Biochemical and Biophysical Research Communications 450 (2014) 762–766

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Effects of celecoxib on proliferation and tenocytic differentiation of tendon-derived stem cells

Kairui Zhang [a,1], Sheng Zhang [a,1], Qianqian Li [d,1], Jun Yang [a,b], Weiqiang Dong [a,c], Shengnan Wang [a], Yirong Cheng [a], Mohammed Al-Qwbani [a], Qiang Wang [e,*], Bin Yu [a,*]

[a] Department of Orthopaedics and Traumatology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China
[b] Department of Orthopaedics, 421 Hospital of PLA, Guangzhou 510318, China
[c] Department of Orthopaedics, The First Affiliated Hospital to Guangzhou Medical University, Guangzhou 510120, China
[d] Cancer Research Institute, Southern Medical University, Guangzhou 510515, China
[e] Department of Orthopaedics, Subei People's Hospital of Jiangsu Province (Clinical Medical College of Yangzhou University), Yangzhou, Jiangsu Province 225001, China

ARTICLE INFO

Article history:
Received 28 May 2014
Available online 19 June 2014

Keywords:
Tendon-derived stem cells
Celecoxib
Tenocytic differentiation
Cell proliferation

ABSTRACT

NSAIDs are often ingested to reduce the pain and improve regeneration of tendon after tendon injury. Although the effects of NSAIDs in tendon healing have been reported, the data and conclusions are not consistent. Recently, tendon-derived stem cells (TDSCs) have been isolated from tendon tissues and has been suggested involved in tendon repair. Our study aims to determine the effects of COX-2 inhibitor (celecoxib) on the proliferation and tenocytic differentiation of TDSCs. TDSCs were isolated from mice Achilles tendon and exposed to celecoxib. Cell proliferation rate was investigated at various concentrations (0.1, 1, 10 and 100 μg/ml) of celecoxib by using hemocytometer. The mRNA expression of tendon associated transcription factors, tendon associated collagens and tendon associated molecules were determined by reverse transcription-polymerase chain reaction. The protein expression of Collagen I, Collagen III, Scleraxis and Tenomodulin were determined by Western blotting. The results showed that celecoxib has no effects on TDSCs cell proliferation in various concentrations ($p > 0.05$). The levels of most tendon associated transcription factors, tendon associated collagens and tendon associated molecules genes expression were significantly decreased in celecoxib (10 μg/ml) treated group ($p < 0.05$). Collagen I, Collagen III, Scleraxis and Tenomodulin protein expression were also significantly decreased in celecoxib (10 μg/ml) treated group ($p < 0.05$). In conclusion, celecoxib inhibits tenocytic differentiation of tendon-derived stem cells but has no effects on cell proliferation.

1. Introduction

Tendon is a compositionally complex tissue with a predominantly mechanical function: translating muscular contractions into joint movement by transmitting forces from muscle to bone. Owing to the critical role of this tissue in body mechanics, injury and degeneration of tendon can be highly debilitating and can result in substantial pain, disability, and health-care costs [1].

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase (COX) enzymes in the arachidonic acid (ArA) pathway to reduce synthesis of prostaglandins. There are two cyclooxygenase isoforms: COX-1 and COX-2 [2]. COX-1 is constitutively expressed in many cell types while COX-2 is inductively expressed. Celecoxib has been shown to selectively inhibit COX-2 enzyme, while leaving COX-1 function intact [3]. This allows a significant reduction in the occurrence of side effects, especially on the gastrointestinal tract and on platelet function [3]. NSAIDs are often ingested to reduce the pain and improve regeneration of tendon after tendon injury [4,5], even though the effect of NSAID is poorly understood. Although the effects of NSAIDs in tendon healing have been reported, the data and conclusions are not consistent [6–9].

Recently, tendon-derived stem cells (TDSCs) have been isolated from tendon tissues of varies species, including human, horse, rabbit, rat, and mouse [10–13]. These cells expressed stem cell-related markers, formed adherent colonies in culture and showed

* Corresponding authors. Address: Department of Orthopaedics, Subei People's Hospital of Jiangsu Province (Clinical Medical College of Yangzhou University), Yangzhou, Jiangsu Province 225001, China (Q.Wang). Address: Department of Orthopaedics and Traumatology, Nanfang Hospital, Southern Medical University, No. 1838 Guangzhou Avenue North, Guangzhou 510515, China (B. Yu).
E-mail addresses: 1780468505@qq.com (Q. Wang), carryzhang1985@live.com (B. Yu).
[1] Kairui Zhang, Sheng Zhang and Qianqian Li are co-first authors who contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.06.058
0006-291X/

self-renewal potential [10–12]. They could differentiate into osteogenic, chondrogenic and adipogenic lineages upon induction in vitro and could form tendon-like, cartilage-like, bone-like and tendon-bone junction-like tissues after subcutaneous transplantation in nude mouse or nude rat models[10,11]. Recent study also suggest that the involvement of TDSCs in tendon repair and tendinopathy [10,14]. To our knowledge, the effects of selective COX-2 inhibitor (celecoxib) on the proliferation and tenocytic differentiation of TDSCs have never been addressed in the literature.

In this study we investigated the effects of celecoxib on the proliferation and tenocytic differentiation in isolated TDSCs in vitro.

2. Materials and methods

2.1. Isolation and culture of rat TDSCs

We used male C57 mouse (aging 6 weeks) which were obtained from the experimental animal research center of Southern Medical University. All animal experimental protocols were approved by the Animal Care and Use Committee of Southern Medical University and followed principle expressed in Declaration of Helsinki. Tendon-derived stem cells were isolated from Achilles tendon following the method previously reported [11]. Briefly, after removing the paratenons, the core portions of the patellar tendons were cut into small pieces and digested with collagenase type I (3 mg/ml) and dispase (4 mg/ml) at 37 °C for 1 h. After centrifugation at 1500 rpm for 15 min and removal of the enzyme-containing supernatant, a single-cell suspension was obtained, which was cultured in growth medium (DMEM plus 20% FBS) at 37 °C with 5% CO_2. After 10 days in culture dishes, TDSCs formed colonies. The stem cell colonies were then isolated and cultured in DMEM with 20% FBS. These TDSCs at passage 1 were used in the following experiments.

2.2. Cell proliferation assay

TDSCs were plated at 10^4/well in 6 well plates and allowed to adhere overnight. FBS free DMEM medium was supplemented with four different concentrations of celecoxib (0.1, 1, 10 and 100 μg/ml) for the treatment group (4 plates) whilst the medium of control group (1 plates) was supplemented with bovine serum albumin (BSA). Proliferation rates were determined at 7 days. The cell count of treatment and control group were performed by hemocytometer.

2.3. Cell differentiation and treatment group

Cells plated in 12 well plates at 20×10^4/well. Ascorbic acid was also added to a final concentration of 50 μg/ml to support proper collagen synthesis. Differentiating cells were exposed to 0.1, 1, 10 and 100 μg/ml celecoxib for 7 days and then analyzed for gene expression and Western blot.

2.4. RNA isolation and quantitative real-time polymerase chain reaction

Total RNA was isolated from TDSCs using TRIzol (Sigma–Aldrich) according to the standard protocol provided by the manufacturer. cDNA was synthesized from total RNA using a Superscript III first-strand synthesis kit (Invitrogen). qRT-PCR was performed using a SYBR Green PCR Master Mix (Applied Biosystem) and an ABI 7900 HT machine (Applied Biosystem) according to the manufacturer's protocols. Average threshold cycle value (Ct value) was calculated. Standard curves were generated using 10-fold serial dilutions of cDNA of each gene with a correlation coefficient of >98%. Relative expression levels were calculated based on a standard curve and normalized to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). 5 independent RNA samples from TDSCs were analyzed in triplicate. The primer sequences used in this research are listed in Table 1.

2.5. Protein extraction and Western blotting

Cells were washed twice with cold PBS and dissolved with lysis buffer containing 50 mM Tris–HCl (pH 8.0), 150 mM NaCl, protease inhibitor and 10% NP-40. Equal amounts of proteins (20 μg) were electrophoresed by SDS–PAGE, then transferred onto PVDF membranes and blotted with antibodies against Collagen I, Collagen III, scleraxis, tenomodulin or GAPDH (Cell Signaling Technology), followed by incubation with secondary antibodies (GE Healthcare). Results were visualized and images captured using a LiCoR Odyssey imager (Li-COR Biosciences). Relative quantification of protein bands were analyzed using Image J software.

2.6. Statistical analysis

All data were expressed as mean ± SD. The data were analyzed using one-way ANOVA or Independent-samples *T* Test. A p value of less than 0.05 was considered statistically significant.

3. Results

To address the effect of celecoxib on TDSCs proliferation, we counted the cell number after treatment with different concentration of celecoxib. The data showed that the cell number after the treatment of different concentration of celecoxib were similar with control group in day 7 (Control group: 4.0417 ± 0.83662, Celecoxib 0.1 μg/ml group: 3.66 ± 0.26, Celecoxib 1 μg/ml group: 4.03 ± 0.87, Celecoxib 10 μg/ml group: 4.51 ± 0.66, Celecoxib 100 μg/ml group: 3.86 ± 0.86, $p > 0.05$) (Fig. 1).

We next examined the effects of different concentration of celecoxib on TDSCs tenocytic differentiation by determining the expression of Scx and Col1a1, two tenocyte-associated genes. The results showed that treatment of TDSCs with two high concentrations (10 and 100 μg/ml) of celecoxib significantly ($p < 0.05$) reduced the expression of both Scx and Col1a1.

Then we used 10 μg/ml celecoxib to investigate the effect of celecoxib on TDSCs differentiation at transcriptional level by analyzing the mRNA expression of tendon associated transcription factors, tendon associated collagens and tendon associated molecules in both control and celecoxib group. The data showed that levels of mRNAs for the tendon associated transcription factor, Scx and Egr1 significantly ($p < 0.05$) decreased in celecoxib group compared to control group (Fig. 2). The relative mRNA levels were lower ($p < 0.05$) for main tendon associated collagen, Col1a1, Col3a1 and Col6a1, in celecoxib group (Fig. 2). The relative mRNA levels were also lower ($p < 0.05$) for tendon associated molecules, Tnmd, Bgn, Dcn, Fmod, Tnc and Eln, in celecoxib group (Fig. 2).

These results were confirmed by Western blot analysis in protein level. The data of Western blot showed that protein expression of Collagen I, Collagen III, scleraxis and tenomodulin significantly decreased in celecoxib (10 μg/ml) group compared to control group (data are presented as mean ± SD; Celecoxib group/control group: 0.76 ± 0.05-fold, $p < 0.05$; Celecoxib group/control group: 0.79 ± 0.04-fold, $p < 0.05$; Celecoxib group/control group: 0.54 ± 0.05-fold, $p < 0.05$; Celecoxib group/control group: 0.83 ± 0.69-fold, $p < 0.05$) (Fig. 3).

4. Discussion

Tendons are composed mostly of parallel arrays of collagen fibers closely packed together, which play the role translating

Table 1
Primers used for quantitative real-time PCR.

Gene	Forward primers	Reverse primers	Accession No.
Scx	5′-CCTTCTGCCTCAGCAACCAG-3′	5′-GGTCCAAAGTGGGGCTCTCCGTGACT-3′	NM_198885.3
Mkx	5′-AGTAAAGACAGTCAAGCTGCCACTG-3′	5′-TCCTGGCCACTCTAGAAGCG-3′	NM_177595
Egr1	5′-CAGCGCCTTCAATCCTCAAG-3′	5′-GCGATGTCAGAAAAGGACTCTGT-3′	NM_007913
Col1a1	5′-TGGAGAGAGCATGACCGATG-3′	5′-GAGCCCTCGCTTCCGTACT-3′	NM_007742
Col3a1	5′-CTAAAATTCTGCCACCCCGAA-3′	5′-AGGATCAACCCAGTATTCTCCACTC-3′	NM_009930
Col5a1	5′-CCTGGCATCAACTTGTCCGATGG-3′	5′-GTGGTCACTGCGGCTGAGGAACTTC-3′	NM_015734
Col6a1	5′-CTACACCGACTGCGCCATTA-3′	5′-CCCCCTATGAGCAGCTCCT-3′	NM_009933
Col14a1	5′-GAGCAGAGACCACATTGGCC-3′	5′-CGTACAGCTCGAGGTCGGAA-3′	NM_181277
Tnmd	5′-AACACTTCTGGCCCGAGGTAT-3′	5′-AAGTGTGCTCCATGTCATAGGTTTT-3′	NM_022322.2
Bgn	5′-TTTCTGAGCTTCGCAAGGATG-3′	5′-GGGCGTAGAGGTGCTGGAG-3′	NM_007542.4
Dcn	5′-CTATGTGCCCCTACCGATGC-3′	5′-CAGAACACTGCACCACTCGAAG-3′	NM_001190451.1
Fmod	5′-CTCCAACCCAAGGAGACCAG-3′	5′-GGATCCACCAGTGAGAGTCTTC-3′	NM_021355.3
Lum	5′-TCGAGCTTGATCTCTCCTAT-3′	5′-TGGTCCCAGGTCTTACAGAA-3′	NM_008524.2
Tnc	5′-AACCATCAATGCGGCCAC-3′	5′-TGTCGTCCAGAAAAACGTCAGA-3′	NM_011607
Fn1	5′-CACGTACCTCTTCAAAGTCTTTGC-3′	5′-GGATTGCTTTCCCTGCCCT-3′	NM_010233.1
Fbn1	5′-GGACACGATGCGCTGAAAGG-3′	5′-CAGGAATGCCGGCAAATGGG-3′	NM_007993.2
Eln	5′-CAAGTCGGAGCTGGCATCGG-3′	5′-GTGGGAACTCCAGGGAGCAC-3′	NM_007925.3
Gapdh	5′-TTGTGGAAGGGCTCATGACC-3′	5′-TCTTCTGGGTGGCAGTGATG-3′	NM_008084.2

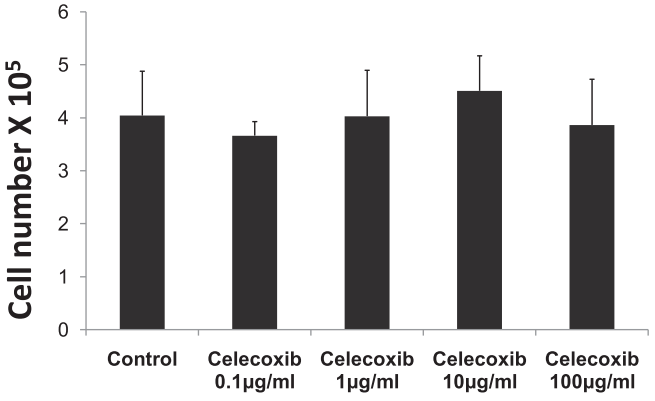

Fig. 1. Proliferation of TDSCs treated by various concentrations of celecoxib. Data represents the mean ± SD of the 6 samples. $^{*}P < 0.05$.

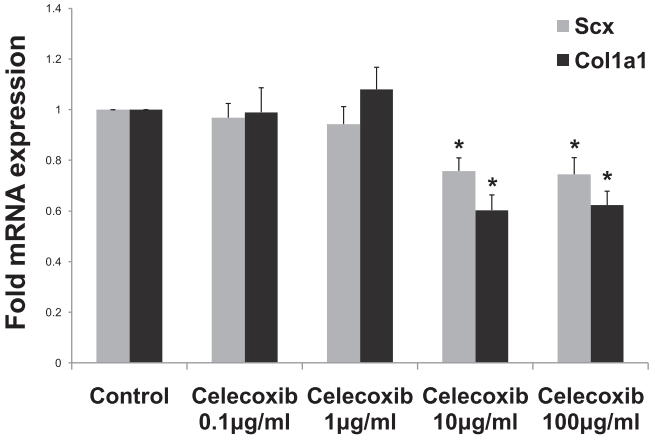

Fig. 2. Expression of Scx and Col1a1 in TDSCs treated with various concentrations (0.1, 1, 10 and 100 μg/ml) of Celecoxib. Data represents the mean ± SD of the 5 samples. $^{*}P < 0.05$.

muscular contractions into joint movement by transmitting forces from muscle to bone. The dry mass of normal tendons, which makes up about 30% of the total mass with water, is composed of about 86% collagen. The collagen portion is made up of 97–98% Col I, with small amounts of other types of collagen [15]. Our results showed that the expression of Col1a1, Col3a1 and Col6a1 significantly decreased with exposure to celecoxib. This was further confirmed by the analysis of Western blotting for Col1a1 and Col3a1 in protein level. Carlstedt et al. reported that there is significant decrease in the amount of soluble collagen in the indomethacin treated group comparing with control group [16], which is consist with our results. Fig. 4 However Tsai et al. reported that mRNA and protein expression of types I and III collagen in tendon cells were not changed after celecoxib treatment [17], which may indicate that the different effects of celecoxib in TDSCs and tendon cells.

The majority of macromolecules present in the extracellular matrix of tendons can be classified into three groups: (1) collagen, (2) proteoglycans, and (3) glycoproteins [18]. Recent studies have shown that the assembly of the chief structural component of the tendon, type I collagen, is regulated by proteoglycans and glycoproteins present in the ECM [19–24]. Decorin is considered a key regulator of matrix assembly because it limits collagen fibril formation and thus directs tendon remodeling due to tensile forces [19]. Evidence that decorin plays a role in collagen fibrillogenesis in animals was provided by the observation that decorin-deficient mice have fragile skin that is not able to withstand sudden tensile strain [19,20]. Similarly to decorin biglycan also binds to type I collagen and the interaction appears to be independent of the presence of N-linked oligosaccharides of the biglycan core protein [21]. Ameye et al. reported that collagen fibrils in the quadriceps tendons of biglycan-deficient mice had smaller diameter and abnormal morphology and this knockout mice also developed ectopic tendon and joint ossification accompanied by osteoarthritis after 3 months of age [22]. Tenascin C is expressed predominately in regions transmitting high levels of mechanical force, such as the myotendinous and osteotendinous junctions [24]. Fibromodulin is also expressed at high levels in the tendon. Knockout mice for fibromodulin show marked increase in small diameter immature collagen fibrils without progression to mature large diameter fibrils and the tendon stiffness of the knockout mice is reduced [23]. Our results showed that celecoxib significantly downgrade the mRNA expression of main tendon associated molecules, Bgn, Dcn, Fmod, Tnc and Eln. During the tendon healing, reduced proteoglycans and glycoproteins which present in the tendon ECM may induce type I collagen loss its hierarchical structure and thus impair the biomechanical function of tendon. Dimmen et al. found a significantly lower tensile strength and stiffness in rats Achilles tenotomy model given both parecoxib and indomethacin compared to the control group [25], which is consist with our results.

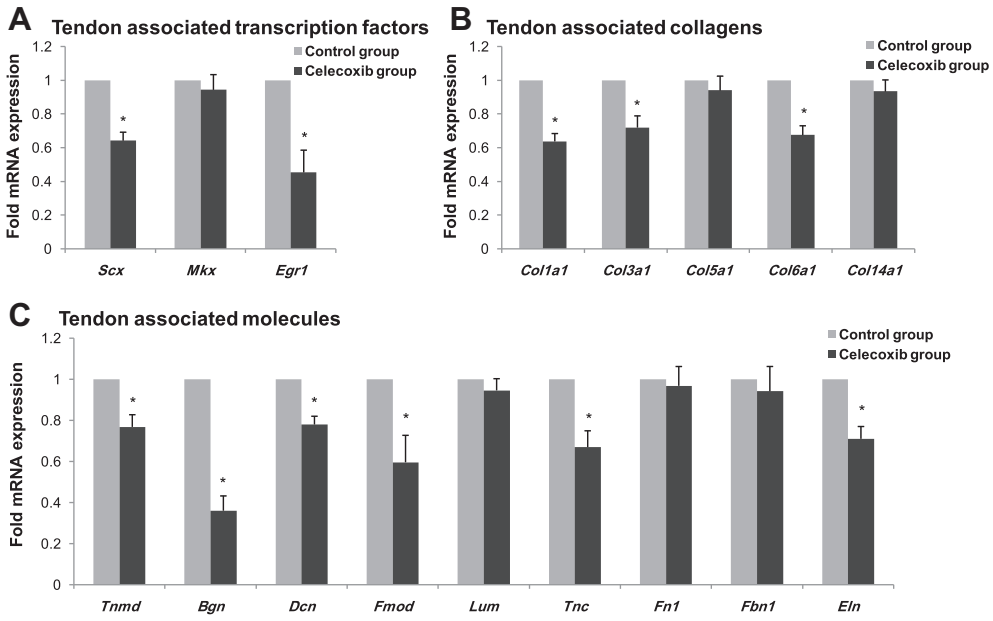

Fig. 3. qRT-PCR analyses of TDSCs gene expression in control and celecoxib (10 μg/ml) treatment group. (A) The tendon associated transcription factors, Scx, Mkx and Egr1; (B) The tendon associated collagens, Col1a1, Col3a1, Col5a1, Col6a1 and Col14a1; (C) The tendon associated molecules, Tnmd, Bgn, Dcn, Fmod, Lum, Tnc, Fn1, Fbn1 and Eln. mRNA levels in control group were normalized to 1. Error bars represent SD. $^{*}P < 0.05$.

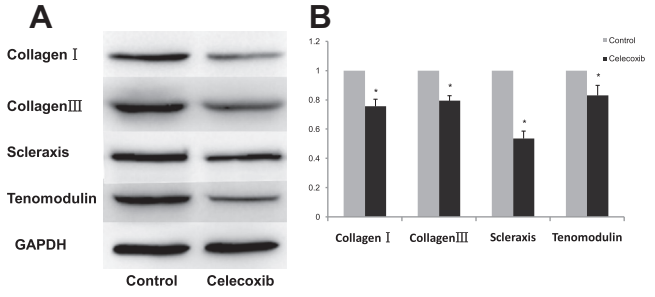

Fig. 4. Western blotting analyses of protein expression in control and celecoxib (10 μg/ml) treatment group. Proteins were separated by SDS -PAGE and transferred on the PVDF membranes to develop bands after incubation with Collagen I, Collagen III, Scleraxis and Tenomodulin antibodies. (B) Relative quantification of protein bands in control and celecoxib treatment group. Relative quantification of protein bands in control group were normalized to 1. Error bars represent SD. $^{*}P < 0.05$.

Scleraxis has been reported as a marker of the progenitor populations of tendons and tenocytes [26]. Tenomodulin is a type II transmembrane glycoprotein containing a C-terminal anti-angiogenic domain and is predominantly expressed in tendons and ligaments. Shukunami et al. reported that tenomodulin expression is closely associated with the appearance of tenocytes during chick development and is positively regulated by Scleraxis [27]. And they suggested that Scleraxis as an early marker of tendon formation positively regulates Tenomodulin, which is a late marker of tendon formation, expression in a tendon cell lineage-dependent manner [27]. Our results showed that the expression of Scx and Tnmd significantly decreased with exposure to celecoxib, which indicated that celecoxib could inhibit the tenocytic differentiation of TDSCs. This was further confirmed by the analysis of Western blotting for Scleraxis and Tenomodulin in protein level.

In conclusion, celecoxib inhibits tenocytic differentiation of tendon-derived stem cells but has no effects on cell proliferation. Our results indicated that the frequent use of NSAIDs in the treatment of tendon injury and tendinopathy may have profound negative effects on tendon regeneration and should be used in caution.

References

[1] P.B. Voleti, M.R. Buckley, L.J. Soslowsky, Tendon healing: repair and regeneration, Annu. Rev. Biomed. Eng. 14 (2012) 47–71.
[2] W.L. Smith, Y. Urade, P.J. Jakobsson, Enzymes of the cyclooxygenase pathways of prostanoid biosynthesis, Chem. Rev. 111 (2011) 5821–5865.
[3] P.T. Leese, R.C. Hubbard, A. Karim, Effects of celecoxib, a novel cycloxygenase-2 inhibitor, on platelet function in healthy adults: a randomised, controlled trial, J. Clin. Pharmacol. 40 (2000) 124–132.
[4] L.C. Almekinders, J.D. Temple, Etiology, diagnosis, and treatment of tendonitis: an analysis of the literature, Med. Sci. Sports Exerc. 30 (1998) 1183–1190.
[5] M.A. Childress, A. Beutler, Management of chronic tendon injuries, Am. Fam. Physician 87 (2013) 486–490.
[6] O. Virchenko, B. Skoglund, P. Aspenberg, Parecoxib impairs early tendon repair but improves later remodeling, Am. J. Sports Med. 32 (2004) 1743–1747.
[7] S.T. Ferry, L.E. Dahners, H.M. Afshari, P.S. Weinhold, The effects of common anti-inflammatory drugs on the healing rat patellar tendon, Am. J. Sports Med. 35 (2007) 1326–1333.
[8] C. Forslund, B. Bylander, P. Aspenberg, Indomethacin and celecoxib improve tendon healing in rats, Acta Orthop. Scand. 74 (2003) 465–469.
[9] O. Sandberg, P. Eliasson, T. Andersson, F. Agholme, P. Aspenberg, Etanercept does not impair healing in rat models of tendon or metaphyseal bone injury, Acta Orthop. 83 (2012) 305–310.
[10] Y. Bi, D. Ehirchiou, T.M. Kilts, C.A. Inkson, M.C. Embree, W. Sonoyama, L. Li, A.I. Leet, B.M. Seo, L. Zhang, S. Shi, M.F. Young, Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche, Nat. Med. 13 (2007) 1219–1227.
[11] J. Zhang, J.H. Wang, Characterization of differential properties of rabbit tendon stem cells and tenocytes, BMC Musculoskelet. Disord. 11 (2010) 10.
[12] Y.F. Rui, P.P. Lui, G. Li, S.C. Fu, Y.W. Lee, K.M. Chan, Isolation and characterization of multi-potent rat tendon-derived stem cells, Tissue Eng. Part A 16 (2010) 1549–1558.
[13] A.B. Lovati, B. Corradetti, A. Lange Consiglio, C. Recordati, E. Bonacina, D. Bizzaro, F. Cremonesi, Characterization and differentiation of equine tendon-derived progenitor cells, in: J. Biol. Regul. Homeost. Agents 25 (2011) S75–S84.
[14] J. Zhang, T. Pan, Y. Liu, J.H. Wang, Mouse treadmill running enhances tendons by expanding the pool of tendon stem cells (TSCs) and TSC-related cellular production of collagen, J. Orthop. Res. 28 (2010) 1178–1183.
[15] S.C. Juneja, C. Veillette, Defects in tendon, ligament, and enthesis in response to genetic alterations in key proteoglycans and glycoproteins: a review, Arthritis (2013) 154812.
[16] C.A. Carlstedt, K. Madsen, T. Wredmark, The influence of indomethacin on tendon healing. A biomechanical and biochemical study, in: Arch. Orthop. Trauma. Surg. 105 (1986) 332–336.
[17] W.C. Tsai, C.C. Hsu, S.W. Chou, C.Y. Chung, J. Chen, J.H. Pang, Effects of celecoxib on migration, proliferation and collagen expression of tendon cells, Connect. Tissue Res. 48 (2007) 46–51.
[18] J.H. Yoon, J. Halper, Tendon proteoglycans: biochemistry and function, J. Musculoskelet. Neuronal Interact. 5 (2005) 22–34.

[19] R.J. McCormick, Extracellular modifications to muscle collagen: implications for meat quality, Poult. Sci. 78 (1999) 785–791.
[20] K.G. Danielson, H. Baribault, D.F. Holmes, H. Graham, K.E. Kadler, R.V. Iozzo, Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility, J. Cell Biol. 136 (1997) 729–743.
[21] E. Schönherr, P. Witsch-Prehm, B. Harrach, H. Robenek, J. Rauterberg, H. Kresse, Interaction of biglycan with type I collagen, J. Biol. Chem. 270 (1995) 2776–2783.
[22] L. Ameye, D. Aria, K. Jepsen, A. Oldberg, T. Xu, M.F. Young, Abnormal collagen fibrils in tendons of biglycan/fibromodulin-deficient mice lead to gait impairment, ectopic ossification, and osteoarthritis, FASEB J. 16 (2002) 673–680.
[23] K.J. Jepsen, F. Wu, J.H. Peragallo, J. Paul, L. Roberts, Y. Ezura, A. Oldberg, D.E. Birk, S. Chakravarti, A syndrome of joint laxity and impaired joint integrity in lumican- and fibromodulin-deficient mice, J. Biol. Chem. 277 (2002) 35532–35540.
[24] T.A. Järvinen, L. Jozsa, P. Kannus, T.L. Järvinen, M. Kvist, T. Hurme, J. Isola, H. Kalimo, M. Järvinen, Mechanical loading regulates tenascin-C expression in the osteotendinous junction, J. Cell Sci. 112 (1999) 3157–3166.
[25] S. Dimmen, L. Engebretsen, L. Nordsletten, J.E. Madsen, Negative effects of parecoxib and indomethacin on tendon healing: an experimental study in rats, Knee Surg. Sports Traumatol. Arthrosc. 17 (2009) 835–839.
[26] R. Schweitzer, J.H. Chyung, L.C. Murtaugh, A.E. Brent, V. Rosen, E.N. Olson, A. Lassar, C.J. Tabin, Analysis of the tendon cell fate using Scleraxis, a specific marker for tendons and ligaments, Development 128 (2001) 3855–3866.
[27] C. Shukunami, A. Takimoto, M. Oro, Y. Hiraki, Scleraxis positively regulates the expression of tenomodulin, a differentiation marker of tenocytes, Dev. Biol. 298 (2006) 234–247.