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Original Article

JAK/STAT and VEGF/PAK1 signaling as emerging targets for topical treatment of psoriasis: a pilot study

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Abstract: Psoriasis is reportedly modulated by the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) or vascular endothelial growth factor/p21-activated kinase 1 (VEGF/PAK1) pathways. However, no research has evaluated the expression of JAK/STAT and VEGF/PAK1 signaling pathway molecules in human psoriasis skin tissue concurrently. We investigated the expression of autocrine STAT1, STAT3, VEGF, suppressor of cytokine signaling-1 (SOCS1), SOCS3, and PAK1 in psoriatic tissues. Skin biopsies were retrospectively collected from 55 patients with psoriasis from the tissue biobank. Skin biopsies from 40 healthy volunteers undergoing plastic surgery were used as controls. Immunohistochemical staining revealed that STAT1, STAT3, SOCS1, SOCS3, VEGF, and PAK1 were present at significantly higher levels in the psoriasis samples compared to the control group. Similarly, the mRNA expression of these signaling molecules was also significantly upregulated in psoriatic skin. Additionally, some of the molecules in these two signaling pathways exhibited significant positive correlations. In summary, we present pilot evidence that JAK/STAT and VEGF/PAK1 signaling molecules are expressed in psoriasis, which may provide topical treatment targets for this disease.

Keywords: STAT, SOCS, VEGF, PAK1, psoriasis

Introduction

Psoriasis is an immune-mediated, genetic disease affecting the skin, joints, or both. When involving the skin, this chronic inflammatory disease is characterized by the development of scaly, red, and well-demarcated skin lesions because of the hyperproliferation of epidermal keratinocytes [1]. Cytokines from multiple sources (e.g., activated tissue-resident immune cells, T-cell infiltrates, dendritic cells, innate immune cells, keratinocytes) mediate this proliferation [2]. Genome-wide association studies identified genetic variations within or near some genes which encode cytokines, cytokine receptors, or members of their signal transduction pathways, which suggests that these cytokines play a key role in the pathogenesis of psoriasis [3, 4].

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway [5, 6] affects cell growth, differentiation, and death in response to growth factors and cytokines [7]. This pathway is also associated with the expression of vascular endothelial growth factor (VEGF) [8, 9], which is involved in psoriasis development and the regulation of the expression of p21-activated kinases (PAKs) [10-12]. Previous studies demonstrated that the expression of inflammatory mediators in psoriatic skin can be suppressed by interfering with the JAK/STAT signaling pathway [13, 14]. Furthermore, JAK1 plays a pro-inflammatory role in the pathogenesis of psoriasis, presumably by increasing STAT3 expression. Thus, tissue expression of JAK1 and STAT3 could represent markers of psoriasis severity [15]. However, no studies have evaluated the expression of JAK/STAT and VEGF/PAK1 signaling pathway molecules in human psoriasis skin tissues concurrently. Therefore, the present pilot study investigated the classic molecules of the JAK/STAT and VEGF/PAK1 signaling pathways (i.e., STAT1, STAT3, suppressor of cytokine signaling-1 [SOCS1], SOCS3, VEGF, and PAK1)

using immunohistochemistry and real-time quantitative polymerase chain reaction (qPCR) to identify possible targets for topical treatment of psoriasis.

Materials and methods

Patient biopsies

Skin biopsies from psoriasis patients (n = 55) with unequivocal disease diagnosis (clinical and histopathologic) were retrospectively retrieved from the tissue biobank of the 7th People's Hospital of Shenyang. Skin biopsies of healthy volunteers (n = 40) undergoing plastic surgery were used as controls. Among these samples, lesional skin biopsies from patients with psoriasis (n = 20) and controls (n = 20) were preserved in Trizol (RNAiso Plus, Takara Bio Inc., Kusatsu, Japan) at -80°C. Included patients did not receive systemic or topical immunosuppresants or immunomodulators less than 15 days before the biopsy. The Declaration of Helsinki principles were followed in this study. The protocol was approved by the Ethics Committee of the 7th People's Hospital of Shenyang (Number: 2015-12-007). Written informed consent was granted from subjects prior to the skin biopsy. For retrospective material, patients were informed of the research.

Immunohistochemistry

Briefly, biopsy tissue was fixed first in 4% paraformaldehyde and then subsequently embedded in paraffin. Antigen retrieval was performed on deparaffinized tissue sections (4 μm) in near-boiling sodium citrate buffer for 10 min. EnVision FLEX peroxidase-blocking reagent (Dako, Glostrup, Denmark) was used to block the fixed biopsy tissue, which was then incubated overnight with primary antibodies (Bioss, Beijing, China) at 4°C. EnVision FLEX/HRP detection reagent with AEC+ (Dako) was used to visualize the staining after subsequent counterstaining with EnVision Flex Haematoxylin (Dako, Glostrup, Denmark). Pictures were taken on an Axiophot fluorescence microscope (Zeiss, Wetzlar, Germany) with an Axiocam camera and Axiovision software (Zeiss, Zaventem, Belgium) [16].

The complete antibody panel was used to stain the skin biopsies (n = 20 healthy controls, 35 psoriasis patients). The optical density of each slide was blindly analyzed using ImageJ software.

RNA isolation and qPCR analysis

Frozen skin tissue (100 mg) was pulverized, and total RNA was isolated with Trizol (RNAiso Plus, Takara Bio Inc.). RNA was quantified with a Nanodrop (Thermo Fisher Scientific, Waltham, MA, USA). cDNA was prepared using the PrimeScript™ RT reagent kit (Takara Bio Inc.) in the following reaction mixture: 2 μL 5× PrimeScript Buffer, 0.5 μL Random 6-mers (100 μM), 0.5 μL Oligo dT Primer (50 μM), 4.5 μL dH_2O, 0.5 μL PrimeScript RT Enzyme Mix I, and 2 μL total RNA (400 ng). Reverse transcription was carried out in the GeneAmp® PCR System 9700 (Life Technologies, Carlsbad, CA, USA) using the following thermal conditions: 37°C for 15 min, 85°C for 5 s, and 4°C for 5 min. The qPCR was performed in triplicate in a LightCycler®480 Instrument II (F. Hoffmann-La Roche Ltd., Basel, Switzerland) using reaction mixtures containing 6 μL dH_2O, 10 μL SYBR®Premix Ex Taq™ II (2×, Takara Bio Inc.), 0.8 μL forward primer (10 μM), 0.8 μL reverse primer (10 μM), 0.4 μL ROX Reference Dye II (50×), and 2 μL cDNA, as previously described [17, 18]. Relative expression of STAT1, STAT3, SOCS1, SOCS3, VEGF, and PAK1 was quantified using the comparative CT, $2^{-(\Delta\Delta CT)}$ method, with β-actin as the endogenous reference. The primers are listed in **Table 1**.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows version 22.0 software (IBM Corp., Armonk, NY, USA). Differences between psoriasis and control groups were determined with the independent sample t-test. Correlations were assessed by Pearson correlation analysis. $P < 0.05$ indicated statistical significance.

Results

Demographic and clinical information

The complete demographic and clinical data of the patients in this study are presented in **Table 2**.

STAT1, STAT3, SOCS1, SOCS3, VEGF, and PAK1 expression in psoriatic skin

All six JAK/STAT and VEGF/PAK1 signaling molecules investigated were expressed in the psoriatic epidermis and dermis (**Figure 1**). All six

Table 1. Primer sequences and conditions

Gene	Primer sequences (5' → 3')	Annealing-cycles	Restriction enzyme conditions
STAT1	Forward Primer CAGCTTGACTCAAAATTCCTGGA Reverse Primer TGAAGATTACGCTTGCTTTTCCT	95°C 5 min; 95°C 30 s, 59°C 30 s, 72°C 30 s (40 cycles); 72°C 5 min	37°C 15 min
STAT3	Forward Primer CAGCAGCTTGACACACGGTA Reverse Primer AAACACCAAAGTGGCATGTGA	95°C 5 min; 95°C 30 s, 59°C 30 s, 72°C 30 s (40 cycles); 72°C 5 min	37°C 15 min
SOCS1	Forward Primer CACGCACTTCCGCACATTC Reverse Primer TAAGGGCGAAAAAGCAGTTCC	95°C 5 min; 95°C 30 s, 59°C 30 s, 72°C 30 s (40 cycles); 72°C 5 min	37°C 15 min
SOCS3	Forward Primer CCTGCGCCTCAAGACCTTC Reverse Primer GTCACTGCGCTCCAGTAGAA	95°C 5 min; 95°C 30 s, 59°C 30 s, 72°C 30 s 40 cycles); 72°C 5 min	37°C 15 min
VEGF	Forward Primer GAGGAGCAGTTACGGTCTGTG Reverse Primer TCCTTTCCTTAGCTGACACTTGT	95°C 5 min; 95°C 30 s, 59°C 30 s, 72°C 30 s (40 cycles); 72°C 5 min	37°C 15 min
PAK1	Forward Primer GTCCTCTTTGGGTTCGCTGTA Reverse Primer CCAGTGACCACAAAACGACTAT	95°C 5 min; 95°C 30 s, 59°C 30 s, 72°C 30 s (40 cycles); 72°C 5 min	37°C 15 min

Table 2. Demographic and clinical characteristics of patients

Data	Psoriasis	Control
Female	26	18
Male	29	22
Mean age (years)	43.25±11.37	41.64±13.21
PASI		
Mild (PASI ≤ 10)	0	40
Moderate-high (PASI > 10)	55	0
Psoriasis arthritis		
Yes	23	
No	32	40
Family history		
Yes	29	2
No	26	38

PASI, psoriasis area and severity index.

signaling molecules were present in the cytoplasm, cell membrane, and nucleus of keratinocytes. A comparison of the average optical density results between the two study groups revealed that STAT1, STAT3, SOCS1, SOCS3, VEGF, and PAK1 were at significantly higher levels in the psoriasis group compared with the control group (**Figure 2**).

Relative STAT1, STAT3, SOCS1, SOCS3, VEGF, and PAK1 mRNA expression in psoriatic skin

The mRNA expression of the JAK/STAT and VEGF/PAK signaling molecules in the two study groups was evaluated using qPCR. Compared to healthy skin, STAT1, STAT3, SOCS1, SOCS3, VEGF, and PAK1 were significantly overexpressed in psoriatic skin (**Figure 3**).

Correlation analysis for JAK/STAT and VEGF/PAK1 signaling molecules

The relative mRNA expression values for the different JAK/STAT and VEGF/PAK1 signaling molecules were analyzed to assess the correlation between the two signaling pathways. STAT1 exhibited significant positive correlations with SOCS1, SOCS3, and PAK1, whereas STAT3 had significant positive correlations with VEGF and PAK1, and SOCS1 with SOCS3 and VEGF. Moreover, SOCS3 was significantly positively correlated with PAK1 (**Table 3**).

Discussion

Currently, there are no reports regarding the simultaneous expression of the JAK/STAT and VEGF/PAK1 signaling pathways in psoriasis. The present study investigated whether these two signaling pathways are involved in the pathogenesis of psoriasis from a molecular biologic perspective and explored possible mechanisms of action to identify potential targets for the treatment of this disease.

Wilks et al. [19] identified the Janus kinases (JAKs) as a new family of protein-tyrosine kinases. This family consists of JAK1-3 and non-

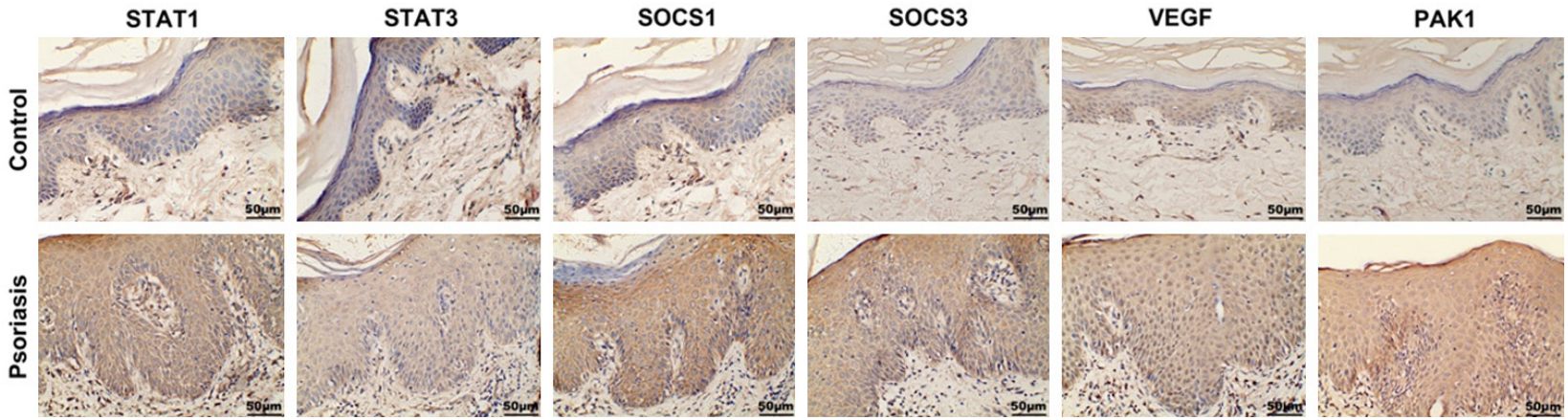

Figure 1. STAT1, STAT3, SOCS1, SOCS3, VEGF, and PAK1 immunohistochemistry. STAT1, STAT3, SOCS1, SOCS3, VEGF, and PAK1 showed positive staining in the epidermis and dermis of psoriatic tissues (200×).

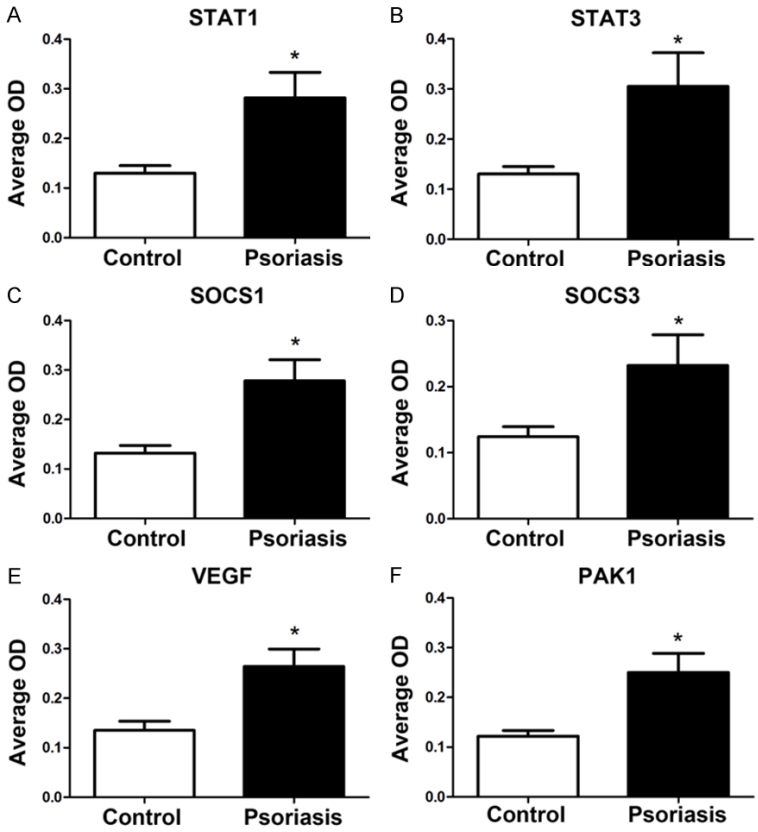

Figure 2. A-F. Average optical density (OD) of STAT1, STAT3, SOCS1, SOCS3, VEGF, and PAK1 immunohistochemical staining. The ODs of STAT1, STAT3, SOCS1, SOCS3, VEGF, and PAK1 were significantly elevated in psoriatic tissue compared to control tissues (*P < 0.05).

receptor tyrosine-protein kinase 2 (TYK2). The JAKs contain seven conserved JAK homology (JH) domains and are 120 to 140 kDa [20, 21]. Signaling through the JAK/STAT pathway by ligand binding results in autoactivation of the JAKs, leading to receptor dimerization, cross-phosphorylation of the associated JAKs, and activation of these proteins in a sliding manner. Specifically, the domains JH1 and JH2 of the juxtaposed JAKs move away to let kinase activate, leading to phosphorylation in many other pathways, including the STATs. Following tyrosine phosphorylation, the STAT transcription factors dimerize in parallel and then rapidly translocate into the nucleus. Sometimes, STATs can restrain gene transcription as well [22]. In healthy tissues, JAK/STAT pathway signaling is normally a process well-regulated at various levels [23]. Protein inhibitors of the activated STATs down regulate STAT signaling through interfering STAT DNA binding or promoting STAT proteolysis [23]. SOCS proteins lessen JAK/STAT signaling by modulating the JAK kinase activity, competing binding sites with STATs, or targeting STATs for proteasomal degradation [24, 25]. Therefore, selectively inhibiting the JAK/STAT signaling pathway might be an approach for treating common skin disorders. Thus, we focused on the expression statuses of STAT1, STAT3, SOCS1, as well as SOCS3 in psoriatic skin.

Endothelial cell proliferation and migration induced by angiogenic growth factors (e.g., VEGF) are crucial components of angiogenesis [26]. VEGF receptor 2 (VEGFR2) is a type II transmembrane tyrosine kinase receptor expressed on circulating bone marrow-derived endothelial progenitor cells and endothelial cells [REF]. Stimulation of VEGFR2 by VEGF results in the activation of a few signaling pathways that regulate various endothelial functions [27, 28]. There are two groups of PAKs (Group 1, PAK1-3; Group II, PAK4-6) differentiated by their structural organization and type of regulation [29]. Autophosphorylation occurs at multiple sites in the PAKs [30, 31], which regulates cell motility and signal transduction as well as cell death and survival [32-34]. Normally, PAK1 is required for endothelial migration and permeability [35, 36]. Its inhibition suppresses angiogenesis through its autoinhibitory domain [37]. PAK1 is activated by growth factors and cytokines (e.g., VEGF) [38]. Thus, evaluating the role of PAK1 signaling in autocrine VEGF-induced psoriasis is of importance [39]. Therefore, we investigated the expression of VEGF and PAK1 in psoriatic skin.

Strong positive immunohistochemical staining of STAT1, STAT3, SOCS1, and SOCS3 in keratinocytes was observed in the psoriasis group, which is consistent with the significantly higher mRNA expression levels for these signaling molecules in the psoriatic lesions compared to the controls. Based on these data, we specu-

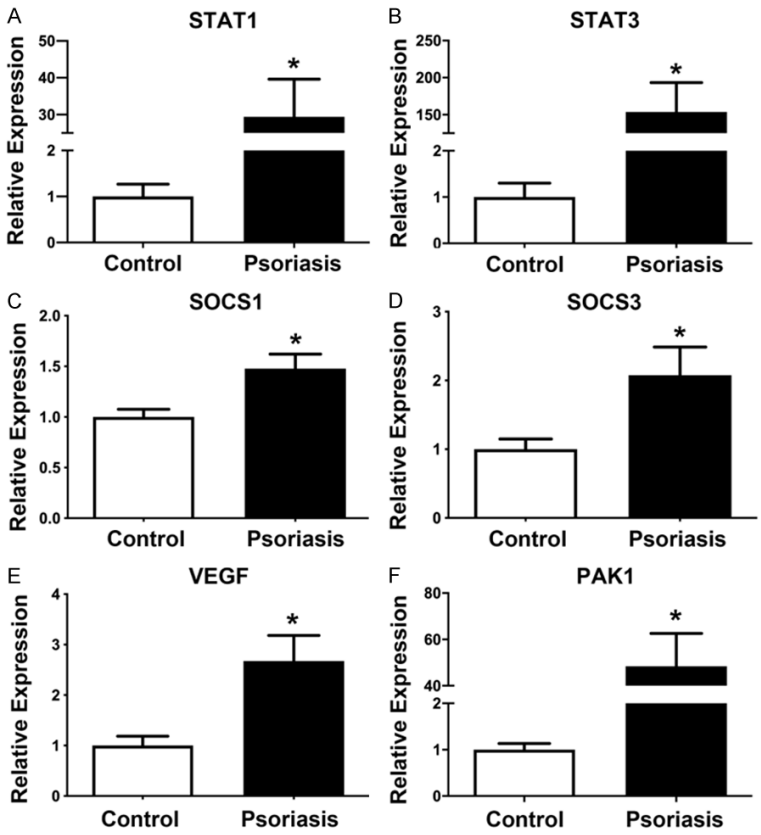

Figure 3. A-F. Relative STAT1, STAT3, SOCS1, SOCS3, VEGF, and PAK1 mRNA expression in psoriatic skin biopsies. The STAT1, STAT3, SOCS1, SOCS3, VEGF and PAK1 mRNA expression were significantly elevated in psoriatic tissues compared to control tissues (*P < 0.05).

Table 3. Correlation analysis for JAK/STAT and VEGF/PAK1 signaling pathway molecules

		STAT1	STAT3	SOCS1	SOCS3	VEGF	PAK1
STAT1	R	1	.838	.881*	.978**	.673	.907*
	p	—	.076	.048	.004	.213	.034
STAT3	R	.838	1	.849	.807	.902*	.963**
	p	.076	—	.069	.099	.037	.009
SOCS1	R	.881*	.849	1	.931*	.885*	.832
	p	.048	.069	—	.022	.046	.081
SOCS3	R	.978**	.807	.931*	1	.698	.881*
	p	.004	.099	.022	—	.190	.048
VEGF	R	.673	.902*	.885*	.698	1	.782
	p	.213	.037	.046	.190	—	.118
PAK1	R	.907*	.963**	.832	.881*	.782	1
	p	.034	.009	.081	.048	.118	—

*P < 0.05, **P < 0.01.

lated that the JAK/STAT signaling pathway was activated in these patients, triggering the development of psoriatic lesions and the induction of SOCS1 and SOCS3 in keratinocytes. Thus, blocking cytokines from triggering JAK/STAT pathway signaling might inhibit the development of psoriasis. IFN-c, the main cytokine involved in psoriasis pathogenesis, initiates STAT1 signaling pathway activation [40]. IL-6 can activate the STAT3 signaling pathway, which constitutes a complex cytokine regulation network with IL-17 and IL-23. STAT3 levels in human psoriatic lesions, especially in the nuclei of keratinocytes, are higher compared to the healthy epidermis [41]. Cytokine signaling is negatively regulated by SOCS proteins. Indeed, SOCS1 and SOCS3 are components of a typical negative-feedback loop in cytokine-induced signaling. SOCS1 inhibits the JAKs by direct binding, whereas SOCS3 inhibits signaling through its interaction with activated cytokine receptors. There is no perfect matching between cytokines and SOCS [42]. SOCS1 maintains immune homeostasis. SOCS3 influences the degree of an IL-6 signaling response. The basal expression levels of SOCS1 and SOCS3 are low; however, they rapidly increase in cytokine-treated cells in an activated STAT-dependent manner. Thus, SOCS1 and SOCS3 expression reflect the activation of STAT [43]. In psoriatic keratinocytes, upregulated SOCS1 and SOCS3 expression may represent a self-protection mechanism through which these cells try to protect themselves from adverse effects mediated by INF-γ and other cytokines. However, the rash in patients with psoriasis demonstrates a progressive change, with obvious red plaques, scales, and infiltration. Therefore, we hypothe-

sized that increased SOCS1 and SOCS3 levels in psoriatic keratinocytes might not sufficiently inhibit the JAK/STAT pathway, and the expression levels of STAT1, STAT3, SOCS1, and SOCS3 in psoriatic keratinocytes are potential targets for psoriasis treatment.

Polymorphism analysis of the VEGF gene in patients with psoriasis in southern India showed that serum VEGF levels in psoriatic patients were significantly elevated compared to that in healthy individuals. VEGF overexpression in skin tissues for a certain period is related to symptom severity in psoriasis [44]. PAK1 can modulate endothelial cell migration, permeability, and angiogenesis. Rac1 controls tumor migration and invasion through PAK1 [45]. Furthermore, the expression of a constitutively active PAK1 in endothelial cells induces the rapid formation of lamellipodia, filopodia, and dorsal ruffles, and increases the reorganization of the actin cytoskeleton and cell migration [46]. Therefore, we investigated whether VEGF induced endothelial cell migration through PAK1 activation. The results of this study, which demonstrated strong positive expression of VEGF and PAK1 in keratinocytes at both the protein and mRNA expression levels in the psoriasis group, suggested that the VEGF/PAK1 pathway might be involved in the onset of psoriasis. Thus, expression levels of VEGF and PAK1 in psoriatic keratinocytes may provide targets for psoriasis treatment.

As a chronic skin disease, psoriasis is manifested by keratinocyte hyperproliferation and cutaneous inflammation. Here, we present the first pilot evidence of cell migration using the JAK/STAT and VEGF/PAK1 signaling pathways in psoriasis. The study findings reveal a possible molecular mechanism responsible for worsening psoriasis symptoms induced by JAK/STAT and VEGF/PAK1, which may lead to new therapies to treat this pathologic condition.

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Disclosure of conflict of interest

None.

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