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

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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.

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 nuclear 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

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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 α-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...
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 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 mannered (39). Conversely, 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 HNRNPQ. 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). HNRNPQ 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...
method for transcriptome-wide m\textsuperscript{6}A localization, and m\textsuperscript{6}A methylated RNA was immunoprecipitated, followed by next-generation sequencing. m\textsuperscript{6}A-seq and MeRIP-seq identified thousands of mRNAs with m\textsuperscript{6}A modification and revealed that m\textsuperscript{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\textsuperscript{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\textsuperscript{6}A sites could not be identified.

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

Other revolutionary technological methods include photo-crosslinking-assisted m\textsuperscript{6}A-seq (69), m\textsuperscript{6}A individual-nucleotide-resolution cross-linking and immunoprecipitation (70) and ultraviolet (UV) CLIP (71). In these methods, UV strengthens the interaction between m\textsuperscript{6}A-modified RNA and m\textsuperscript{6}A antibodies, and affinity purification was performed. m\textsuperscript{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\textsuperscript{6}A modification site and the m\textsuperscript{6}A modification level of the whole transcriptome. However, although highly accurate, it is also costly and time-consuming, limiting its application in m\textsuperscript{6}A methylation detection (72). Other methods include MAZTER-seq, RNAmod, FunDMDeep-m\textsuperscript{6}A and m\textsuperscript{6}A-REF-seq (72-76). Table I summarizes the methods of m\textsuperscript{6}A methylation detection (7). These findings have provided insight into the mammalian genome-wide mapping of m\textsuperscript{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\textsuperscript{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\textsuperscript{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...
4. m^A and cardiovascular disease

There is emerging evidence to indicate that m^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^A modification in CVD is briefly reviewed.

**m^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 stimulation initially induces adaptive hypertrophic response 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).

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

| Methods           | Features                        |
|-------------------|---------------------------------|
| m^A-Seq           | High throughput                 |
| MeRIP-seq         | High throughput                 |
| m^A-LAIC-seq      | High throughput, precise        |
| PA-m^A-Seq        | High throughput, single site    |
| mCLIP             | High throughput, single site    |
| m^A-CLIP          | High throughput, single site    |
| SCARLET           | High throughput, single site    |
| MAZTER-seq        | High throughput, single site    |
| RNAmod            | High throughput, single site    |
| FunDMDeep-m^A     | High throughput, single site    |
| DART-seq          | High throughput, single site    |
| RNA sequencing    | High throughput, single site    |
| m^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).

**m^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^A in autophagy and discovered that m^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^A-modified RNA (25), and an increased 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^A modification in ischemic heart disease.

**m^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^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^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 infarction. 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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{6}A modified transcripts in heart failure are mainly involved in metabolism and cardiac signaling (92). Previous research has reported that m\textsuperscript{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\textsuperscript{6}A modification, while Calm1 protein expression is significantly decreased in failed heart tissue, indicating that m\textsuperscript{6}A methylation affects Calm1 translation rather than transcription in heart failure development. m\textsuperscript{6}A-seq has revealed differentially methylated transcripts of epigenetic proteins, transcription factors and upstream regulators of signaling pathways, indicating that m\textsuperscript{6}A methylation is possibly involved in the regulation of gene expression in heart failure (92). These data suggest that the modulation of m\textsuperscript{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\textsuperscript{6}A modification in dilated cardiac tissue and observed an increased m\textsuperscript{6}A methylation in mRNAs isolated from dilated cardiomyopathy samples. m\textsuperscript{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 C57BL/6/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\textsuperscript{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\textsuperscript{6}A methylation in failing hearts, as compared with healthy hearts, indicating that the aberrantly m\textsuperscript{6}A methylation level may serve as a potential biomarker and therapeutic target by modulating m\textsuperscript{6}A-modulators and downstream genes.

m\textsuperscript{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\textsuperscript{6}A modification in AAA and provided a potential epigenetic mechanism in AAA (96). They discovered that m\textsuperscript{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\textsuperscript{6}A modification. A higher m\textsuperscript{6}A level was also found to be associated with a higher risk of rupture. They subsequently discovered significant correlations between the m\textsuperscript{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\textsuperscript{6}A level was found to be positively associated with the AAA diameter and hematological parameters (96). Hence, abnormal m\textsuperscript{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\textsuperscript{−/−} 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\textsuperscript{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\textsuperscript{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
A significantly elevated m⁶A 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 m⁶A 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).

m⁶A 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 m⁶A methylation and may promote tumor occurrence and development. The effects of hypoxia on m⁶A modification are cell type-dependent (86,105-107). Little was known of m⁶A modification in circular RNAs (circRNAs), until thousands of m⁶A-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 m⁶A level of circRNAs in HPH rat lung tissue was lower than that in healthy rat lung tissue. m⁶A abundance in circRNAs was also decreased in hypoxia in vitro. The distribution of total circRNAs, m⁶A-circRNAs and non-m⁶A circRNAs was similar between the HPH and control groups. m⁶A 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 m⁶A level. m⁶A-modified circRNAs 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 miRNAs 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 m⁶A 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 m⁶A-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 m⁶A methylation in HPH remain to be elucidated.

m⁶A-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 m⁶A methylation by altering the RNA sequences of target sites or key flanking nucleotides (118). Putative m⁶A-related SNPs (m⁶A-SNPs), which are close to or at the exact methylation site, can disrupt m⁶A modification and corresponding biological processes through multiple mechanisms, due to the location of m⁶A-SNPs, and can subsequently affect disease progression (119). m⁶A-SNPs have been recognized as a fundamental class of crucial multifunctional genetic variants associated with CVDs, which is concerning. Numerous m⁶A-SNPs associated with CVDs have been identified using genome-wide association studies (GWAS) and the genetic functional mechanisms have been explored. These m⁶A-SNPs and genes may be candidates and promote novel therapeutic approaches (120,121). The present study reviewed the relevant research on m⁶A-SNPs and CVD.

m⁶A-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 (m⁶Avar), which is a useful tool for the investigation of the association between m⁶A-related variants and disease. A previous study first made efforts to investigate the association between m⁶A-SNPs and BP by excavating data from a large-scale GWAS, and demonstrated that m⁶A-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 m⁶A-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.
m6A-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 m6A-SNPs on CAD was previously explored and 304 m6A-SNPs were found to be associated with CAD (120). Some m6A-SNPs not only alter m6A methylation and local gene expression, but also regulate protein binding, indicating that m6A-SNPs may be functional variants of CAD. Among these, rs12286 was markedly associated with CAD. Mechanistically, rs12286 may influence the m6A methylation level and regulate the expression of downstream gene ADAMTS7 to exert its function (120). On the whole, these findings elucidated the role of m6A-SNPs in CAD; however, the specific regulatory mechanisms remain to be explored.

m6A-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 m6A modification in lipid metabolism has been reported (133). Mo et al (121) investigated the effect of m6A-SNPs on lipid levels using GWAS summary datasets of 188,578 individuals. m6A-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 m6A-SNPs (121). Further studies are required to elucidate the mechanisms.

Further large-scale GWASs are required to identify m6A-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 m6A 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 m6A editing through specific METTL3 inhibition contributed to eliciting circadian period elongation and RNA processing delay (10).

m6A as a potential biomarker. A number of studies have suggested that aberrant m6A modifications affect the progression of CVD. For instance, significantly elevated METTL3-modulated m6A methylation has been found in cardiac hypertrophy, ischemic heart disease, AAA, etc. (81,86,97). In addition, innovations in the detection technology for m6A modification render it a potential diagnostic biomarker of CVD. The early detection of upregulated m6A 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 infarction (90). On the whole, m6A methylation may serve as a prospective non-invasive diagnostic biomarker and be used for the diagnosis and prognosis of CVD.

m6A as a therapeutic target of CVD. Accumulated research has indicated that m6A methylation is of importance in CVD, providing novel insight into the innovative therapeutic strategy of targeting related methyltransferases, demethylases, m6A-binding proteins or m6A-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 m6A 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 m6A modification. Therefore, WTAP and m6A 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 m6A 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 m6A-related modulators, including METTL3, METTL14, ALKBH5, FTO and YTHDF2. Further upregulated m6A 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 m6A RNA modification (139). The precise mechanisms of how curcumin affects gene-specific m6A modification require further investigation. Collectively, the findings indicate that m6A could be a promising therapeutic target for hyperlipidemia.

The development of m6A 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 m6A-containing substrate binding.

Of note, Li et al (141) successfully constructed an in vivo manipulation CRISPR-Cas13b-based tool for the study of m6A-related biological functions and targeted demethylation of specific mRNA. They created a gratifying fusion protein, dm6ACRISPR, by linking a catalytically inactive enzyme to ALKBH5. dm6ACRISPR could specifically demethylate transcripts with m6A 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 dm6AcRISPR, by linking a catalytically inactive enzyme to ALKBH5. dm6ACRISPR 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 m6A-modulated enzymes is still in its infancy, and several issues remain to be resolved. First, the
high selectivity in transcripts and methylated sites needs to be tackled. Methylation patterns on transcripts may be molecular markers that recruit distinct m6A readers to enter downstream metabolism, respectively. Subsequently, side-effects caused by the complex mRNAs targeted by m6A 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 m6A modification on cell phenotypes and gene expression, aiming to develop novel approaches for the treatment of cardiovascular diseases. Unveiling the mechanism of regulation of m6A methylation in CVD is of significance for further understanding the pathogenesis and contribute to the diagnosis of cardiovascular disease and may pave the way for the development of novel special drugs targeting the altered epigenetic marks. In addition, the rapid development of m6A-seq provides a more precise localization of adenine methylation. However, the development and use of m6A-targeting drugs requires further exploitation in the future.

6. Conclusions and future perspectives

m6A methylation is a type of RNA epigenetics and the most abundant internal RNA modification type. m6A methylation is an emerging research field that is growing rapidly, especially in CVD. The present article reviewed the role of m6A and m6A-SNPs in the mechanisms of various types of CVD. The observations highlight the potential role of m6A methylation in the diagnosis of CVD by serving as an invasive biomarker, and lay the foundation for the accurate treatment of CVD.

m6A 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 m6A methylation as a hallmark of the development of CVDs. m6A modification promotes or inhibits the development of m6A-modified mRNA levels. The dysregulation of methyltransferase, demethylases and m6A-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 m6A regulators in CVD. Table III contains a brief introduction of m6A-related SNPs and CVD.

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| MAP3K6, MAP4K5, MAPK14 | - | (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| 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)  |
Figure 3. m^6A modulators involved in cardiovascular disease and bioprocess. METTL3-mediated m^6A 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^6A-modified Klotho mRNA plays a crucial role in vascular calcification. TPMS alleviated arterial restenosis through the downregulation of m^6A methylation of p16. Decreased m^6A 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^6A associated plays a role in blood pressure, hyperlipidemia and coronary artery disease.
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.

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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

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Competing interests

The authors declare that they have no competing interests.
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