Dear editor,

We appreciate a lot for the reviewer's insightful and valuable comments, and give us the opportunity to submit a revised version of the manuscript. We have indicated the changes with red font in the revised version, and included a "clean" version of the revised manuscript. Point-by-point responses to reviewer's comments are listed below.

Response to reviewers' comments

Reviewer #1

This is an interesting and meaningful review paper. There are some issues that must be addressed, in order to show that the manuscript support the conclusions.

Comments

Since this is the review and summary paper, I would like to suggest you to make 2-3 tables for the better and fast understanding for the readers, especially to distinguish the TSPCs from other BM-MSC and ASC including their advantages and disadvantages.

Response: Thank you very much for kind suggestions. In the section **Endogenous and exogenesis of stem cells/progenitors for tendon repair**, we have added Difference among TSPCs, BM-MSCs and ASCs for tendon repair (Table 1), which helps to distinguish these stem cells including advantages and shortcoming. Also, we modified the description of these stem cells to highlight the characteristics and distinction of these stem cells.

Several types of endogenous and exogenous stem cells have proven effective for tendon repair. TSPCs are fibroblast-like shaped cells[22], which have been identified in mice, rabbits and humans, with typical

stem-cell makers.[4, 22, 23] Nevertheless, the exact source location of TSPCs remains unclear. TSPCs have been isolated and differentiated from tendon,[4] peritenon,[24] and perivascular sources.[25, 26] A recent study reported that a PDGFRA$^+$ cell population expressing tubulin polymerization-promoting protein family member 3 (TPPP3$^+$), which is located in peritenon, has stem-cell characteristics, such that it may generate new tenocytes and self-renew upon injury.[27] TSPCs share some common markers with tenocytes, such as collagen I, collagen III, tenascin C, and tenomodulin (TNMD), but they express still more markers, like Oct-4, SSEA-1/4, and nucleostemin.[4, 5] Both TSPCs isolated from the tendon and peritenon regions of mouse Achilles tendons have the Sca1, CD90, and CD44 markers,[26] but progenitor cells from the tendon and peritenon regions can be distinguished with genes such as Scx, Mkx, Thbs4, and Wnt10a.[24] Moreover, perivascular stem cells isolated and cultured from human supraspinatus tendon biopsies express both tendon-like and stem/precursor-cell-like markers, including musashi-1, nestin, prominin-1/CD133, CD29, CD44, Scx, and Smad8.[25] TSPCs from tendons proper lack of CD133 markers, however, which may help distinguish TSPCs from tendons proper and perivascular sources. TSPCs have shown a high capacity for proliferation and multipotential differentiation into tenocytes, osteoblasts, chondrocytes, and adipocytes.[4, 28] Although TSPCs show multipotential differentiation, they also show spontaneous tenogenic

differentiation, which can be beneficial for tendon repair.[20] In a tendon-window-wound study, TSPCs participated in tendon repair by proliferation and activation of tenogenesis.[29] The therapeutic effect of TSPCs has also been confirmed by using animal models. It has been reported that TSPCs promote tendon repair by improving cell and collagen-fiber alignment, collagen birefringence, and Young's modulus typical of tendon, as well as by increasing ultimate stress capacity.[30] Similarly, ultimate failure load and the expression of collagen I and collagen III of the ruptured Achilles tendon have been much improved by TSPC transplantation.[31] Nevertheless, the abnormal differentiation of TSPCs into nontendon cells has a negative effect on tendon development, homeostasis, and repair. For instance, tendon progenitor cells of injured tendon have strong chondrogenic potential, which may cause endochondral ossification as a result of ectopic mineralization.[32] To date, very scant clinical research has been performed using TSPCs for tendon-related diseases.

As shown in Table 1, stem cells/progenitors derived from other tissues, such as bone marrow-derived mesenchymal stem cell (BMSCs) and adipose-derived stem cells (ASCs), are much easier to acquire than TSPCs[33, 34], and they have been proven efficient for tendon repair.[14, 19] BMSCs are spindle-shaped[35], have the potential of tenogenic differentiation[36, 37] and high proliferation[4, 38]. Several mechanisms may contribute to tendon repair with exogenic BMSCs.

First, BMSCs can differentiate into certain new cells (tenocytes) to replace lost normal cells;[19, 39] second, BMSCs can secrete various cytokines and growth factors to promote the proliferation of cells in injured tissue;[40] and third, BMSCs can increase the deposition of collagenous proteins.[41] BMSC-based therapy has been found to improve histological and biomechanical properties and to increase the expression of collagen in animal injury.[42, 43] But the application of BMSCs may also carry the risk of nontendon differentiation and of forming ectopic bone during tendon repair.[44] The clinical application of BMSCs was started very early, and four clinical trials (NCT03688308, NCT01788683 and NCT02484950 NCT01687777) using BMSCs for rotator-cuff repair are at the stage of recruiting, but the results have not yet been released. ASCs are spindle-shaped [45] with stem-cell marks[23, 35, 46]; these cells commonly being isolated from subcutaneous adipose tissue[34] and liposuction aspirates[47], have shown the multipotential ability of differentiation including tenogenic cells [48-50] and high proliferation[23, 38, 51]. ASCs transplantation could enhance the secretion of collagen I and tenascin-C during healing and improve the mechanical strength of tendon,[52, 53] as well as improve the pathological changes of tendinopathy and the normalize of collagen ratios within the affected tendon.[54] Recently, a study indicated that ASCs improved tendon repair in tendinopathy by inhibiting inflammation and inducing neovascularization at the early stage of tendon healing, and ASCs are

also effective for the inhibition of ectopic ossification *in vivo*.[55] Additionally, the clinical safety and efficacy of ASCs therapy have been reported. After allogeneic ASC treatment, patients with lateral elbow epicondylosis self-reported outcomes with reduced pain and improved function, without safety issues, as well as demonstrated decreased tendon defect areas in ultrasound images at fifty-two weeks post-injection.[56] However, the application of ASCs may give rise to fibrotic tissue formation and scarring[57] as well as forming adipocytes[58] during tendon repair. In addition, induced pluripotent stem cells (iPSCs) can be reprogrammed from adult somatic cells. It has been found that human iPSC-derived neural crest stem cells (iPSC-NCSCs) can differentiate into mesenchymal-lineage tenocytes, which accelerate the process of tendon repair.[59] In a rat patellar-tendon window-defect trial, iPSC-NCSCs promoted healing by improving matrix synthesis and mechanical properties and by increasing fetal tendon-related matrix proteins, stem-cell recruitment factors, and the tenogenic differentiation factor.[60]

Compared with exogenic stem cells/progenitors, TSPCs possess higher regenerative potential for tendon repair. For instance, during treatment of rat Achilles tendon injury, TSPCs have a greater positive effect on morphological and histological alteration and biomechanical strength when compared to BMSC transplantation.[31] This distinction may be because TSPCs proliferate more rapidly and have a greater capacity for colony formation;[41, 61, 62] additionally, TSPCs undergo

spontaneous tenogenic differentiation, whereas BMSCs do not.[20] It has been demonstrated that mouse TSPCs express higher levels of tenogenic markers, such as Scx, Comp, Sox9, and Runx2, than mouse BMSCs; similarly, human TSPCs express more TNMD than BMSCs do.[4] Thus, TSPCs more rapidly differentiate to functional tenocytes. Moreover, the expressions of collagen I and collagen III are higher in TSPCs, which results in greater biomechanical strength at the early stage of repair.[31] However, the limited number of resident TSPCs hinders the large-scale clinical application.[63] Hence, both endogenous and exogenous stem cells have therapeutic potential. According to current evidence, TSPCs possess some advantages for tendon repair, but the efficacy of endogenic and exogenic stem cells requires further investigation.

Comments

Please give more detailed information for "normal loading" and "abnormal loading" in tendon injuries in "Mechanical response of stem cell/progenitors for tendon repair" section (page 8, line 11-14).

Response: Thanks for your kind comments. We added some detailed information of "normal loading" and "abnormal loading" for easy understanding for readers in "Mechanical response of stem cell/progenitors for tendon repair" section.

A number of factors influence the homeostasis of tendon, in which mechanical loading plays a critical role.[64] Under normal or physiological loading, the magnitude of loading is much less than the ultimate tensile

strength (UTS). Typically, tendon could return to its original length when the strain is less than 4 percent elongation; but tendon will have macroscopic tearing and eventually rapture when the strain is beyond 8-10 percent elongation.[65] Researchers usually use 4 percent cyclic uniaxial stretching to mimic this loading condition in *vitro*, and to moderate treadmill running model of rats (13 m/min, 15 min/day, and 5 days/week in the first week; 13 m/min, 50 min/day, 5 days/week for another 3 weeks) *in vivo*[21]. In normal or physiological loading, tendon can maintain homeostasis and respond to loading through cellular anabolic adaptation[3, 21]. By contrast, abnormal loading may be different from normal mechanical loading in magnitude, frequency, duration, and/or direction; typically, abnormal loading of tendon can be unload, overload or high repetitive low load.[66] Compared with explants tensioned with constant 4 percent strain, nontensioned rabbit patellar tendon decreased linear stiffness, elongation to failure, and maximum failure force after 20h;[67] undergoing cyclic loading at approximately 35 percent of the UTS led to tendon rapture in 15 min;[68] also, cyclic loading at under 5 percent UTS (around 1 percent strain) resulted in rupture within 15 h.[69] *In vitro*, researchers usually use 8 percent cyclic uniaxial stretching to mimic the over loading condition, as well as intensive treadmill running (13 m/min, 15 min/day, and 5 days/week in the first week; 13 m/min, 3 hrs/day, 4 hrs/day, and 5 hrs/day in the second, third, and fourth weeks for 5 days) [21].

Comments

In conclusion section, (page 15 line 2) the word “method” does not seem to fit this paper, since authors are describing the all sources of stem cells and their molecular mechanism for tendon repair.

Response: We appreciate a lot for your excellent comment. we have replaced the term “method” with the term “strategies” to make the expression more in line with our thoughts in the **conclusion** section.

The response of stem cells to strain stress

These findings promise a bright future with new therapeutic strategies for tendon repair. Further studies are necessary to identify mechanosensors and deeply understand the signaling pathways of stem cells.

Comments

Please choose anther term. Please add more explanations the differences between TSPCs and TDSCs.

Response: Thank you very much for valuable suggestions. In the field of tendon stem cell research, the terminology has not been unified. Commonly, the term “TSPCs” and the term “TDSCs” refer the same thing. We clarified this point to avoid confusion of readers in the **Introduction** section.

Additionally, tendon stem/progenitor cells (TSPCs), also commonly termed tendon-derived stem cells (TDSCs) or tendon stem cells (TSCs), located in the fascicular matrix, are responsible for replenishing tendon cells through differentiation and proliferation.