Round-1

University of Thessaly
School of Health Sciences
Faculty of Medicine
Department of Biology

Panepistimiou 3 (Biopolis), 41500 Larisa
Tel: 2410685624-5570 Fax. 2410685549
www.med.uth.gr biologymed@uth.gr

Dr VARVARA TRACHANA
Ass. Professor of Cell Biology
Department of Biology, Faculty of Medicine
School of Health Sciences, University of Thessaly
41550, Biopolis, Larisa, Greece
Email1: vtrachana@med.uth.gr
Email2: vtrachana@gmail.com

Larisa, May 1st, 2021

Dear Professor Tong Cao,

Editor-in-Chief, *World Journal of Stem Cells (WJSC)*

Please find enclosed our revised manuscript entitled **"Stem cells' centrosomes: How can the organelles identified 130 years ago contribute to the future of regenerative medicine?"** by Goutas and Trachana, that we would like to resubmit for publication by the "*World Journal of Stem Cells (WJSC)*".

Also find below our comprehensive **point-by-point-reply** to the valuable reviewers' comments that accompanies our revised manuscript.

I look forward to hearing from you in due course.

Yours sincerely,

Varvara Trachana

Point-by-point reply to Specific Comments to Authors

(Reviewers' comments in italics/ numbers in reviewer's comments were added by authors for clarity/Changes in the revised version of the manuscript are tracked and extended changes are highlighted in yellow/text that addresses reviewers' comments also highlighted in yellow)

- **Reviewer 1-SPECIFIC COMMENTS TO AUTHORS**

1. *In general, the review is innovative in terms of ideas and topics, and the authors have summarized the existing studies and presented their own views, which have some significance to the field. However, I would like to make some suggestions.*

We would like to thank the reviewer for the overall positive comments for our manuscript. Below we give a point-by-point reply to their comments and suggestions.

2. *As a review, it is certain that the literature published in recent years needs to be cited enough. And in this article, it is suggested that authors need to update the literature.*

The reviewer is right. We have now kept older research to the absolute necessary minimum, and added literature published in recent years (preferably the last 5 years). Pease see revised text throughout the manuscript and revised reference list.

3. *The format of the article I suggest needs to be changed. There are too many paragraphs under the headings, for example, "CENTROSOMES AND ASYMMETRIC STEM DIVISION", so I suggest to create subheadings for discussion to make the organization and format clearer.*

That is a very helpful comment. Based on the reviewer's suggestion we have now added subheadings. Please see revised manuscript, especially the section "CENTROSOMES AND ASYMMETRIC STEM CELL DIVISION".

4. *There are some linguistic errors in the article, so I suggest the authors to check and correct them.*

We have checked and corrected all linguistic errors that we were previously missed. Please see revised manuscript. Thank you for this comment.

- **Reviewer 2-SPECIFIC COMMENTS TO AUTHORS**

The review focuses on centrosomes and centrosome primary cilium control the asymmetric cell division of stem cells. The authors also hint that in future this organelle would play a critical role in regenerative medicine. Major comments:

1. *I ran the through plagiarism checking software turnitin, it showed a similarity index of 84% and the software flagged the document for the word replacements that were done in the document to reduce similarity index. The submitted documents is 84% identical to one student paper submitted in University of Thessaly. I give benefit of doubt that one of the authors themselves would have submitted the paper to University of Thessaly. However, it is unethical to submit same paper to two different journals for publication. Authors should give an explanation regarding the Similarity index (SI).*

   As the reviewer already assumed, it is indeed our manuscript that *I* put it on Turnitin, as I usually do for review articles, especially when I have co-authors. As it obvious by the file submitted by the reviewer, it is our manuscript that he found similarity with on Turnitin (the file has our names and the name of the *only* journal that we have submitted the paper to: World Journal of Stem Cells). The University of Thessaly is the university I work, and I used my university account to perform the plagiarism check on Turnitin. I have now deleted the manuscript from Turnitin repository (and will be permanently deleted after a certain period based on Turnitin policy). I am sorry if we have caused some kind of inconvenience.

2. *The title seems misleading, authors address the centrosomes are 130 years old, however were identified 130 years ago, but the organelle is much older than 130 years. So kindly modify the title.*

   Thank you for this comment. The title has been changed accordingly. Please see revised manuscript (please also note that all changes in the revised manuscript are highlighted in yellow).

3. *Page 6, the authors mention that role of centrosome based on review by Januschke et al 2014, Semin Cell Dev Biol. However, this review by Januschke et al was based on original articles. The researchers who uncovered role of centrosomes need to be credited and cited. There are several instances, where authors should cite original articles and not just reviews.*

The reviewer is right. On several occasions we opted in citing review articles, in order for our readers to be able to find as many articles as possible covering an issue. But, following the reviewer's suggestion in the revised manuscript we have added citation of mainly original articles (see revised text throughout the manuscript and revised reference list). As for the specific work mentioned here by the reviewer, that was cited previously as "Januschke et al 2014, Semin Cell Dev Biol", we have corrected it as follows: "Several studies over the last 20 years have revealed the, previously unappreciated, multifaceted role of centrosomes in interpreting signals from the extracellular as well as the intracellular environment that govern cellular asymmetry (Piel et al., 2000; Yamashita et al., 2007; Conduit and Raff, 2010; Poulson and Lechler, 2010; Williams et al., 2011; Das and Storey, 2012; Shitamukai and Matsuzaki, 2012; Januschke and Näthke, 2014)". **Please see, page 5, lines 176-177, page 6, lines 184-185**.

4. *The authors have not described the mechanisms by which centrosomes asymmetric cell division is established. It would help the reader immensely, if authors can incorporate it detailed mechanisms, since the focus is on centrosome and how if affects stem cell function and division.*

We would like to thank the reviewer for this suggestion. We believe that the manuscript is benefit drastically by adding the detailed description of biased centrosome segregation that was previously missing. **Please see page 11, lines 382-400.**

5. *The studies on asymmetric cell division are mostly from drosophila, since the focus of the review is regenerative medicine, I would assume authors allude to regenerative medicine in humans. In that case, studies who have investigated role of centrosome in asymmetric cell division in mammalian system should be cited.*

We find this comment very important. Studies in Drosophila were included so that we present how most of the important advances on the topic were originally discovered in this organism. But, based on the reviewer's suggestion, we have now included more recent information on mammalian systems and especially added research performed in human cells where possible. **Please see paragraphs added, page 12 (the whole page), page 13, lines 448-461. Also see page 17, lines 642-649, page 18, lines 653-654 and page 18, lines 658-666, page 20, lines 745-750, page 23, lines 838-847.**

Also note that some of the studies included in the previous version of our manuscript were actually performed in human cells, but we haven't mentioned it. This is now corrected.

6. *Authors have given generalized function of centrosomes, but what protein constitute the centrosome needs to be mentioned. In addition, the studies (drosophila/chick/mice) that have performed knockout/mutations/knockdown of the proteins that make up centrosomes would really make the case for role of centrosomes much stronger.*

We share the reviewer's view on the importance of providing information on the role of specific centrosome proteins and tried to include relevant research (see revised text throughout the manuscript). Also, as a matter of fact, an extended paragraph provides exactly this type of information: Please see highlighted text: "As a matter of fact, more direct evidence supporting this asymmetric nature is accumulating recently, as the molecular composition of the centrosomes is being revealed. Several proteins...and this phosphorylation is important not only for centrosome asymmetry but also for proper mitotic spindle positioning (Gambarotto *et al.*, 2019)", **page 15, lines 551-576 and page 16, lines 582-583.** Moreover, more research on specific related protein has been added to the revised manuscript, such as the research that addresses the issue of in situ co-translation of mRNAs of key centrosome proteins (**please see page 12, lines 439-443, and page 13, lines 448-461)** and the asymmetry in protein composition between mother and daughter centriole **(please see page 11, lines 374-381).**
Also note that in the section "PRIMARY CILIA AND STEM CELL DIFFERENTIATION" most of the studies cited have performed knockout/mutations/knockdown of cilium specific proteins in order to demonstrate their role in stem cell differentiation.

7. *Authors cited Vestergaard et al 2015, as evidence that OCT4, NANOG and SOX2 co-localized to the cilia. Surprisingly, OCT4, NANOG and SOX2 signal was clearly in nucleus, they show only one cilia per cells, which is curious. Authors have not discussed the strong and weak points of such studies, and I request authors to do the same.*

We think that the research by Vestergaard et al. is an important study, but the reviewer is right about its limitations. The fact that the authors show the localization of NANOG and SOX2 only on one cilium of one cell and not showing more cells (by adding, for example, pictures at smaller magnification) is problematic. We speculate that this is due to the fact that

this localization signal was observed -as mentioned by the authors- only on a percentage of cilia and not all of them. We thank the reviewer for this suggestion and we now discuss this issue in a paragraph that was added to the revised manuscript. **Please see, page 18, lines lines 658-666.**

8. *Overall the manuscript seemed like a literature review, the authors have not critically discussed some key references and their results, this review does not ask questions such as how centriole or centrosome proteins are regulated, how are the centrioles duplicated prior to cell division. Reviews also should highlight key questions that need to be investigatesd and what are the ways the critical questions can be addressed, hence, authors need to incorporate these suggestions.*

We share this view with the reviewer. For key issues such as the centriole duplication, for example, information was included: **please see highlighted text in page 7, lines 224-241 and also Figure 1 and Figure legend 1.** As for specific centriole/centrosome proteins please see our answer to comment 6. We have tried to address as many of the important issues related to our theme as possible, without making the manuscript too long. Also, we included, based on the reviewer's suggestion, comments on critical questions that remain answered (**please check revised manuscript, page 13, lines 458-461, page 14, lines 511- 513, page 14, lines 517-518, page 14, lines 524-526, page 18, lines 661-666**). In general, as explained in detail under all the reviewer's previous comments, we tried to address all their suggestions/concerns, and we hope that the manuscript is now raised to their standards.

Round-2

University of Thessaly
School of Health Sciences
Faculty of Medicine
Department of Biology

Panepistimiou 3 (Biopolis), 41500 Larisa
Tel: 2410685624-5570 Fax. 2410685549
www.med.uth.gr biologymed@uth.gr

Dr VARVARA TRACHANA
Ass. Professor of Cell Biology
Department of Biology, Faculty of Medicine
School of Health Sciences, University of Thessaly
41550, Biopolis, Larisa, Greece
Email1: vtrachana@med.uth.gr
Email2: vtrachana@gmail.com

Larisa, August 3rd, 2021

Dear Dr Chen-Chen Gao,
Science editor, *World Journal of Stem Cells (WJSC)*

Please find enclosed our revised manuscript entitled **"Stem cells' centrosomes: How can the organelles identified 130 years ago contribute to the future of regenerative medicine?"** by Goutas and Trachana, that we would like to resubmit for publication by the "*World Journal of Stem Cells (WJSC)*".

Also find below our comprehensive **point-by-point-reply** to the valuable reviewer's comments that accompanies our revised manuscript.

I look forward to hearing from you in due course.

Yours sincerely,

Varvara Trachana

Point-by-point reply to Specific Comments to Authors

(Reviewers' comments in italics/ numbers in reviewer's comments were added by authors for clarity/Changes in the revised version of the manuscript are tracked are highlighted in yellow.

Reviewer

1) *The authors have improved the manuscript but there are some minor issues that need to be addressed.*

   We would like to thank the reviewer for the overall positive comments for our manuscript. Below we give a point-by-point reply to their comments and suggestions.

2) *I feel that figures do not depict what the authors have described in the review. The figures seem crude representation. I request authors to provide better quality illustrations.*

   The figures that are included in our manuscript are there in order to provide a general description of the centrosome cycle (Figure 1) and the basic organisation of the primary cilium (Figure 2). Their main purpose is to depict the basic structures (i.e., the mother and the daughter centriole, the appendages or the axoneme) so that the information provided, or claims made in the text are easy to be followed by all the readers. We provide better quality figures (in a power point file), as requested.

3) *There are some sentences with spelling errors and grammatical errors and these will require rectification for example line no 396,416,425,428,451,455,526,660 and 843.*

   All the above-mentioned errors have been addressed. Note that line numbers have been changed as follows:

   396 =>345
   416=>356
   425=>365
   428=>368
   451=>391
   455=>395
   526=>450
   660=>560
   843=>724

   All the above changes are highlighted in yellow in the revised manuscript. The manuscript has been sent to a native English speaking expert for further polishing the text (highlighted by track changes).