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CCL19 promotes TNF-alpha/IFN-gamma-induced production of cytokines by targeting CCR7/NF-κB signalling in HaCaT cells

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ABSTRACT

Introduction. Atopic dermatitis (AD) is the most common allergic skin disease. The dysfunction of keratinocytes is closely associated with AD progression. Nevertheless, the specific functions of CC chemokine ligand 19 (CCL19) and its receptor CC chemokine receptor 7 (CCR7) in human HaCaT keratinocytes are still unclear.

Material and methods. AD cell models *in vitro* were established by treating HaCaT cells with *TNF-alpha* **(TNF-α, 10 ng/mL) and IFN-gamma (IFN-γ, 10 ng/mL)**. Cell viability was estimated by MTT assay. The protein levels of CCL19 and CCR7 were tested *via* Western blotting. The expression of CCL19 protein was knocked down by transfecting si-CCL19 into HaCaT cells. The contents of inflammatory factors *i.e.* thymus and activation-regulated chemokine (TARC), interleukin 6 (IL-6), and prostaglandin E2 were measured by ELISA, and the nitric oxide content was detected by Griess reagent. The protein levels of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) were tested *via* Western blotting.

Results. TNF-α and IFN-γ induced cytotoxicity and upregulated the expression of CCL19 and CCR7 in HaCaT cells. CCL19 knockdown alleviated cytokines-induced cytotoxicity and the release of TARC, IL-6, PGE2 and nitric oxide in TNF-α + IFN-γ-treated HaCaT cells. Furthermore, the protein levels of iNOS and COX-2 were also repressed by CCL19 knockdown. In addition, knockdown of CCL19 decreased CCR7 protein content and inhibited the phosphorylation of IκBα and p65, implying that knockdown of CCL19 inactivated CCR7/NF-κB signalling in HaCaT cells. Rescue assays validated that CCR7 overexpression reversed the effects of CCL19 silencing on the viability and levels of inflammatory factors in TNF-α + IFN-γ-induced HaCaT cells.

Conclusions. This study proves that CCL19 can promote TNF-α + IFN-γ-induced skin inflammatory responses by targeting CCR7/NF-κB pathway in HaCaT cells.

Keywords: atopic dermatitis; CCL19; CCR7; inflammatory responses; TNF-α; IFN-γ

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INTRODUCTION

Atopic dermatitis (AD) is the most common allergic skin disease [1]. The initial manifestations of AD usually appear early in life and often precede other allergic diseases such as asthma or allergic rhinitis [2]. In recent years, the incidence rate of AD has been increasing, and the recurrence of AD brings a psychological burden and economic pressure to bear on patients and their families [3]. AD is a chronic inflammatory disease caused by genetic predisposition, epidermal barrier disruption, and dysregulation of the immune system [4]. At present, there are no treatments that are suitable for all AD patients [5]. Common drugs such as glucocorticoids

and tacrolimus may be effective in some patients, but they are accompanied by serious side effects [5].

Therefore, we aimed to conduct an in-depth study of the pathogenesis of AD and to explore more effective treatment methods, with the goal of improving the life quality of AD patients.

Keratinocytes are the major cell types in the epidermis and participate in the processes of assorted inflammatory skin diseases [6]. Epidermal barrier dysfunction induces keratinocytes to release inflammatory mediators such as proinflammatory cytokines and chemokines, resulting in T lymphocytes infiltration and inflammatory response activation [7, 8]. The inflammatory factors TNF-alpha (TNF-α) and IFN-gamma (IFN-γ) have been shown to activate keratinocytes to induce the production of inflammatory cytokines and chemokines, including thymus and activation-regulated chemokine (TARC), IL-6, and IL-8 [9, 10].

Chemokines are crucial factors in the recruitment of inflammatory cells into positions of allergic inflammation [11]. Some chemokines have been demonstrated to express at a high level on keratinocytes in the epidermis of AD patients, thereby developing local inflammation [12, 13]. For example, in infantile AD patients, the levels of CCL-17, CCL-20, and CCL-27 have been found to be upregulated, and these are closely correlated with AD progression [14]. CC chemokine ligand 19 (CCL19) is a member of the chemokine family, and it can function as a chemotactic signal for assorted immune cells, such as dendritic cells, T cells, and macrophages [15]. CCL19 and its receptor CC chemokine receptor 7 (CCR7) regulate a series of migratory events in adaptive immunity following antigen encounter by immunocytes [16]. It has been reported that CCR7 is overexpressed in T cells and dendritic cells in AD lesions [17] and may play a proinflammatory role in the development of atherosclerotic lesions [18]. CCL19 has also been shown to facilitate inflammation in HIV-infected patients with ongoing viral replication [19]. Furthermore, it has been reported that CCL19 interacts with CCR7 to accelerate inflammation response in mice with asthma [20]. Importantly, studies have indicated that AD-related genes are markedly enriched in the components of the chemokine signalling pathway, and AD-relevant proteins widely interact with chemokines CCR7, CCL19, STAT1 and PIK3R1 [21, 22]. However, the specific role of CCL19 in AD is not yet known, nor is the interaction between CCL19 and CCR7 in AD.

The main aim of this study was to investigate the specific function and underlying mechanism of CCL19 and CCR7 in HaCaT keratinocytes. We hypothesised that CCL19 may promote inflammatory responses by regulating CCR7 in HaCaT keratinocytes. Our results have enhanced a deeper understanding of the complex pathogenesis and intricate mechanisms underlying allergic dermatitis.

Materials and methods

Cell culture and treatment

Human immortalised keratinocytes (HaCaT) obtained from the Chinese Academy of Sciences (Kunming, China) were cultured in DMEM (Gibco, Grand Island, NY, USA) added to 10% foetal bovine serum (FBS) and 1% penicillin and streptomycin (Gibco) at 37°C with 5% CO_2. To establish an *in vitro* AD cell model, different concentrations of TNF-α (1 ng/mL) + IFN-γ (1 ng/mL), TNF-α (5 ng/mL) + IFN-γ (5 ng/mL), TNF-α (10 ng/mL) + IFN-γ (10 ng/mL), and TNF-α (15 ng/mL) + IFN-γ (15 ng/mL) (R&D Systems, Minneapolis, MN, USA) were used to treat HaCaT cells for 48 h. Cytokines and vectors were added into HaCaT cell medium (2×10^6 cells/ml in a 6-well plate) at 60–70% confluence. The final cell volume was at 90% confluence.

RT-qPCR

Total RNAs were subjected to extraction from HaCaT cells with TRIzol (Invitrogen, Carlsbad, CA, USA) in line with the manufacturer's instructions. Then, RNAs were subjected to reverse transcription to cDNA with a PrimeScript Reverse Transcriptase Kit (Takara, Shiga, Japan), and qPCR was conducted using a SYBR Green PCR Master Mix (Invitrogen) on an ABI 7500 Real-time PCR System (Applied Biosystems, Foster City, CA, USA). The levels of CCL19 and CCR7 mRNAs were calculated by the $2^{-\Delta\Delta CT}$ method and normalised to GAPDH. PCR amplification was carried out as follows: denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. The sequences of primers used were as follows:

- CCL19 forward, 5′-GAAGACTGCTGCCTGTCTGT-3′
- CCL19 reverse, 5′-GCAGTCTCTGGATGATGCGT-3′
- CCR7 forward, 5′-GTCATGGACCTGGGGAAACC-3′
- CCR7 reverse, 5′-GCTGTAGGTGACGTCGTAGG-3′
- GAPDH forward, 5′-GGAGTCCCTGCCACACTCA-3′
- GAPDH reverse, 5′-GCCCCTCCCCTCTTCAAG-3′.

Cell transfection

Double-stranded siRNAs for CCL19 (si-CCL19) and a negative control (si-NC) were synthesised by GenePharma (Shanghai, China). The full-length sequence of CCR7 was inserted into the pcDNA3.1 vector (Geenseed Biotech, Guangzhou, China) and the empty pcDNA3.1 vector served as NC. HaCaT cells were placed in a 6-well plate, and then transfected with plasmids by Lipofectamine 3000 (Invitrogen) for 48 h according to the manufacturer's instructions. The transfection efficiency was checked by RT-qPCR and Western blotting analyses.

MTT assay

On the basis of the manufacturer's instructions, an MTT test (Sigma-Aldrich, St. Louis, MO, USA) was used to detect cell viability. HaCaT cells (2×104 cells/well) were put into

96-well plates and treated with different concentrations of TNF-α plus IFN-γ for 24 h as described above. After that, 0.5 mg/mL MTT dye was supplemented for an incubation of 3 h. Then, the medium was removed, and the insoluble formazan was solubilised in DMSO (Sigma-Aldrich). The optical density at 560 nm was detected with a PowerWave™XS spectrophotometer (BioTek, Winooski, VT, USA).

Detection of TARC, IL-6, PGE2 and nitric oxide

HaCaT cells (1×10^5 cells/well) put in 6-well plates were cultured with TNF-α (10 ng/mL) + IFN-γ (10 ng/mL) for 24 h. Then, the cells were centrifuged (13,200 rpm, 10 min, 4°C) and supernatants were collected. The concentrations of PGE2 (R&D Systems), TARC (eBioscience, San Diego, CA, USA), and IL-6 (eBioscience) in the cell supernatants were tested using their corresponding ELISA kits, in line with the manufacturer's instructions.

Nitric oxide was detected by a Griess reagent (Molecular Probes, Eugene, OR, USA) in line with the manufacturer's instructions. Griess reagent was used to mix with the cell's supernatant for half an hour, and then the absorbance at 540 nm was estimated by a spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Western blotting

Total protein was obtained from HaCaT cells by lysis with RIPA (Sigma-Aldrich, Shanghai, China), followed by determination of protein concentration with the BCA protein kit (Beyotime, Shanghai, China). Proteins were isolated by 10% SDS-PAGE and then transferred to PVDF membranes (Millipore, Burlington, MA, USA). Next, 5% non-fat milk was used to blockade the membranes, which were then incubated with the following primary antibodies: anti-CCR7 (Abcam, Cambridge, UK, ab32527, 1:10,000), anti-CCL19 (Abcam, ab192877, 1:10,000), anti-iNOS (Abcam, ab178945, 1:1,000), anti-COX-2 (Abcam, ab179800, 1:1,000), anti-p-IκBα (Abcam, ab133462, 1:10,000), anti-p-p65 (Abcam, ab76302, 1:1,000), and anti-GAPDH (Abcam, ab8245, 1:1,000) at 4°C overnight. GAPDH was used as the loading control. After that, membranes were rinsed with TBST and incubated with HRP-conjugated secondary antibody (Abcam, ab6789, 1:2,000) for 2 h. Protein bands were detected with an ECL kit (Millipore) and analysed by an ImageJ (v1.8.0; National Institutes of Health, Bethesda, MD, USA).

Statistical analyses

Statistical analysis was performed using GraphPad Prism software (version 7.0, GraphPad Software, San Diego, CA, USA). Data was displayed as the means ± SD from three individual repeats. Student's *t*-test was applied for comparison between two groups. Comparisons among multiple groups were analysed by one-way ANOVA followed by Tukey's *post hoc* analysis. $P < 0.05$ was considered to indicate statistical significance.

Results

TNF-α plus IFN-γ induce cytotoxicity and upregulate expression of CCL19 and CCR7 in HaCaT cells

To establish the *in vitro* AD cell model, HaCaT cells were stimulated with different concentrations of TNF-α + IFN-γ (for both cytokines 1 ng/mL, 5 ng/mL, 10 ng/mL, or 15 ng/mL). As shown by MTT assay, cell viability was suppressed by the addition of TNF-α + IFN-γ at 5 ng/mL, 10 ng/mL, and 15 ng/mL concentrations (Fig. 1A). Then CCL19 and CCR7 levels were tested in HaCaT cells with different concentrations of TNF-α + IFN-γ (for both cytokines 1 ng/mL, 5 ng/mL, 10 ng/mL, or 15 ng/mL) *via* Western blotting. We found that the protein levels of CCL19 and CCR7 in the HaCaT cells were elevated at TNF-α + IFN-γ 5-15 ng/mLconcentrations (Fig. 1B, C). Overall, CCL19 and CCR7 were highly expressed in the TNF-α + IFN-γ-treated HaCaT cells.

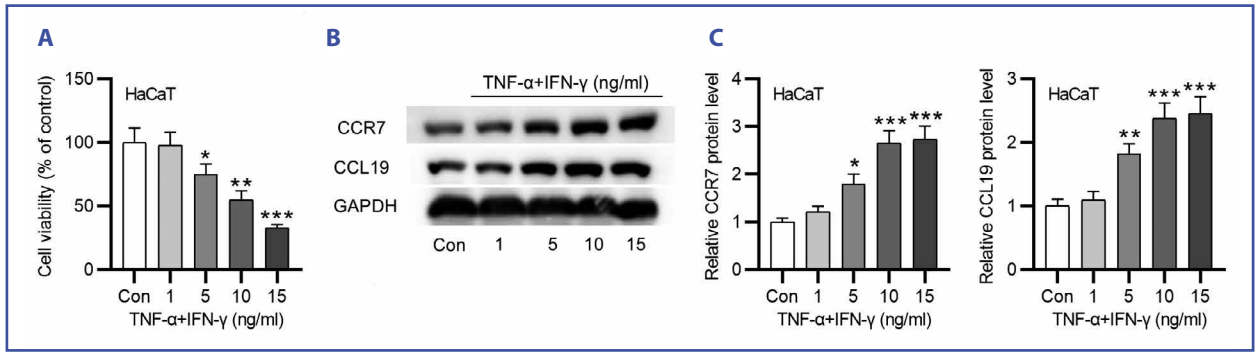

Figure 1. TNF-α + IFN-γ induces cytotoxicity and upregulates expression of CCL19 and CCR7 in HaCaT cells. **A.** HaCaT cell viability under treatment of TNF-α + IFN-γ (for both cytokines 1 ng/mL, 5 ng/mL, 10 ng/mL, and 15 ng/mL) measured by MTT assay. **B, C.** Western blots of protein levels of CCR7 and CCL19 in HaCaT cells stimulated with different concentrations of TNF-α + IFN-γ. $^{*}P < 0.05$, $^{**}P < 0.01$. Data displayed as means ± SD. Abbreviations: GAPDH — glyceraldehyde-3-phosphate dehydrogenase; HaCaT — human immortalised keratinocytes; SD — standard deviation.

CCL19 knockdown alleviates cytotoxicity and inflammatory response in TNF-α + IFN-γ-treated HaCaT cells

The release of proinflammatory mediators plays an important role in skin inflammatory response [23]. Thus, we explored the role of CCL19 in TNF-α + IFN-γ-treated HaCaT cells. Firstly, CCL19 was silenced in HaCaT cells, and the transfection efficiency in different groups (the control + si-NC group, the control + si-CCL19 group, the 10 ng/mL of TNF-α + IFN-γ + si-NC group, and the 10 ng/mL of TNF-α + IFN-γ + si-CCL19 group) was tested. We discovered that the protein level and the mRNA level of CCL19 were markedly reduced in the two si-CCL19 transfection groups (Fig. 2A).

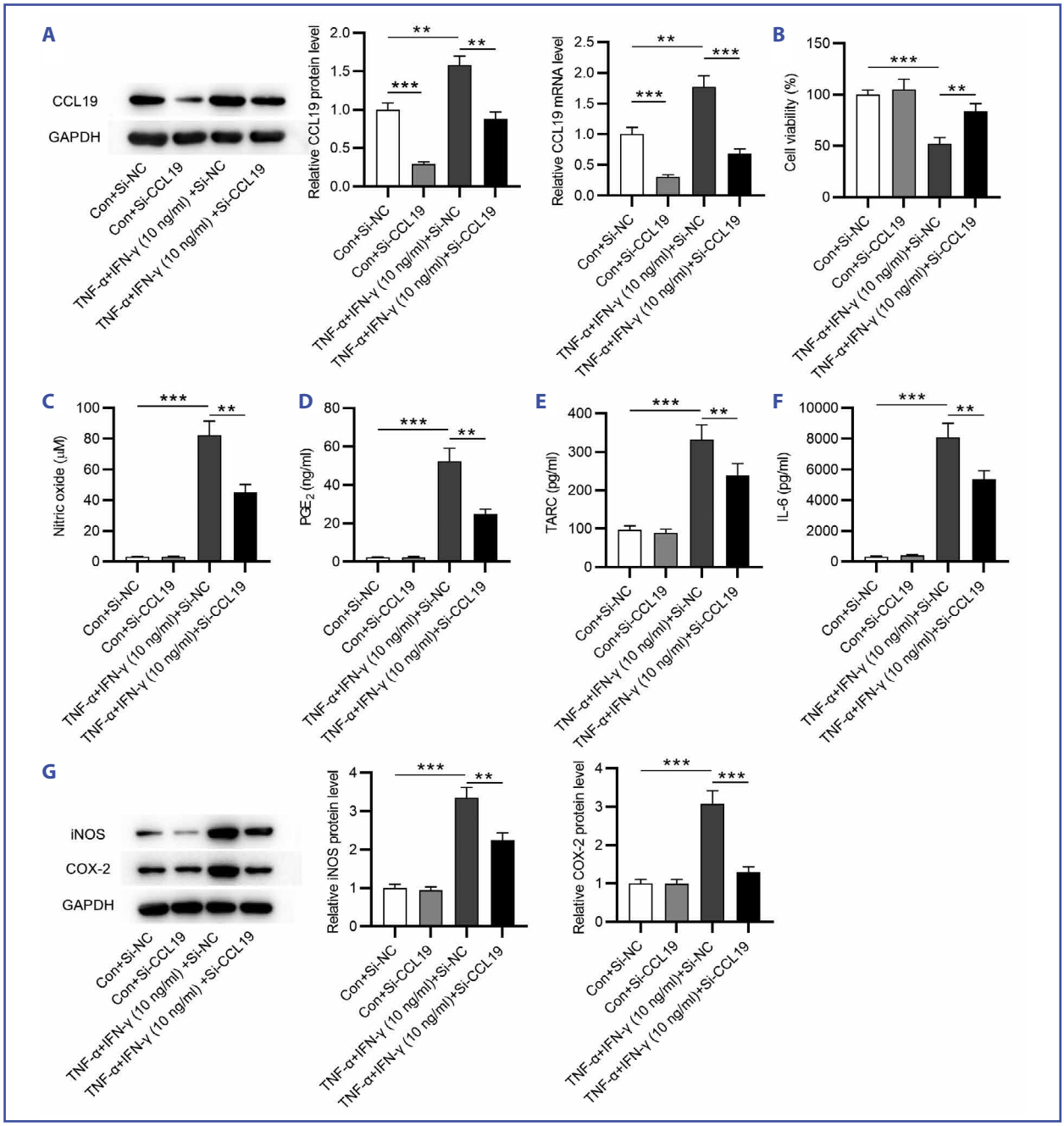

Figure 2. CCL19 knockdown alleviates cytotoxicity and inflammatory response in control (con) and TNF-α + IFN-γ-treated HaCaT cells. **A.** CCL19 protein level determined by Western blotting in following cell groups: control + si-NC group, control + si-CCL19 group, TNF-α (10 ng/mL) with IFN-γ (10 ng/mL) and si-NC group, and TNF-α (10 ng/mL) + IFN-γ (10 ng/mL) and si-CCL19 group. **B.** MTT assay used to detect HaCaT cell viability in four abovementioned cell groups. **C–F.** Content of nitric oxide (C), PGE2 (D), TARC (E) and IL-6 (F) levels in supernatants of HaCaT cells detected by ELISA. **G.** Protein levels of iNOS and COX-2 in HaCaT cells measured by Western blotting. $^{**}P < 0.01$, $^{***}P < 0.001$. Data are displayed as means ± SD. Abbreviations: COX-2 — cyclooxygenase 2; GAPDH — glyceraldehyde-3-phosphate dehydrogenase; HaCaT — human immortalised keratinocytes; iNOS — induced nitric oxide synthase; SD — standard deviation; TARC — thymus and activation-regulated chemokine.

By using the MTT assay, we documented that there was no obvious change of cell viability between the control + si-NC group and the control + si-CCL19 group. However, the transfection of si-CCL19 significantly promoted cell viability which was repressed by the treatment with TNF-α (10 ng/mL) + IFN-γ (10 ng/mL) in HaCaT cells (Fig. 2B). Then, we measured the effects of CCL19 knockdown on the content of proinflammatory mediators (nitric oxide and PGE2). We found that the concentrations of nitric oxide and PGE2 were elevated by treatment with 10 ng/mL of TNF-α + IFN-γ, while CCL19 knockdown decreased their concentrations, suggesting the inhibitory effect of CCL19 silencing on inflammatory mediators secretion by TNF-α + IFN-γ-stimulated HaCaT cells (Figs. 2C, D). Furthermore, ELISA illustrated that the increased concentrations of inflammatory factors (TARC and IL-6) caused by TNF-α + IFN-γ treatment were recovered by CCL19 silencing (Figs. 2E, F). Moreover, we estimated the inflammation-related proteins (iNOS and COX-2) in TNF-α + IFN-γ-stimulated HaCaT cells. Moreover, by using Western blotting we found that TNF-α plus IFN-γ treatment markedly increased the cellular levels of iNOS and COX-2, while CCL19 silencing reversed levels of these proinflammatory factors (Fig. 2G).

We concluded that CCL19 knockdown alleviates cytotoxicity and the inflammatory response in TNF-α + IFN-γ-treated HaCaT cells.

CCL19 activates CCR7/NF-κB signalling in TNF-α + IFN-γ-treated HaCaT cells

CCL19 is a well-characterised ligand of CCR7. It has been reported that CCR7 can activate NF-κB signalling in oesophageal squamous carcinoma cells [24]. Thus, we suspected that CCL19 may activate CCR7/NF-κB signalling in TNF-α + IFN-γ-treated HaCaT cells. Then we detected the levels of important proteins of the NF-κB signalling pathway in different groups (Fig. 3A; the control + si-NC group, the control + si-CCL19 group, the 10 ng/mL of TNF-α + IFN-γ + si-NC group, and the 10 ng/mL of TNF-α + IFN-γ + si-CCL19 group).

Western blots manifested that CCR7, p-IκBα, and p-p65 levels were almost unchanged in the control + si-NC group and the control + si-CCL19 group, while TNF-α + IFN-γ treatment markedly upregulated CCR7, p-IκBα, and p-p65 proteins, and co-transfection of si-CCL19 reversed this promotive effect (Fig. 3A). These results confirm that CCL19 activates CCR7/NF-κB signalling in TNF-α + IFN-γ-treated HaCaT cells.

CCL19 promotes inflammatory response in TNF-α + IFN-γ-induced HaCaT cells by upregulating CCR7

Finally, we performed rescue assays to verify the influence of CCL19 and CCR7 on the inflammatory response in TNF-α + IFN-γ-stimulated HaCaT cells. Firstly, we overexpressed CCR7 by transfecting the pcDNA3.1-CCR7 vector in cells. Western blots and RT-qPCR showed that the protein level and mRNA level of CCR7 were elevated in the pcDNA3.1-CCR7 transfection group (Fig. 4A). MTT assay illustrated that the viability of TNF-α + IFN-γ-induced HaCaT cells was promoted by CCL19 depletion, while it was reversed by co-transfection with pcDNA3.1-CCR7 (Fig. 4B). Furthermore, we observed that the concentrations of nitric

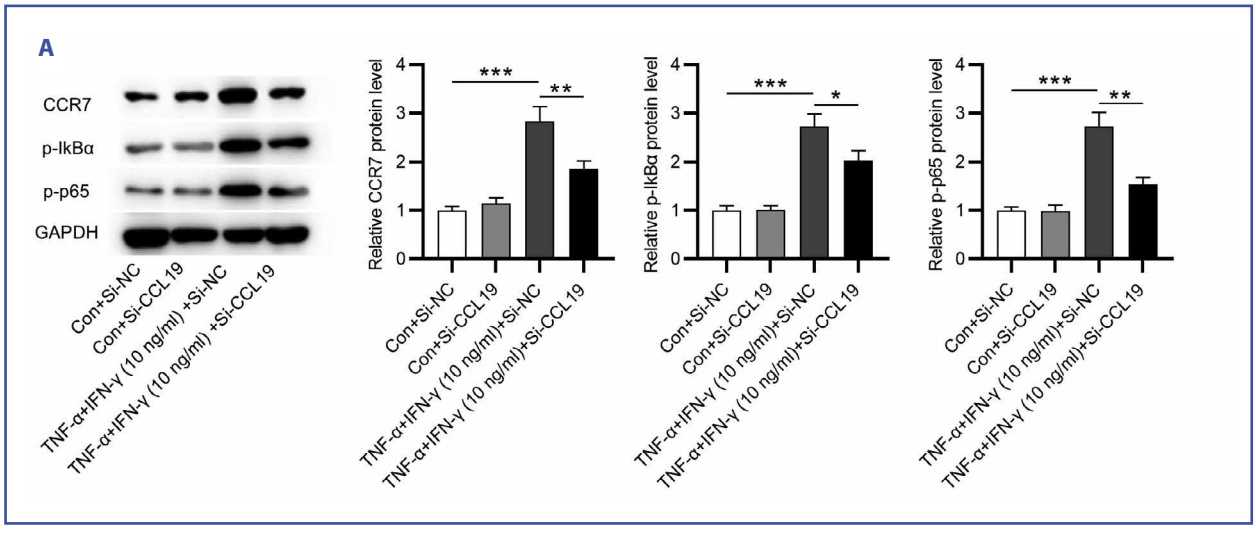

Figure 3. CCL19 activates CCR7/NF-κB signalling in TNF-α + IFN-γ-treated HaCaT cells. **A.** CCR7, p-IkBα and p-p65 levels in con + si-NC group, con + si-CCL19 group, TNF-α (10 ng/mL) + IFN-γ (10 ng/mL) + si-NC group, and TNF-α (10 ng/mL) + IFN-γ (10 ng/mL) + si-CCL19 group detected by Western blotting. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data displayed as means ± SD. Abbreviations: GAPDH — glyceraldehyde-3-phosphate dehydrogenase; SD — standard deviation.

oxide and PGE2 were significantly decreased by CCL19 knockdown, while CCR7 overexpression reversed that effect (Figs. 4C, D). Moreover, CCR7 overexpression offset the effect of CCL19 downregulation on the concentrations of TARC and IL-6 in TNF-α + IFN-γ-stimulated HaCaT cells (Figs. 4E, F). Similarly, Western blotting indicated that CCL19 silencing inhibited iNOS and COX-2 proteins, while CCR7 overexpression enhanced these proteins (Fig. 4G). Overall, we showed that CCL19 promotes the inflammatory response in TNF-α + IFN-γ-induced HaCaT cells by upregulating CCR7.

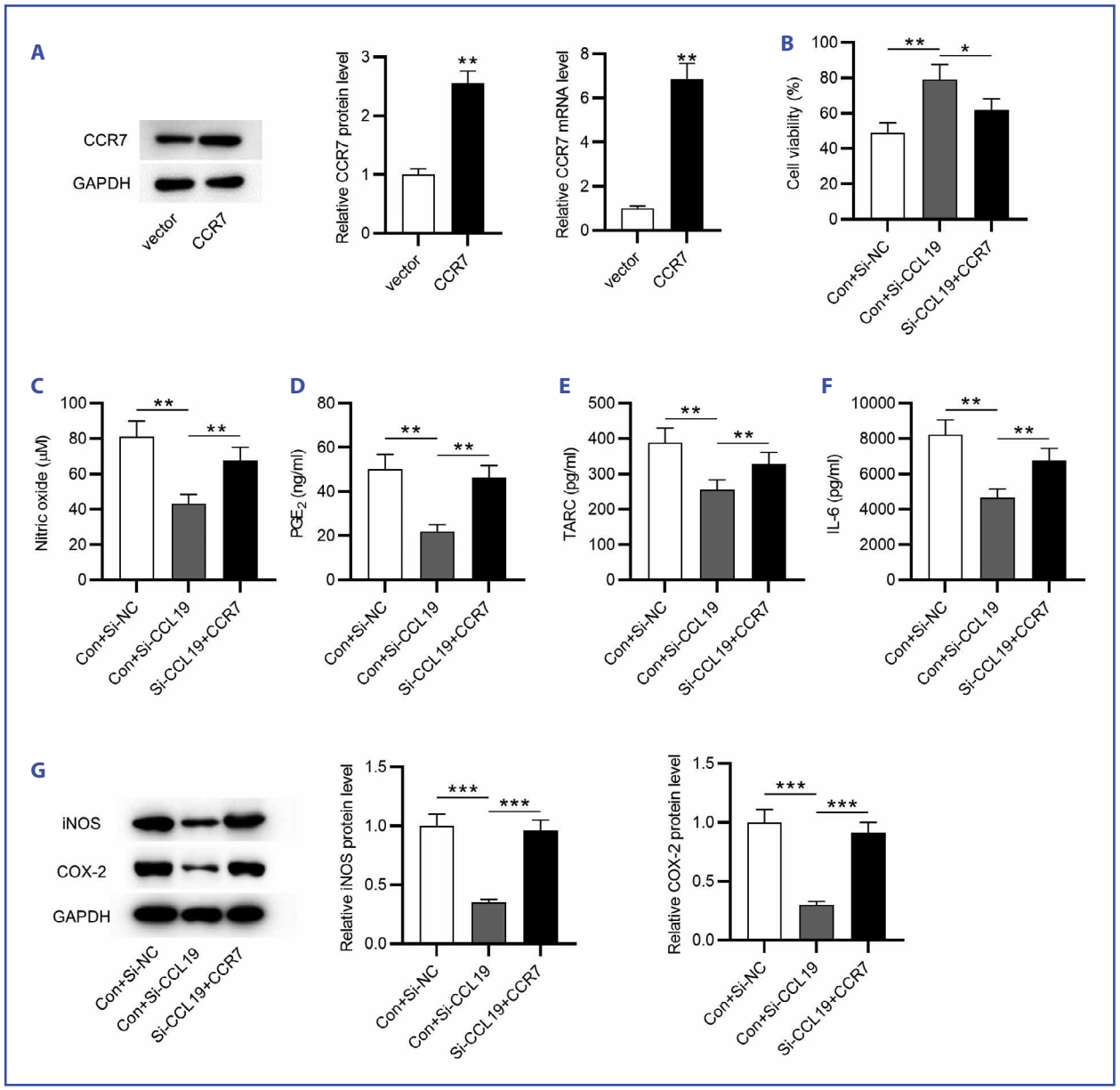

Figure 4. CCL19 promotes inflammatory response in TNF-α+IFN-γ-induced HaCaT cells by upregulating CCR7. **A.** Western blotting and RT-qPCR were used to estimate protein and mRNA levels of CCR7, respectively, in TNF-α + IFN-γ-induced HaCaT cells transfected with empty vector denoted as 'vector' or pcDNA3.1-CCR7 vector (denoted as 'CCR7'). **B.** HaCaT cells' viability assessed by MTT assay in transfected cells: con + si-NC group, con + si-CCL19 group, and si-CCL19 + CCR7 group. **C, D.** Content of nitric oxide and PGE2 in HaCaT cells were detected by ELISA. **E, F.** Concentrations of TARC and IL-6 in cell supernatants of cells treated as described in B and C were measured by ELISA. **G.** Protein level of iNOS and COX-2 in cells described in B and C were determinated by Western blotting. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data are displayed as means ± SD. Abbreviations: COX-2 — cyclooxygenase 2; GAPDH — glyceraldehyde-3-phosphate dehydrogenase; HaCaT — human immortalised keratinocytes; iNOS — induced nitric oxide synthase; SD — standard deviation; TARC — thymus and activation-regulated chemokine.

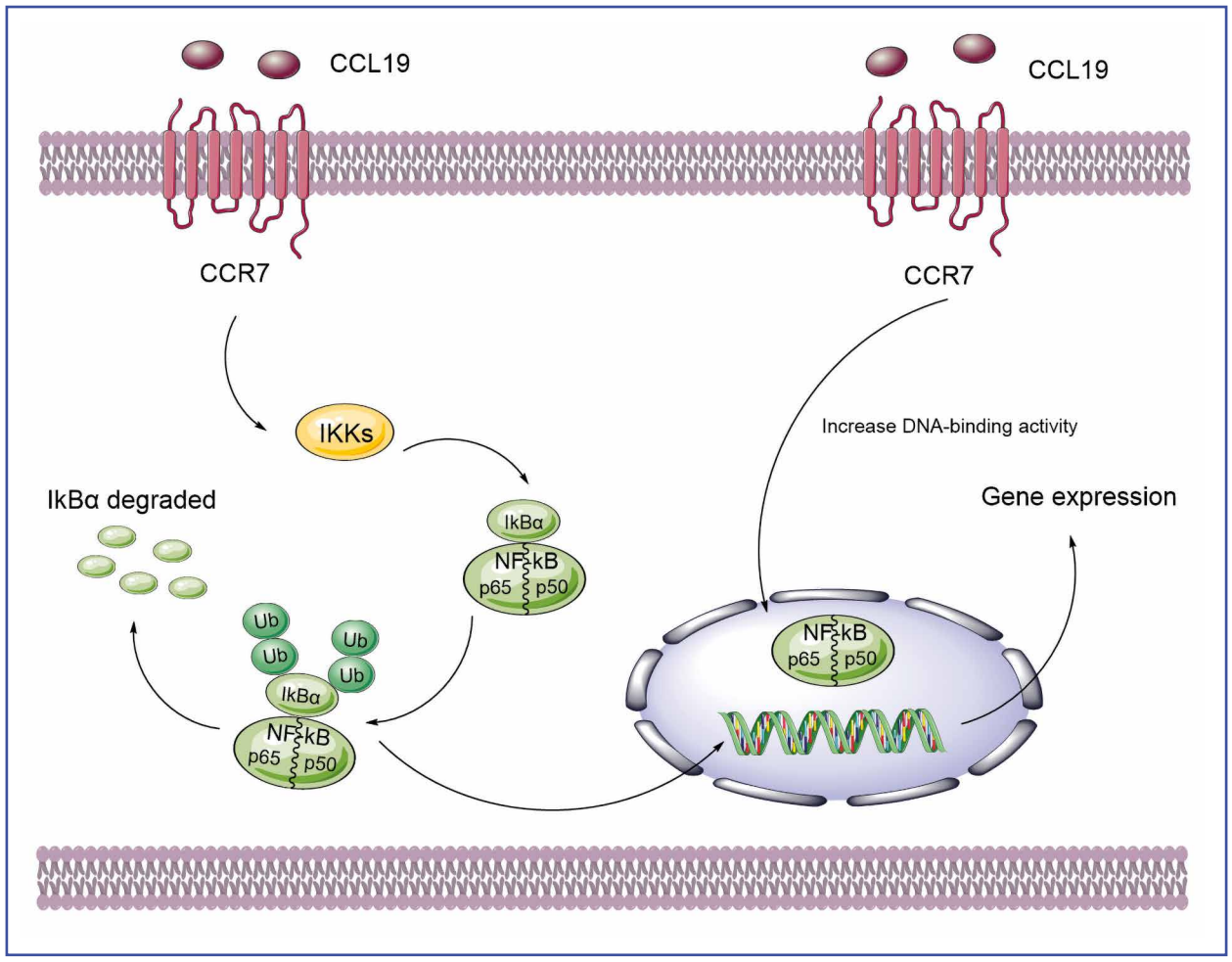

Figure 5. Schematic diagram of interactions between CCL19, CCR7 and NF-κB signalling. In human immortalised keratinocytes (HaCaT cells) induced with TNF-α and IFN-γ, CCL19 interacted with CCR7 to activate I-kappaB kinases (IKKs). IKKs phosphorylated IκBα in IκBα-NF-κB complex in cells, and as a result IκBα was ubiquitinated and degraded. Thereafter, NF-κB complex made of two subunits entered nucleus, thereby promoting DNA-binding capacity and gene expression.

Interaction between CCL19, CCR7 and NF-κB signalling

Our results can be summarised in the following way: in the TNF-α + IFN-γ-induced HaCaT cells, CCL19 interacted with CCR7 to activate I-kappaB kinases (IKKs). IKKs phosphorylated IκBα in the IκBα-NF-κB complex in cells, so that IκBα was ubiquitinated and degraded. Then, NF-κB entered the nucleus, thereby promoting DNA-binding capacity and gene expression (Fig. 5).

Discussion

Atopic dermatitis is a chronic pruritus and inflammatory skin disease related to heredity [25]. Like asthma and allergic rhinitis, it belongs to the category of allergic diseases [25, 26]. Skin inflammation is caused by skin barrier injury, anomalous cells, and the infiltration of T cells into the dermis [27]. A growing number of reports have indicated that IFN-γ and TNF-α can stimulate keratinocytes, so as to activate different signalling pathways and participate in promoting inflammation [28]. Thus, IFN-γ/TNF-α treatment is frequently used as the inducing method for studying *in vitro* models of skin inflammatory responses [28, 29]. When the skin barrier is injured, stimulated keratinocytes promote the production of abundant cytokines and chemokines [30]. In our study, we stimulated human immortalised keratinocytes using TNF-α together with IFN-γ. The results proved that the treatment of TNF-α + IFN-γ markedly induced cytotoxicity and repressed cell viability of HaCaT cells, suggesting that an *in vitro* AD cell model was effectively established.

Chemokines are small proteins which stimulate the recruitment of leukocytes [31]. Many chemokines have been suggested to be maladjusted in AD and have the potential to be biomarkers of AD [11]. For example, CCL17 has been shown to function as a key chemokine in the progression of AD and can serve as a dependable biomarker [32].

CCR4 can facilitate AD development by promoting Th2 cell infiltration [33]. Although previous studies have confirmed that CCL19 and CCR7 can interact with AD-relevant proteins [21, 22], their specific roles in AD have not yet been elucidated. We detected the levels of CCL19 and CCR7 in HaCaT cells. The results showed that treatment with both TNF-α and IFN-γ significantly upregulated levels of CCL19 and CCR7 in HaCaT cells. Some chemokines are considered to play a pro-inflammatory role, and their release can be induced during an immune response at a site of infection [34]. Thus, we further estimated the effect of CCL19 on inflammation response.

We found that CCL19 knockdown repressed the protein levels of the proinflammatory enzymes, iNOS and COX-2, and the levels of their products (nitric oxide and PGE2), and the release of proinflammatory factors (TARC and IL-6) caused by TNF-α + IFN-γ stimulation of HaCaT cells. Moreover, cytotoxicity induced by TNF-α + IFN-γ was also repressed by CCL19 knockdown. Therefore, we showed that CCL19 accelerated TNF-α + IFN-γ-induced inflammatory responses in HaCaT cells. CCR7 is mainly expressed on the surface of dendritic cells, T-lymphocytes and B-lymphocytes and it can regulate cell survival and migration [35]. It has been reported that CCR7 can be considered as a potential therapeutic target in allergies [20].

In this study, we found that overexpression of CCR7 offset the inhibitory effects of CCL19 knockdown on nitric oxide, PGE2, iNOS, COX-2, TARC and IL-6 levels in TNF-α + IFN-γ-induced HaCaT cells. Our findings suggest that CCL19 exerts a proinflammatory effect in TNF-α + IFN-γ-induced HaCaT cells by upregulating CCR7. The interaction of CCL19 and CCR7 has been confirmed in a colorectal cancer model [36]. For example, CCL17 combined with CCL19 as a nasal adjuvant enhances the immunogenicity of an anti-caries DNA vaccine in rodents [37]. CCL19 has been shown to inhibit gastric cancer cell proliferation *via* the CCL19/CCR7/AIM2 pathway [38]. We have demonstrated an interaction between CCL19 and CCR7 in an *in vitro* model of AD for the first time.

NF-κB is a crucial transcription factor in immune and inflammatory reactions, and it participates in skin diseases by regulating the transcription of different proinflammatory factors in AD [39, 40]. For example, diisononyl phthalate expedites AD development in mice by activating NF-κB [40]. Paeoniflorin facilitates the progression of AD by repressing the NF-κB/IκBα signalling pathway in T lymphocytes [41]. A previous study revealed that CCR7 promoter includes the potential binding site for NF-κB, which plays a key role in various inflammatory diseases [42]. For example, SIRT1 can suppress atherosclerosis formation in a U937 cell model regulating the CCR7/NF-κB pathway [43]. The IKKs, p65/p50 complex, and IκB, are key factors controlling the NF-κB pathway [44]. In our study, we discovered that CCL19 depletion repressed p-IkBα and p-p65 levels in TNF-α + IFN-γ-induced HaCaT cells *via* regulating CCR7, suggesting that the NF-κB signalling pathway was activated by CCL19.

Overall, our study has shown that CCL19 promotes TNF-α + IFN-γ-induced inflammatory responses by targeting CCR7/NF-κB signalling in cultured keratinocytes. This new finding provides a robust theoretical and experimental foundation for the investigation of CCL19's role in animal models of atopic dermatitis. The main limitation of our study is the lack of an *in vivo* animal assay, and this will become the focus of our future research.

Article information and declarations

Data availability statement

The datasets used or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics statement

Not applicable.

Authors' contributions

YZ: conceived and designed study; YZ, XN, YD: analysed data; YZ, YD: drafted manuscript. All authors agreed to be accountable for all aspects of work. All authors read and approved final manuscript.

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Conflicts of interest

No conflicts of financial interest are enclosed in this study.

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