Follistatin mitigates myofibroblast differentiation and collagen synthesis of fibroblasts from scar tissue around injured flexor tendons

Su-Keon Lee

Department of Medicine

The Graduate School, Yonsei University

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Directed by Professor Ho-Jung Kang

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Su-Keon Lee

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This certifies that the Doctoral Dissertation
of Su-Keon Lee is approved.

Thesis Supervisor : Ho Jung Kang

Thesis Committee Member#1 : Seong Hwan Moon

Thesis Committee Member#2 : Yun Rak Choi

Thesis Committee Member#3: Yong Jung Park

Thesis Committee Member#4: Dong Suk Yoon

The Graduate School
Yonsei University

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Su Keon Lee

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Ho Jung Kang)

Purpose: The aim of this study was to investigate the effect of the FST gene on the inhibition of fibrosis in fibroblastic cells from scar tissue around the repaired zone II flexor tendons.

Materials and Methods: Immunohistochemistry was conducted on fibroblast cells transduced with adenovirus-LacZ (Ad-Mock) as a marker gene (control) or adenovirus-FST (Ad-FST) as a therapeutic gene. Fibroblast cultures, without adenoviral exposure, served as controls.

Results: Fibroblastic cells transduced with Ad-FST demonstrated significant decreases in collagen type I, *MMP-1*, *MMP2*, and *α-SMA* mRNA expressions compared to those transduced with Ad-LacZ. In addition, fibroblastic cells with Ad-FST exhibited significant decreases in MMP-1, TIMP-1, fibronectin, PAI-1, TRPV4, α-SMA, desmin, and PAX7 protein expression. Conclusion: Based on these findings, we conclude that FST may be a novel therapeutic strategy for preventing scar adhesions around repaired tendons by inhibiting fibroblasts from differentiating into myofibroblasts and producing type I collagen and regulating extracellular matrix turnover via the downregulation of MMP-1 and TIMP-1. FST may also decrease contracture of the scar by inhibiting Ca^{2+}-dependent cell contraction.

Key words : follistatin; tendon injuries; tendon adhesion; fibrosis; gene therapy

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I. INTRODUCTION

Although acute flexor tendon injuries are rare, restoration of hand function following such trauma has been challenging[1]. Adhesions may form between the repaired tendons and the surrounding tendon sheath, especially in the tight fibroosseous spaces of zone II injuries, despite meticulous surgical techniques and aggressive postoperative therapy[2]. Several innovative approaches to avoid adhesion formation after tendon repair have been proposed, including anti-inflammatory agents such as steroids and non-steroidal anti-inflammatory drugs[3,4]; natural polymers such as hyaluronic acid[5-7], collagen solution[8], and amniotic fluid[8]; anti-metabolites such as 5-fluorouracil[9,10]; and barrier methods such as polytetrafluoroethylene membrane[11] and porcine gelatin[12]. However, these anti-adhesion therapies remain unsuccessful and have not progressed beyond preclinical studies.

It has been shown that the transforming growth factor-beta (TGF-β) pathway is involved in the formation of adhesions[13]. Studies in which the TGF-β pathway is inhibited have reported an improvement in the range of digital motion as well as alteration of collagen production[14,15]. In vitro studies using the natural TGF-β inhibitors decorin and mannose 6-phosphate (M6P) have reported a reduction in TGF-β induced collagen production[16]. However, other studies have reported that TGF-β pathway inhibition is associated with a decrease in mechanical strength[17,18], and a clinical trial

of M6P did not demonstrate a beneficial effect on finger movements[19].

It has been reported that activin A in fibroblasts, which is induced by TGF-β, stimulates inflammation and fibrosis in many different models, including in inflammatory bowel disease, rheumatoid arthritis, and wound healing and following burn injuries [20,21]. A key regulator of activin is follistatin (FST), a glycoprotein that binds activin. This binding is virtually irreversible. The resulting complex targets a lysosomal degradation pathway[22]. FST can block the fibrogenic actions of TGF-β despite its inability to bind TGF-β1, suggesting that TGF-β1 is exerting its fibrogenic actions through its stimulation of activin A[21]. In addition, as FST binds to activin A, it attenuates the capacity of activin A to stimulate fibrosis, as demonstrated in hepatic fibrosis[23], pulmonary fibrosis[24], and radiation-induced fibrosis[25].

In this study, we hypothesized that the activin/FST pathway offers a novel biological target for preventing the formation of tendon adhesions. We used an adenovirus system to transfer a follistatin gene construct into fibroblasts isolated from scar tissue around the repaired flexor tendons of patients who underwent tenolysis after a zone II tendon repair. The goal of this experiment was to investigate the effect of this gene on the inhibition of fibrosis.

II. MATERIALS AND METHODS

The Human Subjects Institutional Review Board of our institution approved all of the experimental protocols in this study.

Study design

To test the anti-fibrotic effect of the adenovirus-FST construct (Ad-FST) on fibroblasts *in vitro*, we utilized cells from the scar tissue of patients who underwent tenolysis following zone II flexor tendon repairs. The fibroblasts were activated by TGF-β1 (2 ng/mL), exposed to Ad-FST as a therapeutic gene and adenovirus-LacZ construct (Ad-

Mock) as a marker gene, and cultured for 24 hours. A second set of fibroblast cultures that were not exposed to Ad-FST or Ad-Mock served as the control. The mRNA expression levels of collagen types I, III, IV, V, and XI, as well as MMP-1, -3, -8, -9, and -13 were analyzed by reverse-transcription polymerase chain reaction (RT-PCR). In addition, protein expression levels of fibronectin, PAI-1, TRPV4, MMP-1, -2, -13, TIMP-1, -2, -4, and alpha smooth muscle actin (α-SMA) were estimated using Western blot analysis.

Isolation and cell culture

The scar tissues around the repaired zone II flexors were harvested from seven patients in whom adhesions had formed between the tendon and its sheath during tenolysis. Minced tissues were digested at 37℃ under gentle agitation with type IV collagenase (250 unit/mL; Sigma, St. Louis, MO, USA). Cells were placed in culture plates (TPP, Trasadingen, Switzerland) at 4×10^5 cells/mL. Primary cultures were sustained for 2–3 weeks in Dulbecco's Modified Eagle Medium (DMEM; Life Technologies Corporation, Carlsbad, CA, USA) containing 10% fetal bovine serum, 1% v/v penicillin, streptomycin, and nystatin (Life Technologies Corporation) in a 37℃ incubator with 5% CO_2 and humidity.

Follistatin constructs and transductions

For this study, Ad-FST expressing the human follistatin gene was prepared and Ad-LacZ expressing the LacZ gene was used as a viral control. Each recombinant adenoviral vector originated from replication-deficient adenovirus type 5 lacking E1 and E3 regions (Fig. 1). The FST gene was cloned into the E1 region under the control of the human cytomegalovirus early promoter. Recombinant virus was grown in transformed human embryonic kidney 293 (HEK293) cells and purified with Vivapure AdenoPACK 100 (Sartorius Stedim Biotech, Goettingen, Germany). Titers were

determined by optical density at 260 nm. At confluence, the fibroblastic cells were rinsed with Dulbecco's Phosphate Buffered Saline (PBS; Life Technologies Corporation), and then exposed to DMEM containing one dose of Ad-LacZ or Ad-FST with a viral multiplicity of infection of 80.

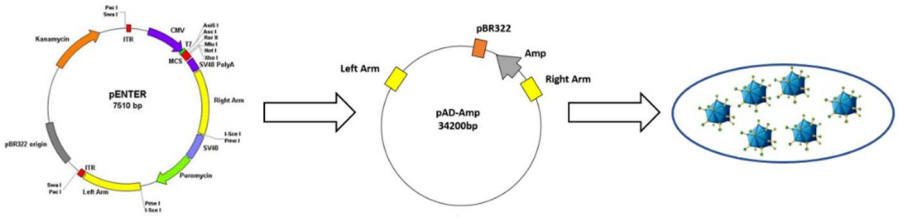

Fig 1. Recombinant adenovirus was made by insertion of follistatin (FST) gene under control of human cytomegalovirus early promoter.

RT-PCR analysis for matrix metalloproteinases and collagens

Total RNA was isolated from the fibroblasts transduced with Ad-FST for 24 hours using the QIAGEN RNeasy mini kit (QIAGEN, Germantown, MD, USA). In addition, cDNA was prepared using the QuantiTect Reverse Transcription Kit (QIAGEN). Collagen and MMP genes were amplified, and relative expressions were normalized to the average beta-actin levels. Resulting data were analyzed using ImageJ software version 1.6 (National Institutes of Health, Bethesda, MD, USA).

Protein extraction and Western blot analysis

Fibroblasts at a density of 2×10^5 cells per well were transduced with Ad-FST for 24 hours, and lysed in RIPA lysis buffer (ATTO Corp., Tokyo, Japan) containing protease inhibitor (Pierce Mini Tablets, IL, USA). Meanwhile, the culture medium was collected to measure FST protein expression by Ad-FST. Lysates and culture medium were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and proteins then were transferred to polyvinylidene difluoride membranes(Merck Millipore Ltd., Darmstadt, Germany) using a transfer system (Mini Trans-Blot Cell and

systems, Bio-Rad, Hercules, CA, USA). The blots were incubated with specific antibodies against MMP-1, MMP-13, TIMP-1, TIMP-2, TIMP-4, fibronectin, PAI-1, TRPV4, and α-SMA (Abcam, Cambridge, UK). After reacting with secondary antibodies, immunoreactive bands were visualized by a Western blot detection system (EzWestLumi Plus, ATTO Corp., Japan). To verify the amounts of loaded proteins, blots were stripped of bound antibodies and re-probed using antibodies to actin (Abcam).

Immunocytochemistry

The cells were transduced with Ad-FST for 24 hours at a density of 2×10^5 cells per well in culture medium and were fixed in 4% formaldehyde. The cells then were permeabilized in 0.1% Triton X-100 for 20 minutes and incubated in PBS with 1% bovine serum albumin for 1 hour. The cells were incubated with the antibodies overnight at 4°C. The secondary antibodies (stained red and green) were used at a 1:1,000 dilution for 1 hour. DAPI was used to stain the cell nuclei (blue).

Collagen Membrane Study

The cells were transduced with Ad-FST for 24 hours at a density of 5×10^5 cells per collagen membrane (5m x 5m x 3m) in culture medium and were fixed in 4% formaldehyde. Then the cells were permeabilized 0.1% triton x100 for 20 minutes and incubated in PBS with 1% bovine serum albumin for 1hours. The cells were incubated with the actin and follistatin antibodies overnight at 4°C. The secondary antibodies (red and green) were used at a 1:100 dilution for 1 hour. DAPI was used to stain the cell nuclei (blue). Images were recorded on a LSM710 confocal microscope (Carl Zeiss MicroImaging GmbH, Germany) equipped with a 63x oil-emersion objective.

Statistical analyses

All data are expressed as the mean ± standard deviation (SD) compiled from three

independent experiments of separate cultures isolated from seven donors. A two-tailed Student's t-test was used to compare the means of the two groups. A value of $p < 0.05$ was considered statistically significant.

III. RESULTS

FST protein expression from Ad-FST transduced fibroblasts

Fibroblasts from the scar tissue of patients with adhesions around a zone II flexor tendon repair that were transduced with Ad-FST exhibited increased staining of FST protein on confocal images compared to fibroblasts transduced with a control (Fig. 2), indicating the FST gene construct was efficiently transduced into the fibroblasts.

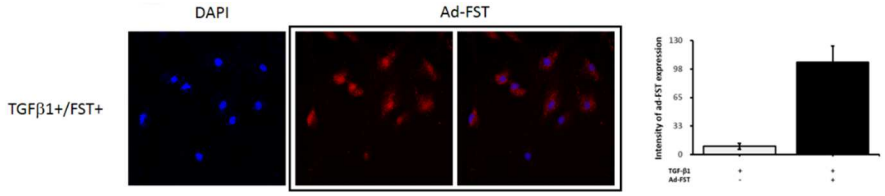

Fig 2. Immunocytochemistry analysis shows highly efficient transduction rate of adenoviral marker gene when activated by transforming growth factor beta-1 (TGF-β1). 4, 6-di-amidino-2-phenylindole (DAPI) shows blue nuclei and FST stain can be seen in red. Graph shows AD-FST + group shows significant expression of FST compared to AD-FST– group.

Expression of collagen type I, Mmp-1, and Mmp-2 mRNAs

Fibroblasts transduced with Ad-FST exhibited a significant (25%) decrease in collagen type I mRNA expression at 24 hours compared to Ad-LacZ controls (TGF- β1+) ($p < 0.05$) (Fig. 3). Fibroblasts transduced with Ad-FST also exhibited significant decreases in *Mmp-1* and *Mmp-2* mRNA expression (31% and 59%, retrospectively) at 24 hours compared to Ad-LacZ controls (TGF- β1+) (Fig. 4). The expression of *α-SMA* mRNA also was significantly decreased (23%) in fibroblasts transduced with Ad-FST at 24

hours compared to Ad-LacZ controls (TGF- β1+) ($p < 0.05$) (Fig. 5). The effect of TGF-β1 on these fibroblasts did not change when treated with a selective small molecule inhibitor, SB505124, of the canonical (i.e., Smad-dependent) TGF-β signaling pathway. These findings suggest that fibroblasts may be activated by TGF-β1 via the non-canonical TGF-β signaling pathway.

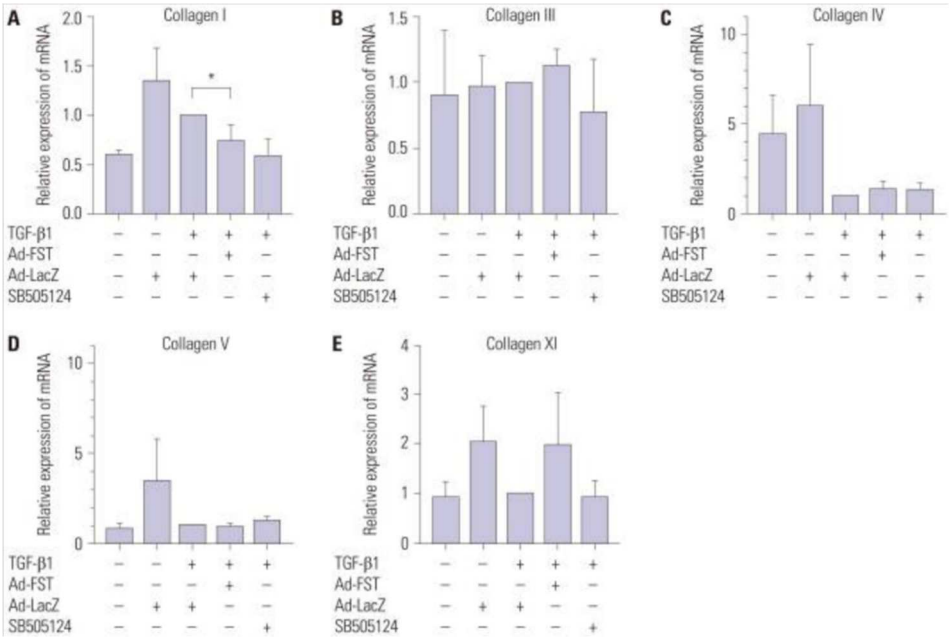

Fig3. Gene expression of collagens in fibroblasts from adhesion tissue in patients with zone II flexor repair and transduction with/without adenovirus follistatin gene construct (Ad-FST/AD-LacZ). Expression of collagen type I mRNA in fibroblasts with Ad-FST showed a 25% decrease at 24 hours compared to control (Ad-LacZ). + means 'treatment' and – means 'no treatment'. (* indicates $p<0.05$. TGF-β1 transforming growth facto-beta 1, Ad-LacZ adenovirus LacZ gene construct, SB505124 selective inhibitor of transforming growth factor-β type 1 receptor).

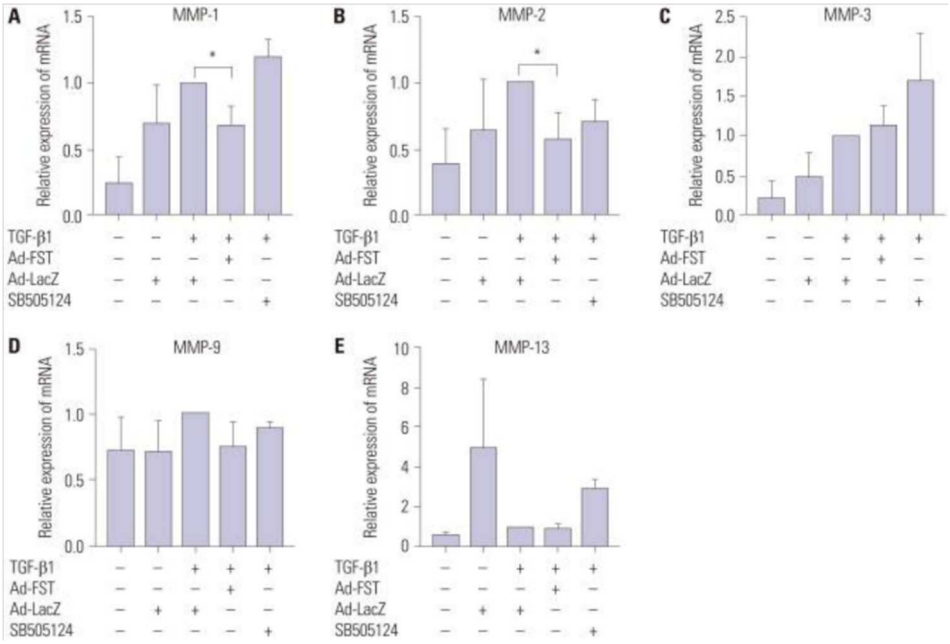

Fig4. Gene expression of MMPs in fibroblasts from adhesion tissue in patients with zone II flexor repair and transduction with/without adenovirus follistatin gene construct (Ad-FST/AD-LacZ). Expressions of MMP-1 and MMP-2 mRNA in fibroblast with Ad-FST showed a 31% and 59% decrease, respectively at 24 hours compared to control (Ad-LacZ). + means 'treatment' and – means 'no treatment'. (* indicates $p<0.05$. TGF-β1 transforming growth facto-beta 1, Ad-LacZ adenovirus LacZ gene construct, SB505124 selective inhibitor of transforming growth factor-β type 1 receptor).

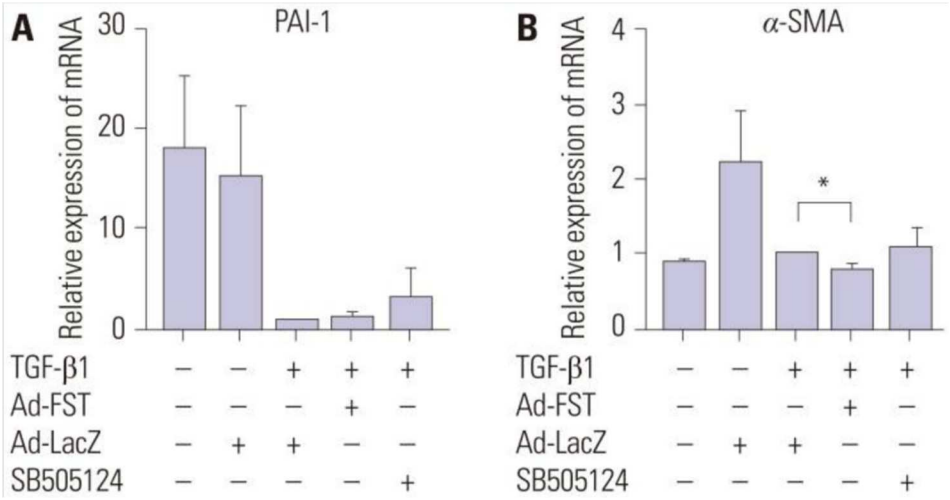

Fig5. Gene expression of PAI-1 and α-SMA in fibroblasts from adhesion tissue in patients with zone II flexor repair and transduction with/without adenovirus follistatin gene construct (Ad-FST/AD-LacZ). Expression of α-SMA mRNA in fibroblasts with Ad-FST showed a 23% at 24 hours compared to control (Ad-LacZ). + means 'treatment' and – means 'no treatment'. (* indicates $p<0.05$. TGF-β1 transforming growth factor-beta 1, Ad-LacZ adenovirus LacZ gene construct, SB505124 selective inhibitor of transforming growth factor-β type 1 receptor).

Expression of MMP-1, TIMP-1, fibronectin, PAI-1, TRPV4, and α-SMA

Fibroblasts transduced with Ad-FST exhibited significant decreases in MMP-1, TIMP-1 and fibronectin (24%, 23%, and 24%, retrospectively) at 24 hours compared to Ad-LacZ controls (TGF- β1+) ($p < 0.05$) (Figs. 6 & 7). Fibroblasts transduced with Ad-FST also demonstrated significant inhibition of PAI-1, TRPV4, and α-SMA protein expressions (23%, 23% and 28%, retrospectively) at 24 hours compared to Ad-LacZ controls (TGF- β1+) ($p < 0.05$) (Fig. 8).

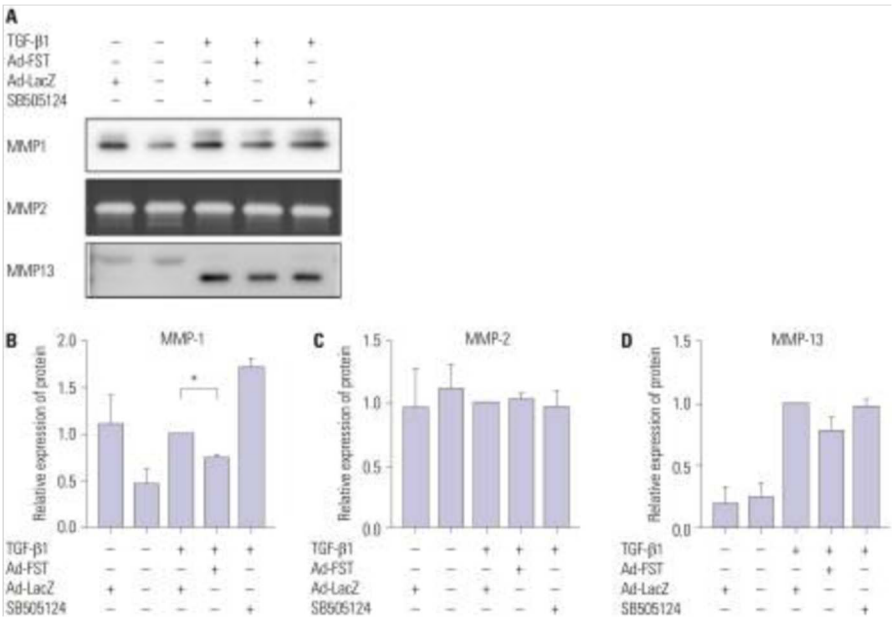

Fig6. Protein expressions of MMPs in fibroblasts from adhesion tissue in patients with zone II flexor repair and transduction with/without adenovirus follistatin gene construct (Ad-FST/AD-LacZ). Expression of MMP-1 protein in fibroblasts with Ad-FST showed a 24% decrease at 24 hours compared to control (Ad-LacZ). + means 'treatment' and –

means ‘no treatment’. (* indicates p<0.05. TGF-β1 transforming growth factor-beta 1, Ad-LacZ adenovirus LacZ gene construct, SB505124 selective inhibitor of transforming growth factor-β type 1 receptor).

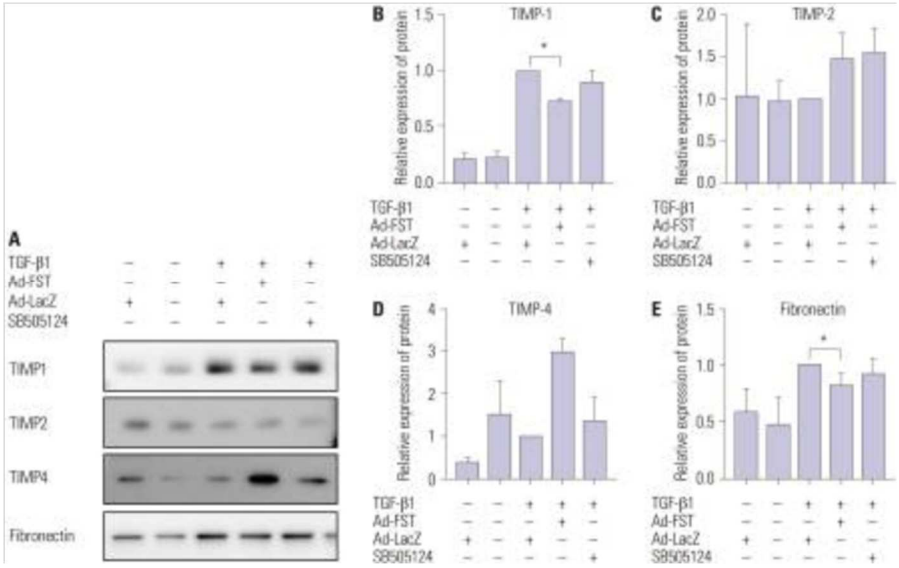

Fig7. Protein expressions of TIMPs and fibronectin in fibroblasts from adhesion tissue in patients with zone II flexor repair and transduction with/without adenovirus follistatin gene construct (Ad-FST/AD-LacZ). Expressions of TIMP-1 and fibronectin protein in fibroblasts with Ad-FST showed a 23% and 24% decrease, respectively at 24 hours compared to control (Ad-LacZ). + means ‘treatment’ and – means ‘no treatment’. (* indicates p<0.05. TGF-β1 transforming growth factor-beta 1, Ad-LacZ adenovirus LacZ gene construct, SB505124 selective inhibitor of transforming growth factor-β type 1 receptor).

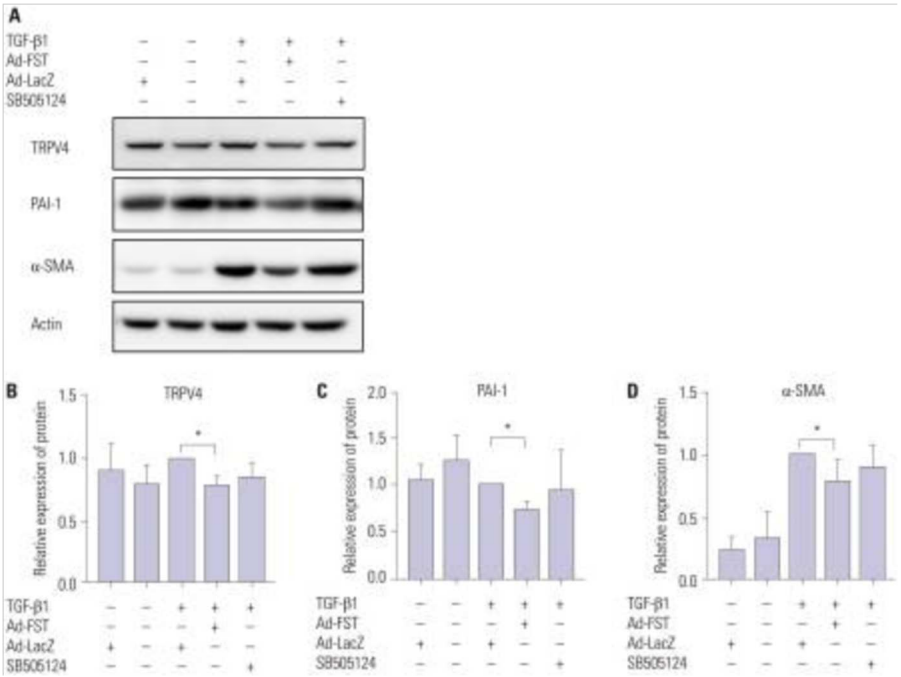

Fig 8. Protein expressions of TPRV4, PAI-1 and α-SMA in fibroblasts from adhesion tissue in patients with zone II flexor repair and transduction with/without adenovirus follistatin gene construct (Ad-FST/AD-LacZ). Expressions of TPRV4, PAI-1 and α-SMA protein in fibroblasts with Ad-FST showed a 23%, 23% and 28% decrease, respectively at 24 hours compared to control (Ad-LacZ). + means 'treatment' and – means 'no treatment'. (* indicates $p<0.05$. TGF-β1 transforming growth factor-beta 1, Ad-LacZ adenovirus LacZ gene construct, SB505124 selective inhibitor of transforming growth factor-β type 1 receptor).

Immunocytochemistry analysis of α-SMA, desmin, and PAX7

Fibroblasts with Ad-FST exhibited decreases in α-SMA, desmin, and PAX7 expression compared to Ad-LacZ controls (Fig.9).

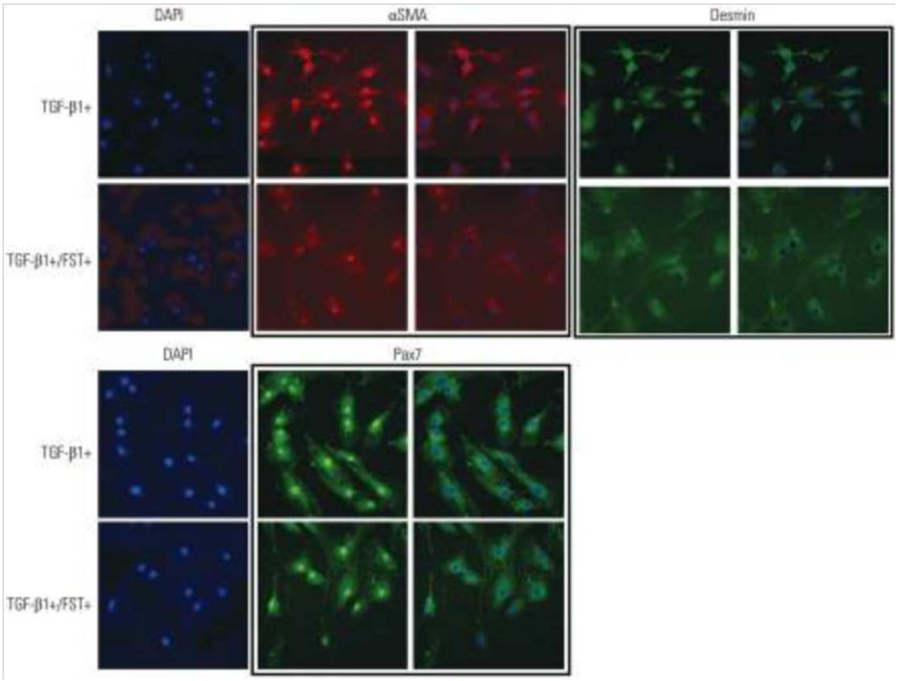

Fig 9. Fibroblasts with adenovirus follistatin gene construct (TGF-β1+/FST+) showed decrease in α-SMA, desmin and PAX7 expressions compared to control culture (TGF-β1+). (TGF-β1 transforming growth factor-beta 1; DAPI 4, 6-di-amidino-2-phenylindole)

Collagen membrane study

Fibroblasts with Ad-FST exhibited decrease in fibroblast size compared to Ad-LacZ controls (Fig.10).

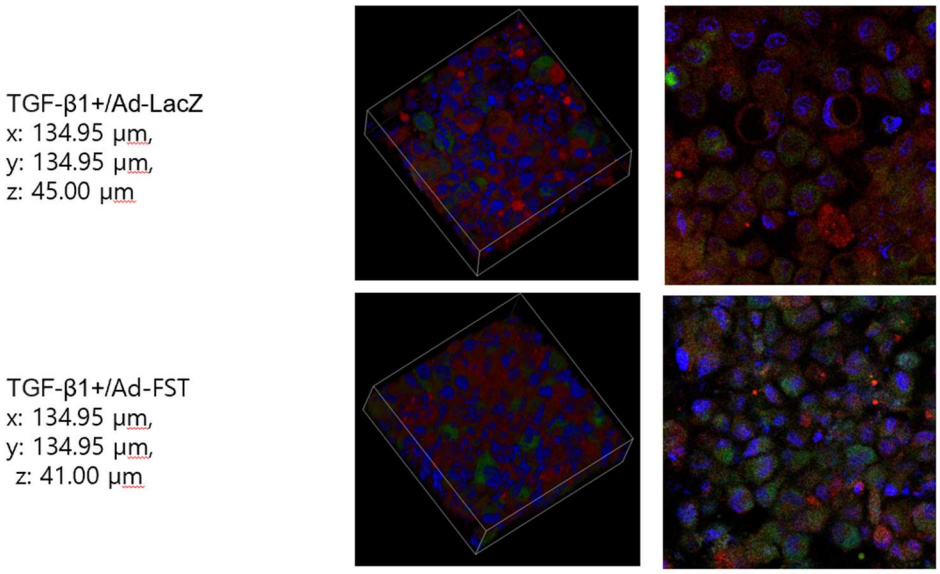

Fig 10. Fibroblasts with adenovirus follistatin gene construct (TGF-β1+/FST) showed significant decrease in cell size compared to control (TGF-β1+/Ad-LacZ).

IV. DISCUSSION

Following repair, tendons heal via both intrinsic and extrinsic pathways. Intrinsic healing involves the proliferation of tenocytes and the production of the extracellular matrix (ECM)[26]. Extrinsic healing involves an invasion of cells from the surrounding sheath and synovium, which envelop the tendon structure and contribute to the formation of adhesions[26]. Extrinsic healing dominates when the tendon or tendon sheath is injured because the tendon has poor cell density and growth factor activity. Although several approaches have been proposed to prevent the formation of adhesions, there is limited evidence that these approaches are successful, and none have been widely accepted for clinical use[27]. Because tendon injury stimulates increased expression of TGF-β1 in the production of collagen type I and type III, fibronectin, and glycosaminoglycans[28], blocking TGF-β1 has been reported to decrease tendon adhesion in animal tendon injury models. However, blocking TGF-β1 also can result in reduced

tendon strength because another role of TGF-β1 is to modulate immune responses and tissue repair[13,17,29]. It is therefore necessary to investigate methods that selectively isolate and block the pro-fibrosis effects of TGF-β1.

FST, a systemically expressed circulating glycoprotein, is the endogenous ligand-binding partner for activin A[30]. It is well recognized that FST can inhibit the activity of activin A, which is overexpressed in TGF-β1-induced fibrosis[21,31]. In the present study, the anti-fibrotic effect of FST was investigated via genetic modification of fibroblastic cells from patients with zone II flexor tendon adhesion after tendon repair. We found that fibroblastic cells from scar adhesion tissue transduced with Ad-FST exhibited a decrease in expression levels of collagen type I mRNA compared to fibroblasts transduced with Ad-LacZ controls. These findings indicate that the FST gene inhibits the transcription of collagen type I. We also showed that the inserted FST gene decreased the transcription and translation of MMP-1 and TIMP-1. Tissue inhibitors of metalloproteinases (TIMPs) are pleiotropic extracellular proteins, and are recognized as endogenous regulators of matrix metalloproteinases (MMPs), which comprise a large family of extracellular enzymes with proteolytic activities that participate in cellular homeostasis, adaptation, and tissue remodeling[32]. The findings of the present study suggest that FST restores the pathological imbalance of ECM turnover from zone II flexor tendon adhesions via the downregulation of MMP-1 and TIMP-1, which may contribute to the decrease of scar formation.

It is now accepted that myofibroblasts go through a precursor stage in which large stress fibers are expressed. These large stress fibers are not observed in quiescent fibroblasts. These prominent bundles of microfilaments permit some contraction, pre-stressing, and remodeling of the surrounding ECM[33]. At a later stage, fully differentiated myofibroblasts exhibit expression of α-SMA, which is usually an SM-specific cytoskeletal protein and is often used to define the myofibroblast phenotype[34]. In the present study, the synthesis of fibronectin, a non-collagenous protein, as well as PAI-1

and α-SMA, were suppressed in fibroblasts transduced with Ad-FST compared to Ad-LacZ controls at the protein level. The significant decrease in α-SMA expression suggests that FST may inhibit the transformation of fibroblasts to myofibroblasts. The decrease of transcription of collagen type I gene also may be associated with a decrease in the transformation of fibroblasts to myofibroblasts.

In the present study, the synthesis of TRPV4 was suppressed at the protein level in fibroblasts transduced with Ad-FST compared to Ad-LacZ controls. It has been proposed that the myofibroblasts contract via Ca^{2+}-dependent mechanisms similar to those present in smooth muscle cells, with increased free Ca^{2+} regulating the phosphorylation of the myosin light chain[35]. The findings from our study suggest that FST may decrease the contractibility of fibroblasts from tendon adhesion tissue by suppressing the translation of TRPV4. Collagen membrane study showed that cellularity was reduced in fibroblasts transduced with Ad-FST compared to Ad-LacZ controls. Myofibroblast has shown to be larger in size compared to fibroblasts[36]. The decrease in cellular size of fibroblasts seen in the collagen membrane study may also indicate that follistatin may reduce the phenotype differentiation of fibroblasts into myofibroblasts.

There are a few limitations to this study. The direct effect of FST on tenocytes after injury was not observed. Further investigation should be followed how FST would affect tendon healing and strength when tenocytes are tested. Another limitation was that this study was an in-vitro study. Further animal studies and clinical studies need to be performed to fortify the findings of this study.

Although many in-vitro and animal studies have been performed in various injury models[23, 24, 25] currently there are no clinically approved diseases for follistatin treatments. However, a clinical trial with promising results has been performed on patients with sporadic inclusion body myositis[37] and an ongoing clinical trial using direct intramuscular gene transfer is currently being performed on patients with

Duchene Muscular Dystrophy.

V. CONCLUSION

In conclusion, FST may be a novel therapeutic strategy for preventing scar adhesions around repaired tendons by inhibiting fibroblasts from differentiating into myofibroblasts and producing type I collagen and regulating ECM turnover via the downregulation of MMP-1 and TIMP-1. FST may also decrease the contracture of the scar produced from myofibroblasts by inhibiting Ca^{2+}-dependent contraction of the cells.

REFERENCES

1 Clayton RA, Court-Brown CM. The epidemiology of musculoskeletal tendinous and ligamentous injuries. *Injury* 2008;39:1338-1344.

2 Dy CJ, Hernandez-Soria A, Ma Y, Roberts TR, Daluiski A. Complications after flexor tendon repair: a systematic review and meta-analysis. *J Hand Surg Am* 2012;37:543-551.

3 Ketchum LD. Effects of triamcinolone on tendon healing and function. A laboratory study. *Plast Reconstr Surg* 1971;47:471-482.

4 Kulick MI, Brazlow R, Smith S, Hentz VR. Injectable ibuprofen: preliminary evaluation of its ability to decrease peritendinous adhesions. *Ann Plast Surg* 1984;13:459-467.

5 Amiel D, Ishizue K, Billings E Jr, Wiig M, Vande Berg J, *et al.* Hyaluronan in flexor tendon repair. *J Hand Surg Am* 1989;14:837-843.

6 Salti NI, Tuel RJ, Mass DP. Effect of hyaluronic acid on rabbit profundus flexor tendon healing in vitro. *J Surg Res* 1993;55:411-415.

7 Wiig M, Abrahamsson SO, Lundborg G. Tendon repair--cellular activities in rabbit deep flexor tendons and surrounding synovial sheaths and the effects of hyaluronan: an experimental study in vivo and in vitro. *J Hand Surg Am* 1997;22:818-825.

8 Nyska M, Porat S, Nyska A, Rousso M, Shoshan S. Decreased adhesion formation in flexor tendons by topical application of enriched collagen solution--a histological study. *Arch Orthop Trauma Surg* 1987;106:192-194.

9 Akali A, Khan U, Khaw PT, McGrouther AD. Decrease in adhesion formation by a single application of 5-fluorouracil after flexor tendon injury. *Plast Reconstr Surg* 1999;103:151-158.

10 Karaaltin MV, Ozalp B, Dadaci M, Kayikcioglu A, Kecik A, *et al.* The effects of 5-fluorouracil on flexor tendon healing by using a biodegradable gelatin,

slow releasing system: experimental study in a hen model. *J Hand Surg Eur Vol* 2013;38:651-657.

11 Bartle BK, Telepun GM, Goldberg NH. Development of a synthetic replacement for flexor tendon pulleys using expanded polytetrafluoroethylene membrane. *Ann Plast Surg* 1992;28:266-270.

12 Golash A, Kay A, Warner JG, Peck F, Watson JS, *et al.* Efficacy of ADCON-T/N after primary flexor tendon repair in Zone II: a controlled clinical trial. *J Hand Surg Br* 2003;28:113-115.

13 Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med* 2012;18:1028-1040.

14 Chang, J., Thunder, R., Most, D., Longaker, M. T. & Lineaweaver, W. C. Studies in flexor tendon wound healing: neutralizing antibody to TGF-beta1 increases postoperative range of motion. *Plast Reconstr Surg* 105, 148-155 (2000).

15 Chen CH, Zhou YL, Wu YF, Cao Y, Gao JS, *et al.* Effectiveness of microRNA in Down-regulation of TGF-beta gene expression in digital flexor tendons of chickens: in vitro and in vivo study. *J Hand Surg Am* 2009;34:1777-1784.

16 Bates SJ, Morrow E, Zhang AY, Pham H, Longaker MT, *et al.* Mannose-6-phosphate, an inhibitor of transforming growth factor-beta, improves range of motion after flexor tendon repair. *J Bone Joint Surg Am* 2006;88:2465-2472.

17 Katzel EB, Wolenski M, Loiselle AE, Basile P, Flick LM, *et al.* Impact of Smad3 loss of function on scarring and adhesion formation during tendon healing. *J Orthop Res* 2011;29:684-693.

18 Jorgensen HG, McLellan SD, Crossan JF, Curtis AS. Neutralisation of TGF beta or binding of VLA-4 to fibronectin prevents rat tendon adhesion following transection. *Cytokine* 2005;30:195-202.

19 Lees VC, Warwick D, Gillespie P, Brown A, Akhavani M, *et al.* A multicentre, randomized, double-blind trial of the safety and efficacy of mannose-6-phosphate in patients having Zone II flexor tendon repairs. *J Hand Surg Eur Vol* 2015;40:682-694.

20 Sugino K, Kurosawa N, Nakamura T, Takio K, Shimasaki S, *et al.* Molecular heterogeneity of follistatin, an activin-binding protein. Higher affinity of the carboxyl-terminal truncated forms for heparan sulfate proteoglycans on the ovarian granulosa cell. *J Biol Chem* 1993;268:15579-15587.

21 Hedger MP, Winnall WR, Phillips DJ, de Kretser DM. The regulation and functions of activin and follistatin in inflammation and immunity. *Vitam Horm* 2011;85:255-297.

22 Hashimoto O, Nakamura T, Shoji H, Shimasaki S, Hayashi Y, *et al.* A novel role of follistatin, an activin-binding protein, in the inhibition of activin action in rat pituitary cells. Endocytotic degradation of activin and its acceleration by follistatin associated with cell-surface heparan sulfate. *J Biol Chem* 1997;272:13835-13842.

23 Patella S, Phillips DJ, Tchongue J, de Kretser DM, Sievert W. Follistatin attenuates early liver fibrosis: effects on hepatic stellate cell activation and hepatocyte apoptosis. *Am J Physiol Gastrointest Liver Physiol* 2006;290:G137-144.

24 Aoki F, Kurabayashi M, Hasegawa Y, Kojima I. Attenuation of bleomycin-induced pulmonary fibrosis by follistatin. *Am J Respir Crit Care Med* 2005;172:713-720.

25 Forrester HB, Li J, Leong T, McKay MJ, Sprung CN. Identification of a radiation sensitivity gene expression profile in primary fibroblasts derived from patients who developed radiotherapy-induced fibrosis. *Radiother Oncol* 2014;111:186-193.

26 Koob TJ, Summers AP. Tendon--bridging the gap. *Comp Biochem Physiol A Mol Integr Physiol* 2002;133:905-909.

27 Khanna A, Friel M, Gougoulias N, Longo UG, Maffulli N. Prevention of adhesions in surgery of the flexor tendons of the hand: what is the evidence? *Br Med Bull* 2009;90:85-109.

28 Thomopoulos S, Parks WC, Rifkin DB, Derwin KA. Mechanisms of tendon injury and repair. *J Orthop Res* 2015;33:832-839.

29 Wu YF, Mao WF, Zhou YL, Wang XT, Liu PY, *et al.* Adeno-associated virus-2-mediated TGF-beta1 microRNA transfection inhibits adhesion formation after digital flexor tendon injury. *Gene Ther* 2016;23:167-175.

30 DePaolo LV. Inhibins, activins, and follistatins: the saga continues. *Proc Soc Exp Biol Med* 1997;214:328-339.

31 Harrington AE, Morris-Triggs SA, Ruotolo BT, Robinson CV, Ohnuma S, *et al.* Structural basis for the inhibition of activin signalling by follistatin. *EMBO J* 2006;25:1035-1045.

32 Moore CS, Crocker SJ. An alternate perspective on the roles of TIMPs and MMPs in pathology. *Am J Pathol* 2012;180:12-16.

33 Hinz B. Formation and function of the myofibroblast during tissue repair. *J Invest Dermatol* 2007;127:526-537.

34 Sandbo N, Dulin N. Actin cytoskeleton in myofibroblast differentiation: ultrastructure defining form and driving function. *Transl Res* 2011;158:181-196.

35 Follonier Castella L, Gabbiani G, McCulloch CA, Hinz B. Regulation of myofibroblast activities: calcium pulls some strings behind the scene. *Exp Cell Res* 2010;316:2390-2401.

36 Baum J, Duffy H. Fibroblast and Myofibroblast: What are we talking about? J Caridovas Pharmacol. 2011 Apr; 57(4): 376-379

37 Mendell JR, Sahenk Z, Al-Zaidy S, Rodino-Klapac L. Follistatin gene thereapy for sporadic inclusion body myositis improves functional outcomes. Mol Ther. 2017 Apr 5:25(4): 870-879

ABSTRACT(IN KOREAN)

Follistatin의 굴곡건 손상 반흔 조직의 섬유모세포의 근섬유모세포 분화 및 콜라겐 합성 감소에 관한 연구

<지도교수 강호정>

연세대학교 대학원 의학과
이 수 건

연구 목적: Follistatin (FST) 유전자가 굴곡건 2 구역 손상 봉합 후 유착 조직에서 얻은 섬유모세포의 섬유화 예방에 대한 연구

연구 방법: 아데노바이러스-LacZ 유전자가 이입된 섬유모세포 대조군과 아데노바이러스-FST 유전자가 이입된 섬유모세포 실험군을 면역조직화학적 검사로 비교.

결과: 아데노바이러스-FST 유전자 이입된 섬유모세포 실험군이 아데노바이러스-LacZ 로 유전자 이입된 섬유모세포 대조군에 비하여 제 1형 콜라겐, 금속단백분해효소-1, 금속단백분해효소-2, 알파-평활근 액틴 전령리보핵산 (mRNA)의 발현이 유의하게 감소하였다. 또한, 아데노바이러스-FST 실험군에서는 금속단백분해효소-1, 금속단백질분해효소조직억제제-1, 섬유결합소, 플마스미노겐 활성화 억제제-1, TRPV4, 알파-평활근 액틴, 데스민, PAX7 단백질의 발현이 유의하게 감소하였다.

결론: Follistatin은 섬유모세포가 금섬유모세포로 분화를 억제, 제 1 형콜라겐 형성 감소, 금속단백분해효소-1 및 금속단백질분해효소조직억제제-1 억제를 통한 세포외기질 형성 조절을 통해 봉합술 후 건의 유착을 예방 할 수 있는 새로운 치료제가 될 수 있을 것으로 사료 된다.

핵심되는 말 : follistatin; 건손상; 건 유착; 섬유화; 유전자 치료

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