Clinical & Experimental Immunology
The Journal of Translational Immunology

British Society for immunology

Clinical and Experimental Immunology ORIGINAL ARTICLE doi: 10.1111/cei.13373

Experimental atopic dermatitis is dependent on the TWEAK/Fn14 signaling pathway

Q. Liu,*[,1] H. Wang,*[,1] X. Wang,*[,1] M. Lu, X. Tan, L. Peng, F. Tan, T. Xiao, S. Xiao and Y. Xia

Department of Dermatology, The Second Affiliated Hospital, Xi'an Jiaotong University, Xi'an, China

Summary

Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) acts through its receptor fibroblast growth factor inducible 14 (Fn14), and participates in skin inflammation. Both TWEAK and Fn14 are highly expressed in skin lesions of patients with atopic dermatitis. The purpose of this study was to further explore the effect of Fn14 inhibition on experimental atopic dermatitis. Experimental atopic dermatitis was induced in the wild-type and Fn14 knock-out BALB/c mice. The effect of TWEAK/Fn14 interaction on keratinocytes was studied in an *in-vitro* model of atopic dermatitis. Fn14 deficiency ameliorates skin lesions in the mice model, accompanied by less infiltration of inflammatory cells and lower local levels of proinflammatory cytokines, including TWEAK, TNF-α and interleukin (IL)-17. Fn14 deficiency also attenuates the up-regulation of TNFR1 in skin lesions of atopic dermatitis. Moreover, topical TWEAK exacerbates skin lesion in the wild-type but not in the Fn14 knock-out mice. *In vitro*, TWEAK enhances the expressions of IL-17, IL-18 and IFN-γ in keratinocytes under atopic dermatitis-like inflammation. These results suggest that Fn14 deficiency protects mice from experimental atopic dermatitis, involving the attenuation of inflammatory responses and keratinocyte apoptosis. In the context of atopic dermatitis-like inflammation, TWEAK modulates keratinocytes via a TNFR1-mediated pathway.

Keywords: atopic dermatitis, fibroblast growth factor-inducible 14 (Fn14), keratinocyte, tumor necrosis factor receptor (TNFR), tumor necrosis factor-like weak inducer of apoptosis (TWEAK)

Accepted for publication 5 September 2019

Correspondence: Y. Xia, Department of Dermatology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710004, China.
E-mail: xiayumin1202@163.com

[1]These authors contributed equally to this work.

Introduction

Atopic dermatitis is a common, chronic inflammatory skin disease with a prevalence of up to 25% in children, ranging from 2·1 to 4·9% in adults across countries [1,2]. Although the specific pathogenesis remains unclear, cutaneous inflammation, which results from defective skin barrier and penetration of microbes and allergens, is known as a main contributor to the development of atopic dermatitis via a complex immune network [3].

Tumor necrosis factor (TNF)-related weak inducer of apoptosis (TWEAK) acts through binding to its sole receptor fibroblast growth factor inducible 14 (Fn14) and is associated with various biological activities, including inflammation. TWEAK is mainly synthesized by inflammatory cells such as macrophages and monocytes [4]. Fn14 can be expressed in epidermal keratinocytes, especially those under certain inflammatory conditions [5]. TWEAK/Fn14 interaction participates in various types of skin inflammation, including Henoch–Schönlein purpura [6], psoriasis [7], cutaneous lupus erythematosus [8] and bullous pemphigoid [9]. Recent studies showed that both TWEAK and Fn14 are highly expressed in the skin lesion of patients with eczema or atopic dermatitis [10–12]. Moreover, TWEAK deficiency limits the severity of atopic dermatitis in a murine model [12]. These findings strongly suggest that TWEAK/Fn14 activation participates in the development of atopic dermatitis.

Previous studies have shown that Fn14 is more strictly expressed in resident cells under inflammatory conditions, while TWEAK can be released by inflammatory

 © 2019 British Society for Immunology,

cells infiltrating affected tissue or activated in circulation [5,8]. Targeting Fn14 in resident cells has become an efficient approach to suppress exacerbated inflammatory responses in skin diseases, including cutaneous lupus erythematosus and psoriasis [8,13]. In patients with atopic dermatitis the serum levels of TWEAK are comparable with healthy controls [10,11], indicating that the TWEAK/Fn14 signals prefer local regulation of inflammatory responses in atopic dermatitis. Therefore, Fn14 inhibition might be more specific as a therapeutic strategy for atopic dermatitis.

Previous studies have demonstrated that TWEAK co-operates with other cytokines, such as TNF-α, interleukin (IL)-13 or IL-17, in the induction of keratinocyte apoptosis or the production of chemokine (C-C motif) ligand (CCL) 2, CCL5 and CCL17 [11,12], which is an important event in the processes of atopic dermatitis. Also, TNF-α, IL-13 and IL-17 are up-regulated in skin tissue under atopic inflammation [14]. Although the interplay between TWEAK and other cytokines has been implicated in the modulation of keratinocytes, the effect of TWEAK/Fn14 activation on these cells remains unclear in the context of atopic inflammation. The purpose of this study aimed to explore the effect of Fn14 deficiency on experimental atopic dermatitis as well as the effect of TWEAK on keratinocytes in the atopic inflammatory microenvironment.

Materials and methods

Patients

Skin tissue and sera were collected from patients with atopic dermatitis (age = 18–60 years) who had received neither systemic nor topical therapies within the recent 4 weeks. Normal skin tissue and sera were collected from healthy donors (age = 18–60 years). There were no statistical differences in gender or age between patients and normal controls ($P > 0{\cdot}05$). This study was approved by the University Research Ethics Committee and written informed consent were obtained from all subjects.

Murine model

Fn14 deficiency was introduced to BALB/c mice by using the clustered, regularly interspaced, short palindromic repeats (CRISPR)/CRISPR-associated (Cas) protein 9 method [13,15]. Mice were housed at the Medical Animal Center of Xi'an Jiaotong University. Male mice aged 8 weeks were used in this study. Atopic dermatitis-like lesion was evoked by a 2,4-dinitrofluorobenzene (DNFB) method, with some modification [16]. On day 0, 0·5% DNFB in acetone/olive oil (4 : 1, 50 µl) was applied to the shaved abdomen for sensitization. Then 0·2% DNFB solution (20 µl) was applied to the inner and outer surfaces of the ears on days 5, 8, 11 and 14. From the third week, the same DNFB solution was applied three times weekly to maintain inflammation. Some mice were treated with the same volumes of vehicle. In other experiments, the DNFB-treated mice received topical administration of bovine serum albumin (BSA) or TWEAK in normal saline (2·5 µg/ml, 20 µl), which was performed 10 min after each DNFB application from the third week. The skin lesion was assessed by measuring the modified scoring atopic dermatitis (SCORAD) index scores [17]. This protocol was approved by the University Research Ethics Committee.

Immunohistochemistry and histological evaluation

Some paraffin sections were processed for hematoxylin and eosin staining. The others were processed for immunohistochemistry, as described previously [18]. After deparaffinization and rehydration, paraffin sections were blocked with dual endogenous enzyme block (Dako, Glostrup, Denmark). Primary antibodies were rabbit anti-TWEAK [or Fn14, IL-18, regulated upon activation normal T cell expressed and secreted (RANTES), monocyte chemotactic protein (MCP)-1, interferon gamma-induced protein (IP)-10, TNF-α, TNF receptor (TNFR) 1 or TNFR2 IgG (2 µg/ml; Abcam, Cambridge, MA, USA]. Secondary antibody was polymer-horseradish peroxidase-labeled goat anti-rabbit IgG (Dako). Some sections were stained with isotype control antibodies (Abcam, Cambridge, MA). Finally, sections were incubated with 3,3-diaminobenzine-chromogen substrate (Dako) and counterstained with hematoxylin and eosin solution in order. The positively stained epidermis was quantified by ImageJ software (National Institutes of Health, Bethesda, MD, USA), according to a previous method [9,13].

Immunofluorescence

Immunofluorescent detection was performed on frozen sections. Apoptotic cells were detected by a terminal deoxynucleotidyl transferase dUTP nick end labeling kit (TUNEL; Beyotime Biotech, Beijing, China) [13]. The percentages of apoptotic cells in epidermis were calculated under an Axioskop II fluorescence microscope (Carl Zeiss, Jena, Germany).

Cell culture

As described previously [13], human primary keratinocytes were cultured in EpiLife® medium supplemented with Supplement S7® (Life Technologies). Cell cultures were added with an inflammatory cocktail [polyinosinic–polycytidylic acid, TNF-α, interleukin (IL)-4 and IL-13], which induces inflammation recapitulating numerous features of atopic dermatitis [19]. The final concentrations of polyinosinic–polycytidylic acid (Sigma-Aldrich, St Louis, MO,

USA), TNF-α (Life Technologies,), IL-4 (R&D Systems, Minneapolis, MN, USA) and IL-13 (R&D Systems) were 10, 10, 50 and 50 ng/ml, respectively. In some experiments, cells were also treated with TWEAK (100 ng/ml) alone or plus this cocktail for 48 h.

Quantitative real time–polymerase chain reaction (qRT–PCR)

Total RNA was extracted from fresh tissue and cell cultures using Trizol reagent (Ambion, Carlsbad, CA, USA). cDNA was generated using a commercial kit (Takara Bio, Kyoto, Japan). qRT–PCR was performed in triplicate using the SYBR Green stain (Takara Bio) and the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Waltham, MA, USA). The PCR primers (AuGCT DNA-SYN; Biotech, Beijing, China) are listed in Supporting information, Table S1. Glyceraldehyde-3-phosphate dehydrogenase was used as the reference gene to normalize the gene expression. The expression levels of the objective genes were calculated using the $2^{-\Delta Ct}$ method.

Enzyme-linked immunosorbent assay (ELISA)

ELISAs were performed with culture supernatants or tissue lysates. Protein lysates were extracted from fresh tissue, and then dissolved in phosphate-buffered saline. These lysate samples were carefully normalized according to the weight of the original tissue. The protein levels of RANTES, MCP-1, IP-10, IL-17A, IL-18 and interferon (IFN)-γ were determined using commercial immunoassay kits (R&D Systems).

Western blotting

Protein lysates were prepared from fresh tissue or cell cultures [20]. Samples were separated on electrophoresis gels, and then transferred onto polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). Rabbit antibodies to TWEAK, Fn14, RANTES, MCP-1, IP-10, IL-17A, IL-18, TNF-α, TNFR1, TNFR2 or β-actin (2 μg/ml; Abcam) and horseradish peroxidase-labeled goat anti-rabbit IgG (1 μg/ml; Abcam) were used in order. Signal was developed by using the ECL chemiluminescence kit (Millipore). The intensities of blot bands were quantitated by ImageJ software, and then normalized to β-actin values.

Statistical analysis

All data were expressed as means ± standard error of the mean. GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA, USA) was used for statistical analysis. Analysis of variance (ANOVA) was used for the comparison of more than two groups. Then, a two-tailed Student's *t*-test was used for comparing the differences in two groups.

Statistical differences were considered at $P < 0{\cdot}05$.

Results

Both TWEAK and Fn14 are up-regulated in skin lesions of patients

The mRNA expression levels of TWEAK, Fn14, RANTES, MCP-1 and IP-10 were determined in skin tissue, showing higher values in the samples of patients with atopic dermatitis compared with healthy controls (Fig. 1a). Using immunohistochemistry, stronger expressions of TWEAK and Fn14 were confirmed accordingly in the patients (Fig. 1b). The proteins of TWEAK and Fn14 were further detected by Western blotting, mirroring the results of mRNA and immunohistochemical analysis (Fig. 1c–d). The levels of TWEAK, RANTES, MCP-1 and IP-10 were also determined in serum samples by ELISA. However, there were no significant differences in these cytokines between patients and healthy controls (Fig. 1e).

Fn14 deficiency ameliorates atopic dermatitis-like lesion in mice

Atopic dermatitis-like lesion was monitored on the DNFB-treated mice. Wild-type (WT) mice showed more severe lesions than Fn14 knock-out (KO) mice after DNFB treatment (day 28), although there was a similarity between the two strains on day 0 (Fig. 2a). SCORAD indices were assessed accordingly, revealing higher scores in the WT mice on day 28 (Fig. 2b). We further evaluated the hematoxylin and eosin-stained sections, and found that both epidermis and dermis were thinner in the KO mice on day 28 (Fig. 2c–e). Moreover, the mRNA expression levels of TWEAK and Fn14 were elevated in both WT and KO mice on day 28 compared with those on day 0; however, the KO mice exhibited a lesser increase (Fig. 2f,g). Accordingly, TWEAK and Fn14 were more expressed in the WT mice on day 28 after DNFB treatment (Fig. 2h). There were no significant differences in SCORAD score, epidermal (or dermal) thickness and TWEAK (or Fn14) expression levels between the two strains that were treated with vehicle (Fig. 2b–h).

Proinflammatory cytokines are less produced in the skin lesion of Fn14-deficient mice

RANTES, MCP-1 and IP-10 are downstream cytokines regulated by TWEAK/Fn14 activation [4,5]. TNF-α, IL-17 and IL-18 are pivotal cytokines contributing to the inflammatory processes of atopic dermatitis [21]. We determined the levels of these cytokines in lesional skin of the vehicle or DNFB-treated mice. It showed that, on day 28, the KO mice had lower mRNA expression levels of these cytokines upon DNFB treatment (Fig. 3a). The protein expression levels of these cytokines were also attenuated in the KO mice compared with the WT mice (Fig. 3b–e). We observed no significant differences in the expression levels of these cytokines between the two strains upon vehicle treatment (Fig. 3).

 © 2019 British Society for Immunology,

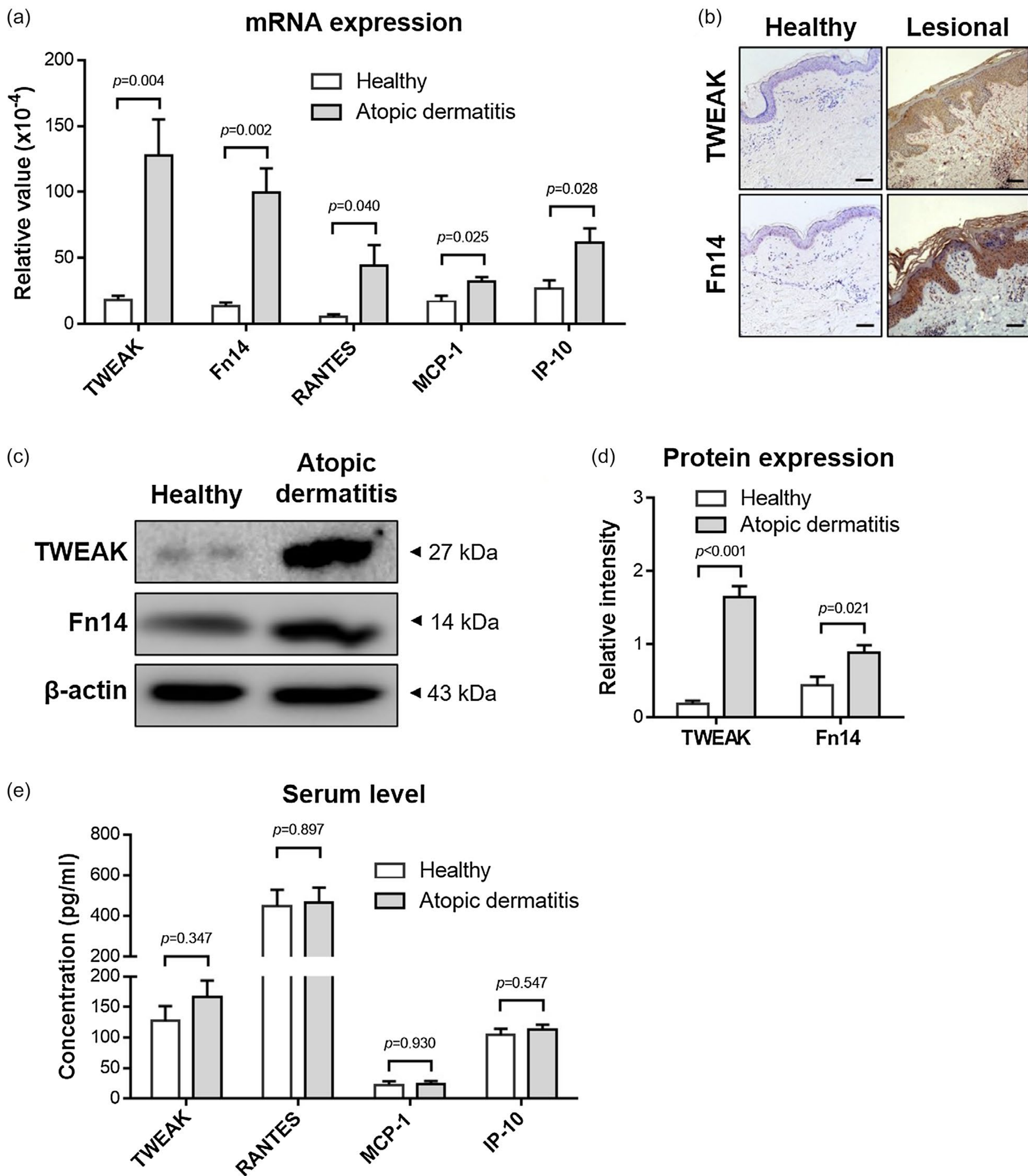

Fig. 1. The expression of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) and fibroblast growth factor inducible 14 (Fn14) in skin lesion of patients with atopic dermatitis. (a) The mRNA expression levels of TWEAK, Fn14, regulated upon activation normal T cell expressed and secreted (RANTES), monocyte chemotactic protein-1 (MCP-1) and interferon gamma-induced protein 10 (IP-10) were determined in skin tissue of patients or healthy controls. (b) Immunohistochemical staining was performed on paraffin sections. Bar = 40 µm. (c,d) The proteins of TWEAK and Fn14 were detected by Western blotting. (e) The levels of cytokines were determined in serum samples of patients (n = 12) or healthy controls (n = 20). (a–d) There were five samples in each group. Representative images are shown.

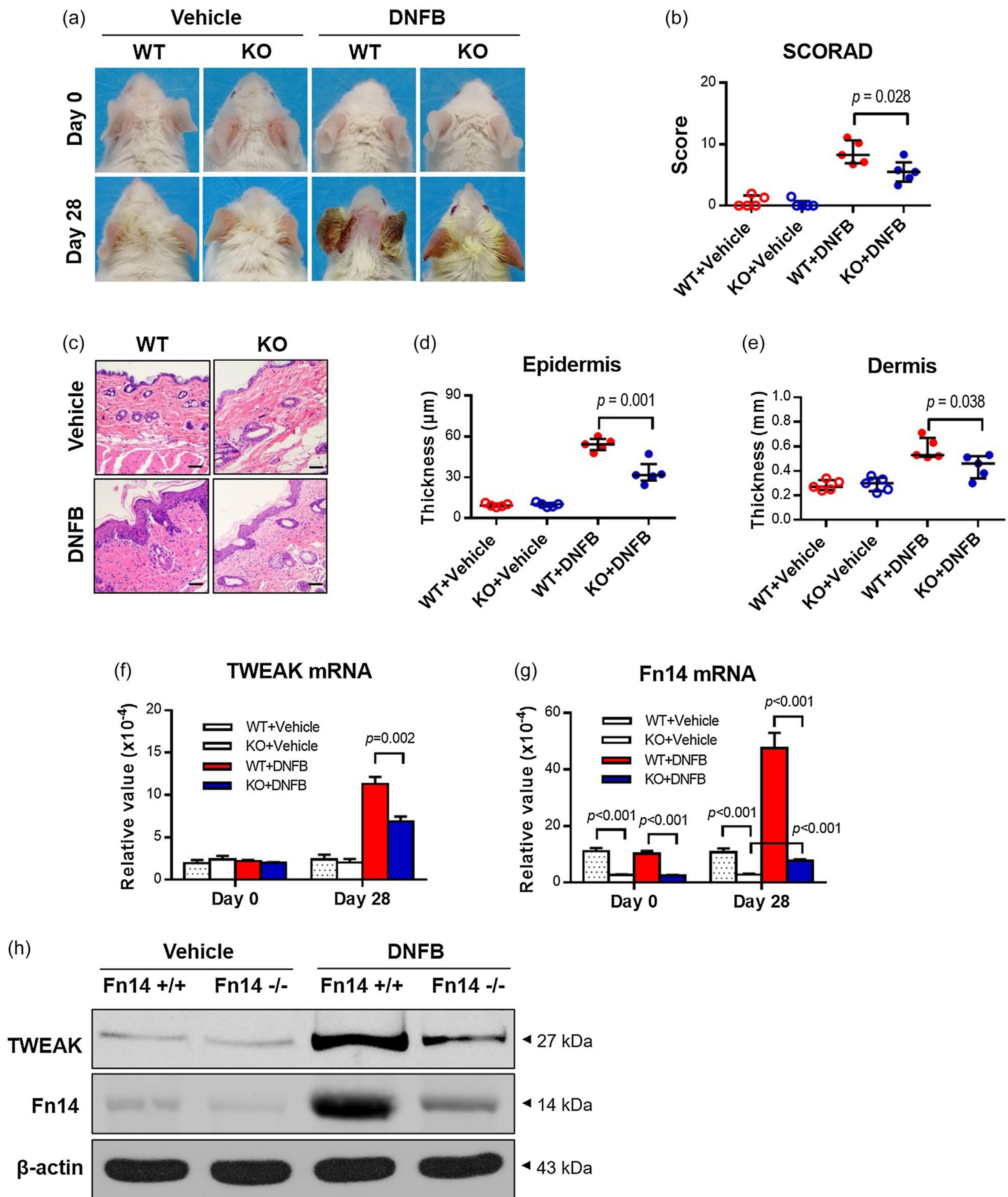

Fig. 2. Fibroblast growth factor inducible 14 (Fn14) deficiency attenuates atopic dermatitis-like lesion in a murine model. Atopic dermatitis-like disease was induced by topical application of 2,4-dinitrofluorobenzene (DNFB) solution to the ears. Skin tissue was harvested on day 28. (a,b) Skin lesion was observed on days 0 and 28, and evaluated for scoring atopic dermatitis (SCORAD) scores. (c) Hematoxylin and eosin staining was performed on paraffin sections. Bar = 40 μm. (d,e) The epidermal and dermal thickness was measured on hematoxylin and eosin-stained sections. (f,g) The mRNA expression levels of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) and Fn14 were determined with fresh tissue. (h) The protein levels of TWEAK and Fn14 were measured by Western blotting. Representative images are shown; $n = 5$ in each group. (b,d,e,f) The DNFB-treated mice had values higher than the two vehicle groups on day 28 ($P < 0·05$). WT = wild-type; KO = knock-out; n.s. = not significant.

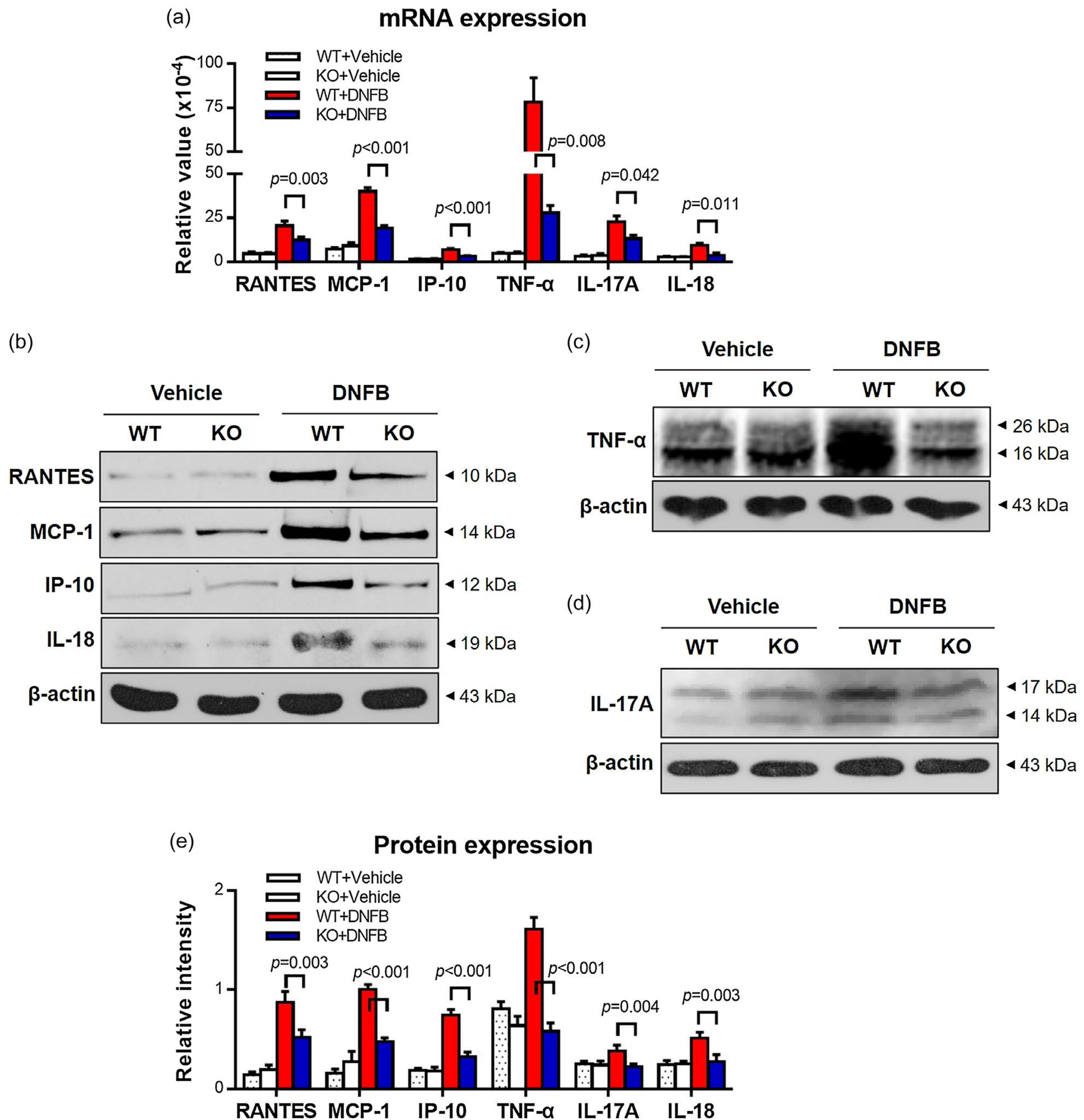

Fig. 3. Fibroblast growth factor inducible 14 (Fn14) deficiency reduces the production of inflammatory cytokines in lesional tissue. Atopic dermatitis-like disease was induced by application of 2,4-dinitrofluorobenzene (DNFB) solution to the ears. Skin tissue was harvested on day 28. (a) The mRNA expression levels of regulated upon activation normal T cell expressed and secreted (RANTES), monocyte chemotactic protein-1 (MCP-1), interferon gamma-induced protein 10 (IP-10), tumor necrosis factor-α (TNF-α), interleukin (IL)-17A and IL-18 were determined in fresh tissue. (b–d) The protein levels of these cytokines were determined by Western blotting. Representative images are shown. (e) The intensities of bands were measured by ImageJ software; $n = 5$ in each group. WT = wild-type; KO = knock-out.

Fn14 deficiency decreases cell apoptosis and alters the TNFR expression profile in the skin lesion of mice

Both proliferative and apoptotic cells were evaluated in epidermis of mice on day 28. Using immunohistochemistry, the proliferative keratinocytes (Ki67-positive) were more observed in the WT mice after DNFB treatment (Fig. 4a,b). TUNEL was performed on frozen sections, revealing that the KO mice had fewer apoptotic keratinocytes after DNFB treatment (Fig. 4a,c). At the same time-point, these two strains had far fewer proliferative

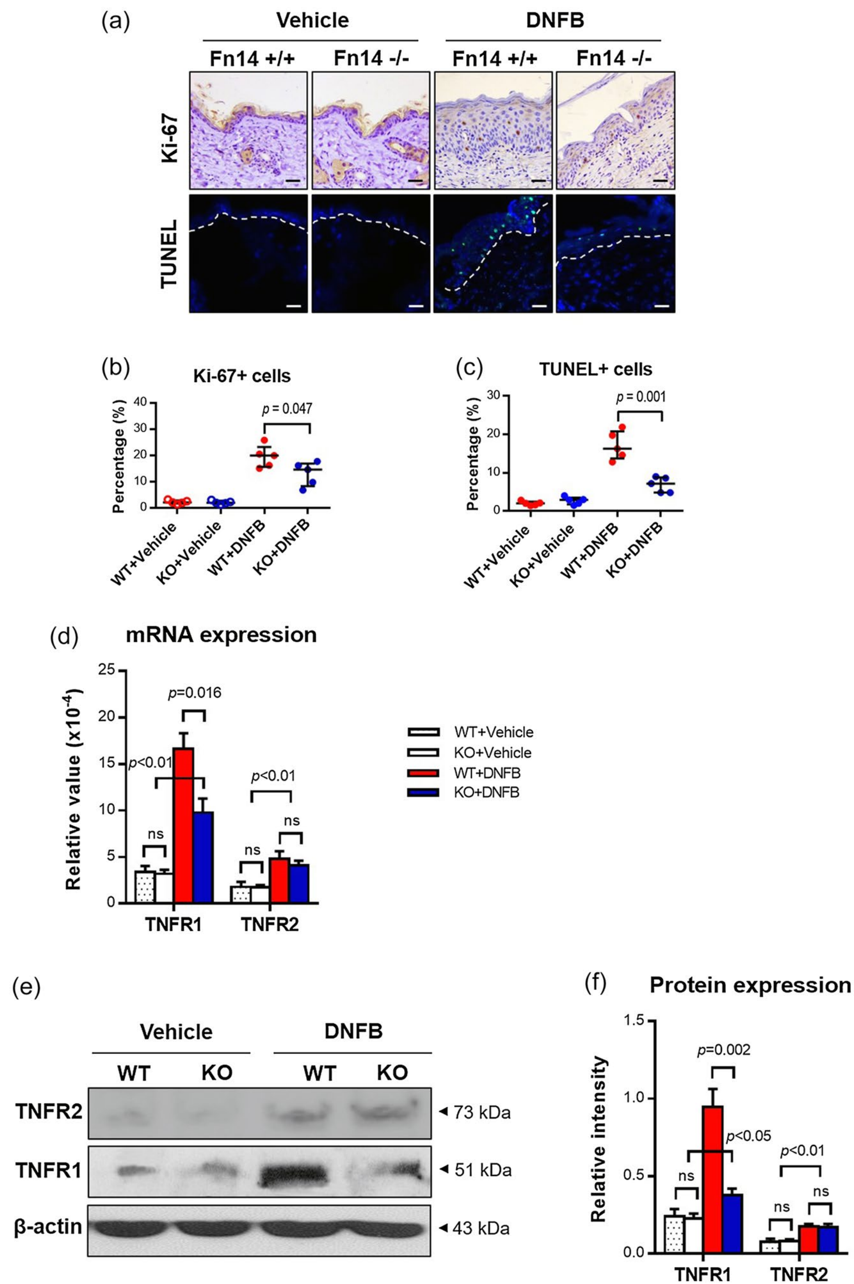

Fig. 4. Fibroblast growth factor inducible 14 (Fn14) deficiency is associated with less apoptotic cells in lesional epidermis and modulates the tumor necrosis factor receptor (TNFR) expression profile in lesional tissue. Atopic dermatitis-like disease was induced by application of 2,4-dinitrofluorobenzene (DNFB) solution to the ears. Skin tissue was harvested on day 28. (a) Using immunohistochemistry, tissue sections were detected for proliferative (Ki67-positive) cells. Apoptotic cells were detected by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Representative images are shown. (b,c) The positive cells were counted according to the areas of epidermis. The DNFB-treated groups had values higher than the vehicle groups ($P < 0{\cdot}05$). (d) The mRNA expression levels of TNFR1 and TNFR2 were determined in fresh tissue. (e,f) The protein levels of TNFR1 and TNFR2 were determined by Western blotting. Representative images are shown; $n = 5$ in each group. Bar = 20 µm. WT = wild-type; KO = knock-out; n.s. = not significant.

and apoptotic cells upon vehicle treatment and showed similar changes (Fig. 4a–c).

Several studies have demonstrated that the predominant expression of TNFR2 accounts for the TWEAK-induced proliferation of keratinocytes [7,8,13,22]. In this study, we further determined the expression of TNF receptors in lesional skin of these mice on day 28. Consistently, the mRNA expression levels of TNFR1 were significantly

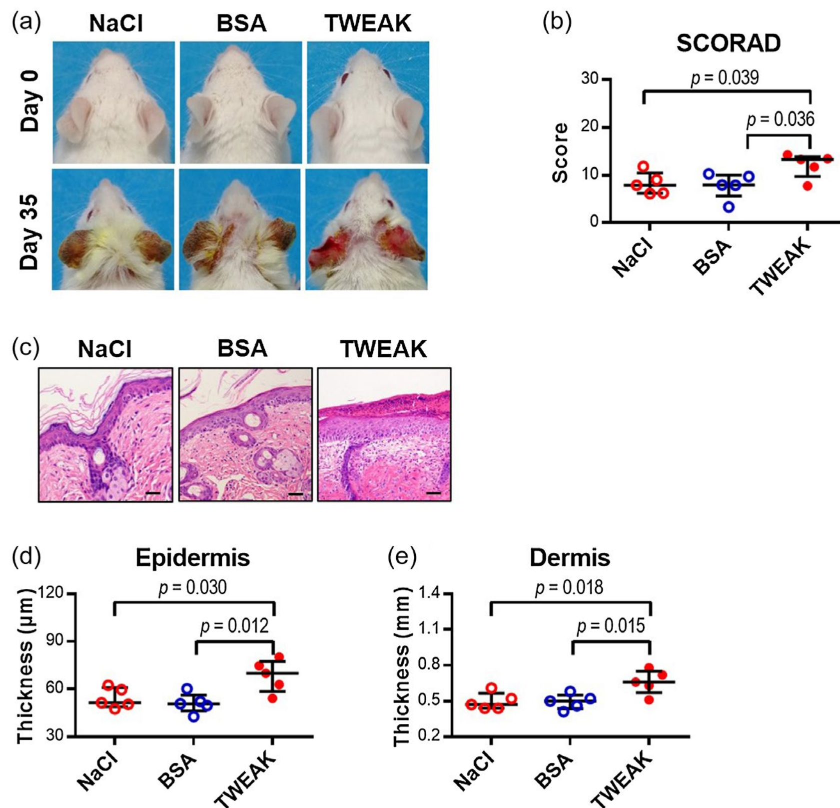

Fig. 5. Exogenous tumor necrosis factor-like weak inducer of apoptosis (TWEAK) exacerbates skin lesion in wild-type mice. Atopic dermatitis-like disease was induced by application of 2,4-dinitrofluorobenzene (DNFB) solution to the ears. Some mice received additional administration of bovine serum albumin (BSA) or TWEAK in normal saline (NaCl) from the third week. Skin tissue was harvested on day 35. (a) Skin lesion was observed on day 35. (b) Skin lesion was measured for scoring atopic dermatitis (SCORAD) scores. (c) Hematoxylin and eosin staining was performed on tissue sections. Bar = 30 µm. (d,e) The epidermal and dermal thickness was measured on hematoxylin and eosin-stained sections. (b,d,e) There were no significant differences between the two control groups ($P < 0{\cdot}05$); $n = 5$ in each group. Bar = 40 µm.

increased in the DNFB-treated WT and KO mice compared with those receiving vehicle treatment (Fig. 4d). There was no significant difference in the TNFR1 (or TNFR2) level between the vehicle-treated strains or in the TNFR2 level between the DNFB-treated strains (Fig. 4d). Using Western blotting, such differences in TNFR1 expression were also observed in protein lysates of lesional skin tissue (Fig. 4e,f).

Exogenous TWEAK exacerbates skin lesion in the WT strain

To further demonstrate the role of TWEAK/Fn14 signaling in the development of atopic dermatitis, we investigated the effect of exogenous TWEAK on this murine model (DNFB-treated WT mice). Interestingly, we found that the topical application of recombinant TWEAK exacerbated skin lesion and increased SCORAD scores compared with the bovine serum albumin (BSA) and normal saline (NaCl) groups (Fig. 5a,b). Moreover, the TWEAK group exhibited thicker epidermis and dermis compared with the other groups (Fig. 5c–e). These two control groups showed similar changes in skin appearance, SCORAD score and epidermal (dermal) thickness (Fig. 5).

TWEAK up-regulates IL-17A, IL-18 and IFN-γ in keratinocytes under atopic dermatitis-like inflammation

We studied the effect of TWEAK stimulation on the keratinocytes under atopic dermatitis-like inflammation. Primary keratinocytes were cultured with the addition of an inflammatory cocktail, which simulated the microenvironment of atopic dermatitis. The results showed that the mRNA and protein expression levels of Fn14 increased with the addition of this cocktail but not with TWEAK stimulation (Fig. 6a–c). Moreover, this cocktail enhanced the mRNA expression level of IL-17A, which was further promoted upon TWEAK stimulation (Fig. 6a). TWEAK alone exhibited no effect on the mRNA expression levels of IL-18 and IFN-γ; however, it favored the promotion effect of this cocktail (Fig. 6a). Such differential effect of TWEAK on these three cytokines was mirrored by the

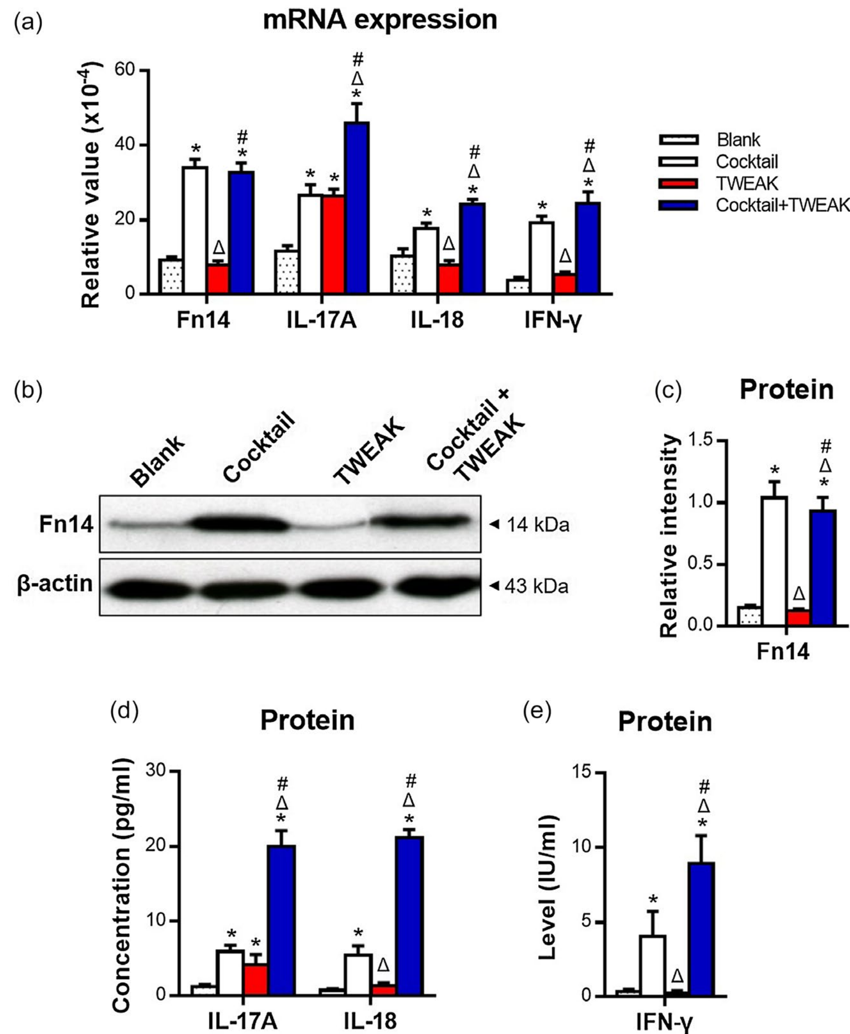

Fig. 6. Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) enhances inflammatory responses of keratinocytes under atopic dermatitis-like inflammation. Human primary keratinocytes were cultured *in vitro* with addition of an inflammatory cocktail or plus TWEAK. (a) The mRNA expression levels of fibroblast growth factor inducible 14 (Fn14), interleukin (IL)-17A, IL-18 and interferon (IFN)-γ were determined in cell lysates. (b,c) Using Western blotting, Fn14 was determined in cell lysates. (d,e) The protein levels of IL-17A, IL-18 and IFN-γ were assessed by enzyme-linked immunosorbent assay (ELISA) in the culture supernatants. Data were obtained from five independent experiments. $*P < 0{\cdot}05$, compared with the blank group; $\Delta P < 0{\cdot}05$, compared with the cocktail alone group; $\#P < 0{\cdot}05$, compared with the TWEAK alone group.

ELISA determination of protein concentrations in culture supernatants (Fig. 6d,e).

Discussion

In this study, we demonstrated that both TWEAK and Fn14 are up-regulated in skin lesions of patients with atopic dermatitis. Moreover, Fn14 deficiency significantly attenuates atopic dermatitis-like disease in mice. The protective effect of Fn14 deficiency is associated with less keratinocyte apoptosis, proinflammatory cytokine production and TNFR1 expression in affected skin. Furthermore, such skin disease can be exaggerated by additional application of TWEAK. Finally, TWEAK up-regulates IL-17A, IL-18 and IFN-γ in keratinocytes under atopic dermatitis-like inflammation. Therefore, TWEAK/Fn14 activation participates in the pathogenesis of atopic dermatitis.

Previous studies showed that TWEAK and Fn14 are up-regulated in skin lesions of patients with atopic dermatitis [10–12], although the serum level of TWEAK is not elevated [6,10,11]. Moreover, subcutaneous injection of TWEAK into mice induces histological and inflammatory signs of atopic dermatitis [12]. TWEAK deficiency protects mice from atopic dermatitis-like disease induced by house dust mite allergen and staphylococcal enterotoxin

B [12]. This study provided additional evidence that both TWEAK and Fn14 are more expressed in lesional skin of atopic dermatitis. Meanwhile, we observed no significant difference in serum TWEAK levels between patients and healthy controls. In most cases, the discrepancy between the TWEAK levels in lesions and sera might be due to the absence of systemic inflammatory responses. In fact, TWEAK is synthesized by infiltrating cells including macrophages. The diffuse distribution may confer TWEAK more systemic effects. However, Fn14 is more restricted to injured tissue. Our results clearly showed that Fn14 inhibition ameliorates atopic dermatitis-like disease in a murine model. Moreover, exogenous TWEAK exacerbates skin lesion in this model. Therefore, these findings strongly demonstrated that TWEAK/Fn14 activation contributes to the development of atopic dermatitis, and inhibition of Fn14 but not TWEAK is a more specific strategy in alleviating skin lesion.

Many proinflammatory cytokines participate in the inflammatory processes of atopic dermatitis. RANTES, IP-10 and MCP-1 are over-expressed by keratinocytes derived from patients with atopic dermatitis [23]. IFN-γ, IL-17 and IL-18 contribute to the inflammatory processes of atopic dermatitis [21]. We observed that TNF-α, RANTES, IP-10, MCP-1, IL-17 and IL-18 are produced less in skin lesions upon Fn14 deficiency in this model. In an *in-vitro* model of atopic dermatitis, IFN-γ, IL-17 and IL-18 are more synthesized by keratinocytes after TWEAK stimulation. These findings revealed that TWEAK/Fn14 signals participate in the pathogenesis of atopic dermatitis through up-regulating these relevant cytokines in skin. An important phenomenon was that IL-17 is increased upon the stimulation of the cytokine cocktail of atopic dermatitis, TWEAK alone or their combination, while IFN-γ and IL-18 are exclusively affected by this cocktail or their combination. In fact, TWEAK directly induces the production of IL-17 in keratinocytes [12]. Thus, these cytokines mediate the function of TWEAK/Fn14 signals in the pathogenesis of atopic dermatitis.

As well as these proinflammatory effects, extensive apoptosis of keratinocytes is one of the histological features in atopic dermatitis [24]. We found that apoptotic cells decrease in skin lesions of Fn14-deficient mice. Meanwhile, proliferative cells are less observed in epidermis of skin lesions upon Fn14 deficiency. This might be due to the attenuation of skin inflammation, which chronically causes the proliferation of keratinocytes. The cytoplasmic domain of Fn14 contains binding sites for TNFR- associated receptor (TRAF)-1, TRAF-2, TRAF-3 and TRAF-5 [25]. Previously, we have proved that TWEAK regulates cell fate via the Fn14–TRAF–TNFR axis and induces the death or proliferation of keratinocytes, depending on the expression profile of TNF receptors [7,8,13,22]. In psoriasis, another immune-mediated skin disorder with chronic cutaneous inflammation, the TWEAK/Fn14 pathway prefers to enhance proliferation but not apoptosis of keratinocytes [7]. We showed that TNFR2 is up-regulated in psoriasis. Keratinocytes express TNFR2 predominantly with the addition of TWEAK and the TNFR2 expression decreases more obviously upon Fn14 inhibition in a psoriasis mouse model [7,13]. In this study we found that, instead of TNFR2, TNFR1 is up-regulated in skin lesion of atopic dermatitis and Fn14 deficiency reduces the TNFR1 expression profile. The predominance of TNFR1 is associated with apoptosis of keratinocytes under TWEAK/Fn14 activation [8,11]. Moreover, TWEAK and TNF-α co-operate in the induction of keratinocyte apoptosis [11]. Considering that TNF-α is abundant in skin tissue of atopic dermatitis [11,24], our results explained the reduction of keratinocyte apoptosis in Fn14-deficient skin of an atopic dermatitis model. We further elucidated that, as well as the anti-inflammatory role, the inhibition of TWEAK/Fn14 interaction exerts its therapeutic effects by altering the TNFR1/TNFR2 expression profile, which may generate cell-fate diversity of keratinocytes under different inflammatory contexts.

Atopic dermatitis is a complex inflammatory condition, involving the defects of epidermal barrier as well as T helper type 2 (Th2) immune response dysregulations [26]. Currently, different *in-vitro* models have been developed to mimic the features of atopic dermatitis [26–28]. These models adopt the cytokine stimulation of cultured keratinocytes or epidermal equivalents, the latter of which may exhibit certain barrier properties. In this study, we utilized an *in-vitro* model by stimulating keratinocytes with a cocktail, which recapitulates inflammatory features of atopic dermatitis [19]. Although the results convincingly demonstrated the pivotal role of TWEAK/Fn14 signals in the pathogenesis of atopic dermatitis, the effect of TWEAK on the barrier function of skin remains unelucidated in this disease. As TWEAK/Fn14 interaction may injury the junction proteins and even the integrities of blood–brain barrier or glomerular filtration barrier [29,30], the exact relationship between TWEAK/Fn14 signals and skin barrier should be investigated in further studies.

Conclusively, Fn14 deficiency ameliorates skin lesion in the murine model of atopic dermatitis, possibly through suppressing the production of inflammatory cytokines. Fn14 inhibition also alters the expression profile of TNF receptors, leading to less apoptosis of keratinocytes. Moreover, exogenous TWEAK exacerbates skin lesion in this murine model and promotes the synthesis of relevant cytokines in keratinocytes under atopic dermatitis-like inflammation. Targeting the TWEAK/Fn14 pathway may be a promising therapeutic approach for patients with atopic dermatitis.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Project no. 81630081), the Natural Science Foundation of Shaanxi Province (Project no. 2017ZDJC-06), and the Innovation Capability Support Plan of Shaanxi Province (no. 2019TD-034).

Disclosures

The authors declare no conflicts of interest.

References

1 Flohr C, Mann J. New insights into the epidemiology of childhood atopic dermatitis. Allergy 2014; **69**:3–16.

2 Barbarot S, Auziere S, Gadkari A *et al*. Epidemiology of atopic dermatitis in adults: results from an international survey. Allergy 2018; **73**:1284–93.

3 Elias PM, Hatano Y, Williams ML. Basis for the barrier abnormality in atopic dermatitis: outside-inside-outside pathogenic mechanisms. J Allergy Clin Immunol 2008; **121**:1337–43.

4 Wang X, Xiao S, Xia Y. Tumor necrosis factor receptor mediates fibroblast growth factor-inducible 14 signaling. Cell Physiol Biochem 2017; **43**:579–88.

5 Liu Q, Xiao S, Xia Y. TWEAK/Fn14 activation participates in skin inflammation. Mediat Inflamm 2017;**2017**:6746870.

6 Chen T, Guo ZP, Li MM *et al*. Tumour necrosis factor-like weak inducer of apoptosis (TWEAK), an important mediator of endothelial inflammation, is associated with the pathogenesis of Henoch–Schonlein purpura. Clin Exp Immunol 2011; **166**:64–71.

7 Cheng H, Xu M, Liu X, Zou X, Zhan N, Xia Y. TWEAK/Fn14 activation induces keratinocyte proliferation under psoriatic inflammation. Exp Dermatol 2016; **25**:32–7.

8 Liu Y, Xu M, Min X *et al*. TWEAK/Fn14 activation participates in Ro52-mediated photosensitization in cutaneous lupus erythematosus. Front Immunol 2017; **8**:651.

9 Liu Y, Peng L, Li L *et al*. TWEAK/Fn14 activation contributes to the pathogenesis of bullous pemphigoid. J Invest Dermatol 2017; **137**:1512–22.

10 Chen Y, Lind Enoksson S, Johansson C *et al*. The expression of BAFF, APRIL and TWEAK is altered in eczema skin but not in the circulation of atopic and seborrheic eczema patients. PLOS One 2011; **6**:e22202.

11 Zimmermann M, Koreck A, Meyer N *et al*. TNF-like weak inducer of apoptosis (TWEAK) and TNF-α cooperate in the induction of keratinocyte apoptosis. J Allergy Clin Immunol 2011; **127**:200–207.e10.

12 Sidler D, Wu P, Herro R *et al*. TWEAK mediates inflammation in experimental atopic dermatitis and psoriasis. Nat Commun 2017; **8**:15395.

13 Peng L, Li Q, Wang H *et al*. Fn14 deficiency ameliorates psoriasis-like skin disease in a murine model. Cell Death Dis 2018; **9**:801.

14 Jeong NH, Yang EJ, Jin M *et al*. Esculetin from *Fraxinus rhynchophylla* attenuates atopic skin inflammation by inhibiting the expression of inflammatory cytokines. Int Immunopharmacol 2018; **59**:209–16.

15 Liu J, Liu Y, Peng L *et al*. TWEAK/Fn14 signals mediate burn wound repair. J Invest Dermatol 2019; **139**:224–34.

16 Kim TH, Kim GD, Ahn HJ, Cho JJ, Park YS, Park CS. The inhibitory effect of naringenin on atopic dermatitis induced by DNFB in NC/Nga mice. Life Sci 2013; **93**:516–24.

17 Choi YJ, Lee HJ, Lee DH *et al*. Therapeutic effects and immunomodulation of suanbo mineral water therapy in a murine model of atopic dermatitis. Ann Dermatol 2013; **25**:462–70.

18 Zou XY, Ding D, Zhan N, Liu XM, Pan C, Xia YM. Glyoxalase I is differentially expressed in cutaneous neoplasms and contributes to the progression of squamous cell carcinoma. J Invest Dermatol 2015; **135**:589–98.

19 Rouaud-Tinguely P, Boudier D, Marchand L *et al*. From the morphological to the transcriptomic characterization of a compromised three-dimensional *in vitro* model mimicking atopic dermatitis. Br J Dermatol 2015; **173**:1006–14.

20 Hu G, Liang L, Liu Y *et al*. TWEAK/Fn14 interaction confers aggressive properties to cutaneous squamous cell carcinoma. J Invest Dermatol 2019; **139**:796–806.

21 Szegedi K, Lutter R, Res PC *et al*. Cytokine profiles in interstitial fluid from chronic atopic dermatitis skin. J Eur Acad Dermatol Venereol 2015; **29**:2136–44.

22 Cheng H, Zhan N, Ding D *et al*. HPV type 16 infection switches keratinocytes from apoptotic to proliferative fate under TWEAK/Fn14 interaction. J Invest Dermatol 2015; **135**:2427–36.

23 Giustizieri ML, Mascia F, Frezzolini A *et al*. Keratinocytes from patients with atopic dermatitis and psoriasis show a distinct chemokine production profile in response to T cell-derived cytokines. J Allergy Clin Immunol 2001; **107**:871–7.

24 Szymanski L, Cios A, Lewicki S, Szymanski P, Stankiewicz W. Fas/FasL pathway and cytokines in keratinocytes in atopic dermatitis - Manipulation by the electromagnetic field. PLoS ONE 2018; **13**:e0205103.

25 Brown SA, Richards CM, Hanscom HN, Feng SL, Winkles JA. The Fn14 cytoplasmic tail binds tumor necrosis factor receptor-associated factors 1, 2, 3 and 5 and mediates nuclear factor-kappa B activation. Biochem J 2003; **371**:395–403.

26 De Vuyst E, Salmon M, Evrard C, Lambert de Rouvroit C, Poumay Y. Atopic dermatitis studies through *in vitro* models. Front Med 2017; **4**:119.

27 An HJ, Kim JY, Kim WH *et al*. Therapeutic effects of bee venom and its major component, melittin, on atopic dermatitis *in vivo* and *in vitro*. Br J Pharmacol 2018; **175**:4310–24.

28 Sriram G, Bigliardi PL, Bigliardi-Qi M. Full-thickness human skin equivalent models of atopic dermatitis. Methods Mol Biol 1879; **2018**:367–83.

29 Wen J, Doerner J, Weidenheim K *et al*. TNF-like weak inducer of apoptosis promotes blood brain barrier disruption and increases neuronal cell death in MRL/lpr mice. J Autoimmun 2015; **60**:40–50.

30 Xia Y, Herlitz LC, Gindea S *et al*. Deficiency of fibroblast growth factor-inducible 14 (Fn14) preserves the filtration barrier and ameliorates lupus nephritis. J Am Soc Nephrol 2015; **26**:1053–70.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web site:

Table S1. Primer sets used in PCR