Reviewer’s report

Title: Human amniotic fluid stem cell injection therapy for urethral sphincter regeneration in animal model

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Reviewer: Arnulf Stenzel

Reviewer’s report:

Comments to the editor/authors:
The authors here present substantial data concerning in vitro analysis of human amniotic fluid stem cells (hAFSCs) and their in vivo behavior in mice. At first hAFSCs were isolated and analyzed for stem cell characteristics. Three different media were evaluated for myogenic differentiation of the afore-mentioned cells. In a mouse model of urinary incontinence hAFSCs were injected into the sphincter muscle and PCR, histological and immunohistological investigations were performed as well as urodynamics. Furthermore a new cell imaging method was tested in addition to immunogenicity and tumorigenicity evaluation.

As SUI treatment by periurethral injection of stem cells of different sources is a current topic in regenerative medicine and could offer new therapeutic options this manuscript is of considerable importance.

The title should be rephrased to make it sound more eloquent. The abstract is well structured with regard to contents but requires major linguistic editing. The content and structure of this paper are fine but it could be helpful to divide the methods more precisely into in vitro and in vivo parts. The presented tables and figures are well selected and substantial. However photographs of the surgical procedure should be added maybe in the expense of in vitro data.

All together the manuscript is well composed and presents considerable data but should undergo major linguistic revision including spaces and punctuation.

Abstract

• Discretionary Revisions: Please, rephrase/correct the following terms!
  1. “…received sham operation for a normal control group.”
  2. “…into subcapsular space of kidney …“
  3. “…resulted histologically and functionally similar …”

• Minor Essential Revisions:
  4. “The amniotic fluids were isolated …” -> Isn’t it rather the cells that were isolated? Please, clarify.
  5. “Optical images were followed. “ -> What kind of technique did you use? Please, clarify by adding more information and rephrase.
Background

- Discretionary Revisions: Please, rephrase the following terms!
  9. “SUI … are a common and embarrassing problem”
  10. “…can restore urethral sphincter histologically and functionally similar to normal.”

- Minor Essential Revisions: Please, rephrase/correct the following terms!
  11. “…remains as major problems.”
  12. “…most commonly used cell is bone marrow stromal cells.”
  13. “…share with embryonic stem cell property.”
  14. “…stemness and myogenic differentiation potency in vitro.”

Methods

In general, it should be made clear that not all the injected cells were labelled by RITC (but only in nine mice) and what kind of cells were injected (differentiated or not).

It has to be made clear how many animals served for which investigation (LPP/CP measurement, human nuclei IHC, CD8 IHC, real-time PCR, human fibroblast injection) and when the animals were sacrificed. This is quite confusing. [Minor Essential Revisions]

Maybe a table would be helpful. It could be helpful to divide this section clearly into in vitro and in vivo investigations! [Discretionary Revisions]

- Discretionary Revisions: Please, rephrase the following terms!
  15. “was taken as the LLP“
  16. “until visual identification of ceasing the leak”
  17. “immunohistochemical stain”

- Minor Essential Revisions: Please, rephrase/correct the following terms!
  18. “The amniotic fluid was obtained from 4 women …“ -> Meaning collected and pooled afterwards? How many fluids were investigated, separately? Please, clarify.
  19. “supernatant were discarded“
  20. “placed in petri dish“
  21. „to find optimum myogenic condition“
  22. „fibroblasts were served as a positive and negative control“
  23. “The 5-azaC and TGF-ß were treated …“
24. “into a myogenic lineage”
25. “received sham operation”
26. “externalsphincter”
27. “human nuclei-specific antibody were followedat”
28. “immunohistochemical”
29. “were anlalyzed”
30. “whether injection of hAFSCs induce host myogenic response”
31. “with the filters set”
32. “in SUI model”

Results
• Minor Essential Revisions: Please, correct the following term!
33. “ICC stain was confirmed that hAFSCs”

Discussion
• Minor Essential Revisions: Please, rephrase/correct the following terms!
34. “hAFSCs differentiated into muscle cells” -> Due to the lack of overall evident data (not only in this manuscript) that the cells turned into fully differentiated muscle cells instead of expressing only a certain kind of surface markers this phrase is the reviewers point of view to enthusiastic expressed! Please, rephrase this sentence to a bit more uncommon one.
35. “… source for various cell therapy and tissue engineering.”
36. “we could isolate homogeneous genotypic profile with a double sorting procedure”
37. “the expression level were similar“
38. “negative for hematopoietc stem cell marker”
39. “the expression …were gradually decreased”
40. “cell viability was significantly lower than CM treatment“
41. “were injected into animal“
42. “resulted normal-appearing sphincter muscle regeneration with time”
43. “hAFSCs did not made teratoma”
44. “Introduction of nanoparticle had no cytotoxicity“ -“ … in this setting” or something like this should be added!

Conclusion
• Minor Essential Revisions: Please, rephrase/correct the following terms!
45. “were able to differentiate myogenic lineage”
46. “paracrine effect of hAFSCs” -> This is speculative! Which here presented or already published data give conclusive evidence for that? Please, give a short
explanation to the audience or rephrase or delete.

Figures

In general, the reviewer suggests that all microscopic pictures should be scaled by bars and the written information about their sizes in the figure legends, instead of “200x” only. In this way the given magnifications should be checked carefully. [Discretionary Revisions]

Whenever the use of any antibody is stated the reader misses details about specific conjugates and isotypes as well as the corresponding information whether a primary or secondary antibody staining was performed! [Minor Essential Revisions]

• Discretionary Revisions:
47. Fig. 3B: The given magnification “200x” is misleading. Could you please check it once again?
48. Fig. 4C: Is this a necessary figure?

• Minor Essential Revisions: Please, rephrase/correct the following terms!
49. Fig.1: “were strong positive for mesenchymal markers”
50. Fig. 2C: For staining the positive control obviously another antibody setting (both anti-mouse?) was used. Why and what kind of antibody/conjugate were used? That is quite a bit confusing. Please clarify!
51. Fig. 3, legend: “immunohistochemical stain”
52. Fig. 6: This is the most interesting figure. It is urgently suggested that more details and explanations concerning the coloring of the sections and the special areas i.e. ‘atrophic sphincter’ should be given to let the readers retrace the authors’ conclusions! In addition a marking would be helpful.

Quality of written English: Needs some language corrections before being published

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.