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ERK2 small interfering RNAs prevent epidural fibrosis via the efficient inhibition of collagen expression and inflammation in laminectomy rats

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ABSTRACT

Laminectomy is a widely accepted treatment for lumbar disorders. Epidural Fibrosis (EF) is a common post-laminectomy or post-discectomy complication, which is thought to cause recurrent pain. RNA interference (RNAi) is a process by which double-stranded RNA triggers the destruction of mRNAs sharing the same sequence. Previously, extra-cellular signal-regulated kinase (ERK) 2 plays crucial roles in suppressing the collagen expression. To investigate the effects of lentiviral ERK2 siRNA on the prevention of post-laminectomy EF formation in a rat model, a controlled double-blinded study was conducted in 75 healthy adult Wistar rats that underwent laminectomy. They were divided randomly into 3 groups according to the treatment method: (1) control group; (2) ERK scrRNA group; (3) ERK siRNA group. All rats were euthanized humanely 4 weeks post-laminectomy. The hydroxyproline content, Rydell score, vimentin cells density, fibroblasts density, inflammatory cells density and inflammatory factors expressions were performed. The hydroxyproline content, Rydell score, vimentin cells density, fibroblasts density, inflammatory cells density and inflammatory factors expressions all suggested better results in ERK siRNA group than other two groups. None of the rats expired and no obvious adverse effects were observed. Local delivery of a lentiviral siRNA targeting ERK2 can prevent epidural scar adhesion in post-laminectomy rat via inhibiting collagen expression and inflammation.

1. Introduction

Epidural fibrosis (EF) is the development of the deposition of dense scar tissue adjacent to the dura matter, which distort normal tissue architecture with leading failed back surgery syndrome (FBSS) post-laminectomy [1]. With the exhibition of a series of symptoms including significant functional disability and recurrent radicular pain, FBSS was reported to attack in 8–40% of patients who undergo lumbar disc surgery [2]. According to the previous literature, EF, leading compression or tethering the nerve roots, is one of the causative factors in FBSS [3].

Currently, different kinds of biological and non-biological agents or mechanical barriers have been administered to prevent EF formation, such as Adcon-L, anti-inflammatory agents, animal collagen membranes, Gelofoam, honey and others [4–9]. However, no satisfactory solutions for treating EF have been found to date.

Small interfering RNAs (siRNAs), comprising short duplexes of 19–23 nucleotide single-stranded RNAs, is able to lead the degradation of the target RNAs [10–12]. Therefore, siRNAs could silence the expression of specific post-transcriptional gene. With a series of advantages of significant and long-lasting inhibitory effects, siRNAs have been reported to be administrated in many orthopedics diseases, such as osteoarthritis, rheumatoid arthritis and periprosthetic osteolysis [13–15]. To date, whether gene therapy can be applied to prevent EF formation has not been reported.

Previously, extra-cellular signal-regulated kinase (ERK) 2 plays crucial roles in suppressing the collagen expression and fibroblasts proliferation [13,14]. In our rat laminectomy model, we investigated whether lentiviral ERK2 siRNA attenuated EF by regulating the expressions of interleukin 6 (IL6), transforming growth fac-

Abbreviations: FBSS, failed back surgery syndrome; EF, epidural fibrosis; RNAi, RNA interference; ERK2, extra-cellular signal-regulated kinase 2; siRNAs, small interfering RNAs; TGF-β1, transforming growth factor-β1; IL6, interleukin 6; GFP, green fluorescent protein; DMEM, Dulbecco's Modified Eagle's Medium; SEPs, sensory-evoked potentials; BBB locomotion test, Basso, Beattie, and Bresnahan locomotion test; HPC, hydroxyproline content.

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tor-β1 (TGF-β1) and collagen, which are reported to play an important role in the promotion and/or development of EF.

2. Material and methods

2.1. Animals

Subjects were 75 Wistar, young-adult, female rats (Radiation Study Institute-Animal Center, Tianjin, China) that weighed approximately 250 g at the time of surgery. The rats were housed in the vivarium of the Tianjin Medical University on a 12:12 h light:dark cycle with clean food and water available ad libitum. Experiments were carried out in compliance with the principles of EU Directive 2010/63/EU and were approved by the local ethical committee. All rats were randomly divided into three groups (25 rats per group): (1) control group (laminectomy with DMEM treatment); (2) ERK scrRNA group (laminectomy with lentiviral ERK2 scramble RNA treatment); (3) ERK siRNA group (laminectomy with lentiviral ERK2 siRNA treatment).

2.2. Lentiviral vector construction and virus packaging

The pFG12 lentiviral vector, constructed with green fluorescent protein (GFP) for detection, was used as the siRNA expression system [16]. The siRNA targeting rat ERK2 whose sequence is 5′-GCACCTCAGCAATGATCAT-3′. A scramble siRNA with the sequence 5′-TGCAGTTCGGAATCAGCTT-3′ was established as a control. Pairs of complementary oligonucleotides containing these sequences were synthesized (Invitrogen) and cloned into the pFG12 lentiviral vector. 293T producer cells were cotransfected with pFG12 and helper plasmids. Transfection was performed as described in the Invitrogen siRNA Transfection Manual using Lipofectamine 2000 (Invitrogen). Then 48 h post transfection, the cultured supernatant was harvested, and concentrated with ultracentrifugation. The lentivirus was stocked in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen). The titer of the lentiviral stocks was assessed using serial dilutions to HeLa cells. The viral titer was 4×10^8 TU/mL for lentiviral ERK2 siRNA and 5×10^8 TU/mL for lentiviral ERK2 scramble RNA control.

2.3. Surgery

All surgical procedures were performed under aseptic conditions. Rats were anesthetized with 10% chloral hydrate (0.3 ml/100 g body weight, ip) and restrained on a warm pad in the prone position. Dorsal incisions were made in the skin and underlying muscles. Muscles retracted and a partial laminectomy was performed at lumbar segment 1 (L_1). Gauze was used for hemostasis. Close attention was paid not to traumatize the dura or the nerve roots. After the different disposes for three groups, the muscles of the incision were then closed with 5–0 absorbable braided suture, and the skin was closed. All rats were placed into an incubator for 2–3 h to recover from anesthesia and then returned to their home cages in the colony room.

To verify that the nerve roots and spinal cord were not intraoperatively injured, all rats underwent pre- and post-operative neurobehavioral assessment using the Basso, Beattie, and Bresnahan (BBB) locomotion test [17]. This scale assesses posture, weight support, and coordination during open field locomotion. Sensory-evoked potentials (SEPs) were measured in rats both pre- and post-operatively to evaluate neurological deficits.

2.4. Lentiviral siRNA deliver system

In the ERK siRNA treatment group, 10 μl lentiviral ERK2 siRNA was injected into the paraspinal muscle before incision closure. Similarly, 10 μl lentivirual ERK2 scramble RNA was applied in the ERK scrRNA group, and the same volume of DMEM (serum free) was applied in the control group. To reduce the risk of infection, the rats were postoperatively given the antibiotic (Baytril; Bayer AG Leverkusen) for 7 days. The dose of lentiviral ERK2 siRNA was chosen based on previous studies [18–20].

2.5. Detection of virus delivery and histological analysis

GFP detecting and histological analysis were performed 4 weeks after surgery in ten rats randomly selected from each group.

Intracardial perfusion with ice-cold saline followed by 4% paraformaldehyde was performed in five rats. The entire L_1 vertebral column, including the paraspinal muscles and epidural scar tissue, was resected en bloc. The fixed samples were serially cryosectioned at 10 μm. The expression of GFP was directly viewed using an epifluorescent microscopy system (Leica CM3050S, Germany).

The same perfusion and samples harvest were performed in other five rats. After decalcification and dehydration with Cal-Ex II solution (Thermo Fisher Scientific, USA) for 2 days, samples were embedded in paraffin, and 5-μm axial sections of the laminectomy site were stained with hematoxylin and eosin (H&E). Epidural scar adhesion was evaluated under a light microscope. Three counting areas were selected at the center and the margins of the laminectomy sites. The numbers of fibroblasts were calculated as follows: cells in the three different areas were counted, and the mean was calculated [21]. The fibroblast and inflammatory cell densities were graded as following: Grade 1, <100 fibroblasts/inflammatory cells per ×400 field; Grade 2, 100–150 fibroblasts/inflammatory cells per ×400 field, Grade 3, >150 fibroblasts/inflammatory cells per ×400 field. To more stringently assess fibrotic situation, immunohistochemistry was performed with a vimentin antibody, and vimentin staining intensity was evaluated.

2.6. Real-time PCR

The mRNA analyses of IL-6 and TGF-β1 were performed 4 weeks after surgery in five rats randomly selected from each group. All rats were humanely euthanized, and the scar tissue from the laminectomy sites was resected. Total RNA was extracted using TRIzol reagent (Invitrogen), and the RNA (2 μg) was transcribed into cDNA by use of AMV Reverse Transcriptase. Quantitative real-time PCR (RT-PCR) was performed using the Bio-Rad MYIQ2 (Hercules, CA, USA) [22]. Based on our previous study [21], the primer sequences used in the present study were as folloing: TGF-β1 (148 bp), forward, 5′-GCCCTGCCCCTACATTTGG-3′, reverse, 5′-CTTGCGACCCACGTAGTAGACGAT-3′; IL-6 (131 bp), forward, 5′-ACCCCAACTTCCAATGCTCT-3′, reverse, 5′-TGCCGAGTAGACCTCATAGTGACC-3′; GAPDH (169 bp), forward, 5′-TCACCACCATGGAGAAGGC-3′, reverse, 5′-GCTAAGCAGTTGGTGGTGCA-3′. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) amplification was performed as an internal control.

2.7. Macroscopic assessment of EF

Macroscopic assessment was performed 4 weeks after surgery in five rats randomly selected from each group. The surgical sites were carefully reopened, and epidural scar adhesion was evaluated in a double-blind fashion, with the results based on the Rydell classification. Grade 0, Epidural scar tissue was not adherent to the dura mater; Grade 1, Epidural scar tissue was adherent to the dura mater, but easily dissected; Grade 2, Epidural scar tissue was adherent to the dura mater, and difficultly dissected without disrupting the dura matter; Grade 3, Epidural scar tissue was firmly adherent to the dura mater, and could not be dissected [23,24].

2.8. Determination of hydroxyproline content in epidural scar tissue

Hydroxyproline content (HPC) analysis was performed 4 weeks after surgery in five rats randomly selected from each group. Approximately 5 mg wet weight scar tissue was obtained from the surgical area. The HPC analysis was performed as previously reported [21,25]. Briefly, the samples were lyophilized, ground, and hydrolyzed with 6 mol/l HCl at 110 °C for 24 h. Then 1 ml hydroxyproline developer (β–dimethylaminobenzaldehyde solution) was added to the samples and standards. The absorbances were evaluated at 550 nm using a spectrophotometer. In the end, the HPC/mg scar tissue was calculated according to the standard curve constructed with serial concentrations of commercial hydroxyproline.

2.9. Statistical analysis

Data are expressed as mean ± standard error of mean (SEM) values of the mean, median, and minimum–maximum. Differences among groups were assessed with one-way analysis of variance (ANOVA) using SPSS 13.0 statistical package (SPSS Inc., Chicago, IL, USA). Bonferroni correction, as post hoc test was performed. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. ERK2 siRNA in vitro and vivo detection

In vitro, the expression of GFP could be observed in HeLa cells which were infected with the lentiviral ERK scramble RNA or ERK2 siRNA. However, the GFP expression is unavailable in the control cells (Fig. 1).

In vivo of the laminectomy site, the expression of GFP could be available in both the ERK scrRNA group and the ERK siRNA group. However, the GFP cannot be observed in the control group (Fig. 1).

3.2. Macroscopic assessment of epidural fibrosis

Pre- and post-operation comparisons of posture, weight support, and coordination according to the BBB locomotion test did not show significant changes (BBB score = 21). Pre- and post-operative SEP values were not significantly different. These results confirm that nervous tissues were not injured intra-operatively. None of the rats expired intra- or post-operatively, and no obvious adverse effects were observed.

Gross examination of the wound site revealed severe epidural scar adhesions in the control group, and it was difficult to dissect the adhesion without disrupting the integrity of the dura mater along with hemorrhages (Fig. 2A and B). The fewest epidural scar adhesions were observed in the ERK siRNA group (Fig. 2C). The grades of epidural scar adhesions in rats were classified according to Rydell's classification (Table 1).

3.3. Hydroxyproline content (HPC)

The HPC in ERK siRNA group (36.39 ± 4.65 μg/mg) was less than those in the control group (53.42 ± 3.16 μg/mg) ($P = 0.001$) and ERK scrRNA group (50.85 ± 3.55 μg/mg) ($P = 0.008$). The content in the control group showed no significant difference compared with that of the ERK scrRNA group ($P = 0.089$).

3.4. Histological analysis

In the control group and ERK scrRNA group, dense epidural scar tissue with widespread adhesions to dura mater and dorsal muscle were observed in the laminectomy sites (Fig. 3A and B). A large quantity of fibroblasts and inflammatory cells appeared in the scar tissue around the laminectomy sites (Fig. 3D, E and G, H). However, loose or little scar adhesion was observed in the laminectomy sites in the ERK siRNA group (Fig. 3C) and the numbers of fibroblasts and inflammatory cells were significantly less than those of the control and ERK scrRNA group (Fig. 3F and I). In an effort to confirm this

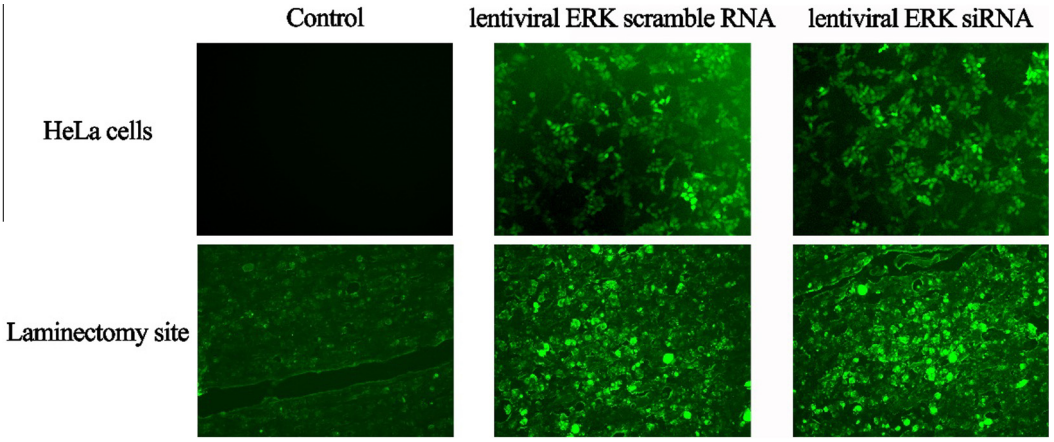

Fig. 1. The expression of GFP with lentiviral siRNA in HeLa cells and laminectomy sites. Original magnification, 100×.

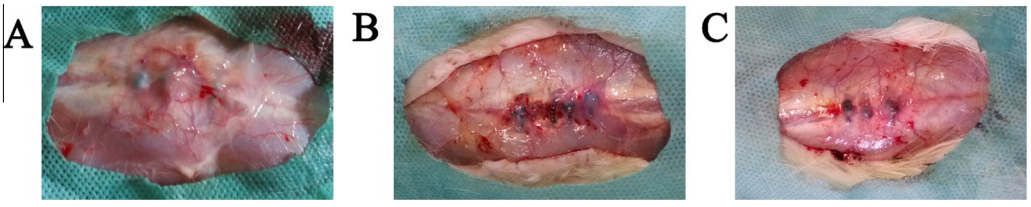

Fig. 2. Gross examination of the wound site. (A and B) More inflammatory fibrous tissue was observed in the wound sites of the control and ERK scrRNA group. (C) The inflammatory reaction was minimal in the ERK siRNA group.

Table 1
Grades of epidural scar adhesion in rats, according to the Rydell standard.

Group	Grade			
	0	1	2	3
Control	5	0	0	0
ERK scrRNA	0	0	1	4
ERK siRNA	4	1	0	0

finding, we conducted an additional immunohistochemistry analysis for vimentin. The data showed less vimentin expression in the ERK siRNA group (Fig. 3L) versus the control group and ERK scrRNA group (Fig. 3J and K).

The fibroblasts and inflammatory densities grades of epidural scar tissue in each group are set out in Table 2. The fibroblasts density in ERK siRNA group was less than those of the control group and ERK scrRNA group (Fig. 3D–F). At the same time, the inflammatory cells density in the ERK siRNA group was less than those of the control group and ERK scrRNA group (Fig. 3G–I). Both fibroblasts and inflammatory cells densities did not show significant difference between the control group and ERK scrRNA group.

Table 2
Grades of cell density.

Groups	Fibroblast density	Inflammatory cell density
Control		
Control 1	3	3
Control 2	3	3
Control 3	3	3
Control 4	3	3
Control 5	3	3
ERK scrRNA		
ERK scrRNA 1	3	3
ERK scrRNA 2	2	3
ERK scrRNA 3	3	3
ERK scrRNA 4	3	3
ERK scrRNA 5	3	3
ERK siRNA		
ERK siRNA 1	1	1
ERK siRNA 2	1	1
ERK siRNA 3	2	1
ERK siRNA 4	1	1
ERK siRNA 5	1	1

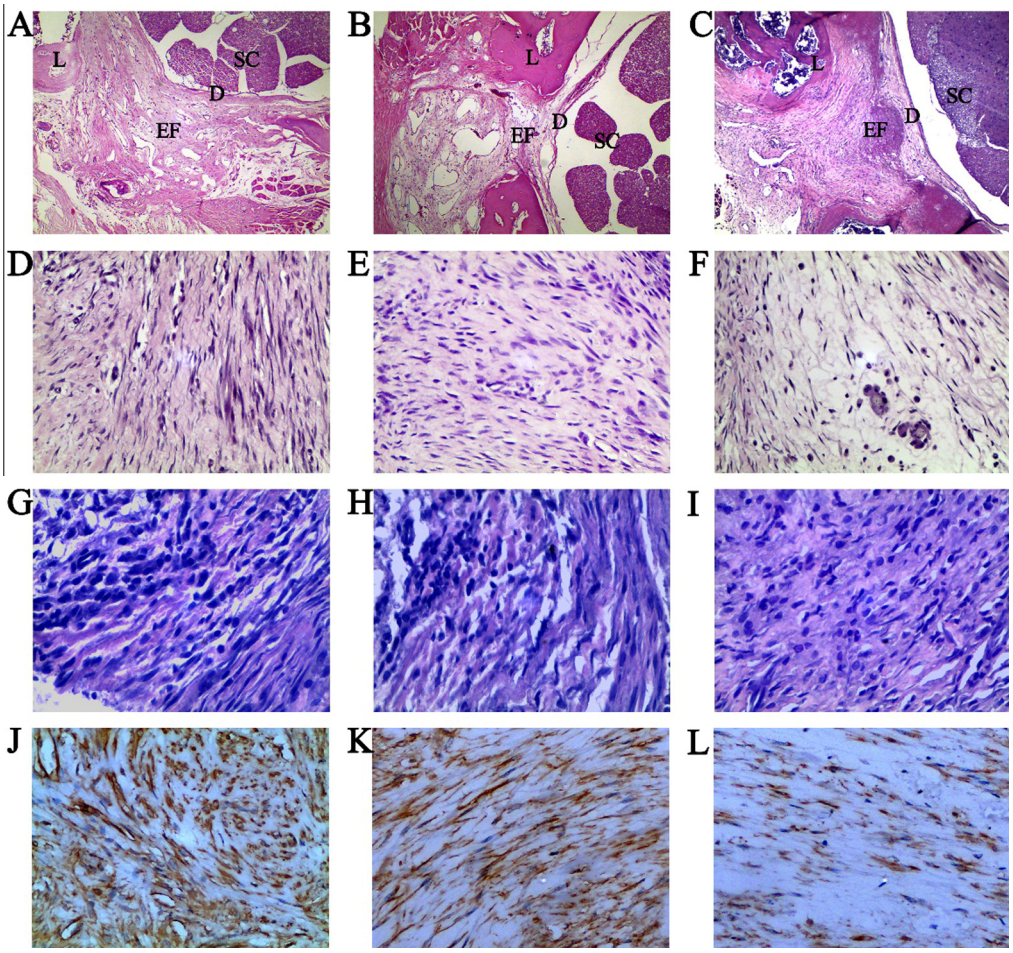

Fig. 3. H&E staining and immunohistochemistry analysis of vimentin cells in the laminectomy sites applied with DMEM (A, D, G, J), lentiviral scramble RNA (B, E, H, K) and lentiviral ERK2 siRNA (C, F, I, L). (A and B) Dense scar tissues adhered to dura maters were found in the control and ERK scrRNA group. (C) Loose scar tissues without adherence to dura mater were found in ERK siRNA group. The density of fibroblast and inflammatory cell in the ERK siRNA group (F and I) was less than those of other 2 groups (D, E and G, H). The density of fibroblast and inflammatory in the ERK scrRNA group (E and H) was similar with that of the control group (D and G). The density of vimentin cells in the ERK siRNA group (L) was less than those of other 2 groups (J and K). The density of vimentin cells in the ERK scrRNA group (K) was similar with that of the control group (J). S = spinal cord, L = laminectomy defect, D = dura mater, EF = epidural fibrosis. (A–C, D–L original magnification, 40× and 100×, respectively).

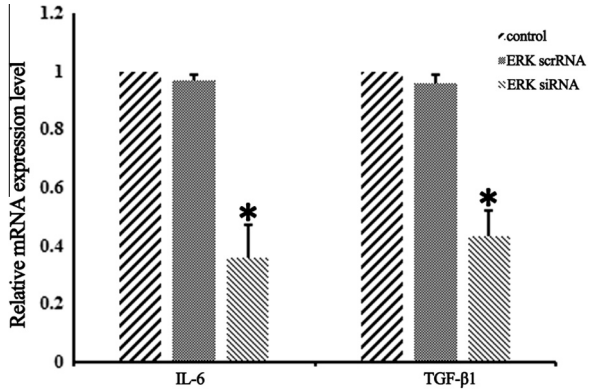

Fig. 4. The mRNA expression level of IL-6 and TGF-β1 in epidural scar tissue in each group. RT-PCR testing was conducted to evaluate. $^{*}P < 0.05$, compare with the control group.

3.5. Effect of lentiviral ERK2 siRNA on suppressing TGF-β1 and IL-6 expression

In our study employing EF rats, ERK2 siRNA has shown its ability to suppress the inflammatory activity during gross examination. This inspired us to determine whether lentiviral ERK2 siRNA is able to suppress TGF-β1 and IL-6 expression in EF rats. In order to find out, we conducted RTPCR to examine the mRNA expression levels of both TGF-β1 and IL-6. The results of mRNA expression levels of both TGF-β1 and IL-6 are shown in Fig. 4: the ERK siRNA group was lower than those of the control group ($P = 0.003$) and ERK scrRNA group ($P = 0.004$); the expressions between the control group and ERK scrRNA group did not show significant differences ($P = 0.092$).

4. Discussion

EF is thought to be a nature process of wound healing post-laminectomy [26]. With the loss of epidural fat intra-operation, the fibroblasts from the adjacent musculature generate fibrosis post-operation. And the inflammatory factors accelerate this pathological progress. The physiologic scar formed a hypertrophic enveloping film which can lead FBSS. Besides the recurrent symptoms including radicular pain this extradural fibrotic tissue can lead, it is also able to bring the next exploration of the operative field technically challenging, such as nerve root damage, dural tears, and iatrogenic injuries [27–29].

The current treatments for EF involve rebuilding lost physical barriers with autologous fat grafts and topical gel foams. However, autologous fat grafts can cause complications, including infection and hematoma in the donor area [30]. Previously, a prospective study reported that the employment of either a fat barrier or gel foam as a post-operative treatment of the nerve roots and dura mater did not produce improvements in the clinical setting, actual improve functional status, or significant changes in follow-up MRI [31]. Therefore, we are against using either a fat barrier or gel foam as the preferred choice of treatment because of the likelihood of infection due to fat liquefaction.

In the present study, we report the development of the RNAi strategy to knockdown ERK2 expression in treating epidural fibrosis post-laminectomy. To our knowledge, this is the first study to successfully demonstrate that inhibition of the MEK/ERK pathway in vivo attenuates the progression of fibrosis and physiologic alterations in EF rat. The data showed significant decrease of Hydroxyproline levels in scar tissues in lentiviral ERK2 siRNA group. In the laminectomy rat, both the inflammatory cells and fibroblast cells decreased significantly after topical injection of lentiviral ERK2 siRNA. The scar adhesions showed better Rydell Classification scores when treated with lentiviral ERK2 siRNA. RTPCR analyses showed a significant decrease of both IL-6 and TGF-β1, suggesting a reduction of inflammation. All of the above support our theory of lentiviral ERK2 siRNA could be a potential therapy for EF, with the characterizations on anti-fibrotic, anti-inflammatory, and anti-proliferative roles. The clinical relevance for this finding is the strong evidence demonstrating up-regulation of ERK/MAPK in human fibrotic disease [32,33].

Combining our previous studies and the latest literatures, treating EF remains a medical challenge and developing new therapeutic strategies is clinically important [21,34,35]. The present study provides a proof-of-concept that ERK2 knockdown can inhibit collagen expression and inflammation, thereby revealing a detrimental role for ERK2 pathway in EF and paving the way for further investigation with post-laminectomy administration of lentiviral siRNA targeting ERK2 in the pathophysiology and treatment of EF.

In conclusion, local delivery of a lentiviral siRNA targeting ERK2 can inhibit fibroblasts proliferation, TGF-β1 and IL-6 expressions and prevent epidural scar adhesion in post-laminectomy rat, which reflects the predominant role of ERK2 in EF formation. This study provides a novel and promising strategy that will serve as an alternative for the prevention of EF formation. More research on safety, effective safe concentration, long-term effects, and possible side- and adverse effects of lentiviral ERK2 siRNA are warranted before clinical trials and application.

Acknowledgments

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References

[1] H. Emmez, O. Kardes, F. Dogulu, G. Kurt, L. Memis, M.K. Baykaner, Role of antifibrotic cytokine interferon-gamma in the prevention of postlaminectomy peridural fibrosis in rats, Neurosurgery 62 (2008) 1351–1357.
[2] J. Xu, Y. Chen, Y. Yue, J. Sun, L. Cui, Reconstruction of epidural fat with engineered adipose tissue from adipose derived stem cells and PLGA in the rabbit dorsal laminectomy model, Biomaterials 33 (2012) 6965–6973.
[3] J. Andrychowski, M. Frontczak-Baniewicz, D. Sulejczak, T. Kowalczyk, T. Chmielewski, Z. Czernicki, T.A. Kowalewski, Nanofiber nets in prevention of cicatrisation in spinal procedures. Experimental study, Folia Neuropathol. 51 (2013) 147–157.
[4] G.G. Gill, M. Scheck, E.T. Kelley, J.J. Rodrigo, Pedicle fat grafts for the prevention of scar in low back surgery. A preliminary report on the first 92 cases, Spine 10 (1985) 662–667.
[5] M.A. Sandoval, D. Hernandez-Vaquero, Preventing peridural fibrosis with nonsteroidal anti-inflammatory drugs, Eur. Spine J. 17 (2008) 451–455.
[6] B. Cemil, K. Tun, E. Kaptanoglu, F. Kaymaz, B. Cevirgen, A. Comert, I. Tekdemir, Use of pimecrolimus to prevent epidural fibrosis in a postlaminectomy rat model, J. Neurosurg. Spine 11 (2009) 758–763.
[7] H.M. Lee, K.H. Yang, D.Y. Han, N.H. Kim, An experimental study on prevention of postlaminectomy scar formation, Yonsei Med. J. 31 (1990) 359–366.
[8] J. Barberá, J. Gonzalez, J. Esquerdo, J. Broseta, J.L. Barcia-Salorio, Prophylaxis of the laminectomy membrane, an experimental study in dogs, J. Neurosurg. 49 (1978) 419–424.
[9] M.R. Farrokhi, M. Vasei, S. Fareghbal, A. Bakhtazad, Effect of honey on peridural fibrosis formation after laminectomy in rats: a novel experimental study, Evid Based Compl. Alternat. Med. (2011), http://dx.doi.org/10.1155/2011/504967.
[10] S.M. Elbashir, J. Harborth, W. Lendeckel, A. Yalcin, K. Weber, T. Tuschl, Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells, Nature 411 (2001) 494–498.
[11] A. Fire, S. Xu, M.K. Montgomery, S.A. Kostas, S.E. Driver, C.C. Mello, Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*, Nature 391 (1998) 806–811.
[12] G.J. Hannon, RNA interference, Nature 418 (2002) 244–251.
[13] Q. Wang, Z. Peng, S. Xiao, S. Geng, J. Yuan, Z. Li, RNAi-mediated inhibition of COL1A1 and COL3A1 in human skin fibroblasts, Exp. Dermatol. 16 (2007) 611–617.

[14] H. Nakamura, S.S. Siddiqui, X. Shen, A.B. Malik, J.S. Pulido, N.M. Kumar, B.Y. Yue, Interference targeting transforming growth factor-beta type II receptor suppresses ocular inflammation and fibrosis, Mol. Vis. 10 (2004) 703–711.
[15] 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.
[16] X.F. Qin, D.S. An, I.S. Chen, D. Baltimore, Inhibiting HIV-1 infection in human T cells by lentiviral-mediated delivery of small interfering RNA against CCR5, Proc. Natl. Acad. Sci. USA 100 (2003) 183–188.
[17] D.M. Basso, M.S. Beattie, J.C. Bresnahan, A sensitive and reliable locomotor rating scale for open field testing in rats, J. Neurotrauma 12 (1995) 1–21.
[18] F. Li, H. Ruan, C. Fan, B. Zeng, C. Wang, X. Wang, Efficient inhibition of the formation of joint adhesions by ERK2 small interfering RNAs, Biochem. Biophys. Res. Commun. 391 (2010) 795–799.
[19] C.G. Yu, R.P. Yezierski, A. Joshi, K. Raza, Y. Li, J.W. Geddes, Involvement of ERK2 in traumatic spinal cord injury, J. Neurochem. 113 (2010) 131–142.
[20] Y.Q. Cai, S.R. Chen, H.D. Han, A.K. Sood, G. Lopez-Berestein, H.L. Pan, Role of M2, M3, and M4 muscarinic receptor subtypes in the spinal cholonergic control of nociception revealed using siRNA in rats, J. Neurochem. 111 (2009) 1000–1010.
[21] C. Zhang, X. Kong, H. Zhou, C. Liu, X. Zhao, X. Zhou, Y. Su, H.S. Sharma, S. Feng, An experimental novel study: angelica sinensis prevents epidural fibrosis in laminectomy rats via downregulation of hydroxyproline, IL-6, and TGF- β 1, Evid Based Compl. Alternat. Med. (2013), http://dx.doi.org/10.1155/2013/291814.
[22] C. Liu, R. Chang, X. Yao, W.T. Qiao, Y.Q. Geng, ISG15 expression in response to double-stranded RNA or LPS in cultured fetal bovine lung(FBL) cells, Vet. Res. Commun. 33 (2009) 723–733.
[23] N. Rydell, Decreased granulation tissue reaction after installment ofhyaluronic acid, Acta Orthop. Scand. 41 (1970) 307–311.
[24] L. Zhu, B. Ni, J. Liu, J. Yang, Q. Guo, W. Zhou, Hydroxycamptothecin liposomes inhibit collagen secretion and induce fibroblast apoptosis in a postlaminectomy rabbit model, Eur. J. Orthop. Surg. Traumatol. 23 (2013) 85–91.
[25] N. Fukui, T. Tashiro, H. Hiraoka, H. Oda, K. Nakamura, Adhesion formation can be reduced by the suppression of transforming growth factor-beta1 activity, J. Orthop. Res. 18 (2000) 212–219.
[26] M.S. Bryant, A.M. Bremer, T.Q. Nguyen, Autogenic fat transplants in the epidural space in routine spine surgery, Neurosurgery 13 (1983) 367–370.
[27] K. Chandler, R. Cappello, Laminectomy membrane formation in dogs: is the answer still elusive?, Vet J. 172 (2006) 1–2.
[28] I.M. Jou, T.W. Tai, C.L. Tsai, T.M. Tsai, W.S. Yung, Y.C. Jung, Spinal somatosensory evoked potential to evaluate neurophysiologic changes associated with post-laminectomy fibrosis: an experimental study, Spine 32 (2007) 2111–2118.
[29] T. Kato, H. Haro, H. Komori, K. Shinomiya, Evaluation of hyaluronic acid sheet for the prevention of postlaminectomy adhesions, Spine J. 5 (2005) 479–488.
[30] J.M. Cabezudo, A. Lopez, F. Bacci, Symptomatic root compression by a free fat transplant after hemilaminectomy. Case report, J. Neurosurg. 63 (1985) 633–635.
[31] M.A. MacKay, J.S. Fischgrund, H.N. Herkowitz, L.T. Kurz, B. Hecht, M. Schwartz, The effect of interposition membrane on the outcome of lumbar laminectomy and discectomy, Spine (Phila Pa 1976) 20 (1995) 1793–1796.
[32] K.M. Antoniou, G.A. Margaritopoulos, G. Soufla, E. Symvoulakis, E. Vassalou, R. Lymbouridou, K.D. Samara, D. Kappou, D.A. Spandidos, N.M. Siafakas, Expression analysis of Akt and MAPK signaling pathways in lung tissue of patients with idiopathic pulmonary fibrosis (IPF), in: J. Recept Signal Transduct Res. 30 (2010) 262–269.
[33] K. Yoshida, K. Kuwano, N. Hagimoto, K. Watanabe, T. Matsuba, M. Fujita, I. Inoshima, N. Hara, MAP kinase activation and apoptosis in lung tissues from patients with idiopathic pulmonary fibrosis, J. Pathol. 198 (2002) 388–396.
[34] C. Zhang, X. Kong, G. Ning, Z. Liang, T. Qu, F. Chen, D. Cao, T. Wang, H.S. Sharma, S. Feng, All-trans retinoic acid prevents epidural fibrosis through NF-κB signaling pathway in post-laminectomy rats, Neuropharmacology 79 (2013) 275–281.
[35] Z. Wang, Y. Wang, P. Xie, W. Liu, S. Zhang, Calcium channel blockers in reduction of epidural fibrosis and dural adhesions in laminectomy rats, Eur. J. Orthop. Surg. Traumatol. (2013). Epub ahead of print.