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**ABOUT COVER**

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## Understanding cellular and molecular mechanisms of pathogenesis of diabetic tendinopathy

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### Abstract

There is accumulating evidence of an increased incidence of tendon disorders in people with diabetes mellitus. Diabetic tendinopathy is an important cause of chronic pain, restricted activity, and even tendon rupture in individuals. Tenocytes and tendon stem/progenitor cells (TSPCs) are the dominant cellular components associated with tendon homeostasis, maintenance, remodeling, and repair. Some previous studies have shown alterations in tenocytes and TSPCs in high glucose or diabetic conditions that might cause structural and functional variations in diabetic tendons and even accelerate the development and progression of diabetic tendinopathy. In this review, the biomechanical properties and histopathological changes in diabetic tendons are described. Then, the cellular and molecular alterations in both tenocytes and TSPCs are summarized, and the underlying mechanisms involved are also analyzed. A better understanding of the underlying cellular and molecular pathogenesis of diabetic tendinopathy would provide new insight for the exploration and development of effective therapeutics.

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**Core Tip:** Tendinopathy is an important cause of chronic pain, restricted activity, and even tendon rupture in individuals with diabetes. Both tenocytes and tendon stem/progenitor cells (TSPCs) play essential roles in tendon maintenance, regeneration, and repair. In this review, we describe the biomechanical properties and histopathological changes in diabetic tendons. Then, we summarize the cellular and molecular alterations and the underlying mechanisms involved in tenocytes and TSPCs that might be associated with the development and progression of diabetic tendinopathy, providing clues for potential effective therapeutics for diabetic tendinopathy.

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## INTRODUCTION

Tendons are dense and highly organized fibrous connective and collagenous tissues that are dominated by type I collagen, connect muscle to bone, and function to efficiently deliver muscle forces during musculoskeletal movement. There is an increased incidence of tendon disorders, which are characterized by impaired tendon structure, functions, and healing capacity in people with diabetes mellitus (DM)<sup>[1-4]</sup>. An epidemiological investigation demonstrated a higher prevalence of tendinopathy in patients with DM than in age- and sex-matched nondiabetic populations<sup>[5]</sup>. Tendinopathy is an important cause of chronic pain, limited range of motion (ROM) of the joints, and even tendon rupture in patients with DM<sup>[6-9]</sup>.

Although some studies have focused on the structure<sup>[10,11]</sup>, composition<sup>[10,12]</sup>, imaging characteristics<sup>[13,14]</sup>, biomechanical properties<sup>[12,15-17]</sup>, and histopathological features<sup>[13,16,18]</sup> of tendons of both clinical patients and animal models of DM, clinical treatments for diabetic tendinopathy are still unsatisfactory due to a lack of understanding of the pathological changes and pathogenic mechanisms of diabetic tendinopathy<sup>[9]</sup>. The majority of practical treatments are based on current limited clinical experience rather than pathogenic changes during the course of diabetic tendinopathy. These findings emphasize the significance of understanding the pathogenic alterations in diabetic tendinopathy to explore effective therapeutic strategies.

In this review, we aim to summarize the current cellular and molecular alterations, and analyze the potential pathogenic mechanism involved in the pathogenesis of diabetic tendinopathy. The changes in biomechanical properties and histopathological features are also presented. A better understanding of the cellular and molecular pathogenesis of diabetic tendinopathy might provide new insight for the exploration and development of effective therapeutics.

## CHANGES IN BIOMECHANICAL PROPERTIES IN DIABETIC TENDINOPATHY

A few studies have focused on the biomechanical properties of tendons in both human and animal models of DM. Significantly impaired biomechanical properties of diabetic Achilles tendons, such as reduced elasticity (Young's modulus), maximum load, stress tensile load, stiffness, and toughness, have been reported<sup>[16,17,19,20]</sup>. Stiffness was also decreased in both intact tendons and healing tendons after injury in diabetic rats<sup>[21]</sup>. Evranos *et al*<sup>[22]</sup> found significantly reduced stiffness of the medial and distal third, but not the proximal third of diabetic tendons. This finding indicated that there might also

be variations in stiffness between different regions of the tendon. However, Petrovic *et al.*<sup>[23]</sup> showed reduced elongation and increased stiffness and hysteresis of Achilles tendons during walking in people with diabetes. This may indicate reduced storage and release of elastic energy from the tendon and a decrease in the energy saving capacity of the diabetic tendon as important factors contributing to the increased metabolic cost of walking in individuals with diabetes. Couppé *et al.*<sup>[24]</sup> reported that regardless of hyperglycemia severity, Achilles tendon material stiffness was greater in diabetic patients than in age-matched healthy controls, but no significant differences in biomechanical properties were observed between diabetic patients with good and poor glycemic control. However, except for the decrease in the elastic modulus, de Oliveira *et al.*<sup>[25,26]</sup> showed increases in specific strain, maximum strain, and energy/tendon areas in diabetic tendons compared with nondiabetic tendons. Bezerra *et al.*<sup>[27]</sup> reported increases in the elastic modulus and maximum tension of diabetic tendons. In addition to the ultimate stress and modulus in diabetic tendons at the material level, the nanoscale modulus of tendon fibrils was also significantly higher and more variable than that of nondiabetic tendons<sup>[28]</sup>. The glycosylated Achilles tendon showed significant increases in maximum load, Young's modulus of elasticity, energy to yield, and toughness<sup>[29]</sup>. Diabetic flexor digitorum longus (FDL) tendons had significantly decreased ROM and a reduced maximum load at failure but increased stiffness<sup>[30]</sup>. Ackerman *et al.*<sup>[31]</sup> also showed reduced yield load and energy to maximum force in diabetic FDL tendons, while stiffness was not altered. Although diabetes did not alter the mechanical properties of uninjured FDL tendons, the maximum force, work to maximum force, and stiffness of diabetic tendons were significantly decreased during injured tendon healing<sup>[32]</sup>. Significantly reduced stiffness at the insertion site was shown in diabetic supraspinatus, Achilles, and patellar tendons, and the modulus at the insertion site of the diabetic Achilles tendon was also decreased, but no significant difference in stiffness or modulus of mid-substance was observed in any tendon<sup>[33]</sup>. The tendon-bone complex of the supraspinatus tendon in diabetic rats also showed decreased mean load-to-failure and stiffness, impairing tendon bone healing<sup>[34]</sup>. The diabetic patellar tendon had significantly decreased stiffness with a reduced Young's modulus<sup>[35]</sup>. However, Thomas *et al.*<sup>[35]</sup> showed that hyperglycemia did not alter mechanics (stiffness and modulus) at either insertion site or mid-substance of the supraspinatus tendon, while some studies showed no significant changes in biomechanical properties between diabetic and nondiabetic tendons<sup>[12,36]</sup>.

Based on these findings, the parameters of some biomechanical properties may be inconsistent among these studies due to different species used for models and tendon sources, *in vivo* and *in vitro* mechanical measurements, various measurement methods and assessment protocols, the duration of DM and hyperglycemia severity, and other accompanying chronic complications associated with diabetes. In addition, biomechanical tests on complete tendon specimens may provide more valuable and accurate data on the biomechanical properties of the specimens than analysis of tendon segments, which might also lead to differences in measurement results. However, although there are few disagreements on the mechanical changes, the majority of studies revealed inferior biomechanical properties of diabetic tendons in human and animal models and during the healing of injured tendons. The main findings are summarized in Table 1. These impaired biomechanical properties may lead to limited ROM of the affected joints and increased susceptibility to tendon injury, reinjury, and even rupture in patients with DM.

## HISTOPATHOLOGICAL ALTERATIONS IN DIABETIC TENDINOPATHY

Compared with healthy tendons, diabetic tendons appear yellowish, fragile, and atrophied<sup>[15,34]</sup>. The thickness of the Achilles tendon in a diabetic rat model was significantly increased<sup>[37]</sup>. Histopathological changes were also observed in diabetic tendons. In diabetic Achilles tendons, some collagen fibers were separated and lost their parallel orientation, with a decrease in fiber diameter and collagen density, and unequal and irregular crimping, loosening, increased waviness, and degeneration of tendon cells and chondrocyte-like cells were observed; otherwise, diabetic tendons also exhibited obvious ruptures in the insertion area, degeneration of tenocytes, collagen fiber microtears, and vascular proliferation<sup>[38]</sup>. The density of fibrocytes and total cellularity, blood vessels, and mast cells were significantly increased in diabetic tendons compared with nondiabetic tendons; immunohistochemical (IHC) staining of diabetic tendons showed an increase in the density of collagen I (Col I), which was associated with disorganized fibers and the expression of vascular endothelial growth

**Table 1 Biomechanical property changes of diabetic tendinopathy**

Species	Tendon(s)	Groups	Duration of DM	Biomechanical properties of diabetic tendons	Ref.
Human	Achilles tendon	CG and DG	An average of 14 yr	Significantly less tendon elongation, higher tendon stiffness and hysteresis, and lower tendon forces in DM group during walking compared with CG.	Petrovic <i>et al</i> <sup>[23]</sup> , 2018
Human	Achilles tendon	CG and DG	An average of 13 yr	No significance in maximum force including max force, stiffness, stress, strain, and modulus between DG and CG, but a trend towards reduced tendon strain in DG; significantly higher tendon modulus in common force in DG than in CG.	Couppé <i>et al</i> <sup>[24]</sup> , 2016
Human	Achilles tendon	CG and DG	Stage V type II diabetes patients	Significantly inferior biomechanical properties of diabetic tendons in DG including decreased elasticity (Young's modulus), maximum load, stiffness, toughness, load at yield point, energy, strain, and elongation at break point, tenacity, and strain at automatic load drop.	Guney <i>et al</i> <sup>[17]</sup> , 2015
Human	Achilles tendon	CG, DG I (with foot ulcer), and DG II (without foot ulcer).	An average of 15 yr in DG I and an average of 6 yr in DG II.	Significantly higher thickness of proximal, medial, and distal third tendon in DG I than in DG II and CG, higher tendon thickness in DG II than in CG but no significance; significantly reduced stiffness of medial and distal third tendon in DG I.	Evranos <i>et al</i> <sup>[22]</sup> , 2015
Male C57Bl/6J mice	FDL tendon	CG (low fat diet) and DM (high fat diet)	High or low-fat diet for 48 wk; at 12, 24, and 48 d post-injury.	Significantly decreased tendon range of motion at 40 and 48 wk in high fat diet group relative to low fat diet group; reduced max load at failure at 48 wk and increased stiffness at 24 wk in high fat diet group.	Studentsova <i>et al</i> <sup>[30]</sup> , 2018
Male C57Bl/6J mice	FDL tendon	CG (low fat diet) and DM (high fat diet)	High or low-fat diet for 12 wk; at 10, 14, 21, and 28 d post-diet initiation.	Significantly lower maximum load, yield load, and energy to maximum force of tendon in DM compared with CG at 28 d; no differences in stiffness between the two groups.	Ackerman <i>et al</i> <sup>[31]</sup> , 2017
Male C57BL/KsJ ( <i>db/db</i> ) mice	Achilles tendon	CG and DG	16 wk of DM	Significantly decreased maximum load, elastic modulus, maximum stress, and stiffness of tendons in DG; no significance in tensile strain.	Boivin <i>et al</i> <sup>[16]</sup> , 2014
<i>db/db</i> Diabetic mice and <i>db/+</i> non-diabetic heterozygous control mice	Supraspinatus, Achilles, and patellar tendons.	CG and DG	60 days for DM	Significantly reduced stiffness at the insertion site of tendons in DG for all three tendons and reduced modulus at the insertion site of Achilles tendons in DG; no significance in stiffness or modulus of mid-substance in any tendon between DG and CG.	Connizzo <i>et al</i> <sup>[33]</sup> , 2014
Male C57BL/6J mice	FDL tendon	CG (low fat diet) and DG (high fat diet)	High or low-fat diet for 12 wk for uninjured tendons; high or low-fat diet for 24 wk for injured tendons, at 7, 14, and 28 d post-injury.	No significance in biomechanical parameters including maximum force, work to maximum force, and stiffness of uninjured FDL tendon at 12 wk; reduced maximum force of uninjured FDL tendon at 24 wk; significantly decreased biomechanical parameters of injured tendons in DG at 28 d.	David <i>et al</i> <sup>[32]</sup> , 2014
Wistar rats	Achilles tendon	CG and DG	4 wk post-induction; 3 wk post-operation.	No significance in ultimate load, ultimate elongation, stiffness, ultimate strength, ultimate strain, elastic modulus, and cross-sectional area.	de Oliveira <i>et al</i> <sup>[34]</sup> , 2019
Wistar rats	Achilles tendon	CG and DG	5 wk post-induction	Significantly increased elastic modulus and maximum tension, reduced transverse area in DG; no significance in maximum strength between DG and CG.	Bezerra <i>et al</i> <sup>[27]</sup> , 2016
SD rats	Achilles and tail tendon	CG, acute DG (1 wk), and chronic DG (10 wk)	10 wk post-induction	No significance in biomechanical properties of Achilles and tail tendons between groups, including maximum force, deformation, stiffness, stress, strain, and Young's modulus.	Volper <i>et al</i> <sup>[12]</sup> , 2015
Wistar rats	Achilles tendon	CG and DG	30 d post-induction; at days 10 post-surgery.	Significantly decreased stress tensile load and Young's modulus of stiffness of tendons in DG than in CG.	Mohsenifar <i>et al</i> <sup>[20]</sup> , 2014
ZDSD and control rats (CD: SD-derived)	Tail tendon	CG and DG	High fat diet for 12 wk	Significantly higher nanoscale modulus at tendon fibrils level in DG and more variable compared with CG; at the fascicle level, no significance in mechanical properties	Gonzalez <i>et al</i> <sup>[28]</sup> , 2014

SD rats	Supraspinatus tendon	Hyperglycemia group and control group	8 wk following hyperglycemia induction	between DG and CG; at the material level, significantly greater ultimate stress and modulus in DG tendon than in CG.	Thomas <i>et al</i> <sup>[35]</sup> , 2014
Lewis rats	Achilles tendon	CG and DG	5 d post-induction	No significance in stiffness and modulus at both the insertion site and mid-substance of tendon between hyperglycemia group and control group.	Lehner <i>et al</i> <sup>[19]</sup> , 2012
Male diabetic GK rats and control Wistar rats	Achilles tendon	CG and DG	1 year of DM; at 14 d post-rupture.	Significantly reduced maximum tensile load of tendon in DG.	Ahmed <i>et al</i> <sup>[21]</sup> , 2012
Wistar rats	Achilles tendon	CG and DG	70 d post-induction	No significance in biomechanical properties as peak load, energy at peak load and stress, except for lower stiffness of intact tendons in DG; lower stiffness of injured tendons in DG compared with the injured tendons in CG.	de Oliveira <i>et al</i> <sup>[26]</sup> , 2012
Wistar rats	Achilles tendon	CG and DG	70 d post-induction	Significantly decreased elastic modulus of tendon in DG; increased specific deformation, deformation at maximum force and energy/tendon area of tendon in DG.	de Oliveira <i>et al</i> <sup>[25]</sup> , 2011
Lewis rats	Patellar tendon	CG and DG	12- and 19-d post-induction	Significantly decreased elastic modulus of tendon in DG; increased specific strain, maximum strain and energy/tendon area of tendon in DG.	Fox <i>et al</i> <sup>[15]</sup> , 2011
Lewis rats	Supraspinatus tendon	CG and DG	1 and 2 wk post-operation	Significantly reduced Young's modulus of tendon in DG at both time points.	Bedi <i>et al</i> <sup>[34]</sup> , 2010
New Zealand rabbits	Achilles tendon	Non-glycated group and glycated group	60 d following glycation	Significantly reduced mean load-to-failure and stiffness of tendon-bone complex in DG at both time points.	Reddy <i>et al</i> <sup>[29]</sup> , 2003

FDL: Flexor digitorum longus; CG: Control group; DG: Diabetes group; DM: Diabetes mellitus; SD: Sprague-Dawley; ZDSD: Zucker diabetic SD; GK: Goto-Kakizaki.

factor (VEGF) and NF- $\kappa$ B, which might play roles in these vascular changes<sup>[37]</sup>. Compared with those of nondiabetic and acute diabetic tendons, total cell density and cell proliferation were increased in chronic diabetic tendons<sup>[12]</sup>. However, Thomas *et al*<sup>[35]</sup> showed that although cell density at the insertion site of hyperglycemic supraspinatus tendons was not altered, nor was cell shape at the insertion and mid-substance, hyperglycemic tendons had a higher cell density at the mid-substance than those of the normal hyperglycemic groups. The increase in cell density may indicate an increase in the metabolic activity of the tendon. There were also increased levels of interleukin (IL) 1- $\beta$  and advanced glycation end-products (AGEs) localized to the insertion and mid-substance of hyperglycemic tendons, indicating increased AGEs and chronic inflammatory responses induced by hyperglycemia. In addition, mild neutrophil infiltration was also observed in diabetic tendons<sup>[16]</sup>. Prolonged exposure to inflammatory conditions may have a negative effect on the biomechanical properties of diabetic tendons. Significant fiber disorganization, as well as increased interfibrillar spaces, were observed in diabetic tendons<sup>[16,17,38,39]</sup>. Focal collagen degeneration<sup>[17]</sup>, uneven glycoprotein deposition<sup>[39]</sup>, lipid deposits<sup>[30]</sup>, and increased nuclear size/rounding<sup>[16]</sup> were observed in diabetic tendons, which was consistent with degenerative tendons. Our previous work<sup>[40]</sup> demonstrated microtears, red blood cells,

small blood vessels, and changes in the rounding of tendon cells surrounding the tear sites in diabetic tendons, and IHC staining of diabetic patellar tendons showed increased expression of osteochondrogenic differentiation markers, including osteopontin (OPN), osteocalcin (OCN), Sox9, and Col II, and reduced expression of tenogenic markers, including Col I and tenomodulin (Tnmd). In addition, Wu *et al.*<sup>[41]</sup> reported increased peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ )-positive, rounded cells residing in diabetic tendons, which were aligned along the collagen fibrils. Phenotypic variations in tendon cells may accelerate the degeneration of diabetic tendons, deteriorating the biomechanical properties of the tendons. Additionally, stenosing flexor tenosynovitis in diabetic patients was characterized by fibrocartilage metaplasia, including the presence of fibrocartilage-like cells, and granulation tissue contained newly formed microvessels, stromal cells, a small number of inflammatory cells, and the extracellular matrix (ECM) that exhibited myxomatous degeneration<sup>[18]</sup>. However, Ahmed *et al.*<sup>[21]</sup> reported that diabetic tendons exhibited slightly smaller transverse areas than normal tendons, but showed no apparent alterations in the structural organization of collagen fibers. Ueda *et al.*<sup>[42]</sup> showed that no significant difference was observed in fiber structure, fiber arrangement, rounding of the nuclei, or regional variations in cellularity between diabetic and nondiabetic Achilles tendons, but IHC staining of diabetic tendons demonstrated markedly increased NADPH oxidase (NOX) 1 expression within tenocytes compared with those of nondiabetic tendons, activating the production of reactive oxygen species (ROS) and resulting in tendon tissue damage.

The repair and regenerative capacity of diabetic tendons is compromised during the healing process after injury. Fibroblast proliferation<sup>[43,44]</sup>, vascularity<sup>[21,44,45]</sup>, and collagen levels<sup>[36,44]</sup> were reduced in injured diabetic tendons during healing. Egemen *et al.*<sup>[43]</sup> showed that although similar collagen deposition and vessel proliferation were observed in both injured diabetic and nondiabetic tendons during healing, lymphocyte infiltration and osteochondroid metaplasia of some tenocytes were observed in the injured diabetic tendons. Diabetic tendons displayed a significant increase in inflammation and significantly decreased fibrosis compared with nondiabetic tendons, resulting in the delayed healing of tenotomized Achilles tendons<sup>[20]</sup>. Furthermore, after rupture, diabetic tendons had reduced reparative activities, as illustrated by a much smaller transverse area, poor structural organization with fewer longitudinally oriented collagen fibers along the functional loading axis, and decreased vascularity than injured nondiabetic tendons. Most fibers were yellowish and arranged irregularly, indicating ruptured Col I structures. IHC staining showed that reduced expression of Col I, Col III, and biglycan (Bgn) and weak VEGF, thymosin  $\beta$  (T $\beta$ )-4, transforming growth factor beta 1 (TGF $\beta$ 1), and insulin-like growth factor-1 (IGF-1) immunoreactivity were observed, and strong cyclooxygenase-2 (COX-2), hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), inducible nitric oxide synthase (iNOS), and IL-1 $\beta$  expression at the injured site and increased matrix metalloproteinase (MMP)-13 staining were observed around blood vessels and cells in the callus in healing diabetic tendons compared with injured nondiabetic tendons<sup>[21,45]</sup>. Additionally, MMP-9 and IL-6 Levels were also increased in the torn tendons of diabetic patients<sup>[46]</sup>. These findings indicate that increased inflammation, reduced ECM deposition, and accelerated degradation are involved in the development and progression of diabetic tendinopathy. After surgical transection associated injury, diabetic tendons induced by a high-fat diet exhibited excessive and prolonged scar tissue formation and fibrotic tendon healing, resulting in compromised biomechanical properties<sup>[31]</sup>. Smaller cellular and fibrous repair tissue were also observed at the injury site in diabetic tendons than in nondiabetic tendons, while the degree of collagen remodeling and fiber alignment in the injured area was reduced in the diabetic tendons, which was consistent with compromised biomechanical properties and was associated with decreased repair tissue size and cellularity<sup>[32]</sup>.

In summary, diabetic tendons exhibit degenerative and injured features, which may be accelerated by diabetic conditions, inflammation, AGEs accumulation, and oxidative stress. The healing process of diabetic tendons after injury is also delayed and compromised, indicating the impaired repair and regenerative abilities of diabetic tendons. The main findings are summarized in [Table 2](#). These histopathological changes in both intact and healing diabetic tendons after injury may account for the reduced biomechanical properties observed, which ultimately lead to increased risk of tendon injury and even rupture.

**Table 2 Histopathological features of diabetic tendinopathy**

Species model	Tendon(s)	Groups	Duration of DM	Histopathological feature of diabetic tendons	Ref.
Human	Achilles tendons	CG and DG	Stage V diabetes patients	Diabetic tendons had mild impairment of collagen organization and focal collagen degeneration.	Guney <i>et al</i> <sup>[17]</sup> , 2015
Human	Stenosing flexor tenosynovitis	CG and DG	DM for an average of 17 yr	SFTS in diabetic patients had fibrocartilage metaplasia including fibrocartilage-like cells, and granulation tissue contained newly formed microvessels stromal cells, a small number of inflammatory cells, and extracellular matrix that showed myxomatous degeneration.	Kameyama <i>et al</i> <sup>[18]</sup> , 2013
Human	Rotator cuff tendon	CG and DG	DM for at least 5 yr	IHC showed increased MMP-9 and IL-6 in the torn tendon of diabetic patients.	Chung <i>et al</i> <sup>[16]</sup> , 2017
Male C57Bl/6J mice	FDL tendons	CG (low fat diet) and DG (high fat diet)	At 40 wk post-induction	Lipid deposits were observed in the mid-substance of high fat diet-induced diabetic tendons.	Studentsova <i>et al</i> <sup>[30]</sup> , 2018
C57BL/6 mice	Achilles tendons	CG and DG	DM for one-year post-induction	Fiber disorganization, uneven glycoprotein deposition, and increased interfibrillar spaces in diabetic tendon.	Wu <i>et al</i> <sup>[39]</sup> , 2017
Male <i>db/db</i> C57BL/KsJ mice and wild type control C57BL/6 mice.	Achilles tendons	CG and DG	DM for 11 wk	Mild neutrophil infiltration, mild disorganization of the collagen fibers, mild increased basophilia of the tenocytes, and mildly increased nuclear size/rounding were observed in diabetic tendon. These pathologic changes are consistent with degenerative tendon.	Boivin <i>et al</i> <sup>[16]</sup> , 2014
C57BL/6J Ob mice and wild-type mice	Achilles tendons	CG and DG (obese group, leptin-deficient)	12 wk	In diabetic Achilles tendon, some collagen fibers separated and lost their parallel orientation, with a decrease in fiber diameter and in density of collagen. Unequal and irregular crimping, loosening, increased waviness, lots of degeneration of tendon cells and chondrocyte-like cells were observed. Otherwise, diabetic tendons also showed obvious ruptures in insertion area, degeneration of tendinocytes, collagen fibers microtears, and vascular proliferation.	Ji <i>et al</i> <sup>[38]</sup> , 2010
Male C57Bl/6J mice	FDL tendon	CG (low fat diet) and DG (high fat diet)	Surgery at 12 wk post-induction; days 7-28 post-repair.	After surgical transection injury, the diabetic tendons induced by high fat diet exhibited excess and prolonged scar tissue formation.	Ackerman <i>et al</i> <sup>[31]</sup> , 2017
Male C57BL/6 mice	FDL tendons	CG (low fat diet) and DG (high fat diet)	Surgery at 12 wk post-induction; days 14 and 28 post-surgery.	Smaller cellular and fibrous repair tissue was observed at the injury site of diabetic tendons relative to non-diabetic tendons. The degree of collagen remodeling and fiber alignment in the injured area was less in the diabetic tendons.	David <i>et al</i> <sup>[32]</sup> , 2014
SD rats	Patellar tendons	CG and DG	At 1, 2, and 4 wk post-induction.	Disordered arrangement of collagen fibers, micro-tears, red blood cells and small blood vessels, and the rounding changed tendon cells surrounding the tear sites were observed in the diabetic tendons. IHC staining of diabetic patellar tendons showed increased expression of osteo-chondrogenic differentiation markers including OPN, OCN, SOX9, and Col II, and reduced expression of tenogenic markers including Col I and TNMD.	Shi <i>et al</i> <sup>[40]</sup> , 2019
SD rats	Achilles tendons	CG and DG	At 6 wk post-induction	No significant difference was observed in fibre structure, fibre arrangement, rounding of the nuclei, and regional variations in cellularity between diabetic and non-diabetic Achilles tendons. Immunohistochemical staining of the diabetic Achilles tendon showed markedly increased NOX1 expression within the tenocytes compared with the non-diabetic tendons.	Ueda <i>et al</i> <sup>[42]</sup> , 2018
SD rats	Achilles tendons	CG and DG	DM for over 1 year post-induction	IHC: Increased PPAR $\gamma$ -positive, rounded cells were found to reside in the diabetic tendons, aligning along the collagen fibrils.	Wu <i>et al</i> <sup>[41]</sup> , 2017
SD rats	Achilles tendons	CG, acute DG and chronic DG	1 wk for acute DM, 10 wk for chronic DM.	Total cell density and Achilles tendon cell proliferation were greater in the chronic diabetic tendons compared with the non-diabetic and acute diabetic tendons.	Volper <i>et al</i> <sup>[12]</sup> , 2015

SD rats	Supraspinatus tendons	CG and hyperglycemia group	8 wk following hyperglycemia induction	Cell shape at the insertion site and mid-substance of the hyperglycemic tendon did not alter, nor did cell density at the insertion site; however, the hyperglycemic tendon had a greater cell density at the mid-substance of the tendon compared to the non-hyperglycemic group. Immunohistochemistry staining of the tendon demonstrated significantly increased IL1-β and AGE staining localized to the insertion and mid-substance of the hyperglycemic tendon.	Thomas <i>et al</i> <sup>[45]</sup> , 2014
Wistar rats	Achilles tendons	CG and DG	DM for 24 d post-induction	Tendon thickness, the density of fibrocytes and total cellularity, blood vessels and mast cells were significantly increased in diabetic tendons compared with non-diabetic tendons. IHC showed increased density of type I collagen, associated with the disorganization of the fibers in the diabetic tendons, and expression of VEGF and NF-κB.	de Oliveira <i>et al</i> <sup>[37]</sup> , 2013
Male diabetic GK rats and Wistar control rats	Achilles tendons	CG and DG	DM for 1 yr	Diabetic tendons exhibited slightly lesser transverse area, but showed no apparent alteration in structural organization of collagen fibers.	Ahmed <i>et al</i> <sup>[21]</sup> , 2012
Male white rats	Achilles tendons	CG and DG	Surgery at two weeks post-induction; four weeks post-surgery.	Fibroblasts, capillary and collagen were reduced during the healing process of diabetic tendons after transection injury.	Sananta <i>et al</i> <sup>[44]</sup> , 2019
Wistar rats	Achilles tendons	CG and DG	Surgery at one-week post-induction; 21 d post-surgery.	The fibroblasts in injured diabetic tendons were significantly increased. IHC of the injured diabetic Achilles tendons showed nearly no Col I expression in comparison with injured non-diabetic tendons.	de Oliveira <i>et al</i> <sup>[36]</sup> , 2019
Wistar rats	Achilles tendons	CG (low fat diet) and DG (high fat diet)	Surgery at 30 d post-induction; days 5, 10 and 15 post-surgery.	The diabetic tendons displayed a significant increase in inflammation and a significant decrease in fibrosis compared to the non-diabetic tendons.	Mohsenifar <i>et al</i> <sup>[20]</sup> , 2014
Male GK rats and control Wistar rats	Achilles tendons	CG and DG	DM for one year; two weeks post-surgery.	After rupture, the diabetic tendons had a reduced reparative activity with decreased transverse area, poor structural organization and decreased vascularity. IHC of injured diabetic tendons showed weaker VEGF, Tβ-4, TGF-β1 and IGF-1 immunoreactivity and fewer positively stained tenocytes, but strong COX-2, HIF-1α, iNOS and IL-1β at the injured site compared with injured non-diabetic tendons.	Ahmed <i>et al</i> <sup>[45]</sup> , 2014
Male GK rats and control Wistar rats	Achilles tendons	CG and DG	DM for one year; two weeks post-surgery.	After rupture, the diabetic tendons had a reduced reparative activity illustrated by a much smaller transverse area, poor structural organization with fewer longitudinally oriented collagen fibers along the functional loading axis, and decreased vascularity, compared with injured non-diabetic tendons. Most fibers were yellowish and arranged irregularly, denoting ruptured Col I structures. IHC showed that less Col I, Collagen III and biglycan were observed, but increased MMP-13 around blood vessels and cells in the callus in the healing diabetic tendons.	Ahmed <i>et al</i> <sup>[21]</sup> , 2012
Wistar Albino rats	Achilles tendons	CG and DG	Surgery at 3 d post-induction; 2-, 4- and 6-wk post-surgery.	Although similar collagen deposition and vessels proliferation were observed in both injured diabetic and non-diabetic tendons during healing, the injured diabetic tendons exhibited a significantly smaller amount of fibroblast proliferation and lymphocyte infiltration, and osteochondroid metaplasia of some tenocytes.	Egemen <i>et al</i> <sup>[43]</sup> , 2012

SFTS: Stenosing flexor tenosynovitis; IHC: Immunohistochemical; FDL: Flexor digitorum longus; CG: Control group; DG: Diabetes group; DM: Diabetes mellitus; OPN: Osteopontin; OCN: Osteocalcin; Col II: Collagen II; Col I: Collagen I; TNMD: Tenomodulin; PPARγ: Peroxisome proliferator-activated receptor γ; SD: Sprague-Dawley; AGE: Advanced glycation end-products; VEGF: Vascular endothelial growth factor; GK: Goto-Kakizaki; COX-2: Cyclooxygenase-2; HIF-1α: Hypoxia-inducible factor-1α; iNOS: Inducible nitric oxide synthase; TGF-β: Transforming growth factor beta; IGF: Insulin-like growth factor; MMP: Matrix metalloproteinase; IL-6: Interleukin-6; Tβ: Thymosin β; NOX: NADPH oxidase.

### CYTOLOGICAL AND MOLECULAR ALTERATIONS IN TENOCYTES

Tenocytes are the primary cellular components of tendons and are important for maintaining tendon tissue function, mechanics, homeostasis, and capacity to remodel the ECM by generating collagen and repairing proteins and matrix proteoglycans (PGs)<sup>[47-49]</sup>. In recent years, some studies have focused on the alterations in tenocytes in diabetic tendinopathy. The main findings are summarized in **Table 3**. Some

Table 3 Cellular and molecular alterations in tenocytes and tendon stem/progenitor cells

Cell type	Cell source	Study type	Groups	Main results	Ref.
Tendon-derived fibroblasts	SD rat, Achilles tendon	<i>In vitro</i>	NG (5.5 mmol/L) and HG (25 mmol/L) with different concentrations of AGEs (0, 50, 100, and 200 µg/mL).	HG had no effect on cell proliferation and expressions of genes associated with extracellular matrix remodeling. AGEs impaired proliferative capacity, ATP production, and electron transport chain efficiency, coupled with alterations in mitochondrial DNA content and expression of genes associated with extracellular matrix remodeling, mitochondrial energy metabolism, and apoptosis. While HG condition did impact some mitochondrial parameters, AGEs appear to be the primary insult and may be responsible for the development of the diabetic tendon phenotype.	Patel <i>et al</i> <sup>[51]</sup> , 2019
Tenocytes	SD rat, Achilles tendon	<i>In vitro</i> and <i>in vivo</i>	CG (12 mmol/L) and HG (33 mmol/L)	Significantly higher gene expressions of <i>NOX1</i> , <i>NOX4</i> , <i>MMP-2</i> , <i>TIMP-1</i> , <i>TIMP-2</i> , <i>IL-6</i> , <i>Col III</i> and ROS accumulation but lower cell proliferation and type I collagen expression in HG than those in CG.	Ueda <i>et al</i> <sup>[42]</sup> , 2018
Tenocytes	SD rat, Achilles tendon	<i>In vitro</i>	LG (5.5 mmol/L) and HG (25 mmol/L)	High glucose-treated tenocytes expressed higher levels of the adipogenic transcription factors PPARγ and C/EBPs. Increased adipogenic trans-differentiation and decreased cell migration induced by high glucose.	Wu <i>et al</i> <sup>[41]</sup> , 2017
Tenocytes	SD rat, Achilles tendon	<i>In vitro</i>	LG (5.5 mmol/L) and HG (25 mmol/L)	No significant effect on cell growth and apoptosis between LG and HG. Increased glucose uptake and consumption in HG condition. Significantly decreased expression of tendon-related genes, including <i>Egr1</i> , <i>Mkx</i> , <i>TGF-β1</i> , <i>Col1a2</i> , and <i>Bgn</i> , in HG culture.	Wu, <i>et al</i> <sup>[39]</sup> , 2017
Tenocytes	Human, hamstring tendon	<i>In vitro</i>	LG (5 mmol/L) and HG (17.5 mmol/L)	Apoptosis level of tenocytes was 1.5 times greater in peroxide-treated cells cultured in HG compared with untreated controls, while apoptosis level of tenocytes was not increased in peroxide-treated cells cultured in low glucose. Peroxide-treated tenocytes cultured in low glucose expressed higher RNA levels of <i>col1a1</i> and <i>col1a2</i> .	Poulsen, <i>et al</i> <sup>[57]</sup> , 2014
Tenocytes	SD rat, Achilles tendon.	<i>In vitro</i>	LG (6 mmol/L) and HG (12 mmol/L and 25 mmol/L)	The glucose concentration did not affect tendon cell proliferation. The mRNA expression of <i>MMP-9</i> and <i>MMP-13</i> was up-regulated by treatment with 25 mmol/L glucose, whereas the mRNA expression of type I and III collagen was not affected. 25 mmol/L glucose increased the enzymatic activity of <i>MMP-9</i> .	Tsai <i>et al</i> <sup>[50]</sup> , 2013
Tenocytes	Porcine, patellar tendon.	<i>In vitro</i>	LG (5.5 mmol/L) and HG (25 mmol/L)	Exposure to HG or AGEs did not affect cell viability. Significantly decreased PG levels in tendons exposed to HG. Relative mRNA levels of biglycan and versican were unchanged in HG. Levels of fibromodulin were modestly increased, whereas mRNA for decorin and lumican were significantly decreased. High glucose media decreased PG production by tenocytes whereas AGE-modified type I collagen and free radical scavengers had no effects. High glucose conditions increase TGFβ1 levels in tenocyte.	Burner <i>et al</i> <sup>[52]</sup> , 2012
Tenocytes	Porcine, patellar tendon	<i>In vitro</i>	NG (5.5 mmol/L) and HG (25 mmol/L)	Significantly higher Tgase activity in tenocytes incubated in HG. CML-Collagen stimulated Tgase activity in tenocytes in both normal and high glucose media but did not induce markers of apoptosis or alter cell viability. Antioxidants reduced the effect of CML-Collagen on tenocytes Tgase activity.	Rosenthal <i>et al</i> <sup>[58]</sup> , 2009
TDSCs	Human, patellar tendon, rotator cuff and hamstring tendons.	<i>In vitro</i>	LG (5.5 mmol/L) and HG (11.1 mmol/L)	HG stimulated inflammation and weakened pro-resolving inflammation response in TDSCs.	Kwan <i>et al</i> <sup>[57]</sup> , 2020
TDSCs	SD rats, Achilles tendon	<i>In vitro</i> and <i>in vivo</i>	CG and AGEs-treated group	AGEs decreased the cell viability, induced apoptosis and senescence of TDSCs, exacerbated osteogenic differentiation of TDSCs and led to more ectopic calcification in Achilles tendon.	Xu <i>et al</i> <sup>[53]</sup> , 2020
TPCs	Horses, superficial digital flexor tendons	<i>In vitro</i>	CG and insulin-treated group (insulin concentrations: 0, 0.07, 0.7 nmol/L)	Insulin increased proliferation and osteogenic differentiation of TPCs <i>in vitro</i> , with the increased ALP activity and elevated expression of osteogenic genes including <i>Runx2</i> , <i>ALP</i> and <i>osteonection</i> .	Durgam <i>et al</i> <sup>[54]</sup> , 2019
TDSCs	SD rats, patellar tendon	<i>In vitro</i>	Non-diabetic TDSCs and diabetic TDSCs	Significantly decreased colony-forming ability, cell proliferation and tenogenic differentiation ability and increased osteogenic and chondrogenic differentiation ability were demonstrated in diabetic TDSCs.	Shi <i>et al</i> <sup>[40]</sup> , 2019

TDSCs	SD rats, patellar tendon	<i>In vitro</i>	LG (5.5 mmol/L) and HG (15 mmol/L, 25 mmol/L)	High glucose could inhibit proliferation, induce cell apoptosis and suppress the tendon-related markers expression of TDSCs.	Lin <i>et al</i> <sup>[52]</sup> , 2017
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SD: Sprague-Dawley; NG: Normal glucose; LG: Low glucose; HG: High glucose; AGEs: Advanced glycation end-products; CG: Control glucose; ROS: Reactive oxygen species; PPAR $\gamma$ : Peroxisome proliferator-activated receptor  $\gamma$ ; Egr1: Early growth response factor 1; Mx: Mohawk; TGF- $\beta$ 1: Transforming growth factor beta 1; Bgn: Biglycan; PG: Proteoglycans; Tgase: Transglutaminase; CML: Carboxymethyl-lysine; TDSCs: Tendon derived stem cells; ALP: Alkaline phosphatase; Runx2: Runt-related transcription factor 2; NOX: NADPH oxidase; TPCs: Tendon progenitor cells; MMP: Matrix metalloproteinase; IL-6: Interleukin-6; TIMP: Tissue inhibitor of metalloproteinases; Col III: Collagen III.

researchers have shown that the proliferation of tenocytes is significantly decreased<sup>[42]</sup> and that cell migration, a crucial tenocyte function, is retarded under high glucose conditions<sup>[41]</sup>. Therefore, hyperglycemia might inhibit the ability of cells to repair damaged or degenerated tendons. In contrast, it was reported that tenocyte proliferation<sup>[50,51]</sup>, viability<sup>[52]</sup>, and apoptosis<sup>[39]</sup> were not affected by high glucose, and that tenocytes had increased glucose uptake and consumption in high glucose conditions in which insulin further elevated glucose consumption but did not affect either glucose uptake or expression of glucose transporter (Glut) 4, an insulin-sensitive glucose transporter<sup>[39]</sup>. This indicates that tenocytes do not rely on Glut 4 for glucose uptake. However, Wu *et al*<sup>[39]</sup> demonstrated that the expression of Glut 1 was approximately 15 thousand times higher than that of Glut 4, suggesting that tenocytes mainly relied on Glut 1, which is the transporter responsible for basal glucose uptake, rather than Glut 4. Therefore, due to the excessive cellular glucose overload and pronounced toxic side effects of glycolysis and oxidative phosphorylation<sup>[53]</sup>, tenocytes may suffer from adverse effects associated with hyperglycemia.

The expression of tendon-related genes and matrix metabolism in tenocytes are essential for maintaining homeostasis and the physiological functions of tendons. PGs are significant components and regulators of tendon structure and play key roles in tendon biomechanics and function<sup>[52,54,55]</sup>. However, Burner *et al*<sup>[52]</sup> reported that high glucose conditions but not N-carboxymethyl-lysine (CML)-collagen, a receptor for AGEs (RAGE)-activating AGE-modified collagen, reduced PG levels in both tendons and primary tenocytes with variations in mRNA expression of PG protein backbones, including unchanged levels of Bgn and versican, increased fibromodulin, and decreased decorin and lumican, and these effects were not reversed by inhibiting free radicals. In diabetic rat models, the transcription of tendon-related genes, such as *Col1a1*, *Col3a1*, *scleraxis (Scx)*, and *Tnmd*, were lower in acute and chronic diabetic tendons than in nondiabetic tendons<sup>[56]</sup>. In addition, the mRNA expression of Col I in tenocytes incubated in high glucose conditions was significantly lower than that under control glucose conditions, and the mRNA expression of Col III was significantly higher in high glucose conditions<sup>[42]</sup>. In contrast, Tsai *et al*<sup>[50]</sup> reported that the mRNA expression of Col I and Col III was not altered in tenocytes treated with high glucose. Such inconsistent results may be due to the different glucose concentrations used. However, Wu *et al*<sup>[39]</sup> showed that the expression of tendon-related genes, including *mohawk (Mx)*, *early growth response factor 1 (Egr1)*, *TGF $\beta$ 1*, *Col1a2*, and *Bgn*, was downregulated in high glucose conditions at day 14; considering that

hyperinsulinemia occurs in the early phase of type 2 diabetes, the expression of *Egr1*, *TGFβ1*, and *Bgn* was also suppressed by insulin under high glucose conditions at day 7, and the expression of *Scx* and *Col1a1* was suppressed by insulin at day 14. The downregulation of *Mxk*, *Egr1*, and *Scx*, which are crucial transcription factors associated with tendon development and repair, may alter homeostasis and impair the maintenance and remodeling of tendons by regulating the expression of downstream tendon-related genes and matrix molecule genes, which might be involved in the pathogenic mechanism of diabetic tendinopathy. Burner *et al*<sup>[52]</sup> detected increased *TGFβ1* levels in tenocytes under high glucose conditions. *TGFβ1* has important and varied effects on the metabolism of ECM components in tendons and tenocytes. *TGFβ* is necessary for tendon development and may participate in tendon healing, but high *TGFβ* levels may result in scar formation and tenocyte death<sup>[49]</sup>. These results indicate that both diabetes and insulin may result in rapid and large changes in the expression of tendon-related genes and transcription factors that are vital for ECM remodeling, maintenance, and maturation. Alterations in either PG levels or Col I and III affect the formation of collagen fibrils and the ECM, which globally impairs tendon structure, biomechanics, and tendon repair and may contribute to the pathogenesis and progression of diabetic tendinopathy.

Furthermore, Wu *et al*<sup>[41]</sup> also demonstrated that increased rounded, PPARγ-positive cells were observed in diabetic tendons, and high glucose conditions increased the adipogenic transdifferentiation potential of tenocytes, with elevated levels of the adipogenic transcription factors PPARγ, C/EBPα, and C/EBPβ in tenocytes *in vitro*. This finding may indicate a fibroblast-to-adipocyte phenotypic transition that might generate lipid deposits in diabetic tendons. The adipogenic transdifferentiation of tenocytes under high glucose conditions could cause histological, organizational, and structural changes and further impair biomechanical properties and may also be involved in the pathologic mechanism of diabetic tendinopathy.

In addition, there was a significant increase in ROS accumulation and mRNA expression of *NOX1*, *NOX4*, and *IL-6* in tenocytes treated with high glucose *in vitro*<sup>[42]</sup>. Similarly, an *in vivo* study showed that *NOX1* and *IL-6* were also highly expressed in the tenocytes in diabetic rats<sup>[42]</sup>. These results indicate that high glucose conditions induce oxidative stress and stimulate subsequent inflammatory processes within diabetic tendons. Moreover, an *in vitro* study by Poulsen *et al*<sup>[57]</sup> demonstrated that the level of apoptosis was significantly increased in primary human tenocytes exposed to oxidative stress, and apoptosis was markedly elevated in oxidative stress-treated tenocytes cultured in high glucose but not in low glucose conditions, while compared with untreated controls, oxidative stress-treated tenocytes cultured in low glucose expressed higher levels of *Col1a1* and *Col1a2*, the gene products of which form Col I. These results suggest that a level of oxidative stress that is normally anabolic in low glucose conditions may be pathological and promotes apoptosis of tenocytes in high glucose conditions. In addition, extracellular glucose has a profound effect on the cellular response of tenocytes to oxidative stress and is a critical determinant of tenocyte fate following oxidative stress exposure.

Although CML-Collagen did not induce apoptosis or alter the viability of tenocytes, CML-Collagen stimulated transglutaminase (Tgase) activity, catalyzing the formation of highly protease-resistant intra- and inter-molecular crosslinks and mediating modifications that altered the structure and function of the ECM and contributed to tissue fibrosis by interacting with substrates, including many key ECM proteins, such as collagen, fibronectin, OPN, and microfibrillar proteins, in primary porcine tenocytes in both normal and high glucose conditions<sup>[58]</sup>. In addition, high glucose, insulin, and ROS also significantly increased Tgase activity<sup>[58]</sup>. Tgase can generate highly protease-resistant crosslinks that alter the structure and function of affected extracellular matrices, impairing the remodeling and repair abilities of tendons and reducing the capacity of tendons to respond to injury. Tgase may also contribute to the excessive thickness, stiffness, and calcification in diabetic tendons. Additionally, *Col1a1* mRNA transcript counts were significantly reduced and *Col3a1* mRNA transcript counts and total ROS/superoxide production were markedly increased in tenocytes treated with AGEs<sup>[51]</sup>.

The balance of ECM generation and degradation is regulated by MMPs and their inhibition by tissue inhibitor of metalloproteinases (TIMPs)<sup>[59,60]</sup>. Altered levels of various types of MMPs have been detected in chronic tendon disorders and tendon healing<sup>[61-64]</sup>. Tsai *et al*<sup>[50]</sup> showed that the mRNA expression of *MMP-9* and *MMP-13*, as well as the enzymatic activity of *MMP-9*, was upregulated in tenocytes treated with high glucose. In addition, Ueda *et al*<sup>[42]</sup> reported that high glucose increased the mRNA expression of *MMP-2*, as well as the expression of *TIMP-1* and *TIMP-2*. Patel *et al*<sup>[56]</sup> showed decreased transcription of *MMP-2* and *TIMP-1* in acute and chronic diabetic

rat tendons. However, the MMP-9 transcript counts were significantly reduced in tenocytes treated with AGEs, but the expression of MMP-2 was increased<sup>[51]</sup>. MMPs are a family of ECM-degrading enzymes that are inhibited by MMP inhibitors called TIMPs. An imbalance between MMPs and TIMPs could result in excessive degradation of tendon ECM. Therefore, increased ECM degradation due to enhanced MMP expression, together with decreased ECM generation by tenocytes in high glucose and diabetic conditions, may weaken the mechanical properties of tendons and accelerate the development and progression of diabetic tendinopathy, predisposing individuals to tendon injury and rupture.

## CYTOLOGICAL AND MOLECULAR ALTERATIONS IN TENDON STEM/PROGENITOR CELLS

In addition to tenocytes, which are traditionally considered to be the only cellular component in normal tendon tissues, a small population of tendon stem/progenitor cells (TSPCs) has also been identified in tendons from mice<sup>[65]</sup>, rats<sup>[66]</sup>, rabbits<sup>[67]</sup>, turkeys<sup>[68]</sup>, pigs<sup>[69]</sup>, fetal cattles<sup>[70]</sup>, and human<sup>[65,71]</sup>. These TSPCs possess stem cell characteristics including clonogenicity, proliferation, self-renewal, and multidifferentiation potential, and play significant roles in tendon repair, regeneration, and the maintenance of tendon homeostasis<sup>[72-76]</sup>. However, the characteristics and fate of TSPCs are altered under some pathological conditions, which might be involved in the pathogenesis of tendinopathy<sup>[40,77-80]</sup>. Some studies have focused on the alterations in TSPCs in diabetic tendinopathy. The main findings are summarized in [Table 3](#).

### **Specific cluster differentiation markers**

TSPCs derived from normal tendons are positive for Sca-1<sup>[65]</sup>, Stro-1<sup>[65]</sup>, cluster differentiation (CD) 44<sup>[40,65,66,68,70,71]</sup>, CD90<sup>[40,65,66,68,69,71]</sup>, CD105<sup>[68,69,71]</sup>, and CD146<sup>[65,71]</sup>. In addition, TSPCs derived from diabetic rat tendons are also positive for CD44 and CD90. However, the expression of CD44 was markedly reduced in diabetic TSPCs compared with healthy TSPCs<sup>[40]</sup>. CD44, a cell surface glycoprotein, participates in cell growth, survival, differentiation, and motility processes<sup>[81]</sup>. Thus, the reduction in CD44 expression in diabetic TSPCs might be related to the altered cell growth, survival, multidifferentiation potential, and motility and result in a decrease in the self-regeneration and self-repair abilities of TSPCs in diabetic tendinopathy.

### **Cell clonogenicity and proliferation**

The colony-forming ability of TSPCs from diabetic rat tendons was significantly decreased compared with that of TSPCs from healthy rat tendons<sup>[40]</sup>. Hyperglycemia is a typical clinical manifestation of DM. *In vitro*, high glucose could inhibit the proliferation of TSPCs from rat patellar tendons<sup>[82]</sup>. Furthermore, significantly decreased proliferation capacities of TSPCs isolated from DM rat tendons were demonstrated compared with those of TSPCs from non-DM rat tendons<sup>[40]</sup>. The accumulation of AGEs, which are oxidative derivatives resulting from diabetic hyperglycemia, is a significant feature of DM-related changes in tendinopathy<sup>[1,3]</sup>. Xu *et al*<sup>[83]</sup> showed that treatment with high doses of AGEs resulted in reduced proliferation of TSPCs, and pretreatment with pioglitazone significantly attenuated this effect. However, Durgam *et al*<sup>[84]</sup> reported that systemic hyperinsulinemia secondary to impaired insulin sensitivity could occur during early DM and that insulin could significantly increase TSPC proliferation *in vitro*.

### **Cell viability, apoptosis, and senescence**

Cell apoptosis was induced when TSPCs were cultured in a high glucose environment *in vitro*<sup>[82]</sup>. Additionally, Xu *et al*<sup>[83]</sup> reported that AGEs could decrease viability and induce apoptosis of TSPCs and significantly increase the expression of cleaved caspase-3 (C-Cas3) and cleaved caspase-9 (C-Cas9), which are key factors in apoptosis execution. These results indicate that apoptosis of TSPCs may occur in diabetic tendinopathy, and the TSPC pool may become exhausted during the progression of diabetic tendinopathy. TSPCs treated with AGEs exhibited increased expression of P53 and P21, and there was an increase in the percentage of SA-β-Gal-positive TSPCs, indicating that AGEs induced cell senescence in TSPCs<sup>[83]</sup>. However, the function of TSPCs is impaired or lost during senescence<sup>[80]</sup>. Thus, AGE-induced senescence of TSPCs may play an important role in diabetic tendinopathy. The proliferative capacity and viability of TSPCs play important roles in maintaining the physiological function

of tendons. The increased apoptosis and senescence and decreased viability, together with the aforementioned reduced colony-formation and proliferative abilities, may reduce the TSPC pool and result in cellular deficits associated with tendon repair in diabetic tendinopathy.

### **Inflammation and the pro-resolving response**

There is accumulating evidence that inflammatory cells are present, and chronic inflammation is a feature of tendinopathy<sup>[85,86]</sup>, especially diabetic tendinopathy<sup>[3]</sup>. Kwan *et al*<sup>[87]</sup> showed that the mRNA expression of COX2 was increased in both healthy and tendinopathic TSPCs under high glucose conditions, while the upregulation of FPR1, ChemR23, and ALOX15 mRNA was significantly weakened in tendinopathic TSPCs upon IL-1 $\beta$  stimulation compared with that of healthy TSPCs, and the upregulation of ALOX15 mRNA was also weakened in IL-1 $\beta$  stimulated healthy TSPCs after preincubation in a high glucose environment. These results suggest that high glucose conditions may stimulate inflammation in tendinopathy and weaken the ability of pro-resolving response in TSPCs. However, a weakened pro-resolving response may lead to persistent chronic inflammation and prolonged exposure of tendon tissues to nonselective digestive enzymes associated with inflammation, resulting in excessive injury and degenerative changes in diabetic tendons.

### **Cell differentiation**

Our previous study found that high glucose could suppress the expression of tendon-related markers in TSPCs *in vitro*<sup>[82]</sup>. The inhibition of tendon-related marker expression in TSPCs suggests impaired or suppressed tenogenic differentiation abilities of TSPCs in diabetic tendons. Furthermore, our previous study<sup>[40]</sup> also demonstrated that the osteogenic and chondrogenic differentiation abilities of diabetic TSPCs (dTSPCs) were significantly increased compared with those of healthy TSPCs (hTSPCs), while the tenogenic differentiation ability of dTSPCs was significantly decreased. In addition, the expression of osteochondrogenic markers such as bone morphogenetic protein 2 (BMP2), alkaline phosphatase (ALP), OPN, OCN, Col II, and Sox9 was also significantly increased, while the expression of the tenogenic markers Col I and Tnmd was decreased in dTSPCs. Xu *et al*<sup>[83]</sup> showed that AGEs exacerbated the osteogenic differentiation of TSPCs. Moreover, insulin could also increase osteogenic differentiation of TSPCs *in vitro*, with increased mineralized nodules, increased expression of osteogenic genes *runt-related transcription factor 2* (*Runx2*), *ALP*, and *osteonectin* (*OSN*), and increased ALP bioactivity<sup>[84]</sup>. Taken together, these results suggest that the tenogenic differentiation ability of TSPCs is inhibited and that the osteogenic and chondrogenic differentiation abilities of TSPCs are increased in diabetic tendons. In 2011, Rui *et al*<sup>[77]</sup> first proposed the erroneous differentiation theory of TSPCs and expounded the potential roles in the pathogenesis of chronic tendinopathy. Under the pathological condition of DM, inhibited tenogenic differentiation and increased osteogenic and chondrogenic differentiation, namely, the altered fate of TSPCs, are likely to affect tendon repair and facilitate the development and progression of diabetic tendinopathy. However, the cellular and molecular mechanisms of erroneous differentiation of TSPCs in diabetic tendinopathy are unclear and still need to be further illuminated.

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## **MECHANISMS INVOLVED IN DIABETIC TENDINOPATHY**

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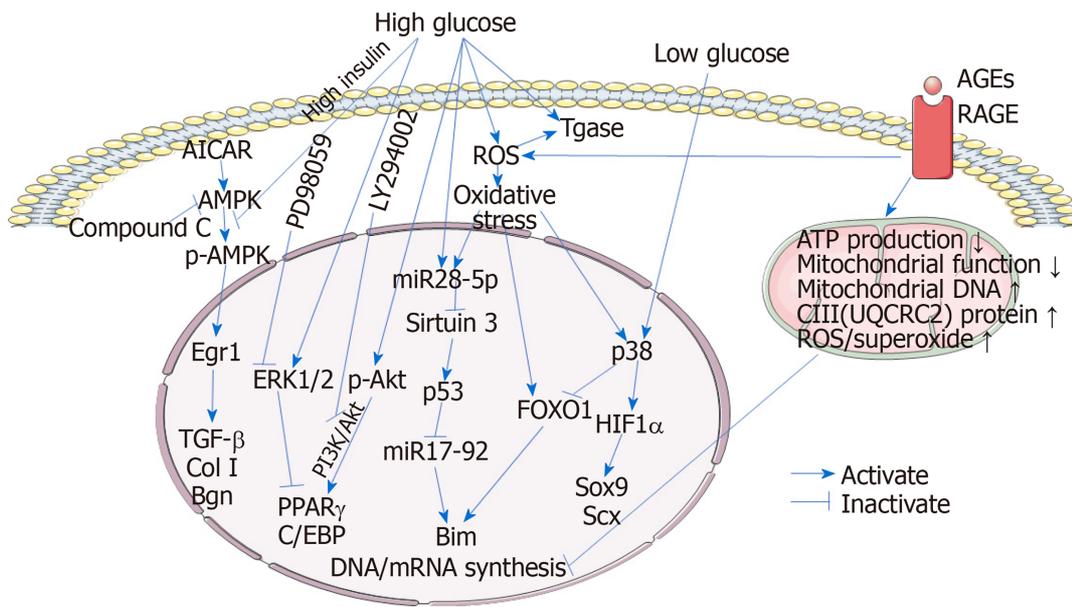
The occurrence of diabetic tendinopathy is a complicated process that is also affected by various factors, including a high glucose environment, inflammation, cytokines, hormones, enzymes, AGEs, oxidative stress, and mechanical stretch. Although epigenetic, cellular, and molecular alterations have been observed and detected in diabetic tendinopathy, the underlying mechanisms have yet to be uncovered. Based on recent progress in the cellular and molecular alterations and pathways involved in diabetic tendinopathy, the mechanisms are summarized and listed below.

Glucose consumption in cells exposed to high glucose conditions tends to be increased and thus inactivates AMPK signal<sup>[88,89]</sup>. Wu *et al*<sup>[39]</sup> demonstrated that glucose uptake and consumption by tenocytes were markedly increased under high glucose conditions, and insulin further increased the glucose consumption of tenocytes under high glucose conditions, although the expression of glucose transporters was unaffected. Additionally, the expression of p-AMPK $\alpha$ , which is a transcriptional regulator, was dramatically downregulated in high glucose conditions, and insulin

also further reduced p-AMPK $\alpha$  expression in the high glucose condition to some extent, indicating that increased glucose consumption in tenocytes in high glucose conditions resulted in inactivation of AMPK signaling. In addition, the mRNA expression of Egr1, a crucial transcription factor associated with tendon development and repair, was also significantly decreased in tenocytes under high glucose conditions. Furthermore, inhibiting AMPK signaling in tenocytes with the AMPK inhibitor compound C under low glucose conditions, markedly suppressed the protein expression of p-AMPK $\alpha$  and the mRNA expression of Egr1. Knockdown of Egr1 with siRNA significantly suppressed the expression of downstream target genes, including Col1a1, Col1a2, TGF $\beta$ 1, and Bgn, and the expression of the transcription factors Scx and Mxk, suggesting that knockdown of Egr1 downregulated tendon-related gene expression. However, activation of AMPK with AICAR, an AMPK activator, under high glucose conditions could increase Egr1 expression and alleviate these changes. These results indicate that the expression of tendon-related genes in tenocytes under high glucose conditions was downregulated by inactivation of the AMPK/Egr1 signaling pathway. Previous studies have shown that knockout of Egr1 in mice attenuates tendon mechanical strength, increases interfibrillar spaces, and suppresses the tendon-related gene expression<sup>[90,91]</sup>. Thus, high glucose alters tendon homeostasis through downregulation of the AMPK/Egr1 signaling pathway and the expression of downstream tendon-related genes in tenocytes, which may be involved in the pathological mechanism of diabetic tendinopathy. In addition, Wu *et al*<sup>[41]</sup> also showed that high glucose elevated the mRNA expression of the adipogenic transcription factors PPAR $\gamma$ , C/EBP $\alpha$ , and C/EBP $\beta$  in tenocytes and augmented the adipogenic transdifferentiation potential of tenocytes. The protein expression of p-Akt was significantly increased, while the protein expression of p-ERK1/2 was slightly increased in tenocytes under high glucose conditions. Inhibition of PI3K/Akt signaling with the PI3K inhibitor LY294002 significantly suppressed the expression of the downstream adipogenic genes PPAR $\gamma$ , C/EBP $\alpha$ , and C/EBP $\beta$ . Inhibition of ERK1/2 signaling with the inhibitor PD98059 markedly upregulated the mRNA expression of PPAR $\gamma$  but downregulated the mRNA expression of C/EBP $\alpha$ . These results indicated that activation of the PI3K/Akt pathway may play an essential role in maintaining adipogenic factor expression and promoting fibroblast-to-adipocyte phenotypic changes, whereas the activation of ERK signaling downregulates PPAR $\gamma$  expression, highlighting the possible pathological mechanisms of diabetic tendinopathy. Intriguingly, mechanical stretch significantly induced the phosphorylation of ERK while simultaneously repressing that of Akt in tenocytes, indicating the activation of ERK and the concomitant inactivation of Akt. In addition, the mRNA expression of PPAR $\gamma$  and C/EBP $\beta$ , as well as the adipogenic differentiation, was markedly reduced after tenocytes were treated with mechanical stretch<sup>[41]</sup>. These findings not only suggest the activation of ERK1/2 and PI3K/Akt signaling in tenocytes cultured in high glucose conditions but also provide new therapeutic strategies for diabetic tendinopathy. Ueda *et al*<sup>[42]</sup> measured ROS accumulation in tenocytes under high glucose conditions and found upregulation of NOX1 and NOX4 expression. Oxidative stress was triggered by ROS in high glucose conditions<sup>[92]</sup>. Poulsen *et al*<sup>[57]</sup> demonstrated that oxidative stress enhanced the tenocyte phenotype and characteristics in low glucose conditions, whereas, in high glucose conditions, tenocyte apoptosis was induced. Oxidative stress upregulated the expression of both FOXO1 and HIF1 $\alpha$  in tenocytes. Under high glucose conditions, the level of miR28-5p was also upregulated, especially in oxidative stress-treated tenocytes. miR28-5p directly inhibited the expression of the p53 deacetylase sirtuin 3, resulting in an increase in acetylated p53. p53 inhibited the expression of miR17-92, which is a cluster of miRNAs including the Bim repressor miR17-5p, repressing the degradation of the proapoptotic protein Bim. Moreover, FOXO1 promoted the transcription of *Bim*, the gene product of which was a proapoptotic protein. Inhibition of Bim degradation and upregulation of Bim transcription synergistically resulted in an increase in Bim, facilitating tenocyte apoptosis. However, under low glucose conditions, the miR28-5p-sirt3-p53 pathway was not stimulated. Instead, p38 MAPK was activated and acted on both FOXO1 and HIF1 $\alpha$ , resulting in the inhibition of FOXO1 transcriptional activity and activation of HIF1 $\alpha$ . HIF1 $\alpha$  enhanced the expression of the tendon-related genes *Sox9* and *Scx*, two genes whose products are essential for tenocyte differentiation. These factors may account for the pathogenic mechanisms of diabetic tendinopathy. Rosenthal *et al*<sup>[58]</sup> showed that high glucose and insulin levels increased Tgase activity, and CML-collagen, an AGE-modified protein associated with hyperglycemia, also stimulated Tgase activity in tenocytes, which could be attenuated by antioxidants. The substrates of Tgase include many key ECM proteins, such as collagen, fibronectin, OPN, and microfibrillar proteins. Tgase produces highly protease-resistant crosslinks that alter

the structure and function of affected extracellular matrices. Increased production of Tgase crosslinks may impair the ability of the tendon to remodel and repair, rendering the tendon less able to respond to injury. Tgase activity may also contribute to the excessive thickness, stiffness, and calcification observed in diabetic tendinopathy. In addition, ROS could also increase Tgase activity. Patel *et al*<sup>[51]</sup> demonstrated that after AGEs interact with RAGE on the tenocyte membrane, a harmful effect was initiated inside the cells. AGEs could impair the function of tenocytes by reducing ATP production, basal respiration, electron transport efficiency, and coupling efficiency, decreasing transcript counts of mitochondrial complexes, increasing mitochondrial DNA levels and complex III (UQCRC2) protein levels, and further affecting nucleus DNA synthesis with reduced *Col1a1* mRNA expression and increased *MMP2* mRNA expression. In addition, mitochondrial and total ROS/superoxide production were also markedly increased in tenocytes that were treated with AGEs. Although high glucose conditions also affected some parameters representing mitochondrial functions, AGEs appeared to be the primary insult. Thus, AGEs, together with high glucose, ultimately altered mitochondrial energy metabolism, reduced the proliferative capacity, restricted the biosynthesis of tendon ECM, and stimulated the degradation of ECM, contributing to the development and degenerative process of diabetic tendinopathy. The potential mechanisms associated with tenocytes during the progression of diabetic tendinopathy are summarized in **Figure 1**.

Our previous study<sup>[52]</sup> showed that high glucose could suppress proliferation, induce apoptosis, and inhibit the tenogenic differentiation ability of TSPCs, with decreased mRNA expression of the tendon-related genes *Scx* and *Col1a1* and protein expression of *Tnmd* and *Col I*. In addition to the decreased proliferative and clonogenicity abilities of diabetic TSPCs, it was also demonstrated that the osteogenic and chondrogenic differentiation abilities of diabetic TSPCs were significantly stimulated, with increased expression of osteochondrogenic markers, including *BMP2*, *ALP*, *OPN*, *OCN*, *Col II*, and *SOX9*, while the tenogenic differentiation ability was remarkably suppressed, with decreased expression of the tenogenic markers *Col I* and *Tnmd* in dTSPCs<sup>[40]</sup>. The depleted population and erroneous differentiation of TSPCs might account for the pathological alterations in diabetic tendons and severely affect the tendon generation and repair potential, which may be involved in the pathogenesis of diabetic tendinopathy. However, the underlying molecular mechanism of the erroneous differentiation of TSPCs in diabetic tendons has not yet been elucidated, and further research is needed. Insulin is a conventional medicine that is used in the clinical management of DM, and systemic hyperinsulinemia secondary to impaired insulin sensitivity can occur during early DM. Durgam *et al*<sup>[84]</sup> demonstrated that insulin increased the proliferation and osteogenic differentiation of TSPCs *in vitro* and increased *ALP* activity and the mRNA expression of osteogenic genes, including *Runx2*, *ALP*, and *OSN*. In addition, the mRNA expression of IGF-I receptor was upregulated during insulin-induced osteogenic differentiation of TSPCs, and inhibition of the IGF-I receptor could reduce the insulin-induced osteogenic differentiation of TSPCs, with decreased osteogenic gene expression. These results suggest that insulin might interact with the IGF-I receptor to promote the osteogenic differentiation of TSPCs. However, the specific mechanisms involved in the insulin-mediated proliferation and osteogenic differentiation of TSPCs were not further investigated. The accumulation of AGEs, which are oxidative derivatives resulting from diabetic hyperglycemia, is a feature of DM-related changes in tendinopathy. Xu *et al*<sup>[83]</sup> showed that AGEs decreased cell viability and induced apoptosis of TSPCs and increased the expression of C-Cas3 and C-Cas9, suggesting an increase in TSPC apoptosis induced by AGEs. In addition, AGEs increased the ratio of LC3B/LC3A and decreased P62 expression, indicating that AGEs could induce autophagy. Pharmacological activation of autophagy with rapamycin (an autophagy agonist) could decrease C-Cas3 and C-Cas9, and inhibition of autophagy with 3-MA (an autophagy antagonist) had the opposite effect, indicating that autophagy played a protective role against AGE-induced apoptosis. Pioglitazone could decrease AGE-induced apoptosis in TSPCs by activating the AMPK/mTOR pathway to stimulate autophagy. In addition, AGEs also induced TSPC senescence, with elevated expression of P53 and P21, which was defined as limited regenerative potential, decreased self-renewal capacity, and impaired tenogenic differentiation capacity of TSPCs, enhanced osteogenic differentiation of TSPCs, and ectopic ossification, and pioglitazone could reverse these effects. These results not only reveal the pathological mechanisms of diabetic tendinopathy but also provide a new potential treatment strategy. Kwan *et al*<sup>[87]</sup> showed that high glucose conditions stimulated an inflammatory response and increased COX2 in both healthy and tendinopathic TSPCs; high glucose also weakened the pro-resolving responses, with decreased expression of FPR1, ChemR23, and

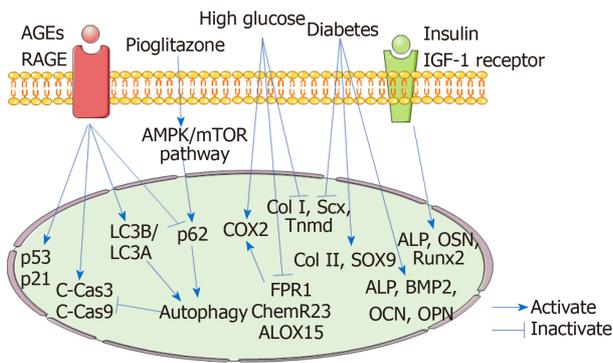


**Figure 1 Mechanisms involved in the tenocytes during the process of diabetic tendinopathy.** High glucose and high insulin inhibit the expression of the tendon-related genes *TGF-β*, *collagen I*, and *biglycan* in tenocytes by inactivating the AMPK/Egr1 pathway. In addition, high glucose promotes the adipogenic transdifferentiation of tenocytes and increases the protein expression of p-Akt and p-ERK1/2. Activation of the PI3K/Akt pathway plays an essential role in maintaining the expression of the adipogenic transcription factors peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) and C/EBP, however, activation of ERK signaling downregulates PPAR $\gamma$  expression, suggesting the fibroblast-to-adipocyte phenotypic transition induced by high glucose which can be inhibited by Akt inhibitor LY294002 and promoted by ERK inhibitor PD98059. High glucose stimulates transglutaminase (Tgase) activity, leading to an increase in extracellular matrix degradation. Reactive oxygen species (ROS) accumulation is also increased in tenocytes under high glucose conditions, triggering the oxidative stress and increasing Tgase activity. Oxidative stress increases both FOXO1 and hypoxia-inducible factor-1 $\alpha$  (HIF1 $\alpha$ ) expression in tenocytes. Under high glucose conditions, miR28-5p is also upregulated, especially in oxidative stress-treated tenocytes. miR28-5p directly inhibits the expression of the p53 deacetylase sirtuin 3, resulting in an increase in acetylated p53. p53 inhibits the expression of miR17-92, repressing the degradation of the proapoptotic protein Bim. Meanwhile, FOXO1 promotes the transcription of *Bim*, the gene product of which is a proapoptotic protein. Inhibition of Bim degradation and upregulation of Bim transcription synergistically result in an increase in Bim, facilitating the tenocyte apoptosis. However, under low glucose conditions, miR28-5p-sirt3-p53 pathway is not stimulated. Instead, p38 MAPK is activated and acts on both FOXO1 and HIF1 $\alpha$ , resulting in the inhibition of FOXO1 transcriptional activity and activation of HIF1 $\alpha$ . HIF1 $\alpha$  enhances the expression of the tendon-related genes *Sox9* and *Scx*. Advanced glycation end-products (AGEs), which bind to receptor for AGEs, impair the mitochondrial functions, affecting the DNA and mRNA synthesis in tenocytes. In addition, mitochondrial and total ROS/superoxide production are also remarkably increased in AGEs-treated tenocytes. AGEs: Advanced glycation end-products; RAGE: Receptor for AGEs; ROS: Reactive oxygen species; Tgase: Transglutaminase; Col I: Collagen I; Bgn: Biglycan; PPAR $\gamma$ : Peroxisome proliferator-activated receptor  $\gamma$ ; HIF1 $\alpha$ : Hypoxia-inducible factor-1 $\alpha$ .

ALOX15 in tendinopathic TSPCs and ALOX15 in healthy TSPCs, which may cause persistent chronic inflammation in tendinopathic tendons and increase the risk of diabetic tendinopathy. The potential mechanisms associated with TSPCs during the progression of diabetic tendinopathy are summarized in Figure 2.

## CONCLUSION

In summary, previous studies in both patients with DM and diabetic animal models have shown the compromised biomechanical properties and histopathological alterations in diabetic tendons. Suppressed expression of collagen and PGs and transdifferentiation of tenocytes in high glucose or diabetic environments could affect the formation of collagen fibrils and the ECM and alter tendon homeostasis, which may globally impair tendon structure, biomechanics, and tendon repair. In particular, the reduced proliferation, increased apoptosis, and erroneous differentiation of TSPCs indicate intrinsic cellular deficits and an altered degenerative capacity in diabetic tendons, which eventually results in deficient of tendon repair, maintenance, and remodeling. In summary, these factors, as well as the altered expression of MMPs, may synergistically contribute to the occurrence and accelerate the progression of diabetic tendinopathy. Although limited studies have been performed the partial results regarding the alterations in tenocytes and TSPCs and the mechanisms involved are controversial, high glucose conditions, AGEs, altered inflammatory responses, oxidative stress, and hyperinsulinemia may be associated with the cellular and molecular changes in diabetic tendons. Further studies are needed to explore the underlying mechanisms of diabetic tendinopathy, particularly the cellular and



**Figure 2 Mechanisms involved in the tendon stem/progenitor cells during the process of diabetic tendinopathy.** High glucose inhibits the expression of the tenogenic genes *collagen I* (*Col I*), *scleraxis* (*Scx*), and *tenomodulin* (*Tnmd*) in tendon stem/progenitor cells (TSPCs), indicating the suppressed tenogenic differentiation. Moreover, diabetic TSPCs exhibit increased osteogenic and chondrogenic differentiation abilities and decreased tenogenic differentiation ability with increased expression of osteochondrogenic markers alkaline phosphatase (ALP), bone morphogenetic protein 2, osteocalcin, osteopontin, Col II, and SOX9 and reduced expression of tenogenic genes *Col I*, *Scx*, and *Tnmd*. Insulin interacts with insulin-like growth factor-1 receptor to promote osteogenic differentiation of TSPCs by activating the expression of ALP, OSN, and runt-related transcription factor 2. These findings reveal that the erroneous differentiation of TSPCs plays important roles in the development and progression of diabetic tendinopathy. High glucose upregulates the expression of COX2 and downregulates the expression of FPR1, ChemR23, and ALOX15 in TSPCs, suggesting that high glucose stimulates an inflammatory response and weakens the pro-resolving responses, which results in persistent chronic inflammation in diabetic tendons. Advanced glycation end-products (AGEs) increase the expression of cleaved caspase-3 and cleaved caspase-9, suggesting an increase in TSPC apoptosis induced by AGEs. AGEs elevate the ratio of LC3B/LC3A and decrease P62 expression, indicating AGEs-induced autophagy. Autophagy plays protective roles against the AGE-induced apoptosis. AGEs also induced the TSPC senescence, with elevated expression of P53 and P21. Pioglitazone could decrease AGE-induced apoptosis by stimulating autophagy via activating the AMPK/mTOR pathway. AGEs: Advanced glycation end-products; RAGE: Receptor for AGEs; HIF1 $\alpha$ : Hypoxia-inducible factor-1 $\alpha$ ; C-Cas3: Cleaved caspase-3; C-Cas9: Cleaved caspase-9; Col I: Collagen I; Scx: Scleraxis; Tnmd: Tenomodulin; ALP: Alkaline phosphatase; OSN: Osteonectin; Runx2: Runt-related transcription factor 2; BMP2: Bone morphogenetic protein 2; OPN: Osteopontin; OCN: Osteocalcin.

molecular mechanisms associated with erroneous differentiation of TSPCs in diabetic tendons, which would have profound implications for the exploration and development of new and effective therapeutics for diabetic tendinopathy.

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