Loureirin B Inhibits Hypertrophic Scar Formation via Inhibition of the TGF-β1-ERK/JNK Pathway

Ting He  Xiaozhi Bai  Longlong Yang  Lei Fan  Yan Li  Linlin Su  Jianxin Gao  Shichao Han  Dahai Hu

Department of Burns and Cutaneous Surgery, Xijing Hospital, the Fourth Military Medical University, Xi’an, Shaanxi, China

Key Words
Loureirin B • Hypertrophic Scar • Fibroblasts • MAPK Pathway • Extracellular Matrix

Abstract
Background/Aims: Our previous study confirmed that Loureirin B (LB) can inhibit hypertrophic scar formation. However, the mechanism of LB-mediated inhibition of scar formation is still unknown. Methods: Immunohistochemistry was used to detect expression of Col1, FN and TGF-β1 in skin and scar tissue. Fibroblasts were stimulated with TGF-β1 to mimic scar formation. LB or MAPK inhibitors were used to study the pathways involved in the process. Western blotting was used to evaluate the expression of p-JNK, p-ERK, p-p38, Col1 and FN. The contractile capacity of fibroblasts was evaluated using a gel contraction assay. Tissues were cultured ex vivo with LB to further investigate the participation of ERK and JNK in the LB-mediated inhibition of scar formation. Results: FN and Col1 were upregulated in hypertrophic scars. LB downregulated p-ERK and p-JNK in TGF-β1-stimulated fibroblasts, while levels of phosphorylated p38 did not change. The down regulation of p-ERK and p-JNK was associated with a reduction of Col1 and FN. Similarly, inhibition of ERK and JNK downregulated the expression of Col1 and FN in TGF-β1-stimulated fibroblasts. LB downregulated protein levels of p-ERK and p-JNK in cultured hypertrophic scar tissue ex vivo. Conclusions: This study suggests that LB can inhibit scar formation through the ERK/JNK pathway.

Introduction
A hypertrophic scar is the pathological outcome of wound healing due to dermal injury [1]. Fibroblasts are an important type of effector cell activated during this process. During the wound-healing process, fibroblasts from the border of the wound migrate to the centre...
and transdifferentiate into myofibroblasts that abundantly synthesize extracellular matrix (ECM), leading to hypertrophic scar formation [1-3]. The mechanism of hypertrophic scar formation is still unclear. Current studies suggest that the abnormal expression of several cytokines is associated with hypertrophic scar formation. One of the most important cytokines associated with fibrotic disease and hypertrophic scarring is transforming growth factor β1 (TGF-β1) [1, 4, 5]. Through multiple cellular processes, TGF-β1 regulates tissue homeostasis, including cell proliferation, migration, apoptosis, and ECM remodeling [6]. During wound healing, increased TGF-β1 improves tissue regeneration, while a persistent increase in TGF-β1 activates several intracellular signals, such as the Sma- and Mad-related proteins (Smads) [7] as well as those of the mitogen-activated protein kinase (MAPK) pathway. The activation of these pathways promotes the transcription of fibrosis-related molecules [8] and stimulates autocrine release of TGF-β1, leading to a persistent autocrine-positive feedback loop that may result in the overproduction of matrix proteins and subsequent fibrosis [9, 10]. Our previous study showed that Loureirin B (LB) can suppress hypertrophic scar formation, and we validated this effect in a rabbit ear scar model [11]. In addition, we found that the phosphorylation of Smad2 and Smad3 induced by TGF-β1 was suppressed by LB. Given that MAPKs are one group of important intracellular proteins that transduce extracellular signals from TGF-β1 [12, 13], we investigated whether the inhibitory effect of LB on hypertrophic scarring is associated with the MAPK pathway. In the present study, TGF-β1-stimulated fibroblasts were used to study the response of the MAPK pathway to LB. We found that in TGF-β1-stimulated fibroblasts, the phosphorylation of extracellular signal-regulated protein kinase (p-ERK) and the phosphorylation of c-Jun N-terminal kinase (p-JNK) were suppressed by LB, while the phosphorylation of p38 MAPK kinase (p-p38) was not affected. The contractile capacity of fibroblasts, as well as ECM synthesis, was attenuated through the down regulation of p-ERK and p-JNK. These results suggest that the anti-fibrotic effect of LB is closely associated with inhibition of the ERK/JNK pathway.

Materials and Methods

Ethics Statement

All of the experimental procedures were conducted under protocol No: XJYLL-2013190, which was reviewed and approved by the Institutional Ethical Committee of the Fourth Military Medical University.

Cell Culture and Treatment

Paired normal skin and hypertrophic scar tissue, which were used in our previous experiments [11], were collected from four patients who received no treatment before surgery. The age of the four patients ranges from 18 to 44 years old. Written consent was obtained from patients or their legal guardians. Dermal fibroblasts were isolated and cultured as described previously. Briefly, tissues were trimmed to remove excessive adipose and then rinsed with phosphate buffer solution (PBS) three times. Then, tissues were minced into small pieces and incubated in Dulbecco’s Modified Eagle Medium (DMEM, Gibco, Grand Island, New York, USA) containing 0.1% collagenase type I (Sigma, St. Louise, Missouri, USA) at 37 °C for 3 hours. The isolated fibroblasts were then cultured in DMEM containing 10% foetal calf serum (Gibco), 1% penicillin and 1% streptomycin at 37 °C in a humidified atmosphere of 5% CO2. Fibroblasts from the 3rd to the 5th passages were used in all experiments. Before any treatment, fibroblasts reaching 70~80% confluence were incubated in serum-depleted medium for another 12 hours.

Several 60-mm dishes of normal skin fibroblasts were randomly arranged into different groups (n = 4). LB was obtained from the National Institute for the Control of Pharmaceutical and Biological Products of China the same as we have reported before, and reconstituted in DMSO at a final stock concentration of 20 mg/mL. Recombinant human TGF-β1 was purchased from PeproTech (London, UK) and dissolved in 10 mM citric acid (pH 3.0), yielding a final stock concentration of 10 ng/mL. Previous reports suggest that 5 ng/mL of TGF-β1 can significantly induce transdifferentiation of fibroblasts to myofibroblasts, and our previous experiments suggest that 25 μg/mL of LB can effectively improve scar formation in vivo. Thus, in the present experiment, TGF-β1 was diluted to 5 ng/mL, while LB was diluted to 25 μg/mL.
Western Blotting

Forty micrograms of total protein was subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto a PVDF membrane (Millipore, Bedford, MA). After blocking with 5% non-fat milk, membranes were incubated at 4°C overnight with a specific primary antibody, such as rabbit anti-human FN (1:1000, GeneTex, TX, USA), rabbit anti-human Col1α2 (1:1000, Abcam, Cambridge, UK), rabbit anti-human JNK (1:1000, Cell Signaling Technology, Beverly, MA), rabbit anti-human ERK1/2 (1:1000, Cell Signaling Technology), rabbit anti-human p38 (1:1000, Cell Signaling Technology), rabbit anti-human phospho-JNK (1:1000, Cell Signaling Technology), rabbit anti-human phospho-ERK1/2 (1:1000, Cell Signaling Technology), or rabbit anti-human phospho-p38 (1:1000, Cell Signaling Technology). The next day, membranes were washed three times with TBST (Tris-buffered saline with 0.1% Tween-20) and then incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibodies (1:3000, Santa Cruz, CA, USA) at 37 °C for 1 hour at room temperature. Antibodies against β-actin (1:1000, Abcam) were used as loading controls. The proteins were visualized with an ECL Kit (Millipore, USA) and Fluor Chem FC (Alpha Innotech, USA).

Immunohistochemistry

Scar tissues and autologous skin tissues were embedded in paraffin and cut into 4-μm-thick sections for immunohistochemical staining. Sections were subjected to deparaffinization, dehydration and antigen retrieval. Sections were then separately incubated with primary antibodies such as rabbit anti-human FN antibody (1:200, GeneTex), rabbit anti-human Col1α2 antibody (1:200, Abcam) and rabbit anti-human TGF-β1 antibody (1:200, Gene Tex) at room temperature overnight. The next day, sections were incubated with biotinylated secondary antibody; then, streptavidin–biotin–horseradish peroxidase was used for signal amplification and diaminobenzidine (DAB) for staining, following the instructions of the SP-9001 Histostain TM Kit (ZSJQ, Beijing, China). The images were obtained and analysed using the Image-Pro Plus system 6.0.

Gel Contraction Assay

The contractile capacity of fibroblasts was measured using a gel contraction assay. Fibroblast-embedded collagen gels were prepared, as described previously in the literature [14]. Briefly, four 24-well plates were pre-treated with 0.2% BSA for 1 hour. A 0.5-mL suspension containing $1 \times 10^6$ fibroblasts and 1.4 mg/mL collagen were added into the wells. Then, fibroblasts in the wells were treated with TGF-β1 (5 ng/mL), LB (25 μg/mL), TGF-β1 (5 ng/mL) + LB (25 μg/mL), TGF-β1 (5 ng/mL) + SP600125/PD98059 (30 μmol/L), TGF-β1 (5 ng/mL) + LB (25 μg/mL) + SP600125/PD98059 (30 μmol/L) or dimethyl sulfoxide (DMSO, control). The gel solutions were added to 24-well plates (600 μL per well) and incubated at 37 °C for 24 hours for polymerization, followed by mechanical detachment from the sides of the wells. The images of gels were captured at 0, 24 and 48 hours after the gels were released, and the images were analysed using Image Pro Plus 6.0 software.

Statistical Analysis

Results were presented as the mean ± SEM. Data were analysed for significance by analysis of variance (ANOVA) using SPSS 17.0 software (Chicago, USA). p<0.05 was considered statistically significant.

Results

The expression of collagen 1 (Col1), fibronectin (FN) and TGF-β1 were significantly increased in hypertrophic scar tissue compared with normal skin tissue

Expression of ECM is higher in hypertrophic scar tissue than in normal skin [15]. During this experiment, we detected the expression of Col1, FN and TGF-β1 by immunohistochemistry. The results showed that expression of all three proteins increased in hypertrophic scar tissue compared with autologous skin (n = 4), which suggested that the samples we selected were consistent with the pathological standard of hypertrophic scar tissue (p<0.05).
He et al.: Loureirin B Attenuates Scar Formation Through the TGF-β1-ERK/JNK Pathway

Fig. 1. The expression level of FN, Col1, and TGF-β1 was increased in hypertrophic scar tissue. Scar tissue was acquired from patients who never received any treatment before surgery, while the normal skin was autologous. The first row shows that the expression of TGF-β1 was significantly increased in hypertrophic scar tissue. Similar to TGF-β1, FN and Col1 levels were increased in hypertrophic scar tissue, as shown in the right image. Scale bar = 50 μm. The results represent the mean ± SEM of four independent experiments. *p<0.05.

Fig. 2. LB down regulated the expression of p-JNK in TGF-β1-stimulated fibroblasts. Fibroblasts were divided into five groups and stimulated with TGF-β1. (a) TGF-β1 significantly increased phosphorylation of JNK 30 minutes after stimulation. Next, fibroblasts were divided into four groups and stimulated with TGF-β1 (5 ng/mL), LB (25 μg/mL), both or none. (b) LB significantly reduced the phosphorylation of JNK that was induced by TGF-β1. Then, fibroblasts were divided into six groups and treated with TGF-β1 (5 ng/mL), LB (25 μg/mL), TGF-β1 (5 ng/mL) + LB (25 μg/mL), TGF-β1 (5 ng/mL) + SP600125 (30 μmol/L), TGF-β1 (5 ng/mL) + LB (25 μg/mL) + SP600125 (30 μmol/L) or dimethyl sulfoxide (DMSO, control). (c) SP600125 or LB significantly inhibited the expression of FN and Col1 that was induced by TGF-β1 (p<0.05) (n=4). The results represent the mean ± SEM of four independent experiments. *p<0.05 compared with the TGF-β1 group, **p<0.01 compared with the TGF-β1 group, ***p<0.01 compared with the control group.

LB inhibited the expression of Col1 and FN, as well as the TGF-β1-mediated up regulation of p-JNK

Given that the MAPK pathway is one of the most important pathways in fibrosis and TGF-β1 is the most important cytokine involved in hypertrophic scar formation, we examined whether TGF-β1 stimulation could separately increase phosphorylation of ERK, JNK, and p38 in normal-skin-derived fibroblasts. As shown in Fig. 2, thirty minutes after stimulation with
TGF-β1, the expression of p-JNK was up regulated. Next, we stimulated the fibroblasts with TGF-β1, LB, both or none for thirty minutes. The results show that LB significantly reduced the phosphorylation of ERK that was induced by TGF-β1. Then, fibroblasts were divided into six groups and treated with TGF-β1 (5 ng/mL), LB (25 μg/mL), TGF-β1 (5 ng/mL) + LB (25 μg/mL), TGF-β1 (5 ng/mL) + PD98059 (30 μmol/L), TGF-β1 (5 ng/mL) + LB (25 μg/mL) + PD98059 (30 μmol/L) or dimethyl sulfoxide (DMSO, control). The expression of FN and Col1 was significantly reduced by PD98059 (p<0.05) (n=4). The results represent the mean ± SEM of four independent experiments. *p<0.01 compared with the TGF-β1 group, **p<0.01 compared with the TGF-β1 group, ***p<0.01 compared with the control group.

**LB inhibited the up regulation of p-ERK that was induced by TGF-β1**

As shown in Fig. 3, similar to the results for the JNK pathway, 5 minutes after stimulation with TGF-β1, the expression of p-ERK was up regulated. LB suppressed the up regulation of p-ERK that was induced by TGF-β1. During this process, the expression of Col1 and FN was also reduced. PD98059, an inhibitor of ERK, had a similar effect as that of LB (n=4) (p < 0.05).

**p38 does not respond to LB stimulation during TGF-β1-induced fibrosis**

As shown in Fig. 4, similar to the results of the ERK pathway, 5 minutes after stimulation with TGF-β1, the expression of p-p38 was up regulated. However, LB did not suppress the up regulation of p-p38 that was induced by TGF-β1, which indicated that the p38 MAPK pathway participates in TGF-β1-stimulated fibrosis, while there seems to be no relationship between the anti-fibrosis effect of LB and the p38 MAPK pathway (n=4) (p < 0.05).

**LB inhibited the contraction of TGF-β1-stimulated fibroblasts through the down regulation of p-ERK and p-JNK**

Given that the hypertrophic scar tissue shows increased contraction compared to normal skin, it is important to know whether LB affects the contraction capacity of TGF-β1-stimulated fibroblasts.
He et al.: Loureirin B Attenuates Scar Formation Through the TGF-β1-ERK/JNK Pathway

Fig. 4. p38 did not respond to the inhibitory effect of LB in TGF-β1-stimulated fibroblasts. Fibroblasts were divided into five groups and stimulated with TGF-β1. (a) TGF-β1 significantly increased phosphorylation of p38 5 minutes after stimulation. Then, fibroblasts were divided into four groups and stimulated with TGF-β1 (5 ng/mL), LB (25 μg/mL), both or none for thirty minutes. (b) LB did not reduce the phosphorylation of p38 that was induced by TGF-β1 (p>0.05) (n=4). The results represent the mean ± SEM of four independent experiments. *p<0.01 compared with the TGF-β1 group, **p<0.01 compared with the TGF-β1 group, ***p<0.01 compared with the control group.

LB inhibited phosphorylation of ERK and JNK in human hypertrophic scar tissue ex vivo

To further investigate the role of ERK and JNK in LB-mediated inhibition of scar formation, we cultured human hypertrophic scar tissue ex vivo [16]. The tissues were incubated in medium containing LB solution. As shown in Fig. 6, the detection of p-ERK and p-JNK protein levels by western blotting showed that both of these two proteins, as well as FN and Col1, were downregulated in hypertrophic scar tissue after LB stimulation (n=4).

Discussion

We demonstrated that LB down regulated expression of p-JNK and p-ERK in TGF-β1-stimulated fibroblasts, which can then inhibit ECM synthesis. Recent studies suggest that compared with normal skin tissue, fibroblasts in hypertrophic scar tissue tend to transdifferentiate into myofibroblasts [17], which results in an increase in myofibroblast contractions and synthesis of ECM. The major components of ECM in hypertrophic scar tissue include Col1, collagen 3, FN and proteoglycan. FN participates in cell-to-cell adhesion, cell to ECM adhesion, cell migration and differentiation. FN, which is important for the maintenance of cell structure, also has some affinity to collagen [18]. In the present study, we used FN and Col1 expression to investigate the ECM. Cytokines play an important role in hypertrophic scar formation, and TGF-β1 is the most important of these cytokines. A TGF-β1-
mediated signalling pathway is believed to be closely associated with scar formation. TGF-β1 binds to a receptor, which then facilitates cell transdifferentiation and ECM deposition. TGF-β1-stimulated fibroblasts were used here to simulate the process of scar formation.

During this period, the expression of Col1 and FN significantly increased. However, LB inhibited the expression of these two molecules. In a previous study, we confirmed that LB improved scar appearance and reduce collagen synthesis in a rabbit ear scar model. In this study, we collected hypertrophic scar samples from the adults who were included in the previous study. These cases were established using the clinical standards of hypertrophic scar diagnoses. Then, we examined the expression of FN and Col1 in scar tissues using
He et al.: Loureirin B Attenuates Scar Formation Through the TGF-β1-ERK/JNK Pathway

immunohistology. As shown in Fig. 1, the expression of TGF-β1, FN and Col11 increased in hypertrophic scar tissue. The results further confirmed the diagnosis of the samples and laid a foundation for further experiments.

To clarify the possible mechanism of LB-mediated inhibition of hypertrophic scar formation, we examined the phosphorylation of Smad2 and Smad3 in TGF-β1-stimulated fibroblasts before and after LB stimulation. We found that the phosphorylation of Smad2 and Smad3 was inhibited by LB. In addition to the Smad pathway, the MAPK pathway also plays an important role in hypertrophic scar formation and fibrosis [19-22]. MAPK is a serine/threonine kinase composed of extracellular signal regulated kinases (ERK), c-Jun N-terminal kinase (JNK) and p38 MAP kinase (p38 MAPK), which can be activated by TGF-β1 and participates in cell proliferation, differentiation and apoptosis. MAPKs can be activated in liver fibrosis, increasing Col1α2 transcription [23, 24]. The MAPK pathway also plays a key role in myocardial fibrosis, lung fibrosis and renal fibrosis [25-28]. In normal skin, exogenous TGF-β1 can activate p38, which can combine with the Col1α2 promoter and increase the transcription of Col1α2. JNK is a key factor in TGF-β1-stimulated lung fibrosis [29]. Inhibition of JNK, ERK and p38 can partially reverse TGF-β1-induced epithelial-to-mesenchymal transition (EMT) and fibrosis [5, 30]. We are interested in knowing whether MAPK plays a role in the inhibitory effects of LB on scar formation. However, we should first clarify whether the phosphorylation of MAPKs is increased in TGF-β1-stimulated fibroblasts. As shown in the results, TGF-β1 stimulation elevated the expression levels of p-ERK, p-JNK and p-p38. Next, we investigated whether MAPK participates in the LB-mediated inhibition of scar formation. The experiments showed that LB significantly inhibited the TGF-β1-mediated
He et al.: Loureirin B Attenuates Scar Formation Through the TGF-β1-ERK/JNK Pathway

Fig. 7. A summary diagram showing that Loureirin B reduced fibronectin and collagen 1 expression as well as the transdifferentiation of fibroblasts into myofibroblasts in TGF-β1-stimulated fibroblasts through the inhibition of both phosphorylation of ERK and JNK, result in the improve of hypertrophic scar formation.

induction of Col1 and FN expression. During this period, the expression of p-ERK and p-JNK decreased while p-p38 did not change, which indicates that ERK and JNK responded to LB stimulation.

To further verify our results, TGF-β1-stimulated fibroblasts were separately co-cultured with an inhibitor of ERK and JNK. As a result, the expression of both Col1 and FN was significantly reduced. It is clear that ERK and JNK are associated with TGF-β1-stimulated ECM expression in fibroblasts. Overall, our cellular experiments demonstrate that LB inhibited the expression of ECM in fibroblasts through the ERK and JNK pathway.

Previous research suggests that enhanced contractile capacity is related to a high level of α-SMA expression in myofibroblasts [8, 30]. The contractile capacity of TGF-β1-stimulated fibroblasts was significantly increased compared to that of normal fibroblasts [31], which is one of the most important characteristics of myofibroblasts in hypertrophic scar tissue [32, 33]. We used a gel contraction assay to examine the contractile capacity of fibroblasts after exposure to LB. As shown in Fig. 5, LB, as well as an inhibitor of JNK and ERK, significantly inhibited the fibroblast contraction that was induced by TGF-β1. All of these results suggest that LB inhibits hypertrophic scar formation through the inhibition of ERK and JNK phosphorylation.

To further clarify the response of ERK and JNK to LB stimulation in hypertrophic scar formation, we designed experiments at the tissue level. There are several animal models used for scar research, including a heterologous scar transplantation model, a chemically induced scar model and animal models with scarring in specific locations [34-37]. All of these models do not completely simulate hypertrophic scar formation in humans. Yasuoka et al reported that tissue culture better mimics in vivo environments [16]. Scar tissues were cultured in medium that included LB. The protein expression levels of p-ERK and p-JNK were observed using western blotting. The results demonstrated that compared with the control group, LB significantly down regulates the expression of p-ERK and p-JNK as well as the expression of Col1 and FN.

Overall, we have shown that in both TGF-β1-stimulated fibroblasts and scar tissue, the stimulation of LB suppressed phosphorylation of ERK and JNK (Fig. 7). Similarly, the inhibitors of either ERK or JNK down regulated the expression of ECM and inhibited cell contraction. These results suggest that LB can inhibit scar formation though the ERK and JNK pathway.
Acknowledgements

The National Natural Science Foundation of China supported this study (Grant Number: 81171811, 81372069).

Disclosure Statement

The authors state no conflict of interest.

References

1. Honardoust D, Kwan P, Montazi M, Ding J, Tredget EE: Novel methods for the investigation of human hypertrophic scarring and other dermal fibrosis. Methods Mol Biol 2013;1037:203-231.
2. Wang J, Dodd C, Shankowsky HA, Scott PG, Tredget EE; Wound Healing Research Group: Deep dermal fibroblasts contribute to hypertrophic scarring. Lab Invest 2008;88:1278-1290.
3. Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillesen D, Gabbiani G: A monoclonal antibody against alpha-smooth muscle actin: A new probe for smooth muscle differentiation. J Cell Biol 1986;103:2787-2796.
4. Goldberg MT, Han YP, Yan C, Shaw MC, Garner WL: Tnf-alpha suppresses alpha-smooth muscle actin expression in human dermal fibroblasts: An implication for abnormal wound healing. J Invest Dermatol 2007;127:2645-2655.
5. Ding ZY, Jin GN, Liang HF, Wang W, Chen WX, Datta PK, Zhang MZ, Zhang B, Chen XP: Transforming growth factor beta induces expression of connective tissue growth factor in hepatic progenitor cells through smad independent signaling. Cell Signal 2013;25:1981-1992.
6. Massague J, Blain SW, Lo RS: Tgfbeta signaling in growth control, cancer, and heritable disorders. Cell 2000;103:295-309.
7. Qi Q, Mao Y, Yi J, Li D, Zhu K, Cha X: Anti-fibrotic effects of astragaloside iv in systemic sclerosis. Cell Physiol Biochem 2014;34:2105-2116.
8. Cho JS, Kang JH, Shin JM, Park IH, Lee HM: Inhibitory effect of delphinidin on extracellular matrix production via the mapk/nf-kappab pathway in nasal polyp-derived fibroblasts. Allergy Asthma Immunol Res 2015;7:276-282.
9. Liang CJ, Yen YH, Hung LY, Wang SH, Pu CM, Chien HF, Tsai JS, Lee CW, Yen FL, Chen YL: Thalidomide inhibits fibronectin production in tgf-beta1-treated normal and keloid fibroblasts via inhibition of the p38/smad3 pathway. Biochem Pharmacolarch. 2013;83:1594-1602.
10. Wolfram D, Tzankov A, Pulal P, Piza-Katzer H: Hypertrophic scars and keloids--a review of their pathophysiology, risk factors, and therapeutic management. Dermatol Surg 2009;35:171-181.
11. Bai X, He T, Liu J, Wang Y, Fan L, Tao K, Shi J, Tang C, Su L, Hu D: Loureirin b inhibits fibroblast proliferation and extracellular matrix deposition in hypertrophic scar via tgf-beta/smadd pathway. Exp Dermatol 2015;24:355-360.
12. Guo X, Wang XF: Signaling cross-talk between tgf-beta/bmp and other pathways. Cell Res 2009;19:71-88.
13. Lim MJ, Ahn J, Yi JY, Kim MH, Son AR, Lee SL, Lim DS, Kim SS, Kang MA, Han Y, Song JY: Induction of galectin-1 by tgf-beta1 accelerates fibrosis through enhancing nuclear retention of smad2. Exp Cell Res 2014;326:125-135.
14. Bell E, Ivarsson B, Merrill C: Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. Proc Natl Acad Sci USA 1979;76:1274-1278.
15. Viera MH, Amini S, Valins W, Berman B: Innovative therapies in the treatment of keloids and hypertrophic scars. J Clin Aesthet Dermatol 2010;3:20-26.
16. Yasuoka H, Larregina AT, Yamaguchi Y, Feghali-Bostwick CA: Human skin culture as an ex vivo model for assessing the fibrotic effects of insulin-like growth factor binding proteins. Open Rheumatol J 2008;2:17-22.
17. Honardoust D, Varkey M, Marcoux Y, Shankowsky HA, Tredget EE: Reduced decorin, fibromodulin, and transforming growth factor-beta3 in deep dermis leads to hypertrophic scarring. J Burn Care Res 2012;33:218-227.
He et al.: Loureirin B Attenuates Scar Formation Through the TGF-β1-ERK/JNK Pathway

18 Nagata H, Ueki H, Moriguchi T: Fibronectin. Localization in normal human skin, granulation tissue, hypertrophic scar, mature scar, progressive systemic sclerotic skin, and other fibroblastic dermatoses. Arch Dermatol 1985;121:995-999.

19 Chen JY, Zhang L, Zhang H, Su L, Qin LP: Triggering of p38 mapk and jnk signaling is important for oleanolic acid-induced apoptosis via the mitochondrial death pathway in hypertrophic scar fibroblasts. Phytother Res 2014;28:1468-1478.

20 Javelaud D, Mañuel A: Crosstalk mechanisms between the mitogen-activated protein kinase pathways and smad signaling downstream of tgf-beta: Implications for carcinogenesis. Oncogene 2005;24:5742-5750.

21 Sullivan BP, Kassel KM, Manley S, Baker AK, Luyendyk JP: Regulation of transforming growth factor-beta1-dependent integrin beta6 expression by p38 mitogen-activated protein kinase in bile duct epithelial cells. J Pharmacol Exp Ther 2011;337:471-478.

22 Gui T, Sun Y, Shimokado A, Muragaki Y: The roles of mitogen-activated protein kinase pathways in tgf-beta-induced epithelial-mesenchymal transition. J Signal Transduct 2012;2012:289243.

23 Chen A, Davis BH: Uv irradiation activates jnk and increases alphai(i) collagen gene expression in rat hepatic stellate cells. J Biol Chem 1999;274:158-164.

24 Ji L, Xue R, Tang W, Wu W, Hu T, Liu X, Peng X, Gu J, Chen S, Zhang S: Toll like receptor 2 knock-out attenuates carbon tetrachloride (ccl4)-induced liver fibrosis by downregulating mapk and nf-kappab signaling pathways. FEBS Lett 2014;588:2095-2100.

25 Ma FY, Tesch GH, Nikolic-Paterson DJ: Ask1/p38 signaling in renal tubular epithelial cells promotes renal fibrosis in the mouse obstructed kidney. Am J Physiol Renal Physiol 2014;307:F1263-1273.

26 Park JH, Yoon J, Lee KY, Park B: Effects of geniposide on hepatocytes undergoing epithelial-mesenchymal transition in hepatic fibrosis by targeting tgfbeta/smad and erk-mapk signaling pathways. Biochimie 2015;113:26-34.

27 Ulm S, Liu W, Zi M, Tsui H, Chowdhury SK, Endo S, Satoh Y, Prehar S, Wang R, Cartwright EJ, Wang X: Targeted deletion of erk2 in cardiomyocytes attenuates hypertrophic response but provokes pathological stress induced cardiac dysfunction. J Mol Cell Cardiol 2014;72:104-116.

28 Chen L, Liu JP, Tang KL, Wang Q, Wang GD, Cai XH, Liu XM: Tendon derived stem cells promote platelet-rich plasma healing in collagenase-induced rat achilles tendinopathy. Cell Physiol Biochem 2014;34:2153-2168.

29 Kasabova M, Joulin-Giet A, Lecaille F, Gilmore BF, Marchand-Adam S, Saidi A, Lalmanach G: Regulation of tgf-beta1-driven differentiation of human lung fibroblasts: Emerging roles of cathepsin b and cystatin c. J Biol Chem 2014;289:16239-16251.

30 Rouabhia M, Park H, Meng S, Derbal H, Zhang Z: Electrical stimulation promotes wound healing by enhancing dermal fibroblast activity and promoting myofibroblast transdifferentiation. PLoS One 2013;8:e71660.

31 Peters AS, Brunner G, Krieg T, Eckes B: Cyclic mechanical strain induces tgfbeta1-signalling in dermal fibroblasts embedded in a 3d collagen lattice. Arch Dermatol Res. 2015;307:191-197.

32 Sakota Y, Ozawa Y, Yamashita H, Tanaka H, Inagaki N: Collagen gel contraction assay using human bronchial smooth muscle cells and its application for evaluation of inhibitory effect of formoterol. Biol Pharm Bull 2014;37:1014-1020.

33 Au K, Ehrlich HP: When the smad signaling pathway is impaired, fibroblasts advance open wound contraction. Exp Mol Pathol 2010;89:236-240.

34 Seo BF, Lee JY, Jung SN: Models of abnormal scarring. Biomed Res Int 2013;2013:423147.

35 Ramos ML, Gragnani A, Ferreira LM: Is there an ideal animal model to study hypertrophic scarring? J Burn Care Res 2008;29:363-368.

36 Xiao Z: A novel murine model of hypertrophic scarring using subcutaneous infusion of bleomycin. Plast Reconstr Surg 2014;134:481e.

37 van den Broek LJ, Limanderjaja GC, Niessen FB, Gibbs S: Human hypertrophic and keloid scar models: Principles, limitations and future challenges from a tissue engineering perspective. Exp Dermatol 2014;23:382-386.