REPLY to EDITOR

12 March 2015/ 24 April 2015

Dear Professor Ji,

Please accept my sincere apologies for the very late return of the final revision of manuscript No. 10652.

Name of journal: *World Journal of Biological Chemistry*

One of the original authors in the initial paper submitted, Dr Jing Cheng, felt that his contribution was not significant and has asked to be removed as an author.

In regard to the comments of the reviewers, we have revised the text extensively, included the addition of significant additional discussion of the roles of miR-1 and miR-206 as well as content on the cellular processes affected by the myomiRs and their role in various diseases.

In particularly we have also increased the content on the roles of miR-1 and -206 in cell development and disease states. Sections relating to the roles of myomiRs in nerve development, fat cell development and stress/ immunological responses have also been expanded.

We have restricted the scope to the three cistons encoded miR-1, miR-133 and miR-206 as the literature and biology of these myomiRs alone is overwhelming; and these myomiRs cover much of the new roles of the myomiRs in cancers.

As suggested by reviewers, we have rearranged the section order extensively and changed many of the section headings to better present the material and to emphasize common aspects of the function of myomiRs.

Furthermore, the abbreviations have been rationalized in their use, and a long list of the definitions of abbreviations used in the main text, as well as additional abbreviations used the supplementary section are now included.

New surveys of expressed myomiRs in tissues determined by deep sequencing are also contrasted to the more numerous earlier studies undertaken using microarrays, and based in contrasting findings recommendations for future surveys are made.

One purpose of this review is to reflect on the emerging cellular roles of the classical myomiR microRNAs, miR-1, miR-133 and miR-206 families. These miRs were initially identified and associated with muscle development and associated disease, but more recently they are reported to be expressed in a deregulated manner in a wide variety of tissues in association with different cancers, where frequently the aberrant expression of the myomiR(s) is significant to cancer progression or metastasis.

We have rearranged the discussion of roles of the miRs in cancers, emphasizing common targets identified for the various myomiRs, particularly emphasizing common key cell factors with altered expression in several cancers, which are influenced in their expression by myomiRs, and for which a myomiR has been identified as associated with increased severity of the cancer. This rearrangement helps to identify common paths linking the myomiRs to disease development.

Finally, in contrast to the suggestion by one referee to reduce our discussion of cancers, we have instead added significantly more information and a more focused discussion. We have also added greatly to the references, with many more recent papers, to include the wider biology of these miRs and their roles in different cancers. In the Supplement section we present a broad overview of the wider literature in Table format.

Because of the extensive changes to the text, and the extensive additional material and the rearrangement of the section order of the main manuscript, we found it impractical to indicate these alterations. Please consider that all of the text is altered compared to the original submitted paper. For your convenience the lines of the main text are numbered, as requested by several reviewers.

Yours most sincerely,

For the authors:

Keith Mitchelson

Email: keith_mitchelson@hotmail.com

RESPONSE TO THE REFEREES:

May we thank the referees for their diligent reviews and very constructive and useful comments. Their summaries were broad in scope.

	Referee 1	Referee 2	Referee 3	
	00238092	***00057400***	***00063723***	
Classification Grade	C (Good)	D (Fair)	A (Excellent)	
Language evaluation	B (Minor language polishing)	C (a great deal of language polishing)	C (a great deal of language polishing)	
Conclusion	Major revision	Major revision	rejection	

REFEREE 1

Reviewed by 00238092

Comment 1: In this review manuscript, Mitchelson K et al. summarized recent findings on the potential contribution of myomiRs to various biological contexts. This reviewer strongly encourages the authors to assign page and line numbers throughout for a smooth reviewing process. This reviewer designated the title page as the 1st page.

Response 1: Line numbers have been added to the main text and figure legends.

Comment 2: The authors may need to include miR-499 in their detailed description as another major contributor to the muscle development and cardiac stem cell biology as well as to certain cancer progression/inhibition; this miR is encoded in a myosin gene and generally considered as a family member of myomiRs.

Response 2: Although miR-499 is important for cardiac stem cell biology, it has limited cancer biology at present. In the interests of space, we have continued to restrict this paper to the three canonical myomiR cistronic genes, the miR-1, miR-206 and miR-133 isomers, with significant expansion of the content about these miRs.

Comment 3: Besides, miR-133s dominates the overall discussion, which makes the manuscript unbalanced. Similar proportions should be spent to explore miR-1, 206, and 499, unless corresponding information is lacking on these miRs.

Response 3: The manuscript has been expanded considerably to explore the developmental cell biology and cancer biology of miR-1 and -206 more completely.

Comment 4: The subheadings are not well organized or helpful for the readers to track the contents. Please reconsider the whole structure.

Response 4: We have taken this comment to heart, and have rearranged contents and reorganized sub-headings extensively. The paper is still organized into two main sections: normal developmental cell biology of each of the myomiRs, followed by their cancer biology.

Comment 5: As minor comments, on page 2, regarding the gene locations, the chromosome numbers would be corresponding to those of mouse. This should be clarified.

Response 5: The chromosomal location of the three human myomiR cistrons has been confirmed from the NIH Gene database.

Comment 6: Sporadic typographical errors were found. As examples, on page 9, “which than” should read “which then”. On page 11, “Haem” should read “Heme”. Also, the typing font is not unified. Please thoroughly edit the manuscript prior to potential resubmission.

Response 5: We thank the referee for these detailed observations. We have corrected the spelling errors, unified the typing fonts and typographical faults as far as possible.

REFEREE 2

Reviewer 00057400

Comment 1: First of all thank you for your contribution to the understanding of microRNA in disease and development. I have read your manuscript with great interest. I though think that it needs some revisions. 1. Methodology: I miss a section that explains how you searched literature for this paper. What databases and keywords did you use in order to ensure a complete list of background literature?

Response 1: The primary search database used was PubMed. In brief, the search keywords included each the microRNAs, defined cells and tissues, named cancers, and specific genes. Key deregulated genes were independently searched by name in PubMed. The nomenclature of genes was also searched in the NIH Gene databases.

Comment 2. Language: I think that a revision of the manuscript focusing on the language could improve it and make the make it more readable. There are many interposed phrases and some sentences are very long, for example on page 14: “Interestingly, the increasing level of miR-133b as cervical cancers increase in metastatic potential parallels the association of decreasing levels in miR-1/ miR-206 with increased metastatic potential, discussed above, suggesting that the worsening change in the levels of these miR regulators increases the cellular progression towards cell proliferation and mobility and reduced apoptotic potential”.

Response 2: We have reviewed the manuscript and have simplified some complex sentences, refine the language and improved the grammar and syntax.

REVIEWER 3:

Reviewed by 00063723

We thank the reviewer for these helpful comments.

Comment 1: Major points: First of all, there is no page number the whole manuscript, bringing great reading inconvenience to reviewers. Each text line should be numbered consecutively from the start to the end of the manuscript. Secondly, the structure of the manuscript is not clear to be understood the content that the authors described and the subtitles need to be named again.

Response 1: The text has been line numbered and some subtitles have been renamed more appropriately.

Comment 2: The text form needs to be readjusted to a unified format and there are a number of grammatical errors and instances of badly worded/constructed sentences. Descriptive language is too cumbersome and complicated for reviewers to understand the exact meaning that the authors wanted to convey.

Response 2. The manuscript has been rewritten and the content simplified and reworked extensively. Care has been taken to explain cumbersome detail more simply and directly.

Comment 3: In terms of content, the types of cancer were listed too much, but the roles and mechanisms of myomiRs in the process of cancer and muscle development were not clarified and explained definitely. The physiological function of different myomiRs should be classified instead of putting together various functions of different myomiRs disorderedly.

Response 3: We thank the reviewer for these helpful comments. The purpose in listing the numerous cancers in which myomiRs play significant biological roles was to (1) show the broad relevance of these particular miRs to cancer biology, (2) to capture common deregulated targets in different cancers, and (3) to reveal the similarity in pathways affected in cancers to the pathways involved in normal cell processes under myomiR regulation. In the revised manuscript, the physiological roles of the myomiR targets in cancers have been unified. We have also attempted to identify common cellular factors altered in target cancers for which the myomiRs have an identified role.

Comment 4: Minor points: (1) The terms were not written in a unified form. For instance, miRNA, miRs, microRNA. (2) Text format was not written in a unified form. For instance, miR-133 and miR-133

Response 4. Terms have been unified throughout the text. Some referenced work by

authors have not distinguished miR-133a and miR-133b, hence miR-133 is used where appropriate, as well as the individual isomers in other instances.

Comment 5: (3) Grammatical mistakes. For instance, Yin and Poss (2008) found miR-133 controls complex biological processes involving formation and function of the regeneration blastema. (4) Badly worded/constructed sentences. For instance, The miRs -1 and -206 have closely homologous sequences and target some genes in common, as well as other independent targets. The discovery that miRs also impact on the regenerative capacity of mammalian tissues also provides insights into tissue degenerative processes that occur if the normal regulation of these factors is altered.

Response 5: The text has been extensively rewritten and care has been taken to eliminate grammatical mistakes and to provide simpler, clearer descriptions.

Comment 6: (5) Abbreviated terms can use abbreviations when mentioned again.

Response 6: In the revised manuscript the abbreviations used are defined and listed. These abbreviations are then used in the main text and throughout the supplement. Additional abbreviations found only in the Supplement are also defined there.

REVIEWER 4

Reviewed by 00238092

Comment 1: In this review manuscript, Mitchelson K et al. summarized recent findings on the potential contribution of myomiRs to various biological contexts. This reviewer strongly encourages the authors to assign page and line numbers throughout for a smooth reviewing process. This reviewer designated the title page as the 1st page. The authors may need to include miR-499 in their detailed description as another major contributor to the muscle development and cardiac stem cell biology as well as to certain cancer progression/inhibition; this miR is encoded in a myosin gene and generally considered as a family member of myomiRs. Besides, miR-133s dominates the overall discussion, which makes the manuscript unbalanced. Similar proportions should be spent to explore miR-1, 206, and 499, unless corresponding information is lacking on these miRs. The subheadings are not well organized or helpful for the readers to track the contents. Please reconsider the whole structure. **As minor comments, on page 2, regarding the gene locations, the chromosome numbers would be corresponding to those of mouse. This should be clarified**. Sporadic typographical errors were found. As examples, on page 9, "which than" should read "which then". On page 11, "Haem" should read "Heme". Also, the typing font is not unified. Please thoroughly edit the manuscript prior to potential resubmission.

Response: The points mentioned by reviewer 00238092 have each been addressed in the revised manuscript.

Regarding the stated chromosomal location of the myomiRs. Referring to the US national institute of health, data base [http://www.ncbi.nlm.nih.gov/gene/] the stated locations of the human and mouse myomiR genes are as follows. In the revised manuscript we present the location of the human genes and state this clearly.

Human gene	Human chromosome		Mouse gene	Mouse chromosome
Hsa-miR-1-1	20q13.33		mmu- miR-1-1	2
Hsa-miR-133a2	20q13.33		mmu-miR-133a2	2
Hsa-miR-1-2	18q11.2		mmu-miR-1-2	18
Hsa-miR-133a1	18q11.2		mmu-miR-133a1	18
Hsa-miR-206	6p12.2		mmu-miR-206	1
Hsa-miR-133b	6p12.2		mmu-miR-133b	1