Role of m^{6}A RNA methylation in cardiovascular disease (Review)

YUHAN QIN, LINQING LI, ERFEI LUO, JIANTONG HOU, GAOLIANG YAN, DONG WANG, YONG QIAO and CHENGCHUN TANG

Department of Cardiology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu 210009, P.R. China

Received June 20, 2020; Accepted September 29, 2020

DOI: 10.3892/ijmm.2020.4746

Abstract. N^6-methyladenosine (m^{6}A) is the most prevalent and abundant type of internal post-transcriptional RNA modification in eukaryotic cells. Multiple types of RNA, including mRNAs, rRNAs, tRNAs, long non-coding RNAs and microRNAs, are involved in m^{6}A methylation. The biological function of m^{6}A modification is dynamically and reversibly mediated by methyltransferases (writers), demethylases (erasers) and m^{6}A binding proteins (readers). The methyltransferase complex is responsible for the catalyzation of m^{6}A modification and is typically made up of methyltransferase-like (METTL)3, METTL14 and Wilms tumor 1-associated protein. Erasers remove methylation by fat mass and obesity-associated protein and ALKB homolog 5. Readers play a role through the recognition of m^{6}A-modified targeted RNA. The YT521-B homology domain family, heterogeneous nuclear ribonucleoprotein and insulin-like growth factor 2 mRNA-binding protein serve as m^{6}A readers. The m^{6}A methylation on transcripts plays a pivotal role in the regulation of downstream molecular events and biological functions, such as RNA splicing, transport, stability and translatability at the post-transcriptional level. The dysregulation of m^{6}A modification is associated with cancer, drug resistance, virus replication and the pluripotency of embryonic stem cells. Recently, a number of studies have identified aberrant m^{6}A methylation in cardiovascular diseases (CVDs), including cardiac hypertrophy, heart failure, arterial aneurysm, vascular calcification and pulmonary hypertension. The aim of the present review article was to summarize the recent research progress on the role of m^{6}A modification in CVD and give a brief perspective on its prospective applications in CVD.

Correspondence to: Dr Chengchun Tang, Department of Cardiology, Zhongda Hospital, School of Medicine, Southeast University, 87 Dingjiaqiao, Nanjing, Jiangsu 210009, P.R. China
E-mail: tangchengchun@hotmail.com

Key words: m^{6}A, cardiovascular disease, mechanism, regulation, single nucleotide polymorphism

Contents

1. Introduction

Epitranscriptomics is an emerging research field of biology, in which a recently discovered novel mechanism of post-transcriptional regulation of RNA has been suggested to play a vital role in the regulation of RNA function and to be involved in various biological processes, including disease progression (1). RNA methylation modification is the most common type of RNA modification in eukaryotes, accounting for 60% of total RNA modification. The identified RNA modifications have been found to contribute to the structural complexity of RNA and participate in multiple biological functions (2).

In the early 1970s, a novel RNA epigenetic modification, N^6-methyladenosine (m^{6}A), was first discovered and proposed in eukaryotic messenger RNA (mRNA) from Novikoff hepatoma cells (3). m^{6}A modification is regarded as the most prevalent, reversible and dynamic eukaryotic mRNA transcriptional modification among >170 types of RNA modification, accounting for ~50% of all methylated ribonucleotides. More than 7,000 mRNAs in mammalian cells are m^{6}A-modified, and it is estimated that m^{6}A exists in 0.1-0.4% of adenosines (4). Recent studies have found that, in addition to mRNA, m^{6}A also occurs in rRNA, tRNA, small nucleolar RNAs, microRNAs (miRNAs or miRs) and long non-coding RNAs (5,6). The rapid development of next-generation sequencing technology and epigenetic research have assisted in the precise assessment of the *in vivo* methylation state of m^{6}A sites at single-nucleotide resolution (7). Approximately 1/4 of transcripts harbor m^{6}A modifications, which are mainly enriched around the stop codons, within long internal exons in the 5'and 3' untranslated regions (UTRs) at the consensus motif RRACH (R=A or G, H=A, C or U) (8). Understanding the functionality and mechanism of m^{6}A is essential for understanding the implications of m^{6}A in molecular governance. The m^{6}A modification is involved in multiple procedures throughout a number of

biological processes in mammals, such as splicing regulation (9), translocation (10), RNA stability (11), translation (12) and miRNA maturation (13).

m^{6}A is reversibly and dynamically regulated by methyltransferase and demethylases. m^{6}A binding proteins play the role by recognizing and binding with the m^{6}A sites of target RNAs (14). m^{6}A modification has become a popular research field of molecular biology, due to its crucial regulatory role in biological processes and the pathogenesis of a variety of diseases (15-18). Despite recent progress in m^{6}A modification research, the presence and functionality of m^{6}A remains largely unknown. Recent studies have reported the emerging roles of m^{6}A in the development of cardiovascular diseases (CVDs). The present review focuses on the latest progress in made m^{6}A modification research and provides an up to date summary of the association between m^{6}A modification and CVDs, which may provide insight into m^{6}A-related molecular biomarkers and therapeutic targets in CVD.

2. m^{6}A methylation

Molecular mechanisms of m^{6}A methylation. Adenosine methylation at the N^6-position is regarded as the most pervasive internal post-transcriptional chemical modification in mammalian mRNA and non-coding RNAs (19,20). RNA decoration by m^{6}A plays a fundamental role in the regulation of mRNA stability, translational efficiency and gene expression during normal cellular bioprocess or under disease conditions (21,22). m^{6}A is essential for RNA processing during mammalian development and disease progression. m^{6}A RNA methylation affects a variety of cellular biological processes, including splicing, processing, nuclear export, stability and decay, translation, cellular differentiation and metabolism (23,24). Dysregulated m^{6}A modification is an important hallmark of various diseases, including cancer (25-27), neurological disorders (28,29) and osteoporosis (30). Recently, an increasing number of studies have reported the role of m^{6}A methylation in the occurrence and development of CVD. m^{6}A modifications are mainly enriched around the 3'UTR, near stop codons and within long internal exons at the consensus motif RRACH (R=A/G, H=A/C/U) (31).

Epitranscriptomic m^{6}A modification is dynamically and reversibly regulated by modulators characterized as dedicated methyltransferases (writers), dedicated demethylases (erasers) and m^{6}A binding protein (readers), according to their functions (32). The aberrant expression of methyltransferases and demethylases results in the dysregulation of m^{6}A. Methyltransferases are responsible for the erection of m^{6}A modification, and m^{6}A methylation is removed by demethylases. Of note, m^{6}A binding proteins have been shown to recognize target m^{6}A-modified RNAs and participate in the biological process and development of human disease (33).

Writers [i.e., methyltransferase-like 3 (METTL3), METTL14 and Wilms tumor 1-associated protein (WTAP)] and erasers [i.e., fat mass and obesity-associated protein (FTO) and a-ketoglutarate-dependent dioxygenase (ALKB) homolog 5 (ALKBH5)] are responsible for catalyzing and removing m^{6}A, respectively (34-37). The regulatory mechanisms of m^{6}A are complex. Moreover, an increasing number of m^{6}A modulators have been discovered, particularly methyltransferases and m^{6}A binding proteins. m^{6}A readers have both stimulatory and inhibitory effects on translation dynamics. YTHDF2 can accelerate the degradation of m^{6}A-modified maternal transcripts, while YTHDF1 increases the translation efficiency of m^{6}A-marked dynamic transcript (38-40). The site-specific m^{6}A maps of transcripts and the role of site-specific methylation in translation warrant further investigation.

m^{6}A methyltransferases. m^{6}A methyltransferases are responsible for catalyzing the formation of m^{6}A modification (18). The multicomponent methyltransferase complex consists of a METTL3/METTL14 heterodimer and various other methyltransferases (41). METTL3 has been described as the core enzyme exerting methyltransferase activity in the methyltransferase complex through its combination with S-adenosyl methionine. METTL14 acts as a second supporting enzyme to strengthen the catalytic effect of m^{6}A RNA methylation (42,43). C-terminal arginine-glycine repeats in METTL14 are secondary RNA substrate binding sites and indispensable for METTL3-METTL14 catalytic activity (44). The heterodimer preferentially methyltransferases a GGACU motif (45).

WTAP is a mammalian splicing factor responsible for the interaction with the METTL3-METTL14 complex. WTAP is critical for initiating and guiding the localization of nuclear speckles, which is required for m^{6}A methylation activation. WTAP also regulates their recruitment to mRNA targets (46). Numerous regulatory enzymes have been found to interact with the heterodimer, such as Vir-like METTL16, KIAA1429 (47), RNA-binding motif 15 (RBM15)/RBM15B (48), Vir-like m6A methyltransferase associated (49), E3 ubiquitin-protein ligase Hakai and zinc finger CCCH domain containing protein 13 (50). The above-mentioned enzymes bind to mRNA and recruit the METTL3-METTL14 complex, guiding the heterodimer to target regions (Fig. 1).

m^{6}A demethylases. The discovery in 2011 of demethylases FTO and ALKBH5 revealed that m^{6}A modification is dynamic and reversible (34,36). The two identified demethylases both belong to the AlkB-related family of proteins. The conserved α-ketoglutarate/iron-dependent domain is indispensable for demethylation modifications. FTO and ALKBH5 perform potent functions in the splicing, processing, stability and translation of RNA (51). The downregulation of FTO or ALKBH5 has been shown to lead to an elevated m^{6}A modification in mRNA (34).

FTO was the first identified demethylase of m^{6}A; it localizes into the nucleus and has been recognized as a member of the AlkB-related family of non-heme FeII/α-KG-dependent dioxygenases. FTO has an efficient oxidative demethylation activity in targeting m^{6}A residues in RNA (52). FTO is essential for the biological development of cardiovascular systems (52). FTO-dependent m^{6}A demethylation can contribute to the increase or decrease of protein levels, due to the regulation of mRNA stability, and degradation and translation efficiency (53).

A number of AlkB protein family members serve as RNA methylation erasers through the oxidative demethylation of m^{6}A marks; however, only ALKBH5 has been identified

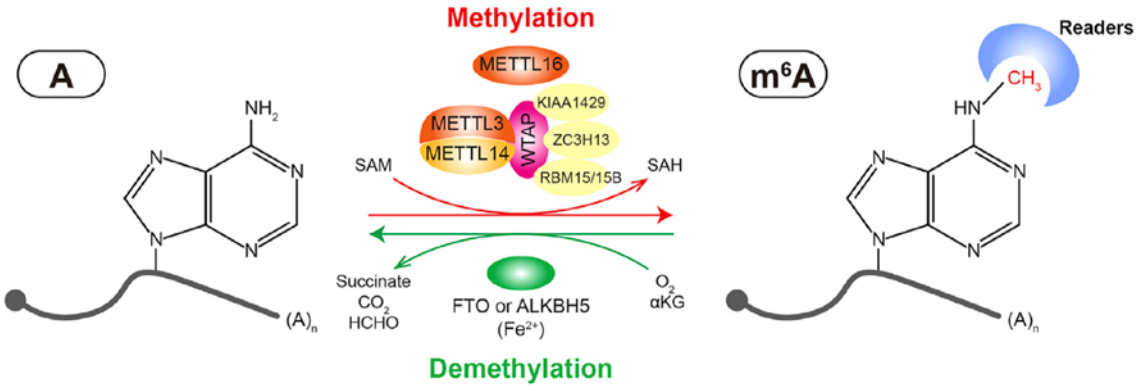

Figure 1. Reversible m^{6}A modification on mRNA. The adenosine (A) bases reside in mRNA could be methylated to form N^6-methyladenosine (m^{6}A) by the large MTC writer complex composed of the METTL3-METTL14-WTAP core component and other regulatory cofactors or by METTL16 alone. This enzymatic reaction uses S-adenosylmethionine (SAM) as a methyl donor. m^{6}A could be recognized by m^{6}A binding proteins (readers) to affect mRNA fate, or could be reversibly removed by m^{6}A eraser proteins (i.e., FTO and ALKBH5). The demethylation process requires a-ketoglutaric acid (a-KG) and molecular oxygen (O_2) as co-substrates and ferrous iron (Fe^{2+}) as a cofactor (2). METTL, methyltransferase-like; FTO, fat mass and obesity-associated protein; ALKBH5, ALKB homolog 5.

to exhibit efficient demethylation activity towards m^{6}A in animals (54). ALKBH5 has a tight interaction with mRNA and other RNA substrates. The alanine-rich sequence and potential coiled-coil structure in the N-terminus of ALKBH5 are important for its localization. ALKBH5 can affect both the synthesis and splicing rate of mRNAs (36) (Fig. 1).

m^{6}A RNA binding protein. The 'reading' of m^{6}A methylation marks is achieved through m^{6}A RNA binding proteins (RBP), which specifically recognize target m^{6}A-modified mRNA and subsequently play a role in the regulation of RNA splicing, fold, transport, translocation, degradation and translation. m^{6}A RBPs are involved in the regulation of RNA metabolism and bioprocess (33). m^{6}A-RBPs include YT521-B homology (YTH), heterogenous nuclear ribonucleoprotein A2/B1 (HNRNPA2B1) and insulin-like growth factor 2 mRNA-binding protein (IGF2BP) domain (39,55,56) (Fig. 2).

The YTH domain was identified in the human splicing factor YT521-B and is typically found in 174 different proteins expressed in eukaryotes, with a residue size of 100-150. It is featured by 14 invariant residues with an α-helix/β-fold to bind to RNA (57,58). The YTH domain-containing family can be divided into two subfamilies, YTHDF (YTHDF1-3) and YTHDC (YTHDC1-2) (59). YTHDF1 is known as an important m^{6}A reader promoting the cap-independent translation regulation of m^{6}A-modified RNA transcripts in the 5'UTR region. YTHDF1 relocates from the cytoplasm to the nucleus and initiates and augments translation in an eIF3 initiation factor-dependent manned (39). Coversely, YTHDF2 transports the mRNA targets to the cytoplasmic processing body and promotes its degradation (18). Specifically, Du *et al* (60) reported the specific mechanism of deadenylation mediated by YTHDF2 in mammalian cells. YTHDF2 distinguishes m^{6}A-bearing RNAs, and then interacts with CNOT1 to recruit the CCR4-NOT complex, which is critical for the demethylation of m^{6}A-marks by CAF1 and CCR4. YTHDF3 plays a cooperative role in RNA stability and translation among proteins from the YTHDF family to influence the metabolism of m^{6}A-methylated mRNA (24).

Nuclear binding protein YTHDC1 regulates alternative nuclear mRNA splicing by recruiting SRSF3 splicing factor and inhibiting SRSF10 binding to mRNA (61). YTHDC1 has also been found to be involved in nuclear transport (62) and gene translation silencing (48). YTHDC2 has several defined domains, including the YTH domain, R3H domain and ankyrin repeats (63). Phillip reported that YTHDC2 is a critical m^{6}A reader involved in spermatogenesis. YTHDC2 selectively binds to m^{6}A residues, decreases their mRNA abundance and enhances the translation efficiency of mRNAs (64).

Several proteins from the HNRNP family have been found to have the potential of recognizing m^{6}A-modified mRNA, including HNRNPA2B1, HNRNPC and HNRNPG. HNRNPA2B1 is a nuclear m^{6}A-reader that directly binds to nuclear transcripts, elicits regulatory effects on RNA splicing (55) and promotes primary microRNA processing (13). HNRNPC plays a regulatory role in the acceleration of pre-miRNA processing (65). HNRNPG interacts with RNA polymerase II and m^{6}A-modified pre-mRNAs to modulate the alternative splicing and expression of target mRNAs (66).

IGF2BPs (IGF2BP1-3) belong to conserved m^{6}A-binding proteins, whose RNA-binding domains consist of two RNA recognition motif domains and four K homology (KH) domains, with KH 3-4 being indispensable in recognizing m^{6}A marks (67). As m^{6}A readers, IGF2BPs are almost exclusively expressed in the cytoplasm, preferentially recognize m^{6}A-bearing mRNAs and fortify the stability of mRNA, therefore promoting translational efficiency (56). Dysregulated IGF2BPs have been found in several aggressive cancer cells and play oncogenic roles by enhancing the stability of methylated oncogenic mRNAs (67).

Methods of assessing the methylation of m^{6}A sites. The rapid emergence of next-generation sequencing technology has increased our understanding of epigenetic research and helped us assess the *in vivo* methylation state of m^{6}A sites. The two novel m^{6}A modification site detection technologies, m^{6}A sequencing (m^{6}A-seq) and m^{6}A-specific methylated RNA immunoprecipitation (MeRIP-seq) were created. m^{6}A-seq, presented by Dominissini *et al* (8), is a novel approach based on m^{6}A antibody-mediated capture and high-throughput sequencing. m^{6}A-Modified are highly conserved between human and mice (8). Meyer *et al* (68) presented the MeRIP-seq

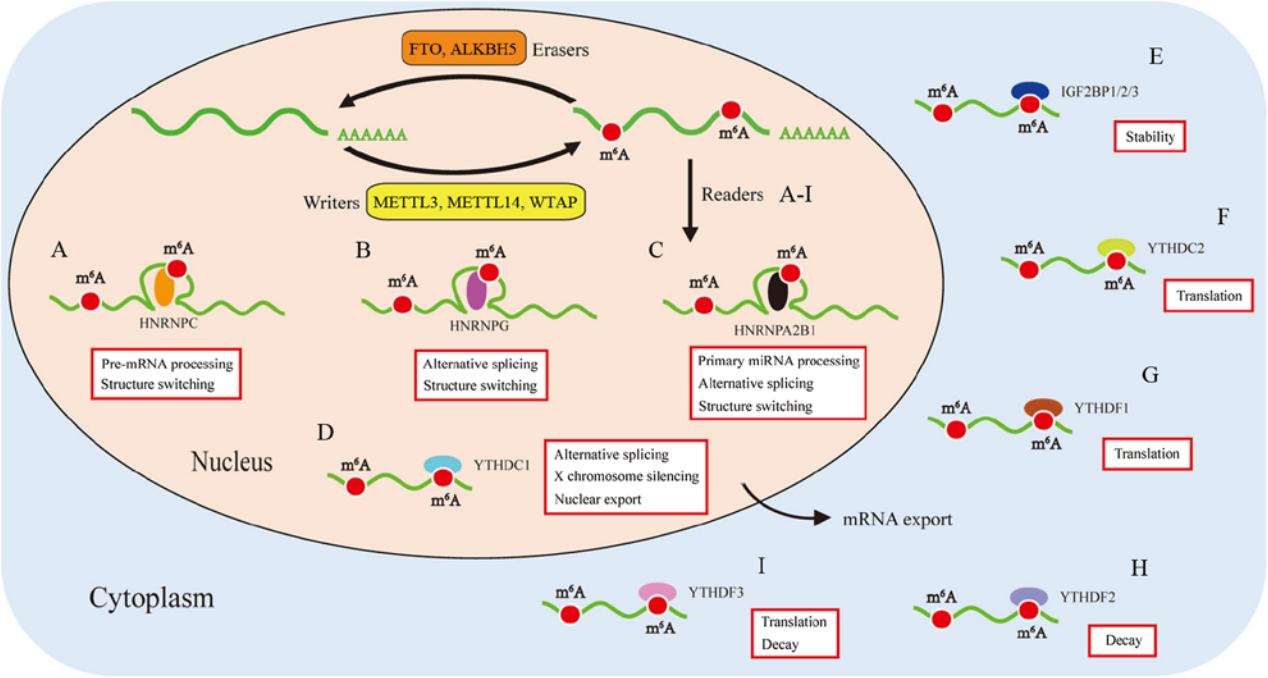

Figure 2. The regulation of m^{6}A modification. m^{6}A is established by m^{6}A methyltransferases ('writers') and removed by m^{6}A demethylases ('erasers'). m^{6}A readers are involved in multiple procedures of RNA metabolism through recognizing and binding to the m^{6}A sites of RNAs. (A) HNRNPC plays an important role in the pre-mRNAs processing and structure switching. (B) HNRNPG modulates alternative splicing and structure switching. (C) HNRNPA2B1 accelerates primary miRNAs processing, alternative splicing, and structure switching. (D) YTHDC1 participates in the alternative splicing, nuclear export, and X chromosome silencing. (E) IGF2BP1/2/3 have a function to increase the stability of targeted mRNAs. (F) YTHDC2 promotes mRNAs translation. (G) YTHDF1 augments mRNAs translation. (H) YTHDF2 facilitates mRNAs decay. (I) YTHDF3 cooperates with YTHDF1 to increase mRNAs translation, and strengthens mRNAs decay mediated by YTHDF2 (33).

method for transcriptome-wide m^{6}A localization, and m^{6}A methylated RNA was immunoprecipitated, followed by next-generation sequencing. m^{6}A-seq and MeRIP-seq identified thousands of mRNAs with m^{6}A modification and revealed that m^{6}A is a pervasive and dynamically reversible internal chemical modification of mRNA. However, there exist some limitations in the detection technology. This method has a relatively low accuracy in the identification of m^{6}A modification sites in the whole transcriptome for the following reasons: First, only RNA fragments 100-200 nt long could be captured through these mapping approaches. Besides, two similar m^{6}A sites could not be identified.

m^{6}A-level and isoform-characterization sequencing is another m^{6}A modification detection technique (9). Different from m^{6}A-seq and MeRIP-seq, intact full-length transcripts, rather than fragmented RNA, were isolated and sequenced. The quantification of m^{6}A modification helps examine differential isoform usage in transcripts with/without methylation.

Other revolutionary technological methods include photo-crosslinking-assisted m^{6}A-seq (69), m^{6}A individual-nucleotide-resolution cross-linking and immunoprecipitation (70) and ultraviolet (UV) CLIP (71). In these methods, UV strengthens the interaction between m^{6}A-modified RNA and m^{6}A antibodies, and affinity purification was performed. m^{6}A modification sites can be detected more precisely at single-nucleotide resolution on a transcriptome-wide level. Site-specific cleavage and radioactive-labeling followed by ligation-assisted extraction and thin-layer chromatography can accurately detect a single m^{6}A modification site and the m^{6}A modification level of the whole transcriptome. However, although highly accurate, it is also costly and time-consuming, limiting its application in m^{6}A methylation detection (72). Other methods include MAZTER-seq, RNAmod, FunDMDeep-m^{6}A and m^{6}A-REF-seq (72-76). Table I summarizes the methods of m^{6}A methylation detection (7). These findings have provided insight into the mammalian genome-wide mapping of m^{6}A modifications and unveiled the importance of dynamic mRNA modifications on gene expression. However, numerous difficulties and challenges remain in single-nucleotide detection and quantitative sequencing, and further research is required in the future.

3. m^{6}A and vascular smooth muscle cell differentiation

Adipose-derived stem cells (ADSCs) are a source of mesenchymal stem cells that can be used to develop biological treatment strategies for tissue regeneration. ADSCs can differentiate into vascular smooth muscle cells (VSMCs) under certain conditions. METTL3 mRNA and protein expression are increased in the VSMC differentiation of ADSCs. The downregulation of METTL3 contributes to a decrease in the m^{6}A modification of VSMC-specific markers, thus leading to a decline in their mRNA and protein expression. The silencing of METTL3 inhibits ADSC differentiation into VSMCs. In addition, an increased expression of paracrine factors has been observed in ADSC differentiation into VSMCs, including vascular endothelial growth factor, hepatocyte growth factor, granulocyte-macrophage colony-stimulating factor, basic fibroblast growth factor and stromal cell-derived factor-1 (77). An investigation of the role of METTL3 in the regulation of

Table I. Methods for the detection of m^{6}A methylation.

Methods	Features
m^{6}A-Seq	High throughput
MeRIP-seq	High throughput
m^{6}A-LAIC-seq	High throughput, precise
PA-m^{6}A-Seq	High throughput, single site
miCLIP	High throughput, single site
m^{6}A-CLIP	High throughput, single site
SCARLET	High throughput, single site
MAZTER-seq	High throughput, single site
RNAmod	High throughput, single site
FunDMDeep-m^{6}A	High throughput, single site
DART-seq	High throughput, single site
RNA sequencing	High throughput, single site
m^{6}A-REF-seq	High throughput, single site

VSMC differentiation has suggested that METTL3 is involved in ADSC differentiation to VSMCs, providing a promising perspective for novel therapeutic strategies for vascular network regeneration (77).

4. m^{6}A and cardiovascular disease

There is emerging evidence to indicate that m^{6}A modification is closely related to the occurrence and progression of CVDs, including cardiac hypertrophy, heart failure, ischemic heart disease, aortic aneurysm, vascular calcification, pulmonary hypertension etc. Herein, the recent progress in m^{6}A modification in CVD is briefly reviewed.

m^{6}A and cardiac hypertrophy. Cardiac remodeling occurs in the heart following stress stimulation or injury, and it involves molecular, cellular and interstitial alterations, clinically manifested as changes in size, shape and function (78). Stress stimulation initially induces adaptive hypertrophic response to produce sufficient force to match increased wall tension or increased pressure/volume overload in cardiomyocytes (79). Currently, post-transcriptional regulation is recognized as similarly critical to transcriptional regulation in cardiac hypertrophy (80).

Dorn *et al* (81) demonstrated that METTL3-mediated m^{6}A modification is significant for maintaining cardiac homeostasis and normal cardiac function and revealed increased m^{6}A methylation in cardiomyocytes under hypertrophic stimulation. Of note, m^{6}A peaks are specifically enriched in mRNAs encoding protein kinases and modifiers, such as mitogen-activated protein kinase kinase kinase 6, mitogen-activated protein kinase kinase kinase kinase 5 and mitogen-activated protein kinase 14. METTL3-overexpressing mice exhibited marked cardiac hypertrophy but not accelerated dysfunction during pressure overload stress. The inhibition of m^{6}A blocked cardiomyocyte hypertrophy by deleting METTL3. *In vivo*, METTL3 knockout mice exhibited signs of failure, both morphologically and functionally. Collectively, they demonstrated that METTL3-mediated m^{6}A methylation is dynamic. The enhanced METTL3-mediated m^{6}A modification caused by hypertrophic stimuli resulted to cardiac hypertrophy, whereas downregulated m^{6}A methylation led to cardiomyocyte remodeling, highlighting the importance of the mechanism in the maintenance of cardiac homeostasis, function and adaptation to stress responses (81).

m^{6}A and ischemic heart disease. The lysosomal-mediated degradation pathway (autophagy) is an important evolutionarily conserved degradation mechanism in eukaryotic cells that removes unnecessary and harmful parts to maintain homeostasis (82). Autophagy is associated with numerous human diseases, including CVD (83). Song *et al* (83) first researched the role of m^{6}A in autophagy and discovered that m^{6}A modification is significantly upregulated in hypoxia/reoxygenation (H/R)-treated cardiomyocytes and ischemia/reperfusion (I/R)-treated mouse hearts. The key member of the methyltransferase complex, METTL3, was abnormally upregulated in infraction heart tissue, as compared with healthy heart tissue. Consistently, an increased expression of METTL3 was identified in cardiomyocytes treated with H/R. The silencing of METTL3 increased the I/R-impaired autophagic flux and inhibited apoptosis (83).

It has been well documented that transcription factor EB (TFEB) is an important gene in lysosomal biogenesis, which also drives the expression of autophagy and lysosomal genes (84,85). Decreased TFEB mRNA stability and TFEB protein expression were previously discovered in H/R-induced cardiomyocytes. The silencing of METTL3 significantly promoted the expression and transcriptional activity of TFEB and translocation to the nucleus. TFEB knockdown inhibited the enhanced autophagy induced by the downregulation of METTL3, indicating that the mediation of autophagic flux by METTL3 is TFEB-dependent (86). The RNA binding protein heterogeneous nuclear ribonucleoprotein D (HNRNPD) was reported to bind to m^{6}A-modified RNA (25), and an increased HNRNPD was found in patients with heart failure (87). RIP-PCR confirmed the interaction between HNRNPD and TFEB pre-mRNA. H/R further enhanced the binding (86). In accordance with previous research (88), the H/R-induced HNRNPD elevation has been shown to promote TFEB mRNA degradation, and reduce the protein expression and transcriptional activity of TFEB. Furthermore, TFEB can suppress METTL3 expression by downregulating the stability of METTL3 mRNA, thereby forming negative feedback between METTL3 and TFEB (86). On the whole, research has revealed that the METTL3-TFEB feedback loop plays an essential role in autophagy, and has laid a foundation for dynamic m^{6}A modification in ischemic heart disease.

m^{6}A and heart failure. There is currently no effective approach available for adverse cardiac remodeling post-ischemia. mRNA and protein expression levels in the right ventricles during heart failure are inconsistent, suggesting a role for post-transcriptional regulation in failing hearts (89). Mathiyalagan *et al* (90) discovered that FTO played a vital role in cardiac contractile function during homeostasis and remodeling. An increased m^{6}A modification and significantly decreased FTO expression were found in infarct and peri-infarct regions of failing hearts, as compared with healthy heart tissues. FTO overexpression attenuated the ischemia-induced elevation in m^{6}A modification.

FTO-knockdown exhibited aberrantly increased arrhythmic events in cardiomyocytes. *In vivo*, FTO-overexpression significantly improved cardiac function at the chronic stage of post-myocardial infraction. Enhanced angiogenesis and alleviated fibrosis were also observed. A more rapid progression of heart failure was witnessed with a lower ejection fraction and more severe dilatation in FTO knockout mice, indicating the indispensable role of FTO in heart failure (90). Mechanistically, the Ca^{2+}-handling transcript Serca2a has been shown to be hypermethylated in failing hearts, and FTO demethylated Serca2a and enhanced the stability of Serca2a mRNA (91) or exerted co-transcriptional regulation (38), leading to an increase in SERCA2a expression, ultimately improving cardiac contractile function. Collectively, these results provide compelling evidence that FTO-mediated m^{6}A methylation plays an important role in cardiac function during heart failure and recommend FTO as a novel therapeutic approach (90).

Differently expressed genes of m^{6}A methylation are involved in heart failure development (92). Hypermethylated and hypomethylated transcripts have been linked to different biological processes. Gene ontology (GO) analysis has revealed that the differentially m^{6}A modified transcripts in heart failure are mainly involved in metabolism and cardiac signaling (92). Previous research has reported that m^{6}A modification can affect mRNA translation efficiency by regulating ribosome occupancy (12). The Calm1 mRNA level, a member of the CaMKII signaling pathway, is unaltered during m^{6}A modification, while Calm1 protein expression is significantly decreased in failed heart tissue, indicating that m^{6}A methylation affects Calm1 translation rather than transcription in heart failure development. m^{6}A-seq has revealed differentially methylated transcripts of epigenetic proteins, transcription factors and upstream regulators of signaling pathways, indicating that m^{6}A methylation is possibly involved in the regulation of gene expression in heart failure (92). These data suggest that the modulation of m^{6}A methylation may be a potential target for the treatment of heart failure.

Kmietczyk *et al* (93) explored the critical role of internal m^{6}A modification in dilated cardiac tissue and observed an increased m^{6}A methylation in mRNAs isolated from dilated cardiomyopathy samples. m^{6}A methylation is highly dynamic in stress-induced cardiomyocytes. They demonstrated the opposite result to that of the study by Dorn *et al* (81), demonstrating that METTL3 knockdown promoted cardiac hypertrophy and remodeling in cardiomyocytes. METTL3-overexpression attenuated pathological cardiac hypertrophy (93). The different study designs may explain the contrasting data. A transgenic mouse model and a transgenic approach driven by alpha myosin heavy chain-promoter for METTL3 overexpression were used in the study by Dorn *et al* (81), while C57Bl6/N mice and a adeno-associated virus-based approach for METTL3 overexpression were used in the study by Kmietczyk *et al* (93). Further studies are required to fully elucidate the mechanisms of stress response in the heart, for the maintenance of normal cardiac homeostasis and function. The above-mentioned study (93) revealed a novel mechanism underlying stress response, with the aim of maintaining normal cardiac function. Once elucidated, the manipulations of METTL3-mediated m^{6}A may provide a novel therapeutic strategy for the prevention of maladaptive and worsening of cardiac function. However, research to date has not elucidated the exact mechanisms underlying the regulation of cardiomyocyte growth by Mettl3 and identify the specific downstream targets.

The aforementioned research results suggested abnormal m^{6}A methylation in failing hearts, as compared with healthy hearts, indicating that the aberrantly m^{6}A methylation level may serve as a potential biomarker and therapeutic target by modulating m^{6}A modulators and downstream genes.

m^{6}A and abdominal aortic aneurysm (AAA). AAA is a common vascular condition among the elderly with a high mortality rate (94). AAA is characterized by chronic inflammation in the tunica media and adventitia, with the upregulation of numerous cytokines activating a large amount of proteolytic enzymes, ultimately leading to the rapid expansion and rupture of AAA (95). The exact mechanisms contributing to AAA have not yet been elucidated. He *et al* first investigated the role of m^{6}A modification in AAA and provided a potential epigenetic mechanism in AAA (96). They discovered that m^{6}A modification significantly increased in AAA, as compared to healthy aortic tissues. METTL14 was shown to be associated with inflammatory infiltrates and neovascularization in AAA, and increased FTO was also involved in aberrant m^{6}A modification. A higher m^{6}A level was also found to be associated with a higher risk of rupture. They subsequently discovered significant correlations between the m^{6}A modification level and the mRNA expression level of the writers, erasers and readers, indicating a tight crosstalk among these modulators. In clinical data, a higher m^{6}A level was found to be positively associated with the AAA diameter and hematological parameters (96). Hence, abnormal m^{6}A modification is crucial to the occurrence and progression of AAA.

Zhong *et al* (97) further explored METTL3-modulated methylation and the development of AAA. They revealed that the downregulation of METTL3 suppressed AAA formation in both ApoE$^{-/-}$ mice treated with angiotensin II, and a calcium chloride-induced mouse model. Of note, the downstream target gene they focused on, which was regulated by METTL3 in AAA, was not an mRNA, but miR-34a. Mechanistically, METTL3 mediated m^{6}A modification and promoted miR-34a maturation from pri-miR-34a, and then miR-34a negatively regulated SIRT1 expression. Collectively, the METTL3/miR-34a/SIRT1 axis plays a role in AAA formation and may serve as a diagnostic biomarker and novel therapeutic target of AAA treatment (97).

m^{6}A and vascular calcification. Chronic kidney disease (CKD) is a worldwide public health concern. Patients with CKD have a 2-fold higher risk of suffering from CVD and also exhibit a higher mortality rate, as compared to the healthy population (98). Patients with CKD are more likely to suffer from vascular calcification, and vascular calcification is recognized as the main cause of increased mortality from CVD (99). It has been confirmed that indoxyl sulfate (IS), a vital protein-bound uremic toxin, is associated with the development of renal and vascular progression (100). Chen *et al* investigated the epigenetic translation underlying IS-induced vascular calcification and noted marked *in vitro*, *in vivo* and translational evidence, indicating the essential role of METTL14 in IS-induced

vascular calcification (101). A significantly elevated m^{6}A level of total RNA was discovered in the radical arteries of patients with end-stage renal disease and preclinical calcified mouse arteries. An increased METTL14 mRNA and protein expression was detected in calcified arteries, indicating an IS-induced increase in the levels of METTL14 and METTL14, which mediated the increase in global m^{6}A modification, which may be a hallmark of vascular calcification. *In vitro*, the overexpression of METTL14 caused the loss of repair function. Mechanistically, MeRIP analysis revealed that the vascular-protecting transcript Klotho mRNA was hypermethylated in calcified arteries, resulting in the degradation and decreased mRNA expression of Klotho. Therefore, this investigation demonstrated the functional significance of METTL14 in IS-induced vascular calcification and provided proof of concept for anti-vascular calcification therapy through the modulation of METTL14 (101).

m^{6}A and pulmonary hypertension. Pulmonary hypertension (PH) is a complex and multidisciplinary pathophysiological disorder defined as a resting mean pulmonary artery pressure(PAP) of >25 mmHg, as assessed by right heart catheterization (102,103). Hypoxic PH (HPH) is a progressive disease due to lung diseases and categorized as group III PH. Chronic obstructive pulmonary disease and interstitial lung diseases are common causes (2). Currently there is no specific therapy for the increased PAP and structural abnormalities of HPH (104).

Previous studies have reported that hypoxia may lead to the dysregulation of m^{6}A methylation and may promote tumor occurrence and development. The effects of hypoxia on m^{6}A modification are cell type-dependent (86,105-107). Little was known of m^{6}A modification in circular RNAs (circRNAs), until thousands of m^{6}A-modified cell-specific expressed circRNAs were identified (108). Wang *et al* first identified a transcriptome-wide circRNA expression profile in lung tissues from a mouse model of HPH using microarray analysis (109). The whole m^{6}A level of circRNAs in HPH rat lung tissue was lower than that in healthy rat lung tissue. m^{6}A abundance in circRNAs was also decreased in hypoxia *in vitro*. The distribution of total circRNAs, m^{6}A-circRNAs and non-m^{6}A cicRNAs was similar between the HPH and control groups. m^{6}A methylation was enriched in circRNAs originating from single exons. GO and Kyoto Encyclopedia of Genes and Genomes pathway analysis identified different host genes of circRNAs with a hyper- and hypomethylated m^{6}A level. m^{6}A-modified cicRNAs tended to decrease in hypoxia (110).

circRNAs mostly play the role of a sponge for miRNAs, and are involved in the regulation of target miRNAs (111). A circRNA-miRNA-mRNA network was previously constructed to explore the regulation of gene expression in HPH. Key mRNAs associated with the Wnt (112) and FoxO signaling pathways (113), and miRNAs reported to be involved in HPH were filtered, and the most enriched novel circRNA Xpo6 and Tmtc3 were then identified (110). RIP-PCR confirmed that the expression of circXpo6 and circTmtc3 were significantly decreased in both hypoxia-induced pulmonary artery smooth muscle cells and endothelial cells (110). Collectively, the study (110) first revealed that m^{6}A affected the stability of circRNAs, subsequently influencing the circRNA-miRNA-mRNA network, leading to the activation of the Wnt and FoxO signaling pathways and ultimately promoting HPH. The results of that study (110) suggested that m^{6}A-modified circRNAs were associated with pulmonary hypertension. However, the research was just an expression file of circRNAs in lung tissues from HPH and control mice. The exact molecular mechanisms underlying the role of m^{6}A methylation in HPH remain to be elucidated.

m^{6}A-related single nucleotide polymorphisms (SNPs) and CVDs. High-throughput sequencing technology has identified millions of SNPs across multiple genomes. Distinguishing the disease-associated functional variants, which can regulate amino acids at the protein level (114), RNA secondary structure (115), RNA-protein interactions and RNA splicing (116) or editing (117) at the transcriptional or post-transcriptional level, is a major challenge. SNPs can interfere with m^{6}A methylation by altering the RNA sequences of target sites or key flanking nucleotides (118). Putative m^{6}A-related SNPs (m^{6}A-SNPs), which are close to or at the exact methylation site, can disrupt m^{6}A modification and corresponding biological processes through multiple mechanisms, due to the location of m^{6}A-SNPs, and can subsequently affect disease progression (119). m^{6}A-SNPs have been recognized as a fundamental class of crucial multifunctional genetic variants associated with CVDs, which is concerning. Numerous m^{6}A-SNPs associated with CVDs have been identified using genome-wide association studies (GWAS) and the genetic functional mechanisms have been explored. These m^{6}A-SNPs and genes may be candidates and promote novel therapeutic approaches (120,121). The present study reviewed the relevant research on m^{6}A-SNPs and CVD.

m^{6}A-SNPs and blood pressure (BP). Genetic factors are significant, and heritability contributes to 40-60% of BP cases (122). A large number of SNPs for BP have been identified by a GWAS (123). Zheng *et al* (118) reported a comprehensive database (m^{6}Avar), which is a useful tool for the investigation of the association between m^{6}A-related variants and disease. A previous study first made efforts to investigate the association between m^{6}A-SNPs and BP by excavating data from a large-scale GWAS, and demonstrated that m^{6}A-SNP may play a pivotal role in BP regulation (124). Some identified BP-related SNPs in genes have been found to be associated with coronary artery disease (CAD) (125). The majority of m^{6}A-SNPs were closely associated with gene expression. For example, rs9847953 and rs197922 were identified as potential functional variants and strongly associated with gene expression, ultimately contributing to the regulation of blood pressure.

The FTO gene has been found to be enriched in the hypothalamus (126), a brain structure involved in the control of blood pressure (127). Caucasian populations with the FTO-risk genotype (AA genotype) have been shown to have a higher systolic blood, which may result from a higher sympathetic modulation of vasomotor tone (128). Another study revealed that the most common genetic variant of the FTO gene (rs9939609 A/T) (129) was not associated with blood pressure in either sex (130). Further studies are required to elucidate the role of the FTO genetic variant in blood pressure.

m^{6}A-SNPs and coronary artery disease. CAD is the leading cause of mortality and disability worldwide, with both genetic and environmental factors playing an essential role in its pathogenesis (131). The effect of m^{6}A-SNPs on CAD was previously explored and 304 m^{6}A-SNPs were found to be associated with CAD (120). Some m^{6}A-SNPs not only alter m^{6}A methylation and local gene expression, but also regulate protein binding, indicating that m^{6}A-SNPs may be functional variants of CAD. Among these, rs12286 was markedly associated with CAD. Mechanistically, rs12286 may influence the m^{6}A methylation level and regulate the expression of downstream gene ADAMTS7 to exert its function (120). On the whole, these findings elucidated the role of m^{6}A-SNPs in CAD; however, the specific regulatory mechanisms remain to be explored.

m^{6}A-SNPs and lipid metabolism. Blood cholesterol is one of the most important heritable risk factors for CAD, and is positively associated with mortality due to CAD (132). The role of m^{6}A modification in lipid metabolism has been reported (133). Mo *et al* (121) investigated the effect of m^{6}A-SNPs on lipid levels using GWAS summary datasets of 188,578 individuals. m^{6}A-SNP rs6859 at the 3'UTR of poliovirus receptor-related 2 was found to be significantly expressed at the genome-wide level and associated with triglycerides, total cholesterol and high/low-density lipoprotein cholesterol, suggesting that rs6859 may be a lipid metabolism-related multifunctional SNP and an important candidate for further functional studies. That study found a large amount of lipid-related m^{6}A-SNPs (121). Further studies are required to elucidate the mechanisms.

Further large-scale GWASs are required to identify m^{6}A-SNPs in CVD, and further technical and biological experiments through single-nucleotide gene editing of SNPs to determine the detected SNPs functionalities.

5. Potential applications of m^{6}A in cardiovascular disease

Characteristic biomarkers play a crucial role in the prevention, diagnosis and treatment of disease. Previous studies have demonstrated that methylation serves as a potential biomarker and therapeutic target in cancer (134). Fustin *et al* demonstrated that m^{6}A editing through specific METTL3 inhibition contributed to eliciting circadian period elongation and RNA processing delay (10).

m^{6}A as a potential biomarker. A number of studies have suggested that aberrant m^{6}A modifications affect the progression of CVD. For instance, significantly elevated METTL3-modulated m^{6}A methylation has been found in cardiac hypertrophy, ischemic heart disease, AAA, etc. (81,86,97). In addition, innovations in the detection technology for m^{6}A modification render it a potential diagnostic biomarker of CVD. The early detection of upregulated m^{6}A modification may help monitor and prevent the occurrence and development of CVD. A higher FTO was detected in failing hearts, as compared with healthy hearts. The overexpression of FTO has been shown to markedly improve the cardiac function of post-myocardial infraction (90). On the whole, m^{6}A methylation may serve as a prospective non-invasive diagnostic biomarker and be used for the diagnosis and prognosis of CVD.

m^{6}A as a therapeutic target of CVD. Accumulated research has indicated that m^{6}A methylation is of importance in CVD, providing novel insight into the innovative therapeutic strategy of targeting related methyltransferases, demethylases, m^{6}A-binding proteins or m^{6}A-modified RNA.

Total Panax notoginseng saponin (TPNS) has been reported to inhibit balloon injury-induced intimal hyperplasia and VSMC proliferation (135). Zhu *et al* (136) observed a reduced WTAP expression and decreased m^{6}A modification in balloon catheter-injured rat carotid artery. Furthermore, TPNS alleviated the proliferation and migration of VSMCs through the upregulation of WTAP and downstream target p16 m^{6}A modification. Therefore, WTAP and m^{6}A regulatory p16 expression may serve as a novel molecular target for the assessment of arterial stenosis risk and a novel therapeutic target of arterial stenosis.

It is widely acknowledged that lipopolysaccharide (LPS) is a trigger for inflammation and metabolic diseases (137). Rao *et al* (138) first found that curcumin reduced the blood cholesterol level in normal animals. Lu *et al* discovered that the lipid metabolism disorder in the liver and increase in total cholesterol induced by LPS injection were all attenuated after dietary supplementation with curcumin (139). Mechanistically, the LPS injection increased the m^{6}A methylation level, accompanied by an increased METTL3 level, and decreased the mRNA of ALKBH5 and FTO in the liver. Of note, curcumin affected the expression of some crucial m^{6}A-related modulators, including METTL3, METTL14, ALKBH5, FTO and YTHDF2. Further upregulated m^{6}A methylation in the liver was discovered in piglets that had received dietary curcumin, as compared with the LPS injection group, suggesting that the protective effect of curcumin in LPS-induced hepatic lipid metabolism may result from increased m^{6}A RNA modification (139). The precise mechanisms of how curcumin affects gene-specific m^{6}A modification require further investigation. Collectively, the findings indicate that m^{6}A could be a promising therapeutic target for hyperlipidemia.

The development of m^{6}A modulation inhibitors provides an opportunity for the treatment of CVD. For instance, Huang *et al* (140) identified meclofenamic acid (MA), an inhibitor of demethylase FTO. MA exhibited an efficient and selective inhibitory effect on FTO demethylation in Hela cells by competition on m^{6}A-containing substrate binding.

Of note, Li *et al* (141) successfully constructed an *in vivo* manipulation CRISPR-Cas13b-based tool for the study of m^{6}A-related biological functions and targeted demethylation of specific mRNA. They created a gratifying fusion protein, dm^{6}ACRISPR, by linking a catalytically inactive enzyme to ALKBH5. dm^{6}ACRISPR could specifically demethylate transcripts with m^{6}A marks through the regulation of mRNA stability or translation. In addition, the manipulation tool is safe for a limited off-target effect. The decreased epidermal growth factor receptor and MYC expression edited by dm^{6}ACRISPR suppressed cancer cell proliferation. In the near future, dm^{6}ACRISPR targeting RNA demethylation might be used for gene repression and regulation of cellular functions in CVD. Despite the achievements, the clinical practice of small-molecule inhibitors targeting m^{6}A modulated enzymes is still in its infancy, and several issues remain to be resolved. First, the

Table II. Methyltransferases/demethylases and corresponding target RNAs in cardiovascular disease and cardiac bioprocess.

Cardiovascular diseases/bioprocess	Methyltransferases/ demethylases	Expression	Target RNAs	Mechanism	(Refs.)
VSMC differentiation	METTL3	Upregulated	-	Affecting the expression of paracrine factors	(77)
Heart failure	FTO	Upregulated	Calm1	Influencing RNA–ribosome interaction and changing Calm1 protein expression	(92)
Heart failure	FTO	Downregulated	Serca2a	Involving in regulation of calcium and contractile function during cardiac remodeling	(90)
Ischemic heart disease	METTL3	Upregulated	TFEB	Promoting the association of HNRNPD with TFEB pre-mRNA and decreasing TFEB expression, eventually impairing autophagic flux and enhancing apoptosis	(86)
Abdominal aortic aneurysm	METTL14, FTO	Upregulated	-	-	(96)
Cardiac hypertrophy and homeostasis	METTL3	Upregulated	MAP3K6, MAP4K5, MAPK14	Affecting kinase-regulated signaling pathway during cardiac hypertrophy	(81)
Vascular calcification	METTL14	Upregulated	Klotho	METTL14 promotes the degradation of arteries preventing transcripts Klotho mRNA	(101)
Hypoxic pulmonary hypertension	-	Downregulated M6a level	circXpo6, circTmtc3	M6A methylation could influence circRNA-miRNA-mRNA network	(110)
Arterial restenosis	WTAP	Upregulated	p16	VSMC proliferation and migration	(136)
Lipid metabolism	METTL3	Upregulated	-	-	(139)

high selectivity in transcripts and methylated sites needs to be tackled. Methylation patterns on transcripts may be molecular markers that recruit distinct m^{6}A readers to enter downstream metabolism, respectively. Subsequently, side-effects caused by the complex mRNAs targeted by m^{6}A enzymes cannot be ignored. Moreover, heterogeneity in human individuals also needs to be taken into account (142).

Scientists have made numerous attempts to explore the effect of m^{6}A modification on cell phenotypes and gene expression, aiming to develop novel approaches for the treatment diseases. Unveiling the mechanism of regulation of m^{6}A methylation in CVD is of significance for further understanding the pathogenesis and contribute to the diagnosis of CVD and may pave the way for the development of novel special drugs targeting the altered epigenetic marks. In addition, the rapid development of m^{6}A-seq provides a more precise localization of adenine methylation. However, the development and use of m^{6}A-targeting drugs requires further exploitation in the future.

6. Conclusions and future perspectives

m^{6}A methylation is a type of RNA epigenetics and the most abundant internal RNA modification type. m^{6}A methylation is an emerging research field that is growing rapidly, especially in CVD. The present article reviewed the role of m^{6}A and m^{6}A-SNPs in the mechanisms of various types of CVD. The observations highlight the potential role of m^{6}A methylation in the diagnosis of CVD by serving as an invasive biomarker, and lay the foundation for the accurate treatment of CVD.

m^{6}A methylation presents across different species and plays a fundamental role in cardiac biological processes and the pathogenesis of CVD. An increasing number of studies have uncovered the novel dysregulation of m^{6}A methylation as a hallmark of the development of CVDs. m^{6}A modification promotes or inhibits the development of CVD by regulating the targeted m^{6}A-modified mRNA levels. The dysregulation of methyltransferase, demethylases and m^{6}A-binding proteins plays a role in the occurrence and progression of CVD. Studies have demonstrated that the regulation of these 'writers', 'erasers' and 'readers' may alleviate the progression of cardiac diseases; these modulators might provide a novel insight into potential diagnostic biomarkers or therapeutic strategies for CVD (143). Table II and Fig. 3 summarize the role of m^{6}A modulators in CVD. Table III contains a brief introduction of m^{6}A-related SNPs and CVD.

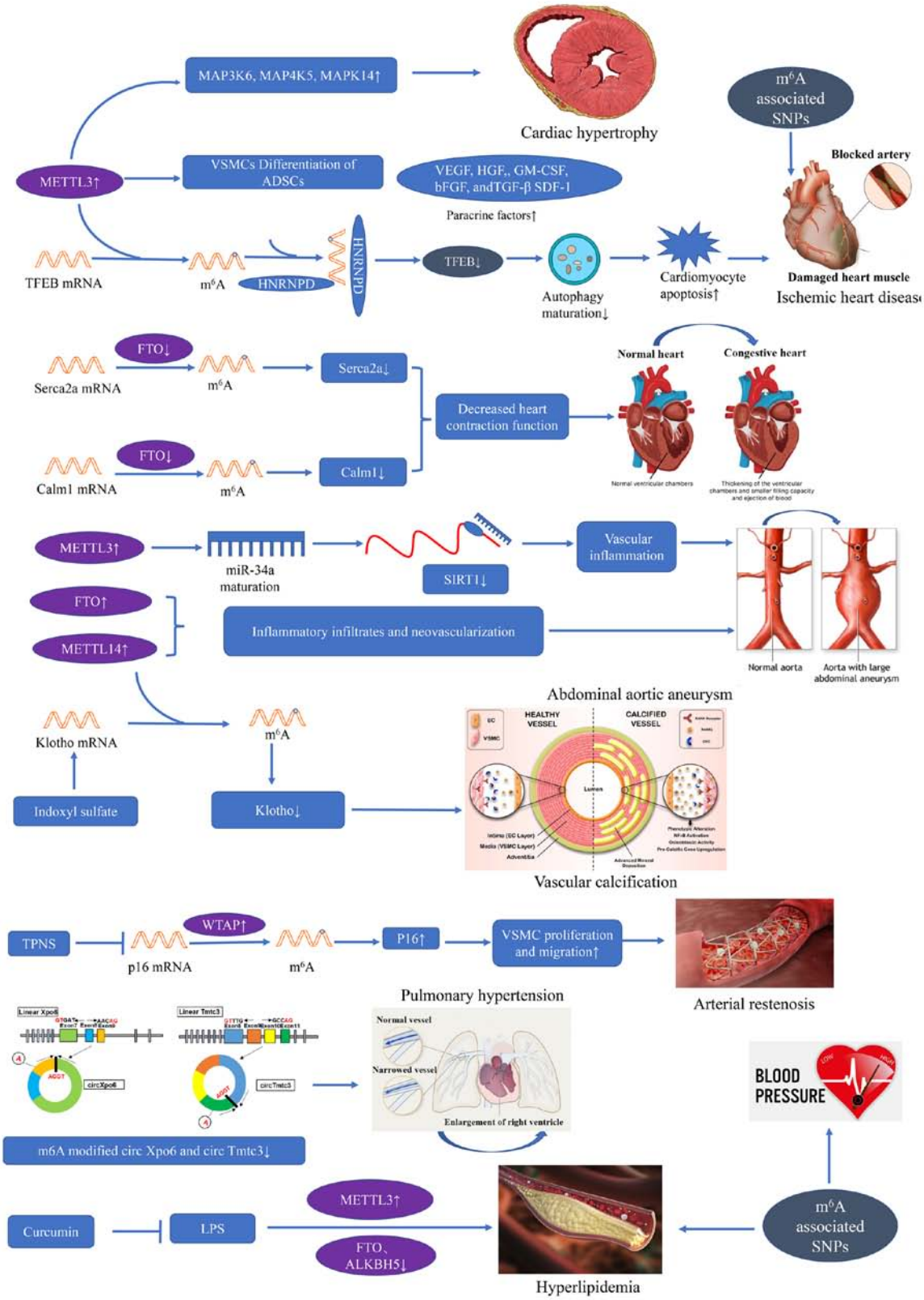

Figure 3. m^{6}A modulators involved in cardiovascular disease and bioprocess. METTL3-mediated m^{6}A modification is involved in cardiac hypertrophy by affecting kinase-regulated signaling pathway. Upregulated METTL3 and increased expression of paracrine factors, including VEGF, HGF, TGF-β, GM-CSF, bFGF and SDF-1 were found in ADSCs undergoing VSMC differentiation induction. The upregulation of METTL3 promoted the association of RNA binding protein HNRNPD with TFEB pre-mRNA and decreased TFEB expression, eventually impaired autophagic flux and enhancing apoptosis in ischemic heart disease. FTO mediated decreased expression of Calm1 and Serca2a are responsible for decreased heart contraction function and heart failure. Both elevated METTL14 and FTO are associated with inflammatory infiltration and neovascularization in abdominal aortic aneurysm. In addition, METTL3 mediated miR-34a maturation from pre-miR-34a, and then miR-34a negatively regulated the expression of SIRT1. m^{6}A-modified Klotho mRNA plays a crucial role in vascular calcification. TPNS alleviated arterial restenosis through the downregulation of m^{6}A methylation of p16. Decreased m^{6}A modified circ Xpo6 and circ Tmtc3 are found in hypoxic pulmonary hypertension. Elevated METTL3, decreased FTO and ALKBH5 are involved in the development of hyperlipidemia resulted from LPS. Curcumin exerts a protective effect on LPS induced abnormal lipid metabolism. m^{6}A associated plays a role in blood pressure, hyperlipidemia and coronary artery disease.

Table III. m^{6}A-associated SNPs and cardiovascular disease.

Cardiovascular diseases	RNP rsID	Gene	(Refs.)
Blood pressure	rs56001051	C1orf167	(124)
	rs9847953	ZNF589	
	rs197922	GOSR2	
	rs740406	DOT1L	
Hypertension and sympathetic modulation of vasomotor tone	rs9939609	FTO	(128)
	rs9302652		
Coronary artery disease	rs12286	ADAMTS7	(120)
Lipid metabolism	rs6859	PVRL2	(121)

Transcriptome-wide mapping of m^{6}A in mRNA helped us catalog m^{6}A targets and demonstrated potential epigenetic mechanisms. The functional significance of m^{6}A methylation in physiological and biological processes is gaining appreciation and has been well described, yet studies on m^{6}A and pathological conditions, especially in cardiovascular organs and tissues are still lacking and the precise role of m^{6}A in CVD remains largely unknown (81). In addition, the current research on m^{6}A methylation has species limitations, with several areas of the mechanism and function of m^{6}A methylation still unknown. The role of different modulator-mediated types of m^{6}A methylation in CVDs remains to be addressed. It still remains to be investigated whether there are interplay, synergistic and competitive effects between these modulators. The mechanisms of how m^{6}A regulates gene expression are complex and differ among different cells, and more studies need to be performed to investigate the role of m^{6}A modification in pathological conditions of CVD and the exact mechanisms in the future. Furthermore, the clinical significance of m^{6}A in CVD should be investigated further.

To date, the majority of studies focus on the mechanism of m^{6}A methylation; however, few studies have focused on m^{6}A application, particularly m^{6}A-targeting drug therapy in CVD. Future prospects need to be further explored. In addition, the detailed and precise function, as well as the specificity and sensitivity are all necessary in the development of therapeutic approaches for CVD (140). Studies on m^{6}A-SNP and CVDs may help further explore the mechanism underlying SNPs and CVDs. More m^{6}A-SNPs associated with CVD will be identified in the future using the emerging high-throughput data, which will broaden the current understanding of m^{6}A-SNPs and the pathogenesis of CVD. These m^{6}A-SNPs may become potential candidates for the detection of CVD.

In conclusion, m^{6}A modification is the most prevalent RNA methylation type. Both m^{6}A methylation and m6A-SNPs are closely associated with CVD. Previous studies have provided proof of concept for acting as biomarkers and therapeutic targets. More research is required to explore the mechanism of m^{6}A in CVD and translate the existing results into clinical application.

As a newly identified type of post-transcriptional regulation, the dynamic and reversible m^{6}A modification is the most prevalent type of internal modification of RNA methylation. m^{6}A modification plays a significant role not only in various cellular biological processes but also in the pathogenesis of CVDs. The dysregulated m^{6}A methylation has enhanced our understanding of epigenetic regulation in the pathogenesis in cardiovascular disorders. However, the investigation of m^{6}A modification in CVDs remains in its infancy and a more comprehensive understanding of the biological function of m^{6}A is needed. m^{6}A modification may further contribute to the recognition of molecular mechanisms underlying cardiovascular pathogenesis, and might provide novel insight into the potential biomarkers and therapeutic approaches for CVD in the future.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant nos. 81970237 and 81600227).

Availability of data and materials

Not applicable.

Authors' contributions

YQin, LL, EL, JH, GY, DW, YQiao and CT contributed to the conception and design of the study. YQin, LL, EL and JH searched the relevant literature. YuQ wrote the manuscript. GY, YQiao and DW provided advice and were responsible for revising the manuscript. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Stellos K: The rise of epitranscriptomic era: Implications for cardiovascular disease. Cardiovasc Res 113: e2-e3, 2017.
2. Huang H, Weng H and Chen J: m^{6}A modification in coding and non-coding RNAs: Roles and therapeutic implications in cancer. Cancer Cell 37: 270-288, 2020.
3. Desrosiers R, Friderici K and Rottman F: Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. Proc Natl Acad Sci USA 71: 3971-3975, 1974.
4. Wei CM, Gershowitz A and Moss B: Methylated nucleotides block 5' terminus of HeLa cell messenger RNA. Cell 4: 379-386, 1975.
5. Lee M, Kim B and Kim VN: Emerging roles of RNA modification: m(6)A and U-tail. Cell 158: 980-987, 2014.
6. Bhat SS, Bielewicz D, Jarmolowski A and Szweykowska-Kulinska Z: N^6-methyladenosine (m^{6}A): Revisiting the old with focus on new, an arabidopsis thaliana centered review. Genes (Basel) 9: 596, 2018.
7. Zhao W, Qi X, Liu L, Ma S, Liu J and Wu J: Epigenetic regulation of m^{6}A modifications in human cancer. Mol Ther 19: 405-412, 2020.
8. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec M, *et al*: Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature 485: 201-206, 2012.
9. Molinie B, Wang J, Lim KS, Hillebrand R, Lu ZX, Van Wittenberghe N, Howard BD, Daneshvar K, Mullen AC, Dedon P, *et al*: m(6)A-LAIC-seq reveals the census and complexity of the m(6)A epitranscriptome. Nat Methods 13: 692-698, 2016.
10. Fustin JM, Doi M, Yamaguchi Y, Hida H, Nishimura S, Yoshida M, Isagawa T, Morioka MS, Kakeya H, Manabe I and Okamura H: RNA-methylation-dependent RNA processing controls the speed of the circadian clock. Cell 155: 793-806, 2013.
11. Wang X, Lu Z, Gomez A, Hon GC, Yue Y, Han D, Fu Y, Parisien M, Dai Q, Jia G, *et al*: N6-methyladenosine-dependent regulation of messenger RNA stability. Nature 505: 117-120, 2014.
12. Meyer KD, Patil DP, Zhou J, Zinoviev A, Skabkin MA, Elemento O, Pestova TV, Qian SB and Jaffrey SR: 5' UTR m(6) A promotes cap-independent translation. Cell 163: 999-1010, 2015.
13. Alarcón CR, Lee H, Goodarzi H, Halberg N and Tavazoie SF: N6-methyladenosine marks primary microRNAs for processing. Nature 519: 482-485, 2015.
14. Chen XY, Zhang J and Zhu JS: The role of m^{6}A RNA methylation in human cancer. Mol Cancer 18: 103, 2019.
15. Edupuganti RR, Geiger S, Lindeboom RGH, Shi H, Hsu PJ, Lu Z, Wang SY, Baltissen MPA, Jansen PWTC, Rossa M, *et al*: N^6-methyladenosine (m^{6}A) recruits and repels proteins to regulate mRNA homeostasis. Nat Struct Mol Biol 24: 870-878, 2017.
16. Visvanathan A and Somasundaram K: mRNA traffic control reviewed: N6-Methyladenosine (m^{6}A) takes the driver's seat. Bioessays: Dec 4, 2017 (Epub ahead of print). doi: 10.1002/bies.201700093.
17. Meyer KD and Jaffrey SR: The dynamic epitranscriptome: N6-methyladenosine and gene expression control. Nat Rev Mol Cell Biol 15: 313-326, 2014.
18. Fu Y, Dominissini D, Rechavi G and He C: Gene expression regulation mediated through reversible m^{6}A RNA methylation. Nat Rev Genet 15: 293-306, 2014.
19. Tong J, Flavell RA and Li HB: RNA m^{6}A modification and its function in diseases. Front Med 12: 481-489, 2018.
20. Chen K, Lu Z, Wang X, Fu Y, Luo GZ, Liu N, Han D, Dominissini D, Dai Q, Pan T and He C: High-resolution N(6)-methyladenosine (m(6) A) map using photo-crosslinking-assisted m(6) A sequencing. Angew Chem Int Ed Engl 54: 1587-1590, 2015.
21. Zhou J, Wan J, Gao X, Zhang X, Jaffrey SR and Qian SB: Dynamic m(6)A mRNA methylation directs translational control of heat shock response. Nature 526: 591-594, 2015.
22. Barbieri I, Tzelepis K, Pandolfini L, Shi J, Millán-Zambrano G, Robson SC, Aspris D, Migliori V, Bannister AJ, Han N, *et al*: Promoter-bound METTL3 maintains myeloid leukaemia by m^{6}A-dependent translation control. Nature 552: 126-131, 2017.
23. Wang X and He C: Dynamic RNA modifications in posttranscriptional regulation. Mol Cell 56: 5-12, 2014.
24. Shi H, Wang X, Lu Z, Zhao BS, Ma H, Hsu PJ, Liu C and He C: YTHDF3 facilitates translation and decay of N-methyladenosine-modified RNA. Cell Res 27: 315-328, 2017.
25. Zhang S, Zhao BS, Zhou A, Lin K, Zheng S, Lu Z, Chen Y, Sulman EP, Xie K, Bögler O, *et al*: m^{6}A demethylase ALKBH5 maintains tumorigenicity of glioblastoma stem-like cells by sustaining FOXM1 expression and cell proliferation program. Cancer Cell 31: 591-606.e6, 2017.
26. Zhu S, Wang JZ, Chen D, He YT, Meng N, Chen M, Lu RX, Chen XH, Zhang XL and Yan GR: An oncopeptide regulates m^{6}A recognition by the m^{6}A reader IGF2BP1 and tumorigenesis. Nat Commun 11: 1685, 2020.
27. Wu Y, Yang X, Chen Z, Tian L, Jiang G, Chen F, Li J, An P, Lu L, Luo N, *et al*: m^{6}A-induced lncRNA RP11 triggers the dissemination of colorectal cancer cells via upregulation of Zeb1. Mol Cancer 18: 87, 2019.
28. Hess ME, Hess S, Meyer KD, Verhagen LA, Koch L, Brönneke HS, Dietrich MO, Jordan SD, Saletore Y, Elemento O, *et al*: The fat mass and obesity associated gene (Fto) regulates activity of the dopaminergic midbrain circuitry. Nat Neurosci 16: 1042-1048, 2013.
29. Du K, Zhang L, Lee T and Sun T: m(6)A RNA methylation controls neural development and is involved in human diseases. Mol Neurobiol 56: 1596-1606, 2019.
30. Wu Y, Xie L, Wang M, Xiong Q, Guo Y, Liang Y, Li J, Sheng R, Deng P, Wang Y, *et al*: Mettl3-mediated m^{6}A RNA methylation regulates the fate of bone marrow mesenchymal stem cells and osteoporosis. Nat Commun 9: 4772, 2018.
31. Kane SE and Beemon K: Precise localization of m6A in Rous sarcoma virus RNA reveals clustering of methylation sites: Implications for RNA processing. Mol Cell Biol 5: 2298-2306, 1985.
32. Roignant JY and Soller M: m^{6}A in mRNA: An ancient mechanism for fine-tuning gene expression. Trends Genet 33: 380-390, 2017.
33. Zhao Y, Shi Y, Shen H and Xie W: m^{6}A-binding proteins: The emerging crucial performers in epigenetics. J Hematol Oncol 13: 35, 2020.
34. Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, Yi C, Lindahl T, Pan T, Yang YG and He C: N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol 7: 885-887, 2011.
35. Yao QJ, Sang L, Lin M, Yin X, Dong W, Gong Y and Zhou BO: Mettl3-Mettl14 methyltransferase complex regulates the quiescence of adult hematopoietic stem cells. Cell Res 28: 952-954, 2018.
36. Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ, Vågbø CB, Shi Y, Wang WL, Song SH, *et al*: ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. Mol Cell 49: 18-29, 2013.
37. Ping XL, Sun BF, Wang L, Xiao W, Yang X, Wang WJ, Adhikari S, Shi Y, Lv Y, Chen YS, *et al*: Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. Cell Res 24: 177-189, 2014.
38. Slobodin B, Han R, Calderone V, Vrielink JAFO, Loayza-Puch F, Elkon R and Agami R: Transcription impacts the efficiency of mRNA translation via co-transcriptional N6-adenosine methylation. Cell 169: 326-337.e12, 2017.
39. Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, Ma H, Weng X, Chen K, Shi H and He C: N(6)-methyladenosine modulates messenger RNA translation efficiency. Cell 161: 1388-1399, 2015.
40. Choi J, Ieong KW, Demirci H, Chen J, Petrov A, Prabhakar A, O'Leary SE, Dominissini D, Rechavi G, Soltis SM, *et al*: N(6)-methyladenosine in mRNA disrupts tRNA selection and translation-elongation dynamics. Nat Struct Mol Biol 23: 110-115, 2016.
41. Bujnicki JM, Feder M, Radlinska M and Blumenthal RM: Structure prediction and phylogenetic analysis of a functionally diverse family of proteins homologous to the MT-A70 subunit of the human mRNA:m(6)A methyltransferase. J Mol Evol 55: 431-444, 2002.
42. Śledź P and Jinek M: Structural insights into the molecular mechanism of the m(6)A writer complex. Elife 5: e18434, 2016.
43. Wang P, Doxtader KA and Nam Y: Structural basis for cooperative function of Mettl3 and Mettl14 methyltransferases. Mol Cell 63: 306-317, 2016.
44. Schöller E, Weichmann F, Treiber T, Ringle S, Treiber N, Flatley A, Feederle R, Bruckmann A and Meister G: Interactions, localization, and phosphorylation of the m^{6}A generating METTL3-METTL14-WTAP complex. RNA 24: 499-512, 2018.

45. Yue Y, Liu J and He C: RNA N6-methyladenosine methylation in post-transcriptional gene expression regulation. Genes Dev 29: 1343-1355, 2015.
46. Liu J, Yue Y, Han D, Wang X, Fu Y, Zhang L, Jia G, Yu M, Lu Z, Deng X, *et al*: A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. Nat Chem Biol 10: 93-95, 2014.
47. Schwartz S, Mumbach MR, Jovanovic M, Wang T, Maciag K, Bushkin GG, Mertins P, Ter-Ovanesyan D, Habib N, Cacchiarelli D, *et al*: Perturbation of m6A writers reveals two distinct classes of mRNA methylation at internal and 5' sites. Cell Rep 8: 284-296, 2014.
48. Patil DP, Chen CK, Pickering BF, Chow A, Jackson C, Guttman M and Jaffrey SR: m(6)A RNA methylation promotes XIST-mediated transcriptional repression. Nature 537: 369-373, 2016.
49. Yue Y, Liu J, Cui X, Cao J, Luo G, Zhang Z, Cheng T, Gao M, Shu X, Ma H, *et al*: VIRMA mediates preferential m^6A mRNA methylation in 3'UTR and near stop codon and associates with alternative polyadenylation. Cell Discov 4: 10, 2018.
50. Knuckles P, Lence T, Haussmann IU, Jacob D, Kreim N, Carl SH, Masiello I, Hares T, Villaseñor R, Hess D, *et al*: Zc3h13/Flacc is required for adenosine methylation by bridging the mRNA-binding factor Rbm15/Spenito to the m^6A machinery component Wtap/Fl(2)d. Genes Dev 32: 415-429, 2018.
51. Tang C, Klukovich R, Peng H, Wang Z, Yu T, Zhang Y, Zheng H, Klungland A and Yan W: ALKBH5-dependent m6A demethylation controls splicing and stability of long 3'-UTR mRNAs in male germ cells. Proc Natl Acad Sci USA 115: E325-E333, 2018.
52. Boissel S, Reish O, Proulx K, Kawagoe-Takaki H, Sedgwick B, Yeo GS, Meyre D, Golzio C, Molinari F, Kadhom N, *et al*: Loss-of-function mutation in the dioxygenase-encoding FTO gene causes severe growth retardation and multiple malformations. Am J Hum Genet 85: 106-111, 2009.
53. Su R, Dong L, Li C, Nachtergaele S, Wunderlich M, Qing Y, Deng X, Wang Y, Weng X, Hu C, *et al*: R-2HG exhibits anti-tumor activity by targeting FTO/m^6A/MYC/CEBPA signaling. Cell 172: 90-105.e23, 2018.
54. A Alemu E, He C and Klungland A: ALKBHs-facilitated RNA modifications and de-modifications. DNA Repair (Amst) 44: 87-91, 2016.
55. Alarcón CR, Goodarzi H, Lee H, Liu X, Tavazoie S and Tavazoie SF: HNRNPA2B1 is a mediator of m(6)A-dependent nuclear RNA processing events. Cell 162: 1299-1308, 2015.
56. Huang H, Weng H, Sun W, Qin X, Shi H, Wu H, Zhao BS, Mesquita A, Liu C, Yuan CL, *et al*: Recognition of RNA N^6-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. Nat Cell Biol 20: 285-295, 2018.
57. Zhang Z, Theler D, Kaminska KH, Hiller M, de la Grange P, Pudimat R, Rafalska I, Heinrich B, Bujnicki JM, Allain FH and Stamm S: The YTH domain is a novel RNA binding domain. J Biol Chem 285: 14701-14710, 2010.
58. Stoilov P, Rafalska I and Stamm S: YTH: A new domain in nuclear proteins. Trends Biochem Sci 27: 495-497, 2002.
59. Liao S, Sun H and Xu C: YTH domain: A family of N^6-methyladenosine (m^6A) readers. Genomics Proteomics Bioinformatics 16: 99-107, 2018.
60. Du H, Zhao Y, He J, Zhang Y, Xi H, Liu M, Ma J and Wu L: YTHDF2 destabilizes m(6)A-containing RNA through direct recruitment of the CCR4-NOT deadenylase complex. Nat Commun 7: 12626, 2016.
61. Xiao W, Adhikari S, Dahal U, Chen YS, Hao YJ, Sun BF, Sun HY, Li A, Ping XL, Lai WY, *et al*: Nuclear m(6)A reader YTHDC1 regulates mRNA splicing. Mol Cell 61: 507-519, 2016.
62. Roundtree IA, Luo GZ, Zhang Z, Wang X, Zhou T, Cui Y, Sha J, Huang X, Guerrero L, Xie P, *et al*: YTHDC1 mediates nuclear export of N^6-methyladenosine methylated mRNAs. Elife 6: e31311, 2017.
63. Kretschmer J, Rao H, Hackert P, Sloan KE, Höbartner C and Bohnsack MT: The m^6A reader protein YTHDC2 interacts with the small ribosomal subunit and the 5'-3' exoribonuclease XRN1. RNA 24: 1339-1350, 2018.
64. Hsu PJ, Zhu Y, Ma H, Guo Y, Shi X, Liu Y, Qi M, Lu Z, Shi H, Wang J, *et al*: Ythdc2 is an N^6-methyladenosine binding protein that regulates mammalian spermatogenesis. Cell Res 27: 1115-1127, 2017.
65. Zarnack K, König J, Tajnik M, Martincorena I, Eustermann S, Stévant I, Reyes A, Anders S, Luscombe NM and Ule J: Direct competition between hnRNP C and U2AF65 protects the transcriptome from the exonization of Alu elements. Cell 152: 453-466, 2013.
66. Liu N, Zhou KI, Parisien M, Dai Q, Diatchenko L and Pan T: N6-methyladenosine alters RNA structure to regulate binding of a low-complexity protein. Nucleic Acids Res 45: 6051-6063, 2017.
67. Bell JL, Wächter K, Mühleck B, Pazaitis N, Köhn M, Lederer M and Hüttelmaier S: Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): Post-transcriptional drivers of cancer progression? Cell Mol Life Sci 70: 2657-2675, 2013.
68. Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE and Jaffrey SR: Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. Cell 149: 1635-1646, 2012.
69. You X, Vlatkovic I, Babic A, Will T, Epstein I, Tushev G, Akbalik G, Wang M, Glock C, Quedenau C, *et al*: Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. Nat Neurosci 18: 603-610, 2015.
70. Linder B, Grozhik AV, Olarerin-George AO, Meydan C, Mason CE and Jaffrey SR: Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. Nat Methods 12: 767-772, 2015.
71. Ke S, Alemu EA, Mertens C, Gantman EC, Fak JJ, Mele A, Haripal B, Zucker-Scharff I, Moore MJ, Park CY, *et al*: A majority of m6A residues are in the last exons, allowing the potential for 3' UTR regulation. Genes Dev 29: 2037-2053, 2015.
72. Liu N, Parisien M, Dai Q, Zheng G, He C and Pan T: Probing N6-methyladenosine RNA modification status at single nucleotide resolution in mRNA and long noncoding RNA. RNA 19: 1848-1856, 2013.
73. Garcia-Campos MA, Edelheit S, Toth U, Safra M, Shachar R, Viukov S, Winkler R, Nir R, Lasman L, Brandis A, *et al*: Deciphering the 'm^6A Code' via antibody-independent quantitative profiling. Cell 178: 731-747.e16, 2019.
74. Liu Q and Gregory RI: RNAmod: An integrated system for the annotation of mRNA modifications. Nucleic Acids Res 47: W548-W555, 2019.
75. Zhang SY, Zhang SW, Fan XN, Zhang T, Meng J and Huang Y: FunDMDeep-m6A: Identification and prioritization of functional differential m6A methylation genes. Bioinformatics 35: i90-i98, 2019.
76. Zhang Z, Chen LQ, Zhao YL, Yang CG, Roundtree IA, Zhang Z, Ren J, Xie W, He C and Luo GZ: Single-base mapping of mA by an antibody-independent method. Sci Adv 5: eaax0250, 2019.
77. Lin J, Zhu Q, Huang J, Cai R and Kuang Y: Hypoxia promotes vascular smooth muscle cell (VSMC) differentiation of adipose-derived stem cell (ADSC) by regulating Mettl3 and paracrine factors. Stem Cells Int 2020: 2830565, 2020.
78. Cohn JN, Ferrari R and Sharpe N: Cardiac remodeling-concepts and clinical implications: A consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. J Am Coll Cardiol 35: 569-582, 2000.
79. Kehat I and Molkentin JD: Molecular pathways underlying cardiac remodeling during pathophysiological stimulation. Circulation 122: 2727-2735, 2010.
80. Maier T, Guell M and Serrano L: Correlation of mRNA and protein in complex biological samples. FEBS Lett 583: 3966-3973, 2009.
81. Dorn LE, Lasman L, Chen J, Xu X, Hund TJ, Medvedovic M, Hanna JH, van Berlo JH and Accornero F: The N^6-Methyladenosine mRNA methylase METTL3 controls cardiac homeostasis and hypertrophy. Circulation 139: 533-545, 2019.
82. Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo Arozena A, Adachi H, Adams CM, Adams PD, Adeli K, *et al*: Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy 12: 1-222, 2016.
83. Song H, Pu J, Wang L, Wu L, Xiao J, Liu Q, Chen J, Zhang M, Liu Y, Ni M, *et al*: ATG16L1 phosphorylation is oppositely regulated by CSNK2/casein kinase 2 and PPP1/protein phosphatase 1 which determines the fate of cardiomyocytes during hypoxia/reoxygenation. Autophagy 11: 1308-1325, 2015.
84. Pastore N, Brady OA, Diab HI, Martina JA, Sun L, Huynh T, Lim JA, Zare H, Raben N, Ballabio A and Puertollano R: TFEB and TFE3 cooperate in the regulation of the innate immune response in activated macrophages. Autophagy 12: 1240-1258, 2016.
85. Zhao E and Czaja MJ: Transcription factor EB: A central regulator of both the autophagosome and lysosome. Hepatology 55: 1632-1634, 2012.
86. Song H, Feng X, Zhang H, Luo Y, Huang J, Lin M, Jin J, Ding X, Wu S, Huang H, *et al*: METTL3 and ALKBH5 oppositely regulate m^6A modification of TFEB mRNA, which dictates the fate of hypoxia/reoxygenation-treated cardiomyocytes. Autophagy 15: 1419-1437, 2019.

87. Misquitta CM, Iyer VR, Werstiuk ES and Grover AK: The role of 3'-untranslated region (3'-UTR) mediated mRNA stability in cardiovascular pathophysiology. Mol Cell Biochem 224: 53-67, 2001.
88. Gratacós FM and Brewer G: The role of AUF1 in regulated mRNA decay. Wiley Interdiscip Rev RNA 1: 457-473, 2010.
89. Su YR, Chiusa M, Brittain E, Hemnes AR, Absi TS, Lim CC and Di Salvo TG: Right ventricular protein expression profile in end-stage heart failure. Pulm Circ 5: 481-497, 2015.
90. Mathiyalagan P, Adamiak M, Mayourian J, Sassi Y, Liang Y, Agarwal N, Jha D, Zhang S, Kohlbrenner E, Chepurko E, *et al*: FTO-Dependent N^6-Methyladenosine regulates cardiac function during remodeling and repair. Circulation 139: 518-532, 2019.
91. Wang Y, Li Y, Toth JI, Petroski MD, Zhang Z and Zhao JC: N6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. Nat Cell Biol 16: 191-198, 2014.
92. Berulava T, Buchholz E, Elerdashvili V, Pena T, Islam MR, Lbik D, Mohamed BA, Renner A, von Lewinski D, Sacherer M, *et al*: Changes in m6A RNA methylation contribute to heart failure progression by modulating translation. Eur J Heart Fail 22: 54-66, 2020.
93. Kmietczyk V, Riechert E, Kalinski L, Boileau E, Malovrh E, Malone B, Gorska A, Hofmann C, Varma E, Jürgensen L, *et al*: m^{6}A-mRNA methylation regulates cardiac gene expression and cellular growth. Life Sci Alliance 2: e201800233, 2019.
94. Hirsch AT, Haskal ZJ, Hertzer NR, Bakal CW, Creager MA, Halperin JL, Hiratzka LF, Murphy WR, Olin JW, Puschett JB, *et al*: ACC/AHA 2005 Practice Guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): A collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease): Endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. Circulation 113: e463-e654, 2006.
95. Reeps C, Pelisek J, Seidl S, Schuster T, Zimmermann A, Kuehnl A and Eckstein HH: Inflammatory infiltrates and neovessels are relevant sources of MMPs in abdominal aortic aneurysm wall. Pathobiology 76: 243-252, 2009.
96. He Y, Xing J, Wang S, Xin S, Han Y and Zhang J: Increased m6A methylation level is associated with the progression of human abdominal aortic aneurysm. Ann Transl Med 7: 797, 2019.
97. Zhong L, He X, Song H, Sun Y, Chen G, Si X, Sun J, Chen X, Liao W, Liao Y and Bin J: METTL3 induces AAA development and progression by modulating N6-methyladenosine-dependent primary miR34a processing. Mol Ther Nucleic Acids 21: 394-411, 2020.
98. Thomas B, Matsushita K, Abate KH, Al-Aly Z, Ärnlöv J, Asayama K, Atkins R, Badawi A, Ballew SH, Banerjee A, *et al*: Global Cardiovascular and Renal Outcomes of Reduced GFR. J Am Soc Nephrol 28: 2167-2179, 2017.
99. Fang Y, Ginsberg C, Sugatani T, Monier-Faugere MC, Malluche H and Hruska KA: Early chronic kidney disease-mineral bone disorder stimulates vascular calcification. Kidney Int 85: 142-150, 2014.
100. Cao XS, Chen J, Zou JZ, Zhong YH, Teng J, Ji J, Chen ZW, Liu ZH, Shen B, Nie YX, *et al*: Association of indoxyl sulfate with heart failure among patients on hemodialysis. Clin J Am Soc Nephrol 10: 111-119, 2015.
101. Chen J, Ning Y, Zhang H, Song N, Gu Y, Shi Y, Cai J, Ding X and Zhang X: METTL14-dependent m6A regulates vascular calcification induced by indoxyl sulfate. Life Sci 239: 117034, 2019.
102. McLaughlin VV, Archer SL, Badesch DB, Barst RJ, Farber HW, Lindner JR, Mathier MA, McGoon MD, Park MH, Rosenson RS, *et al*: ACCF/AHA 2009 expert consensus document on pulmonary hypertension a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association developed in collaboration with the American College of Chest Physicians; American Thoracic Society, Inc.; and the Pulmonary Hypertension Association. J Am Coll Cardiol 53: 1573-1619, 2009.
103. Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk Noordegraaf A, Beghetti M, *et al*: 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). Eur Heart J 37: 67-119, 2016.
104. Weitzenblum E, Sautegeau A, Ehrhart M, Mammosser M and Pelletier A: Long-term oxygen therapy can reverse the progression of pulmonary hypertension in patients with chronic obstructive pulmonary disease. Am Rev Respir Dis 131: 493-498, 1985.
105. Shi Y, Fan S, Wu M, Zuo Z, Li X, Jiang L, Shen Q, Xu P, Zeng L, Zhou Y, *et al*: YTHDF1 links hypoxia adaptation and non-small cell lung cancer progression. Nat commun 10: 4892, 2019.
106. Zhang C, Samanta D, Lu H, Bullen JW, Zhang H, Chen I, He X and Semenza GL: Hypoxia induces the breast cancer stem cell phenotype by HIF-dependent and ALKBH5-mediated m^{6}A-demethylation of NANOG mRNA. Proc Natl Acad Sci USA 113: E2047-E2056, 2016.
107. Fry NJ, Law BA, Ilkayeva OR, Holley CL and Mansfield KD: N^6-methyladenosine is required for the hypoxic stabilization of specific mRNAs. RNA 23: 1444-1455, 2017.
108. Zhou C, Molinie B, Daneshvar K, Pondick JV, Wang J, Van Wittenberghe N, Xing Y, Giallourakis CC and Mullen AC: Genome-wide maps of m6A circRNAs identify widespread and cell-type-specific methylation patterns that are distinct from mRNAs. Cell Rep 20: 2262-2276, 2017.
109. Wang J, Zhu MC, Kalionis B, Wu JZ, Wang LL, Ge HY, Chen CC, Tang XD, Song YL, He H and Xia SJ: Characteristics of circular RNA expression in lung tissues from mice with hypoxiainduced pulmonary hypertension. Int J Mol Med 42: 1353-1366, 2018.
110. Su H, Wang G, Wu L, Ma X, Ying K and Zhang R: Transcriptome-wide map of m^{6}A circRNAs identified in a rat model of hypoxiamediated pulmonary hypertension. BMC Genomics 21: 39, 2020.
111. Chan JJ and Tay Y: Noncoding RNA: RNA regulatory networks in cancer. Int J Mol Sci 19: 2018.
112. Baarsma HA and Königshoff M: 'WNT-er is coming': WNT signalling in chronic lung diseases. Thorax 72: 746-759, 2017.
113. Savai R, Al-Tamari HM, Sedding D, Kojonazarov B, Muecke C, Teske R, Capecchi MR, Weissmann N, Grimminger F, Seeger W, *et al*: Pro-proliferative and inflammatory signaling converge on FoxO1 transcription factor in pulmonary hypertension. Nat Med 20: 1289-1300, 2014.
114. Haraksingh RR and Snyder MP: Impacts of variation in the human genome on gene regulation. J Mol Biol 425: 3970-3977, 2013.
115. Mao F, Xiao L, Li X, Liang J, Teng H, Cai W and Sun ZS: RBP-Var: A database of functional variants involved in regulation mediated by RNA-binding proteins. Nucleic Acids Res 44: D154-D163, 2016.
116. Wu X and Hurst LD: Determinants of the usage of splice-associated cis-Motifs predict the distribution of human pathogenic SNPs. Mol Biol Evol 33: 518-529, 2016.
117. Ramaswami G, Deng P, Zhang R, Anna Carbone M, Mackay TFC and Billy Li J: Genetic mapping uncovers cis-regulatory landscape of RNA editing. Nat Commun 6: 8194, 2015.
118. Zheng Y, Nie P, Peng D, He Z, Liu M, Xie Y, Miao Y, Zuo Z and Ren J: m6AVar: A database of functional variants involved in m6A modification. Nucleic Acids Res 46: D139-D145, 2018.
119. Yang N, Ying P, Tian J, Wang X, Mei S, Zou D, Peng X, Gong Y, Yang Y, Zhu Y, *et al*: Genetic variants in m6A modification genes are associated with esophageal squamous-cell carcinoma in the Chinese population. Carcinogenesis 41: 761-768, 2020.
120. Mo XB, Lei SF, Zhang YH and Zhang H: Detection of m^{6}A-associated SNPs as potential functional variants for coronary artery disease. Epigenomics 10: 1279-1287, 2018.
121. Mo X, Lei S, Zhang Y and Zhang H: Genome-wide enrichment of m^{6}A-associated single-nucleotide polymorphisms in the lipid loci. Pharmacogenomics J 19: 347-357, 2019.
122. Kupper N, Willemsen G, Riese H, Posthuma D, Boomsma DI and de Geus EJC: Heritability of daytime ambulatory blood pressure in an extended twin design. Hypertension 45: 80-85, 2005.
123. Evangelou E, Warren HR, Mosen-Ansorena D, Mifsud B, Pazoki R, Gao H, Ntritsos G, Dimou N, Cabrera CP, Karaman I, *et al*: Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. Nat Genet 50: 1412-1425, 2018.

124. Mo XB, Lei SF, Zhang YH and Zhang H: Examination of the associations between m^{6}A-associated single-nucleotide polymorphisms and blood pressure. Hypertens Res 42: 1582-1589, 2019.
125. Lee JY, Lee BS, Shin DJ, Woo Park K, Shin YA, Joong Kim K, Heo L, Young Lee J, Kyoung Kim Y, Jin Kim Y, *et al*: A genome-wide association study of a coronary artery disease risk variant. J Hum Genet 58: 120-126, 2013.
126. Gerken T, Girard CA, Tung YC, Webby CJ, Saudek V, Hewitson KS, Yeo GS, McDonough MA, Cunliffe S, McNeill LA, *et al*: The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 318: 1469-1472, 2007.
127. Guyenet PG: The sympathetic control of blood pressure. Nat Rev Neurosci 7: 335-346, 2006.
128. Pausova Z, Syme C, Abrahamowicz M, Xiao Y, Leonard GT, Perron M, Richer L, Veillette S, Smith GD, Seda O, *et al*: A common variant of the FTO gene is associated with not only increased adiposity but also elevated blood pressure in French Canadians. Circ Cardiovasc Genet 2: 260-269, 2009.
129. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, *et al*: A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316: 889-894, 2007.
130. Marcadenti A, Fuchs FD, Matte U, Sperb F, Moreira LB and Fuchs SC: Effects of FTO RS9939906 and MC4R RS17782313 on obesity, type 2 diabetes mellitus and blood pressure in patients with hypertension. Cardiovasc Diabetol 12: 103, 2013.
131. O'Donnell CJ and Nabel EG: Genomics of cardiovascular disease. N Engl J Med 365: 2098-2109, 2011.
132. Prospective Studies Collaboration, Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J, Qizilbash N, Peto R and Collins R: Blood cholesterol and vascular mortality by age, sex, and blood pressure: A meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. Lancet 370: 1829-1839, 2007.
133. Yadav PK, Rajvanshi PK and Rajasekharan R: The role of yeast m^{6}A methyltransferase in peroxisomal fatty acid oxidation. Curr Genet 64: 417-422, 2018.
134. Ma S, Chen C, Ji X, Liu J, Zhou Q, Wang G, Yuan W, Kan Q and Sun Z: The interplay between m6A RNA methylation and noncoding RNA in cancer. J Hematol Oncol 12: 121, 2019.
135. Zhang W, Chen G and Deng CQ: Effects and mechanisms of total Panax notoginseng saponins on proliferation of vascular smooth muscle cells with plasma pharmacology method. J Pharm Pharmracol 64: 139-145, 2012.
136. Zhu B, Gong Y, Shen L, Li J, Han J, Song B, Hu L, Wang Q and Wang Z: 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. Biomed Pharmacother 124: 109935, 2020.
137. Nakarai H, Yamashita A, Nagayasu S, Iwashita M, Kumamoto S, Ohyama H, Hata M, Soga Y, Kushiyama A, Asano T, *et al*: Adipocyte-macrophage interaction may mediate LPS-induced low-grade inflammation: Potential link with metabolic complications. Innate Immun 18: 164-170, 2012.
138. Rao DS, Sekhara NC, Satyanarayana MN and Srinivasan M: Effect of curcumin on serum and liver cholesterol levels in the rat. J Nutri 100: 1307-1315, 1970.
139. Lu N, Li X, Yu J, Li Y, Wang C, Zhang L, Wang T and Zhong X: Curcumin attenuates lipopolysaccharide-induced hepatic lipid metabolism disorder by modification of m^6 A RNA methylation in piglets. Lipids 53: 53-63, 2018.
140. Huang Y, Yan J, Li Q, Li J, Gong S, Zhou H, Gan J, Jiang H, Jia GF, Luo C and Yang CG: Meclofenamic acid selectively inhibits FTO demethylation of m6A over ALKBH5. Nucleic Acids Res 43: 373-384, 2015.
141. Li J, Chen Z, Chen F, Xie G, Ling Y, Peng Y, Lin Y, Luo N, Chiang CM and Wang H: Targeted mRNA demethylation using an engineered dCas13b-ALKBH5 fusion protein. Nucleic Acids Res 48: 5684-5694, 2020.
142. Lawson DA, Kessenbrock K, Davis RT, Pervolarakis N and Werb Z: Tumour heterogeneity and metastasis at single-cell resolution. Nat Cell Biol 20: 1349-1360, 2018.
143. Paramasivam A, Vijayashree Priyadharsini J and Raghunandhakumar S: N6-adenosine methylation (m6A): A promising new molecular target in hypertension and cardiovascular diseases. Hypertens Res 43: 153-154, 2020.