Biological Augmentation for Tendon Repair: Lessons to Be Learned from Development, Disease, and Tendon Stem Cell Research

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Abstract

Tendons resemble connective tissues rich in highly organized collagen fibers, displaying a remarkably high tensile strength. However, partly due to the low number of tissue-resident cells and their more or less avascular nature, tendons heal relatively slowly. As there is a growing socio-economic need for effective and reproducible treatments to repair injured tendons, researchers and clinicians are challenged to develop strategies to restore native tendon structure and functionality.

This chapter highlights the features and functions of tendon-resident cells and their niche, beginning with a general view on tendon structure. It further gives an overview of tendon development and the cellular and molecular events underlying tendon aging. Finally, we will close the chapter by briefly outlining current strategies to augment tendon repair, aiming at reaching the ambitious goal of functional tendon regeneration.

1 Introduction

Tendons enable musculoskeletal forces to be transmitted and redirected across skeletal joints, facilitating a wide range of joint motion and locomotor movement. Due to their remarkable tensile strength, stiffness, and viscoelastic properties, tendons not only allow the safe transmission of muscle forces over long lengths, but partially also enable the storage and release of elastic energy, reducing energy costs and minimizing the risk of injury.

Tendon disorders are frequent, debilitating conditions affecting both the working population and recreational athletes, placing an enormous burden on healthcare systems worldwide. Despite the high prevalence of tendon injuries due to overuse and/or aging (Maffulli et al. 2003), possible therapeutic interventions remain limited compared to other musculoskeletal tissues, such as bone, muscle, or cartilage. Acute tendon injuries and chronic tendinopathies remain clinically challenging, in part due to very few of low activity tendon-resident cells. Further, the avascular nature of tendons delays healing, while the innate reparative processes are incomplete and often are associated with the formation of scar tissue that compromises the mechanical function (Benjamin and Ralphs 1997). Despite significant advancements in tissue engineering (e.g., sophisticated combination of scaffolds, cells biologics), the clinical impact for the functional regeneration of tendons remains limited. Currently, tendon injuries are either treated by a conservative approach (e.g., eccentric training, pain management) or by surgical intervention. However, irrespective of the methods employed, tendons heal slowly and rarely full function is regained due to the

formation of scar tissue, the formation of adhesions, or ectopic bone formation. To that end, for the development of functional reparative tendon therapies, we need to pin down the molecular and cellular mechanisms amenable to modulate endogenous (or exogenous) cell behavior towards functional tendon repair and regeneration.

Advancements in the fields of biomaterial and stem cell research present promising avenues for the development of alternative treatments. While autografts remain the gold standard for tendon repair augmentation, allografts, xenografts, and synthetic materials are gaining more interest to overcome limited supply and donor site morbidity associated with autograft harvesting (Lomas et al. 2015). However, despite promising preclinical results, scaffold materials do not fully recapitulate native tendon structure, biomechanical properties, or overall composition. Alternatively, the use of adult stem cell therapies has received tremendous attention hoping to successfully repair and/or replace injured or damaged tendon tissue (Lui 2015; Veronesi et al. 2016), as the investigation of the heterogeneous population of tendon-resident cells continues to provide valuable insights into the cellular and molecular mechanism driving tendon disease and healing. The bulk of cells present in the tendon proper are elongated, specialized fibroblast-like cells, termed tenocytes, and their precursor cells, termed tenoblasts (Kannus 2000). In addition, tendons harbor a population of tendon stem and progenitor cells (TSPCs) (Bi et al. 2007; Salingcarnboriboon et al. 2003; Tempfer et al. 2009), synovial cells located in the connective tissue sheaths surrounding the tendon (Banes et al. 1988), and vascular endothelial cells (Lehner et al. 2016). The multipotency and high proliferation rate of TSPCs makes them attractive candidates for tendon repair; however, a better understanding of their functions in situ in health and disease is needed to fully harness their therapeutic potential.

Finally, by investigating the developmental programs driving tendon tissue formation and, on the other hand, the mechanisms contributing to the senescence of tendons, ultimately resulting in decreased quality of tendons in the elderly, novel targets for clinical intervention potentially can be discovered.

2 Tendon Structure and ECM

Tendons contain a range of fibrous, soft tissue structures, endowing them with their biomechanical properties to transmit force from muscle to bone. Their ability to provide rigidity combined with flexibility is made possible by the nonlinear, viscoelastic properties, the biomechanical behavior reflecting the properties of their main building block – type I collagen fibrils. Tendons are organized in a highly hierarchical manner with collagen being bundled into progressively larger subunits (see Fig. 1). The smallest units are the collagen molecules – after secretion from tendon-resident cells, the procollagen molecules are being processed and 300-nm-long triple-helical molecules remain. These molecules then undergo a self-assembly process leading to a staggered arrangement of parallel molecules, with a periodicity of $D = 67$ nm, also known as the "D" period (Canty and Kadler 2002). These insoluble collagen molecules then assemble into microfibrils, which in turn form larger, moderately twisted, lattice-type fibrils by lateral and longitudinal stacking ranging in size from

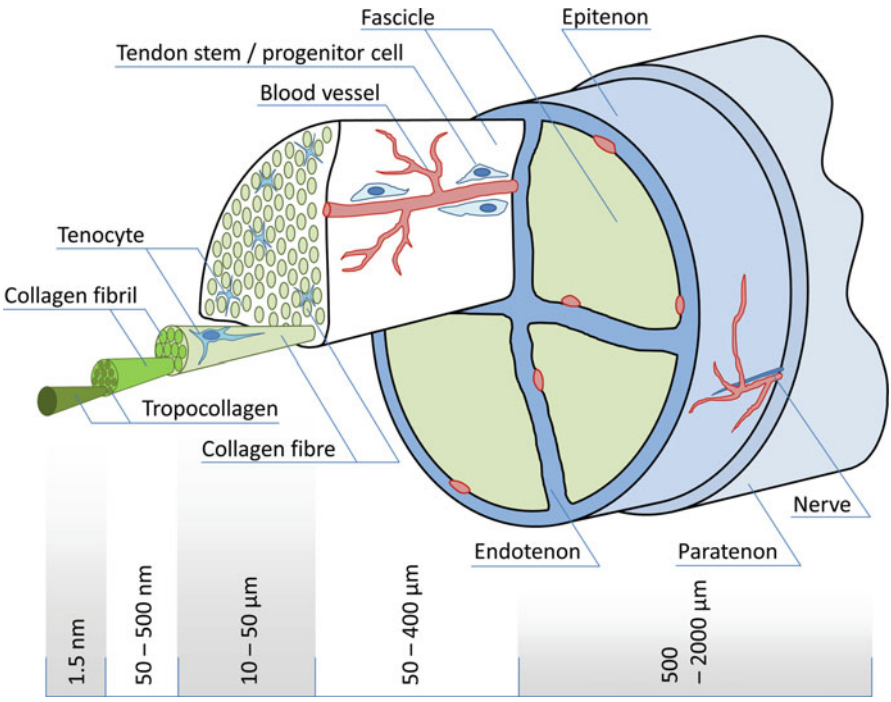

Fig. 1 Hierarchical tendon structure in which mainly collagen type I molecules assemble to form increasingly large subunits of the anisotropic tendon ECM. Tendons are further mainly populated by terminally differentiated tenocytes and a small population of tendon stem and progenitor cells. The tendon fiber bundles are further ensheathed in a loose connective tissue termed endotenon, harboring vessels, lymphatics, and nerves. Finally, the entirety of the tendon is surrounded by the epitenon and paratenon

approximately 10 nm to 500 nm (Canty and Kadler 2005). Fibrils continue to pack together to larger fibers, which are then bound together to fascicles. At each level of this hierarchy, collagens are interspersed with a varying amount of matrix rich in proteoglycans which also significantly contribute to the mechanical behavior of tendons (Thorpe et al. 2013). Finally, fascicles are then bundled by a connective tissue sheath termed the endotenon, forming the intrasubstance, and the epitenon, which encircles the periphery of the full tendon. Both facilitate and lubricate tendon movement and embed blood vessels, nerves, and lymphatics which run to the deeper portion of the tendon (Benjamin et al. 2008).

The majority of the tendon matrix is comprised by collagens, elastin, proteoglycans (PGs), and glycosaminoglycans (GAGs). Collagen type I is the major constituent, accounting for around 60% of the dry mass and about 95% of the total collagen (Sharma and Maffulli 2006; Wang 2006). Other less abundant collagen isotypes include collagen types III, V, VI, XI, XII, and XIV. In healthy tendons, collagen type III is mainly found in the endotenon and epitenon (Kannus 2000). It is thought to be essential for collagen fibril formation regulating collagen type I fibril size (Kadler

et al. 1990). Further, an increase of collagen type III has been reported for overloaded or injured tendons (Pajala et al. 2009; Pingel et al. 2014), and it is believed to have a role in the healing response. Collagen type V and the nonfibrillar collagens XII and XIV also serve a regulatory role during fibrillogenesis (Ansorge et al. 2009; Wenstrup et al. 2004). Elastin is present in tendon as elastic fibers which make up around 1–2% of the total dry mass (Kannus 2000), providing tissue flexibility, extensibility, and passive recoil (Kielty et al. 2002).

Next to collagens, tenocytes also produce glycoproteins and proteoglycans (PG). PGs are core proteins attached to one or several polysaccharide chains, commonly referred to glycosaminoglycans (GAGs). Generally, in the tensile region of tendons, the majority of PGs belong to the family of small leucine-rich proteoglycans (SLRPs), including decorin, biglycan, fibromodulin, and lumican. SLRPs bind to collagen molecules of fibrils and facilitate fibril assembly (Thorpe et al. 2013). For example, mice lacking decorin, which is the most abundant PG in tendons, develop structurally impaired tendons with irregular fibrils. A rather similar phenotype is seen for biglycan knockout mice (Gordon et al. 2015; Robinson et al. 2005). Interestingly, PG concentrations and type differ for tendons loaded in tension, compression, and shear (Berenson et al. 1996; Riley et al. 1994), and also quantitative difference has been demonstrated within a single tendon, comparing the compressed regions and the tensile regions (Carvalho et al. 2000; Vogel et al. 1993). Fibromodulin (Fmod) and lumican are also involved in collagen fibrillogenesis, potentially stabilizing small-diameter fibrils by preventing their fusion (Chakravarti 2002). Fmod and biglycan have further been shown to be important for the formation and maintenance of the TSPC niche (Bi et al. 2007).

Besides SLRPs, other glycoproteins are important constituents of tendons, such as cartilage oligomeric matrix protein (COMP), fibronectin, tenascin C (TNC), and tenomodulin (Tnmd). Although COMP, a large pentameric protein providing a link to neighboring collagen fibrils (Smith et al. 1997), is the most abundant glycoprotein in tendons, its function remains largely unclear. Tnmd is a type II transmembrane glycoprotein predominantly expressed in tendons and ligaments, but can also be found in other tissue types such as muscle, skin, fat, or nervous tissue (Dex et al. 2016). Next to Scleraxis (Scx) and mohawk homeobox (Mkx), tenomodulin has received increasing attention as a putative tendon-marker protein. Further, Tnmd knockout mice show a reduced proliferation rate and an abnormal collagen fibril phenotype with pathologically thicker fibrils (Docheva et al. 2005).

The physicochemical properties of the SLRPs and PGs allow water uptake, which makes up to 60–80% of the total weight of tendons, whereas proteoglycans themselves account only for 1–2% (Kannus 2000). Generally, tendons grow stiffer as they mature due to increased collagen fibril thickness and the formation of covalent cross-links, which are primarily driven by the enzyme lysyl oxidase (Lox) (Bailey 2001). Beyond these enzymatic cross-links, SLRPs have been demonstrated to directly bond collagen fibrils together or to influence Lox-driven cross-linking reactions (Kalamajski et al. 2014).

Taken together, the structure and composition of a tendon is tightly linked to its function and the hierarchical structure exhibiting a highly anisotropic, nonlinear, and viscoelastic mechanical behavior is fundamental for normal tendon function.

3 Tendon-Resident Cells

Mature tendons are populated by several cell types and so far, mainly due to the lack of reliable tendon-specific markers, our knowledge about their identity remains incomplete. The primary resident cells are tendon fibroblasts, termed tenocytes, which comprise approximately 90% of the tendon cellular compartment (Kannus 2000). These flat, elongated differentiated cells are proposed to arise from tenoblasts (Chuen et al. 2004). However, tenoblasts have also been regarded as an activated form of tenocytes in the case of intrinsic healing of tendon injuries (Davidson et al. 1997). The remaining 10% is composed of synovial cells from the endo−/epitenon, chondrocytes located in close proximity of tendon-to-bone insertions (entheses), and vascular endothelial cells. In addition, a population of multipotent tendon stem and progenitor cells (TSPCs) has been identified in tendons (Bi et al. 2007; Salingcarnboriboon et al. 2003; Tempfer et al. 2009).

TSPCs have been described to reside in two different locations. Most studies describe a population of adult stem cells within the tendon proper, suggesting a mainly nonvascular source of stem cells in tendons (Bi et al. 2007; Lui 2013). In a subsequent study, Mienaltowski et al. reported the isolation of stem and progenitor cells from the peritenon of mouse Achilles tendons (Mienaltowski et al. 2013). Most of these data are deduced from in vitro experiments by isolating TSPCs from different tendon regions and subsequent analysis of clonogenicity, self-renewal capacity, multilineage differentiation potential, and BrdU or IdU-retention over time indicating slow cycling cells which is an accepted stem cell feature (Bi et al. 2007). Generally, adult stem cells often reside in a perivascular niche, and indeed in human supraspinatus tendons a population of perivascular cells expressing tendon and stem cell markers has been described (Tempfer et al. 2009). However, studies unequivocally demonstrating the exact location of TSPCs during tendon maintenance and healing are urgently required (see also further below – tendon stem cell niche).

TSPCs exhibit classical adult mesenchymal stromal cell (MSC) criteria and have been described for human, equine, bovine, rabbit, rat, and mouse tendons. They express specific surface antigens and show self-renewal, clonogenicity, and trilineage differentiation capacity, giving rise to adipogenic, osteogenic, and chondrogenic cells. They meet the marker panel defined by the International Society for Cellular Therapy for MSCs as they express CD90, CD73, and CD105 but are negative for CD31, CD34, CD45, HLA-DR, CD11b, CD14, and CD19. In addition, they express tendon-related proteins such as scleraxis and tenomodulin and are able to form tendon and enthesis-like tissues when implanted in vivo (Bi et al. 2007; Rui et al. 2010). Importantly, due to the absence of standardized protocols, the population of isolated tendon-resident stem and progenitor cells most likely varies due to differences in tendon type, donor age, and isolation protocol. Therefore, direct comparison of published results can be problematic. TSPCs have also been reported to express markers typical for embryonic stem cells (ESCs), such as Oct-4, Nanog, Sox2, c-myc, SSEA-4, and nucleostemin (Tan et al. 2013; Zhang and Wang 2010a), however fail to form teratomas (Tempfer H.; unpublished results). In vivo expression of these markers could only be detected at the tendon injury site, but not within intact tendons (Tan et al. 2013).

Taken together, our knowledge on the heterogeneous TSPC population is rather fragmentary. It is unclear if TSPCs represent a residual population of the embryonal tendon progenitors and the relationship of TSPCs to tenoblasts and tenocytes remains to be determined. This is mainly hampered by the lack of TSPC-specific markers, such as Tnmd, Scx, Mkx, and ColI, are expressed by both tenocytes and tendon stem and progenitor cells. Therefore, more studies tracking TSPCs in vivo and investigating their niche are imperative to make use of this cell source for treating injured tendons or other musculoskeletal disorders.

4 Tendon Stem Cell Niche

In human tendons, stem cells have been detected by immunohistochemical staining in the perivascular area expressing tendon (Scx, ColI, ColIII, Smad8) as well as stem/precursor cell (CD133, Musashi-1, Nestin, CD44, CD29) and pericyte-associated markers (αSMA) (Crisan et al. 2008; Tempfer et al. 2009). Some of these markers could also be identified in cells residing within the dense part of the tendon. Since TSPCs share many markers with mesenchymal stromal cells (MSCs), it is still not clear whether they are also pericytes as has been suggested by Caplan et al. for MSCs or whether they represent an own cell entity within the same perivascular niche (Caplan 2008; de Souza et al. 2016). This perivascular niche is defined by a variety of different factors providing signaling cues crucial for maintaining a balance of quiescence, self-renewal, and cell-fate commitment of the stem cell population residing in it. The stem cells are embedded within a very complex fibrous three-dimensional extracellular matrix consisting of a multitude of structural proteins such as collagens, glycoproteins, proteoglycans, and glycosaminoglycans. Besides the crosstalk with neighboring cells including tenocytes, pericytes, macrophages, mast, and endothelial cells via direct physical contact or paracrine signaling also soluble factors from the blood such as growth factors, cytokines, and oxygen contribute to establishing the TSPC-niche (see Fig. 2). Anchorage of the cells to the extracellular matrix via integrins, gap junction, and cadherin-based connections between tenocytes allow transmission of mechanical stimuli of the extracellular matrix to the cells eliciting respective signals which instruct TSPCs to either maintain their stem cell nature or direct them to differentiate into tenocytes or nontenocytes (McNeilly et al. 1996; Popov et al. 2015a; Schiele et al. 2013; Schwartz 2010; Stanley et al. 2007). Perturbing a single factor within this intricate network might therefore be sufficient to disturb/perturb tissue homeostasis.

4.1 Extracellular Matrix within the Niche

Regarding the influence of the extracellular matrix on stem cell biology not only the matrix composition is of relevance, but also its topography, nanostructure, stiffness/tissue elastic modulus, and strength (Ahmed and Ffrench-Constant 2016; Das and Zouani 2014; Tsimbouri 2015). Experiments using scaffolds with disoriented fiber

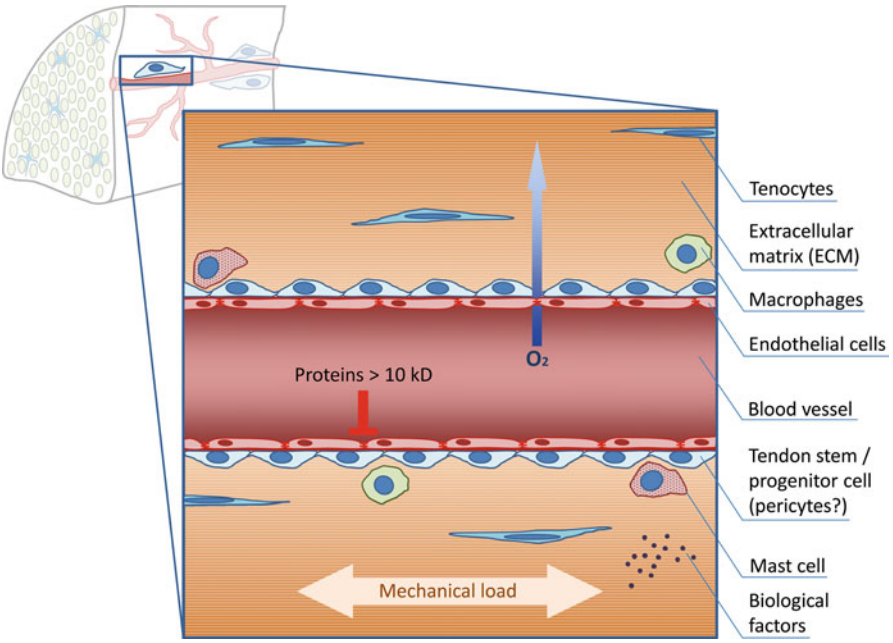

Fig. 2 Perivascular tendon stem and progenitor cell niche. The exact location of the tendon stem cell niche and the cellular and acellular components has not been fully defined yet. Tendon blood vessels are lined by endothelial cells forming a size selective barrier, most likely controlling the passage of blood-borne products into the tendon proper. The perivascular stem cells themselves most likely receive input from soluble factors, the extracellular matrix, neural inputs (?), the vascular network, and other cells (e.g., mast cells). Further, they perceive topographical information, such as ECM alignment and mechanical stress

alignment revealed that seeded stem cells differentiated into the osteogenic lineage, whereas parallel aligned scaffolds promoted tenogenic differentiation (Yin et al. 2010b). Interestingly, it has been shown that the fiber diameter had an even more significant effect on cellular behavior than fiber alignment, a fiber diameter of >2 μm appearing more suitable for tenogenic differentiation (Cardwell et al. 2014). Also, matrix stiffness seems to play an important role for stem cell fate, the stiffness of the ECM which favors tenogenic differentiation lying between values inducing myogenic and osteogenic differentiation (Das and Zouani 2014). By using polyacrylamide substrates functionalized with either fibronectin, collagen, or a combination of both and approximating the elastic modulus of tendon granulation tissue and the osteoid of healing bone with values ranging between 10 and 90 kPa, Sharma and Snedeker observed that seeded bone marrow stromal cells differentiated into the osteogenic lineage at a rigidity of around 80 kPa. In contrast, tenogenic differentiation occurred only on collagen substrate under moderate rigidity conditions (~30–50 kPa) (Sharma and Snedeker 2012).

The impact of the composition of the ECM has impressively been demonstrated by Bi et al. who by using $Bgn^{-/0}$ $Fmod^{-/-}$ knock out animals identified biglycan and

fibromodulin as two critical components that organize the niche (Bi et al. 2007). Mice deficient of these two matrix proteins showed impaired tendon formation and their tendons displayed ectopic ossification due to a change of TSPC fate from tenogenesis to osteogenesis. The authors speculate that changes in TSPC niche-associated ECM composition may perturb the balance of certain cytokines and growth factors stored within the EMC, which could be responsible for the altered TSPC cell fate.

Also, degradation of the ECM proteins by metalloproteinases known to be secreted upon extensive loading or progressive aging may liberate bound growth factors (e.g., VEGF, TGFß1), thereby modulating their bioactivities, and thus impact upon stem cell fate regulation (Koskinen et al. 2004; Spiesz et al. 2015).

4.2 Biomechanical and Biochemical Inputs Driving TSPC Fate

Mechanical loading, being an inherent part of the tendon environment, likely functions as a niche factor regulating the fate of TSPCs. Maintaining the pool of TSPCs and increasing the number of tenocytes from stretching-induced TSPC differentiation are two mechanisms that together provide an effective way for the maintenance of tendon homeostasis. Mechanical stimulation has been shown to upregulate Scx expression under physiological loading both in vitro and in vivo, suggesting that it might be required for tissue homeostasis (Maeda et al. 2011). Along these lines, 3 weeks of treadmill running for 50 min/day, 5 days a week, nearly doubled the proliferation rate of mouse Achilles and patellar TSPCs (Mendias et al. 2012; Zhang et al. 2010a).

There are several studies indicating that mechanical stimulation regulates stem cell proliferation and differentiation in a stretching magnitude-depending manner (Zhang and Wang 2010b). Although there is consensus about the fact that mechanical stimulation does have an effect on tenogenic differentiation, conflicting data exist regarding the extent of strain needed to be applied to be beneficial. While moderate running led to an upregulation of tenocyte-related genes, intensive running induced an increase in both tenocyte and nontenocyte-related genes (Zhang et al. 2010a). Whereas Zhang et al. report that in vitro low mechanical stretching (4%) increased the expression of only the tenocyte-related genes, and high mechanical stretching (8%) increased the expression of both tenocyte and nontenocyte-related genes, Rui et al. demonstrated that repetitive tensile loading at already 4% strain induced osteogenic differentiation (Rui et al. 2013b; Zhang and Wang 2013a). These differences observed might be due to differences in species used, duration, strain, and frequency of mechanical stretching that has been applied. As to the type of force acting on TSPCs, it is still not known whether increased tensile strain or compressive strain induces differentiation of TSPCs into nontenocytes (Lui and Chan 2011).

There are many studies investigating the effect of growth and differentiation factors such as GDF-5 (BMP 14), GDF-6 (BMP 13), GDF-7 (BMP 12), and insulin on mesenchymal stem cells demonstrating their potency to drive stem cell differentiation towards the tenogenic lineage (Hankemeier et al. 2005; Helm et al. 2001; Mazzocca et al. 2011; Park et al. 2010; Sassoon et al. 2012; Violini et al. 2009). Assuming that TSPCs represent specialized MSCs (or pericytes?) displaying a

tissue-specific phenotype, it is likely that TSPCs behave in a similar manner when exposed to the growth factors mentioned, but there are only few studies demonstrating this directly (Tokunaga et al. 2015). Incubation of rat patellar TSPCs with BMP-2 promoted glycosaminoglycan deposition, aggrecan expression, and enhanced nontenocyte differentiation of TSPCs (Rui et al. 2013b). Treatment of three clonal tendon cell lines with bFGF and TGFß significantly enhanced their proliferation (Salingcarnboriboon et al. 2003). Treatment of rat TSPCs with GDF-5 or connective tissue growth factor reduced differentiation along adipogenic and chondrogenic pathways and showed significantly enhanced tenogenic differentiation (Holladay et al. 2016; Lee et al. 2015; Ni et al. 2013). Moreover, IGF-1 treatment at 10 or 100 ng/ml maintained TSPC multipotency and phenotype (Holladay et al. 2016).

As for many tissues, in healthy human tendons macrophages and mast cells are localized in close proximity to blood vessels (Dakin et al. 2015). Whether these immune cells contribute to tendon homeostasis under physiological conditions or whether they solely serve as cellular guards monitoring their environment for stress signals is not known. Under inflammatory conditions or upon excessive loading, mast cells and macrophages become activated and secrete cytokines and inflammatory mediators such as prostaglandin E2 (PGE2). These cytokines lead to the recruitment of additional immune cells including leukocytes which in turn propagate cytokine production.

TSPCs have been shown to be affected by inflammatory mediators and biomechanical stress, driving them down paths of adipogenic and osteogenic differentiation. Prostaglandin E2, a major inflammatory mediator in tendons, decreased TSPC proliferation in vitro and induced both adipogenesis and osteogenesis of TSPCs in a dose-dependent manner (Zhang and Wang 2010c; Zhang and Wang 2012). Examination of the effects of IL-1β on the function of TSPC isolated from mouse injured Achilles tendons revealed that IL-1 β strongly reduced expression of tendon cell markers such as scleraxis and tenomodulin and irreversibly inhibited tenogenic differentiation (Zhang et al. 2015). Recently, Zhang and colleagues showed that leukocyte-enriched platelet-rich plasma (L-PRP) significantly reduced the proliferation of TSPCs in a concentration-dependent manner. Moreover, TSPCs grown in L-PRP differentiated into nontenocytes and produced more inflammatory factors such as membrane-associated prostaglandin synthase (mPGES) and IL-1β (Zhang et al. 2016).

Overall, our understanding of the various mechanical and biochemical stimuli driving and maintaining the tenogenic lineage in health and disease remains very limited, leaving ample room for future research.

4.3 Oxygen Tension

Oxygen tension has been identified as an important factor for maintaining stem cell stemness. The actual oxygen tension in tendons has not been unequivocally determined, but in most tissues it is estimated to be about 10% under physiological conditions; the pO_2 of human blood normally ranging between 75 and 100 mmHg, which is equivalent to O_2 gas levels of 10–13% (Holzwarth et al. 2010). Given that there are TSPCs residing in close proximity to the blood vessels and stem cells

located between aligned collagen fibers in some distance to the vasculature, it can be assumed that these two populations encounter different O_2 tensions, which might have an impact on their behavior. Lee et al. showed that culture of TSPCs under 2% O_2 tension increased cell number, colony number, and mRNA expression of tendon-related markers but reduced the osteogenic, adipogenic, and chondrogenic differentiation potentials (Lee et al. 2012). In line with these findings, it was shown that the culture of porcine tenocytes under hypoxic conditions significantly enhanced their expansion capacity (Zhang et al. 2010b). The important role of hypoxia is further underscored by the observation that human TSPCs maintain their stemness under hypoxic culture conditions by upregulating stem cell markers such as nucleostemin, Oct-4, Nanog, and SSEA-4 (Zhang and Wang 2013b).

4.4 Blood-Tendon Barrier

The recent description of a structural barrier formed by the endothelium lining blood vessels residing in tendons, which size-selectively controls the paracellular passage of macromolecules from the blood stream into the tendon proper, adds a new component to the network of structures potentially forming the tendon stem cell niche (Lehner et al. 2016). Experiments using dextran-labeled tracers of different size revealed that molecules larger than 10 kD are retained within the blood vessels. In vitro differentiation experiments using TSPCs further revealed that the presence of 10% serum in the differentiation-inducing medium promoted the formation of lipid droplets and increased the number of calcium deposits compared to cells cultured under serum-free adipogenic and osteogenic conditions. Further, in mature, healthy tendons very little turnover and thus proliferation occurs (Heinemeier et al. 2013). Upon injury or in tendinopathic tendons, going along with ruptured or leaky blood vessels, proliferation strongly increases most likely due to contact with serum components (e.g., growth factors), which under physiological conditions is restricted (Rolf et al. 2001). Further, serum-derived components potentially also drive the erroneous differentiation of TSPCs in situ, promoting tendon degeneration (see Fig. 3).

5 Tendon Development and Maturation

Tendon healing upon injury or long-term degeneration is a slow process which usually fails to restore tendon quality to the original status before injury. A concept gaining more and more acceptance is that successful interventions to improve (tendon) regeneration will have to recapitulate the developmental events that establish the native structure (Thomopoulos et al. 2010; Yang et al. 2013). Therefore, understanding the fundamental processes involved in embryonic tendon formation and identifying factors controlling these is a prerequisite for the development of novel and innovative treatment strategies. Similarly to most tissues and organs of the body, tendons do not develop independently from their surroundings, but they

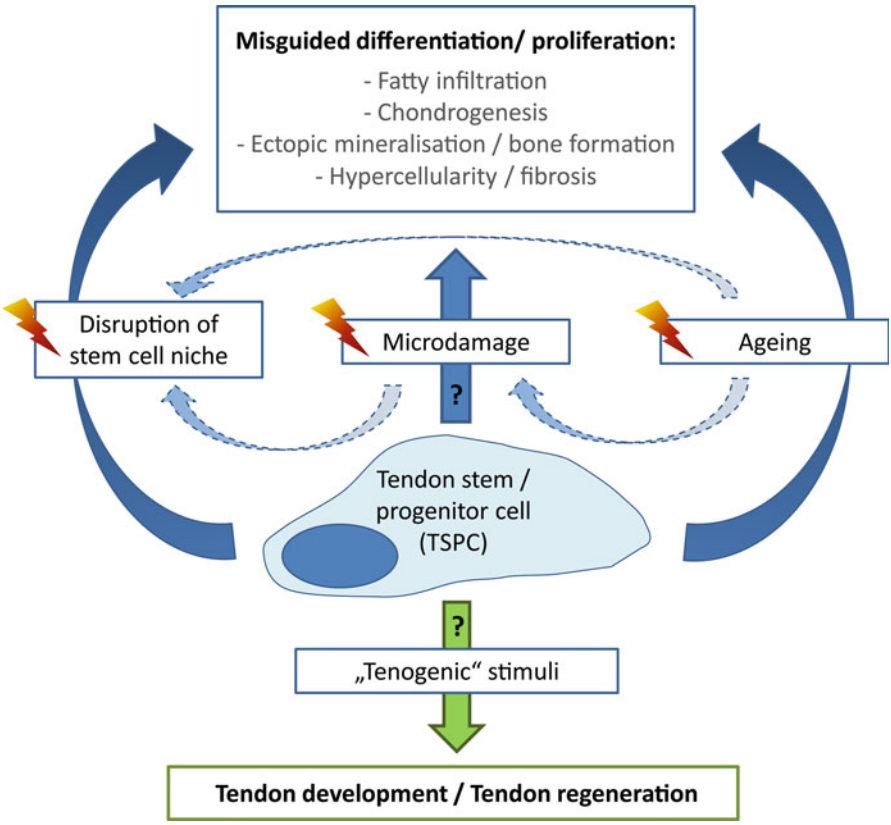

Fig. 3 Tendon stem/progenitor cells (TSPC) are suspected to participate in degenerative processes in aging or upon damage, due to their capacity to both proliferate and differentiate. The disruption of the stem cell niche in aged or damaged tendon is a key event in these unwanted processes. If and how TSPCs participate in tendon development and/or regeneration is still unclear, so are potential physiological tenogenic stimuli driving the maturation of tendon cells

strongly depend on signals from their neighboring tissues, mainly muscle and cartilage or bone (Deries and Thorsteinsdottir 2016; Kardon 1998).

As mentioned before, molecular characterization of tendon cells and/or tendon stem and progenitor cells is challenging and still a matter of debate (Lui 2013). As the master regulator gene(s) of the tendon lineage remain(s) to be identified, the intrinsic and extrinsic programs driving tenogenesis in vertebrates are yet to be discovered.

The identification of the basic helix-loop-helix transcription factor Scleraxis (Scx) as a crucial player in tendon development in 2001 (Schweitzer et al. 2001) was a major leap forward in understanding tendon development and significantly contributed to our current knowledge on early tendon formation. During early somite development, cartilage and muscle emerge from the myotome and sclerotome, responding to signals from surrounding tissues. The axial tendon lineage is established within the dorsolateral sclerotome as the somite matures, adjacent to

and beneath the myotome (Brent et al. 2005). This somatic compartment has also been termed syndetome (Brent et al. 2003). In contrast, craniofacial tendons have been shown to originate from the mesectoderm in mouse, chick, and zebrafish (Chen and Galloway 2014; Grenier et al. 2009).

The molecular program driving tendon development also depends on the anatomical location within the tendon and is influenced by the close association with muscle (myotendinous junction) or cartilage and bone (entheses). At the axial level, muscle is required for the initiation of tendon development (Brent et al. 2005), whereas for craniofacial and limb tendons muscles are not required for early initiation, but maintenance of tendons by inducing Scx expression (reviewed in (Gaut and Duprez 2016). Similarly, the formation of the bone-tendon unit requires complex signaling mechanisms. Zelzer E and Blitz E. et al. propose a so-called "segregation model," according to which a common multipotent "tenochondral progenitor," which is positive for both Scx and the chondrocyte associated transcription factor SRY (Sex Determining Region Y)-Box 9 (Sox9), gives rise to both cartilaginous bone primordia and early tendons (Zelzer et al. 2014). Interestingly, depletion of Sox9 in mice leads to a complete lack of cartilage, and tendon development, however, remains unaffected, indicating that Sox9 does not have a functional role in tendon cell differentiation (Blitz et al. 2013; Sugimoto et al. 2013).

Finally, mechanical load is also a crucial factor for tendon formation. Both FGF/ERK/MAPK and TGF-ß/SMAD2/3 signaling independently induce tendon formation downstream of mechanical loading, as it was shown in a chick embryo model (Havis et al. 2016).

5.1 Genes Involved in Tendon Development

So far, only two DNA-binding proteins strongly influencing tendon formation have been described: Scleraxis was identified to be a crucial factor in early development, regulating tendon progenitor cell fate (Brent et al. 2005; Schweitzer et al. 2001). Scleraxis is fundamental for tendon formation as depletion of this gene results in developmental tendon abnormalities varying between different tendons. However, it does not lead to complete tendon loss. Particularly muscle anchoring tendons remain largely unaffected, underlining the heterogeneity of tendon tissue development (Murchison et al. 2007).

The other transcription factor involved in tendon formation is the IRX family-related homeobox protein Mohawk (Mkx). It is expressed in various musculoskeletal progenitors and is required for neural crest cell migration during development (Chuang et al. 2010). Depletion of Mkx in mice leads to hypoplastic tendons throughout the body. Despite the reduction in tendon mass, the cell number in tail tendon fiber bundles remains similar between wild type and Mkx−/− mice. Again, no complete loss of tendons has been observed in Mkx−/− mice (Ito et al. 2010; Kimura et al. 2011).

Next to vertebrates, also invertebrates such as Drosophila possess tendons, of course displaying major differences, i.e., vertebrate tendons originating from the

mesoderm, whereas fly-tendons are ectodermal derivatives. In Drosophila tendon precursor are characterized by the expression of the transcription factor Stripe, an Egr (early growth response)-like transcription factor. Analysis of loss- and gain-of-function Stripe mutant phenotypes shows that Stripe is a key regulator of tendon cell specification and differentiation in fruit flies. (Frommer et al. 1996; Vorbruggen and Jackle 1997). The vertebrate homologues of Stripe, Early Growth response 1 and 2 (Egr1 and 2) are also involved in vertebrate tendon development: Adult tendons of Egr1−/− mice displayed a deficiency in the expression of tendon genes, including Scx, Col1a1, and Col1a2, and were mechanically weaker compared to their wildtype littermates. However, the observed effects are not severe enough to conclude that these genes are key drivers in vertebrate tendon cell differentiation. Nevertheless, the loss of Egr1 impairs tendon regeneration following injury in rodents and is thus considered a potential target to improve regeneration (Guerquin et al. 2013; Lejard et al. 2011).

Finally, tenomodulin (Tnmd) is one of the most common tendon-related marker proteins used for characterization (Dex et al. 2016) and its expression is closely associated with tendon differentiation during chick development. Loss of Tnmd in mice does not result in an embryonic phenotype, besides a modest decrease in tenocyte proliferation around birth and slightly altered collagen fibril size. However, at 6 months of age, no severe effects are obvious by depleting Tnmd (Docheva et al. 2005).

Taken together, many questions regarding tendon development remain unanswered and the underlying complex temporospatial expression patters ultimately resulting in the formation of mature tendons need to be unraveled to provide a basis for the effective treatment of tendon injury.

5.2 Vasculature and Cell Density in Developing Tendon

The formation of a vascular bed is a crucial factor for the development of virtually every tissue. Also under the aspect of blood vessels supplying a niche for stem and progenitor cells, vascularization during development is highly relevant (see Fig. 4). Unfortunately, for tendons the role of vascularization in development and maturation is still poorly defined (Tempfer and Traweger 2015). Peacock (1959) described embryonic tendons to be "supplied with a rich capillary network," based on the analysis of sections prepared from an 8-month-old human embryo (Peacock 1959). A study in postnatal, immature sheep describes a massive decline in both cellularity and vessel density in the tendon of the extrinsic flexor muscles of the fingers (musculus flexor digitorum superficialis), the meniscus and the cruciate ligaments between 1 and 40 weeks postnatally, with the strongest reduction occurring within the first 8 weeks. Remarkably, also the expression of vascular endothelial growth factor (VEGF) and of smooth muscle actin massively declines in the cells residing in the dense, collagenous tissue (Meller et al. 2009). In line with these findings, several studies point out the (relative) decrease of cell density during tendon maturation (Ippolito et al. 1980; Oryan and Shoushtari 2008). Given the fact that very little turnover of the extracellular matrix occurs in human tendons after termination of linear growth after ~17–18 years of age, low vascular supply seems appropriate (Heinemeier et al. 2013). However, in acute or

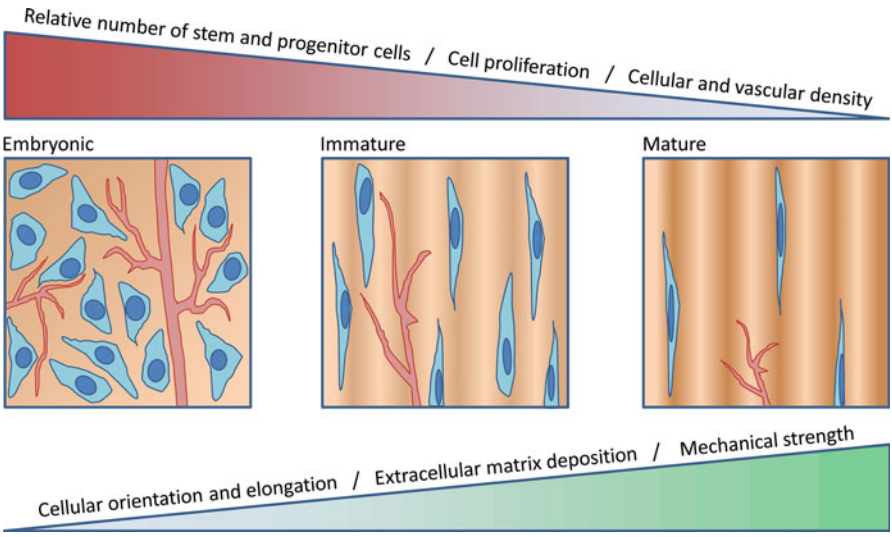

Fig. 4 The transition from embryonic to mature tendon is hallmarked by a decrease in cell density, a decline in the relative number of stem/progenitor cells, and massively reduced density of blood vessels. During this process, the amount of deposited extracellular matrix, the degree of cell alignment, and the mechanical strength of the tissue increase

chronic tendon injuries, hypervascularity does not seem to support functional recovery of tendons. Therefore, scarless tendon regeneration potentially requires a balanced manipulation of the angiogenic response.

6 Tendon Aging

With aging of tendons to withstand force declines and age-related tendon injuries are thought to be a result of changes in structure and mechanical properties of tendons, thus leading to physical frailty, reduced activity in the elderly, and a loss in general quality of life. Additionally, aged tendons only poorly respond to classical treatment strategies. The decline in functional integrity is based on changes to structural and mechanical properties of tendons. Furthermore, tendon-resident cells show qualitative and quantitative alterations dependent on the age of the tendon. The most characteristic age-related degenerations seen on the histological level are morphological changes of tendon cells, an altered collagen fiber structure, as well as accumulations of lipids and calcium depositions (Kannus et al. 2005). Together, these changes promote microdamages in the ECM, ultimately weakening tendons and increasing the risk for overuse injuries and full-thickness ruptures (Oliva et al. 2012).

Increasing age is related to a number of tendinopathies; a recent systematic review on rotator cuff degeneration revealed a prevalence of about 10% in patients aged 20 years or younger, increasing up to 40% in patients older than 60 years and up to 62% in patients 80 years and older (Teunis et al. 2014). Overall, the mechanisms

responsible for the functional decline in aged tendons are poorly described. Most studies on human tendon aging have been published based on degenerated tendon samples, revealing most likely changes due to degenerative processes (tendinopathy, tendinosis, ruptured tendons). However, virtually no data are available for healthy-aged tendons. A recent study demonstrated an age-related decrease of Secreted protein acidic and rich in cysteine (Sparc) in healthy-aged tendons. In vitro studies suggested that Sparc modulates the cell-ECM interaction of tendon-resident stem and progenitor cells and together with a change in ECM properties potentially impacts upon adipocyte differentiation as in aged and Sparc knockout tendons the accretion of lipids was observed (Gehwolf et al. 2016). Therefore, Sparc seems to have a limiting effect on adipogenesis in tendons, and its decreased expression with age might be an underlying cause for the formation of fatty depositions in aged tendons, ultimately increasing the risk of tendon degeneration and/or rupture.

Another common age-related phenomenon in tendons is ectopic mineralization, characterized by inappropriate depositions of calcium hydroxyapatite (De Vilder and Vanakker 2015; Kannus et al. 2005), frequently resulting in painful calcific tendinopathies (e.g., at the rotator cuff). The calcium deposits lead to changes in the mechanical properties of tendons and mineralized deposits often correlate with sites of rupture (Grases et al. 2015). However, it remains largely unclear how this mineralization occurs and whether some tendons are more affected than others. Alterations in the ECM composition, e.g., in the biglycan and decorin content, were described to promote ectopic ossification (Ameye et al. 2002). This is further supported by the reports of Bi et al. (2007) and Rui et al. (2013b) showing that the reduction in these small leucine-rich proteoglycans (SLRPs) at the tendon stem cell niche results in an aberrant differentiation of TSPCs, promoting degenerative calcification processes.

6.1 Cellular Senescence, Aging Tendon Stem Cells

Several studies have demonstrated that tendon resident stem and progenitor cells show age-related changes in vitro, including a reduced proliferation rate, lower colony-forming capacity, a diminished self-renewal capacity, and altered cell fate patterns. Generally, the number of tenocytes and TSPCs is reduced in aged tendons in vivo (see Fig. 4) and the progenitor cells show a higher tendency to differentiate towards the adipogenic lineage, which is supported by an increased expression of adipogenic marker genes such as Pparγ, Cebpα, perilipin, and leptin (Gehwolf et al. 2016; Zhou et al. 2010).

A study of Kohler et al. (2013) on cellular and molecular changes on human tendon progenitor cells derived from young-healthy and aged human Achilles tendons, however mostly displaying signs of degeneration, revealed that genes associated with cell adhesion, migration, or actin cytoskeleton, were significantly changed in their expression, resulting in dysregulated cell-matrix interactions. They provide evidence that the ROCK kinase pathway signaling is involved in tendon stem/progenitor cell aging/senescence (Kohler et al. 2013). The same group showed that the loss of tenomodulin (Tnmd) results in a reduced self-renewal and augmented

cell senescence of tendon progenitor cells (Alberton et al. 2015). Finally, it was also demonstrated that the downregulation of ephrin receptors 4, B2, and B4 (Eph4, EphB2, EphB4) in aged tendon cells limits the establishment of TSPC cell-cell interactions (Alberton et al. 2015).

Along the same lines, tendon-derived cells from healthy-aged mouse Achilles tendons showed age-related changes in cell morphology and their competence to contract matrices in 2D and 3D cell culture assays. Further, they were less spread on collagen type I-coated surfaces, formed larger focal adhesion complexes, and were characterized by an increase in the expression of paxillin when compared to young tendon cells. Additionally, a strong cortical cytoskeletal rearrangement was observed for healthy-aged tendon cells (Gehwolf et al. 2016).

6.2 Tendon Matrix Composition, Remodeling, Glycation, Collagen Turnover

As described in more detail further above, the tendon extracellular matrix is predominantly composed of hierarchically arranged collagen type I fibers. The ECM undergoes age-related changes in structure, composition and organization, and protein turnover. A well-accepted method assessing aging on the protein level is the analysis of amino acid racemization. Amino acids are incorporated in the tissue in their L-form and with time undergo racemization to their D-form. The most rapidly converting amino acid is aspartic acid, which is easily detectable in aged biological tissues, and therefore, increased levels of D-Asp are an indicator for a reduced/slower protein turnover. Together with the determination of collagen degradation, amino acid racemization is a valuable tool to analyze matrix aging and turnover. The reduced matrix turnover rate indicates a decreased ability/activity of tenocytes to repair microdamages potentially leading to their accumulation with age.

On the ultrastructural level, in aged C57BL/6 mouse tendons, the collagen fibril diameters are increased and fibrils are more densely packed (Gehwolf et al. 2016; Goh et al. 2008, 2012). Further, the fibers are less well oriented/aligned in old mouse tendons (Dunkman et al. 2013; Gehwolf et al. 2016) and variations in collagen crimping have been reported (Gautieri et al. 2016; Legerlotz et al. 2014). Also, a reduction in extracellular water content (Kannus et al. 2005), the reduced expression of collagen type I and of several SLRPs (e.g., decorin, biglycan, fibromodulin) and glycoproteins (Dunkman et al. 2014; Dunkman et al. 2013; Gehwolf et al. 2016), a higher collagen crosslinking rate, and the accumulation of advanced glycation end-products (Snedeker and Gautieri 2014) are typically seen for aged tendons. Interestingly, decorin seems to promote age-related changes in collagen fibril maturation and biomechanics, as in decorin knock out mice these effects were ameliorated (Dunkman et al. 2013).

Finally, several studies demonstrated an increase in tendon matrix degrading enzymes with age. Next to matrix-metalloproteinase-1 (MMP-1) (Riley et al. 2002), an enhanced activity of MMP-2 and MMP-9 has been associated with tendon aging (Dudhia et al. 2007; Yu et al. 2013).

Table 1 Cellular and extracellular changes in aged tendons

cellular level	cell number ↓	(Birch et al., 1999; Gehwolf et al., 2016)
	DNA content ↓	(Birch et al., 1999)
	stem/progenitor cell number ↓	(Zhou et al., 2010)
	self-renewal capacity ↓	(Popov et al., 2015b; Zhou et al., 2010)
	colony forming capacity ↓	(Kohler et al., 2013; Zhou et al., 2010)
	Tenomodulin expression ↓	(Alberton et al., 2015)
	Scleraxis expression ↓	(Zhou et al., 2010)
	inflammatory response ↓	(Dakin et al., 2012; Humbert et al., 1976; Kietrys et al., 2012; Speed, 2016)
	metabolic activity ↓	(Almekinders and Deol, 1999; Floridi et al., 1981)
	proliferation ↓	(Kohler et al., 2013; Tsai et al., 2011)
	cell-cell interactions ↓	(Alberton et al., 2015)
	adipogenic differentiation ↑	(Gehwolf et al., 2016; Zhou et al., 2010)
	inflammatory processes ↑	(Dakin et al., 2012; Speed, 2016)
	senescence ↑	(Kohler et al., 2013; Popov et al., 2015b)
extracellular matrix	total collagen content ↔	(Birch et al., 2016; Gehwolf et al., 2016)
	fibre alignment ↓	(Dunkman et al., 2013; Gehwolf et al., 2016)
	crimp angle ↓	(Legerlotz et al., 2014)
	collagen turnover ↓	(Birch et al., 1999; Birch et al., 2016)
	lipid accumulation ↑	(Gehwolf et al., 2016; Kannus et al., 2005)
	collagen type 3 ↑	(Birch et al., 1999; Birch et al., 2016; Gehwolf et al., 2016)
	collagen fibril diameter ↑	(Dunkman et al., 2013; Gehwolf et al., 2016; Goh et al., 2012)
	collagen crosslinking ↑	(Bank et al., 1999)
	collagen degradation ↑	(Birch et al., 2016; Thorpe et al., 2010)
	collagen glycosylation ↑	(Gautieri et al., 2016; Snedeker and Gautieri, 2014)
	ectopic mineralization ↑	(Bi et al., 2007; Rui et al., 2011)
	MMP-1, -2, -9 activity ↑	(Dudhia et al., 2007; Riley et al., 2002)

In summary, these changes in tendon matrix and the associated biomechanical changes underlie the increased risk of tendon injury with age (see also Table 1).

6.3 Mechanical Properties

The mechanical properties of tendons depend on their anatomical location, load, training, and structural characteristics. Material properties and structure of tendons depend mainly on the anisotropic organization of the ECM which improve from birth to maturity and deteriorate with age (Wang et al. 2012). However, published results on the mechanics of aged tendons are partially contradictory; some studies show that aged tendons display a higher tensile strain (Onambele et al. 2006), whereas others report a decreased tensile strain (Kubo et al. 2007), or no impact on most tendon mechanical properties with age (Arampatzis et al. 2007). Similarly discrepant results have been published for tendon stiffness (Flahiff et al. 1995; Gehwolf et al. 2016; Lewis and Shaw 1997; Stenroth et al. 2012; Wu et al. 2015). However, the comparability of these results has to be questioned since different tendons from various species and fresh or embalmed tendon tissues were used for these studies. Furthermore, one must also consider whether altered mechanical properties of healthy-aged tendons are comparable to tendons undergoing

degenerative processes due to other extrinsic or intrinsic factors such as lifestyle, chronic diseases, a specific genetic background, or whether aging alone can account for these changes.

6.4 Tendon Aging and Inflammatory Processes

Cellular aging/senescence has also been linked to a decreased ability to manage inflammatory processes resulting in a chronic low-level inflammation, termed "inflammaging." These changes lead to an increase in the systemic pro-inflammatory status with a concomitantly elevated inflammatory cytokine production/secretion in aged tissues. As a consequence, organs and tissues are more susceptible to frailty and age-related diseases. The correlation of age and the increase in tendon pathologies and injury risk are well recognized. However, the ability of old tendons to cope with inflammation and the contribution of immune-senescence to the pathogenesis of tendon disorders in the elderly are poorly understood.

Dakin and colleagues described an age-related reduced expression of FPR2/ALX and a higher secretion of PGE2 in old tendons. Additionally, in tendons of old horses treated with IL-1ß, the expression of FPR2/ALX and PGE2 secretion, indicating "inflammaging" might be present in aging tendons and old tendons, have a reduced capacity to cope with inflammation. Further, recent studies demonstrated that inflammatory events affecting tendon homeostasis and healing may be responsible for an inappropriate function of the tendon, an increased risk of tendon rupture and re-ruptures (Morita et al. 2016). Along the same lines, healthy-aged mouse Achilles tendons displayed a differential expression of immune response-related genes (Gehwolf et al. 2016). Taken together, a decreased ability to manage inflammatory processes may contribute to the reduced tendon healing capacity in the elderly.

7 Tendon Regeneration: Current Challenges and Future Strategies

Unlike highly regenerative tissues, such as skin or bone, tendons do not functionally regenerate but merely heal by forming a scar tissue with inferior mechanical properties and an increased risk of (re)rupture. Generally, tendon ruptures are believed only to occur upon prior damage to the tissue, unless an acute laceration takes place. This predamage can have a variety of etiologies, such as microdamage due to overload, inflammation, tissue weakening due to systemic diseases such as diabetes or obesity, or as discussed previously due to age-related changes. However, in many cases the exact etiology remains unclear (Abate et al. 2009; Oliva et al. 2011). Many terms have been used to describe these disorders, including tendinitis, tendinosis, and paratenonitis. Commonly, they are described by their symptoms, which usually encompass pain, swelling, hypervascularization, deposition of calcific minerals, and long-term loss of fiber orientation. Likely, these symptoms are the results of a failed healing response (Longo et al. 2009). The role of inflammation in

these events is still a matter of debate; however, at least in the initial phase of disease, involvement of inflammatory cells seems likely (Abate et al. 2009; Dean et al. 2016). In order to circumvent the unclear and probably heterogeneous etiology, the umbrella term "tendinopathy" has been introduced (Maffulli et al. 1998).

The role of tendon stem progenitor cells (TSPC) in the progression of tendinopathy as well as their potential role in regeneration is currently under investigation. As mentioned above, these cells have the potential to differentiate into osteoblasts, chondrocytes, and adipocytes as well as into tendon cell-like cells (Bi et al. 2007; Rui et al. 2010). There is some evidence from in vitro experiments using human and rodent TSPC that cell fate, senescence, and clonogenicity are altered by aging and degeneration, leading to speculations that the TSPC pool is becoming exhausted in terms of size and functional fitness and potentially are the origin of various tendinopathies (Kohler et al. 2013; Rui et al. 2013a). However, to date there are no solid in vivo data available describing the fate of TSPCs during tendinopathies.

7.1 Cellular Mechanisms of Tendon Repair

The tendon repair process follows three distinct phases: (1) An early inflammatory phase, with macrophage invasion and/or proliferation. At this early phase, which lasts about 1 week, macrophages display a proinflammatory M1-polarization, secreting proinflammatory cytokines such as IL-1ß, IL-6 and TNF (Voleti et al. 2012). (2) In the second phase, fibroblasts proliferate within the wound area, a process guided by M2-polarized macrophages. At this stage, also erroneous differentiation of stem cells is potentially initiated (Runesson et al. 2015). (3) In the third phase, the so-called "remodeling phase," the fibroblasts begin to produce, deposit, orient, and crosslink fibrillar collagens. During this process, a disturbance of the fine-tuned balance between M1 and M2 macrophages may lead to improper tendon repair (Sugg et al. 2014). Therefore, guided immunomodulation to prevent unfavorable scar formation may be an interventional strategy to improve tendon healing. One option currently pursued to modulate the inflammatory response is the use of mesenchymal stem cells (see below).

The origin or source of cells involved in tendon repair remains poorly characterized. They potentially originate from blood, surrounding adipose tissue or epitenon sheaths (Dyment et al. 2013; Nourissat et al. 2015). It has also been proposed that TSPCs are activated after tendon injury. In a rat Achilles tenotomy model, an increased number of TSPC positive for the stem cell markers Oct3/4 and nucleostemin are found at early time points after injury. Their distribution becomes more distinct at later time points, with proteoglycan-rich areas containing clusters of more chondrocyte-like cells and containing higher proportions of nucleostemin-positive cells. This could be an indication of a stem cell-related coordination problem in the healing processes, which potentially has a negative impact on the mechanical function after an Achilles mid-tendon rupture (Runesson et al. 2015). In another study making use of a rat model of Achilles tendon injury, perivascular cells

were shown to contribute to tendon repair. These cells with neural crest cell-like characteristics, such as the expression of p75, vimentin, and Sox10, migrate from the vessel to the interstitial space upon injury, where they deposit extracellular matrix (Xu et al. 2015). However, if and to what extent the contribution of these cells during tissue repair is beneficial for tendon quality and their role in tendon development remains elusive.

7.2 Use of Stem Cells for Tendon Treatment

Tissue engineering involves the use of cells, biomaterials, growth factors, enzymatic antagonists, or a combination thereof with the aim of promoting tissue repair. Terminally differentiated cells as well as stem/progenitor cells have been used in tendon tissue engineering approaches. Among these different cell types, stem cells have attracted a great interest in tissue engineering as they can continuously reproduce themselves while maintaining the ability to differentiate into various cell types (Lui 2015). However, several factors need to be considered for choosing the ideal cell type for regenerative approaches: The cells need to be free of ethical concerns, safe in terms of tumor risk, must not cause adverse immunoreactions, must have the potential to functionally replace lost tendon cells, should be obtainable with minimal morbidity and pain for the patient/donor, and should have a high proliferation rate in vitro, in order to obtain a satisfying number of cells in a reasonable time span. Moreover, the cells should not undergo uncontrolled differentiation after implantation. As ectopic osteogenesis was frequently observed, following stem cell implantation to tendons remains a major concern (Harris et al. 2004). Another question related to the debate on the best cell source is whether autologous or allogeneic cells should be used. The main advantage of the latter is faster and more controlled availability, the main concern being their potential immunogenicity (Hilfiker et al. 2011).

Various cell types have been considered to augment tendon repair and have been evaluated in preclinical animal studies over the last two decades. Next to mesenchymal stromal cells of various tissue origin (e.g., bone marrow, adipose tissue), skin fibroblasts, embryonic stem cells, and induced pluripotent stem cells (iPSC) have been evaluated (Docheva et al. 2015; Gaspar et al. 2015; Yin et al. 2010a). In addition, the identification of tendon resident stem/progenitor cells (Bi et al. 2007; Salingcarnboriboon et al. 2003) set the stage for their application as a therapeutic tool to treat diseased tendons. In various small and large animal models, autologous TSCPs were tested for their potential to improve tendon quality. In a rabbit rotator cuff defect model, the authors concluded that the implantation of autologous tenocytes on collagen-based, biodegradable scaffolds resulted in improved rotator cuff tendon healing and remodeling when compared with implantation of the scaffold alone, suggesting their use might be beneficial to treat massive rotator cuff tears (Chen et al. 2007). Similarly, in a rabbit Achilles tendinopathy model, implanted TSPCs were found to have beneficial effects on tendon remodeling,

histological outcomes, collagen content, and tensile strength of the treated tendons (Chen et al. 2011).

Besides dermal fibroblasts, nonbulbar dermal sheath (NBDS) cells isolated from the hair follicle, and adipose-derived stem cells (Usuelli et al. 2017), TSPCs have also been applied in clinical trials to treat diseased tendons. A case series including 20 patients suffering from severe, chronic resistant lateral epicondylitis shows that the application of autologous, in vitro expanded tendon cells obtained from the patient's patella tendon is safe. Also MRI-scores significantly improved over a 12-month period after the injection (Wang et al. 2013). The same patient cohort was followed up to 5 years, showing the initial benefit was persisting (Wang et al. 2015). However, efficacy of this treatment remains to be confirmed in a randomized, controlled trial. A potential limitation of this approach is the relatively high donor site morbidity as punch biopsies taken from tendons such as the patella can cause tendinopathies themselves, even though no adverse events were reported in these studies.

Currently, the therapeutic use of vesicles released from cells, named extracellular vesicles or EVs, has gained tremendous attention (Camussi et al. 2010; Ratajczak et al. 2006). EVs are a heterogeneous population of small vesicles constituted by a circular fragment of membrane containing cytoplasm components which are released by different cell types. Extracellular vesicles derived from MSCs seem to mediate beneficial therapeutic effects in a variety of different diseases. Besides immunomodulation, also angiogenesis and tumor growth is modulated by EVs (Lener et al. 2015). The research on the mechanisms exerted by these vesicles and their potential clinical application is still an emerging field; however, a variety of clinical trials are already on their way, including treatment of dermal wounds, psoriasis, and type I diabetes. As EVs elicit several of the biological actions also observed for adult stem cells, they resemble an attractive option to treat diseased tendons, as they eliminate some of the potential disadvantages when using active, replicating cells that may undergo mal-differentiation or mutation (Tetta et al. 2012).

In summary, the discovery of a population of stem/progenitor cells in tendons has permitted a rapid progress in the study of tendon biology. However, the role of these cells in tendon development and homeostasis remains largely unclear. Also, our understanding of their role in tendon degeneration and aging is far from complete. Therefore, great efforts are required to establish a solid foundation for successfully targeting endogenous TSPCs to improve tendon repair or make use of these cells as a tissue engineering tool.

8 Conclusions

The current options for conservative or surgical treatment of tendon injuries often do not provide satisfactory, long-term outcomes. Therefore, new therapeutic approaches are needed to augment tendon repair, or even allow functional regeneration of tendon tissue. However, although quite some progress has been made in repairing tendon, the clinical impact remains limited. Also, research progress has been hampered by

our incomplete understanding of tendon development, physiology, and healing, mainly due to the absence of marker proteins specific for the tenogenic lineage. Next to the development of sophisticated scaffolds to support tendon repair, strategies employing cell and gene therapy and other biologicals (e.g., growth factors) are actively being pursued. Most likely a combination thereof will be required to achieve scarless healing and full restoration of the biomechanical properties of tendon tissue after injury or disease and rigorous scientific investigations and scrutiny will be required to translate their full therapeutic potential.

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