**Wnt/β-Catenin and Wnt5a/Ca$^{2+}$ Pathways Regulate Proliferation and Apoptosis of Keratinocytes in Psoriasis Lesions**

Yanfei Zhang, Chen Tu, Dingwei Zhang, Yan Zheng, Zhenhui Peng, Yiguo Feng, Shengxiang Xiao, Zhengxiao Li

Department of Dermatology, The Second Affiliated Hospital, College of Medicine, Xi’an Jiaotong University, Xi’an, Shaanxi Province, China

**Key Words**
Psoriasis • Keratinocyte • Wnt5a/Ca$^{2+}$ pathway • Wnt/β-catenin

**Abstract**

**Background/Aims:** Wnt5a is overexpressed in psoriasis lesions, however the mechanism by which Wnt5a is involved in the pathogenesis of psoriasis is not clear. To address this, the expression of Wnt5a in psoriatic lesions and its effect on keratinocyte cell proliferation and apoptosis was examined *in vitro*. **Methods:** The expression levels of *WNT5A*, and genes encoding its receptors frizzled2 (*FZD2*) and frizzled5 (*FZD5*) were examined in samples obtained from individuals with psoriasis and healthy controls. Knockdown of Wnt5a with short interfering (si)RNAs was performed in cultured HaCaT keratinocytes and normal human keratinocytes (NHK), and the expression of Wnt5a, protein kinase C (PKC), and β-catenin were determined, and cell cycle activity, proliferation and apoptosis were assessed. **Results:** The expression of *WNT5A, FZD2* and *FZD5* mRNA and protein were increased in psoriatic lesions. Wnt5a knockdown suppressed proliferation and induced apoptosis in HaCaT and NHK cells. Additionally, expression of *PCNA, MKI67, CCND1, BCL2, CTNNB1*, and genes encoding PKC and survivin were downregulated, whereas *CASP3* was upregulated. The mRNA levels of the Wnt pathway inhibitors *DKK1* and *SFRP1* were upregulated, Western blotting analyses demonstrated reduction in β-catenin and PKC protein levels. **Conclusion:** Knockdown of Wnt5a suppresses the proliferation of keratinocytes and induces apoptosis by inhibiting the Wnt/β-catenin or Wnt5a/Ca$^{2+}$ pathways.

**Introduction**

Psoriasis is characterized by abnormal growth, differentiation, and apoptosis of keratinocytes. Although the molecular basis of these physiologic alterations is not completely understood, T cells are activated to produce cytokines that mediate epidermal hyperplasia,
acanthosis, hyperparakeratosis, and orthohyperkeratosis [1, 2]. The development of therapeutic measures for psoriasis has remained a challenge due to the complex pathophysiology of the disease.

Wnt proteins affect cellular homeostasis by regulating cell proliferation, cell fate determination, and differentiation [3]. Within this family of proteins, Wnt5a is one of the most extensively studied and is known to impact many developmental processes and promote the proliferation of endothelial and glioblastoma cells in adult tissues [4, 5]. Wnt5a also induces apoptosis in JAR choriocarcinoma and HEK293 cells in vitro [6, 7]. Indeed, Wnt5a is expressed in the basal layer of the skin in adults and is overexpressed in psoriatic lesions [8].

Previous research has shown that interleukin-1, tumor necrosis factor-α, interferon-γ, and transforming growth factor-α markedly induce WNT5A expression [9]. Although Wnt5a was significantly upregulated in keratinocytes from psoriasis patients, these cells were absent from the inflammatory microenvironment [8]. These data suggest that upregulation of Wnt5a is a consequence of inflammation and may constitute a cell-autonomous feature of psoriatic keratinocytes. Therefore, we hypothesized that Wnt5a promotes psoriasis by regulating the proliferation of human keratinocytes and inhibiting apoptosis. To our knowledge, only three studies have addressed the role of Wnt5a in the pathogenesis of psoriasis [8–10]. However, it is not clear how Wnt5a regulates the proliferation of human keratinocytes. The aim of this study was to confirm that Wnt5a is overexpressed in psoriatic tissue, and to investigate the mechanisms by which Wnt5a regulates the proliferation and apoptosis of human keratinocytes.

Materials and Methods

Ethics statement

All experimental protocols were approved by the Institutional Review Board of the Xi'an Jiaotong University, and performed according to guidelines governing ethics care in China. Written informed consent was obtained from all participants for collection, storage, and analysis of biopsy samples.

Psoriasis samples

Paraffin-embedded samples were taken from 35 patients with psoriasis who had not received systemic or topical treatment and from 20 normal healthy controls at the Department of Dermatology of the Second Hospital of Xi'an Jiaotong University. All biopsies were 1 cm in diameter and 3 mm in thickness excised from the trunk (samples were taken from the center of the plaques in patients).

Immunohistochemistry

The following antibodies were purchased from Abcam (Cambridge, UK): anti-Wnt5a (ab110073; final dilution 1:250), anti-Frizzled-2 (ab109094; final dilution 1:300), and anti-Frizzled-5 (ab75234; final dilution 1:250). Specimen sections were incubated with the primary antibody overnight at 4 °C, then incubated with an appropriate biotinylated streptavidin-horseradish peroxidase-conjugated secondary antibody (ZSGB-BIO, Beijing, China), followed by hematoxylin counterstaining and finally visualized with 3′-diaminobenzidine (ZSGB-BIO, Beijing, China). PBS replaced the primary antibody in negative control samples. Positive staining was confirmed in all samples based on layer-specific staining within the hair follicle. Stained sections were examined on NanoZoomer Digital Pathology (Hamamatsu, Japan). For quantitative analysis, five randomly selected images of high-power fields (200× magnification) were digitally captured using a DP 70 CCD camera (Olympus Corp., Tokyo, Japan). Immunopositive cells were counted using Image Pro-Plus software (version 6.0; Media Cybernetics, CA, USA).

Real-time reverse transcription (RT)-PCR

Real-time RT-PCR was performed on samples taken from 15 healthy controls and 15 psoriasis patients and on cDNA prepared from cultured HaCaT keratinocytes or normal human keratinocytes (NHK). RNA was extracted using an RNA extraction kit (Beijing ComWin Biotech, China) according to the manufacturer’s instructions. Briefly, cDNA was synthesized from 0.5 µg of RNA template using anchored-oligo(dT)18 primers
(Takara, Shiga, Japan) according to the manufacturer's recommendations. Real-time RT-PCR was performed using an Applied Biosystems one-step system (Applied Biosystems of Thermo Fisher Scientific, Waltham, MA, USA) using the following conditions: 95°C for 30 s (initial denaturation), followed by 45 cycles of 95°C for 10 s, 60°C for 30 s (annealing), and 72°C for 10 s (extension). The fluorescence was captured at 530 nm at the end of the extension step of each cycle. The primers for Wnt5a were synthesized by Shanghai Generay Biotech (Shanghai China). Primers used for measuring mRNA levels of WNT5A, FZD2, FZD5, PCNA, MKI67, CCND1, BCL2, CTNNB1, DKK1, SFRP1, and CASP3, as well as PKC and survivin are show in Supplementary Table 1. Results were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which served as an internal control. To quantify transcript levels, target-gene Ct values were normalized using the delta Ct method (ΔCt = Ct reference gene - Ct target gene). Fold changes were calculated by using the differences of medians of the two groups.

HaCaT and NHK cultures and siRNA transfection

HaCaT cells were obtained as described previously [11] and cultured in RPMI 1640 medium (Hyclone of Thermo Fisher Scientific) containing 10% fetal bovine serum. NHK were obtained from healthy adult skin and cultured with defined keratinocyte serum-free medium (containing keratinocyte growth factor). Wnt5a siRNA and negative control siRNA sequences were designed and synthesized by GenePharma (GenePharma, Shanghai, China). The primer sequences of Wnt5a-specific siRNAs are: siRNA001, sense, 5'-GUGGUCGCUAGGAUGAAUTT-3' and anti-sense, 5'-AUUCAUACCUAGCGACCACTT-3'; and siRNA003: sense, 5'-GUCAGCUAGCAAGGAAATT3 and anti-sense, 5'-UUUUCGAGCUAGGAAAGCCTT3. Preliminary studies showed that knockdown was more effective with siRNA003, which was therefore used for subsequent experiments. Shortly before transfection, 1 × 10^5 cells/ml per well were seeded in a 12-well plate and cultured under normal growth conditions (37°C with 5% CO₂). siRNA (40 pmol) was diluted in 100 µL culture medium without serum and mixed gently with 2 µL of Lipofectamine 2000 (Invitrogen of Thermo Fisher Scientific), which was found to be the least toxic and yield the best transfection efficiency for HaCaT cells or NHK in 12-well plates as previously observed [12]. The mixture was incubated at room temperature for 20 min and then added slowly to the cells. Cells were then incubated at 37°C at 5% CO₂ for 6 h, and the culture medium was then changed.

Analysis of cell cycle and cell apoptosis by flow cytometry

Cultured HaCaT keratinocytes and NHK were seeded at a density of 1 × 10^5 cells/well in a 12-well plate. Following siRNA transfection, the cell cycle stage was determined by treating the cells with 0.5% propidium iodide (Sigma-Aldrich, St. Louis, MO, USA) for 20 min followed by flow cytometry detection at 488 nm. For detection of apoptosis, the cells were treated with 1 µg/mL Annexin-V-PI (Beyotime, China) for 15 min, followed by flow cytometry detection at 488 nm.

DNA agarose gel electrophoresis

Following siRNA transfection, HaCaT cells were harvested and washed twice with PBS. Chromosomal DNA was extracted using an Apoptotic DNA Ladder Detection Kit (Beyotime) according to the manufacturer's instructions. The DNA sample was incubated at 37°C for 30 min and electrophoresed at 40 V/cm on a 1% agarose gel containing 1 mg/mL ethidium bromide. Finally, the apoptotic DNA fragments were visualized under a UV transilluminator and photographed.

Methyl thiazolyl tetrazolium (MTT) assay

The MTT assay (Sigma-Aldrich) was used to determine cell viability. HaCaT or NHK cells were seeded into 96-well plates at a density of 5 × 10^3 cells/mL and transfected with siRNAs as described above. MTT was dissolved in PBS at concentration of 5 mg/mL, and 10 µl of the reagent was added to each well and the plates were incubated at 37°C for 4 h. The media was then removed and the purple formazan product was dissolved in 100 µL DMSO. The absorption was read at 570 nm using a spectrophotometer (Sunrise; Tecan Trading, Switzerland).

Western blotting

Protein lysates were extracted from healthy controls (n = 5) and psoriatic patients (n = 5) by pulverizing the frozen biopsy samples in a 2 mL glass tissue grinder in 500 µL of RIPA
lysis buffer (Sigma-Aldrich). Samples from five individuals were run separately on SDS-PAGE for Western blotting analyses. Average values obtained from the five samples in each group were then used for comparison and statistical analyses. For cultured HaCaT or NHK cells, 50 µL of RIPA lysis buffer was used. The samples were centrifuged at 12,000 rpm for 20 min and the supernatants were collected. The protein concentration was measured, and all the samples were diluted to obtain a final concentration of 1 mg/mL. Proteins were separated on 10% sodium dodecyl sulfate polyacrylamide gels and transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). The blots were blocked with 5% nonfat dry milk in Tris-buffered saline with 0.1% Tween-20 for 2 h at room temperature, and then incubated overnight at 4°C with the following antibodies purchased from Abcam: anti-Wnt-5a (ab110073; final dilution 1:1000), anti-Frizzled-2 (ab109094; final dilution 1:500), anti-Frizzled-5 (ab75234; final dilution 1:500), anti-β-catenin (ab6301; final dilution 1:1000), and anti-PKC (α, β, and γ isoforms, ab59363; final dilution 1:500). The blots were washed three times and then incubated with horseradish peroxidase-conjugated goat/mouse/rabbit secondary antibodies at a final concentration of 1:5000 (Pioneer, Wuhan, China) for 1 h at room temperature. The blots were washed again and visualized by chemiluminescence using ECL (Millipore). Anti-β-actin (sc-47778; Santa Cruz Biotechnology, Dallas, TX, USA) at a final dilution of 1:500 was used as a loading control.

Statistical analysis
All analyses were performed using SPSS v.13.0 statistical software (SPSS Inc., Chicago, IL, USA). The mRNA expression levels were compared between psoriatic lesions and healthy controls using a Student’s t-test. For Western blot and siRNA transfections, three or five independent biologic samples were used to establish statistical significance using two-sample independent t tests. The results are presented as mean ± SD. P < 0.05 was considered statistically significant.

Results

Overexpression of Wnt5a and frizzled receptors (Fzd2 and Fzd5) in psoriasis
The expression levels of WNT5A, FZD2, and FZD5 mRNA were measured in psoriatic lesions and skin samples from healthy controls. The results showed that WNT5A was upregulated by 5.8-fold in psoriasis lesions compared to control samples (7.08 ± 0.68 vs 1.22 ± 0.53; P=2.7E-21). In the psoriatic lesions, FZD2 and FZD5 were upregulated by 1.5-fold compared to controls (1.62 ± 0.71 vs 1.08 ± 0.45; P = 0.02) and 1.4-fold (1.3 ± 0.51 vs 0.92 ± 0.31; P = 0.02), respectively (Fig. 1).

Immunohistochemical analysis showed that expression of Wnt5a was confined to the stratum basale in the control samples (Fig. 2a,b), but was highly expressed in all the epidermal layers in psoriasis lesions (Fig. 2c,d). Fzd5 was also highly expressed in all the
epidermal layers in the psoriasis lesions (Fig. 2g,h), but was expressed only in the stratum granulosum in healthy skin (Fig. 2e,f). The primary site of Fzd2 expression in controls was the stratum basale (Fig. 2i,j). However, Fzd2 was highly expressed in all the epidermal layers in psoriasis lesions (Fig. 2k,l). In psoriasis lesions, the mean positive cell numbers of Wnt5a, Fzd2, and Fzd5 in the epithelial cells were significantly higher than controls (6.85, 6.07, and 13.15 times, respectively; all $P < 0.001$). In the dermis of the lesions, Wnt5a, Fzd2, and Fzd5 were mainly expressed in inflammatory and endothelial cells, with mean positive cell numbers significantly higher than controls (4.95, 4.27, and 18.66 times, respectively; all $P < 0.001$) (Table 1).

Similar expression patterns were observed with Western blotting analyses (Fig. 3a). Wnt5a protein levels were increased by 2.5-fold ($P = 0.03$) in the psoriasis lesions, and the levels of Fzd5 and Fzd2 were upregulated by 7.4-fold ($P = 0.003$) and 2.1-fold ($P = 0.03$), respectively (Fig. 3b), compared to healthy controls.

Knockdown of Wnt5a expression inhibits proliferation of keratinocytes

$WNT5A$ expression was monitored at 12, 24, 48, and 72 h following transfection of HaCaT and NHK cells with Wnt5a siRNA or a negative control. $WNT5A$ mRNA was downregulated

Table 1. Immunostaining results from psoriasis lesions and healthy controls. Values are expressed as the mean number of positive cells ± standard error.

| Variable     | Healthy skin mean ± SEM | Psoriasis vulgaris mean ± SEM | $P$ value |
|--------------|-------------------------|-----------------------------|----------|
| Wnt5a        |                         |                             |          |
| Epidermis    | 67.03 ± 15.73           | 459.44 ± 26.47              | <0.001   |
| Dermis       | 36.34 ± 10.11           | 170.42 ± 15.98              | <0.001   |
| Frizzled2    |                         |                             |          |
| Epidermis    | 58.95 ± 13.21           | 357.54 ± 32.62              | <0.001   |
| Dermis       | 56.97 ± 9.58            | 243.36 ± 20.71              | <0.001   |
| Frizzled5    |                         |                             |          |
| Epidermis    | 20.28 ± 5.32            | 378.39 ± 35.14              | $<0.001$ |
| Dermis       | 10.81 ± 5.13            | 142.12 ± 21.22              | $<0.001$ |
55% in the HaCaT keratinocytes ($P=0.0029$) (Fig. 4a), and 75% in NHK cells compared to the control ($P=0.007$) compared to the negative control siRNA and the vehicle at 24 h, and 45% at 48 h after transfection ($P=0.036$) (Fig. 4b). Furthermore, there was a significant decrease in Wnt5a protein expression in HaCaT and NHK cells after transfection with Wnt5a-specific siRNA (Fig. 4c,d).

Cell cycle stage was analyzed by flow cytometry at 24 h following transfection of HaCaT and NHK cells with Wnt5a or negative control siRNA or vehicle reagent. Propidium iodide...
staining was used to monitor the cell cycle (Fig. 5a). Transfection with Wnt5a siRNA resulted in an increased fraction of cells in the G0/G1 stage, at the expense of cells in S phase ($P < 0.05$), which is indicative of cell cycle arrest in G0/G1 (Fig. 5b). Furthermore, the expression levels of $PCNA$, $MKI67$, and $CCND1$ were measured by real-time RT-PCR. $CCND1$ was downregulated 7.9-fold compared to the control ($P=0.009$), and $PCNA$ and $MKI67$ were downregulated 2.3-fold ($P=0.022$) and 3.9-fold ($P=0.005$), respectively, following Wnt5a knockdown (Fig. 5c). An MTT assay demonstrated that Wnt5a siRNA significantly inhibited the proliferation of HaCaT cells at 24 and 48 h after transfection ($P=0.006$ and $P=0.001$ respectively) (Fig. 5d). Proliferation of NHK cells was similarly inhibited at 24, 48, and 72 h after transfection ($P=0.02$, $P=0.02$ and $P=0.04$ respectively) (Fig. 5e).
Wnt5a knockdown induces apoptosis of keratinocytes

Apoptosis was analyzed by flow cytometry 24 h following transfection using the fluorescent dye, Annexin V-PI. For keratinocytes treated with Wnt5a siRNA, the rate of apoptosis was increased by 1.58-fold ($P=0.03$) compared to the vehicle reagent and 1.52-fold ($P=0.022$) compared to the negative control siRNA (Fig. 6a). The rate of apoptosis of siRNA-treated NHK cells was increased by 1.68-fold compared to the vehicle control ($P=0.002$) (Fig. 6b). Real-time RT-PCR analysis showed that CASP3 was upregulated 2.6-fold ($P=0.004$), whereas both BCL2 and survivin genes were downregulated 2.0-fold with Wnt5a siRNA ($P=0.013$ and $P=0.01$ respectively) (Fig. 6c). The integrity of DNA from HaCaT cells was assessed by agarose gel electrophoresis 24, 48, and 72 h after transfection was electrophoresed through 1% agarose gels and stained with 0.5 µg/mL ethidium bromide. Data are presented as mean ± SD of three independent experiments; *$P<0.05$, **$P<0.01$.

Wnt5a knockdown inhibits the expression of β-catenin and PKC

Real-time RT-PCR analyses revealed that siRNA knockdown of Wnt5a downregulated the expression CTNNB1 and PKC genes 3.4-fold ($P=0.032$) and 7.7-fold ($P=0.00097$), respectively, in HaCaT cells (Fig. 7a), whereas DKK1 and SFRP1 were upregulated 2.2-fold and 6.5-fold, respectively ($P=0.002$ and $P=0.00055$ respectively) (Fig. 7b). This downregulation was confirmed by Western blotting, which showed the protein levels for β-catenin and PKC.
Zhang et al.: Wnt5a Pathways in Psoriasis

were downregulated 1.6-fold and 2.3-fold, respectively ($P=0.02$ and $P=0.012$ respectively) (Fig. 7d).

**Discussion**

Previous studies have shown that Wnt5a is upregulated in psoriasis and binds Fzd5 and Fzd6 [8–10]. The expression level of Fzd2, another receptor for Wnt5a, was also shown to be upregulated in psoriasis lesions [9]. Consistent with these reports, the results of the present study confirms that Wnt5a and Fzd5 are expressed in healthy skin, but overexpressed in psoriasis lesions. Wnt5a was also shown to be upregulated in cutaneous squamous cell carcinoma [13], which suggests that Wnt5a plays an important role in the pathogenesis of some skin disorders. Importantly, we show that Fzd2 is expressed in the basal layer and in a few cells of the upper spinous and granular layers of healthy skin in adults. Immunohistochemical and Western blotting analyses showed that Fzd2 was also upregulated in psoriasis lesions, suggesting a role for this receptor in the pathogenesis of psoriasis. Together, the overexpression of Wnt5a, Fzd5, and Fzd2 in psoriasis lesions suggests that these ligand–receptor pairs may mediate epidermal hyperplasia, acanthosis, hyperparakeratosis, hemangiectasis, and inflammatory responses, which are very important for the onset of psoriasis. However, further study is needed to confirm this.

Wnt proteins control various cellular functions, including cell proliferation. Previous studies have shown that Wnt5a inhibits the proliferation of human dental papilla cells,
B cells, and melanocytes [14–16]. Wnt5a has also been shown to prevent apoptosis in uncommitted osteoblast progenitors, differentiated osteoblasts [17], dermal fibroblasts [18], and endothelial cells [4]. Based on these data, we hypothesized that Wnt5a may regulate proliferation and apoptosis in healthy skin as well as psoriatic skin lesions. Indeed, the results of the present study support this, as transfection of keratinocytes with Wnt5a siRNA suppressed cell proliferation and induced apoptosis. Wnt5a knockdown downregulated the expression of PCNA and MKI67, proliferation markers that can be used to assess epidermal hyperproliferation in psoriasis and malignancy potential for in cutaneous lesions [19], as well as cyclin D1, which is a key regulator of cell cycle progression in psoriasis lesions [20]. These results are in contrast to those reported by Gudjonsson et al. [9], who found that exogenous Wnt5a suppressed the growth of keratinocytes. However, Romanowska et al. [8] found that Wnt5a was overexpressed in hyperproliferative keratinocytes obtained from psoriasis patients and expanded in vitro for 14 days. Exogenous Wnt5a transfected into HaCat keratinocytes can induce the expression of APP, which is upregulated and redistributed in psoriasis and stimulates both proliferation and mobility of keratinocytes [21]. Thus, these data indicate that Wnt5a promotes the proliferation of keratinocytes in psoriasis.

Apoptosis offsets keratinocyte proliferation and regulates the formation of stratum corneum, and improper functioning of apoptotic pathways leads to the development of several skin diseases. Psoriatic keratinocytes exhibit an enhanced ability to resist apoptosis, which may be one of the key factors in the pathogenesis of psoriasis. However, we found that caspase-3, which is an apoptosis-inducing factor, is upregulated in cells after Wnt5a siRNA transfection whereas anti-apoptotic factors, such as Bcl-2 and survivin are downregulated.

Wnt5a plays a dual role in the Wnt/β-catenin pathway. A previous study has shown that the β-catenin pathway is enhanced in Wnt5a-knockout mice [22], though Wnt5a also stimulates the β-catenin pathway [23]. The high proliferative potential of cultured keratinocytes is attributed to elevated levels of cytoplasmic β-catenin [24], which is essential for regulating cell proliferation and apoptosis. The expression of β-catenin releases cell cycle arrest mediated by exogenous E-cadherin, and regulates the cell cycle at the G2/M phase in normal and transformed epidermal keratinocytes and Sodium iodate (NaI03) increased apoptosis, inhibited mitosis, proliferation through inhibition of the Wnt/β-catenin and noggin pathways [24–27]. But upregulated the expression of β-catenin can induced apoptosis in vascular smooth muscle cells (VSMCs) and colon tumor cells [28, 29]. Our results show that mRNA and protein levels of β-catenin are downregulated following Wnt5a knockdown in HaCaT keratinocytes. In contrast, DKK1, an inhibitor of the canonical Wnt pathway [30], and SFRP1, were upregulated. These data indicate that β-catenin is a key factor for proliferation and apoptosis of keratinocytes, and is regulated by Wnt5a.

The relationship between Wnt5a and β-catenin signaling in psoriasis is not well defined. Hampton et al. [31] showed increased nuclear β-catenin staining in the suprabasal layer in psoriatic lesions, but Yamazaki et al. [32] found only membrane staining, indicating a lack of β-catenin activation in psoriatic skin. Our previous studies showed the β-catenin expression was decreased membrane staining and increased cytoplasm/nuclear β-catenin staining in the lesions of patients with active psoriasis [33]. In addition, Gudjonsson et al. [9] failed to observe nuclear translocation of β-catenin, indicating suppression of canonical Wnt signaling in psoriatic lesions. These data strongly suggest that there is a decrease in β-catenin levels in psoriatic skin, but increased cytoplasm/nuclear β-catenin staining, which may promote the proliferation of keratinocytes [26]. In this study, we found that Wnt5a knockdown inhibits the expression of β-catenin, suggesting that a complex interplay of Wnt5a and β-catenin regulates the pathogenesis of psoriasis and the proliferation of keratinocytes. Further research is needed to clarify this relationship between Wnt5a and β-catenin signaling in psoriasis.

It has been suggested that Wnt5a also regulates the Ca\(^{2+}\) pathway [34]. In mammalian cells, Wnt5a induces the release of Ca\(^{2+}\), which activates PKC and Ca\(^{2+}\)/calmodulin-dependent protein kinase II, which are important regulators of various cellular functions [35]. The PKC pathway, which is downstream of the Wnt5a/Ca\(^{2+}\) pathway, plays a pivotal role in regulating...
the proliferation and differentiation of HaCaT keratinocytes. PKCα and PKCδ are known to stimulate cellular differentiation and increase susceptibility for apoptosis, as well as inhibit cellular proliferation and tumor growth in immunodeficient mice. In contrast, PKCβ and PKCε increase growth of cells in vitro and in vivo, and inhibit differentiation and apoptosis [36]. We found that PKC gene and protein expression levels were downregulated in the HaCaT keratinocytes that were transfected with Wnt5a siRNA. These results suggest that Wnt5a knockdown suppresses the Wnt5a/Ca\(^{2+}\) pathway. However, further experiments are needed to confirm this finding.

A recent study by Zhu et al. [37] reported that mice overexpressing Wnt5a in the epidermis do not develop psoriatic-like plaques. There are several explanations for the discrepancy between the observations in human psoriatic lesions and the normal appearance of Wnt5a transgenic epidermis. First, there are differences between human and mouse skin regarding epidermal proliferation and differentiation and the skin immune systems. T lymphocytes and/or dendritic cells are believed to be indispensable for the pathogenesis of human psoriasis [38], and K14-promoter-driven Wnt5a overexpression may not affect these cells in murine skin. We found an overexpression of Wnt5a and its receptors in the epidermis and superficial dermis in psoriasis lesions, and that Wnt5a regulates the proliferation of keratinocytes. Therefore, activation of these may also regulate hemangiectasis and inflammatory responses in psoriasis, whereas Wnt5a receptor expression in murine epidermis might not be strong enough to promote the development of these phenotypes in the epidermis of transgenic mice.

In this study, we verify the distribution of Wnt5a and the receptor Fzd5 in psoriatic lesion and normal skin. Interestingly, we provide the first reported description of Fzd2 distribution in psoriatic lesions and normal skin. Furthermore, we propose a new mechanism by which Wnt5a may regulate cell proliferation and apoptosis in human keratinocytes, namely via the Wnt/β-catenin or Wnt5a/Ca\(^{2+}\) pathways. It is possible that some cytokines, such as interleukin-1 and tumor necrosis factor, induce cell-autonomous or non-autonomous expression of Wnt5a, which then binds to its receptors to regulate the proliferation and apoptosis of psoriatic keratinocytes via canonical or non-canonical pathways. However, further studies are needed to elucidate the precise function of Wnt5a in psoriasis pathogenesis.

**Disclosure Statement**

The authors have no conflicts of interest to declare.

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