Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
E-mail: jzus@zju.edu.cn

Review:

Epigenetic role of N^6-methyladenosine (m^6A) RNA methylation in the cardiovascular system*

Kun ZHAO, Chuan-xi YANG, Peng LI, Wei SUN, Xiang-qing KONG†‡

Department of Cardiology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

†E-mail: xiangqing_kong@sina.com

Received Dec. 5, 2019; Revision accepted Feb. 28, 2020; Crosschecked June 5, 2020

Abstract: As the most prevalent and abundant transcriptional modification in the eukaryotic genome, the continuous and dynamic regulation of N^6-methyladenosine (m^6A) has been shown to play a vital role in physiological and pathological processes of cardiovascular diseases (CVDs), such as ischemic heart failure (HF), myocardial hypertrophy, myocardial infarction (MI), and cardiomyogenesis. Regulation is achieved by modulating the expression of m^6A enzymes and their downstream cardiac genes. In addition, this process has a major impact on different aspects of internal biological metabolism and several other external environmental effects associated with the development of CVDs. However, the exact molecular mechanism of m^6A epigenetic regulation has not been fully elucidated. In this review, we outline recent advances and discuss potential therapeutic strategies for managing m^6A in relation to several common CVD-related metabolic disorders and external environmental factors. Note that an appropriate understanding of the biological function of m^6A in the cardiovascular system will pave the way towards exploring the mechanisms responsible for the development of other CVDs and their associated symptoms. Finally, it can provide new insights for the development of novel therapeutic agents for use in clinical practice.

Key words: N^6-methyladenosine (m^6A); RNA methylation; Cardiovascular system; Metabolic disorder
https://doi.org/10.1631/jzus.B1900680 **CLC number:** R541.9

1 Introduction

Epigenetics has attracted considerable attention in the biomedical field. Accumulating evidence has shown that epigenetic alterations can affect a variety of common pathological reactions, including ischemia, inflammation, aging, and tumorigenesis (He et al., 2013). Epigenetics can also have a vital impact on gene expression and function without altering the base sequence of DNA. Moreover, these effects are reversible, heritable and are influenced by the external environment (Gibney and Nolan, 2010; Mazzio and Soliman, 2012).

Current epigenetic mechanisms include mainly DNA methylation, post-translational histone modification, chromatin remodeling, and deployment of non-coding RNA. Recent advances in the field of epigenetics can aid our understanding of a series of complicated biological processes associated with aging (Rodriguez-Rodero et al., 2010), development (Smith et al., 2012), inflammation (Bayarsaihan, 2011), immunity (Fernández-Morera et al., 2010), stem cell biology (Calvanese and Fraga, 2012), and angiogenesis (Buysschaert et al., 2008).

The role of reversible RNA methylation has just begun to attract worldwide scientific attention compared with the processes of DNA methylation and histone modification which are now well-established

‡ Corresponding author
* Project supported by the Grants-in-Aid from the Graduate Research and Innovation Projects of Jiangsu Province (No. KYCX18_1461), China
ORCID: Xiang-qing KONG, https://orcid.org/0000-0001-8245-867X

in genetics and cell biology (He, 2010; Panneerdoss et al., 2018). As an important part of developing RNA epigenetics, the modification of N^6-methyladenosine (m^6A) has recently been explored in the cardiovascular field (Liu and Pan, 2015). It has been shown that the m^6A modification is the most universal and abundant internal modification of eukaryotic messenger RNA (mRNA) and long non-coding RNA (lncRNA) and occurs mainly in the 3'-untranslated regions (3'-UTRs) and nearby the stop codons of the mRNA molecules (Parashar et al., 2018; Gan et al., 2019). Furthermore, several independent lines of evidence have revealed that the continuous and dynamic regulation of m^6A may have a far-reaching impact on modulating the expression of specific genes (Niu et al., 2013). In this review, we outline recent advances and discuss potential therapeutic strategies for managing m^6A in relation to several common cardiovascular diseases (CVDs), related metabolic disorders, and external environmental factors.

2 Biological function of m^6A modification

2.1 Methyltransferase and demethylases of m^6A

It has been shown that m^6A methylation is catalyzed by the multicomponent RNA methyltransferase complex, RNA demethylases, and m^6A readers (Liu et al., 2014). The core components of the RNA methyltransferase complex include methyltransferase-like 3/14 (METTL3/14) and Wilms tumor 1 associated protein (WTAP) (Liu et al., 2014). Among them, the spliceosome-associated protein WTAP has recently been found to play its role by recruiting RNA METTL3/14 to sites of methylation in the nucleus (Liu et al., 2014). Although the methyltransferase complex of m^6A methylation has not been fully elucidated, remarkable progress including the discovery of ALKB homolog 5 (ALKBH5) and fat mass- and obesity-related proteins (FTOs) has recently been made. The so-called erasers of specific markers operate on target transcripts via an α-ketoglutarate- and Fe^{2+}-dependent way in the m^6A and N^6,2'-*O*-dimethyladenosine (m^6A_m) of mammalian polyadenylated RNA (Gerken et al., 2007; Niu et al., 2013; Mauer et al., 2017). In addition to the methyltransferase complex and demethylase enzyme, m^6A is also regulated by the activity of m^6A readers, such as YT521-B homology (YTH) m^6A RNA-binding proteins (YTHDF), which can regulate the stability and degradation of RNAs and affect the efficiency of target mRNA translation (Wang and He, 2014; Kennedy et al., 2016). Therefore, m^6A regulates its effects mainly by recruiting a variety of proteins containing the YTH domains and via its assembly by methyltransferases that are located in relative consensus motifs following transcription (Wei and Moss, 1977; Patil et al., 2018). The m^6A modification may not only affect the secondary structure of mRNA and the interactions between mRNA-protein and mRNA-small nuclear RNA (snRNA), but may also be involved in modulating translatability, RNA transport and splicing, and susceptibility to post-transcriptional silencing (Zhong et al., 2008).

2.2 m^6A regulation in the eukaryotic genome

It is believed that m^6A methylation plays an important role in the regulation of gene expression by affecting the stability, degradation, and translation of RNA (Fu et al., 2014; Meyer et al., 2015). Several studies have confirmed that alteration of m^6A levels may have a significant impact on various biological processes in mammals, such as the maintenance and differentiation of embryonic stem cells (ESCs), transcriptional splicing, nuclear RNA export, protein translation control, cell fate determination, circadian rhythm modification, heat shock response, meiotic progression, and neuronal function (Guo et al., 2017; Roignant and Soller, 2017; Wei et al., 2017). Moreover, a lack of m^6A modification can lead to the development of several diseases, such as obesity, cancer, type 2 diabetes mellitus (T2DM), infertility, and developmental arrest (Wei et al., 2017).

Therefore, attention has been focused on the role and importance of m^6A regulation in the eukaryotic genome in relation to physiological and pathological processes. The m^6A profiles of full transcriptomes have shown that m^6A modifications occur in countless RNA transcripts with unique patterns of distribution. Although the role of m^6A editors in the processing, synthesis, and degradation of mRNAs and proteins has been discovered (Patil et al., 2018), the functions and mechanisms of most RNA modifications found in mRNA molecules remain poorly understood, especially with regard to the development of CVDs.

3 m^6A RNA modification in CVDs

Due to improvements in the standard of living and medical care, CVD mortality has declined markedly in certain high-income countries of Western Europe and North America (Libby et al., 2013). However, CVD remains the leading cause of death worldwide in both developed and developing countries (Iyen et al., 2019). Accumulating evidence has shown the contribution of several important but underappreciated issues, such as air pollution, to the genesis and development of CVDs (Cosselman et al., 2015; Peña and Rollins, 2017). Epigenetics plays an effective and leading regulatory role in modulating cardiovascular repair functions (Jin et al., 2019). Therefore, new concepts and therapeutic measures must be developed for myocardial repair and regeneration to improve cardiac function. However, although certain transcription factors and co-activators have been studied in heart failure (HF), post-transcriptional modifications that mediate specific mRNAs affecting the main protein expression and cardiac function have not been explored in detail.

As a key enzyme of m^6A, FTO is widely expressed in cardiac ventricular tissues, with a high level of expression in humans (Boissel et al., 2009). Therefore, particular attention must be paid to the possible associations between m^6A and the physiological and pathological processes of different CVDs. Emerging evidence has identified epigenetic regulation of m^6A and suggested possible therapeutic strategies for treating the development of cardiac defects, including ventricular septal and atrio-ventricular defects (Boissel et al., 2009), arrhythmias (Carnevali et al., 2014), coronary heart disease (Gustavsson et al., 2014), and cardiomyocyte hypertrophy (Dorn et al., 2019) (Fig. 1).

3.1 Ischemic heart diseases

By altering the oxygenation capacity of cardiomyocytes directly, ischemic heart disease caused by stenosis or occlusion of the myocardial coronary artery is considered one of the main causes of morbidity and mortality worldwide, including myocardial infarction (MI) and HF (Ge et al., 2019).

Several studies have shown that cardiomyocytes under chronic anoxic conditions, such as anemia, chronic intermittent hypoxia, and sleep apnea, can retain the homeostasis of heart contractile function and energy metabolism by triggering the corresponding regulation pathways and mediating cardiac metabolic remodeling via induction of transcription (Essop, 2007; Cole et al., 2016).

As one of the main drivers of metabolic disturbance in ischemic disease and MI, hypoxia is the result of multiple physiological and pathological stimuli (da Luz Sousa Fialho et al., 2019). Previous studies of cardiac ventricular biopsies from patients who had undergone coronary artery bypass surgery have shown that hypoxia inducible factor (HIF) is a major transcription factor with significantly increased activity in the early period of post-MI (Lee et al., 2000; Smith et al., 2008). It is well-established that HIF-1 activates most of the regulatory factors involved in oxygen homeostasis in mammals by binding to hypoxia response elements (HREs) and activating the transcription of multiple target genes which may play a critical role in the hypoxic response at both the cellular and the systemic levels. Subsequently, this process regulates the relevant signal transduction pathways and the corresponding microenvironments of the pathological blood vessels induced by hypoxia and ischemia (Ortiz-Barahona et al., 2010).

Due to the deficiency in oxygen availability, cellular metabolism of cardiomyocytes is one of the major changes that occur during chronic adaptation to hypoxia (Dang and Semenza, 1999). In addition to metabolism, the binding between HIF and HREs can be affected by epigenetic mechanisms, such as CpG methylation and oxidative DNA damage caused by hypoxia and reactive oxygen species (ROS) (Rodriguez et al., 1997). It has also been reported that HIF-1 is associated with hypoxia and may be regulated by histone methylation directly. With regard to certain common methylases, several studies have shown that hypoxia induces the expression of ALKBH5 in various types of breast cancer cells (Essop, 2007). In addition, el Azzouzi et al. (2013) confirmed the binding sites of *FTO* mRNA for several hypoxia-induced cardiac microRNAs (miRs) well-known to be up-regulated in HF, such as miR-21, miR-24, miR-488, miR-224, and miR-489. These results suggest that epigenetic regulation of hypoxia may play a significant role in the pathogenesis of CVDs.

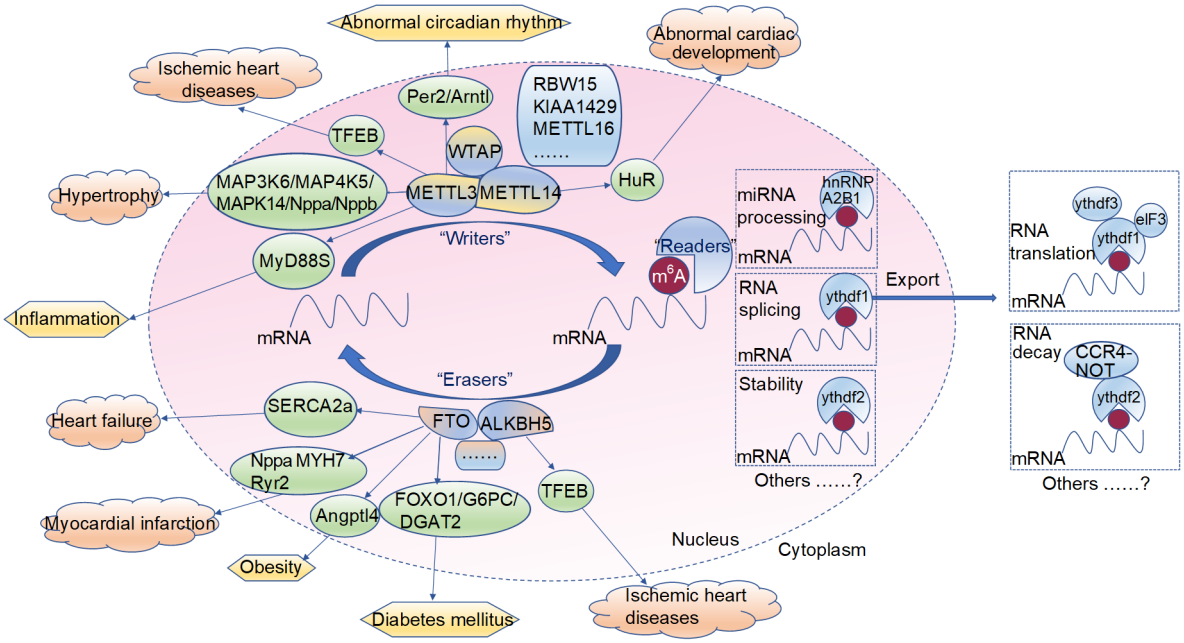

Fig. 1 Molecular roles of N^6-methyladenosine (m^6A) enzymes in different cardiovascular diseases, related metabolic disorders, and external environmental factors

m^6A methylation is catalyzed by the multicomponent RNA methyltransferase complex (methyltransferase-like 3/14 (METTL3/14), Wilms tumor 1 associated protein (WTAP), and KIAA1429), RNA demethylases (ALKB homolog 5 (ALKBH5) and fat mass- and obesity-related protein (FTO)), and m^6A readers (YT521-B homology (YTH) domain family 1/2/3 (YTHDF1/2/3), YTH domain containing 1 (YTHDC1), heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1), and E74-like factor 3 (eIF3)). Modification of m^6A may not only affect the secondary structure of messenger RNA (mRNA) and the interactions between mRNA-protein and mRNA-small nuclear RNA (snRNA), but may also be involved in modulating translatability, RNA transport and splicing, and susceptibility to post-transcriptional silencing. Furthermore, emerging evidence has identified the epigenetic regulation of m^6A and suggested possible therapeutic strategies for treating the development of cardiac defects and internal metabolic disorders, including heart failure, myocardial infarction, cardiomyocyte hypertrophy, obesity, and diabetics mellitus. TFEB: transcription factor EB; MAP3K6: mitogen-activated protein kinase (MAPK) kinase kinase 6; MAP4K5: MAPK kinase kinase kinase 5; Nppa: natriuretic peptide precursor (Npp)-A; MyD88S: splice variant of myeloid differentiation factor 88; SERCA2a: sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 2a; MYH7: β-myosin heavy chain 7; Ryr2: ryanodine receptor 2; Angptl4: angiopoietin-like 4; Per2: period 2; Arntl: aryl hydrocarbon receptor nuclear translocator like; FOXO1: forkhead box O1; G6PC: glucose-6-phosphatase (G6Pase) catalytic subunit; DGAT2: diacylglycerol acyltransferase 2; RBW15: RNA-binding motif protein 15; HuR: human antigen R; hnRN: heterogeneous nuclear ribonucleoprotein; CCR4-NOT: carbon catabolite repressor protein 4-negative on TATA

In hypoxia/reoxygenation (H/R)-treated cardiomyocytes and ischemia/reperfusion (I/R)-treated mouse heart tissues, the modification of m^6A was increased by METTL3, which is the main factor of abnormal m^6A regulation. This was caused by promoting the translation of specific mRNAs (Lin et al., 2016; Song et al., 2019).

The silencing of METTL3 can increase autophagic flux and inhibit apoptosis in H/R-treated cardiomyocytes, suggesting that METTL3 is a negative regulator of autophagy, which determines the cellular and systemic function of H/R-treated cardiomyocytes (Song et al., 2019). METTL3 methylates transcription factor EB (*TFEB*), a key gene involved in lysosomal biogenesis during the autophagic process, at two m^6A residues in the 3'-UTR. METTL3 enhances the binding of the protein heterogeneous nuclear ribonucleoprotein D (hnRNPD) to *TFEB* pre-mRNA, thereby decreasing the expression level of *TFEB* (Pastore et al., 2016). Further experiments have shown that the autophagic flux enhanced by METTL3 deficiency is TFEB-dependent. Therefore, it is reasoned that *METTL3* knockdown can increase the levels of the green fluorescent protein (GFP)-TFEB protein by up-regulating the endogenous levels of *TFEB* via the adenosine monophosphate (AMP)-activated protein

kinase (AMPK)-mammalian target of rapamycin (mTOR) pathway (Song et al., 2019).

3.1.1 Heart failure

Current evidence shows that dysregulation of m^6A levels in RNA can lead to the development of several pathological mechanisms, such as HF, at the molecular, cellular, and organic levels (Jia et al., 2011; Meyer et al., 2012). Mathiyalagan et al. (2019) confirmed that the increased expression of m^6A induced by ischemia and hypoxia may be reduced by altering the expression of FTOs. This evidence was reported in a study of different models of HF and hypoxic primary cardiomyocytes (Mathiyalagan et al., 2019). In addition to improving cardiomyocyte function and attenuating cardiac remodeling in HF by restoring the expression of relative proteins, such as sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a), experimental evidence has shown that FTO overexpression decreases cardiac fibrosis and promotes angiogenesis in models of MI (Mathiyalagan et al., 2019). Furthermore, it has been shown that cardiac contractile transcripts associated with ischemia and hypoxia are demethylated selectively by FTO, which can increase the stability of mRNA and protein expression (Hess et al., 2013).

3.1.2 Myocardial infarction

MI is a heart attack caused by the formation of plaque in the interior walls of the arteries that reduces blood flow to the heart and damages the heart muscles due to tissue inflammation and scarring (Lu et al., 2015). Epicardial specificity in response to injury occurs by down-regulating a group of genes expressed differentially in the epicardium (Bochmann et al., 2010). This is considered one of the main mechanisms of MI development.

During embryogenesis, the proepicardium differentiates into cardiac vasculature and interstitial cells and eventually becomes a single epithelial cell layer covering the heart, which is essential for normal development (Reese et al., 2002; Cai et al., 2008; Zhou et al., 2008; van Wijk et al., 2009). However, it remains controversial whether the epicardium can differentiate into endothelial cells of the vasculature (Smart et al., 2007; Winter and Groot, 2007) and whether it can contribute directly to the formation of the myocardium (Zhou et al., 2008). Although previous study has shown that the epicardium can regulate cardiac regeneration following injury (noted in lower vertebrates), it has also been reported that epicardial cell genes may be a promising therapeutic target following the injury of left coronary artery (LCA) ligation by the application of transcriptomics (Bochmann et al., 2010). Therefore, these studies suggest that the involvement of epigenetics should be explored with regard to MI.

Mathiyalagan et al. (2019) demonstrated that the levels of FTO in mice were down-regulated within 4 h following MI induction by LCA. Furthermore, they examined cardiac remodeling soon after MI and demonstrated that maintenance of FTO expression for nearly one week could be helpful in protecting and repairing the function of cardiomyocytes by improving cardiac homeostasis. Overexpression of FTO was caused by adenoviral transfection. These data suggested the use of FTO or FTO mimics for therapeutic targeting.

3.2 Hypertrophy

Hypertrophic cardiomyopathy is one of the common causes of myocardial injury accompanied by pathological phenomena, such as myocardial hypertrophy (Hou et al., 2017; Liu et al., 2019).

In addition to other common stimuli, Dorn et al. (2019) confirmed that methylation regulated by METTL3 on N^6-adenosines is essential for the pathological process of hypertrophy in vivo and in vitro. Although the increase in the expression level of METTL3, which acts with METTL14, WTAP, or other regulatory subunits, may cause cardiomyocyte remodeling, it is notable that the inhibition of its activity can abrogate the ability of cardiomyocytes to cause cardiac compensatory hypertrophy in response to hypertrophic stimuli, such as aging and stress. Furthermore, cardiac-specific METTL3 knockout mice were shown to promote eccentric cardiomyocyte remodeling and dysfunction in vivo (Liu et al., 2014; Dorn et al., 2019). These data highlight that the dynamic methylation and modification mediated by METTL3 are necessary for regulating the myocyte growth response.

Since both METTL3 and FTO are localized in the nucleus of isolated cardiac myocytes, FTO knockdown was found to decrease the augmented size of neonatal rat cardiac myocytes (NRCMs) induced by

α-adrenergic stimulation in vitro (Kmietczyk et al., 2019). Taken collectively, these results suggest that m^6A methylation may be a novel and vital epigenetic mechanism that should be studied in relation to the maintenance of cardiac homeostasis.

In addition, due to the tissue specificity of different ALKBH proteins, it is still debatable whether they can be used with similar therapeutic effects as those of the METTL3-containing protein, which is expressed ubiquitously (Fedeles et al., 2015).

3.3 Abnormal cardiac development

The consensus sequence contained in m^6A is highly homologous between human and mouse species, and their mRNA levels are regulated in a tissue-specific manner from the embryonic period to adulthood (Dominissini et al., 2012; Meyer et al., 2012). Higher m^6A expression in embryogenesis than in adult tissues has suggested the critical importance of m^6A during growth and development at the cellular and organic levels.

Mouse ESCs (mESCs) are well-known for their roles in embryonic development. Wang et al. (2014) reported that METTL3 and METTL14 can modify m^6A to maintain and even improve the self-renewal capability of mESCs. Their enrichment analysis indicated that m^6A editing might be associated with the stability of RNA, which was in turn negatively associated with regulatory proteins by blocking the binding of human antigen R (HuR)'s RNA, thereby retaining the biological function of mESCs (Wang et al., 2014).

Moreover, in a study of growth retardation and developmental delay, FTO and its homozygous mutant were shown to play a vital role in the early development of the human central nervous and cardiovascular systems by maintaining the stability of 2-oxoglutarate turnover and *N*-methyl-nucleoside demethylase activity (Daoud et al., 2016).

3.4 Downstream gene expression

Alteration in the expression levels of myocardial genes in response to different pathological stimuli plays a major role in the occurrence and progression of HF in several animal models (Tan et al., 2002). A recent study has highlighted the importance of specific modifications that occur in reversible mRNA sequences that control gene expression at the epigenetic level (Tan et al., 2002). As an increase in the levels of m^6A methylation is associated with cardiomyopathy, it is meaningful to investigate the role of internal m^6A modification on the expression of the associated genes. Dynamic m^6A methylation can influence the stability of transcription by regulating the efficiency of mRNA translation in stressed cardiomyocytes. For example, in addition to the size and cardiac function of cardiomyocytes, the expression of the downstream markers natriuretic peptide precursor (Npp)-A (Nppa) and -B (Nppb) that are associated with hypertrophy can be influenced by the enzymatic activity of METTL3. This suggests that m^6A modification plays an integrative role in the regulation of cardiac gene expression and cellular growth response in vivo and in vitro (Kmietczyk et al., 2019).

Overall, the engineering and manipulation of m^6A enzymes or any other type of small molecule at specific sites may be a novel therapeutic avenue for several cardiac diseases caused by dysregulation of m^6A, such as HF and MI (James et al., 2003).

4 m^6A RNA modification is an internal metabolic disorder caused by external environmental factors

Different studies have attempted to understand the important and necessary role of m^6A in relation to metabolism and environmental effects that can, in turn, lead to the development of pathophysiology and abnormal heart function noted in CVDs (Table 1).

4.1 Obesity

The improvement in the general standard of living has rendered obesity a sub-health symptom. Several clinical and basic research studies have shown that the increasing incidence and mortality of various CVDs and even cancers are closely associated with obesity, suggesting it contributes to the severe financial burden associated with the treatment of these conditions (Scuteri et al., 2007; Koliaki et al., 2019).

In addition to dietary habits, the *FTO* gene, which is closely linked to obesity, was identified by the present study as a positive regulator of energy homeostasis, for example via lipid metabolism, by modulating m^6A levels in RNA and DNA sequences

Table 1 Multiple functions exerted by m^{6}A RNA methylation in various cardiovascular diseases

Disease	Molecule	Target gene	Function	Regulation	Mechanism	Reference
Ischemic heart diseases	METTL3	*TFEB*	Writer	Up-regulation	Silencing METTL3 enhances autophagic flux and inhibits apoptosis in H/R-treated cardiomyocytes by TFEB	Pastore et al., 2016
	ALKBH5	*TFEB*	Eraser	Down-regulation	ALKBH5 overexpression enhances autophagic flux and inhibits apoptosis in H/R-treated cardiomyocytes by TFEB	Pastore et al., 2016
Heart failure (HF)	FTO	*SERCA2a*	Eraser	Down-regulation	FTO overexpression improves cardiomyocyte function and attenuatis cardiac remodeling in HF by regulating SERCA2a	Mathiyalagan et al., 2019
Myocardial infarction (MI)	FTO	*Nppa*/*MYH7*/*Ryr2*/*SERCA2a*	Eraser	Down-regulation	FTO overexpression protects and repairs cardiomyocyte function through improving cardiac homeostasis after MI	Mathiyalagan et al., 2019
Hypertrophy	METTL3	*MAP3K6*/*MAP4K5*/*MAPK14*/*Nppa*/*Nppb*	Writer	Up-regulation	Silencing METTL3 maintains normal cardiac function in response to hypertrophic stimuli through stress-response mechanism	Dorn et al., 2019
	FTO		Eraser	Up-regulation	Silencing FTO blunts hypertrophy of NRCM in response to α-adrenergic stimulation	Kmietczyk et al., 2019
Abnormal cardiac development	METTL3/14	*HuR*	Writer	Down-regulation	The level of METTL3 expression maintains and even improves the self-renewal capability of mESCs	Wang et al., 2014
	FTO		Eraser	Mutation	FTO plays an important role in early development of human central nervous and cardiovascular systems	Daoud et al., 2016
Obesity	FTO	*Angptl4*	Eraser	Up-regulation	FTO influences triglyceride metabolism in adipocytes and by post-transcriptional regulation of *Angptl4*	Wang et al., 2015
	YTHDF2	*PPARγ*/*C/EBPα*	Reader	Down-regulation	Adipogenesis of porcine adipocytes can be led by a loss of m^{6}A on FAM134B through m^{6}A-YTHDF2-dependent way	Cai et al., 2019
Diabetes mellitus	FTO	*FOXO1*/*G6PC*/*DGAT2*	Eraser	Up-regulation	FTO expression is highly related to the impairment of plasma glucose and even multiple complications by altering target genes	Yang et al., 2019
Abnormal circadian rhythm	METTL3	*Per2*/*Arntl*	Writer	Down-regulation	Silencing METTL3 can elicit circadian period elongation and RNA processing delay by distributing clock genes *Per2* and *Arntl*	Fustin et al., 2013
Inflammation	METTL3	*MyD88S*	Writer	Up-regulation	Silencing METTL3 attenuates the inflammatory response via regulating MyD88S in LPS-induced hDPCs	Liu et al., 2019

m^{6}A: N^6-methyladenosine; METTL3: methyltransferase-like 3; ALKBH5: ALKB homolog 5; FTO: fat mass- and obesity-related protein; YTHDF2: YT521-B homology (YTH) domain family 2; *TFEB*: transcription factor EB; *SERCA2a*: sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 2a; *Nppa*: natriuretic peptide precursor (Npp)-A; *MYH7*: β-myosin heavy chain 7; *Ryr2*: ryanodine receptor 2; *MAP3K6*: mitogen-activated protein kinase (MAPK) kinase kinase 6; *MAP4K5*: MAPK kinase kinase kinase 5; *HuR*: human antigen R; *Angptl4*: angiopoietin-like 4; *PPARγ*: peroxisome proliferator-activated receptor γ; *C/EBPα*: CCAAT enhancer-binding protein α; *FOXO1*: forkhead box O1; *G6PC*: glucose-6-phosphatase (G6Pase) catalytic subunit; *DGAT2*: diacylglycerol acyltransferase 2; *Per2*: period 2; *Arntl*: aryl hydrocarbon receptor nuclear translocator like; *MyD88S*: splice variant of myeloid differentiation factor 88; H/R: hypoxia/reoxygenation; NRCM: neonatal rat cardiac myocyte; mESCs: mouse embryonic stem cells; FAM134B: family with sequence similarity 134 member B; LPS: lipopolysaccharide; hDPCs: human dental pulp cells

in humans (Bartosovic et al., 2017; Chen and Du, 2019). The physiological and pathological development of obesity may be associated with *FTO* variants, which carry the at-risk alleles of single-nucleotide polymorphisms (SNPs), including rs9939609, rs8050136, rs3751812, rs7202116, and rs9930506, in the first intron of the *FTO* gene (Church et al., 2010; Wang et al., 2015). Variants of *FTO* were reported to have enhanced *FTO* expression and/or activity in previous study performed on homozygous individuals carrying the at-risk alleles (Church et al., 2010). Moreover, most studies demonstrated that these at-risk SNPs appeared to promote energy intake and a preference for energy-dense foods (Cecil et al., 2008; Speakman et al., 2008; Timpson et al., 2008; Haupt et al., 2009), while in two independent genome-wide association studies, the *FTO* gene was shown to be a high risk factor contributing to early-onset and severe obesity of European and Indonesian subjects (Dina et al., 2007; Daya et al., 2019).

An analysis of different phenotypes of mice has shown that adipocyte-specific *FTO* can play an important role in influencing triglyceride metabolism in adipocytes and consequently body weight. This is caused partly by post-transcriptional regulation of downstream genes, such as *Angptl4*. This may decrease energy expenditure and promote fatty acid storage in adipose tissues, regardless of the intake from a high-fat or standard diet (Wang et al., 2015).

Furthermore, a loss of m^6A on family with sequence similarity 134 member B (FAM134B) can lead to the adipogenesis of porcine adipocytes through the m^6A-YTHDF2-dependent pathway (Cai et al., 2019).

The epigenetic modification of m^6A can be a risk factor of CVDs related to obesity, although the exact molecular mechanism remains unclear.

4.2 Diabetes mellitus

Pathoglycemia induced by diabetes mellitus, including hypoglycemia and hyperglycemia, has been identified to be the main risk factor contributing to CVDs and all-cause mortality in diabetic patients. In addition to controlling blood glucose, an increase in insulin sensitivity has become a new auxiliary or main treatment strategy of diabetes, notably in cases with insulin resistance (Khunti et al., 2015; Abdul-Ghani et al., 2019).

Furthermore, hyperglycemia may lead to the down-regulation of m^6A levels by enhancing the expression of FTO or other methyltransferases in patients with T2DM, suggesting an important epigenetic role of m^6A in regulating glucose and lipid metabolism disorders (Gilbert and Liu, 2012; Yang et al., 2019). In addition to dysregulated oxidation of glucose, increased FTO expression induced by diabetes is highly associated with the impairment of plasma glucose and even multiple complications caused by altering the expression of target functional genes, such as forkhead box O1 (*FOXO1*), glucose-6-phosphatase (G6Pase) catalytic subunit (*G6PC*), and diacylglycerol acyltransferase 2 (*DGAT2*) (Souness et al., 1982; Kursawe et al., 2016; Li et al., 2017; Yang et al., 2019).

Recently, it has been shown that an altered content of m^6A can be used as a novel biomarker of T2DM since significantly lower levels of m^6A are noted in both T2DM patients and diabetic rats. At present, *FTO* expression is the only indicator found to be positively correlated with the risk of diabetes by correlation analysis (Shen et al., 2015).

Nevertheless, whether m^6A modification in diabetes mellitus is locus-specific remains to be elucidated.

4.3 Abnormal circadian rhythm

Previous study has suggested that the circadian rhythm (day/night cycle) is one of the basic and vital features of the natural environment due to its contribution to the regulation of normal development and function of the cardiovascular system (Melkani and Panda, 2017). It affects the expression of related genes and the levels of hormones associated with cardiac function, such as angiotensin II, renin, aldosterone, growth hormone, and atrial natriuretic peptide (McNamara et al., 2001; Martino et al., 2004; Martino and Sole, 2009).

Moreover, it has been suggested that the occurrence and the severity of certain CVDs are highly associated with the circadian rhythm via specific intrinsic mechanisms that are currently unknown (Mukamal et al., 2000; Henriques et al., 2003).

The circadian clock may regulate metabolism in an epigenetic feedback loop by modifying the expression levels of certain genes coding for metabolic switches or other important components. This modification is caused at the level of transcription. The acetylation/deacetylation of histones occurs on the promoters related to protein complexes or on clock proteins directly. In addition to this process, methylation

of histone proteins has been shown to play a vital role in maintaining the normal function of the circadian rhythm in vivo (Asher et al., 2008; Nakahata et al., 2008; Katada and Sassone-Corsi, 2010).

A recent study has shown that the transcriptional functions of several clock genes, such as period 2 (*Per2*) and aryl hydrocarbon receptor nuclear translocator like (*Arntl*), play an important role in extending the circadian rhythm period by inhibiting the m^6A modification of methylase METTL3, thereby promoting the uncoupling between steady-state pre-mRNA and cytoplasmic mRNA rhythm (Fustin et al., 2013). It has also been predicted that the period of the circadian rhythm is positively associated with the efficiency of degradation and the nuclear import and export of *Per2* mRNA, as determined by network model analysis (Wilkins et al., 2007; Miki et al., 2012). Moreover, *Arntl* is much more sensitive than *Per2* with regard to the inhibition of METTL3 due to its higher mRNA nuclear accumulation and longer period of transcription (Fustin et al., 2013).

The decrease in RNA methylation induced by the pharmacological action of 3-deazaadenosine (3-DZA) might elongate the period of circadian rhythm in vivo and in vitro via the decreased hydrolysis of *S*-adenosylhomocysteine (SAH) (Fustin et al., 2013; Singhal et al., 2013; Lokody, 2014).

It has been suggested that m^6A modification affects the circadian rhythm and may regulate cardiac function to a certain degree.

4.4 Inflammation

CVDs and diabetes mellitus are defined as metabolic syndromes and have recently been investigated as chronic inflammatory diseases triggered by long-term stimulation of abnormal levels of plasma glucose, free fatty acids, and blood pressure. These abnormal biochemical profiles are promoted via the oxidative stress pathway and the interaction between the cytokines and the renin-angiotensin system (Wong et al., 2008; Grandl and Wolfrum, 2018; Fiechter et al., 2019).

Inflammation, including innate and adaptive immunity, is one of the major and fundamental risk factors of all stages of atherosclerosis (Weber and Noels, 2011; Legein et al., 2015; Ketelhuth et al., 2019). Recent studies have shown that the pathological development of atherosclerosis is driven by immune reactions and is related mainly to immune effector mechanisms, which are involved in the infiltration and accumulation of lipoproteins in the arterial intima (Libby et al., 2011, 2013; Ketelhuth and Hansson, 2016).

In addition to the epigenetic regulation of SNPs that is related to different degrees of CVD risks, hyperacetylation at the 27th lysine residue of the histone H3 protein (H3K27ac) is linked to lower expression of proinflammatory genes in the reversible process of inflammation and aging. This may also explain certain unclear mechanisms in the link between inflammation and the progression of CVDs (Smith and Humphries, 2009; IL6R Genetics Consortium Emerging Risk Factors Collaboration, 2012; Xuan et al., 2016; Mitrokhin et al., 2017; Cheng et al., 2018).

Furthermore, telomere shortening and modulation of certain microRNAs associated with DNA damage are considered another epigenetic mechanism accounting for inflammation, since they can promote the secretion of proinflammatory cytokines through several proinflammatory pathways (Olivieri et al., 2015).

Since the specific mechanism of the association between inflammation and atherosclerosis has not been fully clarified, m^6A has become a new focus for the development of novel clinical therapeutic strategies against CVD.

Recent studies have demonstrated that the loss of N^6-adenosine methyltransferase METTL3 can attenuate the increased expression levels of several inflammatory cytokines and various genes associated with the inflammatory response, mainly by changing the phosphorylation levels of related signaling pathways and by regulating the mRNA splicing of splice variant of myeloid differentiation factor 88 (MyD88S) in lipopolysaccharide (LPS)-induced human dental pulp cells (HDPCs). This suggests the possible role of epitranscriptomic modification of m^6A during the process of inflammation in pathophysiological mechanisms leading to CVDs (Feng et al., 2018).

5 Conclusions

Several independent studies have confirmed that the continuous and dynamic regulation of m^6A in different internal biological metabolic processes has a profound impact on modulating the expression of cardiac genes in the pathological development of

CVDs. Moreover, these risk factors can be combined with several other environmental factors that affect the development of these diseases. This occurs by regulation of RNA transcription, splicing, processing, translation, and decay (Niu et al., 2013; Guo et al., 2017; Roignant and Soller, 2017; Wei et al., 2017). Therefore, certain m^6A enzymes, including FTO and METTL3, have been shown to control epitranscriptomic regulation of specific cardiac defects caused by distinct mechanisms. However, the possible association between m^6A and other CVDs and even between cardiovascular-associated diseases, such as high blood pressure and congenital heart disease, remains unexplored. Although the use of specific inhibitors in different models of animals or cardiocytes has established the cardioprotective role of RNA-modifying enzymes in m^6A modification, additional therapeutic agents for CVDs should be assessed in future studies.

Contributors

Kun ZHAO wrote and edited the manuscript. Chuan-xi YANG and Peng LI collated the literature. Wei SUN edited and revised the manuscript. Kun ZHAO and Xiang-qing KONG designed the study. All authors have read and approved the final manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Kun ZHAO, Chuan-xi YANG, Peng LI, Wei SUN, and Xiang-qing KONG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Abdul-Ghani MA, Jayyousi A, Defronzo RA, et al., 2019. Insulin resistance the link between T2DM and CVD: basic mechanisms and clinical implications. *Curr Vasc Pharmacol*, 17(2):153-163. https://doi.org/10.2174/1570161115666171010115119

Asher G, Gatfield D, Stratmann M, et al., 2008. Sirt1 regulates circadian clock gene expression through PER2 deacetylation. *Cell*, 134(2):317-328. https://doi.org/10.1016/j.cell.2008.06.050

Bartosovic M, Molares HC, Gregorova P, et al., 2017. N6-methyladenosine demethylase FTO targets pre-mRNAs and regulates alternative splicing and 3'-end processing. *Nucleic Acids Res*, 45(19):11356-11370. https://doi.org/10.1093/nar/gkx778

Bayarsaihan D, 2011. Epigenetic mechanisms in inflammation. *J Dent Res*, 90(1):9-17. https://doi.org/10.1177/0022034510378683

Bochmann L, Sarathchandra P, Mori F, et al., 2010. Revealing new mouse epicardial cell markers through transcriptomics. *PLoS ONE*, 5(6):e11429. https://doi.org/10.1371/journal.pone.0011429

Boissel S, Reish O, Proulx K, et al., 2009. Loss-of-function mutation in the dioxygenase-encoding *FTO* gene causes severe growth retardation and multiple malformations. *Am J Hum Genet*, 85(1):106-111. https://doi.org/10.1016/j.ajhg.2009.06.002

Buysschaert I, Schmidt T, Roncal C, et al., 2008. Genetics, epigenetics and pharmaco-(epi)genomics in angiogenesis. *J Cell Mol Med*, 12(6b):2533-2551. https://doi.org/10.1111/j.1582-4934.2008.00515.x

Cai CL, Martin JC, Sun YF, et al., 2008. A myocardial lineage derives from *Tbx18* epicardial cells. *Nature*, 454(7200):104-108. https://doi.org/10.1038/nature06969

Cai M, Liu Q, Jiang Q, et al., 2019. Loss of m^6A on FAM134B promotes adipogenesis in porcine adipocytes through m^6A-YTHDF2-dependent way. *IUBMB Life*, 71(5):580-586. https://doi.org/10.1002/iub.1974

Calvanese V, Fraga MF, 2012. Epigenetics of embryonic stem cells. *Adv Exp Med Biol*, 741:231-253. https://doi.org/10.1007/978-1-4614-2098-9_16

Carnevali L, Graiani G, Rossi S, et al., 2014. Signs of cardiac autonomic imbalance and proarrhythmic remodeling in FTO deficient mice. *PLoS ONE*, 9(4):e95499. https://doi.org/10.1371/journal.pone.0095499

Cecil JE, Tavendale R, Watt P, et al., 2008. An obesity-associated *FTO* gene variant and increased energy intake in children. *N Engl J Med*, 359(24):2558-2566. https://doi.org/10.1056/NEJMoa0803839

Chen JL, Du B, 2019. Novel positioning from obesity to cancer: FTO, an m^6A RNA demethylase, regulates tumour progression. *J Cancer Res Clin Oncol*, 145(1):19-29. https://doi.org/10.1007/s00432-018-2796-0

Cheng H, Xuan HW, Green CD, et al., 2018. Repression of human and mouse brain inflammaging transcriptome by broad gene-body histone hyperacetylation. *Proc Natl Acad Sci USA*, 115(29):7611-7616. https://doi.org/10.1073/pnas.1800656115

Church C, Moir L, McMurray F, et al., 2010. Overexpression of *Fto* leads to increased food intake and results in obesity. *Nat Genet*, 42(12):1086-1092. https://doi.org/10.1038/ng.713

Cole MA, Jamil AHA, Heather LC, et al., 2016. On the pivotal role of PPARα in adaptation of the heart to hypoxia and why fat in the diet increases hypoxic injury. *FASEB J*, 30(8):2684-2697. https://doi.org/10.1096/fj.201500094R

Cosselman KE, Navas-Acien A, Kaufman JD, 2015. Environmental factors in cardiovascular disease. *Nat Rev Cardiol*, 12(11):627-642. https://doi.org/10.1038/nrcardio.2015.152

da Luz Sousa Fialho M, Jamil AHA, Stannard GA, et al., 2019. Hypoxia-inducible factor 1 signalling, metabolism and its

therapeutic potential in cardiovascular disease. *Biochim Biophys Acta Mol Basis Dis*, 1865(4):831-843. https://doi.org/10.1016/j.bbadis.2018.09.024

Dang CV, Semenza GL, 1999. Oncogenic alterations of metabolism. *Trends Biochem Sci*, 24(2):68-72.

Daoud H, Zhang D, McMurray F, et al., 2016. Identification of a pathogenic *FTO* mutation by next-generation sequencing in a newborn with growth retardation and developmental delay. *J Med Genet*, 53(3):200-207. https://doi.org/10.1136/jmedgenet-2015-103399

Daya M, Pujianto DA, Witjaksono F, et al., 2019. Obesity risk and preference for high dietary fat intake are determined by FTO rs9939609 gene polymorphism in selected Indonesian adults. *Asia Pac J Clin Nutr*, 28(1):183-191. https://doi.org/10.6133/apjcn.201903_28(1).0024

Dina C, Meyre D, Gallina S, et al., 2007. Variation in *FTO* contributes to childhood obesity and severe adult obesity. *Nat Genet*, 39(6):724-726. https://doi.org/10.1038/ng2048

Dominissini D, Moshitch-Moshkovitz S, Schwartz S, et al., 2012. Topology of the human and mouse m^{6}A RNA methylomes revealed by m^{6}A-seq. *Nature*, 485(7397):201-206. https://doi.org/10.1038/nature11112

Dorn LE, Lasman L, Chen J, et al., 2019. The *N*^{6}-methyladenosine mRNA methylase METTL3 controls cardiac homeostasis and hypertrophy. *Circulation*, 139(4):533-545. https://doi.org/10.1161/circulationaha.118.036146

el Azzouzi H, Leptidis S, Dirkx E, et al., 2013. The hypoxia-inducible microRNA cluster *miR-199a~214* targets myocardial PPARδ and impairs mitochondrial fatty acid oxidation. *Cell Metab*, 18(3):341-354. https://doi.org/10.1016/j.cmet.2013.08.009

Essop MF, 2007. Cardiac metabolic adaptations in response to chronic hypoxia. *J Physiol*, 584(3):715-726. https://doi.org/10.1113/jphysiol.2007.143511

Fedeles BI, Singh V, Delaney JC, et al., 2015. The AlkB family of Fe(II)/α-ketoglutarate-dependent dioxygenases: repairing nucleic acid alkylation damage and beyond. *J Biol Chem*, 290(34):20734-20742. https://doi.org/10.1074/jbc.R115.656462

Feng ZH, Li QM, Meng RS, et al., 2018. METTL3 regulates alternative splicing of MyD88 upon the lipopolysaccharide-induced inflammatory response in human dental pulp cells. *J Cell Mol Med*, 22(5):2558-2568. https://doi.org/10.1111/jcmm.13491

Fernández-Morera JL, Calvanese V, Rodríguez-Rodero S, et al., 2010. Epigenetic regulation of the immune system in health and disease. *Tissue Antigens*, 76(6):431-439. https://doi.org/10.1111/j.1399-0039.2010.01587.x

Fiechter M, Haider A, Bengs S, et al., 2019. Sex differences in the association between inflammation and ischemic heart disease. *Thromb Haemost*, 119(9):1471-1480. https://doi.org/10.1055/s-0039-1692442

Fu Y, Dominissini D, Rechavi G, et al., 2014. Gene expression regulation mediated through reversible m^{6}A RNA methylation. *Nat Rev Genet*, 15(5):293-306. https://doi.org/10.1038/nrg3724

Fustin JM, Doi M, Yamaguchi Y, et al., 2013. RNA-methylation-dependent RNA processing controls the speed of the circadian clock. *Cell*, 155(4):793-806. https://doi.org/10.1016/j.cell.2013.10.026

Gan HL, Hong L, Yang FL, et al., 2019. Progress in epigenetic modification of mRNA and the function of m^{6}A modification. *Chin J Biotechnol*, 35(5):775-783 (in Chinese). https://doi.org/10.13345/j.cjb.180416

Ge L, Cai Y, Ying F, et al., 2019. miR-181c-5p exacerbates hypoxia/reoxygenation-induced cardiomyocyte apoptosis via targeting PTPN4. *Oxid Med Cell Longev*, 2019:1957920. https://doi.org/10.1155/2019/1957920

Gerken T, Girard CA, Tung YCL, et al., 2007. The obesity-associated *FTO* gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science*, 318(5855):1469-1472. https://doi.org/10.1126/science.1151710

Gibney ER, Nolan CM, 2010. Epigenetics and gene expression. *Heredity (Edinb)*, 105(1):4-13. https://doi.org/10.1038/hdy.2010.54

Gilbert ER, Liu DM, 2012. Epigenetics: the missing link to understanding β-cell dysfunction in the pathogenesis of type 2 diabetes. *Epigenetics*, 7(8):841-852. https://doi.org/10.4161/epi.21238

Grandl G, Wolfrum C, 2018. Hemostasis, endothelial stress, inflammation, and the metabolic syndrome. *Semin Immunopathol*, 40(2):215-224. https://doi.org/10.1007/s00281-017-0666-5

Guo MJ, Liu XH, Zheng XT, et al., 2017. m^{6}A RNA modification determines cell fate by regulating mRNA degradation. *Cell Reprogram*, 19(4):225-231. https://doi.org/10.1089/cell.2016.0041

Gustavsson J, Mehlig K, Leander K, et al., 2014. *FTO* genotype, physical activity, and coronary heart disease risk in Swedish men and women. *Circ Cardiovasc Genet*, 7(2):171-177. https://doi.org/10.1161/circgenetics.111.000007

Haupt A, Thamer C, Staiger H, et al., 2009. Variation in the *FTO* gene influences food intake but not energy expenditure. *Exp Clin Endocrinol Diabetes*, 117(4):194-197. https://doi.org/10.1055/s-0028-1087176

He C, 2010. Grand challenge commentary: RNA epigenetics? *Nat Chem Biol*, 6(12):863-865. https://doi.org/10.1038/nchembio.482

He SK, Li XH, Chan N, et al., 2013. Review: epigenetic mechanisms in ocular disease. *Mol Vis*, 19:665-674.

Henriques JPS, Haasdijk AP, Zijlstra F, et al., 2003. Outcome of primary angioplasty for acute myocardial infarction during routine duty hours versus during off-hours. *J Am Coll Cardiol*, 41(12):2138-2142. https://doi.org/10.1016/S0735-1097(03)00461-3

Hess ME, Hess S, Meyer KD, et al., 2013. The fat mass and obesity associated gene (*Fto*) regulates activity of the

dopaminergic midbrain circuitry. *Nat Neurosci*, 16(8):1042-1048.
https://doi.org/10.1038/nn.3449

Hou N, Wen Y, Yuan X, et al., 2017. Activation of Yap1/Taz signaling in ischemic heart disease and dilated cardiomyopathy. *Exp Mol Pathol*, 103(3):267-275.
https://doi.org/10.1016/j.yexmp.2017.11.006

IL6R Genetics Consortium Emerging Risk Factors Collaboration, 2012. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *Lancet*, 379(9822):1205-1213.
https://doi.org/10.1016/s0140-6736(11)61931-4

Iyen B, Qureshi N, Kai J, et al., 2019. Risk of cardiovascular disease outcomes in primary care subjects with familial hypercholesterolaemia: a cohort study. *Atherosclerosis*, 287:8-15.
https://doi.org/10.1016/j.atherosclerosis.2019.05.017

James K, Weitzel LRB, Engelman CD, et al., 2003. Genome scan linkage results for longitudinal systolic blood pressure phenotypes in subjects from the Framingham Heart Study. *BMC Genet*, 4:S83.
https://doi.org/10.1186/1471-2156-4-s1-s83

Jia GF, Fu Y, Zhao X, et al., 2011. N^6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. *Nat Chem Biol*, 7(12):885-887.
https://doi.org/10.1038/nchembio.687

Jin J, Liu YF, Huang LH, et al., 2019. Advances in epigenetic regulation of vascular aging. *Rev Cardiovasc Med*, 20(1):19-25.
https://doi.org/10.31083/j.rcm.2019.01.3189

Katada S, Sassone-Corsi P, 2010. The histone methyltransferase MLL1 permits the oscillation of circadian gene expression. *Nat Struct Mol Biol*, 17(12):1414-1421.
https://doi.org/10.1038/nsmb.1961

Kennedy EM, Bogerd HP, Kornepati AVR, et al., 2016. Post-transcriptional m^6A editing of HIV-1 mRNAs enhances viral gene expression. *Cell Host Microbe*, 19(5):675-685.
https://doi.org/10.1016/j.chom.2016.04.002

Ketelhuth DFJ, Hansson GK, 2016. Adaptive response of T and B cells in atherosclerosis. *Circ Res*, 118(4):668-678.
https://doi.org/10.1161/circresaha.115.306427

Ketelhuth DFJ, Lutgens E, Bäck M, et al., 2019. Immunometabolism and atherosclerosis: perspectives and clinical significance: a position paper from the Working Group on Atherosclerosis and Vascular Biology of the European Society of Cardiology. *Cardiovasc Res*, 115(9):1385-1392.
https://doi.org/10.1093/cvr/cvz166

Khunti K, Davies M, Majeed A, et al., 2015. Hypoglycemia and risk of cardiovascular disease and all-cause mortality in insulin-treated people with type 1 and type 2 diabetes: a cohort study. *Diabetes Care*, 38(2):316-322.
https://doi.org/10.2337/dc14-0920

Kmietczyk V, Riechert E, Kalinski L, et al., 2019. m^6A-mRNA methylation regulates cardiac gene expression and cellular growth. *Life Sci Alliance*, 2(2):e201800233.
https://doi.org/10.26508/lsa.201800233

Koliaki C, Liatis S, Kokkinos A, 2019. Obesity and cardiovascular disease: revisiting an old relationship. *Metabolism*, 92:98-107.
https://doi.org/10.1016/j.metabol.2018.10.011

Kursawe R, Dixit VD, Scherer PE, et al., 2016. A role of the inflammasome in the low storage capacity of the abdominal subcutaneous adipose tissue in obese adolescents. *Diabetes*, 65(3):610-618.
https://doi.org/10.2337/db15-1478

Lee SH, Wolf PL, Escudero R, et al., 2000. Early expression of angiogenesis factors in acute myocardial ischemia and infarction. *N Engl J Med*, 342(9):626-633.
https://doi.org/10.1056/nejm200003023420904

Legein B, Janssen EM, Theelen TL, et al., 2015. Ablation of $CD8\alpha^+$ dendritic cell mediated cross-presentation does not impact atherosclerosis in hyperlipidemic mice. *Sci Rep*, 5:15414.
https://doi.org/10.1038/srep15414

Li Y, Ma ZQ, Jiang S, et al., 2017. A global perspective on FOXO1 in lipid metabolism and lipid-related diseases. *Prog Lipid Res*, 66:42-49.
https://doi.org/10.1016/j.plipres.2017.04.002

Libby P, Ridker PM, Hansson GK, 2011. Progress and challenges in translating the biology of atherosclerosis. *Nature*, 473(7347):317-325.
https://doi.org/10.1038/nature10146

Libby P, Lichtman AH, Hansson GK, 2013. Immune effector mechanisms implicated in atherosclerosis: from mice to humans. *Immunity*, 38(6):1092-1104.
https://doi.org/10.1016/j.immuni.2013.06.009

Lin SB, Choe J, Du P, et al., 2016. The m^6A methyltransferase METTL3 promotes translation in human cancer cells. *Mol Cell*, 62(3):335-345.
https://doi.org/10.1016/j.molcel.2016.03.021

Liu JZ, Yue YN, Han DL, et al., 2014. A METTL3–METTL14 complex mediates mammalian nuclear RNA N^6-adenosine methylation. *Nat Chem Biol*, 10(2):93-95.
https://doi.org/10.1038/nchembio.1432

Liu N, Pan T, 2015. RNA epigenetics. *Transl Res*, 165(1):28-35.
https://doi.org/10.1016/j.trsl.2014.04.003

Liu X, Lin L, Li Q, et al., 2019. ERK1/2 communicates GPCR and EGFR signaling pathways to promote CTGF-mediated hypertrophic cardiomyopathy upon Ang-II stimulation. *BMC Mol Cell Biol*, 20:14.
https://doi.org/10.1186/s12860-019-0202-7

Lokody I, 2014. Gene regulation: RNA methylation regulates the circadian clock. *Nat Rev Genet*, 15(1):3.
https://doi.org/10.1038/nrg3638

Lu L, Liu M, Sun RR, et al., 2015. Myocardial infarction: symptoms and treatments. *Cell Biochem Biophys*, 72(3):865-867.
https://doi.org/10.1007/s12013-015-0553-4

Martino T, Arab S, Straume M, et al., 2004. Day/night rhythms in gene expression of the normal murine heart. *J Mol Med (Berl)*, 82(4):256-264.
https://doi.org/10.1007/s00109-003-0520-1

Martino TA, Sole MJ, 2009. Molecular time: an often overlooked dimension to cardiovascular disease. *Circ Res*, 105(11):1047-1061. https://doi.org/10.1161/circresaha.109.206201

Mathiyalagan P, Adamiak M, Mayourian J, et al., 2019. FTO-dependent N^6-methyladenosine regulates cardiac function during remodeling and repair. *Circulation*, 139(4):518-532. https://doi.org/10.1161/circulationaha.118.033794

Mauer J, Luo XB, Blanjoie A, et al., 2017. Reversible methylation of m^6A_m in the 5' cap controls mRNA stability. *Nature*, 541(7637):371-375. https://doi.org/10.1038/nature21022

Mazzio EA, Soliman KFA, 2012. Basic concepts of epigenetics: impact of environmental signals on gene expression. *Epigenetics*, 7(2):119-130. https://doi.org/10.4161/epi.7.2.18764

McNamara P, Seo SB, Rudic RD, et al., 2001. Regulation of CLOCK and MOP4 by nuclear hormone receptors in the vasculature: a humoral mechanism to reset a peripheral clock. *Cell*, 105(7):877-889. https://doi.org/10.1016/S0092-8674(01)00401-9

Melkani GC, Panda S, 2017. Time-restricted feeding for prevention and treatment of cardiometabolic disorders. *J Physiol*, 595(12):3691-3700. https://doi.org/10.1113/jp273094

Meyer KD, Saletore Y, Zumbo P, et al., 2012. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. *Cell*, 149(7):1635-1646. https://doi.org/10.1016/j.cell.2012.05.003

Meyer KD, Patil DP, Zhou J, et al., 2015. 5' UTR m^6A promotes cap-independent translation. *Cell*, 163(4):999-1010. https://doi.org/10.1016/j.cell.2015.10.012

Miki T, Xu ZX, Chen-Goodspeed M, et al., 2012. PML regulates PER2 nuclear localization and circadian function. *EMBO J*, 31(6):1427-1439. https://doi.org/10.1038/emboj.2012.1

Mitrokhin V, Nikitin A, Brovkina O, et al., 2017. Association between interleukin-6/6R gene polymorphisms and coronary artery disease in Russian population: influence of interleukin-6/6R gene polymorphisms on inflammatory markers. *J Inflamm Res*, 10:151-160. https://doi.org/10.2147/jir.s141682

Mukamal KJ, Muller JE, Maclure M, et al., 2000. Increased risk of congestive heart failure among infarctions with nighttime onset. *Am Heart J*, 140(3):438-442. https://doi.org/10.1067/mhj.2000.108830

Nakahata Y, Kaluzova M, Grimaldi B, et al., 2008. The NAD^+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. *Cell*, 134(2):329-340. https://doi.org/10.1016/j.cell.2008.07.002

Niu YM, Zhao X, Wu YS, et al., 2013. N^6-methyl-adenosine (m^6A) in RNA: an old modification with a novel epigenetic function. *Genomics Proteomics Bioinformatics*, 11(1):8-17. https://doi.org/10.1016/j.gpb.2012.12.002

Olivieri F, Albertini MC, Orciani M, et al., 2015. DNA damage response (DDR) and senescence: shuttled inflammamiRNAs on the stage of inflammaging. *Oncotarget*, 6(34):35509-35521. https://doi.org/10.18632/oncotarget.5899

Ortiz-Barahona A, Villar D, Pescador N, et al., 2010. Genome-wide identification of hypoxia-inducible factor binding sites and target genes by a probabilistic model integrating transcription-profiling data and *in silico* binding site prediction. *Nucleic Acids Res*, 38(7):2332-2345. https://doi.org/10.1093/nar/gkp1205

Panneerdoss S, Eedunuri VK, Yadav P, et al., 2018. Cross-talk among writers, readers, and erasers of m^6A regulates cancer growth and progression. *Sci Adv*, 4(10):eaar8263. https://doi.org/10.1126/sciadv.aar8263

Parashar NC, Parashar G, Nayyar H, et al., 2018. *N*^{6}-adenine DNA methylation demystified in eukaryotic genome: from biology to pathology. *Biochimie*, 144:56-62. https://doi.org/10.1016/j.biochi.2017.10.014

Pastore N, Brady OA, Diab HI, et al., 2016. TFEB and TFE3 cooperate in the regulation of the innate immune response in activated macrophages. *Autophagy*, 12(8):1240-1258. https://doi.org/10.1080/15548627.2016.1179405

Patil DP, Pickering BF, Jaffrey SR, 2018. Reading m^6A in the transcriptome: m^6A-binding proteins. *Trends Cell Biol*, 28(2):113-127. https://doi.org/10.1016/j.tcb.2017.10.001

Peña MSB, Rollins A, 2017. Environmental exposures and cardiovascular disease: a challenge for health and development in low-and middle-income countries. *Cardiol Clin*, 35(1):71-86. https://doi.org/10.1016/j.ccl.2016.09.001

Reese DE, Mikawa T, Bader DM, 2002. Development of the coronary vessel system. *Circ Res*, 91(9):761-768. https://doi.org/10.1161/01.RES.0000038961.53759.3C

Rodriguez H, Drouin R, Holmquist GP, et al., 1997. A hot spot for hydrogen peroxide-induced damage in the human hypoxia-inducible factor 1 binding site of the *PGK 1* gene. *Arch Biochem Biophys*, 338(2):207-212. https://doi.org/10.1006/abbi.1996.9820

Rodriguez-Rodero S, Fernández-Morera JL, Fernandez AF, et al., 2010. Epigenetic regulation of aging. *Discov Med*, 10(52):225-233.

Roignant JY, Soller M, 2017. m^6A in mRNA: an ancient mechanism for fine-tuning gene expression. *Trends Genet*, 33(6):380-390. https://doi.org/10.1016/j.tig.2017.04.003

Scuteri A, Sanna S, Chen WM, et al., 2007. Genome-wide association scan shows genetic variants in the *FTO* gene are associated with obesity-related traits. *PLoS Genet*, 3(7):e115. https://doi.org/10.1371/journal.pgen.0030115

Shen F, Huang W, Huang JT, et al., 2015. Decreased *N*^{6}-methyladenosine in peripheral blood RNA from diabetic patients is associated with FTO expression rather than

ALKBH5. *J Clin Endocrinol Metab*, 100(1):E148-E154. https://doi.org/10.1210/jc.2014-1893

Singhal A, Arora G, Sajid A, et al., 2013. Regulation of homocysteine metabolism by *Mycobacterium tuberculosis* S-adenosylhomocysteine hydrolase. *Sci Rep*, 3:2264. https://doi.org/10.1038/srep02264

Smart N, Risebro CA, Melville AAD, et al., 2007. Thymosin β-4 is essential for coronary vessel development and promotes neovascularization via adult epicardium. *Ann N Y Acad Sci*, 1112(1):171-188. https://doi.org/10.1196/annals.1415.000

Smith AJP, Humphries SE, 2009. Cytokine and cytokine receptor gene polymorphisms and their functionality. *Cytokine Growth Factor Rev*, 20(1):43-59. https://doi.org/10.1016/j.cytogfr.2008.11.006

Smith TG, Robbins PA, Ratcliffe PJ, 2008. The human side of hypoxia-inducible factor. *Br J Haematol*, 141(3):325-334. https://doi.org/10.1111/j.1365-2141.2008.07029.x

Smith ZD, Chan MM, Mikkelsen TS, et al., 2012. A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature*, 484(7394):339-344. https://doi.org/10.1038/nature10960

Song HW, Feng X, Zhang H, et al., 2019. METTL3 and ALKBH5 oppositely regulate m^{6}A modification of *TFEB* mRNA, which dictates the fate of hypoxia/reoxygenation-treated cardiomyocytes. *Autophagy*, 15(8):1419-1437. https://doi.org/10.1080/15548627.2019.1586246

Souness JE, Stouffer JE, de Sanchez VC, 1982. Effect of N^6-methyladenosine on fat-cell glucose metabolism: evidence for two modes of action. *Biochem Pharmacol*, 31(24):3961-3971. https://doi.org/10.1016/0006-2952(82)90642-6

Speakman JR, Rance KA, Johnstone AM, 2008. Polymorphisms of the *FTO* gene are associated with variation in energy intake, but not energy expenditure. *Obesity (Silver Spring)*, 16(8):1961-1965. https://doi.org/10.1038/oby.2008.318

Tan FL, Moravec CS, Li JB, et al., 2002. The gene expression fingerprint of human heart failure. *Proc Natl Acad Sci USA*, 99(17):11387-11392. https://doi.org/10.1073/pnas.162370099

Timpson NJ, Emmett PM, Frayling TM, et al., 2008. The fat mass- and obesity-associated locus and dietary intake in children. *Am J Clin Nutr*, 88(4):971-978. https://doi.org/10.1093/ajcn/88.4.971

van Wijk B, van den Berg G, Abu-Issa R, et al., 2009. Epicardium and myocardium separate from a common precursor pool by crosstalk between bone morphogenetic protein-and fibroblast growth factor-signaling pathways. *Circ Res*, 105(5):431-441. https://doi.org/10.1161/circresaha.109.203083

Wang CY, Shie SS, Wen MS, et al., 2015. Loss of FTO in adipose tissue decreases *Angptl4* translation and alters triglyceride metabolism. *Sci Signal*, 8(407):ra127. https://doi.org/10.1126/scisignal.aab3357

Wang X, He C, 2014. Reading RNA methylation codes through methyl-specific binding proteins. *RNA Biol*, 11(6):669-672. https://doi.org/10.4161/rna.28829

Wang Y, Li Y, Toth JI, et al., 2014. N^6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. *Nat Cell Biol*, 16(2):191-198. https://doi.org/10.1038/ncb2902

Weber C, Noels H, 2011. Atherosclerosis: current pathogenesis and therapeutic options. *Nat Med*, 17(11):1410-1422. https://doi.org/10.1038/nm.2538

Wei CM, Moss B, 1977. Nucleotide sequences at the N^6-methyladenosine sites of HeLa cell messenger ribonucleic acid. *Biochemistry*, 16(8):1672-1676.

Wei WQ, Ji XY, Guo XQ, et al., 2017. Regulatory role of N^6-methyladenosine (m^{6}A) methylation in RNA processing and human diseases. *J Cell Biochem*, 118(9):2534-2543. https://doi.org/10.1002/jcb.25967

Wilkins AK, Barton PI, Tidor B, 2007. The Per2 negative feedback loop sets the period in the mammalian circadian clock mechanism. *PLoS Comput Biol*, 3(12):e242. https://doi.org/10.1371/journal.pcbi.0030242

Winter EM, Groot ACGD, 2007. Epicardium-derived cells in cardiogenesis and cardiac regeneration. *Cell Mol Life Sci*, 64(6):692-703. https://doi.org/10.1007/s00018-007-6522-3

Wong C, Kanetsky P, Raj D, 2008. Genetic polymorphisms of the RAS-cytokine pathway and chronic kidney disease. *Pediatr Nephrol*, 23(7):1037-1051. https://doi.org/10.1007/s00467-008-0816-z

Xuan Y, Wang LN, Zhi H, et al., 2016. Association between 3 IL-10 gene polymorphisms and cardiovascular disease risk: systematic review with meta-analysis and trial sequential analysis. *Medicine (Baltimore)*, 95(6):e2846. https://doi.org/10.1097/md.0000000000002846

Yang Y, Shen F, Huang W, et al., 2019. Glucose is involved in the dynamic regulation of m^{6}A in patients with type 2 diabetes. *J Clin Endocrinol Metab*, 104(3):665-673. https://doi.org/10.1210/jc.2018-00619

Zhong SL, Li HY, Bodi Z, et al., 2008. MTA is an *Arabidopsis* messenger RNA adenosine methylase and interacts with a homolog of a sex-specific splicing factor. *Plant Cell*, 20(5):1278-1288. https://doi.org/10.1105/tpc.108.058883

Zhou B, Ma Q, Rajagopal S, et al., 2008. Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart. *Nature*, 454(7200):109-113. https://doi.org/10.1038/nature07060

中文概要

题　目：N^6-甲基腺苷（m^{6}A）RNA 甲基化在心血管系统的表观遗传学作用

概　要：作为真核生物基因组中最普遍和最丰富的转录修

饰，N^6-甲基腺苷（m^6A）持续而动态性的调节在缺血性心力衰竭、心肌肥大和心肌梗死等心血管系统疾病的生理和病理过程中发挥着重要作用。此外，m^6A RNA 甲基化通过改变多种 m^6A 酶及下游靶基因的表达，对与心血管系统疾病发生发展相关的内在生物代谢和外在环境因素起着重要的调节作用。但是，目前仍不清楚 m^6A 表观遗传调节具体的分子生物学机制。在此，我们概述了 m^6A RNA 甲基化最新的研究进展及其在常见心血管系统疾病和心血管相关代谢紊乱病理发展中的作用。这将有助于我们正确了解 m^6A 在心血管系统中的生物学作用，并为进一步探索心血管疾病及其相关临床症状的发生机制和开发临床治疗药物，提供新的理论依据和思路。

关键词：N^6-甲基腺苷（m^6A）；RNA 甲基化；心血管系统；代谢紊乱