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REVIEW

Mitochondrial RNA modifications in gene expression and cancer biology

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Abstract **RNA modifications are essential in regulating gene expression at the post-transcriptional level. Recent studies, including our own, have highlighted that RNA modifications, such as N^6-methyladenosine (m^6A) and methyl-5-cytosine (m^5C), play a crucial role in tumorigenesis, metabolism, and anti-tumor immunity. Targeting RNA modification machinery may represent a promising therapeutic strategy in cancer. Intriguingly, emerging evidence reveals numerous modifications in mitochondrial RNA (mt-RNA), expanding the concept of epitranscriptomics to mitochondria. The mammalian mitochondrion possesses its own genome, which encodes 22 transfer RNAs (tRNAs), 2 ribosomal RNAs (rRNAs), and 13 proteins necessary for energy production via oxidative phosphorylation (OxPhos). The mitochondrial transcriptome is produced from large polycistronic transcripts, implying that mitochondrial gene expression is predominantly regulated post-transcriptionally. In this review, we summarize all currently known mt-RNA modifications, their potential regulatory machinery, as well as their biological functions in tumorigenesis and metabolism. Additionally, given that this field is still in its infancy, we discuss several critical knowledge gaps and propose future research directions to clarify the mechanistic and clinical significance in the study of mt-RNA modifications.**

Keywords mitochondria; RNA modifications; epitranscriptomics; cancer; metastasis; metabolism; tumor immunity

Introduction

Mitochondria, the powerhouses of eukaryotic cells, not only supply energy for cellular functions but also act as central hubs for diverse signal pathways, including cell apoptosis, calcium homeostasis, and the regulation of inflammation and immunity [1–3]. Accumulating evidence has established that mitochondrial dysfunction and metabolic reprogramming are fundamental drivers of a wide range of human pathologies, including cancer. In tumors, functional mitochondria sustain oncogenesis by supporting biosynthesis, maintaining redox balance, and enabling metabolic flexibility [4–6]. Consequently, mitochondria are now increasingly recognized as key contributors to therapy resistance and immune modulation [7–11].

To understand the crucial role of mitochondria in cancer biology, it is essential first to consider their evolutionary origins and genomic features. Mitochondria originated from free-living bacterial ancestors that were engulfed by eukaryotic cells via endocytosis. Although more than 99% of the original genetic information from these bacterial ancestors was transferred to the nuclear genome, mitochondria retain their own independent genome. Unlike the linear DNA of nuclear genome, the mitochondrial DNA (mt-DNA) is a circular, double-stranded molecule. Human mt-DNA spans 16 569 base pairs and comprises two strands that can be distinguished by their nucleotide composition: a guanine/thymidine-rich heavy (H) strand and its complementary light (L) strand [12,13] (Fig. 1).

Transcription of mt-DNA occurs from both strands, producing long polycistronic transcripts processed

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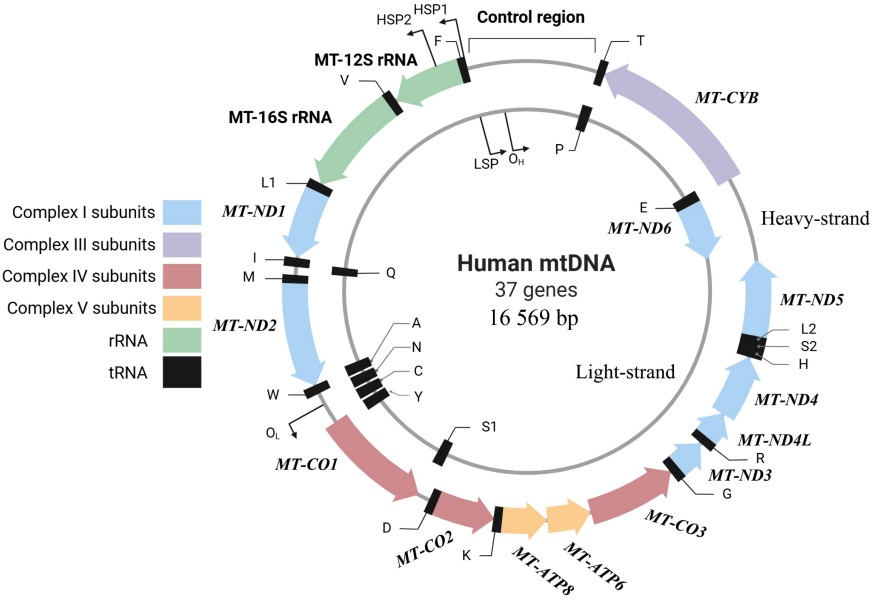


Fig. 1 Schematic map of the human mitochondrial genome. The double-stranded circular mt-DNA contains 37 genes: 13 OxPhos subunits (color-coded by respiratory complex), 2 rRNAs (green), and 22 tRNAs (black). Outer and inner circles represent heavy (H) and light (L) strands. The D-loop region includes LSP, HSP1, and HSP2 promoters, replication origins (O_H, O_L), with arrows showing the direction of transcription. Protein-coding genes: *MT-ND1*, *MT-ND2*, *MT-CO1*, *MT-CO2*, *MT-ATP8*, *MT-ATP6*, *MT-CO3*, *MT-ND3*, *MT-ND4L*, *MT-ND4*, *MT-ND5*, *MT-ND6*, and *MT-CYB*. rRNA genes: *MT-RNR1* (12S rRNA) and *MT-RNR2* (16S rRNA). tRNA genes (letter codes in the figure): *MT-TF* (tRNA-Phe, F), *MT-TV* (tRNA-Val, V), *MT-TL1* (tRNA-Leu^{UUR}, L1), *MT-TI* (tRNA-Ile, I), *MT-TQ* (tRNA-Gln, Q), *MT-TM* (tRNA-Met, M), *MT-TW* (tRNA-Trp, W), *MT-TA* (tRNA-Ala, A), *MT-TN* (tRNA-Asn, N), *MT-TC* (tRNA-Cys, C), *MT-TY* (tRNA-Tyr, Y), *MT-TS1* (tRNA-Ser^{UCN}, S1), *MT-TD* (tRNA-Asp, D), *MT-TK* (tRNA-Lys, K), *MT-TG* (tRNA-Gly, G), *MT-TR* (tRNA-Arg, R), *MT-TH* (tRNA-His, H), *MT-TS2* (tRNA-Ser^{AGY}, S2), *MT-TL2* (tRNA-Leu^{CUN}, L2), *MT-TE* (tRNA-Glu, E), *MT-TP* (tRNA-Pro, P), and *MT-TT* (tRNA-Thr, T). Created with BioRender.

through a unique transfer RNA (tRNA) punctuation model. The H-strand transcript encodes 2 mitochondrial rRNAs (mt-rRNAs), 14 mitochondrial ribosomal RNAs (mt-tRNAs), and 12 mitochondrial messenger RNAs (mt-mRNAs), while the L-strand transcript contains 8 mt-tRNAs and a single mt-mRNA [14,15]. Altogether, human mt-DNA encodes 13 protein-coding mt-mRNAs, 22 mt-tRNAs, and 2 mt-rRNAs [3,14,16,17]. These 13 proteins encoded by the mitochondrial genome serve as key oxidative phosphorylation (OxPhos) subunits that are essential for respiratory chain biogenesis and ATP production [18]. This streamlined system, lacking introns, relies heavily on precise RNA processing to yield mature, functional molecules essential for mitochondrial protein synthesis and energy production.

Unlike nuclear transcripts which are monocistronic, the entire mitochondrial transcriptome is produced from large polycistronic transcripts. Specifically, the H strand is transcribed from a single promoter called heavy strand promoter (HSP), while the L strand is driven by two promoters called light strand promoter (LSP) and light strand promoter 2 (LSP2) [19–21]. Due to the shared promoters, the striking differences in expression levels among individual mitochondrial genes underscore that post-transcriptional regulation is the primary determinant of mitochondrial gene expression [21].

This brings us to an emerging area of research: RNA modifications as key post-transcriptional regulators. Eukaryotic RNAs can be modified by over 170 chemical modifications, which play a crucial role in post-transcriptional regulation [22–24]. Dysregulation of RNA modifications contributes to human diseases, including tumorigenesis. For instance, our recent studies [25–28] and those of others [29–33] suggest that N^6-methyladenosine (m^6A), the most prevalent modification in mRNA, plays a crucial role in tumorigenesis, cancer stem cell self-renewal, and metabolism. Targeting the machinery responsible for RNA modifications has shown promising therapeutic efficacy against cancer [34–38]. While these insights have largely arisen from studies of cytosolic RNAs, recent studies reveal that mt-RNAs also harbor diverse chemical modifications [39–42], extending the concept of RNA epigenetics into mitochondria. Specifically, mt-tRNAs are the most extensively modified, carrying 137 modification sites encompassing 18 distinct chemical types [39–41]. These include marks such as m^1A, m^5C, τm^5U, Ψ, and t^6A, which ensure proper tRNA folding, accurate codon-anticodon pairing, and efficient mitochondrial translation. The mt-rRNAs also contain approximately 10 modification sites involving nine different types [41], including m^1A, m^5C, and Ψ, catalyzed by enzymes such as NOP2/Sun RNA

methyltransferase 4 (NSUN4), transcription factor B1, mitochondrial (TFB1M), and methyltransferase-like protein 15 (METTL15), which are essential for ribosome assembly and function. In addition, mt-mRNAs, though less extensively characterized, harbor multiple modifications, including m^5C, m^1A, and m^6A, distributed across coding and untranslated regions [43–46].

In addition to energy production, mitochondria serve as central hubs for cellular metabolism and immunity. Emerging evidence indicates that mt-RNA modifications act as crucial modulators of mitochondrial function under oncogenic stress, linking transcriptional regulation to metabolic adaptation, immune evasion, and therapy resistance. Thus, mt-RNA modifications-mediated dysregulation of mitochondrial functions might exert profound effects on tumorigenesis, metabolic reprogramming, and anti-tumor immunity. Given the central roles of mitochondria in cancer biology and immunity, together with the growing recognition of RNA epigenetics, mt-RNA modifications represent a largely unexplored but potentially transformative frontier in oncology. This review provides a comprehensive overview of mt-RNA modifications and their regulatory enzymes, and discusses their roles in tumorigenesis, metastasis, immune regulation, and drug resistance. We also discuss the outstanding knowledge gaps, challenges, and future perspectives in the field of mitochondrial epitranscriptomics.

mt-RNA modifications, enzymes, and functions

mt-RNAs, including mt-rRNA, mt-tRNA, and mt-mRNA, are extensively decorated with many chemical modifications that fine-tune their processing and function [47]. These modifications are introduced by nuclear-encoded mitochondrial-localized enzymes in a tightly coordinated manner, thereby safeguarding mitochondrial function and cellular homeostasis. Disruption of mt-RNA modification has been increasingly linked to defective OxPhos, metabolic disorders, and human diseases. Within this conceptual framework, the following sections provide a systematic overview of currently known mt-RNA modifications, including mt-mRNA, mt-rRNA, and mt-tRNA modifications, and their functions in determining mt-RNA fate.

mt-mRNA modifications and functions

Compared with the densely modified mt-tRNAs and mt-rRNAs, mammalian mt-mRNAs carry a relatively sparse but functionally important set of post-transcriptional marks. Advances in high-throughput sequencing, mass spectrometry-based nucleoside mapping, and chemical mapping approaches have generated nucleotide-resolution catalogs of mt-RNA modifications. These studies reveal that mt-mRNA chemistry and processing are tightly integrated with transcript maturation and translation [45,46]. Although fewer in number, mt-mRNA modifications are not randomly distributed; rather, they occur at positions with high potential to influence decoding, ribosome dynamics, or RNA stability.

The chemical modifications reported in mt-mRNAs include base methylations, such as N^1-methyladenosine (m^1A), and pseudouridine (Ψ), both can alter base-pairing or local RNA structure [48,49] (Fig. 2, Table S1). These modifications are deposited by nuclear-encoded enzymes that operate in the mitochondrial matrix, often as part of larger ribonucleoprotein assemblies. The deposition of modification is regulated in a transcript-specific and temporally controlled manner during mt-mRNA maturation. The functional impact of a given modification depends not only on its chemistry but also on its precise transcript and positional context.

Among the emerging epitranscriptomic regulators of mt-mRNA function, m^1A and Ψ have attracted particular attention. Traditionally associated with tRNAs and rRNAs, m^1A has now been detected at internal positions within several mt-mRNAs. Mapping studies [45,46] reported variable site counts, ranging from numerous candidates to a small set of high-confidence positions identified with reverse transcriptase-stalling approaches. This underscores a critical need for orthogonal validation. One reproducible site is m^1A1374 in *MT-ND5*, which is abundant in early human embryos and oocytes but decreases during later development [46], pointing to a potential role in developmental control of mitochondrial translation. Functionally, m^1A disrupts Watson–Crick base pairing and can impede codon–anticodon recognition, leading to ribosomal pausing and reduced translation. Enzymes involved in mt-mRNA m^1A methylation include tRNA methyltransferase 61B (TRMT61B) and tRNA methyltransferase 10C (TRMT10C, also known as mitochondrial RNase P protein 1 (MRPP1)). TRMT61B modifies specific sites in *MT-CO1/2/3* [45,46], whereas TRMT10C, a component of mitochondrial RNase P complex, is associated with methylation of *MT-ND5* [46]. Importantly, the m.13708G>A polymorphism (haplogroup J) in *MT-ND5* gene, linked to Leber's hereditary optic neuropathy (LHON), interferes with m^1A formation at the corresponding site, providing an example of how sequence variation can perturb mitochondrial epitranscriptomic regulation [50].

In addition to m^1A, Ψ has also been progressively mapped in mt-mRNAs [44]. Long recognized as the most abundant RNA modification in nature, Ψ was previously mapped to mt-rRNAs and mt-tRNAs but has since been identified in mt-mRNAs such as *MT-CO1* and *MT-CO3*. However, some earlier reports of Ψ in *MT-CO2* and

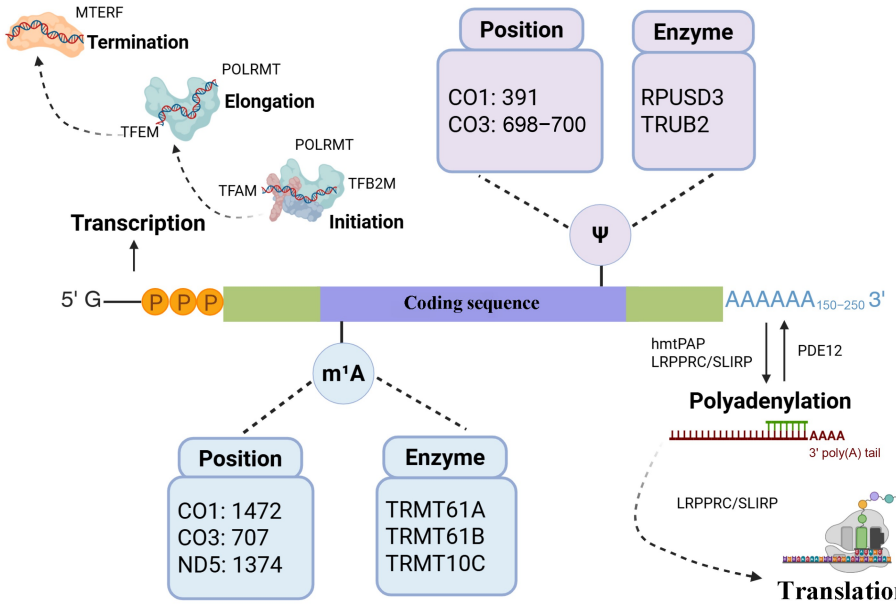


Fig. 2 Schematic representation of mt-mRNA transcription, processing and post-transcriptional modifications. Transcription of mt-mRNAs is initiated by POLRMT together with transcription elongation factor TEFM, producing polycistronic precursors that undergo endonucleolytic cleavage. Polyadenylation by mitochondrial poly(A) polymerase (hmtPAP) completes UAA stop codons in a subset of transcripts, enabling translation termination; poly(A) tail length is modulated by PDE12, while *MT-ND6* lacks polyadenylation. Post-transcriptional modifications include N^1-methyladenosine (m^1A; e.g., m^1A1374 in MT-ND5) introduced by TRMT61B (*MT-CO1/3*) and TRMT10C (*MT-ND5*), and Ψ (e.g., in *MT-CO1* and *MT-CO3*) catalyzed by RPUSD3 and TRUB2. Created with BioRender.

MT-ND4 have not been consistently confirmed by follow-up studies, highlighting the need for targeted validation [44,51,52]. Recent studies suggest that RNA pseudouridine synthase domain-containing protein 3 (RPUSD3) and TruB pseudouridine synthase family member 2 (TRUB2) contribute to mt-mRNA pseudouridylation, though the mitochondrial localization of TRUB2 requires further validation [52]. Depletion of RPUSD3 reduces Ψ levels in specific mt-mRNAs and impairs mitochondrial protein synthesis, suggesting that Ψ may support translational efficiency or RNA stability in mitochondria. Although the precise roles of Ψ in mitochondria remain under investigation, insights from cytosolic systems indicate that it can stabilize RNA secondary structure, improve decoding fidelity, and protect transcripts from endonucleolytic cleavage. These cytosolic insights may offer a conceptual framework for understanding potential roles of Ψ in mitochondria, where coordinated expression of respiratory chain subunits is essential for OxPhos and ATP production.

Beyond covalent modification, canonical mRNA processing steps provide an additional regulatory layer in mitochondria. Transcription elongation, mediated by polymerase RNA mitochondrial (POLRMT) and transcription elongation factor, mitochondrial (TEFM), promotes read-through of promoter-distal regions and influences the availability of full-length polycistronic precursors. Following endonucleolytic cleavage, polyadenylation by the mitochondrial poly(A) polymerase (hmtPAP) is essential for the maturation and translational competence of a subset of mitochondrial transcripts. For at least 7 of the 13 protein-coding mt-mRNAs, addition of a poly(A) tail completes a UAA stop codon, enabling productive termination. Mitochondrial poly(A) tails are typically ~45 nucleotides, though transcript-specific heterogeneity exists, for example, the L-strand–encoded ND6 lacks polyadenylation. Tail length is partly regulated by phosphodiesterase 12 (PDE12), a mitochondrial exonuclease that shortens poly(A) tails; pathogenic variants in mitochondrial poly(A) polymerase (MTPAP) (e.g., N478D) impair tail synthesis, leading to unstable or truncated transcripts with reduced protein output. Although polyribonucleotide nucleotidyltransferase 1 (PNPase1) has been implicated in mt-mRNA metabolism, its localization to the intermembrane space argues against a direct role in matrix-localized mt-mRNA polyadenylation.

The spatial and transcript-specific distribution of mt-mRNA modifications raises several intriguing questions. How dynamically are these modifications regulated in response to metabolic stress, hypoxia, or nutrients? Do they act as an active control signal that directs transcripts toward translation or decay? The observation that some enzymes, such as TRMT10C, participate in both mt-tRNA maturation and mt-mRNA modification [53–55], suggests crosstalk between RNA classes and a shared regulatory infrastructure. Addressing these questions will require quantitative, locus-specific mapping in defined

genetic models, combined with biochemical dissection of enzyme specificity and functional readouts of mitochondrial translation and respiration. Such integrative studies will be critical for clarifying how mt-mRNA modifications contribute to physiologic regulation and mitochondrial disease.

mt-rRNA modifications and functions

Mitochondrial ribosomes synthesize the 13 essential polypeptides encoded by the mitochondrial genome, all of which are core components of the OxPhos system [56,57]. Mitochondrial ribosomes, distinct from cytosolic 80S ribosomes, consist of a 28S small subunit (mtSSU; 12S rRNA) and a 39S large subunit (mtLSU; 16S rRNA), forming the 55S mitoribosome. They have evolved a specialized architecture with a lower rRNA-to-protein ratio, reflecting adaptation to the organelle's compact genome and unique translational requirements [41,57,58]. The mt-rRNA modifications are pivotal for maintaining ribosomal integrity and translational efficiency [59]. These modifications include 2′-O-methylation, base methylations (e.g., $m^{62}A$, m^5C, m^4C, and m^1A), and Ψ, each catalyzed by specific enzymes [46,60–62] (Fig. 3, Table S2). Although limited to approximately 10 distinct modification sites in human mt-rRNAs, these modifications are strategically positioned in functionally critical domains of the ribosome, particularly the decoding center and peptidyl transferase center, underscoring their importance in ensuring mitochondrial translation fidelity and efficiency. Notably, recent work revealed that mitochondrial methylation potential, governed by S-adenosylmethionine (SAM), is essential for proper rRNA methylation and mitoribosome assembly, further linking cellular methylation capacity to mitochondrial translation competence [63].

Modifications in mt-12S rRNA

mt-12S rRNA harbors several well-characterized modifications essential for mtSSU assembly and maturation. Among the most functionally significant is the N^6, N^6-dimethylation of two adjacent adenosines ($m^6{}_2A936$ and $m^6{}_2A937$), catalyzed by TFB1M. TFB1M

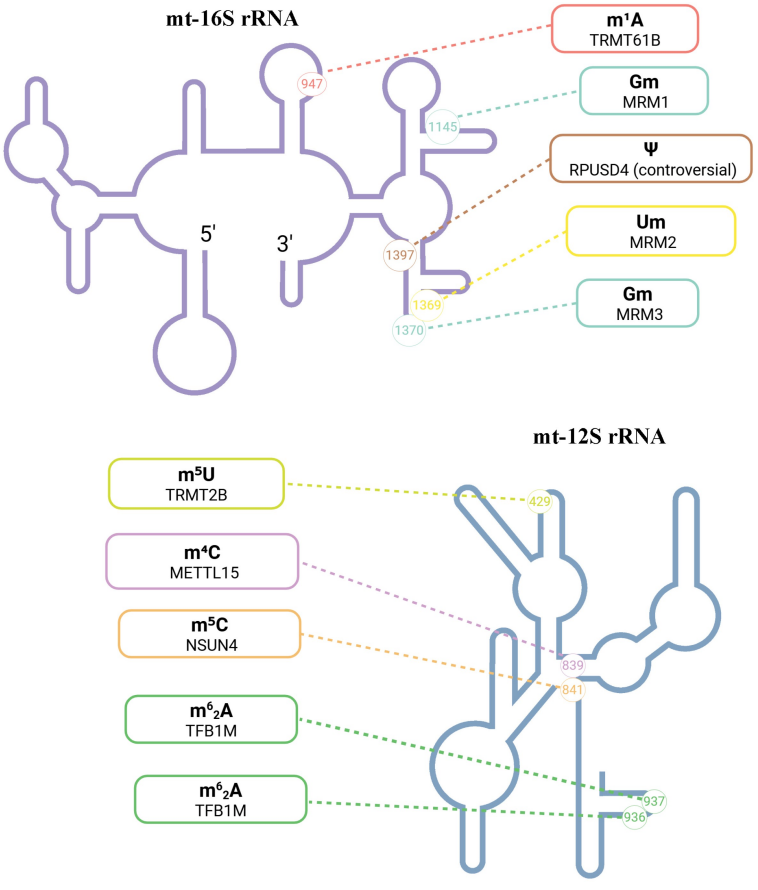


Fig. 3 Schematic representation of human mt-rRNA modifications. The small subunit (12S rRNA) contains $m^{62}A936/m^{62}A937$ installed by TFB1M, m^5C841 introduced by NSUN4, m^4C839 by METTL15, and m^5U429 by TRMT2B. The large subunit (16S rRNA) carries 2′-O-methylations Gm1145 (MRM1), Um1369 (MRM2), and Gm1370 (MRM3), as well as m^1A947 catalyzed by TRMT61B and Ψ1397 probably introduced by RPUSD4. Created with BioRender.

localizes to mitochondrial matrix and functions as a dedicated dimethyltransferase for these two sites, whereas its paralog TFB2M (transcription factor B2, mitochondrial) serves a distinct but coordinated role as a transcription initiation factor acting with POLRMT. The exclusive mitochondrial localization of both proteins ensures tight coupling between rRNA dimethylation and transcription initiation [59,64,65]. These residues are located within the A-loop of mt-12S rRNA, forming a functional decoding center and facilitating recruitment of assembly factors such as ribosome binding factor A (RBFA), which stabilizes mtSSU and prepare it for subunit joining [66]. Loss-of-function mutations or knockdown of TFB1M in human cells or mouse models severely compromise mitochondrial translation and OxPhos [67,68]. Moreover, *TFB1M/TFB2M* variants have been linked to sensorineural hearing loss, impaired mitochondrial function in pancreatic β cells, reduced insulin secretion, and increased type 2 diabetes risks, underscoring the physiologic importance of this methylation event in mitochondrial homeostasis [68–70].

Another important modification site is m^5C841, probably introduced by NSUN4, a dual-function enzyme that not only methylates this cytosine but also serves as a chaperone-like factor for ribosomal subunit joining in cooperation with mitochondrial rRNA methyltransferase 3 (MRM3) [61,71,72]. *NSUN4* deficiency disrupts mitoribosome assembly and reduces mitochondrial protein synthesis [61,71].

Adjacent to C841 is C839, which is methylated at the N4 position to form m^4C839 by METTL15 and/or methyltransferase-like protein 17 (METTL17) [62,73–75]. Recent studies reveal that METTL15 recognizes 12S rRNA with the assistance of the assembly factor RBFA to ensure accurate C839 methylation during early mtSSU biogenesis [76]. In parallel, METTL17 operates at a later stage, facilitating the maturation of pre-mitoribosomal intermediates [77]. Consistent with these roles, *METTL15* loss destabilizes 12S rRNA and impairs early mtSSU assembly, whereas *METTL17* deficiency blocks later maturation steps, collectively compromising mitochondrial translation and OxPhos [62,73,74]. Additionally, tRNA Methyltransferase 2 Homolog B (TRMT2B) catalyzes 5-methyluridine at position 429 (m^5U429) [78]. Although *TRMT2B*-deficient cells show only mild under basal conditions, this modification may contribute to RNA structural stability or interact with other rRNA binding proteins under stress conditions.

Modifications in mt-16S rRNA

mt-16S rRNA of the mtLSU contains a higher density of 2′-O-methyl modifications, primarily located in regions involved in tRNA accommodation and peptide bond formation. A key modification is 2′-O-methylguanosine at position 1145 (Gm1145), situated in the P-loop and catalyzed by mitochondrial rRNA methyltransferase 1 (MRM1) [79]. This modification maintains the geometry of the catalytic core where peptidyl-tRNA resides. Depletion of MRM1 disrupts rRNA architecture and leads to global mitochondrial translation defects.

Two additional 2′-O-methylations occur in the A-loop: 2′-O-methyluridine at position 1369 (Um1369), methylated by mitochondrial rRNA methyltransferase 2 (MRM2), and Gm1370, methylated by MRM3 [60,80,81]. These residues coordinate the accommodation of aminoacylated tRNAs and ensure high-fidelity translation elongation. Um1369 is involved in the final maturation steps of the mtLSU. Loss of its writer, MRM2, leads to severe respiratory defects, impaired mitochondrial protein synthesis, and has been associated with human mitochondrial encephalomyopathies [81,82].

N^1-methyladenosine at position 947 (m^1A947), introduced by TRMT61B [59,60], resides in helix 71 near the intersubunit interface. Its methylation stabilizes tertiary rRNA structure via altered base pairing and charge interactions. Evolutionary conservation of m^1A947 among primates suggests its importance, with non-primate mammals' methylation having a fixed adenine at this site, implying a compensatory adaptation in humans.

Ψ, the isomerization of uridine, enhances base-stacking potential and introduces an additional hydrogen bonding donor. In human mt-16S rRNA, only one Ψ site, Ψ1397, has been unequivocally identified [42,52]. The synthase responsible for this Ψ site remains under debate. An initial study in 143B osteosarcoma cells implicated that RNA Pseudouridine Synthase Domain-Containing Protein 4 (RPUSD4) was responsible for its generation [52]; RPUSD4 depletion leads to a reduction of the steady-state level of mt-16S rRNA, and suppressed biogenesis of mtLSU. However, a subsequent study in HEK293T cells did not demonstrate that RPUSD4 directly catalyzed Ψ1397 formation [83]. This discrepancy highlights the importance of determining which enzyme is truly responsible for pseudouridylation at this site, and whether a common synthase operates across cell types or the process is context dependent. Equally important is determining whether RPUSD4 possesses bona fide enzymatic activity to catalyze Ψ formation and elucidating the mechanism by which Ψ1397 contributes to the steady-state levels of mt-16S rRNA. Does this effect depend on the pseudouridylation activity of RPUSD4, or could it instead involve other, yet uncharacterized, Ψ synthases?

Although human mt-rRNAs contain far fewer modifications than their cytosolic or bacterial counterparts, those present are functionally indispensable. Their relative scarcity likely reflects the necessity to conserve energy in the metabolically constrained

mitochondrial matrix. Nevertheless, their precise localization of these modifications suggests a model in which each modification acts as a checkpoint or scaffolding anchor, guiding ribosomal maturation and safeguarding translational fidelity [84,85]. In addition, several modifying enzymes also serve as ribosome assembly factors, such as NSUN4, MRM2, and RPUSD4 [81,86,87]. The dual functions couple chemical modification with structural maturation, ensuring that ribosome quality control is tightly integrated with translational output. Disruption of this coordination may lead to selective translation defects or destabilization of mitoribosomal components.

mt-tRNA modifications and functions

mt-tRNAs are key components of the mitochondrial translation machinery. They interpret the unique mitochondrial genetic code and ensure accurate synthesis of mitochondria-encoded proteins [40]. Compared with their cytosolic counterparts, mt-tRNAs are smaller and often contain non-canonical secondary structures. Despite these structural peculiarities, they undergo diverse, intricate, and functionally critical post-transcriptional modifications (Fig. 4, Table S3). These modifications support tRNA structural stability, decoding accuracy, and translational efficiency, many of which are evolutionarily conserved [41,88]. Recent studies have considerably expanded our understanding of the chemical nature, enzymes, and physiologic significance of mt-tRNA modifications.

One of the best characterized modification sites is position 9 in mt-tRNAs, where adenine or guanine undergoes N^1-methylation of adenine (m^1A9) or guanine (m^1G9). Their formation is catalyzed by a subcomplex of mitochondrial RNase P, composed of TRMT10C and hydroxysteroid 17-beta dehydrogenase 10 (HSD17B10; also known as MRPP2), together with the endonuclease PRORP (proteinaceous RNase P; also known as MRPP3), which completes the RNase P holoenzyme and is required for efficient 5′-end processing and proper positioning of substrates for methylation [54,89–91]. TRMT10C serves as the primary S-adenosylmethionine (SAM)-dependent methyltransferase, whereas HSD17B10 stabilizes

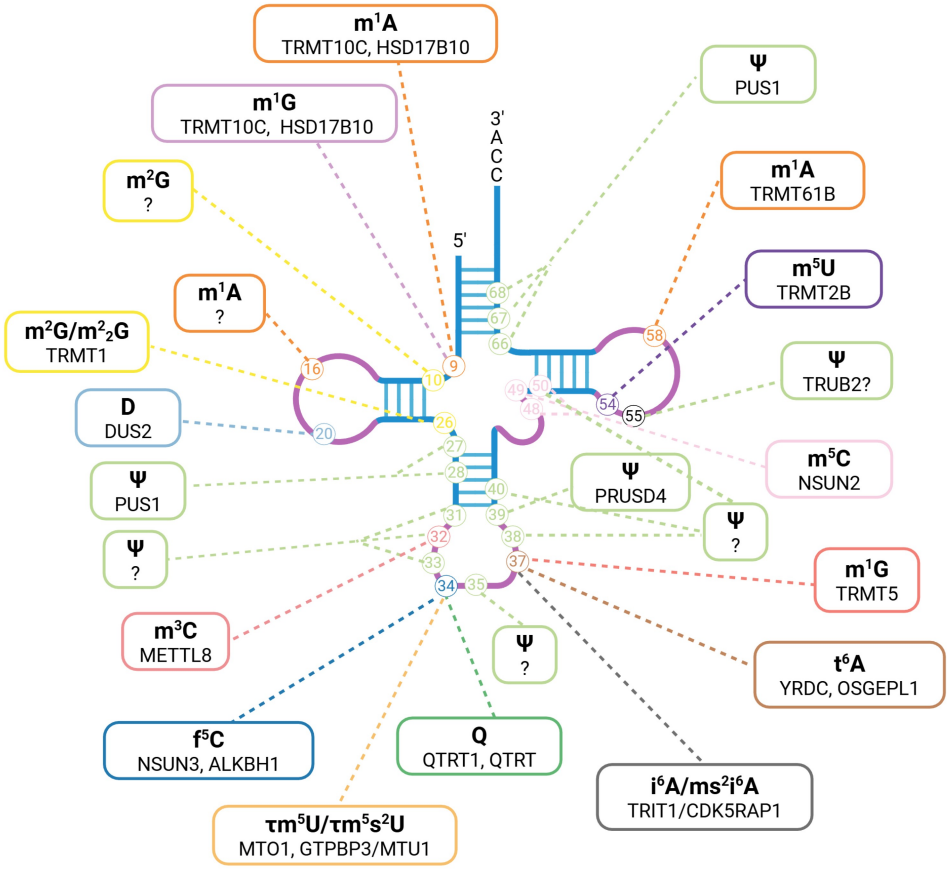


Fig. 4 Schematic representation of human mt-tRNA modifications. Representative mt-tRNAs carry m^1A9 installed by TRMT10C and HSD17B10, m^1A58 by TRMT61B, $m^5C48/49$ by NSUN3, $\tau m^5U34/\tau m^5s^2U34$ by GTPBP3/MTO1 and MTU1, f^5C34 by NSUN3-ALKBH1, Q34 by QTRT1/QTRT2, m^1G37 by TRMT5, i^6A37 by TRIT1, ms^2i^6A37 by CDK5RAP1, t^6A37 by YRDC-OSGEPL1, m^5U54 by TRMT2B, Ψ27/28 by PUS1, and D20 by DUS2. Created with BioRender.

TRMT10C and ensures efficient methylation [92]. The methylation at position 9 is present in most mt-tRNAs and acts as a structural anchor that promotes proper folding and prevents mispairing, thereby preserving the functional tRNA conformation. For example, unmodified A9 in mt-tRNA^{Lys} can mispair with U64, leading to structural misfolding. Mutations in HSD17B10 impair its interaction with TRMT10C, reduce methylation efficiency, and cause mitochondrial disorders such as HSD17B10 disease, characterized by neurodegeneration and cardiomyopathy [93–95]. This modification not only ensures correct tRNA folding but also influences its interaction with mitochondrial elongation factors and aminoacyl-tRNA synthetases. Misfolded tRNAs are prone to degradation by mitochondrial surveillance systems, leading to reduced tRNA pools and impaired translation. Reduced m^{1}G9 levels have been linked to translational stalling and defects in OxPhos complex I assembly.

Another critical modification site is position 58 in the T-loop, where TRMT61B installs m^{1}A58 in several human mt-tRNAs, including mt-tRNA^{Lys}, mt-tRNA^{Leu}, and mt-tRNA^{Ser} [96,97]. The m^{1}A58 modification introduces a positive charge that stabilizes tertiary interactions and promotes tRNA structural integrity. Interestingly, m^{1}A58 might be dynamically regulated by the demethylase AlkB Homolog 1, Histone H2A Dioxygenase (ALKBH1) [98], which responds to oxidative stress and metabolic imbalance. This reversible mechanism suggests that certain mt-tRNA marks could act as rapid sensors of metabolic state, enabling mitochondria to rapidly fine-tune translational output in response to environmental stimuli or disease states.

The anticodon loop of mt-tRNAs, particularly at the wobble position (position 34), is also a hotspot for modifications to decoding fidelity. This position carries a range of modifications, such as 5-formylcytosine (f^{5}C), queuosine (Q), and taurine-containing uridines, including 5-taurinomethyluridine (τm^{5}U) and 5-taurinomethyl-2-thiouridine (τm^{5}s^{2}U), which modulate codon recognition and maintain translation accuracy. A striking case is mt-tRNA^{Met}, in which NOP2/Sun RNA methyltransferase 3 (NSUN3) installs 5-methylcytosine (m^{5}C34) that is subsequently oxidized by ALKBH1 to 5-formylcytosine (f^{5}C34) [99–101]. This two-step modification exemplifies how mitochondria use modular chemical pathways to broaden the decoding capacity of a single tRNA species [102]. Loss of NSUN3 or ALKBH1 impairs mitochondrial translation and respiratory chain function, resulting in embryonic lethality in mice. Consistent with these findings, NSUN3 mutations in humans have been associated with mitochondrial encephalomyopathy-like phenotypes, further underscoring the pathological relevance of this mt-tRNA^{Met} modification axis [103–105].

Q at position 34, installed by queuine tRNA-ribosyltransferase catalytic subunit 1 (QTRT1)-QTRT2 complex, modifies mt-tRNAs, including mt-tRNA^{Tyr}, mt-tRNA^{His}, mt-tRNA^{Asn}, and mt-tRNA^{Asp} [40]. Q34 enhances decoding fidelity and reduces frameshifting [40]. Although direct evidence for Q34 dynamics in mitochondria is still lacking, cellular studies indicate that queuosine incorporation into tRNAs depends on extracellular queuine availability and influences translational accuracy, codon decoding, and global protein synthesis rates [106,107]. These findings raise the possibility that nutrient-dependent variations in queuine levels could indirectly modulate mitochondrial translation through effects on Q-tRNA formation, though this remains to be experimentally demonstrated.

Taurine-containing modifications at U34, including τm^{5}U and its thiolated derivative τm^{5}s^{2}U, are present in mt-tRNA^{Lys}, mt-tRNA^{Gln}, mt-tRNA^{Glu}, mt-tRNA$^{Leu(UUR)}$, and mt-tRNA^{Trp} [108,109]. These modifications are catalyzed by GTP binding protein 3, mitochondrial (GTPBP3) and mitochondrial tRNA translation optimization 1 (MTO1), with thiolation mediated by tRNA mitochondrial 2-thiouridylase (TRMU; also known as MTU1) [108,109]. Functionally, τm^{5}U/τm^{5}s^{2}U enhance base-pairing specificity with purine-ending codons, ensuring accurate decoding of lysine, glutamine, and glutamate codons. Recent studies have revealed that the mitochondrial folate enzyme serine hydroxymethyltransferase-2 (SHMT2) provides essential one-carbon units for τm^{5}U modification [110]. Loss of SHMT2 activity impairs τm^{5}U formation, leading to codon-specific ribosome stalling and defective synthesis of respiratory chain proteins [110]. These findings not only underscore the functional importance of U34 modifications but also reveal that their efficiency is influenced by cellular metabolic state, raising the possibility that τm^{5}U/τm^{5}s^{2}U levels may vary across tissues or disease contexts with distinct one-carbon metabolic profiles. Yet this possibility remains largely untested. Mutations in these modifying enzymes or the tRNAs themselves are associated with classical mitochondrial diseases, such as MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) and MERRF (myoclonic epilepsy with ragged red fibers), indicating the clinical significance of τm^{5}U/τm^{5}s^{2}U modifications. For example, the mt-tRNA^{Leu} mutation m.3243A>G impairs τm^{5}U34 modification, reducing MT-ND6 expression and contributing to MELAS pathology [111]. Moreover, TRMU mutations have been linked to infantile liver failure and can modulate the severity of phenotypes caused by primary mt-DNA mutations [112], highlighting a genetic interaction between nuclear-encoded tRNA modifiers and mt-DNA mutations.

Position 37, adjacent to the anticodon, is also heavily modified to preserve the reading frame and facilitate

accurate codon-anticodon pairing. The characterized modifications include m^1G37 (methylated guanine), i^6A37 (N6-isopentenyladenosine), ms^2i^6A37 (2-methylthio-i6A), and t^6A (N6-threonylcarbamoyladenosine). tRNA methyltransferase 5 (TRMT5) installs m^1G37 methylation in mt-tRNA^{Gln} and mt-tRNA^{Leu}, preventing + 1 frameshifting; mutations in TRMT5 caused lactic acidosis and impaired mitochondrial protein synthesis and OxPhos. tRNA isopentenyltransferase 1 (TRIT1) generates i^6A37 in mt-tRNA^{Phe}, mt-tRNA^{Ser}, mt-tRNA^{Trp}, and mt-tRNA^{Tyr}, which can be further thiolated ms^2i^6A37 by tRNA methylthiotransferase CDK5RAP1 (CDK5 regulatory subunit associated protein 1), enhancing decoding of UNN codons [113]. Defects in this pathway have been implicated in cardiomyopathy and skeletal muscle diseases in mouse models. Mutations in TRIT1 or defects in the tRNA substrates can abolish this modification, contributing to mitochondrial pathologies in humans [114]. Another essential modification is N^6-threonylcarbamoyladenosine (t^6A37), occurring in mt-tRNA^{Ile}, mt-tRNA^{Lys}, mt-tRNA^{Asn}, mt-tRNA^{Ser}, and mt-tRNA^{Thr}. YrdC domain-containing protein (YRDC) synthesizes the intermediate threonylcarbamoyl-AMP, which serves as a substrate for O-sialoglycoprotein endopeptidase-like 1 (OSGEPL1) that transfers the threonylcarbamoyl moiety to the A37 position of tRNA to generate t^6A37 [40,115,116]. This modification enhances stacking interactions with codons during translation initiation and improves decoding fidelity.

In addition, several other sites within mt-tRNAs are chemically altered to stabilize structure and function. N^2-methylguanosine (m^2G) occurs at positions 10 and 26, while m^5C is frequently found at positions 48 to 50 [117,118]. Although NOP2/Sun RNA methyltransferase 2 (NSUN2) is best known for its nuclear and cytosolic functions, recent studies have revealed a mitochondrial pool of NSUN2 that catalyzes m^5C formation at these positions in specific mt-tRNAs [40,118]. The 5-methyluridine (m^5U54) modification, catalyzed by TRMT2B, is another stabilizing modification [78,119]. Recent work has further expanded this repertoire by identifying 3-methylcytidine (m^3C) as a mitochondria-specific tRNA modification. The mitochondrial methyltransferase-like protein 8 (METTL8) installs m^3C on mt-tRNA^{Thr} and mt-tRNA^{Ser}, and *METTL8* loss reduces mitochondrial translation and respiratory capacity, underscoring m^3C as a functionally important mt-tRNA modification [120,121]. Ψ is abundant at conserved sites such as Ψ27, Ψ28, and Ψ39 [52,83,122]. These modifications, probably catalyzed by enzymes such as Pseudouridine Synthase 1 (PUS1) and RPUSD4, are essential for maintaining mt-tRNA structural stability and ensuring accurate mitochondrial translation [52,83,123,124]. Notably, PUS1 is dual-localized, and only its mitochondrial pool contributes to mt-tRNA pseudouridylation, underscoring its specific role in sustaining mitochondrial translation fidelity. The pathological relevance of tRNA pseudouridylation is exemplified by mutations in PUS1, which cause MLASA (mitochondrial myopathy, lactic acidosis, and sideroblastic anemia) syndrome [124,125]. Recent studies have further demonstrated that loss of PUS1-mediated pseudouridylation reduces mitochondrial tRNA levels, impairs translation, and disrupts erythropoiesis, establishing a direct link between tRNA modification defects and anemia phenotypes [126]. Dihydrouridine (D), catalyzed by dihydrouridine synthase 2 (DUS2) at position 20 in mt-tRNA^{Leu} and mt-tRNA^{Ser}, increases the flexibility of the D-loop region and supports proper tRNA folding [40,41,127,128].

Although mt-tRNAs harbor fewer modification types than their cytosolic counterparts, about 18 types across 137 positions, their functional impact is disproportionately significant [39–41]. Anticodon modifications directly shape decoding scope and fidelity, while modifications within core and stem-loop regions modulate tRNA folding, recognition, and translational coordination [41]. Increasing evidence supports the notion that these modifications are not merely static chemical decorations but are dynamically regulated in response to environmental, cellular, or metabolic cues. Moreover, some mt-tRNA-modifying enzymes also act on rRNAs or coordinate with other mitochondrial gene expression machinery, pointing to broader regulatory networks.

While the studies summarized above describe the fundamental roles of mt-RNA modifications in mitochondrial transcript processing and translation, accumulating evidence suggests that these processes may vary substantially across physiologic contexts. Multiple transcriptomic profiling studies in buffalo have consistently shown pronounced tissue-specific differences in nuclear-encoded mitochondrial genes across amino acid, lipid, and carbohydrate metabolism, ATP synthase assembly, and mitochondrial biogenesis [129–132]. Likewise, mtDNA-encoded tRNAs and rRNAs display marked variation in abundance across tissues, indicating that the mt-RNA substrate landscape itself is tissue dependent [133]. In line with these observations, large-scale analyses of human tissues have revealed substantial tissue-specific heterogeneity in m^1A/G modification levels and demonstrated that such variation is shaped by nuclear genetic regulators, including TRMT61B, MRPP3, and LONP1, with downstream associations to mitochondrial RNA processing efficiency and disease-related traits [134]. Notably, human multi-tissue analyses further showed that the functional consequences of these modifications also differ across tissues. For example, the negative correlation between m^1A947 or m^1A1812 modification levels and mtDNA gene expression

observed in many tissues is absent in the brain, and both modification levels and their expression coupling can shift in response to environmental stimuli such as sunlight or UV exposure [135]. These findings underscore that mt-RNA modifications vary across different physiologic contexts and may shift further under disease conditions. This provides an important basis for understanding their emerging roles in cancer.

Role of mt-RNA modifications in cancer

Mitochondria, traditionally regarded as the cell's powerhouses, are now recognized as integrative hubs for signal transduction, biosynthesis, metabolic reprogramming, and immune regulation, functions intertwined with cancer initiation and progression [4,136,137]. Among the multiple layers governing mitochondrial gene expression and function, post-transcriptional modifications of mt-RNAs, particularly mt-tRNAs and mt-rRNAs, represent a relatively understudied yet functionally critical mechanism. These chemical modifications fine-tune RNA structure and translation fidelity, facilitate respiratory chain assembly, and enhance metabolic plasticity, thereby enabling cancer cells to survive within hostile microenvironments and to withstand therapeutic stress.

Recent large-scale genomic studies have revealed widespread alterations in mt-RNA modifications across various tumor types, with m^1A and m^1G modifications in mt-tRNAs showing strong associations with nuclear genetic variation and patient outcomes [138]. These findings indicate that mt-RNA modification states are neither static nor uniform across cancers but instead reflect tumor-specific selective pressures on mitochondrial function. Building on these findings, accumulating evidence links dysregulated mt-RNA modifications to key oncogenic traits, including metastatic adaptation, immune evasion, and chemotherapy resistance [39,139–142]. This section synthesizes current insights into how mt-RNA modifications contributes to these hallmarks of tumor aggressiveness, highlighting its potential as a translational target.

mt-RNA modifications and metabolic plasticity in metastasis

Metastasis, the primary cause of cancer mortality, is a complex, multistep process that requires tumor cells to detach from the primary tumor, invade surrounding tissue, survive in the circulatory system, and colonize distant organs [143–145]. A defining feature of metastatic cells is their remarkable metabolic plasticity, which enables survival in fluctuating microenvironments marked by hypoxia, nutrient deprivation, and oxidative stress. Mitochondria, as key regulators of energy production and redox balance, are central to this adaptability.

Among various mt-RNA modifications, the NSUN3 (NOP2/Sun domain family member 3)–ALKBH1 (alkB homolog 1) axis plays a pivotal role in supporting metabolic plasticity during metastasis. This pathway sequentially converts m^5C to f^5C at the wobble position of mt-$tRNA^{Met}$, thereby expanding codon recognition and enhancing mitochondrial translation efficiency during the glycolysis-to-OxPhos transition [39]. Metastatic cancer cells with high m^5C and f^5C levels display increased OxPhos and invasive potential, whereas loss of these modifications restricts cells to glycolytic metabolism and impairs metastatic dissemination.

Beyond the tRNA modification network, the mitochondrial RNA methyltransferase METTL17 has emerged as another key regulator of mitochondrial translation and tumor metabolism. Elevated METTL17 expression promotes OxPhos and tumor progression, while its loss disrupts mitochondrial rRNA methylation, impairs translation, and sensitizes colorectal cancer cells to ferroptosis [140]. Our recent work [146] further revealed that METTL17 catalyzes m^4C and m^5C modifications on mt-12S rRNA, sustaining mitochondrial translation and oxidative metabolism in acute myeloid leukemia (AML). Genetic or pharmacologic inhibition of METTL17 abrogated mitochondrial function and suppressed leukemogenesis.

Thus, these findings support a model in which mt-RNA modifications and their modifiers modulate metabolic switches, enabling tumor cells to meet energetic and redox demands during metastatic progression and thereby sustain invasion and colonization.

mt-RNA modifications and anti-tumor immunity

The mt-RNA modifications are emerging as critical modulators of anti-tumor immunity. Multi-omics analyses across diverse tumor types now demonstrate that variation in mt-rRNA and mt-tRNA modification pathways aligns with distinct immune infiltration states, T cell activation profiles, and responses to immune checkpoint blockade [147]. These observations suggest a potential role for mt-RNA modifications in shaping the immunological landscape of tumor microenvironment (TME).

In gliomas, a comprehensive analysis of nine mt-rRNA modification regulators identified two subtypes, mt-rRNA modification 1 (RM1) and RM2, that display distinct immune landscapes [147]. RM2 tumors exhibited an immune-inflamed phenotype with enriched adaptive immune signatures and improved anti-PD-1 responsiveness, whereas RM1 tumors displayed an immune-excluded phenotype marked by the presence of innate immune cells. Building on these findings, an

mt-rRNA modification-based scoring model was developed to stratify glioma patients by immune infiltration, molecular features, and predicted therapeutic outcomes. Additional integrative bulk and single-cell transcriptomic analyses of lower-grade gliomas (LGGs) further revealed that several mt-RNA modification regulators, such as GTPBP3 and NSUN4, exhibit cell-type-specific expression and correlate with key immune checkpoints (e.g., HAVCR2, PVR, TNFRSF25, and TNFSF15) [141]. Higher mt-RNA modification scores were associated with enhanced immune cell infiltration and improved checkpoint blockade response [141]. These correlative data sets suggest that mt-RNA modification programs may track with or contribute to immune remodeling, though whether these modification patterns are causal modulators or downstream reflections of metabolic state remains unresolved.

Mechanistic studies now provide independent support for a causal connection between mt-RNA modification and immune activation. NSUN4, traditionally known for its role in mitoribosome maturation, was recently shown to methylate mitochondrial double-stranded RNAs (mt-dsRNAs), promoting their degradation through the C1QBP (complement C1q binding protein)–PNPT1(polynucleotide phosphorylase 1) pathway. Loss of NSUN4 results in cytosolic accumulation of mt-dsRNAs and activation of innate immune signaling [148]. Extending these insights into cancer, our recent work identified that RPUSD4, a mitochondrial pseudouridine synthase, contributes to immune evasion in AML via suppressing "viral-mimicry" pathways [149].

Beyond tumor-intrinsic mechanisms, mitochondria are also pivotal for immune cell function, particularly in T cell activation, differentiation, and memory formation [150–154]. These processes rely on coordinated remodeling of OxPhos, glycolysis, and mitochondrial dynamics [155–158]. Whether mt-RNA modifications themselves tune mitochondrial translation to support the metabolic demands of distinct T cell states remains an open question. Notably, several mt-RNA modifying enzymes exhibit cell-type-specific expression within immune subsets in single-cell data sets, raising the possibility of immunocyte-intrinsic epitranscriptomic regulation. However, direct experimental evidence in T cells is currently lacking, and this area represents an important direction for future investigation.

Collectively, these findings indicate the emerging paradigm wherein mt-RNA modifications influence tumor-immune interactions through their impacts on mitochondrial translation, metabolism, and signaling within both cancer and immune cells. This positions the mitochondrial epitranscriptome as a previously underappreciated yet potentially targetable layer of immune regulation. Future mechanistic studies dissecting the cell-type-specific and context-dependent roles of distinct mt-RNA modifiers will be crucial for harnessing this axis to overcome immune resistance and improve immunotherapeutic outcomes.

mt-RNA modifications in tumor aggressiveness and drug resistance

Beyond metastasis and immune evasion, mt-RNA modifications also promote tumor aggressiveness and therapeutic resistance. By sustaining mitochondrial translation and respiration, they enable cancer cells to adapt to therapeutic stress, maintain bioenergetic flexibility, and evade cell death. mt-RNA modifications actively contribute to the malignant phenotype by enhancing cellular fitness under conditions of selective pressure. Recent studies have begun to delineate how specific mt-RNA modification enzymes orchestrate these adaptive responses across diverse cancer types.

Similarly, METTL8 introduces m^3C modifications in mt-$tRNA^{Ser}$ and mt-$tRNA^{Thr}$, maintaining translational fidelity and preventing ribosome stalling, particularly during synthesis of essential OxPhos components such as MT-ND6 [121,159–161]. In pancreatic cancer [120], METTL8 is frequently overexpressed and correlates with poor prognosis. It enhances mitochondrial respiration and confers chemotherapy resistance. In glioblastoma [162], METTL8 expression is driven by H2AZ-mediated HIF1α (hypoxia-inducible factor 1 alpha) activation, linking mt-RNA modifications to stemness and hypoxia signaling.

In lung cancer, reduced dihydrouridine levels upon DUS2 knockout correlate with impaired mitochondrial protein synthesis and increased apoptosis [163,164]. In AML, therapy-resistant cells rely on TRMT5-mediated m^1G37 modification in mt-tRNAs to sustain OxPhos during cytarabine or venetoclax treatment [142]. Loss of m^1G37 impairs mitochondrial protein synthesis and re-sensitizes resistant AML cells to chemotherapy. These observations establish a direct mechanistic link between mt-tRNA modifications and therapy-induced metabolic adaptation, highlighting that epitranscriptomic plasticity may underlie the persistence of chemoresistant tumor cell populations.

Therapeutic targeting of mitochondrial RNA modifiers in cancer

The mt-RNA-modifying enzymes are increasingly recognized as potential vulnerabilities in cancer, given their essential roles in sustaining mitochondrial translation, OxPhos capacity, redox homeostasis, and stress adaptation in tumor cells. Although the field is still emerging, integrative transcriptomic and epitranscriptomic analyses suggest that several mt-RNA modifiers, including NSUN3, NSUN4, TRMT5, METTL8, METTL17, and RPUSD4, are dysregulated or

functionally essential in diverse malignancies, highlighting the therapeutic potential of selectively disrupting mt-RNA modification pathways.

A key challenge for exploiting these vulnerabilities is specific and efficient delivery of agents into mitochondria. Exogenous RNA molecules do not naturally traverse mitochondrial membranes, and mitochondria lack conventional RNA-import machinery. Nonetheless, several experimental strategies have begun to address this barrier. Mitochondria-targeted delivery systems based on mitochondria-penetrating peptides, cationic moieties such as triphenylphosphonium (TPP^+), and MITO-Porter–type lipid nanoparticles have been used to concentrate nucleic acids and small molecules within mitochondria, enabling efficient silencing of mitochondrial genes or modulation of mitochondrial non-coding RNAs in preclinical models [165–168]. In parallel, exosome-based platforms have been engineered to carry antisense oligonucleotides against mitochondrial antisense noncoding RNAs (ASncmtRNAs), achieving mitochondrial accumulation and potent antitumor activity in breast cancer models [169]. Building on this line of work, an antisense oligonucleotide targeting ASncmtRNAs (Andes-1537) has already advanced to a first-in-human phase I trial in patients with advanced solid tumors, providing proof-of-concept that mitochondria-directed RNA therapeutics are clinically feasible [170].

A second consideration is enzyme selectivity and therapeutic window. Many mt-RNA modifiers carry out essential functions in healthy tissues, raising concerns regarding on-target toxicity if they are systemically inhibited. For example, pathogenic loss-of-function mutations in NSUN3 disrupt $m^5C \rightarrow f^5C$ modification at the wobble base of mt-$tRNA^{Met}$, impairing mitochondrial translation and causing combined respiratory-chain deficiencies and early-onset mitochondrial disease [99,100,104]. Similarly, mutations in TRMT10C lead to fatal infantile mitochondrial encephalomyopathy with multiple OXPHOS deficits [54]. TRMT5, which installs m^1G37 on both mitochondrial and nuclear tRNAs, is also necessary for proper mitochondrial translation, and its deficiency leads to respiratory-chain dysfunction in human disease models [171]. Despite these essential physiologic roles, accumulating evidence indicates that cancer cells may exhibit disproportionately elevated reliance on specific mt-RNA modifiers. NSUN3 is upregulated in several human cancers and promotes tumor cell invasion, mitochondrial metabolic reprogramming, and immunomodulatory remodeling [172,173]. METTL8, which installs m^3C on selected mt-tRNAs, is required for mitochondrial protein synthesis and neural stem-cell maintenance *in vivo* [174]. In glioblastoma, METTL8 overexpression sustains glioma stem-cell self-renewal and tumorigenicity [162]. METTL17, a mitoribosome-associated RNA binding protein, stabilizes 12S mt-rRNA and acts as an Fe–S–cluster–dependent checkpoint for translation of mitochondrial OxPhos subunits; its loss impairs mitochondrial translation and proliferation [75,175]. Together, these findings suggest that tumors with high reliance on mitochondrial translation, elevated OxPhos activity, or enrichment of stem-like cell states may be particularly vulnerable to perturbation of mt-tRNA and mt-rRNA modification pathways. Our own recent work further demonstrates that targeting METTL17 or RPUSD4 with CpG-conjugated siRNAs selectively disrupts mitochondrial translation in leukemia cells but not normal hematopoietic stem cells, highlighting ligand-directed, tumor-restricted delivery as a viable path toward clinical translation [146,149].

Taken together, these advances point to mt-RNA modifications and their regulatory enzymes as a promising, but still nascent, frontier for anticancer therapy. Continued development of mitochondria-targeted delivery systems (e.g., MITO-porter nanoparticles, mitochondria-penetrating peptides, exosome-based carriers), along with selective inhibitors or degraders of mitochondrial RNA modifiers and rational combination strategies with standard-of-care agents, will be critical to convert mitochondrial epitranscriptomic vulnerabilities into clinically actionable interventions.

Discussion and future directions

Epitranscriptomics, or RNA epigenetics, refers to the diverse post-transcriptional RNA modifications, adding a sophisticated layer of gene expression regulation beyond canonical central dogma. The discovery of chemical modifications in mt-RNAs, including mt-mRNAs, extends this concept to mitochondria. Those mt-RNA modifications are particularly important because the mitochondrial gene expression is predominantly controlled post-transcriptionally. While various mt-RNA modifications have been identified, our current understanding of mitochondrial epitranscriptomics remains in its infancy. Emerging evidence suggests that dysregulation of mt-RNA modifiers contributes to tumorigenesis, yet the precise underlying mechanisms remain largely undefined.

We highlight several key directions and knowledge gaps for advancing mt-RNA modifications in cancer. (1) Systematic characterization of mt-RNA modification machinery: as we summarize above, for the majority of the detected mt-RNA modifications, their responsible regulators, especially potential “readers” and “erasers” (if there are), remain unknown. Defining these components is essential to understand the dynamics and reversibility of mt-RNA modification machinery and to reveal how they determine mt-RNA fate, translation efficacy, and

mitochondrial function. To move this field forward, more systematic discovery strategies will be required. Clustered regularly interspaced short palindromic repeats (CRISPR)-based loss-of-function or CRISPR interference (CRISPRi) perturbation screens, particularly when coupled with mitochondrial translation or metabolic reporters, represent a promising approach to uncover nuclear-encoded factors whose perturbation influences mt-RNA metabolism and may point to candidate regulators of specific mt-RNA modifications. Complementary methods such as APEX2 (engineered ascorbate peroxidase 2)-mediated mitochondrial proximity labeling and RNA–protein interaction profiling (e.g., CLIP-seq (cross-linking immunoprecipitation sequencing) or RNA pulldown–mass spectrometry) can further identify proteins that bind, interpret, or modulate modified mt-RNAs. Integrating insights from these complementary approaches will help reconstruct a more complete mt-RNA modification machinery and clarify how individual components influence mitochondrial gene expression and function. (2) Development of accurate, quantitative profiling methodologies for mt-RNA modifications: high variability in reported mt-RNA modifications highlights the need for sensitive and standardized methods. For example, the role of RPUSD4 in catalyzing Ψ1397 is still debated [52,83], likely reflecting methodological differences or context-dependent modification patterns. Thus, accurate and high-resolution, especially single-base resolution, detection is critical for dissecting the functional consequences of mt-RNA modifications. Approaches such as direct RNA nanopore sequencing, chemical-assisted single-base mapping, and liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based quantification can provide more precise and quantitative profiling of individual mt-RNA modifications. For example, Nano-tRNAseq uses nanopore direct RNA sequencing to measure tRNA abundance and modification dynamics in native molecules, while MoDorado enhances RNA-modification detection accuracy on direct RNA and tRNA nanopore data, and together these method developments illustrate how nanopore-based platforms can be adapted to systematically map mt-RNA modifications [176,177]. (3) Elucidation of mt-RNA modifications-mediated retrograde signaling pathways: over 99% of mitochondrial proteins, including all mt-RNA modifiers, are nuclear-encoded, synthesized in the cytoplasm, and then imported into mitochondria. A fundamental and intriguing question is whether, and if so, how dysregulation of mt-RNA modifiers and/or their corresponding modifications can retrogradely regulate nuclear gene expression, coordinating mitochondrial and nuclear activities? Addressing this question will require experimental systems that allow precise perturbation of individual mt-RNA modifiers together with comprehensive profiling of downstream cellular responses. Conditional knockout or inducible perturbation systems in defined cell types or tumor models can help define their roles in mito-nuclear communication. Integrating bulk and single-cell RNA-seq, ATAC-seq (assay for transposase-accessible chromatin sequencing), and chromatin profiling with these models can reveal how changes in mt-RNA modifications reshape nuclear transcriptional programs and chromatin states. Together, these strategies can begin to map the pathways through which mt-RNA modifications regulate nuclear gene expression and cellular phenotypes. (4) Therapeutic targeting mt-RNA modifications in cancer: targeting mt-RNA modifications represents a promising avenue for translational medicine, with potential to inhibit tumor growth, overcome chemotherapy resistance, and enhance anti-tumor immune responses. Building on emerging evidence that several mt-RNA modifiers exhibit context-dependent cancer vulnerabilities, their therapeutic potential will depend on strategies that achieve greater selectivity, efficient delivery, and an acceptable therapeutic window. Given the potential toxicity of broad mitochondrial inhibition, developing approaches that enable tumor-preferential and mitochondria-directed modulation are essential. These may include mitochondria-targeted peptides, ligand- or antibody-conjugated nanoparticles, or oligonucleotide-based therapeutics optimized for mitochondrial import. Engineering drug activation or modifier inhibition through tumor- or microenvironment-responsive mechanisms may further reduce off-tumor toxicity. Systematic evaluation of these delivery platforms, together with careful assessment of on-target activity and safety in normal tissues, will be critical for advancing mt-RNA modification–based therapies.

Collectively, these challenges outline a roadmap for mitochondrial epitranscriptomics in both fundamental biology and oncology. Integrating mechanistic insights with advanced detection technologies and targeted therapeutic strategies holds the potential to transform our basic understanding of mitochondrial biology into cancer and to unlock a new class of precision therapies.

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Compliance with ethics guidelines

Conflicts of interest Haixia Wang, Lili Ren, Dongling Zou, and Rui Su declare no conflicts of interest.

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