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PEER-REVIEW REPORT

Name of journal: World Journal of Stem Cells

Manuscript NO: 57270

Title: Enrichment of retinal ganglion and Müller glia progenitors from retinal organoids derived from human induced pluripotent stem cells - possibilities and current limitations

Reviewer's code: 02934076
Position: Peer Reviewer
Academic degree: PhD
Professional title: Doctor
Reviewer's Country/Territory: India
Author's Country/Territory: Denmark

Manuscript submission date: 2020-06-11
Reviewer chosen by: AI Technique
Reviewer accepted review: 2020-06-11 10:12
Reviewer performed review: 2020-06-12 06:16
Review time: 20 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [Y] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[ ] Yes [Y] No
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

This study reports the generation of retinal organoids from human iPSC by adapting a method reported earlier in 2014. The protocol seem to work well and generates authentic appearing retinal organoids within 56 days, for downstream applications. This can be appreciated in Fig 1. However, there are many concerns with respect to the characterizations done, which needs more rigor. Specific comments: For organoids: Fig 2 1. IHCs are sub-optimal, as stated by the authors themselves. Apart from trying tissue clearing, it would have been simpler to take sections for IHCs. This doesn't require any special chemical treatments. Even muller processes across the retina can be clearly visualized. Fig 2F shows a beautiful optic cup with RPE margins, as confirmed by TEM. It will be useful to give the experimental replicates assessed. For MACS enriched cells: Fig 3 1. ICC with multiple markers will be required. Also, it can be clubbed with RT-PCR assay. 2. Enrichment efficiency need to be checked by FACS or by comparing the positive and negative cell pools. 3. Chx10 can't be cytosolic and overlap with Nestin. Infact they are exclusive. Anti-Chx10 staining may be just the background. 4. Was the sorting efficiency 100% that all the cells in the field are positive for all the markers tested? Either the antibodies have non-specificity or the markers chosen are not-specific to RGC & Muller glia. Overall, it is an useful study, but requires rigorous characterization of retinal organoids and isolated RGCs/Muller glia. General comments: 1. Title is not appropriate and should be revised. Retinal neurodegeneration is diverse, while the authors are focusing on RGC pathology in this MS. Retinal organoid protocol development, their characterization and an RGC isolation method has formed the main body of the manuscript. It doesn't explain any possibilities or limitations. 2. Why referencing Fig 2A later than 2C. Better rearrange the figure order otherwise.

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PEER-REVIEW REPORT

Name of journal: World Journal of Stem Cells

Manuscript NO: 57270

Title: Enrichment of retinal ganglion and Müller glia progenitors from retinal organoids derived from human induced pluripotent stem cells - possibilities and current limitations

Reviewer's code: 02595715
Position: Peer Reviewer
Academic degree: DSc

Professional title: Professor

Reviewer's Country/Territory: Russia

Author's Country/Territory: Denmark

Manuscript submission date: 2020-06-11

Reviewer chosen by: AI Technique

Reviewer accepted review: 2020-06-13 09:41

Reviewer performed review: 2020-06-16 08:57

Review time: 2 Days and 23 Hours

Scientific quality	[Y] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[Y] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [ ] Major revision [ ] Rejection
Re-review	[ ] Yes [Y] No
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The msnuscript entitled “Retinal organoids derived from human induced pluripotent stem cells - possibilities and limitations for studying retinal neurodegeneration” by Freude et al., addresses very interesting issue of the pluripotent stem cells application to model diseases in 2D and 3D tissue formats. Manuscripts describes 3D retinal organoid culture and very simple and robust approach for Muller glia and retinal progenitors isolation using magnetic activated cell sorting. The paper is well and clearly written, title corresponds to the topic of the manuscript. All Figures have a very good quality supporting the accuracy of the data presented. However, I did not find what Pax6 antibody Authors used in their experiments.

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PEER-REVIEW REPORT

Name of journal: World Journal of Stem Cells

Manuscript NO: 57270

Title: Enrichment of retinal ganglion and Müller glia progenitors from retinal organoids derived from human induced pluripotent stem cells - possibilities and current limitations

Reviewer's code: 02446120
Position: Peer Reviewer
Academic degree: PhD

Professional title: Associate Professor, Doctor, Research Scientist

Reviewer's Country/Territory: Argentina

Author's Country/Territory: Denmark

Manuscript submission date: 2020-06-11

Reviewer chosen by: AI Technique

Reviewer accepted review: 2020-06-11 19:14

Reviewer performed review: 2020-06-17 00:22

Review time: 5 Days and 5 Hours

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

Comments to the authors The manuscript by Freude KK et al., demonstrates that it is possible to generate RGCs and Müller glial cells from retinal organoids obtained from human induced pluripotent stem cells, by following protocols previously established. The expression of specific markers for these cells in their retinal organoids, was confirmed by RT-PCR and immunocytochemical determinations. The use of magnetic-activated cell sorting (MACS) method, a useful top-notch technique, allowed the authors to isolate and expand both cell types from the organoids. In general, the manuscript represents a valuable piece of information which potentially could be useful for treating retinal diseases compromising survival of RGC such as Glaucoma Minor points 1) The abstract is not very well written, and confusing and should be rewritten. The phrase the authors wrote in the "aim" of the manuscript is much more precise; I would suggest expanding the phrase the authors wrote in the aim and use it as an abstract. 2) Relevant contributions of other authors to obtain retinal organoids (i.e: V. Canto Soler´s group) and about the crosstalk between MGC and retinal neurons should be included in the references.