Tendon stem/progenitor cell ageing: Modulation and rejuvenation

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Abstract
Tendon ageing is a complicated process caused by multifaceted pathways and ageing plays a critical role in the occurrence and severity of tendon injury. The role of tendon stem/progenitor cells (TSPCs) in tendon maintenance and regeneration has received increasing attention in recent years. The decreased capacity of TSPCs in seniors contributes to impaired tendon functions and raises questions as to what extent these cells either affect, or cause ageing, and whether these age-related cellular alterations are caused by intrinsic factors or the cellular environment. In this review, recent discoveries concerning the biological characteristics of TSPCs and age-related changes in TSPCs, including the effects of cellular epigenetic alterations and the mechanisms involved in the ageing process, are analyzed. During the ageing process, TSPCs ageing might occur as a natural part of the tendon ageing, but could also result from decreased levels of growth factor, hormone deficits and changes in other related factors. Here, we discuss methods that might induce the rejuvenation of TSPC functions that are impaired during ageing, including moderate exercise, cell extracellular matrix condition, growth factors and hormones; these methods aim to rejuvenate the features of youthfulness with the ultimate goal of improving human health.
Tendon stem/progenitor cell ageing

**INTRODUCTION**

Ageing is an intricate physiological progress caused by multiple factors that result in variations in the structure and composition of cells, organs and tissues and a decrease in the capacity and activity of mammals. The global population over the age of 60 years is growing rapidly, and the occurrence of tendon-related injuries increases upon ageing. Moreover, the consequences of tendon damage in elderly patients are more severe, and older populations also experience a higher occurrence of sport-related tendon injuries and more difficulties in healing processes, which places a heavy burden on the health systems of individual countries. 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.

Recently, studies focused on stem cells have become emerging areas in regenerative medicine 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. Pluripotent stem cells 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. 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 2007, and they have been found to possess self-renewal ability, clonogenicity and multidifferentiation potential. Compared with bone marrow stromal cells, TSPCs show express higher level of Oct4, which is known to positively modulate mesodermal lineage differentiation, and have greater ability of proliferative and clonogenicity. Thus, TSPCs potentially represent an more appropriate cell source for the regeneration of musculoskeletal tissue, particularly tendon tissue, which has limited repair and healing abilities with traditional tenocytes. Based on these findings, scholars have a strong interest in identifying the potential role of TSPCs in tendon regenerative medicine and the injury healing process; thus, numerous related studies have been published on this topic in recent years.

However, ageing exerts negative effects on TSPCs functions, which could limit the application of TSPCs in tendon injury repair and the choice of cell sources for regenerative medicine. Ageing also affects cell the genetics of cells, through a series of pathways involved in both accelerating and delaying the ageing process. In the MSC ageing process, the P16/RB pathway and P53/P21 pathway have vital roles in modulating the cellular senescence by regulating telomere length and function. In addition to telomeres, DNA damage, mitochondria dysfunction and reactive oxygen species are involved in suppressing the expression of genes that promote the stem cell cycle progression of stem cells and induce the expression of cell cycle inhibitors.

**Key words:** Tendon stem/progenitor cell; Ageing; Mechanisms; Modulation; Rejuvenation

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**Core tip:** Tendon stem/progenitor cells (TSPCs) play an essential role in tendon maintenance, regeneration and repair. Recent studies indicate that an association between the decreased capacities of aged TSPCs and the impaired tendon functions observed with increasing age. In this review, we briefly discuss novel updates in research investigating TSPCs characteristics. Then, we summarize the epigenetic variations in TSPCs that occur with ageing and provide a detailed description of the pathways that play essential roles in the cellular ageing process. Finally, we propose potential methods to rejuvenate ageing TSPCs and provide additional therapeutic targets for the treatment of age-related tendon diseases.
Up-regulation of P53, P16, P14 and P21 genes related to cell cycle arrest and activation of the P53 pathway and P21 pathway have also been observed in aged TSPCs, which are thought to function as accelerators of the cellular ageing process[14]. What’s more, stem cell markers expression declines with age in TSPCs, indicating potential causes of the alterations in cell differentiation ability[15]. In this regard, a novel hypothetical model of altered TSPCs fates in the ageing process has been formulated based on the observation of ectopic metaplasia and the decline in the tenogenic differentiation capacity of tendon tissue during the ageing process, which ultimately increases the occurrence of age-related tendon diseases[16]. 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 youth. Thus, iPSCs provided inspiration to reverse the stem cell fate by modulating the factors that influence cell growth[16-18].

With limited treatment options for tendon diseases and unsatisfactory healing outcomes, studies aimed to explore the biological link between tendon ageing and TSPCs are very meaningful for the development of age-related diseases treatments. In this review, we initially discuss recent studies addressing the characteristics of TSPCs. Then, we summarize the epigenetic variations in TSPCs that occur with age and provide a detailed description of the pathways that play essential roles in the cellular ageing process. Finally, we propose potential pathways to rejuvenate ageing TSPCs, providing further therapeutic targets for the treatment of age-related tendon diseases.

TENDON STEM/PROGENITOR CELLS

Traditionally, tenocytes were considered the only cell present in tendon tissue and play a critical role in tendons metabolism. This hypothesis did not change until the isolation and identification of TSPCs in many tendon fascicles, including mouse[11], human[18], rat[20], rabbit[21], turkey[22], porcine[23] and fetal bovine tendon fascicles[24] in recent years. Although TSPCs represent a minor percentage of the tendon cell composition, these cells possess features such as self-renewal, clonogenicity and multidifferentiation and TSPCs are distinguished by the presence of stem cell markers[25]. 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.

Compared with tenocytes, TSPCs express stem cell markers, proliferate faster, exhibit multidifferentiation potential and express tenogenic markers at higher levels[21-23]. Although Berglund et al[24] proposed a different hypothesis that major histocompatibility complex (MHC) mismatched MSCs were not immune privileged because they induced both cell-mediated and humoral immune responses, the majority of studies consistently shown that MSCs display low immunogenicity and immuno-modulatory properties, which avoid immunological rejection. Thus MSCs are a potential allogeneic cell source for transplantation, and TSPCs, a subtype of MSCs, may possess features similar to MSCs[26]. According to Lui et al[27], TSPCs expressed lower levels of MHCI/II, cluster differentiation 86 and cluster differentiation 80 on the cell surface; these proteins are essential for inducing a T-cell response. Additionally, the infiltration of inflammatory cells was not observed in tendon injuries treated with allogeneic TSPCs, revealing the low immunogenicity of TSPCs in vitro and in vivo[28-29]. Based on these facts, researchers have confirmed that these active TSPCs are immune-privileged and can be used for allogeneic transplantation. Benefiting from the positive aspects, particularly the multi-differentiation capacities and immune-privilege, TSPCs potentially represent an ideal cell source for musculoskeletal tissue regenerative medicine and therapeutic targets for numerous related diseases. Although important research has shown that TSPCs might reside within the tendon fascicles, others researchers have suggested that the epitenon might be another source of TSPCs[30]; subsequent studies have confirmed this hypothesis[31-33]. Although all TSPCs generally exhibit the characteristics of tendon stem cells, they have their own unique features when isolated from different sites in the tendon. These findings reveal the presence of more than one source of distinct TSPCs in tendon tissue, and these populations represent a seed cells source for application in different tendon injuries according to the different cellular characteristics[30-33].

Moreover, numerous studies have confirmed that TSPCs play an essential role in the progression of tendon diseases and/or tendon injuries. In this regard, a novel hypothetical model of altered TSPCs fates in the ageing process has been formulated based on the observation of ectopic metaplasia and the decline in the tenogenic differentiation capacity of tendon tissue during the ageing process, which ultimately increases the occurrence of age-related tendon diseases[16]. 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 youth. Thus, iPSCs provided inspiration to reverse the stem cell fate by modulating the factors that influence cell growth[16-18].

With limited treatment options for tendon diseases and unsatisfactory healing outcomes, studies aimed to explore the biological link between tendon ageing and TSPCs are very meaningful for the development of age-related diseases treatments. In this review, we initially discuss recent studies addressing the characteristics of TSPCs. Then, we summarize the epigenetic variations in TSPCs that occur with age and provide a detailed description of the pathways that play essential roles in the cellular ageing process. Finally, we propose potential pathways to rejuvenate ageing TSPCs, providing further therapeutic targets for the treatment of age-related tendon diseases.
attributed to increased DNA synthesis, increased cellular migration velocity and the supplements of TSPCs. In several cases, growth and differentiation factor-5 was reported to promote the tenogenic differentiation of TSPCs, and transforming growth factor-β1 and insulin-like growth factor 1 (IGF-1) promotes TSPC proliferation and phenotype maintenance. Additionally, the expression of inflammatory cytokines is dramatically upregulated in injured tendons, some of which inhibit the proliferation and tenogenic and osteogenic differentiation of TSPCs. Moreover, TSPCs are essential for tendon healing and the regulation of inflammation, and the production of the pro-inflammatory cytokine Interleukin-6 (IL-6) and anti-inflammatory cytokine Interleukin-10 (IL-10), is significantly up-regulated at the late stage of inflammation in injured tendons. Based on these findings, IL-6 and IL-10 evidently up-regulate cell proliferation, and IL-10 significantly enhances cell migration. However, both IL-6 and IL-10 inhibit the production of gene and protein functioning as tenocytes markers, including scleraxis and tenomodulin, and dramatically activate the JAK/Stat3 signaling pathway, which has a crucial role in modulating inflammation in TSPCs, indicating that IL-6 and IL-10 may exert dual effects on TSPCs in vitro, and connective tissue growth factor plays a role in anti-inflammatory by regulating the IL-6 and IL-10 expression. Decreased annexin A1 (an anti-inflammation protein) expression resulted in elevation of inflammation during the mouse tendon injury process; thus, annexin A1 potentially represents a novel curative target in clinical applications. In addition, many drugs and proteins exert effects on TSPCs that promote tendon healing. Celastrol exerts beneficial effects on human TSPCs stemness and the vital role of HIF1α-Smad7 signaling in the process is elucidated. Celecoxib inhibits the tenogenic differentiation of TSPCs but has no effects on cell proliferation, and a high concentration of aspirin induces apoptosis in TSPCs by delaying the activation of Wnt/β-catenin pathway. All these factors might affect the quality of tendon healing by targeting TSPCs, regardless of whether the effects are positive or negative. The recent main factors are summarized in Table 1.

In addition, an altered fate of TSPCs was observed in a collagenase-induced tendon injury model of tendinopathy due to the presence of tenocytes lacking the multidifferentiation capacity, 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. Thus, TSPCs are considered to play a crucial role in maintaining tendon homeostasis by affecting tendon repair and regeneration. Recently, Li et al. 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. 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.

**AGEING AND ALTERATIONS IN EPIGENETIC AND THE UNDERLYING MECHANISMS**

**Age-related markers in TSPCs**

TSPCs undergo a series of significant cellular epigenetic alterations with age, which are viewed as age-related markers in TSPCs for that can be used in future studies, and these results are consistent with similar results obtained from other types of stem cells. The main findings are summarized in Table 2.

**Ageing and cell morphology**

*In vitro* aged-TSPCs (A-TSPCs) exhibit cell shape of star-like flattened, while young-TSPCs (Y-TSPCs) exhibit spindle-shaped morphology. In addition, aged TSPCs are obviously larger in size, have more podia, spread further, and exhibit more robust actin stress fibers and a higher actin content that distorts the balance of the actin cytoskeleton organization, which has also been confirmed by analyses of microarray data in aged TSPCs. Additionally, aged TSPCs display a large, flat and heterogeneous morphology, while younger cells exhibit the morphology of uniform elongated. An increase in the size is often associated with cell senescence. In addition, the number of heterogeneous and cobblestone-shaped TSPCs is dramatically down-regulated with ageing, and the oldest TSPCs have only a few percent displaying the cobblestone shape. Kohler et al. reported an important role for...
## Table 1 Recent main factors for regulating tendon stem/progenitor cells biological features

| Factor | Cell source | Interventional details | Results | Ref. |
|--------|-------------|------------------------|---------|------|
| PRP    | SD rats     | 10% PRP                | 10% PRP augments and accelerates the effects of TSPCs on the healing process | [37] |
| BMACs and PRP complex | Human | A T75 flask (450 μL of BMACs and PRP) | BMAC–PRP enhances the proliferation and migration of TSPCs | [38] |
| PRP    | SD rats     | 2% PRGF                | PRP can activate TSPCs to improve the quality of Achilles tendon rupture healing | [39] |
| IGF-1, GDF-5 and TGFβ1 | Lewis rats | Each growth factor (1, 10, and 100 ng/mL) | GDF-5 promotes TSPCs tenogenic differentiation, and TGFβ1 and IGF-1 increase TSPCs proliferation and are beneficial for phenotype maintenance | [40] |
| IL-1β  | Dogs        | -                      | The expression of inflammatory cytokines is dramatically up-regulated in injured tendon | [41,42] |
| IL-1β  | Mouse       | IL-1β (1, 5 or 10 ng/mL) | IL-1β strongly and irreversibly impairs tenogenic and osteogenic differentiation potentials of TSPCs | [43] |
| IL-6   | SD rats     | IL-6 (0, 0.1, 1, 10, and 100 ng/mL) | IL-6 enhances proliferation and inhibits tenogenic differentiation in TSPCs via the JAK/Stat3 pathway | [44] |
| IL-10  | SD rats     | IL10 (0, 0.1, 1, 10 or 100 ng/mL) | IL10 enhances cell proliferation and migration, and inhibits tenogenic differentiation in TSPCs | [45] |
| CTGF   | SD rats     | CTGF (100 ng/mL)       | CTGF plays a role in anti-inflammatory, leading to enhanced tendon healing | [46] |
| Annexin A1 | WT and DF508 mice | -                     | Decreased annexin A1 expression resulted in elevation of inflammation during the mouse tendon injury process | [47] |
| Celastrol | Human | celastrol (0, 1, 2, and 4 μM) | Celastrol exerts beneficial effects on human TSPCs stemness and the vital role of the HIF1α-Smad7 pathway in the process is elucidated | [48] |
| Celecoxib | C57 mouse | celecox (0.1, 1, 10 and 100 μg/mL) | Celecoxib inhibits tenogenic differentiation of TSPCs but has no effects on cell proliferation | [49] |
| Aspirin | SD rats     | Aspirin (1, 2, and 5 mM) | A high concentration of aspirin induces apoptosis in TSPCs by delaying the activation of Wnt/β-catenin pathway | [50] |

PRP: Platelet-rich plasma; SD: Sprague–Dawley; TSPCs: Tendon stem/progenitor cells; BMACs: Bone marrow aspirate concentrates; PRGF: Platelet-rich growth factors; CTGF: Connective tissue growth factor; IL-10: Interleukin-10; IL-1β: Interleukin1β; TGFβ1: Transforming growth factor-β1; GDF-5: Growth and differentiation factor-5; IGF-1: Insulin-like growth factor1.

Increased Rho associated coiled-coil forming protein kinase (ROCK) activity in accelerating the ageing process of A-TSPC, and A-TSPCs revert to a morphology similar to Y-TSPCs upon treatment with Y-27632, an common ROCK inhibitor. Similar results have also been detected in aged tenocytes as well as in other types of stem cells.[58,59].

### Ageing and cell proliferation

**Growth rate:** A-TSPCs showed a proliferation deficit after 120 d of culture and had an
| Object          | Species model | Groups                          | Tendon type          | Main findings                                                                                                                                                                                                 | Ref.  |
|-----------------|---------------|---------------------------------|----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| Cell morphology | Human         | Y-TSPC group: 28 ± 5 yr; A-TSPC group: 63 ± 14 yr | Achilles tendon     | A-TSPC exhibit cell shape of star-like flattened, while Y-TSPCs exhibit spindle-shaped                                                                                                                      | [14]  |
| Human           |                | Y-TSPC: 28 ± 5 yr and A-TSPC: 63 ± 14 yr | Achilles tendons    | Aged TSPCs are obviously larger in size, have more podia, spread further, and exhibit more robust actin stress fibers, and exhibit higher actin content                                                                 | [15]  |
| Rat             | old rats: 20 mo and young rats: 8 wk | Achilles tendons             | Aged TSPCs display a morphologies of large, flat and heterogeneous morphology, while younger cells exhibit the morphology of uniform elongated                                                                          | [17]  |
| Mice            | young (2.5, and 5 mo) and aging (9 and 24 mo) mice | Patellar tendons        | The number of heterogeneous and cobbledstone-shaped TSPCs is dramatically down-regulated with ageing, and the oldest TSPCs have only a few percent displaying the cobbledstone shape | [15]  |
| Growth rate     | Human         | Y-TSPC group: 28 ± 5 yr; A-TSPC group: 63 ± 14 yr | Achilles tendon     | A-TSPCs showed a proliferation deficit after 120 d of culture and had an early plateau phase, while Y-TSPCs didn’t exhibit the plateau                                                                 | [14]  |
| Rat             | 3–4 (young) and 24–26 mo (aged) | Patellar tendons             | Proliferation rate is decreased and cell cycle progression is delayed with increasing age                                                                                                                   | [13]  |
| Rat             | three different postnatal stages: 1 d, 7 d and 56 d | Achilles tendon          | TSPCs-7d displayed that a higher proliferation rate than the groups of TSPCs-1d and TSPCs-5d                                                                                                                     | [16]  |
| Cell clonogenicity | Human              | Y-TSPC group: 28 ± 5 yr; A-TSPC group: 63 ± 14 yr | Achilles tendon     | Age-dependent clonogenic deficits in TSPCs are based on a decreased in the colony number and CFU efficiency with ageing                                                                                   | [14]  |
| Human           | Group 1: aged 20 (female) and 22 (male); group 2: aged 28 (female) and 31 (male) and Group 3: aged 49 (male) and 50 (female) | Hamstring tendons        | The clonogenic potential is dramatically decreased with age; in addition, the size of the colonies was heterogeneous in patients, as the size of colonies produced by cells from aged patients was obviously larger than the colonies composed of cells from younger patients | [18]  |
| Cell Type | Species | Time Points | Tissue | Findings |
|-----------|---------|-------------|--------|----------|
| Rat       | three different postnatal stages: 1 d, 7 d and 56 d | Achilles tendon | TSPCs-7d have an obviously higher clonogenic ability than TSPCs-1d and TSPCs-56d |
| Rat       | early P5, mid P10, and late P20 and P30 passages were used | Patellar tendons | The colony numbers of TSPCs increase with passaging, |
| Human     | Y-TSPC group: 28 ± 5 yr; A-TSPC group: 63 ± 14 yr | Achilles tendon | The migration of TSPCs exhibits a decreasing trend with advanced age |
| Rat       | three different postnatal stages: 1 d, 7 d and 56 d | Achilles tendon | TSPCs from different time groups displays multidifferentiation capability, while the ability of TSPCs-7d is higher than TSPCs-1d and TSPCs-56d, and a similar trend is observed in the tenogenic differentiation capacity |
| Human     | Y-TSPC: 25 ± 8yr, and A-TSPC: 65 ± 10 yr | Achilles tendon | Tenogenic differentiation capacity of TSPCs significantly decreases with ageing |
| Mice      | young (2.5, and 5 mo) and aging (9 and 24 mo) mice | Patellar tendons | Aged TSPCs formed adipocytes more readily than younger cells and expressed higher levels of adipogenic markers |
| Rat       | early P5, mid P10, and late P20 and P30 passages were used | Patellar tendons | TSPCs tend to differentiate into osteoblasts, while the adipogenic, chondrogenic and tenogenic differentiation capacities in TSPCs decline during in vitro subculture |
| Mice      | early P0, and late P5 passages were used | Achilles tendon | The TSPCs experiences a gradual loss of tenogenic differentiation with passaging due to increased expression and activity of Hdac |
| Human     | Y-TSPC group: 28 ± 5 yr; A-TSPC group: 63 ± 14 yr | Achilles tendon | A-TSPC have been reported to display an evident self-renewal and clonogenic decrease, multipotency is maintained in vitro |
| Human     | Group 1: aged 20 (female) and 22 (male); group 2: aged 28 (female) and 31 (male) and Group 3: aged 49 (male) and 50 (female) | Hamstring tendons | Multi-potency assays were not influenced by advanced ageing, although Y-TSPCs produced higher levels of some osteogenic and adipogenic genes, while chondrogenic genes were expressed at high levels in A-TSPCs |
| CD marker | Rat 3–4 (young) and 24–26 mo (aged) | Patellar tendons | Aged TSPCs express lower levels of CD90.1 than young cells, but higher levels of CD44 |
| CD marker | early P5, mid P10, and late P20 and P30 passages were used | Patellar tendons | CD90 and CD73 is down-regulated with increasing numbers of passaging |
| **Cell stemness marker** | **Mice** | **young (2.5, and 5 mo) and aging (9, and 24 mo) mice** | **Patellar tendons** | **The expression of the stem cell markers Oct-4, NS, Sca-1 and SSEA-1 in TSPCs decreased in an age-dependent manner** |
|--------------------------|-----------|------------------------------------------------------|---------------------|--------------------------------------------------------------------------------------------------|

| **Cell viscoelasticity** | **Rat** | **old rats: 20 mo and young rats: 8 wk** | **Achilles tendons** | **An overall increase in G′, G″ and hTSPC with ageing, revealing an important increase in stiffness of aged TSPCs** |
|--------------------------|----------|----------------------------------------|---------------------|--------------------------------------------------------------------------------------------------|

| **Cell senescence markers** | **human** | **Y-TSPC: 28 ± 5 yr and A-TSPC: 63 ± 14 yr** | **Achilles tendon** | **A-TSPCs undergo an early appearance of cellular senescence, as determined by quantifying the number of β-gal- positive cells at different time points** |
|---------------------------|-----------|----------------------------------------|---------------------|--------------------------------------------------------------------------------------------------|

| **rat** | **Early P5, mid P10, and late P20 and P30 passages were used** | **Patellar tendons** | **The significant up-regulation of β-gal activity in TSPCs with increasing passaging** |
|-----------|-------------------------------------------------|---------------------|--------------------------------------------------------------------------------------------------|

Y-TSPC: Young-TSPC; A-TSPC: Aged-TSPCs; TSPCs-7d: TSPCs-7days; P5: Passage 5; CFU: Colony-forming unit; Hdac: Histone deacetylase; CD: Cluster differentiation; NS: Nucleostemin.

early plateau phase, while Y-TSPCs didn’t exhibit the plateau[14]. Zhou et al[53] also observed this decrease in TSPCs proliferation with increasing age, consistent with similar results observed in TSPCs from other aged vertebrate animals[15,30,36,60]. Additionally, TSPCs-7 day (TSPCs-7d) displayed that a higher proliferation rate than the groups of TSPCs-1 day (TSPCs-1d) and TSPCs-56 day (TSPCs-56d)[61]. However, Tan et al[62] observed more rapid proliferation of TSPCs at late passage 20 (P20) and P30 than cells at an early P5 and middle P10, revealing a different perspective of the increased proliferation with additional passaging. Moreover, the increased proliferation of aged TSPCs was restored by treatment with ephrin receptor A4-Fc (EphA4-Fc), a moderate treadmill running (MTR) intervention and other factors, revealing that the proliferation rate of TSPCs can be modulated[15,56,60].

**Ageing and cell clonogenicity (colony-forming unit numbers and colony size):** Age-dependent clonogenic deficits in TSPCs are based on a decreased in the colony number and colony-forming unit efficiency with ageing[14,56,60]. Ruzzini et al[50] observed a dramatic decrease in the clonogenic potential with ageing; in addition, the size of the colonies was heterogeneous in patients, as the size of colonies produced by cells from aged patients was obviously larger than the colonies composed of cells from younger patients. Another study reported an obviously higher clonogenic capacity of TSPCs-7d than TSPCs-1d and TSPCs-56d[61]. In summary, the mainstream hypothesis is that ageing exerts negative impact on the clonogenicity of TSPCs. However, Tan et al[62] revealed an increase in the numbers of TSPCs colonies with passaging, in contrast to the findings from other studies.

**Ageing and cell migration**

The migration of TSPCs exhibits a decreasing trend with advanced age in a series of studies[14,56,60]. Popov et al[56] observed a significant decrease in the migratory of aged TSPC, and EphA4-Fc and ephrin receptor B2-Fc (ephB2-Fc) restore the decreased migration of A-TSPCs by inducing cell motility. Additionally, young hypoxic conditioned culture medium (HCCM) and inhibition of ROCK, a factor related to accelerate ageing, promote the restoration of cell migration[14,60].

**Ageing and cell differentiation**

The TSPC pool becomes exhausted considering the size and functional fitness with ageing. However, the maintenance of the multidifferentiation capacities of TSPCs from animals and humans is widely accepted, although a consensus on the direction of alteration has not been reached.

TSPCs from different time groups display multidifferentiation potential, while the ability of TSPCs-7d was greater than TSPCs-1d and TSPCs -56d, and a similar trend was observed in the tenogenic differentiation capacity[61]. The capacity of TSPCs to differentiate into tenocytes is reduced with ageing[31], consistent with the observation that the tenogenic differentiation capacity of TSPCs is profoundly diminished during ageing[45]. Moreover, aged TSPCs are not sensitive to transforming growth factor-β3, a
sublineage of the TGF-β superfamily that regulates cell growth and differentiation \[66\]. However, A-TSPCs transformed into adipocytes more readily than younger cells and produced higher levels of adipogenic markers that further resulted in the appearance of adipose tissue, which is generally related to aged tendons, while they presented no obvious difference in the capacity to transform into osteoblasts or chondrocytes \[15\]. Moreover, TSPCs tend to differentiate into osteoblasts as the number of passages in vitro increases, while the adipogenic, chondrogenic and tenogenic differentiation capacities in TSPCs decline during in vitro subculture \[62\]. Furthermore, Can Zhang et al \[57\] detected that a gradual loss of the tenogenic differentiation capacity of TSPCs with passaging due to the increased expression and activity of histone deacetylase (Hdac). Additionally, conflicting evidence shows a lack of age-related changes. Although, A-TSPCs have been reported to display an evident decrease in self-renewal and clonogenic capacities, multipotency is maintained in vitro \[57\]. Another research concluded that the multipotency assays were not influenced by advanced ageing, although Y-TSPCs produced higher levels of some osteogenic and adipogenic genes, while chondrogenic genes were expressed at high levels in A-TSPCs \[50\]. Overall, researchers have concluded that the multidifferentiation capacities of TSPCs are maintained during the ageing process without a conclusive determination of the trends in their variations, but most studies conclude that ageing impairs the tenogenic differentiation capacity of TSPCs.

### Ageing and cell specific cluster differentiation (CD) markers

Greater than 98% of TSPCs are positive for CD73, CD90, CD105, STRO-1, CD146, Musashi-1 and CD44, but are negative for CD19, CD34, CD45 and HLA-DRA \[14,50\]. Compared with young TSPCs, aged cells exhibit lower CD90.1 level, but higher CD44 expression \[57\]. CD44 is involved in the healing processes of numerous tissues and its levels are reduced in the process of scar less fetal tendon healing \[66\]; moreover, an improvement in mouse patellar tendon healing might attributed to a deficiency in CD44 \[54\]. Based on these findings, the up-regulation of CD44 in A-TSPCs might result in a decrease in the self-repair ability of TSPC with ageing. Additionally, the production of CD90 and CD73 decreases with increasing numbers of passage in vitro \[62\].

### Ageing and cell stemness markers

Approximately all TSPCs are positive for stem cell markers, including nucleostemin, Oct-4, and SSEA-4 in different age groups, revealing that the cells still maintained stemness features with age \[53\]. However, the levels of stem cell markers are dramatically decreased with ageing. Additionally, moderate mechanical stretching (4%) dramatically upregulated the stem marker NS expression of A-TSPCs in vitro, but 8% stretching reduced its production; similarly, 4% stretching also upregulated the production of another stem cell marker, Nanog \[53\].

### Ageing and cell viscoelasticity

One study revealed an overall increase in $G'$, $G''$ and $H_{\text{vec}}$ with ageing, which are valuable indicators of the cellular viscoelasticity that correspond to the storage modulus ($G'$), loss modulus ($G''$) and average thickness ($H_{\text{vec}}$), respectively. A dense cytoskeletal organization might result in a larger cell size and anomalous cell shape and is the cause of the increase in stiffness and viscosity \[68\]. Other authors had also detected an increase in the cell stiffness and size of A-TSPCs, as well as a denser and well-structured actin cytoskeleton. Moreover, treatment with a ROCK inhibitor rejuvenated these age-related variations in morphology and stiffness \[57\]. 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 \[50\]. Although, Kostrominova et al \[64\] showed alterations of ECM protein expression in rat tendons with ageing, while composition of ECM related to the cell adhesion was not analyzed. 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.

### Ageing and cell senescence markers

A-TSPCs undergo cellular senescence at an early stage, as determined by quantifying the number of β-gal-positive cells at different time points, and at P4, more A-TSPCs displayed positive staining. In addition, the quantity of β-gal positive A-TSPCs was dramatically increased at later passages. Moreover, the P16 protein was already detected in the P1 A-TSPCs, and its expression was evidently upregulated at P14 \[57\], accompanied by the evident upregulation of β-gal activity in TSPCs with increasing passaging \[14\]. In addition, an up-trend in the levels of senescence-related markers was observed in A-TSPCs in other studies \[13,68,28\], and the inhibition of ROCK, up-regulation of Pin1 (peptidyl-prolylcis-transiso merase NIMA-interacting1) or miRNA...
(miR)-135a, down-regulation of P16, or modulation of other molecules involved in the ageing process reversed the senescence of TSPCs and effectively delayed the ageing process\cite{14,63,69}.

**Mechanisms involved in the ageing process**

Because TSPCs ageing is an intricate process, its progression is also affected by multiple factors, including hormones, cytokines, enzymes, the oxygen content, mechanical force and exercise. Although the occurrence of epigenetic alterations in TSPCs with ageing has been observed, few scholars have focused on the underlying mechanisms partially because of the ambiguous conclusion regarding changes in aged TSPCs. The following section summarizes recent progress in the discovery of molecules and pathways involved in the TSPC ageing process and their various roles in mediating the ageing process, providing future research directions for TSPCs ageing and potential treatment targets for age-related tendon diseases. The mechanisms involved in the TSPC ageing process are listed in Figure 1.

Compared with Y-TSPCs, cAMP-responsive element-binding protein/p300-interacting transactivator with ED-rich tail 2 (CITED2) was dramatically down-regulated in older-TSPCs (O-TSPCs) at both the mRNA and protein levels and O-TSPCs showed reduced proliferation and elevated senescence. Furthermore, upon induction with TGFB\&-2, the nuclear expression of CITED2 and SP1 was significantly decreased, indicating that TGFB\&-2 mainly suppresses nuclear expression of CITED2. At the same time, P21 expression was increased, and melanocytomatis viral oncogene homolog (MYC) was up-regulated following the silencing of CITED2, revealing that the TGFB\&2-CITED2-MYC-SP1/P16 pathway mediates TDSC senescence. These findings were further supported by the results of a previous study showing that MYC functions as a transcriptional activator or repressor in regulating cell cycle progression and that the TGFB\& receptor kinase inhibitor SB525334 modulates the activity of this pathway\cite{71}. By comparing genome-wide RNA microarray data obtained from human Y-TSPCs and A-TSPCs, an intriguing difference was found: Altered genes were mainly distributed in categories such as cell–cell contact, cell adhesion, motility, migration, cytoskeleton and actin-associated transcripts, which might be the cause of the phenotypic and behavioral variations in A-TSPCs. In addition, the changes in features related to actin in A-TSPCs also significantly disrupted the formation of actin stress fibers and cell-matrix interactions\cite{14,72}. Moreover, collagen I expression and the corresponding integrins was decreased\cite{14}, while Rho-associated coiled-coil protein kinase1/2 (ROCK1/2), a downstream molecule that modulates the stabilization of actin filaments by phosphorylating LIMK, was up-regulated in A-TSPCs\cite{63}. Recently, another study illustrated an apparent increase in cell stiffness in aged TSPCs, which was associated with an increase in the activation of ROCK and a satisfactory rejuvenating effect of ROCK inhibition with Y-27632, because A-TSPCs exhibited similar features to Y-TSPCs after the intervention\cite{38}. Based on these findings, ROCK activity plays an essential role in TSPC ageing, primarily by regulating actin stress fibers and/or cell stiffness. Chen et al\cite{63} detected an obvious decrease in miR-135a level in A-TSPCs through direct bind to the 3'-untranslated region of ROCK1 compared with Y-TSPCs. Overexpression of miR-135a inhibits cell senescence, increases proliferation, and enhances migration and tenogenic differentiation of Y-TSPCs, while the inhibition of miR-135a produces the opposite results in A-TSPCs. The effects of miR-135a on TSPCs were attributed to its interaction with the ROCK1 mRNA, which was confirmed by a series of functional studies. Overall, miR-135a-Rock1 plays a crucial role in TSPC senescence. Han et al\cite{14} showed a substantial decrease in the A-TSPC tenogenic differentiation capacity, along with a decrease in the expression of P16 and the senescence-associated β-gal with age. P16 overexpression was responsible for the decrease in the tenogenic differentiation capacity of young TSPCs, and an analysis of the underlying mechanism revealed that this effect was mediated by P16, which enhanced the expression of miR-217 and subsequently inhibited the product of its direct target EGR1. According to these studies, A P16-miR-217-EGR1 pathway modulates TSPC the tenogenic differentiation and senescence of TSPCs. In addition, the EphA4, EphB2 and EphB4 and ephrin ligand B1 (EFNB1) in A-TSPCs is decreased compared with Y-TSPCs, which accelerates the decrease in self-renewal, migration, and actin turnover in A-TSPCs caused by advanced age. Upon stimulation with recombinant EphA4-Fc and EphB2-Fc proteins, significant effects on the key downstream signaling pathways mediated by ephrin-EPN binding were observed, including the activation of the cellular kinases focal adhesion kinase (FAK), extracellular signal-regulated kinase (ERK), Akt, c-Jun N-terminal kinase (JNK), and P38 in A-TSPCs; however, stimulation with EphB4-Fc and EFNB1-Fc did not exert an obvious effect on kinase activity in A-TSPC. Moreover, following stimulation with EphA4-Fc, FAK and JNK activity increased in A-TSPCs and more importantly, ERK phosphorylation was reduced to levels similar to the
TGFβ2 promotes the expression of CITED2, which inhibits the expression of MYC, ultimately regulating cell senescence. 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. MiR-140-5p reduces the expression of pin1 that downregulates the expression of P16 and ultimately delays TSPCs ageing. P16-miR-217-EGR1 pathway negatively modulates the cell senescence process.

JAK/FAK pathways are involved in the modulation of Ephrin A/B and EphB2 by affecting cell self-renew, migration and actin dynamics. GH/IGF-I pathways may participate in TSPCs ageing process by increasing the expression of decorin and scleraxis, resulting in delaying TSPCs ageing. Additionally, there are many cell external environment conditions, such as moderate treadmill running, moderate exercise, young decellularized extracellular matrix and young hypoxic-conditioned culture medium, can rejuvenate age-related alterations in aged-TSPCs. TD: Tenogenic differentiation; ED: Erroneous differentiation; MYC: Myelocytomatosis viral oncogene homolog; ROCK: Rho associated coiled-coil forming protein kinase; TGF-β2: Transforming growth factor-β2; TSPCs: Tendon stem/progenitor cells; IGF: Insulin-like growth factor; Pin1: Peptidyl-prolylcis-transiso merase NIMA-interacting1; miR: miRNA; CITED2: cAMP-responsive element-binding protein/p300-interacting transactivator with ED-rich tail; JAK: Janus kinase; FAK: Focal adhesion kinase; P16/21: Passage 16/21.
REJUVENATION OF AGED TENDON STEM/PROGENITOR CELLS

As a result of in-depth explorations of age-related changes in TSPCs during the cellular ageing process, scholars are now more likely to develop methods to reverse the deficits in TSPC function that result from advanced age. 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 ageing, and these alterations are mainly deleterious to TSPC function and the maintenance of tendon homeostasis. Furthermore, repair might be achieved by adjusting these factors, which have potential roles in the rejuvenation of aged TSPCs and are listed in Figure 1.

MTR has also been studied to determine the effects of motion on wound healing in aged tendons\(^\text{[59]}\), resulting in faster healing and a better healing quality through the restoration of the TSPC pool, which is beneficial for delaying TSPC senescence, enhancing the production of collagen fibers and reversing the erroneous differentiation of TSPCs. This approach eventually reverses the histopathological alterations that observed in subjects with age-related tendon diseases\(^\text{[76-78]}\). Furthermore, the role of moderate exercise in the effects of ageing on TSPCs has been investigated. Moderate exercise ameliorates the depletion of the TSPC pool by up-regulating the expression of cell proliferation and stem cell markers coupled with decreased lipid deposition, proteoglycan accumulation and calcification formation, and it is beneficial for delaying and reversing the age-related reductions of TSPCs. The impaired proliferative capacities of aged TSPCs were rejuvenated in a recent study by culturing cells with young decellularized extracellular matrix (DECM) because the young DECM increased the proliferation and tenogenic differentiation of aged TSPCs. Moreover, the expression of senescence-related marker in aged TSPCs was decreased and that of stem cell markers was increased after culture with young DECM, suggesting that the ECM is an important factor contributing to TSPCs ageing and the modulation of the ECM might be a promising anti-ageing approach\(^\text{[93]}\). Similar results were also obtained from young TSPCs cultured with HCCM, which restored the impaired function of aged TSPCs\(^\text{[60]}\). Pin 1 plays an important role in delaying the TSPC senescence process, which was confirmed by the decreased production of senescence markers and P16 and increased telomerase activity coupled with the opposite results following transfection with the Pin1-siRNA. Overexpression of Pin1 also effectively deferred late-stage TSPC senescence progression, but had no evident effect on the progression of early-stage cellular senescence, and miR-140-5p was involved in regulating Pin1 production, leading to a substantial decrease in Pin1 expression. Thus, Pin1 might be an anti-senescence target in TSPCs, together with miR-140-5p\(^\text{[60]}\). Numerous studies have reported an important role for ROCK activity in the TSPCs ageing process, and after inhibition of ROCK, A-TSPCs re-established a phenotype and cell stiffness similar to Y-TSPCs\(^\text{[43,45]}\). Notably, miR-135a also has a crucial role in modulating TSPCs senescence by facilitating the proliferation, migration and tenogenic differentiation of these cells and decreasing the expression of senescence markers inhibiting target downstream molecules of ROCK1 activity\(^\text{[43]}\), revealing that the blockade of ROCK activity is another promising strategy for combating TSPC ageing. A similar process is modulated by CITED2, providing an additional novel direction for fighting TSPC ageing\(^\text{[93]}\). Culture-expanded TSPCs (an in vitro ageing process) tend to exhibit a loss of phenotype, resulting in impaired function of TSPC, and Zhang et al\(^\text{[60]}\) found that altered gene expression was related to the increased activity and expression of Hdac subtypes with passaging. Overall, these molecules and their functional states represent potential therapeutic targets for reversing age-related pathological changes in TSPCs.

As shown in the study by Popov et al\(^\text{[69]}\), the ephrin receptors EphA4, EphB2 and EphB4 and ligand EFN B1 is decreased in A-TSPCs, and the down-regulation of EphA4 and EphB2 playes crucial roles in the age-associated reductions of the self-renewal, migration, and actin turnover in human TSPCs. Moreover, the activation of EphA4 or EphB2-dependent pathways reverses these harmful consequences, further revealing essential roles in preventing TSPC ageing. According to another study, ageing induces a progressive loss of activity of the GH/IGF-I axis, and the level of IGF-1 decreases with age\(^\text{[14,55]}\). At the same time, IGF-1 promotes the proliferation and maintenance of TSPC phenotypes by increasing the expression of decorin and scleraxis\(^\text{[46]}\), indicating that the altered fate of TSPCs is able to be reversed by modulating the relative expression levels of hormones. In addition, rapamycin slows ageing in mice\(^\text{[32,33]}\), and metformin, pentosidine and multiflorum increase the lifespans of animals and humans\(^\text{[34-44]}\). However, the relationships between these drugs...
and the mechanisms underlying the increase in lifespans are unknown due to the limited and insufficient number of studies conducted in this area\cite{84-86}. Additionally, based on most recent development in regenerative medicine, Dale et al.\cite{87} induced human embryonic stem cells to differentiate into tendon-like cells in the presence of exogenous bone morphogenetic protein (BMP) 12 and BMP 13 and directed parthenogenetic stem cells to differentiate into tenocytes. Moreover, mechanical stretching improved the tenogenic differentiation of pMSCs\cite{89,90}. Similar results were also obtained using iPSCs\cite{89,90}. Thus, these cells may represent an exogenous supplementation to TSPCs or tenocytes, which is also an ideal way to method for rejuvenating ageing of tendons and provides alternative healing strategies for reversing tendon ageing in the future.

CONCLUSION

As a result of advanced studies on tendons and the ageing of TSPCs, tendon ageing can be considered to be partially due to the ageing of TSPC. TSPCs sustain regeneration at the site of tendon injury, and the loss of their function with advanced age causes aged-related tendon diseases. Although limited studies have been performed and the conclusions regarding the altered differentiation capacities and mechanisms involved are controversial, particularly regarding the erroneous differentiation, researchers generally agree that the cell number and tenogenic differentiation decrease with ageing, providing future directions for studies of TSPCs ageing. In particular, alterations in the ECM environment have been shown to re-establish the regenerative capacity of aged TSPCs, indicating that alterations in stem cell activity may be tractable for intervention, a hypothesis that is supported by the effects of alterations in cell-intrinsic pathways involved in TSPC ageing. Because humans are living longer, improvements in our understanding of the mechanistic networks underlying the age-associated in TSPCs and the tendon repair ability are critical for combating age-related tendon diseases.

REFERENCES

1. Li Y, Dai G, Shi L, Lin Y, Chen M, Li G, Rui Y. The Potential Roles of Tendon Stem/Progenitor Cells in Tendon Ageing. Curr Stem Cell Res Ther 2019; 14: 34-42 [PMID: 30329376 DOI: 10.2174/1574888X136618107112233]
2. Agabalyan N. Tendinopathy – from basic science to treatment. Int J Exp Pathol 2013; 94: A1 [PMID: 24003459 DOI: 10.1111/ije.12043]
3. Gumina S, Carbone S, Campagna V, Candela V, Sacchetti FM, Giannicola G. The impact of aging on rotator cuff tear size. Musculoskelet Surg 2013; 97 Suppl 1: 69-72 [PMID: 23588834 DOI: 10.1007/s12306-013-0263-2]
4. Dressler MR, Butler DL, Boivin GP. Age-related changes in the biomechanics of healing patellar tendon. J Biomech 2006; 39: 2205-2212 [PMID: 16120443 DOI: 10.1016/j.jbiomech.2005.07.003]
5. Howel D, Moffatt S, Haughton C, Bryant A, Becker F, Steer M, Lawson S, Aspray T, Milne EMG, Vogel G. Induced pluripotent stem cells derived from human blastocysts. Sci Transl Med 2011; 3: 89ra45 [PMID: 21700516 DOI: 10.1126/scitranslmed.3002344]
6. de Aro AA, Carneiro GD, Teodoro LFR, da Veiga FC, Ferrucci DL, Simões PF, Alvares LE, de Oliveira ALR, Vicente CP, Gomes CP, Pesquero JB, Esquisatto MAM, de Campos B Pimentel. Injured Achilles Tendons Treated with Adipose-Derived Stem Cells Transplantation and GDF-5. Cells 2016; 7 [PMID: 2009560 DOI: 10.3390/Cells7090127]
7. Deng G, Li K, Chen S, Chen P, Zheng H, Yu B, Zhang K. Interleukin10 promotes proliferation and migration, and inhibits tendon differentiation via the JAK/Stat3 pathway in tendonderived stem cells in vitro. Mol Med Rep 2018; 18: 5044-5052 [PMID: 30202084 DOI: 10.4061/2018.9474]
8. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science 1998; 282: 1145-1147 [PMID: 9804558 DOI: 10.1126/science.282.5391.1145]
9. Vogel G. Breakthrough of the year: Capturing the promise of youth. Science 1999; 286: 2238-2239 [PMID: 10636772 DOI: 10.1126/science.286.5448.2238]
10. Biel JK, Russell B. Introduction to stem cell therapy. J Cardiovasc Nurs 2009; 24: 98-103 [PMID: 19242274 DOI: 10.1097/JCN.0b013e318197faa65]
11. Bi Y, Ehiriouchi D, Kiliş TM, Inkson CA, Embree MC, Sonoymaya W, Li L, Leet AI, Seo BM, Zang L, Shi S, Young MF. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat Med 2007; 13: 1219-1227 [PMID: 17828634 DOI: 10.1038/nm1630]
12. Tan Q, Liu PP, Rui YF, Wong YM. Comparison of potentials of stem cells isolated from tendon and bone marrow for musculoskeletal tissue engineering. Tissue Eng Part A 2012; 18: 841-851 [PMID: 22011320 DOI: 10.1089/tissue.2011.0362]
13. Wang F, Cai F, Shi R, Wang XH, Wu XT. Aging and age related stresses: a senescence mechanism of intervertebral disc degeneration. Osteoarthritis Cartilage 2016; 24: 398-408 [PMID: 26453958 DOI: 10.1016/j.joca.2015.09.010]
14. Kohler J, Popov C, Klotz B, Alberton P, Prall WC, Haasters S, Ebert S, Klein-Hitpass L, Jakob F, Schieker M, Docheva D. Uncovering the cellular and molecular changes in tendon
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stem/progenitor cells attributed to tendon aging and degeneration. Aging Cell 2013; 12: 988-999 [PMID: 23826690 DOI: 10.1111/ace.12124]

Zhang J, Wang JH. Moderate Exercise Mitigates the Detrimental Effects of Aging on Tendon Stem Cells. PLoS One 2015; 10: e0136454 [PMID: 26086850 DOI: 10.1371/journal.pone.0136454]

Shrestha R, Wen YT, Ding DC, Tsai RK. Aberrant hPSCs-Derived from Human Keratinocytes Differentiates into 3D Retinal Organoids that Acquire Mature Photoreceptors. Cells 2019; 8 [PMID: 30634512 DOI: 10.3390/cells8010036]

McGrath M, Yam E, Sladkova M, Al Manaia A, Zimmer M, de Peppo GM. GMP-compatible and xenofree cultivation of mesenchymal progenitors derived from human-induced pluripotent stem cells. Stem Cell Res Ther 2019; 10; 11 [PMID: 30635059 DOI: 10.1186/s13287-018-1119-3]

Marchetto MC, Gage FH. Your brain under the microscope: the promise of stem cells. Cerebrum 2014; 1 [PMID: 25090991]

Yin Z, Chen X, Chen JL, Shen WL, Hieu Nguyen TM, Gao L, Ouyang HW. The regulation of tendon stem cell differentiation by the alignment of nanofibers. Biomaterials 2010; 31: 2163-2175 [PMID: 19995669 DOI: 10.1016/j.biomaterials.2009.11.083]

Rui YF, Lui PP, Li G, Fu SC, Lee YW, Chan KM. Isolation and characterization of multipotent rat tendon-derived stem cells. Tissue Eng Part A 2010; 16: 1549-1558 [PMID: 20000222 DOI: 10.1089/ten.TEA.2009.0529]

Zhang J, Wang JH. Characterization of differential properties of rabbit tendon stem cells and tenocytes. BMC Musculoskelet Disord 2010; 11: 10 [PMID: 20082706 DOI: 10.1186/1471-2474-11-10]

Liu Q, Zha Y, Amadio PC, Moran SL, Gingerly A, Zhao C. Isolation and Characterization of Multipotent Turkey Tendon-Derived Stem Cells. Stem Cells Int 2018; 2018: 9687971 [PMID: 29773306 DOI: 10.1155/2018/9687971]

Yang J, Zhao Q, Wang K, Ma C, Liu H, Liu Y, Guan W. Isolation, culture and biological characteristics of multipotent porcine tendon-derived stem cells. Int J Mol Med 2018; 41: 3611-3619 [PMID: 29512747 DOI: 10.3829/ijmm.2018.3545]

Yang J, Zhao Q, Wang K, Liu H, Ma C, Huang H, Liu Y. Isolation and biological characterization of tendon-derived stem cells from fetal bovine. In Vitro Cell Dev Biol Anim 2016; 52: 846-856 [PMID: 27130678 DOI: 10.1007/s11626-016-0043-z]

Lee KJ, Clegg PD, Comerford EJ, Canty-Laird EG. A comparison of the stem cell characteristics of murine tenocytes and tendon-derived stem cells. BMC Musculoskelet Disord 2018; 19: 116 [PMID: 29650048 DOI: 10.1186/s12891-018-2038-2]

Berglund AK, Fortier LA, Antczak DF, Schnabel LV. Unimmunoprivileged no more: measuring the immunogenicity of allogeneic adult mesenchymal stem cells. Stem Cell Res Ther 2017; 8: 288 [PMID: 29273086 DOI: 10.1186/s13287-017-0742-8]

Ryan JM, Barry FP, Murphy JM, Mahon BP. Mesenchymal stem cells avoid allogeneic rejection. J Inflamm (Lond) 2005; 2: 8 [PMID: 16045881 DOI: 10.1186/1476-9255-2-8]

Lui PP, Kong SK, Lau PM, Wong YM, Lee YW, Tan C, Wong OT. Immunogenicity and escape mechanisms of allogeneic tendon-derived stem cells. Tissue Eng Part A 2014; 20: 3010-3020 [PMID: 24813640 DOI: 10.1089/ten.TEA.2013.0714]

Ni M, Lui PP, Rui YF, Lee YW, Lee YW, Tan Q, Wong YM, Kong SK, Lau PM, Li G, Chan KM. Tendon-derived stem cells (TDCs) promote tendon repair in a rat patellar tendon window defect model. J Orthop Res 2012; 30: 613-619 [PMID: 21928428 DOI: 10.1002/jor.21559]

Walia B, Huang AH. Tendon stem progenitor cells: Understanding the biology to inform therapeutic strategies for tendon repair. J Orthop Res 2019; 37: 1270-1280 [PMID: 30270569 DOI: 10.1002/jor.24456]

Dymant NA, Hagwara Y, Matthews BG, Li Y, Kalazizic I, Rowe DW. Lineage tracing of resident tendon progenitor cells during growth and natural healing. PLoS One 2014; 9: e96113 [PMID: 24759953 DOI: 10.1371/journal.pone.0096113]

Howell K, Chien C, Bell R, Launder D, Tufa SF, Keene DR, Andarawis-Puri N, Huang AH. Novel Model of Tendon Regeneration Reveals Distinct Cell Mechanisms Underlying Regenerative and Fibrotic Tendon Healing. Sci Rep 2017; 7: 45338 [PMID: 28332620 DOI: 10.1038/srep45338]

Dymant NA, Liu CF, Kazemi N, Aschbacher-Smith LE, Kenter K, Breidenbach AP, Shearn JT, Wylie C, Rowe DW, Butler DL. The paratenon contributes to scleraxis-expressing cells during patellar tendon healing. PLoS One 2013; 8: e59944 [PMID: 23558841 DOI: 10.1371/journal.pone.0059944]

Mianaltowski MJ, Adams SM, Birke DE. Regional differences in stem/progenitor cell populations from the mouse achilles tendon. Tissue Eng Part A 2013; 19: 199-210 [PMID: 22871318 DOI: 10.1089/ten.TEA.2012.0182]

Mianaltowski MJ, Adams SM, Birke DE. Tendon proper- and peritenon-derived progenitor cells have unique tenogenic properties. Stem Cell Res Ther 2014; 5: 86 [PMID: 25005797 DOI: 10.1186/s13287-014-0047-z]

Gumucio JP, Sugg KB, Mendias CL. TGF-β superfamily signaling in muscle and tendon adaptation to resistance exercise. Exerc Sport Sci Rev 2015; 43: 93-99 [PMID: 25607281 DOI: 10.1249/JES.0000000000000411]

Chen L, Liu JP, Tang KL, Wang Q, Wang GD, Cai XH, Liu XM. Tendon-derived stem cells promote platelet-rich plasma healing in collagenase-induced rat Achilles tendinopathy. Cell Physiol Biochem 2014; 34: 2153-2168 [PMID: 25562162 DOI: 10.1159/000396659]

Kim SJ, Song DH, Park JW, Park S, Kim SJ. Effect of Bone Marrow Aspirate Concentrate-Platelet-Rich Plasma on Tendon-Derived Stem Cells and Rotator Cuff Tendon Tear. Cell Transplant 2017; 26: 867-878 [PMID: 28105983 DOI: 10.3727/096368917X76894705]

Xu K, Al-Ani MK, Sun Y, Xu W, Pan L, Song Y, Xu Z, Pan X, Yang L. Platelet-rich plasma activates tendon-derived stem cells to promote regeneration of Achilles tendon rupture in rats. J Tissue Eng Regen Med 2017; 11: 1173-1184 [PMID: 25758330 DOI: 10.1002/ten2016]

Holladay CA, Abbah SA, O'Dowd C, Pandit A, Zeugolis D. Preferential tendon stem cell response to growth factor supplementation. J Tissue Eng Regen Med 2016; 10: 783-798 [PMID: 24474722 DOI: 10.1002/ten.1852]

Nouriati G, Berebauma F, Duprez D. Tendon injury: from biology to tendon repair. Nat Rev Rheumatol 2015; 11: 223-233 [PMID: 25734975 DOI: 10.1038/nrrheum.2015.26]

Manning CN, Havioglu N, Knutsen E, Sakayama-Elbert SE, Silva MJ, Thomopoulos S, Gelberman RH. The early inflammatory response after flexor tendon healing: a gene expression and histological analysis. J Orthop Res 2014; 32: 645-652 [PMID: 24464937 DOI: 10.1002/jor.22575]

Zhang K, Asai S, Yu B, Inomoto-Iwamoto M. IL-1β irreversibly inhibits tenogenic differentiation and
alters metabolism in injured tendon-derived progenitor cells in vitro. Biochem Biophys Res Commun 2015; 463: 667-672 [PMID: 26051275 DOI: 10.1016/j.bbrc.2015.05.122]

44 Chen S, Deng G, Li K, Zheng H, Wang G, Yu B, Zhang K. Interleukin-6 Promotes Proliferation but Inhibits Tenogenic Differentiation via the Janus Kinase/Signal Transducers and Activators of Transcription 3 (JAK/STAT3) Pathway in Tendon-Derived Stem Cells. Med Sci Monit 2018; 24: 1567-1573 [PMID: 29547593 DOI: 10.12659/MSM.908802]

45 Tarefder S, Chen E, Jun Y, Kao K, Sim KH, Back J, Lee FY, Lee CH. Tendon stem/progenitor cells regulate inflammation in tendon healing via ANK and STAT3 signaling. FASEB J 2017; 31: 3991-3998 [PMID: 28533228 DOI: 10.1096/fj.201700718R]

46 Liu Y, Feng L, Wang H, Wang YJ, Chan HC, Jiang XH, Fu WM, Li G, Zhang JF. Identification of an Anti-Inflammation Protein, Annexin A1, in Tendon Derived Stem Cells (TDSCs) of Cystic Fibrosis Mice: A Comparative Proteomic Analysis. Proteomics Clin Appl 2018; 12: e1700162 [PMID: 29781578 DOI: 10.1002/pca.201700162]

47 Wu T, Liu S, Wen G, Xu J, Yu Y, Chai Y. Celastrol improves self-renewal and differentiation of human tendon-derived stem cells by suppressing Smad7 through hypoxia. Stem Cell Res Ther 2017; 8: 274 [PMID: 29208212 DOI: 10.1186/s13287-017-0724-x]

48 Zhang K, Zhang S, Li Q, Yang J, Dong W, Wang S, Cheng Y, Al-Qwabmi M, Wang Q, Yu B. Effects of celecoxib on proliferation and tenogenic differentiation of tendon-derived stem cells. Biochem Biophys Res Commun 2014; 450: 762-766 [PMID: 24953691 DOI: 10.1016/j.bbrc.2014.06.058]

49 Wang Y, Tang H, He G, Shi Y, Kang X, Lyu J, Zhou M, Zhu M, Zhang J, Tang K. High Concentration of Aspirin Induces Apoptosis in Rat Tendon Stem Cells via Inhibition of the Wnt/β-Catenin Pathway. Cell Physiol Biochem 2018; 50: 2046-2059 [PMID: 30415260 DOI: 10.1159/000495050]

50 Ruzzini L, Abbruzzese F, Rainer A, Longo UG, Trombetta M, Maffulli N, Denaro V. Characterization of age-related changes of tendon stem cells from human tendons. Knee Surg Sports Traumatol Arthrosc 2014; 22: 2856-2866 [PMID: 25359346 DOI: 10.1007/s00167-013-2457-4]

51 Ni M, Rui YF, Tan Q, Liu Y, Xu LL, Chen KM, Wang Y, Li G. Engineered scaffold-free tendon tissue produced by tendon-derived stem cells. Biomaterials 2013; 34: 2024-2037 [PMID: 23246605 DOI: 10.1016/j.biomaterials.2012.11.046]

52 Tan Q, Lui PP, Lee YW. In vivo identity of tendon stem cells and the roles of stem cells in tendon healing. Stem Cells Dev 2013; 22: 3128-3140 [PMID: 23815595 DOI: 10.1089/scd.2013.0073]

53 Zhou Z, Akinbiniyi T, Xu L, Ramcharan M, Leong DJ, Ros SJ, Colvin AC, Schafferl MB, Majeska RJ, Flatow EL, Sun HB. Tendon-derived stem/progenitor cell aging: defective self-renewal and altered fate. Aging Cell 2010; 9: 911-915 [PMID: 20509237 DOI: 10.1111/j.1474-9726.2010.00506.x]

54 Han W, Wang B, Liu J, Chen L. The p16/miR-217/EGFR pathway modulates age-related tenogenic differentiation in tendon stem/progenitor cells. Acta Biochim Bio phys Sin (Shanghai) 2017; 49: 1015-1021 [PMID: 28036495 DOI: 10.1007/s00167-017-0518-3]

55 Kiderlen S, Polec C, Rädler JO, Docheva D, Clausen-Schaumann H, Sudhop S. Age related changes in cell stiffness of tendon stem/progenitor cells and a rejuvenating effect of ROCK-inhibition. Biochem Biophys Res Commun 2019; 509: 839-844 [PMID: 30638929 DOI: 10.1016/j.bbrc.2019.01.027]

56 Popov C, Kohler J, Docheva D. Activation of EphA4 and EphB2 Reverse Signaling Restores the Age-Associated Reduction of Self-Renewal, Migration, and Actin Turnover in Human Tendon Stem Cells. Front Aging Neurosci 2016; 7: 246 [PMID: 26779014 DOI: 10.3389/fnagi.2015.00246]

57 Wu H, Zhao G, Zu H, Wang JH, Wang QM. Aging-related viscoelasticity variation of tendon stem cells (TSCs) characterized by quartz thickness shear mode (TSM) resonators. Sens Actuators (Warrendale Pa) 2015; 210: 369-380 [PMID: 26251564 DOI: 10.1016/j.snb.2014.12.117]

58 Gehwolf R, Wagner A, Lohner C, Bradshaw AD, Scharfer C, Niestrawska J, Holzapfel GA, Bauer HC, Tempfer H, Tragwein A. Pleiotropic roles of the matricellular protein Spint-2 in tendon maturation and ageing. Sci Rep 2016; 6: 32635 [PMID: 27586416 DOI: 10.1038/srep32635]

59 Baxter MA, Wynn RF, Jowitt SN, Wraith JE, Fairbairn LJ, Bellantuono I. Study of telomere length in tendon-derived stem/progenitor cells. Sci Rep 2016; 6: 32915 [PMID: 27586416 DOI: 10.1038/srep32915]

60 Chen L, Wang GD, Liu JP, Wang HS, Liu XM, Wang Q, Cai XH. miR-135a modulates tendon/stem/progenitor cell senescence via suppressing ROCK1. Bone 2015; 71: 210-216 [PMID: 25460182 DOI: 10.1016/j.bone.2014.11.001]

61 Kovacevic D, Rodeo SA. Biological augmentation of rotator cuff tendon repair. Clin Orthop Relat Res 2008; 466: 622-633 [PMID: 18264850 DOI: 10.1097/01.blo.0000315022.2-5875]

62 Zhang C, Zhang E, Yang L, Tu W, Lin J, Yuan C, Bumpetch V, Chen E, Yang H. Histone deacetylation inhibitor treated cell sheet from mouse tendon/stem/progenitor cells promotes tendon repair. Biomaterials 2018; 172: 66-82 [PMID: 29723756 DOI: 10.1016/j.biomaterials.2018.03.043]

63 Pavata M, Beredjiklian PK, Zgonis MH, Beason DP, Crombleholme TM, Jawad AF, Soslowsky LJ. Regenerative properties of fetal sheep tendon are not adversely affected by transplantation into an adult environment. J Orthop Res 2006; 24: 2128-2132 [PMID: 16944731 DOI: 10.1002/jor.20271]

64 Ansoor HL, Beredjiklian PK, Soslowsky LJ. CD44 deficiency improves healing tendon mechanics and increases matrix and cytokine expression in a mouse patellar tendon injury model. J Orthop Res 2009; 27: 1386-1391 [PMID: 19382192 DOI: 10.1002/jor.20891]

65 Kostrominova TV, Brooks SV. Age-related changes in structure and extracellular matrix protein expression levels in rat tendons. Age (Dordr) 2013; 35: 2203-2214 [PMID: 23354684 DOI: 10.1007/s11357-013-9514-2]

66 Chen L, Liu J, Tao X, Wang G, Wang Q, Liu X. The role of Pin1 protein in aging of human tendon stem/progenitor cells. Biochem Biophys Res Commun 2015; 464: 487-492 [PMID: 26150353 DOI: 10.1016/j.bbrc.2015.06.162]

67 Xu H, Liu F. Downregulation of FOXP1 correlates with tendon/stem/progenitor cells aging. Biochem
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Biophys Res Commun 2018; 504: 96-102 [PMID: 30170733 DOI: 10.1016/j.brc.2018.08.136]  
71 Hu C, Zhang Y, Tang K, Luo Y, Liu Y, Chen W. Downregulation of CITED2 contributes to TGFβ-mediated senescence of tendon-derived stem cells. Cell Tissue Res 2017; 368: 93-104 [PMID: 28084522 DOI: 10.1007/s00441-016-2552-1]  
72 Schmitz AA, Govek EE, Böttner B, Van Aelst L. Rho GTPases: signaling, migration, and invasion. Exp Cell Res 2000; 261: 1-12 [PMID: 11082269 DOI: 10.1006/excr.2000.5049]  
73 Zadzik Z, Chaliew SA, McCarter RJ, Meistas M, Kowarski A. The influence of age on the 24-hour integrated concentration of growth hormone in normal individuals. J Clin Endocrinol Metab 1985; 60: 513-516 [PMID: 3729964 DOI: 10.1210/jcem-60-3-513]  
74 Heinemeyer KM, Mackey AL, Doessing S, Hansen M, Bayer ML, Nielsen RH, Herchenhan A, Malengaard-Claussen NM, Kjaer M. GH/IGF-1 axis and matrix adaptation of the musculoskeletal tissue to exercise in humans. Scand J Med Sci Sports 2012; 22: e1-e7 [PMID: 22429205 DOI: 10.1111/j.1600-0838.2012.01459.x]  
75 Zhang J, Yuan T, Wang JH. Moderate treadmill running exercise prior to tendon injury enhances wound healing in aging rats. Oncotarget 2016; 7: 8498-8512 [PMID: 26885754 DOI: 10.18632/oncotarget.7381]  
76 Rui YF, Lui PP, Wong YM, Tan Q, Chan KM. Altered fate of tendon-derived stem cells isolated from a failed tendon-healing animal model of tendinopathy. Stem Cells Dev 2013; 22: 1076-1085 [PMID: 23106341 DOI: 10.1089/scd.2012.0555]  
77 Rui YF, Lui PP, Chan LS, Chan KM, Fu SC, Li G. Does erroneous differentiation of tendon-derived stem cells contribute to the pathogenesis of calcifying tendinopathy? Chin Med J (Engl) 2011; 124: 606-610 [PMID: 21162289 DOI: 10.3760/cnma.issn.0366-6999.2011.04.022]  
78 Adams CW, Baylos OB. Acid mucosubstances underlying lipid deposits in ageing tendons and atherosclerotic arteries. Atherosclerosis 1973; 18: 191-195 [PMID: 4355127 DOI: 10.1016/0021-9150(73)90100-7]  
79 Jiang D, Xu B, Gao P. Effects of young extracellular matrix on the biological characteristics of aged tendon stem cells. Adv Clin Exp Med 2018; 27: 1625-1630 [PMID: 30063128 DOI: 10.17219/acecm/75503]  
80 Leitke E, Gorenoi V, Wichers C, Von Zur Mühlen A, Von Büren E, Brabant G. Age-related changes of serum sex hormones, insulin-like growth factor-I and sex-hormone binding globulin levels in men: cross-sectional data from a healthy male cohort. Clin Endocrinol (Oxf) 2000; 53: 689-695 [PMID: 11155090 DOI: 10.1046/j.1365-2265.2000.01159.x]  
81 Rudman D, Kutner MH, Rogers CM, Lubin MF, Fleming GA, Bain RP. Impaired growth hormone secretion in the adult population: relation to age and adiposity. J Clin Invest 1981; 67: 1361-1369 [PMID: 7194888 DOI: 10.1172/JCI110164]  
82 Zaseck LW, Miller RA, Brooks SV. Rapamycin Attenuates Age-associated Changes in Tibilias Anterior Tendon Viscoelastic Properties. J Gerontol A Biol Sci Med Sci 2016; 71: 858-865 [PMID: 26809496 DOI: 10.1093/gerona/glv307]  
83 Spong A, Barlert A, Rapamycin slows aging in mice. Cell Cycle 2012; 11: 845 [PMID: 22356747 DOI: 10.4161/cc.11.5.19607]  
84 Menendez JA, Cufí S, Oliveras-Ferraros C, Vellon L, Joven J, Vazquez-Martin A. Gerosuppressant metformin: less is more. Aging (Albany NY) 2012; 4: 348-362 [PMID: 21483040 DOI: 10.18632/aging.100316]  
85 Sun YN, Li W, Song SB, Yan XT, Yang SY, Kim YH. Nuclear Factor Kappa B Activation and Peroxisome Proliferator-activated Receptor Transactivational Effects of Chemical Components of the Roots of Polygonum multiflorum. Pharmacogn Mag 2016; 12: 31-35 [PMID: 27019559 DOI: 10.4103/0973-1296.176019]  
86 Sell DR, Monnier VM. Age-related association of tail tendon break time with tissue pentosidine in DBA/2 vs C57BL/6 mice: the effect of dietary restriction. J Gerontol A Biol Sci Med Sci 1997; 52: B277-B284 [PMID: 9310078 DOI: 10.1093/gerona/52A.5.B277]  
87 Dale TP, Mazher S, Webb WR, Zhou J, Maffulli N, Chen GQ, El Haj AJ, Forsyth NR. Tenogenic Differentiation of Human Embryonic Stem Cells. Tissue Eng Part A 2018; 24: 361-368 [PMID: 28548630 DOI: 10.1089/ten.TEA.2017.0117]  
88 Liu W, Yin L, Yan X, Cui J, Liu W, Rao Y, Sun M, Wei Q, Chen F. Directing the Differentiation of Parthenogenetic Stem Cells into Tenocytes for Tissue-Engineered Tendon Regeneration. Stem Cells Transl Med 2017; 6: 196-208 [PMID: 28170171 DOI: 10.5966/sctm.2015-0334]  
89 Yang F, Zhang A, Richardson DW. Regulation of the tenogenic gene expression in equine tenocyte-derived induced pluripotent stem cells by mechanical loading and Mohawk. Stem Cell Res 2019; 39: 101489 [PMID: 31277043 DOI: 10.1016/j.scr.2019.101489]  
90 Bavin EP, Smith O, Baird AE, Smith LC, Guest DJ. Equine Induced Pluripotent Stem Cells have a Reduced Tendon Differentiation Capacity Compared to Embryonic Stem Cells. Front Vet Sci 2015; 2: 55 [PMID: 26664982 DOI: 10.3389/fvets.2015.00055]
