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RNA modification by M6A methylation in cardiovascular diseases: Current trends and future directions

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Abstract

N6-methyladenosine (M6A) is the most common modification in eukaryotic RNAs for the regulation of RNA transcription, processing, splicing, degradation, and translation. RNA modification by M6A is dynamically reversible, involving methylated transferase, demethylase, and methylated reading protein. M6A-mediated gene regulation involves cell differentiation, metastasis, apoptosis, and proliferation. Dysregulation of M6A can lead to various diseases. Cardiovascular disease (CVD) seriously endangers human health and brings great social burden. Seeking effective prevention and treatment strategies for CVD is a challenge to both fundamentalists and clinicians. Substantial evidence has suggested the key role of M6A modification in the development of CVDs. This review summarizes the mechanism of M6A RNA modification and the latest research progress in respect with its role in CVDs, including atherosclerosis, coronary artery disease, myocardial infarction and cardiac remodeling, myocardial ischemia-reperfusion injury, heart failure, hypertension, and aortic aneurysm, and the potential applications of the findings to CVDs, thereby providing new ideas and approaches for the diagnosis and therapy of CVDs.

Keywords

RNA modification; M6A methylation; cardiovascular disease; epigenetics

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1 Introduction

Cardiovascular disease (CVD) is the world's biggest killer, accounting for 16% of all deaths worldwide. In 2019, deaths from CVDs increase to 8.9 million. By 2020, the number of CVDs in China has reached 330 million[1-2]. The prevalence of unhealthy diets, physical inactivity, smoking and other lifestyle risk factors, as well as the absolute number of people with hypertension, dyslipidemia, diabetes, and obesity continues to climb, will further increase the incidence and mortality of CVDs[2]. CVDs are caused by the long-term combined effects of many adverse factors. Traditional risk factors include high blood pressure (BP), hyperlipidemia, smoking, alcohol abuse, diabetes, physical inactivity, overweight/obesity, and genetic factors. In recent years, increasing evidence that certain environmental risk factors, such as air pollution and extreme temperature, can also increase morbidity and mortality of CVDs. A significant seasonal pattern exists in CVDs, that is, morbidity and mortality are higher in winter than in summer[3-5]. Cold weather is a major contributor to the burden of temperature-related cardiovascular deaths[6]. The low temperature in the Czech Republic in the winter of 1987 resulted in 274 excess deaths from CVDs[7]. The diurnal temperature range (DTR) is commonly used to evaluate the effect of temperature on CVDs. The relationship between DTR and CVD morbidity and mortality is non-linear[8]. Statistically notable differences in the incidence of myocardial infarction (MI) were noticed and attributed to geographic and weather variations. The short-term risk of MI was significantly increased at lower temperatures[9], and the incidence of acute myocardial infarction (AMI) increased by 1.6% with temperature drop by 1°C below 15°C[10]. In addition, low temperature can aggravate lung disease and promote pulmonary arterial hypertension (PAH)[11]. Considering that climate also plays a significant role in CVDs, some experts suggest that the effect of temperature should be considered in the prevention of cardiac events[9]. CVDs pose a significant risk to human health, and the costs associated with the treatment of CVDs are increasing rapidly, resulting in a growing global social and economic burden. CVD prevention and treatment have become a major public health challenge[12]. Research on the prevention and treatment of CVDs is of great significance in reducing the incidence of CVDs and improving patients' quality of life. With the development of science and

technology, basic research and clinical treatment of CVDs have been rapidly advancing. However, the pathogenesis of CVDs remains yet to be thoroughly elucidated and the currently available treatment regimens are limited to only some of the conditions, so new therapeutic strategies and targets are still in urgent need.

Heredity and epigenetic inheritance are important causes of CVDs[13]. To date, many pathogenic or susceptibility genes for CVDs have been identified[14], and many of them have been developed as effective therapeutic targets for clinical treatment, such as *Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9)*[15-16], *interleukin 6 (IL-6)*[17]and *interleukin-1β (IL-1β)*[18-19]. Epigenetics is a new area of scientific research and application on how cells control gene activity without changing the DNA sequence and increasing studies have confirmed the important role of epigenetics in CVDs[20]. Epigenetics involves modifications of DNA, RNA, and protein, with DNA and histone modifications as early focuses and RNA modification as an emerging field[13]. Although RNA modifications have been known for decades, progress in this field has been slow and little has been known about their specific functionalities. Not until recent years with the advances in highly specific antibody, high throughput sequencing technology, methyled RNA immunoprecipitation (MeRIP), cross-linking immunoprecipitation (CLIP), m6A individual-nucleotide-resolution cross-linking and immunoprecipitation (miCLIP), and liquid chromatography tandem mass spectrometry (LC-MS/MS), has the study of RNA modification, especially N6-methyladenosine (M6A) RNA modification, led us to better understanding of the underlying mechanisms of diseases, such as cancer, neurological diseases, metabolic abnormalities as well as CVDs[21-22]. Meanwhile, a growing number of studies has also highlighted the important role of M6A modification in CVDs[23]. This review summarizes the mechanism of M6A RNA modification and its latest research progress in CVDs and prospects its potential applications of the findings in CVDs, thereby providing new ideas and approaches for the diagnosis and therapy of CVDs.

2 M6A RNA modification

Fifty years ago, a variety of base modifications RNA began to be uncovered[24]. Most of these base modifications were initially found in non-coding RNAs such as tRNA and rRNA. Later, it was found that many base modifications also exist in mRNAs[25]. Now, more than 100 different RNA modifications have been discovered. In eukaryotes, 5' cap and 3' poly A modifications take part in transcriptional regulation, while the internal modification of mRNA help maintain the stability of mRNA. Earlier studies on RNA modification focused on mRNA 5' cap, which contributes to the maintenance of mRNA stability, shearing of mRNA precursors, polyadenylation, mRNA transport, and translation initiation. The modification of 3' poly A contributes to nuclear extranuclear transport and translation initiation, as well as structure stability of mRNA in conjunction with poly A-binding proteins. In recent years, internal modification of mRNAs has begun to attract more and more attentions, including M6A, N1-methyladenosine (M1A), 5-methylcytidine (M5C), and ψ, *etc*. M6A is a common base modification on mRNA, accounting for 80% of the RNA base methylation modification and it has recently become a research hotspot. Although research on RNA methylation began in the 1970s, it has been hampered due to the lack of efficient analytical tools. It is only in recent years that breakthroughs in methylation modification particularly M6A were witnessed[25-26]. As the most common modification in eukaryotes, M6A regulates RNA transcription, processing, splicing, degradation, and translation. M6A modification is reversible, involving methylated transferase, demethylase, and methylated reading protein. Up to now, many enzymes involved in M6A modification have been identified, of which methyltransferases include METTL3/14/16, RBM15/15B, WTAP and KIAA1429/Virilizer, whose main role is catalyzing M6A methylation, while demethylases mainly include FTO and ALKHB5, which demethylate the M6A modified base[27]. Reading proteins include YTH domain proteins, heterogeneous nuclear ribonucleoprotein (hnRNP), eukaryotic initiation factors (eIF) and insulin like growth factor 2 mRNA-binding proteins (IGF2BPs), which recognize the M6A modified base, thus activating downstream regulatory pathways such as RNA degradation and processing. More than 7 000 genes in humans have M6A methylation locus[28] in various classes of RNAs, including mRNA, miRNA, snRNA and lncRNA[29]. M6A regulates gene expression and participates in cell differentiation, metastasis, apoptosis, and proliferation. Dysregulation of M6A may lead to various diseases such as cancer and CVDs[30-31].

2.1 M6A methyltransferase

M6A methyltransferase, designated as a Writer, is a vital catalyticase that enables M6A methylation of bases in RNA. METTL3, METTL14, WTAP and KIAA1492 are the core proteins of M6A methyltransferase[32-33]. These proteins form complexes to perform their catalytic functions together. METTL3 and METTL14 proteins have key catalytic domains and can form hetero complexes, of which, METTL3 accounts for the catalytic activity and METTL14 plays a key role in substrate recognition[32]. WTAP, RBM15/15B, KIAA1429/Virilizer and other factors are also important components of this complex, in which WTAP plays key roles in the recruitment of METTL3 and METTL14. Another methyltransferase, METTL16, can target U6 snRNA and MAT2A mRNA[34]. These proteins work together to methylate adenosine both *in vivo* and *in vitro*. In addition to

mammals like humans and mice, similar homologous proteins have been found in fruit flies, yeast and even Arabidopsis thaliana. M6A methyltransferase is extensively involved in tumorigenesis and metastasis, immunity, stem cell renewal, and adipose differentiation, as well as other biological and cellular processes.

2.2 M6A demethylase

M6A demethylase, designated as an Eraser, was initially identified in eukaryotes as FTO and ALKBH5. FTO is a member of Alkb protein family and is closely related to fat development. In 2007, three separate cohort studies identified FTO gene mutation as a risk factor of obesity[35-37]. Similarly, in mouse models, *FTO* knockout or overexpression significantly changed the body weight of mice[38-39]. Until 2011, professor Chuan He firstly proposed that FTO protein is an important demethylase for both DNA and RNA[40]. FTO protein is similar to the Alkb protein family in the core domain, but the unique long loop at the C-terminal is different from other Alkb proteins. It is this long-loop domain that enables FTO to demethylate methylated single-stranded DNA/RNA, primarily at the M6A site within the gene. Transcriptional anomalies of FTO can result in a variety of diseases. ALKBH5 is the second important M6A demethylase discovered after FTO. ALKBH5, like FTO, is also a member of Alkb protein family, but different from other Alkb family members in substrate specificity, ALKBH5 only demethylated M6A modification on single-stranded DNA/RNA. It has an alanine rich zone at its N-end and a unique coiled-coil structure, which is capable of demethylation modification of mRNA in the nucleus[41]. Knocking down *ALKBH5* significantly increased M6A modification level on mRNA in cell lines. It plays important regulatory roles in the processing of mRNA and the development of sperm in mice[41].

2.3 M6A reading proteins

To execute specific biological functions, M6A-modified RNAs require specific RNA-binding protein, the methylated reading protein, namely Reader. RNA pull-down experiments have identified multiple reading proteins, including YTH domain proteins, HNRNPA2B1, eIF3 and IGF2BPs[42-43]. These reading proteins specifically bind to the M6A methylated region, weakening homologous binding to RNA-binding proteins, and changing RNA secondary structure to alter protein-RNA interactions. Proteins with YTH domain include YTHDC1-2 and YTHDF1-3, which all contain YTH domain at the C-terminal that overlaps with the M6A motif to mediate RNA-specific binding, while the proline/glutamine/asparagine enrichment (P/Q/N-rich) domain is associated with subcellular localization. Among them, YTHDF1-3 mainly recognizes M6A-modified mRNAs in cytoplasm, while YTHDC1-2 mainly acts in nucleus[44]. YTHDF1, YTHDF3 and YTHDC2 accelerate protein synthesis by increasing efficiency of mRNA translation, while YTHDF2, YTHDF3 and YTHDC1 mediate the degradation of mRNA. As a member of the hnRNP family, HNRNPA2B1 can perform the function of reading protein, but it does not bind directly to M6A-modified bases. HNRNPA2B1 is not only associated with the activation of the pre-miRNA downstream pathways, but also associated with pre-miRNA processing. Another reader protein eIF3 could bind to M6A-modified base on the 5' UTR of RNA, and the RNA recruits 43S ribosomes to form protein complexes at the 5' cap under the action of eIF3, thus promoting the translation of mRNA[45]. IGF2BPs, including IGF2BP1-3, as a novel M6A reader protein nearly exclusively expressed in cytoplasm, can recognize the conserved GG (M6A) C sequence in mRNA, thus enhancing the stability of the target mRNA and improving the efficiency of mRNA translation[46].

M6A is the most common and abundant methylation modification in eukaryotic mRNAs, affecting the splicing, translation and degradation of mRNA and other RNA metabolic processes. In addition, some non-coding RNAs, such as lncRNA, tRNA, rRNA and splicing RNA, can also be modified by M6A. M6A plays a crucial role in regulating gene expression and it is widely involved in embryo development, apoptosis, sperm development, and circadian rhythms[47-49]. M6A modification is dynamically and reversibly regulated by both methyltransferase and demethylase. Dysfunction of M6A modification enzyme can well result in various diseases, such as tumors, neurological diseases, CVDs, embryonic development retardation and the like[50]. Although the number of studies on M6A RNA modification has been increasing in recent years, more rigorous investigations are required for our better understanding of in-depth mechanisms. Collectively, the dynamic modifications in coding/non-coding RNAs represent a novel molecular mechanism for the regulation of genetic information.

3 M6A RNA methylation in cardiovascular diseases

Gene expression and its regulation play an important role in cardiac homeostasis and stress response, and M6A-modified transcripts have recently been identified as key post-transcriptional mechanisms for CVDs. Thousands of M6A-modified mRNAs are known in mammals, and about a quarter of transcripts from healthy mouse and human hearts undergo M6A RNA methylation[51]. Increased methylation levels have also been found in human cardiomyopathy. In both *in vitro* and *in vivo* models, knockdown or overexpression of M6A methylase METTL3 affects myocardial cell size and remodeling process.

In cardiomyocytes, mRNA methylation is highly dynamic, and changes in mRNA methylation regulate translation efficiency by affecting the stability of transcripts[52]. This suggests that M6A modification landscape has important implications in cardiac pathophysiology and once clarified, the specific M6A site may be a therapeutic target for the development of strategies for CVDs. Of late years, studies on the role of M6A methylation in various CVDs, *e.g*., atherosclerosis (AS), coronary artery disease (CAD), MI and cardiac remodeling, myocardial ischemia-reperfusion injury (MIRI), heart failure (HF), hypertension, and aortic aneurysm, have been increasing exponentially.

3.1 Atherosclerosis and coronary artery disease

AS is widely regarded as a chronic inflammatory disease, characterized by dysfunction of vascular endothelial cells (EC) and interaction between inflammation and lipids. Narrowing or occlusion of the lumen caused by AS in the coronary arteries can lead to CAD. AS and its complications pose a serious threat to human health due to population aging and increasing prevalence of obesity. Published studies indicate that epigenetic modification of mRNA, especially M6A methylation, affects the occurrence and development of AS[53-54]. Quiles-Jiménez *et al*. used mass spectrometry to analyze the changes of M6A modification and M6A related enzymes in carotid atherosclerotic of different degrees and non-atherosclerotic control and discovered for the first time an abnormal regulation of M6A mRNA and reduced M6A level in total RNA in human atherosclerotic lesions[54]. M6A levels were significantly lower in early and late AS than in the control group, and were particularly low in early AS. The authors suggested that the decrease in M6A primarily occurs in 18S rRNA. M6A modification enzymes have different regulatory roles during atherosclerosis development. Protein levels of WTAP, METTL3 and eIF3a were found significantly increased in advanced atherosclerotic lesions, while the levels of FTO and YTHDF2 were significantly decreased in early AS. METTL14 and YTHDF3 showed no obvious differences between the three sample groups, and ALKBH5 was not detected[54]. Wu *et al*. found that leukocyte M6A levels, rather than 5mC DNA modification levels, reduced with increasing carotid plaque size and thickness in patients with AS, whereas the opposite alterations were observed in the controls[55]. There was an independent negative correlation between serum low density lipoprotein cholesterol (LDL-C) level and carotid plaque thickness with M6A level. Decreased M6A levels in leukocytes and ECs have also been demonstrated in AS mice and ox-LDL treated human ECs and monocytes. The decrease of M6A level is associated with up-regulation of ALKBH1. Silencing *ALKBH1* or hypoxia inducible factor 1α (*HIF1α*) rescues the hypoxia response gene MIAT level. Ox-LDL induces transfer of HIF1α to the nucleus, and nucleus HIF1α binds to the ALKBH1-demethylated MIAT promoter and upregulates its transcription. Increased ALKBH1 levels of ECs and leukocyte reduces M6A levels, which may be a susceptive biomarker for the progression of AS[55]. Vascular endothelial dysfunction and inflammation are the main pathological features of AS, and mononuclear/macrophages play key roles in AS by regulating inflammation. The levels of RNA M6A in peripheral blood mononuclear cell (PBMC) of CAD patients were significantly reduced by colorimetry, and differential methylation M6A sites were found in mRNAs and lncRNAs of CAD and control groups by Methylated RNA Immunoprecipitation Sequencing (MeRIP-seq). Further bioinformatics analysis suggested that differential methylation genes are involved in the pathogenesis of AS[56]. Mitochondrial biogenesis and energy metabolism are necessary to regulate monocyte inflammation. Peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1α) is a coactivator that regulates mitochondrial biogenesis and energy metabolism. Zhang *et al*. found METTL3 contributed to ox-LDL-induced monocyte inflammation. METTL3 interacts with YTHDF2 to modify and degrade PGC-1α mRNA, thereby decreasing PGC-1α protein, ATP production, and oxygen consumption rate (OCR), which in turn increases the accumulation of cellular and mitochondrial reactive oxygen species (ROS) and the level of pro-inflammatory cytokines in monocytes, enhancing the inflammatory response to AS[57]. M6A modification also plays a role in ox-LDL-induced macrophage inflammation. Li *et al*. found that 40 μg/mL ox-LDL obviously increased M6A modification in RAW264.7, and knockdown of METTL3 inhibited ox-LDL-induced M6A modification and inflammatory response[58]. METTL3 affects the expression and activation of signal transducer and activator of transcription 1 (STAT1) by M6A modification of *STAT1* mRNA, thus promoting ox-LDL induced macrophage inflammation. Ox-LDL stimulation strengthens the interaction between METTL3 and STAT1, and ultimately promotes the transcriptional regulation of STAT1 on the expression of macrophage inflammatory factors[58]. M6A modifications also take part in macrophage pyroptosis. IFN regulatory factor-1 (IRF-1) is known to effectively promote macrophage pyroptosis in patients with acute coronary syndrome (ACS). Guo *et al*. found circRNA, HSA_circ_0029589 was decreased in PBMC derived macrophages from ACS patients, but expression of METTL3 and the M6A modification of HSA_circ_0029589 were significantly increased[59]. IRF-1 overexpression inhibited HSA_circ_0029589 expression but induced the METTL3 expression and M6A modification in macrophages. In addition, overexpression of HSA_circ_0029589 or inhibition of METTL3 distinctly up-regulated HSA_circ_0029589 expression and weakened macrophages pyroptosis. Collectively, IRF-1 inhibits circ_0029589 by promoting M6A modification of circ_0029589, thereby promoting pyroptosis and inflammatory response of macrophages in ACS and AS,

which is a novel mechanism[59].

It is currently accepted that atherosclerotic lesions begin with EC injury. M6A plays vital roles in ECs. METTL3 is up-regulated in ox-LDL-induced human umbilical vein endothelial cell (HUVEC). In ox-LDL-induced HUVEC, down-regulation of METTL3 inhibits cell proliferation, migration, tube formation, and vascular endothelial growth factor (VEGF) expression/secretion and impedes angiogenesis of developing embryo and AS progression. In HUVECs, METTL3 positively regulates janus kinase 2 (JAK2) expression and the JAK2/signal transducer and activator of transcription 3 (STAT3) pathway in an M6A-dependent manner, and IGF2BP1 positively regulates JAK2 expression by directly binding to the M6A site of JAK2 mRNA. *METTL3* knockdown attenuates the interaction between JAK2 and IGF2BP1 and inhibits the JAK2/STAT3 pathway, thereby preventing AS progression[60]. Chien *et al*. identified METTL3 as the response center for hemodynamic and atherosclerotic stimulation in ECs[61]. Oscillatory stress (OS) up-regulates METTL3 expression, which is accompanied by an increase in M6A modification, elevated phosphorylation of NF-κB p65 Ser536, and increased monocyte adhesion. Deletion of *METTL3* eliminates OS-induced hypermethylation of M6A, while METTL3 overexpression produces qualitatively the same effects as OS. RNA sequencing (RNA-seq) and M6A-enhanced cross-linking and immunoprecipitation (eCLIP) assay identified NLRP1 and KLF4 as targets of METTL3. METTL3-mediated RNA hypermethylation up-regulates NLR family pyrin domain containing 1 (NLRP1) and down-regulates kruppel-like factor 4 (KLF4) transcripts via YTHDF1 and YTHDF2, respectively. In AS models, partial carotid artery ligation results in plaque formation, up-regulation of METTL3 and NLRP1, and down-regulation of KLF4. Down-regulation of METTL3 by shRNA prevents AS process, up-regulates NLRP3 and down-regulates KLF4[61]. In a latest study, OS reduced METTL3 expression. M6A caused EC dysfunction by modifying the epidermal growth factor receptor (EGFR) 3'UTR region and accelerating its mRNA degradation. METTL3 overexpression obviously reduced EGFR activation and EC dysfunction in the presence of OS. In addition, EGFR ligand thrombospondin 1 (TSP-1) is specifically expressed in atherosclerotic areas, independent of METTL3. Blocking the TSP-1/EGFR axis obviously improves AS. Overall, METTL3 mediates the EGFR signal pathway during EC activation, thus regulating AS[62]. METTL14, another methylase, was also found to play a role in AS. Zhang *et al*. discovered that M6A modification levels and METTL14 expression were obviously elevated in atherosclerotic vascular endothelial cells (ASVEC)[63]. Silencing *METTL14* inhibited the proliferation and invasion of ASVEC. Low expression of METTL14 inhibited the binding of methylated RNA to RNA splicing related protein DGCR8 and the expression of miR-19a, while promoting the expression of primary pre-miR-19a. In contrast, high METTL14 expression significantly increased DGCR8 expression and methylation of M6A. In addition, silencing miR-19a inhibited the proliferation and invasion of ASVEC. In general, METTL14 increased M6A modification of pri-miR-19a and promoted the processing of mature miR-19a, thereby promoting proliferation and invasion of ASVEC[63]. In addition, Jian *et al*. demonstrated that METTL14 participated in TNF-α induced EC inflammation in a TNF-α induced EC inflammation model[64]. M6A modification of forkhead box O1 (FOXO1), an important transcription factor, was significantly increased during EC inflammation. Down-regulation of METTL14 significantly reduced TNF-α induced FOXO1 expression. RNA Binding Protein Immunoprecipitation (RIP) revealed that METTL14 directly binds FOXO1 mRNA, increasing its M6A modification and enhancing its translation through subsequent YTHDF1 recognition. In addition, METTL14 has been shown to interact with FOXO1 and directly acted on the promoter regions of VCAM1 and intercellular adhesion molecule 1 (ICAM1) to promote their transcription, thus inducing endothelial inflammatory response and atherosclerotic plaque formation. *METTL14* knockdown inhibited EC inflammation and significantly reduced the occurrence of mouse atherosclerotic plaques[64]. Zinc finger NFX1-Type1 (ZNFX1) antisense RNA1 (ZFAS1) and downstream disintegrin and metalloprotease 10 (ADAM10)/ras-related protein Rab-22A (RAB22A) are involved in vascular inflammation and the development of cholesterol metabolism[65]. Chen *et al*. found that the M6A modification of ZFAS1 in AS patients was significantly higher than that in the control group[66]. METTL14 affected the expression of ADAM10/RAB22A by affecting the M6A modification of ZFAS1 to regulate cholesterol metabolism and vascular inflammation thereby AS[66]. Gong *et al*. suggested that METTL14 may play a role in AS by mediating M6A modification of ZFAS1/RAB22A[67]. Since METTL14 participates in the development of AS through multiple mechanisms, it may be a promising therapeutic target for AS. In addition, balloon injury model of carotid artery in rats revealed the role of FTO in neointima formation[56].

3.2 Myocardial infarction and cardiac remodeling

MI is one of the biggest threats to human health. M6A modification plays important roles in cardiac fibrosis, cardiomyocyte contractile function, and the development of cardiac remodeling. A quantitative method was established for the quantitative analysis of epigenetic modified nucleosides (5mdC, 5mrC, M6A) at 1, 4 and 8 weeks after MI in rats. The concentrations of 5mdC, 5mrC, and M6A were significantly increased at 8 weeks after surgery. Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS) was helpful to observe the concentration changes of these three methylation biomarkers in peripheral blood after

8 weeks. Moreover, the dynamic process of these three methylation biomarkers in peripheral blood was related to the content of methylation biomarkers in heart tissue. Chang *et al*. also demonstrated that methylation of genetic material in peripheral blood is similar to that in heart tissue of MI[68]. The relationship between them showed that peripheral blood may be a promising alternative to heart tissue for monitoring levels of methylation as auxiliary diagnosis of MI. The levels of DNA and RNA methylation biomarkers in heart tissue and peripheral blood of rats after MI were significantly increased, and were positively correlated with the degree of HF, suggesting its potential diagnostic value[68]. Shi *et al*. used GSE5406 database to analyze the expression of M6A regulatory factor in human MI and normal myocardium[69]. In comparison with the control group, expression of FTO, YTHDF3 and ZC3H13 in MI tissues were significantly lower while the expression of WTAP in MI tissues was significantly higher than in healthy myocardium. Bioinformatic analysis indicates that differentially expressed genes in MI are related to the regulation of calcium signaling and chemokine signaling, while FTO and IGFBP2 are associated with increased blood glucose levels[69]. Alarcón *et al*. proposed in 2015 that the generation of miRNAs induced by the selective cleavage of M6A RNA mediated by HNRNPA2B1 might be related to cardiac fibrosis[70]. METTL3-mediated modification of M6A has recently been found to be critical for the development of cardiac fibrosis. METTL3 expression is increased in cardiac fibrotic tissue of chronic MI mice and cultured cardiac fibroblasts treated with TGF-β1. Overexpression of METTL3 promotes fibroblast proliferation and myofibroblast transformation, as well as collagen accumulation, while silence of METTL3 produces the opposite effects. Cardiac fibrosis is significantly reduced in MI mice after METTL3 silencing by siRNA, and the expression of collagen-associated genes and M6A level are decreased[71]. Dorn *et al*. found that METT3-mediated M6A methylation is a dynamic modification which is essential for the normal hypertrophic response of cardiomyocytes and enhanced response to hypertrophic stimuli[72]. They established a mouse model of cardiac restricted gain-and-loss-of-function and found that M6A methylation levels were increased significantly in hypertrophic cardiomyocytes and enriched with genes involving in protein kinases and the intracellular signaling pathways[72]. METTL3, via M6A, regulates cardiac homeostasis and hypertrophic responses in mice. Increased METTL3 expression in the heart causes spontaneous compensatory hypertrophy without affecting cardiac function, while heart-specific METTL3 knockout induces poor concentric remodeling and results in morphological and functional phenotypes of HF. These alterations indicate that M6A RNA methylation is necessary for maintaining cardiac homeostasis. METTL3 is also thought to have a detrimental effect on pathological cardiac hypertrophy. Stress can induce pathological cardiac hypertrophy, and ubiquitin-proteasome system (UPS) plays important roles in maintaining protein homeostasis and cardiac function. Deubiquitylation enzyme ubiquitin specific proteinase 12 (USP12) can accentuated Ang II-induced cardiac hypertrophy via METTL3. In neonatal rat cardiomyocytes (NRCMs), upregulation of METTL3 reverses USP12 silencing induced reduction in myocardial hypertrophy. In contrast, in USP12-overexpressing NRCMs, knockdown of METTL3 alleviates cardiac hypertrophy. Mechanically, USP12 binds and stabilizes P300 to upregulate P300 expression, activate METTL3 transcription, and thus promotes myocardial hypertrophy[73]. PiRNA was found highly expressed during cardiac hypertrophy, and piRNA-mediated RNA epigenetic participates in regulating cardiac hypertrophy. Gao *et al*. identified a piRNA (CHAPIR) which is associated with cardiac hypertrophy[74]. CHAPIR promotes hypertrophy and cardiac remodeling by targeting METTT3-mediated M6A methylation of Parp10 mRNA to regulate Parp10 expression. Its deficiency significantly reduces cardiac hypertrophy and restores cardiac function, while its mimics enhances the pathological hypertrophy response in pressure overload mice[74]. Other studies found YTHDC1 plays a key role in regulating the normal systolic function of myocardium and the development of dilated cardiomyopathy (DCM). Loss of YTHDC1 decreases contractility of myocardium cells and disorder of sarcomere arrangement. Furthermore, defects in YTHDC1, instead of other members of the YTH family, cause DCM in mice. Heart-specific knockout of YTHDC1 results in marked left ventricular enlargement and severe systolic dysfunction. By integrating M6A-MeRIP, RIP-seq, and mRNA-seq, 42 genes were identified as potential downstream targets of YTHDC1. Among them, Titin mRNA is modified by M6A, and deletion of YTHDC1 modification leads to the abnormal splicing of Titin[75].

M6A modification is dynamically reversible which is critical in tissue development and pathogenesis. Recently, researchers have found that M6A also plays a role in myocardial regeneration after cardiac injury. Gong al. found upregulation of METTL3 expression in the heart after birth, contrary to changes in myocardial cell proliferation. In addition, both METTL3 heterozygous knockdown mice and administration of METTL3 shRNA adenovirus in mice showed myocardial cell cycle re-entry, reduced infarct area size, as well as improved cardiac function after MI. Silencing METTL3 promotes the proliferation of myocardial cells by reducing primary miR-143 (pri-miR-143) thereby downregulating mature miR-143-3p, which in turn derepresses its target genes yes-associated protein (YAP) and catenin delta 1 (Ctnnd1)[76]. Another study found that down-regulation of ALKBH5 increased M6A methylation in the heart after birth[77]. ALKBH5 knockdown showed reduced cardiac regeneration and cardiac function after neonatal apical resection

of mice. In contrast, forced expression of ALKBH5 significantly reduced the infarct size, restored cardiac function and promoted cardiomyocyte proliferation after myocardial infarction in juvenile (7 days old) and adult (8 weeks old) mice. Mechanically, ALKBH5-mediated M6A demethylation enhances the stability of YTHDF1 mRNA and increases its expression, which in turn promotes YAP translation. Moreover, the regulation of ALKBH5 and YTHDF1 expression in human-induced pluripotent stem cell derived cardiomyocytes consistently produces similar results. These findings highlight the important roles of the ALKBH5-M6A-YTHDF1-YAP axis in regulating myocardial cell re-entry into the cell cycle[77] and provide novel insights into the significance of RNA M6A modification in cardiac regeneration and new strategies for cardiac regeneration therapy.

3.3 Myocardial ischemia-reperfusion injury

After AMI, recovery of myocardial perfusion through thrombolytic or percutaneous coronary intervention (PCI) is the most effective method to reduce the size of MI and improve the clinical prognosis. However, recovery of ischemic myocardial blood flow may cause MIRI and presently there is no effective treatment for MIRI. Accumulating evidence suggests that epigenetic regulation is closely related to the pathogenesis of MIRI, indicating epigenetics as a new therapeutic approach for improving or preventing MIRI[78]. The disturbance of energy metabolism is the initial physiological stage of MIRI. FTO-mediated M6A modification is related to dysregulation of cardiomyocyte energy metabolism. Deng *et al*. found that the expression of FTO and sarcoplasmic/endoplasmic reticulum calcium ATPase 2a (SERCA2a) is downregulated, whereas M6A levels in total RNA and in SERCA2a mRNA are enriched, in cardiomyocytes subject to hypoxia/reoxygenation (H/R)[79]. H/R treatment decreases cell viability, mitochondrial membrane potential and ATP content, but increases cytotoxicity, apoptosis, ROS content and calcium concentration of cardiomyocytes. The up-regulation of FTO reverses all these alterations. FTO promotes SERCA2a expression, maintains calcium homeostasis, and improves energy metabolism of H/R cardiomyocytes by reducing the M6A level of SERCA2a mRNA through demethylation[79]. Other studies found that M6A RNA methylation aggravates acute MIRI through ALKBH5-related metabolic reprogramming, which is characterized by diminished enzyme activity of the tricarboxylic acid cycle. In order to achieve precision treatment, Cheng *et al*. developed a surface modified bioengineered ferritin nanocage named HAfFtO from Archaea using recombinant expression technique based on genetic engineering[80]. Three G4S joints were then used to connect the SpyTag to HAfFtO to synthesize Haffto-ST and reassemble Scarf1, or SC-SF, containing the SpyCatcher structure. According to SpyTag/SpyCatcher technology, HAffto-ST and SC-SF can form a mild and strong combination, namely HSSS, which can be decomposed and self-assembled in neutral solution at different Mg^{2+} concentrations and target dead cells in the infarcted area under the guidance of Scarf1. They loaded ALKBH5 inhibitor IOX1 onto HSSS and found it effectively improves cardiac function and reduces the infarct size of AMI[80]. Su *et al*. found age-related differences in myocardial M6A transcriptome regulation in response to acute MIRI[81]. They examined overall level of M6A RNA methylation and expression of M6A regulators in hearts of young and old female mice undergoing sham surgery or acute MIRI. The levels of M6A RNA and mRNAs of related genes were found to be similar in intact young and old female hearts. However, in acute MIRI, M6A RNAs were significantly reduced in young hearts but only slightly decreased in older hearts. METTL3 mRNA and protein expression was obviously decreased in both young and old hearts, whereas FTO mRNA and protein expression was significantly decreased only in ischemic elderly hearts. It is possible that the age-related FTO downregulation might offset the effect of reduced METTL3 on M6A RNA levels in aging mice with acute MIRI. BCL-2-associated X (BAX) and phosphatase and tensin homolog (PTEN) are the known target genes of METTL3 under MIRI stress as uncovered by M6A RIP-qPCR[81]. Other studies have found that METTL3 is also involved in the process of myocardial apoptosis. METTL3 is down-regulated in mice ischemia/reperfusion (I/R) myocardial tissue and H/R myocardial cells, and METTL3 up-regulation attenuates I/R and H/R induced apoptosis. In cardiomyocytes, miR-25-3p and miR-873-5p are positively regulated by METTL3 in a DGCR8-dependent manner. MiR-25-3p and miR-873-5p are significantly down-regulated in mouse tissues and cardiomyocytes after I/R and H/R treatment, and overexpression of these two miRNAs can effectively improve the viability of cardiomyocytes under H/R stress. When miR-25-3p and miR-873-5p are activated, METTL3 activates the PI3K/Akt pathway in H/R-treated cardiomyocytes and suppresses cell death induced by H/R, thereby alleviating I/R injury. PI3K/Akt inhibitor LY294002 can eliminate the protective effect of METTL3 overexpression in H/R treated cardiomyocytes[82]. Wang *et al*. demonstrated the importance of WTAP in MIRI by uncovering that in human cardiomyocytes (AC16), H/R increased WTAP expression in a time-dependent manner and exacerbated endoplasmic reticulum (ER) stress and apoptosis[83]. Silencing WTAP can block the effects of H/R on ER stress and apoptosis, which can be reverted by WTAP overexpression or ameliorated by ER stress inhibitor 4-PBA. WTAP also increases activating transcription factor 4 (ATF4) 5′UTR M6A levels, upregulating its expression, promoting endoplasmic network stress and apoptosis, and thus aggravating MIRI[83]. Recently, the role of M6A in macrophage autophagy has also been explored. M6A modification was

found to be increased in H/R treated cardiomyocytes and I/R hearts of mice, which could be ascribed to METTL3 as a major factor. In H/R treated cardiomyocytes, silencing METTL3 augmented autophagy flux and inhibited apoptosis, while overexpression of METTL3 or inhibition of ALKBH5 elicited the opposite effects, indicating METTL3 as a negative regulator of autophagy. Mechanically, METTL3 methylates transcription factor EB (TFEB), a master regulator of lysosomal biogenesis and autophagy genes, at two m6A residues in the 3'UTR to promote the association of HNRNPD with TFEB pre-mRNA and subsequently reduce TFEB expression. Deficiency of METTL3 enhances autophagy flux through TFEB. In turn, TFEB regulates METTL3 and ALKBH5 expression in the opposite direction with TFEB upregulating ALKBH5 by binding to its promoter to activate its transcription and inhibiting METTL3 expression by destabilizing mRNAs rather than affecting transcription, thus establishing a negative feedback loop. Song *et al*. revealed a key link between METTL3-ALKBH5 and autophagy, highlighting the functional importance of reversible mRNA M6A methylation and its regulatory factors in ischemic heart disease (IHD)[84]. Wang *et al*. recently proposed that metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) exacerbates inflammation response through regulating prostaglandin-endoperoxide synthase 2 (PTGS2) by targeting miR-26b in MIRI[85]. Furthermore, increasing expression of MALAT1 was found in MI patients which is closely related to I/R-induced inflammation[86], MALAT1 has multiple M6A sites and is highly M6A modified. It is speculated that M6A modification of MALAT1 may also play a key role in MIRI[87].

3.4 Heart failure

HF is the terminal stage in the development of heart disease. Abnormal epigenetic process and the consequent deregulation of gene expression are significant mechanisms for HF. In 2019, Berulava *et al*. identified the role of M6A modification in the development of HF[51]. Changes in M6A modification outweigh changes in gene expression during the progression of HF in both mice and humans. M6A methylated RNAs are primarily associated with the metabolic and regulatory pathways, while changes of RNA expression level are mainly related to structural plasticity. M6A modification is also linked to RNA translation efficiency thereby protein expression. The authors found that it is differentially methylated RNAs rather than differentially expressed RNAs that affect the expression of proteins. That is, changes in M6A modification result in changes of protein abundance, independent of mRNA level, suggesting a novel transcription-independent regulatory mechanism for translation. They accordingly proposed that the regulation of external transcriptome processes like M6A methylation may be an interesting therapeutic target[51]. Hinger *et al*. found increased levels of M6A in human failing hearts[88]. Using genome-wide analysis, they identified all differential M6A sites in between human failing hearts and non-failing hearts and found that target genes involved in histone modification are enriched in HF. In addition, they compared all M6A sites in human heart with those in hypertrophic rat cardiomyocytes to identify cardiomyocyte specific M6A sites that are conserved across species. Finally, 38 shared genes targeted by M6A under stress and 11 events specific to unstressed cardiomyocytes were identified[88]. Heart failure with preserved ejection fraction (HFpEF) is a heterogeneous disease with complex pathogenesis. Zhang *et al*. examined M6A modification in HFpEF patients and mice and mapped total transcriptome M6A in control and HFpEF mice using RNA-seq[90]. They found that the expression patterns of M6A regulators and M6A landscape in HFpEF were altered. The expressions of METTL3, METTL4, KIAA1429, FTO, and YTHDF2 in PBMCS of HFpEF patients were up-regulated compared with those of healthy controls, and in HFpEF mice FTO was also significantly increased. MeRIP-seq uncovered significant alterations of 661 M6A peaks. GO analysis showed that protein folding, the ubiquitin dependent ERAD pathway and positive regulation of RNA polymerase II are the three most obviously changed biological processes in HFpEF. KEGG pathway analysis revealed significant changes in proteasome, endoplasmic reticulum protein processing, PI3K-Akt signaling and other pathways in HFpEF[89]. Zhang *et al*. found that mice with cardiomyocyte restriction knockdown of FTO exhibited impaired heart function compared with control mice[51]. Another study found that FTO alleviated cardiac insufficiency by regulating glucose uptake and glycolysis in stress-overload induced HF mice[90]. FTO has also been found to play a key role in cardiac systolic function during homeostasis, remodeling, and regeneration. Mathiyalagan *et al*. used human clinical samples, preclinical pig and mouse models, and primary cardiomyocyte cultures to investigate the functional roles of M6A and FTO in heart and cardiomyocytes[91]. The authors found that FTO expression decreased in failing mammalian hearts and hypoxic cardiomyocytes, thereby increasing M6A in RNAs and reducing the contractile function of cardiomyocytes. Increased FTO expression in HF mice attenuates the ischemia-induced increase in M6A, and FTO selectively demethylates cardiac contractile transcripts, thereby preventing their degradation under ischemia to maintain the expression of corresponding proteins and ultimately ameliorating the decrease in myocardial systolic function. In addition, they found that overexpression of FTO reduces fibrosis and enhances angiogenesis in MI mice[91]. Recently, FTO was found to affect cardiomyocyte apoptosis. Shen *et al*. built a mouse model of HF by aortic constriction or intraperitoneal injection of Adriamycin and a cell model of H/R in mouse cardiomyocytes[92]. They observed that FTO and MHRT were down-regulated whereas the levels of total M6A

and MHRT M6A were increased in the heart of HF mice. On the other hand, FTO overexpression inhibited apoptosis of H/R cardiomyocytes by regulating M6A modification of MHRT[92]. Moreover, the role of YTHDF2 in myocardial hypertrophy has also been documented in published studies. For example, Xu *et al*. constructed a HF mouse model by cross-sectional aortic coarctation and a cell model of cardiomyocyte hypertrophy by stimulating primary cultured cardiomyocytes with isoproterenol or phenylephrine[93]. Their results demonstrated that the expression of YTHDF2 at both mRNA and protein levels was elevated during HF development, whereas that of YTHDF1 and YTHDF3 remained unaltered. Overexpression of YTHDF2 effectively alleviated cardiac hypertrophy. Isoproterenol stimulation had no significant effect on YTHDF2 interacting proteins, but isoproterenol or phenylephrine obviously strengthened the interaction between YTHDF2 protein and MYH7 mRNA, an important marker of cardiac hypertrophy, in an M6A-dependent manner and enhanced the stability of YTHDF2 mRNA thereby the expression of YTHDF2 protein. Knockdown of MYH7 or deletion of the YTH domain of YTHDF2 reversed the protective effect of YTHDF2 on cardiac hypertrophy. Overall, YTHDF2 inhibits cardiac hypertrophy in M6A dependent manner through MYH7 mRNA bait[93].

IHD and DCM are the two most common causes of HF, which share common features, including the final stage of ventricular dilation. The immune mechanism of HF has received increasing attention and is related to the pathogenesis of IHD and DCM. In the Gene Expression Omnibus database, multiple data sets were selected to analyze differentially expressed genes (DEGs). The results showed that vascular cell adhesion molecule 1 (VCAM1) plays a vital role in the regulation of DEGs. Subsequent Xcell analysis revealed that VCAM1 is differentially expressed in myocardium and involved in the regulation of immune cell infiltration. Further investigations with clinical risk prediction models showed that abnormal VCAM1 expression is associated with increased HF risk. Association was found between M6A RNA modification and VCAM1 expression and immune regulation[94].

3.5 Hypertension

Hypertension is an important risk factor for CVDs. More than 1.2 billion adults worldwide suffer from hypertension in 2019, doubling the number in 1990[95]. In 2019, Wu *et al*. detected the M6A methylation levels in Wistar Kyoto rats and spontaneously hypertensive rats and found that M6A methylation of mRNAs is richer in coding sequence and 3'UTR and 5'UTR regions and is well maintained in varying conditions. The mean abundance of M6A in peripheral cells of spontaneously hypertensive rats is overall reduced[96]. PAH is a common and frequently occurring disease with a high disability rate and a fatality rate characterized by elevated pulmonary artery pressure due to the imbalance of vasoconstriction/dilation signals in the pulmonary vascular system, which ultimately develops into right HF. To date, there has been no effective treatment for hypoxia mediated pulmonary hypertension (HPH).

M6A RNA modification also participates in the development of PAH. MeRIP-seq was used to compare the levels of M6A and the expression of methylation related enzymes in the lung tissues of MCT-PAH and control rats, and the results showed that M6A methylation is principally increased in MCT-PAH rat lung. FTO and ALKBH5 are down-regulated, whilst METTL3 and YTHDF1 are up-regulated, and other methylation related proteins are unchanged in MCT-PAH rat lung. Immunofluorescence showed that FTO expression is decreased whereas YTHDF1 expression is increased in pulmonary arterioles of MCT-PAH rats. In MCT-PAH rats, up-methylated genes are enriched in the processes related to inflammation, glycolysis, ECM-receptor interactions and PDGF signaling, while down-methylated genes are enriched in the TGF-β signal pathway[97]. Recently, elevated levels of M6A and YTHDF1 protein were found in human and rodent PAH samples and hypoxia pulmonary artery smooth muscle cell (PASMCs). Loss of YTHDF1 improves proliferation, phenotypic switch over of PASMC and PAH development both *in vivo* and *vitro*. Immunoprecipitation analysis of M6A RNA showed that melanoma antigen D1 (MAGED1) is a M6A-regulated gene in PAH, and genetic ablation of MAGED1 improves vascular remodeling and hemodynamic parameters in SU5416/hypoxia mice. YTHDF1 recognizes and promotes MAGED1 translation, PASMC proliferation and PAH in an M6A-dependent manner, which is absent in METTL3-deficient PASMCs. Furthermore, silencing of MAGED1 inhibits hypoxia-induced PASMCs proliferation by down-regulating proliferating cell nuclear antigen (PCNA)[98]. In addition, Qin *et al*. found that METTL3 mRNA and protein are abnormally up-regulated in PASMCs model and hypoxia rat model[99]. Down-regulation of METTL3 suppresses PASMCs proliferation and migration. YTHDF2 is obviously increased in PASMCs under hypoxia. YTHDF2 recognizes METTL3-mediated M6A-modification of PTEN mRNA and promotes degradation of PTEN. Decrease of PTEN activates the PI3K/Akt signal pathway and eventually results in excessive proliferation of PASMCs. It is suggested that the METTL3/YTHDF2/PTEN axis plays an important role in the proliferation of PASMC induced by hypoxia[99]. The methyltransferase SET domain-containing 2 (SETD2) can catalyze the trimethylation of lysine 36 on histone 3 (H3K36me3). In the hypoxia-induced PAH mouse model, SMC specific SETD2 deletion improves pulmonary arterial pressure and right ventricular function and decreases pathologic pulmonary arterial remodeling and right

ventricular hypertrophy. SETD2-mediated H3K36me3 and METTL14-mediated M6A RNA modifications are involved in hypoxia-induced PAH. The expression of SETD2 and METTL14 in PASMCs of hypoxia-induced PAH mice is increased, and the level of total RNA with M6A is significantly enhanced. However, the loss of SETD2 in SMCs reduces METTL14 expression and M6A RNA methylation levels in PAH SMCs[100].

CircRNAs play significant roles in physiological processes, which can also be modified by M6A. The abundances of circRNAs are affected by M6A. The transcriptome of circRNAs M6A modification in HPH has been identified. The abundance of m6A in lung of HPH rats is increased, and the abundance of M6A in circRNAs is markedly decreased under *in vitro* hypoxic conditions, and two HPH down-regulated M6A circRNAs: circXpo6 and circTmtc3 have been identified. In the control group and the HPH group, M6A circRNAs are primarily derived from protein-coding genes. M6A affects the circRNA-miRNA-mRNA co-expression network under hypoxia[101]. Early life exposure to hypoxia can change pulmonary blood vessels leading to PAH. The long-term effects of postnatal hypoxia on lung development and function were investigated in a rat model of hypoxia. MeRIP-seq was used to analyze M6A modification in lung of rats subjected to postnatal hypoxia at 2 weeks and 9 weeks of age, respectively. In comparison with the controls, the average pulmonary artery pressure, lung/body weight ratio and Fulton index of hypoxic rats are significantly increased, and the differences persisted into adulthood. In postnatal hypoxia-induced PAH, no statistically significant change of total M6A was observed in lung, while M6A regulators (METTL3 and METTL14, FTO and ALKBH5) were significantly down-regulated. M6A modified peak-related genes were different between the two groups, which were related to lung development. The occurrence and persistence of PAH induced by postnatal hypoxia may be the causes for persistent low expression of M6A methyltransferase and low M6A levels of PAH related genes[102].

3.6 Aortic aneurysm

A pathological dilation of the aorta, greater than 50 percent of the normal blood vessel diameter, is called as aortic aneurysm. The common sites of the disease are thoracic aorta, abdominal aorta, and descending aorta, which can be caused by AS, vascular middle cystic necrosis, syphilis infection, bacterial infection, and rheumatic aortitis and trauma, among which AS is the most common cause. In 2019, He *et al*. firstly observed M6A modification in human abdominal aortic aneurysm (AAA), with significantly elevated M6A levels in the AAA group compared to normal aortic tissues[103]. Moreover, higher M6A levels represent a higher rupture risk in AAA patients compared with unruptured AAA [*OR*, 1.370; 95% *CI*, 1.007-1.870]. It was further revealed that YTHDF1, YTHDF3, FTO and METTL14 participate in abnormal modification of M6A and are obviously related to the proportion of M6A in mRNAs. YTHDF3 is correlated with a greater rupture risk, and METTL14 is associated with inflammatory infiltration and neovascularization. In addition, there is a strong association between FTO and aneurysm SMCs and between YTHDF3 and macrophage infiltration[103]. METTL14 and HNRNPC are down-regulated in human AAA according to the gene expression analyses with human AAA tissues and control ones using public GEO database. METTL14 expression is low in AAA and its rupture type, and low METTL14 expression is related to high white blood cell count and expression of C-reactive protein, while RBM15B is up-regulated. The levels of METTL14, HNRNPC and RBM15B are related to the immune infiltration of macrophages, mast cells, and Tgd and NK CD56 cells. The target genes of METTL14, HNRNPC and RBM15B participate in metabolism and autophagy of AAA[104]. In AAA mouse models ($ApoE^{-/-}$ mouse angiotensin II treatment model and $CaCl_2$-induced model), Zhong *et al*. found that METTL3 knockdown inhibits AAA formation, while METTL3 overexpression enhances it[105]. Mechanically, METT3-mediated M6A promotes the maturation of primary miRNA-34a (miR-34a, pri-miR-34a) through DGCR8, and the increased expression of miR-34a markedly down-regulates sirtuin 1 (SIRT 1) expression and intensifies the formation of AAA. In contrast, knockdown of miR-34a elicits an opposite effect. Knockdown of miR-34a or partial SIRT1 overexpression weakens the protective effect of METTL3 deficiency on AAA. This study suggested that METTL3/M6A-mediated maturation of miR-34a plays vital roles in the formation of AAA[105].

Aortic dissecting aneurysm (ADA) is an aortic remodeling disease with high mortality. FTO is highly expressed in the aorta of ADA patients and is positively correlated with several clinicopathological parameters including body mass index (BMI), triglyceride (TG), and D-Dimer. FTO is also highly expressed in human vascular SMC. Both vascular endothelium-induced FTO expression and forced FTO expression promote, while inhibition of FTO suppresses, the proliferation and migration of human vascular SMCs. Mechanistic studies showed that overexpression of FTO decreases the M6A modification of kruppel-like factor 5 (KLF5) mRNA in vascular SMCs, promotes KLF5 mRNA expression, increases p-GSK3β level, and enhances the GSK3β signaling pathway. Conversely, FTO down-regulation inhibits p-GSK3β and KLF5 expression regardless of AngII treatment[106].

3.7 Other cardiovascular diseases

In addition to the CVDs mentioned above, M6A methylation also takes part in the occurrence or development of other CVDs like diabetic cardiomyopathy (DCM) and hyperlipidemia. DCM

is a metabolic CVD, manifested as a decrease in myocardial glucose consumption, a slight increase in ketone metabolism, as well as a significant increase in fatty acid utilization. Ju *et al*. systematically described the modification pattern of total RNA M6A in hearts of DCM mice[107]. M6A levels in DCM and normal hearts were measured by M6A-specific MeRIP and MeRIP-seq combined with RNA-seq. It was found that total M6A levels in DCM are elevated, while FTO protein levels are mitigated. 973 new M6A peaks and 984 differential methylation sites were found, including 295 hypermethylated M6A sites and 689 hypomethylated M6A sites. Pathway analysis indicated that the unique M6A-modified transcripts in DCM is closely relevant to myocardial fibrosis, hypertrophy, and myocardial energy metabolism. Overexpression of FTO in DCM mice improves cardiac function by reducing myocardial fibrosis and myocardial hypertrophy[107].

Hyperlipidemia is a major risk factor for metabolic disorders and CVDs. Excessive deposition of saturated fatty acids in heart can cause chronic cardiac inflammation, leading to myocardial damage and systolic dysfunction. Effectively inhibiting cardiac inflammations has been considered a new strategy to diminish the negative impact of hyperlipidemia on CVDs. Recently, Yu *et al*. discovered a novel monomer called LuHui Derivative (LHD), which can reduce serum total cholesterol (TC), TG, LDL-C levels, cardiomyocyte lipid deposition, inflammatory infiltration caused by hyperlipidemia, and the release of IL-6 and TNF-α to improve cardiac function[108]. Furthermore, LHD-treated palmitic acid induces cardiomyocyte inflammation through an important cell surface receptor CD36 downstream. LHD specifically binds to FTO and inhibits the demethylation of M6A by FTO. In addition, overexpression of FTO significantly increases CD36 expression and inhibits the anti-inflammatory effect of LHD. Conversely, *FTO* knockdown affects the stability of CD36 mRNA and thus reduces CD36 expression and inhibits palmitic acid-induced cardiac inflammation[108].

Endotoxemia can induce life-threatening immune and cardiovascular responses, resulting in tissue injury, multiple organs failure, as well as death. Dubey *et al*. found that endotoxemia changed RNA methylation in myocardium[109]. They found that increased M6A level and decreased FTO expression in the myocardium of endotoxemic mouse induced by lipopolysaccharide (LPS). These changes are associated with significant increases in IL-6, TNF-α and IL-1β and reduce left ventricular function. LPS-exposed rat cardiomyocytes (H9c2) showed similar changes, with hypermethylation, increased IL-6 and TNF-α expression and down-regulated FTO expression in LPS-treated H9c2 cells compared with non-treated control cells. *FTO* knockdown up-regulates M6A RNA methylation and expression of IL-6 and TNF-α, and M6A methylation of IL-6 and TNF-α mRNA in *FTO* knockdown cells is also significantly increased compared with control cells[109].

3.8 M6A-SNPs and cardiovascular diseases

Genetic variation may influence M6A methylation by altering M6A targets or key nucleotides of RNA sequences. Therefore, M6A-associated single nucleotide polymorphism (M6A-SNP) is considered as an important functional variant. Mo *et al*. investigated the effect of M6A-SNPs on CAD in 185 000 individuals and uncovered that out of 4 390 M6A-SNPs detected, 304 are correlated with CAD ($P < 0.05$)[110]. Rs12286 is correlated with CAD at the genome-wide level ($P < 5.0\times10^{-8}$) and has the potential to affect M6A methylation and the binding affinity of the regulatory motif thereby the expression of ADAMTS7[110]. This study identified various CAD related M6A-SNPs and demonstrated their potential function[110]. Furthermore, Shi *et al*. found that M6A regulator WTAP is localized in the MI pathogenic region of chromosome 6, and SNPs in WTAP is significantly correlated with the progression of MI through data analysis of genome-wide association study (GWAS)[69]. Through analyzing single-cell sequencing data from heart tissue, WTAP was found widely expressed in heart with the highest expression level in EC. WTAP is significantly reduced in MI hearts, suggesting that maintaining normal expression of WTAP might be a means to prevent or improve MI[69]. In 2019, Mo *et al*. reported the effect of M6A-SNPs on lipid levels in a GWAS of 188 578 individuals[111]. A total of 1 655 M6A-SNPs were found, of which 395 are correlated with lipid level ($P < 0.05$) and 22 reaches genome-wide significant level. FGWAS analysis showed that SNPs affecting high density lipoprotein cholesterol (HDL-C) and TG are rich in M6A methylation. M6A-SNP rs6859 in PVRL2 3'UTR is related to HDL-C, LDL-C, TC, TG levels, and CAD, as well as PVRL2 mRNA expression in tibial artery and whole blood. In addition, PVRL2 is differentially expressed in adipose tissues with familial hyperlipidemia. In this study, the authors found many M6A-SNPs associated with lipids and suggested the important roles of M6A-SNPs in lipid metabolism[111]. In addition, large-scale GWASs have also been used to explore the relationship between M6A-SNPs and BP. One study documented that a total of 1 236 M6A-SNPs is correlated with BP, of which 33 reach the genome-wide significant level[112]. The proportion of M6A-SNPs with $P < 0.05$ was found higher than that of non-M6A-SNPs, and diastolic-associated SNPs are enriched in M6A-SNPs. About 10% of BP-associated M6A-SNPs are related to CAD or stroke. Furthermore, most of these M6A-SNPs are closely related to gene expression, with rs56001051, rs9847953, rs197922 and rs740406 being related to the expression of C1orf167, ZNF589, GOSR2 and DOT1L in human PBMCs, respectively[112].

4 Conclusion and perspectives

In a word, the present study suggests that the presence of M6A on cardiovascular transcripts is essential for maintaining cardiac function, M6A modification widely participates in the development of CVDs, and the proteins related to M6A modification play a crucial regulatory role in CVDs (Fig. 1, Table 1). In recent years, M6A modification at the RNA level as a new mechanism for CVDs has aroused great interest of researchers. This field is still in its infancy and needs more investment and exploration.

M6A as an epigenetic regulation is subject to environmental influences. CVDs showed strong regional variations and seasonal differences, and prevalence of CVDs is known to be much higher in extremely cold areas. These differences may be due at least partly to epigenetic alterations. Future research focusing on this aspect is needed; for instance, long-term residence in extremely cold areas may confer epigenetic differences and different patterns of energy metabolism in extremely cold areas may be regulated by M6A. Analysis of epigenetic differences in different regions may help identify new pathogenic mechanisms and therapeutic targets for precise prevention and control of CVDs. In addition, M6A is a dynamically reversible regulation, with large differences in different pathophysiological processes of disease or in different tissues[113]. Future research may focus on the dynamics of M6A regulation and the differences between tissues. Moreover, M6A participates in the process of disease often through modifying downstream target genes, rather than the overall level of M6A modification. Therefore, more attentions should be paid to the changes of M6A in specific genes as well as the changes of tissue levels of M6A. Recently, expression quantitative trait loci (QTL) data from four tissues (brain, lung, muscle, and heart) of 91 individuals were analyzed using the GTEx/eGTEx database to integrate the M6A data with genetic and expression differences between individuals. A total of 8 843 tissue-specific QTLs and 469 tissue-shared M6A-QTLs were

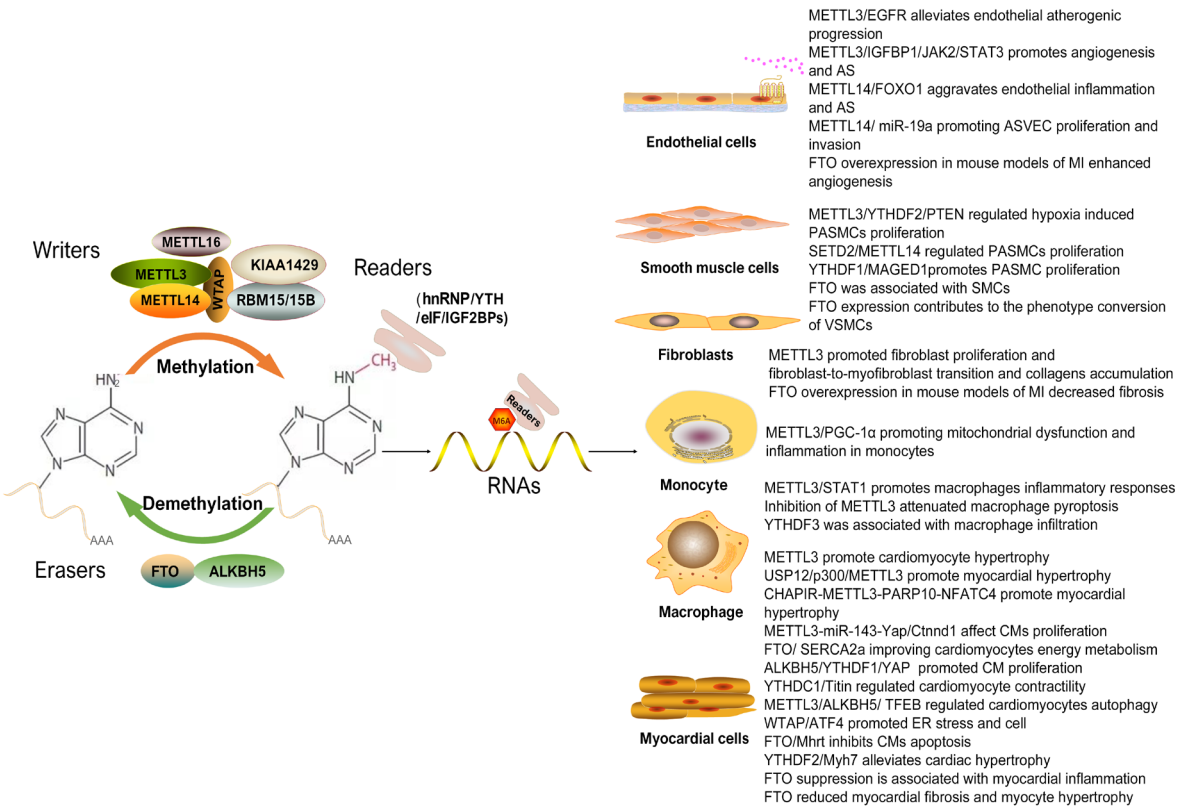

Fig. 1 M6A modification and its mechanisms involved in cardiovascular diseases

hnRNP, (nuclear inhomogeneous ribose protein); eIF, eukaryotic initiation factors; IGF2BPs, insulin like growth factor 2 mRNA-binding proteins; EGFR, epidermal growth factor receptor; JAK2, janus kinase 2; STAT3, (activator of transcription 3) ; FOXO1, forkhead box O1; ASVEC, atherosclerotic vascular endothelial cells; MI, myocardial infarction; PTEN. phosphatase and tensin homolog; SETD2, SET domain-containing 2; PASMCs, pulmonary artery smooth muscle cell; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1-α; STAT1, signal transducer and activator of transcription 1; USP12, ubiquitin specific proteinase 12; Ctnnd1, catenin delta 1; SERCA2a, sarcoplasmic/endoplasmic reticulum calcium ATPase 2a; YAP, yes-associated protein; TFEB, transcription factor EB; ATF4, activating transcription factor 4.

Table 1 The role of M6A regulators in cardiovascular diseases

Cardiovascular disease	M6A regulator	Related gene	Mechanism
Atherosclerosis /coronary artery disease	METTL3	EGFR	METTL3 mitigated endothelial atherogenic progression by M6A-dependent stabilization of EGFR mRNA[62]
	METTL3	NLRP1/ KLF4	In the AS model, partial ligation of the carotid artery resulted in plaque formation and up-regulation of METTL3. Knockdown of METTL3 prevented the atherogenic process[61]
	METTL3	STAT1	METTL3 promotes ox-LDL-triggered inflammatory responses in macrophages by interacting with STAT1 protein and mRNA[58]
	METTL3	IRF-1/ hsa_circ_0029589	Overexpression of IRF-1 suppressed the expression of hsa_circ_0029589, but induced its M6A level along with the expression of METTL3 in macrophages. Overexpression of hsa_circ_0029589 or inhibition of METTL3 significantly increased the expression of hsa_circ_0029589 and attenuated macrophage pyroptosis[59]
	METTL3	PGC-1	METTL3 modifies PGC-1α mRNA promoting mitochondrial dysfunction and ox-LDL-induced inflammation in monocytes[57]
	METTL3	JAK2	METTL3 knockdown prevented AS progression by inhibiting JAK2/STAT3 pathway via IGF2BP1[60]
	METTL14	FOXO1	METTL14 aggravated endothelial inflammation and AS by increasing FOXO1 M6A modification[64]
	METTL14	miR-19a	METTL14 increased the M6A modification of pri-miR-19a and promoted the processing of mature miR-19a, thus promoting the proliferation and invasion of ASVEC[63]
	FTO		Related to neointima formation[56]
	ALKBH1	MIAT/ HIF1α	Silencing of ALKBH1 or HIF1α could rescue the ox-LDL-increased level of MIAT[55]
	YTHDF1	NLRP1	The METTL3-mediated RNA hypermethylation up-regulated NLRP1 transcript through YTHDF1[61]
	YTHDF2	KLF4	The METTL3-mediated RNA hypermethylation down-regulated KLF4 transcript through YTHDF2[61]
	YTHDF2	PGC-1	METTL3 coordinated with YTHDF2 to suppress the expression of PGC-1α, as well as CYCS and NDUFC2 and reduced ATP production and OCR, which subsequently increased the accumulation of cellular and mitochondrial ROS and the levels of proinflammatory cytokines in inflammatory monocytes[57]
	IGF2BP1	JAK2	METTL3 knockdown prevented AS progression by inhibiting JAK2/STAT3 pathway via IGF2BP1[60]
Myocardial infarction/cardiac remodeling	METTL3		Inhibition of METTL3 completely eliminated the ability of cardiomyocytes to undergo hypertrophy when stimulated to grow, whereas increased expression of METTL3 promoted cardiomyocyte hypertrophy both *in vitro* and *in vivo*. Cardiac-specific METTL3 knockout mice shown morphological and functional signs of HF with aging and stress[72]
	METTL3	Collagen-associated genes	Enforced expression of METTL3 promoted proliferation and fibroblast-to-myofibroblast transition and collagens accumulation. Silencing METTL3 reduced cardiac fibrosis induced by MI via inhibiting the activation of cardiac fibroblasts[71]
	METTL3		USP12 was partially dependent on the stabilization of p300 to activate METTL3 expression and promoted myocardial hypertrophy[73]
	METTL3	CHAPIR	The piRNA CHAPIR regulated cardiac hypertrophy and cardiac remodelling by controlling METTL3-dependent M6A of Parp10 mRNA[74]
	METTL3	miR-143-3p	METTL3 deficiency resulted in heart regeneration after MI via METTL3-pri-miR-143-(miR-143)-Yap/Ctnnd1 axis[76]
	ALKBH5	YAP	ALKBH5 regulated cardiomyocyte proliferation and heart regeneration by demethylating the mRNA of YTHDF1[77]
	YTHDF1	YAP	ALKBH5 regulated cardiomyocyte proliferation and heart regeneration by demethylating the mRNA of YTHDF1[77]
	YTHDC1	Titin	Cardiac-specific conditional YTHDC1 knockout led to obvious left ventricular chamber enlargement and severe systolic dysfunction. YTHDC1 induces DCM by abnormal splicing of Titin[75]
Myocardial ischemia-reperfusion injury	METTL3	BAX /PTEN	Down-regulated in both young and elderly hearts. BAX and PTEN are target genes of METTL3 under iH/R stress[81]
	METTL3	miR-25-3p/miR-873-5p	METTL3 up-regulated miR-25-3p and miR-873-5p to activate the PI3K/Akt pathway, leading to the suppression of I/R injury[82]
	METTL3	TFEB	Silencing METTL3 enhanced autophagic flux and inhibited apoptosis in H/R-treated cardiomyocyte[84]
	WTAP	ATF4	WTAP promoted myocardial H/R injury by increasing endoplasmic reticulum stress via regulating M6A modification of ATF4 mRNA[83]
	FTO	SERCA2a	FTO decreased M6A level of SERCA2a mRNA, thus accelerating SERCA2a expression, maintaining calcium homeostasis and improving the energy metabolism of H/R cardiomyocytes[79]
	ALKBH5		Ferritin nanocage loaded with ALKBH5 inhibitor improved the cardiac function and reduced the infarct size in AMI[80]

Continued

Table 1 Continued

Cardiovascular disease	M6A regulator	Related gene	Mechanism
Heart failure	FTO		Cardiomyocyte restricted knockout of FTO impaired mice cardiac function[51]
	FTO		Up-regulated expression in HFpEF patients and HFpEF mice[89]
	FTO		FTO weakened cardiac dysfunction by regulating glucose uptake and glycolysis in mice with pressure overload-induced HF[90]
	FTO		FTO was decreased expression in failing mammalian hearts and hypoxic cardiomyocytes, thereby increasing M6A in RNA and decreasing cardiomyocyte contractile function. FTO overexpression decreased fibrosis and enhanced angiogenesis[91]
	FTO	MHRT	FTO overexpression inhibited apoptosis of hypoxia/reoxygenation-treated myocardial cells by regulating M6A modification of MHRT[92]
	YTHDF2	MYH7	YTHDF2 improved cardiac hypertrophy by regulating MYH7 mRNA decoy[93]
Hypertension	METTL3		Down-regulated in postnatal HPH[97]
	METTL3	PTEN	YTHDF2 recognized METTL3 mediated M6A modified PTEN mRNA and accelerated the degradation of PTEN, which resulting in over-proliferation of PASMC by activating PI3K/Akt signaling pathway[99]
	METTL14		Down-regulated in postnatal HPH[97]
	METTL14	SETD2	SEDT2/METTL14-mediated M6A methylation awakening resulted in hypoxia-induced PAH in mice[100]
	FTO		Decreased expression of FTO in small PA of MCT-PAH rat[97]
	FTO		Down-regulated in postnatal HPH[97]
	ALKBH5		Down-regulated in postnatal HPH[97]
	YTHDF1	MAGED1	YTHDF1 promoted PASMC proliferation and PH by improving MAGED1 translation[98]
	YTHDF1		Increased expression of YTHDF1 in small PA of MCT-PAH rat[97]
	YTHDF2	PTEN	YTHDF2 recognized METTL3 mediated M6A modified PTEN mRNA and accelerated the degradation of PTEN, which resulting in over-proliferation of PASMC by activating PI3K/Akt signaling pathway[99]
Aortic aneurysm	METTL3	miR-34a	METTL3 induced AAA development and progression by modulating M6A-dependent primary miR-34a processing[105]
	METTL14		Down-regulated expression in human AAA, low METTL14 expression was related to high WBC and CRP expression[104]
	METTL14		Associated with inflammatory infiltration and neovascularization[103]
	RBM15B		Up-regulated expression in human AAA[104]
	FTO	KLF5	FTO expression promotes phenotype conversion of VSMC[106]
	FTO		Associated with aneurysm smooth muscle cells[103]
	YTHDF3		YTHDF3 was associated with a greater risk of rupture and a strong association with macrophage infiltration[103]
	HNRNPC		Down-regulated expression in human AAA[104]
Other cardiovascular diseases	FTO		Overexpression FTO can reduce cardiac fibrosis and myocardial cell hypertrophy[107]
	FTO	CD36	FTO knockdown affects the stability of CD36 mRNA and thus reduces the expression of CD36 and inhibits palmitic acid-induced cardiac inflammation[108]
	FTO	IL-6/TNF-α	FTO knockdown in rat cardiomyocyte up-regulated M6A RNA methylation and expression of IL-6 and TNF-α [109]

EGFR, epidermal growth factor receptor; M6A, N6-methyladenosine; NLRP1, NLR family pyrin domain containing 1; KLF4, kruppel-like factor 4; AS, atherosclerosis; STAT1, signal transducer and activator of transcription 1; PGC-1α, proliferator-activated receptor γ coactivator 1-α; JAK2, janus kinase 2; STAT3, signal transducer and activator of transcription 3; IGF2BPs, insulin like growth factor 2 mRNA-binding proteins; FOXO1, forkhead box O1; ASVEC, atherosclerotic vascular endothelial cells; HIF1α, hypoxia inducible factor 1α; OCR, oxygen consumption rate; ROS, reactive oxygen species; YAP, yes-associated protein; HF, heart failure; MI, myocardial infarction; USP12, ubiquitin specific proteinase 12; PTEN, phosphatase and tensin homolog; TFEB, transcription factor EB; H/R: hypoxia/reoxygenation; BAX: BCL-2-associated X; SERCA2a, sarcoplasmic/endoplasmic reticulum calcium ATPase 2a; AMI, acute myocardial infarction; HFpEF, heart failure with preserved ejection fraction; HPH, hypoxia mediated pulmonary hypertension; SETD2, SET domain-containing 2; MAGED1, melanoma antigen D1; AAA, abdominal aortic aneurysm; KLF5, kruppel-like factor 5; M6A, N6-methyladenosine.

found. These M6A-QTLs are enriched in expression QTLs and are mostly orthogonal to expression QTLs. 184 GWAS co-located M6A-QTLs are also identified, including brain M6A-QTLs associated with neuroticism, depression, schizophrenia and anxiety, lung M6A-QTLs correlated with expiratory flow and asthma, and myocardial/cardiac M6A-QTLs correlated with CAD[114]. It is suggested that the differences in the regulation of M6A between tissues play key roles in diseases and exploring tissue-specific regulation of M6A will contribute to understanding the pathological mechanisms and the progression of diseases at deeper levels.

M6A can modify various types of RNAs. Previously, researchers believed that mRNA is the most important RNA species, so the research focus were on mRNA. Now, increasing evidence suggests that non-coding RNA may play important regulatory roles in various pathophysiological processes. For example, piRNAs, as important regulatory factors, take part in the occurrence and development of CVDs[115]. Although M6A is mainly present in mRNA, it also exists in non-coding RNAs such as tRNA, rRNA, circRNA and snRNA[116]. Jakobi *et al*. recently identified shared circRNAs and model-specific circRNAs from human induced pluripotent stem cell-derived cardiomyocyte (HIPSC-CMS), human health and disease (ischemic cardiomyopathy, dilated cardiomyopathy) heart and HUVECs[117]. The integration of M6A data provides evidence for the M6A methylation in these circRNAs, which may be associated with translational regulation. They also described a circRNA subclass (AUG circRNAs) containing an initiation codon in its master transcript in a cardiac model system and enrichment of M6A methylation in AUG circRNAs[117]. The modification of these non-coding RNAs by M6A may also be involved in the pathophysiological process of CVDs, which deserves more attention in the future.

With continuous emergence of new omics technologies, the development of omics research has been accelerated to the directions of quantification and high throughput. The integration and analysis of multiple omics data have become a new direction for exploring the mechanism of life. Multi-omics is an approach to explore the interactions among various substances in living systems, including genomics, epigenomics, transcriptomics, proteomics, metabolomics, and microbiome, which together affect the phenotypes and traits of living systems. Biological phenomena and gene expression regulation are complex and diverse, and the conclusion of a single omics study is often not comprehensive. For example, single cell omics has advanced the research of various diseases including CVDs, but the premise of single-cell sequencing is that organization must be mechanically separated or enzyme digested into single cell suspension, which inevitably loses tissue cells of the original location information and breaks the intercellular communication network. This makes it hard to get the cell composition and gene expression status of different regions in the tissue. However, the recently popular spatial transcriptomics technology utilizes the advantages of conventional in-situ technology and omics technology to combine the spatial information of total mRNAs with morphological content and plots the locations of all gene expression to obtain complex and complete gene expression maps of diseases. This method preserves spatial location while identifying different cell populations, so as to provide important information on the relationships among cell function, phenotype, and location in the tissue microenvironment. Multi-omics combined analysis has also aroused the interest of cardiovascular researchers, and great advances have been achieved in the past two years. Li *et al*. performed a complete "phenotypic" analysis of the left ventricular tissues from the healthy and patients with cardiomyopathy (ICM and DCM) by combining proteomics and metabolomics to identify common and unique signaling pathways and factors sex-specific molecules in these cardiomyopathies[118]. Cui *et al*. found that serum succinic acid levels are significantly increased in aortic aneurysm and dissection (AAD) patients by untargeted and targeted metabolomics analyses with two AAD cohorts and healthy controls, respectively and suggested that by combining with clinical data analysis, succinic acid can be used as a novel diagnostic marker for AAD[119]. By combining 16S rRNA sequencing and metabolomics analysis, Yan *et al*. found that a high-salt diet reduces Bacteroides by changing the composition and metabolism of intestinal microflora and increasing the level of corticosterone to promote hypertension[120]. Barallobre-Barreiro *et al*. integrated single-cell sequencing and proteomics analysis on the left ventricular tissues from HF patients and the control subjects to reveal ECM component of scar myocardium[121]. They found the accumulations of polysaccharides and other proteoglycans in scar myocardium in patients with ischemic HF[121]. Yokota *et al*. analyzed cardiac scar tissue of mice at 3, 7, 14, 21, and 42 days after ischemic heart injury respectively using multiple omics (transcriptomics, proteomics, and single-cell omics), screened out a key collagen gene col5a1 in scar tissue of early MI, and revealed the mechanical properties of its influence on scar tissue fibroblasts and mechanisms[122]. Porritt *et al*. combined single-cell omics and spatial transcriptomics in a mouse model of Kawasaki disease (KD) vasculitis to construct cell maps of inflammatory vascular tissue and found that the infiltration of innate and adaptive immune cells is associated with increased NLRP3 and IL-1β expression, revealing the cellular diversity of IL-1β production and signal transduction in KD-related CVD and providing a theoretical basis for targeting NLRP3 in the treatment of KD-associated CVD[123]. Asp *et al*. selected embryonic heart tissue from 4.5-5.0 weeks, 6.5 weeks, and 9 weeks post gestation for spatial transcriptome and single-

cell transcriptome sequencing to construct a 3D map of the whole heart of human cardiac development[124]. At present, several studies have identified the regulatory targets of CVDs by MeRIP-seq and RNA-seq, and recent clinical IHD epigenomics and biomarkers (IHD-EPITRAN) studies have been carried out to gain more complete understanding of epigenetic properties and its roles in CVDs pathology, as well as to identify epigenome IHD biomarkers and potential drug targets[125]. RNA regulation is complex, and it is difficult to clarify the pathophysiological process from the RNA level alone. Multi-omics analysis allows for systemic analyses and panoramic view of protein/transcription and metabolites, exploration of cause-and-effect relationships of biological processes and human diseases, and delineation of the underlying molecular mechanisms. Whilst the combinations of different omics have made great breakthroughs in the research field of CVDs, the possible combinations of epigenetics with other omics have been rarely studied. Future studies can integrate epigenetics with other omics to systematically explore the regulatory mechanisms of CVDs from multiple dimensions by combining DNA and protein levels. For example, the gut microbiome, is now found to be strongly linked to CVDs. Metabolic effects of gut microbiota play crucial roles in the pathogenesis of cardiometabolic diseases. M6A may be involved in the regulation of the complex interactions between gut microbiota and host metabolism. New discoveries may be made by integrating epigenome and gut microbiome or other omics.

In addition, M6A modification can be combined with other novel biotechnologies, such as bioengineering, an advanced technique that leverages the latest advances in molecular biology to consciously manipulate genetic material targeting organisms or their functions. Cheng *et al*. combined bioengineering technology with M6A modification to construct a surf-modified bioengineered ferritin nanocage and loaded ALKBH5 inhibitor IOX1 into the constructed composition, which could effectively improve cardiac function and reduce infarct area of AMI[80]. This suggests that combining M6A modification and bioengineering technology is effective and feasible and may provide a potential strategy for CVDs treatment in the future.

Moreover, currently available studies suggest that M6A modification is mediated by M6A modification enzyme that plays a core role in M6A-mediated epigenetic modification. However, the M6A-related enzymes identified to date may not be complete and it is possible that some other enzymes are yet-to-be discovered in future studies. Currently, studies on M6A modification in CVDs have been primarily focused on METTL3 and FTO, and the roles of other related enzymes remain poorly understood. Furthermore, multiple mechanisms of epigenetic modifications of RNAs have been discovered. Yet, in this review, we only touch the M6A modification that has been more extensively and intensively studied. Other modifications, like M5C, have been investigated in insects and plants, with few studies involving human diseases due to technical limitations. In the future, with the development of sciences and technologies, more information about other types of RNA modifications related to CVDs may be obtained.

Conflicts of interests

The authors have no conflicts to declare.

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Author contributions

Lingfeng Zha provided ideas. Lingfeng Zha and Jing Lin Wang organized the figure and wrote the manuscript. Lingfeng Zha revised the manuscrip. All authors reviewed the manuscript and agreed to publish.

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