



# Knockdown of CPEB1 and CPEB4 Inhibits Scar Formation via Modulation of TAK1 and SMAD Signaling

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**Background:** Cytoplasmic polyadenylation element binding (CPEB) proteins are sequence-specific RNA-binding proteins that control translation via cytoplasmic polyadenylation. We previously reported that CPEB1 or CPEB4 knockdown suppresses TAK1 and SMAD signaling in an in vitro study.

**Objective:** This study aimed to investigate whether suppression of CPEB1 or CPEB4 expression inhibits scar formation in a mice model of acute dermal wound healing.

**Methods:** CPEB1 and CPEB4 expression levels were suppressed by siRNA treatment. Skin wounds were created by pressure-induced ulcers in mice. Images of the wound healing were obtained using a digital camera and contraction was measured by ImageJ. mRNA and protein expression was analyzed using quantitative real time polymerase chain reaction and western blotting, respectively.

**Results:** Wound contraction was significantly decreased by pre-treatment with CPEB1 or CPEB4 siRNA compared to the control. Suppression of CPEB1 or CPEB4 expression decreased TAK1 signaling by reducing the levels of TLR4 and TNF- $\alpha$ , phosphorylated TAK1, p38, ERK, JNK, and NF- $\kappa$ B-p65. Decreased levels of phosphorylated SMAD2 and SMAD3 indicated a reduction in SMAD signaling as well. Consequently, the expression of  $\alpha$ -SMA, fibronectin, and type I collagen decreased.

**Conclusion:** CPEB1 siRNA or CPEB4 siRNA inhibit scar formation by modulating the TAK1 and SMAD signaling pathways. Our study highlights CPEB1 and CPEB4 as potential therapeutic targets for the treatment of scar formation.

**Keywords:** Cicatrix, CPEB1, CPEB4, MAP3K7 signaling, SMAD signaling, Wound healing

## INTRODUCTION

Wound healing is a complex, well-orchestrated, multicellular process usually characterized by three phases: inflammation, proliferation, and remodeling<sup>1</sup>. These processes are involved in the communication and interplay between several cell types, including keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets<sup>1</sup>. Moreover, multiple signaling pathways participate in the healing process and are regulated by numer-

ous growth factors, cytokines and chemokines<sup>2</sup>. Deregulation of any of these steps can ultimately delay healing or lead to excessive scar formation such as hypertrophic scars (HTS)<sup>1</sup>.

Upon skin injury, when toll-like receptors (TLRs) are activated by danger signals, a series of intracellular signaling pathways involving the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) and mitogen-activated protein kinases (MAPKs), which induce the expression of many TLR target genes, are activated<sup>3</sup>. Transforming growth factor- $\beta$ 1 activated



kinase 1 (TAK1), also known as mitogen-activated protein kinase kinase kinase (MAP3K7), is a downstream molecule of the TLR signaling pathway. It is important for the activation of the transcription factor, NF- $\kappa$ B, and for that of p38 MAPK, c-Jun terminal kinase (JNK), and extracellular signal-regulated kinase 1/2 (ERK1/2) signaling<sup>3,4</sup>. Transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) is a potent inducer of fibroblast proliferation and/or differentiation into myofibroblasts that produce extracellular matrix (ECM), composed of collagen, fibronectin, connective tissue growth factor, etc., to form the clot in the wound<sup>5</sup>. However, excessive TGF- $\beta$ 1 produced from an unbalanced healing process leads to over-scarring, called as HTS, a serious burn sequela in clinical practice<sup>5</sup>. TGF- $\beta$ 1 acts through SMAD-dependent pathways, including phosphorylation and activation of SMAD 2 and SMAD 3 by TGF- $\beta$  receptor 1 (TGFR1)<sup>6</sup>. However, TGF- $\beta$ 1 can also activate SMAD-independent pathways to modify cellular functions, including the TAK1, phosphatidylinositol 3-kinase/AKT, and Rho-like GTPase signaling pathways<sup>6</sup>.

The mammalian cytoplasmic polyadenylation element binding (CPEB) protein is a sequence-specific RNA-binding protein that controls poly(A) tail length and polyadenylation-induced translation of mRNAs. It is important for cell proliferation and differentiation, cellular senescence, neuronal synaptic plasticity, and immune response<sup>7</sup>. In vertebrates, CPEB-like proteins are composed of four paralogs (CPEB1~4), and many studies have elucidated the biological functions of CPEB1 and CPEB4 in health and disease<sup>7,8</sup>.

Our previous work showed that CPEB1 or CPEB4 knockdown inhibits TAK1 and SMAD signaling in lipopolysaccharide (LPS)-induced human macrophages and TGF- $\beta$ 1-induced fibroblasts that are derived from human skin, respectively<sup>9</sup>. Therefore, in the present study, we investigated the effects of suppression of CPEB1 or CPEB4 expression on wound healing in mice models of pressure ulcers. Our findings indicate that suppression of CPEB1 or CPEB4 expression inhibited TAK1 and SMAD signaling led to decreased scar formation. These results, combined with previous *in vitro* studies, indicate that CPEB1 and CPEB4 could be potential targets for the treatment of dermal fibrotic scarring.

## MATERIALS AND METHODS

### siRNA transfection and pressure ulcer mouse model

SMARTpool siRNAs for the negative control, and knockdown of CPEB1 and CPEB4, were purchased from Dharmacon (La-

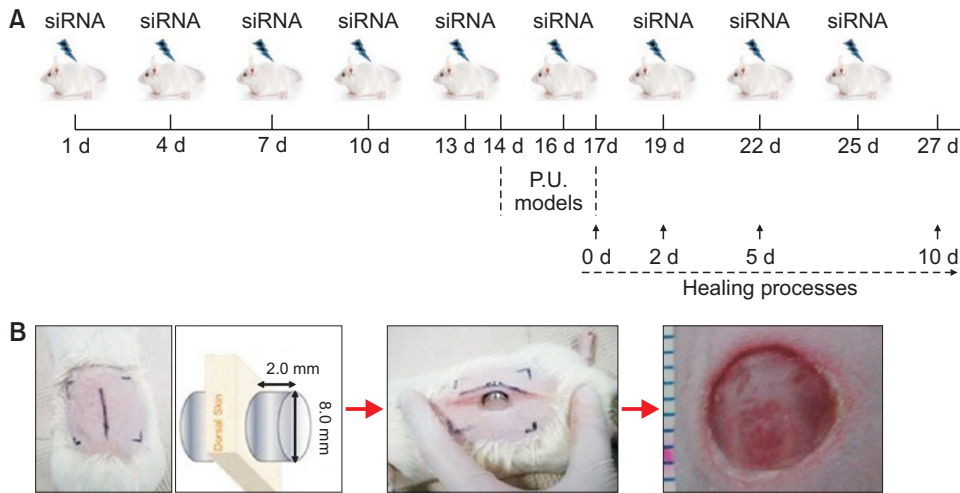
fayette). ICR mice (8-week-old) were purchased from Korean Animal Technology (Koatech). All animal experiments were conducted in the animal laboratory of Hangang Sacred Heart Hospital, Hallym University, in accordance with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal protocols were approved by the Animal Research Ethics Board of Hallym University (HMC2017-2-1121-30). Mice were randomly classified into four groups: (1) without any intervention (NC), (2) treated with the negative control siRNA (si-Ct)/atelocollagen mixture, (3) treated with CPEB1 siRNA (si-CP1)/atelocollagen mixture, and (4) treated with CPEB4 siRNA (si-CP4)/atelocollagen mixture. Each siRNA was mixed with atelocollagen, according to the manufacturer's instructions (Koken)<sup>10</sup>. Mice were injected with 100  $\mu$ l of atelocollagen containing 3  $\mu$ g<sup>11</sup> of CPEB1 or CPEB4 siRNA, or negative control siRNA intravenously (i.v.) via the tail vein, once every three days for nine consecutive times (Fig. 1A). After the fifth injection (day 14), mice were anesthetized and maintained using 100% oxygen with 2.5% isoflurane (Hana Pharm). The dorsal skins of mice were shaved and sterilized with 70% alcohol. Then it was gently pulled up and loaded with two circular ceramic magnetic plates (1.2 g, 1500.0 Gauss, 8.0 mm diameter, and 2.0 mm thickness) as Fig. 1B, to creating a 5.0 mm of skin thick bridge between the two magnets. The magnets were placed for 12 hours, and the removal time was 12 hours as a single ischemia-reperfusion (I/R) cycle. The mice were provided food and water *ad libitum*. After three cycles, a complete pressure ulcer (P. U.) was created<sup>12</sup>. Wounds were allowed to be exposed to air, and dry naturally forming a scab.

### Measurement of wound contraction

To analyze wound contraction, images were captured using a digital camera (Nikon) on days 0, 2, 5, and 10 post-wounding. Quantification of wound closure was carried out by Image J software (National Institutes of Health) and normalized with the wound size at day 0 being taken as 100% (<https://imagej.nih.gov/ij>).

### Quantitative polymerase chain reaction (qPCR)

Wound tissues were obtained 2, 5, and 10 days after wound formation. Total RNA was extracted, concentration was measured and RNA was reverse-transcribed to cDNA, for qPCR as previously described<sup>9</sup>. The following primers were used: CPEB1, 5'-TTTCAAGCCTTCG CATTTC3'-3' (forward)



**Fig. 1.** Design of siRNA-treatment and wound model in mice. (A) Flowchart of atelocollagen-mediated siRNA transfer procedure, modeling of P.U. wound, and tissue preparation times. siRNA/atelocollagen mixture was injected via the tail vein once in three days, nine times in total. On day 14, P.U. was modelled in mice using circular magnets on the dorsal skin for 3 days. Wound tissues were collected on 0, 2, 5, and 10 days after wound creation. (B) Illustration showing the application of magnets for wound creation in mice. P.U.: pressure ulcer.

and 5'-GGACCCAACGCCCATCTTTA-3' (reverse); CPEB4, 5'-CCTTCTTCCTCCACTATA-3' (forward) and 5'-GAGAG-CACCATTAT TAGC-3'. The relative fold mRNA expression of each target gene was evaluated by the 2- $\Delta\Delta$ Ct method, using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the normalization control<sup>13</sup>.

### Western blot

Wound tissue samples were obtained 2 and 5 days after wounding. Total protein sample preparation, electrophoresis, development, and imaging analyses were performed as previously described<sup>9</sup>. The primary antibodies used were rabbit polyclonal anti-CPEB1 and anti-CPEB4 (Thermo Fisher Scientific); rabbit polyclonal anti-TLR4 (Cusabio Biotech); rabbit polyclonal anti-phospho-TAK1, anti-TAK1, anti-p38, anti-phospho-JNK, anti-JNK, anti-phospho-ERK, anti-phospho-NF- $\kappa$ B-p65, anti- $\beta$ -actin, and anti-phospho-SMAD2; rabbit polyclonal anti-phospho-SMAD3 and anti-SMAD3 (ST John's laboratory); rabbit polyclonal anti-SMAD2, mouse polyclonal anti-phospho-p38, and anti-ERK (Cell Signaling Technology); rabbit polyclonal anti-NF- $\kappa$ B-p65, anti-type I collagen, mouse polyclonal anti- $\alpha$ -SMA, and monoclonal anti-fibronectin (Abcam); and mouse monoclonal anti-phospho-I $\kappa$ B $\alpha$ , anti-I $\kappa$ B $\alpha$ , and anti-GAPDH (Santa Cruz Biotechnology). The secondary antibodies used were peroxidase-conjugated anti-mouse IgG and anti-rabbit IgG (Merck Millipore).

### Statistical analysis

All results are presented as the mean  $\pm$  standard error of the mean (SEM). The Mann-Whitney U test was used for

comparisons between two groups. Statistical analyses were performed using PASW Statistics 24 (IBM Corp.), and significance was set to  $p < 0.05$ .

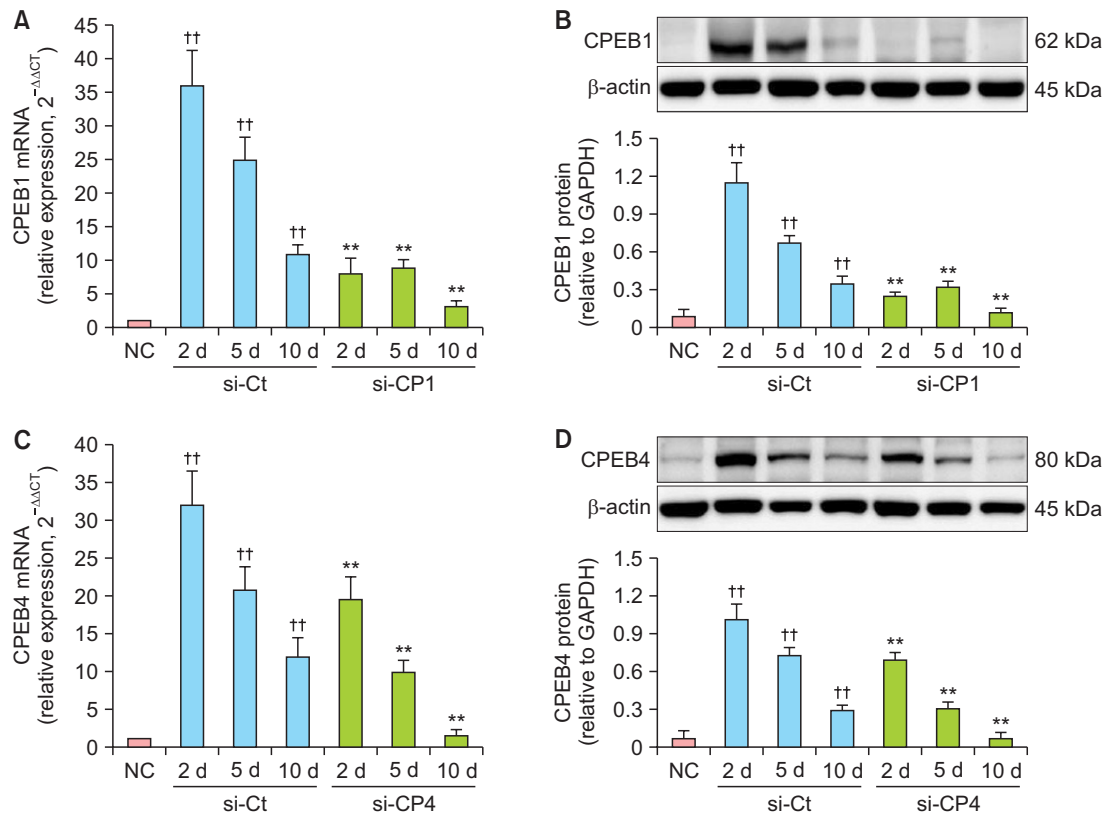
## RESULTS

### Atelocollagen-mediated siRNA transfer suppression of CPEB1 or CPEB4 expression in wound tissues of mice

To confirm efficiency of atelocollagen-mediated siRNA transfer via tail vein on mouse, we examined mRNA and protein expression of CPEB1 and CPEB4 by qRT-PCR and western blotting respectively, in wound tissues obtained on day 2 (inflammation phase), 5 (proliferation phase), and 10 (remodeling phase) after wound creation. The mRNA and protein expression of CPEB1 or CPEB4 was significantly higher in the wound tissue of control mice (si-Ct) than in the normal skin tissue (NC) in each healing phase ( $p < 0.01$ ) (Fig. 2). However, treatment with si-CP1 or si-CP4 significantly suppressed the mRNA and protein expression of CPEB1 or CPEB4 in wound tissues compared to si-Ct corresponding to each healing phase ( $p < 0.01$ ) (Fig. 2).

### Suppression of CPEB1 and CPEB4 decreased wound contraction

Wound contraction was not significantly different on day 2 for the comparison of si-Ct with si-CP1 and si-Ct with si-CP4 groups (Fig. 3). Moreover, on days 5 and 10, contraction was remarkably decreased in the si-CP1 and si-CP4 groups compared to that in the si-Ct group ( $p < 0.05$ ) (Fig. 3). These results suggested that the rate of wound healing was delayed by treatment with si-CP1 or si-CP4.



**Fig. 2.** Treatment with si-CP1 or si-CP4 decreased mRNA and protein expression of CPEB1 or CPEB4 in wound tissues of mice. RT-PCR and western blotting analysis for the expression of CPEB1 or CPEB4 on day 2, 5, and 10 in wound tissues undergoing the healing process. (A) RT-PCR result showing si-CP1 decreased the increased mRNA expression of CPEB1 in wound tissues compared to si-Ct corresponding to each healing phase. (B) Western blot result showing si-CP1 decreased the increased protein expression of CPEB1 in wound tissues compared to si-Ct corresponding to each healing phase. (C) RT-PCR result showing si-CP4 decreased the increased mRNA expression of CPEB4 in wound tissues compared to si-Ct corresponding to each healing phase. (D) Western blot result showing si-CP4 decreased the increased protein expression of CPEB4 in wound tissues compared to si-Ct corresponding to each healing phase. Data are expressed as mean  $\pm$  standard error ( $n=10$ ). RT-PCR: quantitative real-time PCR, NC: normal control, si-Ct: negative control siRNA, si-CP1: CPEB1 siRNA, si-CP4: CPEB4 siRNA. <sup>††</sup> $p<0.01$  for comparison with NC, and <sup>\*\*</sup> $p<0.01$  for comparison with si-Ct.

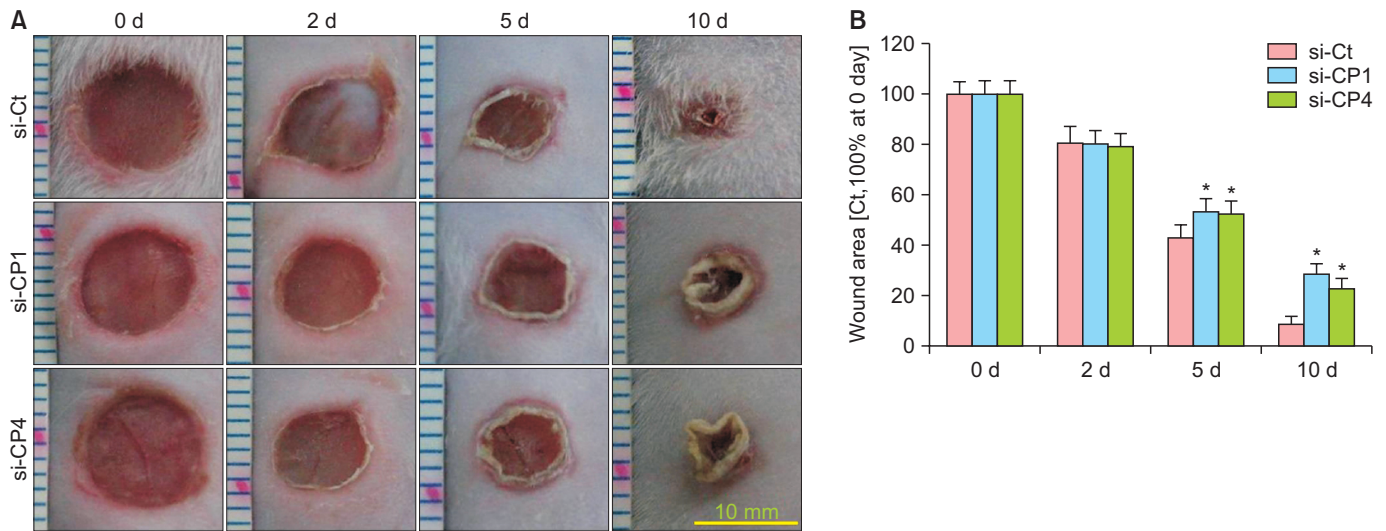
### Suppression of CPEB1 and CPEB4 decreased inflammation related-TAK1 signaling

To explore the functional mechanism of CPEB1 and CPEB4 in inflammation, we measured changes in TLR4-TAK1 signaling on day 2 after the wound creation. TLR4 and TNF- $\alpha$  protein expression levels were significantly decreased in the si-CP1 and si-CP4 groups compared to the si-Ct group ( $p<0.05$ ) (Fig. 4A, B). The phosphorylation levels of TAK1 and p38 were decreased in the si-CP1 and si-CP4 groups compared to those in the si-Ct group ( $p<0.05$ ) (Fig. 4C, D). Although phosphorylation of the JNK isoform at 54 kDa was decreased ( $p<0.05$ ), the levels of the JNK isoform at 46 kDa unaltered in the si-CP4 group (Fig. 4E). Moreover, phosphorylation of isoforms 54 and 46 kDa of JNK was not affected in the si-CP1 group (Fig. 4E). Phosphorylation of ERK was significantly decreased in the si-

CP4 group ( $p<0.05$ ) but was unchanged in the si-CP1 group (Fig. 4F). Phosphorylation of both IKB $\alpha$  and NF- $\kappa$ B-p65 was decreased in the si-CP1 and si-CP4 groups compared to the si-Ct group ( $p<0.05$ ) (Fig. 4G, H). These results indicated that si-CP1 and si-CP4 decreased the inflammatory response of wound healing by suppressing TLR-TAK1 signaling.

### Suppression of CPEB1 and CPEB4 decreased SMAD and TAK1 signaling

We determined the changes in SMAD signaling and the expression of its related scarring markers in tissues obtained on day 5 and 10 after wound creation. The phosphorylation of SMAD2 and SMAD3 was significantly decreased in the si-CP1 and si-CP4 groups compared to that in the si-Ct group ( $p<0.05$ ) (Fig. 5A, B; Supplementary Fig. 1A, B). Moreover, the



**Fig. 3.** Treatment with si-CP1 or si-CP4 decreased wound contraction in mice. (A) Representative images of wound contraction on day 2, 5, and 10 undergoing the healing process. Scale bar=10 mm. (B) Quantification of wound contraction. Wound contraction was expressed as a percentage relative to the initial wound surface area, which was considered to be 100%. Data are expressed as mean  $\pm$  standard error (n=10). si-Ct: negative control siRNA, si-CP1: CPEB1 siRNA, si-CP4: CPEB4 siRNA. \* $p$ <0.05 for the comparison with si-Ct.

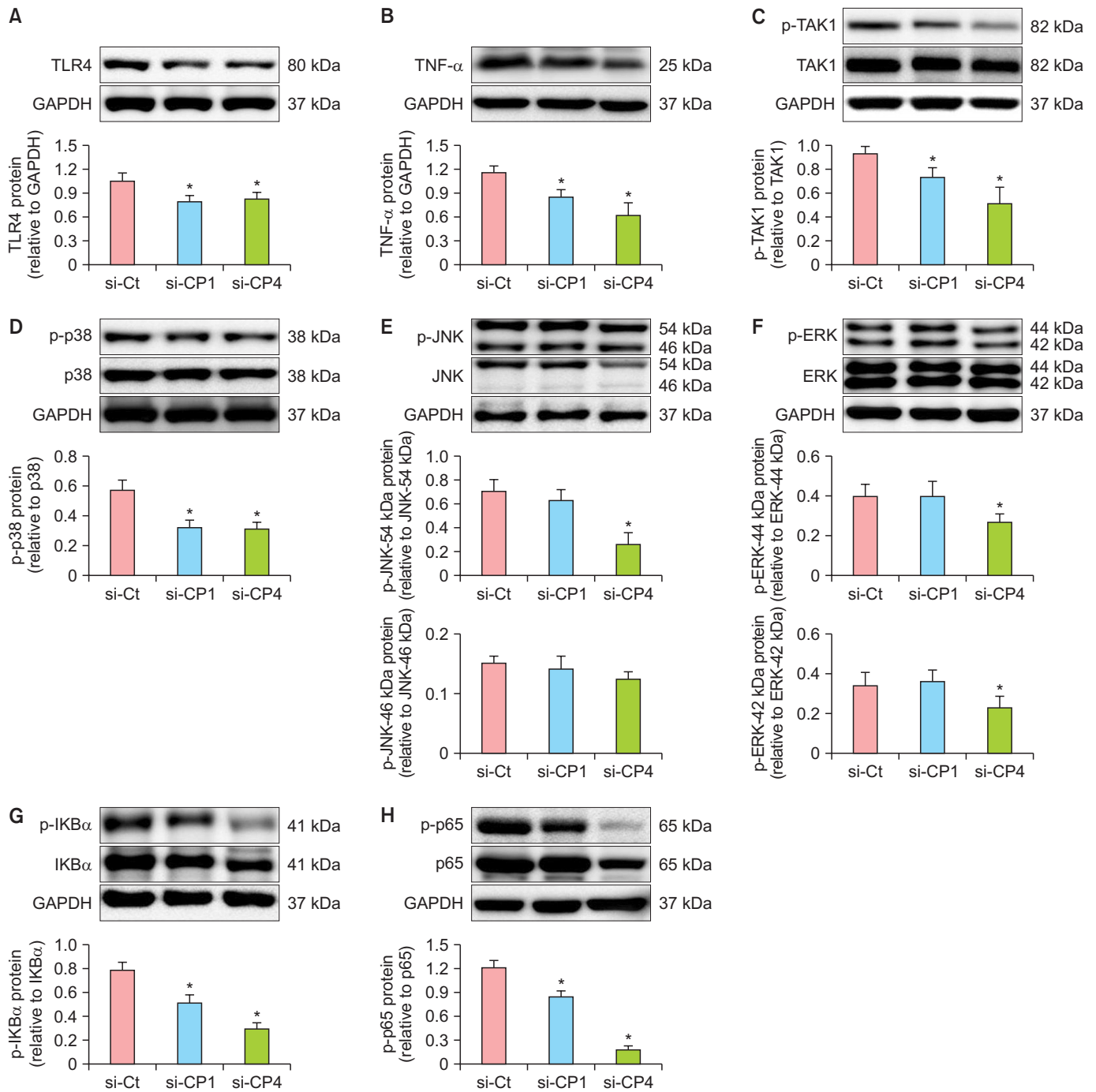
expression of the myofibroblast marker,  $\alpha$ -SMA, was significantly decreased in the si-CP1 and si-CP4 groups compared to that in the si-Ct group ( $p$ <0.05) (Fig. 5C; Supplementary Fig. 1C). Furthermore, the levels of fibronectin and collagen-I, both novel marker of scars, were dramatically decreased in the si-CP1 and si-CP4 groups on day 5 ( $p$ <0.05) (Fig. 5D, E). In addition, the phosphorylation TAK1 was significantly decreased in the si-CP1 and si-CP4 groups compared to that in the si-Ct group on day 10 ( $p$ <0.05) (Supplementary Fig. 1D). The pro-inflammatory cytokines, TNF- $\alpha$  and IL-6 were decreased in the si-CP1 and si-CP4 groups on day 10 ( $p$ <0.05) (Supplementary Fig. 1E, F). These results suggest that si-CP1 or si-CP4 decreased the scarring response of wound healing by suppressing SMAD and TAK1 signaling.

## DISCUSSION

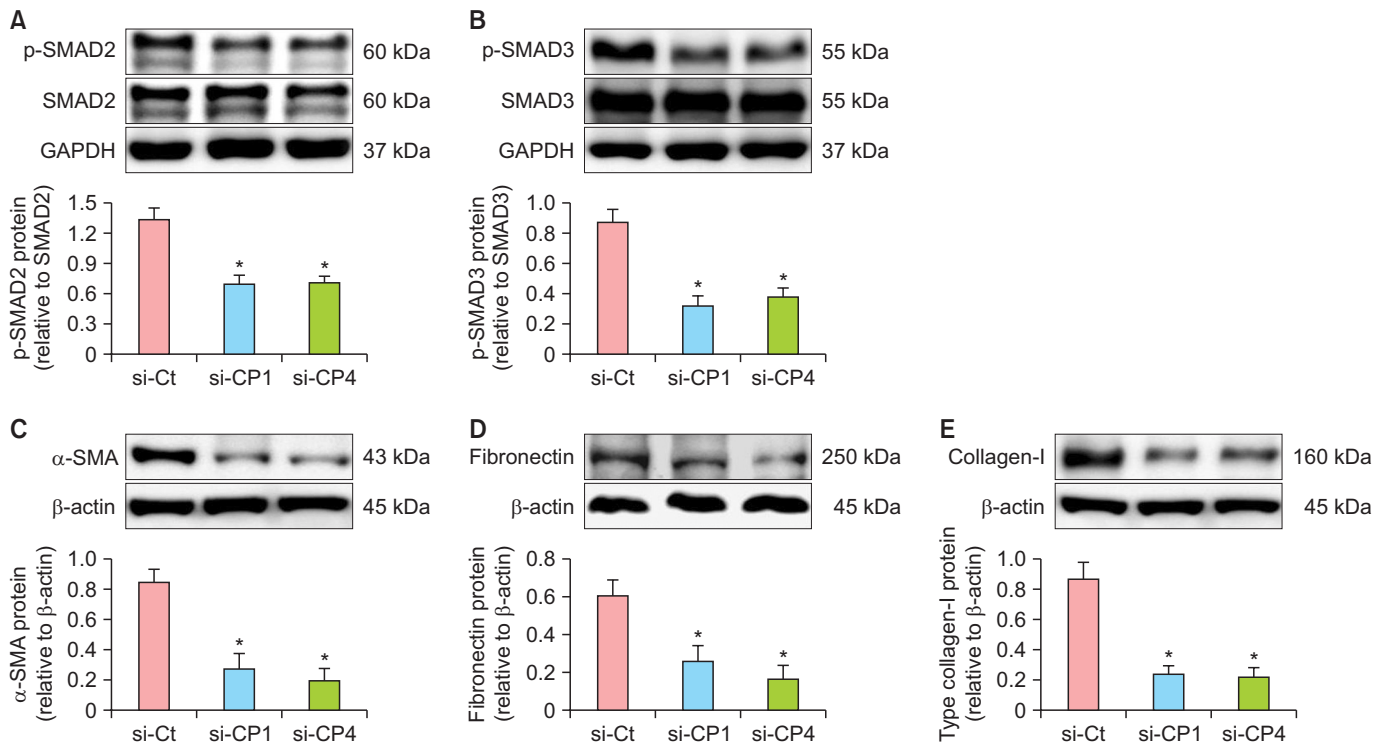
In this study, we found that CPEB1 and CPEB4 were significantly increased in the wound tissues of mice compared to normal tissues suggesting that CPEB1 and CPEB4 are involved in wound healing. However, RNAi-mediated suppression of CPEB1 or CPEB4 expression decreased wound contraction during TLR4-TAK1 signaling in inflammatory phases and SMAD signaling of proliferative phases during wound healing. These results indicate that CPEB1 and CPEB4 play impor-

tant roles in scar formation during wound healing.

In our previous study, we reported that levels of both CPEB1 and CPEB4 were increased in LPS-treated macrophages; however, knockdown of their expression suppressed TAK1 signaling by reducing the phosphorylation levels of TAK1, p38, ERK, JNK, I $\kappa$ B $\alpha$ , as well as NF- $\kappa$ B-p65 in LPS-treated macrophages<sup>9</sup>. Accumulating evidence supports the pathological roles of TAK1 signaling in the inflammatory response, which is becoming a potential therapeutic target for inflammatory disorders<sup>6</sup>. Suppression or deletion of TAK1 prevents neuronal death in cerebral ischemia or inhibits renal and pulmonary inflammation<sup>14-16</sup>. Inflammatory response is the first essential stage and plays an important role in the normal wound healing process. An acute inflammatory reaction generates an antimicrobial host defense, resulting in the elimination of infectious pathogens and wound debris, followed by resolution, which is mediated mainly by tissue-resident and recruited macrophages, both originating from embryonic and circulating monocytes, respectively<sup>4</sup>. M1 macrophages accumulate and are activated in the inflammatory phase of wound healing and produce pro-inflammatory cytokines, including IL-1, IL-6, and TNF- $\alpha$ <sup>7</sup>. These pro-inflammatory cytokines have a positive therapeutic effect on wound healing, particularly TNF- $\alpha$ , which exerts a growth-promoting activity on fibroblasts and enhances collagen deposition<sup>17</sup>. Clinical research



**Fig. 4.** Treatment with si-CP1 or si-CP4 decreased inflammatory signaling in the mouse model of P.U. Protein expression was measured by western blot analysis on day 2 after wound creation. (A) CPEB1 or CPEB4 siRNA decreased protein expression of TLR4 in wound tissues compared to si-Ct. (B) CPEB1 or CPEB4 siRNA decreased protein expression of TNF- $\alpha$  in wound tissues compared to si-Ct. (C) CPEB1 or CPEB4 siRNA decreased phosphorylation of TAK1 in wound tissues compared to si-Ct. (D) CPEB1 or CPEB4 siRNA decreased phosphorylation of p38 in wound tissues compared to si-Ct. (E) si-CP1 not changed phosphorylation of JNK in wound tissues compared to si-Ct. si-CP4 decreased phosphorylation of JNK isoform at 54 kDa, and not changed phosphorylation of isoforms at 46 kDa of JNK in wound tissues compared to si-Ct. (F) si-CP1 not changed phosphorylation of ERK in wound tissues compared to si-Ct. si-CP4 decreased phosphorylation of ERK in wound tissues compared to si-Ct. (G) CPEB1 or CPEB4 siRNA decreased phosphorylation of IKB $\alpha$  in wound tissues compared to si-Ct. (H) CPEB1 or CPEB4 siRNA decreased phosphorylation of NF- $\kappa$ B-p65 in wound tissues compared to si-Ct. Data are expressed as mean  $\pm$  standard error (n=10). si-Ct: negative control siRNA, si-CP1: CPEB1 siRNA, si-CP4: CPEB4 siRNA, P.U.: pressure ulcer. \* $p$ <0.05 for the comparison with si-Ct.



**Fig. 5.** Treatment with si-CP1 or si-CP4 decreased SMAD signaling and expression of scar markers in the mouse model of P.U. Protein expression was measured by western blot analysis on day 5 after wound creation. (A) CPEB1 or CPEB4 siRNA decreased phosphorylation of SMAD2 in wound tissues compared to si-Ct. (B) CPEB1 or CPEB4 siRNA decreased phosphorylation of SMAD3 in wound tissues compared to si-Ct. (C) CPEB1 or CPEB4 siRNA decreased protein expression of  $\alpha$ -SMA in wound tissues compared to si-Ct. (D) CPEB1 or CPEB4 siRNA decreased protein expression of fibronectin in wound tissues compared to si-Ct. (E) CPEB1 or CPEB4 siRNA decreased protein expression of collagen-I in wound tissues compared to si-Ct. Data are expressed as mean  $\pm$  standard error ( $n=10$ ). si-Ct: negative control siRNA, si-CP1: CPEB1 siRNA, si-CP4: CPEB4 siRNA, P.U.: pressure ulcer. \* $p < 0.05$  for comparison with si-Ct.

has indicated that prolonged inflammation is a risk factor for the pathological development of HTS; therefore, they are defined as weakly inflamed pathological scars<sup>18</sup>. It has been observed that most of the intervention modalities for the clinical treatment of HTS are accompanied by reducing inflammation<sup>18</sup>.

The wound healing proliferative phase consists of granulation tissue formation, angiogenesis, and re-epithelialization<sup>4,5</sup>. Granulation tissue formation is characterized by a high density of fibroblasts, macrophages, capillaries, and ECM<sup>4,5</sup>. Scarring is a fibrous tissue that is physiologically essential for dermal wound healing in adults. However, excessive fibrotic scarring often leads to HTS, which has a high incidence of up to 91% in burn patients with deep dermal injuries<sup>7</sup>. It is well accepted in HTS formation that M2 macrophage-produced TGF- $\beta$ 1 is a theoretical basis, which acts through SMAD-dependent or independent pathways and stimulates secretion and deposition of excessive ECM protein by fibroblasts (myofibroblasts),

that raises the normal skin surface area<sup>7</sup>. In addition, HTS-derived fibroblasts overexpress TGF- $\beta$ 1,  $\alpha$ -SMA, type I and III collagen, and fibronectin<sup>19</sup>. A previous in vitro study reported that protein expression of both CPEB1 and CPEB4 increased in TGF- $\beta$ 1-induced human dermal fibroblasts; knockdown of their expression by siRNA decreased phosphorylation levels of SMAD2 and inhibited the expression of  $\alpha$ -SMA, type I collagen, and fibronectin in fibroblasts treated with TGF- $\beta$ 1<sup>9</sup>.

In the present study, we observed that CPEB1 and CPEB4 were upregulated on day 2 (inflammatory stage) and day 5 (proliferative stage) in the process of wound healing. Our previous work indicated that mRNA levels of CPEB1 and CPEB4 in HTS tissues are 1.8 and 2.2 times higher than those in normal skin tissues, respectively<sup>9</sup>. Other studies have also reported that the levels of both CPEB1 and CPEB4 were increased in human cirrhotic liver tissues compared with those in control tissues<sup>20</sup>. These evidences indicate that both CPEB1 and CPEB4 are involved in tissue repair or fibrotic scarring.

CPEB1 depletion inhibits up-regulation of iNOS, NO, and ROS production that are induced by LPS stimulation<sup>21</sup>. CPEB1 activation induces angiogenesis by promoting the expression of vascular endothelial growth factor (VEGF); conversely, knockdown of CPEB1 downregulates angiogenic capacity<sup>20</sup>. A recent study showed that in an LPS-induced sepsis model, depletion of CPEB4 in mouse macrophages impaired resolution of inflammation<sup>22</sup>. However, CPEB4 is necessary for IL-22 production in CD4<sup>+</sup>T and innate lymphoid (ILC3) cells<sup>23</sup>. Moreover, CPEB4 promotes tissue regeneration in acute transient intestinal inflammation and is conducive to the development of colorectal cancer<sup>23</sup>. In addition, knockdown of all the subtypes of CPEB proteins (CPEB1, 2, 3, and 4) increases the phosphorylation levels of TAK1 and p38, activates NF- $\kappa$ B, and augments the production of IL-6 in mouse RAW 264.7 macrophages<sup>24</sup>. It is well known that CPEBs bind to CPEs located in the 3' untranslated region (UTR) of specific mRNAs and control their translation by favoring their poly(A) tail elongation. Furthermore, they modulate the formation of alternative 3'-UTRs, which contain AU-rich elements, and mediate mRNA stability of several pro-inflammatory or anti-inflammatory cytokines<sup>25,26</sup>. However, to date, the mechanism by which CPEB regulates TAK1 and SMAD signaling is unclear and is a task for future studies. In addition, the CPEB2 regulate hippocampus-dependent synaptic plasticity and long-term memory<sup>27</sup>, controls translation of hypoxia-inducible factor 1 $\alpha$  (hif-1 $\alpha$ ) mRNA in response to oxidative stress<sup>28</sup>, and suppress tumorigenesis in breast epithelial cells by inhibit the EMT, migration, invasion, and proliferation<sup>29</sup>. The CPEB3 also involved in synaptic plasticity and spatial memory<sup>27</sup>. Circular RNA, mmu-Cpeb3\_0007 was related to cardiac hypertrophy and cardiac fibrosis based on gene set enrichment analysis in mouse model of cardiac hypertrophy<sup>30</sup>. In addition, CPEB3 suppresses EMT by blocking the interaction between colorectal cancer cells and tumor-associated macrophages via decreasing the IL-6/STAT3 signaling<sup>31</sup>.

In present study, suppression of CPEB1 or CPEB4 expression by RNAi inhibited wound scar formation, which seems to delay the wound healing rather than inhibits HTS formation. In vitro study, we demonstrated that the CPEB1 or CPEB4 knockdown suppressed TGF- $\beta$ 1-induced fibrosis<sup>9</sup>. Moreover, mRNA expression of CPEB1 and CPEB4 are significantly higher in HTS of patients compared to normal skin, they matched with HTS<sup>9</sup>. The results of both in vitro and vivo

study suggest CPEB1 or CPEB4 is related to HTS formation. Wound healing of mice is mediated by the panniculus carnosus muscle, which, importantly, the skin of mice is structurally and functionally different with humans. Therefore, the wound of mice cannot form HTS<sup>32</sup>. Nevertheless, mice models can help to understand the skin diseases and predict treatment efficacy. In the future, it is worthwhile to evaluate the inhibition of CPEB1 or CPEB4 on the formation of HTS in HTS models using medium or large mammals.

In conclusion, this study reported that CPEB1 or CPEB4 were upregulated in the process of wound healing, and siRNA-mediated suppression of CPEB1 or CPEB4 inhibited inflammation and scar formation through TAK1 and SMAD signaling in a mice model of pressure-induced wounds (Supplementary Fig. 2). Our findings indicate that CPEB1 and CPEB4 are involved in the scarring response, providing new insights into the pathogenesis of dermal fibrosis.

## SUPPLEMENTARY MATERIALS

Supplementary data can be found via <http://anndermatol.org/src/sm/ad-22-210-s001.pdf>.

## CONFLICTS OF INTEREST

The authors have nothing to disclose.

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## DATA SHARING STATEMENT

Data supporting the results of this study are available from corresponding authors upon reasonable request.

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