REVIEW

Epitranscriptomic Regulations in the Heart

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Summary

RNA modifications affect key stages of the RNA life cycle, including splicing, export, decay, and translation. Epitranscriptomic regulations therefore significantly influence cellular physiology and pathophysiology. Here, we selected some of the most abundant modifications and reviewed their roles in the heart and in cardiovascular diseases: N^6-methyladenosine (m^6A), N^6,2'-O-dimethyladenosine (m^6Am), N^1-methyladenosine (m^1A), pseudouridine (Ψ), 5-methylcytidine (m^5C), and inosine (I). Dysregulation of epitranscriptomic machinery affecting these modifications vastly changes the cardiac phenotype and is linked with many cardiovascular diseases such as myocardial infarction, cardiomyopathies, or heart failure. Thus, a deeper understanding of these epitranscriptomic changes and their regulatory mechanisms can enhance our knowledge of the molecular underpinnings of prevalent cardiac diseases, potentially paving the way for novel therapeutic strategies.

Keywords

Epitranscriptomics • RNA modifications • Epigenetics • m^6A • RNA • Heart

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Introduction

The original central dogma of molecular biology states that DNA is transcribed into RNA, which is subsequently translated into proteins [1]. However, the whole process is under the control of epigenetic mechanisms. Epigenetic mechanisms involve chemical modifications to the DNA itself, to the proteins that package DNA into chromatin (histones), or to the RNA molecules transcribed from the DNA (Fig. 1). Importantly, the epigenome is responsive to various environmental factors (diet, stress, exposure to toxins, etc.) and can produce heritable phenotypic changes without altering the DNA sequence [2,3].

RNA modifications are specifically known as the epitranscriptome. The research field of epitranscriptomics is rapidly developing. Currently, over 170 chemical RNA modifications are known (common RNA modifications overviewed in Fig. 2) [4]. The largest number of modifications with the widest chemical diversity is present in tRNA; however, various modifications also occur in other RNA types, including mRNA [5]. These modifications may be either irreversible or reversible [6]. Epitranscriptomic regulators can be described according to their function as writers (addition of the epitranscriptomic mark), erasers (removal of the epitranscriptomic mark), and readers (binding to the modified nucleotide). Dynamic regulation of epitranscriptomic modifications can affect key stages of the RNA life cycle, including splicing, export, decay, and translation [7,8].

Remodeling of the cardiac epitranscriptome has been described in several physiological as well as pathological states. This review summarizes the current knowledge and gaps about RNA modifications in cardiac biology and cardiovascular diseases (CVDs). A better understanding of epitranscriptomic regulations in the healthy and diseased heart opens the door for clinically relevant discoveries in the future.

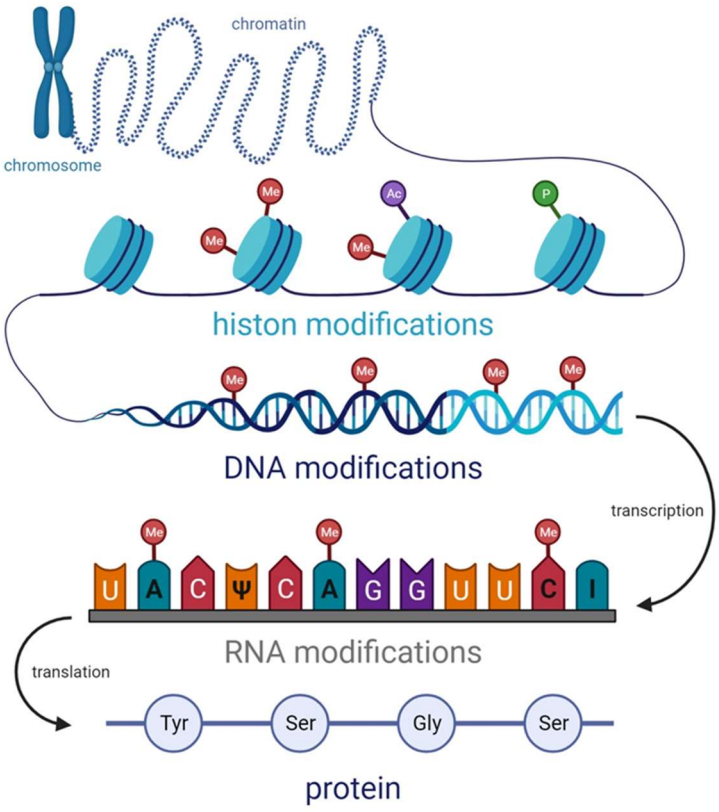

Fig. 1. Basic overview of epigenetic modifications

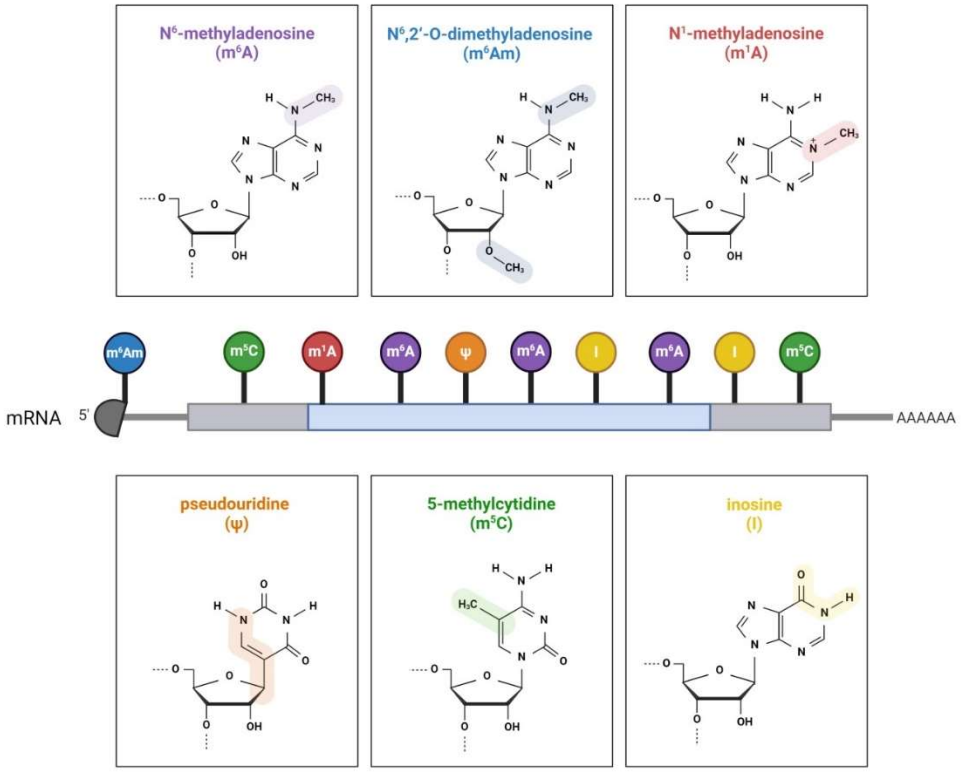

Fig. 2. Common RNA modifications

Common RNA modifications and their role in cardiac physiology

N^6-methyladenosine

N^6-methyladenosine (m^6A) is the most numerous modification in eukaryotic mRNA; however, it also occurs in other RNA types [9-12]. Multicomponent methyltransferase complex (MTC) is responsible for the deposition of the methyl group to adenosine, forming m^6A. The two main regulatory subunits of the MTC are methyltransferase-like 3 (METTL3) and methyltransferase-like 14 (METTL14). The catalytic function of the MTC is carried by METTL3 while METTL14 facilitates RNA binding [13,14]. The removal of the methyl group is mediated by two main demethylases. AlkB homolog 5 (ALKBH5) is the primary m^6A eraser [15]. Fat mass and obesity-associated protein (FTO) is not an m^6A-specific demethylase, however, m^6A is the preferable target of FTO in the nucleus [16-18]. There are many described m^6A readers. The most characterized include YTH domain-containing family proteins 1-3 (YTHDF1-3) and YTH domain-containing proteins 1-2 (YTHDC1-2). While readers YTHDF1-3 mediate primarily mRNA degradation, YTHDC1 regulates mRNA splicing and YTHDC2 promotes translation [19-25].

The heart is affected by m^6A already during its ontogenetic development as m^6A machinery regulates cardiomyocyte growth, proliferation, and differentiation [26-29]. Children born with a loss-of-function mutation in the *FTO* gene (m^6A demethylase) exhibited heart defects (ventricular septal defect, atrioventricular defect, patent ductus arteriosus), hypertrophic cardiomyopathy and died before 3 years of age [30]. Moreover, various gene variants of m^6A regulators were linked with CVDs, including myocardial infarction, acute coronary syndrome, increased risk of rejection in heart transplant patients, and sudden cardiac death [31-37]. It has been reported that m^6A also controls cardiac hypertrophy [38-40]. Dorn et al. [41] suggested that enhanced m^6A RNA methylation results in compensated cardiac hypertrophy, whereas diminished m^6A drives eccentric cardiomyocyte remodeling and dysfunction. Changes in m^6A methylation and dysregulation of m^6A machinery can contribute to the progression of heart failure [42-47]. Altered cardiac m^6A patterns were detected also in diabetic cardiomyopathy with distinct dysregulation of m^6A machinery in the two types of diabetes [48-50]. The heterogeneous role of m^6A modification in CVDs has been reviewed in several recent publications [51-60].

Altered m^6A levels in different CVDs might also serve as useful biomarkers. For instance, it has been described that patients with coronary artery disease (CAD) had significantly lower urine m^6A levels compared to healthy individuals [61].

Since cardiac m^6A machinery is dysregulated under many pathophysiological conditions, targeting m^6A modifiers can also induce cardioprotection. Several studies showed that demethylases FTO and ALKBH5 can protect cardiomyocytes against detrimental effects, such as treatment with cardiotoxic compounds or hypoxia/reoxygenation injury [43,62-69]. On the contrary, loss of METTL3 or METTL14 can alleviate myocardial injury and promote heart regeneration [70,71]. Thus, improving our knowledge of the m^6A regulations in the heart may lead to novel cardioprotective strategies using specific pharmacological activators or inhibitors targeting m^6A modifiers.

N^6,2'-O-dimethyladenosine

N^6,2'-O-dimethyladenosine (m^6Am) is formed by N^6-methylation of 2'-O-methyladenosine (Am). It has been described only in mRNA and snRNA [50,72]. This modification is present at the first transcribed nucleotide and forms the extended cap structure in at least 30-40% of all vertebrate mRNA [73,74]. Moreover, m^6Am is also present at the internal sites of snRNAs [17]. The formation of m^6Am in the cap is mediated by phosphorylated CTD interacting factor 1 (PCIF1), while methyl-transferase-like 4 (METTL4) is responsible for internal m^6Am formation [75-78]. The demethylation of m^6Am takes place mainly in the cytosol where it is mediated by FTO, the same eraser that targets m^6A in the nucleus [17,18,79,80]. There are currently no m^6Am readers mediating the biological functions of this modification described, but it is known that the presence of m^6Am in the cap structure markedly enhances mRNA stability (in mRNA cap) and splicing (in snRNA cap) [79,81].

The function of m^6Am modification in the heart is mostly unknown. There are several problems associated with m^6Am research: 1) many m^6A detection methods do not distinguish between m^6A and m^6Am; 2) FTO is not a specific eraser because it demethylates also m^6A and m^1A; 3) METTL4 can also catalyze 6mA methylation. Thus, the potential effect of m^6Am on cardiac function could be masked as m^6A in many studies [72]. Besides the non-specific demethylase FTO covered in the previous chapter, not much is known about the role of m^6Am and its

regulators in the heart. Publicly available RNA-seq datasets generated from human left ventricles of failing and non-failing hearts reported some degree of regulation of *METTL4* (down-regulation) and *PCIF1* (up-regulation) [72]. Besides that, we recently found that m^6Am writers were regulated also in cardioprotective interventions. METTL4 was decreased in the hearts of rats adapted to chronic hypoxia and PCIF1 was increased in the hearts of rats subjected to fasting [69,72].

N^1-methyladenosine

N^1-methyladenosine (m^1A) is found mainly in tRNA and rRNA, but less numerously also in mRNA [82-85]. The writer proteins responsible for m^1A methylation include tRNA methyltransferase 6 (TRMT6), TRMT61A, TRMT61B, TRMT10C or ribosomal RNA-processing protein 8 (RRP8; also known as NML) [86-90]. Demethylation of m^1A is catalyzed by erasers ALKBH1, and ALKBH3 [85,91-93]. Moreover, FTO (m^6A and m^6Am eraser) also works as a demethylase of m^1A in tRNA [17]. The m^1A modification affects the structure and stability of tRNA and rRNA and its presence in mRNA regulates translation [85,86,94-96].

So far, no association between m^1A and CVDs has been found [97]. Analysis of methylated nucleosides in urine that revealed altered m^6A levels in CAD patients did not find any changes in the case of m^1A [61].

Pseudouridine

Pseudouridine (Ψ), the C5-glycoside isomer of uridine (U), is the first discovered and overall the most prevalent RNA modification that has been identified in almost all known RNA types [98-100]. The conversion of U to Ψ is mediated by the diverse pseudouridine synthase (PUS) family [101]. So far, 13 members of PUSs have been described in eukaryotes [100]. The human homologs of PUSs include PUS1, PUS3, PUS7, PUS10, PUSL1, PUSL7, TRUB1-2 (TruB pseudouridine synthase 1-2), RPUSD1-4 (RNA pseudouridine synthase D1-4), and DKC1 (dyskerin pseudouridine synthase 1) [102]. The formation of Ψ is irreversible (unlike the aforementioned modifications) [103]. The only known Ψ reader is a yeast RNA helicase Prp5 interacting with snRNA [104,105]. The molecular functions of Ψ include stabilization of RNA conformations and destabilization of interactions with RNA-binding proteins; the most well-characterized function of Ψ in mRNA is the promotion of a stop codon read-through [100,106].

Plasma and urine levels of Ψ were linked to CVDs [107]. Patients with heart failure exhibited higher plasma concentrations of Ψ than healthy controls and this modification was suggested as a suitable biomarker for heart failure diagnosis [108-110]. Tetralogy of Fallot, the most common cyanotic congenital heart defect, is associated with decreased Ψ levels in ventricular myocardial tissues, which is under the control of small Cajal body-specific RNAs [111,112].

5-methylcytidine

5-methylcytidine (m^5C) is an abundant RNA modification present in a wide variety of RNA types. The writers responsible for the installation of m^5C in humans are NOL1/NOP2/SUN domain proteins 1-7 (NSUN1-7) and DNA methyltransferase homolog DNMT2 [113,114]. Ten-eleven translocation proteins 1-3 (TET1-3) and ALKBH1 are known as m^5C erasers. TET-mediated oxidation results in a formation of 5-hydroxymethylcytidine (hm^5C), while ALKBH1 is responsible for the oxidation of m^5C in mitochondrial tRNA generating 5-formylcytidine (f^5C) [115,116]. The readers of m^5C include Aly/REF export factor (ALYREF), which influences nuclear-cytoplasmic shuttling [117], and Y-box-binding protein 1 (YBX1), which preserves the stability of its target mRNA by recruiting ELAVL1 [118]. This modification is an important regulator of RNA export, ribosome assembly, translation, and RNA stability [113,119,120].

In mammals, m^5C modification occurs more frequently in the myocardium and skeletal muscle compared to other organs. The enrichment of m^5C is especially present in mitochondrial-related genes, suggesting a particularly important function of m^5C in the high-energy demanding myocardium [121]. Indeed, specific inactivation of the methyltransferase NSUN4 in the heart caused cardiomyopathy with mitochondrial dysfunction [122]. Deficiency of methyltransferase *Dnmt2* gene in mice resulted in cardiac hypertrophy [123]. RNA binding protein and known m^5C reader YBX1 was also identified as a cardiac hypertrophy regulator [124,125]. NSUN2 was found to increase *Nrf2* expression by promoting m^5C methylation of its mRNA and enhancing its antioxidant stress effect, which attenuates doxorubicin-induced myocardial damage [126].

RNA editing

RNA editing includes nucleoside modifications such as adenosine deamination to inosine (A-to-I editing) or cytosine deamination to uridine (C-to-U editing), as

well as insertion and deletion of nucleotides [127,128]. Deamination of A to I is irreversible and it is performed by enzymes belonging to the adenosine deaminase acting on RNA (ADAR) family, which is represented by three ADAR orthologs (ADAR1-3) in mammals. ADAR1 and ADAR2 are widely expressed, while ADAR3 was detected only in the brain [129,130]. C-to-U editing is not as common as A-to-I editing [131]. The deamination of C to U is performed by a multiple-protein editosome, which includes the catalytic subunit apolipoprotein B mRNA editing enzyme catalytic subunit 1 (APOBEC1) and an RNA-binding protein APOBEC1 complementation factor (A1CF) [132]. RNA editing in protein-coding regions of mRNAs can result in the expression of functionally altered proteins while editing in microRNA (miRNA) precursors leads to reduced expression or altered function of mature miRNAs [133].

ADAR1 is an essential enzyme for normal embryonic cardiac growth and development [134]. Cardiomyocyte-specific deletion of *Adar1* in adult mice caused severe ventricular remodeling and spontaneous cardiac dysfunction associated with a significant rise in lethality [135]. ADAR1 was also shown to prevent autoinflammatory processes in the heart [136]. A-to-I RNA editing has been significantly increased among children with cyanotic congenital heart disease compared to acyanotic controls [137]. On the contrary, reduction of A-to-I editing and decreased levels of ADAR2 have been described in the failing human heart [138]. Strong down-regulation of ADAR2 and up-regulation of ADAR1 expression was observed in blood samples of patients with congenital heart disease. The decrease in ADAR2 levels was in line with its down-regulation in ventricular tissues of dilated cardiomyopathy patients. Thus, it has been suggested that ADAR2 activity might play a critical role in preventing cardiovascular disorders [139]. Indeed, Wu et al. [140] described that ADAR2 was up-regulated in the heart during exercise and that this enzyme protects the heart against myocardial infarction as well as doxorubicin-induced cardiotoxicity, supporting the hypothesis of the beneficial effect of ADAR2 on the heart. So far, RNA editing therapeutics have not been established for the treatment of CVDs, however, it is a prospective therapeutic approach that could be implemented in the near future [141].

Conclusion

CVDs remain the leading cause of death worldwide. The search for appropriate cardioprotective strategies is therefore of crucial importance. The significant role of epitranscriptomics in cellular physiology and pathophysiology has been already accepted by the scientific community in the past few years. However, the exact role of complex epitranscriptomic regulations in the heart and CVDs is still far from being understood. It is becoming clear that RNA modifications and their regulators play a vital role in the ontogenetic development of the heart. Many CVDs, such as myocardial infarction, cardiomyopathies, or heart failure, have been also associated with dysregulated epitranscriptomic machinery (Fig. 3). Most importantly, targeting the enzymes responsible for regulating the RNA modifications affected by these diseases proved to be beneficial for the heart. Thus, it is only a matter of time before targeting epitranscriptomic regulations becomes a part of clinical practice.

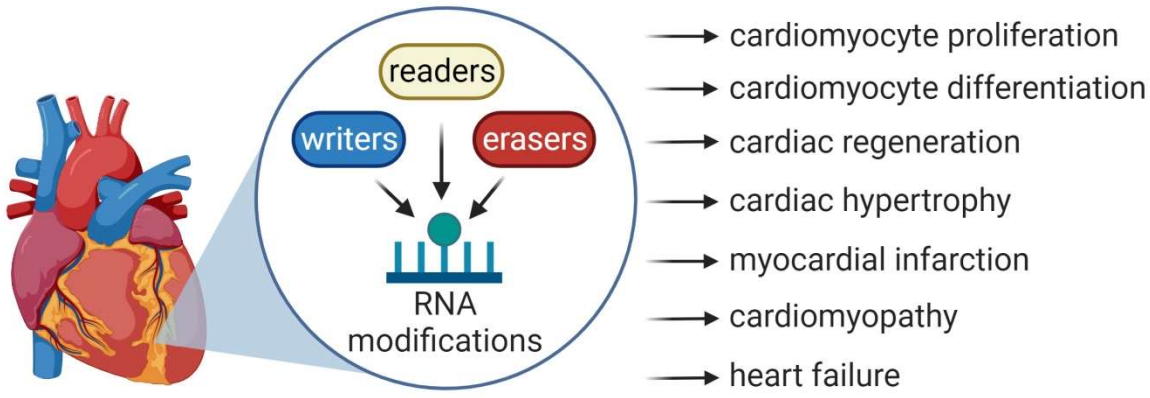

Fig. 3. Role of RNA modifications in the heart

Authors' contributions

B.D. drafted the article, K.F. and H.M. provided substantive revisions.

Conflict of Interest

There is no conflict of interest.

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