [57,59]. Recent studies have highlighted how specific tsRNAs, such as those derived from tRNA-Gly and tRNA-Val, are enriched in EVs and regulate key pathways in immune cell function, including the modulation of inflammatory cytokines and chemokines [60-62]. These findings underscore the role of tsRNAs in immune response regulation and establish their significance in cancer-related immune signaling (Figure 2G). Research by Chiou et al. revealed that 5′-tRFs, packaged in T-cell-derived exosomes, downregulate T-cell receptor signaling and cytokine production, promoting immune tolerance and reducing inflammatory responses [62]. Similarly, Wei et al. demonstrated that exosomes released by glioblastoma cells carry 5′-tRFs derived from tRNA-Gly and tRNA-Val, which aid in immune evasion by altering macrophage activation and driving tumor-supportive polarization [63]. Furthermore, tsRNAs regulate protein synthesis in recipient cells. Cooke et al. reported that syncytiotrophoblast-derived exosomes deliver 5′-tRNA-Gly-GCC during pregnancy, which binds to ribosomal subunits, suppressing global protein translation and mediating maternal-fetal communication [64]. This mechanism highlights the ability of tsRNAs to influence cellular metabolism through translational inhibition.

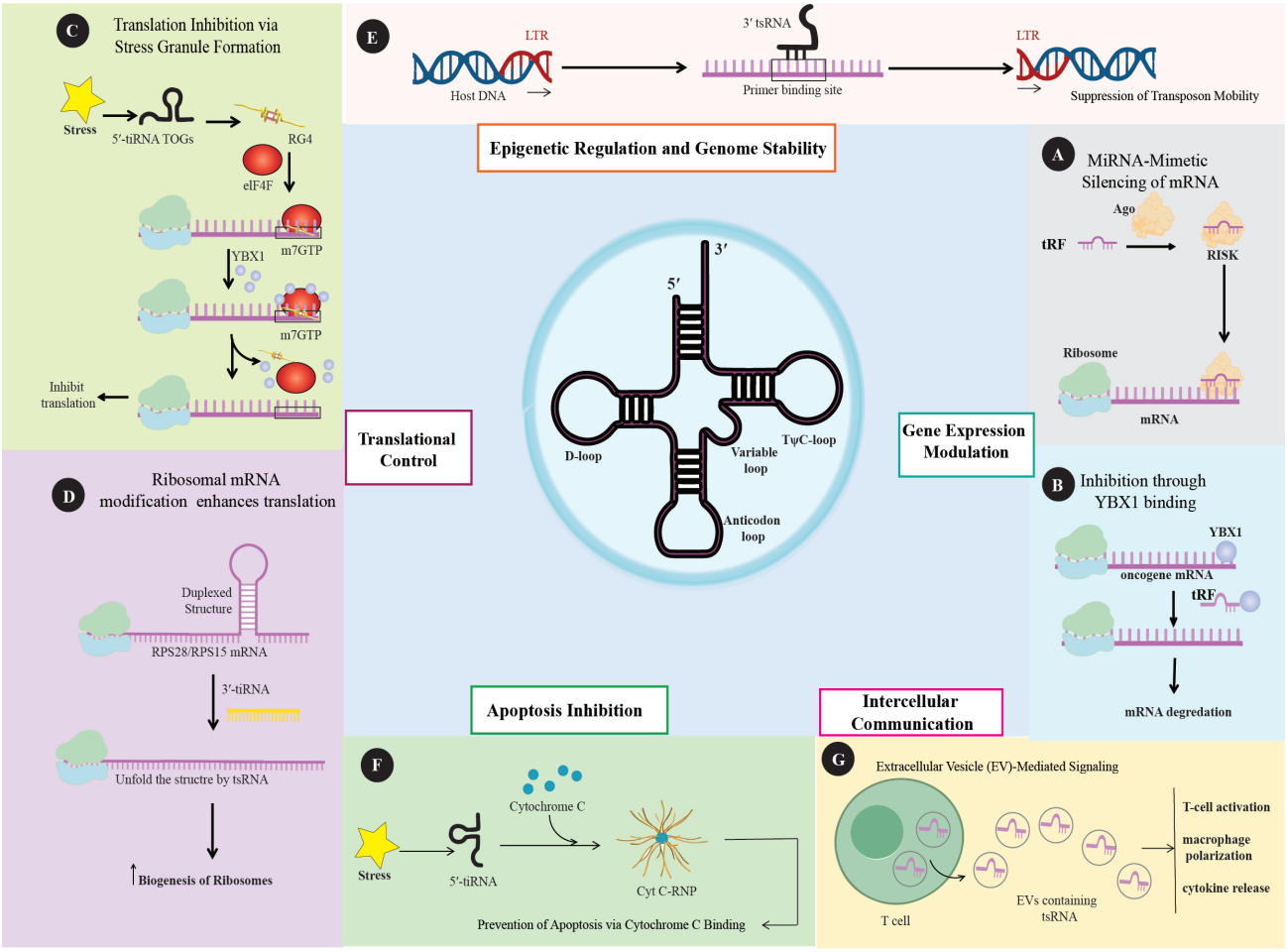

Figure 2. TsRNAs generated through tRNA cleavage participate in numerous biological processes, including A. MiRNA-Mimetic Silencing of mRNA, B. Inhibition through YBX1 binding, C. Translation Inhibition via Stress Granule Formation, D. Ribosomal mRNA modification enhances translation, E. Suppression of Transposon Mobility, F. Prevention of Apoptosis via Cytochrome C Binding, and G. Extracellular Vesicle-Mediated Signaling.

Another critical regulatory role of tsRNAs involves their impact on chromatin remodeling and epigenetic modifications. Sharma et al. found that tsRNAs transferred via epididymosome-derived exosomes target retrotransposons and regulate histone methylation and acetylation during embryogenesis [65]. These interactions underline the epigenetic influence of tsRNAs on recipient cells. In the context of cancer, exosomal tsRNAs play a pivotal role in promoting tumorigenic processes. Zhu et al. identified 5′-tRF-Val and 5′-tRF-Gly in exosomes derived from liver cancer cells, which enhance cellular proliferation by stabilizing oncogenic transcripts and modulating mRNA translation [60]. Additionally, tsRNAs can act as decoys for RNA-binding proteins, disrupting their normal cellular functions and contributing to an oncogenic environment.

These findings collectively showcase the multifaceted roles of tsRNAs in mediating intercellular communication through exosomes. By influencing transcription, translation, and protein activity, tsRNAs are emerging as central regulators of cellular signaling pathways, particularly in immune modulation and tumor progression. Further investigation into their molecular targets and mechanisms could pave the way for novel therapeutic strategies.

3. TsRNAs and Cancers

TsRNAs have garnered attention as vital regulators in cancer biology, impacting critical processes like tumor development, advancement, metastasis, and resistance to therapy. Through their ability to influence gene expression and optimize translational activity, tsRNAs equip cancer cells to adapt to the ever-changing microenvironment and stress conditions inherent in malignancy. These small RNA molecules play a pivotal role in orchestrating the complex signaling pathways that drive cancer progression, making them valuable targets for both diagnostic and therapeutic innovation [66].

3.1 The Roles of tsRNAs in Tumorigenesis and Progression of Cancers

The process of tumorigenesis involves a shift from regulated cellular proliferation to uncontrolled growth and the evasion of apoptosis. This transition places a substantial demand on protein synthesis, one of the most energy-intensive cellular processes. tRNA modifications play a critical role in fine-tuning this translation process, facilitating the synthesis of proteins required for cancer progression.

Key epitranscriptomic marks on tRNAs, such as 7-methylguanosine (m7G), have been implicated in various cancers. For example, METTL1-mediated m7G modifications enhance the translational efficiency of oncogenic genes with codon biases, as seen in esophageal squamous cell carcinoma and bladder cancer. These modifications promote tumor growth and are associated with poor survival outcomes. Additionally, METTL2A, responsible for 3-methylcytosine (m3C32) modifications on specific tRNAs, is upregulated in breast invasive carcinoma, correlating with higher tumor grades and poor prognosis. Therapeutic agents targeting high METTL2A-expressing tumors have shown promise, suggesting potential precision medicine approaches [67-69].

Polymorphisms in tRNA-modifying enzymes such as TRMT6 and PUS7 further highlight their role in tumorigenesis. TRMT6 polymorphisms are linked to an increased risk of hepatocellular carcinoma and Wilms tumor, while PUS7 is associated with worse outcomes in glioblastoma. These enzymes influence codon-specific translation, impacting gene expression networks crucial for cancer progression [70,71].

The NAT10 enzyme, responsible for acetylation of tRNAs, is upregulated in esophageal cancer and contributes to tumor progression. In xenograft models, NAT10 depletion reduced proliferation, invasion, and metastasis while enhancing apoptosis, indicating its therapeutic potential. Similarly, loss of NSUN2, a methyltransferase, impaired translation of oncogenic proteins in thyroid and bladder cancers, providing further evidence for the importance of tRNA modifications in tumor growth [72,73].

During cancer progression, malignant cells develop invasive phenotypes and establish tumor microenvironments to sustain growth and evade immune responses. METTL1-driven m7G modifications are crucial in nasopharyngeal carcinoma and head and neck squamous cell carcinoma, enabling efficient translation of WNT signaling components. Loss of METTL1 reduces global translation and tumor progression, underscoring its role as a key regulator of cancer progression [74,75].

Epitranscriptomic modifications also help cancer cells adapt to hypoxic environments, a common feature of advanced tumors. For instance, METTL1 downregulation under hypoxia modulates HIF1α translation, facilitating metabolic shifts that promote tumor survival. Additionally, polymorphonuclear myeloid-derived suppressor cells in the tumor microenvironment exploit METTL1-mediated modifications to evade immune responses and promote tumor growth [76,77].

Further studies have revealed novel tRNA modifiers such as hTrmt13 and FTSJ1, which regulate cell migration, metastasis, and oxidative stress responses. For example, hTrmt13-dependent methylation of tRNAs impacts global translation and cell migration, while FTSJ1-driven modifications support stress resistance in melanoma. These findings suggest that targeting tRNA modifications could provide new therapeutic strategies for combating aggressive and treatment-resistant cancers [78,79].

In summary, tRNA modifications play an integral role in the tumorigenesis and progression of various cancers by modulating protein translation, enabling metabolic adaptation, and facilitating immune evasion. These modifications represent promising targets for innovative therapeutic interventions aimed at disrupting the translational demands of cancer cells.

3.2 TsRNAs and Tumor Metastasis

Metastasis, the primary cause of cancer-related deaths, refers to the process where malignant cells migrate from their initial site to distant organs via the circulatory or lymphatic systems. This complex phenomenon involves several stages, including local invasion, entry into circulation (intravasation), survival in the bloodstream, exit into distant tissues (extravasation), and colonization to establish secondary tumors [80]. These processes are regulated by mechanisms such as epithelial-mesenchymal transition (EMT), hypoxia, angiogenesis, and the formation of a tumor-supportive microenvironment (TME). Additionally, significant cellular changes, including reduced adhesion, cytoskeletal remodeling, degradation of the extracellular matrix (ECM), and pseudopodia formation, play essential roles in promoting metastasis [81].

A key feature of metastasis is EMT, where epithelial cells transition into a mesenchymal phenotype, enhancing their ability to invade and migrate. This transformation is orchestrated through pathways such as TGFβ, Wnt, and Notch [82]. For instance, tRF/miR-1280 was found to suppress colorectal cancer (CRC) metastasis by targeting the Notch signaling pathway. Mechanistically, it positively regulates miR-200b, which inhibits EMT and controls processes like apoptosis and cell division by downregulating JAG2. Lower levels of tRF/miR-1280 in CRC patients correlate with increased metastatic potential, highlighting its role in disease progression [83]. In ovarian cancer, tRF-03357 has been identified as a significant contributor to tumor spread. By reducing the expression of the tumor suppressor HMBOX1, it facilitates cell proliferation, migration, and invasion. This makes tRF-03357 a potential biomarker for identifying high-grade ovarian cancer and a promising therapeutic target [84].

The role of tsRNAs extends beyond EMT and involves autophagy regulation in metastasis. For example, elevated levels of 5’-tRF-GlyGCC in breast cancer cells promote metastasis by inhibiting autophagy. This tsRNA interacts with the demethylase enzyme FTO, enhancing its activity to inhibit autophagy, which ultimately drives cancer cell invasion and spread. These findings suggest that targeting 5’-tRF-GlyGCC could provide new treatment options for breast cancer [85]. In gastric cancer, another tsRNA, tRF-3017A (derived from tRNA-Val-TAC), is linked to increased lymph node metastasis. It exerts oncogenic effects by silencing the tumor suppressor gene NELL2 through RISC formation with the AGO2 protein, promoting cell invasion and migration.

This highlights its potential as a key player in gastric cancer progression [42]. A distinct tsRNA, tRF-20-M0NK5Y93, has shown promise in suppressing metastasis in colorectal cancer. It interacts with MALAT1, a well-known oncogene associated with metastatic cancers, to downregulate its expression. This tsRNA inhibits MALAT1-mediated pathways by regulating SMC1A, a critical factor in cell division and metastasis. Such findings position tRF-20-M0NK5Y93 as a valuable therapeutic target for reducing tumor spread [86].

In conclusion, tsRNAs influence multiple aspects of metastasis by regulating key signaling pathways, modulating tumor suppressors and oncogenes, and impacting processes like EMT. These small RNAs hold immense potential as diagnostic markers and therapeutic targets, providing new directions for cancer treatment and research.

3.3 The Roles of tsRNAs in Drug Resistance

Drug resistance remains one of the most significant challenges in cancer treatment, often arising from the ability of tumor cells to adapt to therapeutic stress. This adaptation frequently involves translational reprogramming, wherein tsRNAs play a central role by regulating translation efficiency and sustaining oncogenic pathways critical for survival under therapeutic pressure [74].

In hepatocellular carcinoma, tsRNAs contribute to lenvatinib resistance by enhancing the translation of survival-related genes. Modifications such as methylation at specific sites on tRNAs increase their efficiency in decoding codon-biased oncogenic mRNAs. Studies have shown that suppressing these tRNA modifications, particularly those mediated by enzymes like METTL1, restores drug sensitivity and promotes apoptosis, demonstrating the importance of tsRNAs in therapeutic evasion [87].

In esophageal cancer, tRNA modifications are similarly implicated in resistance mechanisms. Loss of modifying enzymes such as NAT10 disrupts the synthesis of oncogenic proteins, sensitizing cells to EGFR inhibitors. Combining NAT10 inhibition with EGFR-targeting drugs has demonstrated synergistic effects, including reduced proliferation, migration, and invasion, while increasing apoptosis. This highlights the potential of targeting tsRNA-modifying pathways to overcome drug resistance in esophageal tumors [72].

In melanoma, ELP3- and CTU1-mediated tRNA modifications enable the efficient translation of HIF1α under hypoxic conditions, facilitating resistance to BRAF inhibitors. These modifications ensure continuous HIF1α expression, which supports metabolic adaptation and survival under treatment stress. Loss of these tRNA modifications sensitizes melanoma cells to therapy, indicating that targeting this axis could improve treatment outcomes [88].

Further studies in hepatocellular carcinoma have shown that sublethal heat stress from insufficient radiofrequency ablation enhances tsRNA-mediated resistance by promoting the translation of EMT regulators like SNAIL and SLUG. This adaptation supports tumor cell migration and metastasis, demonstrating the role of tsRNAs in enabling cancer cells to evade both therapeutic and environmental stressors [89].

The role of wobble U34 modifications in therapy resistance has also been highlighted. These modifications regulate ribosomal dynamics and co-translational folding, particularly under stress conditions. In melanoma, the loss of U34 modifications decreases the translation of survival genes, enhancing sensitivity to BRAF inhibitors. Restoration of these modifications rescues resistance, emphasizing their importance in maintaining cellular fitness during treatment [90,91].

In summary, tsRNAs and their modifying enzymes are central to the development of drug resistance in various cancers. By modulating translation and sustaining oncogenic pathways, tsRNAs enable tumor cells to adapt and survive under therapeutic pressures. Targeting tsRNA-mediated mechanisms offers a promising choice to enhance treatment efficacy and overcome resistance, paving the way for more effective cancer therapies.

3.4 The Dual Roles of tsRNAs in Xenograft Models: Insights into Tumor Progression and Suppression

TsRNAs are increasingly recognized as important players in cancer biology, with significant roles in regulating gene expression, stress response pathways, and tumor progression. Research using xenograft mouse models has highlighted both tumor-promoting and tumor-suppressive functions of tsRNAs, underscoring their potential for translational cancer research and therapeutic applications [38]. Several tsRNAs have been identified as drivers of tumor growth and metastasis in xenograft models. tRF-1001, derived from tRNA-Ser, has been shown to enhance cell proliferation in cancers such as prostate and colon. When tRF-1001 expression was silenced in xenograft mice, a marked reduction in tumor size and proliferation was observed, indicating its role as an oncogenic factor [1,38]. Similarly, 5′-tiRNA-Gly, which is upregulated under hypoxic conditions, has been shown to facilitate cancer cell survival and metastasis. By interacting with YBX1, this tsRNA stabilizes mRNAs that promote tumor growth [43].

In contrast, some tsRNAs function as tumor suppressors, hindering cancer progression by targeting critical oncogenic pathways. For instance, CU1276, a fragment generated from tRNA-Leu, has been demonstrated to suppress tumor growth in lymphoma models by impairing DNA repair mechanisms. The study revealed that the absence of CU1276 led to genomic instability and accelerated tumor growth [92]. In colorectal cancer, tRF/miR-1280, another tRNA fragment derived from tRNA-Leu, effectively downregulates Notch signaling, a pathway essential for tumor growth and metastasis. Xenograft experiments showed that its expression reduced tumor progression, indicating its therapeutic potential as a regulator of cancer signaling [93]. Other studies in xenograft models have also provided insights into the therapeutic potential of targeting tsRNAs. For example, antisense oligonucleotides designed to inhibit

Leu3′tsRNA were used in hepatocellular carcinoma models, resulting in reduced tumor size and cell proliferation. These findings highlight the feasibility of targeting tsRNAs for cancer therapy [94].

Building upon this, tRF-T11, a tsRNA derived from the Chinese yew (Taxus chinensis), has shown significant potential in ovarian cancer research. In studies conducted on ovarian cancer A2780 cells, tRF-T11 displayed anti-cancer effectiveness similar to that of taxol, a widely used chemotherapy drug, but required only one-sixteenth of the dosage. Its mechanism involves directly binding to the 3′ untranslated region (UTR) of the TRPA1 mRNA oncogene and inhibiting its expression through an RNA interference (RNAi) process facilitated by AGO2. This discovery not only emphasizes the therapeutic possibilities of RNA-based treatments but also represents a novel direction in utilizing plant-derived macromolecules for cancer treatment [95]. With continued advancements in research, the investigation of tsRNAs across various cancer models and their application in translational therapies could pave the way for innovative, highly effective, and low-toxicity treatment strategies. This rapidly evolving field has the potential to redefine the future of precision oncology, offering tailored and transformative solutions for cancer management.

3.5 Diagnostic and Therapeutic Applications of tsRNAs in Gynecological Malignancies

TsRNAs have become known as pivotal regulators in the advancement of certain cancers, including gynecological malignancies. These small non-coding RNAs, typically operating via Ago protein-dependent processes, regulate the expression of genes at both pre-transcriptional and post-transcriptional stages. In ovarian cancer, the tRF5Glu fragment modulates the mRNA levels of BCAR3 by binding directly to its 3' UTR, thereby suppressing its expression and restricting the proliferation of cells [96]. The expression of tsRNAs, such as ts-101 and ts-46, has also been correlated with key cellular processes like chromatin structure maintenance, cellular survival, apoptosis, and proliferation in ovarian, breast, and colon cancers [97].

In ovarian cancer, tsRNAs have been implicated in regulating gene expression and modulating tumor behavior. For instance, i-tRF-GlyGCC, a tRNA-derived internal fragment, was recently identified as a novel marker associated with poor prognosis in EOC. Elevated i-tRF-GlyGCC levels correlate with advanced tumor stages, suboptimal surgical outcomes, and early progression following platinum-based chemotherapy. Its integration into multivariate clinical models enhances risk stratification, supporting precision medicine approaches [98].

Similarly, 3'U-tRFValCAC, derived from pre-tRNAValCAC, has been shown to promote cancer cell growth and migration in vitro and is associated with inferior survival outcomes in EOC patients. This tsRNA enhances risk stratification when incorporated into clinical models, offering a more refined assessment of treatment response and disease progression compared to traditional markers [99]. A new study of RNA-sequencing data from 180 samples of serum, including those from healthy individuals, patients with ovarian tumors, and tumors that were non-cancerous or borderline, showed that tsRNAs make up 2.5% to 29.4% of all small RNAs and are mostly found in certain types of tRNA, especially Gly-tRNA. These tsRNAs may accurately predict aberrant cell growth, indicating their potential as diagnostic biomarkers [100]. More research showed that tRF-03357 lowers the tumor suppressor HMBOX1, which makes ovarian cancer cells grow, migrate, and invade. This makes it even more likely that it can be used as a biomarker for high-grade ovarian cancer [84].

Studies have also explored the role of tsRNAs in cervical cancer. Wang et al. found that tRF-Glu49 can stop tumors from growing by targeting FGL1, an inflammatory factor released by the liver that is connected to both tumor growth and apoptosis [101,102]. In addition, 5'-tRF-GlyGCC was found to promote cervical cancer cell proliferation and protein synthesis, contributing to the progression of ALKBH3-mediated cervical cancer [103]. tRF-Glu49 was also reported to be downregulated in cervical cancer tissues, with its low expression correlating to less aggressive disease and better prognosis, highlighting its potential as a prognostic marker [101].

The expression and role of tRF-20-S998LO9D vary significantly across different cancer types, highlighting its context-dependent functions. In endometrial carcinoma, tRF-20-S998LO9D is notably downregulated, where it acts as a tumor suppressor by enhancing apoptosis and inhibiting cell proliferation and metastasis through the upregulation of SESN2. This suggests a protective function in this specific cancer type [104]. On the other hand, in cancers such as breast, lung, kidney, and head and neck cancers, tRF-20-S998LO9D is highly expressed, promoting oncogenic behaviors, including increased proliferation and tumor progression [105]. This disparity likely arises from the unique tumor microenvironments and molecular landscapes of different cancers. Non-coding RNAs, including tRFs, are known to exhibit dual roles, functioning as either tumor suppressors or oncogenes depending on their interactions with specific regulatory molecules, signaling pathways, and cellular contexts.It has also been found that ovarian cancer patients and healthy controls have differential expression of tsRNAs. For example, RNA sequencing identified four tsRNAs derived from tRNA-Gly, with ts-3 showing the best diagnostic performance, with an area under the curve (AUC) between 0.836 and 0.948, supporting its possible use as a diagnostic biomarker [100].

Sequencing tRFs and tiRNAs in high-grade serous ovarian cancer revealed 20 tiRNAs and 20 upregulated TRFs in malignant tissues, with 15 downregulated tiRNAs further detected. These molecules are involved in critical pathways, such as mucin-type O-glycan biosynthesis, glycosphingolipid metabolism, and fatty acid metabolism, which are crucial for tumor progression [106].

Further insights into tsRNA-based therapies have also emerged from plant-derived tsRNAs. For instance, tRF-T11, derived from the Chinese yew, has been shown to inhibit ovarian cancer growth by targeting the oncogene TRPA1. Remarkably, tRF-T11 requires a significantly lower dosage than traditional chemotherapies like taxol, offering an innovative RNA-based therapeutic option for ovarian cancer [95]. Collectively, tsRNAs are implicated in the initiation, progression, and drug response of various cancers. In addition to their roles in ovarian, cervical, and endometrial cancers, tsRNAs such as tRF5-Glu, tRF-03357, and tRF-20-S998LO9D are promising candidates for both diagnostic and therapeutic purposes. As cancer cells adapt to stress by generating tsRNAs to support survival, these small RNAs hold great potential as biomarkers and molecular targets for innovative cancer treatments [107,108].

Further evidence of tsRNAs' potential as non-invasive diagnostic indicators comes from their detection in cancer patients' urine and serum. For instance, studies have connected tsRNAs to the pathology of breast cancer, and the overexpression of hormone-dependent tsRNAs in both breast and prostate cancers promotes the growth of cancer cells [109,110]. As a result, a comprehensive tsRNA database across different tumor types may revolutionize cancer management, supporting earlier diagnosis and more personalized treatment approaches [111,112].

In summary, the significant dysregulation of tsRNAs across various cancer types, their involvement in key regulatory pathways, and their potential as diagnostic biomarkers and therapeutic targets underscore their importance in cancer research and clinical application. Future studies focusing on the mechanistic roles of tsRNAs in cancer may pave the way for novel RNA-based diagnostics and treatments, transforming cancer care.

4. Conclusions

Transfer RNA-derived small RNAs have emerged as influential regulators in the intricate landscape of cancer biology, with notable relevance in gynecological malignancies such as ovarian, cervical, and endometrial cancers. Their multifaceted biogenesis and functional versatility position tsRNAs as pivotal modulators of essential cellular processes, including gene expression, cell proliferation, apoptosis, and stress responses. By interacting with critical molecular pathways, tsRNAs play a dual role in pre- and post-transcriptional gene regulation, underscoring their contribution to tumor progression and adaptation.

Recent advancements in high-throughput RNA sequencing have shed light on the aberrant expression patterns of tsRNAs in various malignancies, highlighting their potential as non-invasive diagnostic biomarkers. Their detectability in body fluids, such as serum and urine, offers a unique advantage for early detection, disease monitoring, and prognostication. This characteristic not only broadens their applicability in clinical diagnostics but also paves the way for the development of precision medicine strategies in gynecological oncology.

In therapeutic contexts, tsRNAs represent a promising frontier for RNA-based interventions. Their involvement in tumor microenvironment adaptation, stress resistance, and metastatic progression highlights their value as therapeutic targets. Ongoing advancements in RNA therapeutics, including the development of miRNA mimics, antisense oligonucleotides, and innovative delivery platforms, create opportunities to leverage tsRNAs for personalized and targeted cancer treatments. These approaches have the potential to mitigate tumor progression while enhancing therapeutic efficacy.

Looking forward, bridging the gap between fundamental research and translational applications will be critical for realizing the clinical potential of tsRNAs. Further mechanistic studies are essential to uncover the intricate roles of tsRNAs in gynecological cancers, offering insights into their therapeutic and diagnostic applications. With their ability to integrate seamlessly into emerging RNA-based technologies, tsRNAs are poised to transform gynecological cancer management, heralding a new era of precision oncology and improved patient outcomes.

Abbreviation List

tRNA: Transfer RNA, tsRNA: Transfer RNA-derived Small RNA, tRF: tRNA-derived Fragment, tiRNA: tRNA-derived Stress-induced RNA, ANG: Angiogenin, GC: Gynecological Cancer, miRNA: MicroRNA, AGO: Argonaute Protein, RISC: RNA-induced Silencing Complex, UTR: Untranslated Region, SG: Stress Granule, Cyt C: Cytochrome C, EV: Extracellular Vesicle, TRF: Transfer RNA-derived Fragment, HGSOC: High-Grade Serous Ovarian Cancer, ERV: Endogenous Retrovirus, RNP: Ribonucleoprotein, RG4: RNA G-quadruplex, YBX1: Y-Box Binding Protein 1, AUC: Area Under the Curve, SHOT-RNA: Sex Hormone-dependent tRNA-derived RNA, SESN2: Sestrin 2, HMBOX1: Homeobox Containing 1, BCAR3: Breast Cancer Anti-estrogen Resistance Protein 3, FGL1: Fibrinogen-like Protein 1, RPS: Ribosomal Protein

Competing Interests

The author declares no competing interests

Authors' Contributions

V.H. contributed to the conceptualization, writing the original draft, review, editing, and approved the final manuscript.

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