Reviewer's report

Title: Instructive role of gut-derived factors on ES cells differentiation.

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Reviewer: Miyako Takaki

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General comments:
This study shows that some factor secreted from LM-MP preparations of the gut can induce differentiation of ES cells into specific neuronal phenotypes in vitro system. However, in vivo system, grafted ES-NS failed to acquire a distinct neuronal phenotype at least 1 week following transplantation. From this result, the authors suggested the presence of local inhibitory influences that prevents ES-NS differentiation. This reviewer has an experience that the gut-like organ differentiated from ES cells transplanted into the host gut failed to survive. The reason for this seems to be deficiency of blood vessels. Therefore, the reason for the present failure to acquire a distinct neuronal phenotype does not seem to be simply the presence of local inhibitory influences.

Specific comments:
1) In introduction, the authors described that “Furthermore, several studies have also shown ES differentiation into neural crest cells, ------- (23-25).” The authors did not mention about Takaki et al’s study where in vitro formation of enteric neural network structure from neural crest cells by BDNF is performed in a gut-like organ differentiated from mouse embryonic stem cells. (Stem Cells 24: 1414-1422, 2006)
2) P13, L7: --with LM-MP (Fig 4D-I) and Fig 5A-D).>>>> This reviewer could not find Fig 4D-I.
3) P13, L19-20: Remarkably, expression of the glial marker GFAP was not observed in either group (LM-MP and control) by all analysis performed (data not shown). >>> If so, how to maintain neural cells without glial cells?
4) In Figure 7A, Dil-labeled cells are neurons or not? Because a failure on the expression of the neuronal markers and non-neuronal markers GFAP and alpha SMA by the engrafted ES-NS was revealed (Fig 7 B-D).
5) Figure 4: BetaIII-tubulin is used as a marker for immature and mature neurons. Which are immature neurons and mature neurons in Figure 4C?
6) In Figure 3 E and F, DAPI-positive nuclei exist in the smooth muscle cells. What are DAPI-positive nuclei in Figure 3A, B, C, D?
7) Figure 6: ES-NS with LM-MP expressed alpha SMA mRNA, but did not alpha SMA protein. How to explain this?
8) Figure 7: Please explain abbreviations used in this figure.
Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.