

Tranexamic acid ameliorates rosacea symptoms through regulating immune response and angiogenesis

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ABSTRACT

Rosacea is a chronic inflammatory cutaneous disease characterized by immune system anomalies and vascular hyperreactivity. Recently, therapy of rosacea has improved substantially with the approval of Tranexamic acid (TXA), an antifibrinolytic agent. However, we know little about the underlying mechanism. In this study, we evaluated the effects of TXA and its molecular mechanism on rosacea by using LL37-induced mouse model and HaCaT cell model. Rosacea-like symptoms including skin erythema and histopathological alterations, as well as the elevated pro-inflammatory cytokines (IL-6 and TNF α) and MMP9 expression were significantly ameliorated by TXA treatment. In addition, TXA reduced the expression levels of innate immune gene (TLR2, KLK5 and Camp) and neutrophils relative gene in rosacea-like lesion. For adaptive immune, CD4⁺ T cell infiltration and the gene expression of Th cytokines and chemokines were regulated by TXA in skin lesion. Furthermore, the anti-inflammatory effects of TXA were associated with the inhibition of TLR2, pro-inflammatory cytokines (IL-6 and TNF α) and chemokines (CCL10) expression in LL37-activated HaCaT cells. Finally, TXA repressed the angiogenesis by reducing the number of CD31⁺ cell and downregulating the expression levels of VEGF in rosacea. In conclusion, our finding defines a treatment mechanism by which TXA ameliorates rosacea symptoms by regulating the immune response and angiogenesis.

1. Introduction

Rosacea is a common, chronic facial inflammatory disease and typically categorized into 4 main subtypes: erythematotelangiectatic rosacea (ETR), papulopustular rosacea (PPR), phymatous rosacea (PHR), and ocular (OR) rosacea [1]. The clinical characteristics of rosacea contain facial erythema, papules, pustules, telangiectasia, and recurrent flushing, that have an adverse effect on quality of life of rosacea patients, although it is not a lethal disease [2–4]. Moreover, recent studies have showed that rosacea is linked to the increase risk of dementia [5] and incident cancer [6]. These findings indicate that rosacea may be a response of a systemic disorder and it deserves our attention.

The pathophysiology of rosacea has yet to be fully elucidated. However, current evidence suggested that rosacea was attribute to the dysfunction of immune, vascular, and nervous for many years [2]. Studies showed that consistently aberrant innate immune response plays critical role in the progression of rosacea [7]. The elevated expression of Toll-like receptor 2 (TLR2), cathelicidin antimicrobial peptide (Camp), and kallikrein 5 (KLK5) were observed in rosacea skin

lesion, and inhibition of KLK5 improved the erythema and papules in rosacea [8]. LL37, an important Camp identified in rosacea, was reported to induce a rosacea-like skin inflammation in mice [9]. The innate immune cells infiltration was also observed in rosacea including macrophages, neutrophils and mast cells [10]. Buhl et al. revealed the involvement of adaptive immune in rosacea including CD4⁺ T cells [10,11]. In addition, as a vascular and neuronal dysfunction skin disease, histopathological features of rosacea were also characterized by blood vessels dilation, angiogenesis and a slightly increase of nerve fibers [11,12]. The gene array and qPCR results showed the elevated levels of vascular endothelial growth factor (VEGF) and neurogenic inflammation gene (TRP) vanilloid receptor 1 (TRPV1) in rosacea [11,13]. The clinical agents for rosacea approved by the FDA include doxycycline and alpha-adrenergic receptor agonists. And the clinical efficacy of these agents were attributed to their anti-inflammatory or anti-angiogenic properties.

Tranexamic acid (TXA) is a lysine derivative that is commonly used as an antifibrinolytic agent to reduce the risk of excessive bleeding in hemophilia, menorrhagia as well as the surgical procedures clinically

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Table 1
List of primers used for Real-time PCR.

Target gene	Forward primers	Reverse primers
Mouse GAPDH	AGGTCGGTGTGAACGGATTG	TGTAGACCATGTAGTTGAGGTCA
Mouse CAMP	GCTGTGGCGGTCACTATCAC	TGTCTAGGGACTGCTGGTTGA
Mouse KLK5	ATGGGCAATGGTACCCTG	GTTCGGTTCCAGAGGGGTT
Mouse TNF- α	CTGAACTTCGGGGTGATCGG	GGCTTGCACTCGAATTTGAGA
Mouse TLR2	TCTAAAGTCGATCCGCGACAT	CTACGGGCAGTGGTAAAAC
Mouse VEGF	TATTCAGCGGACTCACCAGC	AACCAACCTCCTCAAACCGT
Mouse IL6	TAGTCCTTCTACCCCAATTTCC	TGGTCCCTAGCCACTCCTTC
Mouse MMP9	CTGGACAGCCAGACACTAAAG	CTCGGGCAAGTCTTCAGAG
Mouse CXCL1	CTGGGATTCACCTCAAGAACATC	CAGGGTCAAGGCAAGCCTC
Mouse CXCL5	GTTCCATCTCGCCATTCATGC	GCGGCTATGACTGAGGAAGG
Mouse CXCL10	CAAAGTGTGCGCGTCAATTTTC	GGCTCGCAGGGATGATTTC
Mouse CCR3	TCAACTTGGCAATTTCTGACCT	CAGCATGGACGATAGCCAGG
Mouse CCR5	TTTTCAAGGGTCAAGTCCGAC	GGAAAGACCATCATGTTACCCAC
Mouse CCR6	CCTGGGCAACATTATGGTGGT	CAGAAGGTAGGGTGAAGGACA
Mouse CCL20	GCCTCTCGTACATACAGACGC	CCAGTTCTGCTTTGGATCAGC
Mouse Tpsb1	GCCAATGACACTACTGGATG	GCTTACGGAGCTGACTCTGA
Mouse CMA1	CGCCCTACATGGCTATC	AGGAGGACTGTTATAGACCCTTC
Mouse ITGAM	CCATGACCTTCCAAGAGAATGC	ACCGGCTTGTGCTGTAGTC
Mouse ITGB2	CAGGAATGCACCAAGTACAAAGT	GTCACAGGCAAGGAGTCA
Mouse STAT1	TCACAGTGGTTCGAGCTTCAG	GCAAACGAGACATCATAGGCA
Mouse STAT4	TGGCAACAATTCTGCTTCAA AAC	GAGGTCCCTGGATAGGCATGT
Mouse STAT3	CAATACCATTGACCTGCGCAT	GAGCGACTCAAAGTCCCT
Mouse IL20	TCITGCTTTGACTGTTCTCC	GTTTGCAATACACAGCTTC
Mouse IL4	GGTCTCAACCCAGCTAGT	GCCGATGATCTCTCAAGTGA
Mouse FOXP3	CCCATCCCCAGGAGTCTTG	ACCATGACTAGGGGCACTGTA
Mouse TRPV1	CCACTGGTGTGAGACGCC	TCTGGTCTTTGAAGCTGCTG
Human GAPDH	TGTTGCCATCAATGACCCCTT	CTCCACGAGTACTCAGCG
Human TLR2	ATCCTCCAATCAGGCTTCTCT	GGACAGGTCAAGGCTTTTACA
Human TNF- α	CCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
Human CXCL10	GTGGCATTCAAGGAGTAGCTC	TGATGGCCTCGATTCTGGATT
Human IL6	CCTGAACCTTCCAAGATGGC	TTACCAGGCAAGTCTCCTCA
Human KLK5	TCAGACCCATCAACGCTCTCT	GCACGGTGATATCAAGCACT
Human Camp	GGCTGGTGAAGCGGTGTAT	TGGGTACAAGATTCCGAAAAA
Human ET-1	AGAGTGTGCTACTTCTGCCA	CTTCCAAGTCCATAGGAAACA

[14–18]. Moreover, studies showed the potential anti-inflammatory role of TXA in cardiac surgery, acute lung injury [19–21]. Recently, TXA was used as treatment option available for rosacea [22], nevertheless the potential molecular mechanism of its therapeutic effects is still unclear.

In this study we investigated the role and mechanism of TXA on rosacea by using a LL37-induced mouse model and HaCaT cell model, and found that TXA ameliorated rosacea symptom by suppressing of immune response and angiogenesis. Our study provides the first experimental basis for TXA treatment on rosacea.

2. Material and methods

2.1. Compounds

TXA was purchased from Selleck Chemicals (California, USA). The LL37 was synthesized from Shengong (Shanghai, China) with the amino acid sequences: LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLPV-RTE, and then purified by HPLC and their identity was confirmed by mass spectrometry.

2.2. Animal experiments

7-week-old BALB/c mice used for experiments were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China). All animal experiments were approved by the Animal Ethics Committee of the Xiangya Hospital of Central South University.

For the TXA treatment, TXA (Selleck Chemicals, California, USA) was diluted in filtered PBS and 130 mg/kg body by gavage daily for seven consecutive days, LL37 peptide was injected intradermally for the last 2 days to induce rosacea-like skin lesion as previous described [9]. The rosacea-like lesions induced by LL37 were photographed and

evaluated based on the redness score and area as previous described [23].

2.3. Cell culture and treatment

HaCaT cells were cultured in Free-calcium basal media (DMEM; Gibco, ThermoFisher Scientific, USA) medium supplemented with 10% fetal bovine serum (FBS; Gibco) and penicillin/streptomycin (50 U/ml), in an incubator at 37 °C, 5% CO₂. After HaCaT cells reached 90% confluence, HaCaT cell were cultured in high-calcium medium and stimulated with TXA (120 μ g/ml) for 24 h or LL37 (4 μ M) for 12 h. Then RNA was collected stored at –80 °C until analysis.

Human umbilical vein endothelial cells (HUVEC) were cultured in RPMI Medium Modified (1640) supplemented with 10% fetal bovine serum (FBS; Gibco) and penicillin/streptomycin (50 U/ml) in an incubator at 37 °C, 5% CO₂. The cells were starved overnight by serum-free basal media and then media were removed from cells and replaced with media containing stimulation TXA (120 μ g/ml) for 24 h or LL37 (4 μ M) for 12 h. RNA was collected after 12 h stored at –80 °C until analysis.

2.4. Histologic analysis

The full thickness of rosacea-like lesion was fixed with formalin and sectioned at 4 μ m thickness. The sections were stained with hematoxylin and eosin (H&E) stain and their histomorphology was observed under standard light microscopy (OLYMPUS, Japan) as previous described [24].

2.5. Immunofluorescence

We fixed 8-mm frozen sections with paraformaldehyde for 15 min at

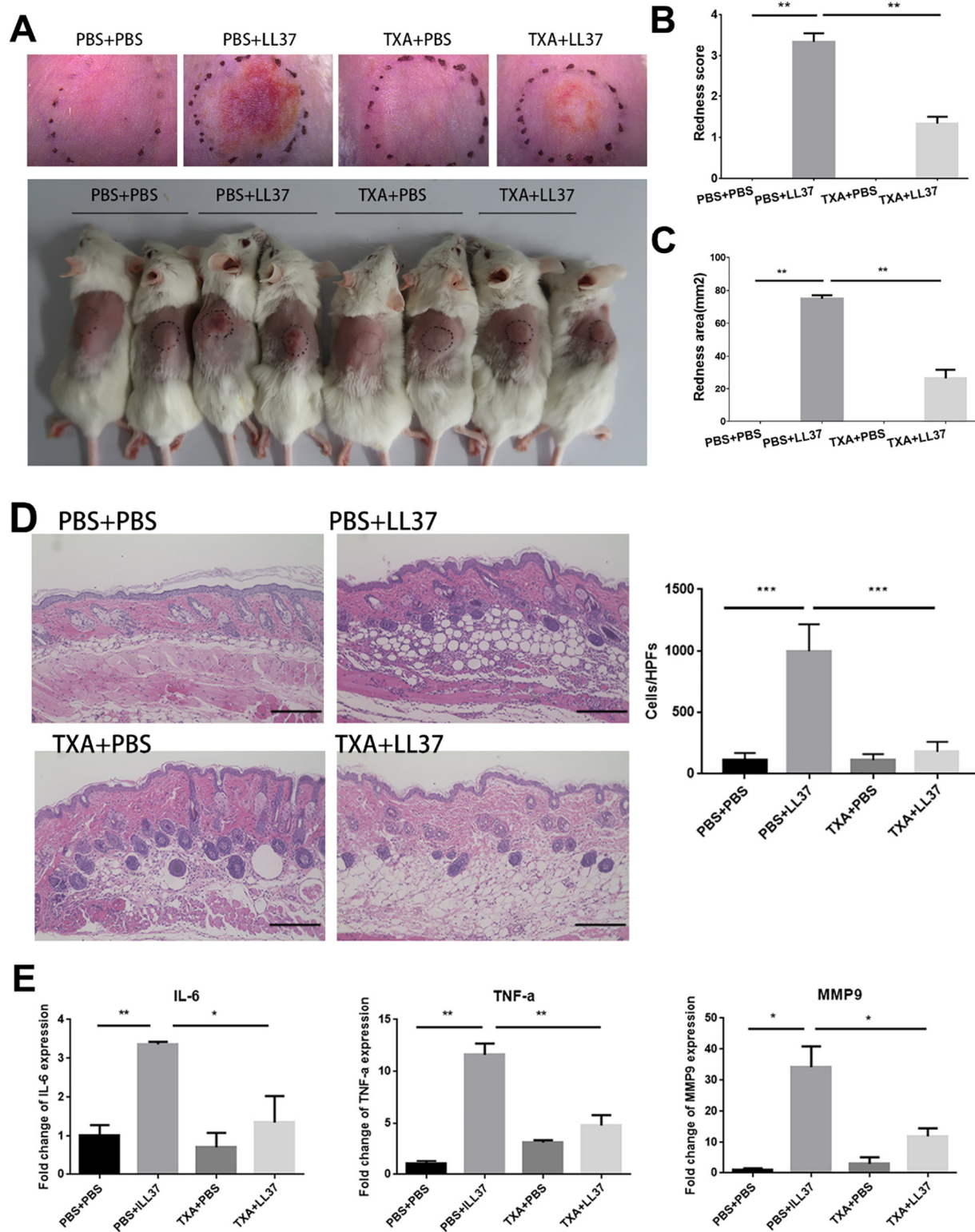


Fig. 1. Treatment with TXA ameliorated the rosacea-like phenotype. (a) After the removal of hair, LL37 was injected intradermally into the dorsal skin to induce rosacea-like phenotype, TXA was pretreated by gavage. The severity of the rosacea-like phenotype was assessed based on the redness score (b) and area (c). (d) H&E for histological analysis of rosacea-like skin. (e) The expression levels of IL-6, TNF- α and MMP9 were detected by qPCR analysis. Results are representative of three independent experiments. Data represent the means \pm SEM, * P < 0.05, ** P < 0.01 and *** P < 0.001.

room temperature, and blocked them with 5% normal donkey serum (NDS) (#017000-121, Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania) and 0.2% TritonX at room temperature for 1 h. Then the skin sections were treated with anti-CD4 (1:100, ebioscience) and anti-CD31 (1:100, ebioscience) antibodies at 4 °C overnight and

then stained with Alexa Fluor 488-labeled anti-goat IgG antibodies (Life technologies) overnight. Images were captured using Zeiss Axio Scope A1 (Zeiss, Germany).

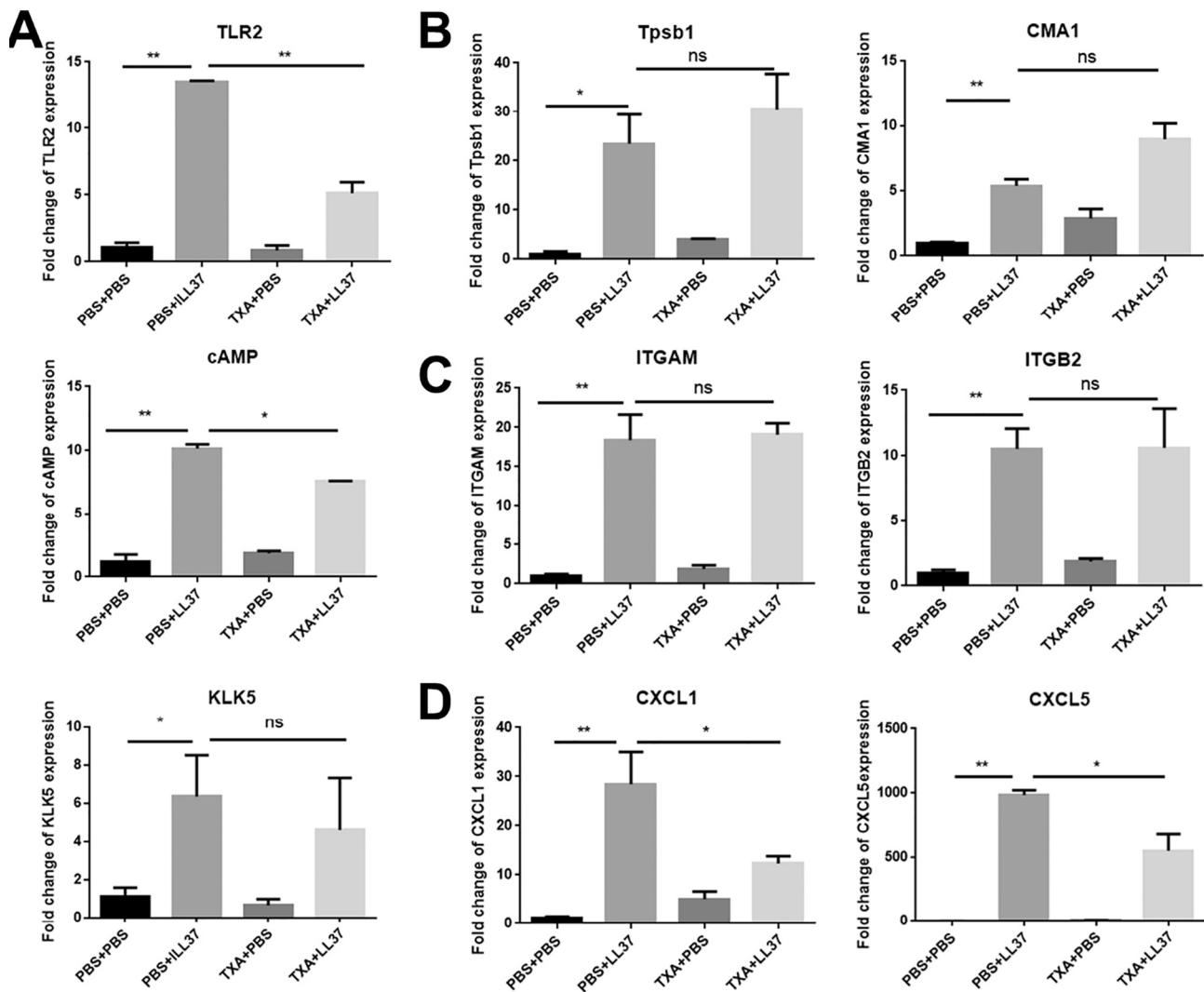


Fig. 2. TXA suppressed innate immune in rosacea-like mice. (a) The expression levels of TLR2, KLK5, Camp, IL-6, TNF- α and MMP9 in rosacea-like mice. The expression levels of mast cell (b) macrophage (c) and neutrophils (d) -related genes in rosacea-like mice. Data represent the means \pm SEM of three independent experiments. * $P < 0.05$ and ** $P < 0.01$.

2.6. Real-time PCR analysis

Total RNA was isolated from HaCaT cells or skin lesion using TRIzol reagent (Invitrogen Life Technologies, USA). 2 μ g RNA was transcribed to cDNA using PrimeScript RT reagent Kit (Takara, Shiga, Japan), and qPCR was performed with iTaq Universal SYBR GREEN Supermix (Bio-Rad, California, USA) on an Applied Biosystems 7500 machine (Life Technologies). The primers were showed in Table 1.

2.7. Statistical analysis

All data are analyzed with GraphPad Prism 6 (GraphPad Software, La Jolla, CA) and presented as means \pm SEM. The Student's *t*-test was used for compare differences between two groups. *, $P < 0.05$ and **, $P < 0.01$ are considered significant.

3. Results

3.1. TXA attenuated rosacea-like dermatitis LL-37-induced mice model

Clinical studies have shown that TXA is an effective therapeutic option for rosacea patients [22,25,26]. Herein, we used a mouse model of rosacea to investigated the pharmacologic mechanisms of TXA. As

shown in Fig. 1a, TXA significantly ameliorated LL37-induced rosacea-like skin erythema. TXA evidently reduced the average redness score and area by $\sim 60.6\%$ and $\sim 54.6\%$ respectively (Fig. 1b and c). The histological analysis showed the rosacea-like dermatitis including inflammatory infiltration as well as vascular dysregulation in rosacea mouse [9] were ameliorated by TXA treatment (Fig. 1d). The pro-inflammatory cytokines (IL-6, TNF- α) and matrix remodeling (MMP9) were upregulated in various inflammatory diseases, recently emerging as significant in rosacea [12]. Thus, we examined the expression levels of IL-6, TNF- α as well as MMP9 by qPCR and showed that the upregulation of IL-6, TNF- α and MMP9 expression in rosacea were attenuated by TXA treatment (Fig. 1e). In conclusion, these results indicate that TXA ameliorates the symptoms of rosacea in LL37-induced mice model.

3.2. TXA reduced in LL37- induced immune dysregulation in rosacea-like mice

To better understand the immunomodulatory of TXA in rosacea, the involvements of innate immune and adopt immune were determined in rosacea-like mice. Innate immune response appears to be important in rosacea with the elevated expression of TLR2, KLK5 and Camp in the lesion of rosacea patient and rosacea-like mice [9,27]. Consistent with

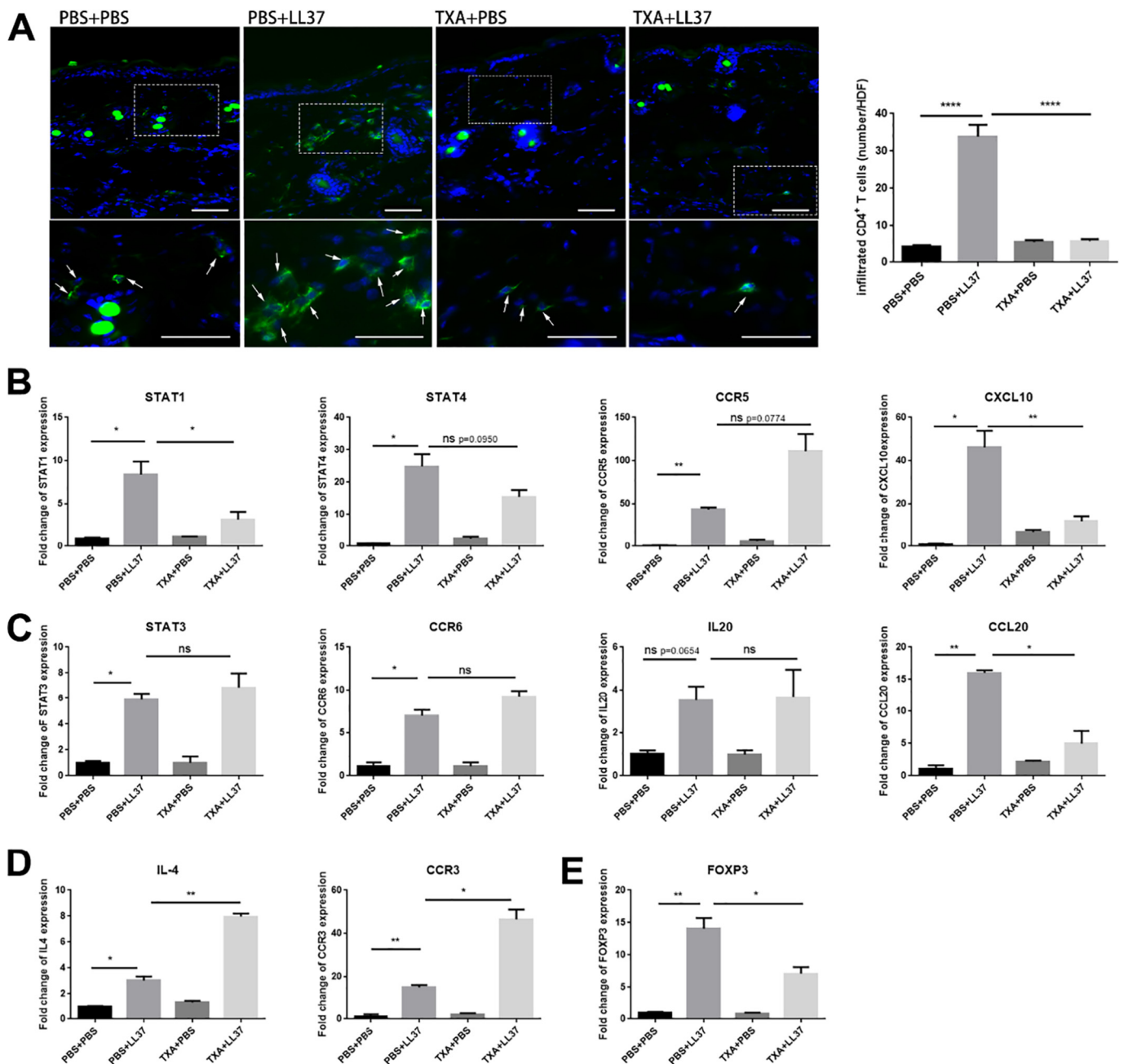


Fig. 3. CD4⁺ cells are activated in the regulation of the immune response in rosacea-like mice. (a) Expression of CD4 in skin visualized by immunofluorescence. Green indicates CD4⁺ T cell. Blue indicates DAPI. Scale bars, 50 μm. Th1 (b), Th17 (c), Th2 (d) and Treg (e) cell-related genes expression significantly modulated in rosacea-like mice. Data represent the means ± SEM. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

these studies, TLR2, KLK5 and Camp expression were evidently upregulated in rosacea-like lesion. TXA treatment significantly attenuated LL37-induced expression of TLR2, KLK5 and Camp (Fig. 2a). The recruitment and activation of innate immune cells including mast cell, macrophages and neutrophils were also observed in rosacea patients [10]. To define an involvement of mast cell in rosacea, we analyzed the mRNA expression levels of Tpsb1 and CMA1, and found the significant upregulation of these mRNAs in rosacea mice (Fig. 2b). Moreover, the involvement of macrophage cell was supported by identical upregulation of macrophage markers (ITGB2 and ITGAM) (Fig. 2c). However, the upregulation of mast cell and macrophage cell markers in rosacea mice were not rescued by the treatment of TXA (Fig. 2b and c). The qPCR analysis of CXC chemokines showed significant upregulation of

neutrophil-recruiting chemokines, including CXCL1 and CXCL5, which were repressed by TXA treatment (Fig. 2d). These results indicate that mast cell, macrophages and neutrophils cells contribute to rosacea-skin inflammation, TXA reduces neutrophils cells instead of mast cells and macrophages infiltration in rosacea-like mice. Collectively, these results indicate that TXA ameliorates rosacea partly by repressing innate immune response.

Previous study showed that CD4⁺ T cells dominate the immune cell infiltrate in rosacea [10]. To investigate the effects of TXA on adopt immune, the CD4⁺ T cells were detected by immunofluorescence in rosacea-like mice. Similar with the results in rosacea patients, the number of CD4⁺ T cell was greatly increased in LL37-induced rosacea-like dermatitis in mice (Fig. 3a). TXA treatment significantly attenuated

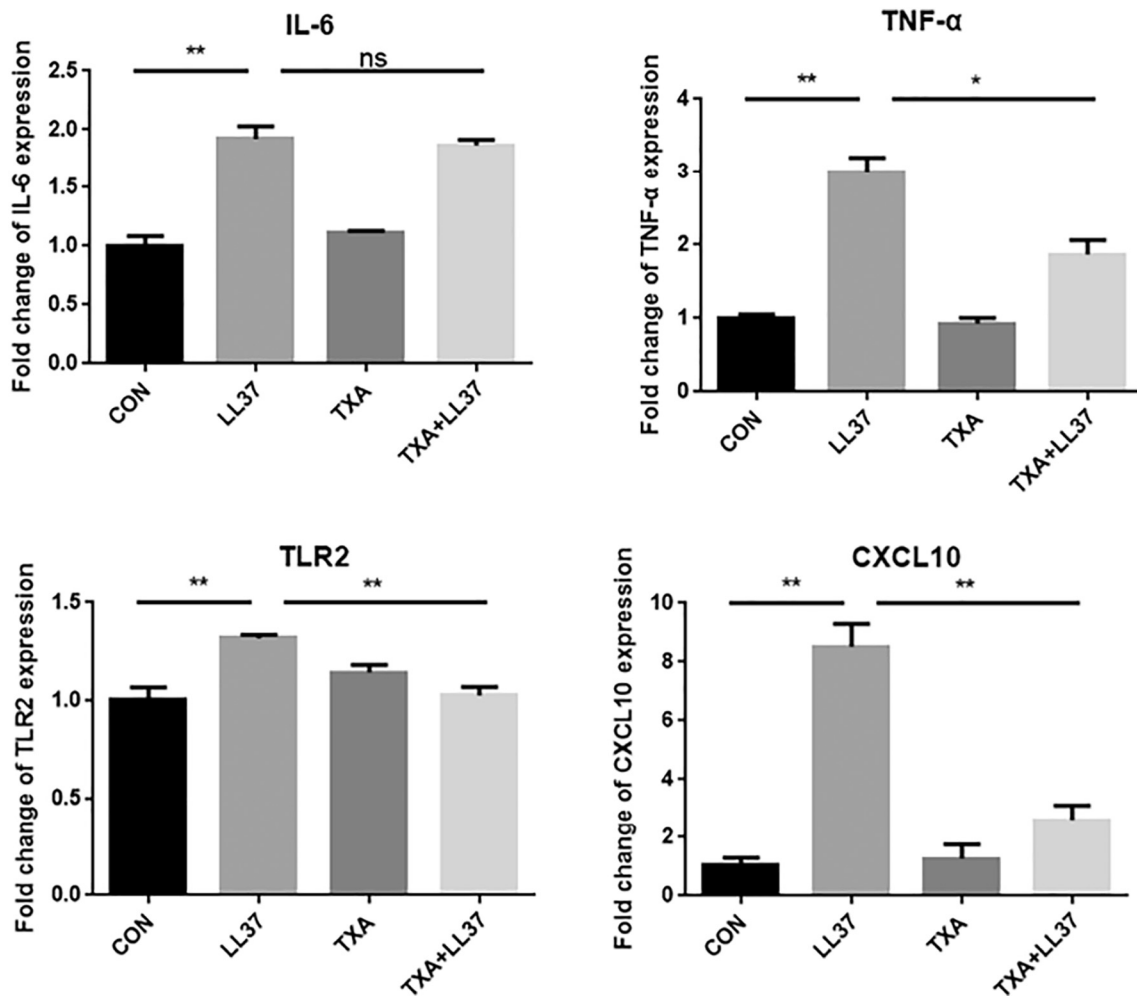


Fig. 4. TXA reversed inflammatory cytokines and chemokines expression in HaCaT cells. Data represent the means \pm SEM. * P < 0.05 and ** P < 0.01.

the LL37-induced CD4⁺ T cell infiltration (Fig. 3a). Because Th1/Th17 polarization was observed in skin lesion of rosacea patients, we speculated that T cell polarization may also be involved in LL37-induced rosacea and could be regulated by TXA [10]. Herein, we detected the expression levels of T polarization-related gene in rosacea-like mice. Consistent with the elevated expression of Th1 and Th17-associated genes observed in rosacea patients, the expression levels of Th1-associated gene (STAT1, STAT4, CCR5 and CXCL10) and Th17-associated gene (STAT3, CCR6 and CXCL20) were significantly upregulated in rosacea-like mice (Fig. 3b and c). The TXA treatment evidently reduced LL37-induced Th1-associated gene (STAT1 and CXCL10) and Th17-associated gene (CXCL20). Moreover, LL37 significantly induced Th2-associated gene (IL4 and CCR3) and Treg-associated gene (FOXP3) (Fig. 3d and e). TXA treatment increased the LL37-induced Th2-associated gene (IL4 and CCR3) expression, but reduced the FOXP3 expression (Fig. 3a and c). Collectively, these results indicate that TXA represses CD4⁺ T cell infiltration and T cell polarization induced by LL37, which may contribute to its anti-inflammation effect for rosacea treatment.

3.3. TXA reduced cytokines and chemokines expression in HaCaT cells

Overexpression of innate immune-related gene in keratinocytes acts as a critical element in the pathogenesis of rosacea. [27]. We next examined the effects of TXA on these gene expression in LL37-induced human keratinocyte (HaCaT) model. The significantly upregulation of TLR2 expression was observed in LL37-induced HaCaT cells, which was

reversed by TXA treatment (Fig. 4). Moreover, KLK5 and Camp expression were slightly induced by LL37 but not affected by TXA treatment (data not shown). To define the underlying anti-inflammatory mechanism responsible for the effects of TXA on rosacea, we detected the effect of TXA on the expression of inflammatory cytokines and chemokines in LL37-activated HaCaT cell. It turned out that the LL37 significantly upregulated TLR2, pro-inflammatory cytokines (IL-6 and TNF α) and Th17 chemokines (CXCL10) expression, all of which except IL-6 were reversed by TXA treatment (Fig. 4). Collectively, these findings support that TXA represses the production of TLR2, cytokines and chemokines in keratinocytes primed by LL37.

3.4. TXA reduced angiogenesis and repressed VEGF expression

To confirm the repression of TXA on the dysregulation of neurovascular in rosacea-like mice, we performed immuno-staining of CD31 (a marker of blood vessels) and qPCR of mRNA expression of VEGF and TRPV1. Consistent with the previous results, LL37 significantly induced angiogenesis with the increase number of CD31 microvessels and VEGF expression (Fig. 5a). Treatment with TXA acutely abrogated the angiogenesis effects of LL37 in the dermis (Fig. 5a and b). In addition, although LL37 upregulated the expression of TRPV1, TRPV1 expression was not significantly changed by TXA treatment (Fig. 5c). These results indicate that TXA represses angiogenesis induced by LL37 in rosacea-like mice. Next, we tried to detect the effects of LL37 and TXA on HUVEC. As shown in Fig. 5d, TXA treatment significantly repressed the expression levels of endothelin-1 (ET1) and TNF α , however, LL37

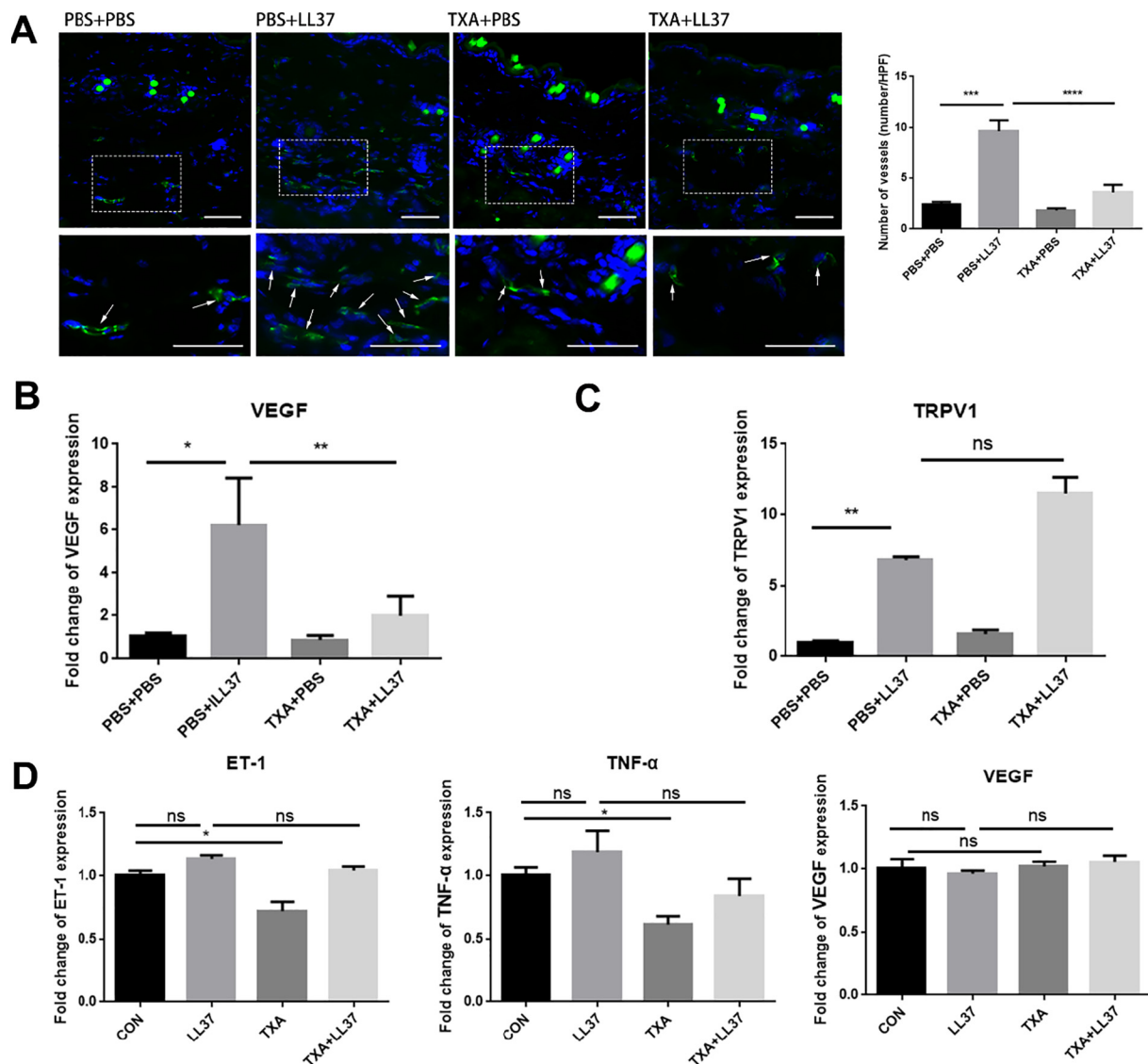


Fig. 5. TXA reduced angiogenesis in LL37-induced rosacea-like mice. (a) Expression of CD31 in skin visualized by immunofluorescence. Green indicates CD31 cell. Blue indicates DAPI. Scale bars, 50 μ m. (b) VEGF expression in rosacea-like mice. (c) TRPV1 expression in rosacea-like mice. (d) The effects of LL37 and TXA on the expression levels of ET1, TNF α and VEGF in HUVEC. Data represent the means \pm SEM. * P < 0.05, ** P < 0.01 and *** P < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

treatment and LL37 + TXA treatment did not affect the expression levels of ET1 and TNF α . Moreover, TXA, LL37 and LL37 + TXA treatment did not affect the expression level of VEGF. Moreover, TXA, LL37 and LL37 + TXA treatment did not affect the expression level of VEGF.

4. Discussion

Rosacea is a chronic inflammatory skin disease that affects approximately 3% of the world's population [28]. Although the exact pathogenesis of rosacea is limited, it is widely accepted that rosacea was attribute to immune, vascular, and nervous dysfunction [12]. TXA is a potent antifibrinolytic agent and widely used for surgical patients with excessive bleeding [29]. Recent studies have described TXA as treatment option available for rosacea [22]. However, the pharmacologic mechanism has not been elucidated. In this study, we have provided the first evidence that TXA treatment ameliorated the rosacea symptoms via suppressing inflammation and angiogenesis.

It is well known that immune response is involved in rosacea pathogenesis. Innate immune was reported to play a critical role on the initiation of rosacea [9]. Environmental stimuli of TLR2 activation in

rosacea induces KLK5 expression and subsequently results in accumulation of LL37, which acts as a critical element in the pathogenesis of rosacea [30]. Azelaic acid is a beneficial agent for rosacea treatment because of the suppression on innate immune [30]. Here, we showed that TXA evidently reduced LL37-induced expression of KLK5, Camp and TLR2. In addition, for the inflammatory infiltration in rosacea [10], our data showed that the neutrophil infiltration was reduced (Fig. 1d) and the expression of neutrophil-recruiting chemokines were repressed by TXA treatment in rosacea-like mice (Fig. 2d). Consistent with these results, the reduced neutrophil infiltration by TXA was also observed in acute lung injury [31]. Although mast cell and macrophages were reported to play important role in rosacea, TXA did not affect the infiltration of mast cell and macrophages. The increased inflammatory cytokines in rosacea including TNF α and IL-6 [12] were also reduced by TXA, which was consistent with the anti-inflammatory role of TXA in previous studies [32,33]. In addition, the adaptive immune system is significantly activated in rosacea with the increase of CD4⁺ T cell as well as the upregulation of Th1 and Th17 cytokines and chemokine in rosacea lesion [10,12]. In the current study, the elevated Th1 and Th17 cytokines and chemokines induced by LL37 were reduced by TXA in

rosacea-like mice. Although no involvement of Th2 and Treg cells was reported in rosacea [10], the gene expression of cytokines and chemokines of Th2 and Treg cells were increased in rosacea-like mice, and Th2 cytokines and chemokines were further induced by TXA. Together, our studies indicated the anti-inflammation and immunomodulatory properties of TXA account for its therapeutic effects on rosacea.

Keratinocytes was reported to produce inflammatory molecules including anti-microbial peptides, cytokines as well as chemokines in response to environmental stimuli [34–36], which is involved in the progression of various inflammatory skin diseases, including psoriasis [37], atopic dermatitis [38], vitiligo [39], as well as lichen planus [40]. Recent study demonstrated that LL37, an antimicrobial peptide produced by keratinocyte, induced chemokines expression and contributes to inflammatory cell infiltration [41]. In this study, LL37 significantly upregulated TLR2, pro-inflammatory cytokines (IL-6 and TNF α) and Th17 chemokines (CCL10) expression, and the expression of TLR2, TNF α and CCL10 were suppressed by TXA treatment (Fig. 4). These findings support that TXA represses the production of TLR2, cytokines and chemokines in keratinocytes primed by LL37 and further reduces the T cell infiltration induced by keratinocytes.

It is well established that rosacea is a vascular and neuronal dysfunction skin disease, with the pathophysiology character of dilated blood vessels and angiogenesis [11,42]. The increase expression levels of VEGF [13] and TRPV1 were also observed in rosacea [11,13]. In this study, an increase of the number of CD31 microvessels induced by LL37 was decreased by TXA treatment. Similar to previous observations that TXA reduced the VEGF expression in Ultraviolet A-induced skin cancer [43], and TXA also attenuated the VEGF expression induced by LL37 in rosacea-like mice. Although the expression levels of ET1 and TNF α were significantly repressed by TXA, these gene expression was not affected by LL37 and LL37 + TXA treatment. Moreover, the expression level of VEGF was also not affected by TXA, LL37 and LL37 + TXA treatment. A recent study demonstrates that TXA inhibits the activity of plasmin, a serine protease which plays a critical role in angiogenesis [31], we speculated that TXA could inhibit angiogenesis partly by suppressing serine proteases activity in rosacea.

In summary, we investigated the role and mechanism of TXA on rosacea and observed that TXA ameliorated rosacea symptoms by regulating immune response and angiogenesis. This study provides the first experimental basis for TXA treatment on rosacea. The exact mechanisms underlying TXA-mediated anti-inflammation and anti-angiogenesis remain to be defined.

Abbreviations

TXA	Tranexamic acid
ETR	erythematotelangiectatic rosacea
PPR	papulopustular rosacea
PHR	phymatous rosacea
OR	ocular rosacea
TLR2	Toll-like receptor 2
Camp	cathelicidin antimicrobial peptide
KLK5	kallikrein 5
VEGF	vascular endothelial growth factor
NDS	normal donkey serum
ET1	endothelin-1

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Author contribution

Yiya Zhang and Ji Li designed research; Yangfan Li, Hongfu Xie, Zhili Deng, Ben Wang performed research; Yan Tang, Zhixiang Zhao, San Xu, Xin Yuan contributed new reagents or analytic tools; Yiya Zhang and Yangfan Li analyzed data; Zhihong Zuo, Yiya Zhang and Ji Li wrote the paper.

Conflicts of interest

The authors have no conflicts of interest to declare.

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