Stretching Promotes Wound Contraction Through Enhanced Expression of Endothelin Receptor B and TRPC3 in Fibroblasts

Kenichiro Kawai, MD, PhD*
Hisako Ishise, MD, PhD*
Tateki Kubo, MD, PhD†
Barrett Larson, MD‡
Toshihiro Fujiwara, MD, PhD*
Soh Nishimoto, MD, PhD*
Masao Kakibuchi, MD, PhD*

Background: One factor that can contribute to the development of hypertrophic scar contracture is mechanical stress. Mechanical cyclic stretch stimuli enhance the secretion of endothelin-1 (ET-1) from keratinocyte. Cyclical stretching of fibroblasts also increases the expression level of the transient receptor potential ion channel (TRPC3), which is known to couple with the endothelin receptor and induce intracellular $Ca2+$ signaling via the calcineurin/nuclear factor of activated T cells (NFAT) pathway. The aim of this study was to investigate the relationship between keratinocytes and fibroblasts when they are stretched.

Methods: The conditioned medium from stretched keratinocyte was added to the fibroblast populated collagen lattice. Then, we analyzed the levels of endothelin receptor in the human hypertrophic scar tissue and stretched fibroblasts. To address the function of TRPC3, we have used an overexpression system with the collagen lattice. Finally, the TRPC3 overexpressing fibroblasts were transplanted to mouse dorsal skin, and the rate of skin wound contraction was assessed.

Results: Conditioned medium from stretched keratinocytes increased the rate of contraction of fibroblast populated collagen lattice. In human hypertrophic scar and stretched fibroblasts, endothelin receptor type B was increased. Cyclic stretching of TRPC3 overexpressing fibroblasts activated NFATc4, and stretched human fibroblasts showed more activation of NFATc4 in response to ET-1. The wound treated with TRPC3 overexpressing fibroblasts showed more contraction than control wound.

Conclusion: These findings suggest that cyclical stretching of wounds have an effect on both keratinocytes and fibroblasts, where keratinocytes secret more ET-1, and fibroblasts develop more sensitivity to ET-1 by expressing more endothelin receptors and TRPC3. *(Plast Reconstr Surg Glob Open 2023; 11:e4954; doi: 10.1097/GOX.0000000000004954; Published online 25 April 2023.)*

INTRODUCTION

Two important phases in the wound healing process are wound contraction and epithelialization. Contraction of skin wounds is caused by the differentiation of fibroblasts to myofibroblasts, and epithelialization is the result of migration and proliferation of keratinocytes.[1,2] Although these are different phenomena carried out by different cell types, numerous reports have shown that fibroblasts and keratinocytes interact with each other and orchestrate the wound healing processes.[3–5] When the process fails, pathological phenomenon such as hypertrophic contracture and scarring can occur. Cutaneous scar contracture tends to occur in areas where the wound is exposed to stretching forces (eg, shoulder, elbow, and mouth). However, the detailed mechanisms by which stretching forces lead to scar contracture are not fully understood.

Disclosure statements are at the end of this article, following the correspondence information.

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Recently, an ion channel group called transient receptor potential channel (TRPC) has been identified as a possible mechanical cell transduction pathway.[6–8] Previously, we found that TRPC3 was upregulated in human hypertrophic scar tissue when compared with normal skin and stretching forces induced TRPC3 expression in cultured fibroblasts.[9] It is well known that TRPCs are involved in the activation of the calcineurin/nuclear factor of activated T cell (NFAT) pathways in response to G protein-coupled receptor signaling.[7,10] Among the cytokines that stimulate G protein-coupled proteins, endothelin-1 (ET-1) is considered to be a stress-responsive regulator working in a paracrine and autocrine manner in a variety of organs.[11–16] Kurita et al reported that the expression of ET-1 in keratinocytes was upregulated when they were exposed to cyclical stretching in vitro.[17]

In this study, we aimed to understand the relationship between fibroblasts and keratinocytes in wound healing. We used a fibroblast populated collagen lattice (FPCL) method to investigate the paracrine factors secreted from stretched keratinocytes. Additionally, we examined the expression levels of endothelin receptors in human scar tissue and stretched fibroblasts in vitro. To further understand the role of ET-1 in FPCL contraction, we used fibroblasts that overexpress TRPC3. Finally, we transplanted these TRPC3 overexpressing cells to nude mice and measured the wound contraction rate.

Our results indicate that keratinocytes communicate with fibroblasts in a paracrine manner via the secretion of ET-1 when they are stretched. Moreover, fibroblasts increase their sensitivity to ET-1 by upregulating endothelin receptor B in response to mechanical stimuli. These findings demonstrate that the interaction between fibroblasts and keratinocytes during the wound healing process contributes to the formation of scar contracture, particularly when wounds are exposed to stretching forces.

MATERIALS AND METHODS

Materials

All procedures were approved by the Hyogo Medical University ethics committee, with informed consent obtained from the involved patients. PlatE packaging cells and pMXs-IRES-GFP plasmids were provided from Dr. Toshio Kitamura at the University of Tokyo.

Animals

Eight-week-old female BALB/c nu/nu mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). All procedures were approved by the Hyogo Medical University ethics committee, and all animals were housed under standard conditions at the Hyogo Medical University animal facility under institution-approved guidelines.

Generation of TRPC3 Overexpressing Fibroblast

Mouse cDNA TRPC3 clones were obtained from Origene Technologies Inc. (Md., USA). We constructed a retroviral plasmid using a pMXs-IRES-GFP by incorporating the TRPC3 gene into the plasmid.[18] Then, the

Takeaways

Question: Is stretching force a risk factor of keloid formation and wound contracture?

Findings: The effect of cyclical stretching on wounds involves both keratinocytes and fibroblasts, with keratinocytes exhibiting increased sectretion of endothelin and fibroblasts becoming more sensitive to it.

Meaning: Repetitive stretching force on a wound increases the risk of keloid formation or wound contracture.

plasmids were transfected into packaging cells (Plat E), which produced retrovirus containing both TRPC3 and GFP.[19] Transfection was carried out using Lipofectamin LTX (Life Technologies, Calif., USA). Mouse skin fibroblasts (NIH3T3) were infected with the virus, which allowed them to express both TRPC3 and GFP. As a control, NIH3T3 cells were transfected with the virus that contained the vector alone. Then, GFP positive cells were collected with a cell sorter (FACS Aria, Becton, Dickinson, N.J.).[9]

Cell Culture

Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma, Mo.) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution (penicillin 100 U/ml and streptomycin 100 µg/ml mixed solution; Nacalai tesque, Kyoto, Japan) in an incubator set at 37℃, 5% CO_2.

Normal human skin fibroblasts were obtained from normal adult human skin discarded after scar revision surgery performed at the Hyogo Medical University. Early passage cells (passage 3–5) were used in the experiment. The isolated fibroblasts were cultured in DMEM containing 10% FBS and antibiotics until they became confluent.

Cyclic Mechanical Stretch

Mechanical stretching experiments were performed as described in our previous report.[9] In each experiment, either primary human dermal fibroblasts or TRPC3 overexpressing cells and control cells were seeded on type I collagen-coated silicon chambers (2×2 cm) at a density of 8×10^5 cells per chamber. After allowing the cells to attach for 24 hours, the medium was replaced with DMEM supplemented with 0.5% BSA and 1% antibiotic solution. Cells were incubated in the serum-starved condition media for 24 hours. Then, the fibroblasts were mechanically stretched to 20% strain for the indicated time period at a frequency of 10 cycles/minute, each cycle consisting of 2 seconds of strain and 2 seconds of relaxation in a cell stretcher (STB-140-10, STREX, Osaka, Japan) that was placed in an incubator set to 37℃ and 5% CO_2.

Immunocytochemistry and Immunohistochemistry

Three different human samples of hypertrophic scar and adjacent normal skin were taken from scar revision procedures performed at the Hyogo Medical University Hospital. The samples were soaked in O.C.T. Compound (SAKURA, Tokyo, Japan) and placed into liquid nitrogen

immediately. Sections 12 μm in thickness were harvested. For immunohistochemistry, tissue sections were blocked with normal goat serum and incubated with rabbit anti-TRPC3 antibody (Alamone labs, Jerusalem, Israel) at a 1:100 dilution for 1.5 hours at room temperature. Sections were incubated with a biotinylated goat anti-rabbit secondary antibody for 30 minutes and developed with ABC complex (Vectastain ABC System, Vector Labs, Calif., USA). Primary antibody binding sites were visualized by Peroxidase Stain DAB kit (Nakalai Tesque, Kyoto, Japan). Sections were then counterstained with hematoxylin. For immunocytochemistry, primary human fibroblasts were stretched for 24 hours as previously described, and the cells were fixed with ice cold 4% paraformaldehyde for 15 minutes. Then, the bottom of the silicon chamber was cut into small pieces (5mm × 5mm) and a piece of the chamber was attached to a slide glass. After blocking with normal goat serum, the cells were incubated with anti-TRPC3 antibody at 1:100 dilution for 1 hour at room temperature. Cells were then developed with Vectastain ABC System and DAB staining. Hematoxylin was used for counterstaining.

Fibroblast Populated Collagen Lattice

For the conditioned medium assay, human keratinocyte HaCaT cells were stretched for 24 hours, as described above. The conditioned medium was harvested and centrifuged at 10,000g for 10 minutes at 4℃ and the supernatant was stocked at –80℃ until use. Human dermal fibroblasts were embedded in type I collagen at a final concentration of $1×10^6$ cells and a collagen concentration of 1.5 mg per milliliter. A volume of 500 μl per gel was fabricated in a 24-well plastic culture plate, which ensured that the gel would remain attached throughout the culture period. The cells were cultured in 10% FBS for 24 hours after polymerization. Then, the FPCLs were serum-starved with 0.5% BSA DMEM for 24 hours, followed by incubation in conditioned media obtained from keratinocytes. After 24 hours in culture, the FPCLs were mechanically released from the plate, and rapid contraction was recorded in 1 hour.

For the ET-1 assay, either TPC3 overexpressing fibroblast cells or control fibroblast cells were embedded in type I collagen at a final concentration of $1×10^6$ cells per milliliter and collagen concentration of 1.5 mg per milliliter. A volume of 500 μl per gel was fabricated in a 24-well plastic culture plate. The cells were cultured in 10% FBS for 24 hours after polymerization. Then, the FPCLs were incubated in 0.5% FBS with ET-1 (100 nM), Pyr3 (3 μM), and a combination of ET-1 (100 nM) and Pyr3 (3 μM) or PD142893 (1 μM). After 24 hours in culture, the treated FPCLs were mechanically released from the plate, and the contraction rate was recorded after 24 hours of incubation.

Quantitative Real-time PCR

Total RNA of cultured cells was isolated using NucleoSpinRNAⅡ (MACHEREY-NAGEL GmbH & Co. KG, Germany) according to the manufacturer's instructions. Aliquots of 900 ng of mRNA were reverse-transcribed to complementary DNA using PrimeScript RT Master Mix (TAKARA, Shiga, Japan). Quantitative real-time PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems, Calif., USA) and the 7900 Real-time PCR system (Applied Biosystems, Calif., USA). The following primers were used: human EDNRA, 5′-CTA CCC AGC AAT GGC TCA AT-3′ (forward) and 5′-CAAGGGCAAGACTGGCTATC-3′ (reverse); human EDNRB, 5′-ATGACGCCACCCACTAAGAC-3′ (forward) and 5′-GAACACAAGGCAGGACACAA-3′ (reverse); human cyclophilin, 5′-CAG ACG CCA CTG TCG CTT T-3′ (forward) and 5′-TGT CTT TGG AAC TTT GTC TGC AA-3′ (reverse). Cyclophilin was used for the internal standards.

Western Blot Analysis

Cells were lysed with RIPA buffer (Nacalai Tesque, Kyoto, Japan). Two micrograms of protein was resolved on a sodium dodecyl sulfate-polyacrylamide gel and transferred to a PVDF membrane. The membranes were incubated with anti-NFATc4 antibody (Santa Cruz Biotechnology, Tex., USA). Then, the membranes were incubated with anti-rabbit IgG, horseradish peroxidase-conjugated secondary antibody before detection of immunoreactivity with ECL Prime Western Blotting Detection Reagent (GE Healthcare, Backinghamshire, UK). All blots were quantified with densitometry using ImageQuant TL (GE Healthcare, Backinghamshire, UK).

Open Mouse Wound Model

For cutaneous injuries, 8-week-old female Balb/c nu/nu mice were anesthetized with pentobarbital. TRPC3 overexpressing fibroblasts were transplanted to the subdermal layer on the back of mice. Ten days post transplantation, wounds were created. To make wound sizes as consistent as possible, a 6-mm punch biopsy was used. The skin, including the panniculus carnosus, was carefully excised just above the myofascial layer with scissors. The wounds were dressed with Silkypore Dressing (Alcare, Tokyo, Japan). Wound dressings were removed carefully with saline, so as not to change the wound size or shape. A standard silicon ring was used as a reference frame. Wound photographs were taken at day 1, day 5, and day 9 with a digital camera (D40, Nikon, Tokyo, Japan). The wound and reference ring areas were measured using Image J software. The ratio of the wound area to the reference ring area was calculated, and the progression of the wound healing was determined by comparing the ratio on each day to the ratio on day 0.

Statistical Analysis

Results are presented as means ± SD of n observations. Data involving two groups were analyzed using two-tailed Student t test assuming unequal variances. When more than two experimental groups were compared, the data were analyzed using the Tukey-Kramer test to compare data between individual experimental groups. A P value of less than 0.05 was considered to be statistically significant for all tests.
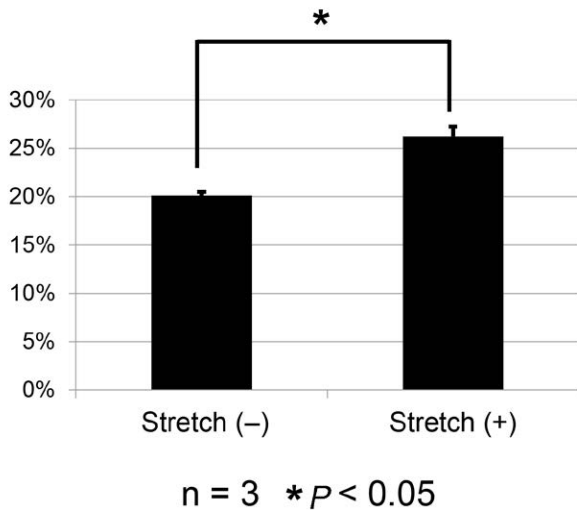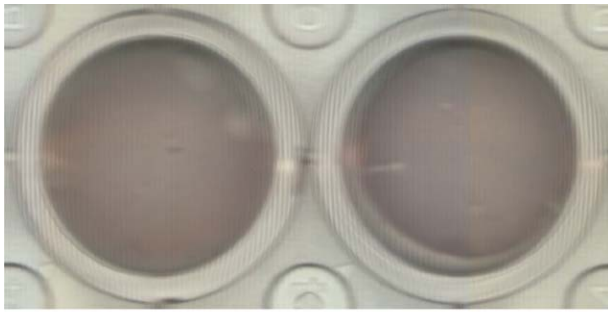
Fig. 1. FPCL treated with the control media from stretched keratinocyte seemed to contract more than control. To investigate the interaction of keratinocyte and fibroblasts, the contractile activities of human primary fibroblasts were analyzed with gel contraction assay. Gels were incubated with either the conditioned media from keratinocytes subject to 24 hour stretch or control media. Gels with the conditioned media from stretched keratinocyte exhibited more contraction than gels with control media. The data are expressed as the mean ± SD (n = 4). *P < 0.05 by Student *t* test.

RESULTS

Conditioned Medium from Stretched HaCaT Human Keratinocytes Enhances the Contraction of FPCLs

Cyclic stretching of skin wounds is known to cause scar contracture. To simulate the microenvironment of dermal wounds, isolated human dermal fibroblasts were embedded in a three-dimensional type-I collagen matrix and then incubated with conditioned media from keratinocytes, which had been subjected to 24 hours of repetitive strain. The FPCLs treated with conditioned medium from stretched HaCaT cells showed significantly more contraction than the control FPCLs (Fig. 1).

Endothelin-1 Promotes Contraction in TRPC3 Overexpressing FPCLs

Keratinocytes produce ET-1 in response to cyclical stretching.[17] Given that the TRPC channel couples with endothelin receptors to induce calcium influx upon stimulation by extrinsic ET-1,[20,21] we speculated that ET-1 might exert its effects on fibroblasts via the TRPC3 channel.

Adding ET-1 to the FPCL increased the contraction rate of TRPC3 overexpressing fibroblasts. In addition, this contraction effect was attenuated by the addition of both a TRPC3 specific inhibitor (Pyr3) and an endothelin antagonist (PD142893) (Fig. 2).

Endothelin Receptor B Expression Are Increased in Human Hypertrophic Scar and Stretched Fibroblasts

To determine whether endothelin receptor expression levels in hypertrophic scar tissue was increased, we investigated human hypertrophic scar samples. The expression level of endothelin receptor B (EDNRB) was increased in human hypertrophic scar tissue, whereas the expression of endothelin receptor A (EDNRA) was rarely different from normal skin (Fig. 3A).

Hypertrophic scars tend to occur in areas where the wound is repeatedly stretched and relaxed. To investigate whether cyclical stretching of fibroblasts also increases the expression of endothelin receptors, human primary fibroblasts were stretched. Cyclical stretching of the cells increased mRNA expression of EDNRB, but had no significant effect on the expression of EDNRA. This result was confirmed via immunohistochemistry (Fig. 3B, C).

NFATc4 in Stretched Fibroblasts Is Activated via Endothelin-1

Because TRPC3 acts as a mechano-stress transducer via the calcineurin/NFATc pathway, we investigated whether stretching stimuli activate NFATc in TRPC3 overexpressing fibroblasts by analyzing the cell lysate from stretched fibroblasts. Western blot analysis demonstrated that cyclic stretching caused the activation of NFATc4 in TRPC3 overexpressing fibroblasts (Fig. 4A).

Given that the stretched fibroblasts express more TRPC3 and the effect of ET-1 was mediated by TPRC3, NFATc4 in the stretched fibroblasts is assumed to be activated with ET-1. To test this, human primary fibroblasts were stretched for 24 hours and followed by treating with ET-1. NFATc4 was found to be upregulated in stretched fibroblasts upon addition of ET-1 (Fig. 4B).

Transplantation of Overexpressing TRPC3 Fibroblasts Accelerates Wound Contraction in Excisional Mouse Wounds

The effect of TRPC3 on wound healing was investigated using an open, full-thickness, excisional mouse skin wound model. TRPC3 overexpressing fibroblasts were transplanted into the subdermal layer on the back of mice. Ten days post transplantation, wounds were created using a 6-mm punch biopsy. For the first 24 hours after injury, the rate of wound closure was found to be significantly faster in mice that had been transplanted with TRPC overexpressing fibroblasts when compared with control mice and saline transplanted mice. By day 7, mice transplanted with TRPC3 overexpressing fibroblasts had healed faster than controls (Fig. 5). (**See figure, Supplemental Digital Content 1,** which displays the representative photographs of mouse cutaneous wounds. To evaluate the impact of
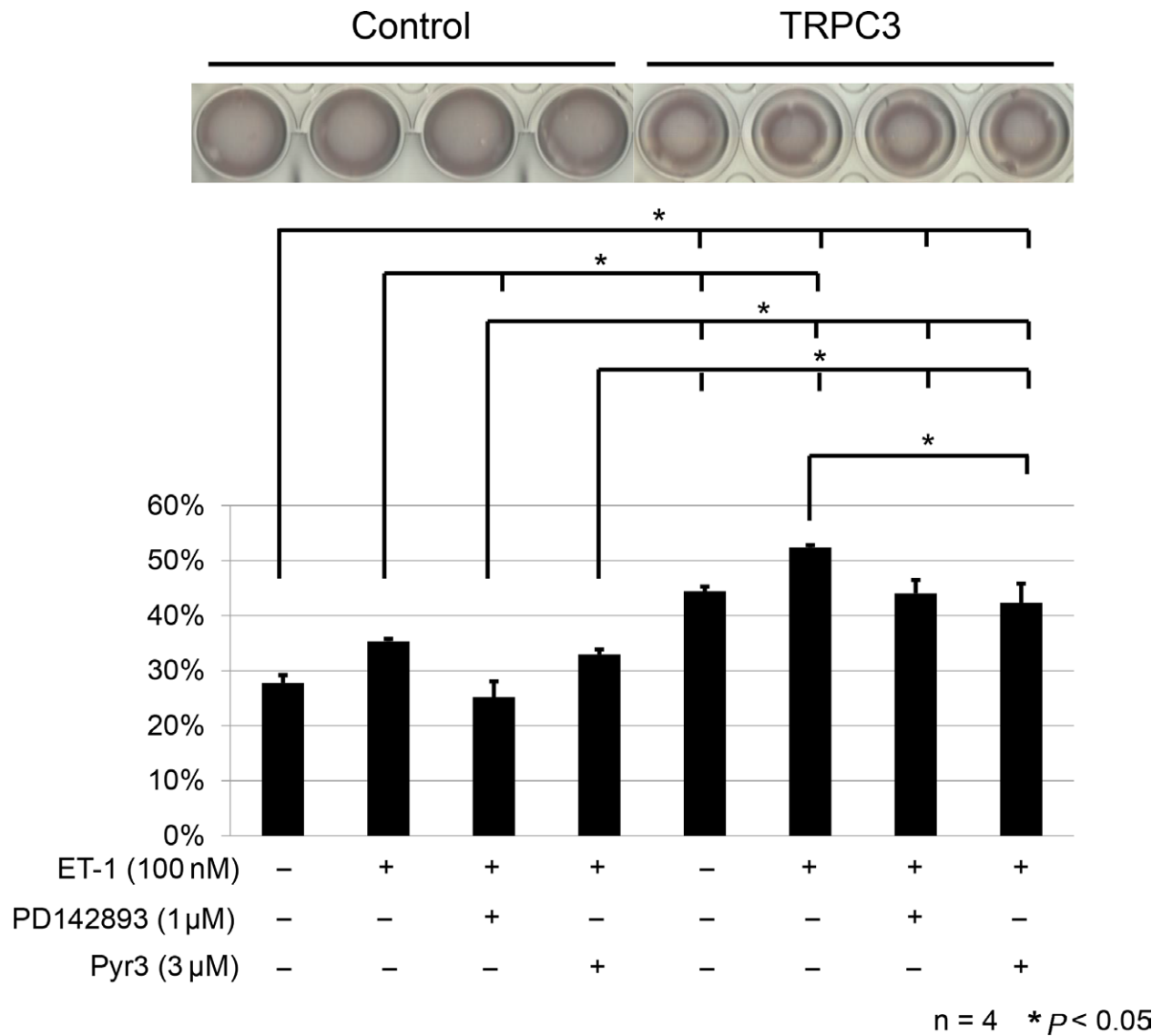
Fig. 2. Fibroblast populated collagen lattice with TRPC3 overexpressing fibroblasts seemed to have more contractile activity than collagen lattice with control fibroblasts. The contractile activities of TRPC3 overexpressing fibroblasts were analyzed with gel contraction assay. Each gel contains either 8×10^5 TRPC3 overexpressing cells or empty vector transfected control cells and incubated for 24 hours. After starvation, the gels were treated with either ET-1, ET-1 antagonist; PD142893 or inhibitor; Pyr3 and the contraction ratio was observed. Gels with TRPC3 overexpressing cells apparently exhibited more contraction than gels with control cells. This contraction was enhanced with ET-1, which was attenuated with PD142893 and Pyr3. The data are expressed as the mean ± SD (n = 4). *$P < 0.05$ by Tukey-Kramer test.

TRPC3 on wound healing in vivo, we transplanted TRPC3-overexpressing fibroblasts, empty-vector transfected control fibroblasts, or an equivalent volume of saline onto the dorsum of nude mice. Ten days post-transplantation, we created 6-mm wounds and measured wound size at specified time points. Differential rates of healing among the three groups were observed. **http://links.lww.com/ PRSGO/C511**.)

DISCUSSION

Clinically, there seems to be a strong correlation between wound contracture and repetitive mechanical stretching. Substantial stretching activates downstream signaling in the fibroblasts through various pathways.[2,9,22–24] Although numerous mechanisms to explain the relationship between mechanical stretching and hypertrophic scar contracture have been proposed, most of these mechanisms focus on a single cell type operating in isolation. In the present study, we focused on the epithelial-mesenchymal *interaction* that occurs when keratinocytes and fibroblasts undergo repetitive stretch stimulation.

It has been shown that several growth factors are responsible for mediating the cross talk between keratinocytes and fibroblasts.[3] In this study, conditioned media from stretched HaCaT human keratinocytes enhanced

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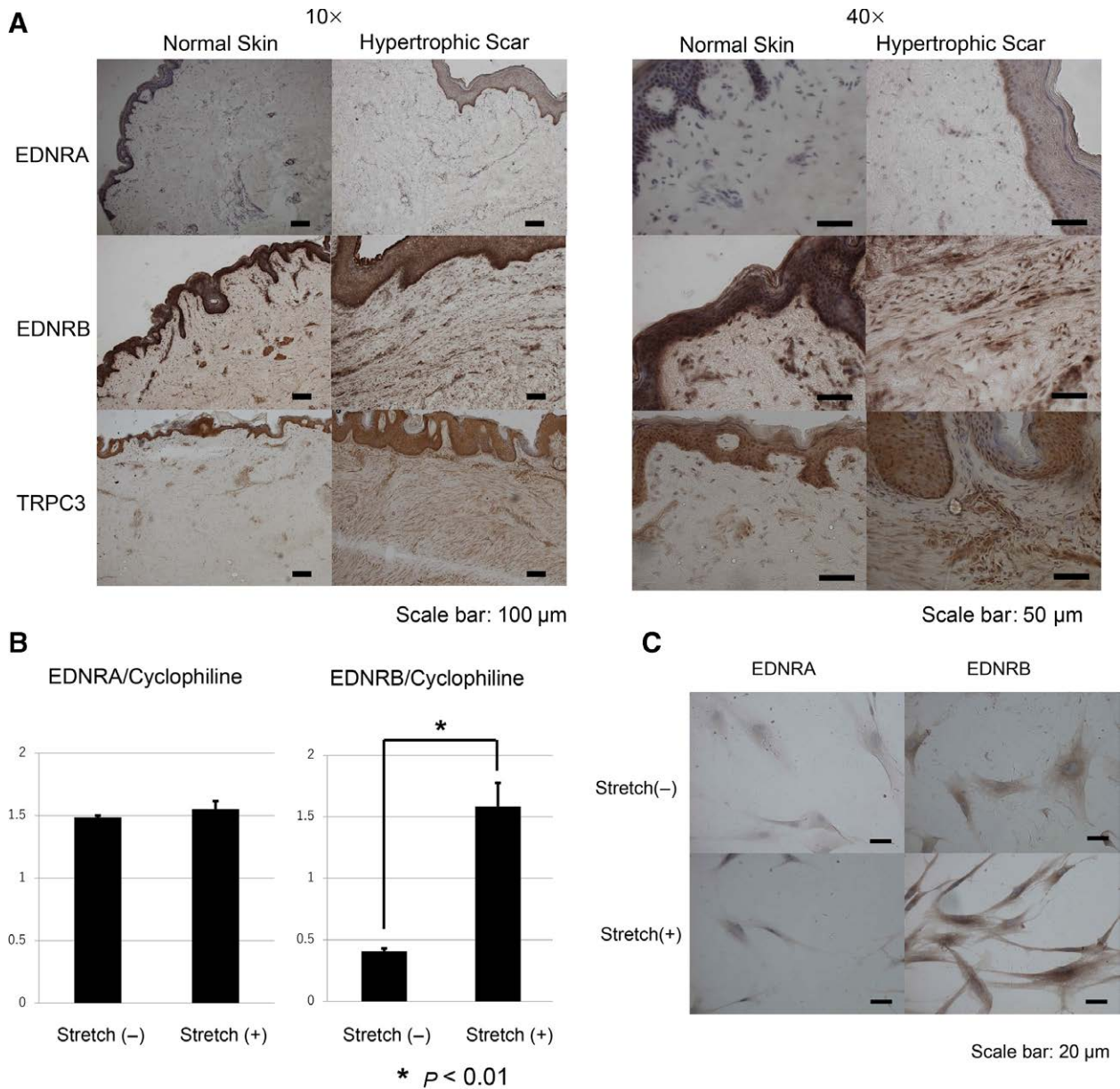
Fig. 3. Expression of EDNRA, EDNRB, and TRPC3 in human hypertrophic scar tissue and expression of EDNRA and EDNRB in stretched human primary fibroblasts. A, Immunohistochemical staining with anti EDNRA, EDNRB, and TRPC3 showed that EDNRB and TRPC3 were strongly stained around the cell cluster in human hypertrophic scar tissue, whereas EDNRA was rarely stained. Scale bars represent 50 μm in 40× images and 100 μm in 10× images. B, mRNA expression of EDNRA and EDNRB in human fibroblasts subjected to mechanical load was measured with qRT-PCR, as described in Materials and methods section. Stretched cells showed more EDNRB expression compared with normal, unstretched fibroblasts, whereas EDNRA did not show significant difference. Data represent means ± SD of three samples. *P < 0.01 by student t test. C, Human primary fibroblasts were stretched (20%, 10 Hz) for 24 hours and then stained with anti EDNRA and EDNRB antibody. Immunocytochemistry confirmed increased EDNRB expression in human fibroblasts in response to mechanical stretch. Scale bar represents 20 μm.

FPCL contraction, thereby demonstrating the presence of factors secreted from keratinocytes in response to mechanical stretching that influence the function of fibroblasts.

ET-1 is a 21 amino acid peptide that has a wide range of biological functions. ET-1 enhances tissue remodeling and causes fibrogenesis in a number of tissue types.[14,25,26] In the skin wound healing process, ET-1 is also thought to play an important role.[16,27] Kurita et al had reported that human keratinocytes increased the secretion of ET-1 in response to cyclic stretching and ET-1 expression increase with increments of stretch length and frequency.[17] ET-1 is known to bind two receptor subtypes: EDNRA and EDNRB. Endothelin receptors are expressed almost ubiquitously in mammals. Distribution and expression of each endothelin receptor depends on the organ, tissue, developmental stages, and physiologic

A

NFATc4 in TRPC3 overexpressing fibroblasts

150 kD →

	Control				TRPC3			
Stretch	−	−	+	+	−	−	+	+
Pvr3 (5 µm)	−	+	−	+	−	+	−	+
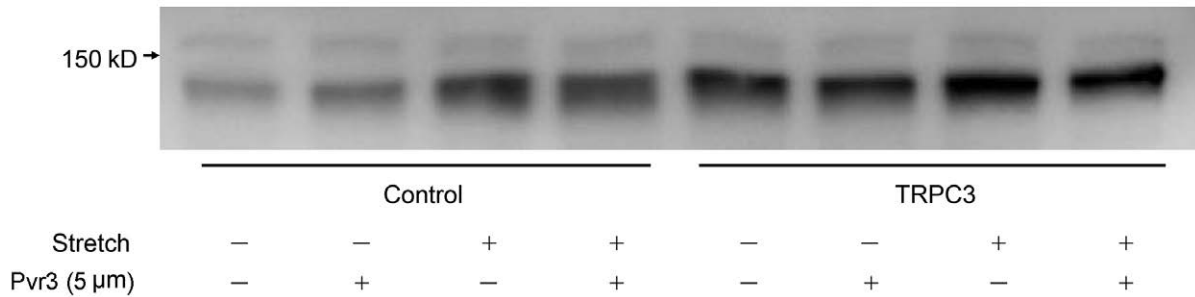
B

NFATc4 in human primary fibroblasts

150 kD →
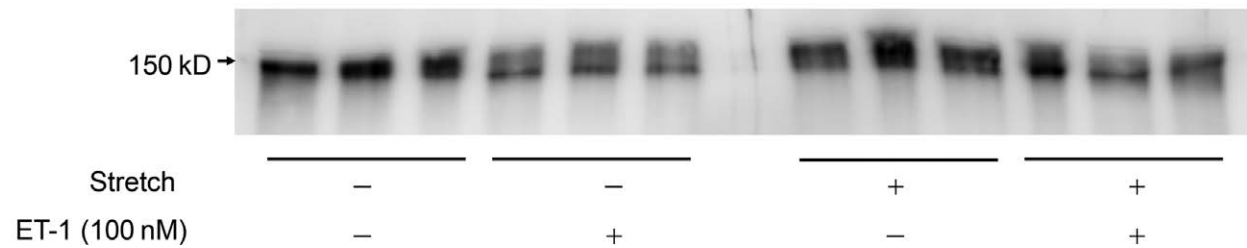
Stretch	−	−	+	+
ET-1 (100 nM)	−	+	−	+

Fig. 4. Stretching force activates NFATc4 in TRPC3 overexpressing fibroblasts, and NFATc4 in stretched human primary fibroblast is activated with ET-1. To reveal whether TRPC3 transmits the mechanical force to cell signaling and to investigate the reactivity of stretched fibroblasts to ET-1, transcriptional factor NFAT was assessed. A, TRPC3 overexpressing fibroblasts and control fibroblasts were stretched for 15 minutes, and the activity of NFAT was assessed with Western blot analysis. The activated form of NFAT (140 kDa) was increased in stretched TRPC3 overexpressing fibroblasts compared with the controls. The increase was attenuated with TRPC3 antagonist; Pyr3. B, Human primary fibroblasts were subject to 24 hour stretch, which presumably increases both TRPC3 and EDNRB expression, followed by the incubation with ET-1. The Western blot analysis demonstrated that NFATc4 was more activated in the stretched fibroblasts in response to ET-1 treatment than controls.

situation.[28] For example, Akashi et al reported that ET-1 EDNRB signaling is involved in skin sclerosis and causes dermal fibroblasts to synthesize collagen.[29] In the present study, immunohistochemical and qRT-PCR analyses demonstrated that cyclic stretching enhanced the expression of EDNRB in fibroblasts (Fig. 4). Immunohistochemical analysis of hypertrophic scar tissue also revealed that the distribution of EDNRB was increased relative to EDNRA (Fig. 3). These findings suggest that mechanical stretching, both in vitro and in vivo, cause fibroblasts to express more EDNRB.

EDNRB is a G-protein-coupled receptor. In general, once ET-1 binds to the receptors, the G-protein subunit dissociates and activates phospholipase C, leading to the cleavage of phosphatidylinositol-4,5-bisphosphate to form inositol-1,4,5-triphosphate and diacylglycerol. The binding of inositol-1,4,5-triphosphate to its receptors on the endoplasmic reticulum cause immediate fast Ca^{2+} release from the endoplasmic reticulum, followed by a store-operated Ca^{2+} entry through store-operated Ca^{2+} channels. On the other hand, diacylglycerol activates receptor-operated Ca^{2+} channels that enhance receptor-operated Ca^{2+} Entry.[11]

TRPCs are potential candidates for store-operated Ca^{2+} channels and receptor-operated Ca^{2+} channels, and the association of TRPC and endothelin receptors has been studied.[7,11,20,21] It is well established that TRPC-mediated calcium influx directly activates calcium-sensitive phosphatase calcineurin, which subsequently activates transcriptional factor NFAT.[7,30] NFAT has been reported to be a profibrotic transcription factor in many tissues.[12,31–33] In this study, we found that NFATc4 expression increased in TRPC3 overexpressing fibroblasts exposed to stretching (Fig. 4A). In addition, stretched human primary fibroblasts showed more NFATc4 activation with ET-1 than unstretched fibroblasts (Fig. 4B). Furthermore, treating a FPCL made of TRPC3 overexpressing fibroblasts with ET-1 resulted in more contraction compared with control FPCLs, and the contraction effect was attenuated by the addition of the endothelin receptor antagonist PD142893 and the TRPC3 antagonist Pyr3 (Fig. 2). These results, taken together, strongly suggest that mechanical stretching of fibroblasts at sites of injury not only enhance the expression of TRPC3 and EDNRB, but also activate these cells.
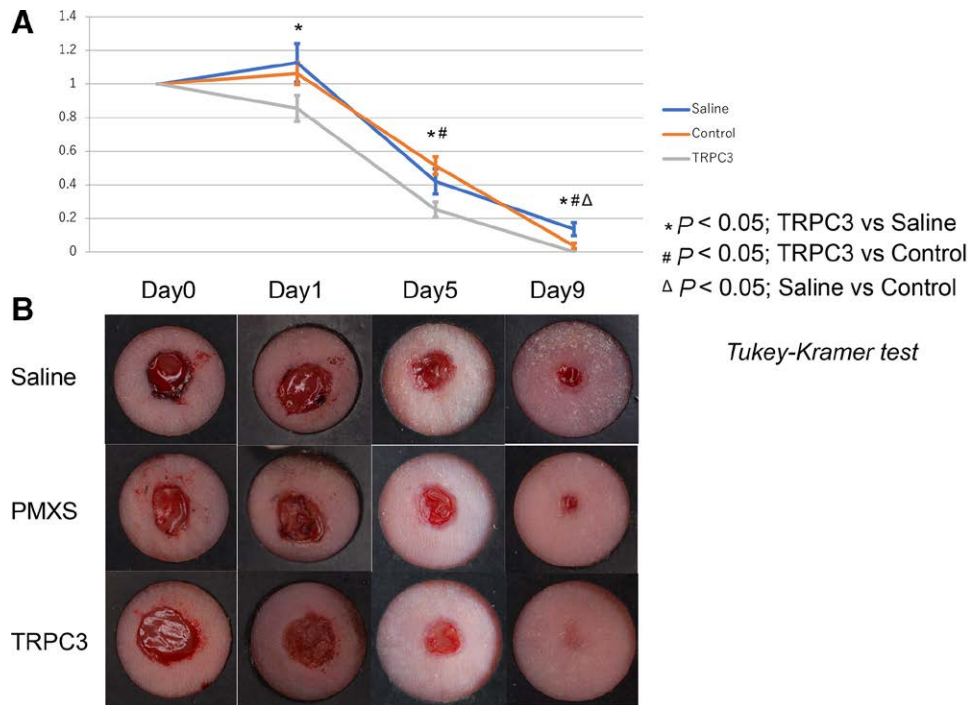
Fig. 5. The transplantation of TRPC3 overexpressing fibroblasts in mice enhanced the wound contraction. To assess the effect of TRPC3 on wound healing in vivo, TRPC3 overexpressing fibroblasts, empty-vector transfected control fibroblasts or the same amount of saline were transplanted in the dorsum of nude mice. Ten days posttransplantation, 6-mm wounds were created, and the size of wound was measured at the indicated time points. A, Quantification of the wound area from days 0-9. The data are expressed as the mean ± SD (n = 6 per group). *$P < 0.05$ TRPC3 vs Saline. #$P < 0.05$ TRPC3 versus control. △$P < 0.05$ control vs Saline by Tukey-Kramer test. B, Representative photographs of mouse cutaneous wounds. Differential healing was observed at all time points checked (n = 6 for each time point).

As mentioned above, TRPC3 is a known mechano-stress transducer. Our results showed that mice transplanted with TRPC3 overexpressing cells exhibited accelerated skin wound contraction compared with controls. These findings suggest that TRPC3 overexpressing fibroblasts, engrafted at the subdermal layer, may have played a role in exerting contractile activity during the wound healing process, providing further evidence for TRPC3's function as a mechano-stress transducer.

Taken together, these results suggest that when fibroblasts in dermal wounds are stretched, TRPC3 channels are not only highly expressed but also activated, and signaling mediated by TRPC3 might be amplified by the stretching forces. ET-1 derived from stretched keratinocytes may also enhance TRPC3 signaling in the fibroblast, which may result in more contraction at the wound due to differentiation. The results presented herein may partly explain the mechanism by which severe scar contracture occurs at areas of the body exposed to repetitive stretching forces (Fig. 6A, B).

Kenichiro Kawai, PhD
Department of Plastic Surgery
Hyogo Medical University
Nishinomiya, Hyogo
Japan 663-8501
E-mail: k-kawai@hyo-med.ac.jp

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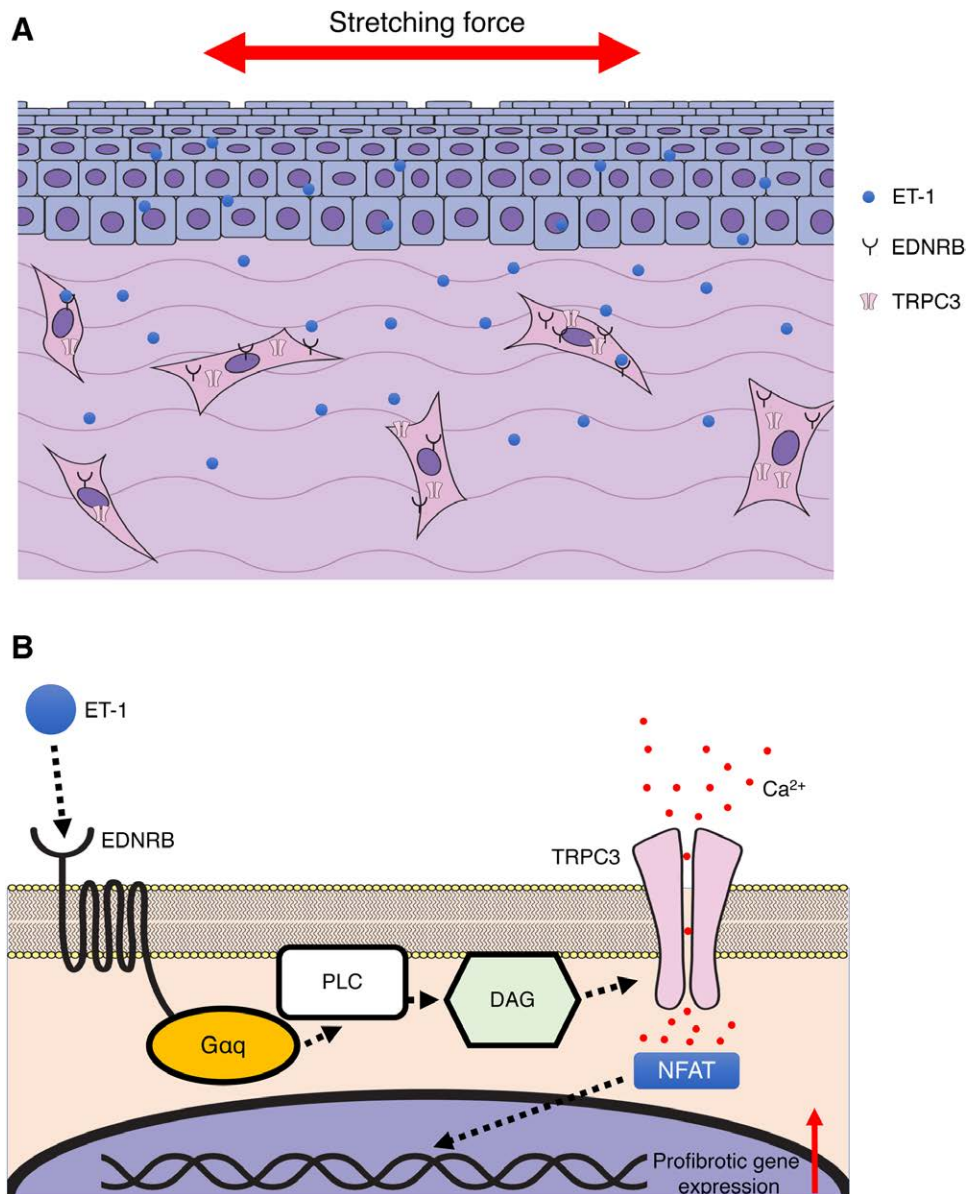
Fig. 6. Proposed model of the role of EDNRB and TRPC3 in hypertrophic scar contracture formation in vivo. A, Keratinocytes secrete more ET-1 in response to the stretching force. On the other hand, repetitive stretching stimuli upregulate the expression of EDNRB and TRPC3 in fibroblasts. B, Increased EDNRB in fibroblasts develops more sensitivity to endothelin. Endothelin signal is transmitted to TRPC3 via Gαq-PLC-DAG, which in turn increases calcium influx and NFATc4 activation. Activated NFAT translocate into nucleus and promote profibrotic gene expression, which may ultimately enhance wound contraction or scar contracture.

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