

All changes have been marked in red highlight.

Reviewer #1

SPECIFIC COMMENTS TO AUTHORS

1. The authors describe three different types of antler stem cells and their role during antler development, but do not combine them with Figure 2 in the beginning of the section 3. It will be easier to understand if the three axes of the antler development could be described in chronological order.

Response: Thanks for your kind suggestion. We have combined Figure 2 with the description in section 3, and described them in chronological order. Please refer to new version.

2. At the end of Page 6, the authors describe “As for the antler itself, it has been confirmed to be regenerated from PPCs”. However, no references are cited here.

Response: Thanks! We have added the reference in the new version.

3. At the end of Page 7 and beginning of Page 8, Some of the mentioned markers that can be detected in AnSCs, including Tert, Nestin, S100A4, nucleostemin, and c-Myc, do not indicate what properties are the cells that specifically express these markers.

Response: Thanks for your kind comments. We have revised it to “Some of the ESC and other stem cell markers” in the new version.

4. The header of Section 4.1 “Wound healing is not a conventional scar in deer”, is unclear and difficult to understand. Please revise your manuscript for clarity and make sure concise and succinct statements are made.

Response: Thanks! We have revised it to “Wound healing in deer is a natural process”.

5. Please cite pictures in the correct place, especially Figure 2 and Figure 4.

Response: We have cited the picture in the correct places.

6. Please give some discussion of Stem Cell Res Ther. 2019 Nov 19;10(1):326.

Response: Thanks for your kind comments. We have added some discussion in the new version according to the AnSCs- CM article (Stem Cell Res Ther. 2019 Nov 19;10(1):326). “Interestingly, topical application of conditioned medium (CM) of AnSCs on cutaneous wounds can also effectively induce regenerative wound healing (Figure 3C). Content

analysis of AnSCs-CM by protein chromatography revealed that relative peak area of the AnSCs-CM was significantly larger than those of the two controls, DMEM and MSCs-CM. Besides, the AnSCs-CM had two extra peaks. ELISA tests showed that EGF concentrations in AnSCs-CM was significantly higher than that in MSCs-CM. Thus, AnSCs-CM, which contains more soluble components and growth factors, has great potential to be developed as a novel cell-free therapeutic approach for cutaneous wound healing.”

Reviewer #2

SPECIFIC COMMENTS TO AUTHORS

Dear Authors, We have read with interest your manuscript, discovering a new field of stem cells. The overall story is well written, and easy to follow and understand (Title, Abstract, Key wordds, Background).

We have some remarks/questions :

1/ Paragraph §2 « State of the art » : As the field will be unknown for many readers, it would be interesting to describe a PubMed research methodology, to give an idea about the abundance of research works on AnSC, based on a PRISMA checklist (i.e « Antler Stem Cell » in PubMed leads to 11 results, while « Adipose derived stem cell » leads to 12048 results).At that stage of AnSC, it seems possible to give an exhaustive overview of the topic.

Response: Thanks for your nice suggestions. We have done a Real-time PubMed searching, and added it to the new version. “Real-time PubMed searching showed that “antler stem cell” led to 11 results, “antler AND stem cell led to 45 results, while “adipose derived stem cell” was 12277 results. Therefore, as a relative new field, a more comprehensive understanding of AnSCs will lay the foundation for developing an effective clinical therapy for regenerative medicine.”. please refer to paragraph 2 in Scetion 1.

2/ Do we have any available data about effect of aging on deer antler formation?

Response: Thanks for your kind comments. Available data indicated that first antler formation was at approach puberty or yearling. A relationship with age in deer showed

that the weight of antler was increasing then decreasing, and the maximum weight was at age 7 to 9 year.

Reference: 1. Goss R J , Powel R S . Induction of deer antlers by transplanted periosteum I. Graft size and shape[J]. Journal of Experimental Zoology Part A Ecological Genetics & Physiology, 2010, 235(3):359-373; 2. Zheng et al,. Relation of weight of antler and age of deer, Chinese Journal of Animal Science,25(3): 31-33. Article in Chinese).

3/ Risk of aberrant bone formation : do we have comprehensive knowledge about the specific process involved in AnSC regulation ? This question might become crucial when considering clinical application (same problem as for the direct use of pluripotent stem cells).

Response: Thanks for your kind comments. Currently, through regulation of AnSCs was studied by many research groups worldwide for decades, the exact mechanism is not clear yet. But in deer, the transplantation of AP can induce a ectopic antler, which is normal and well regulated undergoing regeneration cycles. We believe that AnSCs is risk-controlled in clinical, however AnSCs are heterologous to humans and cannot be directly injected. Therefore, cell-free therapy, as described in our manuscript, is our main focus in this stage.

4/ Potential clinical applications : References 5, 31, 45 show the applicability of AnSC for preclinical use (in rabbit). But translation to human seems impossible (xenogenic graft). The use of AnSC-CM sounds promising (as described). But paragraph §6 « Future Directions » should be much more discussed-detailed, as this is the core of the manuscript : what particularity do we find in AnSC-CM content? is there a specific proteic composition explaining the interesting effects ? or is it cell-based effect ? how could we efficiently recover AnSC in GMP conditions ? Do we have to kill deer, or is it possible to collect with a limited invasive surgery ? These questions will be of prime interest for potential clinical applications.

Response: Thanks for nice comments. We have revised it accordingly. "Collection of AnSCs do not have to kill deer. Preparation of APCs and PPCs just need limited invasive surgeries, while RMCs was originated from tips of fresh commercial antlers. Laboratory

data showed that AnSC can be cultured and passed without differentiation, thus, we can efficiently recover AnSCs in GMP conditions after simple optimization.” And “Notably, functional contents from cell homogenates and cell post-culture supernatants of AnSCs also have the potential for wound healing and bone regenerative repair. Though the exact ingredients haven't been thoroughly studied, these findings undoubtedly offer a potential opportunity to develop a cell-free therapy in the clinic.”. Please refer to section 6.

In addition, more details and discussion of the particularity in AnSC-CM content was added in Section 3 and Section 4.