Eur J Dermatol 2022; 32(1): 24-33

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Calcipotriol inhibits psoriasis-like angiogenic features in K14-VEGF transgenic mice

Background: Angiogenesis is both a notable characteristic of psoriasis and a key driver of its pathogenesis. Calcipotriol has been used for the treatment of psoriasis for several decades, yet few studies have focused on its microvascular mechanism utilizing appropriate animal models. The K14 vascular endothelial growth factor (VEGF) transgenic mouse model is an ideal animal model to study the microvascular mechanism of psoriasis. *Objectives:* To explore the mechanism through which calcipotriol exerts its anti-angiogenic effect in psoriasis using the K14 VEGF transgenic mouse model *Materials & Methods:* Having established the transgenic mouse model, the K14 VEGF mice were randomly divided into three groups with topical treatment with either vehicle cream, calcipotriol ointment or halometasone cream. The skin of the mice was subsequently collected to evaluate the level of VEGF and pigment epithelium-derived factor (PEDF) as well as microvascular density (MVD). Furthermore, the phosphorylation level of JAK/STAT3 was also investigated. *Results:* In contrast to C57 mice, there was an observed increment in the level of VEGF and MVD in the skin of K14 VEGF mice, while the PEDF level was decreased. Moreover, we found that topical calcipotriol treatment affected the ratio of VEGF to PEDF, and decreased MVD in the skin of K14 VEGF mice. In addition, calcipotriol also upregulated vitamin D receptor and inhibited the JAK/STAT3 signalling pathway *Conclusion:* Calcipotriol inhibits psoriasis-like angiogenic features by suppressing VEGF, increasing PEDF and ultimately decreasing MVD in the skin of K14 VEGF mice, possibly involving the JAK/STAT3 pathway.

Article accepted on 06/10/2021

Key words: calcipotriol, MVD, PEDF, psoriasis, VEGF

Psoriasis is a common, chronic, immune-mediated skin disease associated with polygenic inheritance, immunological dysfunction and many environmental factors [1, 2]. The aetiology and pathogenesis of psoriasis have remained largely unclear. It is believed that angiogenesis plays an important role in the early stage of psoriasis and is also significantly involved in its recurrence [3, 4]. Vascular endothelial growth factor (VEGF) is a widely known proangiogenic cytokine that acts as an important mediator in the promotion of neovascularization [5, 6]. Immunocytes in psoriatic lesions, such as plasmacytoid dendritic cells, are reported to be responsible for the activation of keratinocytes, which further leads to the generation of VEGF and subsequently results in neovascularization along with endothelial dysfunction [6]. Pigment epithelium-derived factor (PEDF) is an effective angiogenic inhibitor that could inhibit the expression of VEGF and suppress corneal angiogenesis [7, 8]. The microvascular hyperplasia of psoriasis is considered to be the result of the simultaneous effects of these cytokines. CD31 and ICAM-1 have been found to be overly expressed in psoriatic lesions and are involved in angiogenesis [9-11], making them ideal practical markers of microvessel density (MVD) [12, 13]. Moreover, the microvascular area has been previously quantified to evaluate the level of MVD in tissue sections of mice, as previously reported [14, 15].

Calcipotriol, a synthetic derivative of vitamin D3, has been widely applied as a first-line therapeutic against psoriasis for several decades due to its regulatory effect on epidermal growth, keratinocyte differentiation and T lymphocyte function [16-18]. To date, the mechanism involved in the effects of calcipotriol remains unclear, nevertheless, there are studies suggesting that signalling pathways, such as JAK/STAT3 [19, 20] and NF-κB, could be involved [21, 22]. Calcipotriol has been reported to inhibit vitreous angiogenesis in zebrafish eyes [23] and the secretion of VEGF in TNF-α stimulated synovial stromal cells [16]. Studies have shown that the topical application of calcipotriol results in a significant improvement in the structure of keratinocytes and the capillary plexus in lesions, and that abnormal endothelial cells may be replaced by normal endothelial

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doi:10.1684/ejd.2022.4230

To cite this article: Cao M, Mei L, Xiao M, Li J, Fang F. Calcipotriol inhibits psoriasis-like angiogenic features in K14-VEGF transgenic mice. *Eur J Dermatol* 2022; 32(1): 24-33 doi:10.1684/ejd.2022.4230

cells [24]. Thus, we speculated that the anti-psoriatic effect of calcipotriol could also be related to suppression of angiogenesis.

In this study, K14 VEGF transgenic mice were selected to investigate calcipotriol-related mechanisms. In this model, the human keratin 14 promoter and mouse VEGF coding genes were inserted into the DNA of mice, resulting in the overexpression of VEGF, leading to psoriasis-like chronic inflammation and vascular hyperplasia [25, 26]. The levels of VEGF, PEDF, CD31 and ICAM-1 in skin lesions of K14 VEGF mice after the topical application of calcipotriol ointment were evaluated to examine the anti-angiogenic therapeutic effect of calcipotriol on psoriasis. Moreover, we also conducted experiments to explore whether the expression of vitamin D receptor and phosphorylation of the JAK/STAT3 signalling pathway were affected in mice treated with calcipotriol.

Materials and methods

Materials

Antibodies against VEGF (Cat#66828-1-Ig), ICAM-1(Cat#10020-1-AP) and mouse IgG2b isotype control antibody (Cat#66360-3-Ig) were purchased from ProteinTech (Chicago, USA), anti-PEDF antibody from Abclonal (Cat#A16945, Wuhan, China), antibodies against CD31/PECAM-1 (Cat#77699), Jak2 (Cat#3230), p-Jak2 (Cat#3771), STAT3 (Cat#4904), p-STAT3 (Cat#9131) and rabbit IgG XP® isotype control (Cat#3900) from Cell Signaling Technology (Boston, USA), antibody against vitamin D receptor (Cat#ab109234) from Abcam (Cambridge, England), and goat anti-rabbit IgG-TRITC (Cat#BS10250) was purchased from Bioworld (Nan-Jing, China). DAPI (4',6-diamidino-2-phenylindole) was obtained from Beyotime Biotechnology (Jiangsu, China). qRT-PCR kit was purchased from Takara (Cat#RR420A, Dalian, China). Calcipotriol ointment and halometasone cream were obtained from BrightFurture (Hong Kong, China). Vehicle cream, the Vaseline placebo base cream without active drug substance, was obtained from Shanghai Dermatology Hospital (Shanghai, China).

Animals and treatment

The insertion of the K14 promoter and VEGF-A gene fragment into the C57 mouse genome was accomplished by Suzhou SaiYe biological Co., Ltd. (China), utilizing Crisper/Cas9 technology. After breeding, the homozygous mice were selected and used in this study. The C57 mice, used as the control, were purchased from JieSi-jie Experimental Animal Company (Shanghai, China). All experiments were approved by the animal ethics committee of Jinshan Hospital and conducted in accordance with the principles of Guide for the Care and Use of Laboratory Animals.

For the characterization of the mouse model, an area of 4 cm × 5 cm on the backs of four-month-old K14 VEGF mice and C57 mice was shaved, observed and photographed. Thereafter, the mice were sacrificed to collect the skin samples for further experiments.

Similarly, to investigate the effects of calcipotriol, the K14 VEGF mice were selected and their dorsal skin was shaved. They were then randomly divided into three groups ($n = 5$ for each group): the vehicle cream group (negative control) using a base cream of Vaseline, the calcipotriol group (experiment group) using the calcipotriol ointment, and the halometasone group (positive control) using the halometasone cream. After application for 14 consecutive days, the back skin from the mice was retrieved for quantitative real-time PCR, western blot and immunohistochemistry. A schematic diagram summarising the procedure is presented in *figure 1*.

Immunohistochemistry

For immunohistochemical staining of VEGF, PEDF, CD31 and ICAM-1, the skin tissue from the back lesions was fixed in 4% paraformaldehyde and then embedded in paraffin. After drying, dewaxing, antigen retrieval and sealing, the sections were first stained with the primary antibody at 4 °C overnight, then incubated with anti-rabbit or anti-mouse secondary antibody at 4 °C for 30 minutes, then avidin-biotin complex reagent was used. Areas with brown-yellow spots in the epidermis or dermis were identified as positive staining. The IgG-matched isotype antibodies were utilized for the control sections in order to evaluate non-specific staining for VEGF, CD31, ICAM-1 and PEDF.

For positive staining analysis, five high-power fields were randomly selected for each section, then the percentage of positive nucleated cells and the observed number of vessels were determined. Data for each mouse was based on the average number of high-power fields. For the evaluation of MVD, the dermal vascular area in CD31-stained sections was quantified and then standardized using the area of the control group set as "1", as previously reported [14, 15]. Observed vessel number under high-power (200 ×) was also determined.

Haematoxylin-eosin (H&E) staining

The skin samples from the back lesions of each mouse were fixed in 4% paraformaldehyde and embedded in paraffin. After pre-treatment, sections (thickness of 4 μm) were stained using haematoxylin and eosin.

Immunofluorescence

For immunofluorescence of the vitamin D receptor (Vit DR), the skin tissue from back lesions was fixed in 4% paraformaldehyde and then embedded in paraffin. After antigen retrieval, the sections were first stained with primary antibody at 4 °C overnight, then incubated with secondary antibody at 4 °C for 30 minutes. The nuclei were stained with DAPI.

Real-time PCR

Total RNA was extracted from skin specimens according to the protocol of the RNA extraction kit. Samples containing 0.5 μg of total RNA were utilized to obtain cDNA by reverse transcription using PrimeScriptTM RT Reagent Kit with gDNA Eraser (Perfect Real Time) (Takara). Real-time PCR was performed with a Lightcycler 480 and a SYBR Green

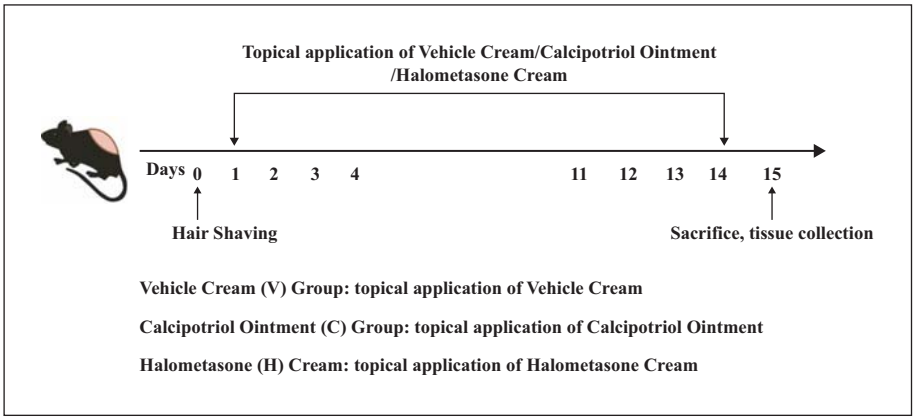

Figure 1. Summary of animal experiments.

Table 1. Mouse primer sequences.

GENE	Primer sequences
VEGF	Forward: 5′-AGGAGTACCCCGACGAGATAGA-3′
	Reverse: 5′-CACATCTGCTGTGCTGTAGGAA-3′
PEDF	Forward: 5′-TTACGATACGGCTTGGACTCTG-3′
	Reverse: 5′-ATGGTCAAGTTCTGGGTCACG-3′
GAPDH	Forward: 5′-CCTCGTCCCGTAGACAAAATG-3′
	Reverse: 5′TGAGGTCAATGAAGGGGTCGT-3′

system (Takara). The primers used in this study are shown in *table 1*.

Western blot analysis

Mouse skin lysates were initially obtained by homogenization. Protein concentrations were then determined and western blot analysis was performed, as previously described [27]. Briefly, skin proteins were obtained and prepared for SDS-PAGE gel electrophoresis. Membranes were transferred, blocked and incubated with primary and secondary antibodies, and then visualized. GAPDH was used as an internal control.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM) and were compared using unpaired two-tailed Student's t-tests or one-way ANOVA. The results were analysed using SPSS version 22.0 software. $P < 0.05$ was considered as statistically significant. Images were produced using Adobe Illustrator 2019 software and GraphPad Prism version 8.0 software.

Results

Establishment of the K14 VEGF transgenic mouse model

The K14 VEGF transgenic mice model was successfully established by the insertion of the K14 promoter and VEGF-A gene fragment into the genome of C57 mice using Crisper/Cas9 technology. Psoriasis-like lesions, including erythema (*figure 2A*), epidermal hyperplasia and dermal microangiosis (*figure 2B*), were observed in K14 VEGF mice, in contrast to C57 mice. Moreover, to investigate the level of VEGF expression, we harvested the dorsal skin from K14 VEGF and C57 mice and then determined the level of VEGF by quantitative real-time PCR and immunohistochemical staining. As shown in *figure 2C-E*, a marked increase in VEGF was detected in K14 VEGF mice compared to C57 mice. Overall, compared with wild-type mice, the K14 VEGF mice overexpressed VEGF-A, mediated by the human keratin 14 promoter, ultimately mimicking the clinical features of psoriasis.

Evaluation of PEDF level and MVD in K14 VEGF mice

PEDF is an angiogenic inhibitor that is not only closely associated with psoriasis [28-30], but also acknowledged as an antagonist against VEGF [29, 31, 32], while MVD is a common indicator of psoriatic lesions [33, 34]. Samples of dorsal skin from K14 VEGF mice and C57 mice were extracted and analysed. As shown in *figure 3A-C*, in contrast to C57 mice, lower expression levels of PEDF were detected in K14 VEGF mice by qRT-PCR and immunohistochemistry, suggesting an increased ratio of VEGF/PEDF. Furthermore, the level of CD31, ICAM-1, along with vessel number and dermal vascular area in the skin of K14 VEGF mice also increased compared with C57 mice (*figure 3D-G*), thus demonstrating increased MVD in the K14 VEGF mouse model.

Calcipotriol down-regulates the ratio of VEGF/PEDF in K14 VEGF mice

To investigate whether calcipotriol exerts its anti-psoriatic effect by regulating the levels of VEGF and PEDF, K14 VEGF mice were grouped and treated with different ointments, as previously described. Immunohistochemistry, qRT-PCR and western blotting were conducted after collecting the dorsal skin samples. Compared to the vehicle cream group, regular topical application of calcipotriol down-regulated the expression of VEGF in the skin of K14 VEGF mice (*figure 4A, C, D, F, G*), which was similar to

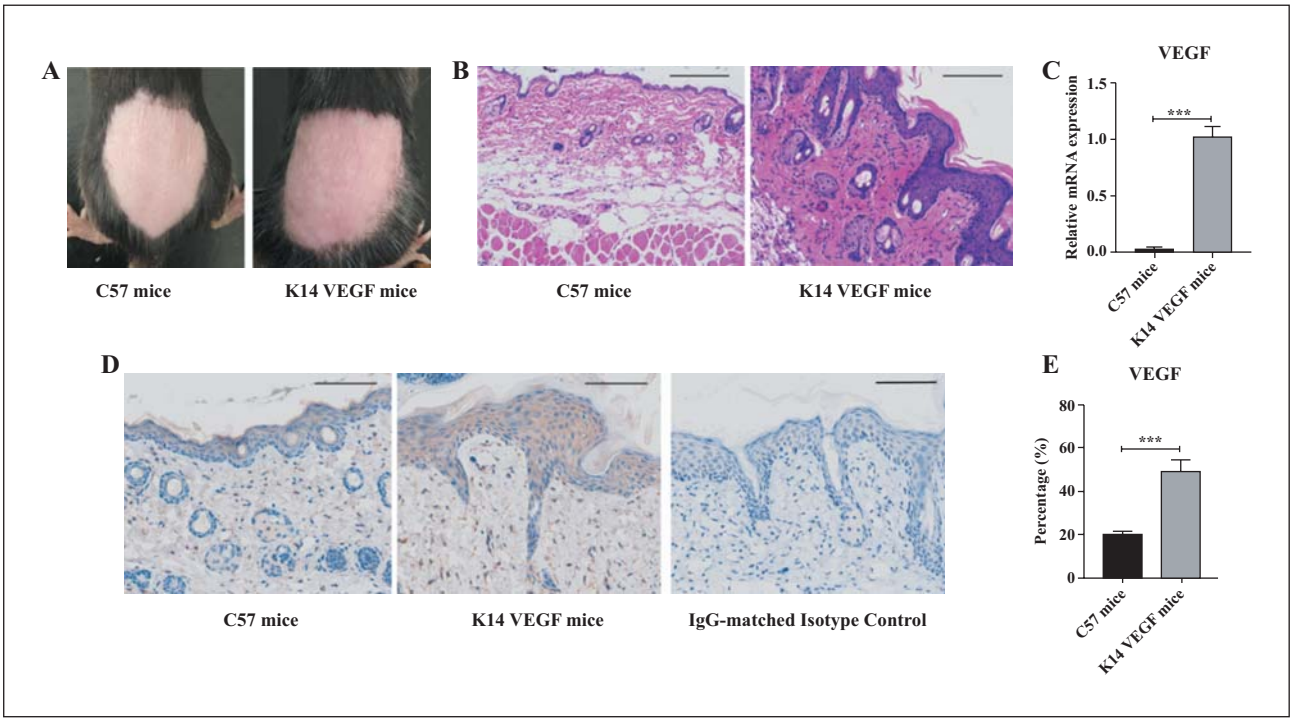

Figure 2. The K14 VEGF mice, in contrast to C57 mice, exhibit human-like psoriatic features. **A**) Erythema and scales observed on the back skin of K14 VEGF mice. **B**) Epidermal hyperplasia and dermal microangiosis observed following H&E staining of K14 VEGF mouse skin samples. **C**) RT-PCR of VEGF. **D**, **E**) Immunohistochemistry for VEGF. Images for H&E staining and immunohistochemistry were all captured at original magnification of ×200 (scale bar represents 100 μm). The data represent the mean ± SEM, $n = 3$, with statistical significance at ***$p < 0.001$.

that of the halometasone group. Moreover, the mRNA and protein level of PEDF were also increased in the calcipotriol group in contrast to the vehicle cream group (*figure 4B, C, E, H, I*). Overall, calcipotriol significantly down-regulated the ratio of VEGF/PEDF, similarly to halometasone, the positive control, demonstrating a strong therapeutic effect against angiogenesis.

Calcipotriol down-regulates MVD in K14 VEGF mice

The effect of calcipotriol on MVD in K14 VEGF mice was also investigated. Topical application of calcipotriol ointment and halometasone cream on the dorsal skin of the K14 VEGF mice led to a significant decline in the expression levels of CD31 and ICAM-1 (*figure 5A, B*). Furthermore, a decrease in the number of dermal vessels and shrinkage in the vascular area based on the immunohistochemical sections were also observed (*figure 5C, D*), demonstrating the effectiveness of calcipotriol in down-regulating MVD in the psoriasis-like lesions of K14 VEGF mice.

Calcipotriol enhances the level of Vitamin D receptor and inhibits JAK/STAT3 signalling in K14 VEGF mice

Since calcipotriol is a vitamin D analogue, its effect on vitamin D receptor (Vit DR) was evaluated. As shown in *figure 6A-C*, the level of vitamin D receptor was up-regulated after treatment with calcipotriol. Vitamin D receptor is known to be an effective drug target for autoimmune diseases as it regulates the expression of numerous associated genes [35, 36], among which those related to the JAK/STAT3 signalling pathway are of particular interest since the production of VEGF is reported to be mediated by STAT3 during angiogenesis [37, 38]. Therefore, the JAK/STAT3 signalling pathway was investigated in mice treated with calcipotriol. Compared with C57 mice, the phosphorylation levels of JAK2 and STAT3 were both enhanced in K14 VEGF mice. In addition, calcipotriol significantly decreased the level of pJAK and pSTAT3 in K14 VEGF mice (*figure 6D-F*).

Discussion

For many decades, calcipotriol has been principally used as topical treatment for psoriasis. However, its mechanism of action remains largely unclear. Several functions have been found to be related to the anti-psoriatic therapeutic effect of calcipotriol. For instance, calcipotriol suppresses the production of IL-6, IL-17/IL-23 and TNF-α, which are secreted by keratinocytes, dendritic cells and T cells [17, 39]. A previous study has shown that calcipotriol also alters the cellular structure and vascular distribution of the basal layer in psoriatic skin [24]. However, the effect of calcipotriol on the vascular system and the mechanism involved are not yet fully elucidated.

Consequently, we studied the effect of calcipotriol on the microvasculature of psoriatic lesions. Halometasone,

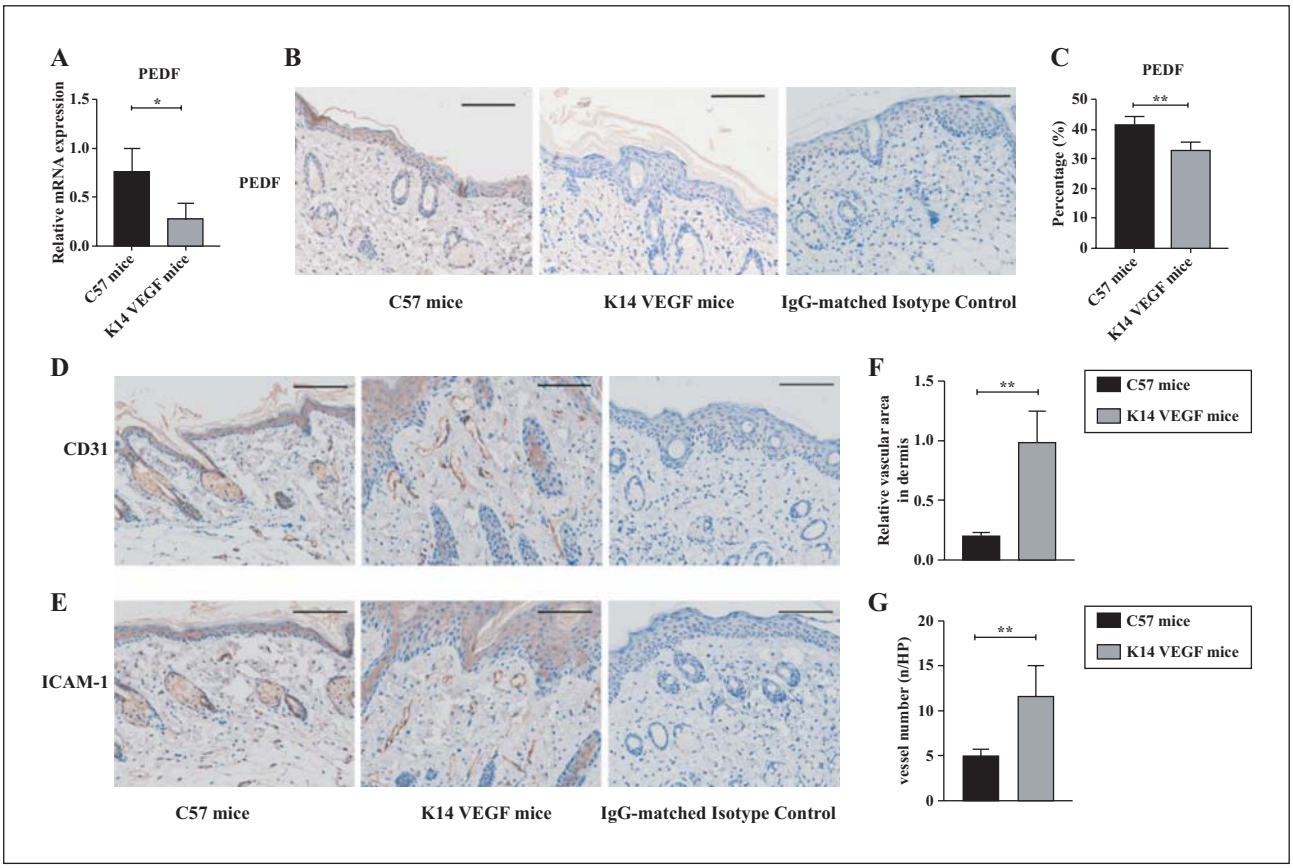

Figure 3. Expression of PEDF and MVD in K14 VEGF and C57 mice. **A**) RT-PCR for PEDF. **B**, **C**) Immunohistochemistry for PEDF (original magnification: ×200; scale bar represents 100 μm). **D**, **E**) Immunohistochemistry for CD31 (**D**) and ICAM-1 (**E**) as indicators of MVD (original magnification: ×200). The dermal vascular area (**F**) and the vessel number (**G**) were determined in CD31 immunohistochemical sections. The data represent mean ± SEM, $n = 3$, with statistical significance at $^{*}p<0.05$, $^{**}p<0.01$.

a medication widely acknowledged to be very useful as treatment for psoriasis, was utilized as a positive control for the evaluation of therapeutic effects of calcipotriol. The K14 VEGF mouse model was established by the insertion of a K14-based expression vector and a mouse cDNA encoding VEGF164 into C57 mice, leading to the overexpression of VEGF. VEGF-mediated micro-angiogenesis is considered to be one of the major drivers of the pathogenesis of psoriasis [40]. Thus, sustained increased levels of VEGF may ultimately reveal characteristic features of psoriasis, such as epidermis hyperplasia and lymphocyte infiltration [25]. Studies have shown that K14 VEGF mice begin to exhibit mild pre-psoriatic features at three months of age, and spontaneously develop the typical psoriasis-like manifestations, including epidermal hyperplasia, abnormal epidermal differentiation and up-regulation of vascular adhesion molecules [25] by the fifth month. Wang *et al*. applied imiquimod externally to both WT and K14 VEGF mice and found that the K14 VEGF mice mimicked more sustained, stable psoriasis-like skin inflammation [26]. Therefore, this model is ideal and practical for the study of micro-angiogenesis associated with psoriasis. Consistent with the above reports, we successfully established the K14 VEGF mouse model, simulating the clinical and pathological features of human psoriasis. Compared with C57 mice, the dorsal skin of K14 VEGF mice exhibited erythema and scaling. Epidermal proliferation, overexpression of VEGF and hyperplasia of blood vessels were also observed as part of the pathogenesis of K14 VEGF mice (*figure 2*).

PEDF is a potent angiogenic inhibitor that produces the opposite effects to VEGF, and there is a general balance between PEDF and VEGF to maintain body homeostasis [29, 31, 32]. Yan *et al.* demonstrated that the imbalance between VEGF and PEDF could be one of the major factors in the transformation of normal skin into diseased skin in psoriasis patients [41]. Therefore, the VEGF/PEDF ratio is an ideal indicator of angiogenic capability. The experimental results from this study show that, compared to C57 mice, the skin of K14 VEGF mice displayed increased expression of VEGF and decreased expression of PEDF, resulting in a higher VEGF/PEDF ratio (*Figures 2*, *3*). Furthermore, this ratio was significantly down-regulated after topical application of calcipotriol, with a decrease in VEGF level and increase in PEDF (*figure 4*). Our experimental results suggest that calcipotriol exerts its anti-angiogenic therapeutic effect against psoriasis through the regulation of the VEGF/PEDF ratio.

CD31, also known as platelet endothelial cell adhesion molecule-1, plays a key role in angiogenesis, neutrophil recruitment and platelet aggregation [12, 42]. It is highly expressed in the endothelial cells of newly formed vessels,

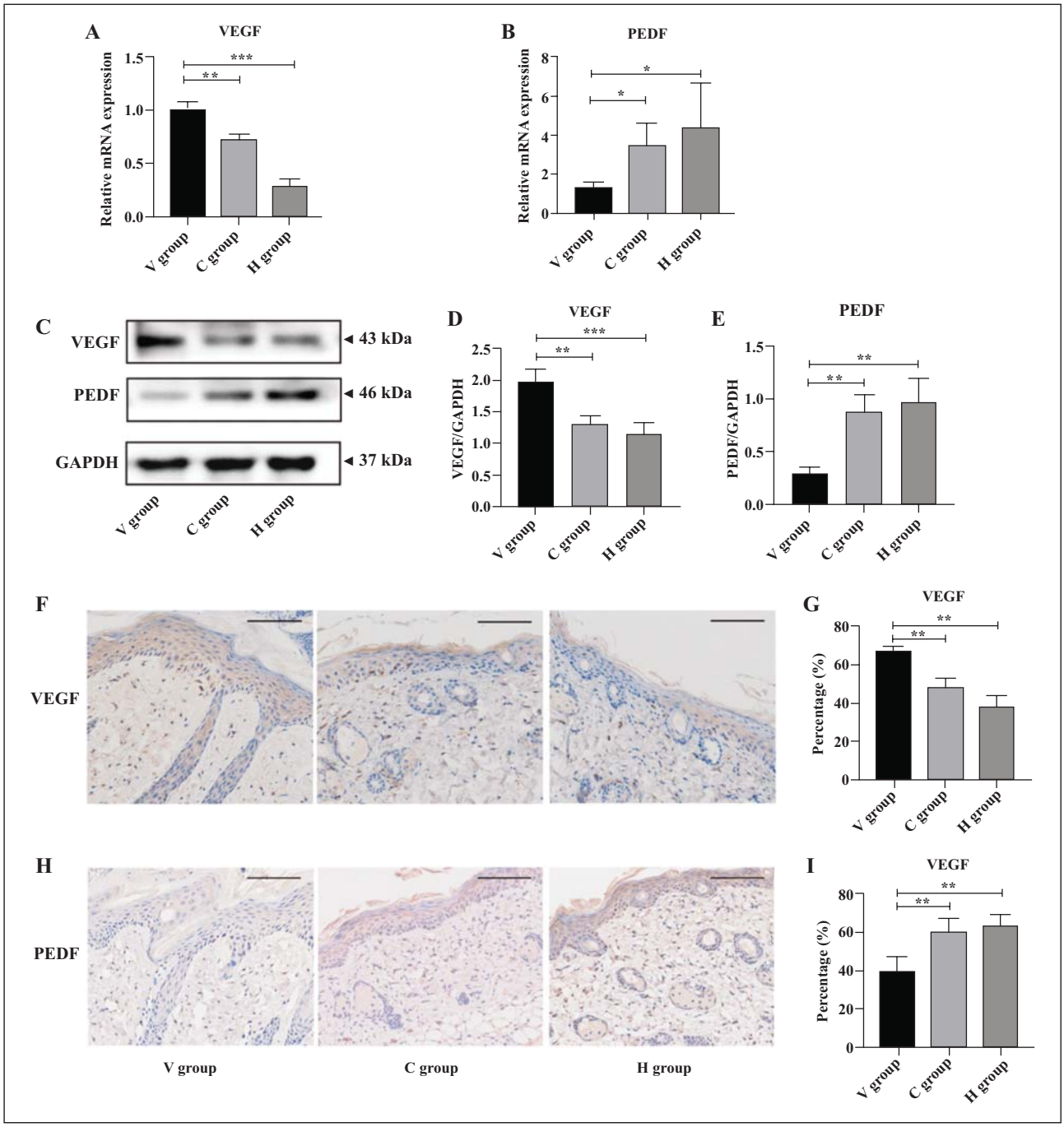

Figure 4. Calcipotriol exhibits anti-angiogenic effects by regulating the ratio of VEGF to PEDF in K14 VEGF mice. The mRNA levels of VEGF and PEDF were determined by RT-PCR (**A**, **B**) and protein levels by western bolt analysis (**C**) and immunohistochemistry (original magnification × 200; scale bar represents 100 μm) (**F-I**). **D**, **E**) Ratio between VEGF and PEDF based on expression levels. V group: vehicle cream group; C group: calcipotriol group; H group: halometasone group. The data represent mean ± SEM, $n = 3$, with statistical significance at $^{*}p<0.05$,$^{**}p<0.01$, $^{***}p<0.001$.

making it a useful marker of neovascularization [43]. Intercellular cell adhesion molecule-1 (ICAM-1) is a member of the cell adhesion molecule family. The continuous and up-regulated expression of ICAM-1 in dermal vascular endothelial cells may result in aggregation of inflammatory cells [13, 44]. In this study, the levels of CD31 and ICAM-1, along with vessel number and dermal vascular area, were used as indicators of MVD, and their levels in K14 VEGF mice lesions all decreased following calcipotriol treatment (*figure 5*).

The underlying mechanism of the effect of calcipotriol on K14 VEGF mice was also investigated. Calcipotriol is a synthetic vitamin D analogue that regulates keratinocyte function through vitamin D receptors [45]. In this study, calcipotriol was found to significantly up-regulate the level of vitamin D receptors (*figure 6*). Vitamin D receptor plays

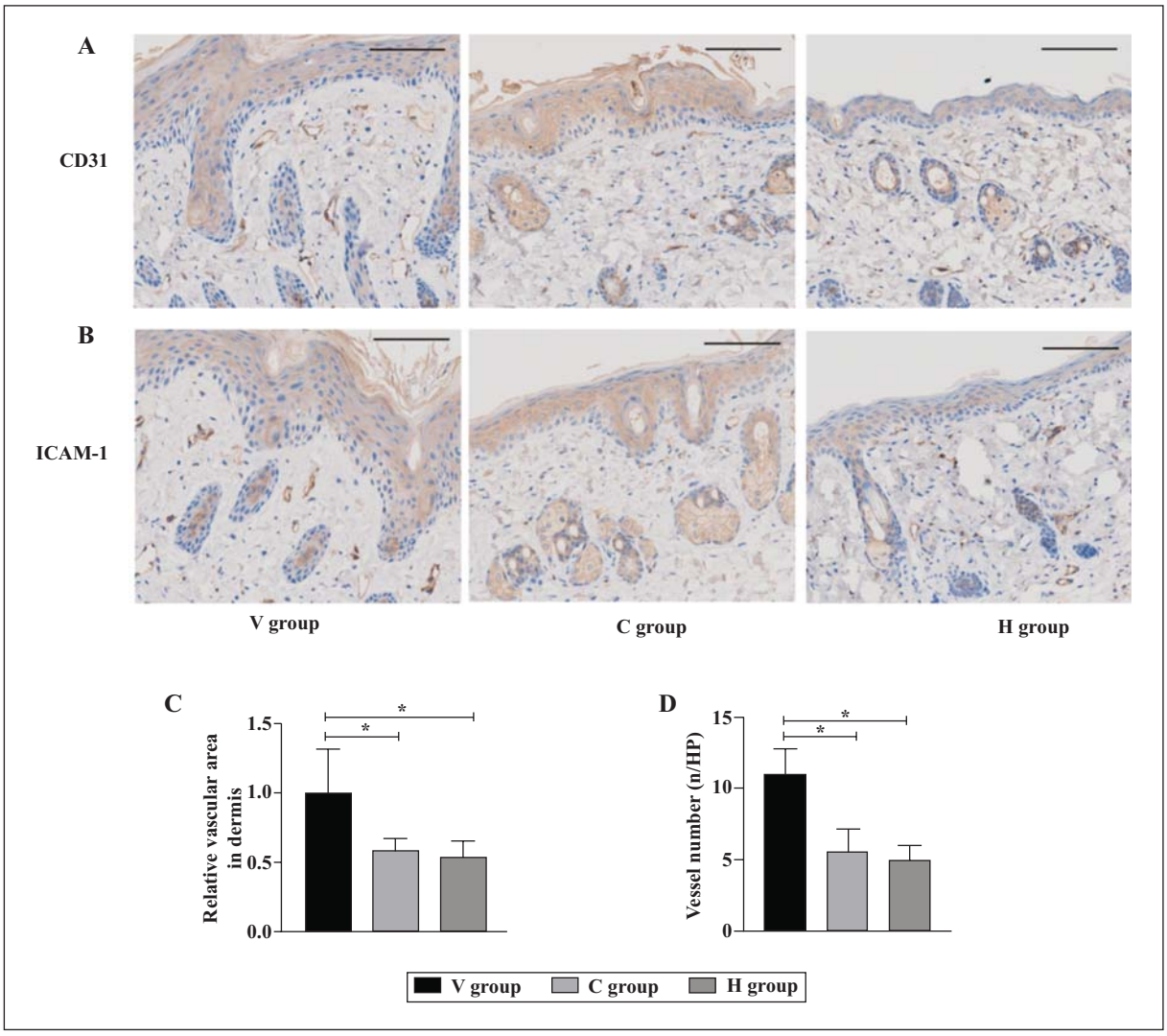

Figure 5. The effect of calcipotriol on MVD in K14 VEGF mice. To evaluate the effect of calcipotriol on MVD, the expression of CD31 (**A**) and ICAM-1 (**B**) were evaluated by immunohistochemistry (original magnification × 200; scale bar represents 100 μm). Dermal vascular area (**C**) and vessel number (**D**) in the CD31 immunohistochemical sections were quantified. V group: vehicle cream group; C group: calcipotriol group; H group: halometasone group. The data represent mean ± SEM, $n = 3$, with statistical significance at $^*p < 0.05$.

a central role in the regulation of many genes involved in calcium/phosphate homeostasis, cellular proliferation, differentiation and the immune response [35]. Many studies have demonstrated that the vitamin D receptor is implicated in the regulation of the JAK/STAT signalling pathway [46-48], which is involved in the pathogenesis of many autoimmune diseases including psoriasis [49, 50]. Moreover, the JAK/STAT signalling pathway is also closely associated with the expression of VEGF, as activation of JAK/STAT signalling effectively leads to the production of VEGF [51-53]. Furthermore, Wei *et al*. found that activated STAT3 directly activated the VEGF promoter, whereas dominant-negative STAT3 inhibited the VEGF promoter [54]. Therefore, JAK/STAT signalling is highly likely to be involved in the effect of vitamin D analogue treatment on the VEGF-induced psoriasis model. The results of this study also indicate that JAK/STAT signalling was enhanced in K14 VEGF mice and inhibited by calcipotriol treatment.

Overall, although the mechanisms through which calcipotriol exerts its effect in K14 VEGF mice may be complicated, due to the diverse and multifunctional role of vitamin D receptor, we identify JAK/STAT signalling as a possible prominent factor. The interactions between PEDF and VEGF are largely unclear, and it has been reported that PEDF may decrease the expression of VEGF through many pathways, including suppression of VEGF promoter activity and inhibiting VEGF-VEGF receptor 2 binding [31]. The down-regulation of JAK/STAT signalling by calcipotriol suggests the existence of an anti-VEGF pathway that may not directly involve PEDF.

Conclusion

Calcipotriol is a safe and effective drug for the treatment of psoriasis, yet its effects on microvascular hyperplasia associated with psoriasis require further clarification.

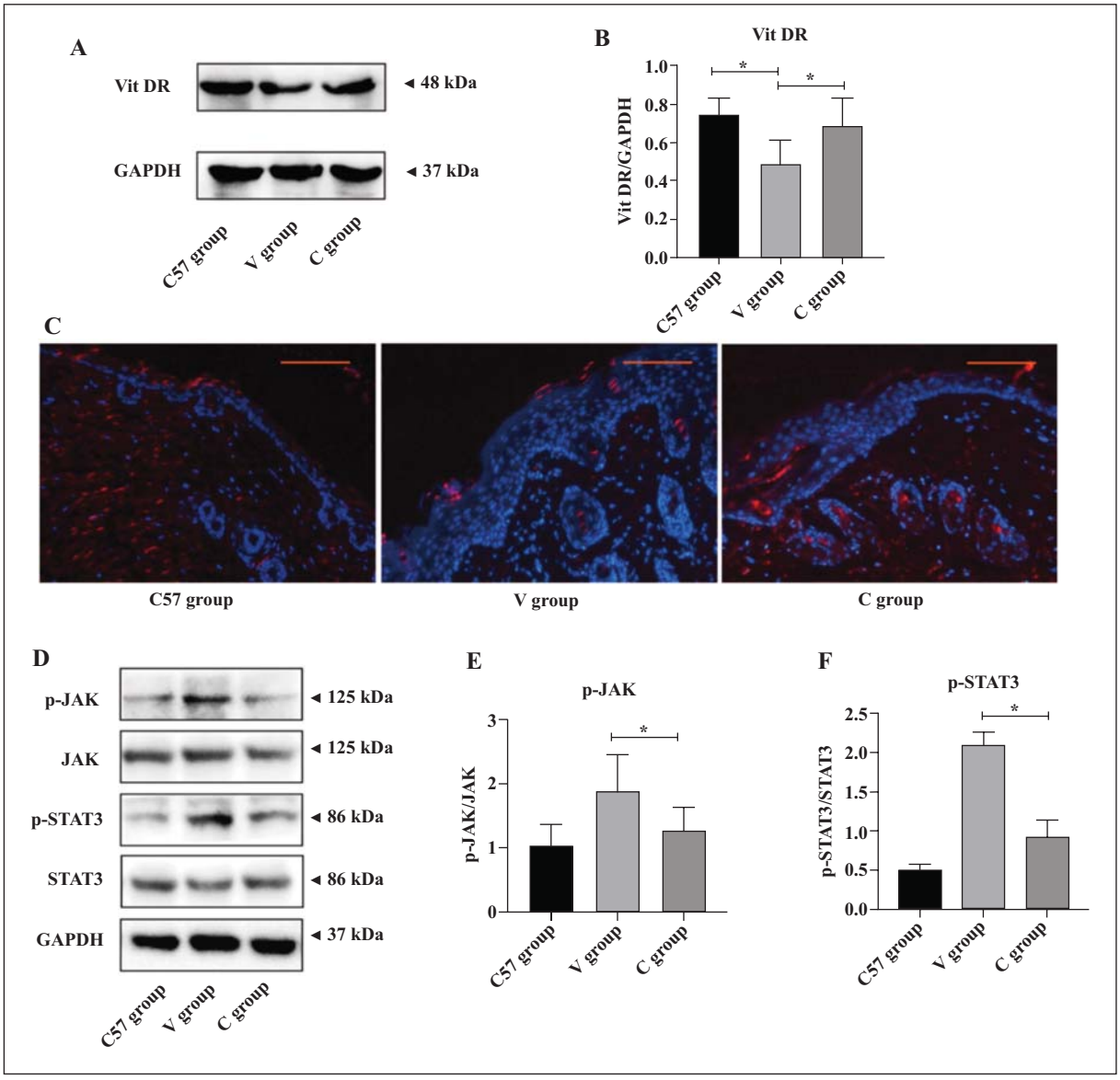

Figure 6. Vitamin D receptor (Vit DR) expression and JAK/STAT phosphorylation in the C57 group, V (vehicle cream) group and C (calcipotriol) group. **A**-**C**) Immunofluoresence for Vit DR; red indicates Vit DR and blue indicates nuclei (original magnification: 200 × ; scale bar represents 100 μm). **D**-**F**) Phosphorylation of JAK and STAT3. The data represent mean ± SEM, $n = 3$, with statistical significance at $*p<0.05$.

This study adopted a proven K14 VEGF mouse model to explore the regulatory effects of calcipotriol on microvascular proliferation associated with psoriasis. We uncovered that calcipotriol may exert an anti-angiogenic effect in psoriasis by down-regulating the expression of VEGF and up-regulating the expression of PEDF, re-balancing the ratio of VEGF to PEDF, and ultimately decreasing MVD. According to the results of further experiments on vitamin D receptor and JAK/STAT3 signalling, we propose that calcipotriol could down-regulate the expression of VEGF by inhibiting the JAK/STAT3 signalling pathway, thus alleviating VEGF-induced psoriasis-like angiogenesis in K14 VEGF mice. ■

Acknowledgments and disclosures. *Acknowledgments: this work was financially supported by the Dermatology Research Fund of China Association of Integrative Medicine (No.2018-2).*
Conflicts of interest: none.

References

1. Lebwohl M. Psoriasis. *Ann Intern Med* 2018; 168: ITC49-64.
2. Kamiya K, Kishimoto M, Sugai J, Komine M, Ohtsuki M. Risk factors for the development of psoriasis. *Int J Mol Sci* 2019; 20: 4347.
3. Bae ON, Noh M, Chun YJ, Jeong TC. Keratinocytic vascular endothelial growth factor as a novel biomarker for pathological skin condition. *Biomol Ther (Seoul)* 2015; 23: 12-8.

4. Bhushan M, McLaughlin B, Weiss J, Griffiths C. Levels of endothelial cell stimulating angiogenesis factor and vascular endothelial growth factor are elevated in psoriasis. *Br J Dermatol* 1999; 141: 1054-60.

5. Marina ME, Roman II, Constantin AM, Mihu CM, Tătaru AD. VEGF involvement in psoriasis. *Clujul Med* 2015; 88: 247-52.

6. Grozdev I, Korman N, Tsankov N. Psoriasis as a systemic disease. *Clin Dermatol* 2014; 32: 343-50.

7. Matsui T, Nishino Y, Maeda S, Yamagishi S. PEDF-derived peptide inhibits corneal angiogenesis by suppressing VEGF expression. *Microvasc Res* 2012; 84: 105-8.

8. Gao G, Li Y, Zhang D, Gee S, Crosson C, Ma J. Unbalanced expression of VEGF and PEDF in ischemia-induced retinal neovascularization. *FEBS Lett* 2001; 489: 270-6.

9. Watabe D, Kanno H, Yoshida A, Kurose A, Akasaka T, Sawai T. Adhesion of peripheral blood mononuclear cells and CD4+ T cells from patients with psoriasis to cultured endothelial cells *via* the interaction between lymphocyte function-associated antigen type 1 and intercellular adhesion molecule 1. *Br J Dermatol* 2007; 157: 259-65.

10. Xiong H, Xu Y, Tan G, *et al.* Glycyrrhizin ameliorates imiquimod-induced psoriasis-like skin lesions in BALB/c mice and inhibits TNF-α-induced ICAM-1 expression via NF-κB/MAPK in HaCaT cells. *Cell Physiol Biochem* 2015; 35: 1335-46.

11. Teixeira G, Mari N, de Paula J, *et al.* Cell adhesion molecules, plasminogen activator inhibitor type 1, and metabolic syndrome in patients with psoriasis. *Clin Exp Med* 2020; 20: 39-48.

12. Musumeci G, Castorina A, Magro G, Cardile V, Castorina S, Ribatti D. Enhanced expression of CD31/platelet endothelial cell adhesion molecule 1 (PECAM1) correlates with hypoxia inducible factor-1 alpha (HIF-1α) in human glioblastoma multiforme. *Exp Cell Res* 2015; 339: 407-16.

13. Bressan A, Picciani B, Azulay-Abulafia L, *et al.* Evaluation of ICAM-1 expression and vascular changes in the skin of patients with plaque, pustular, and erythrodermic psoriasis. *Int J Dermatol* 2018; 57: 209-16.

14. Chen J, Zhu Z, Li Q, *et al.* Neutrophils enhance cutaneous vascular dilation and permeability to aggravate psoriasis by releasing matrix metallopeptidase 9. *J Invest Dermatol* 2021; 141: 787-99.

15. Zhu Z, Chen J, Lin Y, *et al.* Aryl hydrocarbon receptor in cutaneous vascular endothelial cells restricts psoriasis development by negatively regulating neutrophil recruitment. *J Invest Dermatol* 2020; 140: 1233-43.e9.

16. Huhtakangas JA, Veijola J, Turunen S, *et al.* 1,25(OH)(2)D(3) and calcipotriol, its hypocalcemic analog, exert a long-lasting anti-inflammatory and anti-proliferative effect in synoviocytes cultured from patients with rheumatoid arthritis and osteoarthritis. *J Steroid Biochem Mol Biol* 2017; 173: 13-22.

17. Lovato P, Norsgaard H, Tokura Y, Røpke MA. Calcipotriol and betamethasone dipropionate exert additive inhibitory effects on the cytokine expression of inflammatory dendritic cell-Th17 cell axis in psoriasis. *J Dermatol Sci* 2016; 81: 153-64.

18. Germán B, Wei R, Hener P, *et al.* Disrupting the IL-36 and IL-23/IL-17 loop underlies the efficacy of calcipotriol and corticosteroid therapy for psoriasis. *JCI Insight* 2019; 4: e123390.

19. Liang W, Lin Z, Zhang L, Qin X, Zhang Y, Sun L. Calcipotriol inhibits proliferation of human keratinocytes by downregulating STAT1 and STAT3 signaling. *J Investig Med* 2017; 65: 376-81.

20. Mishra SK, Wheeler JJ, Pitake S, *et al.* Periostin activation of integrin receptors on sensory neurons induces allergic itch. *Cell Rep* 2020; 31: 107472.

21. Liu X, Liu Y, Xu M, *et al.* Zinc finger protein A20 is involved in the antipsoriatic effect of calcipotriol. *Br J Dermatol* 2016; 175: 314-24.

22. Tang YJ, Zhang RZ, Liu XM, Xu CX, Cheng S, Liu QI. Effect of the topical application of calcipotriol on the expression levels of zinc finger protein A20 and nuclear factor-kappaB in the skin lesions of patients with psoriasis vulgaris. *Exp Ther Med* 2016; 11: 247-50.

23. Merrigan SL, Kennedy BN. Vitamin D receptor agonists regulate ocular developmental angiogenesis and modulate expression of dre-miR-21 and VEGF. *Br J Pharmacol* 2017; 174: 2636-51.

24. Palleschi GM, Gentili A, Caproni M, Giacomelli A, Falcos D, Fabbri P. Structural alterations of basal keratinocytes and capillary loop in psoriasis during treatment with topical calcipotriol. *Acta Derm Venereol Suppl* 1994; 186: 49-51.

25. Xia YP, Li B, Hylton D, Detmar M, Yancopoulos GD, Rudge JS. Transgenic delivery of VEGF to mouse skin leads to an inflammatory condition resembling human psoriasis. *Blood* 2003; 102: 161-8.

26. Wang X, Sun J, Hu J. IMQ induced K14-VEGF mouse: a stable and long-term mouse model of psoriasis-like inflammation. *PLoS One* 2015; 10: e0145498.

27. Wang ZY, Li YQ, Guo ZW, *et al.* ERK1/2-HNF4α axis is involved in epigallocatechin-3-gallate inhibition of HBV replication. *Acta Pharmacol Sin* 2020; 41: 278-85.

28. He L, Dang L, Zhou J, Bai J, Li YZ. Association of angiopoietin-1, angiopoietin-2 and caspase-5 polymorphisms with psoriasis vulgaris. *Clin Exp Dermatol* 2015; 40: 556-63.

29. Xi L. Pigment epithelium-derived factor as a possible treatment agent for choroidal neovascularization. *Oxid Med Cell Longev* 2020; 2020: 8941057.

30. Abe R, Yamagishi S, Fujita Y, *et al.* Topical application of anti-angiogenic peptides based on pigment epithelium-derived factor can improve psoriasis. *J Dermatol Sci* 2010; 57: 183-91.

31. Zhang SX, Wang JJ, Gao G, Parke K, Ma JX. Pigment epithelium-derived factor downregulates vascular endothelial growth factor (VEGF) expression and inhibits VEGF-VEGF receptor 2 binding in diabetic retinopathy. *J Mol Endocrinol* 2006; 37: 1-12.

32. Yamagishi S, Matsui T, Nakamura K, *et al.* Pigment-epithelium-derived factor (PEDF) inhibits angiotensin-II-induced vascular endothelial growth factor (VEGF) expression in MOLT-3 T cells through anti-oxidative properties. *Microvasc Res* 2006; 71: 222-6.

33. Liu JH, Wu HH, Zhao YK, Wang F, Gao Q, Luo DQ. Thalidomide improves psoriasis-like lesions and inhibits cutaneous vegf expression without alteration of microvessel density in imiquimod- induced psoriatic mouse model. *Curr Vasc Pharmacol* 2018; 16: 510-21.

34. Zemheri E, Karadag AS, Zindanci I, Zerk PE, Ozturk MK. Evaluation of microvessel density with CD31 and CD105 in patients with psoriasis under methotrexate and acitretin therapy. *Postepy Dermatol Alergol* 2020; 37: 422-7.

35. Wang Y, Zhu J, DeLuca HF. Where is the vitamin D receptor? *Arch Biochem Biophys* 2012; 523: 123-33.

36. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. *Nutrients* 2013; 5: 2502-21.

37. Hong C, Lee C, Chen G, Chang K, Yu H. STAT3-dependent VEGF production from keratinocytes abrogates dendritic cell activation and migration by arsenic: a plausible regional mechanism of immunosuppression in arsenical cancers. *Chem Biol Interact* 2015; 227: 96-103.

38. Dong W, Xian Y, Yuan W, *et al.* Catalpol stimulates VEGF production *via* the JAK2/STAT3 pathway to improve angiogenesis in rats' stroke model. *J Ethnopharmacol* 2016; 191: 169-79.

39. Suzuki T, Sakabe J, Kamiya K, Funakoshi A, Tokura Y. The vitamin D3 analogue calcipotriol suppresses CpG-activated TLR9-MyD88 signalling in murine plasmacytoid dendritic cells. *Clin Exp Dermatol* 2018; 43: 445-8.

40. Malecic N, Young H. Novel investigational vascular endothelial growth factor (VEGF) receptor antagonists for psoriasis. *Expert Opin Investig Drugs* 2016; 25: 455-62.

41. Yan BX, Zheng YX, Li W, *et al.* Comparative expression of PEDF and VEGF in human epidermal keratinocytes and dermal fibroblasts: from normal skin to psoriasis. *Discov Med* 2018; 25: 47-56.

42. Kellermair J, Redwan B, Alias S, *et al.* Platelet endothelial cell adhesion molecule 1 deficiency misguides venous thrombus resolution. *Blood* 2013; 122: 3376-84.

43. Zemheri E, Karadağ A, Zindancı I, Zerk P, Ozturk M. Evaluation of microvessel density with CD31 and CD105 in patients with psoriasis under methotrexate and acitretin therapy. *Postepy Dermatol Alergol* 2020; 37: 422-7.

44. Vautrin-Glabik A, Devy J, Bour C, *et al*. Angiogenesis inhibition by a short 13 amino acid peptide sequence of tetrastatin, the α4(IV) NC1 domain of collagen IV. *Front Cell Dev Biol* 2020; 8: 775.

45. Kragballe K. Treatment of psoriasis with calcipotriol and other vitamin D analogues. *J Am Acad Dermatol* 1992; 27: 1001-8.

46. Lange CM, Gouttenoire J, Duong FH, Morikawa K, Heim MH, Moradpour D. Vitamin D receptor and Jak-STAT signaling crosstalk results in calcitriol-mediated increase of hepatocellular response to IFN-alpha. *J Immunol* 2014; 192: 6037-44.

47. Zhang YG, Lu R, Wu S, *et al*. Vitamin D receptor protects against dysbiosis and tumorigenesis *via* the JAK/STAT pathway in intestine. *Cell Mol Gastroenterol Hepatol* 2020; 10: 729-46.

48. Yang Y, Lei Y, Liang Y, *et al*. Vitamin D protects glomerular mesangial cells from high glucose-induced injury by repressing JAK/STAT signaling. *Int Urol Nephrol* 2021; 53: 1247-54.

49. Banerjee S, Biehl A, Gadina M, Hasni S, Schwartz DM. JAK-STAT signaling as a target for inflammatory and autoimmune diseases: current and future prospects. *Drugs* 2017; 77: 521-46.

50. Kvist-Hansen A, Hansen PR, Skov L. Systemic treatment of psoriasis with JAK inhibitors: a review. *Dermatol Ther (Heidelb)* 2020; 10: 29-42.

51. Hwang S, Seong H, Ryu J, *et al*. Phosphorylation of STAT3 and ERBB2 mediates hypoxiainduced VEGF release in ARPE19 cells. *Mol Med Rep* 2020; 22: 2733-40.

52. Zhao J, Du P, Cui P, *et al*. LncRNA PVT1 promotes angiogenesis *via* activating the STAT3/VEGFA axis in gastric cancer. *Oncogene* 2018; 37: 4094-109.

53. Yang M, Wang L, Wang X, Wang X, Yang Z, Li J. IL-6 promotes FSH-induced VEGF expression through JAK/STAT3 signaling pathway in bovine granulosa cells. *Cell Physiol Biochem* 2017; 44: 293-302.

54. Wei D, Le X, Zheng L, *et al*. Stat3 activation regulates the expression of vascular endothelial growth factor and human pancreatic cancer angiogenesis and metastasis. *Oncogene* 2003; 22: 319-29.