Peer Review File

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Reviewer A

The review written by Mirolyuba Ilieva and Shizuka Uchida focused on the three epitranscriptomic markers m6A, A to I RNA editing and m5C, and their effects on cardiovascular physiology and pathology. The review was presented in a very educational, logical and condensed manner which makes readers easy to follow and understand. Each section was supplied with appropriate examples to support the main point. However, here are some minor suggestions: 1. Some phrases were italicized while others are not, for example, Ythdc1 (page 5, line 97) and YTHDC2 was not italicized (page 5, line 105).

Reply 1: Thank you very much for your praise on our manuscript. The reason for italicized words is to follow the international guideline for gene nomenclature, which the gene names should be italic, while protein names should be all in capital letters without being italic for human proteins: https://en.wikipedia.org/wiki/Gene_nomenclature#Symbol_and_name.

2. The paragraph on m6A eraser YTHDC2 could be better explained, it started with discussing YTHDC2 but then switched to Ythdf2, then back to YTHDC2 which is confusing.

Reply 2: Thank you very much for noticing this mistake. This should read, YTHDF2; not YTHDC2. We have corrected as follows to reflect the original report:

Xu H, Wang Z, Chen M, Zhao W, Tao T, Ma L, et al. YTHDF2 alleviates cardiac hypertrophy via regulating Myh7 mRNA decoy. Cell Biosci. 2021;11(1):132.

Changes in the text: Another m6A reader, YTHDF2, is also indicated to be involved in cardiac physiology. In both human and mice, the expression of YTHDF2 mRNA and protein, but not its family members -YTHDF1 or YTHDF3, are upregulated during the progression of heart failure (30). When challenged with transverse aortic constriction, *Ythdf2* overexpressing mice attenuated cardiac hypertrophy. Mechanistically, YTHDF2 suppresses cardiac hypertrophy by recognizing m6A site on the Myh7 mRNA (also known as β-MHC, which is a marker for cardiac hypertrophy) to promote its degradation.

3. Authors should proofread the manuscript to correct for any grammatical errors."

Reply 3: The manuscript has been thoroughly read to correct any grammatical errors.

Reviewer B

The review manuscript by Ileiva & Uchida nicely summarized our current understanding of the functional roles of epictranscriptomic marks in the cardiovascular system. The manuscript

is very well-written and provide an nice overview of the impact of RNA modifications in cardiac development and diseases.
However, there two points that I missed on this review. Firstly it the fact that the functional consequences of such epitranscriptomic modifications is not properly detailed, i.e. what are the functional consequences of m6A, A-to-I or m5C modification, respectively. A short summary should be stated on each case.

Reply 1: Thank you very much for your praise on our manuscript. The following sentences have been modified or added:

Changes in the text: "The m^6A sites are found mostly in mRNAs, especially around stop codon (15), and regulate mRNA metabolism, such as splicing, nuclear export, mRNA stability, and translation (16)."

"RNA editing affects RNA metabolism by alterning splicing, miRNA biogenesis and binding, amino acid conversion, lncRNA binding and structures, RNA stability, and translation."

"Just as DNA, RNA can be marked by 5-methylcytosine (m5C), which are found abundantly in rRNAs and tRNAs but also in mRNAs and ncRNAs, which stabilize RNA folding and structures (52, 53)."

Secondly, the authors highlighted in the abstract and introduction that over 170 RNA modifications have been identified, however they only summarize information of three of them. Thus this discrepancy should be eliminated or at least properly commented (in the case that information for the cardiovascular system is not available for the remaining ones)

Reply 2: The Abstract and Introduction sections have been modified as follow:

Changes in the text: "Abstract: The recent emergence of epitranscriptomics provides an avenue for identifying RNA modifications implicated in the pathophysiology of human disease. To date, over 170 RNA modifications have been identified; these modifications are important because they can affect the fate of RNAs, including their decay, maturation, splicing, stability, and translational efficiency. Although RNA modifications have been reported in many tissues and disease contexts, detailed functional studies in the heart and cardiovascular disease are only beginning to be reported. By searching for relevant articles related to epitranscriptomics by focusing on the cardiovascular system and disease in the PubMed database, we summarize the recent findings of three epitranscriptomic marks– N6-methyladenosine (m^6A), adenosine to inosine (A-to-I) RNA editing, and 5-methylcytosine (m^5C) as other epitranscriptomic marks are not studied extensively in the cardiovascular system and disease. In this narrative review, the current status of cardiac epitranscriptomics is summarized to raise the awareness of this important field of study."

"In this narrative review, we summarize the current status of epitranscriptomic marks in cardiovascular system and disease by focusing on three epitranscriptomic marks – N6-methyladenosine (m^6A), adenosine to inosine (A-to-I) RNA editing, and 5-methylcytosine (m^5C) as other epitranscriptomic marks are not studied extensively in cardiovascular system and disease."

Minor comments

The sentence: "Mechanistically, ALKBH5 stabilizes the m6A reader, YTHDF1, mRNA in a m6A dependent manner, thereby promoting the translation of YAP,…" should be replaced by: "Mechanistically, ALKBH5 stabilizes the m6A reader YTHDF1 mRNA in a m6A dependent manner, thereby promoting the translation of YAP,…"

Reply 3: The above sentence has been modified as suggested:

Changes in the text: "Mechanistically, ALKBH5 stabilizes the m6A reader YTHDF1 mRNA in a m6A dependent manner, thereby promoting the translation of YAP, which is a downstream effector of the Hippo pathway that is important for cardiomyocyte growth."

Similarly, the sentence: "The mutation in the human ADAR1 gene is known to cause of autoimmune disease, Aicardi-Goutières syndrome" should be replaced by "The mutation in the human ADAR1 gene is known to cause the Aicardi-Goutières autoimmune syndrome."

Reply 4: The above sentence has been modified as suggested:

Changes in the text: "The mutation in the human *ADAR1* gene is known to cause of the Aicardi-Goutières autoimmune disease (39), while the accumulation of ADAR1 protein was observed in atherosclerotic plaques (40), which are the most common cause of coronary artery diseases."

Several paragraphs are in double-spaced. Please modify accordingly to single (1.5) space.

Reply 5: The Guidelines for Authors (https://atm.amegroups.com/pages/view/guidelines-for-authors) states as follow:

"3.3 Text
Format: Text should be double-spaced throughout. The pages should be numbered.
Font: A clearly readable font (e.g., Arial, Calibri, Times New Roman, or Verdana) with 10 or 12 pt. font size.
Language: English. British or American spelling is acceptable but must be consistent throughout."

Thus, all texts are double-spaced.

Reviewer C

"Functional roles of epitranscriptomic marks in cardiovascular system and disease: a narrative review" by Ilieva and Uchida summarizes evidence that chemical modifications of RNAs, known as epitranscriptomics, which include mRNAs and noncoding RNAs such as miRNAs and lncRNAs have been detected in cardiac muscle. Specifically, the authors summarize evidence describing differences in RNA modifications between healthy and diseased hearts including rodent models of cardiac development and disease such as cardiomyopathy and heart failure. In addition, they describe the phenotypes of RNA modification enzymes such as the methyltransferases when overexpressed or inhibited in various model systems. This current review paper provides a succinct perspective on RNA modifications in the heart. Three figures are included in this review which effectively complement the information provided in the text. Having said that, the review paper has too many abbreviations. The names of these RNA modification enzymes should be spelled out initially otherwise it is difficult to distinguish one enzyme abbreviation from another. This would be helpful because the RNA modification field nomenclature is not as well-known as the chromatin modification research area. Overall, this is a well written and concise review paper containing pertinent information on RNA modifications in the heart and disease.

Minor concerns:

1. The title should have "the" before cardiovascular system.

Reply 1: Thank you very much for your praise on our manuscript. The title has been modified as follow:

Changes in the text: "Functional roles of epitranscriptomic marks in the cardiovascular system and disease: a narrative review".

2. While the authors do a commendable job on providing clear and artful figures to summarize the three major themes of the review (m6A, A-to-I editing, and m5C pathways), there are numerous abbreviations throughout the text and these figures. It would be worthwhile writing out the names of the various RNA modification enzymes in the text and figure legends.

Reply 1: The abbreviations are spelled out at their first appearances and in the figure legends.

3. Also, a table summarizing each RNA modification enzyme, model system, and phenotype would also enhance the review. A table would provide a nice complement to the figures by summarizing the observed phenotypic effects of various RNA enzymes in the cardiac development, disease models or in cardiomyocytes in vitro (NRVMs).

Reply 2: The new table, Table 2, has been generated as below:

Changes in the text:

Epitrancriptomic Enzyme	Experimental System	Phenotypes/Mechanisms	Reference
m^6A writer, *Mettl3*	neonatal rat ventricular cardiomyocytes, cardiomyocyte-specific *Mettl3* conditional knockout mice	causes cardiac hypertrophy	(27)
m^6A reader, *Ythdc1*	cardiomyocyte-specific *Ythdc1* conditional knockout mice	possibly required for the proper splicing of sarcomeric protein, *Titin*	(29)
m^6A reader, *Ythdf2*	*Ythdf2* overexpressing mice	suppresses cardiac hypertrophy by recognizing m^6A site on the *Myh7* mRNA	(30)
m^6A eraser, *Fto*	*Fto* overexpressing mice	demethylates cardiac contractile mRNAs to prevent their mRNA degradation and promote their protein expression to preserve cardiac functions in the infarcted hearts	(33)
m^6A eraser, *Alkbh5*	*Alkbh5* knockout and overexpressing mice, hiPSC-CM	stabilizes the m^6A reader YTHDF1 mRNA in a m^6A dependent manner, thereby promoting the translation of YAP	(36)
A-to-I RNA editing writer, *Adar1*	*Adar1* knockout mice	results embryonic death due to massive apoptosis and aberrant interferon induction	(41)
A-to-I RNA editing writer, *Adar1*	cardiac-specific *Adar1* conditional knockout mice	regulates the cardiomyocyte survival and proliferation	(45)
A-to-I RNA editing writer, *Adar1*	cardiomyocyte-specific *Adar1* conditional knockout mice	results in increased lethality due to increased endoplasmic stress leading to apoptosis and reduction in miRNA levels	(48)
A-to-I RNA editing writer, *Adar2*	neonatal rat cardiomyocytes, cardiomyocyte-specific *Adar2* overexpresing mice	negatively regulates mature *miR-34a* to protect murine hearts from acute myocardial infarction	(51)
m5C writer, *Trdmt1* (*Dnmt2*)	*Dnmt2* mutant mice	results in cardiac hypertrophy possibly due to decreased methylation and	(57)

		increased dissociation of small nuclear RNA from P-TEFb complex	
m5C writer, *Nsun4*	muscle-specific *Nsun4* conditional knockout mice	methylates 12S rRNA and forms a complex with MTERF4 to regulated mitoribosomal assembly	(58)
m5C writer, *Nsun2*	TALEN-mediated *Nsun2* knockout rats	methylates *Icam1* mRNA to promote its translation	(59)

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