Published By Guangdong Pharmaceutical University, Guangzhou

Journal of Holistic Integrative Pharmacy

2022, 3(4):392-408

REVIEWS

Molecular mechanisms of RNA m^{6}A-modifying enzymes in cardiovascular diseases

GAO Xiangyu[1], XU Mingyi[1], LIU Jiaming[1], XIAO Mingzhu[1,2*]

[1]*School of Traditional Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou 510006, China;*

[2]*Key Laboratory of Pharmaceutical Bioactive Substances, Guangdong Pharmaceutical University, Guangzhou 510006, China*

[Abstract] Cardiovascular diseases are the leading causes of death globally. Their pathological mechanisms are complex and have not yet been fully clarified. With the development of epigenetics research in recent years, the epigenetic modification N^6-methyladenosine (m^{6}A) has been demonstrated to play an important role in the occurrence and development of cardiovascular diseases. Moreover, m^{6}A methyltransferases, demethylases, and recognition proteins can regulate m^{6}A methylation levels in the RNA, affecting various biological processes such as RNA splicing, nuclear export, protein translation, and degradation. In this paper, we focus on the biological functions of m^{6}A and investigate its role in the biological processes of apoptosis, inflammation, oxidative stress, energy metabolism, and lipid metabolism. Furthermore, we describe the current findings on the mechanisms involving m^{6}A in cardiovascular diseases and discuss potential drugs targeting m^{6}A for therapy.

[Key words] m^{6}A; Cardiovascular diseases; Inflammation; Apoptosis; Targeted drug

1 Introduction

Cardiovascular diseases (CVDs) are the leading causes of mortality worldwide, accounting for one-third of current global deaths[1]. Cardiovascular diseases are characterized by morphological and functional abnormalities in the heart and vasculature, with major CVDs including heart failure, myocardial infarction, hypertension, coronary artery disease, and arrhythmia[2-3]. Pathological processes implicated in these diseases include oxidative stress, inflammation, and mitochondrial dysfunction[4]. Although some of the mechanisms of cardiovascular diseases have been elucidated[5-7], much more remains to be explored.

With the development of epigenetics and deep sequencing in recent years, it has been found that N^6-methyladenosine (m^{6}A) modification of RNA plays an important role in the occurrence and development of cardiovascular diseases[8-10]. m^{6}A is an important RNA epigenetic modification that has been validated in over 10 000 m^{6}A peaks in 25% of human transcripts. m^{6}A modifications primarily occur in the RRACH (R=A or G; H=A, U or C) consensus sequence, which is enriched in long exons near the termination codon and 3'-untranslated region (3'-UTR)[11].

The introduction of m^{6}A modifications is

[*Corresponding authors] E-mail:618xiaomingzhu@gdpu.edu.cn.

catalyzed by m^{6}A methyltransferases, including methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14), and Wilms' tumor 1-associating protein (WTAP). In the METTL3-METTL14 methyltransferase domain complex, METTL3 acts as the catalytic subunit, while METTL14 provides an RNA binding scaffold that activates and enhances the catalytic activity of METTL3. Although WTAP lacks a methyltransferase domain, it interacts with METTL3 and METTL14 to regulate m^{6}A levels of RNA transcription[11]. The m^{6}A writer complex, consisting of WTAP, METTL3, and METTL14, was demonstrated to be localized primarily in the nucleus[12]. Dorn et al. found that METTL3-mediated m^{6}A modification of RNA controlled cardiac homeostasis and promoted myocardial hypertrophy[13]. In addition, He et al. found that METTLE14 protein expression and increased m^{6}A levels are associated with the progression of abdominal aortic aneurysm (AAA)[14]. The discovery of m^{6}A demethylases, such as fat mass and obesity-associated protein (FTO) and AlkB homologous protein 5 (ALKBH5), indicates that the modification is reversible and can be eliminated[15]. Mathiyalagan et al. confirmed that the decrease in FTO expression and increase in messenger RNA (mRNA) m^{6}A levels in the cardiac tissue of patients with heart failure led to cardiac dysfunction[16]. m^{6}A-binding protein can specifically recognize these mRNA m^{6}A modifications[17] to control almost every stage of mRNA metabolism directly or indirectly, including splicing, nuclear export, translation, and decay[18] (Fig. 1). Kmietczyk et al. showed that m^{6}A can regulate translation efficiency by increasing or decreasing the mRNA stability of cardiac-specific genes[19]. In addition, m^{6}A can upregulate the expression of specific long non-coding RNA (lncRNA)[20]. Although there is relatively little research on m^{6}A in the field of cardiovascular diseases at present, its role in cardiovascular diseases cannot be ignored.

In this article, we summarize the known molecular mechanisms involving m^{6}A methyltransferase, demethylase, and m^{6}A-binding protein in different cardiovascular diseases and their relationship

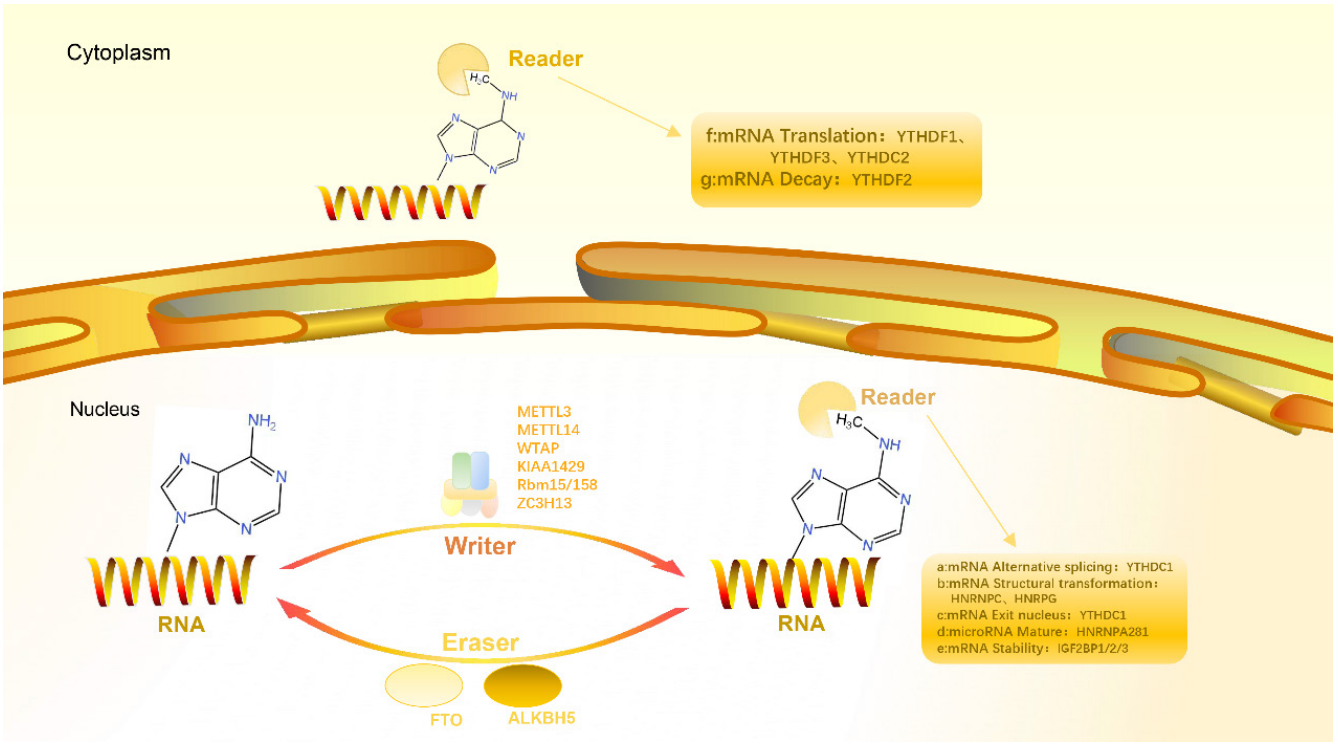

Fig. 1 m^{6}A mechanism of action.

with cardiovascular diseases. We further analyze and discuss the potential role of m^6A level as a biomarker in diagnosing CVDs and therapeutic drugs targeting m^6A metabolism, which can modulate m^6A levels for CVD treatment.

m^6A is an RNA modification "written" by methyltransferase complex and "erased" by demethylases. KIAA1429 protein, zinc finger CCCH-type containing 13 (ZC3H13 protein), methyltransferase-like 16 (METTL16), and RNA binding motif protein 15/15b (RBM15/15b protein), and other cofactor proteins can help enhance the activity and specificity of the methyltransferase complex. Different m^6A-binding proteins recognize the m^6A site and perform various biological functions. These include YTH domain-containing protein 1/2 (YTHDC 1/2), YT521-B homologous domain family protein 1/2/3 (domain family-containing YT521b homologs 1/2/3, YTHDF 1/2/3), heterogeneous nuclear ribonucleoprotein C (HNRNPC), heterogeneous nuclear ribonucleoprotein G (HNRNPG), heterogeneous nuclear ribonucleoprotein A2/B1 (HNRNPA2B1), and insulin-like growth factor 2 mRNA-binding protein 1/2/3 (IGF 2BP1/2/3).

2 The relationship between m^6A methyltransferases and cardiovascular diseases

Methyltransferases, also known as writers, are important enzymes that introduce the m^6A modification on the adenine moieties in mRNA. Writers include METTL3, METTL14, WTAP, and KIAA1492. These proteins do not act in isolation; rather, they form a complex to perform the catalytic functions in a concerted fashion[21]. In the process of m^6A modification, the catalytic activity of the complex requires the methyltransferase domains of METTL3 and METTL14. Several analyses have found that METTL3 plays an important role in cardiovascular phenomena, such as atherosclerosis, cardiovascular response to stress, and myocardial ischemia-reperfusion injury.

2.1 The relationship between METTL3 and cardiovascular diseases

METTL3 is the core methyltransferase that catalyzes the methylation of adenosine in mRNA[11,17]. It is a member of the putative *S*-adenosyl-L-methionine (SAM)-dependent methyltransferase family, which is highly conserved in mammals. The purified METTL3 protein selectively methylates GAC and AAC sequences in single-stranded RNA *in vitro*. Deletion of the METTL3 gene can lead to the complete or near complete loss of m^6A.

2.1.1 METTL3-mediated m^6A modification is closely related to the cardiac stress response

Dynamic regulation of m^6A maintains cardiac homeostasis. The research of Dorn et al. confirmed that METTL3 mediates the methylation of specific mRNA m^6A in kinases and intracellular signaling pathways, such as the nuclear factor kappa-B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways. In response to stress, METTL3 and m^6A are increased, resulting in compensatory myocardial hypertrophy. Inhibition of METTL3 expression and reduction of m^6A can lead to myocardial remodeling and cardiac dysfunction after stress[13]. The research of Kmetczyk et al. found that m^6A can enhance or decrease the stability of specific mRNA in the heart and regulate translation. Increasing METTL3 and m^6A levels inhibited Rho guanine nucleotide exchange factor 3 (Arhgef3 or Xpln) and promoted the expression of myosin light chain 2 (MYL2). Khanna et al. confirmed that Arhgef3 can interact with the mammalian target of rapamycin (mTOR). Inhibition of Arhgef3 expression level can increase the activity of mTOR complex 2 (mTORC2), which further mediates survival in stressed cardiomyocytes. Sheikh et al. found that MYL2 is a sarcomere protein belonging to EF-hand calcium-binding protein superfamily and plays an important role in myocardial contraction[19,22-24].

2.1.2 METTL3-mediated m^{6}A modification plays an important role in myocardial ischemia-reperfusion injury

Myocardial ischemia-reperfusion injury (MIRI) refers to the phenomenon of myocardial cells being further damaged when blood flow is restored following myocardial ischemia. Excess reactive oxygen species (ROS) produced by ischemia/reperfusion (I/R) have been proposed to cause cellular damage through autophagy, necrosis, and apoptosis[25]. Song et al. confirmed that the regulatory action of METTL3 on autophagic flux depends on the two m^{6}A residues in the 3'-UTR of transcription factor EB (TFEB). This promotes the binding of heterogeneous nuclear ribonucleoprotein D (HNRNPD) to TFEB pre-mRNA, reducing TFEB expression and ultimately inhibiting autophagy. Reduction in m^{6}A levels by silencing METTL3 can enhance autophagy, resulting in the inhibition of apoptosis in cardiomyocytes after hypoxia/reoxygenation (H/R) treatment[26]. In addition, Liu et al. confirmed that METTL3-mediated methylation of signal transducer and activator of transcription 1 (STAT1) mRNA in mouse macrophages significantly enhanced its stability, thus upregulating STAT1 protein levels to promote the polarization of M1 macrophages[27]. This polarization-mediated inflammatory response by M1 macrophages plays an important role in myocardial ischemia-reperfusion injury[28].

2.1.3 The potential relationship between METTL3-mediated m^{6}A modification and atherosclerosis

Atherosclerosis is a complex systemic disease. Elevated plasma low-density lipoprotein cholesterol (LDL-C) levels can lead to endothelial damage and infiltration of blood vessels by monocytes, which mature into local macrophages. Macrophages transform into foam cells through lipid intake, resulting in fatty streaks. Chronic inflammation triggers lymphocytes to secrete cytokines and promotes smooth muscle cell migration, proliferation, and collagen formation, resulting in the fibrous cap and finally forming atherosclerotic plaques[29]. Zhao et al. confirmed that oxidized low-density lipoprotein (ox-LDL) induced the expression of the DEAD box protein DDX5 in human peripheral blood-derived macrophages. DDX5 interacts with METTL3, thereby reducing mRNA m^{6}A methylation of macrophage scavenger receptor A (MSR1), stabilizing MSR1 mRNA, and finally promoting lipid uptake in macrophages[30].

In summary, previous data suggest that METTL3 can potentially regulate the development and occurrence of MIRI, cardiac stress, and atherosclerosis through m^{6}A methylation. In addition to METTL3, METTL14, another component of the m^{6}A methyltransferase complex, has been linked to cardiovascular diseases.

2.2 The Relationship between METTL14 and cardiovascular diseases

2.2.1 METTL14-mediated m^{6}A modification is closely related to atherosclerosis

Park et al. found that mono-2-ethylhexyl phthalate (MEHP) can reduce mRNA m^{6}A methylation of scavenger receptor class B type 1 (Sr-B1) mRNA by inhibiting the expression of METTL14 in mouse Raw264.7 macrophage cells. Reduced Sr-B1 expression decreases cholesterol efflux and promotes atherosclerosis[31]. METTL14 can also regulate vascular endothelial function. Chen et al. confirmed that METTL14 was upregulated in calcified arteries, which resulted in an abnormal increase in total RNA m^{6}A level and selective osteoblast-related transcript m^{6}A level, reducing its protein expression. Increasing the expression of METTL14 leads to the loss of repair function in primary human aortic smooth muscle cells (HASMC). In contrast, METTL14 silencing decreased indolyl sulfate-induced HASMC calcification and enhanced vascular repair function[32].

2.2.2 METTL14-mediated m^{6}A modification may be involved in heart failure

METTL14 expression is increased in heart failure, suggesting its role in heart failure pathogenesis[16]. Xu et al. confirmed that METTL14 reduced the mRNA and protein levels of Yes1 associated transcriptional regulator (YAP1) through mRNA m^{6}A methylation. This inhibits the YAP1-TEA domain protein (YAP1-TEAD) pathway and promotes renal ischemia-reperfusion injury (IRI). Furthermore, YAP1 regulates kidney regeneration and fibrosis in the recovery after acute IRI. YAP1 also has a protective effect on myocardial ischemia or injury[33]. These studies suggest that METTL14 may play an important role in atherosclerosis and heart failure.

2.3 The relationship between METTL3-METTL14 complex and cardiovascular diseases

METTL3 and METTL14 are highly conserved in mammals and form a stable core complex of METTL3–METTL14 heterodimers, which plays a role in the m^{6}A modification of mammalian nuclear RNA. The methyltransferase activity of either METTL3 or METTL14 alone is weak *in vitro*, while the METTL3–METTL14 complex has a stronger catalytic activity[11]. In addition, the METTL3–METTL14 complex and the recently discovered m^{6}A RNA demethylases can dynamically regulate m^{6}A levels in the mRNA and other nuclear RNA through their opposing enzymatic functions[34].

2.4 The relationship between WTAP and cardiovascular diseases

Studies in arabidopsis and yeast show that the mammalian pre-mRNA splicing regulatory protein WTAP[35] is involved in RNA methylation[36-37]. WTAP is a nuclear protein related to cell proliferation and apoptosis regulation. Additionally, WTAP is a regulatory subunit of RNA m^{6}A methyltransferase and can directly introduce m^{6}A in RNA[38].

Wang et al. found that the expression of WTAP in arteriovenous malformations (AVM) is decreased, leading to a decrease in m^{6}A methylation and expression of desmoplakin (DSP) mRNA in endothelial cells. On the other hand, it enhances the activity of Wilm's tumor 1 (WT1) protein and inhibits the Wnt signaling pathway, inhibiting endothelial angiogenesis[39]. Intima hyperplasia caused by the proliferation and migration of vascular smooth muscle cells (VSMC) is the key event in arterial restenosis[40]. Zhu et al. found that total *Panax notoginseng* saponin (TPNS) inhibited VSMC proliferation and migration by activating WTAP/p16 signaling axis and modifying RNA m^{6}A. This results in the inhibition of intimal hyperplasia after carotid balloon catheter injury in rats.

These results suggest that WTAP can potentially regulate cerebral arteriovenous malformation, endothelial cell angiogenesis, and intimal hyperplasia. However, the role of WTAP in cardiovascular diseases has yet to be discovered.

2.5 The relationship between WTAP and METTL3-14 complex and cardiovascular diseases

WTAP is a mammalian splicing factor with no methyltransferase activity, but it can interact with METTL3 and METTL14 by mediating their localization in the nucleus. This affects the activity of m^{6}A methyltransferase *in vivo* and, consequently, m^{6}A levels. Furthermore, it was found that an increase in METTL3 expression level, acting together with METTL14, WTAP, or other regulatory subunits, may lead to cardiomyocyte remodeling[34].

3 The relationship between m^{6}A demethylase and cardiovascular diseases

The regulation of m^{6}A depends on the activity of demethylases, primarily FTO and ALKBH5[41]. FTO (or ALKBH9) is the first demethylase to

be discovered, providing evidence that the m^{6}A modification is reversible and dynamic. FTO is mainly located in the nucleus, where it mediates about 5%–10% of mRNA demethylation. In addition, FTO is also abundant in the cytoplasm of some leukemia cells and can demethylate up to 40% of mRNA. FTO protein, a member of the AlkB protein family, is associated with obesity. Knockout or overexpression of FTO significantly changes the body weight in mice. ALKBH5 is another important enzyme that can demethylate mRNA in the nucleus. After knocking down ALKBH5 in a cell line, mRNA m^{6}A levels increased significantly. Thus, m^{6}A demethylases may play important roles in cardiovascular diseases, such as myocardial infarction, myocardial hypertrophy, atherosclerosis, and hypertension.

3.1 the relationship between FTO and cardiovascular diseases

FTO, located on chromosome 16 (16q12.2), was first reported as the demethylase of N3-methylthymidine in single-stranded DNA and N3-methyluridine in single-stranded RNA. *In vivo*, FTO demethylates m^{6}A in both DNA and RNA. The loss of FTO leads to a significant increase in the total amount of m^{6}A in RNA. FTO dysfunction is directly related to the development of many diseases, including obesity, cardiovascular disease, brain malformation, and tumors[11,17].

3.1.1 FTO-mediated m^{6}A demethylation plays an important role in myocardial infarction

Myocardial infarction is defined as the death of myocardial cells due to ischemic injury[42]. Chapman et al. defined the long-term prognosis of patients with type 2 myocardial infarction and myocardial injury and discussed risk stratification in 2017[43]. On this basis, Lindsey et al. proposed an animal model of myocardial ischemia and infarction in 2018[44]. Wang et al. confirmed in 2017 that FTO-mediated m^{6}A demethylation can provide energy for myoblast differentiation by activating mitochondrial mTOR-PPAR-γ coactivator-α (mTOR-PGC-1α)[45]. FTO in rat brain neurons can decrease the mRNA m^{6}A level of BCL-2 and increase its expression, thus inhibiting apoptosis caused by ischemia-reperfusion injury[46]. Mathiyalagan et al. demonstrated in 2019 the functional importance of FTO-dependent m^{6}A group during myocardial contraction in heart failure. This study confirmed that the decrease in FTO expression and increase in RNA m^{6}A levels in infarcted and hypoxic cardiomyocytes in failing mammalian hearts resulted in abnormal calcium and sarcomere dynamics, thus reducing the contractile function of myocardial cells. The expression of FTO in the infarcted mouse heart can attenuate the increase in RNA m^{6}A modification post-ischemia, thus alleviating ischemia-induced myocardial remodeling and fibrosis and enhancing myocardial contractile function and angiogenesis. FTO selectively demethylates the myocardial contraction transcripts *Serca2a* and *Ryr2* to prevent their degradation after ischemia and enhance protein expression. In addition, FTO also regulates energy metabolism and apoptosis in other cells, suggesting that FTO expression has other potential roles in myocardial infarction[16].

3.1.2 FTO plays a role in myocardial hypertrophy

Cardiac hypertrophy is an adaptive response of the heart caused by various pathological stimuli. It is an independent risk factor for heart failure and sudden cardiac death[47]. The onset of cardiac hypertrophy is complex, involving the complex interaction of various cellular signaling pathways. In 2013, Gan et al. first demonstrated leptin-induced FTO upregulation in cardiac myocytes through JAK2/STAT3-dependent upregulation of CUX1[48]. Carnevali et al. found that global knockout of the FTO gene in mice leads to sympathetic overactivation of cardiac function and proarrhythmic remodeling of the heart[49].

Qian et al. found that m^{6}A modification inhibited cardiac development and hypertrophy through the action of specific miRNA[50]. In the same year, Ju et al. determined the m^{6}A RNA modification profile in a diabetic cardiomyopathy (DCM) mouse model and found that the DCM m^{6}A profile is related to myocardial fibrosis, myocardial hypertrophy, and myocardial energy metabolism. Moreover, total m^{6}A levels in DCM were higher, while FTO expression was downregulated. FTO overexpression in this model increased cardiac function by reducing myocardial fibrosis and myocardial hypertrophy[51-52].

Pathological myocardial hypertrophy refers to cardiac remodeling, which results from changes in gene expression and post-transcriptional modification[53]. Mathiyalagan et al. found that the expression of FTO is decreased in mammalian cardiomyocytes during heart failure and hypoxic conditions, thus increasing m^{6}A in the RNA and decreasing the contractile function of cardiomyocytes. Rescuing the expression of FTO in the failing hearts of mice can ameliorate these ischemia-induced cardiac remodeling effects. These suggest that FTO-dependent m^{6}A plays an important role in cardiac contraction, remodeling, and repair in heart failure [54].

3.1.3 FTO-mediated m^{6}A demethylation may potentially regulate the occurrence and development of atherosclerosis

Wang et al. found in 2015 that FTO in pig adipocytes promoted adipogenesis by reducing RNA m^{6}A levels[55]. Wu et al. found in 2017 that AMPK can reduce the expression of FTO and increase mRNA m^{6}A, resulting in reduced lipid accumulation in skeletal muscle cells[56]. In the same year, Chunfen et al. found that upregulation of FTO can significantly reduce the accumulation of cholesteryl ester in ox-LDL-loaded macrophages, while downregulation of FTO can reverse this effect. It is suggested that the expression of FTO can prevent the formation of atherosclerotic plaque and significantly reduce the total plasma cholesterol and LDL-C levels[52].

Wu et al. found in 2018 that FTO in adipocytes decreased m^{6}A levels in cyclin A2 (CCNA2) and cyclin-dependent kinase 2 (CDK2) mRNA, thereby inhibiting CCNA2 and CDK2 mRNA degradation by YT521-B homologous domain family protein 2 (YTHDF2). This increased the expression of CCNA2 and CDK2 and promoted cell cycle progression and adipogenesis[57].

In 2020, Gu, X. et al. found that m^{6}A demethylase FTO gene expression in mouse bone marrow-derived macrophages was downregulated, while the polarization of M1 and M2 macrophages was inhibited simultaneously. After FTO knockout, the increase of RNA m^{6}A levels led to a decrease in STAT1 and peroxisome proliferator-activated receptor-γ (PPAR-γ) mRNA stability and expression[58]. Zhou Yang et al. proposed in 2021 that m^{6}A demethylation by FTO could inhibit lipid intake in macrophages by downregulating PPARγ protein expression and accelerating cholesterol outflow by phosphorylating AMPK[59]. These studies suggest that FTO may play an active role in hindering macrophage activation and foam cell formation in the pathogenesis of atherosclerosis.

3.1.4 FTO-mediated m^{6}A demethylation may also be closely related to the occurrence of hypertension

Hypertension refers to the clinical syndrome in which arterial blood pressure is higher than the normal range, often accompanied by pathological changes in vital organs such as the heart and blood vessels[47]. Krüger et al. found that FTO-mediated m^{6}A demethylation plays a key role in regulating arterial myogenic contraction and vascular resistance. Prostaglandin D_2 (PGD_2) activates G protein-coupled receptors expressed on vascular smooth muscle membrane, promotes the relaxation of vascular smooth muscle (VSMCs), and delays

the progress of hypertension. Lipoprotein-type prostaglandin D synthase (L-PGDS) is the main enzyme for synthesizing PGD_2. In human and mouse blood vessels, FTO inhibited the expression of L-PGDS and blocked the synthesis of PGD_2 in an m^6A-dependent manner, which further increased vascular resistance and promoted the development of hypertension[60]. Lastly, the increase in m^6A in cardiomyocytes following a decrease in FTO expression or FTO gene mutation is positively correlated with hypertension and cardiovascular diseases[8].

In summary, these studies indicate that FTO-mediated m^6A demethylation plays an important role in myocardial infarction, myocardial hypertrophy, atherosclerosis, and hypertension.

3.2 The relationship between ALKBH5 and cardiovascular diseases

ALKBH5 is the second m^6A demethylase to be discovered. It can directly remove the methyl group from m^6A rather than through oxidative demethylation, with its demethylation activity equivalent to that of FTO. ALKBH5 is located in the nucleus, where it regulates mRNA export and metabolism of nuclear RNA and proteins. Silencing ALKBH5 increases m^6A levels in total mRNA and cell type-specific mRNA. ALKBH5 not only demethylates m^6A in mRNA but also in non-coding RNA (ncRNA). Therefore, ALKBH5 can have a wide range of biological functions[11,17].

In 2019, Song et al. found that ALKBH5-mediated m^6A demethylation can reduce autophagic flux and apoptosis in cardiomyocytes after H/R treatment. ALKBH5 can reduce m^6A levels of TFEB pre-mRNA, leading to a decrease in its binding with HNRNPD and an increase in TFEB mature mRNA levels to promote autophagy and inhibit apoptosis in cardiomyocytes[26]. Furthermore, the compensatory elevation of ALKBH5 after cerebral IRI in rats inhibits neuronal apoptosis by decreasing the m^6A levels of BCL-2 and increasing its expression[46].

The above studies suggest that ALKBH5 plays an important role in myocardial infarction and MIRI.

4 The relationship between m^6A binding protein and cardiovascular diseases

At present, a class of proteins containing the YTH functional domain has been identified as m^6A-binding proteins. Among them, YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2 have been demonstrated to bind with m^6A-modified RNA and regulate its metabolism. YTHDF1, YTHDF2, and YTHDC1 mainly affect the translation, degradation, and splicing of m^6A-modified transcripts, respectively. HNRNPC is an abundant nuclear RNA binding protein that participates in the processing of pre-mRNA[61]. Studies have shown that HNRNPC regulates the abundance and selective shearing of target transcripts by binding m^6A RNA[62].

YTHDF2 is the most studied m^6A-binding protein and is abundantly expressed in almost all cell types. As an RNA binding protein (RBP), YTHDF2 can regulate the stability of mRNA transcripts of inflammatory genes to regulate the inflammatory process. Yu et al. found that YTHDF2 gene knockout can stabilize mRNA transcription. Moreover, it increased the mRNA expression levels of mitogen-activated protein kinase kinase 4 (MAP2K4) and mitogen-activated protein kinase kinase kinase kinase 4 (MAP4K4) and activated MAPK and NF-κB signaling pathways. These promote the expression of pro-inflammatory cytokines and aggravate the inflammatory response in RAW 264.7 cells stimulated by LPS[63]. Wang et al. confirmed that autophagy-related 5 (Atg5) and autophagy-related 7 (Atg7) are the targets of YTHDF2. FTO silencing in preadipocytes leads to an increase in m^6A levels, which promotes the degradation of YTHDF2-dependent mRNA of Atg5

and Atg7. This reduces their protein expression and inhibits autophagy and adipogenesis[32]. Compared with unmethylated mRNA, the half-lives of mRNA containing m^{6}A are decreased. In YTHDF2 gene knockout cells, the half-life of mRNA increased. Therefore, YTHDF2 can accelerate the degradation of m^{6}A-modified transcripts[11,17].

These results suggest that the m^{6}A-binding protein YTHDF2 may potentially regulate pathological processes such as inflammation and fat accumulation in cardiovascular diseases. However, the role of other m^{6}A-binding proteins in cardiovascular diseases remains to be discovered.

5 Analysis of potential drugs targeting m^{6}A

Recent studies have shown that methyltransferases and demethylases regulate m^{6}A levels and play important roles in the physiological and pathological processes in various cardiovascular diseases (Fig. 2-3 and 4-5). Different reading proteins perform various biological functions and participate in the occurrence and development of cardiovascular diseases by recognizing RNA m^{6}A.

METTL3 is the core protease that catalyzes mRNA modification with m^{6}A and is a member of the large family of methyltransferases, which is highly conserved in mammals. Deletion of the METTLE3 gene can lead to the complete or near complete loss of m^{6}A. The Fig. 2 shows the effect of METTL3 on cardiovascular diseases such as MIRI, cardiac stress, and atherosclerosis. Green lines indicate promotion/activation, while red lines indicate inhibition.

METTL14 is a member of the methyltransferase family and has been linked to cardiovascular diseases. The Fig. 3 shows the effect of METTL14 on moderate cardiovascular diseases such as atherosclerosis and heart failure.

WTAP is a nuclear protein related to cell proliferation and apoptosis and is a regulatory subunit of RNA m^{6}A methyltransferase. In addition, WTAP can also directly modify RNA with m^{6}A. The WTAP can potentially regulate cerebral arteriovenous malformation, endothelial cell angiogenesis, and intimal hyperplasia as shown in Fig. 3. Green lines indicate promotion/activation, while red lines indicate inhibition.

FTO is the first m^{6}A demethylase to be discovered and is located on chromosome 16 (16q12.2). *In vivo*, FTO demethylates m^{6}A in both DNA and RNA. The loss of FTO leads to a significant increase in the total amount of m^{6}A levels in RNA. The Fig. 4 shows the effects of FTO-mediated m^{6}A demethylation on cardiovascular diseases such as myocardial infarction, myocardial

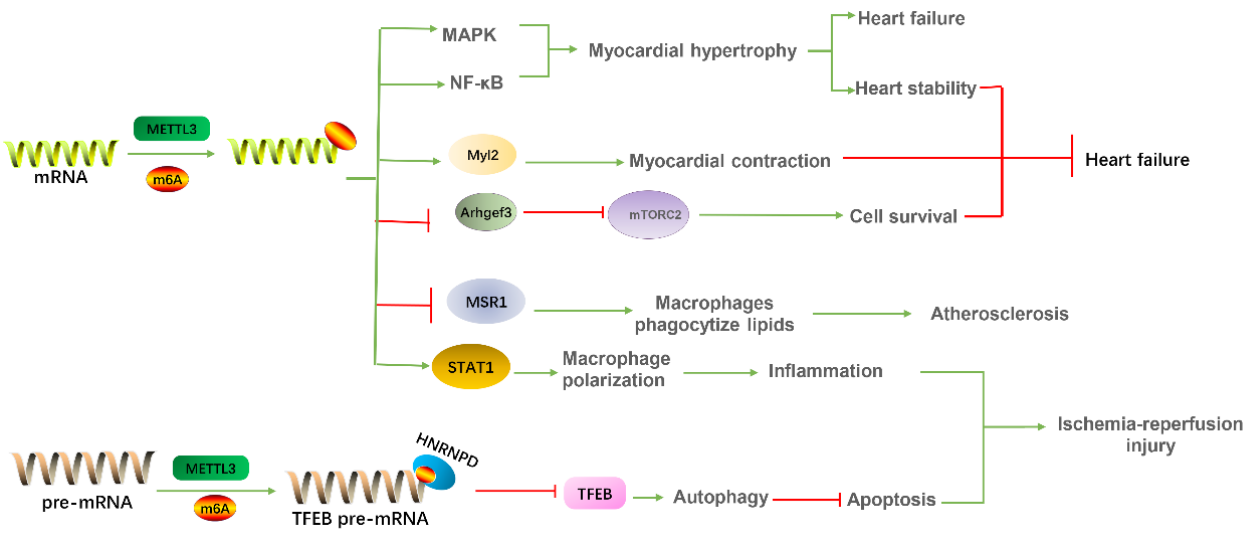

Fig. 2 The role of m^{6}A methyltransferase METTL3 in cardiovascular diseases.

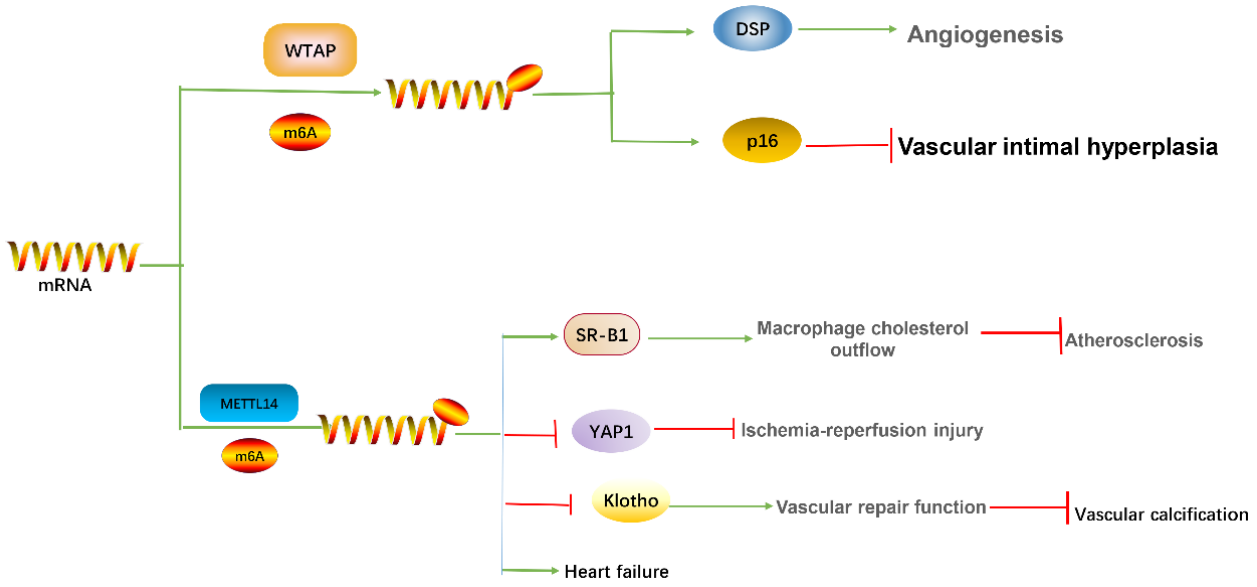

Fig. 3 The role of m^{6}A methyltransferase METTL14 and WTAP in cardiovascular diseases.

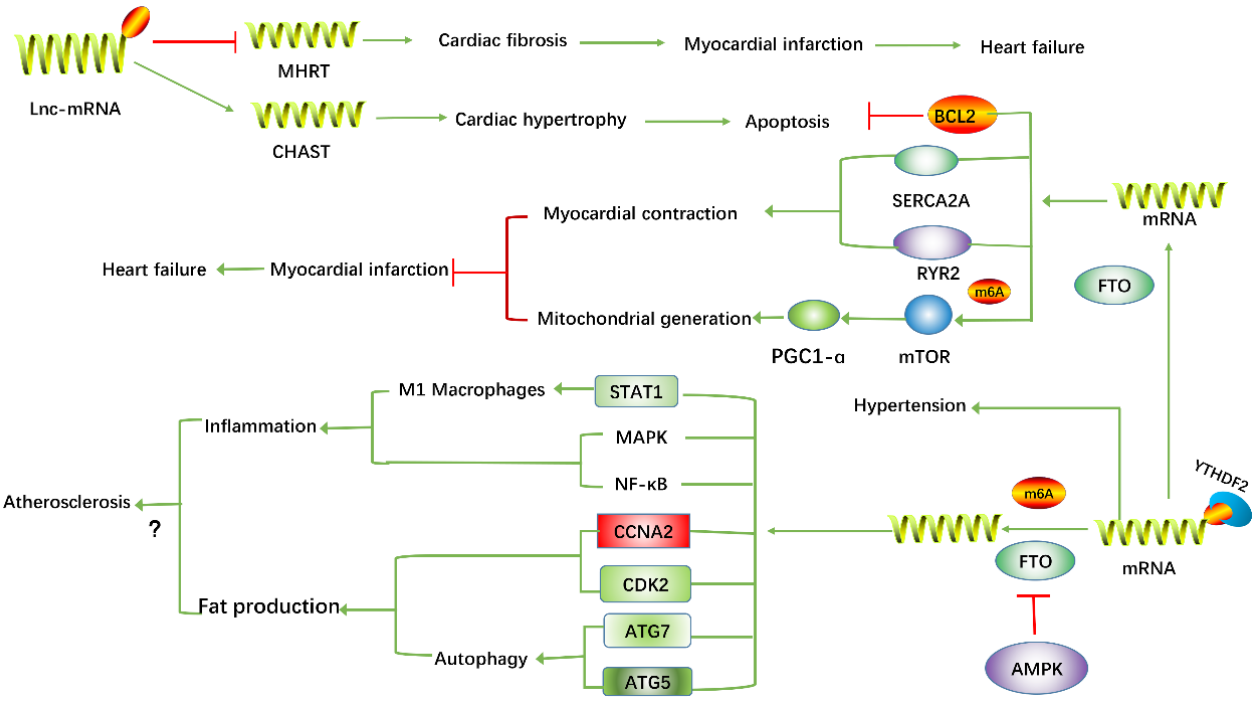

Fig. 4 The role of m^{6}A demethylase FTO in cardiovascular diseases.

hypertrophy, and atherosclerosis. Green lines indicate promotion/activation, while red lines indicate inhibition.

ALKBH5 is the second m^{6}A demethylase to be discovered. ALKBH5 removes the methyl group directly from m^{6}A rather than through oxidative demethylation. Its m^{6}A demethylating activity is comparable to FTO. Silencing of ALKBH5 increased m^{6}A levels in total mRNA and specific transcripts. The Fig. 5 shows the important effects of ALKBH5 on myocardial infarction and MIRI. Green lines indicate promotion/activation, while red lines indicate inhibition.

Increased m^{6}A levels in the heart promote several pathological processes, such as cardiomyocyte hypertrophy, apoptosis, inflammation,

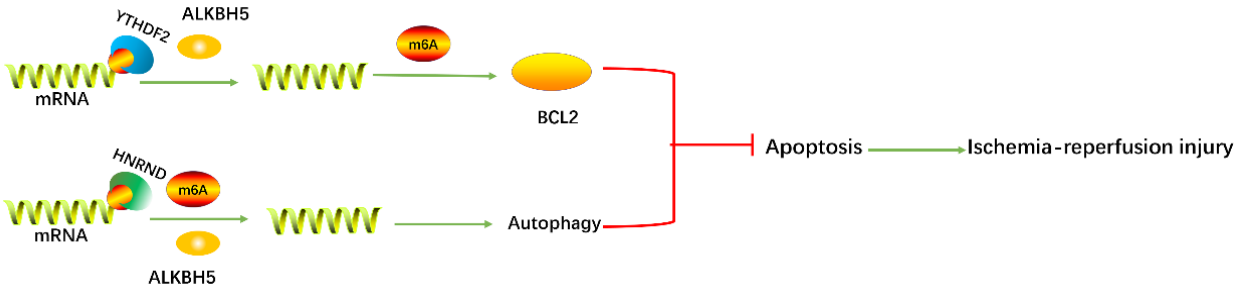

Fig. 5 The role of m^{6}A demethylase ALKBH5 in cardiovascular diseases.

and fibrosis. These contribute to the development of CVDs, including myocardial infarction, MIRI, heart failure, and myocardial hypertrophy. In IRI, METTL3 promotes the binding of HNRNPD to TFEB pre-mRNA by methylating two adenosine residues in TFEB. This reduces the expression level of TFEB, ultimately inhibiting autophagy and increasing apoptosis in cardiomyocytes[26]. METTL3-mediated signal transduction and STAT1 expression were observed in mouse macrophages. mRNA m^{6}A methylation could significantly enhance the stability of STAT1 mRNA, thereby upregulating STAT1 protein expression and promoting M1 macrophage polarization. M1 macrophage polarization-mediated inflammatory response was found to be an important factor causing myocardial ischemia-reperfusion injury[32]. Studies on vascular calcification have pointed out that increased expression of METTL14 leads to loss of repair function in primary HASMC. Silencing METTL14 attenuates indole sulfate-induced calcification of HASMC, resulting in enhanced expression of angioprotective proteins and vascular repair function[20]. Arterial restenosis was associated with WTAP/p16 signaling axis and RNA m^{6}A modification. VSMC proliferation and migration were inhibited with m^{6}A modification, thereby inhibiting intimal hyperplasia after injury to the carotid balloon catheter injury in rats[40]. Cardiomyocyte contractile dysfunction is an important feature of heart failure. Decreased FTO expression increases RNA m^{6}A levels leading to abnormal calcium processing and sarcomere dynamics and reduced cardiomyocyte contractile function. In addition, the expression of METTL14 in heart failure is elevated, suggesting that METTL14 may also be involved in the pathophysiology of heart failure. Both myocardial remodeling and fibrosis in infarcted mouse hearts were shown to be associated with decreased expression of FTO and increased modification of RNA m^{6}A. Increasing the expression of FTO could enhance myocardial contractile function and angiogenesis. FTO selectively demethylates myocardial contractile transcripts *Serca2a* and *Ryr2*, among others, thereby preventing their degradation after ischemia and improving their protein expression[64]. However, the changes in m^{6}A levels in different cells in vascular diseases do not all seem to promote the occurrence of diseases. The decrease in m^{6}A in macrophages can promote lipid uptake and inhibit cholesterol outflow, thus promoting the formation of foam cells and leading to atherosclerosis. In addition, a decrease in m^{6}A levels promotes vascular smooth muscle cell proliferation and migration and fat production and accumulation, all of which contribute to the development of atherosclerosis. Table 1 summarizes the m^{6}A modifications in various cardiovascular pathologies[30]. Changes in m^{6}A levels are expected to become novel biomarkers for cardiovascular disease and function as new diagnostic indices.

6 Analysis of potential drugs targeting m^{6}A

The development of therapies targeting

Table 1 Summary of m^{6}A levels and corresponding target RNAs in cardiovascular pathophysiology

Cardiovascular pathophysiology	Cell category	Methyltransferases / demethylases	Target RNAs	m^{6}A level	[Refs.]
Myocardial ischemia-reperfusion injury	Myocardial cells	METTL3	TFEB	Upregulated	[26]
	Macrophages	METTL3/METTL14	STAT1	Upregulated	[32]
Vascular calcification	Aortic smooth muscle cells	METTL14	Klotho	Upregulated	[20]
Heart failure	Myocardial cells	FTO/METTL14	Serca2a/Ryr2	Upregulated	[64]
Arterial restenosis	Vascular smooth muscle cell	WTAP	p16	Upregulated	[40]
Atherosclerosis	Macrophages	METTL3	DDX5/MSR1	Downregulated	[30]

m^{6}A has great potential. At present, several m^{6}A methylation regulators have been found. Betaine is a methyl donor that can increase m^{6}A levels to reduce body fat and inhibit liver fat production[65]. Chen et al. found reduced m^{6}A levels and increased FTO expression in the liver of adolescent mice fed with a high-fat diet. Betaine supplementation prevented these changes[66]. In addition, betaine has shown promise in the treatment of diabetes, cancer, and Alzheimer's disease. Whether the mechanism of betaine in these diseases is related to m^{6}A has not been proven[67].

Cycloleucine is a methylation inhibitor that can inhibit m^{6}A introduction and increase lipogenesis[68]. Zhang et al. confirmed that cycloleucine negatively regulates the maturation and embryonic development of porcine oocytes by regulating the modification of RNA m^{6}A and histones[69]. Cycloleucine and betaine often serve as positive controls in m^{6}A studies.

Rhein can reversibly bind with FTO and competitively prevent FTO from recognizing m^{6}A substrate[70]. It shows excellent clinical efficacy and is widely used in the treatment of tumors, inflammation, diabetic nephropathy, and viral infections[71]. However, whether the treatment mechanism is related to m^{6}A still needs further verification.

MA, an anti-inflammatory drug, can specifically bind to m^{6}A. This inhibits demethylation by FTO because the enzyme requires binding to the m^{6}A moiety[72]. In a study investigating resistance of non-small cell lung cancer to gefitinib (GE), Chen et al. found that, while downregulating the expression of BCRP and MRP7 in GE-resistant cells, GE co-administration with MA also increased m^{6}A[73].

Epigallocatechin gallate (EGCG), an extract of green tea, can inhibit the protein expression of FTO, thus increasing the total mRNA m^{6}A levels in mouse preadipocytes. EGCG can also decrease the expression of FTO and increase YTHDF2, thus increasing the mRNA m^{6}A levels of CCNA2 and CDK2, promoting their mRNA degradation and protein expression reduction. These inhibit mitotic clonal expansion and fat production[74]. This may be one of the molecular mechanisms of the hypolipidemic effect of EGCG.

Zhu et al. first found that total Panax notoginseng saponins (TPNS) can inhibit intimal hyperplasia after carotid artery injury by activating WTAP-m^{6}A-p16 signaling axis and reversing the decreased mRNA m^{6}A levels after carotid artery injury[40]. The combination of resveratrol and curcumin can reduce m^{6}A level in the mRNA of the tight junction proteins occludin (OCLN), claudin 1 (CLDN1), zonula occludens 1 (ZO-1), and the antioxidant heme oxygenase-1 (HO-1). This results in increased transcript levels and protein expression of these genes, thus inhibiting oxidative stress. The decrease in m^{6}A levels may be due to the decrease in protein expression of METTL3[75]. Increasing the expression of HO-1 can inhibit the oxidative stress of myocardial ischemia-reperfusion injury[76].

The Fig. 6 shows the m^{6}A-related processes targeted by betaine, cycloleucine, and rhein. Green lines indicate promotion/activation, while red lines indicate inhibition.

The Fig. 7 shows the m^{6}A-related processes targeted by Meclofenamic acid (MA), EGCG, and TPNS. Green lines indicate promotion/activation, while red lines indicate inhibition.

7 Summary and prospects

In summary, m^{6}A methyltransferases, demethylases, and m^{6}A-binding proteins play important roles in regulating cardiovascular physiology and pathology, as evidenced by the association of these enzymes and m^{6}A levels with cardiovascular diseases. However, several important questions remain; How do drug molecules regulate m^{6}A levels? Do drugs act upstream of m^{6}A-related

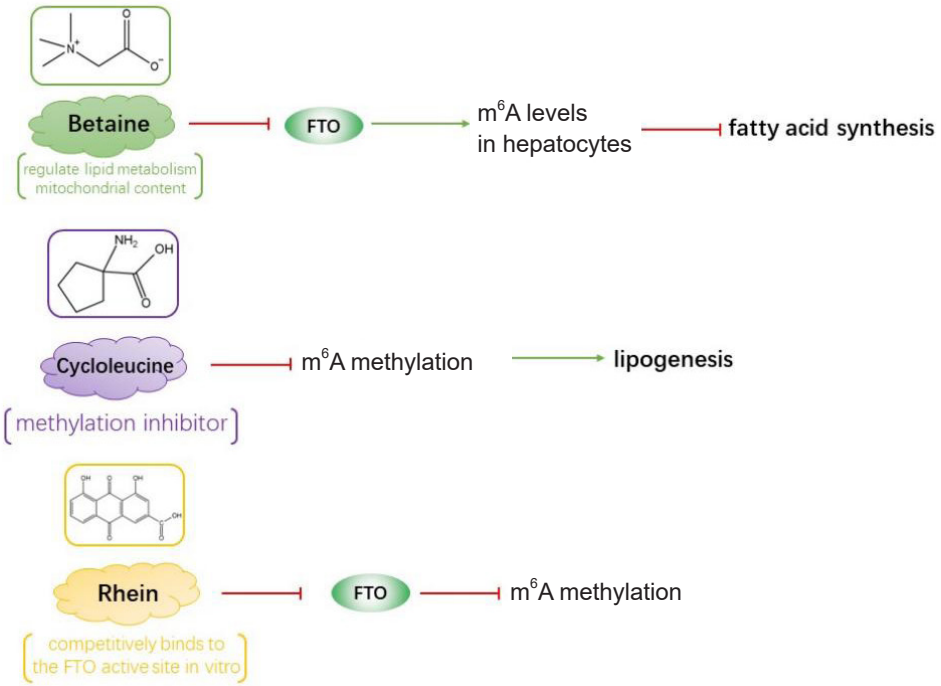

Fig. 6 Analysis of potential drugs targeting m^{6}A: betaine, cycloleucine, rhein.

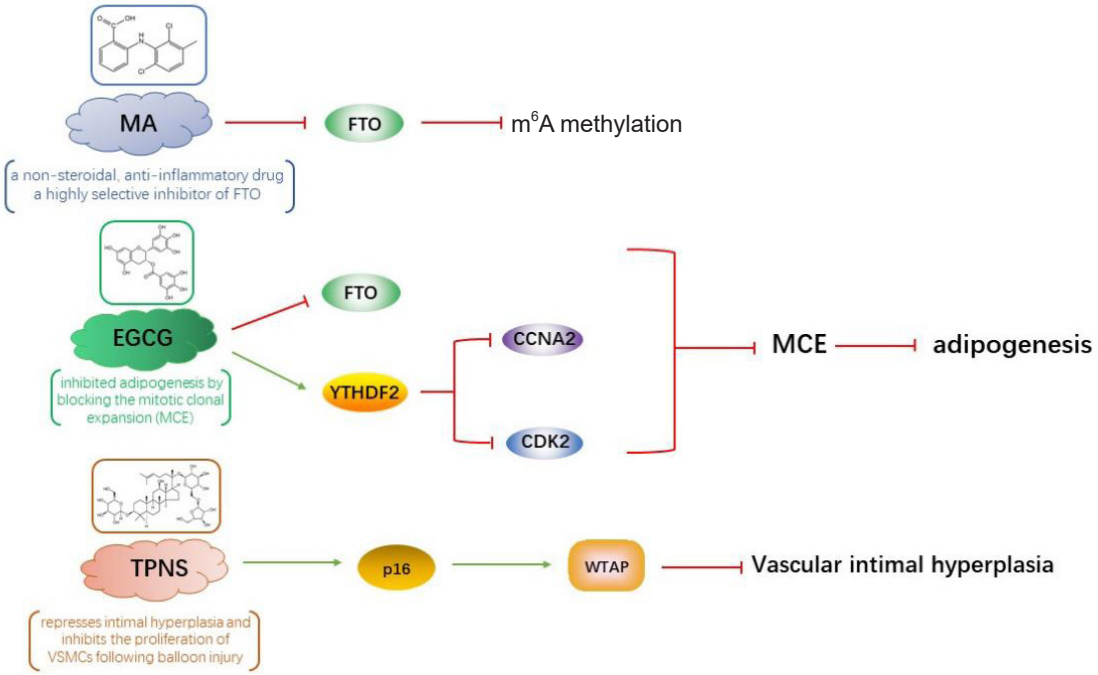

Fig. 7 Analysis of potential drugs targeting m^{6}A: MA, EGCG, TPNS.

enzymes to regulate their expression or directly on m^6A-related enzymes to affect their activity? What are the key chemical structures in drugs that are responsible for their m^6A-regulating bioactivities?

The use of m^6A methylation modification for CVD drug therapy is still in the exploratory stage and needs to be developed further. Therefore, more studies are needed to clarify the mechanisms of action and regulatory networks of m^6A methylation in cardiovascular diseases.

8 Abbreviations

Cardiovascular diseases: CVDs; RNA N6-methyladenosine: m^6A; 3'-untranslated region: 3'-UTR; Methyltransferase-like 3: METTL3; Methyltransferase-like 14: METTLE14; Abdominal aortic aneurysm: AAA; Fat mass and obesity-associated protein: FTO; AlkB homologous protein 5: ALKBH5; Wilms' tumor 1-associating protein: WTAP; Messenger RNA: mRNA; long non-coding-RNA: LncRNA; *S*-Adenosyl-*L*-methionine: SAM; Nuclear factor kappa-b: NF-*κ*B; Mitogen-activated protein kinase: MAPK; Rho guanine nucleotide exchange factor 3: arhgef 3; Myosin light chain 2: MYL2; rapamycin complex 2: mTORC2; Myocardial ischemia-reperfusion injury: MIRI; Reactive oxygen species-ROS: ischemia/reperfusion: I/R: Transcription factor EB: TFEB; heterogeneous nuclear ribonucleoprotein *D*: HNRNPD; hypoxia/reoxygenation: H/R; transcription 1: STAT1; low-density lipoprotein cholesterol: LDL-C; oxidized low-density lipoprotein: ox-LDL; DEAD box protein: DDX5; macrophage scavenger receptor A: MSR1; Mono-2-ethylhexyl phthalate: MEHP; Scavenger receptor class B type 1: Sr-B1; Human aortic smooth muscle cells: HAMCS; Yes 1 Associated Transcriptional Regulator: YAP1; YAP1-TEA domain protein: YAP1-TEAD; ischemia-reperfusion injury: IRI; arteriovenous malformations: AVMS; Desmoplakin: DSP; Wilms' tumor 1: WT1; Panax Noto Ginsenosides: TPNs; chromosome 16: 16q12.2; diabetic cardiomyopathy: DCM; cyclin A2: CCNA2; cyclin-dependent kinase 2: CDK2; homologous domain family protein 2: YTHDF2; Peroxisome proliferator-activated receptor-*γ*: PPAR-*γ*; Prostaglandin D2: PGD2; Lipoprotein-type prostaglandin D synthase: L-PGDS; vascular smooth muscle: VSMCs; non-coding Ribonucleic Acid: ncRNA; RNA binding protein: RPP; Mitogen-activated protein kinase kinase 4: MAP2K4; Autophagy-related 5: Atg5; Autophagy-related 7: Atg7; Meclofenamic acid: MA; Epigallocatechin gallate: EGCG; Total Panax notoginseng saponins: TPNS; Occludin: OCLN, Claudin 1: CLDN1; Zonula Occludens 1: ZO-1; antioxidant Heme oxygenase-1: HO-1.

9 Conflicts of interest

These authors have no conflict of interest to declare.

10 Acknowledgments

This work was supported by the National Natural Science Foundation of China (8217020711); Guangdong Medical Research Fund (A2019446); Guangdong Provincial Science and Technology Department Rural Commissioner Special (No. KTP20200165).

References

[1] Joseph P, Leong D, Mckee M, et al. Reducing the global burden of cardiovascular disease, part 1: The epidemiology and risk factors[J]. *Circ Res*, 2017, 121(6):677-694.

[2] Cheng Y and Rong J. Pro-resolving lipid mediators as therapeutic leads for cardiovascular diseases[J]. *Expert Opin Ther Targets*, 2019, 23(5):423-436.

[3] Mongelli A, Atlante S, Bachetti T, et al. Epigenetic signaling and RNA regulation in cardiovascular diseases[J]. *Int J Mol Sci*, 2020, 21(2):509.

[4] Wang Y, Wang Q, Li C, et al. A review of chinese herbal medicine for the treatment of chronic heart failure[J]. *Curr Pharm Des*, 2017, 23(34):5115-5124.

[5] Kura B, Szeiffova Bacova B, Kalocayova B, et al. Oxidative stress-responsive microRNAs in heart

injury[J]. *Int J Mol Sci*, 2020, 21(1):358.

[6] Liu X, Wang S and Zhao G. Baicalin relieves lipopolysaccharide-evoked inflammatory injury through regulation of miR-21 in H9c2 cells[J]. *Phytother Res*, 2020, 34(5):1134-1141.

[7] Yang M, Linn BS, Zhang Y, et al. Mitophagy and mitochondrial integrity in cardiac ischemia-reperfusion injury[J]. *Biochim Biophys Acta Mol Basis Dis*, 2019, 1865(9):2293-2302.

[8] Paramasivam A, Vijayashree Priyadharsini J and Raghunandhakumar S. N6-adenosine methylation (m^6A): a promising new molecular target in hypertension and cardiovascular diseases[J]. *Hypertens Res*, 2020, 43(2):153-154.

[9] Qin Y, Li L, Luo E, et al. Role of m^6A RNA methylation in cardiovascular disease[J]. *Int J Mol Med*, 2020, 46(6):1958-1972.

[10] Berulava T, Buchholz E, Elerdashvili V, et al. Changes in m^6A RNA methylation contribute to heart failure progression by modulating translation[J]. *Eur J Heart Fail*, 2020, 22(1):54-66.

[11] Yang Y, Hsu PJ, Chen YS, et al. Dynamic transcriptomic m^6A decoration: writers, erasers, readers and functions in RNA metabolism[J]. *Cell Res*, 2018, 28(6):616-624.

[12] Meyer KD, Jaffrey SR. Rethinking m^6A readers, writers, and erasers[J]. *Annu Rev Cell Dev Biol*, 2017, 33:319-342.

[13] Dorn LE, Lasman L, Chen J, et al. The N6-methyladenosine mRNA methylase METTL3 controls cardiac homeostasis and hypertrophy[J]. *Circulation*, 2019, 139(4):533-545.

[14] He Y, Xing J, Wang S, et al. Increased m^6A methylation level is associated with the progression of human abdominal aortic aneurysm[J]. *Ann Transl Med*, 2019, 7(24):797.

[15] Wu J, Frazier K, Zhang J, et al. Emerging role of m6 A RNA methylation in nutritional physiology and metabolism[J]. *Obes Rev*, 2020, 21(1):e12942.

[16] Mathiyalagan P, Adamiak M, Mayourian J, et al. FTO-dependent N6-methyladenosine regulates cardiac function during remodeling and repair[J]. *Circulation*, 2019, 139(4):518-532.

[17] Chang G, Leu JS, Ma L, et al. Methylation of RNA N6-methyladenosine in modulation of cytokine responses and tumorigenesis[J]. *Cytokine*, 2019, 118: 35-41.

[18] Liu J, Harada, BT and He C. Regulation of gene expression by N6-methyladenosine in cancer. Trends Cell Biol, 2019, 29(6):487-499.

[19] Kmietczyk V, Riechert E, Kalinski L, et al. m^6A-mRNA methylation regulates cardiac gene expression and cellular growth[J]. *Life Sci Alliance*, 2019, 2(2):e201800233.

[20] Zuo X, Chen Z, Gao W, et al. m^6A-mediated upregulation of LINC00958 increases lipogenesis and acts as a nanotherapeutic target in hepatocellular carcinoma[J]. *J Hematol Oncol*, 2020, 13(1):5.

[21] Liu J, Yue Y, Han D, et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation[J]. *Nat Chem Biol*, 2014, 10(2):93-95.

[22] Khanna N, Fang Y, Yoon MS, et al. XPLN is an endogenous inhibitor of mTORC2[J]. *Proc Natl Acad Sci USA*, 2013, 110(40):15979-15984.

[23] Sciarretta S, Forte M, Frati G, et al. New insights into the role of mTOR signaling in the cardiovascular system[J]. *Circ Res*, 2018, 122(3):489-505.

[24] Sheikh F, Lyon RC, Chen J. Functions of myosin light chain-2 (MYL2) in cardiac muscle and disease[J]. *Gene*, 2015, 569(1):14-20.

[25] Wu MY, Yiang GT, Liao WT, et al. Current mechanistic concepts in ischemia and reperfusion injury[J]. *Cell Physiol Biochem*, 2018, 46(4):1650-1667.

[26] Song H, Feng X, Zhang H, et al. METTL3 and ALKBH5 oppositely regulate m^6A modification of TFEB mRNA, which dictates the fate of hypoxia/reoxygenation-treated cardiomyocytes[J]. *Autophagy*, 2019, 15(8):1419-1437.

[27] Liu Y, Liu Z, Tang H, et al. The N6-methyladenosine (m^6A)-forming enzyme METTL3 facilitates M1 macrophage polarization through the methylation of STAT1 mRNA[J]. *Am J Physiol Cell Physiol*, 2019, 317(4):C762-C775.

[28] Zhao J, Li X, Hu J, et al. Mesenchymal stromal cell-derived exosomes attenuate myocardial ischaemia-reperfusion injury through miR-182-regulated macrophage polarization[J]. *Cardiovasc Res*, 2019, 115(7):1205-1216.

[29] van der Sluis RJ, Hoekstra M. Glucocorticoids are active players and therapeutic targets in atherosclerotic cardiovascular disease[J]. *Mol Cell Endocrinol*, 2020, 504:110728.

[30] Zhao W, Wang Z, Sun Z, et al. RNA helicase DDX5 participates in oxLDL-induced macrophage scavenger receptor 1 expression by suppressing mRNA degradation[J]. *Exp Cell Res*, 2018, 366(2):114-120.

[31] Park MH, Jeong E, Choudhury M. Mono-(2-ethylhexyl)phthalate regulates cholesterol efflux via microRNAs regulated m^{6}A RNA methylation[J]. *Chem Res Toxicol*, 2020, 33(2):461-469.

[32] Wang X, Wu R, Liu Y, et al. m^{6}A mRNA methylation controls autophagy and adipogenesis by targeting Atg5 and Atg7[J]. *Autophagy*, 2020, 16(7):1221-1235.

[33] Xu Y, Yuan XD, Wu JJ, et al. The N6-methyladenosine mRNA methylase METTL14 promotes renal ischemic reperfusion injury via suppressing YAP1[J]. *J Cell Biochem*, 2020, 121(1):524-533.

[34] Liu J, Yue Y, Han D, et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation[J]. *Nat Chem Biol*, 2014, 10(2):93-5.

[35] Horiuchi K, Umetani M, Minami T, et al. Wilms' tumor 1-associating protein regulates G2/M transition through stabilization of cyclin A2 Mrna[J]. *Proc Natl Acad Sci USA*, 2006, 103(46):17278-17283.

[36] Zhong S, Li H, Bodi Z, et al. MTA is an arabidopsis messenger RNA adenosine methylase and interacts with a homolog of a sex-specific splicing factor[J]. *Plant Cell*, 2008, 20(5):1278-1288.

[37] Agarwala SD, Blitzblau HG, Hochwagen A, et al. RNA methylation by the MIS complex regulates a cell fate decision in yeast[J]. *PLOS Genet*, 2012, 8(6):e1002732

[38] Ping XL, Sun BF, Wang L, et al. Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase[J]. Cell Res, 2014, 24(2):177-189.

[39] Wang LJ, Xue Y, Li H, et al. Wilms' tumour 1-associating protein inhibits endothelial cell angiogenesis by m^{6}A-dependent epigenetic silencing of desmoplakin in brain arteri ovenous malformation[J]. *J Cell Mol Med*, 2020, 24(9):4981-4991.

[40] Zhu B, Gong Y, Shen L, et al. Total Panax notoginseng saponin inhibits vascular smooth muscle cell proliferation and migration and intimal hyperplasia by regulating WTAP/ p16 signals via m^{6}A modulation[J]. *Biomed Pharmacother*, 2020, 124:109935.

[41] Zheng G, Dahl JA, Niu Y, et al. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility[J]. *Mol Cell*, 2013, 49(1):18-29.

[42] Frangogiannis NG. Pathophysiology of myocardial infarction[J]. *Compr Physiol*,2015, 5(4):1841-1875.

[43] Chapman AR, Shah ASV, Lee KK, et al. Long-term outcomes in patients with type 2 myocardial infarction and myocardial injury[J]. *Circulation*, 2018, 137(12):1236-1245.

[44] Lindsey ML, Bolli R, Canty JM Jr, et al. Guidelines for experimental models of myocardial ischemia and infarction[J]. *Am J Physiol Heart Circ Physiol*, 2018, 314(4):H812-H838.

[45] Wang X, Huang N, Yang M, et al. FTO is required for myogenesis by positively regulating mTOR-PGC-1alpha pathway-mediated mitochondria biogenesis[J]. *Cell Death Dis*, 2017, 8(3):e2702.

[46] Xu K, Mo Y, Li D, et al. N6-methyladenosine demethylases Alkbh5/F to regulate cerebral ischemia-reperfusion injury[J]. *Ther Adv Chronic Dis*, 2020, 11:2040622320916024.

[47] Noubiap JJ, Nansseu JR, Nyaga UF, et al. Global prevalence of resistant hypertension: A meta-analysis of data from 3.2 million patients[J]. *Heart*, 2019, 105(2):98-105.

[48] Gan XT, Zhao G, Huang CX, et al. Identification of fat mass and obesity associated (FTO) protein expression in cardiomyocytes: regulation by leptin and its contribution to leptin-induced hypertrophy[J]. *PLOS ONE*, 2013, 8(9):e74235.

[49] Carnevali L, Graiani G, Rossi S, et al. Signs of cardiac autonomic imbalance and proarrhythmic remodeling in FTO deficient mice[J]. *PLOS ONE*, 2014, 9(4):e95499.

[50] Qian B, Wang P, Zhang D, et al. m^{6}A modification promotes miR-133a repression during cardiac development and hypertrophy via IGF2BP2[J]. *Cell Death Discov*, 2021, 7(1):157.

[51] Ju W, Liu K, Ouyang S, et al. Changes in N6-methyladenosine modification modulate diabetic cardiomyopathy by reducing myocardial fibrosis and myocyte hypertrophy[J]. *Front Cell Dev Biol*, 2021, 9:702579.

[52] Mo C, Yang M, Han X, et al. Fat mass and obesity-associated protein attenuates lipid accumulation in macrophage foam cells and alleviates atherosclerosis in apolipoprotein E-deficient mice[J]. *J Hypertens*, 2017, 35(4):810-821.

[53] Shimizu I and Minamino T. Physiological and pathological cardiac hypertrophy[J]. *J Mol Cell Cardiol*, 2016, 97:245-262.

[54] Mathiyalagan P, Adamiak M, Mayourian J, et al. FTO-dependent N6-methyladenosine regulates cardiac function during remodeling and repair[J]. *Circulation*,

2019, 139(4):518-532.

[55] Wang X, Zhu L, Chen J, et al. mRNA m^6A methylation downregulates adipogenesis in porcine adipocytes[J]. *Biochem Biophys Res Commun*, 2015, 459(2):201-207.

[56] Wu W, Feng J, Jiang D, et al. AMPK regulates lipid accumulation in skeletal muscle cells through FTO-dependent demethylation of N_6-methyladenosine[J]. *Sci Rep*, 2017, 7:41606.

[57] Wu R, Liu Y, Yao Y, et al. FTO regulates adipogenesis by controlling cell cycle progression via m^6A-YTHDF2 dependent mechanism[J]. *Biochim Biophys Acta Mol Cell Biol Lipids*, 2018, 1863(10):1323-1330.

[58] Gu X, Zhang Y, Li D, et al. N6-methyladenosine demethylase FTO promotes M1 and M2 macrophage activation[J]. *Cell Signal*, 2020, 69:109553.

[59] Yang Z, Yu GL, Zhu X, et al. Critical roles of FTO-mediated mRNA m^6A demethylation in regulating adipogenesis and lipid metabolism: Implications in lipid metabolic disorders[J]. *Genes Dis*, 2022, 9(1):51-61.

[60] Krüger N, Biwer LA, Good ME, et al. Loss of endothelial FTO antagonizes obesity-induced metabolic and vascular dysfunction[J]. *Circ Res*, 2020, 126(2):232-242.

[61] Cieniková Z, Damberger FF, Hall J, et al. Structural and mechanistic insights into poly(uridine) tract recognition by the hnRNP C RNA recognition motif[J]. *J Am Chem Soc*, 2014, 136(41):14536-14544.

[62] Liu N, Dai Q, Zheng G, et al. N6-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions[J]. *Nature*, 2015, 518(7540):560-564.

[63] Yu R, Li Q, Feng Z, et al. m^6A reader YTHDF2 regulates LPS-induced inflammatory response[J]. *Int J Mol Sci*, 2019, 20(6):1323.

[64] Chen J, Ning Y, Zhang H, et al. METTL14-dependent m^6A regulates vascular calcification induced by indoxyl sulfate[J]. *Life Sci*, 2019, 239:117034.

[65] Zhang L, Qi Y, ALuo Z, et al. Betaine increases mitochondrial content and improves hepatic lipid metabolism[J]. *Food Funct*, 2019, 10(1):216-223.

[66] Chen J, Zhou X, Wu W, et al. FTO-dependent function of N6-methyladenosine is involved in the hepatoprotective effects of betaine on adolescent mice[J]. *J Physiol Biochem*, 2015, 71(3):405-413.

[67] Zhao G, He F, Wu C, et al. Betaine in inflammation: Mechanistic aspects and applications[J]. *Front Immunol*, 2018, 9:1070.

[68] Kang H, Zhang Z, Yu L, et al. FTO reduces mitochondria and promotes hepatic fat accumulation through RNA demethylation[J]. *J Cell Biochem*, 2018, 119(7): 5676-5685.

[69] Zhang M, Zhang S, Zhai Y, et al. Cycloleucine negatively regulates porcine oocyte maturation and embryo development by modulating N6-methyladenosine and histone modifications[J]. *Theriogenology*, 2022, 179:128-140.

[70] Chen B, Ye F, Yu L, et al. Development of cell-active N6-methyladenosine RNA demethylase FTO inhibitor[J]. *J Am Chem Soc*, 2012, 134(43):17963-17971.

[71] Cheng L, Chen Q, Pi R, et al. A research update on the therapeutic potential of rhein and its derivatives[J]. *Eur J Pharmacol*, 2021, 899:173908.

[72] Huang Y, Yan J, Li Q, et al. Meclofenamic acid selectively inhibits FTO demethylation of m^6A over ALKBH5[J]. *Nucleic Acids Res*, 2015, 43(1):373-384.

[73] Chen H, Jia B, Zhang Q, et al. Meclofenamic acid restores gefinitib sensitivity by downregulating breast cancer resistance protein and multidrug resistance protein 7 via FTO/m^6A-demethylation/c-Myc in non-small cell lung cancer[J]. *Front Oncol*, 2022, 12:870636.

[74] Wu R, Yao Y, Jiang Q, et al. Epigallocatechin gallate targets FTO and inhibits adipogenesis in an mRNA m^6A-YTHDF2-dependent manner[J]. *Int J Obes*, 2018, 42(7):1378-1388.

[75] Gan Z, Wei W, Wu J, et al. Resveratrol and curcumin improve intestinal mucosal integrity and decrease m^6A RNA methylation in the intestine of weaning piglets[J]. *ACS Omega*, 2019, 4(17):17438-17446.

[76] Liu K, Wang F, Wang S, et al. Mangiferin attenuates myocardial ischemia-reperfusion injury via MAPK/Nrf-2/HO-1/NF-κB *in vitro* and *in vivo*[J]. *Oxid Med Cell Longev*, 2019, 5(13):7285434.