Review Article

Functional roles of epitranscriptomic marks in the cardiovascular system and disease: a narrative review

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Background and Objective: The recent emergence of epitranscriptomics provides an avenue for identifying RNA modifications implicated in the pathophysiology of human disease. To date, over 170 RNA modifications have been identified; these modifications are important because they can affect the fate of RNAs, including their decay, maturation, splicing, stability, and translational efficiency. Although RNA modifications have been reported in many tissues and disease contexts, detailed functional studies in the heart and cardiovascular disease are only beginning to be reported.

Methods: The search for relevant articles related to epitranscriptomics was conducted by focusing on the cardiovascular system and disease in the PubMed database.

Key Content and Findings: We summarize the recent findings of three epitranscriptomic marks—N6-methyladenosine (m^6A), adenosine to inosine (A-to-I) RNA editing, and 5-methylcytosine (m^5C) as other epitranscriptomic marks are not studied extensively in the cardiovascular system and disease.

Conclusions: In this narrative review, the current status of cardiac epitranscriptomics is summarized to raise the awareness of this important field of study.

Keywords: Cardiovascular; cardiomyocyte; development; epitranscriptomics; ncRNAs; narrative review

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Introduction

Heart is the first organ to mature during the development. It sustains the live of organism by serving as an engine for blood circulation. The main cell types of the heart include cardiomyocytes (heart muscle), endothelial cells, fibroblasts, smooth muscle cells, pericytes, immune cells (myeloid and lymphoid), adipocytes, mesothelial cells, and neuronal cells (1). Unlike fish and amphibians, the mammalian cardiomyocytes do not normally regenerate themselves shortly after birth (2). Thus, the developmental studies of heart as well as how its dysfunction leads to cardiovascular disease are the topics of intensive studies. The protein-centered research could not uncover all the gene regulatory and signaling pathways to sustain the healthy heart. The emergence of non-protein-coding RNAs (ncRNAs), especially microRNAs (miRNAs) and more recent long non-coding RNAs (lncRNAs), has shed a light on further dissecting the gene regulatory networks of the heart (3,4). Yet, the deaths related to cardiovascular disease still ranks the top among all causes of deaths in industrialized countries (5), calling for further understanding of cardiac gene regulatory networks.

RNA is not only a mediator of genomic information encoded in DNA to the final products, proteins. The lifecycle of RNA is more dynamic, where they can be modified by a variety of enzymes. Indeed, more than 170 RNA modifications have been identified across species (6). The recent discoveries of RNA modifications and their

importance in normal and pathophysiological conditions have led to the emergence of new field of study called, epitranscriptomics (7,8). This new field of study extends further understanding of epigenetics from the perspective of RNA, where the terminologies are adopted from epigenetics, including writers, readers, and erasers to categorize epitranscriptomic enzymes. These enzymes are active in many tissues and cell types, whose dyregulations are linked to a variety of disorders and critical diseases, such as cardiovascular disease (9,10). These epitranscriptomic enzymes mark all kinds of RNA species, including mRNAs and ncRNAs, such as ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), miRNAs, and lncRNAs (11). These marks are deposited by epitranscriptomic writers, interpreted by epitranscriptomic readers, and nullified by epitranscriptomic erasers. The lifecycle of RNA affected by these epitranscriptomic marks include splicing, subcellular localization, miRNA binding and biogenesis, mRNA stability and decay, and translation. Many of these marks are found throughout the transcriptome, which regulate downstream signaling pathways. Although modifications of rRNAs and tRNAs have been known over 50 years (12), only recently epitranscriptomic marks in mRNAs and ncRNAs have been identified. In this narrative review, we summarize the current status of epitranscriptomic marks in cardiovascular system and disease by focusing on three epitranscriptomic marks—N6-methyladenosine (m^6A), adenosine to inosine (A-to-I) RNA editing, and 5-methylcytosine (m^5C) as other epitranscriptomic marks are not studied extensively in cardiovascular system and disease. We present the following article in accordance with the Narrative Review reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-1074/rc).

Methods

A literature review was performed using PubMed to search all scientific articles published through to January 1, 2022. The search terms used included: “cardiovascular”, “cardiovascular disease”, “epitranscriptomics”, “RNA modifications”, “2'-O-methylation”, “A-to-I RNA editing” “RNA editing”, “C-to-U RNA editing”, “ac4C”, “m1A”, “m3C”, “m5C”, “m6A”, “m7G”, and “pseudouridylation”. We use a table (*Table 1*) to present detailed search strategy.

N^6-methyladenosine (m^6A)

Among all RNA modifications, the most studied epitranscriptomic mark in recent years is N^6-methyladenosine (m^6A), which is the methylation of adenosine (A) at its nitrogen-6 position. This reversible modification is deposited by m^6A writers [methyltransferase 3, N6-adenosine-methyltransferase complex catalytic subunit (METTL3), methyltransferase 14, N6-adenosine-methyltransferase complex catalytic subunit (METTL4), WT1 associated protein (WTAP), vir like m6A methyltransferase associated (VIRMA, also known as KIAA1429), methyltransferase 16, N6-methyladenosine (METTL16), RNA binding motif protein 15 (RBM15), RNA binding motif protein 15B (RBM15B), and zinc finger CCCH-type containing 13 (ZC3H)], interpreted by m^6A readers [YTH N6-methyladenosine RNA binding proteins 1 (YTHDF1), YTH N6-methyladenosine RNA binding proteins 2 (YTHDF2), YTH N6-methyladenosine RNA binding proteins 3 (YTHDF3), YTH domain containing 1 (YTHDC1), YTH domain containing 2 (YTHDC2), heterogeneous nuclear ribonucleoprotein C (HNRNPC), RNA binding motif protein X-linked (RBMX, also known as HNRNPG), insulin like growth factor 2 mRNA binding protein 1 (IGF2BP1), insulin like growth factor 2 mRNA binding protein 2 (IGF2BP2), and insulin like growth factor 2 mRNA binding protein 3 (IGF2BP3)], and nullified by m^6A erasers [alkB homolog 5, RNA demethylase (ALKBH5) and FTO alpha-ketoglutarate dependent dioxygenase (FTO)] (13,14) (*Figure 1*). The m^6A sites are found mostly in mRNAs, especially around stop codon (15), and regulate mRNA metabolism, such as splicing, nuclear export, mRNA stability, and translation (16). Because of the availability of high-throughput methods to detect m^6A sites {e.g., m^6A-seq [also more commonly known as m^6A RNA immunoprecipitation followed by high-throughput sequencing (MeRIP-seq)], m^6A individual-nucleotide-resolution cross-linking and immunoprecipitation (miCLIP), and m^6A crosslinking immunoprecipitation sequencing (m^6A-CLIP); comprehensively reviewed and compared in (17)}, a number of m^6A profiles were reported in healthy hearts (18) as well as in those compared to diseased ones and/or cardiovascular disease models in mice and rats, including during cardiac development (19,20), cardiomyopathy (21), diabetic cardiomyopathy (22), heart failure (21,23), intermittent fasting in high-fat diet-induced cardiomyopathy (24), and myocardial infarction (25). Although these screening data are informative for the global changes in m^6A profiles under different cardiac conditions, gain/loss-of-function analysis of each m^6A enzyme focusing on cardiac physiology and disease is important to

Table 1 The search strategy summary

Items	Specification
Date of search (specified to date, month and year)	January 2, 2022
Databases and other sources searched	PubMed
Search terms used (including MeSH and free text search terms and filters)	"cardiovascular"
	"cardiovascular disease"
	"epitranscriptomics"
	"RNA modifications"
	"2'-O-methylation"
	"A-to-I RNA editing"
	"RNA editing"
	"C-to-U RNA editing"
	"ac4C"
	"m1A"
	"m3C"
	"m5C"
	"m6A"
	"m7G"
	"pseudouridylation"
Timeframe	July 1, 1980 – January 2, 2022
Inclusion and exclusion criteria (study type, language restrictions etc.)	Inclusion: English, original articles
	Exclusion: review articles
Selection process (who conducted the selection, whether it was conducted independently, how consensus was obtained, etc.)	Shizuka Uchida
Any additional considerations, if applicable	Phenotypic and mechanistic studies

understand the function of each m^{6}A enzyme, which are described below (*Table 2*).

m^{6}A writers

Pathological cardiac hypertrophy is a condition in which the heart enlarges in response to stresses, such as pressure overload or myocardial infarction, which progresses to heart failure (38). Understanding the cascade of events in maladaptive hearts is important for therapeutic purpose. Interestingly, the m^{6}A level increased in hypertrophied cardiomyocytes (26). When a major component of the m^{6}A methyltransferase complex, *Mettl3*, was overexpressed in neonatal rat ventricular cardiomyocytes, cardiomyocytes were hypertrophied, while the opposite effect was observed when silencing *Mettl3*. When *Mettl3* was overexpressed in mice, cardiac hypertrophy was observed, while *Mettl3* floxed mice crossed with the cardiomyocyte-specific cre-driver, Myh7-cre (also known as β-MHC cre), displayed signs of heart failure with aging and stress, suggesting the functional importance of Mettl3 in cardiac physiology.

m^{6}A readers

Just as other readers of epitranscriptomic marks, m^{6}A readers are RNA-binding proteins (RBP), which affects the mRNA stability and decay, leading to translational regulations (39). As these readers bind many RNAs, it is often difficult to pinpoint only one transcript been affected in a particular condition. Nevertheless, there are

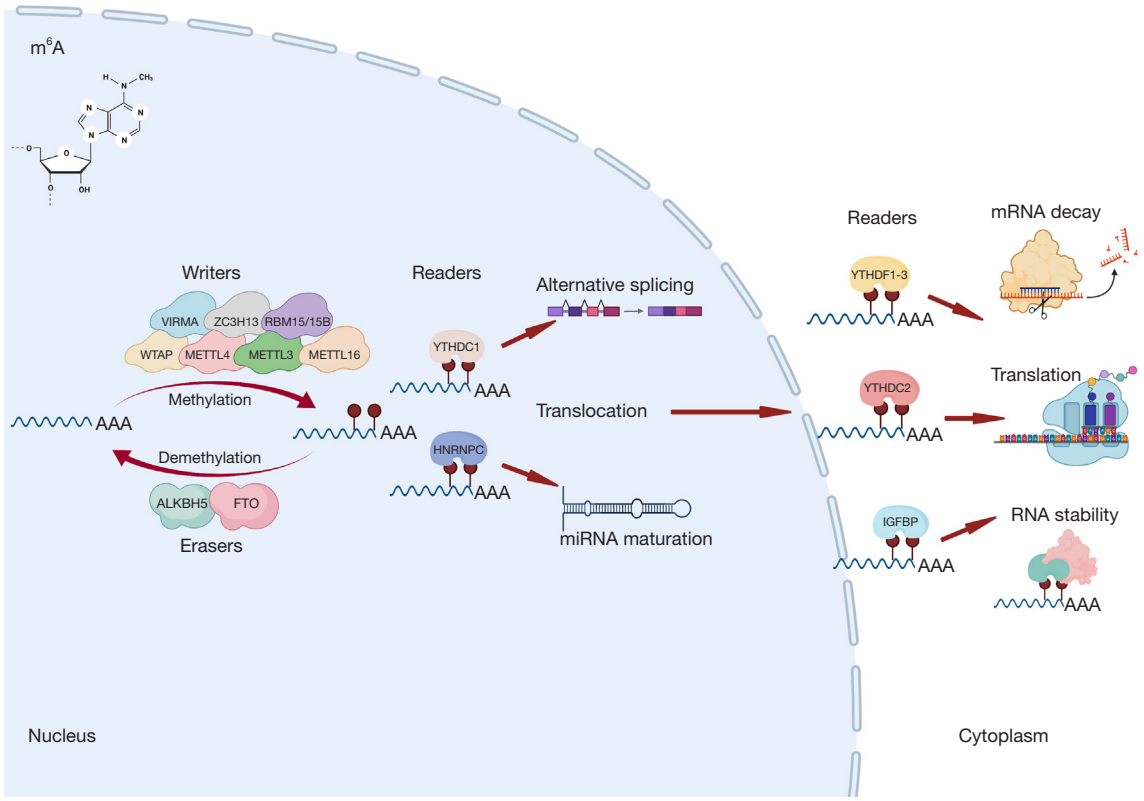

Figure 1 Mechanism of m^{6}A modification. Reversible m^{6}A methylation is catalyzed by the writers METTL3, METTL4, WTAP, VIRMA, METTL16, RBM15/15B, and ZC3H13. The m^{6}A modification is removed by erasers (demethylases), FTO and ALKBH5. The m^{6}A-modified RNA readers include YTHDF1-3, YTHDC1, YTHDC2, HNRNPC, and IGF2BP1-3. m^{6}A modification modulates mRNA splicing, miRNA biogenesis, RNA translocation, RNA translation, RNA decay and RNA stability. Created with BioRender.com. m^{6}A, N6-methyladenosine.

several targeted studies of m^{6}A readers in cardiovascular system and disease. For example, when *Ythdc1* floxed mice were crossed with the constitutive active, cardiomyocyte-specific cre-driver, α-MHC-Cre, homozygous *Ythdc1* conditional knockout mice died around 10 weeks of age (27), suggesting its importance in postnatal cardiac function. The detailed analysis showed that *Ythdc1*-ablated mice displayed left ventricular chamber enlargement and severe systolic dysfunction, which are the hallmark of dilated cardiomyopathy, as well as decrease of cardiomyocyte contractility and disordered sarcomere arrangement. Mechanistically, it was found that Ythdc1 might be required for the proper splicing of sarcomeric protein, Titin.

Another m^{6}A reader, YTHDF2, is also indicated to be involved in cardiac physiology. In both human and mice, the expression of YTHDF2 mRNA and protein, but not its family members—YTHDF1 or YTHDF3, are upregulated during the progression of heart failure (28). When challenged with transverse aortic constriction, *Ythdf2* overexpressing mice attenuated cardiac hypertrophy. Mechanistically, YTHDF2 suppresses cardiac hypertrophy by recognizing m^{6}A site on the myosin heavy chain 7 (*Myh7*) mRNA (also known as β-MHC, which is a marker for cardiac hypertrophy) to promote its degradation.

m^{6}A erasers

Discovered as human obesity-susceptibility gene (40,41), FTO (fat mass and obesity associated gene) is a m^{6}A demethylase that is shown to be involved in cardiac physiology. In failing human, pig, and murine hearts, the expression of FTO was decreased, which coincided with the increased level of m^{6}A marks and decreased cardiomyocyte contractile function (29). When challenged with a

Table 2 List of experimentally validated functions and mechanisms of epitranscriptomic enzymes

Epitrancriptomic enzyme	Experimental system	Phenotypes/mechanisms	Reference
m^6A writer, *Mettl3*	Neonatal rat ventricular cardiomyocytes, cardiomyocyte-specific *Mettl3* conditional knockout mice	Causes cardiac hypertrophy	(26)
m^6A reader, *Ythdc1*	Cardiomyocyte-specific *Ythdc1* conditional knockout mice	Possibly required for the proper splicing of sarcomeric protein, *Titin*	(27)
m^6A reader, *Ythdf2*	*Ythdf2* overexpressing mice	Suppresses cardiac hypertrophy by recognizing m^6A site on the *Myh7* mRNA	(28)
m^6A eraser, *Fto*	*Fto* overexpressing mice	Demethylates cardiac contractile mRNAs to prevent their mRNA degradation and promote their protein expression to preserve cardiac functions in the infarcted hearts	(29)
m^6A eraser, *Alkbh5*	*Alkbh5* knockout and overexpressing mice, hiPSC-CM	Stabilizes the m^6A reader YTHDF1 mRNA in a m^6A dependent manner, thereby promoting the translation of YAP	(30)
A-to-I RNA editing writer, *Adar1*	*Adar1* knockout mice	Results embryonic death due to massive apoptosis and aberrant interferon induction	(31)
A-to-I RNA editing writer, *Adar1*	Cardiac-specific *Adar1* conditional knockout mice	Regulates the cardiomyocyte survival and proliferation	(32)
A-to-I RNA editing writer, *Adar1*	Cardiomyocyte-specific *Adar1* conditional knockout mice	Results in increased lethality due to increased endoplasmic stress leading to apoptosis and reduction in miRNA levels	(33)
A-to-I RNA editing writer, *Adar2*	Neonatal rat cardiomyocytes, cardiomyocyte-specific *Adar2* overexpresing mice	Negatively regulates mature *miR-34a* to protect murine hearts from acute myocardial infarction	(34)
m^5C writer, *Trdmt1* (*Dnmt2*)	*Dnmt2* mutant mice	Results in cardiac hypertrophy possibly due to decreased methylation and increased dissociation of small nuclear RNA from P-TEFb complex	(35)
m^5C writer, *Nsun4*	Muscle-specific *Nsun4* conditional knockout mice	Methylates 12S rRNA and forms a complex with MTERF4 to regulated mitoribosomal assembly	(36)
m^5C writer, *Nsun2*	TALEN-mediated *Nsun2* knockout rats	Methylates *Icam1* mRNA to promote its translation	(37)

permanent ligation of the left anterior descending artery (a mouse model of myocardial infarction), the hearts of *Fto* overexpressing mice attenuated the pathological features of failing hearts (e.g., decreased cardiac contractile function). Mechanistically, Fto demethylates cardiac contractile mRNAs [e.g., natriuretic peptide A (*Nppa*, also known as *ANP*), *Myh*7, ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 2 (*Atp2a2*, also known as *Serca2a*), and ryanodine receptor 2, cardiac (*Ryr2*)] to prevent their mRNA degradation and promote their protein expression to preserve cardiac functions in the infarcted hearts. However, there is still an ongoing debate as to the validity of FTO as m^6A eraser as it targets N6,2'-O-dimethyladenosine (m^6Am, which is a modification found adjacent to the mRNA cap) instead of m^6A (42). Thus, more research is needed to firmly confirm the functional role of FTO in the context of m^6A marks.

Although the adult mammalian cardiomyocytes are terminally differentiated without capacity for regeneration, neonatal murine hearts can regenerate within the first week after birth when challenged with apical resection or myocardial infarction, mediated by cardiomyocyte proliferation (43). A recent study shows that the m^6A eraser, ALKBH5, plays a role in cardiac regeneration as the neonatal *Alkbh5* knockout mice fail to regenerate their hears after apical resection, while overexpression of *Alkbh5* in juvenile (7 days old) and adult (8 weeks old) mice promoted cardiomyocyte proliferation after myocardial infarction (30).

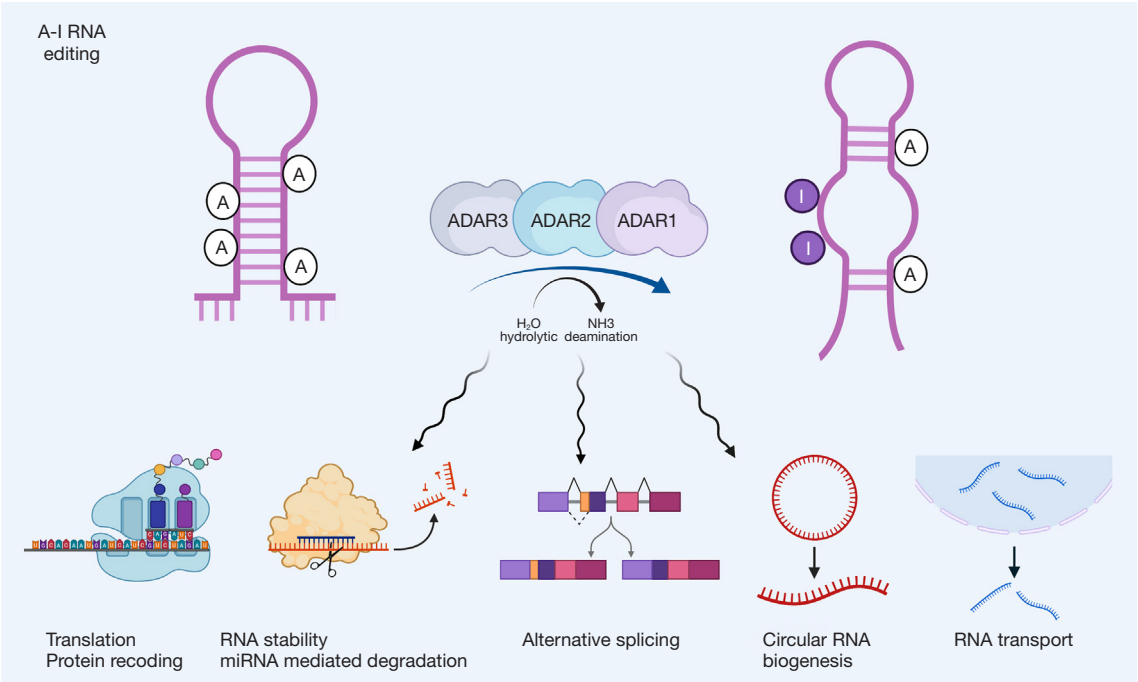

Figure 2 Adenosine to inosine (A-to-I) RNA editing. The ADAR family consists of three members—ADAR1, ADAR2, and the catalytically inactive ADAR3. ADAR proteins catalyze A-to-I RNA editing by binding to double-strand RNA and deaminating adenosine residues by turning it into inosine; thus, inosine no longer pairs with uracil. The main molecular consequences of ADAR-induced RNA editing result in mRNA recoding; regulation of RNA stability via miRNA mediated degradation; alternative splicing that may translate into proteins, with altered function; inhibition of biogenesis of circular RNA, nuclear retention, and RNA translocation. Created with BioRender.com.

In vitro, overexpression of *ALKBH5* enhanced mitosis in human induced pluripotent stem cell-derived cardiomyocytes. Mechanistically, ALKBH5 stabilizes the m^{6}A reader *YTHDF1* mRNA in a m^{6}A dependent manner, thereby promoting the translation of Yes1 associated transcriptional regulator (YAP1, also known as YAP), which is a downstream effector of the Hippo pathway that is important for cardiomyocyte growth.

A-to-I RNA editing

Adenosine to inosine (A-to-I) RNA editing is a conversion of A into I via the catalytic reaction of adenosine deaminase acting on RNAs (ADARs) by binding to double-stranded RNA (44) (*Figure 2*). RNA editing affects RNA metabolism by alterning splicing, miRNA biogenesis and binding, amino acid conversion, lncRNA binding and structures, RNA stability, and translation. Compared to m^{6}A marks, RNA editing occurs most frequently in introns and 3'-untranslated regions (UTR) as both epitranscriptomic marks target the same nitrogen-6 position of A (45); thus, they are in a reciprocal relation. Unlike m^{6}A, RNA editing is an irreversible process with no known reader to date. In mammals, there are three ADAR genes: *ADAR* (*ADAR1*), *ADARB1* (*ADAR2*), and catalytically inactive *ADARB2* (*ADAR3*). The mutation in the human *ADAR1* gene is known to cause of the Aicardi-Goutières autoimmune disease (46), while the accumulation of ADAR1 protein was observed in atherosclerotic plaques (47), which are the most common cause of coronary artery diseases. In mice, the whole body knockout of *Adar1* results in embryonic death due to massive apoptosis and aberrant interferon induction (31), which can be rescued to live birth by ablating the RIG-I-like receptors, mitochondrial antiviral signaling protein (*Mavs*) or interferon induced with helicase C domain 1 (*Ifih1*, also known as *Mda5*) (48-50). Interestingly, these massive apoptotic events are most visible in the embryonic heart, which the recent study crossing *Adar1* conditional knockout mice with the early cardiac specific cre-driver, Nkx2.5-Cre, resulted in embryonic death due to increased apoptosis and reduced proliferation in developing cardiomyocytes (32) (*Table 2*), suggesting that Adar1 is important in regulating the cardiomyocyte survival

and proliferation. Because NK2 homeobox 5 (*Nkx2-5*) gene is also expressed in other cell type (i.e., endothelium) and tissue types (tongue, thymus, and spleen) during embryonic development (51), it is possible that the ablation of *Adar1* in early cardiac field may not be specific for cardiomyocytes. However, another recent study crossing *Adar1* conditional knockout mice with the endothelial-specific cre driver, VE-Cadherin-Cre (Chd5-Cre), demonstrated that the ablating *Adar1* in endothelial cells did not result in embryonic death, instead postnatal death due to growth retardation within three weeks after birth for 75% of neonates (52). To further confirm the important role of Adar1 in cardiomyocytes, another study crossing *Adar1* conditional knockout mice with the tamoxifen-inducible cardiomyocyte specific cre-driver, αMHC-MCM, showed that ablating *Adar1* in adult cardiomyocytes resulted in increased lethality due to increased endoplasmic stress leading to apoptosis and reduction in miRNA levels, especially *miR-199a-5p* that regulates unfolded protein response (UPR) (33). Taken together, these studies demonstrate that Adar1 in murine cardiomyocytes is important for development and physiology of the heart.

Compared to ADAR1, ADAR2 is more specific in its targets [i.e., glutamate ionotropic receptor AMPA type subunit 2 (*GRIA2*, also known as *GluR2*) pre-mRNA editing at Q/R site (53)], which demonstrated to be very useful for RNA-based genome editing by fusing the catalytic domain of ADAR2 to CRISPR-Cas13b enzyme to perform A-to-I conversion in RNA transcripts (54). In mice, the expression of *Adar2* was induced in exercised hearts (34). *In vitro*, overexpression of *Adar2* inhibited doxorubicin-induced apoptosis in neonatal rat cardiomyocytes, while silencing of *Adar2* resulted in increased apoptosis. When *Adar2* was overexpressed in a cardiac specific manner, it protected murine hearts from acute myocardial infarction by negatively regulating mature *miR-34a*, suggesting that *Adar2* could be a potential therapeutic target for cardiovascular disease (34).

5-methylcytosine (m^5C)

Just as DNA, RNA can be marked by 5-methylcytosine (m^5C), which are found abundantly in rRNAs and tRNAs but also in mRNAs and ncRNAs, which stabilize RNA folding and structures (55,56). This reversible modification is deposited by m^5C writers [NOP2 nucleolar protein (NOP2, also known as (NSUN1), NOP2/Sun RNA methyltransferase 2 (NSUN2), NOP2/Sun RNA methyltransferase 3 (NSUN3), NOP2/Sun RNA methyltransferase 4 (NSUN4), NOP2/Sun RNA methyltransferase 5 (NSUN5), NOP2/Sun RNA methyltransferase 6 (NSUN6), NOP2/Sun RNA methyltransferase family member 7 (NSUN7), and tRNA aspartic acid methyltransferase 1 (TRDMT1, also known as DNMT2)], interpreted by m^5C readers [Aly/REF export factor (ALYREF), Y-box binding protein 1 (YBX1), and RAD52 homolog, DNA repair protein (RAD52)], and nullified by m^5C erasers [tet methylcytosine dioxygenase 1 (TET1), tet methylcytosine dioxygenase 2 (TET2), and tet methylcytosine dioxygenase 3 (TET3)] (*Figure 3*). By employing RNA bisulfite sequencing (RNA-BisSeq), 3,575 m^5C sites in 1,574 mRNAs were identified in the murine heart (57), although differential m^5C analysis of diseased hearts compared to the healthy ones has not been performed yet.

The DNA methyltransferase homologue, TRDMT1 (DNMT2), methylates tRNAs rather than DNA (58). Although Dnmt2 mutated flies, plants, and mice are viable and fertile, Dnmt2 morphant zebrafish embryos showed developmental defects in the brain, retina, and liver (59). When the adult (3 months old) Dnmt2 mutant mice were examined carefully, cardiac hypertrophy due to induced cardiac growth with preserved function was observed (35) (*Table 2*). This phenotype was possibly due to decreased methylation and increased dissociation of small nuclear RNA, RNA, 7SK, nuclear (*Rn7sk*), from P-TEFb complex (positive transcription elongation factor), thereby over-activating RNA polymerase II to induce cardiac hypertrophy via gene expression.

Another m^5C writer, Nsun4, is also important in cardiomyocytes. Crossing *Nsun4* conditional knockout mice with the muscle-specific (both cardiac and skeletal muscles) cre-driver, Ckmm-Cre, it was found that ablation of *Nsun4* resulted in shorter lifespan (death within the age of 25 weeks) with progressive cardiomyopathy based on the increased heart to body weight ratio, leading to mitochondrial dysfunction caused by impaired biogenesis of the respiratory chain complexes (36). Mechanistically, Nsun4 is a dual function protein, which methylates 12S rRNA and forms a complex with the mitochondrial protein, mitochondrial transcription termination factor 4 (MTERF4), to regulated mitoribosomal assembly.

Besides cardiomyocytes, m^5C marks are also important in endothelial functions. Using TALEN-mediated knockout of m^5C writer, *Nsun2*, in rats demonstrated that ablation of *Nsun2* impaired the formation of allograft arteriosclerosis

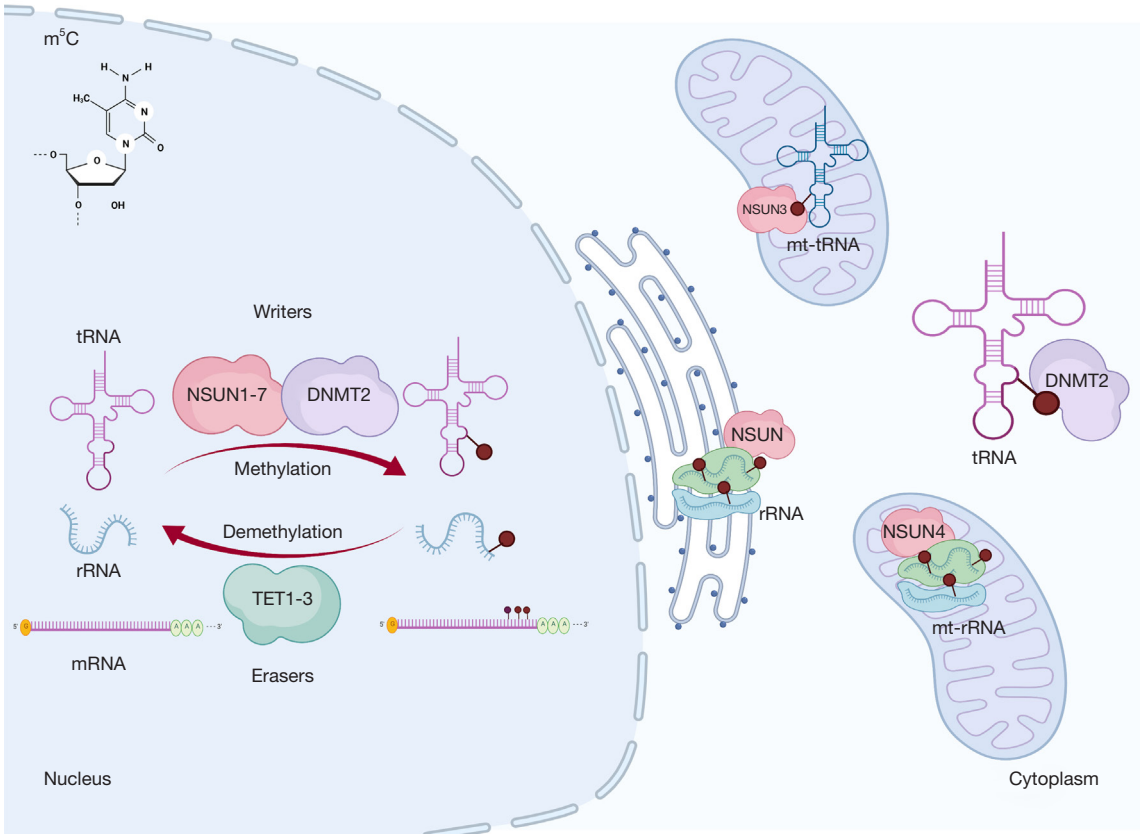

Figure 3 Mechanism of 5-methylcytosine (m^5C) modification. This modification is introduced by RNA m^5C methyltransferases, and it is abundant in rRNA/mt-rRNA, tRNA/mt-tRNA as well as mRNA and ncRNA. The writers DNMT2 and NSUN3 methylate tRNA, while Nsun4 methylates rRNA. The methylation is reversible by the functions of erasers TET1-3. Created with BioRender.com.

(hardening of the arteries) in a model of aortic allograft by transplanting thoracic aortic allografts of wildtype or *Nsun2* knockout rats into the abdominal aorta of donor rats, suggesting that Nsun2 is important for neointima formation (37). Mechanistically, Nsun2 methylates intercellular adhesion molecule 1 (*Icam1*) mRNA to promote its translation, thereby increasing the adhesion of leukocytes to endothelial cells to regulate vascular endothelial inflammation and atherosclerosis.

Conclusions

Compared to m^6A, A-to-I RNA editing, and m^5C, less is known about other epitranscriptomic marks in the heart. Because of the convenience and availability of next generation sequencing, especially RNA sequencing (RNA-seq), high-throughput screening studies should be carried out to understand the changes in epitranscriptomic marks in diseased hearts compared to the healthy ones, for example. Furthermore, more genetic studies, especially those using cell-type-specific knockout mice (60-62), are urgently needed to understand functional roles of epitranscriptomic marks in the heart. Because of the availability of induced pluripotent stem cells (iPSC) and their differentiated cell types (i.e., iPSC-derived cardiomyocytes), more functional studies of epitranscriptomic enzymes should be performed by gain/loss-of-function experiments, especially using CRISPR/Cas9 system to ablate each epitranscriptomic enzyme in iPSC. These functional and mechanistic studies along with screening studies are needed to better characterize epitranscriptomic marks in cardiovascular system and disease.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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