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ORIGINAL ARTICLE

GDF11 Antagonizes Psoriasis-like Skin Inflammation *via* Suppression of NF-κB Signaling Pathway

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Abstract— Growth differentiation factor-11 (GDF11) is a key member of the transforming growth factor β (TGF-β) superfamily, which plays a momentous role in both normal physiological processes and pathophysiology processes. Recently, it was reported that GDF11 was closely associated with several inflammatory conditions and protected against development of inflammation. Psoriasis-like skin inflammation is a common skin inflammatory disease, yet much is unknown about the underlying mechanisms. In this study, we investigated the expression pattern of GDF11 in two psoriasis-like skin inflammation mice models and tumor necrosis factor-α (TNF-α)-induced RAW264.7 macrophages. Furthermore, RAW264.7 cell was cultured, and GDF11 antagonized the inflammatory function of TNF-α *in vitro*. Moreover, imiquimod-induced mice model and IL-23-induced mice model were established to investigate the anti-inflammatory role of GDF11 *in vivo*. As a result, the administration of GDF11 remarkably attenuated the severity of skin inflammation in both two mice models. Additionally, the activation of nuclear NF-κB (nuclear factor κ-light-chain-enhancer of activated B cells) signaling pathway was repressed by GDF11 treatment. Collectively, GDF11 may represent a promising molecular target for the prevention and treatment of psoriasis-like skin inflammation.

KEY WORDS: GDF11; psoriasis; inflammation; NF-κB signaling pathway.

INTRODUCTION

Growth differentiation factor-11 (GDF11) is a key member of the transforming growth factor β (TGF-β) superfamily, which is also known as bone morphogenetic protein-11 (BMP-11) [1, 2]. Since 1999, GDF11 was proved that it played a momentous role in both normal physiological processes and pathophysiology processes, such as embryonic development, erythropoiesis, cardiovascular disease, and diabetes mellitus [3, 4]. Recently, many scholars hold the view that GDF11 was connected with the pathophysiology of aging [5, 6]. Though there was also some evidence argue against it, more and more scholars concentrated themselves on GDF11. Moreover, future studies are needed to prove the potential beneficial effects of GDF11 on pathophysiology processes. Recently, a research confirmed that GDF11 inhibited palmitic acid-induced inflammatory response in RAW264.7 macrophages and it shows a protective role in endotheliocyte [7], which suggested that GDF11 might inhibit

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inflammatory reaction and play a protective role in tissue suffered by inflammatory cytokines.

The skin is the largest organ in the body, which forms a barrier between the body and the environment [8]. It is the first biological barrier to protect the body against attack from bacteria and virus [9, 10]. Thus, the skin is always attacked by physicochemical factors, radial and microorganism [10]. Psoriasis-like skin inflammation is a common skin disorder in clinic which trouble patients with cutaneous pruritus and skin lesions [11, 12]. Although the etiological agent of skin inflammation is always associated with external stimulus and immunoreaction, the underlying mechanism still remains to be elucidated [13]. As previous reported, TNF-α plays a key role in inflammatory diseases, including skin inflammation [7, 14]. Moreover, TNF-α is released by activated macrophage and it is a predominant cytokines for activation of macrophage, which forms a regenerative feedback during inflammatory reaction [15, 16]. Additionally, activation of macrophage has been reported for various autoimmune inflammatory diseases including skin inflammation [17, 18]. Recent research proved that NF-κB pathway, which is associated well with inflammatory reactions, was suppressed by GDF11 in atherosclerosis [19]. Moreover, TNF-α was activated in inflammatory reaction through NF-κB signaling pathway, which has been reported in our early work [14, 20, 21]. The findings from these studies, together with the involvement of TNF-α/NF-κB signaling pathway in psoriasiform skin inflammation, prompted us to study the potential role of GDF11 in skin inflammation. In this study, we examined the involvement of GDF11 in TNF-α mediated inflammation *in vitro*, and determined the potential therapeutic function of GDF11 in psoriasiform skin inflammation mice model.

MATERIALS AND METHODS

Cell Culture

Mouse leukemic monocyte macrophages (RAW264.7) used for this study were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). It was cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) which contain fetal bovine serum (10%) (Gibco, USA), penicillin (1%) (HyClone, UT, USA) streptomycin (1%) (HyClone, UT, USA). RSBiotech (Thermo Scientific, Shanghai, China) provide a situation with 37 °C, 5% CO2, and proper humidity for cells.

Nitric Oxide Production Assay—Griess Method

To verify the anti-inflammatory action of GDF11 in RAW264.7 macrophages, nitric oxide (NO) production was tested by using Griess reagent kit (Sigma Corporation, USA) [14]. In short, RAW264.7 cells were cultured with 10 ng/ml TNF-α (Peprotech Biotechnology, USA) in the 96-well plates (1×10^5 cells/well) for 24 h in the presence or absence GDF11 recombinant protein (50 ng/ml). Next, the medium supernatant was collected and mixed with Griess reagent to incubate for 30 min in dark as protocol. The absorbance was measured by microplate reader (Thermo Corporation, USA) at 550 nm.

Inflammation Biomarker Level Assay *In Vitro*

To verify the anti-inflammatory action of GDF11 in RAW264.7 macrophages, levels of inflammation biomarkers were tested by real-time PCR. After washed by germfree phosphate buffer saline (PBS), cells were planted in 96-well plates (1×10^6 cells/well) and incubated overnight. Then, we divided them into three control groups and three experimental groups. Control group 1 was treated with the culture medium without any addition. Control group 2 was cultured with GDF11 (10 ng/ml). At the same time, experimental groups were treated with GDF11 in concentration of 5, 25, 50, 100, and 200 ng/ml in the presence of TNF-α (10 μg/ml). After 12 h, total RNA was collected from all control and experimental groups, and PCR were performed as described below. Moreover, total protein was extracted from each group after 72 h, and western blot followed.

Transfection of miRNA-GDF11 and SN50 Treatment

RAW264.7 macrophages were transfected with miRNA-GDF11 and negative control (GENECHEM, Shanghai, China) at a final concentration of 100 nM, using the transfection reagent HiPerfect (Qiagen). After transfection, RAW264.7 cells were cultured with 10 μg/ml TNF-α with or without 18 μM SN50 (Medchemexpress, USA). Then, total RNA and protein were collected for the next test.

Animals

Nine-week-old WT BL6/C57 male mice used for these experiments were purchased from Shandong University Laboratory Animal Center (Jinan, Shandong, China). All experimental mice were housed under specific pathogen-free (SPF) conditions with standard rodent food

and water conformed to the Institutional Animal Care and Use Committee of Shandong University.

IMQ-Induced Skin Inflammation Mice Models

Imiquimod (IMQ)-induced skin inflammation mice models were established in 9-week-old WT BL6/C57 male mice as previously reported [22]. Then, the dorsal skin of mice was shaved by razor and depilatory cream. The mice ($n = 7$) in experimental group were daily bepainted with 62.5 mg IMQ cream (5% imiquimod) in shaved dorsal skin and received intraperitoneal injection of recombinant GDF11 (0.1 mg/kg) every day for 1 week. While the mice ($n = 7$) in skin inflammation group were treated with the same methods of IMQ, but injected with same volume of germfree phosphate buffer saline (PBS). At the same times, the mice ($n = 7$) in PBS group were treated with vaseline cream and injected with PBS. Based on Psoriasis Area Severity Index, an evaluation to distinguish the severity of skin inflammation was performed using clinical score as previously reported [23]. Briefly, erythema, scaling, and thickening were scored on a scale from 0 to 4 (0: none; 1: slight; 2: moderate; 3: marked; and 4: severe). The cumulative score (scale 0–12) of these three parameters was showed to evaluate the severity of skin inflammation. Two observers, blinded to the experimental protocol, carried out assessment of tissue changes on coded samples. After 1 week, the mice from all groups were euthanized by excess 10% chloral hydrate (Qilu Hospital, China), and the serum was extracted for ELISA; the ears were collected for protein and total RNA.

IL-23-Induced Skin Inflammation Mice Models

The method to establish interleukin-23 (IL-23)-induced skin inflammation mice models has been reported earlier [24]. The ear from mice ($n = 14$) were injected intradermally with 10 μl IL-23 (50 μg/ml) at day 0, while the mice in PBS group ($n = 7$) were injected intradermally with same volume of germfree phosphate buffer saline (PBS). To verify the therapeutic role of GDF11 in IL-23-induced skin inflammation mice models, the experimental group ($n = 7$) received an intraperitoneal injection of recombinant GDF11 (0.1 mg/kg) every day for 7 days, while the skin inflammation group ($n = 7$) was treated same volume of germfree phosphate buffer saline (PBS). At the same times, the thickness of the ears was measured by Digital Vernier caliper every day till to harvest. After 7 days, the tissue of the mice ears was collected as described above for next test.

Real-Time PCR

Total RNA was extracted from RAW264.7 cells and tissues mentioned above using an RNAeasy kit (Beyotine Biotechnology Corporation, Shanghai, P.R. China). Then, the reverse transcription followed by using a RT-for-PCR kit (Vazyme Corporation, Nanjing, P.R. China) as protocol described. Sequence-specific primers in this experiment are synthesized by (Sangon Company, Shanghai, P.R. China) and listed in Table 1. Each single-specific PCR product was verified by melting curve analysis and the experiments were repeated more than three times.

Western Blot

Total protein was extracted from RAW264.7 cells, ears, and dorsal skins in each experimental and control group by using RIPA Lysis Buffer (Beyotine Biotechnology Corporation, Shanghai, P.R. China) mixed with 1% PMSF (phenylmethanesulfonyl fluoride) (Beyotine Biotechnology Corporation, Shanghai, P.R. China) and 1% protease and phosphatase inhibitor cocktail for general use (Beyotine Biotechnology Corporation, Shanghai, P.R. China). Next, western blot was performed as described in our previous published. Proteins were resolved on a 10% SDS-polyacrylamide gel (Beyotine Biotechnology Corporation, Shanghai, P.R. China) and electroblotted onto a nitrocellulose membrane (Beyotine Biotechnology Corporation, Shanghai, P.R. China). After blocking in 5% nonfat dry milk in Tris buffer-saline-Tween 20 (10 mM Tris-HCl, pH 8.0; 150 mM NaCl; and 0.5% Tween 20), blots were incubated with polyclonal anti-phosphorylated IκB-α (pIκB-α), anti-NIK (diluted 1:2000), or anti-iNOS (diluted 1:1000) antibody for 1 h. After washing, the secondary antibody (horseradish peroxidase-conjugated anti-rabbit immunoglobulin; 1:2000 dilution) was added, and bound antibody was visualized using an enhanced chemiluminescence system (abcam Corporation, USA).

Immunofluorescence

In this study, RAW264.7 cells were selected to verify the expression of p65 with immunofluorescence staining. According to our previous study presented [25], with minor modifications, RAW264.7 cells were stimulated with 10 ng/ml TNF-α in the presence or absence of GDF11 for 1 h. Immunofluorescence staining of NF-κB p65 was then performed on these cells, examined using a confocal fluorescence microscope system.

Table 1. Sequence-Specific Primers in This Experiment Are Synthesized by (Sangon Company, Shanghai, P.R. China)

Gene symbol	Primer sequences
IL-1β (mouse)	5′-AATCTCACAGCAGCACATCA-3′; 5′-AAGGTGCTCATGTCCTCATC-3′
IL-6 (mouse)	5′-CCTTCCTACCCCAATTTCCAAT-3′; 5′-GCCACTCCTTCTGTGACTCCAG-3′
iNOS (mouse)	5′-ACA GGAGGGGTTAAAGCTGC-3′; 5′-TTGTCTCCAAGGGACCAGG-3′
NF-κB2 (mouse)	5′-TACAAGCTGGCTGGTGGGGA-3′; 5′-GTCGCGGGTCTCAGGACCTT-3′
GAPDH (mouse)	5′-AGAACATCATCCCTGCATCC-3′; 5′-AGTTGCTGTTGAAGTCGC-3′
COX-2 (mouse)	5′-AACATCTCAGACGCTCAGGAAATAG-3′; 5′-GCCGTAGTCGGTGTACTCGTAG-3′
TNF-α (mouse)	5′-AGGGTCTGGGCCATAGAACT-3′; 5′-CCACCACGCTCTTCTGTCTAC-3′
NIK (mouse)	5′-TCTCTGGAGGAACAGGAACAA-3′; 5′-GCCATTGAGAGACTGGATCTG-3′

Histology

Mice ears and skins from all groups were fixed in 10% formalin during more than 72 h at room temperature. After embedding by paraffin, at least four consecutive 6 μm sections were obtained from the sagittal planes, and stained using hematoxylin and eosin (HE) for routine morphologic analysis. The epidermal thickness was examined using OsteoMeasure software (OsteoMetrics, Inc., Decatur, GA). For immunohistochemistry of indicated biomarkers, sections were pretreated with Improved Citrate Antigen Retrieval Solution (Beyotine Biotechnology Corporation, Shanghai, P.R. China). To reduce nonspecific staining, sections were blocked with 10% normal goat serum (Beyotine Biotechnology Corporation, Shanghai, P.R. China) for 30 min at room temperature. Next, as protocol described in Immunohistochemistry Kit (ZSGB-BIO Company, Beijing, P.R. China), sections were incubated with polyclonal anti-CD4 or anti-CD68 serum (1:100 dilution; Santa Cruz Biotechnology) at 4 °C overnight, and horseradish peroxidase-conjugated secondary antibody followed for 60 min in room temperature. The signal was detected by using the DAB kit (ZSGB-BIO Company, Beijing, P.R. China).

ELISA Assays both *In Vitro* and *In Vivo*

Conditional medium of RAW264.7 cells was collected after centrifugation at 3600×*g* for 10 min at 4 °C. The serum was extracted from mice blood collected using pro-coagulation tube in all groups by using the centrifuge at 3600×*g* for 10 min at 4 °C. IL-1β, TNF-α, and IL-6 were assayed in all samples described above using a commercial kit (Beyotine Biotechnology Corporation, Shanghai, P.R. China), according to the instructions of the manufacturer. The signal was detected by using the microplate reader (Thermo Fisher Corporation, USA) at 540 nm.

Statistical Analysis

The data were expressed as mean ± standard deviation (SD). The Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL) was used for standard statistical analysis including one-way ANOVA and Student's *t* test. Statistical significance was achieved when a value of $p < 0.05$.

RESULTS

GDF11 Inhibited TNF-α-Mediated Inflammatory Reaction in Macrophage

It is a widely held view that macrophage plays a critical role in development of skin inflammation [26, 27]. TNF-αis released by activated macrophage and it is also one of the most important cytokines for activation of macrophage, which form a regenerative feedback in inflammatory reaction [28, 29]. To investigate the positive effect of GDF11 in inflammatory reaction of macrophage, a stable macrophage cell line, RAW264.7 cells, was cultured and performed as described above. As previously reported, IL-1β, IL-6, iNOS, and COX2 were contributing cytokines in skin inflammation [30]. In Fig. 1a–d, we found that recombinant GDF11 remarkably reduced the mRNA levels of IL-1β, IL-6, iNOS, and COX2 in inflammatory reaction caused by TNF-α in macrophage in a dose-dependent manner. Furthermore, iNOS and COX2 were critical mediators for oxidative stress in skin inflammation, which could synthesize NO to lead to tissue damage in inflammatory reaction. Therefore, to investigate the expression profiling of iNOS and COX2 in presence or absence of recombinant GDF11 in inflammatory reaction, western blot was performed, and showed that recombinant GDF11 largely lowered the expression level of iNOS and COX2 in RAW264.7 macrophage cell line (Fig. 1e, f). Moreover, after centrifugation, conditional medium was

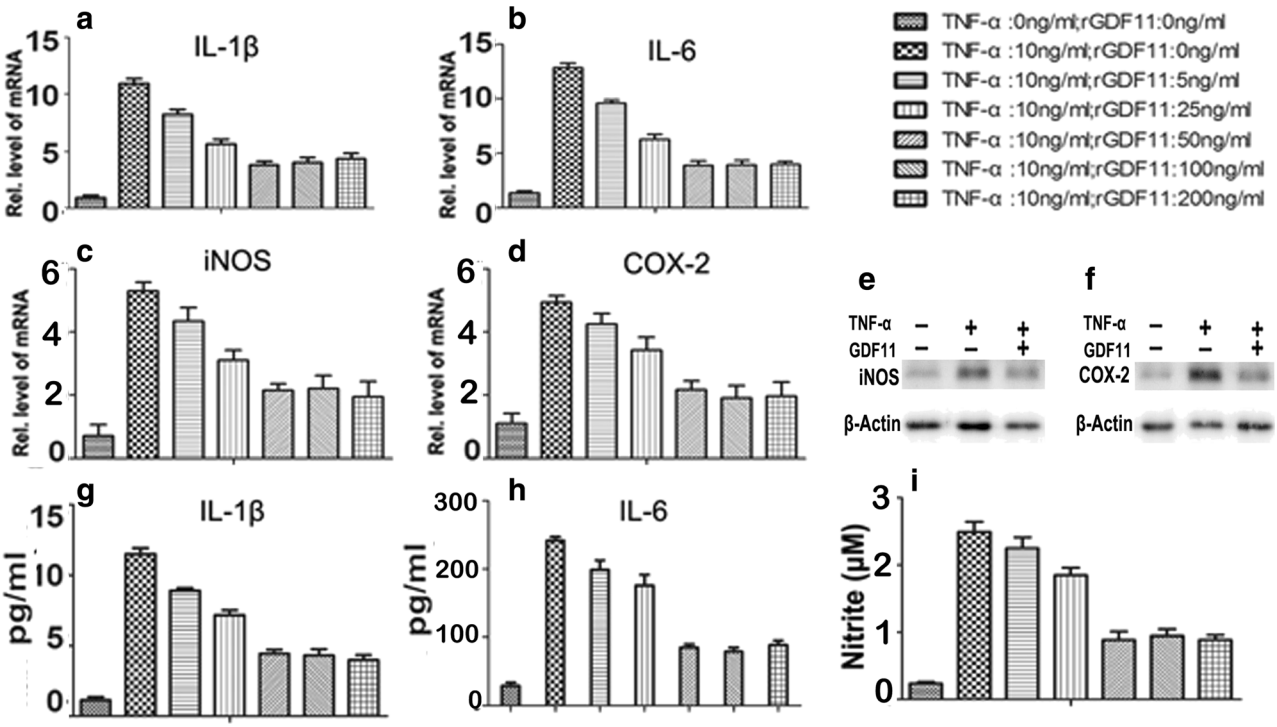

Fig. 1. GDF11 inhibited TNF-α-mediated inflammatory reaction in macrophage. **a**–**d** The mRNA levels of inflammatory cytokines, including IL-1β, IL-6, iNOS, and COX-2, produced after stimulation of TNF-α in presence or absence of rGDF11, were tested by real-time PCR. **e**, **f** The expression of inflammatory cytokines, including iNOS and COX-2, was assayed by western blot. **g**, **h** The levels of circulatory IL-1β and IL-6 in serum were tested by ELISA. **i** The level of NO released in conditional medium was tested by Griess reagent. The values are the mean ± SD. $*p < 0.05$, $**p < 0.01$, and $***p < 0.005$ *vs.* vehicle control group.

performed with ELISA. Figure 1g, h shows lower levels of IL-1β and IL-6 when the RAW264.7 cells were stimulated by TNF-α in presence of recombinant GDF11, which indicated that rGDF11 could suppress the inflammatory factor release caused by TNF-α in macrophage. To evaluate the level of NO released by macrophage, Greiss experiment was performed as described above and Fig. 1i shows diminished NO release in presence of recombinant GDF11 in a dose-dependent manner.

GDF11 Played Therapeutic Role in IMQ-Induced Skin Inflammation Mice Model

In order to assess whether GDF11 play a positive role in inflammatory skin disease, IMQ-induced skin inflammation mice model was established and treated with rGDF11 or PBS as described above. Figure 2a is the representative picture which showed the therapeutic effect of rGDF11 on mice stimulated by IMQ. Moreover, in IMQ-induced skin inflammation mice model, the clinical score of rGDF11-treated group was remarkably lower than PBS group (Fig. 2b). In histological assessment of the skin, Fig. 2c shows that the infiltration of inflammatory cells and thickening of epithelium were inhibited by recombinant GDF11 in IMQ-induced mice model. GDF11-treated group showed less increase of ear thickness than PBS group, which was measured by Digital Vernier caliper (Fig. 2d). Furthermore, after RNA extracted from ear tissue of each group, real-time PCR performed. The results revealed that the levels of inflammatory parameters, such as IL-1β, TNF-α, COX2, iNOS, and IL-6, were lower in recombinant GDF11 injection group remarkably (Fig. 2e–i). Furthermore, total protein was collected from the ears of all groups, followed by western blotting for iNOS and COX2 level. Recombinant GDF11 treatment significantly reduced the level of iNOS and COX2 protein (Fig. 2j, k). Moreover, to assess the circulating levels of IL-1β, TNF-α, and IL-6, sera were collected from all groups and ELISA performed as described above. As revealed in Fig. 2l–n, levels of inflammatory cytokines were significantly diminished through additional treatment of recombinant GDF11.

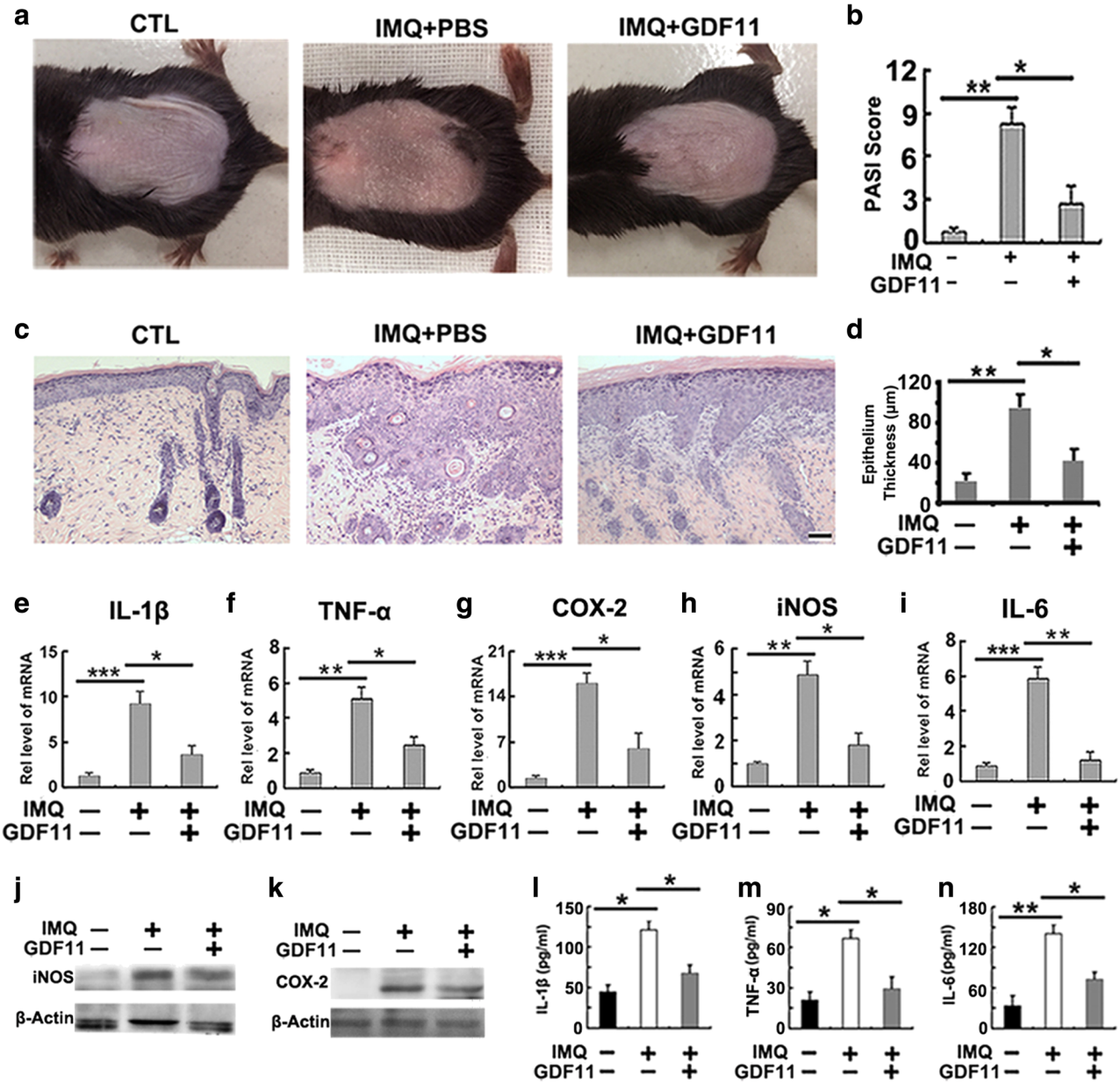

Fig. 2. GDF11 played therapeutic role in IMQ-induced skin inflammation mice model. **a** Representative pictures of dorsal skin following IMQ stimulation and therapy of rGDF11 or PBS. **b** The clinical scores of the skin in the three groups. **c** The severity of inflammation in the skin following IMQ stimulation and therapy of GDF11 or PBS, based on HE staining. Scale bar, 100 μm. **d** The thickness of the skin was measured and compared between PBS and rGDF11 treatment groups. **e**–**i** The mRNA levels of inflammatory cytokines in the skin, including IL-1β, TNF-α, COX-2, iNOS, and IL-6 were measured by real-time PCR. **j**, **k** The expression of inflammatory cytokines, including iNOS and COX-2, was assayed by western blot. **l**–**n** The circulating levels of IL-1β, TNF-α, and IL-6 in serum were measured by ELISA. The values are the mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.005$ *vs.* vehicle control group.

The Therapeutic Effect of GDF11 on IL-23-Induced Skin Inflammation Model

As described above, IL-23-induced skin inflammation model was established to further investigate the anti-inflammatory effect *in vitro*. As Fig. 3a, b indicates, the infiltration of inflammatory cells and thickening of epithelium were inhibited by recombinant GDF11 in histological assessment of ear lobules. To further investigate whether

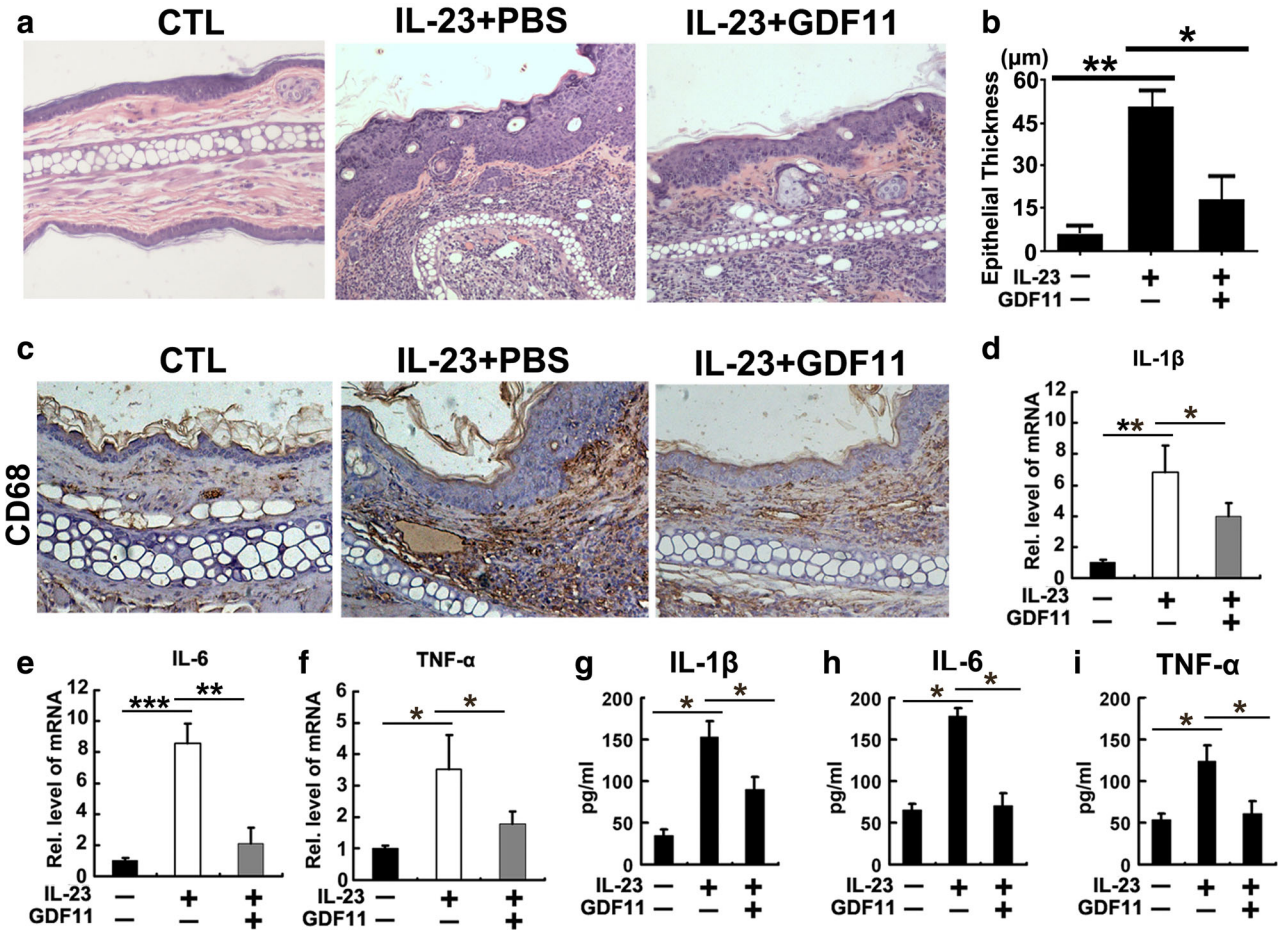

Fig. 3. The therapeutic effect of GDF11 on IL-23-induced skin inflammation model. **a**, **b** GDF11 treatment inhibited increase of ear thickness in IL-23-induced skin inflammation, detected by HE staining. **c** The expression of CD68 was measured by immunohistochemistry. Scale bar, 50 μm. **d**–**f** The mRNA levels of inflammatory cytokines in ear lobes, including IL-1β, IL-6, and TNF-α, were measured by real-time PCR. **g**–**i** The circulating levels of IL-1β, IL-6, and TNF-αin serum were measured by ELISA. The values are the mean ± SD. $*p < 0.05$, $**p < 0.01$, and $***p < 0.005$ *vs.* vehicle control group.

recombinant GDF11 inhibit the release of inflammatory cytokines *in vitro* or not, immunohistochemistry was performed for iNOS. As described above, it is well accepted that in dermatitis, inflammatory cells are accumulated in the skin, especially macrophage. To investigate whether recombinant GDF11 inhibit the infiltration of macrophage in skin inflammation or not, immunohistochemistry was performed for CD68. In Fig. 3c, GDF11 antagonized the accumulation of macrophage in ear samples remarkably. Moreover, after RNA extracted from ear tissue of all groups, real-time PCR performed. The results revealed that the levels of inflammatory parameters, such as IL-1β, IL-6, and TNF-α, were lower in recombinant GDF11 injection group remarkably (Fig. 3d–f). Furthermore, sera were collected from all groups and ELISA performed as described above. As revealed in Fig. 3g–i, levels of IL-1β, IL-6, and TNF-α were significantly diminished through additional treatment of recombinant GDF11.

GDF11 Antagonized Activation of NF-κB Signaling Pathway both *In Vivo* and *In Vitro*

It is well accepted that NF-κB signaling pathway is the key pathway in inflammatory disease include psoriasis-like skin inflammation [31]. Moreover, recent study proved that GDF11inhibit activation of NF-κB signaling pathway in atherosclerosis, which prompted us to determine whether GDF11 affected NF-κB signaling pathway in skin inflammation [7]. RAW264.7 cells were cultured in stimulation of TNF-α for 1 h, with or without treatment of

rGDF11. Total RNA was extracted and the NF-κB2 levels were measured by real-time PCR. As Fig. 4a shows, the mRNA level of NF-κB2 in RAW264.7 cells was significantly lowered by rGDF11. NF-κB-inducing kinase (NIK) is also known as critical component for the activation of NF-κB signaling. In this study, total RNA was extracted from each indicated group, and real-time PCR was performed; the results implied that the mRNA expression of NIK was abolished by rGDF11 addition (Fig. 4b). Western blot analysis also showed that GDF11 was found to reverse the TNF-α-induced enhanced expression of NIK and p52 (Fig. 4c). Moreover, western blot was performed for pIκB, which was a critical parameter for activation of NF-κB signaling. As revealed in Fig. 4d, activation of NF-κB signaling pathway significantly diminished with the rGDF11 administration. Furthermore, cell immunostaining showed the nuclear translocation of NF-κB p65 was inhibited in the presence of rGDF11 (Fig. 4e). To investigate the role of rGDF11 in activity of NF-κB signaling pathway *in vivo*, the dorsal tissues in IMQ-induced skin inflammation mice model were collected, followed by immunohistochemistry for pIκB-α (Fig. 4f). As a result,

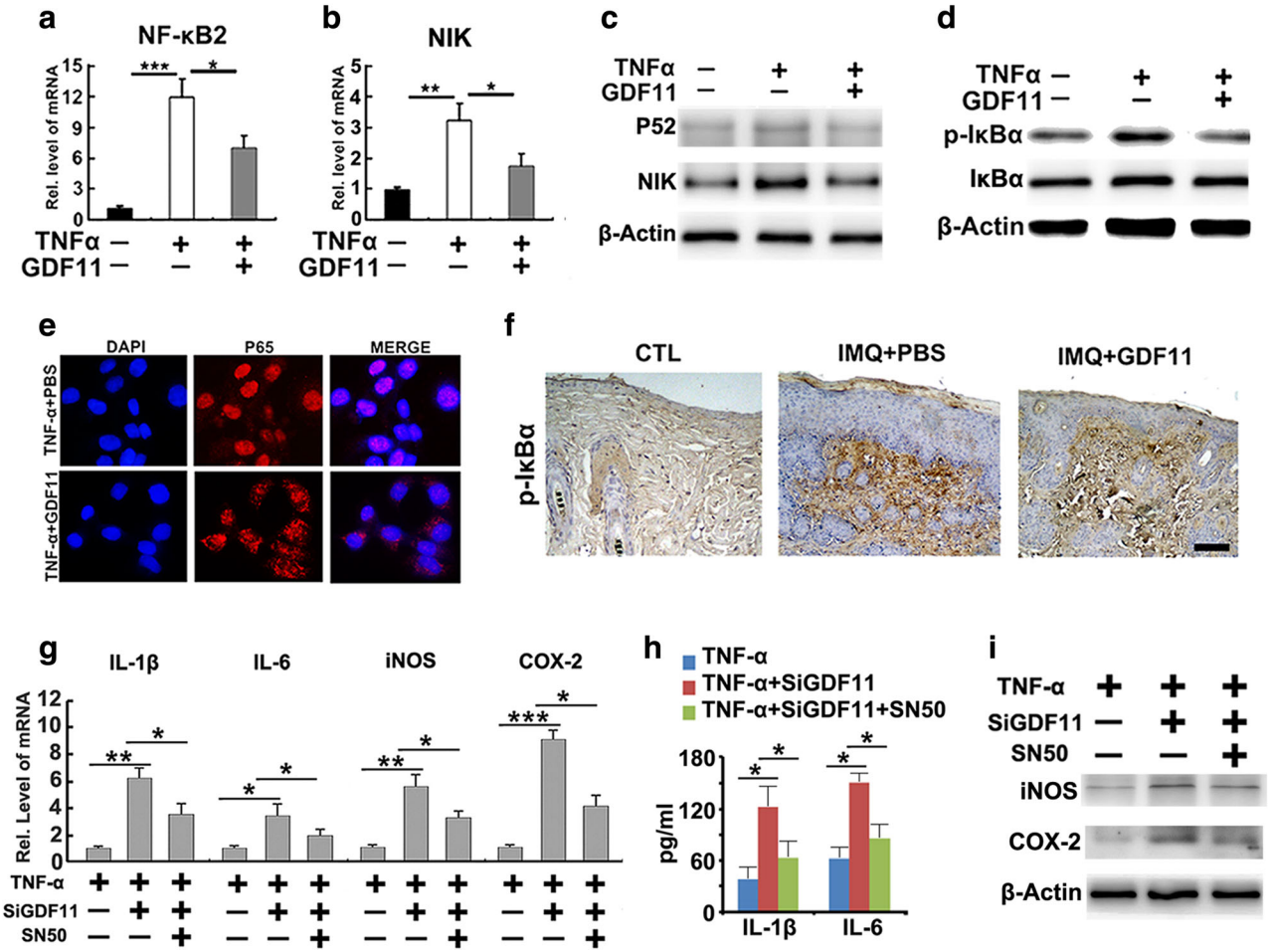

Fig. 4. GDF11 antagonized activation of NF-κB signaling pathway both *in vivo* and *in vitro*. RAW264.7 cells were incubated with TNF-α in the presence or absence of rGDF11. **a**, **b)** The mRNA level of NF-κB2 and NIK in RAW264.7 cells was tested by real-time PCR. **c** The expression of NIK and p52 in RAW264.7 cells, as examined by western blot. **d** The phosphorylation and expression of IκB-α in RAW264.7 cells were determined by western blot. **e** The nuclear translocation of NF-κB p65 in RAW264.7 cells was assayed by immunofluorescence. **f** The expression of phosphorylated IκB-α in the murine skin of each indicated group, performed by immunohistochemistry. **g** In the presence or absence of siRNA-GDF11 and SN50, the mRNA levels of inflammatory biomarkers were tested by real-time PCR. **h** The release of IL-1β and IL-6 in supernatant of RAW264.7 cultured media, as detected by ELISA. **i** The protein expression of iNOS and COX-2 in TNF-α stimulated RAW264.7 cells with selectively co-cultured with SN50 and SiGDF11, as determined by western blot. Scale bar, 50 μm. The values are the mean ± SD. $*p < 0.05$, $**p < 0.01$, and $***p < 0.005$ *vs.* vehicle control group.

rGDF11 administration dampened levels of the mentioned molecules in mice. Furthermore, the mRNA expression levels of inflammatory cytokines comprised IL-1β, IL-6, iNOS, and COX-2 *in vitro* cultured RAW264.7 cells were all examined (Fig. 4g); the data originated from real-time PCR showed that GDF11 knockdown exhibited an exaggerated higher expression of IL-1β, IL-6, iNOS, and COX-2 in TNF-α-stimulated RAW264.7 cells, while SN50, the prominent inhibitor for NF-κB translocation, played an alternative role that reversed these cytokines' expression. Similarly, results from ELISA (IL-1β and IL-6) and western blot (iNOS and COX-2) also were in line with the data above (Fig. 4h, i). Conclusion drawn from the data is presented that GDF11 antagonized activation of NF-κB signaling pathway both *in vivo* and *in vitro*.

DISCUSSION

The major finding of this study described as follows: (1) Exogenous GDF11 could reduce the expression and release of inflammatory cytokines in RAW264.7 cells stimulated by TNF-α. (2) Exogenous recombinant GDF11 play a therapeutic role in skin inflammation in mice induced by imiquimod and IL-23. (3) NF-κB signaling pathway might be closely associated with the therapeutic effect of GDF11 on skin inflammation.

In the early years, GDF11 was considered to have similar effect with GDF-8 because of the 90% protein sequence homology [32]. In 2015, a study was published, which suggested that GDF11 could specifically antagonize aging [33]. Recently, Loffredo et al. proved that GDF11 play an anti-hypertrophic role in aged mice by systemic administration of GDF11 for 28 days [34]. Moreover, Loffredo et al. demonstrated a remarkable dose-dependent decrease in heart weight through a dose titration study in young and aged mice [35]. Furthermore, a recently published article presented that GDF11 decreased endothelial apoptosis *in vivo* and lowered the level of inflammatory biomarkers *in vitro*, which proved the therapeutic effect of GDF11 on atherosclerosis in apoE$^{-/-}$ mice [7]. In the current study, we used both *in vitro* and *in vivo* experiments to investigate the role GDF11 in psoriasis-like skin inflammation.

It is known that macrophage is closely associated with inflammatory reactions including psoriasis-like skin inflammation, which provides a potential therapeutic target for the treatment of psoriasiform skin inflammation [36]. TNF-α is a well-established inflammatory mediator in numerous inflammatory reactions [37]. Moreover, it is well accepted that antagonizition of TNF-α attenuated inflammatory response in various skin inflammatory diseases [7, 38]. In clinical, anti-TNF agents were chosen for treating psoriasis. Furthermore, various crucial biomarkers for skin inflammation such as IL-1β, IL-6, and iNOS can be induced by TNF-α [14, 39]. In this study, RAW264.7 cell, a commonly used macrophage cell line, was chosen to detect the effect of GDF11 in TNF-α-mediated inflammatory reaction, and GDF11 suppressed the mentioned inflammation-associated molecules mediated by TNF-α.

Previous reports demonstrated that psoriasis was displayed in the typical form by exaggerated infiltration of specific immune cells which sustained the inflammatory extent for skin layer that functional destruction of the skin [40, 41]. IMQ-induced psoriasiform mice model, by repeated treatment with IMQ on murine skin, is extensively studied in preclinical psoriasis studies, for its similarity to psoriasiform skin inflammation [42]. To date, IMQ-induced skin inflammation has been established to detect the therapeutic effect of various reagents [43, 44]. IL-23 intradermal injection-induced skin inflammation model is another widely used model for study of psoriasis, which also involves inflammatory reaction and recruitment of immune cells. In the present study, IMQ-induced psoriasis-like skin inflammation mice model and IL-23-induced psoriasis-like skin inflammation mice model were established to determine the function of GDF11 in skin inflammation. As a result, the severity of skin inflammation was significantly alleviated by the treatment of rGDF11. Earlier study has provided that infiltration of macrophage plays a detrimental role in skin inflammation [45]. In this study, the detection of macrophage in skin tissue was largely abolished by rGDF11 treatment in both two mice models, suggesting the reduction of inflammatory cell infiltration. Moreover, treatment of GDF11 inhibited the expression of inflammatory cytokines *in vivo*.

Additionally, we investigated the underlying signaling pathway for the protective effect of GDF11 on psoriasis-like skin inflammation. It has been reported that protective effects of GDF11 against apoptosis could be diminished through NF-κB signaling pathway [7]. Moreover, NF-κB signaling pathway was a key pathway for TNF-α-mediated inflammatory reaction and played a crucial role in progression of psoriasis-like skin inflammation [46]. In the current study, the levels of the downstream molecules of NF-κB signaling pathway were reduced with the treatment of rGDF11 both *in vivo* and *in vitro*, which indicate that the expression and activity of NF-κB signaling pathway were inhibited through GDF11. Moreover, knockdown of GDF11 was performed in RAW264.7 cells,

and SN50, a common inhibitor of NF-κB signaling pathway, attenuated the elevation of inflammatory molecules [47, 48], implying the role of GDF11 might rely on suppression of NF-κB signaling pathway. Therefore, a probable explanation is that the therapeutic effect of GDF11 maybe through repressing activation of NF-κB signaling pathway.

However, there is also limitation in this study comparing with previous studies. Although IMQ and IL-23-induced psoriasis-like skin inflammation mice model are commonly used to investigate psoriasis-like skin inflammation, underlying mechanisms of psoriasis-like skin inflammation still remain to be elucidated. Furthermore, clinical research was not performed in this study, because of the ethical restrictions, so it is unknown whether GDF11 has a therapeutic effect on psoriasis patients. Therefore, further studies are needed to solve all the questions.

In summary, GDF11 plays a protective role in the development of psoriasis-like skin inflammation, which might be associated with NF-κB signaling pathway. Moreover, GDF11 might represent a promising therapeutic agent in treatment of inflammatory skin diseases, including psoriasis.

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AUTHORS' CONTRIBUTIONS

W. Li and Y. Zhao conceived and designed the experiments; W. Wang, R. Qu, X. Wang, M. Zhang, Y. Zhang, C. Chen, X. Chen, C. Qiu, and J. Li performed the experiments; W. Wang, X. Pan, and Y. Zhao analyzed the data; and W. Li and Y. Zhao contributed reagents, materials, and analysis tools.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

Ethics Approval. All animal experiments were approved by the Shandong University Animal Care and Use Committee (Shandong, China).

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