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REVIEW ARTICLE

The critical roles of m6A modification in metabolic abnormality and cardiovascular diseases

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Abstract N6-methyladenosine (m6A) RNA methylation is an emerging area of epigenetics, which is a reversible and dynamic modification mediating by 'writers' (methylase, adding methyl groups, METTL3, METTL14, and WTAP), 'erasers' (demethylase, deleting methyl groups, FTO and ALKBH5), and 'readers' (YTHDF1-3, YTHDC1 and YTHDC2). Recent studies in human, animal models and cell levels have disclosed a critical role of m6A modification in regulating the homeostasis of metabolic processes and cardiovascular function. Evidence from these studies identify m6A as a candidate of biomarker and therapeutic target for metabolic abnormality and cardiovascular diseases (CVD). Comprehensive understanding of the complexity of m6A regulation in metabolic diseases and CVD will be helpful for us to understand the pathogenesis of CVD. In this review, we discuss the regulatory role of m6A in metabolic abnormality and CVD. We will emphasize the clinical relevance of m6A dysregulation in CVD.

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Introduction

Recently, epigenetics has been demonstrated to be closely related to the onset and development of metabolic abnormality and cardiovascular diseases (CVD).[1–5] Epigenetics is a reversible, dynamic modification on the levels of DNA, histone and RNA, mainly including DNA methylation, histone acetylation, RNA modification and chromatin rearrangement.[6] Among these modifications in epigenetics, DNA methylation and histone modification have been well studied, while RNA modification is an emerging field of epigenetics.

There are more than one hundred posttranscriptional modifications of RNA in regulating its stability, decay, translation and splicing.[7] 5′ cap modification plays an important role in RNA metabolism and was drew attention by the early studies.[8] mRNA 7-methylguanylate (m7G) capping is forming by the action of 5′ triphosphatese, guanylyltransferase, and guanine-7 methyltransferase, and is of great important in mRNA translation and cell viability.[9] N6-methyladenosine (m6A) is recognized as most abundant internal post-transcriptional modification in eukaryocytes and is essential for the regulation of various cellular processes and many diseases.[10,11] Following the identification of m6A, multiple post-transcriptional modifications were discovered in mRNA, including 5-methylcytosine (m5C), pseudouridine (Ψ), N1-methyladenosine (m1A), 5-hydroxymethylcytosine (hm5C) and N6, 2′-O-dimethyladenosine (m6Am).[8] Despite these chemical modifications have been identified for decades, the development of researches in the modifications is slow and we know little about their biological functions. In recent years, due to the rapid development of highly specific antibodies and the high-throughput sequencing technologies,[12,13] researches on the RNA epigenetic modifications especially m6A RNA methylation have made great progress in disclosing the potential mechanism of human diseases, such as cancers,[14] neurological disorders,[15] metabolic abnormality and CVD.[16,17]

RNA m6A methylation

As a widely studied chemical modification, m6A methylation is an epigenetic modification of adding a methyl group which provided by S-adenosyl methionine (SAM) to the N6 site of adenosine, which was identified in 1970s.[18–21] It is a reversible and dynamic modification in RNA level, including mRNA, micro-RNA, lncRNA, circ-RNA, transfer RNA (tRNA), ribosomal RNA (rRNA) and small nuclear RNA (snRNA).[22] It is estimated that about 0.1%–0.4% of adenosine in RNA is modified by m6A in eukaryocytes, and 2–3 m6A-modified sites per transcript.[22] It regulates RNA methylation by 'writers' (methylase, adding methyl groups) and 'erasers' (demethylase, deleting methyl groups). And the altered methylation is recognized by readers, then exerts a regulatory function in RNA stability, decay, translation and nuclear export (Fig. 1).

Writers

Installation of methyl groups in m6A methylation is accomplished by a highly conserved RNA methyltransferase complex, including methyltransferase-like 3 (METTL3), METTL14, and Wilms tumor suppressor-1-associated protein (WTAP) in the core of the complex.[23–26] METTL3 and METTL14 contain a SAM-binding motif, and tend to catalyze the m6A in a consensus motif of RRACH (where R = G or A, and H = A, C or U), which usually occurs in 3′UTR and transcription start site.[12,13] In this complicated methyltransferase complex, METTL3 and METTL14 form stable heterodimers, in which METTL3 is the first methyltransferase discovered as a catalytic subunit, and METTL14 promotes the binding with RNA as an RNA-binding platform.[24] WTAP is a regulatory subunit to recruit METTL3- METTL14 complex to bind to mRNA.[26] Subsequently, many methylases have been found, such as KIAA1429,[25] METTL16,[27] and RBM15.[28]

Erasers

The reversal of m6A methylation is mediated by the only two found demethylases FTO (ALKBH9) and ALKBH5, the so-called 'erasers' of m6A. Both of them belong to the AlkB family, and they catalyze the demethylation of m6A in a Fe (II)- and α-ketoglutarate dependent manner.[22] FTO was initially found as an obesity-related gene, and it was first discovered to remove m6A modification of mRNA effectively *in vitro* in 2011.[29] Subsequent studies have shown that FTO oxidizes m6A to two intermediates, N6-hydroxymethyladenosine (hm6A) and N6-formyladenosine (f6A).[30] Due to FTO is a dioxygenase, its enzymatic efficiency is expected to weaken under hypoxic or ischemic conditions,[16] such as myocardial ischemia or infarction. It is also reported that FTO not only removes m6A methylation, but also reverses m6Am modification in cells, which stabilizes the 5′ cap structure of mRNA.[31] This indicates that FTO is also the eraser of m6Am, and plays a role on the stability of mRNA. Shortly after FTO was validated, ALKBH5 was identified as the second mammalian m6A demethylase. Different from FTO, ALKBH5 directly reverses m6A to adenosine, thus no intermediate can be detected.[32]

Readers

It has been demonstrated that the YTH domain can selectively bind to the m6A site in RNA, so the protein with a YTH domain has specific function of recognizing the altered m6A methylation.[33] The proteins that can recognize m6A modified and contain the YTH domain include YTHDF1–3, YTHDC1, and YTHDC2. It is validated that YTHDF2 binds to mRNA to accelerated its degradation.[34] In contrast, YTHDF1 and YTHDF3 enhance translation efficiency by recruiting translation initiation factors in HeLa cells.[35,36] YTHDC1 has a variety of regulatory functions, including regulating mRNA splicing by recruiting specific splicing factors,[37] accelerating mRNA nuclear export[38] and promoting the decay of specific transcripts.[39] Recent studies have shown that YTHDC2 can improve the translation efficiency of *hypoxia-inducible factor-1alpha (HIF-1α)* mRNA and regulate spermatogenesis through its helicase action.[40,41] Meyer et al reported that eukaryotic initiation factor 3 (eIF3), a component of 43S translation initiation complex, could directly bind to the mRNA m6A sites at

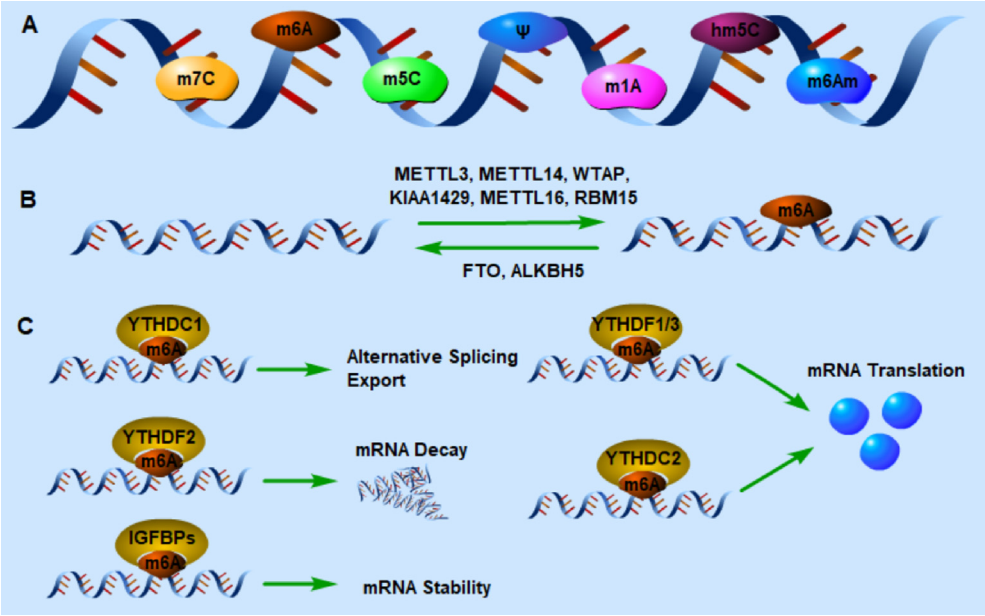

Figure 1 Regulation of gene expression by RNA modifications. **(A)** Chemical modification of eukaryotic mRNA. **(B & C)** Mechanism of m6A modification. Installation of methyl groups in m6A modification is accomplished by writers: METTL3, METTL14, WTAP, KIAA1429, METTL16 and RBM15. And the reversal of m6A methylation is mediated by erasers: FTO and ALKBH5. The altered m6A transcripts are recognized by m6A readers, and then leading to different effects on methylated mRNAs.

5′UTR, which played an important role in translation initiation.[42] Heterogeneous nuclear ribonucleoprotein C (HNRNPC) is a rich nuclear RNA binding protein, which is known to participate in the processing of pre-mRNA.[43] It has been found that m6A can regulate the binding of HNRNPC-RNA, thus affecting the abundance and alternative splicing of target genes.[44] Another kind of reader, insulin-like growth factor 2 mRNA-binding protein 1—3 (IGF2BP1—3), stabilizes target mRNA in a m6A dependent manner.[45] A recent study confirmed that Proline rich coiled-coil 2 A (Prrc2a) was an m6A reader, and the results showed that Prrc2a stabilized the m6A modified mRNA that needed for myelination.[46]

m6A in metabolic and cardiovascular diseases

There are many risk factors for causing CVD. The occurrence of CVD is the result of long-term interaction of the risk factors. Metabolic disease, which results from disrupted metabolic processes of proteins, fats, carbohydrates and other substances,[47] is a great threaten to cardiovascular health, especially hypertension, dyslipidemia, diabetes mellitus, atherosclerosis, obesity and nonalcoholic fatty liver disease (NAFLD). The role of m6A modification in metabolic and CVD has been explored (Table 1, Fig. 2 and 3).

Hypertension

Hypertension is a major risk factor for CVD, and researchers have recently disclosed that epitranscriptomic mechanism of m6A plays a critical role in hypertension.[48] The global level of m6A methylation is reduced in spontaneously hypertensive rat pericytes[49] and hypoxia mediated pulmonary hypertension,[50] and the m6A is distributed mainly in the coding sequence region, 3′UTR and 5′UTR of mRNAs.[49] m6A circXpo6 and m6A circTmtc3 are downregulated in pulmonary hypertension, moreover, m6A connects with circRNA-miRNA-mRNA network to affect the development of pulmonary hypertension.[50] Mo et al found that 1236 m6A-associated single-nucleotide polymorphisms (m6A-SNPs) were related to blood pressure (BP), especially diastolic BP, and approximately 10% of these BP-associated m6A-SNPs were associated with coronary artery disease or stroke.[51] m6A-SNPs rs56001051, rs9847953, rs197922, and rs740406 were strongly associated with BP-related genes expression in Chinese individuals.[51] Meyer et al also found that m6A-SNPs (Lys67Arg, rs197922) was associated with hypertension and BP in Whites in the Atherosclerosis Risk in Communities Study and in the Women's Genome Health Study.[52] A meta-analysis comprising 57,464 hypertensive patients and 41,256 controls demonstrated that FTO variant was related to hypertension in both European and Asian populations.[53] However, FTO gene rs9939609 variant has positive associations with BMI and neck circumference, but does not have an effect on BP in hypertension patients.[54] Patients with gestation-associated arterial hypertension were showed to have higher incidence of heterozygotic genotype AT (FTO gene) than healthy puerperants.[55] These results reveal that m6A is closely related to hypertension, further study its epitranscriptomic mechanisms will provide us more concepts of the cause and treatment of hypertension.

Diabetes mellitus

Patients with diabetes develop a distinct form of atherosclerosis due to endothelial injury, which lead to myocardial ischemia and ultimately to heart failure. Type 2 diabetes (T2D) is characterized by deficient insulin, insulin resistance

Table 1 The role of m6A in metabolic and cardiovascular diseases.

Metabolic and cardiovascular diseases	m6A regulators	Biological function	Mechanism	PMID	References
Hypertension	FTO	—	Gene variant	24641884	53
	m6A	Influences pulmonary hypertension	Influences the circRNA-miRNA-mRNA co-expression network	31931709	50
	m6A	—	m6A-SNPs	31175347, 19057520	51,52
Diabetes mellitus	FTO	Influences glucose metabolism	Induces mRNA expression of FOXO1, G6PC, and DGAT2	30137347	61
	FTO	Loss of FTO protects mice from glucose intolerance and insulin resistance	Loss of FTO increases AKT phosphorylation in endothelial cells and skeletal muscle	31801409	70
	FTO	Influences insulin resistance and adipose tissue inflammation	Silencing FTO induces the transformation of macrophages into M1-type pro-inflammatory macrophages	31709454	71
	METTL 14	Decreases β-cell proliferation and insulin degranulation	Influences insulin/IGF1-AKT-PDX1 pathway	31867565	67
	METTL3	Inhibits hepatic insulin sensitivity	METTL3 silence decreases the m6A methylated and total mRNA level of Fasn	31405565	69
Obesity	FTO	Loss of endothelial FTO antagonizes obesity-induced metabolic and vascular dysfunction	FTO deficiency upregulates L-Pgds and prostaglandin D2 levels	31801409	70
	FTO	Increases in skeletal muscle mass	—	31572457	84
	FTO	FTO depletion blocks adipogenesis	FTO regulates splicing of RUNX1T1	25412662	91
	FTO	Promotes adipogenesis	Inhibits of the Wnt/β-catenin signaling	28267420	130
	FTO	FTO deficiency affects browning of white adipose tissue	—	27827997	131
	FTO	Regulates mitotic clonal expansion	Enhances RUNX1T1	25881961	92
	METTL3	Inhibits adipogenesis	—	25725156	93
	METTL3	METTL3 knockdown promotes mitotic clonal expansion and adipogenesis	Promotes CCND1 expression	31434544	94
	YTHDF2	Prolongs cell cycle progression and suppresses adipogenesis	YTHDF2 inhibits CCNA2 and CDK2	30305247	87
	YTHDF2	Inhibits autophagy and adipogenesis	Inhibits Atg5 and Atg7	31451060	90
	YTHDF2	Inhibits adipogenesis	Inactivates JAK2-STAT3-C/EBPβ signaling	31295563	97
	YTHDF1	Promotes adipogenesis	Targets MTCH2	30339471	99

(*continued on next page*)

Table 1 (*continued*)

Metabolic and cardiovascular diseases	m6A regulators	Biological function	Mechanism	PMID	References
Nonalcoholic fatty liver disease	FTO	Enhances oxidative stress and lipogenesis	—	23329013	105
	FTO	—	The expression of FTO is significantly correlated to FOXO1	25382334	106
	FTO	Induces lipid accumulation	—	32116145	108
Vascular diseases	METTL14	METTL14 de-expression decreases the calcification and enhances the vascular repair function	—	31697949	110
	FTO	Enhances angiogenesis	—	29997116	16
	METTL3	Promotes osteogenic differentiation	Inhibits twist-related protein 1 through a YTHDF2-dependent pathway	31761339	118
	METTL3	Facilitates M1 macrophage polarization	Methylates STAT1 mRNA and upregulates its expression	31365297	114
	WTAP	Inhibits vascular smooth muscle cell proliferation and migration	Regulates p16 via m6A modification	31986407	116
	FTO	Attenuates lipid accumulation in macrophage foam cells and alleviates atherosclerosis	—	28253220	111
Myocardial infarction and ischemia-reperfusion injury	m6A	—	m6A-SNPs	30221544	120
	FTO	Improves cardiac contractile function	Selectively demethylates cardiac contractile transcripts	29997116	16
	METTL3	Silencing METTL3 enhances autophagic flux and inhibits apoptosis	METTL3 methylates TFEB at two m6A residues in the 3′-UTR	30870073	122
	ALKBH5	Inhibition of ALKBH5 inhibits autophagic flux and enhances apoptosis	TFEB binds to the ALKBH5 promoter and activates its transcription	30870073	122
Heart failure	FTO	Knockout of FTO impairs cardiac function	—	31849158	127
	METTL3	Controls cardiac homeostasis and hypertrophy	Enhances the levels of MAP3K6, MAP4K5 and MAPK14	30586742	17
	FTO	Regulates cardiac function	Selectively demethylates cardiac contractile transcripts	29997116	16

and hyperglycemia. FTO rs9939609 and rs9940128 variants are closely related to hyperglycemia, insulin resistance and diabetes mellitus in several populations.[56–60]

Dysfunction of glucose metabolism is one of main characteristics of diabetes mellitus. As an m6A demethylase, FTO post-transcriptionally modulates glucose metabolism via m6A-dependent pathways. It is reported that m6A mRNA methylation involves in glucose metabolism through hepatic gluconeogenesis.[61,62] FTO, METTL3, METTL14, and WTAP are upregulated in T2D patients.[61] The global level of m6A methylation is decreased in T2D and mainly contributed by FTO rather than ALKBH5.[63] Furthermore, FTO specially demethylates some genes and enhances their expression in protein level, such as forkhead box protein O1 (FOXO1),

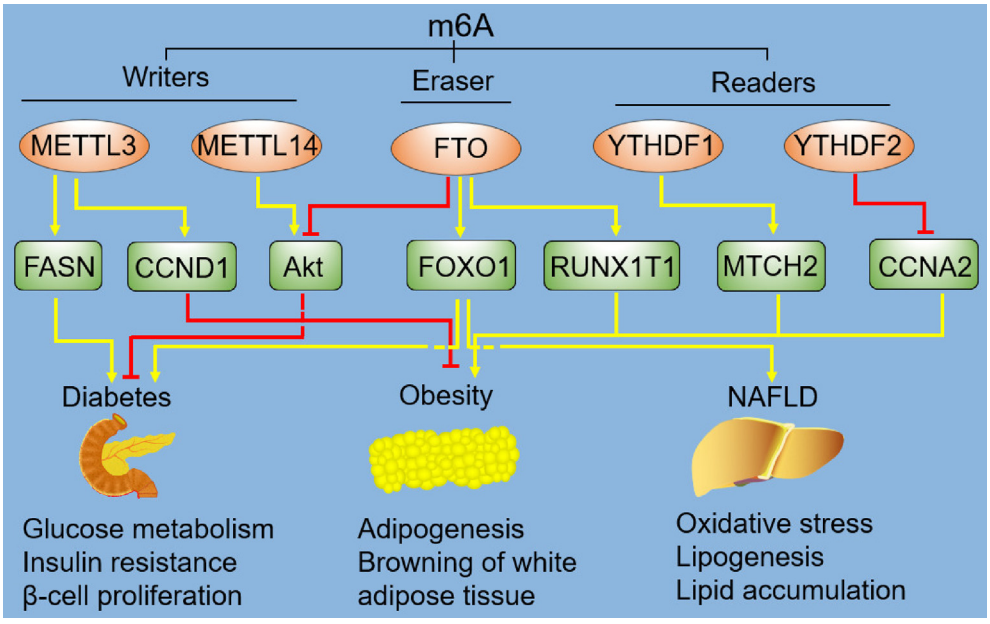

Figure 2 Regulation of metabolic disorders by m6A. The components of m6A methylation includes writers, erasers, and readers, and they regulate the development of diabetes, obesity and NAFLD by various down-stream targets. The dashed lines mean the nodes are non-intersect. m6A, N6-methyladenosine; METTL3, methyltransferase-like 3; METTL14, methyltransferase-like 14; FTO, fat mass and obesity associated protein; YTHDF1, YTH domain family protein 1; YTHDF2, YTH domain family protein 2; FASN, fatty acid synthase; CCND1, cyclin D1; Akt, also known as protein kinase B; FOXO1, forkhead box protein O1; RUNX1T1, runt-related transcription factor 1; MTCH2, mitochondrial carrier homology 2; CCNA2, cyclin A2; NAFLD, nonalcoholic fatty liver disease.

glucose-6-phosphate (G6P) and diacylglycerol O-acyltransferase 2 (DGAT2), which are related to increased blood glucose in patients.[61] As an essential transcription factor for mediating gluconeogenesis through G6P, FOXO1 was identified as a direct substrate of FTO.[62] And entacapone, a potential FTO inhibitor, exerts the glucose-lowering function by acting on the FTO-FOXO1 pathway.[62] In addition, Zhou et al observed the expression of activating transcription factor 4 (ATF4) was

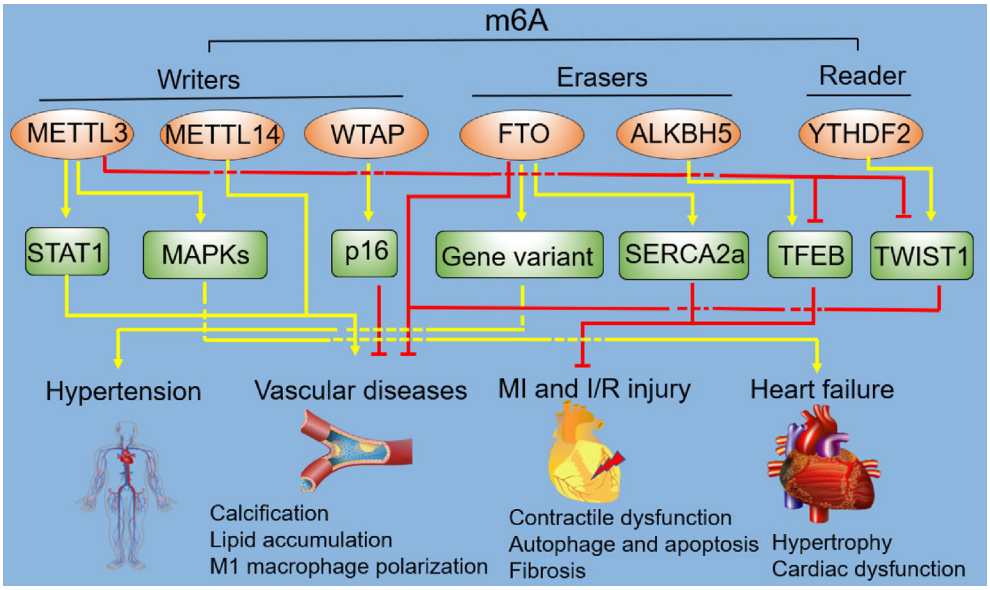

Figure 3 Regulation of cardiovascular diseases (CVD) by m6A. As a novel regulator of CVD, m6A plays various roles in the development of hypertension, vascular diseases, MI and I/R injury, and heart failure, via different pathways. The dashed lines mean the nodes are non-intersect. m6A, N6-methyladenosine; METTL3, methyltransferase-like 3; METTL14, methyltransferase-like 14; WTAP, Wilms' tumor 1-associating protein; FTO, fat mass and obesity associated protein; ALKBH5, Alk B homologue 5; YTHDF2, YTH domain family protein 2; STAT1, signal transducer and activator of transcription 1; MAPKs, mitogen-activated protein kinases; SERCA2a, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 2a; TFEB, transcription factor EB; TWIST1, twist-related protein 1; MI, myocardial infarction; I/R, ischemia/reperfusion.

increased in FTO overexpression transgenic mice.[64] Further research showed that ATF4 was able to increase glucose production by modulating G6P.[65]

The intact β-cell in pancreatic islet is essential for glucose homeostasis.[66] MeRIP-seq indicated a decreased m6A level in T2D human islets.[67] Dysfunction of islet, such as reduced β-cell proliferation and insulin degranulation, was observed in β-cell specific knockout of METTL14 in mice, which accelerated the occurrence of diabetes.[67] It is reported that METTL3/14 are responsible for the functional maturity of neonatal β cells, and depletion of METTL3/14 results in hypo-insulinemia and hyperglycemia.[68] In addition, the role of m6A modification in insulin resistance was also explored. The insulin sensitivity is enhanced in hepatocyte-specific METTL3 knock-out mice fed a high-fat diet, by targeting fatty acid synthase (Fasn) in an m6A-dependent manner.[69] Moreover, silence of endothelial FTO was demonstrated to alleviate glucose intolerance and insulin resistance induced by high-fat diet, via up-regulating AKT phosphorylation in endotheliocytes and skeletal muscle.[70] Contradictorily, Hu et al validated that losing FTO induced the transformation of macrophages into M1-type pro-inflammatory macrophages to aggravate insulin resistance in T2D mice.[71] Mussa et al identified FTO as a potential biomarker and novel therapeutic target for hypoglycemia-associated autonomic failure, a complication of diabetes.[72] These results disclose that m6A is emerging as a regulator of diabetes mellitus.

Obesity

Patients with obesity are more susceptible to CVD and tend to a worse outcome.[73,74] It results from an interaction between the genetic traits and environmental factors, including high-fat diet and lacking of exercise. Epigenetic mechanisms such as m6A modification are essential for the development of obesity.

FTO, as an obesity-related protein before identified as an m6A demethylase, is instinctively related to obesity. Sequence variants of FTO were observed in obesity in many populations, such as European, East Asian, African, Arab and Brazilian populations.[75–81] A study of the connection of parental diet during pregnancy with obesity in offspring by Kaspi et al showed that parental low-fat diet affected the obesity phenotype by altering the expression of FTO and METTL3 in the offspring.[82] Wang et al found that METTL3 played an important role in postnatal maturation of brown adipose tissue (BAT), and BAT-specific knockout of METTL3 leaded to the development of high-fat diet-induced obesity.[83] Moreover, increased expression of FTO is related to elevated skeletal muscle mass in overweight male adolescents,[84] whereas inhibited FTO activity by entacapone decreases body weight and lowers fasting blood glucose in diet-induced obese mice.[62] Similarly, Wang et al found that FTO was involved in the skeletal muscle differentiation by regulating mitochondrial function via mTOR-PGC-1α axis.[85] Furthermore, treatment with FTO inhibitor increase myogenic tone in obesity, which contributed to prevent the development of obesity-induced hypertension.[70] Interestingly, AMPK, the known energy sensor, is found to exert regulatory function of lipid accumulation in skeletal muscle by influencing FTO and m6A modification.[86] Therefore, it is believable that the translational study of controlling lipid accumulation in skeletal muscle by using m6A related medicines will be valuable.[86]

Mechanistically, m6A participates in the development of obesity by influencing the process of adipogenesis and lipid metabolism. Depletion of FTO reduces cyclin A2 (CCNA2) and cyclin dependent kinase 2 (CDK2), the vital regulators of mitotic clonal expansion, thus inhibits the cell cycle progression of preadipocytes and adipogenesis.[87,88] FTO regulates the expression of CCNA2 and CDK2 via m6A-YTHDF2 dependent mechanism.[87,88] Zinc finger protein (Zfp217), a regulator of adipogenesis, was also validated to regulate adipogenesis in an FTO-YTHDF2 dependent manner.[89] This FTO-YTHDF2 axis was also demonstrated to facilitate autophagosome formation and autophagy and thus promoted adipogenesis.[90] Moreover, FTO-dependent m6A demethylation transforms the mRNA splicing of RUNX1T1 (an adipogenic regulatory factor) into the pro-adipogenic short isoform to increase adipocyte proliferation.[91,92] Interestingly, it was reported that METTL3 and FTO played opposite roles in adipogenesis.[93] METTL3 enhances the m6A methylation of cyclin D1 (CCND1) mRNA, which recognized by YTHDF2 and thus degraded, leading to blocked cell-cycle progression and adipogenesis inhibition.[94] CCAAT enhancer binding protein β (C/EBPβ) is a pivotal transcriptional factor regulating adipocyte differentiation in the early stage.[95] Knockdown of METTL3 promotes adipogenesis by activating JAK1-STAT5-C/EBPβ signaling through m6A-YTHDF2-dependent mechanism.[96] Similarly, Wu et al disclosed that FTO deficiency suppressed adipogenesis in porcine and mouse via JAK2-STAT3-C/EBPβ pathway, and YTHDF2 facilitated *JAK2* mRNA decay in an m6A dependent manner in this process.[97] Moreover, YTHDF2 recognizes methylated *FAM134B (Family with Sequence Similarity 134, Member B)* mRNA and leads to its decay and thus reduces its protein abundance, resulting in adipogenesis in porcine adipocytes.[98] YTHDF1 facilitates translation of *mitochondrial carrier homology 2 (MTCH2)* mRNA to promote adipogenesis.[99] Taken together, m6A plays a vital role in adipogenesis and obesity.

Nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD) is characterized by hepatic steatosis, which caused by metabolic disorders of *de novo* lipogenesis, fatty acid uptake, fatty acid oxidation, and triglycerides export.[100] NAFLD increases the risk of CVD, such as atherosclerosis, cardiomyopathy, and arrhythmia, by the pathological mechanisms of systemic inflammation, endothelial dysfunction, insulin resistance, oxidative stress, and altered lipid metabolism.[101–103] Recently, m6A methylation has been reported to play an important role in the development of NAFLD.

A decreased global level of m6A and increased FTO expression are detected in NAFLD.[104–106] Exposure of endocrine disrupting chemicals (EDCs) is related to induction of NAFLD, global m6A level and expression of m6A modulators are alerted when exposure by EDCs in zebrafish.[107] FTO knockdown significantly mitigates dexamethasone-induced fatty liver (a mouse model of

NAFLD).[108] Moreover, it is reported that exenatide therapy ameliorates lipid accumulation and inflammatory changes in NAFLD by decreasing FTO expression in a PI3K-dependent mechanism.[109]

Atherosclerosis

The role of m6A methylation in the development and progression of vascular calcification,[110] obesity-induced vascular dysfunction,[70] atherosclerosis[111] and angiogenesis[16] has been explored. Atherosclerosis is the most prevalent disease threatening the vasculature, and is characteristic by lipid deposition and fiber cap formation.[112] Mo and colleagues found that overexpression of FTO by adeno-associated virus serotype 9 (AAV9) significantly reduced the lipidic profiles including plasma total cholesterol and LDL cholesterol, resulting in preventing the formation of atherosclerotic plaques.[111] However, Kruger et al demonstrated that loss of endothelial FTO prevented obesity-induced vascular dysfunction by using endothelial FTO-deficient mice.[70] It may be reasonable that FTO exerts different effects in different cell types. But there is no doubt that FTO is capable of affecting vascular homeostasis properties.

It is increasingly recognized that inflammation and immunity contribute mainly to the pathological development of atherosclerosis.[113] m6A writer METTL3 is markedly upregulated following the M1 polarization of mouse macrophages, and knockdown of METTL3 inhibits M1, but leaded to M2, macrophage polarization.[114] METTL3 exerts pro-inflammatory effect via METTL3-STAT1 axis.[114] Hu et al also reported that loss of FTO resulted in the transformation of macrophages into M1-type pro-inflammatory macrophages.[71] These findings imply that METTL3 inhibitor or FTO agonist may be potential anti-inflammatory targets. Vascular smooth muscle cells (VSMCs) are the predominant cell type in the arterial wall, and the abnormal proliferation and migration of VSMCs leads to intimal hyperplasia, resulting in arterial restenosis and increasing the risk of atherosclerosis.[115] Zhu et al showed that epigenetic modifications in VSMCs such as m6A played a critical role in atherosclerotic lesion restenosis.[116] The expression of m6A writer WTAP is reduced in balloon catheter-injured rat carotid artery.[116] Total Panax notoginseng saponin upregulates the reduced WTAP-p16 signaling to repress intimal hyperplasia,[116] which provides a concept that we can use the existing medicines or novel inhibitors to regulate m6A modification to treat some diseases such as CVD.

Vascular calcification is characterized by increased stiffness of vascular wall and decreased compliance due to ectopic deposits of calcium phosphate, and has been proved to increase cardiovascular events and mortality.[117] Recent evidence indicates that the global m6A methylation is increased in calcific arteries and in human artery smooth muscle cells induced by indoxyl sulfate, and METTL14 is increased in these settings.[110] Overexpression of METTL14 by adenovirus increases osteoblast conversion of smooth muscle cells.[110] Similarly, METTL3 also plays a positive role in promoting osteogenic differentiation of human aortic valve interstitial cells via targeting twist-related protein 1 (TWIST1) in a YTHDF2-dependent manner.[118] These results provide novel mechanistic insights into osteogenic differentiation and the onset of vascular calcification, further researches are needed to validate the diagnostics and therapeutics value of m6A in vascular calcification.

Myocardial infarction and ischemia-reperfusion injury

Myocardial infarction (MI) is myocardial necrosis caused by acute and persistent ischemia and hypoxia of coronary artery. The enzymatic activity of FTO may be attenuated under hypoxic or ischemic conditions such as MI,[16] because it is a dioxygenase that oxidatively demethylates m6A-containing mRNAs.[29] Not only that, but the expression of FTO was decreased in failing heart due to MI both in human and mice.[16] So, increased m6A modification levels have been observed in mice after 4 weeks of MI.[16] Overexpression of FTO by AAV9 exerts various cardioprotective function in mice after MI. It not only improves cardiac contractile mechanics by targeting Ca^{2+}-ATPase pump SERCA2a, but also induces angiogenesis in the ischemic conditions.[16] It is known that angiogenesis is crucial to the recovery of heart function after MI.[119] The cardiac remodeling such as fibrosis after MI will worsen the heart function and lead to heart failure. FTO is reported to significantly reduce fibrosis and scar area in mouse models of MI.[16] A study investigating about 185,000 CAD patients and controls, and found that 304 out of 4390 m6A-SNPs were associated with CAD.[120]

The recovery of blood flow after myocardial ischemia can cause additional damage to cardiomyocytes through induction of oxidative stress and release of oxidative free radicals.[121] The resulting myocardial ischemia-reperfusion (IR) injury can lead to MI and heart failure. Song et al reported that m6A mRNA methylation was increased in hypoxia/reoxygenation (H/R)-treated cardiomyocytes and IR-treated mice.[122] Silencing METTL3 can attenuate IR injury by enhancing autophagic flux and inhibiting apoptosis in H/R-treated cardiomyocytes.[122] While ALKBH5 has an opposite effect during myocardial IR.[122] The lncRNA MALAT1 induces inflammation response through regulating PTGS2 by targeting miR-26b in myocardial IR injury,[123] so Yang et al assumed that m6A modification to MALAT1 may be essential for myocardial IR injury, and may act as a potential therapeutic target.[124] Saxena et al put forward a hypothesis that optimizing cardiac ischemic preconditioning by regulating m6A modification levels of cardioprotective mRNAs (such as eNOS, SOD, and HO-1) may result in rendering ischemic cardiac preconditioning more robust and reducing infarct size.[125] However, these need to be demonstrated by experimental evidence.

Cardiac hypertrophy and heart failure

Heart failure is the deadly end stage of various CVDs, commonly caused by myocardial infarction, hypertension, degenerative valve disease and dilated cardiomyopathy.[126] The main pathologic basis of heart failure is pathologic hypertrophy of the myocardium and increased fibrotic scar tissue, and blocking the myocardial remodeling is the key to the treatment of heart failure. Recently, m6A mRNA methylation has been reported to be involved in mediating these structural changes in the failing heart.

The global level of m6A modification is increased in isolated primary cardiomyocytes responded to hypertrophic stimulation and hypertrophic myocardium, by the evidence from m6A methylation RNA immunoprecipitation followed by next-generation sequencing.[17,127] Genes involves in regulating kinases and intracellular signaling pathways are enriched by m6A analysis.[17] Inhibition of the m6A RNA methylase METTL3 blocks the ability of cardiomyocytes to undergo hypertrophy when stimulated to grow, while increased expression of the METTL3 is sufficient to promote cardiomyocyte hypertrophy both *in vitro* and *in vivo*.[17] Upregulated m6A methylation leads to compensated cardiac hypertrophy while decreased m6A drives eccentric cardiomyocyte remodeling and dysfunction, highlighting the critical significance of this novel epitranscriptomic mechanism and its potential therapeutic target in cardiac hypertrophy.[17]

The m6A methylation is also increased in heart failure.[16,127] And the decreased FTO mainly contributes to the increased m6A in human and mouse failing hearts post MI, because the loss of FTO is continuous in the development of heart failure.[16] FTO-deficient mice by cardiomyocyte restricted knockout or AAV9 are showed impaired cardiac function under stress stimuli,[16,127] while overexpression of FTO improves the heart function and postpones the development of heart failure.[16] It is also reported that patients with the FTO TT genotype (SNP rs17817449) exhibits a significantly increased risk for organ rejection when undergoing heart transplantation due to end-stage heart failure.[128] FTO demethylates cardiac contractile genes such as *SERCA2A, MYH6/7, RYR2* in a m6A dependent manner, and increases their protein expression to enhance cardiac contraction.[16] Moreover, overexpression of FTO is capable of reversing cardiac fibrosis and inducing angiogenesis in the heart failure post MI.[16] In summary, m6A is considered to be critical in the development of heart failure,[129] and more researches are need to demonstrate its therapeutic effect.

Future perspective

In recent years, due to the rapid development of highly specific antibodies of m6A and the high-throughput sequencing technologies, the m6A RNA methylation research has made great progress in disclosing the potential mechanism of the onset and development of CVD. However, there are many essential issues that need to be addressed.

It is noted that FTO was reported to enhance cardiac constriction in heart failure after MI,[16] whereas Kruger et al revealed that loss of endothelial FTO prevented obesity-induced vascular dysfunction.[70] And it cannot be ignored that FTO is initially found as an obesity-related gene, and plays a role in promoting adipogenesis as a demethylase. These results indicate that the role of FTO in CVD is different in different cell types, different issues, and different pathological conditions. Thus, the clinical translation of FTO is likely to require to design tissue-specific or cell-specific agonist or inhibitor.

Song et al[122] and Ruan et al[123] have investigated the interaction of autophagy and lncRNA with m6A, respectively. However, the connection of m6A with many regulatory mechanisms related to CVD remains unknown, such as DNA methylation, histone deacetylation, or non-coding RNA. That suggests the mechanism of m6A regulates the biological process needs to be identified.

More profound experimental, translational and clinical research evidence is needed to validate the m6A methylation as the diagnostic biomarkers and therapeutic targets for CVD. In the oncology field, m6A has been identified to be related to circulating tumor cells, a biomarker for monitoring and preventing the development of metastatic diseases. Thus, the association of m6A with traditional biomarkers of CVD such as myocardial enzyme in MI, pro-BNP in heart failure, is needed to be assessed.

Conclusion

In summary, a better understanding of the modifications regulated by m6A methylation under different pathologic conditions is valuable in the exploration of novel biomarkers and therapeutic targets for CVDs. And more profound experimental, translational and clinical research are needed to map the complete picture of m6A modification in CVDs.

Conflict of Interests

The authors have no conflicts to declare.

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