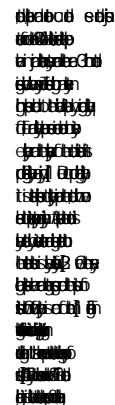


# The Mechanisms and Functions of Transforming Growth Factors- $\beta$ 1 in Tendon Injury

Yujie Li<sup>1</sup>, Yuanqiu Peng<sup>1</sup>, Bin Zhu<sup>1</sup>, Xueli Liu<sup>1</sup>, Xinyue Liu<sup>1</sup>, Sheng Guo<sup>2</sup>, Chenglong Wang<sup>2</sup>, Dingxuan Wang<sup>1</sup>, Sen Li<sup>1,2\*</sup>

<sup>1</sup>Institute of Physical Education, Southwest Medical University, Luzhou, Sichuan, China

<sup>2</sup>



## GF- $\beta$ 1 I

Inflammation is a physiological response to injury and is part of tendon healing. The initial inflammation phase begins shortly after the injury. Inflammatory cells such as neutrophils, monocytes, and macrophages are attracted to the injury site by pro-inflammatory cytokines and produce cytokines and growth factors [7]. The expression level of TGF- $\beta$ 1 increases immediately after tendon injury, released from degranulated platelets, and secreted by all major cell types involved in the repair process, including lymphocytes, macrophages, endothelial cells, and smooth muscle cells, epithelial cells and fibroblasts [8,9]. TGF- $\beta$ 1 attracts inflammatory cells such as white blood cells and macrophages. These cells will release more TGF- $\beta$ 1 and induce a more pronounced inflammatory response [10]. *In vitro* experiments have shown that the addition of exogenous TGF- $\beta$ 1 (2 ng/ml) to mast cells enhances the expression of COX-2 in tendon cells and reaches a peak in 6 hours. The TGF- $\beta$ 1 receptor ALK5 kinase inhibitor A83-01 significantly blocked COX-2 mRNA and COX-2 protein expression [11]. COX-2 is one of the main enzymes that contribute to the production of PGE2, which is the main inflammatory mediator in tendons [12]. Therefore, Mast cell-

derived TGF- $\beta$ 1 plays an important role in enhancing the production of inflammatory mediator PGE2 by human tendon cells. In contrast, neutralizing TGF- $\beta$ 1 with anti-TGF- $\beta$ 1 antibodies can reduce inflammation and angiogenesis. In addition, no inflammatory cells were found at the site of sheep fetal tendon injury. In contrast, there are inflammatory cells in adult tendons. And comparing the fetal tendon with the injured tendon specimen, the injured fetal tendon did not show an increase in TGF- $\beta$ 1 expression, and there was strong TGF- $\beta$ 1 staining around the injured adult tendon and the temporary wound area. The decrease of TGF- $\beta$ 1 in the fetal tendon may be the reason for the lack of inflammatory infiltration in the tendon injury site [13]. It can be concluded from the above that TGF- $\beta$ 1 is a growth factor, but it plays a pro-inflammatory role in the healing of tendon injuries.

Whether inflammation is beneficial to tendon healing is difficult to say. On the one hand, inflammation can promote tendon healing and closure through fibrosis and scar formation, and can prevent tendon infection; on the other hand, scar healing caused by inflammation can hinder tissue regeneration and may further reduce the function of tissues or organs [14]. However, inflammation is an inevitable process of adult tendon injury healing. In most cases, the inflammatory process will gradually subside, but in some case, it will enter the chronic inflammation stage. This chronic tendon inflammation is thought to be a long-term imbalance and maladaptive response to injury [15]. Therefore, inflammation should not be prevented as a necessary step for healing, but inflammation should be regulated and controlled. However, the molecular and cellular mechanisms involved in inflammation are poorly understood and need to be further understood.

**\*Corresponding author:** Sen Li, Institute of Physical Education, Southwest Medical University, Luzhou, Sichuan, China, A fliated Hospital of Southwest Medical University, Lonngmatan City, Luzhou, city, China, Tel: +13679676684; E-mail: jht187@163.com

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## GF-1 A

Tendons are hypocellular tissues mainly composed of tenocytes, and a stem and progenitor cell population [16]. Tenocytes make up the majority of the cellular content of the tendon and are integral in indispensable the healing process following injury [17]. Many previous literatures have studied the role of TGF- $\beta$ 1 after tendon injury to promote the healing of tendon injury. The following summarizes the effect of TGF- $\beta$ 1 on tenocytes.

After the tendon tissue is injured, its natural healing is extremely slow and inefficient when it is severely damaged due to its intrinsic hypocellularity [18,19]. TGF- $\beta$ 1 increases the proliferation of tenocytes. In a recent *in vitro* study, acellular amnion released TGF- $\beta$ 1 to cause the rapid proliferation in tenocytes of adult Leghorn chickens with relatively static properties [20]. Identically after 48 hours of exposure to 1ng/ml TGF- $\beta$ 1 in human tenocytes, there was a significant increase in cell proliferation, as shown with crystal violet method, as compared to control [21]. In addition, TGF- $\beta$ 1 promotes the proliferation of tendon stem cells (TSC, and when TGF- $\beta$  receptors are blocked by TGF- $\beta$  receptor inhibitor (ITD-1), the proliferation of TSC will be significantly reduced [22]. The activation of TGF- $\beta$ /BMP signaling pathway by TGF- $\beta$ 1 may promote proliferation during tendon injury. TGF- $\beta$ 1 may promote the proliferation during tendon injury by activating the TGF- $\beta$ /BMP signaling pathway [23]. The molecular mechanism of the proliferation of tendon cells may be through the up-regulation of proliferating cell nuclear antigen(PCNA) gene and protein expression through TGF- $\beta$ 1[24] (Figure 1).

## M

The migration of tenocytes is a fundamental biological behavior and is a significant factor to promote tendon repair [25]. In the process of tendon healing, tenocytes migrate into the injured site, secreting collagen and reorganizing the extracellular matrix (ECM) to repair the tendon [16]. *In vitro* experiment showed that treatment of TGF- $\beta$ 1 promoted cell migration. However, inhibition of TGF- $\beta$ 1 by transfection of sh-TGF- $\beta$ 1 or treatment of TGFR I/II inhibitor partially reversed the migration of tenocytes [26]. In a wound healing experiment, the migration effect of TGF- $\beta$ 1 carried by the exosomes of TSC on TSC, compared with the control group, the migration ability of TSC was significantly enhanced. In addition, TGF- $\beta$  receptor inhibitors can significantly reduce TSC migration [22]. However, the molecular mechanisms controlling the migration of TSC during tendon repair are not well understood.

## D

It is generally accepted that cellular activation stimulates the conversion of fibroblasts and tenocytes to myofibroblasts represents a key event during wound healing and tissue repair [27]. Because myofibroblasts induce the expression of alpha-smooth muscle actin ( $\alpha$ -SMA), they gain contractility and contribute to the repair of physiological tissues. It is well established that tendon fibroblasts can increase the expression of  $\alpha$ -SMA and activate its transformation into myofibroblasts after treatment with TGF- $\beta$ 1 [28,29]. In addition, the contraction of myofibroblasts also results in the release of TGF- $\beta$ 1 from these cells, which may promote the further trans-differentiation of fibroblasts through a feed-forward mechanism [30,31]. Activation of TGF- $\beta$ 1 causes phosphorylation of smad2/3 to up-regulate the expression of  $\alpha$ -SMA [32]. In addition, *in vitro* experimental studies have shown that TGF- $\beta$ 1 can induce tendon stem cells to differentiate

into tendon cells. During the differentiation process, the levels of phosphorylated Smad1/5/8 and Smad2/3 were increased [23]. Further studies have shown that the inhibitor-Smad seems to be related to tendon Stem cell differentiation is negatively correlated, meaning that TGF- $\beta$ /BMP signaling is involved in the differentiation process of TSC [33]. Further research will be required to determine the extent to which TGF- $\beta$ 1 promotes fibroblast and TSC trans-differentiation, as this may represent an important mechanism through which TGF- $\beta$ 1 may provide benefit to tendon repair.

## GF-1 A M E M

TGF- $\beta$ 1 is known for its effect on the production of extracellular matrix in tendons. TGF- $\beta$ 1 can modulate the production of collagen. Collagen is an essential ECM component of tendons, and their formation and configuration are important factors that determine tendon repair. Although there are numerous collagen types, Type I collagen and Type III collagen seem to play the most important role in tendon healing [34]. A number of *in vitro* experiments showed that compared with control tendon fibroblasts, TGF- $\beta$  treatment of tendon fibroblasts resulted in increased expression of type I collagen and type III collagen [28,35,36]. Eduardo Anitua, et al. [37] showed that regardless of the concentration of TGF- $\beta$ 1, the production of type I collagen is similar. It is suggested that higher TGF- $\beta$ 1 concentration has nothing to do with the further increase of type I collagen. In another experiment, it was shown that TGF- $\beta$ 1 inhibited gene expression of procollagen Type III with fibronectin anchorage only at a low concentration (0.1 ng/mL) [38]. It is suggested that in the later stage of tendon healing, the reduction of TGF- $\beta$ 1 levels can inhibit the accumulation of type III collagen. TGF $\beta$ 1 has been shown to mediate the expression of collagen through Smad and ERK pathways [39]. In a rat Achilles tendon rupture repair model, one week after the injection of exogenous TGF- $\beta$ 1, it was found that the expression of type I and type III procollagen mRNA increased in a dose-dependent manner and increased the mechanical strength of the rat tendon repair [40]. Collagen cross-linking is an important step to impart strength to collagen fibers [7]. This process is mainly promoted by lysyl oxidase, and the synthesis of lysyl oxidase can 0.043 T6 Tw 0 -nS6 (ep

collagen, fibronectin and proteoglycans [41]. Tissue inhibitors of metalloproteinases (TIMPs) are a class of proteins that bind to the active site of the MMP catalytic domain to exert an endogenous inhibitory effect on MMPs [42]. MMPs and TIMPs interact to make ECM in dynamic equilibrium. TGF- $\beta$ 1 can regulate their expression. The cultured flexor tendon cells were treated with TGF- $\beta$ 1, and the effect of TGF- $\beta$ 1 on the expression of MMP and MMP inhibitor was studied. The results showed that TGF- $\beta$ 1 had little effect on the expression of MMP-2, MMP-3 and MMP-14, but it resulted in a significant dose-dependent decrease in MMP-16 gene expression and did not affect TIMP-2 expression [35]. In the rat rotator cuff repair model, the expression of MMP-9 and MMP-13 in the TGF- $\beta$ 1 treatment group was significantly lower than that of the control group at 2 weeks postoperatively [43]. Farhat, et al. [44] proposed that TGF- $\beta$ 1 produces plasminogen activator inhibitor 1 (PAI-1) that inhibits the production of plasmin and MMP-2. These experiments show that the effect of TGF- $\beta$ 1 on the extracellular matrix is inclined to favor the production of ECM. However, the regulation of TGF- $\beta$ 1 on the extracellular matrix is complex and diverse and requires in-depth study (Figure 2).

## GF- $\beta$ 1 A

A well-known feature of TGF- $\beta$ 1 in tendon tissue is its ability to stimulate collagen synthesis. Less attention is paid to the fact that TGF- $\beta$ 1 can indirectly promote angiogenesis. Healthy tendon tissue is an area lacking blood supply. After tendon injury, blood vessels are destroyed, and new blood vessels form new vascular network and granulation tissue to transport nutrients necessary for tendon repair [45]. The formation of new blood vessels is mainly mediated by vascular endothelial cell growth factor (VEGF). The up-regulation of VEGF expression in endogenous tendon cells plays an important role in the process of neovascularization and tendon repair [46]. TGF- $\beta$  has been shown to up-regulate the expression of VEGF in many connective tissues [47-49]. In the rotator cuff tear model, TGF- $\beta$ 1 slows the expression of VEGF in the extracellular matrix of fibroblasts [50]. *In vitro*, experiments have shown that TGF- $\beta$ 1 secreted by platelets leads to an increase in VEGF of tendon cells and induces a potential angiogenic response [37]. The above suggests that TGF- $\beta$ 1 promotes angiogenesis by up-regulating VEGF, which may be one of the mechanisms by which TGF- $\beta$ 1 promotes tendon repair.

## C

TGF- $\beta$ 1 is an important regulator of tendon repair. Accelerate the healing process of tendons by promoting cell proliferation, angiogenesis, collagen remodeling, etc. However, while exogenous TGF- $\beta$ 1 promotes tendon repair, it also promotes inflammation, collagen synthesis, and extracellular matrix deposition, and at the same time causes tendon

adhesion and scar formation. The formation of adhesions causes the tendons to bond with each other and with surrounding structures, thereby preventing normal sliding motion. Scarring causes the tendon tissue to weaken. Studies have shown that anti-TGF- $\beta$ 1 neutralization reduces tendon adhesions and scars, but reduces the mechanical strength of tendons. In the future, it is possible to in-depth study of the mechanism of TGF- $\beta$ 1 on tendon repair, so that the tendon can heal under the condition of intact function, and can reduce the formation of minimal adhesion.

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YJL, XYL designed the present study. YJL and RZ prepared and wrote the manuscript. BZ, XLL, SG and CLW performed a literature search and selected the studies to be included. YJL, DXW and SL revised the manuscript. All authors approved the final version of the article.

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