Tendon Stem Cells: Mechanobiology and Development of Tendinopathy

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Abstract

Millions of people suffer from tendon injuries in both occupational and athletic settings. However, the restoration of normal structure and function to injured tendons still remains as one of the greatest challenges in orthopaedics and sports medicine. In recent years, a remarkable advancement in tendon research field has been the discovery of tendon stem/progenitor cells (TSCs). Unlike tenocytes, the predominant resident cell in tendons, TSCs have the ability to self-renew and multi-differentiate. Because of these distinct properties, TSCs may play a critical role in tendon physiology as well as pathology such as tendinopathy, which is a prevalent chronic tendon injury. Additionally, because TSCs are tendon-specific stem cells, they could potentially be used in tendon tissue engineering *in vitro*, and serve as a promising cell source for cell-based therapy to effectively repair or even regenerate injured tendons in clinical settings.

Keywords

Tendon stem cells • Self-renewal • Multi-differentiation • Mechanobiology

Abbreviations

ATSC	Achilles tendon stem cell
Col. I	Collagen type I
Col. II	Collagen type II
FEM	Finite element method
ITR	Intensive treadmill running
MSCs	Mesenchymal stem cells
MTR	Moderate treadmill running
NS	Nucleostemin
Scx	Scleraxis
Tenom	Tenomodulin
TSCs	Tendon stem/progenitor cells

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Introduction

Tendons are bands of connective tissues that are particularly rich in collagens. The most abundant tendon component is type I collagen, which

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constitutes about 70–80 % of the dry tendon mass and about 95 % of the total collagen in tendons [1–3]. The remaining 5 % consists of type III and V collagens. Within the tendon matrix, collagens are cross-linked [4, 5], which increases the tendon's mechanical strength [6]. Besides collagens, tendons also contain proteoglycans in small quantities, but their amounts vary in tendons in different anatomical locations and also depend on the mechanical loading conditions (e.g., tension vs. compression) on a tendon [7, 8].

Mechanically, tendons are responsible for transmitting muscular forces to bone, and because they are live tissues, tendons also respond to mechanical loads by changing their metabolism as well as their structural and mechanical properties. While appropriate mechanical loading benefits tendons, chronic mechanical loading placed on tendons plays a major role in the development of tendinopathy [9, 10]. This major tendon disorder is often manifested by degenerative changes in the tendon, including lipid deposition, proteoglycan accumulation, and calcification either alone or in combination [11]. Despite years of extensive research, restoration of tendon structure and function after injury still remains as one of the greatest challenges in orthopaedic surgery and sports medicine.

In pursuit of new strategies to promote healing of tendon injuries, significant progress has been made in tendon research in recent years. We and others have identified a new population of tendon cells termed tendon stem/progenitor cells (TSCs) in humans, mice and rabbits [12–15]. Much like other tendon cells, TSCs also respond to the various mechanical loads placed on tendons. This chapter will provide an overview of the recent advancements in TSC mechanobiology. In particular, the role of TSCs in the mechanical loading-induced development of tendinopathy is highlighted. Future research developments in the field are also suggested and discussed.

Discovery of TSCs

Until recently, tenocytes were considered to be the only cell type in tendons. However, about 5 % of tendon cells were recently discovered to be TSCs in the tendons of humans, mice, rats and rabbits [12–15]. TSCs can self-renew and differentiate into tenocytes to maintain tendon homeostasis. Like other adult stem cells, TSCs also have multi-differentiation potential, which allows them to differentiate not only into tenocytes, but also into non-tenocytes including chondrocytes, osteocytes and adipocytes, but only under certain pathophysiological conditions [12, 13].

Unlike TSCs, tenocytes are specialized tendon cells that lack the capacity to differentiate into other cell types. In culture, TSCs proliferate more quickly than their counterparts (tenocytes) and appear as small, cobblestone-shaped cells with large nuclei, while tenocytes grow as highly

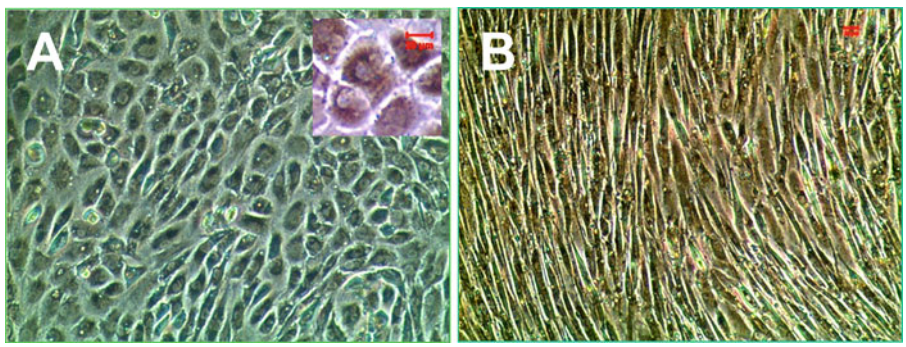

Fig. 5.1 Tendon stem/progenitor cells (TSCs) and tenocytes in culture exhibit a striking difference in morphology. (A) TSCs. The cells are cobblestone-like in culture when grown to confluence. They also have large nuclei (inset). (B) Tenocytes in culture. These cells are fibroblast-like and assume a highly elongated shape in confluent conditions

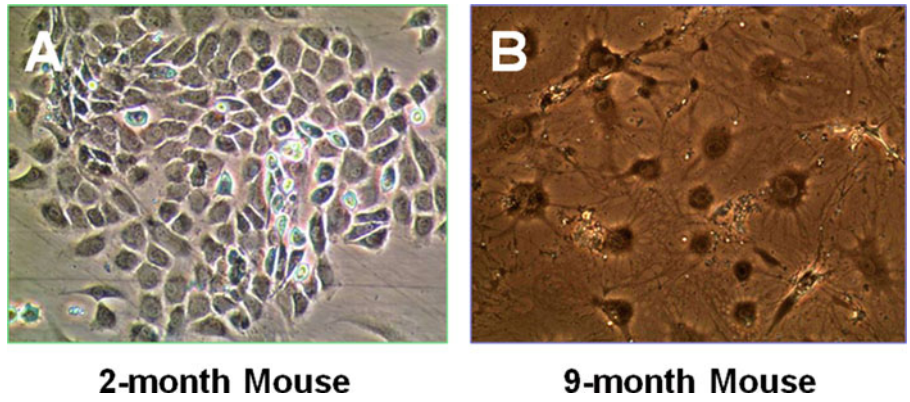

Fig. 5.2 Age affects TSC morphology and growth in culture. (**A**) Shown is a colony of young TSCs derived from 2-months old mouse. The cells are large in numbers and cobble-stone shaped in culture. (**B**) Aging TSCs from 9-months old mouse are lower in numbers, and are flat and spread-out in culture. Their growth is also slower in culture

elongated, fibroblast-like cells with smaller nuclei [13] (Fig. 5.1). Tenocytes also lack a characteristic feature of TSCs, which is the expression of stem cell markers such as Oct-4, SSEA-1/4 and nucleostemin (NS).

The typical features of TSCs in tendons, however, change during aging. In a recent study, we determined the effects of aging on TSCs *in vitro*. TSCs derived from aging mice (9 and 24 months) proliferated significantly slower than TSCs obtained from young mice (2.5 and 5 months) (Fig. 5.2). A likely reason for this was revealed during the analysis of cellular protein expression pattern, i.e., expression of the stem cell markers Oct-4, NS, Sca-1 and SSEA-1 in TSCs decreased in an age-dependent manner, which in part, may contribute to the lowered proliferation rate of TSCs in aging mice. These findings indicate that aging impairs the proliferative ability of TSCs and reduces their stemness [16] (Fig. 5.3).

A Mechanical Loading System to Study TSC Mechanobiology *In Vitro*

To study the mechanobiology of TSCs under well controlled conditions, we developed a novel *in vitro* model system (Fig. 5.4). This *in vitro* system enabled us to examine mechanobiological responses (e.g. proliferation, self-renewal, and differentiation) of TSCs under well controlled, *in vivo*-like multiple mechanical loading conditions [17], which are not possible in an animal model. Therefore, such a system is essential to define the loading-induced, TSC-based mechanisms of degenerative tendinopathy.

Use of Mouse Treadmill Running to Study of Mechanobiology of Tendon *In Vivo*

To study the mechanobiology of tendons *in vivo*, we developed a mouse treadmill running model (Fig. 5.5) and optimized two different running regimens: (1) moderate treadmill running (MTR); and (2) intensive treadmill running (ITR). In the MTR regimen, mice were trained to run at the speed of 13 m/min, 15 min/day and 5 days/week. Then, they ran at the same speed for 50 min/day, 5 days/week for 3 weeks. In the ITR regimen, mice were trained as in MTR in the first week. They then ran at the same speed for 3 h/day, 4 h/day, and 5 h/day for 5 days in the second, third, and fourth weeks, respectively. The mice in the control group did not run on treadmills, but freely moved in cages during treadmill running experiments.

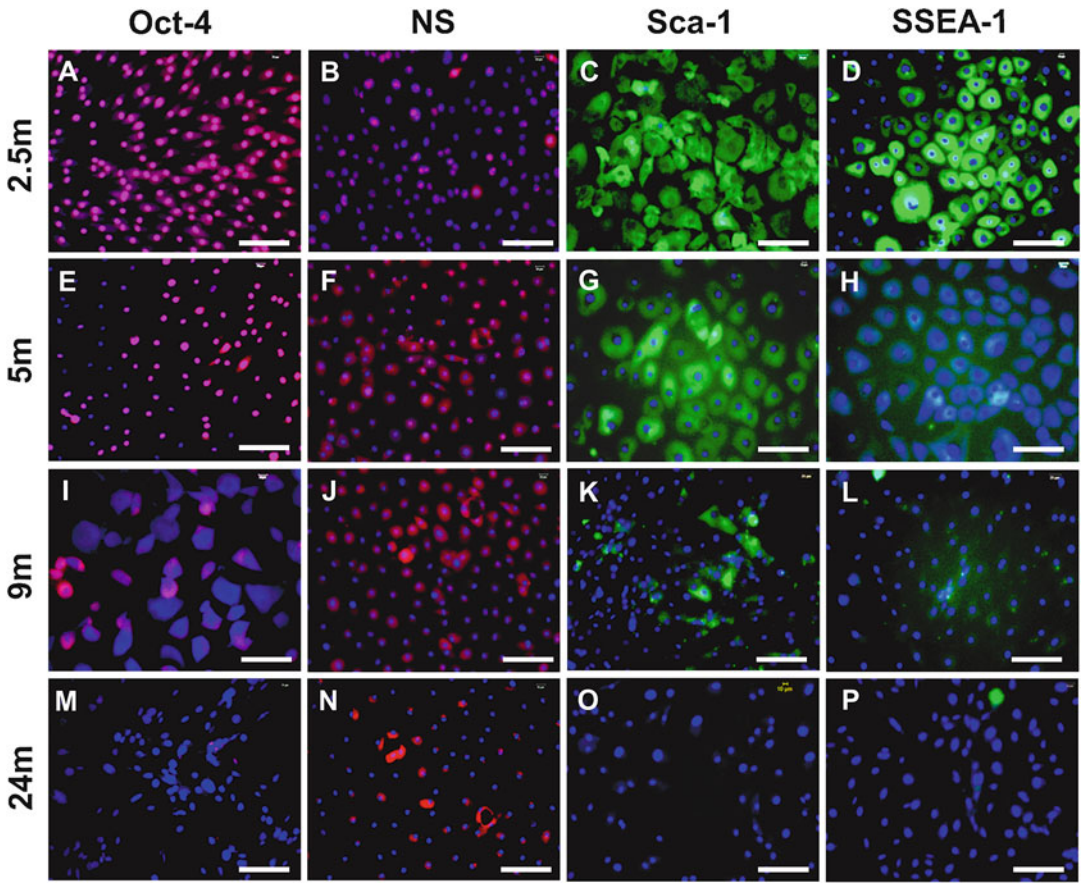

Fig. 5.3 Age reduces TSC stemness. Expression of the stem cell markers, Oct-4, NS, Sca-1, and SSEA-1 in TSCs from 2.5 months (2.5 m), 5 months (5 m), 9 months (9 m) and 24 months (24 m) old mice are shown. Increase in the mouse age decreased the number of TSCs expressing the stem cell markers, which are abundant in TSCs from 2.5 months old mice but scant in TSCs from 24 months old mice. Bar −100 μm

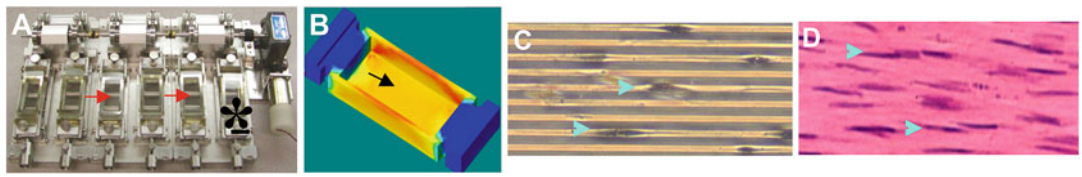

Fig. 5.4 A novel *in vitro* model system to study mechanobiology of TSCs. (**A**) The stretching apparatus. Silicone dishes (*red arrows*) are used to grow and stretch tendon cells. (**B**) Strain distribution in the silicone dish by FEM (finite element method). The surface strains are uniform in the central region of the culture surface (*black arrow*), where cells are plated and stretched. (**C**) Tendon cells (TSCs, tenocytes, or both) on microgrooved culture surfaces (*arrows* point to two tendon cells residing on microgrooves with a width of 10 μm). (**D**) Tendon cells in the tendon section (arrows point to tendon cells). As seen in **C** and **D**, tendon cell density, cell shape and organization can be controlled in this model system to mimic *in vivo* conditions in the tendon

The Role of TSCs in the Development of Tendinopathy

Tendinopathy is a serious healthcare problem for people in both occupational and athletic settings [18–20]. Many occupational activities result in tendinopathy and present with clinical symptoms including pain and signs of inflammation such as redness and swelling; all of these may impair one's ability to work [19]. When tendoninopathy further progresses, one or more of the following degenerative changes are often noticed: lipid deposition, proteoglycan accumulation and calcification [11] (Fig. 5.6). Often times, the etiology for this tendon disorder is recognized as the chronic mechanical loading on tendons [18, 21–28]. However, the precise cellular mechanisms leading to the pathology of tendinopathy remain unclear. As a result, tendinopathy continues to be a nemesis for both physicians and patients alike [10]. Currently, there is no protocol in place that can effectively prevent tendinopathy or restore tendon structure and function after injury [22, 29].

In our quest to address this lacuna, we focused our research on TSCs that play a major role in the maintenance of tendon homeostasis [12, 13, 15]. To explore the possibility that TSCs may also play a critical role in the development of degenerative tendinopathy, we used both *in vitro* and *in vivo* model systems described above to examine the mechanobiological responses of TSCs to various mechanical loading conditions.

Fig. 5.5 Mouse treadmill running is used to study TSC mechanobiology. Mice run on the treadmill with 6 running lanes, according to a specified running regimen; moderate treadmill running (MTR) or intensive treadmill running (ITR)

Mechanobiological Response of TSCs *In Vitro*

Applying small mechanical loading on TSCs using our *in vitro* model system induces tenocyte differentiation, while large mechanical loading induces both tenocyte and non-tenocyte differentiation [30] (Fig. 5.7). Specifically, small mechanical stretching (4 %) significantly increased only the expression of the tenocyte related gene, collagen type I (Col. I), but not the expression of non-tenocyte related genes, PPARγ, collagen type II (Col. II), Sox-9 and Runx-2, which are markers of adipocytes, chondrocytes and osteocytes, respectively. However, large mechanical stretching of 8 %

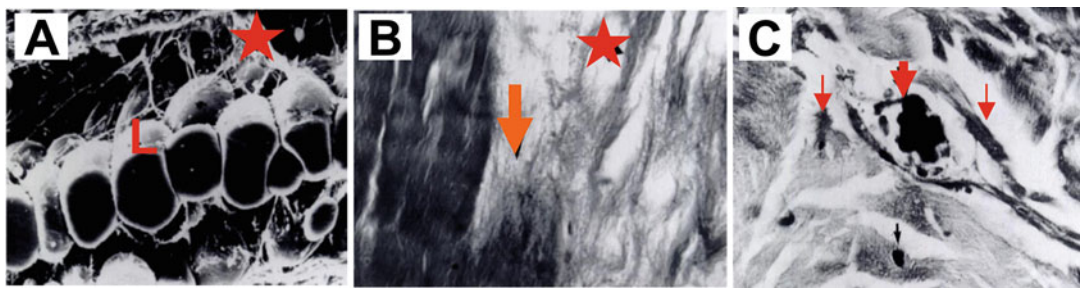

Fig. 5.6 Degenerative tendinopathy in human tendons. Three forms of degenerative changes were observed either alone or in combination in affected tendons: Lipid formation (**A**, L and star), accumulation of proteoglycans (**B**, *arrow* and *star*) and calcified tissue (**C**, *arrows*) [11]

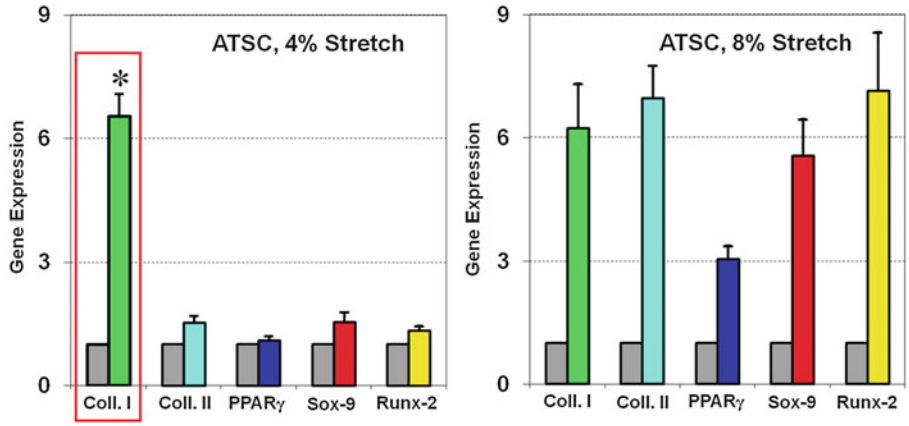

Fig. 5.7 Gene expression profile in TSCs derived from mouse Achilles tendons depends on mechanical loading conditions *in vitro*. TSCs stretched to 4 % (**a**) showed specific up-regulation of collagen type I (Coll. I), which is a tenocyte related gene, but not the non-tenocyte related genes, collagen type II (Coll. II), Sox-9, PPARγ or Runx-2. However, 8 % stretching up-regulated both tenocyte and non-tenocyte genes to varying degrees. *Gray bars* are controls without loading

significantly increased the expression of both tenocyte and non-tenocyte related genes.

Moreover, by performing *in vitro* stretching experiments on isolated tenocytes [30], we further showed that tenocytes do not express non-tenocyte related genes, even under overloading (8 % stretching) conditions [31]. Thus, these findings indicate that TSCs are likely the primary cells responsible for degenerative tendinopathy by undergoing aberrant non-tenocyte differentiation into fatty-, cartilage-like and bone-like tissues in tendons that compromise tendon structure.

In addition to studying the effects of various mechanical loading conditions on TSCs, several studies have investigated the molecular signaling pathways involved in the mechanobiological responses of TSCs. It was reported that Wnt5a/Wnt5b/JNK signaling pathway is involved in the osteogenic differentiation of rat TSCs in response to mechanical loads [32–34]. Particularly, JNK activity was decreased and osteogenic differentiation was inhibited in Wnt5a/Wnt5b/JNK knockdown TSCs after uniaxial mechanical loading. This inhibition was rescued by the JNK activator, anisomycin, or JNK1-cDNA, suggesting that Wnt5a/Wnt5b is located upstream of JNK and regulates the function of JNK in the Wnt5a/Wnt5b/JNK signaling pathway [32]. Wnt3 also promoted osteogenic differentiation of TSCs *in vitro* [35]. Moreover, increased amounts of Wnt3a were observed in human patellar tendons with tendinopathy and animal models of tendinopathy [36]. In another study, mechanical loading of TSCs increased Wnt5a protein levels, but not other Wnt proteins [37]. The discrepancy between these two studies may be due to differences in the mechanical loading variables for TSCs including different amounts of mechanical loads, frequency and duration (see Chap. 7).

To date, only a few studies have investigated the mechanotransduction mechanisms of TSCs. Since TSCs are adult stem/progenitor cells, it is conceivable that the mechano-signaling pathways that have been established in mesenchymal stem cells (MSCs) and tenocytes may also be applied to TSCs. It has been demonstrated that focal adhesion kinase, cytoskeleton and RhoA/ROCK play important roles in mechanical loading-induced tenogenic differentiation of MSCs [38, 39]. Mechanical forces are also known to regulate Scleraxis (Scx) expression through the activation of SMAD2/3-mediated TGF-β signaling in adult mouse tendons [40]. Stretching of tendon constructs *in vitro* also increased ERK/MAPK phosphorylation [41]. Moreover, EGR1 is a known

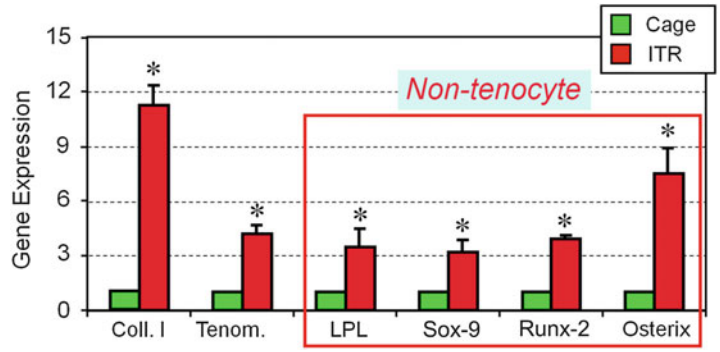

Fig. 5.8 Intensive treadmill running (ITR) up-regulates both tenocyte-related genes, collagen type I (Coll. I) and tenomodulin (Tenom) and non-tenocyte related genes, LPL, Sox-9, Runx-2 and Osterix in mouse Achilles tendons

mechanosensitive transcription factor involved in the tendon cell response [42]. EGR1 is expressed in response to mechanical stimuli, mainly via the MAPK pathways. However, further investigations regarding its role in the mechanotransduction of TSCs are needed.

The Mechanobioological Responses of TSCs *In Vivo*

The effects of mechanical loading on TSCs *in vivo* were also determined. Our results revealed that a moderate treadmill running regimen (MTR, see above for definition) nearly doubled the proliferation rate of TSCs when compared to control mice in cages. Moreover, cellular production of total collagen in the treadmill running group increased by 70 and 200 % in patellar and Achilles tendon cells, respectively, compared to cells from the control group [17].

A continuation of our study to determine the effects of intensive treadmill running (ITR, see above) on mouse tendons [31] revealed that after ITR, all genes related to fatty, cartilage and bony tissues (LPL, Sox-9, Runx-2 and Osterix), and tendon-related genes (Coll. I, and tenomodulin or Tenom) were highly expressed in the mouse patellar tendons (Fig. 5.8). This is consistent with the findings that excessive mechanical loading via treadmill running results in elevated expression of cartilage-related genes (Col. II, aggrecan and Sox-9) in the rat supraspinatus tendon [43] and that long-term treadmill running (up to 16 weeks) leads to degenerative tendinopathy as evidenced by accumulated proteoglycan and chondrocytes present in the rat tendon [26].

Concluding Remarks

Tendon injuries frequently occur among people in all walks of life. Current treatments are largely ineffective and, as a result, the restoration of normal tendon structure and function continues to be a challenge in the clinical treatment of tendons. Therefore, basic scientific studies on tendons are needed so that new treatment strategies can be devised. The recent discovery of TSCs has offered an opportunity for tendon researchers to look into the stem cell mechanisms responsible for chronic tendon injury or tendinopathy. Studies have, indeed, suggested that TSCs may be responsible for the development of degenerative tendinopathy due to excessive mechanical loading constantly placed on tendons. Therefore, effective treatment options for such conditions should not only be anti-inflammatory, but should also include methods to deter degeneration of tendons. This could likely be achieved by blocking the aberrant differentiation of TSCs, even under excessive mechanical loading conditions.

There are several research directions to explore in TSC mechanobiology. First, the *in vivo* characterization of TSCs should be pursued. Currently, TSCs are characterized *in vitro*. The *in vivo* identities and niche of TSCs with respect to anatomical locations and regulatory factors in tendons remain to be investigated [35, 44]. Second, the exact origins and identities

of "TSCs" *in vivo* after a tendon injury are unknown. This requires lineage specific marking/tracing of TSCs. A recent study showed that α-SMA positive progenitor cells in the paratenon migrate into the tendon wound to participate in its healing [45]. Other potential sources of "TSCs" in the healing of tendon injury may include: stem cells in the circulatory system, adipose tissue near tendons, and the bursa, a fluid-filled sac that acts as a cushion between a bone and other moving parts such as muscles, tendons or skin [46, 47].

Third, the effects of aging on TSC mechanobiology should be thoroughly investigated. Although it remains to be demonstrated definitively, aging is presumed to cause tendon cell senescence, which refers to the cell status in which permanent cell cycle arrest cannot be reactivated [48]. Tendon cell senescence may lead to tendon degeneration and impair tendon healing, which are often seen in aging patients. But the relationship between aging and reduced tendon potential to heal needs to be demonstrated. These detrimental effects caused by aging can be reversed by applying moderate physiological loads on tendons that induces anabolic changes by regulating TSCs [49]. Thus, exercise in the form of mechanical loading can improve aging-associated impairment in healing tendons by reactivating senescent tendon cells, especially TSCs. Therefore, this line of research warrants future study.

Finally, it has been well established that stem cells are excellent sources for the engineering of injured tissues. Indeed, implantation of engineered tendon tissue containing TSCs in de-cellularized fibroblast matrix effectively improved the mechanical properties in a patellar tendon injury model [50] and promoted tendon-like tissue formation in rat patellar tendons [51]. However, the role played by moderate mechanical loading in the healing of TSC-treated injured tendons remains to be investigated; devising optimal exercise regimens for tendon injury patients is of great clinical importance.

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