Editors and reviewers,

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Tendon Stem/Progenitor Cell Ageing: Modulation and Rejuvenation

Dear Editors and Reviewers,

First, we would like to thank you for the very useful comments and the time you spent reviewing this manuscript.

We have provided point by point responses to the suggestions and comments and indicate how the suggestions have been incorporated into the revised manuscript when appropriate. The responses to the comments in this letter and the revised portions are shown by blue.

We have revised this manuscript in line accordance with the reviewers' comments, and we believe that the quality of the manuscript has been substantially improved. We would like to resubmit the revised manuscript for your further consideration for publication in the **World Journal of Stem Cells.**

Yours Sincerely,

Dr. Yun-feng Rui, MD., Ph.D.

(On behalf of all co-authors)

Responses to the Reviewers' Comments:

Reviewer 1:

Thank you for reviewing this manuscript.

Reviewer 2:

A well written good conceived manuscript presenting tendon structure metabolism and ageing processes together with potential methods for quenching degradative phenomena due to aging. Comments. Introduction Well written Stem cells can be pluripotent however they are as well varieties that are multipotent, restricted to the embryonic layer of origin (such as MSCs). The phrase starting with "recently discovered IPSCs is unclear and way too long, Please reformulate in a way the message can be conveyed. The myth of MSC immune privilege is somehow contested these days , would be important to note this See Berglund et all Stem Cell Resources and Therapies, 2017. Proliferative capabilities could help TSCs while transplantation, however I am not sure this is the case with multilineage differentiation. One needs TSCs to differentiate to tenocyte when healing tendon not to develop adipo, oste or chondrocytes for example. PRP role in stimulating tendon healing should as well regarded with caution and by discriminating dosage, modalities of use and growth factor profile. High concentration of PRP together with increased VEGF content have been reported to be detrimental for tendon healing (See Gusti et al Biomed Res Int 2014) Maybe inserting a small table summarizing the effect of known

pharmacological agents on TPSCs would improve the clarity of this chapter A morphological description of aging tendons could help the reader visualize what the authors are describing. Is there a corelating between morphological aspects of tendon aging and tendon degeneration (tendinosis) Are tendon stem cells implicated in occurrence of degenerative zones ? What is the current knowledge in this respect? When describing the role of cellular cytoskeleton and surface adhesion markers one should at least mention the interaction with ECM and role of stem cell niche within ageing as the physical biochemical changes that occur in ECM proteins are likely to highly influence both stem cell maintenance and turnover Reader is informed about limited studies regarding the role of several proposed antiageing compounds on tendon stem cells. It is not clear what limited means, are there any (if so please cite) ore there is to date a complete lack of such studies?

1. Introduction Well written Stem cells can be pluripotent however they are as well varieties that are multipotent, restricted to the embryonic layer of origin (such as MSCs).

Answer: Thank you so much for your valuable comment. The reviewer points out an important issue about the embryonic layer of origin of stem cells. We have added relative restrict in the text.

Pluripotent stem stem cells that can differentiate into various tissue types under different conditions and serve as an internal repair system, which is also restricted to the embryonic layer of origin [14].

We have added this information to the revised manuscript.

2. The phrase starting with "recently discovered IPSCs is unclear and way too long, Please reformulate in a way the message can be conveyed.

Answer: Many thanks for your question. As suggested, we have reformulated the pharse in order to make it clear and easy to understand.

Recently, the discovery of induced pluripotent stem cells (iPSCs), particularly cells isolated from mature adult, inspired researchers to develop potential therapies to cure clinical diseases and ponder the eternal topic of regaining our body youth. Thus, it provided inspiration to reverse stem cell fate by modulating the factors that influence cell growth progression [20-22].

We have added this information to the revised manuscript.

3. Proliferative capabilities could help TSCs while transplantation, however I am not sure this is the case with multilineage differentiation. One needs TSCs to differentiate to tenocyte when healing tendon not to develop adipo, oste or chondrocytes for example.

Answer: Thank you so much for your valuable comment. When TSPCs are used to promote tendon healing, the reviewer points out an important question about cell differentiation. Generally, the differentiation of TSPCs into tenocytes can promote tendon healing. Moreover, as it is known, in anatomical structure, tendon tissue usually ends in bone tissue. When tendon tissue is damaged, the osteogenic and chondrogenic differentiation of TSPCs is required in the site near the bone. In this way, the site of tendon-bone

junction can be better repaired and the healing process would be more ideal with satisfied strength and functional recovery. Therefore, it is described as multilineage differentiation in the text. Related research is showed in Tian's article (Tian X et al. Baicalein Accelerates Tendon-Bone Healing via Activation of Wnt/β-Catenin Signaling Pathway in Rats.Biomed Res Int 2018). Thank you again.

4. High concentration of PRP together with increased VEGF content have been reported to be detrimental for tendon healing (See Gusti et al Biomed Res Int 2014) Maybe inserting a small table summarizing the effect of known pharmacological agents on TPSCs would improve the clarity of this chapter.

Answer: Many thanks for your question. We have summarized a small table to improve the clarity of this chapter and added it in the text, and different concentrations of PRP have been identified in the Table1.

We have added this information to the revised manuscript.

4. A morphological description of aging tendons could help the reader visualize what the authors are describing. Is there a corelating between morphological aspects of tendon aging and tendon degeneration (tendinosis) Are tendon stem cells implicated in occurrence of degenerative zones? What is the current knowledge in this respect?

Answer: Thank you so much for your valuable comment. The reviewer pointes out an important view of descripting a morphological description of aging tendons and the correlation between morphological aspects of tendon

aging and tendon degeneration (tendinosis) and the role of tendon stem cells in the process. We briefly introduce their relationship in this paper because we have summarized the related details in our previous review paper from our team (Li Y, etal. The Potential Roles of Tendon Stem/Progenitor Cells in Tendon Aging. Curr Stem Cell Res Ther 2019;14:34-42). So, they are briefly described, and we mainly focus on TSPCs ageing in this paper.

In addition, an altered fate of TSPCs was observed in a collagenase-induced (CI) tendon injury model of tendinopathy due to the presence of tenocytes lacking the multidifferentiation capacity [21], consistent with similar results presented in other studies and supporting the hypothesis that TSPCs might play an essential role in the pathogenesis of tendinopathy. A series of recent studies revealed important roles for TSPCs in tendon healing by replacing mature tendon cells that are lost under normal circumstances, which might be the cause of age-related changes in the pathogenesis of tendon disorders [15, 50]. Thus, TSPCs are considered to play a crucial role in maintaining tendon homeostasis by affecting tendon repair and regeneration [15, 20, 51, 52]. Recently, Li et al. [1] proposed that the altered fate of TSPCs contributes to tendon ageing. Other scholars have also observed alterations in TSPCs features during tendon degeneration and the progression of ageing [14, 15, 50, 53, 54]. Overall, a range of TSPCs functions are altered, and TSPCs might serve as a potential target due to these alterations. Therefore, a relationship between altered TSPCs features and tendon ageing has been hypothesized,

highlighting the importance of TSPCs in the treatment of tendon-related diseases. Thank you again.

5. When describing the role of cellular cytoskeleton and surface adhesion markers one should at least mention the interaction with ECM and role of stem cell niche within ageing as the physical biochemical changes that occur in ECM proteins are likely to highly influence both stem cell maintenance and turnover

Answer: Many thanks for your question. The reviewer pointed a very important view of the role of ECM and cell niche. According to your comment, we have illustrated this part of the relevant content.

As it is known, ECM is another critical factor for the viscoelasticity of TSPCs and intervened in the receptor-substrate ligand interactions of cell adhesion [57]. Although, Tatiana et al. showed alterations of ECM protein expression in rat tendons with age, while composition of ECM related to the cell adhesion was not analyzed [68]. Related experiments can be carried out because ECM proteins and cell niche are likely to highly influence both TSPCs maintenance and turnover in the future.

We have added this information to the revised manuscript.

6. Reader is informed about limited studies regarding the role of several proposed antiageing compounds on tendon stem cells. It is not clear what limited means, are there any (if so please cite) ore there is to date a complete lack of such studies?

Answer: Thank you so much for your valuable comment. The meaning of limited in this article is there any but not sufficient. We have cited relevant literature in the text.

However, the relationships between these drugs and the mechanisms underlying the increase in lifespans are unknown due to the limited and unsufficient number of studies conducted in this area [84-86].

We have added this information to the revised manuscript.

Reviewer 3:

It is a comprehensive narrative review about tendon stem/progenitor cell ageing: modulation and rejuvenation. It is an interesting review, well-constructed but need English language editing.

Answer: Thank you so much for your valuable comment. As suggested, we revised our manuscript with the help of a native English speaker from AJE to improve the read ability of our paper. Thank you again.

Reviewer 4:

Review; Tendon Stem/Progenitor Cell ageing: modulation and rejuvenation Comments to the authors The manuscript by Guang-chun Dai et al, describes the role of Tendon Stem/Progenitor Cells (TSPCs) during aging, or processes leading to deterioration of tendons´s structure and function. More precisely, the authors focused on the incidence of growth factor decreases and hormone

deficits on tendons' diseases. They also gathered evidences that addition of suitable extracellular matrix (ECM), growth factor or hormones improved TSPCs functions. The review is relevant since the isolation and identification of TSPCs in tendon fascicles of many animals is rather recent. Therefore, these cells could be used for regeneration of tendon tissues. Particularly interesting in this review are the facts that moderate mechanical stretching up-regulates stem markers and the importance of controlling environmental cues and signaling mechanisms. In general, the manuscript opens an additional and interesting way of treating tendons, by focusing on reactivation of TSPCs for treatment of tendon-related diseases.

Major concerns - "It is estimated that there are 0.96 billion elderly people over the age of 60 worldwide in 2017, and this population is growing at the fastest rate. Moreover, nearly 150 thousand people will die each day all over the world with two thirds of these deaths caused by age-related reasons, indicating that ageing is a vital risk factor for numerous age and degeneration-related diseases, Authors: it seems obvious the fact that ageing is a vital risk. Please delete the phrase and focus the text on how ageing could affect tendon damages, instead.

- Stem cells are pluripotent cells that can differentiate into various tissue types under different conditions and serve as an internal repair system Authors: the phrase is imprecise, there are different stem cell types and not all of them can differentiate into various tissue types (i.e.: unipotent stem cells) . Please,

rephrase as follows: Pluripotent Stem cells can differentiate into various tissue types under different conditions and serve as an internal repair system

Minor issues - “In adults, TSPCs, a type of MSC, were first confirmed to be present in tendon tissues by Bi et al. in the year of 2007”, Authors please expand “MSC”

- In the MSC ageing process, the p16/RB pathway and p53/p21 pathway have vital roles in affecting the cellular senescence though regulation of telomere length and function

1. “It is estimated that there are 0.96 billion elderly people over the age of 60 worldwide in 2017, and this population is growing at the fastest rate. Moreover, nearly 150 thousand people will die each day all over the world with two thirds of these deaths caused by age-related reasons, indicating that ageing is a vital risk factor for numerous age and degeneration-related diseases, Authors: it seems obvious the fact that ageing is a vital risk. Please delete the phrase and focus the text on how ageing could affect tendon damages, instead.

Answer: Thank you so much for your valuable comment. As suggested, we have deleted the phrase and focused the text on the potential effects of ageing on tendon damage.

The global population over the age of 60 years is growing rapidly [1], and the occurrence of tendon-related injuries increases upon ageing [2]. Moreover, the consequences of tendon damage in elderly patients are more severe [3], and

older populations also experience a higher occurrence of sport-related tendon injuries and more difficulties in healing process [4], which places a heavy burden on the health systems of individual countries [5]. Epidemiological studies have highlighted the importance of obtaining an in-depth understanding of the pathogenesis of aged-related tendon diseases, with the aim of developing appropriate therapeutic approaches.

We have added this information to the revised manuscript.

2. - Stem cells are pluripotent cells that can differentiate into various tissue types under different conditions and serve as an internal repair system Authors: the phrase is imprecise, there are different stem cell types and not all of them can differentiate into various tissue types (i.e.: unipotent stem cells) . Please, rephrase as follows: Pluripotent Stem cells can differentiate into various tissue types under different conditions and serve as an internal repair system.

Answer: Many thanks for your question. We have revised the phrase according to your comments.

Pluripotent stem cells can differentiate into various tissue types under different conditions and serve as an internal repair system.

We have added this information to the revised manuscript.

3. "In adults, TSPCs, a type of MSC, were first confirmed to be present in tendon tissues by Bi et al. in the year of 2007", Authors please expand "MSC"

Answer: Thank you so much for your valuable comment. As suggested, we

have defined "MSC" as "mesenchymal stem cell". Besides, an abbreviation has been added in the text (Table3).

In adults, tendon stem/progenitor cells (TSPCs), as a type of mesenchymal stem cell (MSC), were first confirmed to be present in tendon tissues by Bi et al. in the year of 2007, and have the potentials of self-renewal, clonogenicity and multi-differentiation[15].

We have added this information to the revised manuscript.

4. In the MSC ageing process, the p16/RB pathway and p53/p21 pathway have vital roles in affecting the cellular senescence though regulation of telomere length and function.

Answer: Many thanks for your question. According to your comments, we have revised "…though…" to "…through…"

We have added this information to the revised manuscript.

Reviewer 5:

In this review, the authors summarize the TSPCs characteristics, their epigenetic variations associated with ageing and some rejuvenation methods. There are some comments. 1. The author used different terms to describe the tendon-related stem cells, such as TSPCs, TDSCs and TSCs. The point is, are they the same thing? If so, it is better to use single term to avoid misunderstanding. If not, the authors should give clear definitions of each of them to help the audience to distinguish them. In addition, "TPSCs" was also

found. Is it an error?

2. In the part of “Rejuvenation of aged tendon stem/progenitor cells”, it is mentioned that “Numerous factors, including exercise, estrogen fluctuation, ECM conditions, and drug uses, affect the features that are altered in TSPCs upon the influence of age, and these alterations are primarily harmful to TSPC function and maintenance of tendon homeostasis.” My questions are: 1) Does it mean the exercise is harmful to TSPC function? However, the following citation indicated that “Moderate exercise ameliorates the deteriorative condition of the TSPC”. I think the author must clarify these descriptions. 2) It seems inappropriate to discuss these factors together. “Exercise” is from a macroscopic view while “estrogen fluctuation, ECM conditions, and drug uses” may be from a microscopic view. 3. Due to the latest development of regeneration medicine, the authors should review and add comments on the literature about pluripotent stem cell-derived tenocytes or their progenitor cells. These new experiments should be very informative for the rejuvenation of tendon in the future.

1. The author used different terms to describe the tendon-related stem cells, such as TSPCs, TDSCs and TSCs. The point is, are they the same thing? If so, it is better to use single term to avoid misunderstanding. If not, the authors should give clear definitions of each of them to help the audience to distinguish them. In addition, “TPSCs” was also found. Is it an error?

Answer: Thank you very much for your kind comment. TSPCs, TDSCs and

TSCs are the same cells and we have used a single term to avoid confusion in the text. Moreover, we have revised "TPSCs" to "TSPCs".

We have added this information to the revised manuscript.

2. In the part of "Rejuvenation of aged tendon stem/progenitor cells", it is mentioned that "Numerous factors, including exercise, estrogen fluctuation, ECM conditions, and drug uses, affect the features that are altered in TSPCs upon the influence of age, and these alterations are primarily harmful to TSPC function and maintenance of tendon homeostasis." My questions are: 1) Does it mean the exercise is harmful to TSPC function? However, the following citation indicated that "Moderate exercise ameliorates the deteriorative condition of the TSPC". I think the author must clarify these descriptions. 2) It seems inappropriate to discuss these factors together. "Exercise" is from a macroscopic view while "estrogen fluctuation, ECM conditions, and drug uses" may be from a microscopic view. 3. Due to the latest development of regeneration medicine, the authors should review and add comments on the literature about pluripotent stem cell-derived tenocytes or their progenitor cells. These new experiments should be very informative for the rejuvenation of tendon in the future.

Answer: Many thanks for your question. According to your comments, we have made the following changes.

1) We have clarified these descriptions and revised "exercise" to "uncomfortable exercise intensity"

2) We have discussed these factors separately and corrected “Numerous factors, including exercise, estrogen fluctuation, ECM conditions, and drug use, affect the features that are altered in TSPCs upon the influence of age, and these alterations are primarily harmful to TSPC function and maintenance of tendon homeostasis” to “Numerous factors, including Numerous factors, including macroscopic factors associated with an uncomfortable exercise intensity and microscopic factors associated with an impaired estrogen balance, deteriorated ECM conditions and inappropriate drug use, alter the features of TSPCs, particularly during aging, and these alterations are mainly deleterious to TSPC function and the maintenance of tendon homeostasis.

We have added this information to the revised manuscript.

3. Due to the latest development of regeneration medicine, the authors should review and add comments on the literature about pluripotent stem cell-derived tenocytes or their progenitor cells. These new experiments should be very informative for the rejuvenation of tendon in the future.

Answer: Thank you very much for your valuable comment. You raise an important point about pluripotent stem cell-derived tenocytes or their progenitor cells. According to the reviewer’s suggestion, we have reviewed and commented on previous publications examining these cells.

Additionally, based on most recent development in regenerative medicine, Dale et al. induced human embryonic stem cells (hESC) to differentiate into

tendon-like cells in the presence of exogenous bone morphogenetic protein (BMP) 12 and BMP13 [87] and directed parthenogenetic stem cells (pSCs) to differentiate into tenocytes. Moreover, mechanical stretching improved the tenogenic differentiation of pMSCs [88]. Similar results were also obtained using iPSCs [89, 90]. Thus, these cells may represent an exogenous supplementation to TSPCs or tenocytes, which is also an ideal method for rejuvenating ageing of tendons and provides alternative healing strategies for reversing tendon ageing in the future.

We have added this information to the revised manuscript.

Reviewer 6:

In this manuscript Dai et al have discussed the potential, the nature, and inherent problems of Tendon stem/progenitor cells (TSPCs) for tissue repairing. The content is in general interesting.

However, the text is not reader-friendly, especially for those who are not in the field. For example, there has been no introduction for A-TSPCs and Y-TSPCs in the text (page 8). The same is true for TSPCs 7d, TSPCs 1d, and TSPCs 56 d (page 8-9). Such abbreviations should be clearly defined at the first time when they are used. There are so many in the text.

Besides the above critiques, the reviewer does not agree with the notion that IL-6 is anti-inflammatory cytokines. Since IL-6 is generally considered to be an inflammatory cytokine, while IL-10 to be an anti-inflammatory one (page

6).

In addition, another figure illustrating the problems in the aged TSPCs relative to the young TSPCs would help readers follow the context.

Finally, the reviewer strongly suggests the paper to be edited by (a) professional English editor(s).

1. However, the text is not reader-friendly, especially for those who are not in the field. For example, there has been no introduction for A-TSPCs and Y-TSPCs in the text (page 8). The same is true for TSPCs 7d, TSPCs 1d, and TSPCs 56 d (page 8-9). Such abbreviations should be clearly defined at the first time when they are used. There are so many in the text.

Answer: Thank you so much for your valuable comment. As suggested, we have added definitions for "A-TSPCs", "Y-TSPCs", TSPCs 7d, TSPCs 1d, and TSPCs 56d as well as other terms. Besides, an abbreviation has been added in the text (Table3).

We have added this information to the revised manuscript.

2. Besides the above critiques, the reviewer does not agree with the notion that IL-6 is anti-inflammatory cytokines. Since IL-6 is generally considered to be an inflammatory cytokine, while IL-10 to be an anti-inflammatory one (page 6).

Answer: Many thanks for your question. As suggested, we have corrected "…IL-6…" to "…the production of the pro-inflammatory cytokine Interleukin-6 (IL-6) and anti-inflammatory cytokine Interleukin-10 (IL-10)..."

We have added this information to the revised manuscript.

3. In addition, another figure illustrating the problems in the aged TSPCs relative to the young TSPCs would help readers follow the context.

Answer: Thank you so much for your valuable comment. We have summarized the problems associated with aged TSPCs relative to young TSPCs in Table 2. Initially, we wanted to use a figure to illuminate the problem, but it is was complicated and consuming. Therefore, we decided to use a table to explain the problems more clearly. If the reviewer think it is strongly necessary, we will change it to a figure. Thank you again.

4. Finally, the reviewer strongly suggests the paper to be edited by (a) professional English editor(s).

Answer: Many thanks for your question. According to the comment, we revised our manuscript with the help of a native English speaker from AJE to improve the read ability of our paper. Thank you again.

Responses to the chief editor' comments:

Page 6: "Recently, studies focused on stem cells have become an emerging areas in regenerative and biomedical medicine because these cells have been confirmed to be remarkably important for tissue maintenance, repair and remodeling; and they have also been used to cure various diseases with

satisfactory outcomes [6-9]." Critique: The indefinite article an may not be required with the plural noun "areas" in this sentence. Consider removing the article, or changing the noun to singular. Page 8: It appears that the singular verb has does not agree with the plural compound subject substantial interest and progress in the study of the roles of this cell type in tendon maintenance, repair, remodeling and tendon tissue engineering. Consider changing the verb to the plural form. "Since these discoveries, substantial interest and progress in the study of the roles of this cell type in tendon maintenance, repair, remodeling and tendon tissue engineering has been reported." Figure 1 legend: "ROCK plays an important role in accelerating tendon stem/progenitor cells (TSPCs) senescence and stiffness, and miR-135a reduces the expression of ROCK1, and Y-27632 can inhibit the pathway by targeting the ROCK1/2." It is not logical and misleading. rewrite the structure. Figure 1. the scheme shows that tenogenic differentiation (TD), such as the application of growth factors, mechanical stimulation, biomaterials, coculture, should slow down the aging process, not activate aging as shown in their drawing.

1. Page 6: "Recently, studies focused on stem cells have become an emerging areas in regenerative and biomedical medicine because these cells have been confirmed to be remarkably important for tissue maintenance, repair and remodeling; and they have also been used to cure various diseases with satisfactory outcomes [6-9]." Critique: The indefinite article an may not be

required with the plural noun "areas" in this sentence. Consider removing the article, or changing the noun to singular.

Answer: Thank you so much for your kind comments. We have carefully rechecked this sentence and corrected "Recently, studies focused on stem cells have become an emerging areas in regenerative and biomedical medicine…" to " Recently, studies focused on stem cells have become emerging areas in regenerative and biomedical medicine…"

We have added the information in the revised manuscript.

2. Page 8: It appears that the singular verb has does not agree with the plural compound subject substantial interest and progress in the study of the roles of this cell type in tendon maintenance, repair, remodeling and tendon tissue engineering. Consider changing the verb to the plural form. "Since these discoveries, substantial interest and progress in the study of the roles of this cell type in tendon maintenance, repair, remodeling and tendon tissue engineering has been reported."

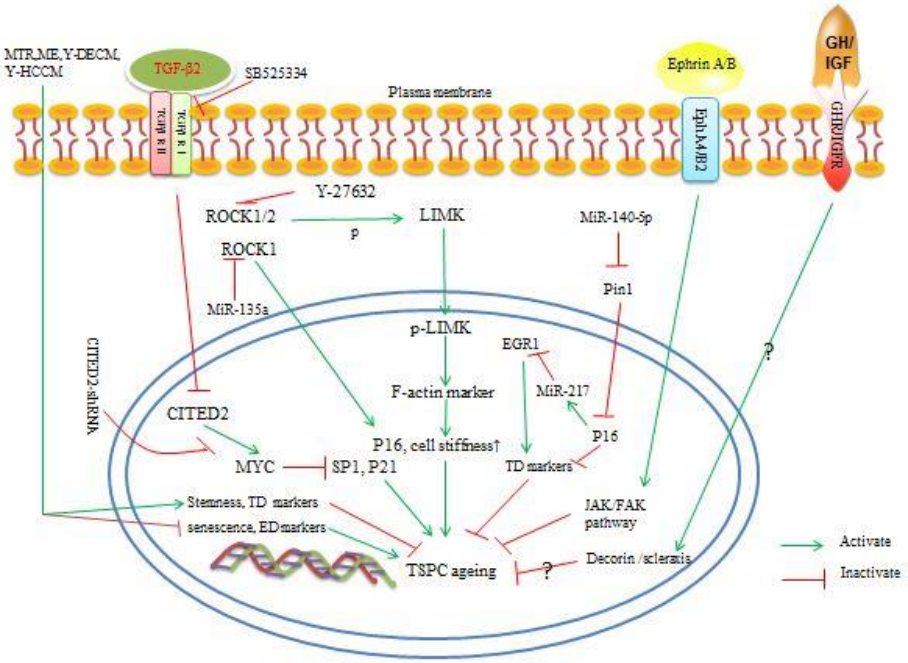
Answer: Many thanks for your questions. We have We have carefully rechecked this sentence and corrected "Since these discoveries, substantial interest and progress in the study of the roles of this cell type in tendon maintenance, repair, remodeling and tendon tissue engineering has been reported" to" Since these discoveries, substantial interest and progress in the study of the roles of this cell type in tendon maintenance, repair, remodeling and tendon tissue engineering have been reported."

We have added the information in the revised manuscript.

3. "ROCK plays an important role in accelerating tendon stem/progenitor cells (TSPCs) senescence and stiffness, and miR-135a reduces the expression of ROCK1, and Y-27632 can inhibit the pathway by targeting the ROCK1/2." It is not logical and misleading. rewrite the structure.

Answer: Thank you so much for your kind comments. We have carefully checked this sentence again and rewrote it to make it more logical.

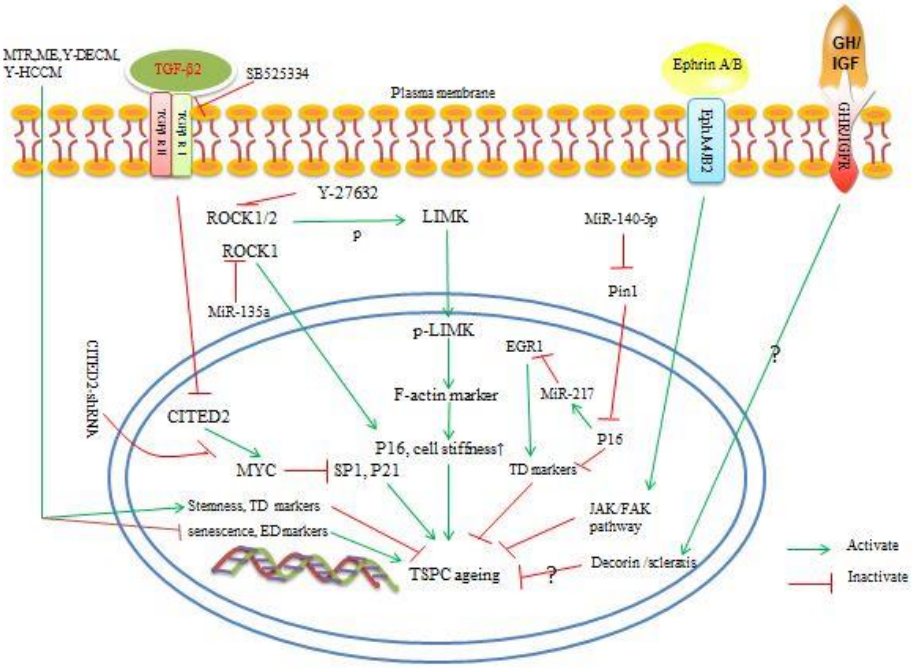
We rewrote "ROCK plays an important role in accelerating tendon stem/progenitor cells (TSPCs) senescence and stiffness, and miR-135a reduces the expression of ROCK1, and Y-27632 can inhibit the pathway by targeting the ROCK1/2." to "Moreover, ROCK1/2 plays an important role in accelerating TSPC senescence and stiffness that can be delayed by the inhibition of Y-27632 on ROCK1/2 and miR-135a on ROCK1."And we also reedited it in the figure.



We have added the information in the revised manuscript.

4. Figure 1. the scheme shows that tenogenic differentiation (TD), such as the application of growth factors, mechanical stimulation, biomaterials, coculture, should slow down the aging process, not activate aging as shown in their drawing.

Answer: Many thanks for your questions. The chief editor raised an important point about the role of delaying aging of tenogenic differentiation (TD) in the figure. We have corrected "the activating aging role of TD" to "the delaying aging role" in the figure.



We have added the information in the revised figure.