Reviewer’s report

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

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Reviewer: Simon Kenny

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MAJOR COMPULSORY REVISIONS

Title: does not fully inform the reader of the subject matter of the paper which is an evaluation of the utility ES cells as a potential source of cells for transplantation

Background: I take issue with the misleading statement that Hirschsprungs disease & pyloric stenosis results in a ‘severe impairment of digestive functions, for which conventional therapeutic approaches (surgical or pharmacological) are mainly palliative and associated with poor quality of life for patients and unacceptably high morbidity and mortality’. As a surgeon who does science I have a responsibility to perform science at the same level as any scientist. I would be grateful if scientists could return the favour. Firstly pyloric stenosis: the mean length of stay for a child after surgery for pyloric stenosis is 48 hours - children are discharged on full feeds and arent routinely followed up. The complication rate in experienced hands is less than 2% and mortality is thankfully a thing of the past. With regards to Hirschsprungs disease many children lead perfectly normal lives with continence that matches their peers. Sadly about 20% experience significant problems but modern surgical management means that mortality is rare. This is not a trivial matter - when for example contemplating stem cell based therapies the authors should fully appreciate that the risks associated with these therapies may outweigh potential therapeutic benefits. It is difficult to imagine that stem cell therapy will play a role in the treatment of pyloric stenosis when surgery is cheap, safe and effective.

Characterization of neurospheres

The authors use the term neurospheres to describe the cell clusters derived by culturing ES cells in non-adherent conditions in neurobasal medium. Expression of nestin is considered enough to merit the neurosphere label but it would be good to know what other markers are expressed at this stage. Are there p75 positive cells present? The TuJ1 neurons seen in Fig 2D-E look like neurons but a 1.8 fold increase is not massive. How does this level of expression compare with neurospheres cultured from eg brain? The lack of expression of GFAP is also worrying - did the authors check with RT-PCR?

RT_PCR Were the PCR products sequenced?

Coculture
This is potentially a very useful method and would shed light on the potential for smooth muscle to influence ENS formation via humoral factors. It is contaminated however by the presence of myenteric plexus - would it be possible to exclude this either by using congenitally aganglionic bowel or by ablating neuronal tissue? As it is there is potential for the neurons to suppress ES neuronal differentiation

Intrapyloric transplantation

Was proliferation assessed in transplanted cells? This would be reassuring to see. The authors quite rightly point out that longer time may be required for ES derived cells to mature to the point that fully differentiated neurons are present in the transplant (and in the coculture)

Figure 4 - It would be good to see PGP9.5 immuno alone as it doesn't look to extend to neurites

Summary

This is a potentially interesting and well conceived paper that is let down at present by poor characterization of putative ES derived neurospheres, the inclusion of myenteric plexus neurons in the coculture experiment, and by not following the behaviour of transplanted cells in vivo for a longer time. The title needs revisiting and the final conclusion that ES-NS may not be suitable candidates for ENS regeneration is in my view nihilistic - the only conclusion is that the methods used by the authors have not worked but I do not believe that they have exhausted this line of enquiry. Thank you for the opportunity to review this potentially excellent paper.

Level of interest: An article whose findings are important to those with closely related research interests

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