Emerging roles of the RNA modifications N6-methyladenosine and adenosine-to-inosine in cardiovascular diseases

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Cardiovascular diseases lead the mortality and morbidity disease metrics worldwide. A multitude of chemical base modifications in ribonucleic acids (RNAs) have been linked with key events of cardiovascular diseases and metabolic disorders. Named either RNA epigenetics or epitranscriptomics, the post-transcriptional RNA modifications, their regulatory pathways, components, and downstream effects substantially contribute to the ways our genetic code is interpreted. Here we review the accumulated discoveries to date regarding the roles of the two most common epitranscriptomic modifications, N6-methyl-adenosine (m6A) and adenosine-to-inosine (A-to-I) editing, in cardiovascular disease.

INTRODUCTION

Cardiovascular diseases (CVDs) cause more than one-third of all deaths worldwide. Almost half of the 18.6 million people that die annually to CVDs are due to ischemic heart disease (IHD), making it the leading single cause of death.1,2 Altogether, a total of 523 million people suffer from these diseases—including 197 million patients with IHD—and their disease burden is manifested as an annual loss of 393 million disability-adjusted life years.3 In the United States, this translates into an annual expense of $352 billion in direct health care costs and lost productivity.3 In the European Union, this cost is approximated as $255 billion.4 Moreover, further contributing factors to the snowballing effect of CVDs are due to ischemic heart disease (IHD), making it the leading single cause of death.1,2 Altogether, a total of 523 million people suffer from these diseases—including 197 million patients with IHD—and their disease burden is manifested as an annual loss of 393 million disability-adjusted life years.2 In the United States, this translates into an annual expense of $352 billion in direct health care costs and lost productivity.3 In the European Union, this cost is approximated as $255 billion.4 Moreover, further contributing factors to the snowballing effect of CVDs are due to ischemic heart disease (IHD), making it the leading single cause of death.1,2 Altogether, a total of 523 million people suffer from these diseases—including 197 million patients with IHD—and their disease burden is manifested as an annual loss of 393 million disability-adjusted life years.2 In the United States, this translates into an annual expense of $352 billion in direct health care costs and lost productivity.3 In the European Union, this cost is approximated as $255 billion.4 Moreover, further contributing factors to the snowballing effect of CVDs are due to ischemic heart disease (IHD), making it the leading single cause of death.1,2 Altogether, a total of 523 million people suffer from these diseases—including 197 million patients with IHD—and their disease burden is manifested as an annual loss of 393 million disability-adjusted life years.2 In the United States, this translates into an annual expense of $352 billion in direct health care costs and lost productivity.3 In the European Union, this cost is approximated as $255 billion.4

Factors such as smoking, hypertension, high cholesterol, obesity, systemic inflammation, and genetics all contribute to the development of CVDs. Nevertheless, the causative factor triggering ACVD development has not yet been identified.5,19 Hypotheses on the etiology of atherosclerosis include, for example, infectious agents,19 as well as gut-microbiota-produced circulating metabolites,21 such as trimethylamine-N-oxide22 and phenylacetylglutamine.23

Ribonucleic acids (RNAs) constitute a critical upstream hub for cellular response control at the intersection of our genetic code and its translation. RNA is subject to multiple levels of processing, including both canonical and alternative splicing,24 tailing,25 and biochemical modifications.26-27 All of these processes are not only...
critical for governing RNA function, cellular homeostasis, and physiological responses but, when dysregulated, they also lead and contribute to the development of disease.

**Epitranscriptomics and the common internal RNA adenosine modifications: m^6^A and A-to-I**

In the 1940s, Conrad Waddington introduced dynamic chemical modifications to nucleic acids, initially recognized in deoxyribonucleic acid (DNA) as epigenetic alterations. However, nitrogen-5^-methylated cytosine was first discovered in 1925 in a living organism as an integral part of tuberculinic acid, a toxic noncanonical nucleic acid produced by *Mycobacterium tuberculosis*. Compared with DNA modifications, the field of research on post-transcriptional biochemical modifications of RNA bases—were obtained decades later in the 1960s and 1970s. First, methionine-dependent methylation of pre-ribosome RNA was identified to be mandatory for its functional maturation in the HeLa cancer cell line. Multiple different types of methylations in messenger RNAs (mRNAs) were first observed in the Novikoff hepatoma cell line. Thereafter, due to methodological limitations, epitranscriptomic research stagnated considerably. Only the methodological breakthroughs of the last decade, first the antibody-based enrichment of methylated RNA prior to sequencing (mERIP-seq), followed by both enzyme-based identifications and recently base-calling algorithms coupled with third-generation direct sequencing methodologies, have made the accurate characterization of some of these epitranscriptomic modifications increasingly feasible. Over 170 post-transcriptional modifications have been identified in nearly all RNA species. However, while numerous RNA decorations have been identified, only a few have been assigned a functional role so far.

Of these, the nitrogen-6-methyl-adenosine (m^6^A) and adenosine-to-inosine (A-to-I) RNA modification and editing, respectively, are the most common and most intensively studied. m^6^A has been shown to favor a consensus sequence DR(A/m^6^A)CH, (D = A,G, or U, R = A or G, and H = A, C or U). On average, three such sites are found in each mammalian mRNA molecule. A-to-I editing primarily occurs in the primate-specific ~300-nucleotide-long *Alu* sequences when such repeats align and pair after transcription to form double-stranded RNA (dsRNA) structures. *Alu* sequences constitute 10% of the human genome and are enriched to gene-rich regions of the genome. The abundance and effects of these modifications are governed by designated enzyme families acting either as writers, erasers, or readers, and are summarized in Figure 1.

Writers of m^6^A to mRNA, methyltransferases, include both methyltransferase 16, N^6^-methyladenosine (METTL16), and the major writer complex that involves methyltransferase 3, N^6^-adenosine-methyltransferase complex catalytic subunit (METTL3), alongside its catalytically inactive methyltransferase 14, N^6^-adenosine-methyltransferase (METTL14) subunit, WT1-associated protein (WTAP),
as well as their interacting partners such as vir-like m^6^A methyltransferase-associated (VIRMA) protein, zinc-finger CCHC-type containing 13 (ZC3H13) protein, E3 ubiquitin-protein ligase hakaí (HAKAI), and RNA-binding motif protein 15 (RBMI5). More-over, a heterodimeric complex of methyltransferase 5, N^6^-adenosine (METTL5) and tRNA methyltransferase activator subunit 11-2 (TRMT112) write m^6^A specifically on 18S ribosomal RNAs (rRNAs). During the methylation process, S-adenosyl methionine (SAM) acts as a methyl donor and converts to S-adenosylhomocys-teine (SAH). To date, three m^6^A erasers, demethylases, have been identified: widespread RNA-acting FTO alpha-ketoglutarate dependent dioxygenase (FTO) and testes-enriched alkB homolog 5, RNA demethylase (ALKBH5), as well as tRNA-targeting alkB homolog 3, alpha-ketoglutarate dependent dioxygenase (ALKBH3). FTO has also been described as a major eraser of N^6^,2'-O-dimethyladenosine (m^6^Am) nucleotide and thus regulator of small nuclear RNA processing. On the other hand, ALKBH3 also demethylates N^4^-methyladenosines in both mRNAs and transfer RNAs (tRNAs). ALKBH5 is currently understood as an m^6^A-dedicated eraser principally localizing to nuclear speckles. All these erasers depend on both alpha-ketoglutarate and molecular oxygen as co-substrates and Fe^2+^ as a cofactor. The readers of m^6^A, crucial for mediating its downstream effects, fall into three major categories: direct binders to the m^6^A, indirect binders to the m^6^A-dependently altered RNA secondary structures, and binders that are speciﬁcally repelled from their binding sites in RNA following m^6^A deposition (Figure 1). The two m^6^A reader families that contain an m^6^A-binding YT521-B homology (YTH) domain; the YTH N^6^-methyladenosine RNA-binding proteins 1, 2, and 3 (YTHDF1, YTHDF2, and YTHDF3, respectively); and YTH domain containing 1 and 2 (YTHDC1 and YTHDC2, respectively) constitute a major set of investigated direct readers. These also include proline-rich (IGF2BP1–3); and fragile X mental retardation protein (FMRP). Last, the lin-28 homolog A (LIN28A), EWS RNA-binding protein 1 (EWSR1), and G3BP stress granule assembly factor 1 (G3BPl) have been described to be repelled from their RNA-binding site following m^6^A methylation.

In vertebrates, A-to-I editing is carried out by three families of deaminases acting on dsRNA: ADAR (adenosine deaminase RNA speciﬁc) family in all tissues, ADAD (adenosine deaminase domain-containing) family principally in testes or brain and ADAT (tRNA adenosine deaminase) family solely targeting tRNAs. While no cofactors for these writers have been identified, inositol hexakisphosphate has been shown to complex within the enzymatic core of adenosine deaminase RNA-specific B1 (ADAR2) and thus to be imperative for its (as well as proper editing function of ADAT1 [adenosine deaminase, tRNA speciﬁc 1]).

While the ADAD family contains two members, ADAD1 (adenosine deaminase domain containing 1) and ADAD2 (adenosine deaminase domain containing 2), the ADAR family consists of three members: ADAR1 (adenosine deaminase RNA speciﬁc), ADAR2, and ADAR3 (adenosine deaminase RNA speciﬁc B2 [inactive]). Only ADAR1 and ADAR2 proteins have catalytic activity. ADAR1 gene is transcribed from two start sites to produce two N-terminally distinct isoforms, a longer and interferon (INF)-inducible ADAR1 p150 and a shorter constitutively expressed ADAR1 p110 isoform. ADAR2 mRNA can undergo extensive alternative splicing in a tissue-specific manner. All ADARs can directly bind dsRNA. For effective deamination, ADAR1 and ADAR2 undergo homodimerization. However, ADAR3 cannot homodimerize, which has been postulated as a reason for its lack of A-to-I editing activity. ADAR2 is predominantly localized to the nucleus, but the ADAR1 isoforms exhibit speciﬁcally regulated nucleocytoplasmic shuttling.

Figure 1. Depiction of the contributors responsible for A-to-I editing and m^6^A modification, respective downstream effectors, and the key effects on RNA biology

*Inositol hexakisphosphate (cofactor). **While ENDOV has been recently suggested to protect inosine-bound transcripts from degradation in vivo, it acts to target them for cleavage in vitro (see section “atherosclerosis”). While red-colored molecules act as non-catalytic subunits. The abbreviations are listed within the text.

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No enzymes converting inosine back to adenosine have been described. However, human antigen R (HuR), or ELAV-like RNA-binding protein 1 (ELAVL1), inosine-dependently binds RNA, and endonuclease V (ENDOV) has been reported to cleave specifically at highly inosine-modified A/U sequences functioning thus as readers or effectors.

Current literature assigns diverse functions to m6A ranging from regulation of RNA secondary structures, stability, translation efficiency, compartmentalization, and degradation to regulation of proliferation, motility, paracrine signaling, phenotype, and cell fate decisions. In addition, m6A RNA has been implicated as a critical contributor to numerous pathologies, including cancer, immunological and metabolic diseases, as well as CVDs. Indeed, m6A has emerged as a tissue- and context-specific hub that mediates cellular stress responses, as recently reviewed. Also, A-to-I modifications participate in a multitude of RNA-related processes, including RNA stability, secondary structure, and accessibility modifications, exon and intron editing, and both microRNA (miRNA) maturation and subsequent target specifications. The formed inosines are capable of altering the RNA secondary structure by disrupting the Watson-Crick base pairing to unwind the dsRNAs and form more immune-tolerable single-stranded RNAs (ssRNAs). Indeed, ADAR1 deficiency has been linked with accumulation of intracellular dsRNAs, activation of interferon production, and various auto-inflammatory diseases. A-to-I editing has also proved essential for the maintenance of hematopoiesis and has been linked with regulation of innate immune responses, development of cancer, and maintenance of neurologic functions.

The key observations on the roles of m6A modification and A-to-I editing in the cardiovascular system are listed in Tables S1 and S2, respectively. Table S3 further details the interventional results regarding m6A regulators in CVD models. Finally, Table S4 provides a molecular-level view into the known interactions and pathways involving the epitranscriptomic m6A and A-to-I modifications in cardiovascular disease.

Cardiogenesis and cardiac regeneration
Heart development begins early during organogenesis, and a four-chamber heart is already established at weeks 5–8 of gestation. While in adult mammals the heart grows in size through hypertrophic adaptation and increased cell volume, in cardiogenesis the cardiomyocyte precursors proliferate and increase in number before differentiating into mature cardiac tissue.

A-to-I editing
The global knockouts of either Adar1/–/– or its cytosolic isoform Adar p150/–/– are nonviable due to multiple organ failures and massive global apoptosis dominating especially in the heart. Cardio-myocyte-directed ADAR1 knockout also die because of massive cardiomyocyte apoptosis. On the other hand, ADAR1 p110 has been shown to be redundant for the viability of human embryonal stem cells. Deletion of either the dsRNA sensor, a melanoma differentiation-associated protein 5 (MDA5), or its downstream effector, a mitochondrial antiviral-signaling protein (MAVS), can extend the survival of Adar1/–/– mice to an immediate postpartum period. Moreover, no cardiac abnormalities were reported in the double-knockout Adar1/–/– Mavs/–/– mice. Hence, ADAR1-induced and A-to-I editing-mediated unwinding of dsRNAs seem to act as a cardiomyocyte survival pathway by keeping the dsRNA-triggered INF-MDA5–MAVS–endoplasmic reticulum (ER) stress-axis activation downstream at bay. El Azzouzi et al. demonstrated that knocking down ADAR1 in a cardiomyocyte-specific manner after birth induced a robust unfolded protein response (UPR)-dependent cardiomyocyte apoptosis and ventricular remodeling, which culminated in rapidly deteriorating cardiac contractile function and death. In light of the above findings and considering activation of ER stress response and UPR is central in not only IHD, but also in CVDs in general, the contribution of ADAR1 p150 in controlling the MDA5–MAVS–INF-axis, ER stress, and activation of UPR in the myocardium warrants further investigation.

Unlike ADAR1, ADAR2 appears redundant for cardiogenesis. Adar2/–/– mice selectively retaining A-to-I modifications only in glutamate ionotropic receptor AMPA type subunit 2 (GluA2) mRNA, mandatory for murine embryogenesis and immediate postpartum development, had no alterations in heart morphology, relative weight, blood pressure, or atrial natriuretic peptide expression. Although the functional role of ADAR3 remains to be characterized in cardiogenesis, its expression in the heart greatly diminishes after birth.

Interestingly, ADAR1 expression is upregulated in the regenerating hearts of tailed amphibians, and the protein is translocated from...
the nucleus to the cytoplasm. Moreover, in ADAR1 knockouts, the ability for cardiac regeneration is lost. In human nerve cells in vitro, analogous exportin-5-dependent nucleus-to-cytoplasm translocation of ADAR p110 (a mammalian counterpart for newts’ ADAR1) is controlled through its phosphorylation by MKK6–p38–MSK1/2 kinases. In the cytoplasm, ADAR p110 then acts as a stress-response mediator preserving antiapoptotic mRNAs from Staufen1-mediated degradation by editing their dsRNA segments. As such, the role of MKK6–p38–MSK1/2–ADAR...
p110–Staufen1 merits further investigation as a putative mechanistic regeneration target pathway. In humans, ADAR p110 expression is enriched in the atria, and its expression is increased most in congenital septal defects. ADAR2 also appears to be a tentative target to instigate cardiac regeneration as its overexpression stimulates proliferation and suppresses apoptosis in rat cardiomyocytes. Regarding a putative underlying molecular mechanism, ADAR2-mediated pri-miR-34a editing, which inhibited the formation of mature miR-34a via a yet veiled mechanism, induced an upregulation of its downstream proliferation-related targets Sirtuin1, Cyclin D1, and B-cell leukemia/lymphoma 2 (Bcl2) protein. The negative regulation of Adar2 promoter was suggested to be due to binding of transcription factor CCAAT/enhancer-binding protein β (C/EBPβ). As discussed later, these effects were later recapitulated in a model of myocardial infarction (MI) in vivo.

m6A modification

Akin to A-to-I editing, m6A has been shown to be imperative for embryogenesis. Without the m6A writer METTL3, embryonal and hematopoietic stem cells (HSCs) lose their self-renewal ability and accumulate cytosolic dsRNA (albeit contrasting roles have also been reported). No such similarity between these modifications is seen during cardiogenesis or imminent postnatal growth. Cardiomyocyte-specific METTL3-knockout mice demonstrate no signs of altered cardiac histopathology, hypertrophy, or dysfunction up to 3 months after birth. At 8 months of age, however, they develop dilated, relatively thin-walled hearts (eccentric hypertrophy), cardiac dysfunction, and major lethality, a classic pathophenotype of dilated cardiomyopathy (DCM). Mettl14+/−/C0 mice have also demonstrated with normal cardiac structure and function at 10 weeks of age. Nonetheless, some focused m6A activity appears indispensable for postnatal cardiac development as heart-specific conditional knockout YTHDC1 m6A reader protein has been described to result in premature death of mice at 2–3 months of age due to disrupted m6A-dependent splicing of Titin pre-mRNA, accompanied by destructed sarcolemne organization, DCM, and ultimately HF. On the other hand, cardiogenesis and postnatal development seem to proceed normally in knockout mice lacking YTHDF1, YTHDF2, YTHDF3, ALKBH5, or either global or cardiomyocyte-targeted FTO knockout. In vitro, however, YTHDF1 promotes embryonic stem cell (ESC)-derived cardiomyocyte differentiation, and YTHDF3 preserves their pluripotency via a mechanism that seems unrelated to the established key transcriptional regulation pathway including transcription factors nanog homeobox (NANOG), SRY-box transcription factor 2 (SOX2), and Pou class 5 homeobox 1 (POU5F1). The expression of METTL3 and METTL4, as well as the abundance of m6A in RNAs, are evenly distributed in embryonic hearts, and their expression is increased by the histone deacetylase inhibitors valproic acid and Trichostatin A.

Figure 3. The unveiled molecular interactions involving m6A and A-to-I or respective key regulators in common vasculopathies and non-malignant angiogenesis

The number of blunted arrows for a given pathway can be used as a guide for assessing the overall effect of the pathway. *METTL3 has been described both as proatherogenic and antiatherogenic factor in endothelium subjected to oscillatory shear stress, see later discussion in section “atherosclerosis.” **The direct role of m6A upregulating the respective downstream mRNAs remains putative. The role of m6A and A-to-I editing in atherosclerosis pathophysiology is presented in greater detail in Figures 6 and 7. References are listed within Table S4 according to molecular pathways illustrated here. PM2.5, fine particulate matter, <2.5 μm; SULF2, sulfatase 2.
The robust cardiac regenerative ability observed in rodents diminishes rapidly during the first week after birth. Within the first postpartum week in C57BL/6J mice, the mRNA m6A content has been measured to triple, METTL3 and YTHDF1 to upregulate, and the levels of Igf2bp1, Igf2bp3, Alkbh5, ALKBH5, FTO, and IGF2BP3 to reduce. In friend leukemia virus B (FVB)-background mice, however, myocardial total RNA m6A content has been reported unchanged all the way from embryonic day 14.5 (E14.5) to 12 months of age, with concurrent, and contrary to the above, upregulation of only FTO, which suggests FTO is the main m6A eraser of adult mice myocardium. Similarly, the adult human myocardium-extracted cardiomyocytes express FTO over the other m6A regulators. In rats, the myocardial METTL3 expression and stromal ALKBH5 and FTO expressions decrease during this time, accompanied by a reduction in total RNA m6A content.

Interestingly, the systematic mapping of mRNA m6A methylome in C57BL/6j-background mice myocardium during the first month after birth exhibited 4,961 m6A peaks in mRNAs from 3,062 annotated genes on their first day postpartum (P1) with corresponding numbers at a week (P7) and a month (P28) after birth soaring to 19,389 and 13,201 peaks in 7,404 and 5,721 genes, respectively. While only 0.26% and 0.12% of the original m6A peaks at P1 were conserved at P7 and P28, of the peaks measured at P28, 76.8% were already present at P7. Yang and colleagues characterized methylated m6A-enriched mRNAs and long non-coding RNAs (lncRNAs) from the P0 and P7 rat myocardia and, well in line with reduced METTL3 and total m6A content, up to 1,553 m6A-peaks were identified downregulated (440 downregulated genes), but only 84 upregulated (520 upregulated genes). Overall, the number of m6A peak differences during P1–P7 in rats appear considerably less than those noted in mice myocardium within the same time frame. Taken together, these observations suggest a major, and thus probably coordinately regulated, reorganization of the murine myocardial m6A methylome concurrent with the closure of the cardioregenerative window during the first week after birth. Future investigations might elucidate both the mechanistic and functional implications of such methylome reorganization, as they may provide novel avenues to rewire the heart’s ability to regenerate also in adulthood.

Mechanistically, Han et al. demonstrated that the actions of ALKBH5 and the YTHDF1 reader converge to promote yes-associated protein 1 (YAP1) expression, a downstream nuclear effector of the Hippo signaling pathway stimulating cardiomyocyte proliferation. In detail, while cardiomyocyte-specific ALKBH5 knockouts presented with reduced regenerative ability at P21 after P1 apex resection with concomitant hypertrophy and reduced cardiomyocyte proliferation, overexpression in both P7 and adult mice enhanced regeneration and functional recovery after MI. In Intriguingly, m6A and YTHDF2 are both crucial for mitotic cytokinesis in mice oocytes. Moreover, an YTHDF family orthologue has been described to restrict endocycling in the plant
These are both links to processes opposing polyplody, which is considered a roadblock for the re-entry of adult cardiomyocytes to the cell cycle, a process associated with cardiac regeneration. The ALKBH5–m^6^A–YTHDF1–Hippo–YAP1 pathway, and regulation of Hippo-mediated S-phase kinase associated protein 2 (Skp2), can link these associations with suppression of polyplody, cytokinesis failure, and thus enhanced cardiac regeneration and cardiomyocyte proliferation after ischemia. Interestingly, downregulation of IGF2BP3, another RNA m^6^A-binding reader that also controls the cell cycle regulator MYC proto-oncogene via m^6^A-dependent respective mRNA stabilization, was observed in cardiac transcriptome profiling of regeneration-competent P1 and regeneration-compromised P8 mice, and was linked with modulated innate immune responses. Its overexpression, on the other hand, extended this 1-week cardioregenerative window. The molecular targets responsible for this IGF2BP3-enhanced regenerative ability were not studied.

Like ALKBH5, overexpression of the FTO m^6^A eraser has also been associated with improved myocardial regeneration in mice. Mathiyalagan et al. reported that FTO overexpression could salvage viable myocardium, increase angiogenesis, and preserve cardiac function after MI. They observed a 96-fold hypermethylation of myocardial periostin mRNA, an integrin ligand supporting cell motility and migration. Intriguingly, prior research has implicated periostin not only to act as a regenerative cardiac mitogen but also to upregulate following MI when simultaneously treated with a regeneration-promoting epicardial patch encasing atrial appendage micrografts. However, it has been also suggested to be a profibrotic mediator in ischemic heart. Mechanistically, periostin has further been shown to be regulated upstream by the interleukin-13–Janus kinase–signal transducer and activator of transcription 3 (IL-13–JAK–STAT3) pathway in regenerating neonatal mice hearts. Combined, it can be speculated that m^6^A hypermethylation of periostin mRNA may promote its stability in ischemic myocardium, but this requires experimental verification. Closing the circle back to the FTO, JAK–STAT3 has been demonstrated to induce nuclear FTO upregulation to ultimately promote cardiomyocyte hypertrophy, as later described in more detail.

Silencing of METTL3 in neonatal rat cardiomyocytes blocked their proliferation and altered the stability of several mRNAs. Of these, ankyrin 2, cardiomyopathy associated 5 (Cmya5), F-box protein 32 (Fbxo32), and 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 (Pfkfb2) mRNAs were stabilized, while 24-dehydrocholesterol reductase (Dhcr24), NAC alpha domain containing (Nacad), and solute carrier family 16 member 3 (Slc16a3) mRNAs were destabilized within hours after METTL3 silencing. In line with this, METTL3 overexpression has recently been unveiled to also promote neonatal rat cardiomyocyte proliferation after hypoxia and to ameliorate ischemic myocardial damage in adult rats by promoting pri-miR-17-3p maturation in a m^6^A-DGCR8 microprocessor complex subunit-(DGCR8)-dependent manner.

On the other hand, in mice, global METTL3 knockout has been described to enhance regeneration-related markers and enhance cardiac function after MI via m^6^A-dependently inhibited pri-miR-143-3p maturation. The muscle-specific cardiac miRNA, miR-133a, was found to harbor a complementary motif CCUG for the DR-m^6^A-CH m^6^A consensus sequence within its seed sequence, thus making it exquisitely prone to bind m^6^A-modified mRNAs. The m^6^A-dependent targets for this m^6^A-oriented miRNA include the cardiomyocyte proliferation regulating cell division cycle 42
(Cdc42) mRNA in its three prime untranslated region (3' UTR). An FTO-regulated m6A- and IGF2BP2-dependent increase in miR-133a repression of Cdc42 mRNA was shown to inhibit mouse neonatal cardiomyocyte proliferation. Interestingly, the myocardial expression of miR-133a increases notably at 1 week after birth, at time of closure of the mouse regenerative window. Taken together, while overexpression of m6A erasers has been shown to increase cardiomyocyte proliferation, preserve myocardial function, and promote cardiac regeneration, the role of the METTL3 m6A writer in these processes appear more complex. While METTL3 knockout decreases RNA m6A content and promotes regenerative cardiac healing in mice, similar to eraser overexpression, the role in rats appears to be the opposite. It is clear that these findings stress the need for species-specific considerations, but further efforts to identifying downstream responsive molecular pathways for potential therapeutic intervention to promote cardiac regeneration are also warranted.

**Congenital heart disease**
Developmental heart malformations are found in approximately 0.8% of births. From whole-blood-derived RNA extracts collected from children with congenital heart disease and cyanosis, Borik et al. linked increased A-to-I levels of mediator complex subunit 13 (MED13) mRNA with reduced ADAR2 expression. MED13 is associated with hypertrophy and angiogenesis, and is regulated upstream by miR-208, which is abundantly expressed in the heart. miR-208 has been further described as a promising target for therapeutic inhibition in failing heart and crucial for cardiac expression of GATA-binding protein 4 (GATA4), a well-established transcription factor regulating cardiomyocyte phenotype, cardiogenesis, and regeneration. In fruit flies, loss-of-function mutation of ADAR abates their ability to survive for hours in severe hypoxia due to impaired editing of various central nervous system (CNS)-expressed ion channel mRNAs. Moreover, ADAR2 is repressed during mammalian CNS hypoxia. Combined, increased A-to-I editing of blood MED13 mRNA might represent a coping mechanism for cyanosis in children with congenital heart disease. Further, the repression in ADAR2 expression could offer access to more editing sites for the ADAR1, thus providing a possible explanation for the increased editing in MED13 but concomitantly reduced ADAR2. MED13 mRNA has later been shown to undergo variable transcript site-specific A-to-I editing within the transcript’s Alu repeat in a lymphoblastoid hypoxia cell model. Consistent with the above findings, a markedly reduced expression of ADAR2 (~90%) and up to 8-fold increases in both ADAR1 p150 and p110 isoforms were reported in the blood cells of children suffering from either cyanotic or acyanotic congenital heart disease. Remarkably, based on mRNA expression analyses from samples derived from the Genotype-Tissue Expression (GTEx) project, the same study also measured both ADAR1 p150 and p110 isoforms to be upregulated 2- to 14-fold and ADAR2 to be markedly downregulated (~75%–95%) specifically within human...
hearts in variable congenital heart diseases. The most pronounced up-regulations have been found in different septal defects.132

**Cardiovascular homeostasis**

Regulation of cardiovascular homeostasis is crucial due to its absolute necessity for complex mammalian life. In humans, regulatory tracts from high- and low-pressure chemo- and baroreceptors converge upstream in the medulla to signal through sympathetic nerves and the cardiac plexus to both the heart133 and its vessels.219 The endocrine and paracrine regulation dominantly comprise myocardium-secreted natriuretic peptides,220 renin-angiotensin-aldosterone axis,221 pituitary antidiuretic hormone, and oxytocin,222 as well as catecholamines from adrenal medullae.223 While the sinus node governs autonomous cardiac contractions,224 the cardiac sarcomeres provide further functional contributions by modulating their contractility based on their level of stretching.225 Moreover, arterial flows are autoregulated in several organs, including brain226 and kidneys,227 to ensure stable flow of oxygen and nutrients despite otherwise varying systemic blood pressure.

**m^6^A modification**

The expression levels of m^6^A writers and erasers in heart have been reported to differ across species and according to age.107,122,124,134 However, a study specifically aiming to characterize the murine baseline cardiac distribution of the various m^6^A writers observed a prominent downregulation of METTL3 and METTL14—and abolished METTL16—expressions within adult myocardium compared with the embryonic state.135 Interestingly, single-cell sequencing has unveiled the m^6^A writing complex’s subunit WTAP to be widely expressed within adult human heart with highest enrichment within myocardial endothelium.135 Moreover, the m^6^A eraser FTO seems to hold the highest expression levels of the core m^6^A governing enzymes within both human and murine myocardium.107,122,124 The abundance of m^6^A-methylated RNAs in the human myocardium (14.6%, 1,239 modified transcripts) is less than that in adult mice (24.1%, 3,208 modified transcripts).121,122 Moreover, myocardial m^6^A residues are potently—up to 10-fold—enriched on mRNAs compared with total RNA.107,122 For the sake of perspective, in adult pig livers,128 mouse brain,129 and isolated basal skin progenitor cells,230 corresponding fractions of m^6^A-methylated mRNAs have measured 33% (~1.3 residues/modiﬁed gene, 4,339 modiﬁed transcripts), 53%–83% (~1.8–2.4 residues/gene, 704–1,392 modiﬁed transcripts), and 11,420 modiﬁed transcripts (~13.8 residues/modiﬁed transcript), respectively. These ﬁndings suggest that the activity of myocardial m^6^A erasers dominate over that of the m^6^A writers in the human adult heart. Such postulation is further supported when considering the preceding stoichiometric estimations suggesting each mRNA to carry 1.3 residues per gene. Hence, for cardiovascular m^6^A erasers to have dominantly regulated substrates, their target mRNA transcripts must carry high m^6^A modiﬁcation levels.232

As myocardial m^6^A modiﬁcations correlate poorly with the overall transcript abundance in the physiological state,212 the role of m^6^A readers is emphasized. Indeed, following METTL3 overexpression, there is an overall increase in cardiomyocyte transcriptome m^6^A content, which induces contrasting effects in terms of transcript stability at level of single transcripts. Namely, both decreased (rho guanine nucleotide exchange factor 3 [Arhgef3]) and increased (myosin light chain 2 [Myl2]) mRNA transcript half-lives have been measured.211

Figure 7. Currently known molecular mechanisms involving m^6^A and its key regulators during macrophage inflammation and foam cell formation

Red upward arrows indicate upregulated expression, red horizontal arrows indicate activation, blue downward arrows denote downregulated expression, and blue blunt-end arrows indicate inhibition. Dashed line represents putative relationship. Brown, green, and blue ellipses denote RNAs, proteins, and m^6^A regulators, respectively. Red ellipse denotes phosphorylation. ABCA1, ATP-binding cassette subfamily A member 1; ABCG1, ATP-binding cassette subfamily G member 1; AMPKα, AMP-activated protein kinase α; CXCL10, C-X-C motif chemokine ligand 10; PPAR-γ, peroxisome proliferator-activated receptor γ; SR-A1, scavenger receptor class A member 1; STAT1, signal transducer and activator of transcription 1.
Similarly, there are variable effects on transcript stability at the single-transcript level in response to METTL3 silencing.\(^5\) Hence, a better understanding is warranted regarding the still considerably veiled functions of the m^\(\text{A}\) readers, such as the YTHDF family and the highly expressed IGF2BP2 within baseline myocardium.\(^2\)

**A-to-I editing**

As discussed above, ADAR1 is imperative for adult cardiac homeostasis, as conditional ADAR1 knockout induces 60% mortality within 3 weeks after knockout induction due to severe cardiac dysfunction with both ER stress and UPR activation as partial underlying mechanisms to the phenotype.\(^3\) Both ADAR p110 and ADAR2 are enriched in the atria.\(^2\) In a comprehensive comparison panel of tissue expressions, ADAR p110 was measured high in the nervous system and ADAR p150 dominated in vascular tissues, including aorta, and coronary as well as tibial arteries.\(^2\) ADAR2 is enriched in arterial tissues\(^3\) and its expression is reduced in various congenital cardiac malformations.\(^2\) In Adar2/−/− mice myocardia, rescued from embryonic lethality via introduction of a pre-edited GluA2 mRNA,\(^2\) multiple heart-related miRNAs were downregulated, but ADARI expression was not induced.\(^2\) The most repressed miRNAs were miR-29b, miR-451b, and miR-451a, leading to increased transcription of genes including collagen type I alpha 2 chain (Col1a2) and insulin-like growth factor 1 (Igf-1).\(^3\) Moreover, based on the decreased A-to-I editing rate of myocardial filamin B, the authors hypothesized filamin B editing to play a still-hidden function in cardiovascular system,\(^2\) similarly as filamin A (FLNA) has been unveiled in hypertension.\(^2\) ADAR2 has been measured with identical expression levels in ex vivo extracted cardiac fibroblasts and cardiomyocytes.\(^2\)

**Hypertension**

Hypertension, or sustainedly elevated blood pressure, either triggers or associates with multiple cardiovascular disease processes, such as atherosclerosis,\(^3\) cardiac hypertrophy,\(^2\) coronary microvascular dysfunction,\(^2\) IHD, MI, stroke, and HF,\(^2\) as well as kidney disease and failure.\(^2\) It is considered the leading cardiovascular disease to cause premature deaths.\(^2\) While hypertension represents a prototypic multifactorial disease with multiple risk factors and varying etiologies, essential hypertension, where no specific etiology is identified, comprises 90% of cases and has been defined as a vascular pandemic due to its estimated staggering worldwide prevalence of 1.39 billion individuals.\(^2\)

**m^\(\text{A}\) modification**

Emerging evidence from human functional genome-wide association studies suggest that m^\(\text{A}\)-related single-nucleotide polymorphisms (m^\(\text{A}\)-SNPs) are associated with elevated blood pressure.\(^2\) These are linked to blood mononuclear cells' expression of hypertension-associated molecules, including zinc-finger protein 589 (ZNF589), \(\beta\)-1-adrenergic receptor, and Golgi SNAP receptor complex member 2 (GOSR2).\(^2\) ZNF589 is a member of Krüppel-associated box domain zinc-finger family of epigenetic regulators known to maintain pluripotency in HSCs,\(^2\) and \(\beta\)-adrenergic \(\beta\)-1-receptor is an independent factor in predicting the treatment outcome for hypertension with \(\beta\)-blockers.\(^2\) The hypertension-associated m^\(\text{A}\)-SNP (Lys67Arg) in the GOSR2 gene is the same as previously associated with the disease.\(^2\) However, experimental approaches are imperative to properly evaluate whether a functional role exists for these target gene m^\(\text{A}\)-SNPs in hypertension.

As an additional link between epitranscriptomics and hypertension, an SNP-variant of FTO has been associated with obesity and elevated systemic blood pressure.\(^2\) The contribution of FTO to vascular tone was hypothesized to be governed by two specific hypothalamic nuclei,\(^2\) which are known to substantially express FTO.\(^2\) However, a more pertinent and peripheral mechanism of action for FTO in hypertension has recently been identified. Conditional endothelium-targeted knockout of FTO during continuous lipid-diet-induced obesity, vascular dysfunction, and hypertension was found to be protective against hypertensive phenotypes via a novel FTO-mediated pathway controlling myogenic tone.\(^2\) Specifically, the loss of FTO upregulated endothelial prostaglandin D\(_2\) (PGD\(_2\)) production via overexpression of its main synthase, lipocalin-type prostaglandin D synthase (L-PGDS), in resistance arteries, and thus alleviated specifically obesity-induced vascular dysfunction and hypertension but did not alter the baseline blood pressure.\(^2\) It is of translational and therapeutic interest that human artery specimens from obese individuals have been reported to overexpress FTO, and its pharmacological inhibition with either rhein or FB23-2 ex vivo also exerted favorable increases in both prostaglandin D\(_2\) production and myogenic tone.\(^2\) In addition, considering that the upstream regulatory pathway responsible for the noted FTO upregulation in endothelium remains veiled, it is interesting to combine a notion that leptin, a major adipocyte-secreted systemic adipokine, has been shown to upregulate FTO in cardiomyocytes.\(^2\) In contrast to its beneficial role in ischemic HF (discussed later), upregulated FTO has been described as detrimental in hyperlipidemia-induced cardiomyopathy.\(^2\) Hence, investigations assessing the role of leptin possibly also regulating endothelial and cardiomyocyte FTO expression in obesity-related hypertension and cardiomyopathy might reveal an unrecognized mechanism within their development.

Last, in pericytes of spontaneously hypertensive rats, the overall m^\(\text{A}\) methylene has been reported to be hypomethylated, which not only suggests either increased m^\(\text{A}\) eraser or decreased writer activity but also underlines the putative role of also other vascular cell types within hypertension development from an epitranscriptomic point of view.\(^2\) Taken together, although reports regarding m^\(\text{A}\) in hypertension remain limited, it is evident that targeted investigations to promote our understanding of m^\(\text{A}\) in hypertension control are needed.

**A-to-I editing**

Interestingly, hypoxic A-to-I editing of miR-27a-3p, which has been established to regulate endothelial GOSR2 mRNA expression,\(^2\) has been shown to induce a major shift on its target gene.\(^2\) In aortas from hypertensive patients and mice, major ADAR2-mediated A-to-I editing events were identified in the vasculature in the actin crosslinking protein Filin mRNA.\(^2\) FLNA A-to-I editing is scarce.
in human fetal hearts (3%) and increases considerably in adulthood (15%). Reduced FLNA mRNA editing, as found in human postmortem aortic-arterial samples, strongly correlated with left ventricular hypertrophy, a strong indicator of significant hypertension during life. Moreover, when Flna mRNA was rendered uneditable by deletion of its 228-bp intronic region, transgenic mice demonstrated increased perivascular fibrosis, diastolic blood pressure, and left ventricular hypertrophy that finally progressed to cardiac dysfunction. In hemizygotic Flnato/− mice, vascular smooth muscle cell (VSMC)-restricted and tamoxifen-induced Flna knockout (smFlna−/−) led to a drop in basal blood pressure due to impaired calcium influx and mechanotransduction. However, global ADAR2 knockout mice have been reported to have unaltered blood pressure profiles.

**Pulmonary hypertension**

Hypertension of the pulmonary circulation is estimated to affect 1% of the global population. Increased pulmonary pressure greatly increases the workload of the right ventricle, which is prone to develop irreversible dilatation and failure, cor pulmonale, which is associated with up to 60% mortality when acute and unstable. Histopathologically, pulmonary hypertension is hallmarked by overt proliferation of VSMCs with consequent muscularization of the pulmonary artery walls. Current drugs fall short in both tackling its underlying pathophysiology and managing its disabling symptoms.

**m6A modification**

While m6A has emerged as a regulator and potential therapeutic target in pulmonary hypertension, excluding an indirectly-relevant report observing ADAR1 to promote VSMCs proliferation and neointima formation—no reports currently exist describing A-to-I editing in pulmonary hypertension. In a hypoxic pulmonary hypertension rat model, Su et al. identified several m6A-modified circular RNAs (circRNAs) affecting circRNA–miRNA–mRNA interactions. Hyperproliferation of rat pulmonary artery smooth muscle cells (PASMCs) was associated with increased METTL3 expression. Increased m6A on phosphatase and tensin homolog (Pten) mRNA led to reduced PTEN expression in a YTHDF2-dependent manner involving the phosphoinositide 3-kinase–AKT serine/threonine kinase 1 (PI3K–Akt) pathway. Moreover, YTHDF1 is upregulated alongside increased m6A contents in hypertensive pulmonary arteries, which were shown to promote hyperproliferation of human PASMCs in vitro as well as pulmonary hypertension development in vivo by increasing m6A-dependently translation of glutathione peroxidase 4 (Gpx4) mRNA and subsequent GPX4 expression. Further, administration of an ferroptosis inhibitor, ferrostatin-1, ameliorated pulmonary hypertension in vivo. Moreover, WTAP has also been implicated in VSMCs hyperproliferation, a key process in pulmonary hypertension by promoting artery wall muscularization. Namely, Panax notoginseng saponin was shown to inhibit VSMC hyperproliferation via upregulating WTAP and m6A.

Histopathologically, while the pulmonary artery-isolated rat PASMCs upregulate METTL3 writer in hypoxia, and the m6A erasers FTO and ALKBH5 are downregulated, m6A writer complex subunits METTL14 and WTAP remain unaltered. On the other hand, no alterations at a level of mRNA in either Mettl4, Wtap, Fto, or Alkbh5 were observed in hypoxic and hypertensive rat pulmonary arteries in vivo. Uptregulation of METTL13 and YTHDF1, and downregulation of FTO and ALKBH5, have been reported in both murine and human adult hypertension-tormented pulmonary arteries and parenchyma. No changes were observed in the expression of the majority of other m6A regulators, including METTL14, VIRMA, RBM15, YTHDF2-3, YTHDC1-2, or IGF2BP1-3. Contrary to the above findings, lung tissue of rat pups with hypoxic pulmonary hypertension showed downregulated levels of m6A residues in RNAs, and decreased expression of METTL3, METTL14, FTO, and ALKBH5.

Together, the above differences delineate age-, tissue-, and cell-specific alterations of m6A regulators in pulmonary hypertension, thus highlighting the need for more cell-type-specific investigations. These investigations could include pulmonary endothelial cells (which seem to be enriched with YTHDF1 in human idiopathic pulmonary hypertension and fibrosis), fibroblasts, resident leukocytes, and pulmonary cells of the lung parenchyma. A recent study by Zhou et al. elegantly unveiled a cell-specific function for an epigenetic regulator SET domain containing 2, histone lysine methyltransferase (SETD2), in hypoxic pulmonary hypertension with its VSMC-targeted knockout as a pulmonary hypertension-promoting regulator and positive upstream regulator of METTL14 in vivo.

Altogether, as the m6A erasers are consistently downregulated within various pulmonary hypertension tissue specimens, and METTL3 knockdown effectively abrogates pathology development, reducing overall m6A content could constitute an avenue for therapeutic benefit.

**Cardiac hypertrophy and failure**

Adult differentiated cardiomyocytes react to increased workload by increasing their size and the number of sarcomeres for better contractility. Over time with, for example, increased ischemic myocardial damage and loss of cells, such hypertrophic compensation for the reduced functionality eventually fails. Decompensated hypertrophy is characterized by interstitial fibrosis, cardiomyocyte apoptosis, inadequate angiogenesis, increased ROS production, mitochondrial dysfunction, and activation of fetal gene expression programmes. This perilous sequence of events often culminates
in HF. The ensuing cardiac dysfunction is often divided into HF with either reduced (systolic dysfunction) or preserved (diastolic dysfunction) ejection fraction (HFrEF and HfPEF, respectively).

**m6A modification**

A rapidly accumulating and prominent body of evidence indicates that epitranscriptomics, and especially m6A, influence not only the initiation of hypertrophy but also progression toward dysfunction and ultimately HF.

**Cardiac hypertrophy.** While transcript hypomethylation has been shown to predominate in pressure-overloaded hypertrophic murine hearts, the number of transcripts with overall changes in m6A modifications measures greater than the amount of differentially expressed transcripts, suggesting notable functional role for m6A regulating machinery in hypertrophy. However, the functional role of METTL3 writer in hypertrophy does not appear to be straightforward. An initial study by Kmietczyk et al. revealed that at the transitory point from the acute phase of adaptation to the early hypertrophic remodeling two days after pressure-overload induction, the expression of METTL3 and the m6A content of several hundred mRNAs were downregulated. In concert, when METTL3 was overexpressed, the hypertrophic response to pressure overload was attenuated. However, pressure-overload-induced hypertrophy has also been associated with increased cardiomyocyte total RNA m6A content in vitro. Here, METTL3 overexpression in vivo, with a different mouse strain and expression method, was demonstrated to act as a spontaneous activator of hypertrophy with no external triggers, but not to affect the hypertrophic adaptation in response to pressure overload. Interestingly, spontaneous hypertrophy also occurs in skeletal muscles following METTL3 overexpression suggesting conserved mechanisms. As the obvious cause(s) responsible for the noted discrepancy within the myocardium remain unknown, validation of the findings with parallel identification of various operant downstream mechanisms can be expected to ultimately shed light on the matter.

The identified molecular mechanisms involving METTL3 in hypertrophy are complex. First, Gao et al. identified and named a novel piwi-interacting RNA, greatly overexpressed in response to cardiac hypertrophy, as cardiac-hypertrophy-associated piwi-interacting RNA (CHAPIR) and reported it to suppress METTL3 expression to ultimately promote development of pathological hypertrophy. Hence, METTL3-mediated m6A methylation was proposed to be cardioprotective against pathologic growth. In finer detail, pressure-overload-induced hypertrophy was associated with increased complexing of CHAPIR with piwi-like RNA-mediated gene silencing 4 (PIWIL4), which subsequently suppressed METTL3 via direct binding, decreased poly(ADP-ribose) polymerase family member 10 (Parp10) mRNA m6A methylation, and consequently upregulated PARP10 protein via relieved YTHDF2-dependent degradation. Further downstream, increased PARP10 promoted mono-ADP-ribosylation of glycogen synthase kinase-3 β (GSK3β), which ultimately resulted in nuclear accumulation of the transcription factor nuclear factor of activated T cells 4 (NFATC4) and transcription induction of hypertrophy-related genes. However, in another experimental study, with yet another stimulus for murine hypertrophy induction via long-lasting subcutaneous infusion of angiotensin II (AngII), Lu et al. pinpointed METTL3 as a potent prohypertrophic downstream player. The authors showed that the deubiquitinating enzyme ubiquitin-specific peptidase 12 (USP12) is upregulated in hypertrophy, stabilizes E1A-binding protein p300 (p300), and enables it to upregulate METTL3. Furthermore, as insights from skeletal muscles also suggest METTL3 to drive spontaneous hypertrophy via an m6A-induced YTHDF2-dependent degradation of activin A receptor type 2A (Acvr2a) mRNA, consequently blocking a muscle-conserved anti-hypertrophic myosin (an ACVR2A ligand) signaling pathway, it is tempting to speculate that such epitranscriptomic control also operates within myocardium. Indeed, ACVR2A inhibition appears therapeutic after MI by (1) promoting early-stage compensatory hypertrophy (concentric hypertrophy) via activated Akt signaling, (2) reducing myocardial fibrosis, and (3) inhibiting dilative late-stage pathologic cardiac remodeling (eccentric hypertrophy).

Aiming to delve further into the methodological differences to pinpoint putative mechanisms for the observed discrepant roles of METTL3 in hypertrophy, the prohypertrophic association for METTL3 arises from studies that used robustly cardiomyocyte-targeted overexpression methods and distinct murine strains from those observing beneficial effects, including opposed pathological hypertrophy, preserved contractility, and post-MI angiogenesis. Additional studies addressing the cell-type-specific nature of the findings in various myocardial cell lineages in hypertrophy are warranted.

In addition, the direct downstream effectors of m6A, the m6A readers, are important as their altered regulation might considerably affect the way METTL3-mediated m6A modification is interpreted by the cells. Albeit recently challenged, the major scheme of YTHDF m6A reader family functions denote YTHDF2 as a repressor of m6A-methylated mRNAs, YTHDF1 a stabilizer of m6A-bound transcripts, and YTHDF3 to act in both directions. Such divergent functions provide a functional basis for a conjecture that these readers might be differently regulated within different hypertrophy models, and thereby observed distinct phenotypes. As an indirect support for such speculation are notions that the YTHDF2-dependent Parp10 mRNA degradation appears protective from pathological hypertrophy, and YTHDF2 has also been denoted with similar protective role in mice and specified in mice primary cardiomyocytes to operate via m6A-dependent Msh7 mRNA decay. In contrast, YTHDF2 was recently revealed to promote rat cardiomyocyte hypertrophy with IncRNA MIAT (MI-associated transcript) acting as a direct positive upstream regulator of YTHDF2-mediated m6A-dependent degradation of carnitine palmitoyltransferase 1A (Cpt-1a) mRNA downregulating subsequently CPT-1a protein, a rate-limiting enzyme in mitochondrial fatty acid oxidation related to PPARx signaling. Such divergent functions for a single YTHDF paralog during qualitatively varied modeling species and conditions add another layer of regulation to...
be considered. Furthermore, as upregulation of both Ythdf2 and YTHDF3 in hypertrophy have yet been reported, not to mention the almost totally veiled role of the litany of other established m6A readers (Figure 1). Indeed, the antihypertrophic effects of miR-133a, targeting effectively m6A-methylated RNAs via its complementary m6A-motif in its seed sequence, has been reported to depend on IGFB2BP2-complexing with the m6A-methylated target transcripts.264 As IGFB2BP2 is a major myocardial paralog of the IGF2BP m6A reader family263 with established upstream regulators IncRNA Aibrn (antisense of IGF2R non-protein coding RNA) in cardiomyocytes265 and high-mobility group AT-hook 2 (HMGA2) protein in skeletal myoblasts266 controlling migration, apoptosis, and proliferation of these cells, targeted investigations toward this m6A reader may also yield some clarification. Last, the role of cardiac hypertrophy-promoting mitogen-activated protein kinase/extracellular regulated MAP kinase (MAPK/ERK) pathway267 also warrants attention, as it was recently shown to positively regulate m6A methylation through phosphorylation-dependent stabilization of the METTL3 writer complex.268 Maslinic acid, a pentacyclic triterpenoid known to inhibit the ERK pathway activation, has recently been unveiled to protect against pressure-overload cardiac hypertrophy via an as-yet unclear mechanism of METTL3 downregulation.268

FTO m6A eraser has also been observed with contrasting but tissue- and cell-type specific functions in hypertrophy. The first report assessing FTO in hypertrophy by Gan et al. pinpointed upregulated FTO in hypertrophic cardiomyocytes treated with leptin, a pro-satiety sensing FTO in hypertrophy by Gan et al. pinpointed upregulated m6A-enriched transcripts, of which only coronin 6, a transcript encoding an actin filament-binding protein,270 emerged as a conserved m6A-modified transcript at a specific sequence locus within its 3’ UTR,271 a known critical RNA regulatory hub.272 Intriguingly, while the function of coronin 6 has not yet been studied in the heart, its protein levels were shown to correlate with those of METTL3 and to be downregulated in hypertrophic cardiomyocytes.273

Last, to identify conserved epitranscriptomic pathways in hypertrophy, Hinger et al. utilized a rat-to-human cross-species comparison approach from myocardium samples of human non-ischemic hypertrophy against that of isolated rat hypertrophied cardiomyocytes. Intriguingly, they found a set of 38 mRNAs with conserved m6A enrichment.274 Of these, five contained conserved m6A sequence loci, and only repressor element silencing transcription factor 1 (Rest1) and splicing factor 3b subunit 4 (Sf3b4) mRNAs were modified at their CDS. Moreover, the baseline comparison of non-hypertrophic human myocardium against rat cardiomyocytes revealed 11 m6A-enriched transcripts, of which only coronin 6, a transcript encoding an actin filament-binding protein,270 emerged as a conserved m6A-modified transcript at a specific sequence locus within its 3’ UTR, a known critical RNA regulatory hub.272 Intriguingly, while the function of coronin 6 has not yet been studied in the heart, its protein levels were shown to correlate with those of METTL3 and to be downregulated in hypertrophic cardiomyocytes.273

Ischemic and hypertrophic cardiomyopathy. Akin to hypertrophy, the roles of post-transcriptional regulation in hypertrophy in both murine HF models and human ischemic HF and DCM specimens are highlighted as the number of differentially m6A-methylated mRNA transcripts seem to outweigh up to 5- to 7-fold the differentially expressed genes.121,122 Further, mice-to-human cross-species-conserved m6A-altered transcripts in HF models have been associated with regulation of calcium fluxes, cardiac contraction, and VSMC differentiation.122

Experimental studies targeting FTO expression suggest it to be cardioprotective against development of HF and fibrosis.107,121,122 While FTO expression has been described as either repressed107,116,131 or unaltered121,122 in HFpEF, it has been reported to be upregulated in HFrEF.131 Based on a combination of measurements from hypoxic cardiomyocytes, ischemic myocardium, and clinical HFpEF samples, such activity has been suggested, at least partially, to relate to demethylation of sarcoplasmic/ER Ca2+ ATPase 2a (Serca2a) mRNA m6A, resulting in increases in the amount of SERCA2A protein and improved Ca2+ signaling.124 Such findings link the m6A-mediated
regulation of mRNA translation and respective protein production to cardiomyocyte contraction kinetics and more generally with Ca\(^{2+}\) dynamics in HF.\(^\text{107}\) According to lessons from neurons, FTO can also demethylate Ca\(^{2+}/\text{calmodulin-dependent protein kinase II (CaMKII)}\) mRNAs, a key mediator of cardiomyocyte Ca\(^{2+}\)-dependent contraction,\(^\text{275}\) to increase its expression.\(^\text{276}\) In addition, decreased m\(^6\)A methylation of both mouse and human Calmodulin 1 mRNAs (a core member of the CaMKII pathway) lead to its reduced protein expression in the failing myocardium.\(^\text{122}\) On the other hand, hypermethylation of the high-conductance intracellular calcium channel Ryanodine receptor 2 (Ryr2) and RYR2 mRNAs has been observed in mice post MI and human ischemic HF/EF myocardial specimens, respectively. These modifications may thus also contribute to disturbances in intracellular calcium signaling during ischemia and proneness for arrhythmias, which is ameliorated with FTO overexpression in hypoxic cardiomyocytes in vitro.\(^\text{107}\) Finally, FTO has recently been proposed to antagonize the development of pressure-overload cardiac dysfunction via duplex mechanism converging to promote glycolysis.\(^\text{175}\) Namely, FTO was shown to upregulate phosphoglycerate mutase 2 (PGAM2) in cardiomyocytes, a key enzyme in glycolysis, via m\(^6\)A hypomethylation of Pgam2 mRNA, and promote AKT phosphorylation, which led to enhanced insulin-responsive glucose transporter type 4 (Glut4) gene transcription, GLUT4 expression, and glucose intake.\(^\text{175}\)

Despite varying ALKBH5 expressions in HF/EF,\(^\text{107,121,122,131,151}\) its overexpression has also been shown to be cardioprotective against the development of ischemic HF.\(^\text{54}\) Taken together with the above notions also for FTO, akin to cardiac regeneration, upregulation of FTO and ALKBH5 emerges as a putative therapeutic handle to antagonize HF development and progression. However, mechanistic insights remain limited.

METTL3 levels have been observed to be repressed in both experimental HFpEF\(^\text{151}\) and pressure-overload hypertrophic HFrEF,\(^\text{122}\) but overexpressed\(^\text{131}\) or unaltered in clinical samples of ischemic HF\(^\text{107}\) or DCM.\(^\text{121,122}\) In preclinical models, knockdown of METTL3 has been shown to reduce fibrosis,\(^\text{60,121}\) preserve cardiac function,\(^\text{60,140}\) and enhance both autophagy\(^\text{134}\) and regeneration-associated markers.\(^\text{140}\) In concert, METTL3 overexpression has been shown to drive progressive eccentric remodeling, ventricular ballooning, and ultimately systolic dysfunction.\(^\text{120}\) Hence, the observed downregulation of METTL3 in murine HF models may act as an active, but insufficient, compensation mechanism. However, the measured both unaltered and upregulated METTL3 in many small sets of human HF samples\(^\text{107,121,122,131}\) highlight the need to keep in mind the probable species-specific differences.

Dominant hypomethylation of the m\(^6\)A-methylomes in both experimental and human HF/EF have been reported.\(^\text{122}\) At the same time, the still-m\(^6\)A-enriched transcripts were positively correlated with polysome occupancy and enhanced translation, an interesting finding not recapitated in the baseline myocardium.\(^\text{122}\) Hence, it can be speculated that the downstream m\(^6\)A reader milieu undergoes notable reorganization within the failing myocardium with as-yet veiled functional consequences.

To date, only the YTHDF2 m\(^6\)A readers have had their protein expression evaluated in failing myocardium in a targeted fashion. Namely, while human failing dilative cardiomyopathy samples upregulate YTHDF2 protein,\(^\text{122}\) the Ythdf2, alongside Ythdf1, Ythdf3, and Ythdc1, mRNA levels have been measured unaltered in experimental models of HF/EF and human DCM.\(^\text{122,131}\) Moreover, YTHDF2 overexpression has been shown to be cardioprotective in pressure-overload failing myocardium.\(^\text{125}\) As such, the dominating hypomethylation in failing murine and human myocardium\(^\text{122}\) may be a consequence of an active compensation mechanism where aberrantly m\(^6\)A-modified transcripts are degraded by YTHDF2 to enable effective positive selection of a smaller subset of cardioprotective m\(^6\)A-methylated mRNAs for recruitment to polysomes and enhanced translation by other m\(^6\)A readers, such as YTHDF1.

Multiple mechanisms, most probably in a synergistic fashion, tend to promote m\(^6\)A in mRNAs in failing myocardium. Indeed, as discussed later in future perspectives, hypoxic metabolism in general may hamper m\(^6\)A eraser function, but FTO and ALKBH5 eraser levels have also been measured to downregulate in ischemic myocardium,\(^\text{107,131,135}\) and their overexpression—as well as METTL3 knockout—has proved beneficial against the development of HF.\(^\text{140}\) Furthermore, the relationship between YTHDF1 protein and Ythdf1 mRNA levels might be complex within the failing myocardium.\(^\text{122}\) For example, the post-MI cardioprotection of ALKBH5 against HF development seems to be conveyed by hypomethylation-dependent stabilization of Ythdf1 mRNA, thus upregulating YTHDF1 protein without altering its transcription.\(^\text{24}\) Moreover, recent evidence suggests most m\(^6\)A to be non-functional enzymatic noise, also in myocardium.\(^\text{277}\) An in vivo HF model with YTHDF1 overexpression, in conjunction with YTHDF2 knockout, and vice versa, come with power to address such speculations.

Despite myocardial YTHDF3-targeted experimental studies remaining to be published, an intersection with HF exists, as bioinformatic reanalysis of published protein expression datasets has revealed YTHDF3 to be downregulated in human ischemic failing myocardium.\(^\text{133}\) Interestingly, YTHDF3 seems to promote translation of m\(^6\)A transcripts common also for YTHDF1 via recruitment to polysomes, but to also perform a contrasting role for other transcripts.\(^\text{278}\) Intriguingly, YTHDF3 has thus been suggested as a modulatory pivot for the effects of YTHDF1 and other m\(^6\)A binders.\(^\text{278,279}\) Furthermore, YTHDF3 has been suggested to suppress YTHDF1 in ESC-derived differentiating cardiomyocytes in vitro with an as-yet veiled mechanism.\(^\text{160}\) Finally, lessons from the fruit fly suggest that its single YTHDF orthologue binds Fmr1, an orthologue of the mammalian m\(^6\)A reader FMRP, and consequently inhibit its translation.\(^\text{280}\) FMRP also associates to polysomes and negatively regulates bound transcript translation.\(^\text{281,282}\) As FMRP has protective effects against inflammatory cardiomyocyte injury\(^\text{283}\) and counteracts myocardial mitochondrial proton leak,\(^\text{284}\) as well as regulating several key
processes against development of cardiac dysfunction, namely RNA splicing and export, FMRP, as a relatively unexplored m6A reader, should be investigated in the failing heart.

Dilated cardiomyopathy. Various causes ranging from toxins and infections to hereditary mutations can disrupt myocardial architecture and develop a pathophenotype of DCM, which is hallmarked by outward enlarged and thin-walled, often poorly contracting, and ultimately failing ventricles. While the myocardial m6A content in clinical DCM samples has been reported to be increased, the expression levels of the major writers and the FTO eraser remain unaltered. However, yet another m6A reader, YTHDC1, has been assigned a key cardioprotective role against DCM development by controlling alternative splicing in mice. Indeed, expression of Titin, a giant myofilament protein that serves as a molecular spring during cardiomyocyte contractions and encoded by a colossal 364 exon-containing Titin gene, was revealed to rely on the m6A reader YTHDC1 for the proper splicing of its m6A modified pre-mRNA. While the m6A-dependent and YTHDC1-guided Titin pre-mRNA splicing produced a shorter and more rigid Titin isoform, N2B, cardiomyocyte-targeted conditional YTHDC1 knockout led to expression dominance of longer and less stiff N2BA isoform manifesting with DCM phenotype and ultimately HF. Considering that Titin gene mutations, which disrupt its proper maturation, underly nearly every fourth case of congenital DCM when the causative mutation can be identified, these findings appear to be of potential therapeutic interest. The N2BA isoform has also been reported to increase at the expense of the stiffer N2B isoform in human end-stage DCM. In sum, this discovery warrants evaluation of YTHDC1 overexpression appearing therapeutic through suppression of nucleotide-binding oligomerization domain-like receptor (NLR) family pyrin domain containing 3 (NLPR3)-mediated cardiomyocyte pyroptosis, which was firmly linked with a m6A-dependent and YTHDF2-mediated degradation of IncRNA terminal differentiation-induced non-coding RNA (TINCR).

As cardiac insulin signaling converges in translocation of GLUT4 receptor to the cardiomyocyte plasma membrane, and its disturbance is a key etiologic factor in diabetic cardiomyopathy, it is worthwhile to reiterate here the notion that FTO protects the murine heart from pressure-overload-induced dysfunction via Akt-mediated GLUT4 upregulation. Hence, the therapeutic role of FTO in diabetic cardiomyopathy, perhaps via regulation of GLUT4 expression, warrants targeted attention. In sum, although metabolic disease causes alterations in the myocardial m6A epitranscriptomic landscape and its regulatory networks, further specific characterizations are required to unleash the therapeutic and biomarker potential of the epitranscriptomic modifications.

A-to-I editing

Cardiac hypertrophy. Reports directly investigating A-to-I editing in hypertrophy remain scarce. While ADAR1 protein levels have been reported to promptly decrease following murine induction of pressure overload and hypertrophy, the Adar1 mRNA levels remain unaltered until the decompensated phase of hypertrophy with HF. Moreover, conditional cardiomyocyte-specific ADAR1 knockout results in hypertrophy and interstitial fibrosis. On the other hand, after ADAR2 knockout, Altai et al. identified myocardial down-regulation of the let-7 miRNA family, known regulators of cardiac hypertrophy, as well as reduced levels of hypertrophy and fibrosis-associated miR-29b. However, ADAR2 has also been reported to be unrelated to the size regulation of unstressed cardiomyocytes when either silenced or overexpressed in vitro. While ADAR2 is reported to upregulate in milder exercise-induced physiological hypertrophy in vivo, the consequent functional assessments corroborated the findings from the cell culture.

Last, miR-1, an abundant and hypertrophy-limiting miRNA in the heart, has been shown to act as an ADAR repressor. As oxidative epitranscriptomic modification of miR-1 at its seed
sequence position 7 guanosine (\(7^\circ\)G-miR-1) changes miR-1 function and provides it with prohypertrophic properties.\(^{302}\) Further studies are required to discover links between A-to-I editing and miRNAs in cardiac hypertrophy. Notably, such functions for miRNA editing have been demonstrated for angiogenesis, as described later in a dedicated section for angiogenesis.

**Heart failure.** A-to-I editing appears to be critical in HF pathophysiology as forced cardiomyocyte ADAR1 knockout during pressure overload accelerated cardiac dysfunction and adverse dilatation, and resulted in massive lethality in an UPR-dependent manner.\(^{130}\) Furthermore, and speculating an underlying mechanism, as the above seems consistent with the ADAR1’s role to keep innate immune response within developing murine myocardium at bay via MDACS-MAVS–INF–(ER stress)–UPR pathway inhibition (described above in section "cardiogenesis and cardiac regeneration"), it appears interesting that case reports have described several unfortunate children with either congenital MDACS gain-of-function\(^{303}\) or ADAR1 loss-of-function mutations,\(^{156}\) both causing a class I interferonopathy, to develop severe cardiac valve calcifications, HF, and ultimately increased premature lethality.

Moreover, it is intriguing to consider the established role of A-to-I editing in control of angiogenesis—a key process in HF\(^{190,304,305}\)—via editing of miRNAs\(^{65-67}\) jointly with miR-1, which has been implicated in cardiogenesis,\(^{296}\) hypertrophy,\(^{297-299,306}\) cardioprotection,\(^{307}\) and atherosclerosis,\(^{308}\) as it represses ADAR1 non-cardiac tissues\(^{300,301}\) and acts oppositely in favor of hypertrophy when modified by oxidation.\(^{302}\) Moreover, ADAR1 knockout downregulated multiple miRNA within failing myocardium.\(^{130}\) Hence, evaluation of an ADAR1-mediated A-to-I editing in controlling, perhaps via mRNA editing, angiogenic responses in myocardium appears an avenue, when better understood, that could ultimately provide feasible molecular targets to ignite therapeutic revascularization to repair the failing heart.

**Atherosclerosis**

Atherosclerosis, described as the fattening and hardening of arteries, is promoted by such factors as aging, hypertension, obesity, smoking, and renal failure.\(^{19,309,311}\) Mechanistically, endothelial shear stress, dysfunction, and inflammation drive atherosclerosis, the pathophysiology of which is characterized by subendothelial deposition of lipids—especially low-density lipoproteins (LDLs)—to arterial walls. The ensuing endothelial dysfunction and increased permeability promote adherence and translocation of immune and inflammatory cells that engulf the deposited lipids and turn into foam cells.\(^{311}\) The plaques calcify and grow over time, may rupture, and eventually obstruct blood flow causing tissue ischemia and MI when occurring in a coronary artery supplying the myocardium. If the patient survives, such infarction is a major risk factor for malignant arrhythmias as well as further infarctions and development of HF in the future.

Several molecular mechanisms in atherosclerosis and involving m\(^6\)A and A-to-I editing have been discovered recently. A summary of these molecular pathways is presented in Figure 3, and a detailed overview involving these modifications with respect to atherosclerosis pathophysiology is presented in Figure 6. In addition, Figure 7 summarizes currently discovered molecular interactions involving m\(^6\)A during macrophage inflammation and formation of foam cells.

**m\(^6\)A modification**

**Disturbed flow, endothelial dysfunction.** The endothelial stress response is considered as the initial step in the pathogenesis toward clinically manifest atherosclerosis.\(^{312}\) Notably, METTL3-mediated m\(^6\)A RNA deposition has been suggested to have a key role in early atherosclerosis. Namely, oscillatory blood flow was found to increase METTL3 expression in endothelial cells predisposed to oscillatory flow and stress.\(^{32}\) The authors identified METTL3 to function as an upstream activator of nuclear factor kappa B (NF-κB), an enhancer of NLR family pyrin domain containing 1 (NLRP1) protein and repressor of Krüppel-like factor 4 (KLF4). They demonstrated NLRP1 mRNA stabilization (upregulating NLRP1) to occur via m\(^6\)A-YTHDF1 and KLF4 mRNA degradation (downregulating KLF4) to be dependent on m\(^6\)A-YTHDF2 interactions. NF-κB is an inflammatory master regulator and NLRP1 is crucial for inflammatory–some activation in endotheliocytes during atherosclerosis.\(^{313}\) While KLF4 is a key regulator of vascular homeostasis and endotheliocyte function.\(^{314}\) However, a contrasting effect for oscillatory-flow-induced endothelial dysfunction has also been described. With a considerably shorter period of oscillatory stimulus, rather than upregulation, Li et al. described endothelial METTL3 downregulation with consequent epidermal growth factor receptor (EGFR) mRNA m\(^6\)A-hypomethylation-mediated EGFR upregulation, which was linked with promoted atherosclerosis both in vitro and in vivo.\(^{95}\) As advocated further later in section “future perspectives”, these drastically contrasting effects for METTL3 require methodologically standardized validations and further profiling of the m\(^6\)A reader expressions and interactions.

**Endothelial and monocyte inflammation, manifest atherosclerosis.** Increased expression of both METTL3 and METTL14 in endothelial cells activated by tumor necrosis factor alpha (TNF-α) have been associated with enhanced monocyte adherence and enhanced vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) expression.\(^{30}\) Binding to METTL14 activates the forkhead box O1 (FOXO1) transcription factor, leading to increased transcription of VCAM-1 and ICAM-1 mRNAs, also independent of m\(^6\)A.\(^{90}\) METTL3/14-dependent m\(^6\)A methylation of FOXO1 mRNA and subsequent binding by YTHDF1 further increase FOXO1 protein expression. Chen et al. linked the increased METTL14 expression with m\(^6\)A-dependent degradation of vasculo-protective Klotho mRNA\(^{315}\) in dysfunctional endothelium.\(^{101}\) Endothelial cells of human cerebrovascular plaques harbor increased METTL14 expression.\(^{91}\) Zhang et al. described a METTL14-DGCR8-m\(^6\)A-miR-19a axis where METTL14 directly binds DGCR8 and enhances its interaction with pri-miR-19a. The resulting m\(^6\)A-dependent maturation of miR-19a then drives proliferation and the invasive capacities of atherosclerotic endothelial cells.\(^{91}\)
Interestingly, knockdown of cardiomyocyte-secreted miR-19a improves angiogenesis after MI via hypoxia-inducible factor 1α (HIF-1α) in vivo.35 Hence, it can be speculated that a m6A-dependent miR-19a maturation regulates, via HIF-1α, endothelial function within myocardium.37

In addition to the endothelial inflammation and dysfunction, activation of the blood monocytes—especially their pro-inflammatory phenotype conversion to endothelium- and plaque-penetrating atherosclerosis-promoting macrophages—is a critical atherosclerosis-promoting event recently shown to be regulated by the m6A methylation (Figure 7). Increased inflammatory activation of oxidized LDL (oxLDL)-stimulated monocytes was associated with METTL3-dependent hypermethylation and YTHDF2-dependent mRNA degradation of peroxisome proliferator-activated receptor gamma co-activator 1α (PGC-1α) protein, a mitochondrial biogenesis-regulating cofactor.109 While pro-inflammatory ROS production was increased, the production of ATP and oxygen consumption decreased alongside downregulation of electron transport chain proteins cytochrome c (CYCS) and NADH:ubiquinone oxidoreductase subunit C2 (NDUFC2).100 In line with the effect on m6A abundance and the adverse METTL3 upregulation, FTO has recently been assigned an anti-atherosclerotic role via inhibiting foam cell formation by controlling cholesterol efflux transporters and scavenger receptors, and suppressing both mature interleukin-1β (IL-1β) synthesis and secretion.94 The suggested atheroprotective role of FTO was further shown in vivo, but the favorable effect interestingly occurred only in male mice.94

However, in contrast to its role in atherosclerotic endothelium, METTL14 has been associated with anti-atherosclerotic activity. Namely, a bioactive metabolite mono-(2-ethylhexyl) phthalate (MEHP) has been shown to inhibit both mRNA m6A content and METTL14 expression to promote intracellular cholesterol accumulation and foam cell formation in vitro through disrupted cholesterol efflux accountable for an m6A-dependent scavenger receptor class B member 1 (Sr-b1) mRNA downregulation with consequent downregulation of SR-B1 protein.318 Furthermore, Gong et al. recently promoted the putative therapeutic importance of METTL14-mediated modification of miR-654 in human atherosclerosis via regulating cholesterol efflux.91 Specifically, they pointed out a putative pathway consisting of METTL14–m6A–(miR-654-3p)–lncRNA ZNFX1 antisense RNA 1 (ZFAS1)–ADAM metallopeptidase domain 10/ RAB22A, member RAS oncogene family (ADAM10/RAB22A). In brief, such a hypothesis stems from a notion that lncRNA ZFAS1, dependent on miR-654-3p, promotes inflammation and diminishes cholesterol efflux from the atherosclerotic plaques by regulating ADAM10/RAB22A.319 Moreover, ZFAS1 is overexpressed in human atherosclerotic plaques320 and is subject to m6A methylation.321

**A-to-I editing**

ADAR1-dependent editing and increased expression of vascular inflammation-associated cathepsin S (CTSS), an extracellular matrix-cleaving protease,50,322,323 was found by Stellos et al. in patient samples from coronary and carotid atherosclerotic arteries and aortic aneurysms as well as in hypoxic and inflamed (TNF-α and interferon gamma [INF-γ]) endothelial cells. Moreover, based on a finding of reduced CTSS expression in atherosclerotic plaques of minipigs treated with anti-oxLDL antibody,324 its atherosclerotic plaque-stabilizing mechanism was hypothesized to be mediated by ADAR1 A-to-I editing of Ctt mRNA.102 In peripheral blood mononuclear cells (PBMCs), the editing of the lncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) by ADAR1 led to its stabilization and increased expression, which then positively correlated with the level of atherosclerotic disease.103 Further, in TNF-α-activated human umbilical vein endothelial cells (HUVECs), NEAT1 knockdown blunted the mRNA expression of proatherosclerotic chemokines C-C motif chemokine ligand 2 (CCL2), C-X-C motif chemokine ligand 8 (CXCL8), and adhesion molecules ICAM-1 and VCAM-1.105 Together with increased Adar1 mRNA levels, the levels of EndoV mRNA and inosine have been shown to be upregulated in human carotid atherosclerotic plaques.73 Moreover, as both reduced atherosclerotic plaque monocyte infiltration and size were observed in double ApoE−/− EndoV−/− knockout mice, the inhibition of the seemingly proatherosclerotic EndoV might prove a therapeutic strategy.73 Also, as the specific interplay between ADAR1, inosines within various RNAs, and EndoV in atherosclerosis remains elusive, such targeted considerations would be of great interest in light of the above findings and the following notions. In fact, cluster of differentiation 47 (CD47), an important macrophage and anti-atherosclerotic activity,73 was measured as potently downregulated in EndoV−/− macrophages ex vivo, despite harboring no sites for A-to-I editing.73 Hence, it justifiable to postulate RNA editing-independent functions for EndoV in atherosclerosis. Indeed, rather than acting as an inosine-specificendonuclease, as established in vitro,54 EndoV has recently been suggested to preferentially bind RNAs to protect them from degradation in vivo.326

**Atherosclerosis pathophysiology as a multiorgan systemic process**

The emerging epitranscriptomic insight regarding atherosclerosis is justifiably heavily plaque focused. However, contributions of other organs and cells to the progression of the atherosclerotic lesions—alongside the emanated paracrine and endocrine signals from these disease foci—are needed to form a more comprehensive view of epitranscriptomic regulation in atherosclerosis. The organs shown to respond to such systemic signals of atherosclerosis are the liver, adipose tissue, bone marrow, and the lymphoid organs. Increased proliferation of the bone-marrow-residing hematopoietic stem cells327,328 and their splenic invasion329 establishing extramedullary hematopoiesis are such key extravascular processes. These processes then seed into circulation pro-inflammatory monocytes with plaque-invasive and plaque-promoting properties328,329. Such a vicious cycle of atherosclerosis has recently been detailed in terms of causation so that the atherosclerotic process itself increases the proliferation rate of hematopoietic stem cells, thus accelerating the efflorescence of proatherogenic clonal hematopoiesis.328 As accumulating research...
also indicates both m⁶A and A-to-I pathways to play crucial roles in regulating the proliferation and differentiation of hematopoietic stem cells, studies focusing on the role of epitranscriptomics within these systemic aspects of atherosclerosis are warranted.

Myocardial hypoxia, infarction, and fibrosis
Although m⁶A has been associated with hypoxia-reoxygenation (H/R) injury, myocardial hypoxia, ischemia, ischemia-reperfusion (I/R) injury, and post-ischemic fibrosis, reports specifically addressing A-to-I editing in these conditions remain very scarce. Stable expression of ADAR2 has been recently reported, however, both shortly and 3 weeks after MI. Remarkably, ADAR2 overexpression unveiled a phenotype of improved cardiac healing after MI. Namely, abolished functional deterioration, infarct size, fibrosis, and necrosis, as well as cardiomyocyte-specific increase in proliferative markers, were demonstrated. As detailed above (section “cardiogenesis and cardiac regeneration”), the underlying mechanism was suggested to operate via an A-to-I editing-dependent inhibition of pri-miR-34a maturation.

m⁶A modification
Reduced myocardial FTO expression has been reported after MI both in humans and mice, and cardiomyocyte-targeted FTO overexpression in mice has been shown to reduce myocardial ischemic damage. In hypoxic cardiomyocytes, FTO overexpression counteracted dysfunctional intracellular Ca²⁺ oscillations, increased contractility, reduced arrhythmic events, and increased both Serca2 mRNA and protein expression. FTO expression reversed the hypermethylation observed in failing cardiomyocytes. Interestingly, Mathiyalagan et al. also showed that the hypermethylation of Ryr2 mRNA, a major mediator of cardiac sarcoplasmic calcium-induced calcium release that is imperative for proper propagation of electrical impulses, was attenuated by FTO overexpression in infarcted myocardium. However, the subsequent impact on the respective protein levels remained elusive.

In response to AngII-induced activation of cardiomyocytes, reduced expression of circular RNA CUGBP Elav-like family member 1 (circCELF1), also seen in plasma samples of MI patients, was reported to drive the downregulation of FTO. It was mechanistically further revealed that the FTO downregulation led to m⁶A hypermethylation of Dickkopf Wnt signaling pathway inhibitor 2 (DKK2) mRNA, which enhanced its miR-636-mediated degradation and promoted a profibrotic cellular phenotype. The therapeutic effect of this pathway was confirmed by DKK2 and miR-636 antagonist overexpression during experimental MI. To identify novel m⁶A-based post-MI angiogenesis-promoting and fibrosis-restricting therapies, it will be of great interest to assess this FTO-dependent molecular pathway, perhaps with an additional focus on the m⁶A-regulated angiogenic Wnt/β-catenin signaling pathway (see discussion in section “angiogenesis”) in myocardial vasculature. Moreover, finer dissection of the revealed positive upstream regulation of FTO by circCELF1, including, for instance, identifying FTO targeting miRNAs sponged by this circRNA, could offer novel druggable targets.

In line with the observed beneficial effects of FTO for ischemic cardiomyocytes, cardiac fibroblasts, and myocardium, similar to the above discussions on hypertrophy and HF, Shen et al. identified FTO to reduce cardiomyocyte apoptosis after H/R injury via demethylation of a lncRNA myosin heavy-chain-associated RNA transcript (Mhrt). Of further therapeutic interest, Mhrt, alongside another myocardium-specific lncRNA cardiac hypertrophy-associated transcript (CHAST), has earlier been measured hypermethylated in a murine infarcted myocardium and to demethylate after local myocardial FTO silencing yielding a beneficial phenotype. Indeed, Mhrt is a cardiac-specific lncRNA transcribed from the antisense strand of Myh7 gene with a protective role against hypertrophy by sequestering the brahma-related gene-1 (Brg1) mRNA to consequently blunt the prohypertrophic transition from myosin heavy chain 6 (MYH6) to myosin heavy chain 7 (MYH7) expression dominance. Interestingly, triiodothyronine upregulates Mhrt in I/R-injury and has been linked to predict MI and HF. Other m⁶A-modified lncRNAs expressed in ischemic myocardium, such as long-chain non-coding RNA metastasis-related lung adenocarcinoma transcript 1 (MALAT1), have also been suggested as future therapeutic targets for myocardial reperfusion injury.

A functional intersection for the METTL3 writer and ALKBH5 m⁶A eraser was established by Song et al. in cardiomyocyte H/R and I/R injuries. The authors reported increased m⁶A levels and expression of METTL3 to adversely associate with decreased autophagic flux and increased apoptosis. Furthermore, a master regulator of apoptosis, transcription factor EB (TFEB), was shown not only to be regulated by METTL3 but also to regulate METTL3 and ALKBH5 in cardiomyocytes and myocardium after I/R injury. Specifically, a negative-feedback loop with two arms was discovered, where first METTL3-mediated m⁶A-methylation of Tfeb pre-mRNA attracts the indirectly m⁶A-binding heterogeneous nuclear ribonucleoprotein D (HNRNPD, alias AUF1 [ARE/poly(U)-binding/degradation factor 1], measured overexpressed in human failing heart to increase its translation and consequently TFEB protein expression. The consequent binding of TFEB to Alkbh5 gene promoter is enhanced, resulting in enhanced Alkbh5 gene transcription and ALKBH5 protein expression. Second, rather than controlling transcription, TFEB was discovered to destabilize Mettl3 mRNA, thus downregulating its own positive upstream regulator.

METTL3 therefore appears to be a detrimental agent in murine hypoxic cardiomyocytes in vitro and in infarcted myocardium, akin to the earlier discussion for cardiac hypertrophy. These findings support a rationale that METTL3 inhibitors may act as putative therapeutic agents in IHD. Such postulation is strengthened by the findings that (1) METTL3 knockout before mice MI preserves cardiac function and structure afterward, and (2) METTL3 promotes cardiomyocyte pyroptosis and myocardial I/R injury in rats via an m⁶A-dependent DGCR8-mediated pri-miR-143-3p maturation to yield miR-143-3p to finally suppress protein kinase C epsilon type (PRKCE). However, myocardial METTL3 overexpression has also been implicated with cardioprotective ability by (1) inducing
therapeutic myocardial angiogenesis in mice shortly after MI, \(^{56}\) (2) lessening post-MI damage in rats by promoting cardiomyocyte proliferation via stimulated pri-miR-17-3p maturation, \(^{147}\) and (3) its non-catalytic METTL14 subunit protecting mice heart from excessive I/R injury by activating Wnt1/ß-catenin signal pathway via an m\(^6\)A-dependent enhanced Wnt family member 1 (Wnt1) mRNA translation. \(^{143}\) Hence, it can be postulated that the resulting final effect on the cardiac phenotype depends considerably on the relative weights of METTL3 activity within the distinct cardiac cell types, ischemic models, as well as the expression profile of the m\(^6\)A readers and transcribed transcriptome available for methylation at a given time, as discussed above (see section “cardiac hypertrophy and failure”).

Hypoxia-inducible m\(^6\)A deposition by METTL3 was recently described to operate specifically in cardiomyocytes, which could possibly provide an operating rationale for some of the above speculations. \(^{144}\) Namely, a hypoxia-inducible, cardiomyocyte-enriched, and mesoderm-restricted upregulation of a nuclear cap-binding subunit 3 (NCPB3) protein was identified to occupy the 5’ UTRs of 85 distinct mRNAs in hypoxic cardiomyocytes with a striking 87.6% congruency to a previously published hypoxic cardiomyocyte dataset of transcripts with incongruent translation activity to their transcriptomic expression. \(^{146}\) NCPB3 was shown to recruit METTL3, promoting the bound mRNA m\(^6\)A methylation and eukaryotic translation initiation factor 4A2 (eIF4A2) to initiate their translation. \(^{144}\)

Furthermore, ALKBH5 overexpression has also been shown beneficial by enhancing cardiac regeneration and salvage myocardial function after MI in both neonatal and adult mice (see section “cardiogenesis and cardiac regeneration”) via m\(^6\)A demethylation-dependent increase in Ythdf1 translation and consequent YTHDF1-dependent enhanced Yap1 translation to YAP1. \(^{54}\) This YTHDF1-m\(^6\)A-Yap1 interaction was confirmed operative irrespective of ALKBH5 activity when YTHDF1 was overexpressed, suggesting incapability of ALKBH5 to demethylate Yap1. FTO, in addition to its anti-apoptotic effects in H/R-treated cardiomyocytes, \(^{135}\) has also been indicated with an age-dependent waning and consequently propagated ischemic myocardial injury. \(^{138}\) In H/R-injured neonatal cardiomyocytes, FTO overexpression upregulated Yap1 via m\(^6\)A-demethylation-mediated protection of Yap1 mRNA from degradation. \(^{145}\) While the phenotype of these H/R-injured neonatal cardiomyocytes appears analogously therapeutic with ALKBH5 overexpression, \(^{145}\) the distinct epitranscriptomic pathways converging at Yap1 translation suggest FTO selectivity as an m\(^6\)A eraser for Yap1 with simultaneous m\(^6\)A reader milieu that promotes the degradation of m\(^6\)A-methylated Yap1. Hence, it will be of interest to assess the capacity of FTO to demethylate Yhdf1 mRNA and consequently regulate the protein.

On the other hand, WTAP, a METTL3 writer complex subunit, \(^{136}\) has been associated with adverse effects in ischemic myocardium. Namely, an ischemic damage and ER-stress-promoting pathway was identified, where WTAP, via activating transcription factor 4 (Atf4) mRNA m\(^6\)A methylation, upregulates ATF4 and promotes cardiomyocyte injury. \(^{136}\) Therapeutically, WTAP knockout effectively restricted the injuries. \(^{136}\) Moreover, the WTAP overexpression-induced cardiomyocyte ER stress and apoptosis during H/R injury was effectively ameliorated in vitro with administration of 4-phenylbutyric acid (4-PBA), an ER stress inhibitor. \(^{136}\) Interestingly, in global Mettl14\(^{144}\) mice with worsened I/R-injury phenotype compared with controls, WTAP was identified as the only differentially expressed, i.e., upregulated, m\(^6\)A writer subunit. \(^{143}\) Finally, based on bioinformatic reanalysis of up to 108 ischemic, 16 non-ischemic, and 86 idiopathic human myocardium specimens, WTAP was also identified as the most consistently upregulated of the m\(^6\)A governing enzymes. \(^{133}\) Hence, WTAP might be unveiled as a biomarker in human ischemic cardiac pathologies.

**Aortic valve calcification**

Aortic valve calcification is the most common progressing cause of aortic stenosis in the industrialized world. \(^{347}\) In Europe and North America, aortic stenosis is estimated to affect up to 12.4% of the population over 75 years of age with a staggering prevalence of 3.4% for such critical disease that surgical intervention is guideline-mandated. \(^{347}\) Macroscopically, progressing stenosis narrows the valve orifice and drives cardiac hypertrophy. \(^{348}\) Microscopically, the valve calcification is characterized by osteoblast-like phenotype conversion of the valve interstitial cells, ROS production, calcium deposition, and activation of resident valve endotheliocytes as well as leukocyte diapedesis. \(^{349}\)

**m\(^6\)A and A-to-I modifications**

Only two studies have addressed m\(^6\)A and A-to-I modifications in the pathophysiology of aortic valve calcification. In the first, the m\(^6\)A modification was described to control the phenotype conversion of human aortic valve interstitial cells to osteoblast-like cells via METTL3-mediated methylation and consequent YTHDF2-dependent degradation of twist family basic helix-loop-helix (bHLH) transcription factor 1 (TWIST1) mRNA, which ultimately downregulated the protein. \(^{157}\) The other case study has described three children, all with tricuspid aortic valves and biallelic loss-of-function mutations in the ADARI gene, who developed systemic class I interferonopathy with pronounced early-age-onset aortic valve calcification, stenosis, and HF. \(^{136}\)

**Angiogenesis**

Angiogenesis has been heavily implicated in epitranscriptomic control of both m\(^6\)A and A-to-I editing. Especially modifications of miRNAs and their altered targetome have been unveiled as important. An overview of the key results from the field of non-malignant angiogenesis is offered below. For more detailed insight into epitranscriptomic control of neovascularization, the reader is directed toward recent reviews for m\(^6\)A \(^{350}\) and A-to-I editing. \(^{68}\)

**m\(^6\)A modification**

Corneal angiogenesis is inhibited in FTO knockout mice. \(^{58}\) Alike, the tube formation of HUVECs in either unstressed or H\(_2\)O\(_2\)-stressed
conditions shrinks via a putative focal adhesion kinase (FAK)-m6A-YTHDF2 axis.58 Accordingly, FTO overexpression has been associated with enhanced post-ischemic myocardial angiogenesis, albeit with slight methodological evidence (see the next paragraph).107 On the other hand, silencing of the m6A eraser, ALKBH5, promoted angiogenesis in a hindlimb ischemia model.104 The angiogenesis-repressing function of ALKBH5 was associated with increased m6A methylation and stability of Wnt family member 5A (Wnt5A) mRNA in hypoxia-treated cardiac microvascular endothelial cells (CMECs).104 No responsible m6A readers were identified, however. Moreover, as the role of WNT5A in angiogenesis remains controversial,51 it is plausible to speculate the molecular network to be more complex. Increased expression of ALKBH5 has also been reported in HUVECs and human microvascular endothelial cells (HMVEs) after lipopolysaccharide and hypoxia, consistent with results for hypoxic CMECs.104 However, rather than disrupting angiogenesis, upregulated ALKBH5 was shown to sustain it. In detail, ALKBH5 was found to maintain sphingosine kinase 1 (SPHK1) expression by reducing SPHK1 mRNA m6A methylation, and to preserve both endothelial nitric oxide synthase (eNOS) and protein kinase B (PKB), alias AKT, phosphorylation.109 Here, it is worth noting the methodological differences. Indeed, with the above lipopolysaccharide and hypoxia protocol,109 the authors could not replicate the well-established vascular endothelial growth factor (VEGF)-A induction in these cells when singly stimulated by these stressors.352,353 At the same time, such findings also suggest a relatively conserved endothelial hypoxia response to upregulate ALKBH5. Such dynamics appear distinct from the measured downregulation of FTO107 and ALKBH551 in infarcted myocardium.

Regarding the role of m6A writers, overexpression of METTL3 in vitro has been shown to increase angiogenic parameters in both HUVECs and human CMECs (HCMECs) during basal conditions,50 and endothelial progenitors under hypoxia.108 Namely, in HUVECs and HCMECs, METTL3 increased the m6A methylation of let-7e-5p and miR-17-92 clusters, which were subsequently shown to down-regulate antiangiogenic thrombospondin 1 (TSP1).56,354-355 METTL3 overexpression was also reported to increase angiogenesis in experimental models of MI and hindlimb ischemia in vivo.6 As the authors pointed out, with regard to general effects on m6A in RNA, their findings are contradictory to the increased myocardial angiogenesis observed with FTO overexpression following MI.107 However, the robust methodological variation limits the interpretation of the results. Specifically, with the FTO overexpression, angiogenesis was assessed with a single-antibody staining against platelet endothelial cell adhesion molecule (PECAM-1), alias cluster of differentiation 31 (CD31), positive endotheliocytes 4 weeks after MI, a time point at which post-MI healing and angiogenesis have mostly taken place already. Notably, HIF-1α has been pinpointed as a positive upstream regulator of proangiogenic METTL3 expression in hypoxic endothelium in vitro.57 Specifically, METTL3 was identified to mediate its proangiogenic role in a YTHDF1-dependent manner by enhancing the translation of m6A-methylated low-density lipoprotein receptor-related protein 6 (LRP6) and disheveled segment polarity protein 1 (DLX1) mRNAs.57 Both targets, alongside the discussed Wnt5a mRNA, encode proteins that are part of the Wnt signaling pathway, a core regulator pathway of angiogenesis in endotheliocytes.356,357

Interestingly, decreased METTL3106 and WTAP105 expression has been associated with larger diameters of human cerebral arteriovenous malformations. Regarding m6A writer METTL3, Wang et al. pinpointed two putative mechanistic pathways for promoting angiogenesis in vitro. The first pathway involves METTL3-mediated stabilization of deltex E3 ubiquitin ligase 3L (DXT3L) mRNA in an m6A-IGF2BP1/3-dependent manner to enable the respective DXT3L protein to heterodimerize with deltex E3 ubiquitin ligase 1 (DXT1) to form a Notch E3 ubiquitin ligase, which suppresses Notch signaling and further downstream hes-related family bHLH transcription factor with YRPW motif 2 (HEY2).106 In the second suggested mechanism, METTL3 represses the transforming growth factor β (TGF-β) pathway via SMAD (homologs of the Drosophila melanogaster protein ‘mothers against decapentaplegic’ (MAD) and Caenorhabditis elegans ‘small body size’ (SMA)) family member 6 (SMAD6) downregulation and increases phosphorylation of its other members, including SMAD1-3, SMAD5, and SMAD9.106 WTAP deficiency was noted also to increase free WT1 expression, which led to Wnt signaling inhibition and increased degradation of β-catenin.105 WTAP has also been reported to maintain angiogenic desmoplakin (DSP)358-360 expression in endothelial cells in an m6A-IGF2BP1/3-dependent manner.105

A-to-I editing
van der Kwast et al. discovered that miR-487b, previously known to maintain the integrity of hypertensive artery walls and post-ischemic blood flow recovery,361 was increasingly edited from its seed sequence in hindlimb ischemia.356 The edited form, miR-487b-ED, was unveiled to have unique proangiogenic functions and a nearly-completely altered targetome compared with the unedited miR-487b. In addition, four other vasoactive and vascular-cell-expressed miRNAs have been established to be the targets for notable A-to-I editing: miR-376a-3p, miR-376c-3p, miR-381-3p, and miR-411-5p.66,67 These miRNAs were shown to contain inosine edits in their seed sequence at the maturation stage and to respond to hypoxia by increased editing. These targetomes of these edited forms acted to seed sequence at the maturation stage and to respond to hypoxia by increased editing. These targetomes of these edited forms acted to

Arterial aneurysms
Arterial aneurysms represent a set of conditions with variable risk factors and etiologies.362-364 All are characterized by the disruption of the structural and mechanical properties of the arterial wall.365-367 This leads to local ballooning of an artery with concurrent thinning of its wall rendering the artery prone to dissection368 and rupture.369-370 In general, aneurysm ruptures are associated with extremely high mortality rates.
A-to-I editing

Increased expressions of the A-to-I editor ADARI and CTSS mRNAs have been described in human aneurysmatic thoracic aortas. The authors identified the RNA-stabilizing HuR to bind the newly formed inosine. Importantly, CTSS has many matrix remodeling functions and participates in both collagenolysis and elastolysis, which are processes also heavily implicated in aneurysm pathophysiology, and could provide a pharmacologically targetable molecular pathway. Based on the discovery for A-to-I editing to control diastolic blood pressure via Flna mRNA editing, producing an actin crosslinking FLNA, heavily implicated in arterial wall integrity (see also section "hypertension"), further investigations regarding A-to-I editing in also controlling aneurysm pathophysiology are warranted and rational.

m6A modification

Aging has been shown to downregulate METTL3 expression in the aorta. The development and progression of abdominal aortic aneurysm has been suggested to be induced through METTL3-mediated maturation of miR-34a and decreased Sirtuin 1 (Sirt1) mRNA expression. Accordingly, knockdown of METTL3 protects from development of abdominal aortic aneurysm, and this therapeutic effect is inhibitable by either miR-34a silencing or SIRT1 overexpression. Interestingly, a recent study highlighted the SIRT1-melanotin axis in a murine thoracic aortic aneurysm model. The authors noted that melatonin administration prevented thoracic aortic aneurysm formation via acting on SIRT1 in a melatonin-receptor-dependent manner. Yang et al. reported melatonin to inhibit METTL3 expression and m^6^A in ESCs specifically via melatonin receptor 1 (MT1) and further through the MT1–Janus kinase 2 (JAK2)–STAT3–zinc-finger protein 217 (Zfp217) pathway, all members of which are implicated to be regulated by m^6^A in CVDs. Moreover, melanotin has been shown to downregulate VEGF in hypoxic retinas, inhibit hypoxic angiogenesis by repressing the HIF-1α-VEGF-Ros axis, and upregulate HIF-1α targeting miR-3195 and miR-374b, thus downregulating VEGF. These findings are of interest considering the upstream roles of melatonin and MT-1 in inhibiting METTL3, since Vegf mRNA has been shown to be m^6^A modified by METTL3, thus modulating a TEK receptor tyrosine kinase (TEK)–PI3K–VEGF axis via increasing its stability and enhancing angiogenesis. Furthermore, the m^6^A-reader IGF2BP3 has been shown to bind m^6^A in Vegf mRNA, increasing its translation. To summarize, as VEGF inhibition has been shown to prevent aortic aneurysm progression, melatonin might, via Vegf mRNA m^6^A methylation, act on aneurysm development.

Dissected aortas were reported to have decreased KIAA1429 (alias VIRMA) and miR-143-3p levels while ALKBH5 was reported upregulated. KIAA1429 was shown, via its increasing m^6^A methylation effect, to enhance pri-miR143-3p maturation by interacting with the important miRNA molecular processor DCGR8 to consequently downregulate its downstream target gene, responsible for observed phenotypes, DEAD-box helicase 6 (DDX6). On the other hand, ALKBH5 was shown to repress such interaction and thus, contrary to the phenotypes observed in KIAA1429 overexpression, promote aortic dissection, suppress human aortic smooth muscle cell (HASMC) proliferation, and promote apoptosis in human aortic endothelial cells (HAECs).

He et al. demonstrated increased m^6^A content and expressions of YTHDF2 and YTHDF3 in abdominal aortic aneurysm, of which YTHDF3 positively correlated with the aneurysm diameter. Another similar associative study has linked reduced METTL14 expression with higher risk of aneurysm rupture. Like ALKBH5, FTO is also upregulated in dissecting and stable aortic aneurysms. Moreover, increased angiotensin-II-induced FTO levels and gain-of-function methods in VSMCs were shown to mediate pathologic phenotype switching. Mechanistically, the FTO-driven demethylation of Krüppel-like factor 5 (KLF5) mRNA and downregulation of glycogen synthase kinase 3β (GSK3β) signaling, leading in combination to upregulated KLF5 protein, were unveiled as the responsible pathways. Interestingly, the adverse role of KLF5 has also been shown in atherosclerosis as a part of oxLDL–KLF5–miR-29a–F-box and WD repeat domain containing 7 (FBW7) positive feedback loop. Specifically, oxLDL-induced upregulation of KLF5, further accelerated via miR-29a accumulation-mediated and FBW7–repression-dependent reduction of KLF5 ubiquitination, increases VSMC proliferation and progression of atherosclerosis, thus stressing miR-29a suppression as a possible therapeutic strategy. Epitranscriptomically, it is interesting that miR-29a has been shown to undergo m^6^A methylation and to be consequently repressed by the HNRNPA2/B1 m^6^A reader. KLF5 has been implicated as a key hub for vascular-injury-induced proliferative responses of VSMCs, neointima formation, as well as both angiotensin II-induced cardiac hypertrophy and fibrosis response. Combined, targeted hypermethylation of either the KLF5 mRNA or miR-29a might prove therapeutic in conditions where VSMC hyperproliferation holds a central pathophysiologic role, including atherosclerosis, arterial aneurysms, and both pulmonary and systemic hypertension.

FUTURE PERSPECTIVES

Taken together, both the m^6^A and A-to-I modifications have emerged as dominant regulators of CVDs. Some future perspectives are discussed below.

Drugging the m^6^A- and A-to-I-related pathways in cardiovascular diseases

A number of compounds targeting m^6^A writers and erasers have been identified. A summary of these small molecules is provided in Table S5. As many of these well-characterized molecules remain untested in cardiovascular models (excluding the FTO inhibitors FB23-2 and Rhein; see section "hypertension") and, based on the above discussions, the function of the m^6^A regulators varies across tissues, cell types, and diseases (as well as their models), their testing within the cardiovascular field appears to be a promising avenue to extend our evolving understanding. Pharmacological evaluation could reveal insightful sum effects of these regulators; for example, for METTL3 in the context of cardiac hypertrophy, MI, and flow-induced
endothelial dysfunction. Consequently, coupled with accumulating new evidence, and methodological standardization, such insights could help us to detangle these currently complex, even controversial, mechanistic landscapes currently hallmarkied by simultaneous and extensive involvement of METTL3-mediated m6A methylation within multiple—even opposing—molecular pathways. Moreover, as these epitranscriptomic regulators have demonstrated both driving and suppressing roles in some of these pathologies, these compounds may also provide novel therapeutic benefits. For example, as a METTL3 inhibitor is emerging a handle to treat acute leukemia, and while YTHDF2 also appears another such target, testing this METTL3 inhibitor, STM2457, during MI or hypertrophy might produce therapeutic effects. Moreover, as upregulation of FTO and ALKBH5 has most consistently proved to be therapeutic during various models of myocardial ischemia, development of activating compounds for these m6A erasers could hold translational therapeutic value to mend ischemic hearts. Finally, the additional discovery of such compounds for the m6A readers and A-to-I editors is awaited.

**m6A readers as a key to clarify the role of epitranscriptomics in CVD pathologies?**

Many of the numerous readers of m6A remain uncharacterized. Due to the nature of these readers, responding to upstream stimuli to initiate the molecular, cellular, and ultimately systemic responses, it is probable that they will prove to be centrally important in CVD epitranscriptomics. Encouragingly, studies exploring the role of these readers in CVDs are being constantly reported (Figures 3, 4, 5, 6, and 7).

Although the YTHDF family of m6A readers has recently attracted considerable scientific interest, the redundancy of their targets and downstream functions remains a matter of recent controversy. Namely, two research groups, led by Jaffrey et al. and Hanna et al., have recently called into question the scheme where YTHDF1 stabilizes the m6A-bound transcripts, YTHDF2 degrades them, and YTHDF3 can act in both directions. According to this view, the YTHDF family shares a virtually identical set of modified target RNAs and functions in unity to promote their degradation. However, many both m6A- and YTHDF1-dependently stabilized mRNAs, as measured in cardiovascular tissues, seem to be in contradiction with such a scheme stemming from HeLa cells and ESCs. These include NLRP1, FOXO1, Myf2, Atf4, MAGED1, and Wnt5a. In addition, YTHDF2, but neither YTHDF1 nor YTHDF3, has been described to bind m6A-modified Acrv2a mRNA in hypertrophic skeletal muscle. Notably, a recent preprint by Zou et al. has brought up major experimental controversies in the original paper published by Zaccara and Jaffrey. Also, based on revised and additional experiments, YTHDF1 was demonstrated to promote translation of its target mRNAs in HeLa cells and to harbor a low-complexity domain notably distinct from that of YTHDF2, which was found to account for their capacity to form different condensates and to act in a paralog-specific manner.

Elucidation of the upstream control of m6A readers may also help to explain the possible discrepancy between YTHDF family members. For example, post-transcriptional YTHDF2 SUMOylation considerably increases its affinity to m6A, thus stimulating the m6A-bound RNA degradation in cancer cells. Also, the importance of post-transcriptional regulation of METTL3, METTL14, WTAP, and YTHDFs in a paralog-specific manner by phosphorylation has been described in non-cardiovascular systems. Finally, to truly unravel the functions YTHDF paralogs, the importance of paralog-specific expressions according to given tissue, cell, and cell state, or even specific molecular signals, cannot be overemphasized. For example, the knockdown of YTHDF1 or YTHDF3 in ESC-derived cardiomyocytes, contrary to ESCs, does not accumulate m6A in RNAs, and tumor protein 63 (p63) seems to upregulate just YTHDF3 in skin. Hence, it is critical that the functions of YTHDF paralogs are also meticulously examined in the cardiovascular system. Such an approach could also provide clarification to the contrasting findings for METTL3-mediated m6A methylation in controlling the cardiac hypertrophy and oscillatory flow-induced endothelial dysfunction discussed above.

**Revealing the upstream control of ADARs in cardiovascular systems**

While the understanding of the role of A-to-I editing in cardiovascular diseases remains limited in general, overall highlighting the need for future investigations, deciphering its upstream control might provide avenues for novel considerations. For example, miR-1 has been shown to target ADARs by repressing their expression in non-cardiac cells. However, although this miRNA is highly expressed in heart and has an established role in many CVDs or related processes, its role from an epitranscriptomic viewpoint remains to be established in heart.

**Cardiometabolism is a putative modulator of the cardiac m6A methylome**

As oxygen and α-ketoglutarate are needed to erase m6A, and the reaction yields succinate, the metabolic state of the myocardium can be expected to affect its RNA m6A content. Indeed, in hypoxic myocardium, α-ketoglutarate depletes downstream in the Krebs cycle into succinate, while it acts to produce high-energy phosphates as substrituents for the deteriorating oxidative metabolism. In addition, α-ketoglutarate acts upstream in the cycle to produce citrate, thus circumventing the tormenting mitochondrial respiration to produce lipids for energy. While originally described in hypoxic cancer cells, such a process may also alter myocardial epigenomes. Last, as α-ketoglutarate levels are supplemented endogenously in hypoxia only via either glutamine or glutamate deaminiation, it seems consistent that exogenous supplementation of these metabolites protects heart from ischemia.

Hence, epitranscriptomically, it appears congruent that m6A levels rise as α-ketoglutarate depletes in myocardial ischemia. Taken together, as overexpression of m6A erasers protects myocardium from ischemic insults, FTO is upregulated in cardiomyocytes by leptin adipokine and conveys cardioprotection also via stimulation of glucose metabolism, the role of hypoxic cardiometabolism...
in controlling cardiac RNA m6A dynamics, and vice versa, emerges as being worthy of future study.

CONCLUSIONS
An epitranscriptomic era is unfolding in translational RNA biology and medicine. Here, we have reviewed the fast-growing body of evidence available regarding both initial associative and experimental findings to establish a firm link for the two most common epitranscriptomic modifications, m6A and A-to-I, to mirror as well as participate in the onset and development of multiple common cardiovascular diseases. As our current mechanistic understandings can be expected to further crystallize in the future, the potential of modifications also hold potential for biomarker discovery. Prospective controlled observational cohort studies, such as the The Ischemic Heart Disease Epitranscriptomics and Biomarkers (IHD-EPITRAN) study (www.ihd-epitrans.com), help us to shed light into this fascinating development.

SUPPLEMENTAL INFORMATION
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AUTHOR CONTRIBUTIONS
V.S. collected the related papers, drafted and wrote the manuscript, and drew illustrations. E.K. wrote and revised the manuscript and provided supervision. A.V. revised the manuscript. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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