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Epitranscriptomics in the Heart: A Focus on m^{6}A

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Abstract

Purpose of Review: Post-transcriptional modifications are key regulators of gene expression that allow the cell to respond to environmental stimuli. The most abundant internal mRNA modification is N6-methyladenosine (m^{6}A), which has been shown to be involved in the regulation of RNA splicing, localization, translation, and decay. It has also been implicated in a wide range of diseases, and here we review recent evidence of m^{6}A's involvement in cardiac pathologies and processes.

Recent Findings: Studies have primarily relied on gain and loss of function models for the enzymes responsible for adding and removing the m^{6}A modification. Results have revealed a multifaceted role for m^{6}A in the heart's response to myocardial infarction, pressure overload, and ischemia/reperfusion injuries. Genome-wide analyses of mRNAs that are differentially methylated during cardiac stress have highlighted the importance of m^{6}A in regulating the translation of specific categories of transcripts implicated in pathways such as calcium handling, cell growth, autophagy, and adrenergic signaling in cardiomyocytes.

Summary: Regulation of gene expression by m^{6}A is critical for cardiomyocyte homeostasis and stress responses, suggesting a key role for this modification in cardiac pathophysiology.

Keywords

"Heart failure"; "m^{6}A"; "RNA methylation"; "Epigenetics"

Introduction

Regulation of cardiac-specific genes plays an important role during normal heart development as well as throughout adulthood when the myocardium undergoes age-related

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Conflict of interest:

Jacob Z. Longenecker, Christopher J. Gilbert, Volha A. Golubeva, Colton R. Martens, and Federica Accornero declare they have no conflict of interest.

Human and Animal rights and Informed Consent

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remodeling. Moreover, changes in gene expression in response to stress or injury are also key in understanding cardiac pathology. The journey to making a functional gene product consists of multiple fundamental steps, all of which are impeccably coordinated by sophisticated regulatory mechanisms. At the epigenomic level, chromatin remodeling and chemical modifications on DNA can regulate the expression of genes important for cardiogenesis and cardiomyocyte stress responses (1, 2). Several systematic studies have highlighted the contribution of DNA methylation, histone modifiers, transcription factors, and long non-coding RNAs to transcriptional regulation in cardiac biology and disease (3–6). Transcriptional regulators, such as histone deacetylase (HDAC) inhibitors, have even shown therapeutic promise to ameliorate cardiac remodeling (7, 8). Indeed, decades of work have clarified the contribution of transcriptional regulation of gene expression for heart remodeling. However, the importance of regulating the life of RNA molecules for fine-tuning cardiac stress responses is only recently emerging.

It is now clear that cardiac gene transcripts are extensively manipulated post-transcriptionally at the level of RNA maturation, transport, stability, and translation. Several studies have recently demonstrated the utility of ribosome profiling, or measuring the ribosomal occupancy sites, to study gene expression control at the post-transcriptional level (9–12). Due to this unique approach, it became evident that translational reprogramming of cardiac gene expression plays a central role in the myocardial response to stress (12). An established mechanism for post-transcriptional regulation of gene expression is represented by microRNAs. Indeed, several stress-inducible microRNAs directly affect cardiac protein levels and can be used as potential biomarkers of pathologic remodeling in the heart (13, 14). However, this is only one of the many complex regulatory layers that can affect mRNA translation in the heart and the critical importance of post-transcriptional gene regulation in cardiac pathophysiology cannot be fully understood without considering the role of RNA modifications.

Similar to regulation by DNA methylation, chemical modification of RNA transcripts can also modulate gene expression, with more than 150 of these structures detected to date (15). The most abundant internal mRNA modification, N6-methyladenosine (m^6A), was first discovered in 1974, but only recently gained scientific attention in cardiac biology. The m^6A modification is dynamic and reversible, with the methyl group catalytically installed by the core enzymatic m^6A methyltransferase complex (the “writer”) and potentially removed by m^6A demethylases (the “erasers”) (16–18). The presence of m^6A sites is recognized by specific RNA binding proteins (the “readers”), which respond to the unique m^6A-encoded messages and modify protein production typically by affecting mRNA splicing, localization, stabilization, or ribosomal accessibility (19–21). The functional and biological relevance of the m^6A RNA modification has been confirmed in diverse processes such as DNA damage response, circadian clocks, and neuronal differentiation, highlighting its role as a master regulator of post-transcriptional gene expression (22–24). In this review, we discuss the most recent studies that focus specifically on the homeostatic ramifications of m^6A RNA modification in the heart.

m⁶A is essential to maintenance of cell homeostasis and stress responses in the heart

The role of m^6A and its associated methyltransferase and demethylase complexes have been studied extensively in the context of cancer and development. However, only recently has its role in the control of cardiac homeostasis started to emerge. Indeed, in the past two years a small number of studies have pointed at an essential role for m^6A in the process of cellular growth, survival, and function in the heart (25–29). In addition to demonstrating how m^6A is central to cardiac pathophysiology, these studies provided the first mechanistic insights into how m^6A exerts its effects on mRNA transcripts, such as modulation of gene expression and translation in the heart. Modulation of cardiac m^6A levels has been possible through genetic or viral manipulation of m^6A modification enzymes. These model systems have shown how changes in m^6A content regulate the degree of cardiac hypertrophy resulting from pressure overload stress as well as the degree of cardiac dysfunction following myocardial infarction or ischemia/reperfusion injuries. These studies incorporated genome-wide sequencing analysis, adopting pull-down of m^6A-modified transcripts using an antibody specific for m^6A to determine what types of genes are differentially methylated during cardiac stress. These results have been very valuable in providing an understanding of the gene programs that are controlled through m^6A in the heart. Overall, as a result of the above-mentioned studies, it is now clear that m^6A plays a critical role in the maintenance of cellular homeostasis and response to stress in the heart, and the specific results obtained will be discussed in more detail below.

The two logical approaches for testing the outcome of modulating m^6A during the stress response are altering the expression of the essential m^6A methylase, Methyltransferase like 3 or METTL3, or either of the m^6A demethylases, Fat mass and obesity-associated protein (FTO) and AlkB homologue 5 (ALKBH5). Notably, FTO experienced intense focus once it was recognized that variations of the gene encoding this protein predispose individuals to obesity and type 2 diabetes (30–32). A result of this metabolic-centric focus is two studies that initially characterized the physiological effects of FTO in cardiomyocytes (33, 34). Gan *et al.* identified a connection between FTO and leptin-induced cardiomyocyte hypertrophy, in a manner independent of angiotensin-II and endothelin-I induced-hypertrophy. Here they utilized cultured neonatal rat cardiomyocytes to show that FTO expression is required for the leptin-dependent hypertrophic response. In the second paper, Carnevali *et al.* utilized a global FTO knock out mouse model to assess the effect that FTO loss has on cardiac function. These authors found that mice lacking FTO exhibited increased cardiac hypertrophy and an increased propensity to develop arrhythmias following stress. Because this group utilized a global knock out model, the contribution of losing FTO in non-cardiac tissues cannot be ignored. An important drawback of these studies is that neither of them assessed changes in m^6A content in cardiomyocytes, and it would be interesting to see what targets are differentially methylated under the conditions tested. The modulation of m^6A on select transcripts following changes to FTO expression could be contributing to the phenotypes seen by these groups, and additional studies on this topic will be discussed later in this section.

A recent study by Dorn *et al.* utilized isolated neonatal rat cardiomyocytes as well as mouse models with cardiomyocyte-specific overexpression or knock out of METTL3, resulting in increased and decreased m^6A levels, respectively (25). Here it was shown that the overall m^6A content of cardiomyocytes is increased following induction of hypertrophy, an outcome similar to the increase of m^6A content seen during heart failure by Mathiyalagan *et al.* (26) and the increase in m^6A content seen during hypoxia/reoxygenation of cardiomyocytes or ischemia/reperfusion of the heart by Song *et al.* (27). Additionally, the overexpression of METTL3 and resultant increase in m^6A promoted spontaneous concentric cardiac remodeling, whereas loss of METTL3 and m^6A promoted eccentric cardiomyocyte remodeling over time and in response to pressure overload stimulation, implying that METTL3 and m^6A are regulators of cardiac geometry and growth (25). Importantly, it was noted in this study that mice without METTL3 in the heart develop cardiac dysfunction beginning at about eight months of age, whereas those overexpressing METTL3 in the heart did not. This indicates that the loss of m^6A is detrimental even in the absence of stress. In agreement with the above studies indicating the important contribution of METTL3 for cardiomyocyte remodeling, a paper published by Kmietczyk *et al.* also showed a connection between m^6A abundance and the hypertrophic response to stress (28). These authors found that in an isolated cardiomyocyte cell system the loss of METTL3 and m^6A exacerbated hypertrophy while loss of FTO increased m^6A and prevented hypertrophic growth. In this same study, adeno-associated vectors were adopted for postnatal overexpression of METTL3 in the heart, which was proven detrimental for pressure overload induced cardiac injury. While the results from Dorn et al. and Kmietczyk et al. make it clear that m^6A is involved in the cardiac hypertrophic response, it must be noted that these studies reached somewhat different conclusions: Dorn et al. described METTL3 as necessary to prevent eccentric cardiomyocyte remodeling and promote adaptation to stress; whereas Kmietczyk et al. found that virus-mediated increase in METTL3 is detrimental to the stressed heart. It is unclear why this outcome differs between the studies; however, there are key methodological differences that may contribute to these differing outcomes. Indeed, each study utilized a different mouse background in combination with a different method of overexpression, likely leading to differential expression patterns for each manipulation as well as strain-dependent differences in how the mice responded to stress. Overall, it is plausible that either globally increasing or decreasing m^6A content in the heart would have pathologic consequences for the heart, and future work dissecting downstream pathways affected by the METTL3-m^6A axis will be essential to therapeutically target the most deleterious branches of the cardiac gene program regulated by METTL3.

The previously mentioned study by Mathiyalagan *et al.* focused on the role of m^6A post-ischemic injury rather than during hypertrophic stress, via modulation of FTO expression (26). Here they showed that overexpression of FTO and consequent reduction of m^6A ameliorated the effects of myocardial infarction-induced heart failure whereas the loss of FTO and consequent increase of m^6A had the opposite effect, indicating that the presence of m^6A contributed to ischemic cardiac damage, a result that is consistent with the idea that m^6A is critical for the stress response. In similar fashion, Berulava *et al.* used a mouse model to test the effect of FTO loss in the context of pressure overload-induced heart failure and found that the loss of FTO led to increase of m^6A in cardiomyocytes and worsened the effect

of pressure overload-dependent stress (29). The outcomes of this study agree with the suggested cardioprotective role of FTO in the context of ischemic injury by Mathiyalagan *et al.*, indicating that key FTO-dependent gene expression events might be shared following either mechanical or ischemic myocardial injuries (26).

The other known eraser of m^6A modifications, ALKBH5, is much less studied than FTO and as a result, only a single paper has focused on how ALKBH5 modulation in the heart contributes to disease (27). Here, Song *et al.* showed that loss of ALKBH5 in cardiomyocytes phenocopied overexpression of METTL3 in the response to hypoxia/reoxygenation. Although these authors did not examine changes to global m^6A levels following ALKBH5 loss, they highlighted a critical role for m^6A in the regulation of autophagy by showing that m^6A was increased on specific relevant transcripts. In fact, this m^6A increase was ALKBH5 specific, evidence that the two m^6A erasers do not share all of the same targets. Specifically, they found that autophagic function is augmented by m^6A loss following ischemic injury. Importantly, Kmietczyk *et al.* provided more evidence supporting a role for m^6A in autophagy when they showed that METTL3 modulates the protein level of Arhgef3 (Rho Guanine Nucleotide Exchange Factor 3), an activator of mTORC1 (mammalian Target of Rapamycin Complex 1), the master regulator of autophagy (28). This is an interesting connection when considering that mTORC1 activity and autophagic flux are affected during cardiac remodeling and heart failure (25–28). However, time courses where m^6A content and autophagy are simultaneously analyzed as the heart remodels post-stress will be needed to better clarify the temporal role of m^6A in this process. Indeed, it is possible that autophagy-related transcripts are not among those hypermethylated during cardiac stresses and it is important to note that the findings of Berulava *et al.* showed that hypomethylation of transcripts is just as relevant to the cardiac stress response as hypermethylation (29). Additionally, it cannot be excluded that regulation of autophagy following manipulation of m^6A content in the heart could be the result of a combination of direct and indirect effects.

An important aspect of studies that modify the m^6A erasers that must be recognized is that in each case the expression of only one of the erasers was altered. It is reasonable to predict that a compensatory change in the expression or activity of the other eraser may occur. This compensation may confound results when taking into consideration the fact that FTO and ALKBH5 do not share all the same targets and have tissue-specific differences in expression. In fact, this exact outcome was noted by Mathiyalagan et al. when they found that global m^6A content was significantly increased beginning one week post-MI, a result of the differential expression of eraser proteins. Even though FTO protein expression is significantly decreased at all time points tested post-MI, the increase of ALKBH5 expression prior to the one week post-MI time point is sufficient to reduce global m^6A content before returning to baseline one week post-MI (26). Thus, when the goal is to increase m^6A through modulation of demethylating enzymes, a double knock-out mouse model of both erasers may represent a most definitive approach for future studies. The converse is also true when the goal is to assess a decrease of m^6A in an eraser-focused approach. A second potential confounder is that changes in m^6A may be mRNA-independent, as m^6A has been found in other RNAs, including long non-coding RNAs (lncRNA), and primary micro RNAs (pri-miRNA) (35, 36). It is indeed possible that

changes to the m^{6}A content of non-coding RNAs may be contributing to the phenotypes seen when modulating m^{6}A during stress.

Regardless of the different approaches used between studies for increasing or decreasing m^{6}A through modulation of METTL3, FTO, or ALKBH5 protein expression in combination with hypertrophic or ischemic insults, all findings collectively provide clear evidence that the presence of m^{6}A on transcripts in the heart is required for maintenance of proper cardiac function.

Mechanistic effects of m^{6}A modification

It has become abundantly clear that m^{6}A is involved in the cardiac stress response, however the prominent question remains of how exactly m^{6}A can exert this modulatory effect. There are a considerable number of studies focused on the mechanistic effects of m^{6}A on mRNA, although these studies were largely not focused on the heart. Generally, non-heart-focused m^{6}A studies have found that m^{6}A regulates splicing (37, 38), differentiation (39, 40), translation (41–43), proliferation (44), RNA structure (21), and mRNA decay (45, 46). Of those focused on m^{6}A in the heart, most have concluded that m^{6}A modulates the ability of methylated transcripts to be translated into protein. Two of these studies provided evidence that genes differentially methylated during disease are typically not simultaneously differentially expressed under the same conditions (28, 29). This represents an important distinction that has been noted in the past: m^{6}A modulation of translation can occur in a separate and distinct process from modulation of transcription. However, coordination between transcription and m^{6}A deposition is also emerging as an important node for gene expression regulation (35, 47).

Although there are numerous mechanisms to modulate translation, two have been shown to be connected to m^{6}A: mRNA decay and ribosome occupancy, the latter of which is determined using polysome profiling. Previous reports on the effect that m^{6}A has on mRNA decay and translation, typically focus on how proteins recognize m^{6}A modifications to facilitate or inhibit these processes. Within the heart specifically, Song *et al.* (27), Mathiyalagan *et al.* (26), and Kmietcyzk *et al.* (28) showed that the addition of m^{6}A modifications to mRNAs can exert a spectrum of effects on mRNA decay, including destabilization of the transcript (27), stabilization (26), or both (28), depending on the transcript analyzed.

Evidence of m^{6}A-mediated modulation of translation in cardiac tissue is presented in two studies, both of which identified transcripts that were methylated following stress (28, 29). These transcripts were then analyzed using polysome profiling, which equates increased occupancy of ribosomes with increased translation. Each of these studies reached the same conclusion that the translation of transcripts was positively correlated with the degree of methylation. This finding is interesting given that m^{6}A modifications differentially affect mRNA stability while seemingly often increasing translation. Understanding how m^{6}A binding proteins (“readers”) work to regulate the fate of m^{6}A-modified transcript in the heart and knowledge on potential tissue-specific function of these proteins will be crucial to better clarify the consequences of methylating mRNAs. Indeed, the most prominent mechanism of

m^{6}A-dependent regulation is the specific recognition of the modification by m^{6}A reader proteins. The functions of these readers are what determine the ultimate outcome of the m^{6}A modification. Notably, m^{6}A can also facilitate local RNA structure destabilization to permit access to adjacent binding sites by other RNA binding proteins (48). Through these mechanisms, RNA methylation functions to regulate gene expression across cell types (18, 49–52). Although studies focused on the reader proteins in the heart are virtually nonexistent, and thus their relative expression patterns in cardiac tissue are not yet known, there have been numerous studies focused on these proteins in other cellular contexts. The reader proteins, which function to preferentially recognize m^{6}A-modified mRNAs, consist of the YT521-B homology (YTH) family (YTHDF) proteins that bind m^{6}A via the C-terminal YTH domain (53). Human YTHDF1 and 3 has been shown to predominantly function in enhancing translational efficiency and, as previously mentioned, interfering with m^{6}A through genetic ablation of writers, erasers or readers generally impacts the translation of specific mRNAs (39, 49, 50). Conversely, selective recognition of m^{6}A by YTHDF2 has been predominantly linked to mRNA degradation, implicating m^{6}A differential control across various tissue systems (19, 54). However, general rules on defined roles for each m^{6}A reader cannot be extrapolated, as tissue-specificity of effects is not excluded, and eventual coordination of mRNA regulation by combination of m^{6}A binding proteins complicates our current ability to unequivocally interpret some of the published results.

Generally, the YTHDF family of proteins modulates vital roles in eukaryotic cell development. YTHDF1 was shown to bind to m^{6}A in the 3’UTR to recruit the 43S pre-initiation complex (55, 56) and to directly interact with initiation factor eIF3 to promote translation in the absence of canonical cap-binding protein eIF4E (55, 57). YTHDF3 interacts with 40S and 60S ribosomal subunits to promote m^{6}A-mRNA translation in cooperation with YTHDF1 (56, 58). Although YTHDF2, as mentioned above, has often been associated with mRNA degradation, it has been shown that this m^{6}A reader can shuttle to the nucleus post-stress where it binds mRNAs with m^{6}A at their 5’-UTR to protect from demethylation by FTO, thus promoting cap-independent mRNA translation (59). Even though these studies were not performed in cardiac tissues, their mechanistic insight into how m^{6}A influences translation undoubtedly extends to the heart and may explain the positive correlation found between m^{6}A and ribosome occupancy seen by Kmietczyk *et al.* and Berulava *et al.* (28, 29).

Beyond translational efficiency, mRNA stability is certainly a critical step in the life of mRNAs that can be regulated by YTHDF proteins. Specifically, cytoplasmic YTHDF2 binds m^{6}A-RNA and localizes to the P-body for mRNA degradation (19, 54). Also, the N-terminus of YTHDF2 promotes deadenylation of mRNA via CNOT1 recruitment (60). Not surprisingly considering the established importance for m^{6}A in regulating entire gene programs in each analyzed cell type, conditional deletion of YTHDF2 leads to lethality during late embryonic developmental stages caused by defects in neural development (61). YTHDF2 also functions to regulate circadian regulation of hepatic lipid metabolism, and innate immune response to infection, implicating YTHDF2 as a key regulator in various cell and tissue systems (62–65). Interestingly, YTHDF3 knockdown reduced RNA-binding specificity of both YTHDF1 and YTHDF2, suggesting dynamic interaction of these three reader proteins (58). Indeed, the varied effects of the different reader proteins underlie the

complex network of m^{6}A regulation, which becomes exponentially more complex when taking the differing tissue-specific expression patterns between reader proteins into consideration. It is possible that expression, localization or function of m^{6}A reader proteins following cardiac stress may be responsible for the differential translation of m^{6}A-modified transcripts. Alternatively, the localization of the m^{6}A modifications within the transcript almost certainly alters recognition of m^{6}A by the reader proteins, thus determining changes in mRNA decay or translation. Further studies identifying the specific effects of m^{6}A localization within a transcript are required to further assess this suggestion. Taken together, these studies make it abundantly clear that the differential effect of m^{6}A on a transcripts stability or translation is highly context-dependent, having a specific effect on one transcript and the opposite effect on another. This highlights the dynamic and highly specific role of m^{6}A in cellular physiology. Highly specific modulation of these parameters can open new doors to exciting new therapeutics that are far more specific than those currently available for the treatment of heart failure. Although effective and safe use of therapeutics that modulate m^{6}A to treat disease are far off into the future, one can speculate that the reader proteins represent the targets most likely to make this endeavor successful. The presence of three related, but unique, proteins to decode the m^{6}A marks on transcripts potentially allows for selective modulation of reader activity without targeting the entire m^{6}A decoding process. Further examination of similarities and differences between the readers are required to make this suggestion possible.

Targets of m^{6}A methylation in the heart post-stress

The recognition of an essential role for m^{6}A modifications in the cardiac stress response as well as the identification of mechanistic outcomes of this modification at the mRNA decay and translation levels begs the question, what pathways are being regulated by m^{6}A to carry out the observed effects during stress? Fortunately, all studies described previously used a genome-wide approach in conjunction with functional protein analysis to identify pathways whose protein transcripts are differentially methylated following cardiac stress. Targets that were found to be differentially methylated included protein kinases and protein modifiers (25), calcium signaling and contractility proteins (26), autophagy regulators (27, 28), cardiac muscle proteins and proteins involved in transcriptional regulation (28). Considering that many of these proteins are involved in pathways integral to proper cardiac tissue signaling and structure, the importance of m^{6}A to the cardiac stress response is abundantly clear. While the previously mentioned studies generalized targets as differentially methylated, in one case the authors described specifically whether certain targets were hypo or hypermethylated post-stress, a distinction that is critically important given that the degree of methylation contributes to the ultimate effect of the modification (29). Amongst those that are hypomethylated in the failing heart the most notable are proteins involved in adrenergic signaling, metabolism, mitochondria, and negative regulation of signal transduction. Considering that the sympathetic nervous system plays a critical role in heart failure, perhaps m^{6}A is a means that cardiomyocytes adopt to cross talk with the sympathetic nervous system and affect the translation of transcripts that can lead to overactivation of the sympathetic nervous system. Additionally, transcripts found to be hypermethylated in the failing heart include those encoding for proteins involved in metabolism, the muscle stretch

response, growth factors, and morphogenesis. The finding that metabolic genes may be hypo or hypermethylated in the failing heart might underlie the known metabolic reprogramming occurring during cardiac remodeling. Collectively, studies focused on m^6A in the cardiac stress response agree that m^6A is essential to these processes, where the dynamic, reversible nature of the modification underlies the ability of m^6A to exert highly specific and varied responses.

Conclusions

Post-transcriptional modifications have emerged as key regulators of RNA maturation, function, and decay. Many RNA modifications are condition-specific and dynamic, which allows a given transcript to adopt multiple distinct functions depending on how it is differentially modified. This functional amplification of genetic information afforded by RNA modifications (and other mechanisms of gene regulation) ultimately increases the suite of tools the cell has access to, which allows cells to respond to many diverse environmental stimuli. The m^6A modification covered in this review is the most abundant internal mRNA modification, and it has been established as a key regulator of virtually all steps in the RNA life cycle. It has also been shown to be highly responsive to environmental stimuli, suggesting that it is involved in stress responses and maintaining cellular and physiological homeostasis. The role of m^6A in cardiac stress responses has recently become a topic of interest, and multiple groups have reported its involvement in various cardiac injury models (Table 1). More specifically, through manipulation of the expression of m^6A writers and erasers in mouse and cell culture models, m^6A has been shown to be important for cardiac hypertrophy, response to ischemia/reperfusion injury, and response to myocardial infarction. The m^6A modification also appears to regulate cardiac metabolism and autophagy. Genome-wide analyses of transcripts methylated during cardiac stress have revealed that genes involved in calcium handling, adrenergic signaling, and growth (among others) are subject to m^6A-dependent regulation in the heart. A key future challenge is to gain an understanding of the site-specific effects of m^6A modifications on a given transcript. Overexpressing or knocking out the writers and erasers is a necessary starting point to understanding the role of m^6A, but such approaches can lead to overgeneralizations as well as underestimations of the complexity and nuance of the m^6A phenomenon. Whether m^6A is “good” or “bad” in a given organ or physiological response likely depends on the specific transcripts involved as well as the cellular context within which a modified transcript is interpreted. To this end, a related future challenge is to understand the mechanisms by which m^6A reader proteins translate a particular m^6A event into a specific functional outcome for that transcript. Gain and loss of function models for the readers, coupled with assays to track aspects of the life cycle of known methylated transcripts will play an essential role in meeting this challenge. We can expect such mechanistic explorations to reveal an even greater importance of m^6A in cardiac physiology and disease.

Abbreviations:

m^6A	N6-methyladenosine
METTL3	methyltransferase like-3

FTO	fat mass and obesity related protein
ALKBH5	AlkB homologue 5
YTHDF1	YT521-B homology domain family protein 1
YTHDF2	YT521-B homology domain family protein 2
YTHDF3	YT521-B homology domain family protein 3

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Table 1:

Summary of studies focused on cardiomyocyte-specific m^6A modulation

Model	Stress	Target	Manipulation	Conclusion	Reference
Isolated neonatal rat cardiomyocytes	None Serum-induced hypertrophy	METTL3	Overexpression Knock down	Spontaneous hypertrophy Inhibited hypertrophy	Dorn et al. (25)
Mouse model	Pressure overload	METTL3	Overexpression Knock down	No change in hypertrophy Accelerated heart failure	Dorn et al. (25)
Isolated neonatal rat cardiomyocytes	Phenylephrine	METTL3 FTO	Overexpression Knock down Knock down	Inhibited hypertrophy Augmented hypertrophy Inhibited hypertrophy	Kmietczyk et al. (28)
Mouse model	Pressure overload	METTL3	Overexpression	Inhibited hypertrophy	Kmietczyk et al. (28)
Isolated adult rat cardiomyocytes	Hypoxia	FTO	Overexpression Knock down	Improved calcium signaling Increased arrhythmic events	Mathiyalagan et al. (26)
Mouse model	Myocardial infarction	FTO	Overexpression	Improved cardiac function	Mathiyalagan et al. (26)
Isolated neonatal mouse cardiomyocytes	Hypoxia	METTL3 ALKBH5	Knock down Overexpression	Enhanced autophagy Inhibited apoptosis	Song et al. (27)
Mouse model	Ischemia	METTL3	Knock down	Enhanced autophagy	Song et al. (27)
Mouse model	Pressure overload	FTO	Knock down	Increased heart failure severity	Berulava et al. (29)
Isolated neonatal rat Cardiomyocytes	Leptin	FTO	Knock down	Inhibited hypertrophy	Gan et al. (33)