Sports Medicine and Rehabilitation

Review Article Open Access

Mechanobiological Response of Tendon Stem Cells in Tendon Injury and Repair [Version 1, Awaiting Peer Review]

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Original Submission

Received: July 20, 2017
Accepted: July 28, 2017
Published: August 03, 2017

Open Peer Review Status: Awaiting Peer Review

How to cite this article: He Huang, Yun Riffle, Jianying Zhang. Mechanobiological Response of Tendon Stem Cells in Tendon Injury and Repair [Version 1, Awaiting Peer Review]. Sports Med Rehabil. (2017) 1: 7.1

Acknowledgements: We gratefully acknowledge the funding support from National Natural Science Foundation of Jiangsu Province (#BK20161111) and Nanjing Science and Technology Commission (#201605079). We also thank Mr. Kevin Riffle for assistance in preparing this manuscript

Abstract

Tendons are the connective tissues interposed between muscles and bones transmitting the force created in the muscle to bone, and making joint movement possible. Intensive and excessive mechanical loading is considered to be a major factor responsible for acute and chronic tendon injuries. Additionally, aging is also known to induce the degenerative changes of the tendons. Traditionally, tenocytes are thought to be the main resident cells of the tendons and are essential for maintaining tendon homeostasis and promoting remodeling or degenerating. However, a new cell type, tendon stem/progenitor cell (TSC) has been identified in the tendons recently, and the mechanobiological response of TSCs to tendon injuries is becoming a hot topic in orthopedic research. The *in vitro* and *in vivo* studies have demonstrated that TSCs play an important role in tendon degeneration and regeneration. This mini review highlights the recent research advances in mechanobiological response of TSCs in tendon injuries and diseases.

Keywords

Tendon Stem Cell; Tendinopathy; Mechanical Loading; Aging; Proliferation; Differentiation

Introduction

Tendons are the connective tissues that attach a muscle to other body parts, usually bones. Tendons are living tissue consisted of collagen, elastin, proteoglycans, and cells. Tendons *in vivo* are constantly subjected to mechanical loading by transmitting muscular forces to bone [1] and respond to mechanical forces by changing the metabolism as well as their structural and mechanical properties. As such, tendon disorders caused by mechanical loading lead to significant disability, pain, health care cost, and lost productivity [2].

Tendon injuries can be acute and chronic caused by intrinsic or extrinsic factors [3]. Acute tendon injuries include lacerations and ruptures, and tendinopathy is a typical chronic tendon injury [4]. Once injured, the healing process of the tendon is very slow and often results in scar tissue formation. It has been reported that degenerative changes can be found in the patients with an Achilles tendon rupture [5]. Tendinopathy is especially prevalent in both occupational and athletic settings as well as in the general population [1]. Aging is known to adversely affect the human body and lead to degenerative changes in tissues and organs [6]. Specifically, aging predisposes tendons to develop tendinopathy and causes tendons to frequently rupture and re-rupture [7]. Despite the prevalence of these injuries, a limited number of investigators are conducting fundamental basic science studies that are focused on precise pathogenic mechanisms of tendinopathy and tendon healing [8].

Histological examination of tendinopathy shows lipoid degeneration, fibrocartilaginous metaplasia, and calcified tissues in tendon lesions [9,10]. However, the cellular and molecular mechanisms about the formation of these non-tenocyte tissues remained largely unknown.

In recent studies, we have demonstrated the critical role of tendon stem/progenitor cells (TSCs) in regulating the beneficial or detrimental effects of tendons in animal models [1,11-13]. Like adult stem cells, TSCs are essential for the maintenance and repair of tendinous tissues when injured. In this mini review, we introduce the differential properties of TSCs and tenocytes, as well as the recent advancements of mechanical and biological response of TSCs in tendon injury, especially in tendinopathy. We also discuss the mechanobiological response of TSCs on wounded tendon healing.

Characterization of TSCs and Tenocytes

The TSCs were first identified from the tendons of human and mice in 2007 [14]. Before that, tendon cell population was used to be considered composed of only tenocytes [8]. After that, TSCs have been isolated from the tendons and ligaments of many animals, such as rabbits and rats by our group and the other researchers [11,15]. We have reported that TSCs differ from resident tenocytes on many aspects such as the morphology, colony formation, proliferation, and differentiation potential [11]. Tenocytes have an elongated spindle phenotype, however, TSCs have a cobblestone shape with large nuclei and smaller cell body [11]. Moreover, TSCs grow more quickly than tenocytes, and TSCs form round colonies, but there is no any colony formed by tenocytes during the culture. Furthermore, TSCs express stem cell markers such as nucleostemin, octamer-binding transcription factor 4 (Oct-4), stage-specific embryonic antigen 1 (SSEA-1), and stage-specific embryonic antigen 4 (SSEA-4). However, tenocytes negatively express stem cell markers. In addition, TSCs have multi-differentiation potential [11,14], they can differentiate into non-tenocyte lineages such as adipocytes, chondrocytes, and osteocytes. However, tenocytes have no the capability to differentiate into any cell lineages [11,14]. The “stemness” of TSCs indicates the possibility that TSCs play a critical role in the development of degenerative tendinopathy, because the fatty tissue, bony tissue, and cartilage-like tissue found inside the tendinopathic tendons may be caused by non-tenocyte differentiation of TSCs.

In Vitro Mechanobiological Responses of TSCs

Previous studies in embryonic stem cells and human bone marrow mesenchymal cells (BMSCs) have demonstrated that mechanical stimulation regulated the proliferation and differentiation of stem cells [16,17]. As mechanical loading is an inherent part of tendon environment, recent researches focus on the mechanobiological response of TSCs. We used an *in vitro* system capable of mimicking *in vivo* loading conditions to determine the effect of mechanical loading on TSCs [1]. We found that mechanical stretching increased the proliferation of TSCs in a stretching magnitude-dependent manner. The differentiation of TSCs into tenocytes was promoted by low mechanical stretching at 4% (clamp-to-clamp engineering stain). Moderate stretching in TSCs enhanced the expression of collagen type I and tenomodulin, two tenocyte-related genes [1]. It has been reported that tenomodulin is a regulator of tenocyte proliferation predominantly expressed in tendon and plays an important role in collagen fibril maturation and organization, and tendon vascularity [18]. These anabolic responses were further confirmed by human TSCs. Moderate stretching of human TSCs also increased matrix proteins (fibromodulin, lumican, versican), collagen type I, and MMPs [19].

While moderate stretching induces anabolic effects in TSCs, excessive stretching causes major catabolic effects. We have found that small stretching (4%) significantly enhanced only the expression of tenocyte-related genes, collagen type I and tenomodulin, while none of the non-tenocyte-related genes, PPARγ (adipocyte marker gene), Runx-2 (osteocyte-related gene), collagen type II and Sox-9 (two chondrocyte-related genes) were affected. However, large stretching at 8% induced the differentiation of TSCs into non-tenocytes such as adipocytes, osteocytes, and chondrocytes, as evidenced by histological analysis and gene expression. The expression

of both tenocyte-related and non-tenocyte-related genes was significantly enhanced by large stretching at 8% [1]. The detrimental effects of excessive mechanical stretching in TSCs were also evident by upregulated expression of BMP-2, and Wnt proteins from numerous studies [20-22]. The *in vitro* studies have indicated that low mechanical stretching may be beneficial to tendons by enabling differentiation of TSCs into tenocytes to maintain tendon homeostasis. However, large mechanical loading may be detrimental, as it induces the differentiation of TSCs into non-tenocytes in tendon, thus, resulting in accumulation of lipid cells, tissue calcification, and cartilage-like tissue formation, which are typical features of tendinopathy at later stage [1].

In Vivo Mechanobiological Response of TSCs

The mechanical loading effect on TSCs has also been studied using treadmill running of rodents as an *in vivo* model [12]. Moderate mechanical loading is achieved by moderate treadmill running (MRT) using a short running protocol (13-15 meter/min, less than an hour/day and do not exceed 6 weeks). Excessive mechanical loading is accomplished either by intensive treadmill running (ITR) using a longer running protocol (at least 13 meter/min, more than 2 hours/day, and over 10-12 weeks) or by one bout of rigorous treadmill running (OTR) using a single run until the rodents are exhausted (at least 13 meter/min, over 3-5 hours). The control rodents are allowed to move freely in their cage without running [23].

We have reported that moderate mechanical loading via MTR increased the presence of myofibroblasts in mouse patellar tendons [24]. As myofibroblasts are activated fibroblasts, their presence in the tendon following MTR indicates that they actively repair and remodel tendon tissue under strenuous mechanical loading, leading to known changes in tendon structure. After MTR treatment, the tendon sections of mice revealed that numerous cells expressed α-SMA, whereas, in the tendon sections of control mice, only a few cells exhibited weak α-SMA signals. Furthermore, the colonies of the TSCs derived from MTR treated mouse tendons were generally larger in culture, proliferated faster, expressed a higher level of α-SMA, produced more amount of collagen, and formed more abundant stress fibers compared to the TSCs derived from cage control mice. In addition, the TSCs from treadmill running mice generated larger traction forces than those from control mice [12,24].

Further studies have demonstrated that MTR treatment elevated the expression of mechanical growth factors (MGF) and enhanced the proliferative potential of TSCs in both patellar and Achilles tendons [25]. In both tendons, MTR enhanced the expression of tenocyte-related genes: collagen type I (10 fold) and tenomodulin (3–4 fold) but did not affect non-tenocyte-related genes: LPL (adipocytes), Sox9 (chondrocytes), Runx2 (osteocytes) and Osterix (osteocytes). However, ITR not only increased tenocyte-related genes but also increased non-tenocyte-related genes. After ITR, the paratenons of mouse tendons were noticeably more vascular with a tendency to bleed [25]. In addition, the production of prostaglandin E_2 (PGE_2) in tendons was markedly increased in response to a bout of rigorous treadmill running (OTR) and the PGE_2 treatment of TSCs in culture decreased cell proliferation and induced non-tenocyte differentiations [23]. The administration of PGE into rat Achilles tendon was used as a possible animal model of chronic tendinopathy [26]. It has been found that repeated exposure of tendon to PGE led to localized tendon degeneration [27]. The classic Achilles overuse model showed extensive inflammatory changes in the paratenon of rabbit tendon, with concomitant structural degeneration in the load-bearing tendon [28].

These studies have indicated that higher levels of PGE_2 are produced by tendons in response to repetitive mechanical loading *in vivo* and high levels of PGE_2 have a detrimental effect on tendons.

Mechanobiological Response of Aging TSCs

Aging is known to adversely affect the human body and lead to degenerative changes in tissues and organs [6]. Specifically, aging predisposes tendons to develop tendinopathy due to prolonged cyclical loading and cumulative damage and causes tendons to frequently rupture and re-rupture [7,29]. Overuse tendinopathies are very common in aging population. The animal study has shown that gene expression of collagen type I, III and V, elastin, and proteoglycan 4 (PRG4) in the Achilles and tibialis anterior tendons of aging rats (22-25 months) are much lower than young rats (3-5 months) [7]. Furthermore, compared to young tendons, in aging tendons, the ratio of nucleus to cytoplasm is higher, proteoglycan content is lower, lipid deposition is increased, and vascularization is decreased. Additionally, aging also decreases the activities of tendon cells including the proliferation of the cells, the integrity of tendon matrices, and the response of tendon cells to cellular stimuli [29,30]. A recent study showed that aging affects the responses of human Achilles and patellar tendons to transverse strain (TS) by reducing the TS ~2.5% every 10 years of life [29]. Thus, tendon injuries appear to be more frequent with aging [31-33] and wounded aging tendons heal poorly.

We have studied the aging effect on TSCs [13]. The TSCs derived from aging mice (9 and 24 months) proliferated significantly slower than TSCs isolated from young mice (2.5 and 5 months). The expression of the stem cell markers nucleostemin, Oct-4, Sca-1, and SSEA-1 in TSCs decreased in an age-dependent manner [13]. Contrary to aging, moderate mechanical loading is well known to exert beneficial effects on tendons [25]. We have found that moderate mechanical loading tested by a cell stretching at 4% *in vitro* model increased the expression of stem cell markers in aging TSCs, but intensive mechan-

ical stretching at 8% *in vitro* decreased the expression of stem cell markers in aging TSCs [13]. It has been reported that in aging tendons collagen content reduces in an age-dependent manner; that is, collagen synthesis as well as collagen turnover diminish with increasing age [9,34]. Higher crosslinking of the tropocollagen [35,36] ensures and increases the mechanical stiffness of tendons [37,38] thus reducing the mechanical properties of aging tendons. However, exercise has the opposite effects on collagen synthesis in tendons. Several studies have shown that acute exercise in young humans increased anabolic processes causing a net increase in collagen I in the Achilles tendons [39,40].

A 5-year follow-up study of Alfredson's heel-drop exercise in 58 patients (average age 50.9 years old) with chronic midportion Achilles tendinopathy has shown that Alfredson's heel-drop exercise significantly increased Victorian Institute of Sports Assessment-Achilles (VISA-A) questionnaire score from 49.2 to 83.6, and 39.7% of the patients were completely pain-free at follow-up. The sagittal tendon thickness decreased from 8.05 mm to 7.50 mm at the 5-year follow-up [41]. This study indicated that mechanical loading through exercise can improve symptoms of tendinopathy.

It is believed that mechanical loading also influences tendons' mechanical properties [4,42]. Some researchers have shown that moderate exercise increased the tendon-aponeurosis stiffness [43], the maximum plantar flexion muscle strength and triceps surae [44]. Similarly, after performing knee extension eccentric exercises, the patellar tendon micro circulation was increased and tendon stiffness was reduced in athletes [45]. Such benefits of moderate exercise have been well established in both human and animal models by a number of studies [46-54].

However, the effect of exercise on wound healing in aging tendons is largely unknown [55]. We have investigated the effect of mechanical loading on wounded aging tendon using an aging rat moderate treadmill running (MTR) model. The rats were divided into a MTR group that ran on a treadmill for 4 weeks and a control group that remained in cages. After MTR, a window defect was created in the patellar tendons of all rats and wound healing was examined. We found that moderate exercise enhances healing of injured aging tendons by increasing anabolic changes and reducing catabolic changes in the TSCs of MTR treated aging rats. When compared to the cage control group, the number of TSCs in MTR treated aging rats was increased, the organization of collagen fibers in aging rat tendons was improved, the number of senescent cells in the wounded tendons was decreased, and the wounds were closed much quicker [13,55]. More studies demonstrated that following tendon injury, the natural healing process forms scar tissue [3], and the exercises augmented tendon healing [56]. Although some investigators have suggested that underuse of a damaged segment of a tendon may be the source of the chronic impairment [57], many studies have demonstrated that mechanical stimulation improves tendon repair [58,59].

Overview

Tendon injuries and tendinopathy are very common especially in athletes as well as in older population. The complete restoration of injured tendons is still a big challenge in clinics due to the treatments are mostly palliate and ineffective. Tendon stem cells (TSCs) are the key cell population of the tendons. The mechanobiological response of TSCs plays an important role in tendon injuries and repair. Although the current studies have revealed the TSC based mechanisms behind the beneficial effects of exercise on tendons and may help devise clinical rehabilitation protocols to treat tendon injuries, especially to treat injured aging tendons, more studies on mechanobiological response of TSCs are still needed so that new effective treatment strategies can be developed.

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