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Pol. J. Sport Tourism **2024**, *31(2), 3-10*
DOI: 10.2478/pjst-2024-0008

Review paper

TGF-B ISOFORMS: TGF-β1, TGF-β2 AND TGF-β3 IN LIGAMENT AND TENDON HEALING

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Abstract

Introduction. Tendon and ligament injuries affect people of all ages, including professional athletes. Tumor Growth Factor β (TGF- β) isoforms play a significant role in the regeneration of tendons and ligaments, such as through the recruitment of macrophages and fibroblasts or the regulation of the formation of extracellular matrix (ECM). Although the TGF- β family regulates signalling pathways related to the healing process, excessive TGF- β activation can lead to fibrosis and the formation of scars and adhesions. This article recapped the significance of TGF- β isoforms TGF- β1, TGF- β2, and TGF- β3 in the physiological healing of injured tendons and ligaments. ***Material and Methods.*** A total of 1434 articles were identified using the PubMed search string (TGF-β isoforms or TGF-β1 or tgfb1 or TGF-β2 or tgfb2 or TGF-β3 or tgfb3) and (tendon or ligament). Human studies, animal models and in vitro cultures were included in the search. Forty-nine published articles were included. ***Results.*** Histologic evidence demonstrated greater ligament and tendon regeneration and collagen type I expression when using TGF-β1, often leading to fibrosis. TGF-β3 inhibits the TGF-β1 and TGF-β2 by switching Smad2/3 signaling to Smad7 and CREB-1 transcription factor. There was a delay in TGF-β3 level peak compared to other isoforms. ***Conclusions.*** All 3 TGF-β isoforms seem to play a significant role in the subsequent stages of healing. We state a hypothesis that during the initial phase of tendon and ligament healing, TGF-β1 levels need to be elevated. Further, TGF-β3 may inhibit the action of TGF-β1 and TGF-β2, which leads to the inhibition of inflammation and changes in ECM production, and consequently to a reduction in the level of scarring.

Key words: TGF-β isoforms, TGF-β1, TGF-β2, TGF-β3, tendon healing, ligament healing

Introduction

Tendon and ligament injuries constitute a significant proportion of musculoskeletal injuries, and they are a prevalent injury, affecting individuals of all ages, including professional and amateur athletes. Tendons and ligaments are classified as soft tissues, primarily composed of connective tissue that connects muscles and bones or bone to bone, thereby stabilizing the joint.

In the case of damage in both tissues, the healing process is similar, divided into 3 main phases. As a result of the injury, initially, up to 7 days, accumulation of blood and immune cells is observed at the site of injury [1]. The infiltration of platelets, macrophages and neutrophils is associated with the initiation of the inflammatory process, resulting in the release of pro-inflammatory cytokines and growth factors. The released factors are a signal for fibroblasts that migrate to the site of injury and to start the synthesis of elements of the extracellular matrix (ECM). The main component of the extracellular matrix (ECM) of tendons is hierarchically organized type I collagen, supplemented by other types of collagen, elastins and proteoglycans [2]. In addition to type I collagen, type III collagen and elastin play a significant role in the construction of ligaments, thereby facilitating some degree of flexibility [3].The proliferation phase follows, recruited tendon progenitor cells and fibroblasts intensively divide and mature, taking part in the production of proteoglycans and collagen, especially type III [4]. Intensified ECM deposition at the injury site increases tendon strength. In the formation phase, the structure and organization of the deposited collagen change, related to its rearrangement, and aimed at recreating the original arrangement of fibers. After about 6-8 weeks after the injury, the reconstruction phase begins, which is estimated to last up to two years [5]. A further reorganization occurs in the ECM of the tendons/ligaments. The predominant type III collagen, which was prevalent in the initial phases, is replaced by type I collagen, which exhibits greater mechanical resistance. The process that leads to the regeneration of injured tendons may be disrupted, for instance, by prolonged inflammation or the proliferation phase. The overproduction of ECM results in the formation of scars and adhesions, significantly affecting the functionality and resistance of the newly formed tissue [4].

The ability to regenerate poorly vascularized tendons or intra-articular ligaments is limited. It has been demonstrated that in the event of significant tears in the rotator cuff, a staggering 73% of patients fail to heal properly following surgery [6]. The result is a tendency to re-injure. Currently, a significant number of researchers are focusing on the identification of appropriate modulators of the biological environment of the injury site, such as the administration of exogenous growth factors, with the aim of enhancing the repair process and preventing the emergence of pathological scars.

Growth factors (GF) play a significant role in all phases of tendon and ligament healing. The most extensively studied are transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) [7]. Among mentioned GF, exogenous PGDF administration is considered in the treatment of difficult to heal injuries of tendons and ligaments, such as rupture of the posterior cruciate ligament [8]. It is worth mentioning that in animal model the administration of exogenous FGF significantly accelerated the healing of tendons and significantly improved their biomechanical resistance [9]. The results of studies reveal various mechanisms of action for isoforms, or the occurrence of negative consequences associated with the administration of growth factors in therapeutic interventions. Therefore, further research on this topic is required.

This article focuses on the significance of TGF-β isoforms TGF-β1, TGF-β2, and TGF-β3 in the physiological healing of injured tendons and ligaments.

Material and methods

The literature was searched through in the PubMed online databases from the inception until August 1, 2023. The main search strategy included terms: (TGF-β or TGF-β1 or tgfb1 or TGF-β2 or tgfb2 or TGF-β3 or tgfb3) and (ligament or tendon). Human studies, animal models and in vitro cultures were included in the search. For the growth factors' section, search strategy included terms: growth factor in tendon healing or growth factors in ligament healing. A restriction was imposed to publications only in English and articles no older than 25 years. The published studies were included in the systematic review if they were peer-reviewed original articles. An initial keyword search yielded 1134 reports, of which 1052 were excluded due to repeat publications in the databases, for inappropriate study design, or results not relevant to the research questions. One reviewer reviewed all studies by title and excluded clearly irrelevant studies, next checked all other abstracts. Ultimately, 49 publications were eligible to provide the contribution to the study aim.

Results

TGF-β role in regeneration

The TGF-β is a cytokine that regulates many processes in the human body, essential for the proper functioning of the immune system, regulating inflammation, tissue regeneration and angiogenesis [10]. The TGFβ-family includes three isoforms: TGF-β1, TGF-β2, and TGF-β3; encoded by different genes. They are present and play a significant role in the regeneration of tendons and ligaments, such as by recruiting macrophages and fibroblasts [11] or regulating the formation of ECM [12] (Tab. 1).

Regulating the above-mentioned processes is carried out via two signaling pathways: canonical and noncanonical [13]. The canonical pathway is well understood – the ligand binds to the TβRI and TβRII receptors present on the surface of the cell membrane. The change in gene expression is regulated by Smad signaling, which is regulated by negative feedback [14]. The signaling pathway mediated by TGF-β/Smad2/3 results in the apoptosis inhibition and proliferation of fibroblasts, there-

Table 1. Comparison of TGF-β isoforms function in tendon healing

	Isoform		
Function	**TGF-β1**	**TGF-β2**	**TGF-β3**
Effect on collagen	1) increases COL1A1 and COL3A1 mRNA in cultured tendon fibroblasts [19] 2) upregulates expression of type I and type III collagen in a rat model of Achilles tendon healing [30]	1) increases COL1A1 mRNA in cultured tendon fibroblasts [19] 2) upregulates of collagen types I, IV, V, VIII, XI, XII, XV, and XVIII in bone marrow-derived Mesenchymal Stem Cells [46]	1) the strongest increase in COL1A1 mRNA in cultured tendon fibroblasts, of all isoforms; increase in COL3A1 mRNA in cultured tendon fibroblasts [19] 2) favorable changes in the ratio of type I to type III collagen [37]
Tenocytes activity	promotes fibrotic activity in rotator cuff tenocytes [65]	promotes tenocytes differentiation into a fibrotic phenotype [43]	promotes tenogenic differentiation of adult adipose derived stem cells and mesenchymal stem cells [66]
Cells migration	promotes migration in rotator cuff tenocytes [65]	increases tenocyte migration [46]	enhances migration of adipose derived mesenchymal stem cells [67]
Proliferation	increases expression of proliferation genes in fibroblasts, promotes tenocytes proliferation [65]	stimulates fibroblasts proliferation, but no data from tendon were found [68]	increased cell proliferation at the repair site only till 28th day post injury in in vivo studies
MMPs and its inhibitors	1) decreases MMP-1 expression and increases MMP3 expression in human tenocytes [51] 2) enhances expression of MMP-1, -2, -3, and -12 genes in fibroblasts [37] 3) decreased expression of MMP-16 [69]	1) downregulates MMP 9, TIMP 3, TIMP4 in bone marrow-derived Mesenchymal Stem Cells [46]	1) reduces expression of MMP2 and MMP9 genes in in vivo studies [63]
ECM components, except collagens	downregulates decorin and upregulates biglycan [69]	upregulates tenascin-C, biglycan, elastin, and fibronectin in bone marrow-derived Mesenchymal Stem Cells [46]	enhances gene expression of decorin, tenascin-C and elastin genes combined with Mohawk overexpression [70]
Scars/ Adhesion formation	Increased risk	Increased risk	Probably decreased risk

by facilitating the repair and production of ECM in damaged tissues [15]. Other signaling pathways activated by TGF-β include all three MAPK signaling pathways: extracellularly activated protein kinase (ERK), p38 mitogen-activated kinase (MAPK), and Jun-N-terminal kinase (JNK) [13]. The activation of TGF-β by TAK1 leads to the induction of NF-B, which persists throughout the healing process of tendons and ligaments [14]. The inhibition of the NF-kB signal has been associated with reducing inflammation and tendon cell proliferation, as well as reducing fibrous tissue formation [16]. The activation of the ERK1/2 signaling pathway by TGF-β has been implicated in the proliferation of cells and fibrosis after injury to the tendon or ligament [17]. In tendon cells, the p38/JNK signaling pathway is associated with the Smad-dependent pathway, which regulates metalloproteinase (MMP) 13 expression. The collagenase MMP 13 levels increase in injured tendons [18]. The p38 signaling pathway is crucial for tendon growth after injury. As can be seen, the TGF-β family regulates the activity of several signaling pathways directly and indirectly related to the healing processes of tendons and ligaments. It is noteworthy that TGF-β excessive activation can result in fibrosis and the formation of scars and adhesions.

TGF-β1 in tendon/ligament healing

Numerous studies conducted on animal models, in vitro cultures, and clinical analyses have confirmed the important role of TGF-β1 in the regeneration of tendons and ligaments. In the acute inflammatory phase, an increase in the concentration of TGF-β1 is observed and is present in all early stages of healing [19, 20].

During the inflammatory phase, immune cells infiltrate the damaged tissue. It has been demonstrated that TGF-β1 at this stage is predominantly derived from external sources and is not produced by tenocytes or fibroblasts, as factor mRNA was detected in a limited number of cells present in the tissue [19]. The regulation of the inflammatory reaction is one of the functions of TGF-β1, for example by changing the phenotype of macrophages from M1 to M2, important during healing and suppression of inflammation [21]. Macrophages might be one of the main sources of TGF-β1 [22]. In a study conducted by de la Durantaye et al. [23], it was observed that the depletion of circulating monocytes/macrophages in a mouse model of Achilles tendon injury resulted in a decrease in the level of detected TGF-β1 and a modification in its distribution as early as 7 days after injury. At day 28, the depletion of circulating macrophages also resulted in a reduction in cell proliferation, ECM formation, and scar formation. The significance of the observed modifications in TGF-β1 is supported by investigations in a transgenic mouse model. The effect of macrophage depletion was abrogated by growth factor overexpression, and pathological fibrotic processes were observed in the damaged tissue. Another potential external source of TGF-β1 during the initial stages of healing could be platelets, which release growth factor through the degranulation of α-granules during inflammation [24]. Although TGF-β1 is an important mediator of angiogenesis and ECM formation, it is known that it contributes to the formation of scar tissue by stimulating collagen synthesis and inhibition of collagen degradation [25].

Depending on the environment, the pattern of release and localization of TGF-β1 may be important for the course of the healing process. Earlier conclusions are confirmed by studies on exogenous factor therapies for the damage to tendons and ligaments. The administration of exogenous platelet-rich plasma (PRP) resulted in significant changes in the localization and level of TGF-β1 in the injured tissue [26]. There was a shift in TGF-β1 levels, which peaked in the first week but decreased significantly by week 4. In this case, the indicated peak shift confirms the important role of platelets, derived from PRP, as a source of TGF-β1. In the groups not treated with PRP, peak values were observed at 2 weeks after injury, while they remained high even at 4 weeks [26]. It should be emphasized that in the period of 2 and subsequent weeks from the injury, the origin of TGF-β1 is cellular, depending on the initial presence of endothelial and inflammatory cells at the site of injury. The enrichment of tenocyte exosomes with TGF-β1 resulted in increased cell proliferation and migration to the site of inflammation [17]. The administration of exogenous TGF-β1 in the replacement of the anterior cruciate ligament increased the binding strength of the graft to the tunnel wall, hence the conclusion that, in the early stages of healing, increasing levels of this protein were beneficial [27]. However, for the patellar tendon, an increased tensile strength of the regenerated fibrous tissue was found [28]. Administration of exogenous TGF-β1 via the Smad3 signaling pathway inhibits the loss of ECM by human utero-sacral ligament fibroblasts [29].

In the advanced stages of healing, exogenous TGF-β1 changes the pattern of expression of type I and type III collagen, which results in better biomechanical properties of healing tendons and ligaments [28, 30]. During week 4, the initial elevation of TGF-β1 throughout the lesion is reduced to being present in the lateral margins of the wound [19]. In the animal model, it was observed that mRNA expression for collagen I and III was observed starting from the 14th day, accompanied by an increase in cellular activity at the site of injury to the patellar tendon [19].

Before the start of ECM reconstruction, TGF-β1 significantly increased the number of fibroblasts by promoting proliferation and apoptosis inhibition [15]. In vitro studies have revealed an increase in the mitogenicity of patellar tendon fibroblasts and an increase in proteoglycan synthesis in explants [31]. The canonical signaling pathway promotes the synthesis of collagens, as well as its proper transformation from collagen III to the more mechanically resistant collagen I [28, 30, 32]. The increased expression of TGF-β1/Smad2/3 in the damaged Achilles tendon significantly enhanced collagen production [33]. Long-term high levels of TGF-β1 are associated with a greater risk of adhesion formation and impairment of tendon/ligament functionality. In vitro studies already indicated TGF-β1 as a pro-fibrotic factor, 30 years ago [34]. Inhibition of the canonical signaling pathway decreased collagen III mRNA expression [35]. Furthermore, TGF-β1 regulates matrix remodeling by also regulating the production of metalloproteinase inhibitors (TIMP) and MMPs within the body [36]. In fibroblasts, TGF-β1 has been shown to enhance the expression of the MMP-1, -2, -3, and -12 genes [37]. The decrease in MMP-1 expression and the rise in MMP3 and collagen III expression were associated with mechanical stress, and the canonical TGF-β signaling pathway was responsible for initiating this process [38, 39]. The importance of the environment in the regulation of the healing process by TGF-β1 is proven by the latest research on a mouse model. Extensive gene expression analysis revealed significant differences between mouse strains in their response to exogenous TGF-β1. Signaling pathways related to ECM organization and cell migration were enriched, and in one of the studied groups, additionally, the regulation of the cell cycle, mitosis and DNA transcription [40]. Various factors influence the level of endogenous TGF-β1,

including the presence of low-density lipoproteins (LDL) in the ECM. LDL reduces the production of TGF-β1 by tenocytes, thereby reducing collagen deposition [41].

TGF-β1 is essential for proper tendon and ligament healing and extracellular matrix reconstruction. Nonetheless, its elevated level in the later stages hinders the regeneration process and leads to scarring and the formation of adhesions. In order to fully exploit its therapeutic properties, further research should focus not only on the dose, but also on the method and time of distribution at the site of damage.

TGF-β2

Unlike the other TGF-β isoforms, research on the role of TGF-β2 has received relatively little attention. This trend has been slowly changing in the last few years, providing interesting data on the effect of TGF-β2 on the healing process of tendons and ligaments. It is known that its level reaches its highest point later than TGF-β1, which may be related to the antagonistic effect of the isoforms on each other [19].

The role of TGF-β2 in tendon development was recognized quite early on. In animal models, embryos with knockouts of the Tgfb2 and Tgfb3 genes lost most of their tendons and ligaments [42]. This indicates the tenogenic properties of these factors. The differentiation of mature tenomodulin-expressing tenocytes is associated with TGF-β2 [43]. The accumulation of type I and III collagens after stimulation with this isoform was demonstrated in vitro, in a dose-dependent manner [12]. Said changes appear to be mediated via canonical signaling by Smad3 phosphorylation. It is interesting that the tenogenic effect of TGF-β2 is weaker in mature cells. The study conducted by Brown et al. on mesenchymal stem cells and embryonic tendon progenitor cells revealed alterations in the activation of identical genes. However, the observed increases in expression were significantly lower in the second group [44]. TGF-β2 affects the expression of the gene for collagen I, i.e. COL1A1, but to a lesser extent than TGF-β3. It was important to note that there was no effect on the expression of collagen III gene – COL3A1 [19]. This demonstrates the importance of TGF-β2 for ECM reorganization and maturation rather than its production. Like TGF- β1, the observed level of the factor in the tissue or organism is also important for TGF-β2. Studies on Emery-Dreifuss muscular dystrophy type 2 and musculoskeletal dystrophy revealed the key role of TGF-β2 in the pathology development. Significantly higher levels of the factor were observed both in patients and animal models. The in vitro culture demonstrated the inhibition of myocyte differentiation and the activation of the fibrogenic program in tenocytes [43]. TGF-β2 has been implicated in scarring and adhesion formation during the healing of tendons and ligaments. Research on microRNA-378a, which inhibits the production of TGF-β2, has confirmed the importance of this gene in the healing of ligaments after injury. The miRNA-378a knock-in mutants showed a decrease in the production of type I collagen and a decrease in the levels of Scleraxis (Scx) and Mohawk (Mkx) transcription factors, as well as an inhibition of tenogenic differentiation, which in turn led to a delay in the healing process [45].

RNA sequencing studies have revealed that TGF-β2 has a wide range of biological activity. After TGF-β2 treatment of bone marrow-derived mesenchymal stem cells, a change in the expression of as many as 2,467 genes was found, including those related to the pathways of extracellular matrix formation (collagens, fibronectin and elastin), tissue development, binding of cell adhesion molecules and growth factor activity [46]. Studies clearly confirm the significant role of TGF-β2 in the healing of tendons and ligaments. However, as with TGF-β1, too high levels of the factor can lead to pathological changes in the advanced stages of regeneration.

TGF-β3

TGF-β3 is essential for the proper development and healing of tendons and ligaments. As previously mentioned, the Tgfb2 and Tgfb3 knockout mutants lacked formed tendons or ligaments [42]. In rotator cuff injury, the administration of exogenous TGF-β3 in vivo has been shown to accelerate the healing process and improve structural properties through changes in cellularity, vascularity, and cell proliferation [47]. Additionally, improvements in collagen organization and biomechanical properties, such as load resistance, are observed [48]. TGF-β3 is also indicated to be a tenogenic factor, especially in an environment rich in other growth factors, such as FGF and insulin-like growth factor 1 [49, 50, 51]. It is noteworthy to mention that radiotherapy, which is widely and efficaciously employed in soft tissue injuries, enhances the expression of TGF-β3 in the damaged tissue [52]. Although numerous studies have posited TGF-β3 as an anti-fibrotic factor, there are studies that do not confirm this effect (Tab. 2).

According to literature data, the level of TGF-β3 in damaged tissue peaks slightly later and is significantly lower than TGF-β1 [19]. It is important to note that in embryos, high levels of TGF-β3 and low levels of TGF-1 are seen in scar healing, while adults have low levels of TGF-β3 and high levels of TGF-β1 [53]. It appears that the ratio of isoform levels is crucial for the healing process. According to Chan et al., a concentration of 1 ng/ml TGF-β1 antagonizes the effect of TGF-β3 on the expression of COL1A1, while in isolated cultures the addition of TGF-β3 at a concentration of 0.1 ng/ml increased the level of COL1A1 mRNA significantly higher than the other isoforms [19]. Furthermore, increased expression of COL3A1 was demonstrated under the influence of TGF-β3 [19,52,54]. Studies confirm a positive correlation between the level of TGF-β3 and the total number of fibroblasts and their activity, which translates into the effectiveness of the healing process and ECM production [42]. Interestingly, increased TGF-β3 levels correlate with decreased Smad3 expression in human and animal mesenchymal stem cells, indicating inhibition of the Smad2/3 pathway associated with TGF-β1 signaling [55]. The decreased expression of Smad2/3 is correlated with a higher level of the transcription factor Scx, which plays a significant role as a tenogenic factor and in the formation of ECM in both embryos and mature animals [56]. At the same time, Smad7 expression increases at the site of injury [57]. This difference in the signaling pathway probably results in the reduction of scarring and the risk of adhesions mentioned at the beginning. Smad7 expression is suggested to be associated with the CREB-1 transcription factor, which is also indicated as antagonizing the pro-fibrotic effect of TGF-β1 [58]. A strong relationship between the level of CREB-1 and the level of TGF-β3 was established. CREB-1 inhibition resulted in a decreased level of TGF-β3 expression and an unfavorable change in the ratio of type I to type III collagen [59]. Research by Orfei et al. suggests an inhibitory effect of TGF-β3 in the early phase on decorin production, necessary for the proper production and arrangement of collagen, while increasing the level of tenogenic markers – decorin, Mkx and COL1A1 – in the later phase of healing. The inhibition of ROCK, strongly associated with increased adhesion, was also indicated by activation of integrins during healing after TGF-β3 stimulation [68]. The collected data allow us to state a hypothesis that during the healing of tendons and ligaments, TGF-β3 inhibits the action of TGF-β1 and

Table 2. Examples of TGF-β3 effects on collagen secretion and scar formation

	Model	Effect	Form of distribution	Ref
1.	In vitro culture of fibroblast from human patellar tendon populated on collagen gels	The administration of TGF-β1 resulted in a greater degree of collagen gel contraction in the fibroblasts compared to those receiving TGF-β3	In vitro culture supplementation	60
2.	Sprague-Dawley rats supraspinatus tendon acute repair by transosseous suture fixation augmented with Ca-P matrix with TGF-β3 use	The administration of TGF-β3 resulted in a greater expression of COLI in comparison with COLIII at the healing enthesis, indicating a more favorable ratio between COLI and COLIII with the administration of TGF-β3	Injectable Ca-P matrix with TGF-b3	47
3.	Sprague–Dawley rats supraspinatus tendons acute repair with fibrin matrices incorporated	The delivery of TGF-β3 to the healing tendon-to-bone insertion resulted in significant improvements in structural property	Fibrin matrices with the heparin/fibrin-based delivery system with TGF-β3	61
4.	Sprague–Dawley rats supraspinatus tendons acute repair	No significant difference between TGF-β isoforms in scar volume	An osmotic pump loaded with TGF-β isoforms s and/or neutralizing antibodies	62
5.	Sprague–Dawley rats toes flexor tendon injury	During flexor tendon tissue healing, type III collagen secretion was significantly decreased, indicating that TGF-β3 reduced the synthesis and secretion of collagen III associated with injury repair	TGF-β3 adenovirus vector treatment	63
6.	Sprague–Dawley rats supraspinatus tendons defect	Histologic scores were improved by adding TGF-β3	Injectable scaffold - gel crosslinked with transglutaminase	64

TGF-β2, both by negative feedback and by switching Smad2/3 signaling to Smad7. It results in the inhibition of inflammation and cell recruitment, and changes in ECM production, collagen deposition and reorganization, and consequently a reduction in the level of scarring.

Conclusions

All 3 TGF-β isoforms seem to play a significant role in the subsequent stages of healing. Most of the currently conducted works focus mainly on the creation of various types of scaffolds providing the selected TGF-β isoform and finding new forms of factor administration to the site of injury. In the literature, there is abundant evidence of both the beneficial effects and the formation of pathological changes within the healing tissue under the influence of exogenous TGF-β. It appears that it is impossible to develop one proper model of treatment, and the process may require individualization of the patient's therapy. The interaction and reciprocal inhibition of TGF-β isoform expression has physiological significance for the regeneration of tendon/ligament injuries, which is difficult to replicate in studies of exogenous administration of TGF-β in vivo. The multitude of animal models and in vitro studies used in studies on the effectiveness of TGF-β in healing pose great difficulties in interpreting the data. It is especially difficult to translate the results of research when interspecies differences are observed, for example, for the phosphorylation of Smad5 and Smad8 under the influence of TGF-β. Both the time of injury and the tissue localization of isoform expression should be considered in further studies. The authors believe that conducting such complex studies may be challenging, but they are necessary for a proper understanding of the healing processes of soft tissues and for the proper and safe implementation of TGF-β therapy in the clinic. In the authors' opinion, the best strategy for the regeneration of functional tissues is to focus on creating a scaffold that releases both TGF-β1 and TGF-β3 isoforms, but separates them temporarily and thus imitates the most important stages of healing.

Funding

This research was funded by the Ministry of Education and Science in 2023/2024 as part of the University Research Project of the University of Physical Education in Warsaw – UPB No. 1.

References

1. Lin T.W., Tony W., Cardenas L., Louis L.J., Soslowsky J. (2004). Biomechanics of tendon injury and repair. *Journal of Biomechanics* 37(6), 865-877. DOI: 10.1016/j.jbiomech.2003.11.005
2. Aparecida de Aro A., de Campos Vidal B., Pimentel E.R. (2012). Biochemical and anisotropical properties of tendons. *Micron* 43: 205–214. DOI: 10.1016/j.micron.2011.07.015
3. Nordin M., Frankel V.H. (2001). *Basic biomechanics of the musculoskeletal system*. Philadelphia: Lippincott Williams & Wilkins.
4. Müller S.A., Todorov A., Heisterbach P.E., Martin I., Majewski M. (2015). Tendon healing: an overview of physiology, biology, and pathology of tendon healing and systematic review of state of the art in tendon bioengineering. *Knee Surgery, Sports Traumatology, Arthroscopy* 23(7), 2097-2105. DOI: 10.1007/s00167-013-2680-z
5. Docheva D., Müller S.A., Majewski M., Evans C.H. (2015). Biologics for tendon repair. *Advanced Drug Delivery Reviews* 84, 222-239. DOI: 10.1016/j.addr.2014.11.015
6. Rashid M.S., Cooper C., Cook J., Cooper D., Dakin S.G., Snelling S. et al. (2017). Increasing age and tear size reduce rotator cuff repair healing rate at 1 year. *Acta Orthopaedica* 88(6), 606-611. DOI: 10.1080/17453674.2017.1370844

7. Liu C.F., Aschbacher-Smith L., Barthelery N.J., Dyment N., Butler D., Wylie C. (2011). What we should know before using tissue engineering techniques to repair injured tendons: a developmental biology perspective. *Tissue Engineering Part B* 17(3), 165-76. DOI: 10.1089/ten.TEB.2010.0662
8. Hildebrand K.A., Woo S.L.Y., Smith D.W., Allen C.R., Deie M., Taylor B.J. et al. (2016). The effects of platelet-derived growth factor-BB on healing of the rabbit medial collateral ligament. *The American Journal of Sports Medicine* 26(4), 549-554. DOI: 10.1177/03635465980260041401
9. Chan B.P., Fu S., Qin L., Lee K., Rolf C.G., Chan K.(2000). Effects of basic fibroblast growth factor (bFGF) on early stages of tendon healing: a rat patellar tendon model. *Acta Orthopaedica Scandinavica* 71(5), 513-518. DOI: 10.1080/000164700317381234
10. Chang H., Brown C.W., Matzuk M.M. (2002). Genetic analysis of the mammalian transforming growth factor-beta superfamily. *Endocrine Reviews* 23, 787-823. DOI: 10.1210/er.2002-0003
11. Amento E.P., Beck L.S. (1991). TGF-beta and wound healing. *Ciba Foundation Symposium* 157, 115-129. DOI: 10.1002/9780470514061.ch8
12. Klein M.B., Yalamanchi N., Pham H., Longaker M.T., Chang J. (2002). Flexor tendon healing in vitro: effects of TGF--beta on tendon cell collagen production. *Journal of Hand Surgery (American Volume)* 27(4), 615-620. DOI: 10.1053/jhsu.2002.34004
13. Zhang Y.E. (2017). Non-smad signaling pathways of the TGF-beta family. *Cold Spring Harbor Perspectives in Biology* 9(2), a022129. DOI: 10.1101/cshperspect
14. Derynck R., Zhang Y.E. (2003). Smad-dependent and smad-independent pathways in tgf-beta family signalling. *Nature* 425(6958), 577-584. DOI: 10.1038/nature02006
15. Zhou H., Jiang S., Li P., Shen H., Yang H., Xu S. et al. (2020). Improved tendon healing by a combination of tanshinone IIA and miR-29b inhibitor treatment through preventing tendon adhesion and enhancing tendon strength. *International Journal of Medical Sciences* 17(8), 1083-1094. DOI: 10.7150/ijms.44138
16. Chen S., Jiang S., Zheng W., Tu B., Liu S., Ruan H. et al. (2017). RelA/p65 Inhibition prevents tendon adhesion by modulating inflammation, cell proliferation, and apoptosis. *Cell Death & Disease* 8(3), e2710. DOI: 10.1038/cddis.2017.135.
17. Li M., Jia J., Li S., Cui B., Huang J., Guo Z. et al. (2021). Exosomes derived from tendon stem cells promote cell proliferation and migration through the TGF β signal pathway. *Biochemical and Biophysical Research Communications* 536, 88-94. DOI: 10.1016/j.bbrc.2020.12.057
18. Minkwitz S., Schmock A., Kurtoglu A., Tsitsilonis S., Manegold S., Wildemann B. et al. (2017). Time-dependent alterations of MMPs, TIMPs and tendon structure in human achilles tendons after acute rupture. *International Journal of Molecular Sciences* 18(10), 2199. DOI: 10.3390/ijms1810
19. Chan K.M., Fu S.C., Wong Y.P., Hui W.C., Cheuk Y.C., Wong M.W. (2008). Expression of transforming growth factor β isoforms and their roles in tendon healing. *Wound Repair and Regeneration* 16(3), 399-407. DOI: 10.1111/j.1524--475X.2008.00379.x
20. Sakai T., Yasuda K., Tohyama H., Azuma H., Nagumo A., Majima T. et al. (2002). Effects of combined administration of transforming growth factor-beta1 and epidermal growth factor on properties of the in situ frozen anterior cruciate ligament in rabbits. *Journal of Orthopaedic Research* 20(6), 1345-1351. DOI: 10.1016/S0736-0266(02)00065-7
21. Kawakubo A., Miyagi M., Yokozeki Y., Nakawaki M., Takano S., Satosh M. et al. (2022). Origin of M2 Mϕ and its macrophage polarization by TGF-β in a mice intervertebral injury model. *International Journal of Immunopathology and Pharmacology* 36, 3946320221103792. DOI: 10.1177/03946320221103792
22. Hays P.L., Kawamura S., Deng X.H., Dagher E., Mithoefer K., Ying L. et al. (2008). The role of macrophages in early healing of a tendon graft in a bone tunnel. *The Journal of Bone and Joint Surgery* 90(3), 565-79. DOI: 10.2106/JBJS.F.00531
23. de la Durantaye M., Piette A.B., van Rooijen N., Frenette J. (2014). Macrophage depletion reduces cell proliferation and extracellular matrix accumulation but increases the ultimate tensile strength of injured Achilles tendons. *Journal of Orthopaedic Research* 32(2), 279-85. DOI: 10.1002/jor.22504
24. Wakefield L.M., Smith D.M., Flanders K.C., Sporn M.B. (1988). Latent transforming growth factor-beta from human platelets. A high molecular weight complex containing precursor sequences. *Journal of Biological Chemistry* 263(16), 7646–54.
25. Werner S., Grose R. (2003). Regulation of wound healing by growth factors and cytokines. *Physiological Reviews* 83(3), 835-870. DOI: 10.1152/physrev.2003.83.3.835
26. Lyras D.N., Kazakos K., Tilkeridis K., Kokka A., Ververidis A., Botaitis S. et al. (2016). Temporal and spatial expression of TGF-b1 in the early phase of patellar tendon healing after application of platelet rich plasma. *The Archives of Bone and Joint Surgery* 4(2), 156-60.
27. Yamazaki S., Yasuda K., Tomita F., Tohyama H., Minami A. (2005). The effect of transforming growth factor-beta1 on intraosseous healing of flexor tendon autograft replacement of anterior cruciate ligament in dogs. *Arthroscopy* 21(9), 1034-41. DOI: 10.1016/j.arthro.2005.05.011
28. Anaguchi Y., Yasuda K., Majima T., Tohyama H., Minami A., Hayasi K. (2005). The effect of transforming growth factor--beta on mechanical properties of the fibrous tissue regenerated in the patellar tendon after resecting the central portion. *Clinical Biomechanics* 20(9), 959-65. DOI: 10.1016/j.clinbiomech.2005.05.012
29. Jabłońska-Trypuć A., Matejczyk M., Rosochacki S. (2016). Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. *Journal of Enzyme Inhibition and Medicinal Chemistry* 31, 177-83. DOI: 10.3109/14756366.2016.1161620
30. Kashiwagi K., Mochizuki Y., Yasunaga Y., Ishida O., Deie M., Ochi M. (2004). Effects of transforming growth factor-beta 1 on the early stages of healing of the Achilles tendon in a rat model. *Scandinavian Journal of Plastic and Reconstructive Surgery* 38(4), 193-197. DOI: 10.1080/02844310410029110
31. Spindler K.P., Imro A.K., Mayes C.E., Davidson J.M. (1996). Patellar tendon and anterior cruciate ligament have different mitogenic responses to platelet-derived growth factor and transforming growth factor beta. *Journal of Orthopaedic Research* 14(4), 542-546. DOI: 10.1002/jor.1100140407
32. Spindler K.P., Murray M.M., Detwiler K.B., Tarter J.T., Dawson J.M., Nanney L.B. et al. (2003). The biomechanical response to doses of TGF-beta 2 in the healing rabbit medial collateral ligament. *Journal of Orthopaedic Research* 21(2), 245-249. DOI: 10.1016/S0736-0266(02)00145-6

33. You T., Yuan S., Bai L., Zhang X., Chen P., Zhang W. (2020). Benzyl alcohol accelerates recovery from achilles tendon injury, potentially via TGF-β1/Smad2/3 pathway. *Injury* 51(7), 1515-1521. DOI: 10.1016/j.injury.2020.03.058
34. Ravitz M.J., Yan S., Dolce C., Kinniburgh A.J., Wenner C.E. (1996). Differential regulation of p27 and cyclin D1 by TGF--Beta and EGF in C3H 10T1/2 mouse fibroblasts. *Journal of Cellular Physiology* 168(3), 510-520.
35. Loiselle A.E., Yukata K., Geary M.B., Kondabolu S., Shi S., Jonason J. H. et al. (2015). Development of Antisense Oligonucleotide (ASO) Technology against Tgf-β Signaling to Prevent Scarring During Flexor Tendon Repair. *Journal of Orthopaedic Research* 33(6), 859-866. DOI: 10.1002/jor.22890
36. Zhang Q., Liu C., Hong S., Min J., Yang Q., Hu M. et al. (2017). Excess mechanical stress and hydrogen peroxide remodel extracellular matrix of cultured human uterosacral ligament fibroblasts by disturbing the balance of MMPs/TIMPs via the regulation of TGF b1 signaling pathway. *Molecular Medicine Reports* 15(1), 423-30. DOI: 10.3892/mmr.2016.5994
37. Xie J., Wang C., Huang, D.Y., Zhang Y., Xu J., Kolesnikov S.S. et al. (2013). TGF-beta1 induces the different expressions of lysyl oxidases and matrix metalloproteinases in anterior cruciate ligament and medial collateral ligament fibroblasts after mechanical injury. *Journal of Biomechanics* 46(5), 890-898. DOI: 10.1016/j.jbiomech.2012.12.019
38. Chen C.H., Lin Y.H., Chen C.H., Wang Y.H., Yeh M.L., Cheng T.L. et al. (2018). Transforming growth factor beta 1 mediates the low-frequency vertical vibration enhanced production of tenomodulin and type i collagen in rat achilles tendon. *PloS One* 13(10), e0205258. DOI: 10.1371/journal.pone.0205258
39. Jones E.R., Jones G.C., Legerlotz K., Riley G.P. (2013). Cyclical strain modulates metalloprotease and matrix gene expression in human tenocytes via activation of TGFβ. *Biochimica et Biophysica Acta* 1833(12), 2596-2607. DOI: 10.1016/j.bbamcr.2013.06.019
40. Kallenbach J.G., Freeberg M.A.T., Abplanalp D. Alenchery R.G., Ajalik R.A., Muscat S. et al. (2022). Altered TGFB1 regulated pathways promote accelerated tendon healing in the superhealer MRL/MpJ mouse. *Scientific Reports* 12, 3026. DOI: 10.1038/s41598-022-07124-4
41. Mousavizadeh R., Waugh C.M., DeBruin E., McCormack R.G., DuronioV., Scott A. (2023). Exposure to oxLDL impairs TGF-β activity in human tendon cells. *BMC Musculoskelet Disorders* 24, 197. DOI: 10.1186/s12891-023-06308-x
42. Pryce B.A., Watson S.S., Murchison N.D., Staverosky J.A., Dünker N., Schweitzer R. (2009). Recruitment and maintenance of tendon progenitors by TGFβ signaling are essential for tendon formation. *Development* 136(8), 1351-1361. DOI: 10.1242/dev.027342
43. Bernasconi P., Carboni N., Ricci G., Siciliano G., Politano L., Maggi L. et al. (2018). Elevated TGF β2 serum levels in Emery-Dreifuss Muscular Dystrophy: Implications for myocyte and tenocyte differentiation and fibrogenic processes. *Nucleus* 9(1), 337-349. DOI: 10.1080/19491034.2018.1467722
44. Brown J.P., Galassi T.V., Stoppato M., Schiele N.R., Kuo C.K. (2015). Comparative analysis of mesenchymal stem cell and embryonic tendon progenitor cell response to embryonic tendon biochemical and mechanical factors. *Stem Cell Research & Therapy* 6(1), 89 DOI: 10.1186/s13287-015-0043-z
45. Liu Y., Feng L., Xu J., Yang Z., Wu T., Zhang J. et al. (2019). MiR-378a suppresses tenogenic differentiation and tendon repair by targeting at TGF-β2. *Stem Cell Research & Therapy* 10, 108 DOI: 10.1186/s13287-019-1216-y
46. Koch D.W., Schnabel L.V., Ellis I.M. Bates R.E., Berglund A.K. (2022). TGF-β2 enhances expression of equine bone marrow-derived mesenchymal stem cell paracrine factors with known associations to tendon healing. *Stem Cell Research & Therapy* 13(1), 477. DOI: 10.1186/s13287-022-03172-9
47. Kovacevic D., Fox A.J., Bedi A., Ying L., Deng X.H., Warren R.F. et al. (2011). Calcium-phosphate matrix with or without TGF-β3 improves tendon-bone healing after rotator cuff repair. *The American Journal of Sports Medicine* 39(4), 811-819. DOI: 10.1177/0363546511399378
48. Reifenrath J., Wellmann M., Kempfert M., Angrisani N., Welke B., Gniesmer S. et al. (2020). TGF–β3 loaded electrospun polycaprolacton fibre scaffolds for rotator cuff tear repair: An in vivo study in rats. *International Journal of Molecular Sciences* 21(3), 1046. DOI: 10.3390/ijms21031046
49. Barsby T., Guest D. (2013). Transforming growth factor beta3 promotes tendon differentiation of equine embryo--derived stem cells. *Tissue Engineering Part A* 19, 2156-2165. DOI: 10.1089/ten.TEA.2012.0372
50. Havis E., Bonnin M.A., Esteves de Lima J., Charvet B., Milet C., Duprez D. (2016). TGFβ and FGF promote tendon progenitor fate and act downstream of muscle contraction to regulate tendon differentiation during chick limb development. *Development* 143(20), 3839-3851. DOI: 10.1242/dev.136242
51. Qiu Y., Wang X., Zhang Y., Carr A.J., Zhu L., Xia Z. et al. (2016). In vitro two-dimensional and three-dimensional tenocyte culture for tendon tissue engineering. *Journal of Tissue Engineering and Regenerative Medicine* 10(3), 216-226. DOI: 10.1002/term.1791
52. Pinheiro N.M., Cardoso F.A.G., Mendonça A.C., Zanier-Gomes P.H., Corrêa R.R.M., Carneiro A.C.D.M. et al. (2020). Effect of radiofrequency on patellar ligament repair of Wistar rats. *Journal of Bodywork and Movement Therapies* 24(4), 164-167.
53. Ferguson M.W., O'Kane S. (2004). Scar-free healing: from embryonic mechanisms to adult therapeutic intervention. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 359(839), 839-850. DOI: 10.1098/rstb.2004.1475
54. Cetik R.M., Yabanoglu Ciftci S., Arica B., Baysal I., Akarca Dizakar S.O., Erbay Elibol F.K. et al. (2022). Evaluation of the effects of transforming growth factor-beta 3 (TGF-β3) loaded nanoparticles on healing in a rat achilles tendon injury model. *The American Journal of Sports Medicine* 50(4), 1066-1077. DOI: 10.1177/03635465211073148
55. Chen L., Yang T., Lu D.W., Zhao H., Feng Y.L., Chen H. et al. (2018). Central role of dysregulation of TGF-β/Smad in CKD progression and potential targets of its treatment. *Biomedicine & Pharmacotherapy* 101, 670-81. DOI: 10.1016/j.biopha.2018.02.090
56. Orfei C.P., Viganò M., Pearson J.R., Colombini A., De Luca P., Ragni E. et al. (2019). In vitro induction of tendon-specific markers in tendon cells, adipose- and bone marrow-derived stem cells is dependent on TGFβ3, BMP-12 and ascorbic acid stimulation. *International Journal of Molecular Sciences* 20(1), 149. DOI: 10.3390/ijms20010149

57. Deng L., Huang L., Guo Q., Shi X., Xu K. (2017). CREB1 and Smad3 mediate TGF-β3-induced Smad7 expression in rat hepatic stellate cells. *Molecular Medicine Reports* 16(6), 8455-62. DOI: 10.3892/mmr.2017.7654
58. Guan S., Wu Y., Zhang Q., Zhou J. (2020). TGF-β1 induces CREB1-mediated miR-1290 upregulation to antagonize lung fibrosis via Napsin A. *International Journal of Molecular Medicine* 46(1), 141-8. DOI: 10.3892/ijmm.2020.4565
59. Wu L.M., Wang Y.J., Li S.F., Wang J.K., Liu J. Fan C.C. et al. (2023). Up-regulation of CREB-1 regulates tendon adhesion in the injury tendon healing through the CREB-1/TGF-β3 signaling pathway. *BMC Musculoskelet Disorders* 24, 325 DOI: 10.1186/s12891-023-06425-7
60. Campbell B.H., Agarwal C., Wang J.HC. (2004). TGF-β1, TGF-β3, and PGE2 regulate contraction of human patellar tendon fibroblasts. *Biomech Model Mechanobiol* 2, 239-245. DOI: 10.1007/s10237-004-0041-z
61. Manning C.N., Kim H.M., Sakiyama-Elbert S., Galatz L.M., Havlioglu N., Thomopoulos, S. (2011). Sustained delivery of transforming growth factor beta three enhances tendon-to--bone healing in a rat model. *Journal of Orthopaedic Research* 29(7), 1099-1105. DOI: 10.1002/jor.21301
62. Kim H.M., Galatz L.M., Das R., Havlioglu N., Rothermich S.Y., Thomopoulos S. (2011). The role of transforming growth factor beta isoforms in tendon-to-bone healing. *Connective Tissue Research* 52(2), 87-98. DOI: 10.3109/03008207.2010.483026
63. Jiang K., Li Y., Xiang C., Xiong Y., Jia J. (2021). TGF-β3 regulates adhesion formation through the JNK/c-Jun pathway during flexor tendon healing. *BMC Musculoskelet Disorders* 22, 843. DOI: 10.1186/s12891-021-04691-x
64. Han B., Jones I.A., Yang Z., Fang W., Vangsness T. (2020). Repair of rotator cuff tendon defects in aged rats using a growth factor injectable gel scaffold. *Arthroscopy: The Journal of Arthroscopic & Related Surgery* 36(3), 629-637. DOI: 10.1016/j.arthro.2019.09.015
65. Li J., Liu Z.P., Xu C., Guo A. (2020). TGF-β1-containing exosomes derived from bone marrow mesenchymal stem cells promote proliferation, migration and fibrotic activity in rotator cuff tenocytes. *Regenerative Therapy* 15, 70-76. DOI: 10.1016/j.reth.2020.07.001
66. Yang G., Rothrauff B.B., Lin H., Yu S., Tuan R.S. (2017). Tendon-derived extracellular matrix enhances transforming growth factor-β3-induced tenogenic differentiation of human adipose-derived stem cells. *Tissue Engineering Part A* 23(3-4), 166-176. DOI: 10.1089/ten.TEA.2015.0498
67. Shojaee A., Parham A., Ejeian F., Hossein Nasr Esfahani M. (2019). Equine adipose mesenchymal stem cells (eq-ASCs) appear to have higher potential for migration and musculoskeletal differentiation. *Research in Veterinary Science* 125, 235-243. DOI: 10.1016/j.rvsc.2019.06.015
68. Cordeiro M.F., Bhattacharya S.S., Schultz G.S., Khaw P.T (2000). TGF-β1, -β2, and -β3 in vitro: Biphasic effects on tenon's fibroblast contraction, proliferation, and migration. *Investigative Ophthalmology & Visual Science* 41(3), 756-763.
69. Farhat Y.M., Al-Maliki A.A., Chen T., Juneja S.C., Schwarz E.M., O'Keefe R.J. et al. (2012). Gene expression analysis of the pleiotropic effects of TGF-β1 in an in vitro model of flexor tendon healing. *PLoS one* 7(12), e51411. DOI: 10.1371/journal.pone.0051411
70. Yang F., Richardson D.W. (2021). Comparative analysis of tenogenic gene expression in tenocyte-derived induced pluripotent stem cells and bone marrow-derived mesenchymal stem cells in response to biochemical and biomechanical stimuli. *Stem Cells International*, 8835576. DOI: 10.1155/2021/8835576

Submitted: November 10, 2023
Accepted: May 22, 2024