Review

The potential of RNA methylation in the treatment of cardiovascular diseases

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SUMMARY

RNA methylation has emerged as a dynamic regulatory mechanism that impacts gene expression and protein synthesis. Among the known RNA methylation modifications, N6-methyladenosine (m^{6}A), 5-methylcytosine (m^{5}C), 3-methylcytosine (m^{3}C), and N7-methylguanosine (m^{7}G) have been studied extensively. In particular, m^{6}A is the most abundant RNA modification and has attracted significant attention due to its potential effect on multiple biological processes. Recent studies have demonstrated that RNA methylation plays an important role in the development and progression of cardiovascular disease (CVD). To identify key pathogenic genes of CVD and potential therapeutic targets, we reviewed several common RNA methylation and summarized the research progress of RNA methylation in diverse CVDs, intending to inspire effective treatment strategies.

INTRODUCTION

Cardiovascular disease (CVD) is one disease with the highest morbidity and mortality, and it has become the number one killer threatening human health.[1] Myocardial injury caused by myocardial infarction (MI), myocardial inflammation, atherosclerosis (AS), hemodynamic overload, and other causes will lead to structural changes and functional dysfunction of myocardial tissue.[2] Currently, the prevention and treatment of CVDs are very limited, and it is urgent to develop targeted and effective treatment strategies.

Epigenetics is an emerging field where dynamic modifications of proteins and RNA are involved in regulating multiple important biological functions. The common epigenetic processes mainly include DNA methylation, histone modification, chromatin remodeling, nucleosome localization, and RNA regulation. In recent years, RNA modification has been widely found to be involved in the pathological process of tumors, metabolic diseases, and CVDs.[3–7] RNA modification can affect RNA folding, embed transcriptional sequences, or form specific recognition elements to play biological functions. It is an important way of post-transcriptional regulation (PTR) of gene expression from the RNA level. At present, about 170 kinds of RNA modifications have been identified and reported, which widely exist in various RNAs, such as mRNA, tRNA, rRNA, and ncRNA.[8] RNA methylation is the predominant form of RNA modification, constituting more than half of the overall modifications observed.[9] Without altering the sequence, RNA methylation can affect cell proliferation, apoptosis, and metabolism.[10] N6-methyladenosine (m^{6}A), N1-methyladenosine (m^{1}A), N6, 2-O-dimethyladenosine (m^{6}Am), N5-methylcytosine (m^{5}C), N7-methylguanosine (m^{7}G), and N3-methylcytosine (m^{3}C) are common forms of RNA methylation.[3] In addition, RNA acetylation of N4-acetylcytidine (ac^{4}C) also plays an important role in RNA epigenetic process.[11] In recent years, m^{6}A has been widely studied in CVDs. This review aims to discuss the research progress of RNA methylation related to diverse CVDs and provide a new vision for the treatment of diseases.

OVERVIEW OF RNA METHYLATION

RNA modification is the key to post-transcriptional regulation, which provides a new way for gene expression regulation. RNA methylation refers to the substitution of original atoms or groups on the substrate to add methyl groups.[12] RNA methylation does not change the original sequence of genes, but changes in RNA structure will affect the relevant functions of these RNAs, resulting in epigenetic changes to regulate gene expression.[13,14] RNA methylation has important contributions to RNA nuclear export, translation initiation, transcript stability, splicing, folding, and localization.[4] With the in-depth study of RNA methylation, enzymes responsible for writing, reading and erasing RNA methylation have been gradually identified. Given the important functions of RNA methylation regulated by these modifying enzymes in a variety of diseases, they have also received significant attention. Disordered RNA methylation can lead to dysfunction of RNA function and trigger a series of pathological changes in the body. Research has confirmed that RNA methylation is closely related to various diseases, including cardiovascular diseases, neurological disorders, metabolic diseases, viral infections, and tumors. Various types of RNA can be modified, and

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understanding the relevant mechanisms of RNA methylation can provide new targets and ideas for the diagnosis and treatment of diseases (Table 1).

m[6]A

m[6]A is a common type of internal modification of mRNA, and nearly 1/4th of the mRNA carries at least one m[6]A site.[5] m[6]A is widely distributed and its modification sites are highly conserved. It can change the stability of mRNA, affect the processing of miRNA, regulate the interaction between RNA-protein, and participate in the degradation of circRNA.[39–42] Meanwhile, m[6]A RNA methylation is regulated by methyltransferases (writers) and demethylases (erasers), which are dynamically reversible.[43] m[6]A writers that have been identified mainly include a structurally stable heterodimer complex composed of catalytic subunit methyltransferase-like 3 (METTL3) and its homologous protein (supporting subunit) methyltransferase-like 14 (METTL14); Wilms' tumor 1-associating protein (WTAP).[44] Among them, METTL3 has methyltransferase activity, which is mainly concentrated in the nucleus and enriched in nuclear speckles.[45] METTL14 does not have methyltransferase activity. And it is mainly used as a scaffold for the combination of a complex with RNA to help better recognize the substrate.[46] WTAP acts as a regulatory subunit that helps recruit the METTL3/METTL14 complex to nuclear speckles. WTAP itself has no methyltransferase activity, and its regulation of the METTL3/METTL14 complex is limited to the *in vivo* environment but has no effect *in vitro*. In addition, vir-like m[6]A methyltransferase associated (VIRMA) could mediate preferential m[6]A in 3′UTR and near stop codon.[16] Meanwhile, the downregulation of VIRMA was accompanied by a decrease in the m[6]A level.[47] RNA-binding motif protein 15 (RBM15) and its paralog RBM15B, acting as additional subunits of the methyltransferase complex, are involved in mediating the formation of m[6]A and guiding the methylated complex to the specific m[6]A sites.[48] METTL16 is a novel writer recently identified which can bind and methylate MAT2A mRNA and U6 small nuclear RNA (snRNA).[20] The m[6]A defect was found in HAKAI mutant flies, suggesting that HAKAI was one of the core members involved in affecting the activity of m[6]A writers.[49] Two main types of m[6]A erasers have been reported, which are fat mass and obesity-associated protein (FTO) and ALKB homolog 5 (ALKBH5).[50,51] FTO can convert m[6]A to N6-formyladenosine by a two-step oxidation reaction, while ALKBH5 is directly demethylated.[51,52] The long non-coding RNA antisense to FOXM1 (FOXM1-AS) promotes the interaction between ALKBH5 and the new transcript of FOXM1, and ALKBH5 further increases the expression of FOXM1 by erasing the methylation of the new transcript of FOXM1.[53] In addition, m[6]A is also regulated by a class of site-reading enzymes (readers), which include the YTH family with YTHDF1-3, YTHDC1, YTHDC2, eukaryotic initiation factor 3 (eIF3), FMR1, insulin-like growth factor-2 mRNA-binding proteins (IGF2BPs), and proline rich coiled-coil 2A (PRRC2A). Their function is to identify m[6]A modified sites and participate in the regulation of RNA processing, transport, translation, and stability[41] (Figure 1). YTHDF2 can specifically recognize m[6]A and promote RNA degradation.[39] The YTH domain in YTHDF2 directly binds to the m[6]A site of Notch1 mRNA to regulate Notch signaling. However, under heat shock conditions, YTHDF2 containing the YTH domain undergoes nuclear translocation, inhibits Notch1 mRNA degradation, increases Notch intracellular domain (NICD), restores Notch signaling, and ultimately affects processes, such as cell proliferation, differentiation, and apoptosis.[54] At the same time, m[6]A participates in RNA-RNA/protein interaction and chromatin remodeling, thus regulating gene expression. YTHDF3 can recognize and bind to m[6]A-modified lncRNA GAS5, promoting its degradation and cell differentiation.[55] In addition, YTHDF3 can also affect the expression and function of specific miRNAs.[56,57]

m[5]C

m[5]C modification is characterized by the addition of a methyl group to the fifth carbon atom of cytosine (Figure 2). It is ubiquitous in diverse types of RNA, including rRNA, tRNA, mRNA, mtRNA, ncRNA, and enhancer RNA (eRNA). m[5]C modification on tRNA is more abundant than other types of RNA. The function of m[5]C modification on diverse types of RNA is different. m[5]C in mRNA is related to mRNA export, maintenance of stability, and translation.[58–60] In rRNA, m[5]C participates in the translation and interpretation of codons.[61] In tRNA, m[5]C is crucial for the maintenance of its structure and stability and ensures the accuracy of translation.[62] In eRNA, it can affect gene transcription.[28] NSUN (1–7), DNMT1/2, and TRDMT1 were identified as m[5]C methyltransferases. Among them, NSUN2 has attracted more attention, which can participate in the modification of ncRNA, mRNA, and tRNA. It has been reported that many DNMTs can bind to RNA and play a role in the recruitment and regulation of enzyme activity.[63] DNMT1 can bind to miR-155-5p and inhibit its enzyme activity, leading to hypomethylation in certain regions.[64] This abnormal methylation state can cause changes in gene expression. At present, only the TET protein family has m[5]C demethylase activity. In addition, Aly/REF export factor (ALYREF) as a nuclear m[5]C reader participates in the mRNA export and PTR.[65] Through the m[5]C reader Y-box binding protein 1 (YBX1) helps maintain the stability of the target mRNA, and enhances translation.[58,66,67]

m[7]G

m[7]G was first discovered and reported at the mRNA start site. It is a methyl group added to the 7th N of RNA guanine and makes it carry a positive charge[68] (Figure 3). m[7]G is widely found in many species, including mRNA, 5′ cap of mRNA, mature miRNA, pri-miRNA, rRNA, and tRNA. The 5′ end of mRNA is modified with m[7]G, which is responsible for recruiting initiation factors and enabling cells to distinguish between self mRNA or viral mRNA.[69] The eukaryotic translation initiation factor 4E (eIF4E), as a mRNA binding protein, forms a complex on the m[7]G-cap structure and participates in the process of mRNA translation initiation.[70] Cap-binding protein 20 (CBP20) is phylogenetically conserved and can interact with the m[7]G cap structure on the RNA polymerase II transcript and participate in the nuclear transmission and splicing of mRNA.[71] Increasing m[7]G modification can help the mRNA resist decay, increase translation efficiency, and reduce ribosome pauses and other functions.[72,30] METTL1-WDR4, as a known m[7]G methyltransferase complex, can help tRNA increase m[7]G modification, which is an important link in the translation process, while WBSCR22 mainly mediates m[7]G modification in rRNA.[73]

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Table 1. List of RNA involved in methylation

| RNA modification	Types of RNA	Function or mechanism	Reference
m6A	mRNA	Bawankar et al. found that E3 ubiquitin ligase Hakai affects mRNA m6A biogenesis and function in Drosophila and human cells	Bawankar et al.[15]
		VIRMA mediates the preferential methylation of mRNA 3′UTR and near termination codon	Yue et al.[16]
	miRNA	METTL3 upregulated the level of m6A and interacted with DGCR8 to promote the high expression of miRNA	Wang et al.[17]
	lncRNA	METTL3 mediates m6A modification of lncRNA PCAT6, leading to upregulation of PCAT6	Lang et al.[18]
	circRNA	The m6A modification of circRNA-SORE enhances its RNA stability and increases tumor drug resistance	Xu et al.[19]
	snRNA	Pendleton and colleagues proposed that METTL16 is a long-term unknown methyltransferase of U6 spliceosome snRNA	Pendleton et al.[20]
	rRNA	Nhan et al. found that METTL5 was the enzyme responsible for 18S rRNA m6A modification and ZCCHC4 was the enzyme responsible for 28S rRNA modification	van Tran et al.[21]
	tRNA	Knockdown of TRMT10A increased the m6A modification of tRNA$^{Gln(TTG)}$, tRNA$^{Arg(CCG)}$ and tRNA$^{Thr(CGT)}$	Ontiveros et al.[22]
m5C	rRNA	NOP2/NSUN1-mediated deposition of m5C on rRNA	Liao et al.[23]
	tRNA	Maintain the stability of tRNA secondary structure	Agris[24]
	mt-tRNA	NSUN2 participates in m5C modification of mt-tRNAs variable loop (V-loop) regions in mice and human cells	Van Haute et al.[25]
	lncRNA	m5C modification of lncRNA H19 enhances its stability	Sun et al.[26]
	mRNA	NSUN6-targeted mRNA m5C modification enhances its transcription efficiency and translation ability	Selmi et al.[27]
	eRNA	Correlated with metabolic stress	Aguilo et al.[28]
m7G	mRNA	METTL1-mediated mRNA m7G enhancement promotes restoration of blood flow and angiogenesis in postischemic injury	Zhao et al.[29]
	tRNA	m7G-modified tRNA$^{Arg(TCT)}$ is associated with oncogenic cell transformation	Orellana et al.[30]
	miRNA	METTL1-mediated m7G enhances miRNA processing by disrupting the secondary structure in pri-miRNA	Pandolfini et al.[31]
m6Am	mRNA	PCIF1-mediated m6Am modification enhances the stability of FOS mRNA and affects tumor progression	Wang et al.[32]
	snRNA	The m6Am regulation of U2 snRNA regulates the splicing of specific pri-mRNA transcripts	Goh et al.[33]
m1A	mRNA	m1A modification increases mRNA translation	Dominissini et al.[34]
	tRNA	m1A demethylated tRNA is more sensitive to angiogenin	Chen et al.[35]
	rRNA	RRP8 mediates m1A modification of 28S rRNA and participates in the formation of ribosomal subunits	Waku et al.[36]
m3C	mRNA	METTL8 contributes m3C to mRNA	Xu et al.[37]
	tRNA	METTL2 and METTL6 contribute m3C in specific tRNA	Xu et al.[37]
	mtRNA	Mettl8 mediates mitochondrial tRNA$^{Chr/Ser (UCN)}$ m3C and affects mitochondrial function	Zhang et al.[38]
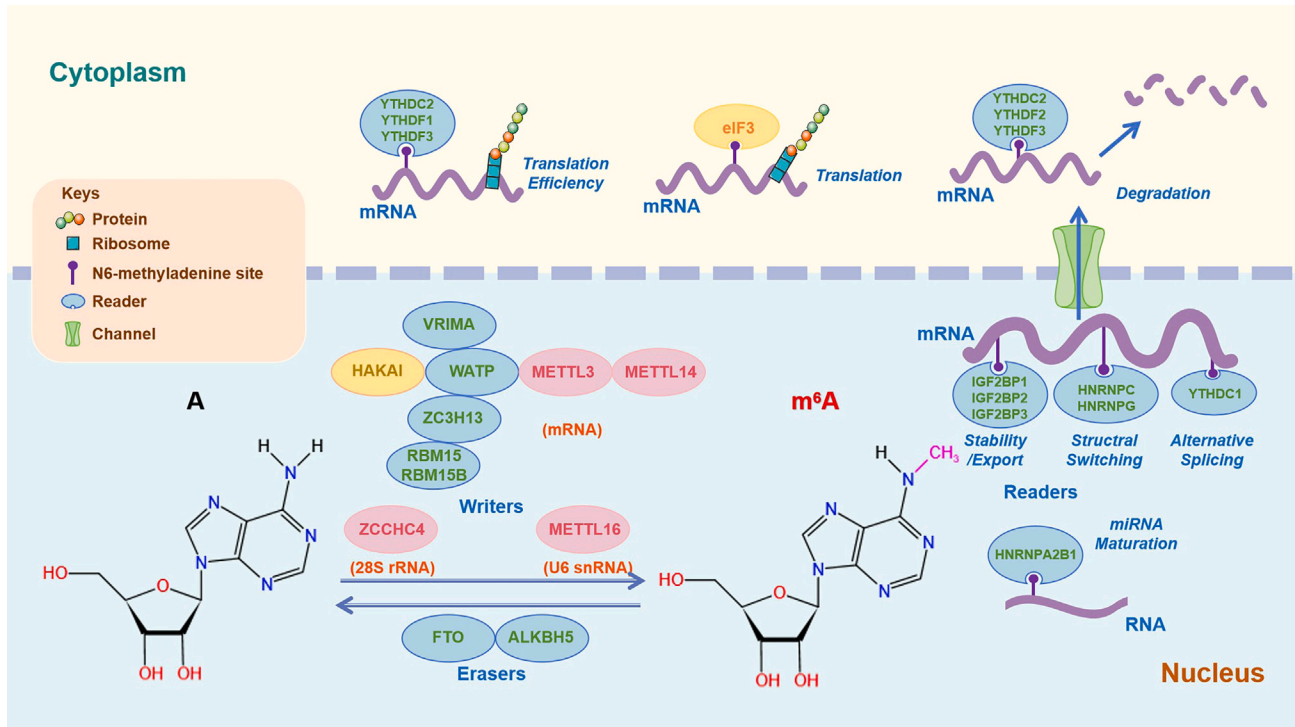
Figure 1. The functional landscape of m⁶A writers, readers, and erasers

RNA methyltransferase complex (METTL3/14/16, WTAP, VIRMA, RBM15/15B, etc.) catalyzes the methylation of m⁶A. RNA demethylases (FTO and ALKBH5) remove m⁶A methylation. The m⁶A readers include YTHDF1-3, YTHDC2, etc., which can recognize m⁶A on mRNA and participate in gene expression regulation.

m⁶Am

m⁶Am modification is mainly distributed around mRNA 5′ cap. Based on m⁶A, a 2′-OH is methylated to 2′-O-methyladenosine (Am) and then further methylated at its N6 position to produce m⁶Am[69,74] (Figure 4A). The methylation of m⁶Am is a reversible dynamic process. When cells encounter heat shock, hypoxia, and other stress stimuli, their modification level will be upregulated.[75] It also suggests that m⁶Am modification may have a potential mechanism of action on the cellular stress response. Deletion of the methyltransferase METTL3 leads to transcription abnormalities of m⁶Am modified RNAs.[76] Using reverse genetics, Akichika et al. demonstrated that phosphorylated CTD-interacting factor 1 (PCIF1) can bind to the serine-5 phosphorylated CTD of RNA pol II and act as a writer for m⁶Am modification.[77] The methylation of m⁶Am catalyzed by CAPAM/PCIF1 promotes the translation of capped mRNA, but other studies have observed an increase in the stability of m⁶Am modified RNA compared to its Am modified RNA.[77–80] METTL4 can mediate m⁶Am modification within snRNA and regulate the selective splicing of some RNAs.[33] FTO can remove the N6 methyl group on m⁶Am modified RNA and participate in the biogenesis of snRNA.[81] Due to the lack of specific antibodies against m⁶Am, m⁶A antibodies are still needed to help with sequencing tasks. Fortunately, a technique called m⁶Am-EXO-SEQ can be used to help researchers conduct m⁶Am-specific immunoprecipitation.[82] However, this technology is to enrich the 5′ terminal of mRNA after depleting the m⁶A modified RNA fragment in the sample, and then use the antibody of m⁶A for the following experiments. Sun et al. reported that m⁶Am-SEQ can reveal high-reliability m⁶Am and 5′-UTR m⁶A methylome by using a similar method.[75] Recently, Dodson and colleagues used liquid chromatography-tandem mass spectrometry (LC-MS/MS) method and analyzed in multiple reaction monitoring mode to observe a decrease in m⁶Am levels in H9c2 cells cultured in a high glucose medium.[83] In this way, the dilemma of lacking an anti-m⁶Am antibody is partly bypassed.

m¹A

m¹A is another common form of RNA post-transcriptional modification (PTM) discovered in recent years. It is an isomer of m⁶A, which participates in the modification of tRNA, rRNA, mRNA, and mitochondrial RNA (mtRNA).[5] The process is similar to m⁶A, which is dynamically reversible. Compared with m⁶A, the abundance of m¹A in mRNA is lower. More and more methods have been developed directly detecting m¹A modification on RNA, helping us to better understand it. Richter et al. detected the modification of mitochondrial tRNA using the demethylase-thermostable group II intron RT tRNA-sequencing (DM-tRNA-seq) method and confirmed that m¹A plays an important role in translation elongation and stability of selected nascent strands.[84] There is also a potential correlation between glucose and m¹A levels in the apex and atrium.[83] The modification of m¹A can interfere with Watson-Crick base pairing, thereby affecting protein-DNA interactions.[77] It is also believed to regulate the stability of tRNA and rRNA. Methyltransferase TRMT6 and TRMT61A can catalyze mRNA to form m¹A.[85]
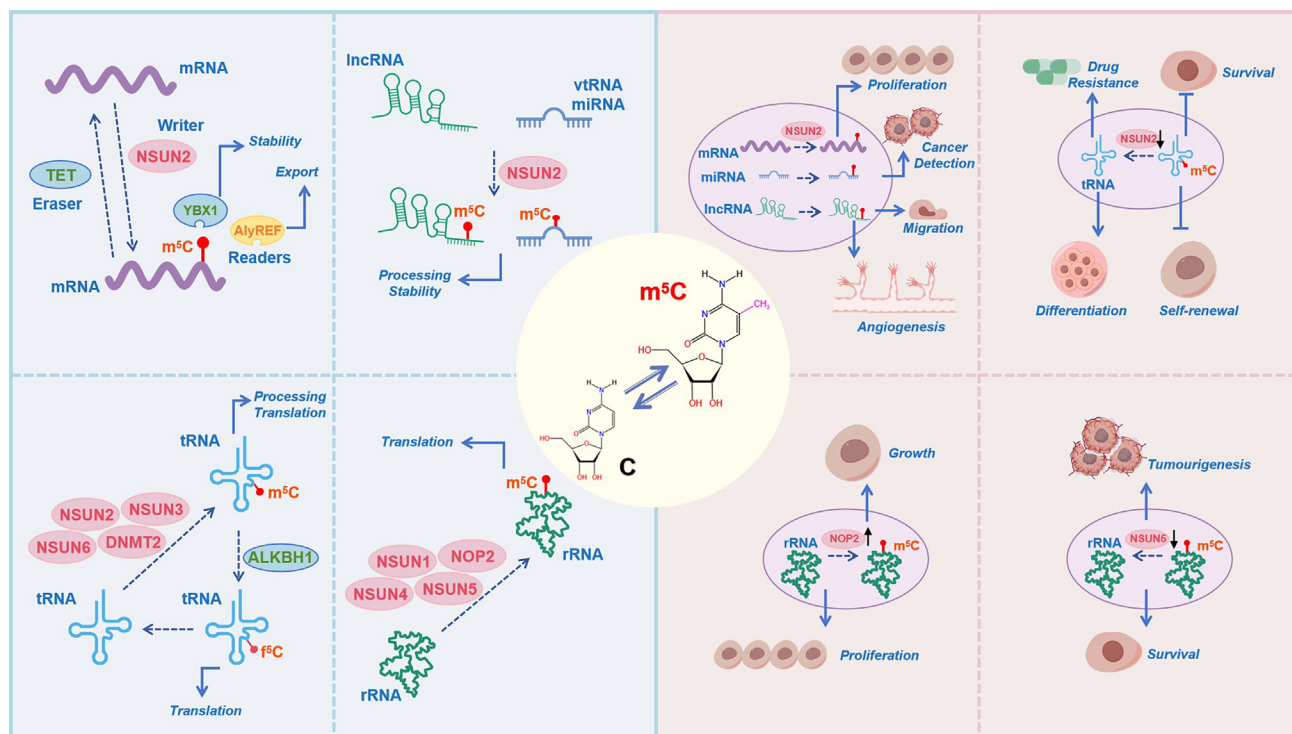
Figure 2. Mechanism of regulation of m⁵C modifications in regulation of various biological processes

RNA methyltransferases (NSUN(1–7), DNMT1/2, TRDMT1) catalyze the methylation of m⁵C. RNA demethylase (TET) removes m⁵C methylation. The m⁵C readers include ALYREF and YBX1, which can recognize m⁵C on mRNA and participate in mRNA export, post-transcriptional regulation and increase stability.

TRMT10C and TRMT61B showed the mitochondrial tRNA (mt-tRNA) adenine-N1-methyltransferase activity.[86] Demethylase FTO and ALKBH1 can demethylate the m¹A modification in tRNA.[86] ALKBH3 could eliminate the modification of m¹A in tRNA and mRNA.[87] ALKBH7 mediates m¹A demethylation of nascent mtRNA, which regulates nascent polycistronic mt-RNA processing and mitochondrial function.[88] YTHDF1-3 and YTHDC1 may act as the readers to recognize and bind m¹A sites.[89] Zheng et al. found that YTHDF3 can recognize m¹A modification,
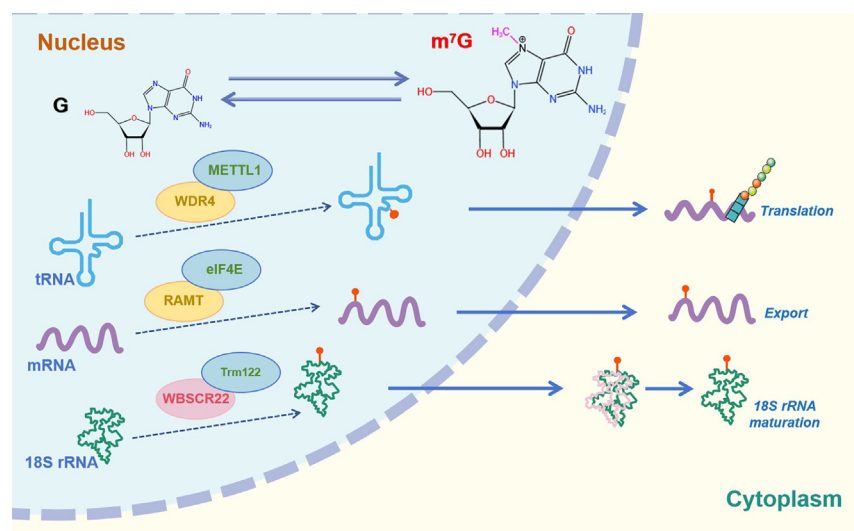

Figure 3. The functional landscape of m⁷G writers, readers, and erasers

m⁷G modifcation in tRNA regulated by METTL1 and WDR4 affects the translation. m⁷G modifcation of mRNA and rRNA regulated by eIF4E, RAMT, Trm112, and WBSCR22 affect their nuclear export and maturate.
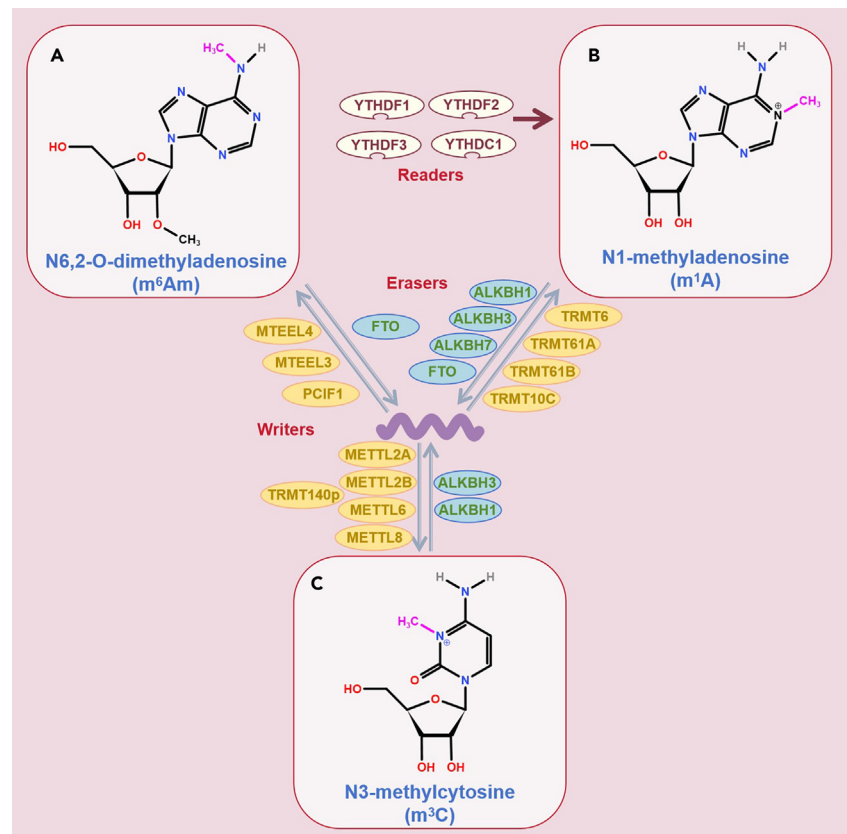
Figure 4. A summary of three different RNA modifications and their regulatory mechanisms; chemical structures, modification enzymes

(A) m^{6}Am exists in mRNA 5′ UTR and is structurally similar to m^{6}A, RNA methyltransferases (METTL3, METTL14, PCIF1) catalyze the methylation of m^{6}Am. RNA demethylase FTO can remove m^{6}Am methylation from RNA.

(B) RNA methyltransferases (TRMT6, TRMT61 A/B, TRMT10C) catalyze the methylation of m^{1}A. RNA demethylases (FTO, ALKBH1/3/7) can remove m^{1}A methylation from RNA. The m^{1}A readers include YTHDF1/2/3, YTHDC1.

(C) RNA methyltransferases (METTL2A/B, METTL6/8, TRMT140p) catalyze the methylation of m^{3}C. RNA demethylases ALKBH1 and ALKBH3 can remove m^{3}C methylation from RNA.

promote IGF1R mRNA degradation and inhibit cell invasion and migration.[90] However, the specific regulatory mechanism of other m^{1}A-related readers is not clear (Figure 4B).

m^{3}C

m^{3}C modification is widely found in the eukaryotic species tRNA^{Ser}, tRNA^{Thr}, and tRNA^{Arg}. TRM140p and MTTL6 exhibit m^{3}C methyltransferase activity, responsible for the formation of m^{3}C in tRNA^{Ser} and tRNA^{Thr} receptors.[91–93] The complex formed by METTL2 and DALRD3 can recognize specific tRNA^{Arg} and promote m^{3}C methylation.[94] METTL2A and METTL2B coexist in some cells and have the same distribution, but the activity of METTL2B is far lower than that of METTL2A. The expression level or proportion of METTL2A and METTL2B may be the key factor affecting the modification level of m^{3}C, which is regulated by tissue or cell specificity.[95] METTL8 can promote m^{3}C methylation of mt-tRNA. Knockout of METTL8 reduces the activity of the cellular respiratory chain, while overexpression of METTL8 increases the activity.[96] ALKBH1, 3 demethylates m^{3}C in tRNA, affecting RNA stability and preventing degradation.[97,98] In addition, ALKBH1 can demethylate the m^{3}C of mammalian mRNA[99] (Figure 4C).

RNA METHYLATION IN CVDs

CVD seriously threatens human life and health. It is urgent to explore its pathogenesis and find new and effective ways of diagnosis and treatment. Recently, many studies indicated that the methylated modifications of RNA play a significant role in the development of CVDs. Interestingly, RNA methylation related enzymes have different manifestations in cardiovascular diseases (Table 2). However, further data support is needed for their clinical application. Here, we summarize the latest research focused on RNA methylation identified in diverse CVDs (Figure 5).

Table 2. The function of RNA methylation in CVDs

| Related enzymes	Expression	Types of RNA methylation	Modification level	Cell type	Diseases type	Function	Reference
METTL3	Down	m^6A	Down	H9c2 cells and neonatal mouse cardiomyocytes;	Ischemic heart disease	Enhance autophagy and inhibit cardiomyocyte apoptosis	Song et al.[100]
	Up	m^6A	Up	Hypoxic rat models and PASMCs	Pulmonary arterial hypertension	Promote the excessive proliferation of PASMCs	Qin et al.[101]
	Up	m^6A	Up	Clinical human samples and HL-1 cells	Cardiomyopathy	Affect cell size and cellular remodeling	Kmietczyk et al.[102]
	Up	m^6A	Up	Mouse model and neonatal mouse cardiomyocytes	Myocardial hypertrophy	Promote the progression of pathological hypertrophy	Gao et al.[103]
FTO	Down	m^6A	Up	Neonatal mouse cardiomyocytes	HF	Alleviate fibrosis symptoms and enhance vascular regeneration	Mathiyalagan et al.[104]
	Down	m^6A	Up	Mouse model and neonatal mouse cardiomyocytes	Reperfusion injury	Attenuate cardiomyocytes apoptosis and inflammatory response	Ke et al.[105]
	Down	m^6A	Up	Mouse model and neonatal mouse cardiomyocytes	HF	Inhibit cardiomyocytes apoptosis	Shen et al.[106]
METTL4/14	Up	m^6A	Up	Clinical human samples	HF	Accelerate the progression of heart failure	Mathiyalagan et al.[104]
METTL14	Up	m^6A	Up	ASVECs	AS	Promote the proliferation and invasion of ASVECs	Zhang et al.[107]
	Up	m^6A	Up	Mouse model	HF	Accelerate the progression of heart failure	Mathiyalagan et al.[104]
ALKBH5	Up	m^6A	Down	Mouse model	MI	The increased m6A due to FTO loss can be offset to some extent in a short time	Mathiyalagan et al.[104]
	Down	m^6A	Up	H9c2 cells and neonatal mouse cardiomyocytes	Ischemic heart disease	Enhance autophagy and inhibit cardiomyocyte apoptosis	Song et al.[100]
NSUN2	Up	m^5C	Up	Human AAA tissues	AAA	May be related to inflammatory infiltration in AAA	He et al.[108]
	Down	m^5C	Down	Human umbilical vein endothelial cells (HUVECs) and rat model	AS	Inhibit the formation of AS	Luo et al.[109]
METTL1	Down	m^7G	Down	Human induced pluripotent stem cells (hiPSCs)	Vascular diseases	Affect gene translation efficiency and slow down the cell cycle	Deng et al.[110]
YTHDF3	Up	m^6A	Up	Human AAA issues	AAA	Associated with a greater risk of AAA rupture	He et al.[111]
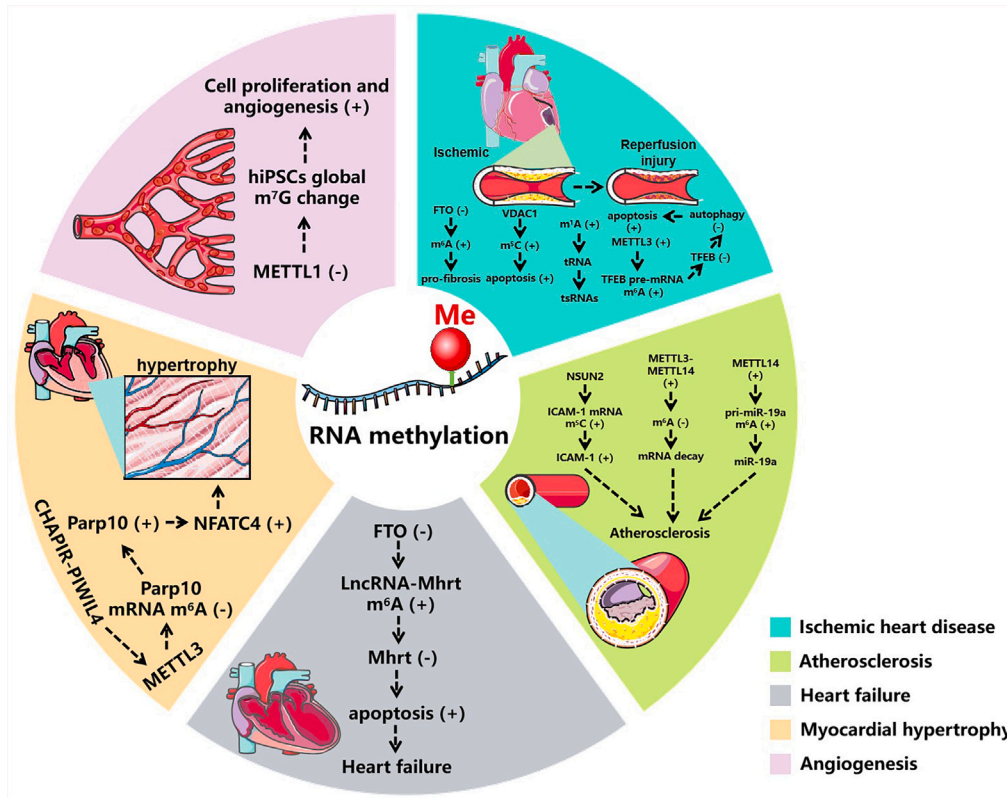
Figure 5. Progress of RNA methylation in CVDs

METTL3 participates in ischemic heart disease, myocardial hypertrophy, and heart failure by catalyzing m^6A methylation. NSUN2 promotes the progression of AS by upregulating the m^5C of ICAM-1 mRNA. m^7G methylation plays an important role in angiogenesis. The (+) indicates increase, and the (−) indicates inhibition.

MI

Ischemic heart disease (IHD) is a common type of CVD. Myocardial injury and heart failure are their main adverse consequences.[112] The m^6A demethylase FTO was underexpressed in hypoxic cardiomyocytes and mouse hearts with abnormal cardiac function, resulting in a dynamic imbalance of m^6A in RNA. Excessive m^6A will limit the systolic function of myocardial cells. Improving the expression of FTO can effectively alleviate the decline of myocardial contractile function. Clearance of m^6A by FTO helped alleviate fibrosis symptoms and enhance angiogenesis in MI mouse hearts.[104] Tong et al. found that acute myocardial infarction (AMI) is related to m^6A, which can be used as a potential early diagnostic marker.[113] After a comparative analysis of the urine of patients with coronary heart disease (CHD) and normal controls, it was found that the levels of m^1A in the two groups were different, suggesting that m^1A may play a potential role in MI.[114] Huang et al. found that m^5C modifications were abundant in mammalian myocardial tissues and highly enriched in mitochondria-related genes.[60] The most abundant protein on the outer membrane of mitochondria, voltage-dependent anion channel 1 (VDAC1), participates in the maintenance of mitochondrial membrane potential and can regulate cardiomyocyte apoptosis.[115] It also has an obvious m^5C modification.[60] Therefore, it is not difficult to speculate that regulating the dynamic balance of m^5C modification will play an important role in the apoptotic pathway of ischemic cardiomyocytes.

Ischemia-reperfusion (I/R) injury

Successive studies have confirmed that m^6A plays an important role in I/R injury. The expression of FTO decreased significantly in cardiomyocytes with hypoxia/reoxygenation (H/R) and I/R injury.[105] Overexpression of FTO enhanced the stability of Yes-associated protein-1 (YAP-1) mRNA by removing the m^6A modification, alleviating H/R-induced cardiomyocyte apoptosis and inflammation.[105] TFEB, an autophagy related factor, can inhibit I/R induced cardiomyocyte apoptosis by enhancing autophagy.[116,117] METTL3 methylates TFEB, at two m^6A residues in the 3′-UTR, which promotes the association of the RNA-binding protein HNRNPD with TFEB pre-mRNA and subsequently downregulates the transcription of TFEB, resulting in the progression of reperfusion injury. In turn, the overexpression of ALKBH5 attenuated cardiomyocyte apoptosis.[100] Decreased cardiomyocyte viability and increased LDH release are associated with METTL14 silencing. METTL14, as an m^6A inhibitor, can significantly reduce myocardial infarct size and apoptosis caused by I/R injury and improve cardiac function after overexpression.[118] In the oxidized damaged samples, Mishima et al. detected the presence of tRNA conformational changes and tRNA fragmentation by m^1A antibody.[119] It is well known that I/R can cause oxidative damage to the myocardial tissue, and tRNA metabolism plays an

important role in oxidative stress response.[120,121] Therefore, targeting m^{1}A-modified tRNA detection may be a potential direction to prevent and identify cardiac oxidative damage.

AS

Atherosclerosis, as a progressive inflammatory disease with complex mechanisms, presents such pathological features as an immune disorder, endothelial cell dysfunction, lipid deposition, and intra-arterial stenosis. AS can induce a variety of CVDs, including CHD and stroke, which seriously threaten human life and health. Many studies have confirmed that RNA methylation plays a potential role in the pathological progression of AS. The expression of intercellular adhesion molecule-1 (ICAM-1) is closely related to vascular endothelial inflammation.[122] NSUN2 can promote the m^{5}C modification of ICAM-1 mRNA, increase the translation of ICAM-1, and then promote the adhesion of leukocytes to endothelial cells. TNFα or homocysteine can activate the methyltransferase activity of NSUN2 by inhibiting Aurora-B phosphorylation of NSUN2. After homocysteine treatment in NSUN2 knockout rats, ICAM-1 induction level and leukocyte adhesion level to vascular endothelium were significantly reduced, and the progression of vascular inflammation and AS was inhibited.[109] In addition, m^{6}A also participates in the occurrence and development of AS. Macrophages play an important role in the development and progression of AS inflammation and plaque formation. Under the stimulation of oxidized low-density lipoprotein (oxLDL), the expression of METTL3 and METTL14 in macrophages increases, while the total m^{6}A modification level decreases. By regulating the formation of METTL3-METTL14 complex and m^{6}A-mediated mRNA decay, inhibiting the activation of pro-inflammatory signals can significantly inhibit oxLDL mediated macrophage inflammatory response, affecting the progression of AS.[123] METTL14 and m^{6}A were significantly increased in atherosclerotic vascular endothelial cells (ASVECs). Through co-immunoprecipitation and RNA immunoprecipitatin experiments, Zhang et al. confirmed that METTL14 can promote the combination of pri-miR-19a and RNA splicing related protein DGCR8 by increasing the m^{6}A modification of pri-miR-19a, increase the generation of miR-19a mature body, and finally enhance the proliferation and invasion of ASVECs.[107] Therefore, regulating RNA methylation via METTL14/m^{6}A/miR-19a signal axis may be a potential way to treat AS.

Myocardial proliferation and cardiac regeneration

Cardiomyocytes have long been considered being highly differentiated cell types with limited proliferation capacity. It is irretrievable that cardiomyocytes are damaged due to various pathological or other adverse factors. Recent studies have found that adult mammalian cardiomyocytes also have the potential to proliferate.[124,125] Although these proliferative abilities are considered being low contributions to cardiac re-development. However, the treatment for patients with ischemic heart disease and severe heart failure is insufficient and costly. Therefore, it is undeniable that regulating the proliferation of cardiomyocytes may be a potential strategy for the treatment of CVDs in the future. The cardiac apical regeneration ability of ALKBH5 knockout mice was impaired. Overexpression of ALKBH5 could save the cardiac function of adult mice and promote the proliferation of cardiomyocytes. Mechanistically, ALKBH5 increased the stability and expression level of YTHDF1 mRNA by erasing m^{6}A modification. YTHDF1 can further activate YAP, a key molecule of myocardial proliferation, and promote the proliferation of cardiomyocytes.[126] By analyzing the cardiac components of rats on days 0 and 7 of the newborn, Yang et al. found that the source of high proliferation and regeneration of neonatal cardiomyocytes may be closely related to the high expression of METTL3.[127] Another study proposed that the number of m^{6}A peaks and the level of m^{6}A modification were the lowest in the heart of 1-day-old mice. On the contrary, the number of m^{6}A peaks and the level of m^{6}A modification were the highest in the heart of 7-day-old mice.[128] The research of RNA modification in the field of cardiac regeneration and myocardial proliferation is still in its infancy, but the current research has proved that it plays an important role in improving myocardial development.

Angiogenesis

Angiogenesis is regulated by a complex and sophisticated signaling network in the cell. It is defined as the formation of new blood vessels from existing capillaries or veins behind them.[129,130] Many studies have confirmed that m^{6}A contributes to the process of angiogenesis. ALKBH5 regulates post-ischemic angiogenesis by affecting m^{6}A modification on WNT5A mRNA, reducing its stability and regulating its post-transcriptional expression.[131] ALKBH5 can also help maintain the angiogenesis of endothelial cells after acute ischemic stress by reducing SPHK1 m^{6}A modification and downstream eNOS-AKT signal pathway.[132] Therefore, targeting ALKBH5 to regulate m^{6}A modification may be a potential therapeutic strategy for ischemic heart disease. The m^{6}A eraser, FTO, can mediate angiogenesis by targeting VEGF-A expression.[133] In addition, overexpression of FTO decreased cardiac fibrosis and enhanced angiogenesis in mice after MI injury.[104] Knockdown METTL1 can regulate the overall expression of m^{7}G related genes and affect gene translation and cell cycle. In vivo, METTL1 knockout mice promoted cell proliferation and angiogenesis, which also provided new insights into m^{7}G modification in vascular development and treatment of vascular diseases.[110]

Myocardial hypertrophy

When cardiomyocytes are affected by various adverse factors (such as injury, pressure overload, etc.), a series of hypertrophy signal pathways will be activated to promote cardiomyocyte hypertrophy. Pathological cardiomyocyte hypertrophy is the pathophysiological basis of myocardial hypertrophy and is easily accompanied by other CVDs, such as ischemic heart disease, myocardial fibrosis, atrial fibrillation, and heart failure.[134] Myocardial hypertrophy also involves epigenetically regulated processes. METTL3-mediated methylation of mRNA m^{6}A is a dynamic modification, which is enhanced in response to hypertrophic stimulation and maintains normal hypertrophic response of

cardiomyocytes. Excess m^{6}A methylation leads to compensatory myocardial hypertrophy, while fewer m^{6}A drives eccentric myocardial cell remodeling and dysfunction, which shows that this dynamic modification mechanism plays an important role in maintaining the normal state of cardiac function.[135] Gao et al. found that when cardiac-hypertrophy-associated piRNA (CHAPIR) was absent, it could significantly reduce the symptoms of myocardial hypertrophy and restore cardiac function. The administration of CHAPIR agomir will aggravate the pathological hypertrophy of the heart in pressure overload mice. Later, they confirmed that the CHAPIR-PIWIL4 complex can directly interact with METTL3, block the m^{6}A methylation of Parp10 mRNA transcripts, and upregulate the expression of Parp10, thus leading to the increase of nuclear NFATC4 and pathological hypertrophy of the heart.[103] In addition, the normal physiological function of myocardial cells is closely related to mitochondria. NSUN4 is a kind of m^{5}C methyltransferase. Its deletion leads to dysfunction of cardiac mitochondria and oxidative phosphorylation damage in mice, from which the symptoms of myocardial hypertrophy appear.[136] Therefore, the regulation of m^{5}C methylation may help us to better maintain the homeostasis of mitochondrial function and improve cardiac function.

HF

HF is not an independent heart disease but the final stage of the development of various CVDs.[106] Due to insufficient systolic and diastolic functions of the heart, it is unable to pump blood normally for the body, thus causing other heart circulation diseases such as pulmonary congestion.[137] Myocardial infarction, reperfusion injury, myocarditis, and cardiac pressure overload can all cause HF, among which pathological myocardial hypertrophy is the main inducement of HF. m^{6}A reader YTHDF2 can inhibit myocardial hypertrophy and reduce the risk of HF by recognizing the m^{6}A modification site on Myh7 mRNA.[138] Zhang et al. hold FTO can exert an important influence on the structure and function of the heart by regulating glucose metabolism.[139] It is suggested that FTO may participate in the process of glucose metabolism by regulating m^{6}A modification and may be a potential therapeutic target for HF. DNMT1 has also been reported to be closely related to heart disease. DNMT1 can inhibit mitochondrial autophagy by enhancing the methylation of the miR-152-3p promoter region and inhibiting its expression; In addition, DNMT1 can upregulate the expression of ETS1, further promoting the expression of RhoH, leading to a decrease in cell viability and ultimately accelerating the progression of HF events.[140]

Others

Diabetic cardiomyopathy (DCM) can cause myocardial remodeling, leading to severe cardiac dysfunction. Shao et al. found that ALKBH5 was upregulated in cardiomyocytes of DCM mice and affected the progression of DCM by activating FOXO3-CDR1as/Hippo signaling pathway after m^{6}A demethylation.[141] YTHDF2 can promote the degradation of phosphatase and tensin homolog (PTEN) by recognizing METTL3-mediated m^{6}A modification on PTEN mRNA, and then activating PI3K/Akt signaling pathway to cause abnormal proliferation of pulmonary artery smooth muscle cells (PASMCs), which is involved in the occurrence and development of hypoxic pulmonary hypertension (HPH).[101] ALKBH5 attenuates m^{6}A methylation of the interlukin-11 (IL-11) mRNA transcript, which increases IL-11 expression level. Inhibition of ALKBH5 can downregulate the transcription of IL-11, and ameliorate cardiac fibrosis and dysfunction under hypertension stress.[142] Bioinformatics analysis shows that m^{6}A modification may be involved in the immune microenvironment homeostasis of patients with coronary heart disease (CHD).[143] m^{6}A regulatory factors, such as YTHDC2, YTHDF1-3, may become key targets for immunotherapy of CHD.[144] He et al. found that compared with the control group, the level of m^{5}C was significantly increased in abdominal aortic aneurysm (AAA) patients.[108] It is speculated that Aly/REF and NUSN2 play a potential role in the pathogenesis of human AAA. Interestingly, another study confirmed that m^{6}A levels were also significantly increased in AAA patients compared with healthy tissue and that high levels of m^{6}A may indicate a greater risk of AAA rupture.[111]

CLINICAL PROSPECT OF RNA METHYLATION IN CVDs

RNA methylation regulates multiple biological processes related to the cardiovascular system, including gene expression, signaling pathways, and cell function. RNA methylation can also serve as an early diagnostic marker for CVDs. The m^{6}A-modified RNA in the blood has potential value in the early diagnosis and treatment of CVDs, such as MI, CHD, and HF.[145] For example, Zhang et al. analyzed peripheral blood mononuclear cells (PBMCs) isolated from the peripheral blood of 16 heart failure with preserved ejection fraction (HFpEF) patients and 24 healthy controls and found that YTHDF2 expression was upregulated in HFpEF patients compared with controls. And the key molecules METTL3, METTL4, KIAA1429, and FTO modified by m^{6}A were also expressed.[146] These data suggest that detecting key biomarkers of RNA methylation may become an important research and application field in the prevention, diagnosis, and treatment of CVDs. Several studies have reported the inhibitors of RNA methyltransferases and demethylase, which have shown potential application value in the treatment of tumors and other diseases.[147–150] These research results provide novel insight and theoretical references for the application of RNA methylation therapy in CVDs. For example, in the treatment of CVDs, we can regulate the level of RNA methylation by targeting RNA methyltransferases and demethylases, thereby affecting relevant biological processes in the cardiovascular system and achieving therapeutic effects. Recent studies have confirmed that non-coding RNA plays a critical role in gene expression regulation, cell proliferation, aging, immune response, cardiovascular system function, and other biological processes.[151–154] miRNA is an important member of non-coding RNA. The m^{6}A modification of miRNA is closely related to the occurrence and development of CVDs. Therefore, in the treatment of CVDs, we can also try to achieve precise treatment by targeting m^{6}A modification of miRNA. The accumulation of miRNA-503 can promote mitochondrial metabolic dysfunction and myocardial cell death, exacerbating myocardial injury. m^{6}A modification of miRNA-503 can alter its stability and function, thereby affecting related biological processes in the cardiovascular system.[155] Therefore, m^{6}A modification targeting miRNA-503
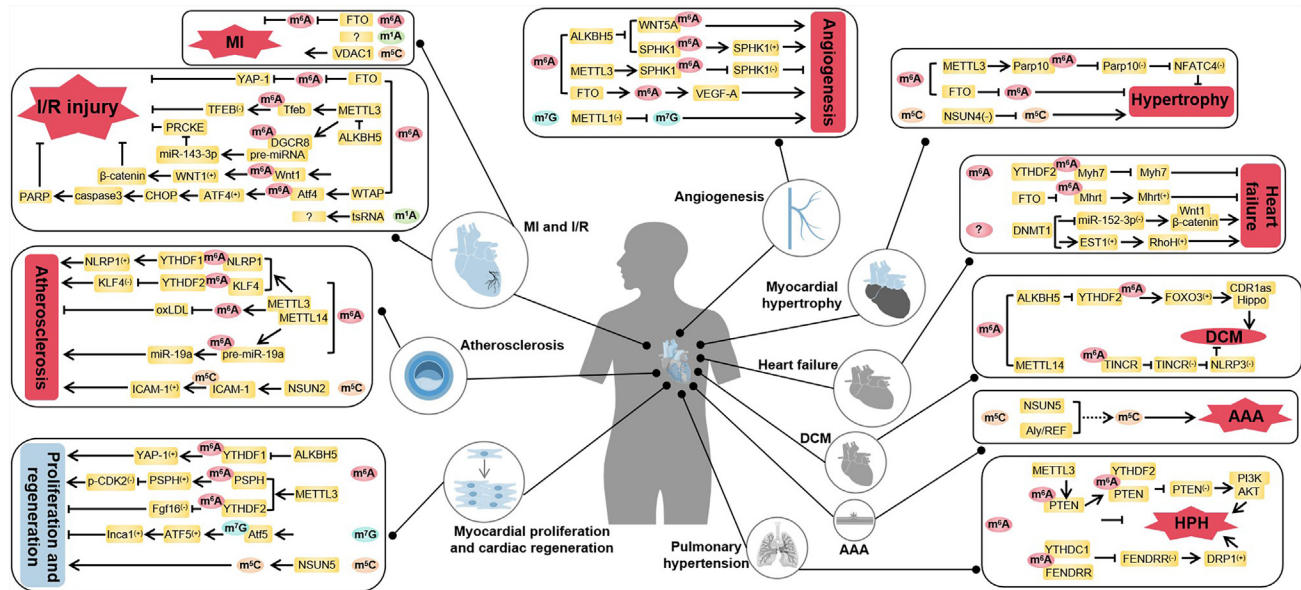
Figure 6. A systematic compendium of the role and mechanism of RNA methylation in CVDs

may become a new strategy for the treatment of CVDs. Here, we review the function and mechanism of RNA methylation in cardiovascular disease and provide a systematic overview (Figure 6). It is foreseeable that after more in-depth research and rigorous clinical verification, RNA methylation will have a very broad application prospect in the treatment of CVDs in the future.

THE MECHANISMS UNDERLYING THE ROLES OF RNA METHYLATIONS

As an emerging field of CVDs, RNA methylation has inspired a series of new therapeutic strategies for us. Several small molecule compounds have been shown to target certain key regulators of RNA methylation.[156–160] However, the pharmacological effects of these compounds have not been officially recognized, and their clinical research in CVDs is still in its infancy. Targeting inflammation, cardiomyocyte death, fibrosis, and adverse cardiac remodeling, as well as stimulating cardiomyocyte regeneration and proliferation, is the theoretical basis for helping us solve CVDs through RNA methylation pathways. Discovering more drugs targeting RNA modification regulators and exploring their mechanisms and therapeutic effects will bring hope for the treatment of CVDs.

Attenuating inflammatory response

More and more studies have shown that RNA methylation is involved in inflammatory response, which is one of the pathological basis of a variety of cardiovascular diseases.[161–163] Macrophages play an important role in a variety of inflammatory diseases. METTL3 is found to be expressed at low levels in monocyte-derived macrophages. METTL3 deficiency ameliorates the activation of M2 macrophages and enhances Th2 cell responses, exacerbating the progression of inflammation.[164] Krishnamurthy et al. found that in LPS stimulated myocardial tissue, RNA methylation levels increased, accompanied by an increase in a series of myocardial inflammatory factors (IL-1β, IL-6, and TNF-α).[165] The lack of FTO can also lead to similar inflammatory reactions. However, they did not provide a specific plan on how to alleviate this type of myocarditis. Similarly, METTL3 levels were positively correlated with the progression of AS in macrophages. Li et al. confirmed that METTL3 deficiency blocks extracellular signal-regulated kinase (ERK) phosphorylation caused by oxLDL and affects inflammatory cytokine levels.[166] In the rat model of pulmonary arterial hypertension (PAH), several key molecules of m6A (FTO and ALKBH5, METTL3 and YTHDF1) were significantly changed. Correspondingly, methylated RNA immunoprecipitation sequencing (MeRIP-seq) technology detected an increase in the methylation level of m6A.[167] The coding genes upregulated by methylation are believed to be mainly enriched in inflammation related pathways. Zhou et al. found that inhibition of FTO blocks high glucose induced endothelial cells inflammation.[168] Li et al. identified that myeloid cell-specific Mettl3 deficiency attenuated hyperlipidemia-induced atherosclerotic inflammation.[166] These studies have all proposed a correlation between m6A methylation and inflammatory response, and inhibiting RNA methylation levels will help suppress the progression of inflammation. Developing inhibitors of METTL3 or activators of FTO and ALKBH5 may be an important research strategy for the treatment of CVDs in the future.

Reducing cell death

Abnormal death of cardiomyocytes can directly lead to dysfunction of the heart and cause adverse myocardial remodeling, including pathological dilation of the ventricle and increased fibrosis, ultimately leading to HF.[169–171] Preventing cell loss can theoretically improve cardiac function. Wang et al. found that heart neoptosis associated piRNA (HNEAP) can directly target DNMT1, a key molecule binding to m5C

methylation, and regulates the expression of Atf7 mRNA.[172] Notably, absence of HNEAP or Atf7 ameliorated necroptosis in cardiomyocytes caused by I/R injury. Reducing cardiomyocyte loss by targeting m5C upstream or downstream signaling in this way provides a rationale for RNA methylation-targeted therapy of CVDs. However, whether this strategy can directly target the site of myocardial injury requires more exploration. Recently, a cross-disciplinary study between medicine and materials science proposed that the complex HSSS-I formed by loading IOX1 (ALKBH5 inhibitor) with ferritin nanocages (HSSS) can target the infarcted area of the heart and inhibit cardiomyocytes apoptosis, significantly improving the cardiac function of AMI patients.[173] Nano-drug delivery systems have better drug loading and targeting properties. Combining it with RNA methylation regulatory factors may be a new approach for clinical precision treatment of CVDs.

Preventing abnormal fibrosis and adverse remodeling

When cardiomyocytes are lost, to maintain the homeostasis of the cardiac system, it will stimulate myocardial hypertrophy and abnormal myocardial fibrosis, resulting in adverse cardiac remodeling. METTL1 expressed in myocardial fibroblasts can facilitate m7G methylation on mRNA of fibrotic genes, such as α-SMA, Col1a1, Col3a1, and Fibronectin1, enhancing their translation efficiency. Artificially silencing METTL1 helps improve myocardial fibrosis and HF.[174] The treatment of forced expression of FTO by injection of adeno-associated virus-9 (AAV9)-FTO effectively improved cardiac fibrosis and myocardial hypertrophy in transverse aortic constriction (TAC) mice, and maintained cardiac metabolic homeostasis.[139] Dorn et al. found that increased expression of METTL3 promoted cardiomyocyte hypertrophy, whereas inhibition of METTL3 abolished the ability of cardiomyocytes to develop hypertrophy in response to specific stimuli.[135] Cheng et al. believe that METTL3 is a key regulatory factor for hypoxia induced apoptosis and fibrosis, and targeting METTL3 may be a future strategy for treating MI.[175] Furthermore, Suo et al. reported that specific deletion of METTL3 in pericytes inhibits diabetes-induced pericytes dysfunction and vascular complications.[176] METTL3 seems to be considered a therapeutic target for CVDs, and inhibiting METTL3 can help patients repair heart function. However, the overall effect of this modality requires further evaluation.

Stimulating cardiomyocyte regeneration and proliferation

Stimulating cardiomyocyte regeneration and proliferation has become a new and important direction for the treatment of CVDs. METTL1 regulated the m7G methylation of Atf5 mRNA, which promoted the transcription of Inca1(an inhibitor of cyclin-dependent kinase) and further inhibited the proliferation of cardiomyocytes.[177] Targeting METTL1-mediated mRNA m7G methylation may be a novel therapeutic strategy to promote cardiac repair and regeneration. Han et al. found that the expression level of ALKBH5 was significantly reduced in postnatal mouse hearts, and cardiac regeneration was limited in ALKBH5$^{-/-}$ (knockout) mice.[126] Injection of AAV9-ALKBH5 significantly improved cardiac function and promoted cardiomyocyte proliferation. Interestingly, ALKBH5 also promoted the proliferation of human cardiomyocytes.[126] Deletion of Nsun5 severely affected the m5C modification and translation efficiency of heart-essential genes, resulting in decreased proliferation of mouse cardiac outflow tract (OFT) cells.[178] Wang et al. suggested that Mettl3 could promote m6A methylation of PSPH mRNA and enhance its transcription, ultimately promoting cardiomyocyte proliferation.[170] However, Jiang et al. also explored the role of m6A in cardiac regeneration. Their results showed that knockdown of Mettl3 significantly increased cardiomyocyte proliferation and accelerated cardiac regeneration, while overexpression of Mettl3 restricted the proliferation and regeneration of neonatal mouse cardiomyocytes.[179] RNA methylation regulators seem to play different functions in different studies, which may be directly related to the different downstream signals they regulate. Unlocking these complex signal regulatory networks may be an effective way to address the current controversial situation.

DETECTION AND IDENTIFICATION OF RNA METHYLATION

RNA modification plays an important regulatory role in gene expression and protein translation.[180] The biological function of RNA methylation varies significantly depending on the type of modified nucleotide and RNA.[181,182] Given the significant role of RNA methylation in CVDs, rational development and utilization of RNA modification detection and sequencing methods can rapidly identify RNA modifications present in various cell types or model organisms and map them to different types of RNA. Based on immunological approaches, RNA modified antibodies, such as m6A, m1A, and m5C are used to detect RNA modifications through MeRIP-seq.[183,184] The third-generation sequencing technology has also been widely used in the detection of RNA methylation.[185–187] Here, we introduce several existing detection schemes of RNA methylation, as well as their respective characteristics, with the expectation of assisting researchers in overcoming difficulties and developing new therapies for CVDs targeted at RNA methylation.

MeRIP-seq

MeRIP-seq is a high-throughput sequencing method that utilizes the principle of antibody-specific binding to methylated bases. It enriches RNA fragments that have undergone methylation through immunoprecipitation with specific antibodies against methylation.[188] Specifically, the collected RNA samples were interrupted into short fragments after quality inspection, and then the methylation modified fragments were selectively enriched using specific antibodies. Finally, the library was constructed for sequencing and data analysis.[189] This technique is convenient, fast, and cost-effective, allowing for a qualitative analysis of highly methylated mRNA regions. However, this method can only identify regions with high levels of methylation and cannot achieve single-base resolution in identifying RNA methylation. MeRIP-seq is commonly used to detect RNA methylation and is a powerful technique for mapping m6A, m1A, m5C, and m7G localization on a whole-transcriptome scale.[183] This technology is limited by specific RNA methylation antibodies.

Methylation-dependent individual-nucleotide resolution cross-linking and immunoprecipitation (miCLIP)

MiCLIP applies to smaller RNAs, and this technique allows for high-resolution detection of individual m^6A residues or m^6A clusters throughout the entire RNA molecule, and to locate m^6A and m^6Am[190] The procedure involves fragmentation of enriched mRNA, followed by specific binding of methylated RNA to m^6A antibodies, UV cross-linking, and reverse transcription to detect whether m^6A exists based on mutation or truncation of the resulting cDNA. However, this method cannot identify the specific position of the modified residue and can only determine the approximate location of the m^6A site. The problem that m^6Am and m^6A are difficult to distinguish can be solved by miCLIP.[191] In addition, other types of RNA modifications, including m^1A, m^7G, and m^5C, can also be specifically detected using miCLIP technology.[27,90,192–194]

Nanopore sequencing

Nanopore sequencing technology is a third-generation sequencing technology based on electrical signal recognition of nucleotide sequences, which can detect methylation sites and abundance in RNA by directly measuring the single-molecule electric current signal of RNA.[195] This technology can obtain methylation on RNA at a single-base resolution without requiring specific antibody binding. The direct RNA nanopore sequencing has been used to analyze m^6A, m^5C, and m^7G in yeast, plant, and human cells.[193,195–198] However, it should be noted that due to the direct reading of RNA by nanopore sequencing technology, there may be a higher error rate when detecting methylation in RNA. Therefore, when using this technology, it is necessary to combine other techniques for validation to ensure the accuracy of the results.

LC-MS/MS

LC-MS/MS is a tandem mass spectrometry technique based on liquid chromatography, which can obtain molecular ion peaks and fragment ion peaks.[199–201] It can perform qualitative and quantitative analysis of bases simultaneously, making it the best method for quantifying RNA modifications within total RNA. With the help of this technology, Dodson et al. found that compared with the control group, there were significant differences in m^5C and m^6Am modification levels between hyperglycemic cells and atrial tissue from diabetic patients.[83] This technology can determine whether RNA modification antibodies have bound to the target modification and whether they have bound to any other nonspecific modification. The procedure involves digesting enriched RNA samples into individual nucleotides using Nuclease P1, followed by incubation with alkaline phosphatase and ammonium bicarbonate. The sample is then injected into a liquid chromatograph to calculate the nucleotide content and finally analyzed by tandem mass spectrometry to calculate the area of m^6A. Based on the ratio of m^6A to total adenosine, the overall level of methylation of m^6A can be calculated on mRNA. However, this technique relies on expensive mass spectrometry equipment and requires high technical expertise for maintenance and operation.

Colorimetry

Compared with LC-MS/MS, colorimetric assays are simpler and can also detect m^6A methylation levels on RNA at the global level.[202–204] The EpiQuik m^6A RNA Methylation Quantification Kit, which uses a reaction principle like that of ELISA, can be used to detect m^6A RNA methylation levels. Deng et al. detected samples from CHD patients by colorimetry and found that the m^6A levels were significantly reduced in RNA from peripheral blood mononuclear cells compared with controls.[205]

Dot blot

Using RNA modification antibodies for dot blot is a quick and simple method for detecting their specificity.[165,166,174,206] The dot blotting method is like a simplified version of a protein blotting assay. By using RNA molecules with the specific modification as a positive control and RNA molecules without the modification as a negative control, it is possible to visually determine whether the tested sample contains the specific modification. Specifically, the total RNA samples were heated to 95°C for 3 min, and then cooled on ice. An appropriate amount of the sample was dropped onto the NC membrane for UV cross-linking. Membrane was blocked with the blocking buffer or 5% BSA, and incubated with the anti-RNA methylation-specific antibody after simple washing. Then incubate with a secondary antibody for a short time (1-2h). The membrane was scanned and analyzed using an enhanced chemiluminescence detection system. Methylene blue staining was used as a reference.

CONCLUSION AND OUTLOOK

Epigenetics has been proven to participate in the pathological process of CVD, and depending on epigenetic therapy, may have good therapeutic significance for CVD. RNA methylation is a key member of RNA epigenetics. The role of RNA methylation in the pathology of CVD is dynamic and reversible, and m^6A, m^1A, m^6Am, m^5C, m^7G, and m^3C modifications have also been confirmed to show abnormal levels in diseases. Writers represent a class of RNA methylation enzymes, which can help to form methylation on RNA. Different types of methylation modification have different writers, and their action sites on RNA are also different. RNA demethylases are called eraser, which can help RNA demethylation and maintain the homeostasis of RNA methylation *in vivo*. Generally, different types of methylation modifications have different Erasers, but there are exceptions, such as FTO, which can remove m^6A, m^1A, and m^6Am methylation on RNA. A series of processes such as mRNA splicing processing, export, translation and degradation are inseparable from the involvement of RNA methylated reading enzyme, namely Reader. It can help functional molecules recognize methylation sites and participate in gene expression regulation.

CVD, a condition characterized by high morbidity and mortality, poses a serious risk to human health by affecting the circulatory system. RNA methylation, an essential epigenetic modification, plays a key role in regulating the expression of genes related to cardiovascular functions. It influences critical biological processes, such as cardiovascular differentiation, proliferation, and apoptosis. This review delves into the molecular intricacies of the dynamic RNA methylation process and provides a comprehensive review of the research progress on RNA methylation in diverse CVDs. According to the current research status, it is intended to stimulate new treatment strategies for CVDs.

m^6A is the most widely studied type of RNA modification, which has been found in many CVDs. The increase in m^6A modification mediated by METTL3 can cause myocardial hypertrophy in mice, and the symptoms of myocardial hypertrophy can be alleviated by inhibiting METTL3.[135] METTL14 can enhance m^6A modification of FOXO1, and induce endothelial cell inflammation and atherosclerotic plaque formation. Inhibiting the expression of METTL14 can inhibit the development of endothelial inflammation and atherosclerosis.[207] The overexpression of demethylases ALKBH5 affects the stability of WNT5A mRNA, reduces the expression of CD31 and α-smooth muscle actin, and negatively regulates angiogenesis after ischemic injury.[131] METTL14 knockout mice had worse cardiac dysfunction after I/R. Overexpression of METTL14 could significantly reduce myocardial infarction area and apoptosis, and improve cardiac function during I/R injury.[118]

Other types of RNA methylation also play potential roles in CVD, with important functions. Although the difference between m6Am and m^6A seems small (one methyl group difference), their effects on mRNA are completely different.[77] DNMT1-mediated methylation of m^5C restricts Atf7 mRNA transcription. Inhibition of ATF7 will further stimulate the transcription of Chmp2a, a necroptosis inhibitor, and inhibit the progression of cardiomyocyte necroptosis.[172] METTL1-mediated m^7G modification enhances Atf5 mRNA transcription and is involved in regulating cardiomyocyte proliferation.[177] Pseudouridine (Ψ), also known as PU or "fifth nucleotide", it is the most abundant RNA modification in human cells, widely present in tRNA, rRNA, snRNA, and mRNA.[208–210] Ψ is produced by a series of Ψ synthase (PUS), which can increase the stability of RNA and alter its translation efficiency.[211,212] Approximately 2,000 Ψ sites have been identified in human mRNA, among which PUS1, PUS7, TRUB1, and DKC1 can catalyze Ψ modification in mRNA.[208,209,213] Through metabolomics studies, plasma and urine Ψ have been confirmed to be associated with CVDs. Especially in patients with symptomatic HF, the plasma Ψ level is significantly increased, and the plasma Ψ level is positively correlated with left ventricular mass index (LVMI), an important indicator of HF.[214,215] Oxyguanine (O^8G) is produced by ROS oxidizing guanine.[216] This special modification results in the generation of O^8G-A pairs that allow the RNA to target new downstream genes. The level of O^8G modification on tRNA derived fragments (tRF-1-AspGTC) was confirmed to be positively correlated with blood pressure in PAH patients, which has the potential to serve as a blood biomarker for the diagnosis of PAH.[217] NAT10-mediated ac^4C acetylation in cardiomyocytes can enhance the stability and translation efficiency of transcription factor EC (TFEC) mRNA to regulate apoptosis.[218] RNA modifications play an important role in various biological processes. Exploration of RNA modification will lead to innovative and effective methods for the diagnosis, treatment, and prevention of CVD.

However, we can see that the methylation modification of RNA is a double-edged sword. The increase of methylation modification on RNA may lead to the deterioration of some CVDs, but it may also inhibit the progress of diseases. RNA methylation is a complex dynamic equilibrium process. Simply regulating the methylation or demethylation of genes may lead to unexpected results. To understand the deeper relationship between RNA methylation in CVD, many clinical samples are needed for validation. Some researchers hope to treat CVD by interfering with the dynamic balance of RNA methylation, such as overexpressing gene levels of RNA methylase or demethylase. However, it should be noted that, as an enzyme, they may not be activated by overexpression. Some RNA modified metabolites can be used as potential markers for the diagnosis of CVDs, but more experimental data are needed before clinical application. In addition, there are still many unknown writers, erasers and readers waiting for us to discover, which is an extremely complex and huge signal regulation network.

Although the research on RNA methylation is still in the exploratory stage, there is no denying that it has shown remarkable therapeutic prospects. It can be predicted that, on this basis, there is a good potential for clinical application to develop new therapeutic drugs and diagnostic markers for CVDs.

LIMITATIONS OF THE STUDY

Application pharmaceutical agents based on regulating RNA methylation may be a potential therapeutic strategy for CVDs to improve outcomes. However, more fundamental research and clinical trials are needed to verify the feasibility and effectiveness of RNA methylation pharmaceutical agents targeting CVDs treatment. Delivering therapeutic pharmaceutical agents precisely to the cardiovascular system is currently the challenge that needs to be addressed. In addition, the stability issues of these pharmaceutical agents in the manufacturing, transportation, and storage processes still need to be further resolved.

AUTHOR CONTRIBUTIONS

All authors provided direction and guidance throughout the preparation of this manuscript. Kai W. is mainly responsible for drafting the manuscript. Y.W. organized the graphics. Y.W., Y.L., B.F., and B.L. assisted in drafting the manuscript and design of ideas. Kai W. and Y.W. contributed equally to this work. W.C., Kun W., and S.Y. reviewed and made significant revisions to the manuscript. All authors have read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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