Peer Review File

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Reviewer A

Comment 1: *"Although expression levels of most circRNAs are low, less than 10% of their cognate linear transcripts [13], recent studies have confirmed several circRNAs are at higher expression levels compared with cognate linear counterparts". Some brief discussion would be helpful to this point.*

Reply 1: Thank you for the comment. In general, back-splicing has a much lower efficiency than canonical splicing in most human gene loci. However, recent studies have confirmed a subset of circRNAs have higher expression levels than their cognate linear counterparts. One possible explanation is that the expressions of these circRNAs are independent of their linear isoforms. In addition, a high Pol II transcription elongation rate (TER) could boost back-splicing and impede linear splicing, partially accounting for the higher expression levels of several circRNAs.

Changes in the text: We added some discussions as described above and relevant references (see Page 4-5, line 86-92).

Comment 2: *"It would be very helpful if the author explain how the process of circRNA biogenesis is regulated and what factors regulate this process.*"

Reply 2: Thank you for the comment. Only one mechanism is not sufficient to explain the biogenesis of all circRNAs. It is a combination of cis-acting elements and trans-acting proteins that control or regulate the occurrence of back-splicing and biogenesis of circRNA in physiological conditions. Moreover, the kinetics of back-splicing can be altered in some pathological conditions, such as inflammation, tumor, or cardiovasular disorders.

Changes in the text: We have modified our text as described above (see Page 7, line 136-140).

Comment 3: *"Some abbreviation should be expanded when used for the first time in the text. For example CDR1as – in page 6 line 127 and check throughout the manuscript. Also, CDR1as,*

sometime written in large letters and in other places in small letters, please be consistent throughout the manuscript."

Reply 3: Thank you for the comment. We are sorry for the carelessness. The full name of CDR1as is cerebellar degeneration-related 1 antisense transcript. CDR1as in human was written in large letters; in mice was written in small letters. Moreover, we have checked and added the full names of other genes or proteins when used for the first time in the text throughout the manuscript.

Changes in the text: We have added the full name of CDR1as (see Page 8, line 159).

Comment 4: *"Of note, the expressions of most circRNAs are not sufficient to adsorb miRNAs and antagonize the effects of miRNAs. Moreover, circRNAs are shorter in length and do not contain many binding sites of miRNAs. Furthermore, most of miRNAs localize in the cytoplasm, while a large part of circRNAs are located in the nucleus" – the mechanisms and stoichiometry of the association between circRNAs and miRNAs should be briefly discussed as there are some concerns that under normal physiological conditions the low abundance and cellular localization (of either) may not be conducive for this to occur for most.*

Reply 4: Thank you for the comment. As you concerned, the low abundance and cellular localization may not be conducive for this to occur for most circRNAs. The model of miRNA sponge is only available for such circRNAs with abundant expression levels or binding sites of miRNAs, for which the most typical examples are *CDR1as* and circular *Sry*. Nevertheless, we have no evidence to deny that administering a therapeutic dose of exogenous circRNAs *in vivo* based on the model of miRNA sponge may be promising for diseases treatment.

Changes in the text: We added some discussions as described above (see Page 8, line 170-174).

Comment 5: *"In page 8 line 172 – obesityassociated protein (FTO) should be written as obesity-associated protein (FTO).*"

Reply 5: Thank you for the kind reminder. We have corrected this error in the revised manuscript.

Changes in the text: We have corrected this typo error (see Page 10, line 210).

Comment 6: *In page 9 – line 186 – "stabilized downstream HMGA2 mRNA" what is the full name of HMGA2? also, check others throughout the manuscript.*

Reply 6: Thank you for the comment. We have added the full name of HMGA2 and checked others throughout the manuscript.

Changes in the text: We have added the full name of HMGA2 (see Page 11, line 224).

Comment 7: "*I suggest the author to have a summary table includes all techniques and methods used for screening and validation of circRNAs, this table can also include the advantage, disadvantage and the limitation of these techniques and what is special or unique for circRNA?*"

Reply 7: Thank you for the comment. We agree with you and believe that a summary table includes all techniques and methods used for screening and validation of circRNAs is very conductive for the readers. However, considering the space limitation and that the revised manuscript focuses on the roles of circular RNAs as potential biomarkers and therapeutic targets for cardiovascular diseases, we added the section "circRNAs as diagnostic biomarkers for CVDs" and enlarged the section "circRNA-based therapeutic strategies for CVDs" through intensifying literature searching and adding Table 2 to list circRNA-based therapeutic strategies for CVDs and their advantages and disadvantages.

Changes in the text: We have added Table 2 to list circRNA-based therapeutic strategies for CVDs and their advantages and disadvantages (see Page 24, line 507-508).

Comment 8: *"circRNAs are resistant to the degradation of Ribonuclease R (RNase R) – please explain how circRNAs are degraded and eliminated."*

Reply 8: Thank you for the comment. Recent studies have suggested several endonucleases are responsible for the degradation of circRNAs. Cytoplasmic endonuclease RNase L can globally degrade circRNAs; ATP-dependent RNA helicase upstream frameshift 1 (UPF1) and its associated endonuclease G3BP1 can target and degrade highly structured circRNAs; ribonuclease complex RNase P/MRP can degrade m6A-modified circRNAs. Furthermore, Hansen et al. found the decay of *CDR1as* depends on miR-671 binding and subsequent

Argonaute 2 (AGO2) cleavage. More detailed mechanisms are needed to explain the decay of circRNAs and their abundance regulation.

Changes in the text: We have added above-mentioned descriptions and relevant references in the revised version (see Page 7, line 143-150).

Comment 9: "*There are a few typos (such as myocardial infarction (MI) ingury model) – ingury should be injury and check others.*"

Reply 9: Thank you for the comment. We are sorry for this typing error and checked others throughout the manuscript.

Changes in the text: We have corrected this typing error (see Page 14, line 299).

Comment 10: "*Figure 1 should include all the abbreviation in the figure legend.*"

Reply 10: Thank you for the comment. We have added all the abbreviations in the figure legend of Figure 1.

Changes in the text: We have added all the abbreviations in the figure legend of Figure 1 (see Page 32, line 776-781).

Reviewer B

Comment 1: *"On the other hand, the focus on CVD, derived from the title, only sets in rather late in the manuscript and should be included more rigorously from the beginning on."*

Reply 1: Thank you for the constructive comment. As you suggested, we added some descriptions on CVDs from the beginning on. In addition, we also added some descriptions on current diagnostic and therapeutic strategies in the section of "circRNAs as diagnostic biomarkers for CVDs" and "circRNA-based therapeutic strategies for CVDs", respectively.

Changes in the text: We added some descriptions on CVDs as described above and relevant references (see Page 3, line 60-68; Page 19, line 393-396; Page 20, line 420-423).

Comment 2: *"In the currently dedicated sections focusing on specific circRNAs in CVD, there*

seems to be a lack of structure; i.e. circRNAs seem to be randomly listed and their reported association with are mentioned, while there is no structure based on i.e. disease or potential clinical application (i.e. biomarker).”

Reply 2: Thank you for the comment. As you suggested, we rearranged the section “circRNAs in CVDs” based on different disease type of CVDs, which improves the structure of the section.

Changes in the text: We rearranged the section “circRNAs in CVDs” (see Page 14-18, line 297-381).

Comment 3: “*Overall, the manuscript attempts to address an important question regarding the potential clinical application of circRNAs in CVD. The current version of the manuscript, on the other hand, needs amending with a more pronounced focus on CVD on the one hand and rigorous structuring according to a defined theme such as i.e. according to CVD disease entities or different fields of clinical application.*”

Reply 3: Thank you for the comment. As you suggested, we added more content on CVDs, including a general introduction and current approaches to diagnosis and treatment. In addition, we rearranged the section “circRNAs in CVDs” based on different CVD disease entities to improve the structure. Moreover, to focus on different fields of clinical application of circRNAs, we added the section “circRNAs as diagnostic biomarkers for CVDs” on the basis of existed section “circRNA-based therapeutic strategies for CVDs”. We believe these two parts may provide new insights for the diagnosis and treatment of CVDs in the future.

Changes in the text: We added more content on CVDs (see Page 3, line 60-68; Page 19, line 393-396; Page 20, line 420-423) and the section “circRNAs as diagnostic biomarkers for CVDs” (see Page 19-20, line 392-417). In addition, we rearranged the section “circRNAs in CVDs” based on different CVD disease entities (see Page 14-18, line 297-381).

Comment 4: *“The current separation of the manuscript into ‘cardio-protective’ vs. ‘cardio-destructive’ circRNAs should be abolished since there is no scientific evidence for the correctness of this approach.”*

Reply 4: Thank you for the comment. As you suggested, we have abolished the terms “cardio-destructive” and “cardio-protective”, and rearranged the section “circRNAs in CVDs” based

on different CVD disease entities. In addition, we revised Table 1 according to the new version of section "circRNAs in CVDs".

Changes in the text: We modified our text (see Page 14-18, line 297-381) and Table 1 (see Page 18-19, line 383-390).

Comment 5: *"Throughout the manuscript, I suggest to include more conclusive statement, putting the highlighted, referenced citations into context with each other and ideally provide additional interpretation of the results; this would drive the manuscript away from a mere listing of references towards a more scientific critical discussion and interpretation."*

Reply 5: Thank you for the constructive comment. We have added more conclusive statements and provided additional interpretation of the references involved throughout the manuscript.

Changes in the text: We added more conclusive statements (see i.e.Page 7, line 136-140 and Page 8, line 170-174).

Comment 6: *"Finally, the title of the manuscript suggests that the main focus will be on circRNAs and their potential as therapeutic target. In this respect the provided information on therapeutic options is rather limited. Therefore, my further suggestion would be to either intensify literature search and provide more and deeper information on this subject and/or broaden the focus away from therapeutic targets. Additionally, I would wish for more details and critical evaluation of the listed therapeutic options in circRNA research and how to overcome the mentioned obstacles in this respect."*

Reply 6: Thank you for the comment. In the current manuscript, we intensified literature search and provided more and deeper information on the section "circRNA-based therapeutic strategies for CVDs". In addition, we evaluated the advantages and disadvantages of the listed therapeutic options, summarized them in Table 2, and further proposed possible solutions to overcome the mentioned obstacles. Moreover, we added a new section "circRNAs as diagnostic biomarkers for CVDs" and changed the title to "*A narrative review of circular RNAs as potential biomarkers and therapeutic targets for cardiovascular diseases*", broadening the focus not only on therapeutic targets and proposing different fields of clinical application.

Changes in the text: We added some points as described above (see Page 20-24, line 420-505

and Page 19-20, line 392-417) and Table 2 (see Page 24, line 507-508).

Comment 7: *"Finally, at the current stage, the first sentence of paragraph 7: 'Recent studies have revealed the involvement of circRNAs in the pathogenesis of CVDs.' Should be emphasised and put more focus on throughout the manuscript."*

Reply 7: Thank you for the comment. In the revised manuscript, we added more content on CVDs, including a general introduction and current approaches to diagnosis and treatment. In addition, we enriched different fields of clinical application of circRNA in CVDs. We believe the current version highlights the roles of circRNAs in CVDs, not only in the pathogenesis of CVDs, but also as potential biomarkers and therapeutic targets.

Changes in the text: We added some content as described above (see Page 3, line 60-68; Page 19, line 393-396; Page 20, line 420-423; Page 19-20, line 392-417; Page 20-24, line 420-508).

Comment 8: *"In line 66 the authors use the terms 'cardio-destructive' and 'cardio-protective'. I suggest to paraphrase, given that biomolecules are unlikely to be per se 'destructive'. This is even more important in paragraph 5.1 – I suggest not to define the listed circRNAs as 'cardio-destructive'."*

Reply 8: Thank you for the comment. We abolished the terms "cardio-destructive" and "cardio-protective", and rearranged the section "circRNAs in CVDs" based on different CVD disease entities.

Changes in the text: We modified our text as described above (see Page 14-18, line 297-381).

Comment 9: *"The sentence in lines 131-134 does not make sense from my understanding. Please revert/explain."*

Reply 9: Thank you for the comment. The expressions of most circRNAs are not sufficient to adsorb miRNAs and antagonize the effects of miRNAs. Moreover, considerable amounts of circRNAs are shorter in length and do not contain many binding sites of miRNAs. Furthermore, most of miRNAs localize in the cytoplasm, while a large part of circRNAs are located in the nucleus. Therefore, the model of miRNA sponge is available for such circRNAs with abundant expression levels or binding sites of miRNAs, for which the most typical examples are *CDR1as*

and circular *Sry*. We have modified our text as described above in the revised manuscript. Given that circular *Sry* is one of the typical examples for miRNA sponge, we consider the description about *Sry* is conductive to strengthen our view.

Changes in the text: We modified our text (see Page 8, line 170-172).

Comment 10: *"There is no explanatory part for Figure 2 legend, only short terms – please add."*

Reply 10: Thank you for the comment. We rewrote Figure 2 legend with more explanatory details.

Changes in the text: We have modified our text (see Page 32, line 784-789).

Comment 11: *"Please put more emphasis on cardiovascular disease from the beginning on; i.e. in 3.3 you briefly mention 'cardioprotective effect' at the end. I suggest to enlarge such paragraphs with respect to CVD in order to better lead the reader, interested in CVD."*

Reply 11: Thank you for the comment. In the revised manuscript, we added more content on CVDs, including a general introduction and current approaches to diagnosis and treatment. In addition, we enriched different fields of clinical application of circRNA in CVDs. We believe the current version highlights the roles of circRNAs in CVDs, not only in the pathogenesis of CVDs, but also as potential biomarkers and therapeutic targets, which accords with the title well.

Changes in the text: We added some content and relevant references (see Page 3, line 60-68; Page 19, line 393-396; Page 20, line 420-423; Page 19-20, line 392-417; Page 20-24, line 420-508).