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Knockdown of FOXM1 inhibits activation of keloid fibroblasts and extracellular matrix production via inhibition of TGF-β1/Smad pathway

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ABSTRACT

Keloid is characterized by overactive fibroblasts. Forkhead box M1 (FOXM1) is transcription factor that plays important roles in the progression of fibrosis. However, the role of FOXM1 in keloid has not been elucidated. In the present study, we examined the expression levels of FOXM1 in clinical keloid tissue specimens and primary keloid fibroblasts (KFs). The results showed that FOXM1 levels were significantly increased in both keloid tissues and KFs. To further investigate the biological functions of FOXM1, FOXM1 was knocked down in KFs by transfection with small interfering RNA targeting FOXM1 (si-FOXM1). Knockdown of FOXM1 inhibited transforming growth factor-β1 (TGF-β1)-induced cell proliferation and migration of KFs. Besides, the increased expressions of collagen (coll I), connective tissue growth factor (CTGF), and α-smooth muscle actin (α-SMA) in TGF-β1-induced KFs were suppressed by si-FOXM1 transfection. Furthermore, TGF-β1-induced increase in p-Smad2 and p-Smad3 expressions was attenuated by FOXM1 knockdown. These data indicated that knockdown of FOXM1 inhibited TGF-β1-induced KFs activation and extracellular matrix (ECM) accumulation, which was attributed to the inhibition of TGF-β1/Smad pathway.

1. Introduction

Keloid, also known as keloid disorder or keloidal scar, is an exuberant response to cutaneous wound healing [1,2]. Keloids is a pathologic fibro-proliferative disease in which proliferative scars grow beyond the confines of the original wound skin, invading into adjacent normal tissue. Although keloid is benign and not contagious, it is commonly accompanied by severe itchiness, pain, and even being ulcerate [3]. It has been surveyed that > 11 million people affect with keloids in the world [4]. Nevertheless, the management of keloid is limited. The most common treatment option is removing the scar, however, it may result in various consequences. For instance, the surgery scar will also become a keloid [5,6]. Therefore, elucidation of the keloid pathogenesis is quite important for developing effective therapeutic strategy. Keloid is characterized by overactive fibroblasts, excessive expression of growth factors and cytokines, effusive extracellular matrix (ECM) component deposition, particularly collagen [5,7]. Several lines of evidence suggest that suppression of fibroblasts activation and ECM component deposition may be potential approach for keloid treatment [8–10].

Forkhead box (FOX) proteins are a family of transcription factors that play important roles in various cellular events including cell differentiation, growth, proliferation, and death [11]. It has been demonstrated that many FOX proteins are involved in the progression of fibrosis, such as FOXA [12], FOXC [13], FOXM [14], and FOXO family [15]. FOXM1 is a member of FOX family that plays important roles in the fibrosis progression. FOXM1 has been demonstrated to be implicated in cardiac fibrosis and lung fibrosis [16,17]. Targeting FOXM1 has the therapeutic potential for the treatment of fibrosis-related diseases. However, the role of FOXM1 in keloid remains unknown. Therefore, the aim of this study was to examine the effects of FOXM1 on keloid fibroblasts (KFs) and explored the underlying mechanism.

2. Materials and methods

2.1. Tissue specimens

Keloid tissue specimens and adjacent normal skin tissues were obtained from ten patients (age range 14–35, mean 25.4 years) who received surgical procedures at the Second Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China) between December 2016 and July 2017. This research was approved by the Institutional Research Ethics

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Committee of the Second Affiliated Hospital of Xi'an Jiaotong University. Informed consent was provided from all participants.

2.2. Cell culture and treatment

Primary KFs and normal human skin fibroblasts (NFs) were isolated from clinical tissues as described previously [18]. The epidermis and subcutaneous adipose tissues were removed. Subsequently, specimens were cut into pieces of 2–4 mm and digested with collagenase type-I (0.5 mg/ml) and trypsin (0.2 mg/ml) for 6 h at 37 °C. After digestion, the suspension was filtered and centrifuged at 1500 rpm for 5 min. The cells were cultured in Dulbecco's Modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco), and 1% penicillin/streptomycin (Life Technologies, Grand Island, NY, USA) in an atmosphere containing 5% CO_2 at 37 °C. Fibroblasts at passages of 3–5 were used in this study. For the human transforming growth factor-beta 1 (TGF-β1) stimulation group, KFs were treated with TGF-β1 (PeproTech, Rocky Hill, New Jersey, USA) at a concentration of 5 ng/ml for 24 h.

2.3. Cell transfection

Small interfering RNA targeting FOXM1 (si-FOXM1) or negative control siRNA (si-con) (GenePharma, Shanghai, China) was transfected into keloid fibroblasts using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Cells transfected with si-con were used as controls. After 48 h post transfection, western blot analysis was performed to assess the transfection efficiency.

The cDNA fragments of FOXM1 expression sequences were inserted into a pcDNA3.1 vector to generate pcDNA3.1/FOXM1 expression vector. Cell transfection was performed with Lipofectamine RNAiMAX Transfection Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

2.4. Cell proliferation assay

Cell proliferation was assessed by MTT assay. Keloid fibroblasts were seeded in 96-well plates at a density of 1×10^4 cells/well. After incubation for an indicated time (24 h, 48 h, and 72 h), 20 μl of 5 mg/ml MTT solution (Sigma, St. Louis, MO, USA) was added to each well and incubated for 4 h, followed by adding 150 μl of dimethyl sulfoxide (DMSO; Sigma) to dissolve the formazan crystals. Then, absorbance was monitored at 490 nm using a microplate reader (Bio-Tek, Winooski, VT, USA).

2.5. Cell migration assay

Cell migration of keloid fibroblast was evaluated by transwell assay using a transwell chamber (8-μm pore size filter; Millipore, Billerica, MA, USA). The upper chamber was plated with keloid fibroblasts (1×10^4 cells/well) in serum-free medium. Then, 500 μl of DMEM with 10% FBS was added to the lower chamber. After incubation for 24 h, cells on the upper surface of the membrane were removed. Cells on the lower surface were fixed in methanol and stained with 1% crystal violet. Then the cells from five randomly selected fields were counted under an inverted microscope (Olympus, Tokyo, Japan).

2.6. Quantitative real-time RT-PCR (qRT-PCR) analysis

Total RNA was extracted from clinical samples and fibroblasts using Trizol reagent (Invitrogen). Then the RNA was used for reverse transcription to synthesize cDNA with the PrimeScript reverse transcriptase reagent kit (Takara, Dalian, China). Subsequently, qRT-PCR was conducted on ABI Prism 7500 (Applied Biosystems, Foster City, CA, USA) using SYBR Green Master Mix (Takara) with the following primers sequences: FOXM1 5′-ATACGTGGATTGAGGACCACT-3′ (forward), 5′-TCCAATGTCAAGTAGCGGTT G-3′ (reverse); α-smooth muscle actin (α-SMA) 5′-TCAAATACCCCATTGAACACGG-3′ (forward), 5′-GGTGC TCT TCAGGTGCTACA-3′ (reverse); type I collagen a1(Col I a1) 5′-TCTGACTGGAAG AGTGGAGAGTAC-3′ (forward), 5′-ATCCATCGGT CATGCTCTCG-3′ (reverse); connective tissue growth factor (CTGF) 5′-GCTGACCTAGAGGAAAACATTAAG A-3′ (forward), 5′-CCGGTAGGT CTTCACACTGG-3′ (reverse); β-actin 5′-CATGTACGTTGCTATCCA GGC-3′ (forward), 5′-CTCCTTAATGTCACGCACGAT-3′ (reverse). β-Actin served as internal reference genes. Relative quantification of FOXM1 mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method.

2.7. Western blot

Total proteins of fibroblasts were extracted using RIPA lysis buffer (Sangon Biotech, Shanghai, China) and the protein concentration was quantified using a BCA protein assay kit (Sangon Biotech). Then, equal amounts of protein samples (50 μg) were subjected to 10% or 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore). The membranes were blocked with 5% dried skimmed milk for 1 h and then incubated with the various primary antibodies including FOXM1 (1:1500; Abcam, Cambridge, MA, USA), Col I (1:1000; Abcam), CTGF (1:1000; Abcam), α-SMA (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA), TGF-β type I receptor (TGF-βRI; 1:1500; Abcam), TGF-βRII (1:2500; Abcam), Smad2 (1:1000, Santa Cruz), p-Smad2 (1:1000, Santa Cruz), Smad3 (1:1000, Santa Cruz), p-Smad3 (1:1000, Santa Cruz) and β-actin (1:1000; Abcam) at 4 °C overnight. The membranes were then incubated with horseradish-peroxidase (HRP) conjugated secondary antibodies (1:5000; Abcam) for 1 h at 37 °C. The protein bands were detected using an enhanced chemiluminescence (ECL) reagent (Thermo Scientific, Waltham, MA, USA).

2.8. Statistical analysis

All data are expressed as the mean ± standard deviation (SD) and analyzed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA). Differences between two groups or more than two groups were carried out using student's *t*-test or one-way ANOVA followed by Dunnett's multiple comparisons test, respectively. $P < 0.05$ was considered statistically significant.

3. Results

3.1. The expression of FOXM1 was highly expressed in keloid tissues and KFs

The FOXM1 levels in keloid tissue specimens and adjacent normal skin tissues were examined using qRT-PCR. The results showed that FOXM1 levels in keloid tissue specimens were significantly higher than that in adjacent normal skin tissues (Fig. 1A). Furthermore, FOXM1 levels in primary keloid fibroblasts (KFs) and normal human skin fibroblasts (NFs) were also evaluated. Results of qRT-PCR and western blot indicated FOXM1 is highly expressed in KFs when compared with NFs (Fig. 1B and C).

3.2. Knockdown of FOXM1 suppressed TGF-β1-induced KFs proliferation and migration

To investigate the biological function of FOXM1 in KFs, FOXM1 was knocked down by transfection with si-FOXM1. Western blot showed that FOXM1 expression was markedly decreased after si-FOXM1 transfection compared to the si-con transfected KFs (Fig. 2A). TGF-β1 treatment significantly induced KFs proliferation, while knockdown of FOXM1 suppressed the cell proliferation (Fig. 2B). Besides, the TGF-β1-induced KFs migration was attenuated by FOXM1 knockdown (Fig. 2C). In addition, FOXM1 knockdown only also suppressed KFs proliferation

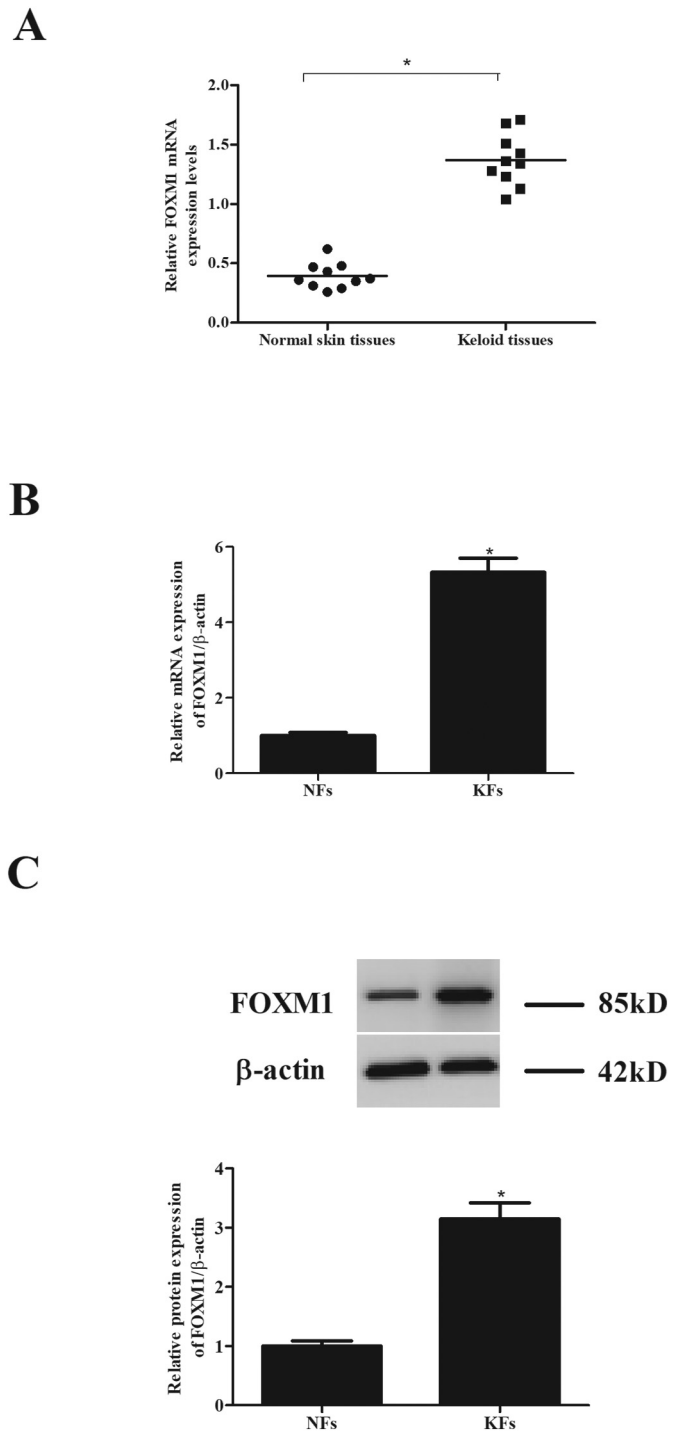

Fig. 1. Upregulation of FOXM1 in keloid tissues and KFs. (A) The mRNA levels of FOXM1 in tissue specimens were examined using qRT-PCR. (B) The mRNA levels of FOXM1 in cultured cells were determined using qRT-PCR. (C) The protein levels of FOXM1 in cultured cells were tested using western blot. * $p < 0.05$ vs. adjacent normal skin tissues. # $p < 0.05$ vs. normal human skin fibroblasts (NFs).

and migration (Fig. 2B and C).

3.3. Overexpression of FOXM1 enhanced TGF-β1-induced KFs proliferation and migration

Then, we overexpressed FOXM1 in KFs by transfecting a FOXM1 expression vector. The increased FOXM1 expression was confirmed by

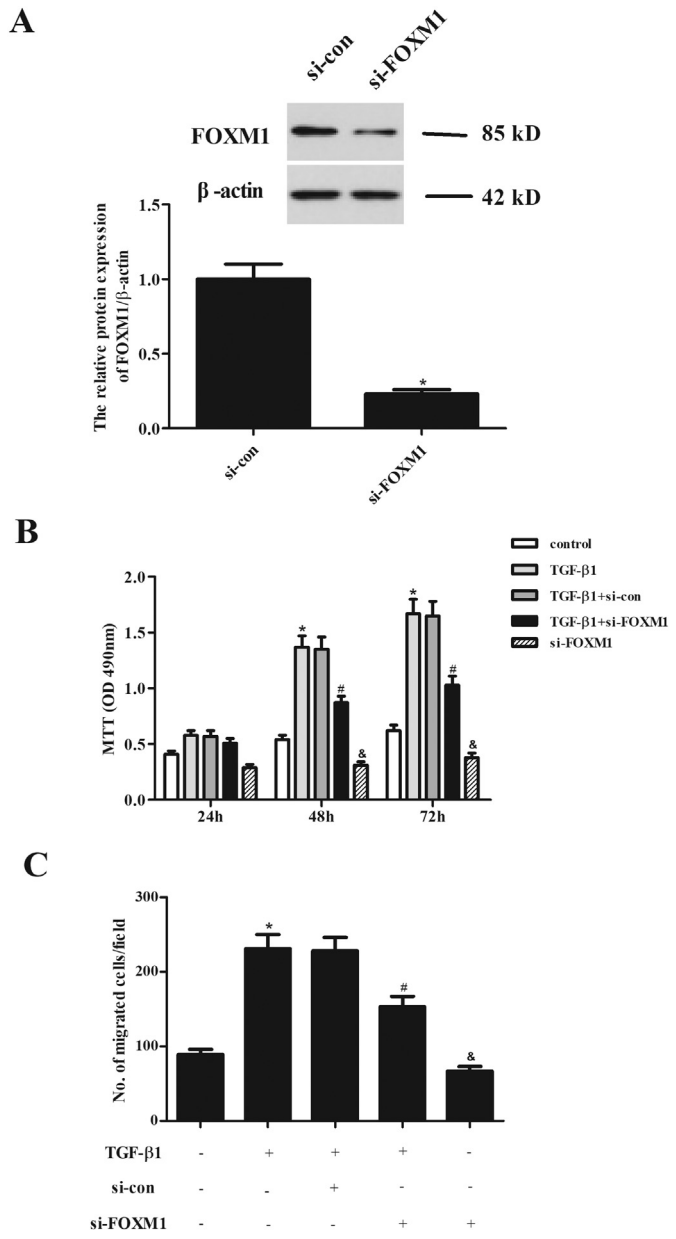

Fig. 2. Impact of FOXM1 knockdown on proliferation and migration in TGF-β1-induced KFs. (A) FOXM1 expressions in KFs after transfection with si-FOXM1 or si-con were examined using western blot. (B) Cell proliferation was assessed by MTT assay. (C) Cell migration was evaluated by transwell assay. * $p < 0.05$ vs. control; # $p < 0.05$ vs. TGF-β1 + si-con group; & $p < 0.05$ vs. control group.

western blot (Fig. 3A). As expected, we demonstrated that FOXM1 overexpression remarkably enhanced TGF-β1-induced KFs proliferation and migration (Fig. 3B and C).

3.4. Silencing FOXM1 also inhibited the expressions of Col I, CTGF and α-SMA in human KFs induced by TGF-β1

Then we explored the effect of FOXM1 knockdown on the expressions of Col I, CTGF and α-SMA using qRT-PCR and western blot. The qRT-PCR analysis showed that TGF-β1-caused increase in the mRNA levels of Col I, CTGF and α-SMA were mitigated by knockdown of FOXM1 (Fig. 4A). Moreover, the increased protein levels of Col I, CTGF and α-SMA in TGF-β1-stimulated KFs were alleviated by FOXM1 knockdown (Fig. 4B). In addition, knockdown of FOXM1 inhibited the expressions of Col I, CTGF and α-SMA in KFs (Fig. 4A and B).

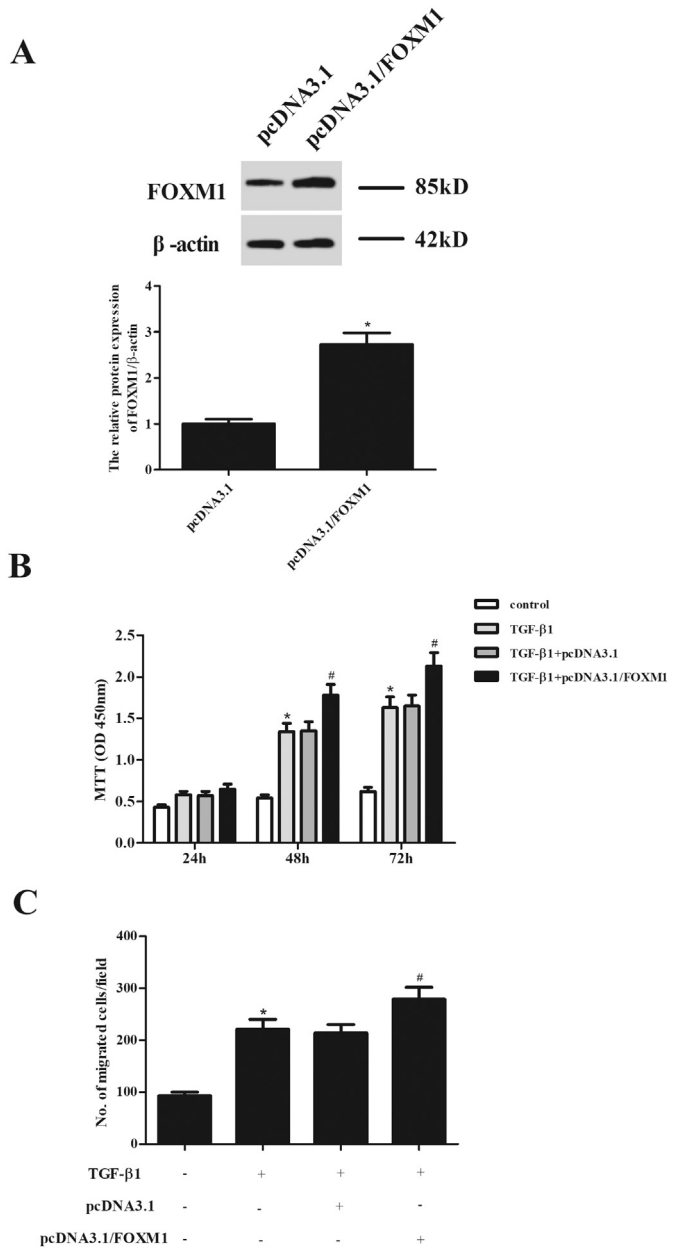

Fig. 3. Effects of FOXM1 overexpression on proliferation and migration in TGF-β1-induced KFs. (A) FOXM1 expressions in KFs after transfection with pcDNA3.1/FOXM1 or pcDNA3.1 were examined using western blot. * $p < 0.05$ vs. pcDNA3.1. (B) Cell proliferation was assessed by MTT assay. (C) Cell migration was evaluated by transwell assay. * $p < 0.05$ vs. control group; # $p < 0.05$ vs. TGF-β1 + pcDNA3.1 group.

3.5. Overexpression of FOXM1 promoted the expressions of Col I, CTGF and α-SMA in human KFs induced by TGF-β1

Furthermore, we also examined the effect of FOXM1 overexpression on the expressions of Col I, CTGF and α-SMA in TGF-β1-stimulated KFs. The results indicated that FOXM1 overexpression significantly promoted the expressions of Col I, CTGF and α-SMA at both mRNA and protein levels induced by TGF-β1 in KFs (Fig. 5A and B).

3.6. Knockdown of FOXM1 suppressed the expression of TGF-βRI and TGF-βRII expressions in TGF-β1-stimulated KFs

TGF-β1 exerts is biological activity by binding to a heterotetrameric receptor complex composed of TGF-βRI and TGF-β type II receptor, so,

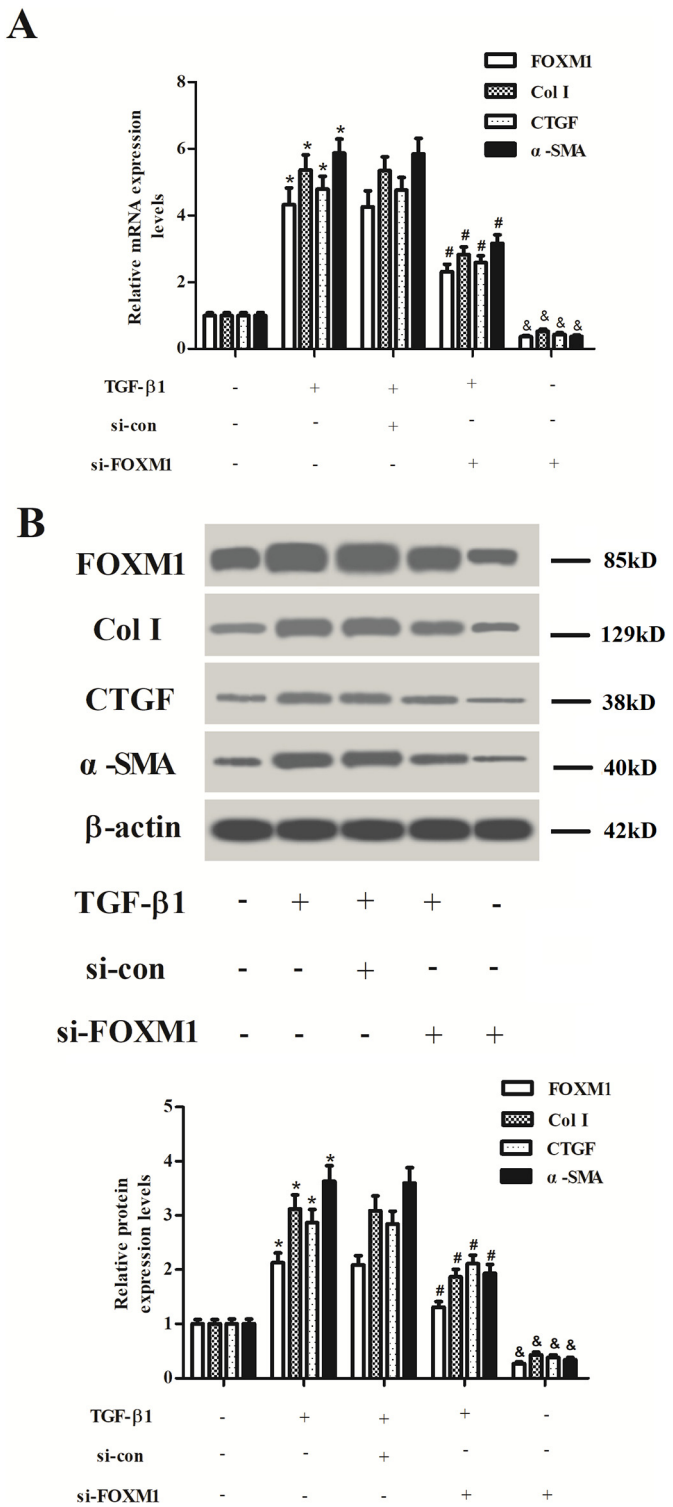

Fig. 4. Impact of FOXM1 knockdown on the expressions of Col I, CTGF and α-SMA in human KFs induced by TGF-β1. The mRNA and protein levels of Col I, CTGF and α-SMA were evaluated by qRT-PCR (A) and western blot (B), respectively. * $p < 0.05$ vs. control group. # $p < 0.05$ vs. TGF-β1 + si-con group; & $p < 0.05$ vs. control group.

we also detected the effect of FOXM1 knockdown on TGF-βRI and TGF-βRII expressions in TGF-β1-stimulated KFs. As shown in Fig. 6, TGF-β1 treatment greatly increased the expressions of TGF-βRI and TGF-βRII in KFs. However, these effects were reversed by FOXM1 knockdown. In addition, the expressions of TGF-βRI and TGF-βRII were also decreased

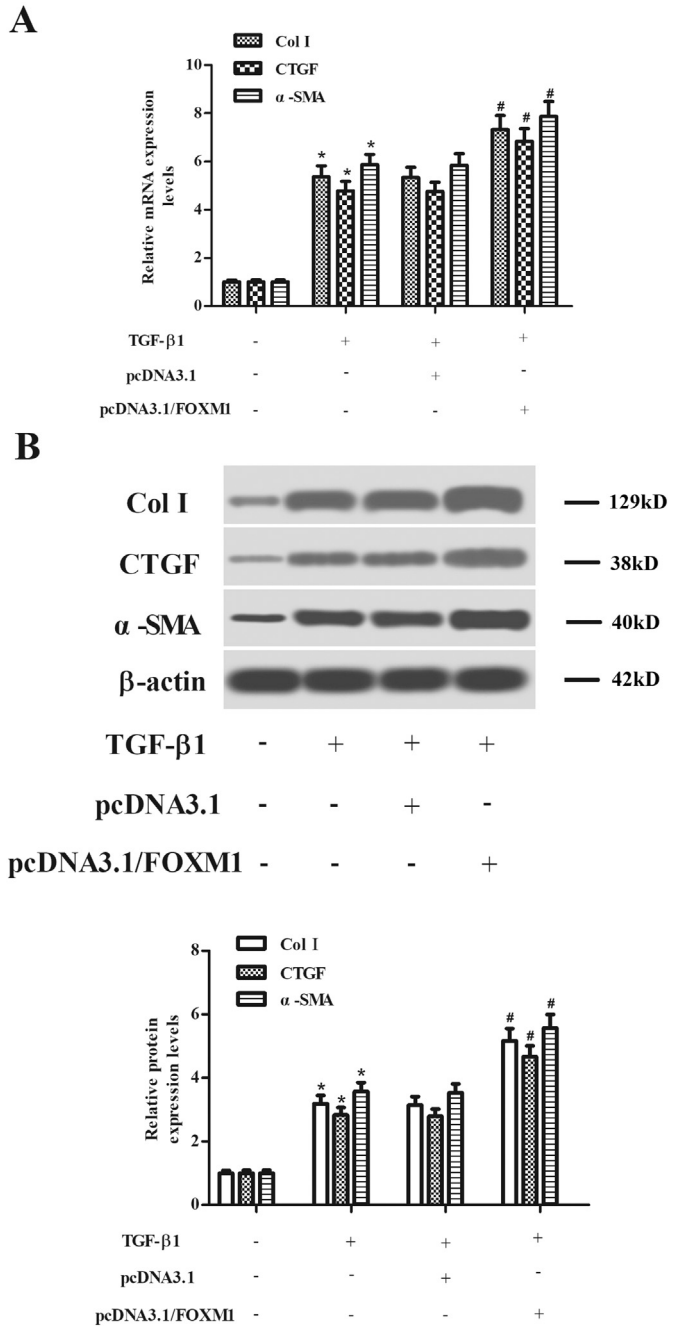

Fig. 5. Effects of FOXM1 overexpression on the expressions of Col I, CTGF and α-SMA in human KFs induced by TGF-β1. The mRNA and protein levels of Col I, CTGF and α-SMA were evaluated by qRT-PCR (A) and western blot (B), respectively. * $p < 0.05$ vs. control; # $p < 0.05$ vs. TGF-β1 + pcDNA3.1.

by knockdown of FOXM1 (Fig. 6A and B).

3.7. Knockdown of FOXM1 suppressed the levels Smad2 and Smad3 phosphorylation in TGF-β1-stimulated KFs

There is convincing evidence that TGF-β1/Smad signaling pathway is involved in keloid [19,20]. In order to investigate the effect of FOXM1 knockdown on Smad signaling pathway, the expressions of Smad2, p-Smad2, Smad3, and p-Smad3 were measured using western blot analysis. As shown in Fig. 7, the expressions of p-Smad2 and p-Smad3 in KFs were obviously increased after TGF-β1 stimulation. However, knockdown of FOXM1 inhibited the expressions of p-Smad2 and p-Smad3 in TGF-β1-stimulated KFs.

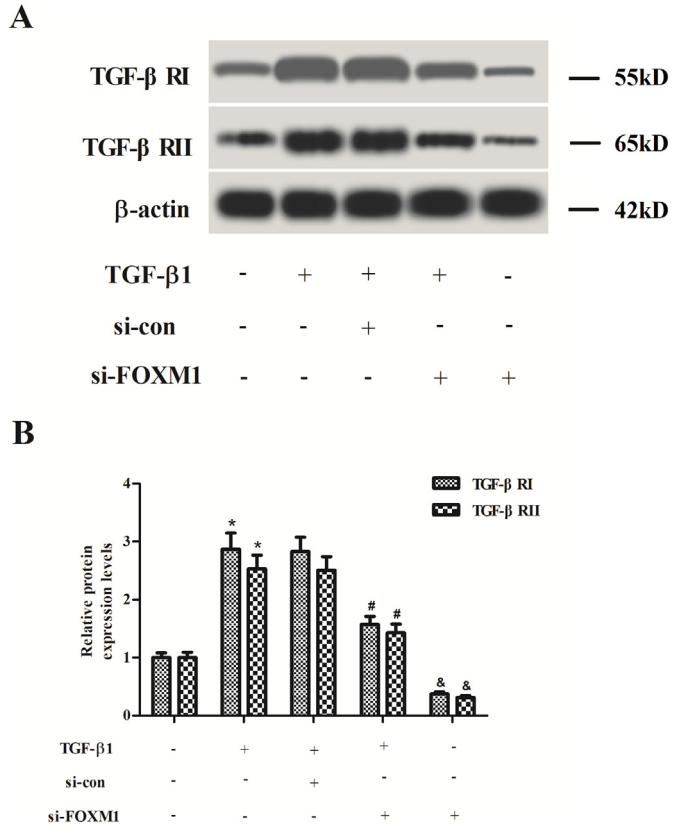

Fig. 6. Impact of FOXM1 knockdown on the expressions of TGF-βRI and TGF-βRII in human KFs induced by TGF-β1. (A) The protein levels of TGF-βRI and TGF-βRII were evaluated by western blot, respectively. (B) Quantification analysis of TGF-βRI and TGF-βRII. * $p < 0.05$ vs. control KFs; # $p < 0.05$ vs. TGF-β1-induced KFs; & $p < 0.05$ vs. control KFs.

4. Discussion

A multitude of cell types including fibroblasts, myofibroblasts, keratinocytes, melanocytes, and mast cells have been identified that contribute to the fibrosis process observed in keloid scarring [1]. In the process of profibrotic events, fibroblasts are the centric cell type. KFs migrative ability and proliferation rates are increased while the apoptotic rates are reduced [7]. Additionally, KFs express increased levels of fibrogenic growth factors such as TGF-β, platelet-derived growth factor (PDGF), fibroblast growth factor β (FGF-β) and insulin-like growth factor I (IGF−I), contributing to ECM deposition and the keloid formation [1]. TGF-β1 is a critical cytokine that has multiple biological functions and plays important roles in many different conditions and disease states. TGF-β1 has been found to serve as a stimulator for wound repair and tissue regeneration [21]. In the case of keloid pathogenesis, TGF-β1 promotes KFs proliferation. In addition, TGF-β1 stimulates the ECM production, while inhibits the collagen-degrading activity of matrix metalloproteinases (MMP), thereby resulting in excessive ECM accumulation [22].

Therefore, we used exogenous TGF-β1 to stimulate KFs in the present study. The results showed that TGF-β1 treatment induced cell proliferation and migration. TGF-β1 promoted the production of coll I, which is an important ECM component. Besides, the expression of CTGF, a fibrotic marker, was also induced by TGF-β1 treatment. In addition, the expression of contractile protein α-SMA, a marker of myofibroblast differentiation was increased in response to TGF-β1 stimulation. These findings suggest that TGF-β1 enhanced the activation and myofibroblast differentiation of KFs and promoted fibrosis process.

FOXM1 has been demonstrated to be more highly expressed in fibrotic fibroblasts isolated from patients with idiopathic pulmonary

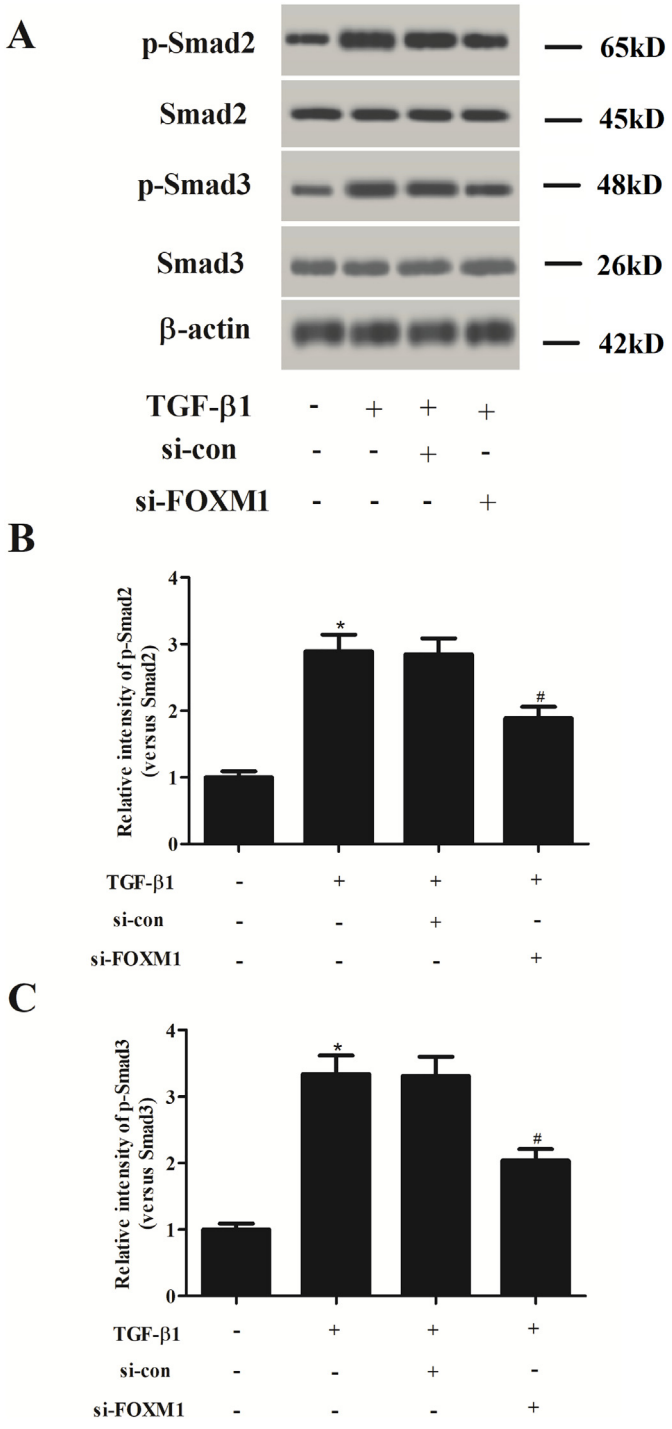

Fig. 7. Impact of FOXM1 knockdown on Smad signaling pathway in TGF-β1-stimulated KFs. The expressions of Smad2, p-Smad2, Smad3, and p-Smad3 were measured using western blot analysis. * $p < 0.05$ vs. control KFs. # $p < 0.05$ vs. TGF-β1-induced KFs.

fibrosis (IPF) when compared to normal lung fibroblasts [17]. The FOXM1 expressions in lung fibroblasts are markedly increased after stimulation with basic fibroblast growth factor (FGF2) and PDGF, which are pertinent fibroblast mitogens. FOXM1 inhibition prevents and reverses TGF-β-induced myofibroblast differentiation and sensitizes myofibroblasts to FasL-induced apoptosis. Further in vivo studies proved that fibroblast-specific deletion of FOXM1 in a therapeutic protocol protects mice from bleomycin-induced fibrosis [17]. In the current study, we found that FOXM1 levels were significantly increased in keloid tissue specimens and KFs. Knockdown of FOXM1 inhibited TGF-β1-induced cell proliferation, migration, as well as the expressions of Col I, CTGF and α-SMA in KFs. By contrast, FOXM1 overexpression promoted cell proliferation/migration, as well as Col I, CTGF and α-SMA expressions induced by TGF-β1 treatment.

Many functions of TGF-β1 are mediated through Smad signal-transduction pathway, a downstream pathway of TGF-β that is critically important for regulating cell development and growth through TGF-β type I receptor [23,24]. Smads are a family of intracellular regulatory proteins that can be categorized into three distinct sub-types receptor-regulated Smads (R-Smad 1, 2, 3, 5 and 8), common partner Smad (Co-Smad 4) and inhibitory Smads (I-Smad 6 and 7) [25,26]. Previous study has proven that elevated intranuclear Smad2/3 accumulation is found in keloid tissues compared with normal skin tissues [27]. Besides, phosphorylation of Smad3 is up-regulated in keloids and plays an important role in the TGF-β induced fibrosis in keloid. Downregulation of Smad3 expression in KFs can significantly reduce ECM deposition and attenuate process of fibrosis [21]. Consistent with the roles of Smad3 in KFs, targeting Smad2 is a promising therapeutic approach for inhibiting progression of fibrotic conditions via interrupting the TGF-β signaling pathway [20]. Therefore, we evaluated whether Smad2/3 pathways were involved in the roles of FOXM1. The results showed that the increased levels of p-Smad2 and p-Smad3 in TGF-β1-stimulated KFs were inhibited by knockdown of FOXM1. Collectively, the inhibition of TGF-β1/Smad2/3 pathways might contribute to the effects of FOXM1 knockdown on KFs.

Overall, our resulted demonstrated that FOXM1 was up-regulated in keloid tissue specimens and KFs. Knockdown of FOXM1 in KFs inhibited TGF-β1-induced KFs activation, ECM accumulation and fibrosis process through inhibiting TGF-β1/Smad2/3 pathways.

Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.116637.

References

[1] J.P. Andrews, J. Marttala, E. Macarak, J. Rosenbloom, J. Uitto, Keloids: the paradigm of skin fibrosis - Pathomechanisms and treatment, Matrix Biol. 51 (2016) 37–46.
[2] J.C. Murray, S.V. Pollack, S.R. Pinnell, Keloids: a review, J. Am. Acad. Dermatol. 4 (1981) 461–470.
[3] R. Ogawa, The most current algorithms for the treatment and prevention of hypertrophic scars and keloids, Plast. Reconstr. Surg. 125 (2010) 557–568.
[4] K. Boahene, A.E. Brissett, L.R. Jones, Facial plastic surgery controversies: keloids, Facial Plast. Surg. Clin. North Am. 26 (2018) 105–112.
[5] A. Al-Attar, S. Mess, J.M. Thomassen, C.L. Kauffman, S.P. Davison, Keloid pathogenesis and treatment, Plast. Reconstr. Surg. 117 (2006) 286–300.
[6] G.G. Gauglitz, H.C. Korting, T. Pavicic, T. Ruzicka, M.G. Jeschke, Hypertrophic scarring and keloids: pathomechanisms and current and emerging treatment strategies, Mol. Med. 17 (2011) 113–125.
[7] G.M. Bran, U.R. Goessler, K. Hormann, F. Riedel, H. Sadick, Keloids: current concepts of pathogenesis (review), Int. J. Mol. Med. 24 (2009) 283–293.
[8] J.H. Mun, Y.M. Kim, B.S. Kim, J.H. Kim, M.B. Kim, H.C. Ko, Simvastatin inhibits transforming growth factor-β1-induced expression of type I collagen, CTGF, and α-SMA in keloid fibroblasts, Wound Repair Regen. 22 (2014) 125–133.
[9] Z.C. Hu, F. Shi, P. Liu, J. Zhang, D. Guo, X.L. Cao, C.F. Chen, S.Q. Qu, J.Y. Zhu, B. Tang, TIEG1 represses Smad7-mediated activation of TGF-β1/Smad signaling in keloid pathogenesis, J. Invest. Dermatol. 137 (2017) 1051–1059.
[10] J.W. Cho, K.J. Il, K.S. Lee, Downregulation of type I collagen expression in silibinin-treated human skin fibroblasts by blocking the activation of Smad2/3-dependent signaling pathways: potential therapeutic use in the chemoprevention of keloids, Int. J. Mol. Med. 31 (2013) 1148–1152.
[11] O.J. Lehmann, J.C. Sowden, P. Carlsson, T. Jordan, S.S. Bhattacharya, Fox's in development and disease, Trends Genet. 19 (2003) 339–344.
[12] R. Dong, Y. Yang, Z. Shen, C. Zheng, Z. Jin, Y. Huang, Z. Zhang, S. Zheng, G. Chen, Forkhead box A3 attenuated the progression of fibrosis in a rat model of biliary atresia, Cell Death Dis. 8 (2017) e2719.

[13] S. Xia, J. Qu, H. Jia, W. He, J. Li, L. Zhao, M. Mao, Y. Zhao, Overexpression of Forkhead box C1 attenuates oxidative stress, inflammation and apoptosis in chronic obstructive pulmonary disease, Life Sci. 216 (2019) 75–84.
[14] J. Im, J. Lawrence, D. Seelig, R.S. Nho, FoxM1-dependent RAD51 and BRCA2 signaling protects idiopathic pulmonary fibrosis fibroblasts from radiation-induced cell death, Cell Death Dis. 9 (2018) 584–599.
[15] I. Norambuena-Soto, C. Nunez-Soto, F. Sanhueza-Olivares, N. Cancino-Arenas, D. Mondaca-Ruff, R. Vivar, G. Diaz-Araya, R. Mellado, M. Chiong, Transforming growth factor-beta and Forkhead box O transcription factors as cardiac fibroblast regulators, Biosci. Trends 11 (2017) 154–162.
[16] C. Bolte, Y. Zhang, A. York, T.V. Kalin, Jel J. Schultz, J.D. Molkentin, V.V. Kalinichenko, Postnatal ablation of Foxm1 from cardiomyocytes causes late onset cardiac hypertrophy and fibrosis without exacerbating pressure overload-induced cardiac remodeling, PLoS One 7 (2012) e48713.
[17] L.R. Penke, J.M. Speth, V.L. Dommeti, E.S. White, I.L. Bergin, M. Peters-Golden, FOXM1 is a critical driver of lung fibroblast activation and fibrogenesis, J. Clin. Invest. 128 (2018) 2389–2405.
[18] Z. Rang, Z.Y. Wang, Q.Y. Pang, Y.W. Wang, G. Yang, F. Cui, MiR-181a targets PHLPP2 to augment AKT signaling and regulate proliferation and apoptosis in human keloid fibroblasts, Cell. Physiol. Biochem. 40 (2016) 796–806.
[19] Z. Gao, Z. Wang, Y. Shi, Z. Lin, H. Jiang, T. Hou, Q. Wang, X. Yuan, Y. Zhao, H. Wu, Y. Jin, Modulation of collagen synthesis in keloid fibroblasts by silencing Smad2 with siRNA, Plast. Reconstr. Surg. 118 (2006) 1328–1337.
[20] Z. Wang, Z. Gao, Y. Shi, Y. Sun, Z. Lin, H. Jiang, T. Hou, Q. Wang, X. Yuan, X. Zhu, H. Wu, Y. Jin, Inhibition of Smad3 expression decreases collagen synthesis in keloid disease fibroblasts, J. Plast. Reconstr. Aesthet. Surg. 60 (2007) 1193–1199.
[21] M. Pakyari, A. Farrokhi, M.K. Maharlooei, A. Ghahary, Critical role of transforming growth factor beta in different phases of wound healing, Adv. Wound Care (New Rochelle) 2 (2013) 215–224.
[22] B. Hinz, The extracellular matrix and transforming growth factor-beta1: tale of a strained relationship, Matrix Biol. 47 (2015) 54–65.
[23] R. Derynck, Y. Zhang, X.H. Feng, Smads: transcriptional activators of TGF-beta responses, Cell 95 (1998) 737–740.
[24] M.J. Macias, P. Martin-Malpartida, J. Massague, Structural determinants of Smad function in TGF-beta signaling, Trends Biochem. Sci. 40 (2015) 296–308.
[25] S. Tao, K. Sampath, Alternative splicing of SMADs in differentiation and tissue homeostasis, Develop. Growth Differ. 52 (2010) 335–342.
[26] J. Massague, J. Seoane, D. Wotton, Smad transcription factors, Genes Dev. 19 (2005) 2783–2810.
[27] Q. Sun, S. Guo, C.C. Wang, X. Sun, D. Wang, N. Xu, S.F. Jin, K.Z. Li, Cross-talk between TGF-beta/Smad pathway and Wnt/beta-catenin pathway in pathological scar formation, Int. J. Clin. Exp. Pathol. 8 (2015) 7631–7639.