September 23, 2013

Dear Editor,
We thank the reviewer for the useful comments. All points have been adduced according to the enclosed checklist and changes are highlighted in the revised version. We really hope that the manuscript can be now accepted for the publication.

Please find enclosed the edited manuscript in Word format (file name: 4279-review2.doc).

Title: Neural stem cells isolated from amyloid precursor protein-mutated mice for drug discovery

Author: Vito Antonio Baldassarro, Giulia Lizzo, Michela Paradisi, Mercedes Fernandez, Laura Calzà, Luciana Giardino

Name of Journal: *World Journal of Stem Cells*

ESPS Manuscript NO: 4279

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer
Peer reviewer 1
The manuscript entitled " Neural stem cells isolated from amyloid precursor protein-mutated mice: a tool for in vitro studies and drug discovery" by Dr. Baldassarro and coworker is a scientific paper that illustrates an in vitro model based of neural stem cells derived from transgenic animals useful for the study of pathological mechanisms of Alzheimer disease (AD) and for testing new molecules for a therapy. The author proposed Neural Stem Cells (NSCs), isolated from the subventricular zone (SVZ) of Tg2576 mice, as a new study tool that represent an appropriate AD in vitro model resembling some cellular alterations observed in vivo. Overall, this is a well-written and well-conceived manuscript. The work is original, well organized and coherent with the title proposal. The methods appear adequate and the paragraphs are complete and exhaustive. The organisation of results and graphs is well structured. The discussion focuses on the validation of the proposed model through the citation of relevant papers.

As is, the only major criticism is that the authors did not sufficiently argument why they selected the SVZ as neurogenesis brain region for obtaining neurospheres. Indeed emerging evidence indicates that altered neurogenesis in the adult hippocampus represents an early critical event in the course of AD. This point should be addressed more thoroughly in the introduction.

We agree with this suggestion. We have introduced arguments for SVZ as source of neurospheres, and this point is new presented in the introduction (page 6 lines from 4 to 14) and related refs have been introduced

Another (minor) point is that sometimes in the manuscript it is not so clear what kind of NSCs have been used for experiments (primary, secondary, undifferentiated, differentiated etc.) a diagram illustrating this point could help to better understand the used methods.

This point is also clarified at page 8 lines 8, 9 and 10.

3 References and typesetting were corrected

Peer reviewer 2

This manuscript characterized a potentially useful in vitro model for Alzheimer's disease. The authors isolated neural stem cells from the subventricular zone of Wild type and Tg2576 mice, and then studied the phenotypes of primary and secondary neurospheres. They found that primary, not the secondary, neurospheres derived from Tg2576 show a decrease in population doubling, and differentiated NSCs from Tg2576 show decreased MAP2+ and increased GFAP+ cells. In addition, a clear decrease in neurites number and length was also observed in differentiated Tg2576 neurons. Furthermore, microarray study found that 11 genes were up-regulated in Tg2576 NSCs.

The data reported in this manuscript are interesting, but the clinical implication is unclear. This is a problem, but actually is not my main concern. My real concern is the clinical relevancy of this model, because Alzheimer's disease is normally **NOT** considered as disease of neural stem cells.

This point has been clarified in the text (Page 13, line 24)

Minor concerns are:

1) Fig 1A needs a negative control to prove the specificity of 6E10.

Figure 1 was updated with the negative control (panel 1B)

2) Can authors explain why only primary, not the secondary, neurospheres derived from Tg2576 show a decrease in population doubling? Technical reasons?

We believe that the decrease in population doubling observed in primary neurospheres derived from Tg2576 animals is not due to technical reasons, starting from the fact that these data derive from independent experiments. Moreover, the difference in the final cell number obtained from individual animals seems to confirm this hypothesis (table 2).
A possible explanation for the difference between primary and secondary neurospheres was added in the text (Page 13, line 16).

3) Are you sure that the fig 2B&D are the pictures of MAP2 staining? They are more like the Tuj staining in fig 2A&C.

Yes, the figures 2B&D were correctly marked as MAP2 stained. We understand the reviewer's point: MAP2 immunostaining is usually punctate along axons, while we obtained a rather diffuse staining. This could be due to the very early differentiation time in our experiments, when MAP2 is synthetized but not yet organized as "microtubule-associated" protein

Thank you again for publishing our manuscript in the *World Journal of Stem Cells*.

Sincerely yours,

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