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ADVANCED REVIEW

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The emergent role of mitochondrial RNA modifications in metabolic alterations

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Abstract

Mitochondrial epitranscriptomics refers to the modifications occurring in all the different RNA types of mitochondria. Although the number of mitochondrial RNA modifications is less than those in cytoplasm, substantial evidence indicates that they play a critical role in accurate protein synthesis. Recent evidence supported those modifications in mitochondrial RNAs also have crucial implications in mitochondrial-related diseases. In the light of current knowledge about the involvement, the association between mitochondrial RNA modifications and diseases arises from studies focusing on mutations in both mitochondrial and nuclear DNA genes encoding enzymes involved in such modifications. Here, we review the current evidence available for mitochondrial RNA modifications and their role in metabolic disorders, and we also explore the possibility of using them as promising targets for prevention and early detection. Finally, we discuss future directions of mitochondrial epitranscriptomics in these metabolic alterations, and how these RNA modifications may offer a new diagnostic and theragnostic avenue for preventive purposes.

This article is categorized under:
RNA Processing > RNA Editing and Modification

KEYWORDS

epitranscriptomics, metabolic alterations, metabolism, mitochondria, RNA modifications

1 | INTRODUCTION

Mitochondria are eukaryotic organelles that evolved through an endosymbiosis process more than 1.45 billion years ago. The peculiarity of mitochondria is that contain unique structures that are involved in the electron transport chain, specializing in energy generation as adenosine triphosphate (ATP) through oxidative phosphorylation. Therefore, the main function of mitochondria is related to cellular respiration and metabolism (Wallace, 2005). Each cell contains many mitochondria, and each mitochondrion contains many copies of its own DNA. The human mitochondrial genome is a compact circular and double-stranded DNA that is 16,569 base pairs in length (Anderson et al., 1981). Except for a few, the majority (approximately 1500) of the proteins required for maintaining mitochondrial structure and processes are encoded by genes in nuclear DNA. These proteins are initially synthesized in the cytoplasm and then transferred to the mitochondria (Wojewoda et al., 2011). Therefore, the cooperation between mitochondrial DNA (mt-DNA) and nuclear DNA is essential for normal cell functioning. The mt-DNA, on the other hand, contains 37 genes coding for their 13 proteins involved in energy-generating processes, 22 transfer RNA (tRNA), and 2 ribosomal RNA (rRNA; Anderson et al., 1981). Faithful expression and regulation of the mitochondrial genes are ensured through several post-transcriptional modifications of bases in the coding and noncoding regions of the mitochondrial transcripts, collectively referred to as epitranscriptome (Mercer et al., 2011).

RNAs in different subcellular locations of eukaryotic cells, including the nucleus, cytoplasm, and mitochondria are subject to such modifications. Currently, more than 170 post-transcriptional modifications have been identified in cellular RNA (Boccaletto et al., 2018) and more than 350 proteins involved in RNA modifications have been included in the MODOMICS database (https://iimcb.genesilico.pl/modomics/; Boccaletto et al., 2018). More than 25 chemical group modifications can occur via the addition of simple (e.g., methyl) or complex groups (e.g., carboxymethylaminomethyl) to the bases. Other modifications such as isomerization (e.g., uridine to pseudouridine [Ψ]), oxidation (e.g., 5-methylcytosine to 5-methylcytidine and 5-hydroxymethylcytosine to 5-hydroxymethylcytidine), reduction (e.g., uridine to dihydrouridine), and substitution (e.g., uridine to 4-thiouridine) have been noted (Rebelo-Guiomar et al., 2019).

Although chemical modifications in mitochondrial RNAs (mt-RNAs) have been observed for a long time in many types of RNAs, tRNAs are heavily modified. In addition, modifications in many different RNA species such as modifications of long noncoding RNAs (lncRNA), circular RNAs (circRNA), microRNAs (miRNAs), and small nucleolar RNAs (snoRNAs) have also been identified (T. Pan, 2018). Some of them are involved in digestive stability, or even in cross-kingdom regulation through diet (T. Pan, 2018; Tomé-Carneiro et al., 2021). Though recent advancements in sequencing technologies combined with improved bioinformatics approaches have accelerated our capability to identify several post-transcriptional modifications in RNA (e.g., N^6-methyladenosine [m^6A]), our understanding of their implication in complex human diseases is still limited (Meyer et al., 2012). Many mutations, directly affecting mt-RNA modifications, play an important role in mitochondrial gene expression. Furthermore, substantial evidence has indicated that RNA modifications regulatory genes such as *writer*, *readers*, and *erasers* are involved in complex biological processes and thus, have been associated with diseases. These modifications may be implicated in the stability and efficiency of RNA translation, suggesting an association between mt-RNAs modifications and diseases (Bohnsack & Sloan, 2018). In this review, we present the current landscape of mt-RNA modifications and their implication for metabolic alterations. We also discuss potential future directions of mitochondrial epitranscriptomics in metabolic diseases could offer relating to this field.

2 | MITOCHONDRIAL RNA MODIFICATIONS

Mitochondrial-RNA modifications represent an important pathway in the regulation of important gene expression in the context of the mitochondria. These modifications can mainly control the structure, stability, and translation efficiency of the mRNAs encoded by the mitochondrial genome. Accumulated evidence indicated the widespread modifications at different mt-RNAs, such as rRNA, tRNA, or mRNA. Each modification is highly regulated and many regulatory proteins are involved (Figure 1).

2.1 | Mitochondrial ribosomal RNA modifications

The content of mitoribosomes differs from cytoplasmic ribosomes in terms of the number of proteins and rRNAs. The mitoribosome 55S consists of the smaller subunit 28S with 12S rRNA and 30 ribosomal proteins, and the larger subunit

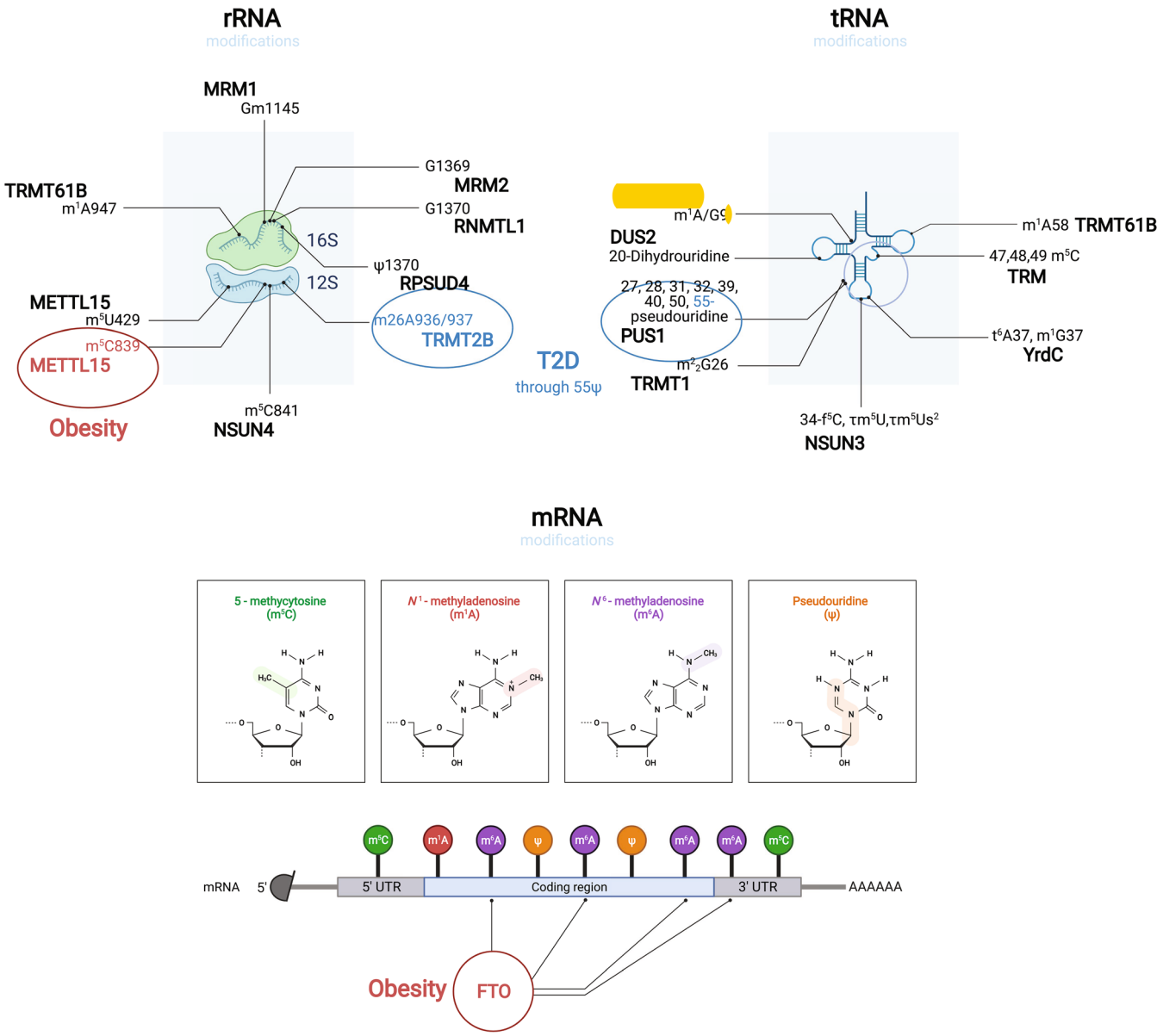

FIGURE 1 Schematic representation of the commonly modified bases in mitochondrial rRNA, tRNA, and mRNA. The most represented modifications and the responsible enzymes are listed for rRNA, tRNA, and mRNA of the mitochondria. The modifications and responsible enzymes linked with obesity are marked in red circles, while those linked with Type 2 diabetes (T2D) are marked in blue circles. Retrieved from BioRender (2021), www.biorender.com/biorender-templates. TRMT5/10A/10B/10C/61A/61B, TRNA Methyltransferase 5/10A/10B/10C/61A/61B/10; MRM1/2/3, Mitochondrial rRNA Methyltransferase 1/2/3; PUS1, Pseudouridine Synthase 1; TRMU, TRNA Mitochondrial 2-Thiouridylase; NSUN2/3/4, NOP2/Sun RNA Methyltransferase 2/3/4; METTL15, Methyltransferase Like 15; RNMTL1 is a synonym for MRM3; DUS2, Dihydrouridine Synthase 2; YrdC, YrdC N6-Threonylcarbamoyltransferase Domain Containing Pseudogene

39S is composed of 16S rRNA, 52 ribosomal proteins, and a mt-tRNA (mt-tRNA^{Val} or mt-tRNA^{Phe}; Bohnsack & Sloan, 2018). Whereas, the mammalian cytoplasmic ribosome 80S consists of a smaller subunit 40S composed of 18S rRNA and 33 ribosomal proteins, and a larger subunit 60S composed of 5S, 5.8S, and 28S rRNAs and 47 ribosomal proteins (Lopez Sanchez et al., 2020). Compared to the cytoplasm, mt-rRNAs contain a smaller number of modified nucleotides. Ten different modifications have been identified in mammalian rRNAs occurring in small and large subunits, which are critical for the functions of mitochondria (Rebelo-Guiomar et al., 2019). Five modifications have been identified in the mammalian mitochondrial smaller subunit 12S rRNAs and other five, in the larger subunit 16S rRNA.

As for the smaller ribosomal subunit, the methylation is the most frequent modification of bases. The most frequent methylations on the 12S rRNA are cytosine N^4-methylation (m^{4}C) and methylation of C5 of cytosine (m^{5}C), being

TABLE 1 Mitochondrial RNA modification-related genes

Mt-RNA	Modification	Position	Modifying enzymes	Biological significance in metabolism	References
12S RNA	m^5U	429	TRMT2B	Mitochondrial translation, mt-tRNA stability, and aminoacylation	(Powell & Minczuk, 2020)
	m^4C	839	METTL15	Required for mitochondrial function	(H. Chen et al., 2020)
	m^5C	841	NSUN4	Functional ribosomes and rRNA maturation	(Metodiev et al., 2014)
	$m^6{}_2A$	936, 937	TFB1M	Maintaining the stability and assembly of the smaller ribosomal unit as well as mitochondrial protein translation	(Cotney & Shadel, 2006; Metodiev et al., 2009; Seidel-Rogol et al., 2003)
16S RNA	m^1A	947	TRMT61B	Potential participation in mitoribosome activity	(Bar-Yaacov et al., 2016)
	Gm, Um, and Gm	1145, 1369 and 1370	MRM1, MRM2, MRM3	Translation of mitochondrial proteins and stabilization of the structure of the loop as well as peptidyl transferase reaction	(Decatur & Fournier, 2002; Dimitrova et al., 2019; Gillis et al., 2014; Lee et al., 2013; Lee & Bogenhagen, 2014; Metodiev et al., 2009)
	Pseudouridine	1397	RPUSD4	Stability of 16S rRNA	(X. Li et al., 2016; Schwartz et al., 2014; Zaganelli et al., 2017)
tRNA	m^1A	9	TRMT10C	Translation repression	(Safra et al., 2017; Vilardo et al., 2012)
	m^1G	9 and 37	TRMT10C	Efficiency and accuracy of the translation	(Urbonavičius et al., 2001)
	m^2G	10, 15	Predicted by TRMT11/112	No reported	(Purushothaman et al., 2005)
	m^1A	14, 16, 58 and 1374	TRMT61B, TRMT10C	Stability of tRNA and regulation of mitochondrial translation	(Chujo & Suzuki, 2012; Kadaba et al., 2004; X. Li et al., 2017; Metodiev et al., 2009; Safra et al., 2017; Suzuki & Suzuki, 2014)
	Dihydrouridine	20	DUS2	Increase in structural flexibility and stability of tRNA	(Dalluge et al., 1996; Suzuki et al., 2020; Suzuki & Suzuki, 2014)
	$m^{2,2}G$ and m^2G	26	TRMT1	Structural stability of tRNA	(Sonawane et al., 2016)
	Pseudouridine	27, 28, 29, 31, 32, 33, 35, 38, 39, 40, 50, 55, 57, 66, 67 and 68	PUS1, RPSUD1	Stability of the secondary structure of tRNA	(Karijolich et al., 2015; Suzuki et al., 2020)
	m^3C	32, 34	Predicted by METTL2A/2B/6/8	Improvement of mitochondrial translation and optimization of tRNA structure	(Kleiber et al., 2022; Suzuki et al., 2020)
	τm^5U	34	GTPBP3, MTO1	Maintenance of mitochondrial genome, tRNA stability,	(Kleiber et al., 2022)

TABLE 1 (Continued)

Mt-RNA	Modification	Position	Modifying enzymes	Biological significance in metabolism	References
				translation, and respiratory function	
	τm^5s^2U	34	GTPBP3, MTO1	Mitochondrial translation and accurate decoding of AAR	(Kamble et al., 2016)
	Q	34	QTRTD1/2	Efficient decoding of UAU and protein folding	(Suzuki et al., 2020)
	f^5C	34	NSUN3	Enhancement of the structure and thermodynamics of the anticodon and the ability to bind to the AUA and AUG codons in translational initiation and elongation	(Lusic et al., 2008; Nakano et al., 2016)
	ms^2i^6A	37	TRIT1, CDK5RAP1	stability of mRNA–tRNA interaction	(Jenner et al., 2010; Reiter et al., 2012)
	i^6A	37	TRIT1	Translational efficiency and fidelity	
	t^6A	37	YRDC/OSGEPL1, YRDC	and required for ribosomal binding	(Lin et al., 2018; Stuart et al., 2000; Suzuki et al., 2020)
	m^1G	37	TRMT5	Essential for reading tRNA CNN codons, thus for efficiency of translation	(Powell et al., 2015; Urbonavičius et al., 2003)
	m^5U	54	TRMT2B	Stability and maturation of tRNA	(Johansson & Byström, 2002; Laptev et al., 2020; Powell & Minczuk, 2020)
	m^5C	48, 49 and 72	NSUN2	Predicted stability of tRNA	(Shinoda et al., 2019; Suzuki et al., 2020; van Haute et al., 2019)
mRNA					
COX1	m^1A	1472	TRMT6/10C/61A	Translational repression, probably through a mechanism involving ribosomal scanning	(Safra et al., 2017)
	Pseudouridine	6294	TRUB2 and RPUSD3	Modulation of the efficiency of mitochondrial protein synthesis	(Antonicka et al., 2017)
COX3	m^1A	707	TRMT6/10C/61A	Translational repression, probably through a mechanism involving ribosomal scanning	(Safra et al., 2017)
COX2	m^1A	297	TRMT6/10C/61A	Translational repression, probably through a mechanism involving ribosomal scanning	(Safra et al., 2017)
	Pseudouridine	9904–9906	TRUB2, RPUSD3	Modulation of the efficiency of mitochondrial protein synthesis	(Antonicka et al., 2017)
ND5	m^1A	1347	TRMT6/10C/61A	Translational repression, probably through a mechanism involving ribosomal scanning	(Safra et al., 2017)

(Continues)

TABLE 1 (Continued)

Mt-RNA	Modification	Position	Modifying enzymes	Biological significance in metabolism	References
ND6	m^1A	449	TRMT6/10C/61A	Translational repression, probably through a mechanism involving ribosomal scanning	(Safra et al., 2017)

Abbreviations: *Modifications*: τm^5U, 5-taurinomethyluridine; τm^5s^2U, dihydrouridine rinomethyl-2-thiouridine; f^5C, 5-Formylcytosine; Gm, 2′-*O*-methylguanosine; i^6A, N6-isopentenyladenosine; m^1A, 1-ethyladenosine; m^1G, 1-methylguanosine; m^2G, *N2*-methylguanosine; $m^2{}_2G$, *N2*,*N2*-dimethylguanosine; m^3C, 3-methylcytosine; m^4C, N4-methyl-cytosine; m^5C, N5-methyl-cytosine; m^5U, 5-methyluridine; $m^6{}_2A$, *N6*,*N6*-dimethyladenosine; ms^2i^6A, 2-Methylthio-N6-isopentenyl modification of adenosine; mt-RNA, mitochondrial RNA; Q, Queuosine; t^6A, *N6*-Threonylcarbamoyladenosine; Um, 2′-*O*-methyluridine. *Enzymes*: CDK5RAP1, CDK5 Regulatory Subunit Associated Protein 1; GTPBP3, GTP Binding Protein 3, Mitochondrial; DUS, tRNA-dihydrouridine synthase; METTL, 12S rRNA N4-methylcytidine methyltransferase; Mod 5, tRNA dimethylallyl transferase; MRM, mitochondrial rRNA methyltransferase; MTO1, mitochondrial TRNA Translation Optimization 1; NSUN, NOP2/Sun RNA Methyltransferase; PUS1, Pseudouridine Synthase, RPUSD, RNA Pseudouridine Synthase Domain; TFB1M, Transcription factor B1, mitochondrial; TRIT1, tRNA Isopentenyltransferase 1; TRMT, tRNA methyltransferase; TRMU, tRNA mitochondrial 2-thiouridylase; TRUB2, Pseudouridylate synthase; QTRTD, Queuine tRNA-ribosyltransferase; YRDC, YrdC N6-Threonylcarbamoyltransferase Domain Containing.

highly regulated (Table 1). For instance, m^4C is mainly catalyzed by the human methyltransferase-like 15 (METTL15), and is required for mitochondrial function, whereas m^5C is catalyzed by NOP2/Sun RNA Methyltransferase 4 (NSUN4), having a role in mt-rRNA maturation (H. Chen et al., 2020; Metodiev et al., 2014). Additionally, transcription factor B type 1 (TFB1), encoding a human mitochondrial DNA transcription factor, functions as a methyltransferase enzyme of $m_2{}^6A936$ and $m_2{}^6A937$ modifications (adenosyl demethylation) at the 3′ terminal of 12S rRNA (Seidel-Rogol et al., 2003). Both TFB1 and TFB2, play a critical role in the expression of the mitochondrial genome (Bohnsack & Sloan, 2018; Cotney & Shadel, 2006). Loss of function of *Tfb1m* in mice in differentiated tissue led to demethylation on specific sites in 12S rRNA (A1583 and A1584) resulting in the instability of the smaller ribosomal unit (Metodiev et al., 2009).

With regards to the larger ribosomal subunit, the most common modification is the 2′-*O*-methylation. 2′-*O*-methyl or Nm (N stands for any base) occurs as a result of the addition of methyl group to the hydroxyl group of the ribose moiety (Dimitrova et al., 2019; Metodiev et al., 2009). Currently, there are three different methyltransferase enzymes of 2′-*O*-methylation reported for 16S sRNAs. Mitochondrial rRNA Methyltransferase type 1 (MRM1) and 2 (MRM2) were identified for the modification of G1145 and G1369 in 16S rRNA, respectively (Lee et al., 2013; Lee & Bogenhagen, 2014). Additionally, RNA methyltransferase-like protein 1 (RNMTL1 or MRM3) was required for the modification of G1370 in 16S rRNA (Bar-Yaacov et al., 2016). Overall, modifications of all these positions are critical for the peptidyl transferase reaction (Decatur & Fournier, 2002). In addition, m^1A modification was identified in mitochondrial 16S rRNA at position 947 in the human cells, catalyzed by methyltransferase (TRMT) type 61B (also responsible for the methylation of A58 position in tRNA; Bar-Yaacov et al., 2016). Finally, pseudouridine is another modification identified in mitoribosomes that occurs by isomerization of uridine (X. Li et al., 2016), observed at the position of 1397 in human cells. The formation of pseudouridine is mainly catalyzed by RPUSD4 (RNA Pseudouridine Synthase D4) contributing to the stability of 16S rRNA (Schwartz et al., 2014; Zaganelli et al., 2017). As a result, most of the rRNA modifications in mitochondria ensure both the stability and function of mitoribosomes as well as fidelity of mitochondrial protein translation. Any dysregulation in the maintenance of such modifications may lead to fatal mitochondrial functions and mitoribosome biogenesis, thereby increasing the risk of metabolic alterations.

2.2 | Mitochondrial transfer and messenger RNA modifications

Decoding of mt-mRNAs is critical for the accurate translation of mitochondrial proteins. In this context, 22 mt-tRNAs encoded from mt-DNA contribute to the translation of 13 proteins (Suzuki & Suzuki, 2014). Two main mechanisms, as mutations and modifications of bases, are known to alter the mitochondrial DNA functions. As for genetic mutations, the mitochondrial genome databases revealed that the pathogenic mutations that commonly occur in mt-tRNAs genes were associated with their biogenesis, stabilities, and functions (Suzuki et al., 2011; Suzuki & Suzuki, 2014). Besides, mutations in mt-tRNAs genes were also correlated with various faithful diseases such as mitochondrial encephalopathy,

lactic acidosis, and stroke-like syndrome (MELAS), as well as myoclonus epilepsy with ragged-red fibers (MERRF; Suzuki & Suzuki, 2014).

In any way, mt-tRNAs are the most extensively modified mt-RNAs (compared to mt-rRNAs and mt-mRNAs). Each tRNAs have unique types of modifications, however, the functions of these modifications have not been fully understood. Compared to the cytoplasm, the mammalian mt-tRNAs contain fewer modifications (15 types of modifications at 118 positions). The presence and location of such modifications have specific roles, such as anticodon loop modifications affecting the decoding capacity and translation fidelity, while core modifications affect the stability and its recognition by aminoacyl-tRNAs synthesizes (Bohnsack & Sloan, 2018; Degoul et al., 1998).

mt-tRNA modifications include isomerization, methylation, pseudouridine, and dihydrouridine modification (originated from pseudouridine). Furthermore, 5-taurinomethyluridine (τm^5U) is another common tRNA modification occurring at the anticodon site which participates in the decoding of codons in mRNA (Grosjean & Benne, 1998). In human mitochondria, 5-formylcytidine (f^5C) modification occurs at the wobble position 34 of $tRNA^{Met(CAU)}$ whereas N6-threonylcarbamoyladenosine (t^6A) is another modification in human tRNAs occurring at position 37 of tRNAs. All those processes are highly regulated (Table 1; Pearce et al., 2017). In addition to the above-mentioned studies, very recently, a comprehensive analysis of human mitochondrial tRNA modifications has been performed and provided all details including modification types and position of modification for each tRNAs (Suzuki et al., 2020). These modifications have a key role in protein synthesis and translation, although the exact in vivo role has yet to be discussed (Perrochia et al., 2013; van Haute et al., 2017).

Regarding modifications in mt-mRNA, many studies have shown the presence of modified nucleotides (pseudouridination and methylation), suggesting the role of an epitranscriptomics. Accordingly, Carlile et al. (2014) revealed hundreds of pseudouridylated sites in mRNAs catalyzed by PUS enzymes (from 1 to 9 types). Notably, the majority of these pseudouridines in mRNA are regulated in response to environmental signals, such as nutrient deprivation and serum starvation, which may be implicated in human mitochondrial-related diseases (Carlile et al., 2014). Additionally, m^1A modification related to translational repression has also been confirmed in mt-mRNA, where it is introduced by TRMT6/61A complex and TRMT10C enzymes (Table 1; Safra et al., 2017). However, the biological function of such modification remains unclear, and further research is needed to understand its functional and pathogenic implications in mitochondria.

3 | BIOLOGICAL FUNCTIONS OF MITOCHONDRIAL RNA MODIFICATIONS

Eukaryotic mt-RNAs, such as rRNA and tRNA are well-known to be modified by methylation and other modifications, playing key roles in mitochondrial ribosome biogenesis as well as accurate protein translation (Bohnsack & Sloan, 2018). These RNA modifications have well-defined biological functions because of their high enzyme specificity. Any disruptions in these RNA modifications—or enzyme modifiers—may result in multiple mitochondria-related diseases. Although these post-transcriptional mechanisms are crucial for protein translation and maturation, and for mitochondrial stability and assembly, their biological functions are a little different and it warrants further exploration. Currently, the association between the role of mt-RNA modifications and disease is mostly inferred from the mutations in mt-RNA modifications affecting both nuclear and mitochondrial DNA encoded genes (Table 2). Overall, more than 400 mutations have been reported to be associated with mitochondrial diseases (www.mitomap.org). Approximately 200 modifications have been identified in mt-tRNAs genes (Lott et al., 2013; Suzuki & Suzuki, 2014).

It is logical to think that modifications in rRNA might offer structural stability and enable correct scaffolding for protein translation. There are 10 most common modifications in mt-rRNA and three of these modifications include nucleobase methylation, 2′-*O*-methylation, and pseudouridination [reviewed in Bohnsack and Sloan (2018), Lopez Sanchez et al. (2020) and Rebelo-Guiomar et al. (2019)]. Overall, modifications in mitochondrial rRNAs are essential for their stability and ensure the normal function of ribosomes in mitochondria. Abnormal phenotypes associated with irregular modifications in rRNA resulted in dysfunction of the ribosome. For example, such phenotypes may manifest as embryonic failure (adenine demethylation of the rRNA of the small mitochondrial ribosomal subunit and abolishing mitochondrial translation; Metodiev et al., 2009), hearing loss (O'Sullivan et al., 2015), or metabolic disorders such as insulin secretion and diabetes (Sharoyko et al., 2014).

Modifications in mt-tRNA are responsible for the highly regulated process of its biogenesis and maturation. Mutations in mitochondrial and nuclear genes that encode for enzymes modifying mt-tRNA can cause crucial errors in the

TABLE 2 Mitochondrial RNA modification and related diseases

Disease	Modification	Biological significance	References
MLASA	Pseudouridine	Pseudourine is essential for the molecular pathway of the MLASA disease. Patients with MLASA did not contain pseudouridination, catalyzed by PUS1 in their tRNAs,	(Fernandez-Vizarra et al., 2007; Patton et al., 2005)
Infantile acute liver failure	2-Thiouridylation	2-Thiouridylation levels in mt-tRNA were found to be significantly reduced due to mutations in the tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase (*TRMU*) gene that encodes for a mt-tRNA modifying enzyme	(Schara et al., 2011; Zeharia et al., 2009)
Metabolic disorders	τm^5U	mutations in *GTPBP3* gene (the *GTPBP3* gene's product catalyzes the formation of the τm^5U in the wobble position of mt-RNAs) resulted in a defect in mitochondrial translation in humans, which was associated with hypertrophic cardiomyopathy, lactic acidosis, and encephalopathy	(Schara et al., 2011)
Metabolic disorders	τm^5s^2U	mutations in *MTO1* gene (*MTO1* gene encodes for enzymes that introduce 5-carboxymethylaminomethylation of the wobble uridine in mt-tRNAs) are responsible for hypertrophic cardiomyopathy and lactic acidosis in humans	(Ghezzi et al., 2012)
MELAS	τm^5U and τm^5s^2U	In mitochondrial encephalopathy, lactic acidosis and stroke-like syndrome (MELAS), A3243G and T3271C mutations in mt-$tRNA^{Leu(UUR)}$ gene inhibit τm^5U (5-taurinomethyluridine) and τm^5s^2U (5-taurinomethyl-2-thiouridine) modifications in mt-tRNA resulting in mitochondrial dysfunction	(Yasukawa et al., 2000)
MERRF	τm^5U and τm^5s^2U	The findings indicated that mutations in specific genes resulted in diseases by altering the presence of the modifications in tRNAs	(Kirino & Suzuki, 2005; Shoffner et al., 1990; Suzuki & Suzuki, 2014; Yasukawa et al., 2000)
MERRF	m^1A	The biological function of this modification relates to the translation, elongation, and stability of peptides. On the other hand, the regulation of post-transcriptional modifications of mt-tRNAs is fine-tune process for the control of mitochondrial gene expression	(Richter et al., 2018)
HSD10 disease	m^1A, m^1G	Mutations of SDR5C1 gene disrupt interaction with TRMT10C, affecting methylation of specific bases on tRNA and leading to mitochondrial alteration observed in HSD10 patients	(Vilardo et al., 2012; Vilardo & Rossmanith, 2015)
Intellectual disability	m^1G, $m^2{}_2G$, m^5C, pseudouridine	Mutations in TRMT1 and NSUN2 induce aberrant methylation of specific position on tRNA, leading to clinical manifestation of intellectual disability	(Khan et al., 2012; Martinez et al., 2012; K. Zhang et al., 2020)
Mitochondrial respiratory chain complex deficiency	f^5C, m^1G	Failure on specific enzymes induces a loss of these modifications, decreasing efficiency of translation	(Powell et al., 2015; van Haute et al., 2016)

Abbreviations: *Modifications*: τm^5U, 5-taurinomethyluridine; τm^5s^2U, dihydrouridine rinomethyl-2-thiouridine; f^5C, 5-Formylcytosine; Gm, 2′-*O*-methylguanosine; i^6A, N6-isopentenyladenosine; m^1A, 1-ethyladenosine; m^1G, 1-Methylguanosine; m^2G, *N2*-Methylguanosine; $m^2{}_2G$, *N2*,*N2*-Dimethylguanosine; m^3C, 3-Methylcytosine; m^4C, N4-methyl-cytosine; m^5C, N5-methyl-cytosine; m^5U, 5-methyluridine; $m^6{}_2A$, *N6*,*N6*-Dimethyladenosine; ms^2i^6A, 2-Methylthio-N6-isopentenyl modification of adenosine; mt-RNA, mitochondrial RNA; Q, Queuosine; t^6A, *N6*-Threonylcarbamoyladenosine; Um, 2′-*O*-methyluridine.

RNA modification process resulting in several mitochondrial diseases (Suzuki & Suzuki, 2014). In addition to modifications in mt-rRNAs and mt-tRNAs, modifications in mt-mRNA have a crucial role in its maturation and stability, as well as function, and may affect both its gene expression and translation. Intriguingly, the m^1A modification in mRNA can block protein translation, and other modifications may also affect mitochondrial protein synthesis and cell viability (reviewed by Zhang and Jia (2018)). Various modifications in mt-RNA may have important implications for both human health and disease. Mutations in RNA modification machinery can compromise the biological function of key mitochondrial genes and the regulatory dysfunction of processes such as inflammation, oxidative stress, and cell damage may result in the pathogenesis of diseases, in which mitochondria plays a relevant role for example cancer, obesity, or diabetes.

4 | MITOCHONDRIAL RNA MODIFICATIONS AND ITS ENZYME MODIFIERS AND METABOLIC ALTERATIONS

Proteins associated with RNA modifications are classified as *writers*, *readers*, and *erasers*, as they are involved in both formation and recognition of RNA modifications. Understanding the role of such proteins provides clues about the functions of modifications. Thus, using the MODOMICS database (https://iimcb.genesilico.pl/modomics), we compiled a list of the proteins catalyzing the various mt-RNA modifications (Boccaletto et al., 2018; Table 1). Abnormal RNA modifications, in addition to potential dysregulation of enzyme modifiers, has been closely related to disorders. That is because they can lead to perturbation in mitochondrial RNA processing and stability and therefore, they are cause of human mitochondrial related-disease. Indeed, coming works now are focusing on functional analysis of mt-RNA modifications—rather than loss of function as a result of genetic mutations—in several metabolic diseases. However, there is a lack of knowledge about the current state of mt-RNA modifications in the context of metabolic disorders/diseases. Therefore, mt-RNA modifications in these metabolic alterations are an emergent issue, which provides new avenues for potential therapy options.

4.1 | The role of mitochondrial RNA modification on metabolism

RNA modifications represent an important layer for the control of gene expression/translation and its dynamic regulation. These modifications are an active process that influences the biogenesis, dynamics, and stability of RNA in order to accurately ensure its translation to protein. Some specific modifications are highly regulated by several proteins which coordinate the core translation machinery, and selectively recognize and bind to specific RNA modifications (Schaefer et al., 2017). The disruption of mt-RNA metabolism via modifications of different types of RNAs may impair the main cellular metabolic pathways and cell homeostasis, such as oxidative phosphorylation stimulation and neutralization of oxidative stress, the Krebs cycle, gluconeogenesis, ketogenesis, and oxidation of fatty acids. Mitochondria also contribute to many additional processes including lipid and aminoacids metabolism, calcium signaling, apoptosis, and programmed cell death, therefore, is worth noting that mt-RNAs modifications may also be involved in such processes and further contribute to metabolism (Bohnsack & Sloan, 2018). The impairment of mt-RNA metabolism can specifically affect the tissues that participate in nutrient metabolism and are highly dependent on aerobic metabolisms such as liver, skeletal muscle, and adipose tissue. The fact that mitochondrial dysfunction can affect important metabolic tissue, such as adipose tissue, has been directly linked to metabolic disorders such as insulin resistance and/or obesity (Woo et al., 2019). For instance, Bournat and Brown (2010) and de Mello et al. (2018) indicated that an excessive caloric intake, metabolic imbalance of specific nutrient input, and defects in oxidative respiration can lead to mitochondrial dysfunction (Bournat & Brown, 2010). This can impair the abovementioned metabolic pathways and also result in a decrease of lipid oxidation, increase glucose levels, and decline in the biogenesis of mitochondria (de Mello et al., 2018). Then, mitochondria play a key role in regulation of homeostatic metabolism, and modification at specific rRNAs could display an interesting contribution to this regulation.

m^6A modification is crucial for mitochondrial biogenesis (Deng et al., 2021; HUGO Gene Nomenclature Committee (HGNC), 2022). Accordingly, Kunovac et al. (2021) found that increase of m^6A in mitochondrial phospholipid hydroperoxide glutathione peroxidase in mice models, may be responsible for diminished antioxidant capacity and resultant mitochondrial and cardiac deficits, which in turn, persisted into adulthood, following gestational maternal nano-TiO_2 aerosols exposure for 8 days (Kunovac et al., 2021), confirming the role of m^6A on the regulation of mitochondrial

biogenesis through the control of mitochondrial antioxidant system. Sometimes, the dysregulations of the mt-RNA modifiers (by their epigenetic silencing, dysregulation of expression status, or harboring mutations in the gene body) may be responsible for the manifestations of several related-metabolic alterations (Jonkhout et al., 2017). For example, the polymorphisms in the fat mass and obesity-associated (*FTO*) gene, known as an *eraser* of RNA m^6A modification, has been widely associated with obesity in several genome-wide association studies (GWAS; Loos & Yeo, 2014). FTO protein works as a demethylase enzyme that remove methyl groups from adenine in mRNA, thereby regulating m^6A levels in the cellular RNAs. The chemical modifications of different mt-RNAs also affect different regulatory mechanisms that might be involved in different disorders (Tomé-Carneiro et al., 2021). In consequence, Wang et al. (2017) evaluated the role of *Fto* in mitochondria biogenesis in C2C12 myoblasts mice cell line. They found that silencing of the *Fto* gene can significantly downregulate *Pgc1α*, a master transcriptional coactivator for mitochondrial biogenesis, and three downstream targets of *Pgc1*α, such as transcription factor A, mitochondrial (TFAM), cytochrome c, and *Cox5a*. The intracellular ATP levels also decreased upon *Fto* silencing, suggesting that RNA mitochondrial methylation through *Fto* silencing may repress mitochondrial biogenesis and function, through *pgc1α* and targeted genes and indicating a potential role of obesity in mitochondria biogenesis (X. Wang et al., 2017). Taking together, FTO (by regulating m^6A) act as controllers of biogenesis of mitochondria. The fact that FTO is an important gene in obesity may suggest a role of m^6A as potential biomarker on the evolution of obesity.

However, m^6A and FTO play an additional role in diverse physiological contexts. For instance, a study conducted by Kang et al. (2018) found that *FTO* was associated with fat accumulation in both in vitro (HepG2 cells) and an in vivo porcine model (Kang et al., 2018), elucidating additional mechanisms related to obesity. In addition, Du et al. (2021) demonstrated a protector effect of *Fto* against hepatic ischemia–reperfusion injury in murine model of the disease. After liver-specific overexpression of *Fto*, the ischemic condition was ameliorated, repressed the elevated level of m^6A mRNA, and alleviated liver oxidative stress, contributing to the hepatic protective effect via demethylating the mRNA of dynamin-related protein 1 (Drp1; Du et al., 2021). This assumption seems to be related to the control of mitochondrial antioxidant system from m^6A and FTO. Therefore, under a strict control of mitochondrial function and antioxidant capacity, it is likely to see that m^6A and its regulation by FTO may exert additional role at the physiological levels (apart from its role on the pathogenesis of obesity). Future studies are needed to clarify whether this epigenetic mark may contribute to other metabolic- and mitochondrial-related disorders such as cardiovascular and neurodegenerative diseases or cancer (Peng et al., 2021; Ghezzi et al., 2012).

4.2 | The role of mitochondrial RNA modifications in obesity and diabetes

As discussed above, metabolic alterations may be similar to obesity phenotype, by inducing important alterations in the mitochondria. Overall, obesity creates an imbalance of mitochondrial functions, which further increases the risk of several related disorders (Ayers et al., 2019). However, there is a lack of knowledge about the current state of mt-RNAs modifications in the context of obesity. Few of the current findings were focused on the dysregulation of the effectors of RNA modifications, rather than on their characterization. In spite of this, RNA modifications in obesity are an emergent issue to consider in the physiopathology of obesity.

Mutations in mitochondrial tRNA have been proposed as a genetic risk factor for obesity in several clinical studies. Accordingly, a novel mutation of the mitochondrial $tRNA^{Cys}$ (5802A>G) in Chinese individuals in which obesity phenotype was observed in matrilineal relatives of a single generation. The A30 site correlated with a destabilized conserved base pair in this tRNA anticodon stem, and remodeled in a molecular dynamics simulation when compared with the isoform of the wild-type, reporting a probable link to obesity in childhood Chinese population (J. Wang, Zhao, et al., 2020). In addition, a study conducted by Wang, Ji, and Fu (2020) reported a pedigree with obesity, which were likely to be caused by mitochondrial $tRNA^{Arg}$ 10461A>G mutation, suggesting a role in obesity. However, further studies are needed, due to the involvement of other modifier risk factors in obesity (J. L. Wang, Ji, & Fu, 2020).

On the other hands, many of regulatory proteins of RNA modifications are usually found dysregulated in the context of obesity. These proteins regulated the mt-RNA frequencies, and therefore, the behind phenotype. Accordingly, *Cdkal1* homolog Cdk5 regulatory subunit-associated protein 1 (CDK5RAP1), an enzyme that is responsible for the modification of tRNA in the mitochondria, by specifically converting i^6A to ms^2i^6A at position A37 of four mitochondrial DNA-encoded tRNAs (Fakruddin et al., 2017), was reduced in adipose tissue from obese mice. By using *Cdkal1* KO mice, the mitochondrial function was found impaired, and the mitochondrial morphology was found abnormal. This suggests that specific modifications introduced by CDKAL1 enzyme is necessary for normal mitochondrial morphology

and function in adipose tissue, in which may contribute to other metabolic disorders related to obesity (Palmer et al., 2017). In addition, Perks et al. (2017) demonstrated in a mouse model that knockout of the protein pentatricopeptide repeat domain protein 1 (*Ptcd1*)—which is required for maintaining the stability and pseudouridination of 16S rRNA—resulted in impaired mitochondrial gene expression and dysregulation of RNA processing that in turn affects the biogenesis of mitochondrial respiratory chain, causing uncoupling and changes in mitochondrial morphology (Perks et al., 2018). The long-term effects were linked to later in life adult-onset of obesity and serious consequences on energy metabolism (Perks et al., 2017). Further, Chen et al. (2020) reported in both in vivo and in vitro assays that human METTL15, encoded by a nuclear gene, was responsible for 12S mt-rRNA methylation at m^4C839 (H. Chen et al., 2020). Since a *METTL15* polymorphism (rs10835310) was associated with childhood obesity (Bradfield et al., 2019), the authors speculated that the METTL15 activity may have an impact on obesity onset, probably due to the ability of METTL15 to regulate mitochondrial function by methylating 12S mt-rRNA (H. Chen et al., 2020). As noted, dysregulated of regulator mitochondrial enzymes have been proposed to show an effect of obesity phenotype, through regulating mitochondrial function. However, the most crucial mitochondrial modifier for mt-mRNA has been the FTO enzyme, as a key gene in the pathogenesis of obesity, but also as a genetic risk factor, although the exact molecular mechanisms remain unknown.

Recent studies have reported that many of mt-mRNA modifications in obesity are as a cause of FTO deregulation. For instance, a study conducted by Shen et al. (2021) found that overexpression of *FTO* inhibits the expression of Bax and mitochondrial unfolded protein response (UPR^{mt}; by reducing HSP60 mRNA m^6A level) and many of proteins related to apoptosis. Particularly, overexpression of *FTO* inhibited mitochondria-dependent apoptosis in adipocytes, by activating JAK2/STAT3 signaling pathway and inhibiting UPR^{mt}, suggesting a potential therapy in obesity and related diseases (Shen et al., 2021). In addition, another study reported that *FTO* protein decreased mitochondrial number, whereas mutations in the *FTO* gene were not able to regulate the mitochondrial contents. Overall, the RNA modifications were related to fat deposition, indicating a local regulation (modulated by m^6A) in hepatocytes, in which obesity term may affect mitochondrial RNA modifications at different tissues, as muscle or liver cells (Kang et al., 2018). Moreover, the DHA supplementation in mice enhanced the expression of *Fto* gene in the muscle tissue and myoblasts, leading to reduced m^6A levels of DNA damage-induced transcript 4 (Ddit4), which finally elevated mitochondria biogenesis and slow muscle fiber formation. These results highlighted the effect of specific DHA-diet in mitochondrial biogenesis and skeletal muscle fiber via *Fto*/m^6A, protecting against obesity-induced decline in skeletal muscle function (W. Chen et al., 2022). Similarly, Wei et al. (2021) treated male piglets with 1 mg/kg of leptin recombinant protein. After 4 weeks treatment, the authors found an upregulation of *FTO*, which in turn leads to the decrease of m^6A methylation of mRNA *Plin5*. An additional in vitro porcine found that overexpression of *FTO* decreased mRNA m^6A methylation and increased the expression of *Plin5* protein in adipocytes. The overexpression in vitro of *Plin5* significantly reduces the size of lipid droplets, promotes the metabolism of triglycerides, and the operation of the mitochondrial respiratory chain, and increases thermogenesis, indicating that *Plin5* m^6A methylation through *FTO* affects lipid metabolism and energy consumption, providing a new preventive mechanism against obesity (D. Wei et al., 2021). Therefore, these studies can conclude that FTO is responsible for mt-mRNA modifications that regulate mitochondrial biogenesis in adipose tissue and specifically, lipid metabolism and energy expenditure in obesity.

On the other hand, the current approach relies on the investigation of mt-RNA modification-related genes in T2D. In this sense, mutations in mt-RNA have been reported, which are responsible for many diabetes types, and affect both mt-RNA and related-processing enzymes for epigenetic modifications. Recently, the association between mitochondrial 12S rRNA modifications and T2D was demonstrated in mouse models and GWAS studies in a human population, with reduced insulin secretion, elevated postprandial glucose levels, and increased future risk of T2D. A genome-wide association study (2007) identified a variant in the *CDKAL1* gene that was associated with subjects with T2D [allele-specific odds ratio (OR) = 1.20, 95% confidence interval, 1.13–1.27]. The insulin response for homozygotes individuals was decreased by up to 20%, suggesting that this variant in the *CDKAL1* gene is responsible for increased risk of T2D through reduced insulin secretion (Steinthorsdottir et al., 2007). An additional familiar study identified an A to G transition was identified at nucleotide 3243, a conserved position in the mitochondrial gene for $tRNA^{Leu(UUR)}$, which co-segregates with the family and it was considered is a pathogenetic factor for noninsulin-dependent type 2 diabetes mellitus (NIDDM; van den Ouweland et al., 1992). This missense mutation has also been largely associated with other different types of diabetes, such as maternally inherited diabetes and deafness (MIDD) (Ohkubo et al., 2001) and Maturity-onset diabetes of the young (MODY) (Vaxillaire et al., 1994), as pathogenic factor. This mutation results in the absence or reduction in the taurinomethyluridine modification level at position 34 in humans. The reduced taurinomethyluridine modification level impacts the mitochondrial protein synthesis and results in mitochondrial

dysfunction in several tissues including pancreatic β-cells, muscles, and neurons (Arroyo et al., 2021; Kirino & Suzuki, 2005; Kobayashi et al., 1990; Yasukawa et al., 2000). Indeed, a study reported two proteins, MTO1 (Mitochondrial TRNA Translation Optimization 1) and GTPBP3 (guanosine triphosphate binding protein 3) that are responsible for the 5-taurinomethyluridine biogenesis. *GTPBP3*-knockout cells exhibited respiratory defects and reduced mitochondrial translation, demonstrating that lack of 5-taurinomethyluridine results in pathological consequences (Asano et al., 2018). Similarly, the mutation 14692A>G in the mitochondrial $tRNA^{Glu}$ gene has been reported in MIDD patients, which resulted in pseudouridine modification at position 55. The mutation 14692A>G in $tRNA^{Glu}$ resulted in reduced pseudouridine levels, thereby altering the structure and function of the tRNA along with the impairment of $tRNA^{Glu}$ metabolism, resulting in impaired mitochondrial translation and mitochondrial dysfunction (Yasukawa et al., 2001). Collectively, these results indicate that mutations in mt-RNA have a potential role in developing several phenotypes in diabetes. Therefore, understanding the effect that mt-RNA modifications on diabetes may provide, not only new strategic therapies but also novel biomarker to identify and monitor specific phenotypes of diabetes.

As for the modifier enzyme of mt-RNA modifications, a member of TRMT family, TRMT10A (responsible for the conversion from adenine to methyladenine and from guanine to methylguanine; Table 1), is closely related to diabetes. Linkage analysis and whole exome sequencing identified a mutation at the position 127 of the tRNA methyltransferase homolog gene *TRMT10A*, in which *TRMT10A* deficiency negatively affects β-cell mass, suggesting a relevance in the pathogenesis of T2D (Igoillo-Esteve et al., 2013), in which further studies confirmed this relationship (Gillis et al., 2014; Narayanan et al., 2015; Yew et al., 2016; Zung et al., 2015). Additionally, Cosentino et al. (2018) demonstrate that *TRMT10A* deficiency induced oxidative stress and triggers the intrinsic pathway of apoptosis in β-cells. A hypomethylation of m^1G leads to $tRNA^{Gln}$ fragmentation and mediates *TRMT10A* deficiency-induced β-cell death, suggesting a tRNA modification may be important in both cytosolic and mitochondrial in the pathogenesis of diabetes (Cosentino et al., 2018). As for TFB1, a methyltransferase enzyme of $m_2{}^6A936$ and $m_2{}^6A937$ modifications at the 3′-terminal of 12S rRNA, a study reported a common variant in the *TFB1* gene in human was associated with reduced insulin release, reduced beta-cell mass, reduced ATP production, and oxygen consumption (Koeck et al., 2011). Verma et al. (2022) reported that dimethyladenosine transferase 1 homolog (DIMT1), a homolog of *TFB1M* and a rRNA methyltransferase, was increased in human islets from patients with T2D, correlated with insulin expression, and negatively association with insulin protein secretion. Next, the authors silenced *DIMT1* in insulin-secreting cells, and observed lower expression of oxidative phosphorylation proteins. This phenotype also led to dysregulate the insulin secretion, indicating that *DIMT1* is responsible for mitochondrial function and insulin pathway, which may participate in potential pathogenic pathways in T2D (Verma et al., 2022). Another study in the *Tfb1* $^{-/-}$ knockout mice also reported an increase of reactive oxygen species (ROS) along with increased apoptosis and necrosis, indicating mitochondrial damage in the beta-cells (Sharoyko et al., 2014). These results show that modifications in 12S rRNA, such as $m_2{}^6A936$ and $m_2{}^6A937$, contributed to the risk of T2D. As shown, apart from genetic mutations that are strongly associated with multiple phenotypes of diabetes, the dysregulation of enzyme modifiers induces aberrant mt-RNA modifications, which, in turn, increase the risk of having several pathogenic pathways in T2D.

In clinical studies, several metabolites from RNA modifications, which may come from mitochondria, can be correlated with T2D. For instance, Chen et al. (2018) analyzed 267 urine samples from healthy subjects and patients with micro- or macroalbuminuria due to nondiabetic disease, and patients with T2D with and without microalbuminuria. The authors found that N^1-methylguanosine levels were lower in macro and micro T2D, when compared to the healthy group and T2D without macroalbuminuria ($p < 0.001$ and 0.001, respectively; C.-J. Chen et al., 2018). In addition, Ottosson et al. (2019) analyzed a case-cohort study from the Malmö Preventive Project, which included 698 metabolically healthy participants, of whom 202 developed T2D (follow-up time of 6.3 years). The authors observed that plasma N^2,N^2-dimethylguanosine levels were associated with an increased risk of T2D. In addition, N^2,N^2-dimethylguanosine and 7-methylguanine were significantly associated with incident T2D (Ottosson et al., 2019). The same authors also found that N^2,N^2-dimethylguanosine and 1-methyladenosine were associated with an increased risk of for all-cause mortality in participants with T2D (Ottosson et al., 2020). Collectively, these findings may suggest plasma or urine as potential source for RNA modifications screening to detect impairment of mitochondria in the T2D context.

Due to limited research on whether mt-RNAs modifications are involved in the pathogenesis of obesity and diabetes, it is important to characterize all the mt-RNAs modifications. It is also essential to understand how many RNA modification regulatory proteins are involved in this process, and how are their translation affected. Finally, it is also crucial to understand if these modifications can be modulated in the pathological scenarios for treatment, and can be used as a potential tool for diagnosis, prognosis, and as a therapeutic target. The above-mentioned studies reported

emergent role of RNA modifications in muscle, liver, and adipose tissues, suggesting a metabolic link between mt-RNAs modifications and obesity and diabetes in their different subtypes.

4.3 | The role of mitochondrial RNA modifications in cancer

One of the hallmarks of cancer is an unbalanced cellular energetics. Mechanisms behind these deregulated cellular energetics are widely associated with mitochondrial impairment, led by key mutations in mitochondrial DNA, over/downexpression of mitochondrial enzymes, or important defects in the oxidative phosphorylation system. Overall, mitochondrial dysfunction induced by up/downexpression in crucial enzymes related to mt-RNA modifications might provide a potential mechanism for energy metabolism dysregulation of tumoral cells, and further, might contribute to cancer progression (Figures 2 and S1) (Hsu et al., 2016). As a result, the main findings of dysregulation of key enzymes responsible for mt-RNA modifications are displayed below, which may contribute to an increased risk of cancer.

Methylation is the most frequent modification in cancer. Accordingly, an integrated genomic analysis conducted by Idaghdour and Hodgkinson (2017) found significant changes to m^1A and m^1G RNA methylation levels in mitochondrial tRNAs in tumor tissues across all cancers, highlighting the potential clinical relevance of altered mitochondrial RNA processing in cancer (Idaghdour & Hodgkinson, 2017). Wei et al. (2018) also reported a dysregulation of m^5C epitranscriptome in breast cancer cell lines in comparison with normal epithelial cell lines. The authors found about 47 differentially methylated genes, related to important biological functions of cancer, such as regulation of apoptosis and programmed cell death. Moreover, m^5C modification was strongly enriched mitochondrial RNA in both normal and breast cancer (Z. Wei et al., 2018). Then, several forms of methylation are detected, indicating a close role in mitochondrial dysregulation in cancer.

In addition to mt-RNA base modification, the modifier enzymes are strongly found imbalanced in cancer, as NSUN family. For instance, a genome-wide meta-analysis of GWAS reported that a polymorphism presented in *NSUN4* (responsible for m^5C modification) gene was associated with increased risk of breast and prostate cancer (Kar et al., 2016). In addition, a study exposed that *NSUN2*, from the Cancer Genome Atlas (TCGA), is highly expressed in various cancers, including breast cancer, colorectal cancer, lung cancer, and others, suggesting a dysregulation of m^5C in cancer [reviewed by Chellamuthu and Gray (2020)]. A similar finding was observed for NSUN3 and NSUN4 in lung squamous cell carcinoma (LUSC) in the TCGA data (J. Pan et al., 2021). Similarly, a study, by using TCGA-LIHC data, found up to seven m^5C RNA methyltransferase-related genes differentially expressed in hepatocellular carcinoma (HCC) tumor tissues. Among them, *NSUN4* and *NSUN5* expression notable varied in different grades, whereas *NSUN4* has shown good prognostic factor properties. Additional pathway analysis displayed that *NSUN4* was linked to typical signaling pathways related to cancer, such as extracellular matrix, mTOR signaling, or RNA degradation (Cui et al., 2021). Also, He et al. (2020) found that high expression of *NSUN4* was significantly correlated with survival outcome for patients with hepatocellular carcinoma (He et al., 2020). Therefore, NSUN family seems to exert important regulatory function in cancer, through the addition of methylated-base modification.

As for the TF family, a study reported by using the bioinformatic approach, that *TFB1M* was upregulated in tissue and HCC cells. This overexpression was related to poor survival, contributed to growth and metastasis, and promoted cell apoptosis, through impairment of oxidative phosphorylation, suggesting that dysregulation of *TFB1M* plays a crucial oncogenic role in HCC progression (Mu et al., 2022). Another study found in patients with Acute myeloid leukemia, that *TFB1M*, *TFB2M*, *TFAM* genes were upregulated, in which they were related to poor overall survival (Wu et al., 2019). In human glioblastoma cell line U87MG, treatment with melatonin disrupted mt-DNA expression and results in cell death due to increased ROS production and mitochondrial damage, through a decrease of *TFAM*. Melatonin also reduced *TFB1M* and *TFB2M*, suggesting a potential role in brain tumors (Franco et al., 2018). Thus, upregulation of TF family has been related to poor survival, probably through its methylation activity on 12S rRNA.

FTO seems to display a potential contribution tp cancer, as in the case of obesity. Hence, Zhuang et al. (2019) demonstrated that *FTO* played a critical role and anti-tumorigenic in clear cell renal cell carcinoma (CCRCC). *FTO*s were found suppressed in CCRCC, which in turn, it was correlated with increased tumor severity and poor patient survival. The *FTO* overexpression restored mitochondrial activity and induce oxidative stress, through increasing *PGC-1α*, and reducing m^6A levels, indicating that the *FTO* metabolism may be crucial in cancer (Zhuang et al., 2019). On the other hands, Liang et al. (2020) reported that methyltransferase-like 3 (METTL3), a methyltransferase responsible for m^6A modification of mRNA, is found highly expressed in ovarian cancer, and associated with poor clinicopathological outcomes, through the AKT signaling pathway. *METTL3* silencing reduced the proliferation and colony formation assay,

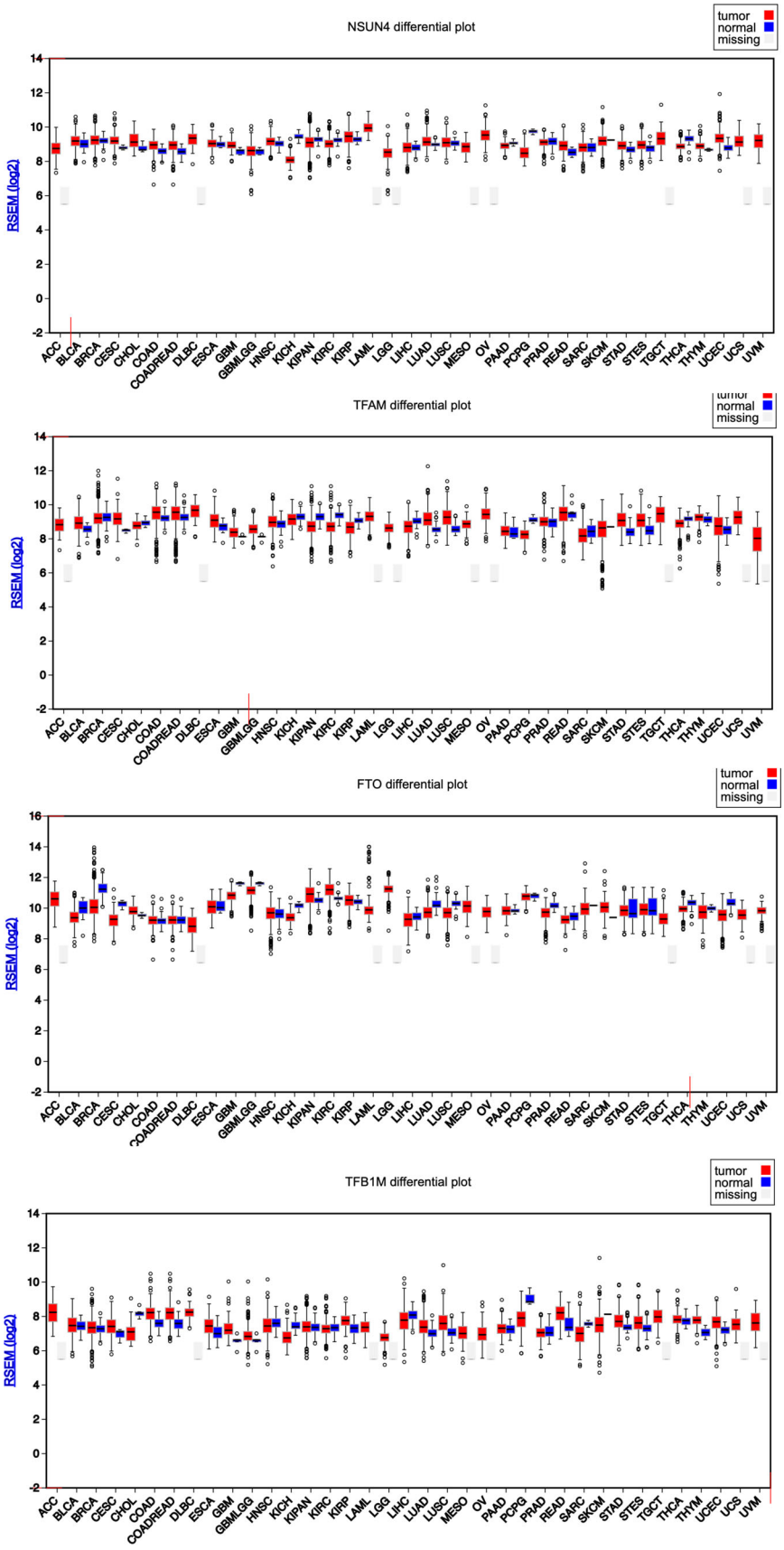

FIGURE 2 Gene expression of the enzyme responsible for mt-RNA modification in cancer, using the TCGA data. Data extracted from http://firebrowse.org/viewGene.html

indicating that this m^6A plays a potential role in carcinogenesis and cancer progression (Liang et al., 2020), in which similar results were also confirmed in esophageal cancer (Hou et al., 2020). Furthermore, Ali et al. (2020), by studying 11,552 samples from 39 tissue/cell types found that genetic variants within *MRPP3* and *TRMT61B* are associated with m^1A and m^1G RNA modification levels across a large number of tissues, and this modification was associated with multiple-related phenotypes, including breast cancer, among others (Ali et al., 2020). Moreover, in human nonsmall cell lung carcinoma, overexpression of *DUS2* has been found and decreased level of dihydrouridine modification in tRNAs has been observed after the knockout of *DUS2* in human lung carcinoma cells (Kato et al., 2005). Finally, Antonicka et al. (2017), by using osteosarcoma cell lines, identified a mitochondrial pseudouridine synthase protein module using BioID. Depletion of the individual enzymes produced specific mitochondrial protein synthesis and oxidative phosphorylation assembly defects without affecting mitochondrial mRNA levels. Further results showed that RPUSD4 plays a role in the pseudouridylation of a single residue in the 16S rRNA, while TRUB2/RPUSD3 were similarly involved in pseudouridylating specific residues in mitochondrial mRNAs. All these results establish essential roles for epitranscriptomic modification of mitochondrial RNA in mitochondrial protein synthesis, oxidative phosphorylation, and cell survival (Antonicka et al., 2017).

The mechanistic role of RNA methyltransferases on cancer is not fully understood, but promising. A study reported that overexpression of RNA guanine-7-methyltransferase (RNMT) promoted human mammary epithelial cell and fibroblast cell transformation (Cowling, 2010), cancer growth, and survival in breast cancer (Manning et al., 2020) and is required for cell proliferation in Hela cells (Aregger & Cowling, 2013). In CRC, a study conducted by Li et al. (2014) found that RNMT (and validated in two CRC cell lines) has been proposed to increase cell proliferation and drug resistance to irinotecan (X. X. Li et al., 2014). The mechanistic role of this phenotype is through the phosphorylation of RNMT (due to CDK1-cyclin). This phosphorylation increases the levels of m^7G at the G1 phase, in which the inhibition of RNMT phosphorylation reduces the cell proliferation rate (Aregger et al., 2016). It was also reported that the phosphorylation of RNMT is recruited by MYC to regulate key genes related to Wnt signaling pathway (Posternak et al., 2017), in which the aberrant activation of Wnt signaling, is so far, considered as the hallmark of cancer (Zhong et al., 2020). Dunn et al. (2019) found in a panel of breast cancer cell lines that by reducing the cellular activity of RNA, the proliferation of a subset of cells were reduced as well as increased the apoptosis rate. All these cells depended on oncogenic mutation in PI3KCA gene, suggesting and PI3KCA signaling pathway is required (Dunn et al., 2019). Therefore, these findings support that RNMT is responsible for cell cancer proliferation as well as they are critical to develop new drug strategies considering RNMT as a promising anti-cancer target.

5 | CONCLUSIONS AND FUTURE PERSPECTIVE

Recent developments in next-generation sequencing methods, together with the developments in the field of bioinformatics, have helped us to better understand the types of RNA modifications (e.g., RNA m^6A) and their distribution in different RNA species (e.g., mt-tRNAs and rRNAs). Each modification found in the mt-RNAs is predicted to have a specific role in the function of the RNA in which it resides. These modifications have several key functions in mitochondrial gene expression and regulation. A thorough understanding of RNA modifications can reveal the underlying molecular mechanisms of diseases, which have not been fully understood. This understanding can help us identify novel biomarkers and design better therapeutic strategies for the treatment of mitochondria-related diseases. From a disease perspective, for example in obesity, T2D, and cancer, we currently know that mitochondrial RNAs, are modified in a diverse and complex manner involving numerous proteins in the process, in which an emergent role is present. Future efforts need to be directed towards systematic mapping strategies to identify mitochondrial RNA modification in individuals with obesity, T2D, cancer, and other diseases compared to healthy controls. This may help us to have a better understanding of the molecular mechanisms affected by such modifications and how it contributes to the disease.

AUTHOR CONTRIBUTIONS

Hatim Boughanem: Conceptualization (equal); data curation (equal); formal analysis (equal); methodology (equal); software (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Yvonne Bottcher:** Conceptualization (equal); data curation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Joao Tome Carneiro:** Formal analysis (equal); validation (equal); visualization (equal); writing – original draft (equal). **Maria del Carmen Lopez de las Hazas:** Software (equal); validation (equal); visualization (equal); writing – original draft (equal). **Alberto Davalos:** Formal analysis (equal); supervision (equal);

visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Manuel Macias-Gonzalez:** Conceptualization (equal); formal analysis (equal); funding acquisition (lead); investigation (equal); methodology (equal); resources (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). **Akin Cayir:** Conceptualization (equal); data curation (equal); formal analysis (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

The authors have declared no conflict of interest for this article.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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