RESPONSE TO REVIEWERS

We thank the reviewer for his very helpful and valuable comments and suggestions.

We have attached in Supplemental Materials a "Tracked changes manuscript" file with changes from the original paper highlighted in the red font to follow changes.

Reviewer #1:

Abstract The sentences appear to be a bit long and complicated. For the readership it may be easier to shorten the sentences.

Core tip The core tip is basically a shortened version of the abstract. The following sentences would suffice: "Aging influences the ability of stem cell renewal, inducing a gradual functional decline of adult tissue-specific stem cells in maintaining homeostasis of the tissue and playing a role in the pathophysiology of various aging-associated disorders. Stem cell rejuvenation strategies may reverse this aging phenotype."

We thank the reviewer for his very helpful and valuable comments and suggestions.

We have re-written the Abstract and the core tip following your suggestions.

Introduction Paragraph 1: - "Because of their location at the bottom of cellular lineages, their dysfunction may have a greater effect than other cell types"

◊ What do you mean with "at the bottom"? I would say the stem cells are at the top of the hierarchy rather than at the bottom. Perhaps also you should explain why their dysfunction has a greater impact than that of more differentiated or committed cells.

We have modified these points as suggested.

Paragraph 2: - Please use "aging" or "ageing" but try to be consistent throughout the manuscript. What do you mean by "the organ level of tissues"??? You mean at the tissue level or at the organ level. There is not organ level of tissues.

We have modified these points as suggested.

Paragraph 3:

- Change "These properties are transferable, and these cells can also reprogram somatic nuclei and presumably confer immortality on donor cells in somatic cell nuclear transfer experiment (nuclear

transfer-embryonic stem, NT-ES)" into "These properties are transferable, and these cells can also reprogram somatic nuclei and presumably confer immortality through somatic cell nuclear transfer (nuclear transfer-embryonic stem, NT-ES)".

- "ESCs protect themselves from senescence-adopting mechanisms aimed at maintaining a high genetic stability because they are very efficient in repairing DNA damage and maintaining epigenetic status." ◊ Do you mean "ESCs protect themselves from senescence through adaptive mechanisms aimed at maintaining a high genetic stability by efficiently repairing DNA damage and maintenance of epigenetic status"? - "…and many of the primary developmental pathways are still involved in these cell populations to maintain postnatal organ homeostasis and regeneration." ◊ ""…and many of the primary developmental pathways are still active or functional in these cell populations to maintain postnatal organ homeostasis and regeneration." - "They maintain their ability to differentiate into the cell types of the organ in which they live, and they play a role in the regeneration and homeostasis of nearly all tissues during life." ◊ "They maintain the ability to differentiate into organ-specific cell types and play a role in regeneration and homeostasis of nearly all tissues during life."

We have modified these points as suggested.

- "They are multicellular organisms' longest-living proliferative cells" ◊ Add a reference source that supports this statement or remove it.

We have removed this sentence.

Paragraph 4: - "..immune to aging" ◊ "..resistant to aging" The influence of aging on the regenerative potential

- "Some studies have shown that the regenerative potential of MSCs is downregulated with age, which limits their therapeutic use" ◊ Explain MSCs. Before going into differentiation capacity and proliferation ability and therapeutic use, explain the basics of these cells (eg therapeutic use for regenerative medicine based on their osteogenic, adipogenic, chondrogenic, myogenic etc differentiation capacity or use in immune modulation)… -

We have added the explanation of MSC in the introduction

"MSCs coming from aged donors" ◊ define aged (which age range) - "displayed senescent markers" ◊ "displayed an increase in senescence markers"

We have now defined "aged donors "(>60 years). We have changed the sentence "displayed senescent markers"

- "Senescence also affects the regenerative capacity of human adipose-derived mesenchymal stem cells (hASCs) that play an important role in many bone and joint degenerative diseases" ◊ hASCs don't play an important role in diseases, they play an important role in the treatment of degenerative diseases

- "hASCs are abundant and easy to remove from patients during surgery" ◊ you don't remove the cells, you obtain the cells

- "Furthermore, the use of this cell line is safe and efficient for regenerative medicine" ◊ hASCs are NOT a cell line. They are primary cells.

- "Periodontal diseases grow with age" ◊ "Periodontal diseases increase with age"

- "Runx2" ◊ RUNX2 (if these are studies done in aged persons and not in mice)

We have modified these points as suggested.

- "Several research groups have studied the impact of aging on bone marrow mesenchymal stem cells (BMSCs), which are essential for promoting hematopoietic cells in addition to contributing to bone formation." ◊ What were the results???? What was the effect of aging on BMSCs???

We have now specified this point

- "Experiments on aged mice show that muscle-derived stem progenitor cells (MDSPCs) have reduced regenerative functions" ◊ As evident from what experiments??? Explain.

We have now described the experiments leading to this evidence

- What do you mean by saying that "All these regenerative properties of ARPCs can be invalidated via renal senescence"? What do you mean by invalidated in this sentence??? Do you mean decrease??? Affected???

- "In rodents, the quantity of senescent proximal tubular cells increases with age but not in the glomeruli." ◊ "In rodents, the quantity of senescent cells in proximal tubules, but not in the glomeruli, increases with age."

- "Prolonged or repeated renal injury leads to maladaptive repair leading to chronic kidney disease" ◊ "Prolonged or repeated renal injury leads to ineffective repair and chronic kidney disease" - Explain AKI (I assume this means acute kidney injury???)

- "Additionally, the level of senescence before kidney transplantation could predict the outcome in terms of graft function" ◊ The level of senescence of what? Graft biopt? Stem Cells? Specific regions? Clarify.

- "Another mechanism involved in premature renal senescence induced by AKI is the deficiency of the Klotho gene that is mainly expressed in kidney tubules (distal and proximal convoluted tubules)[30,31]." This sentence is completely irrelevant to the manuscript (it doesn't concern the effect of senescence on stem cells). It should be removed.

We have modified these points as suggested.

- In general: in this paragraph the effect of aging on regenerative potential of certain stem cells is briefly addressed, but many stem cells or tissues are left out. In particularly the brain (resulting in degenerative diseases) and the hematopoietic system (resulting in differences in differentiation capacity of hematopoiec stem cells, depletion of the stem cell pool, and aplastic anemia, etc) are severely affected by loss of regenerative potential of resident stem cells. These systems should be discussed, and if not in detail should at least be mentioned.

We have now briefly discussed the brain and the hematopoietic system.

How stem cells age - "Many mammalian tissues display a substantial decline in replicative function as they mature" ◊ "Many mammalian tissue-resident stem cells display a substantial decline in replicative function as they mature"

- "The renewal ability of human tissues degenerate with aging of stem cells altering their capacity to differentiate in different types of cells" ◊ "The renewal ability of human tissues declines with aging of stem cells altering their capacity to differentiate in different types of cells"

- "Moreover, self-renewal loss of stem cells occurs with age in certain stem cell compartments, and this is one mechanism that can lead to a reduction in stem cell number as people age" ◊ "Moreover, age-related loss of self-renewal in stem cells leads to a reduction in stem cell number"

- "reverse stem cell ageing" ◊ aging

- "Understanding how stem cells age might have a key role in explaining the normal ageing process, which is important mostly in tissue with continuous regeneration to understand aging at the organ level" ◊ "Understanding how stem cells age may help understanding the normal aging process at the organ level, specifically in tissues with continuous regeneration"

- "Partially through direct association between Nrf2 and C-X-C chemokine receptor type 4, the Nrf2 deficiency induced a cell-intrinsic hyperproliferation and impaired HSC migration and retention in their bone marrow niche (CXCR4)" ◊ "Partially through direct association between Nrf2 and C-X-C chemokine receptor type 4 (CXCR4), Nrf2 deficiency induces cell-intrinsic hyperproliferation and impaired HSC migration and retention in the bone marrow niche"

We have modified these points as suggested.

- "The role of Nrf2 in cell fate determination and cellular ROS control of HSCs and human airway basal stem cells was later discovered in studies on Keap1-knockout mice[39,40]." ◊ This sentence should come before the sentence starting with "Nuclear factor erythroid 2-related factor 2…"

We have modified this sentence as suggested and we have moved this paragraph in 6. MOLECULAR MECHANISMS IMPACTING STEM CELL MARKERS AND PROPERTIES

- "Double strand beats" ◊ "Double strand breaks"

- "which are the most deleterious DNA damage" ◊ "which cause the most…"

- "a decline of protein homeostasis" ◊ "a decline in protein homeostasis"

- "In addition, a reduced capacity of proteostasis can trigger a condition of protein stress that contributes to a loss of regenerative potential of aged hematopoietic stem cells (HSCs)" ◊ Instead of protein stress I would use the term endoplasmic reticulum stress…

- What do you mean with "Moreover, HSCs have an age-dependent decrease in nutrient uptake ability so that aging of the stem cell can also involve nutrient metabolism"? ◊ Maybe you should say "Since HSCs have an age-dependent decrease in nutrient uptake, it is possible that aging of stem cells maybe related to nutrient metabolism as well"

- "…cause maladaptive changes in stem cell function" ◊ "…cause irreversible or detrimental changes…"
- Figure 1: Use aging instead of ageing.

- Figure 1 legend: "…Extrinsic signals are often introduced into epigenetic modifications in stem cells" ◊ ??? what do you mean by saying this. Do you mean: "Extrinsic signals often cause epigenetic modifications in stem cells."?

- "This impairment of the stem cell niche prejudices…" ◊ "This impairment of the stem cell niche precedes…"

- "Overexpression of this signaling" ◊ "Overexpression of the BMP receptor"

- "in aged muscle on old mice" ◊ "in aged muscle of old mice" The aging environment

- "two mice at different ages" ◊ "two mice from different ages"

We have modified these points as suggested.

- "In Drosophila, the number of cap cells and hub cells, which serve as support cells for germline stem cells in the testes and ovaries, respectively, decreases with age[58,65]. This loss of the stem cell niche impairs bone morphogenetic protein (BMP) signaling from the niche that is necessary for germline stem cells maintenance." ◊ This part is redundant, it should be removed. It was more or less explained in the previous section.

We have removed this redundant part.

- "Other circulating factors that have been correlated to a youthful microenvironment were associated to caloric restriction as insulin and IGF-1, as recently demonstrated in growth hormone receptor knockout mice" ◊ "Other circulating factors, such as insulin and IGF-1, that have been correlated to a youthful microenvironment were associated with caloric restriction, as recently demonstrated in growth hormone receptor knockout mice [66]."

- "Nuclear factor κB (NF-κB) appeared as the central molecular regulator of SASP phenotype. Moreover, the levels of the pro-fibrotic TGF-β that increased with aging, impairs the function of neural stem cells, whereas the factor growth differentiation factor 11 (GDF11) has showed beneficial effect on the stemness potential of satellite and neuronal stem cells" ◊ This part was discussed in the last two sentences of the previous section. They should be combined into a single paragraph, rather than two paragraphs stretched out over two different sections.

We have modified these points as suggested.

Epigenetic changes and genomic stability

- "….Also in yeast lower levels" ◊ Shouldn't this be "In contrast"?

Yes, we have modified these points as suggested.

- "a mark associated with proper maintenance of heterochromatin [36]." ◊ This reference appears to be wrong.

We have revised the reference.

- "parabiosis, a surgical technique that has been shown to reverse aging-related degeneration" ◊ this was already explained above - The part concerning TERC and TERT should perhaps be separated from the rest of the paragraph, since the topic changes from epigenetics into genomic instability.

We have modified these points as suggested and separated the part concerning TERC and TERT from the rest of the paragraph

- Also again: ageing related defects ◊ aging-related defects

- And again: related to ageing ◊ related to aging

Molecular mechanisms impacting stem cell markers and properties - "Despite these cells develop different protective mechanisms to counteract aging-related injury and maintain their self-renew property, their functions started to decline with ageing" ◊ "Although these cells develop different

protective mechanisms to counteract aging-related injury and maintain their self-renew property, their functions started to decline with aging"

- "stem cells can loss.." ◊ "…lose.."

- "human mesenchymal stem cells (MSCs)" ◊ this was explained above

- "nuclear factor erythroid 2-related factor (2Nrf2)" ◊ this was explained above (and also abbreviated wrongly in here)

- "Nevertheless, it would be possible to find the mtDNA genes involved in several disorders associated to aging and to discover new therapeutic target" ◊ "Furthermore, it may be possible to find mtDNA genes involved in several disorders associated with aging and to discover new therapeutic targets"

- "Therefore, the combination of mitochondrial impairment and the decrease of biogenesis lead to aggravate aging process" ◊ "Therefore, the combination of mitochondrial impairment and the decrease of biogenesis leads to aggravation of the aging process"

- "nuclear DNA damage, induced by several external factors such as radiations, toxins and endogenous mediators like ROS and error in DNA replication mechanism, is associated to accelerated aging" ◊ "nuclear DNA damage, induced by several external factors such as radiation, toxins and endogenous mediators like ROS and errors in DNA replication mechanisms, is associated with accelerated aging"

- Again: "mimic physiological ageing" ◊ "mimic physiological aging"

- "Another important key player….aging process" ◊ combine this with the TERC/TERT paragraph in the previous section.

- "Interestingly, some miRNA confers to stem cells the capacity to respond to several injury and to prevent the development of" ◊ this sentence is unfinished.

- "Despite several…" ◊ "Although several…"

- "…MSC cellular age and it was significantly associated to increase p21 expression profile …" ◊ "…MSC cellular age and which was significantly associated with increased p21 expression…" Stem cell aging under physiological and pathological conditions: differences

Cell rejuvenation strategies "There a limited studies on rejuvenation of aged stem cells targeting mitochondrial functions" ◊ "There are a limited number of studies on rejuvenation of aged stem cells targeting mitochondrial functions"

"targeting sirtuins" ◊ "targeting of sirtuins" Again: "It can delay ageing" ◊ aging

We have modified these points as suggested

"Another method is heterochronic parabiosis" ◊ This would certainly not be a viable treatment option for human beings. Perhaps you can think of a manner that would result in a similar effect, but without the parabiosis per se, eg plasma exchange or something the like

As suggested, we have specified that "Another method could be plasma exchange to obtain the same effects of heterochronic parabiosis"

Conclusions and future perspectives Again: "organism ageing" ◊ aging

We have modified these points as suggested

Reviewer#2:

Specific Comments to Authors: In this review manuscript, the authors discussed the aging of adult stem cells, the association between stem cells function and aging, and the possible cell rejuvenation strategies. This is a very comprehensive review paper which may enhance the understanding of stem cells aging. The logic of the manuscript is fine, but the content of each section is a little confused. I suggest the authors revised the content and focus on limited kinds of adult tissue-specific stem cells.

We thank the reviewer for his very helpful and valuable comments and suggestions.

As suggested by you and other reviewers, we have re-organized the review removing redundant parts and combined some paragraph.

We have split '4. Aging environment' in '4. Aging enviroment' based on systemic factors and 5. "The niche microenvironment".

We have combined '5. Epigenetic change' with '6. Molecular mechanism' and we have reorganized the paragraph.

We have expanded the paragraph '9. Conclusions and future perspectives' re-summarizing the contents, pointing out the potential target for improving rejuvenation, and adding some limits that so far maybe a challenge to overcome stem cell aging.

We have moved the Figure 1 in "4. The Aging Environment".

We have re-written the Abstract

Reviewer #3:

Specific Comments to Authors: This review paper summarized the potential mechanisms involved in stem cells aging including extinct and intrinsic factors, physiological and pathological status, and rejuvenation strategies. It is valuable for people work on stem cells therapy. Before it can be published, I have some comments here for author to consider and improve their paper.

1. Although the structure of the paper looks clear, the content under each subtitle is somehow redundant. For example, under How stem cells age, author mentioned eight aspects such as accumulation of epigenetic remodeling, DNA damage, mitochondrial dysfunction etc. However, under Molecular mechanism, DNA damage, epigenetic modification, mitochondrial DNA were discussed again.

We thank the reviewer for his very helpful and valuable comments and suggestions.

As suggested by you and other reviewers, we have re-organized the review removing redundant parts and combined some paragraph. We have eliminated the redundant paragraphs in How stem cells age.

2. Under '3. How stem cells age' please compare the general age in general cells with age in stem cells. Are they all the same or some of them are specific to stem cells?

As suggested, we have now added in 3. a paragraph comparing the age in general cells with age in stem cells.

3. Figure 1 is not consistent with the content of second paragraph of '3. How stem cells age'.

We have moved the Figure 1 in 4. The Aging Environment.

4. '4. Aging enviroment' is not very clear to me. I suggest reorganize the content based on systemic factors (body environment) and niche mediators (micro-environment).

As suggested, we have split '4. Aging environment' in '4. Aging environment' based on systemic factors and 5. "The niche microenvironment".

5. I suggest combine '5. Epigenetic change' with '6. Molecular mechanism' because these are all intrinsic factors (molecule changes in cells). Then focusing on the molecular changes of cells such as signaling pathways, key transcription factors, functional related genes or proteins alterations, epigenetic changes.

As suggested, we have combined '5. Epigenetic change' with '6. Molecular mechanism' and we have reorganized the paragraph.

6. '9. Conclusions and future perspectives' needs to re-summerize all the contents and point out the potential target for improving rejuvenation. Also, please think it and add some limits here that so far maybe a challenge to overcome stem cell aging.

As suggested, we have expanded the paragraph '9. Conclusions and future perspectives' re-summarizing the contents, pointing out the potential target for improving rejuvenation, and adding some limits that so far maybe a challenge to overcome stem cell aging.

EDITORIAL OFFICE'S COMMENTS

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

(1) *Science editor:*

5 Issues raised: (1) The title is too long, and it should be no more than 18 words;

We thank the Science Editor for his very helpful and valuable comments and suggestions.

The title is 7 words

(2) The "Author Contributions" section is missing. Please provide the author contributions;

We have added author contributions

(3) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor; and

We have now provided original pictures.

(4) Please obtain permission for the use of picture(s). If an author of a submission is re-using a figure or figures published elsewhere, or that is copyrighted, the author must provide documentation that the previous publisher or copyright holder has given permission for the figure to be re-published; and correctly indicating the reference source and copyrights. For example, "Figure 1 Histopathological examination by hematoxylin-eosin staining (200 ×). A: Control group; B: Model group; C: Pioglitazone hydrochloride group; D: Chinese herbal medicine group. Citation: Yang JM, Sun Y, Wang M, Zhang XL, Zhang SJ, Gao YS, Chen L, Wu MY, Zhou L, Zhou YM, Wang Y, Zheng FJ, Li YH. Regulatory effect of a Chinese herbal medicine formula on non-alcoholic fatty liver disease. World J Gastroenterol 2019; 25(34): 5105-5119. Copyright ©The Author(s) 2019. Published by Baishideng Publishing Group Inc[6]". And please cite the reference source in the references list. If the author fails to properly cite the published or copyrighted picture(s) or table(s) as described above, he/she will be subject to withdrawal of the article from BPG publications and may even be held liable.

Figures have been drawn by us through Biorender software

6 Recommendation: Conditional acceptance.