Epitranscriptomic regulations in the heart

Benak Daniel[1,2*], Kolar Frantisek[1], Hlavackova Marketa[1]

[1]Laboratory of Developmental Cardiology, Institute of Physiology of the Czech Academy of Sciences, 14220 Prague, Czech Republic

[2]Department of Physiology, Faculty of Science, Charles University, 12844 Prague, Czech Republic

*Correspondence:

Benak Daniel

daniel.benak@fgu.cas.cz

Keywords: epitranscriptomics, RNA modifications, epigenetics, m^6A, RNA, heart

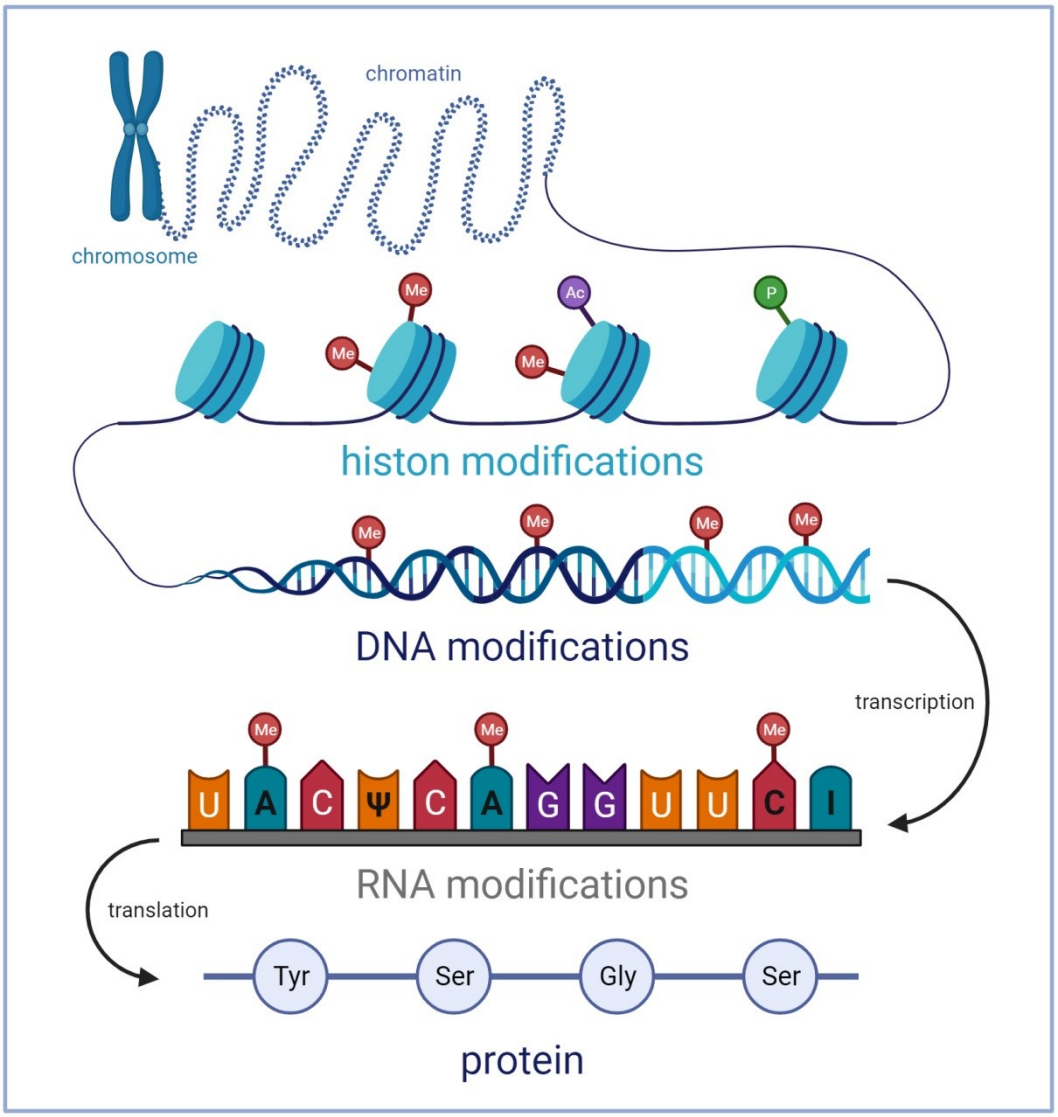
Abstract

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-methylcytosine (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.

1. Introduction

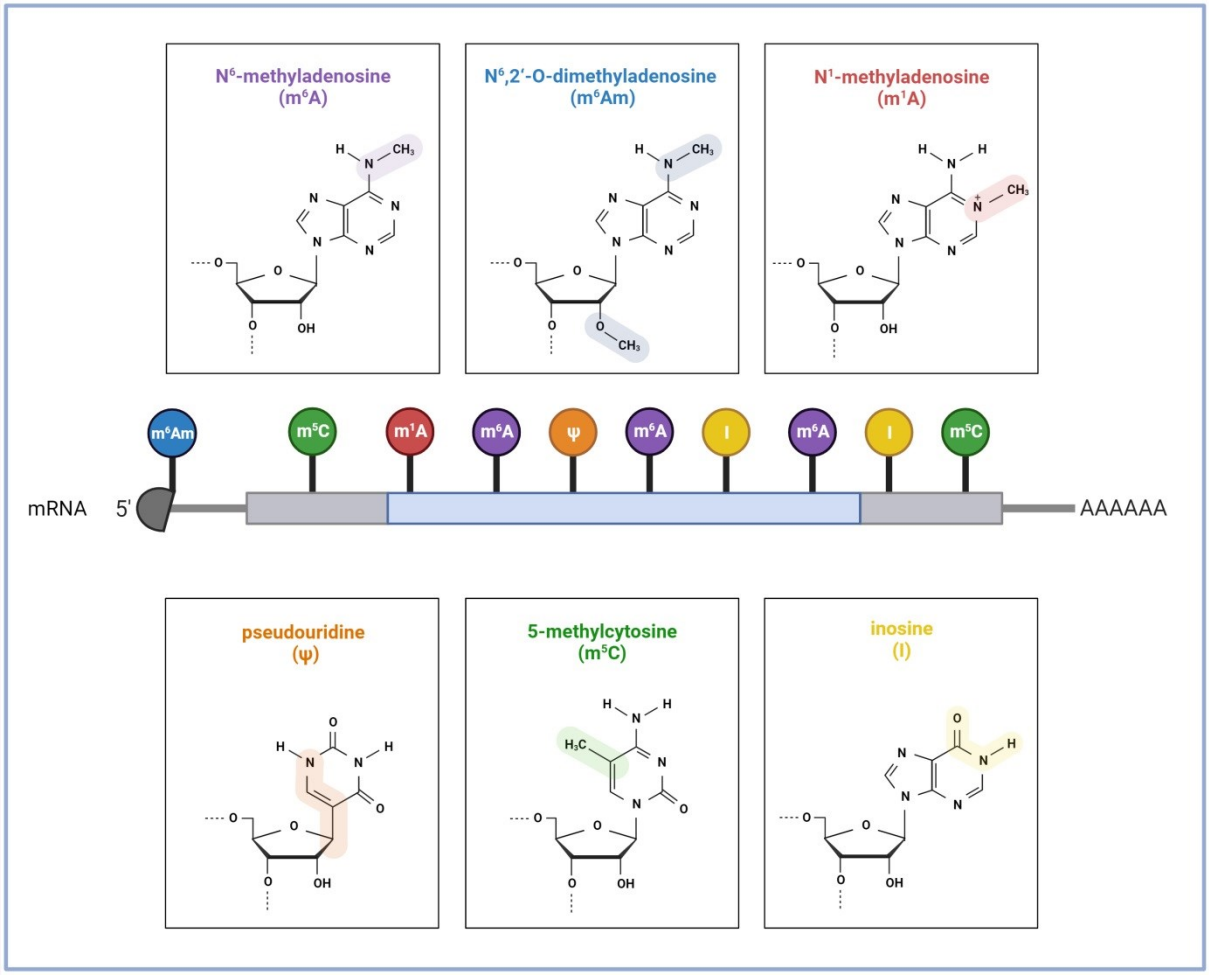
The original central dogma of molecular biology states that DNA is transcribed into RNA, which is subsequently translated into proteins [1]. However, the whole process is under the control of epigenetic mechanisms. Epigenetic mechanisms involve chemical modifications to the DNA itself, to the proteins that package DNA into chromatin (histones), or to the RNA molecules transcribed from the DNA (Figure 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].

Fig. 1: Basic overview of epigenetic modifications



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 Figure 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].

Fig. 2: Common RNA modifications



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.

2. Common RNA modifications and their role in cardiac physiology

2.1. 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^{6}A also controls cardiac hypertrophy [38-40]. Dorn et al. [41] suggested that enhanced m^{6}A RNA methylation results in compensated cardiac hypertrophy, whereas diminished m^{6}A drives eccentric cardiomyocyte remodeling and dysfunction. Changes in m^{6}A methylation and dysregulation of m^{6}A machinery can contribute to the progression of heart failure [42-47]. Altered cardiac m^{6}A patterns were detected also in diabetic cardiomyopathy with distinct dysregulation of m^{6}A machinery in the two types of diabetes [48-50]. The heterogeneous role of m^{6}A modification in CVDs has been reviewed in several recent publications [51-60].

Altered m^{6}A 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^{6}A levels compared to healthy individuals [61].

Since cardiac m^{6}A machinery is dysregulated under many pathophysiological conditions, targeting m^{6}A 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, 62-68]. On the contrary, loss of METTL3 or METTL14 can alleviate myocardial injury and promote heart regeneration [69, 70]. Thus, improving our knowledge of the m^{6}A regulations in the heart may lead to novel cardioprotective strategies using specific pharmacological activators or inhibitors targeting m^{6}A modifiers.

2.2. N^6,2'-O-dimethyladenosine

N^6,2'-O-dimethyladenosine (m^{6}Am) is formed by N^6-methylation of 2'-O-methyladenosine (Am). It has been described only in mRNA and snRNA [50, 71]. This modification is present at the first transcribed nucleotide and forms the extended cap structure in at least 30-40% of all vertebrate mRNA [72, 73]. Moreover, m^{6}Am is also present at the internal sites of snRNAs [17]. The formation of m^{6}Am in the cap is mediated by phosphorylated CTD interacting factor 1 (PCIF1), while methyltransferase-like 4 (METTL4) is responsible for internal m^{6}Am formation [74-77]. 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, 78, 79]. 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) [78, 80].

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 [71]. 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) *[71]*. 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 [71, 81].

2.3. 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 catalysed 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].

2.4. 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].

2.5. 5-methylcytosine

5-methylcytosine (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-hydroxymethylcytosin (hm^5C), while ALKBH1 is responsible for the oxidation of m^5C in mitochondrial tRNA generating 5-formylcytosine (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].

2.6. 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].

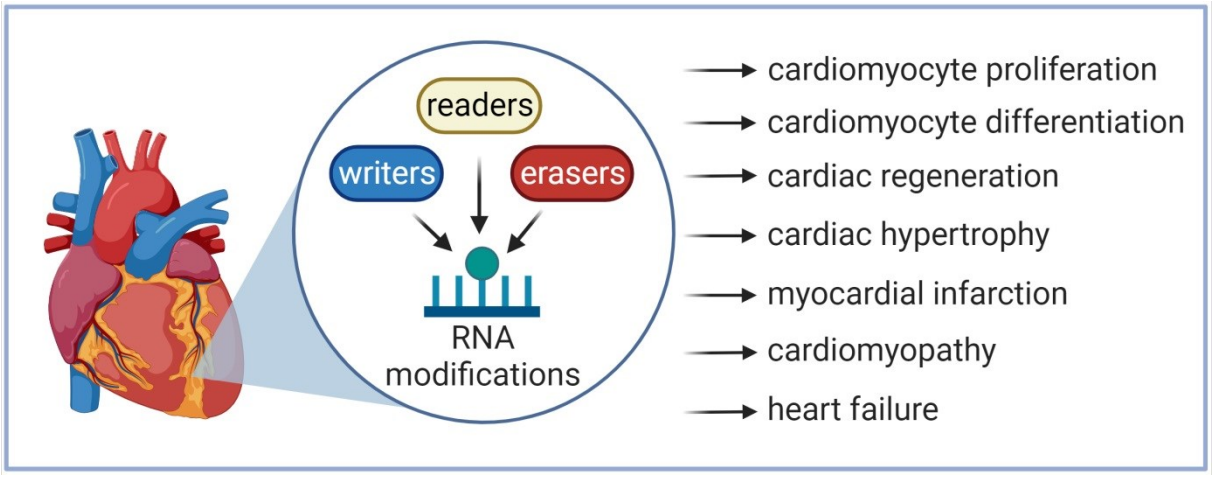
ADAR1 is an essential enzyme for normal embryonic cardiac growth and development [134]. Cardiomyocyte-specific deletion of *Adar1* in adult mice caused severe ventricular remodeling and spontaneous cardiac dysfunction associated with a significant rise in lethality [135]. ADAR1 was also shown to prevent 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 (Figure 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.

Declaration of conflicting interests

The authors declare that they have no conflict of interest.

Acknowledgments

Figures were created with BioRender.com.

Funding

This work was supported by the Czech Science Foundation under Grant (24-10497S) to H.M.; the Charles University Grant Agency under Grant (GA UK 668220) to B.D.; and the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) to K.F. - Funded by the European Union – Next Generation EU.

References:

1. Crick, F.H., *On protein synthesis.* Symp Soc Exp Biol, 1958. **12**: p. 138-63.
2. Devaux, Y. and E.L. Robinson, *Preface*, in *Epigenetics in Cardiovascular Disease*, Y. Devaux and E.L. Robinson, Editors. 2021, Academic Press. p. XXI-XXVI.
3. Zhang, L., Q. Lu, and C. Chang, *Epigenetics in Health and Disease*, in *Epigenetics in Allergy and Autoimmunity*, C. Chang and Q. Lu, Editors. 2020, Springer Singapore: Singapore. p. 3-55.
4. Boccaletto, P., et al., *MODOMICS: a database of RNA modification pathways. 2017 update.* Nucleic Acids Res, 2018. **46**(D1): p. D303-d307.
5. Roundtree, I.A., et al., *Dynamic RNA Modifications in Gene Expression Regulation.* Cell, 2017. **169**(7): p. 1187-1200.
6. Dieterich, C. and M. Völkers, *Chapter 6 - RNA modifications in cardiovascular disease—An experimental and computational perspective*, in *Epigenetics in Cardiovascular Disease*, Y. Devaux and E.L. Robinson, Editors. 2021, Academic Press. p. 113-125.
7. Lee, Y., et al., *Molecular Mechanisms Driving mRNA Degradation by m(6)A Modification.* Trends Genet, 2020. **36**(3): p. 177-188.
8. Boo, S.H. and Y.K. Kim, *The emerging role of RNA modifications in the regulation of mRNA stability.* Exp Mol Med, 2020. **52**(3): p. 400-408.
9. Desrosiers, R., K. Friderici, and F. Rottman, *Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells.* Proc Natl Acad Sci U S A, 1974. **71**(10): p. 3971-5.
10. Dominissini, D., et al., *Transcriptome-wide mapping of N(6)-methyladenosine by m(6)A-seq based on immunocapturing and massively parallel sequencing.* Nat Protoc, 2013. **8**(1): p. 176-89.
11. Meyer, K.D., et al., *Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons.* Cell, 2012. **149**(7): p. 1635-46.
12. Oerum, S., et al., *A comprehensive review of m6A/m6Am RNA methyltransferase structures.* Nucleic Acids Res, 2021. **49**(13): p. 7239-7255.
13. Wang, P., K.A. Doxtader, and Y. Nam, *Structural Basis for Cooperative Function of Mettl3 and Mettl14 Methyltransferases.* Mol Cell, 2016. **63**(2): p. 306-317.
14. Wang, X., et al., *Structural basis of N(6)-adenosine methylation by the METTL3-METTL14 complex.* Nature, 2016. **534**(7608): p. 575-8.
15. Zheng, G., et al., *ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility.* Mol Cell, 2013. **49**(1): p. 18-29.
16. Jia, G., et al., *N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO.* Nat Chem Biol, 2011. **7**(12): p. 885-7.
17. Wei, J., et al., *Differential m(6)A, m(6)A(m), and m(1)A Demethylation Mediated by FTO in the Cell Nucleus and Cytoplasm.* Mol Cell, 2018. **71**(6): p. 973-985.e5.
18. Relier, S., et al., *FTO-mediated cytoplasmic m(6)A(m) demethylation adjusts stem-like properties in colorectal cancer cell.* Nat Commun, 2021. **12**(1): p. 1716.
19. Zaccara, S. and S.R. Jaffrey, *A Unified Model for the Function of YTHDF Proteins in Regulating m(6)A-Modified mRNA.* Cell, 2020. **181**(7): p. 1582-1595.e18.
20. Lasman, L., et al., *Context-dependent functional compensation between Ythdf m(6)A reader proteins.* Genes Dev, 2020. **34**(19-20): p. 1373-1391.
21. Wang, X., et al., *N6-methyladenosine-dependent regulation of messenger RNA stability.* Nature, 2014. **505**(7481): p. 117-20.
22. Wang, X., et al., *N(6)-methyladenosine Modulates Messenger RNA Translation Efficiency.* Cell, 2015. **161**(6): p. 1388-99.
23. Xiao, W., et al., *Nuclear m(6)A Reader YTHDC1 Regulates mRNA Splicing.* Mol Cell, 2016. **61**(4): p. 507-519.
24. Hsu, P.J., et al., *Ythdc2 is an N(6)-methyladenosine binding protein that regulates mammalian spermatogenesis.* Cell Res, 2017. **27**(9): p. 1115-1127.

25. Shi, H., et al., *YTHDF3 facilitates translation and decay of N(6)-methyladenosine-modified RNA.* Cell Res, 2017. **27**(3): p. 315-328.
26. Liu, X.H., et al., *Co-effects of m6A and chromatin accessibility dynamics in the regulation of cardiomyocyte differentiation.* Epigenetics Chromatin, 2023. **16**(1): p. 32.
27. Han, Z., et al., *ALKBH5 regulates cardiomyocyte proliferation and heart regeneration by demethylating the mRNA of YTHDF1.* Theranostics, 2021. **11**(6): p. 3000-3016.
28. Yang, C., et al., *Comprehensive Analysis of the Transcriptome-Wide m6A Methylome of Heart via MeRIP After Birth: Day 0 vs. Day 7.* Front Cardiovasc Med, 2021. **8**: p. 633631.
29. Semenovykh, D., et al., *Myocardial m6A regulators in postnatal development: effect of sex.* Physiol Res, 2022. **71**(6): p. 877-882.
30. Boissel, S., et al., *Loss-of-function mutation in the dioxygenase-encoding FTO gene causes severe growth retardation and multiple malformations.* Am J Hum Genet, 2009. **85**(1): p. 106-11.
31. Liu, C., S. Mou, and C. Pan, *The FTO gene rs9939609 polymorphism predicts risk of cardiovascular disease: a systematic review and meta-analysis.* PLoS One, 2013. **8**(8): p. e71901.
32. Doney, A.S., et al., *The FTO gene is associated with an atherogenic lipid profile and myocardial infarction in patients with type 2 diabetes: a Genetics of Diabetes Audit and Research Study in Tayside Scotland (Go-DARTS) study.* Circ Cardiovasc Genet, 2009. **2**(3): p. 255-9.
33. Hubacek, J.A., et al., *Gene variants at FTO, 9p21, and 2q36.3 are age-independently associated with myocardial infarction in Czech men.* Clin Chim Acta, 2016. **454**: p. 119-23.
34. Hubacek, J.A., et al., *A FTO variant and risk of acute coronary syndrome.* Clin Chim Acta, 2010. **411**(15-16): p. 1069-72.
35. Hubacek, J.A., et al., *The fat mass and obesity related gene polymorphism influences the risk of rejection in heart transplant patients.* Clin Transplant, 2018. **32**(12): p. e13443.
36. Zhen, X., et al., *Genetic Variations Within METTL16 and Susceptibility to Sudden Cardiac Death in Chinese Populations With Coronary Artery Disease.* Am J Cardiol, 2023. **202**: p. 90-99.
37. Wakil, S.M., et al., *A genome-wide association study reveals susceptibility loci for myocardial infarction/coronary artery disease in Saudi Arabs.* Atherosclerosis, 2016. **245**: p. 62-70.
38. Zhang, R., et al., *METTL3 mediates Ang-II-induced cardiac hypertrophy through accelerating pri-miR-221/222 maturation in an m6A-dependent manner.* Cell Mol Biol Lett, 2022. **27**(1): p. 55.
39. Carnevali, L., et al., *Signs of cardiac autonomic imbalance and proarrhythmic remodeling in FTO deficient mice.* PLoS One, 2014. **9**(4): p. e95499.
40. Gan, X.T., et al., *Identification of fat mass and obesity associated (FTO) protein expression in cardiomyocytes: regulation by leptin and its contribution to leptin-induced hypertrophy.* PLoS One, 2013. **8**(9): p. e74235.
41. Dorn, L.E., et al., *The N(6)-Methyladenosine mRNA Methylase METTL3 Controls Cardiac Homeostasis and Hypertrophy.* Circulation, 2019. **139**(4): p. 533-545.
42. Kmietczyk, V., et al., *m(6)A-mRNA methylation regulates cardiac gene expression and cellular growth.* Life Sci Alliance, 2019. **2**(2): p. e201800233.
43. Mathiyalagan, P., et al., *FTO-Dependent N(6)-Methyladenosine Regulates Cardiac Function During Remodeling and Repair.* Circulation, 2019. **139**(4): p. 518-532.
44. Berulava, T., et al., *Changes in m6A RNA methylation contribute to heart failure progression by modulating translation.* Eur J Heart Fail, 2020. **22**(1): p. 54-66.
45. Zhang, B., et al., *Alteration of m6A RNA Methylation in Heart Failure With Preserved Ejection Fraction.* Front Cardiovasc Med, 2021. **8**: p. 647806.
46. Zhang, B., et al., *m6A demethylase FTO attenuates cardiac dysfunction by regulating glucose uptake and glycolysis in mice with pressure overload-induced heart failure.* Signal Transduct Target Ther, 2021. **6**(1): p. 377.

47. Komal, S., et al., *ALKBH5 inhibitors as a potential treatment strategy in heart failure-inferences from gene expression profiling.* Front Cardiovasc Med, 2023. **10**: p. 1194311.
48. Ju, W., et al., *Changes in N6-Methyladenosine Modification Modulate Diabetic Cardiomyopathy by Reducing Myocardial Fibrosis and Myocyte Hypertrophy.* Front Cell Dev Biol, 2021. **9**: p. 702579.
49. Shao, Y., et al., *CircRNA CDR1as promotes cardiomyocyte apoptosis through activating hippo signaling pathway in diabetic cardiomyopathy.* Eur J Pharmacol, 2022. **922**: p. 174915.
50. Benak, D., et al., *The role of m6A and m6Am RNA modifications in the pathogenesis of diabetes mellitus.* Front Endocrinol (Lausanne), 2023. **14**: p. 1223583.
51. Zhang, B., et al., *The critical roles of m6A modification in metabolic abnormality and cardiovascular diseases.* Genes Dis, 2021. **8**(6): p. 746-758.
52. Longenecker, J.Z., et al., *Epitranscriptomics in the Heart: a Focus on m(6)A.* Curr Heart Fail Rep, 2020. **17**(5): p. 205-212.
53. Wu, S., et al., *m(6)A RNA Methylation in Cardiovascular Diseases.* Mol Ther, 2020. **28**(10): p. 2111-2119.
54. Qin, Y., et al., *Role of m6A RNA methylation in cardiovascular disease (Review).* Int J Mol Med, 2020. **46**(6): p. 1958-1972.
55. Paramasivam, A., J. Vijayashree Priyadharsini, and S. Raghunandhakumar, *N6-adenosine methylation (m6A): a promising new molecular target in hypertension and cardiovascular diseases.* Hypertens Res, 2020. **43**(2): p. 153-154.
56. Kumari, R., et al., *mRNA modifications in cardiovascular biology and disease: with a focus on m6A modification.* Cardiovasc Res, 2022. **118**(7): p. 1680-1692.
57. Leptidis, S., et al., *Epitranscriptomics of cardiovascular diseases (Review).* Int J Mol Med, 2022. **49**(1).
58. Chen, Y.S., et al., *N6-Adenosine Methylation (m(6)A) RNA Modification: an Emerging Role in Cardiovascular Diseases.* J Cardiovasc Transl Res, 2021. **14**(5): p. 857-872.
59. Zhou, W., et al., *RNA Methylations in Cardiovascular Diseases, Molecular Structure, Biological Functions and Regulatory Roles in Cardiovascular Diseases.* Front Pharmacol, 2021. **12**: p. 722728.
60. Xu, Z., et al., *Emerging Roles and Mechanism of m6A Methylation in Cardiometabolic Diseases.* Cells, 2022. **11**(7).
61. Li, Y., et al., *Analysis of urinary methylated nucleosides of patients with coronary artery disease by high-performance liquid chromatography/electrospray ionization tandem mass spectrometry.* Rapid Commun Mass Spectrom, 2014. **28**(19): p. 2054-8.
62. Ma, Y., et al., *Alteration of N(6)-Methyladenosine mRNA Methylation in a Human Stem Cell-Derived Cardiomyocyte Model of Tyrosine Kinase Inhibitor-Induced Cardiotoxicity.* Front Cardiovasc Med, 2022. **9**: p. 849175.
63. Deng, W., Q. Jin, and L. Li, *Protective mechanism of demethylase fat mass and obesity-associated protein in energy metabolism disorder of hypoxia-reoxygenation-induced cardiomyocytes.* Exp Physiol, 2021. **106**(12): p. 2423-2433.
64. Shen, W., et al., *FTO overexpression inhibits apoptosis of hypoxia/reoxygenation-treated myocardial cells by regulating m6A modification of Mhrt.* Mol Cell Biochem, 2021. **476**(5): p. 2171-2179.
65. Ke, W.L., et al., *m(6)A demethylase FTO regulates the apoptosis and inflammation of cardiomyocytes via YAP1 in ischemia-reperfusion injury.* Bioengineered, 2022. **13**(3): p. 5443-5452.
66. Zhang, X., et al., *Dexmedetomidine Postconditioning Alleviates Hypoxia/Reoxygenation Injury in Senescent Myocardial Cells by Regulating lncRNA H19 and m⁶A Modification.* Oxidative Medicine and Cellular Longevity, 2020. **2020**: p. 9250512.
67. Cui, Y., et al., *Cinnamic acid mitigates left ventricular hypertrophy and heart failure in part through modulating FTO-dependent N(6)-methyladenosine RNA modification in cardiomyocytes.* Biomed Pharmacother, 2023. **165**: p. 115168.

68. Yu, P., et al., *RNA m(6)A-Regulated circ-ZNF609 Suppression Ameliorates Doxorubicin-Induced Cardiotoxicity by Upregulating FTO.* JACC Basic Transl Sci, 2023. **8**(6): p. 677-698.
69. Gong, R., et al., *Loss of m(6)A methyltransferase METTL3 promotes heart regeneration and repair after myocardial injury.* Pharmacol Res, 2021. **174**: p. 105845.
70. Wu, C., et al., *The m(6)A methylation enzyme METTL14 regulates myocardial ischemia/reperfusion injury through the Akt/mTOR signaling pathway.* Mol Cell Biochem, 2023.
71. Benak, D., et al., *RNA modification m(6)Am: the role in cardiac biology.* Epigenetics, 2023. **18**(1): p. 2218771.
72. Wei, C., A. Gershowitz, and B. Moss, *N6, O2'-dimethyladenosine a novel methylated ribonucleoside next to the 5' terminal of animal cell and virus mRNAs.* Nature, 1975. **257**(5523): p. 251-3.
73. Bokar, J.A., *The biosynthesis and functional roles of methylated nucleosides in eukaryotic mRNA*, in *Fine-Tuning of RNA Functions by Modification and Editing*, H. Grosjean, Editor. 2005, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 141-177.
74. Akichika, S., et al., *Cap-specific terminal N (6)-methylation of RNA by an RNA polymerase II-associated methyltransferase.* Science, 2019. **363**(6423).
75. Sun, H., et al., *Cap-specific, terminal N(6)-methylation by a mammalian m(6)Am methyltransferase.* Cell Res, 2019. **29**(1): p. 80-82.
76. Chen, H., et al., *METTL4 is an snRNA m(6)Am methyltransferase that regulates RNA splicing.* Cell Res, 2020. **30**(6): p. 544-547.
77. Goh, Y.T., et al., *METTL4 catalyzes m6Am methylation in U2 snRNA to regulate pre-mRNA splicing.* Nucleic Acids Res, 2020. **48**(16): p. 9250-9261.
78. Mauer, J., et al., *Reversible methylation of m(6)A(m) in the 5' cap controls mRNA stability.* Nature, 2017. **541**(7637): p. 371-375.
79. Mauer, J. and S.R. Jaffrey, *FTO, m(6) A(m) , and the hypothesis of reversible epitranscriptomic mRNA modifications.* FEBS Lett, 2018. **592**(12): p. 2012-2022.
80. Mauer, J., et al., *FTO controls reversible m(6)Am RNA methylation during snRNA biogenesis.* Nat Chem Biol, 2019. **15**(4): p. 340-347.
81. Benak, D., et al., *Myocardial epitranscriptomics in fasting.* Journal of Molecular and Cellular Cardiology, 2022. **173**: p. S52.
82. Dunn, D.B., *The occurrence of 1-methyladenine in ribonucleic acid.* Biochim Biophys Acta, 1961. **46**: p. 198-200.
83. Helm, M., R. Giegé, and C. Florentz, *A Watson-Crick base-pair-disrupting methyl group (m1A9) is sufficient for cloverleaf folding of human mitochondrial tRNALys.* Biochemistry, 1999. **38**(40): p. 13338-46.
84. Sharma, S., et al., *Identification of a novel methyltransferase, Bmt2, responsible for the N-1-methyl-adenosine base modification of 25S rRNA in Saccharomyces cerevisiae.* Nucleic Acids Res, 2013. **41**(10): p. 5428-43.
85. Dominissini, D., et al., *The dynamic N(1)-methyladenosine methylome in eukaryotic messenger RNA.* Nature, 2016. **530**(7591): p. 441-6.
86. Safra, M., et al., *The m1A landscape on cytosolic and mitochondrial mRNA at single-base resolution.* Nature, 2017. **551**(7679): p. 251-255.
87. Li, X., et al., *Base-Resolution Mapping Reveals Distinct m(1)A Methylome in Nuclear- and Mitochondrial-Encoded Transcripts.* Mol Cell, 2017. **68**(5): p. 993-1005.e9.
88. Chujo, T. and T. Suzuki, *Trmt61B is a methyltransferase responsible for 1-methyladenosine at position 58 of human mitochondrial tRNAs.* Rna, 2012. **18**(12): p. 2269-76.
89. Bar-Yaacov, D., et al., *Mitochondrial 16S rRNA Is Methylated by tRNA Methyltransferase TRMT61B in All Vertebrates.* PLoS Biol, 2016. **14**(9): p. e1002557.
90. Waku, T., et al., *NML-mediated rRNA base methylation links ribosomal subunit formation to cell proliferation in a p53-dependent manner.* J Cell Sci, 2016. **129**(12): p. 2382-93.

91. Liu, F., et al., *ALKBH1-Mediated tRNA Demethylation Regulates Translation.* Cell, 2016. **167**(3): p. 816-828.e16.
92. Li, X., et al., *Transcriptome-wide mapping reveals reversible and dynamic N(1)-methyladenosine methylome.* Nat Chem Biol, 2016. **12**(5): p. 311-6.
93. Chen, Z., et al., *Transfer RNA demethylase ALKBH3 promotes cancer progression via induction of tRNA-derived small RNAs.* Nucleic Acids Res, 2019. **47**(5): p. 2533-2545.
94. Oerum, S., et al., *m1A Post-Transcriptional Modification in tRNAs.* Biomolecules, 2017. **7**(1).
95. Shima, H. and K. Igarashi, *N 1-methyladenosine (m1A) RNA modification: the key to ribosome control.* J Biochem, 2020. **167**(6): p. 535-539.
96. Zhao, B.S., I.A. Roundtree, and C. He, *Post-transcriptional gene regulation by mRNA modifications.* Nat Rev Mol Cell Biol, 2017. **18**(1): p. 31-42.
97. Wu, Y., et al., *RNA modifications in cardiovascular diseases, the potential therapeutic targets.* Life Sci, 2021. **278**: p. 119565.
98. Cohn, W.E., *Some results of the applications of ion-exchange chromatography to nucleic acid chemistry.* J Cell Physiol Suppl, 1951. **38**(Suppl. 1): p. 21-40.
99. Xue, C., et al., *Role of main RNA modifications in cancer: N(6)-methyladenosine, 5-methylcytosine, and pseudouridine.* Signal Transduct Target Ther, 2022. **7**(1): p. 142.
100. Sun, H., et al., *Regulation and functions of non-m(6)A mRNA modifications.* Nat Rev Mol Cell Biol, 2023.
101. Rintala-Dempsey, A.C. and U. Kothe, *Eukaryotic stand-alone pseudouridine synthases - RNA modifying enzymes and emerging regulators of gene expression?* RNA Biol, 2017. **14**(9): p. 1185-1196.
102. Li, X., S. Ma, and C. Yi, *Pseudouridine: the fifth RNA nucleotide with renewed interests.* Curr Opin Chem Biol, 2016. **33**: p. 108-16.
103. Zhao, B.S. and C. He, *Pseudouridine in a new era of RNA modifications.* Cell Res, 2015. **25**(2): p. 153-4.
104. Wu, G., et al., *Pseudouridines in U2 snRNA stimulate the ATPase activity of Prp5 during spliceosome assembly.* Embo j, 2016. **35**(6): p. 654-67.
105. Levi, O. and Y.S. Arava, *Pseudouridine-mediated translation control of mRNA by methionine aminoacyl tRNA synthetase.* Nucleic Acids Res, 2021. **49**(1): p. 432-443.
106. Borchardt, E.K., N.M. Martinez, and W.V. Gilbert, *Regulation and Function of RNA Pseudouridylation in Human Cells.* Annu Rev Genet, 2020. **54**: p. 309-336.
107. Jalan, A., et al., *Decoding the 'Fifth' Nucleotide: Impact of RNA Pseudouridylation on Gene Expression and Human Disease.* Mol Biotechnol, 2023.
108. Razavi, A.C., et al., *Pseudouridine and N-formylmethionine associate with left ventricular mass index: Metabolome-wide association analysis of cardiac remodeling.* J Mol Cell Cardiol, 2020. **140**: p. 22-29.
109. Alexander, D., et al., *Metabolomic distinction and insights into the pathogenesis of human primary dilated cardiomyopathy.* Eur J Clin Invest, 2011. **41**(5): p. 527-38.
110. Dunn, W.B., et al., *Serum metabolomics reveals many novel metabolic markers of heart failure, including pseudouridine and 2-oxoglutarate.* Metabolomics, 2007. **3**(4): p. 413-426.
111. Nagasawa, C.K., et al., *scaRNA1 Levels Alter Pseudouridylation in Spliceosomal RNA U2 Affecting Alternative mRNA Splicing and Embryonic Development.* Pediatric Cardiology, 2020. **41**(2): p. 341-349.
112. Patil, P., et al., *scaRNAs regulate splicing and vertebrate heart development.* Biochim Biophys Acta, 2015. **1852**(8): p. 1619-29.
113. Bohnsack, K.E., C. Höbartner, and M.T. Bohnsack, *Eukaryotic 5-methylcytosine (m^5C) RNA Methyltransferases: Mechanisms, Cellular Functions, and Links to Disease.* Genes (Basel), 2019. **10**(2).
114. Wang, Y.Y., et al., *The role of m5C methyltransferases in cardiovascular diseases.* Front Cardiovasc Med, 2023. **10**: p. 1225014.

115. Haag, S., et al., *NSUN3 and ABH1 modify the wobble position of mt-tRNAMet to expand codon recognition in mitochondrial translation.* Embo j, 2016. **35**(19): p. 2104-2119.
116. Fu, L., et al., *Tet-mediated formation of 5-hydroxymethylcytosine in RNA.* J Am Chem Soc, 2014. **136**(33): p. 11582-5.
117. Yang, X., et al., *5-methylcytosine promotes mRNA export - NSUN2 as the methyltransferase and ALYREF as an m(5)C reader.* Cell Res, 2017. **27**(5): p. 606-625.
118. Chen, X., et al., *5-methylcytosine promotes pathogenesis of bladder cancer through stabilizing mRNAs.* Nat Cell Biol, 2019. **21**(8): p. 978-990.
119. Squires, J.E. and T. Preiss, *Function and detection of 5-methylcytosine in eukaryotic RNA.* Epigenomics, 2010. **2**(5): p. 709-15.
120. Chen, Y.S., et al., *Dynamic transcriptomic m(5) C and its regulatory role in RNA processing.* Wiley Interdiscip Rev RNA, 2021. **12**(4): p. e1639.
121. Huang, T., et al., *Genome-wide identification of mRNA 5-methylcytosine in mammals.* Nature Structural & Molecular Biology, 2019. **26**(5): p. 380-388.
122. Metodiev, M.D., et al., *NSUN4 is a dual function mitochondrial protein required for both methylation of 12S rRNA and coordination of mitoribosomal assembly.* PLoS Genet, 2014. **10**(2): p. e1004110.
123. Ghanbarian, H., et al., *Dnmt2/Trdmt1 as Mediator of RNA Polymerase II Transcriptional Activity in Cardiac Growth.* PLoS One, 2016. **11**(6): p. e0156953.
124. Varma, E., et al., *Translational control of Ybx1 expression regulates cardiac function in response to pressure overload in vivo.* Basic Res Cardiol, 2023. **118**(1): p. 25.
125. Yang, R., et al., *Long non-coding RNA KCND1 protects hearts from hypertrophy by targeting YBX1.* Cell Death Dis, 2023. **14**(5): p. 344.
126. Wang, Y., et al., *NSUN2 alleviates doxorubicin-induced myocardial injury through Nrf2-mediated antioxidant stress.* Cell Death Discov, 2023. **9**(1): p. 43.
127. Brennicke, A., A. Marchfelder, and S. Binder, *RNA editing.* FEMS Microbiol Rev, 1999. **23**(3): p. 297-316.
128. Gott, J.M. and R.B. Emeson, *Functions and mechanisms of RNA editing.* Annu Rev Genet, 2000. **34**: p. 499-531.
129. Ganem, N.S. and A.T. Lamm, *A-to-I RNA editing - thinking beyond the single nucleotide.* RNA Biol, 2017. **14**(12): p. 1690-1694.
130. Dominissini, D., et al., *Adenosine-to-inosine RNA editing meets cancer.* Carcinogenesis, 2011. **32**(11): p. 1569-1577.
131. Bhakta, S. and T. Tsukahara, *C-to-U RNA Editing: A Site Directed RNA Editing Tool for Restoration of Genetic Code.* Genes (Basel), 2022. **13**(9).
132. Sowden, M.P., et al., *The editosome for cytidine to uridine mRNA editing has a native complexity of 27S: identification of intracellular domains containing active and inactive editing factors.* J Cell Sci, 2002. **115**(Pt 5): p. 1027-39.
133. Nishikura, K., *A-to-I editing of coding and non-coding RNAs by ADARs.* Nat Rev Mol Cell Biol, 2016. **17**(2): p. 83-96.
134. Moore, J.B.t., et al., *The A-to-I RNA Editing Enzyme Adar1 Is Essential for Normal Embryonic Cardiac Growth and Development.* Circ Res, 2020. **127**(4): p. 550-552.
135. El Azzouzi, H., et al., *Cardiomyocyte Specific Deletion of ADAR1 Causes Severe Cardiac Dysfunction and Increased Lethality.* Front Cardiovasc Med, 2020. **7**: p. 30.
136. Garcia-Gonzalez, C., et al., *ADAR1 Prevents Autoinflammatory Processes in the Heart Mediated by IRF7.* Circ Res, 2022. **131**(7): p. 580-597.
137. Borik, S., et al., *Increased RNA editing in children with cyanotic congenital heart disease.* Intensive Care Med, 2011. **37**(10): p. 1664-71.
138. Kokot, K.E., et al., *Reduction of A-to-I RNA editing in the failing human heart regulates formation of circular RNAs.* Basic Res Cardiol, 2022. **117**(1): p. 32.
139. Altaf, F., et al., *Modulation of ADAR mRNA expression in patients with congenital heart defects.* PLoS One, 2019. **14**(4): p. e0200968.

140. Wu, X., et al., *ADAR2 increases in exercised heart and protects against myocardial infarction and doxorubicin-induced cardiotoxicity.* Mol Ther, 2022. **30**(1): p. 400-414.
141. Birgaoanu, M., M. Sachse, and A. Gatsiou, *RNA Editing Therapeutics: Advances, Challenges and Perspectives on Combating Heart Disease.* Cardiovasc Drugs Ther, 2023. **37**(2): p. 401-411.