In Vitro Effect of Transforming Growth Factor-β1 (TGF-β1) on Gene Expression in Human Flexor Digitorum Profundus Tendon Cells

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Abstract

Among hand injuries, flexor tendon lacerations remain a challenge for hand surgeons. There are presently no therapeutic agents available for the prevention of tendon adhesions. It is already known that TGF-β1 plays a role in tendon healing as well as in adhesion formation. Anti-TGF-β1 therapies are not effective in preventing adhesion formation. The goal of the present study was to identify possible genes that are affected by TGF-β1 in human Flexor Digitorum Profundus (FDP) tendon cells (tenocytes) in vitro. Tenocytes were isolated from human FDP tendons and treated with TGF-β1 in low-serum cell culture medium. Gene expression was assessed at 6h and 24h using RT-PCR. TGF-β1 caused upregulation of several genes (SERPIN1, PLA2U, ACTA2, CTGF, FN1, COL1A1, COL3A1, LOX, COMP, MMP13, TIMP1, TIMP3, BGN, SCX, POSTN, SMAD7, IL6, IGF1), downregulation of MMP9, DCN and ACAN, and had no effect on MMP2 and TIMP2. Targeting TGF-β1-affected genes may be an alternative therapeutic approach in controlling adhesion formation that may lead to optimal healing of injured FDP tendon or FDP tendon graft.

Keywords: Tendon; Gene expression; TGF-β1; Adhesion formation; Tendon healing

Introduction

Each year, millions of North Americans injure their hands resulting in significant morbidity and lost workdays [1,2]. In the USA, among all the injuries reported at private, state, and local government, 12.7% were hand injuries; and the average days away from work was 5 days in 2014 [2]. Among hand injuries, flexor tendon lacerations remain a challenge for hand surgeons. While flexor tendons heal with reduced mechanical strength, the most clinically relevant issue is adhesion formation, which impairs hand activity. There are presently no therapeutic agents available for the prevention of tendon adhesions [3]. In fact, the only accepted means of preventing adhesion formation in flexor tendons is physical therapy [3]. Even with physical therapy, the strength of healed tendons is markedly less than uninjured tendon, and debilitating adhesions have been reported to occur in as many as 50% of zone II flexor tendon injuries [4]. TGF-β1 is a cytokine that plays multiple roles in wound healing and is also implicated in the pathogenesis of excessive scar formation [5]. TGF-β1 stimulates chemotaxis, promotes angiogenesis, and regulates a wide spectrum of matrix proteins. It accelerates the wound-healing process in several animal models. However, this effect may proceed uncontrollably and result in pathological fibrosis, with excessive disordered collagen deposition resulting in tendon adhesions [6]. Hence, inhibitors of TGF-β1 have been tried to reduce adhesion formation.

Biologic strategies to inhibit TGF-β signaling have been reported, e.g., administration of neutralizing antibodies, application of soluble receptors, usage of antisense nucleotides, and chemically-synthesized inhibitors of the receptor serine/threonine kinases. Clinical trials have been performed with neutralizing antibodies, especially in fibrotic diseases, including TGF-β2 neutralizing antibody (lerdelimumab), which effectively decreased the amount of scarring after glaucoma surgery [7]. A TGF-β1 neutralizing antibody (CAT-192, metelumumab) has been administered intravenously to patients with systemic sclerosis, which causes scarring in skin and internal organs [8]. In rat cutaneous wound healing, exogenous addition of neutralizing antibody to TGF-β1 plus neutralizing antibody to TGF-β2 reduced the monocyte and macrophage profile, neovascularization, fibronectin, collagen III and collagen I deposition in the early stages of wound healing compared to control wounds. This reduced the scarring while the control wounds healed with scar formation [9]. In transected and repaired rabbit flexor tendons, neutralizing antibody to TGF-β1 increased range of motion [5,10]. Mannose-6-phosphate reduced TGF-β1-upregulated collagen production in rabbit flexor tendon cells in vitro, and in vivo application of mannos-6-phosphate to transected repaired rabbit zone II flexor tendon significantly improved the range of motion [11]. Using anti-sense oligonucleotides to reduce the expression of TGF-β1 superfamily members has been applied in clinical trials for cancer treatment [12]. The TGFBR1 inhibitors have shown to be efficient in mouse tumor models [13]. ALK-5 inhibitor (SB-505124) blocked TGF-β1-induced CTGF expression in gingival fibroblasts [14] and suppressed the in vivo and in vitro action of TGF-β in rabbit subconjunctival fibroblasts [15]. None of the foregoing anti-TGF-β therapies addressed or resolved the prevention of adhesion formation in tendon injury or tendon graft repair. The purpose of this study is to understand the effect of TGF-β1 on the expression of multiple genes in vitro in human FDP tenocytes in cell culture. Instead of anti-TGF-β1 therapy to control adhesion formation, targeting individual molecules regulated by TGF-β1 could be an alternative option to control adhesion or scar formation in vivo.

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Methods and Materials

Isolation of tendon cells from FDP and their culture in vitro

Human FDP tendon specimens, discarded for surgical reasons, were obtained from the University of Rochester Medical Center (Rochester NY USA) from patients following a protocol that was approved by University ethics committee. The normal part of the tendon specimen was used to isolate tendon cells known as tenocytes or tendinocytes. The tissue was transported to the laboratory in sterile DPBS (ThermoFisher Scientific #14287072) at room temperature. Specimen were stripped of surrounding tissue, washed in DPBS containing 1% Penicillin-Streptomycin, and 100 µM 2-mercaptoethanol (Sigma-Aldrich, #M7522). Medium was changed gently every 3 days without losing minced tissue pieces. Tenocytes proliferated from tendon pieces by day-10, and the monolayer of cells was obtained by day-16.

We used 5 ng/ml concentration since it is in the accepted physiological range as shown in literature [16].

RNA extraction and real-time reverse transcription polymerase chain reaction

For total RNA isolation, medium was removed from the dishes. The monolayer of tenocytes was first scraped with a tissue scraper and then resuspended in 1 ml of Trizol reagent (ThermoFisher Scientific #15596018) and homogenized using a hand-driven glass homogenizer, and total RNA was isolated using manufacturer’s protocol. Complementary DNA (cDNA) was prepared from 1 μg total RNA in a 20 μL of reaction mixture in 0.2-mL tubes (Bio-Rad) using MMLV Reverse Transcriptase system (ThermoFisher Scientific#28025013) and following the manufacturer’s protocol. A fixed volume of 0.5 μL cDNA was used for real-time reverse transcription polymerase chain reaction (RT-PCR) using SYBR Green (Applied Biosystems#: 4309155) and specific primers for human genes (Table 1). The mRNA expression of several genes at different time-points was assessed (Figures 2-4). The amplification was monitored real time using the 96-well iCycter iQTM Real-Time PCR Detection System (Bio-Rad, Hercules, California, USA). The threshold cycle (Ct) values were related to a standard curve made with the cloned PCR products, and specificity was confirmed by melting curve analysis after amplification. The general range of Ct values were 15-30. Beta-actin gene Actb was chosen as an internal control. Data at different time-points (6h and 24h) of TGF-β1-treatment groups, in triplicates, are presented as the mean fold induction ±SD; p-value less than 0.05 to differ treatment from control, was considered as significant.

| Gene               | Primer sequence (5’è3’)    | NCBI Ref. No. Sequence | Size |
|--------------------|---------------------------|------------------------|------|
| ACAN (aggrecan)    | F  CCCAACCCAGCTGACAACCTTT | NM_013227.3            | 216  |
|                    | R  GTACCCGACACGGAGGATGTAT |                       |      |
| ACTA2 (alpha 2, smooth muscle, aorta) | F  CAGGGCTGTGTCCCATCCAT | NM_00114945.1          | 142  |
|                    | R  GGCATGGCTCTCGGGTACCTT  |                       |      |
| ACTB (actin, beta) | F  CATGTACGGTCTACCCAGGC   | NM_001101.3            | 250  |
|                    | R  CTCCTTAATGTCAGCAGAT    |                       |      |
| BGN (biglycan)     | F  GAAACGTGCTTCTACCCAGGC  | NM_001711.5            | 178  |
|                    | R  CAGCTTTGAAGTGGGAAAGCA  |                       |      |
| COLIA1 (collagen, type I, alpha 1) | F  GCCGTCACCTCAGAGATGT | NM_000088              | 208  |
|                    | R  GCACACCGACATGCT       |                       |      |
| COL3A1 (collagen, type III, alpha 1) | F  GTGGTCGCGGTAATGACG   | NM_000090.3            | 84   |
|                    | R  TCCAGGGATCCGGCAGTT    |                       |      |
| COMP (cartilage oligomeric matrix protein) | F  CGAGTCGCCGATCAACACC   | NM_000095.2            | 170  |
|                    | R  CAGTTGGGAGCAGGTTAGTT  |                       |      |
| CTGF (connective tissue growth factor) | F  GCCAAAATGCGATCGTACT   | NM_001901.2            | 113  |
|                    | R  CCGTGGTACATCTCCACAG   |                       |      |
| Gene (Gene Description) | Forward Primer | Reverse Primer | GeneBank Accession Number | Sequence Length |
|-------------------------|----------------|----------------|---------------------------|-----------------|
| DCN (decorin)           | AGTTGGAACGACCTTTAATCTGTCC | GTGCCCATTTCTATGAAATCA | NM_133503.3 | 160 |
| FN1 (fibronectin)       | GAAGGCTTGAACCAACCTACG | TGGATTCGACATCTGTCCTC | GeneBank: AB191261.1 | 96 |
| IGF1 (insulin-like growth factor 1) | GGAGCCTGTGACTAAGGAGGC | GGGCTGATCCTTTGGGTTTCTTT | NM_001111283.1 | 119 |
| IL6 (interleukin 6)     | AAATCCGTACATCCTGACGG | GGAAGGTTCAGGTTGTTTGTCTGC | NM_0000600.3 | 112 |
| LOX (lysyl oxidase)     | GCCCGTCCTGTTCCAAG | TGGGTTGTAAGGTCGTCCG | GeneBank: EF089438.1 | 164 |
| MMP2 (matrix metalloproteinase 2) | GCCCGAGACAGGTGATCTTG | GCTTGCGAGGGAAGAAGTTGT | NM_001302510.1 | 101 |
| MMP9 (matrix metalloproteinase 9) | TGGCGAGAGTGTGTCGTGGGA | GGGCTGATCCTTTGGGTTTCTTT | NM_004994.2 | 229 |
| MMP13 (matrix metalloproteinase 13) | ACTGAGGAGCCTCCAGAGAAATG | GAAACCCGCTCTCTGCGTT | NM_002427.3 | 103 |
| PLA2 (plasminogen activator, urokinase type) | GTAGCGGACTCCAAGAAGCA | GCAGTTGCACAGTGATGTT | NM_002658.3 | 117 |
| POSTN (periostin, osteoblast specific factor) | CCTGGGCTCATAGTGATCACGG | CCCAAAATCTTGGAGGAGCA | NM_006475.2 | 69 |
| SCX (basic helix-loop-helix transcription factor scleraxis) | GACGCCTGACATCCCCCAGGAG | CAGTGGCCAGGTTCCAGG | NM_001080514.2 | 95 |
| SERPINE1 or PAI1 (serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1) | CATCCCCCATCTTCAGTGG | CCCCATAGGGTGAGAAAACCA | NM_000602.4 | 109 |
| SMAD7 (SMAD family member 7) | TTCTCCGCTGAAACAGGG | CCTCCCAATGATCCCAACCAC | NM_001190821.1 | 116 |
| TIMP1 (TIMP metalloproteinase inhibitor 1) | CTTCTGGCAATCCGACCTGCGT | CCGTGATCTGAGCTATGG | NM_003254.2 | 127 |
| TIMP2 (TIMP metalloproteinase inhibitor 2) | AAGCGGAGGCGAGGAAAGG | GGGCGGTAGGAATCAAACCAACTTAT | NM_003255.4 | 136 |
| TIMP3 (TIMP metalloproteinase inhibitor 3) | CCCAGGTAGTCTTTGTTGAGG | GGCAATGTTTAAAGCTTCC | NM_000362.4 | 101 |

**Table 1:** Human primer sequences used in RT-PCR.

The experiment was repeated three times to observe the consistency of RNA data. Data were analyzed using one-way ANOVA followed by Tukey's all-pair comparisons at alpha = 0.05. A computer software KaleidaGraph was used to analyze the data and MS office Excel was used to draw graphs.
**Results**

A diagrammatic sketch of a human finger is shown to demonstrate the location of FDP tendon (Figure 1A). Also hematoxylin & Eosin-stained paraffin section of human FDP tendon is shown (Figure 1B). Gene expression data is shown as below and in Table 2.

| Gene                                      | Expression at 6h (fold change) | Expression at 24h (fold change) |
|-------------------------------------------|--------------------------------|---------------------------------|
| ACAN (aggrecan)                           | 2.9*↓                          | 3.9*↓                           |
| ACTA2 (alpha 2, smooth muscle, aorta)     | NS                             | 6.5*↑                           |
| BGN (biglycan)                            | NS                             | 2.0**↑                          |
| COLIA1 (collagen, type I, alpha 1)        | NS                             | 2.5↑                            |
| COL3A1 (collagen, type III, alpha 1)      | NS                             | 1.6**↑                          |
| COMP (cartilage oligomeric matrix protein)| 3.4**↑                         | 17.1↑                           |
| CTGF (connective tissue growth factor)    | 5.2*↑                          | 13.0↑                           |
| DCN (decorin)                             | 2.9*↓                          | 2.9*↓                           |
| FN1 (fibronectin)                         | NS                             | 2.0**↑                          |
| IGF1 (insulin-like growth factor 1)       | 5.3*↑                          | 14.1*↑                          |
| IL6 (interleukin 6)                       | 9.6*↑                          | 12.3*↑                          |
| LOX (lysyl oxidase)                       | NS                             | 2.2*↑                           |
| MMP2 (matrix metalloproteinase 2)         | NS                             | NS                              |
| MMP9 (matrix metalloproteinase 9)         | 2.2*↓                          | 2.1**↑                          |
| MMP13 (matrix metalloproteinase 13)       | 2.2*↑                          | 3.0*↑                           |
| PLAU (plasminogen activator, urokinase type)| 2.9**↑                         | 4.2*↑                           |
| POSTN (periostin, osteoblast specific factor)| NS                         | 1.8*↑                           |
| SCX (basic helix-loop-helix transcription factor scleraxis) | 5.0*↑                         | 11.3*↑                          |
| SERPINE1 or PAI1 (serpent peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1)| 6.7*↑                         | 17.0*↑                          |
| SMAD7 (SMAD family member 7)              | 1.9*↑                          | 2.0*↑                           |
| TIMP1 (TIMP metalloproteinase inhibitor 1)| NS                             | 1.3**↑                          |
| TIMP2 (TIMP metalloproteinase inhibitor 2)| NS                             | NS                              |
| TIMP3 (TIMP metalloproteinase inhibitor 3)| 1.9*↑                          | 6.6*↑                           |

**Table 2:** Summary of fold change in gene expression in FDP tendon cells in culture in response to TGFβ1 treatment.

Note: Significance: *P<0.01; **P<0.05

**TGF-β1 caused an increase in SERPINE1, PLAU, ACTA2 and CTGF gene expression**

SERPINE1 gene expression increased 6.7 fold at 6h and 17.0 fold at 24h of TGF-β1 treatment to FDP tenocytes in cell culture (P<0.01, Figure 2a). PLAU gene expressed 2.9 fold higher at 6h (P<0.05) and 4.2 fold higher at 24h (P<0.01) respectively (Figure 2b). ACTA2 gene expression was higher at 24h and that was 6.5 fold (P<0.01, Figure 2c). CTGF gene expression was 5.2 fold higher at 6h and 13.0 fold higher at 24h as compared to their respective controls (P<0.01, Figure 2d).
TGF-β1 caused an increase in FN1, COL1A1, COL3A1, LOX and COMP gene expression

TGF-β1 caused 2-fold increase in FN1 gene expression at 24h (p<0.05; Figure 2e). It caused 2.5-fold increase in COL1A1 gene expression at 24h (p<0.01; Figure 2f), and 1.6-fold increase in COL3A1 at 24h (p<0.05, Figure 2g). LOX gene expression increased 2.2 fold at 24h due to TGF-β1 treatment to FDP tenocytes in cell culture (P<0.01, Figure 2h). TGF-β1 caused a sharp increase of COMP gene expression. The level raised 3.4 fold at 6h (P<0.05) and 17.1 fold at 24h (P<0.01, Figure 2i).

TGF-β1 caused decrease in MMP9, increase in MMP13, whereas it has no effect on MMP2 gene expression

MMP2 gene expression was not affected by TGF-β1 treatment (Figure 3a). TGF-β1 caused a 2.2- and 2.1-fold reduction in expression reduction in MMP9 gene expression at 6h (P<0.01) and 24h (P<0.05) as compared to their respective control values (Figure 3b), respectively. On the other hand, TGF-β1 caused a 2.2 and 3.0 fold increase in expression of MMP13 at 6h (P<0.01) and 24h (P<0.01), respectively (Figure 3c).

TGF-β1 caused an increase in TIMP1 and TIMP3 but did not affect TIMP2 gene expression

TGF-β1 caused 1.3-fold increase in TIMP1 gene expression at 24h (P<0.05, Figure 3d). TIMP3 gene expression increased 1.9 fold at 6h (P<0.01) and 6.6 fold at 24h (P<0.01) in response to TGF-β1 treatment to FDP tenocytes (Figure 3f). TIMP2 gene expression was not affected by TGF-β1 treatment to FDP tenocytes in culture (Figure 3e).

TGF-β1 caused increased BGN and decreased DCN gene expression

BGN gene expression increased 2-fold (P<0.05) at 24h of TGF-β1 treatment (Figure 3g), whereas, DCN gene expression decreased 2.9-fold at 6h and at 24h of TGF-β1 treatment to FDP tenocytes in cell culture (P<0.01, Figure 3h).
Figure 3: mRNA expression of MMP2 (a), MMP9 (b), MMP13 (c), TIMP1 (d), TIMP2 (e), TIMP3 (f), BGN (g), and DCN (h), at 6h and 24h of post-TGF-β1 treatment (5ng/mL) of human FDP tenocytes, in monolayer cell culture. ±S.D., n = 3. *P<0.01; **P<0.05.

TGF-β1 caused varied effect on the expression of other genes

TGF-β1 caused sharp increase of SCX gene expression (P<0.01). The level raised 5.0 fold at 6h and 11.3 fold at 24h (Figure 4a). POSTN gene expression increased 1.8 fold at 24h (P<0.01, Figure 4b). ACAN gene expression was lower at 6h (P<0.01) and 24h (P<0.01) due to TGF-β1 treatment. The expression was 2.9 fold and 3.9 fold lower than their respective control (Figure 4c). SMAD7 was expressed 1.9 times higher at 6h (P<0.01) and 2 times higher at 24h (P<0.01) in response to TGF-β1 treatment (Figure 4d). Interleukin 6 (IL6) was expressed 9.6-fold higher at 6h (P<0.01) and 12.3-fold higher at 24h (P<0.01) due to TGF-β1 treatment (Figure 4e). TGF-β1 caused an increase in IGF1 gene expression (Figure 4f). The increase was 5.3-fold at 6h and 14.1-fold at 24h (P<0.01).

Discussion

SERPINE1, PLAU

Plasminogen activator inhibitor 1 (PAI-1) is a single-chain glycoprotein and is encoded by SERPINE1 gene. It is present in plasma as well as synthesized by many tissues. It inhibits uPA and tPA (tissue-type plasminogen activator) and is a regulator of plasminogen activation and plays primary role in fibrinolysis and is involved in the regulation of cell adhesion, cell migration, and invasion [17]. Skin wound healing is accelerated in PAI-1-deficient mice [18]. PAI-1 deficiency reduces hepatic fibrosis after bile duct obstruction mainly through the activation of tPA and Hepatocyte Growth Factor (HGF) [19]. Earlier we showed that during inflammatory phase of mouse Flexor Digitorum Longus (FDL) tendon graft healing, Serpine1 gene expression was higher along with TGF-β1 gene expression [20]. In the current study, TGF-β1 upregulated SERPINE1 gene expression in FDP tenocyte cell culture (Figure 2a). This may indicate that SERPINE1 gene is regulated by TGF-β1 and may be involved in FDP tendon repair and adhesion in vivo.

Urokinase-type plasminogen activator (uPA; gene: PLAU) specifically cleaves the zymogen plasminogen to form the active enzyme plasmin. Specific cleavage of Arg-|-Val bond, in plasminogen to form plasmin, is inhibited by PAI-1 [21]. Urokinase-type plasminogen activator plays a vital role in early phases of wound healing by aiding fibrin dissolution, promoting migration, proliferation, and adhesion of various cells to the wound bed [17]. In wounded gingival granulation tissue, TGF-β1 caused an enhanced expression of uPA in cells expressing α-SMA indicating its role during wound healing [22]. Expression of PLAU mRNAs was maximal at day 4 and 7 following Achilles tendon injury [23]. In our earlier study, mouse Plau mRNA expression was highest at day-3 of FDL tendon graft healing as compared to other days (day-4 onwards), indicating its role in early phase of healing. Farhat et al. [24] showed that TGF-β1 (at 10 ng/ml) did not affect Plau gene expression significantly at 48h in mouse FDL tendon cells grown on collagen-coated dishes. In the present study, TGF-β1-induced a sharp increase in PLAU gene expression at 6h and 24h in human FDP tenocytes (Figure 2b), indicating that TGF-β1’s effects on PLAU expression could be more pronounced in the first 24h. TGF-β1-induced PLAU gene expression may play a role in the modulation of FDP tendon in lamination, adhesion and fibrosis in vivo.

ACTA2

Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells. α-actin-2 or α-SMA (gene: Acta2 in mouse or ACTA2 in human), though mainly expressed in muscle, has been identified in fibroblastic cells of normal tendons, ligaments, and myofibroblasts [25]. α-actin-2-
expressing cells (myofibroblasts), in injured rabbit ligament (day 3 to 12-weeks post-injury), were identified at day 3 in medial collateral ligament, and their density increased to day 21 [26,27]. In mouse FDL tendon graft healing, Acta2 mRNA expression remained higher on all the days as compared to day 3 post-surgery indicating its role in the post-inflammatory events [20,28]. Cultured fibroblasts acquire myofibroblast phenotype in the presence of TGF-β1 [29]. Myofibroblasts are characterized by large focal adhesions, prominent stress fibers, and enhanced expression of contractile marker proteins such as a-SMA [30]. TGF-β1 (at 10 ng/ml) caused an increase in ACTA2 gene expression in rat embryonic fibroblasts in vitro at 48h [31]. Subcutaneous administration of TGF-β1 to rats results in the formation of a granulation tissue in which α-SMA expressing myofibroblasts were particularly abundant [32]. A universal process in fibrosis is the formation of myofibroblasts and the subsequent collagen deposition by these cells. TGF-β1 plays a major role in the formation of myofibroblasts, e.g., by activating fibroblasts. TGF-β1 caused upregulation of ACTA2 gene at 48h in PDL (periodontal ligament) stem/progenitor cell lines (PDLSCs) undergoing fibroblastic differentiation [33]. In the current study, we showed that TGF-β1 caused an increase in ACTA2 gene expression at 24h in FDP tenocytes, in cell culture (Figure 2c). That suggests a role for TGF-β1-induced ACTA2 in FDP tendon healing and fibrosis in vivo.

CTGF

Connective tissue growth factor (CCN2/CTGF) is a growth factor of 38-40 kDa that acts as a potent fibroblast mitogen and angiogenic factor. CTGF belongs to the CTGF, Cyr61 and Nov (CCN) family of growth factors [34]. CTGF is best known as a molecule that mediates the development of fibrotic disorders in a variety of tissues and organs [35,36]. Inflammation, wound healing and fibrosis are mutually related biological events. Inflammation occurs upon tissue injury and/or invasion of pathogenic factors, which usually causes additional damage to the tissues. The involvement of CTGF in this phase is well represented by the fact that CTGF regulates the behavior of the mediators of inflammation and vice versa. For example, CTGF is induced by TGF-β1 and is repressed by TNF-α, whereas, this gene product induces inflammatory IL-6, monocyte chemoattractant protein-1 (MCP1) and Extracellular Matrix (ECM)-remodeling MMPs [36-38]. In fact, elevated CTGF expression is observed in the inflamed joints of patients with rheumatoid arthritis and osteoarthritis [39]. TGF-β1 stimulated the transcriptional activity of CTGF gene promoter in NIH/3T3 fibroblasts [40]. After exposure to TGF-β1 (5 ng/ml), the maximal level of luciferase activity reached at 12h and maintained to 24h by 2.76- and 2.20-fold, respectively. TGF-β1 stimulated CTGF expression in airway smooth muscle cells [41]. In a lung fibrosis mouse model, CTGF inhibition using an inhibitory antibody (FG-3019) resulted in less fibrosis [42]. In human gingival fibroblasts and Periodontal Ligament (PDL) cells, the expression of CTGF mRNA and protein was significantly increased in a dose- and time-dependent manner in the presence of TGF-β1 [43]. The current study showed that TGF-β1 plays a role in the modulation of CTGF gene expression of cultured human FDP tenocytes, suggesting that CTGF may play role in FDP tendon healing and fibrosis in vivo (Figure 2d).

FNI

Fibronectin (FN) is a high-molecular weight (~440kDa) glycoprotein of the ECM that binds to membrane-spanning receptor proteins called integrins [44]. The FNI gene in humans encodes it. Similar to integrins, fibronectin binds ECM components such as collagen, fibrin, and heparan sulfate proteoglycans (e.g., syndecans). Fibronectin plays a major role in cell adhesion, growth, migration, and differentiation, and it is important for processes such as wound healing and embryonic development [44]. Altered fibronectin expression, degradation, and organization are associated with a number of pathologies, including cancer and fibrosis [45]. TGF-β1 increases the expression of fibronectin. Based upon the 48-72h period required for a maximal fibroproliferative response to dermal injections of TGF-β1, human fetal lung fibroblasts were exposed to TGF-β1 for periods up to 48h in vitro. A 6-fold increase in fibronectin synthesis was observed at 24h [46]. Similar observations were also reported for fibroblastic cells [47]. TGF-β1 induces fibroblast proliferation and transformation into myofibroblasts and stimulates the accumulation of matrix proteins, including laminin, collagens I and 3, as well as fibronectin [48]. In the current study, TGF-β1 enhanced the FNI mRNA expression at 24h in human FDP tenocytes, in cell culture (Figure 2e). TGF-β1-induced fibronectin may play role in physiology and pathology of FDP tendon healing in vivo.

COL1A1, COL3A1, LOX

Collagens contribute to tensile strength to tendon and other tissues. Collagen, type I or [α1(I)], type II or [α2(II)], and type III or [α1(III)], lays an important role in tendon healing process. During wound healing, one of the fibroblasts’ dominant functions is the production of collagen. Higher gene expression of all the collagen genes (COL1A1, COL1A2, and COL3A1) indicated their role during maturation and remodeling phase of FDL tendon graft healing [20,28]. In rat flexor tendon healing study, COL1A1 mRNA expression increased from day 3 to 28 with a peak at day 28, whereas COL3A1 expression increased from day 3 to the peak value at day 14 and then sharply decreased [49].

TGF-β1 at 1 ng/ml to 100 ng/ml caused significant increase in COL1A1 gene expression at 24h in FDL tendon cells in collagen gels [50]. Varga et al. [51] reported that TGF-β1 caused a marked enhancement in the production of type I and III collagen, and fibronectin by cultured normal human dermal fibroblasts. In another instance, TGF-β1 increased the expression of COL1A1 in human dermal fibroblasts and in human lung fibroblasts [52]. TGF-β1 caused an increase in collagen I expression at 48h in two PDL stem/progenitor cells lines [33]. Lung fibroblast cultures maintained in medium containing TGF-β1 sustained an activated rate of collagen production at 5 nmol/ml/24h for at least 72h [53]. We showed that TGF-β1 enhanced COL1A1 and COL3A1 mRNA expression at 24h in cultured FDP tenocytes indicating the importance of TGF-β1-induced collagen production in FDP tendon physiology, pathology and repair (Figure 2f, g).

Lysyl Oxidase (LOX) is a key extracellular enzyme responsible for the post-translational modification of collagen I, III to form mature fibrillar collagen. LOX plays a key role in the post-translational modification of collagens and elastin, catalyzing inter- and intracrosslinking reactions. Since the cross-linked ECMs are highly resistant to degradative enzymes, it is considered that the over-expression of LOX may cause severe fibrotic degeneration. Goto et al. [54] showed that TGF-β1 upregulated the production of LOX in kidney tubular epithelial cells of ICGN (The Institute of Cancer Research (ICR)-derived glomerulonephritis) mice. As a result, the highly cross-linked collagens induce an irreversible progression of chronic renal tubulointerstitial fibrosis in the kidneys of ICGN mice. Transfection studies showed that the Lox and Col1a1 promoters may...
be regulated by similar negative and positive cis-acting elements, which include TGF-β1 response element, reported for rat Col1a1 [55] and for mouse Col1a2 promoters [56]. In vitro studies have shown that TGF-β1 caused an increase in Lac mRNA expression in murine tail tenocytes [57]. Increased expression of LOX is associated with fibrosis and cardiac dysfunction [58]. Adult cardiac fibroblasts were isolated from male rat hearts and were treated with TGF-β1. TGF-β1 treatment upregulated LOX mRNA, and protein expression in cardiac fibroblasts. Concomitant increases in collagen types I and III, and bone morphogenetic protein 1 expression were found in response to TGF-β1 [58]. The current study showed that TGF-β1 increased LOX gene expression at 24h in human FDP tendon cells in culture (Figure 2h). That indicates that TGF-β1-induced LOX gene expression may play role in FDP tendon healing in vivo.

**COMP**

Cartilage Oligomeric Matrix Protein (COMP) is a non-collagenous glycoprotein expressed in the ECM of articular cartilage, tendon and ligaments [59], and is normally produced by chondrocytes, osteoblasts and synovial fibroblasts [60]. COMP plays role in the structural integrity of cartilage via its interaction with other ECM proteins such as the collagens and fibronecrtin. It mediates the interaction of chondrocytes with the ECM through interaction with cell surface integrin receptors. It plays role in the pathogenesis of osteoarthritis [61,62]. In the presence of TGF-β1, human dermal fibroblasts have been shown to increase COMP production in vitro suggesting a correlation between TGF-β1 and COMP production [63]. COMP accumulates in Systemic Sclerosis (SSc) skin and is upregulated by TGF-β1. TGF-β1 treatment increased COMP and SMA-expressing cells. COMP mRNA expression in lesional skin from patients with diffuse cutaneous SSc (dSSc) correlated with TGF-β1 staining [64]. Immunohistochemical analysis revealed that COMP was expressed in dense fibrotic regions of IPF lungs and co-localized with vimentin and around pSMAD3 expressing cells. Stimulation of normal human lung fibroblasts with TGF-β1 increased COMP mRNA and protein expression [65]. In cultured human FDP tenocytes, we showed that TGF-β1 induced COMP mRNA expression 3.4 and 17.1 fold at 6h and 24h respectively indicating its possible role in FDP tendon healing and fibrosis in vivo (Figure 2i).

**MMP2, MMP9, MMP13, TIMP1, TIMP2 and TIMP3**

Matrix metalloproteinase-2 (MMP-2) protein, encoded by MMP2 gene, is a 72kDa type IV collagenase (72kDa gelatinase or gelatinase A). It contains three-fibronectin type II repeats (FNII) in its catalytic site that allow binding of denatured type IV collagen (the major structural component of basement membrane), type V collagen and elastin. Unlike most MMP family members, activation of this protein can occur on the cell membrane. This enzyme can be activated extracellularly by proteases, or intracellularly by its S-glutathiolation. This protein is thought to be involved in multiple pathways including roles in the nervous system, endometrial menstrual breakdown, regulation of vascularization, and metastasis. Mutations in this gene have been associated with Winchester syndrome and Nodulosis-Arthropathy-Osteolysis (NAO) syndrome [66,67]. During wound healing, fibroblasts transition from quiescence to a migratory state, then to a contractile myofibroblast state associated with wound closure. Howard et al. [31] found that the myofibroblast phenotype, characterized by the expression of high levels of contractile proteins, suppresses the expression of the pro-migratory gene, MMP2.

Fibroblasts cultured in a 3-D collagen lattice and allowed to develop tension showed increased contractile protein expression and decreased MMP-2 levels in comparison to a stress-released lattice. In 2-D cultures, factors that promote fibroblast contractility, including serum or TGF-β1, down regulated MMP-2. The current study showed that TGF-β1 did not cause any significant effect on MMP2 gene expression in FDP tenocytes, in cell culture (Figure 3a). In a previous study, in mouse FDL tenocytes in collagen gel culture, the authors showed that MMP2 gene expression was also not affected by TGF-β1 (at 1, 10 and 100 ng/ml) treatment at 6h until 48h post-treatment [50].

Matrix metalloproteinase-9 (MMP-9) protein, encoded by the MMP9 gene, plays an essential role in local proteolysis of the ECM and in leukocyte migration. It plays a role in bone osteoclastic resorption. It cleaves type IV and type V collagen into large C-terminal three-quarter fragments and shorter N-terminal one-quarter fragments. It degrades fibronectin [68]. The decreased degradation of ECM is a potential mechanism of renal fibrosis. Normal kidneys produce proteases responsible for the hydrolysis of ECM, among which MMPs are the most important ones, and their activity is subject to the regulation of the TIMPs (Metalloproteinase inhibitors). MMP-9 is one of the most important MMPs in the human body inducing enzymatic degradation of ECM molecules; and TIMP-1 is specific for the inhibition of MMP-9. The MMP-9/TIMP-1 ratio regulates the aggregation and degradation of the ECM, which are closely related to renal fibrosis [69]. A normal ratio of MMP-9 and TIMP-1 plays an important role in the regulation of ECM secretion and accumulation in glomerular mesangial cells [70]. The activation of hypoxia-inducible factor 1α (HIF-1α)-dependent HGF-signaling can promote the expression of TIMP-1 [71]. MMP-9/TIMP-1 ratio imbalance, by either reduced MMP-9 expression or increased TIMP-1 expression, promotes the progression of renal fibrosis. The mRNA and protein expression of TIMP-1 decreased when TGF-β1 was low in Human amniotic (WISH) cells, whereas those of MMP-9 elevated when TGF-β1 was low. The disruption in the ratio of TIMP-1 and MMP-9 was related to the pathology of the premature rupture of membrane [72]. The current study showed that TGF-β1 decreased MMP9 gene expression and increased TIMP1 gene expression in FDP tenocytes, in cell culture (Figure 3b,3d), indicating that fibrotic behavior in FDP tendon healing or repair may be modulated in vivo by TGF-β1 level.

Matrix metallopeptidase-13 (collagenase 3; MMP-13) is encoded by MMP13 gene in humans. MMP-13 plays role in the degradation of ECM proteins including fibrillar collagen, fibronecrtin, TNC and ACAN. It cleaves triple helical collagens, including type I, type II and type III collagen, but has the highest activity with soluble type II collagen. It can also degrade collagen type IV, type XIV and type X and may also function by activating or degrading key regulatory proteins, such as TGF-β1 and CTGF. MMP-13 plays role in wound healing, tissue remodeling, cartilage degradation, bone development, bone mineralization and ossification. It is required for normal embryonic bone development and ossification [73-75]. TGF-β1 induced a rapid decrease in MMP13 mRNA within first 6h post-cytokine administration and that was accompanied by a 2-fold increase in gene transcription and reached maximum values by 48h [76]. Expression of MMP-13 by human gingival fibroblasts cultured in monolayer or in collagen gel was induced by TGF-β1 [77]. Leivonen et al. [78] reported in Squamous Cell Carcinoma (SCC) cells of the head and neck that specifically express MMP-13, the expression of which correlates with their invasion capacity. TGF-β1 enhanced MMP-13 and MMP-1 expression and invasion of SCC cells. The current study showed that TGF-β1 enhanced MMP13 gene expression at 6h and 24h post-TGF-

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β1 treatment in human FDP tenocytes, in cell culture. This may indicate an active role of TGF-β1-induced MMP13 in FDP tendon healing and fibrosis in vivo (Figure 3c).

Metalloproteinase inhibitor 2 (TIMP-2) complexes with metalloproteinases (such as collagenases) and irreversibly inactivates them by binding to their catalytic zinc cofactor. It is known to act on MMP-1, -2, -3, -7, -8, -9, -10, -13, -14, -15, -16 and -19 [79,80]. MMP-2 and TIMP-2 expression were not altered by TGF-β1 [81]. In mouse FDL tenocytes, Farhat et al. [50] showed that TGF-β1 did not affect Timp2 gene expression at 48h, in collagen gel culture. The current study also showed that TGF-β1 did not alter human TIMP2 gene expression at 6h and 24h in FDP tendon tenocytes, in culture (Figure 3e). Metalloproteinase inhibitor 3 (TIMP-3) is encoded by the gene TIMP3 and forms part of a tissue-specific acute response to remodeling stimuli. It is known to act on MMP-1, -2, -3, -7, -9, -13, -14 and -15 [82,83]. Idiopathic Pulmonary Fibrosis (IPF) is characterized by fibroblast expansion and ECM accumulation. TGF-β1 induced strong upregulation of TIMP-3 at the mRNA and protein levels. In IPF tissues, TIMP3 gene expression was increased and the protein was localized to fibroblastic foci and ECM. Induction of TIMP-3 by TGF-β1 could be a mediator in lung fibrogenesis [84]. TGF-β1 induced expression of TIMP-3 in mouse embryonic fibroblasts. Inhibition of Smad signaling by expression of Smad7 and dominant negative Smad3 completely abolished TGF-β1-elicted expression of TIMP-3 in human fibroblasts, whereas overexpression of Smad3 enhanced it [85]. In the current study, TGF-β1 increased TIMP3 gene expression at 6h and 24h in human FDP tenocytes, in cell culture (Figure 3f). The study supports that FDP tendon may be affected by TGF-β1-induced TIMP3 gene expression for inflammation, repair and fibrosis.

**BGN and DCN**

Biglycan and decorin belong to the SLRPs class I subfamily and are encoded by BGN and DCN gene respectively in human. Both contain 12 LRRs [86]. Biglycan has two attached GAG chains and decorin has one. Biglycan is found in several connective tissues, predominantly in articular cartilage. It is a homodimer and forms a ternary complex with microfibrillar-associated protein 2 and elastin and may be involved in collagen fiber assembly. Biglycan binds to Col I in the gap zone of the fibrils, and decorin competes for that interaction [86]. TGF-β1 differentially regulates DCN and BGN gene expression in skin and gingival fibroblasts. TGF-β1 (at 5 ng/ml) increased BGN 24-fold and inhibited DCN gene expression up to 70% [87]. Farhat et al. [50] also reported that TGF-β1 increased BGN and decreased DCN gene expression in mouse FDL tenocytes in collagen gels [50]. We showed that TGF-β1 increased BGN and decreased DCN gene expression in cultured human FDP tenocytes (Figure 3g), indicating that FDP tendon function can be modulated by TGF-β1-induced biglycan expression in vivo.

TGF-β1 and decorin have important interactions in tendon and other tissues. Decorin has the ability to bind to TGF-β1, which is involved in the regulation of cell proliferation, differentiation, ECM production, wound healing, and tissue repair [88]. TGF-β1 is of crucial importance in triggering excessive formation and deposition of connective tissue matrix molecules. Decorin-TGF-β1 complex formation may lead to inactivation of some cytokines and TGF-β1 itself in connective tissue [89]. To investigate, if scar formation could be prevented by controlling decorin in tenocytes, rabbit Achilles tendon cells were transfected with antisense decorin; the authors found that it suppressed TGF-β1 production. The results showed that the antisense approach is an attractive therapeutic strategy, not only for preventing decorin deposition in scar tissue, which decreases collagen fibril diameter, but also for controlling TGF-β1 production, which leads to organ fibrosis [90]. In vitro studies showed that TGF-β1 suppressed DCN gene expression at 6h and 24h in human FDP tenocytes, in cell culture (Figure 3h), indicating a possible modulatory role of TGF-β1 in tendon function, scarring and pathology. Baghy et al. [91] sought to take advantage of this TGF-β1-decorin relation in treating hepatic fibrosis and cirrhosis. One of the natural inhibitors of TGF-β1 is decorin, which binds with high affinity to TGF-β1 and prevents its interaction with pro-fibrotic receptors. Decorin has a protective role in liver fibrogenesis insofar as its genetic ablation in mice leads to enhanced matrix deposition, impaired matrix degradation, and “activation” of hepatic stellate cells, the main producers of fibrotic tissue. Moreover, TGF-β1 exerts a stronger effect when functional decorin is absent. Endogenous decorin prevents and retards hepatic fibrosis; and thus boosting the endogenous production of decorin, or systemic delivery of recombinant decorin could represent an additional therapeutic modality against hepatic fibrosis [91], and possibly could have a similar effect in the prevention of TGF-β1-mediated tendon adhesions.

**SCX**

The basic helix-loop-helix transcription factor, scleraxis, is a specific marker for all the connective tissues that mediate attachment of muscle to bone, including limb tendons, and its expression marks the progenitor cell populations for these tissues. Scleraxis is encoded by SCX gene in humans [92,93]. Tendon healing is a regenerative process, and tendon progenitor cells are expected to play a role in the healing process. In our earlier study, we demonstrated higher Scx gene expression during mouse FDL tendon graft healing, and that indicated that scleraxis plays a role in the healing process [20]. In a murine patellar tendon injury model, Scx mRNA expression was measured at 1-, 4- and 8-week time-points. The authors observed increased expression at 4- and 8-week time-points [94]. Zeglinski et al. [95] showed that TGF-β1 regulates scleraxis expression in primary cardiac myofibroblasts by a Smad-independent mechanism. TGF-β1 (at 5 ng/ml) caused 6-fold higher expression at 24h [95]. TGF-β1 at 10ng/ml has been shown to cause 4-fold induction of Scx gene expression in mouse FDL tenocytes in collagen gels at 24h [50]. In the current study, we showed that TGF-β1 (at 5 ng/ml) caused an increase in SCX gene expression in human FDP tenocytes, in cell culture at 6h and 24h (Figure 4a). That suggests that scleraxis is modulated by TGF-β1 in injured FDP tendon and contributes to the healing and regeneration process.

**POSTN**

Periostin, a secreted cell adhesion protein, is a matricellular protein and is encoded by POSTN gene in human [86,96]. Periostin is predominantly expressed in collagen-rich fibrous connective tissues that are subjected to constant mechanical stress including heart valves, tendons, and PDL [86,96]. Periostin binds to collagen I and plays a role in collagen fibrillogenesis as evidenced by periostin knockout mice [97]. We showed earlier that periostin plays a role in healing of FDL tendon graft in mouse since Postn mRNA level is modulated by cyclic strain as well as...
TGF-β1 via FAK-dependent pathways [98]. In one study, to understand the importance of masticatory forces, Manokavinchoke et al. [99] showed that an intermittent compressive force regulates SOST7 POSTN gene expression by hPDL cells via TGF-β1 signaling pathway. The current study showed that TGFβ1 increases POSTN gene expression at 24h in human FDP tenocytes, in cell culture (Figure 4b), supporting that TGFβ1 may be a regulator of periostin in FDP tendon in vivo.

ACAN

Aggrecan, a core protein of a proteoglycan, is a major component of cartilaginous ECM. It is encoded by gene ACAN in human. The major function of this proteoglycan is to resist compression in cartilage. The protein binds avidly to hyaluronic acid via an N-terminal globular region [100]. The expression of ACAN decreased in nucleus pulposus cells with the addition of 2.5 ng/ml TGFβ1 [101]. Aggrecan expression was increased by the presence of TGF-β1 [102] or β3 [103] during chondrogenic differentiation of mesenchymal stem cells. In human FDP tendon cells, in cell culture, TGF-β1 suppressed ACAN gene expression indicating that it has opposite effect than differentiating cartilage cells (Figure 4c). One possible explanation of this is that chondrocytes are required to impart cushionary properties to cartilage in order to absorb repeated joint shock, whereas tendon needs to be stiff and strong to tolerate the tension of daily activity.

SMAD7

Mothers against decapentaplegic homolog 7 (SMAD7), is an antagonist of signaling by TGF-β type I receptor superfamily members. It has been shown to inhibit TGF-β and activin signaling by associating with their receptors thus preventing SMAD2/3 access [104,105]. TGF-β1, bone morphogenetic protein 4, and oocyte-derived growth differentiation factor 9 were capable of inducing Smad7 expression, suggesting a modulatory role of SMAD7 in a negative feedback loop. Using a siRNA approach, this was further demonstrated that SMAD7 was a negative regulator of TGF-β1. SMAD7 seemed to play role during follicular development via preferentially antagonizing and/or fine-tuning essential TGF-β superfamily signaling, which is involved in the regulation of oocyte–somatic cell interaction and granulosa cell function [106]. In mouse confluent dermal fibroblast, TGF-β1 (at 12.5 ng/ml) caused an increase in Smad7 gene expression for 24h [107]. In the current study, we showed that TGF-β1 (5 ng/ml) caused an increase in the Smad7 gene expression in cultured FDP tenocytes, suggesting that TGF-β1 modulates SMAD7 in FDP tendon in vivo (Figure 4d).

IL6

Interleukin-6 (IL-6) is an interleukin that acts both as a pro-inflammatory cytokine and an anti-inflammatory myokine. In human, it is encoded by IL6 gene [108]. IL-6 is secreted by T-cells and macrophages to stimulate immune response [109]. In addition, osteoblasts secrete IL-6 to stimulate osteoclast formation [110]. IL-6 expression is stimulated by tumor-producing TGF-β1 in human prostate cancer cells through multiple signaling pathways and enhanced expression of IL-6 contributes to the oncogenic switch of TGF-β1 role for prostate tumorigenesis [111]. TGF-β1 induced Smad2 phosphorylation, and blockade of Smad2/3 prevented both the TGF-β1 induced IL-6 increase in asomatic and non-asmatic cells. Understanding the mechanism of aberrant pro-inflammatory cytokine production in asthmatic airways allows the development of alternative ways to control airway inflammation [112]. While studying the mechanism of subconunctival fibrosis, in human Tenon’s fibroblasts, TGF-β1 stimulates the expression of a-SMA protein and increase mRNA expression levels of IL6. The autocrine IL-6 may participate in the TGF-β1-induced trans differentiation of human Tenon’s fibroblasts to myofibroblasts, which is known to be an essential step for subconunctival fibrosis [113]. IL-6 is an essential mediator of growth factor-induced proliferation of lung fibroblasts. Eickelberg et al. [114] showed that TGF-β1 is a potent inducer of IL-6 mRNA and protein in primary human lung fibroblasts. The current study showed that TGF-β1 enhanced IL6 gene expression multi-fold at 6h and 24h, in FDP tenocytes cell culture, indicating possible in vivo role of TGF-β1-induced IL6 in FDP tendon physiology or pathology (Figure 4e).

IGF1

Insulin-like growth factor I (IGF-1) has been shown to play a role in wound healing and regeneration. Expression levels of IGF1 mRNA and IGF-1 protein increased in healing rabbit medial collateral ligament [115], and in canine flexor tendon after laceration [116]. Mouse Igf1 mRNA expression upregulated, during day-7 through 35 of FDL tendon graft healing, indicating its possible role in angiogenesis and growth of cells [20]. During healing of deep flexor tendon repair in rabbit, the expression of Igf1 mRNA was higher in tendon and sheath at all the time-points (day-6 to 42) as compared to day-3 [117]. TGF-β1 is a potent modulator of IGF-1 production in mouse bone cells where it is thought to act in the local regulation of bone remodeling [118]. In liver, TGF-β1 has been postulated to play a role in fibrogenesis related to disease [119]. Voci et al. [120] showed that TGF-β1 increases IGF-1 production in hepatocytes. The current study showed that TGF-β1 caused an increase in IGF1 gene expression in FDP tenocytes, in cell culture (Figure 4f); indicating that IGF-1 may play a role in tendon metabolism, regeneration or pathology, and may be modulated by TGF-β1 in vivo.

Summary and Conclusion

The present study investigated the effect of TGF-β1 (5 ng/ml) on the expression of several genes in FDP tenocytes in cell culture at 6h and 24h. The results showed that TGF-β1 modulates the expression of genes involved in fibrinolysis (SERPINE1, PLAU), contraction (ACTA2), angiogenesis, inflammation and fibrosis (CTGF), cell adhesion, growth, migration, and differentiation (FN1), tensile strength, maturation, remodeling and healing (COL1A1, COL3A1), cross-linking in ECM fibrils (LOX), ECM (ACAN), mechanical strength and fibrosis (COMP), remodeling (MMP9, MMP13, TIMP1, TIMP3), collagen fiber assembly (BGN), cell proliferation, differentiation, ECM production, wound healing, and tissue repair (DCN), differentiation and neotendon formation (SCX), cell adhesion and collagen fibrillogenesis (POSTN), regulation and fine tuning of TGF-β signaling as a negative regulator (SMAD7), inflammation (IL6), and wound healing and regeneration (IGF1). The expression of MMP2 and TIMP2 was not affected under these conditions. Future studies are needed to identify whether the therapeutic modulation of these downstream targets of TGF-β1 can improve the results of tendon healing. In conclusion, TGF-β1 plays a pleotropic role in human FDP tendon physiology, structure, regeneration and adhesion formation, and the therapeutic targeting of these TGF-β1 affected genes may be a novel approach to help improve FDP healing and reduce the formation of adhesions.
Declaration

Authors have nothing to declare.

Contribution

SCJ designed the experiment. SCJ, TC and YMF acquired the data. SCJ, TC, YMF and CV analyzed the data. SCJ, TC, YMF and CV drafted the article and approved the final version.

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