PIN1-silencing mitigates keratinocyte proliferation and the inflammatory response in psoriasis by activating mitochondrial autophagy

SHUANG XIA, JIN LI, HONGSHAN YUAN and WENLIANG YAN

Department of Dermatology, Jinling Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing, Jiangsu 210001, P.R. China

Received February 7, 2024; Accepted April 24, 2024

DOI: 10.3892/etm.2024.12691

Abstract. Peptidyl-prolyl cis/trans isomerase, NIMA-interacting 1 (PIN1) has been suggested to be a critical regulator in skin-related diseases. However, the role and molecular mechanism of PIN1 in psoriasis remain unclear. HaCaT cells were stimulated with five cytokines (M5) to induce psoriatic inflammation-like conditions. Reverse transcription-quantitative PCR and western blotting were performed to examine PIN1 expression in M5-induced HaCaT cells. A Cell Counting Kit-8 assay and 5-ethynyl-2'-deoxyuridine staining were employed to examine cell proliferation. Inflammatory factors were evaluated using ELISA kits and western blot analysis. Mitochondrial autophagy was examined by immunofluorescence staining, western blotting and a JC-1 assay. Western blot analysis was adopted to assess the levels of psoriasis marker proteins. PIN1 expression was markedly elevated in M5-induced HaCaT cells. Silencing of PIN1 inhibited M5-induced hyperproliferation and the inflammatory response, while it promoted mitochondrial autophagy in HaCaT cells. The addition of the mitochondrial autophagy inhibitor mitochondrial division inhibitor-1 reversed the effects of PIN1 interference on proliferation, the inflammatory response and mitochondrial autophagy in M5-induced HaCaT cells. The present study revealed that PIN1 inhibition protected HaCaT cells against M5-induced hyperproliferation and inflammatory injury through the activation of mitochondrial autophagy.

Correspondence to: Dr Wenliang Yan, Department of Dermatology, Jinling Hospital, Affiliated Hospital of Medical School, Nanjing University, 305 Zhongshan Road, Nanjing, Jiangsu 210001, P.R. China
E-mail: yanwldoctor@163.com

Key words: peptidyl-prolyl cis/trans isomerase, NIMA-interacting 1, psoriasis, keratinocyte, proliferation, inflammatory response, mitochondrial autophagy

Introduction

Psoriasis is a chronic inflammatory skin disease with the characteristics of hyperkeratosis, thickening of the stratum spinosum and dermal inflammation (1). Psoriasis usually contributes to the chronic inflammatory response in the joints, nails and other organs (2). The inflammatory response increases the incidence of systemic inflammatory diseases and may lead to severe arthritic dysfunction (3). In addition, adhesive inflammation occurs in the collateral ligaments near the joints, resulting in frequent spread of the inflammation to the nail bed, leading to nail involvement (4). Long-term chronic inflammation may increase the risk of depression of patients and is associated with numerous diseases, including hypertension, diabetes and metabolic syndrome that pro-inflammatory cytokines and adipocytokines from psoriasis contribute to, which markedly lowers the quality of life of most patients with psoriasis (3,5). Keratinocytes stimulated by initial triggers release their own nucleotides and antimicrobial peptides, participating in the initiation of psoriasis. After cytokine stimulation, activated keratinocytes influence the psoriasis process in terms of inflammatory infiltration, epidermal proliferation, innate immunity and tissue reorganization. In addition, keratinocytes also act as amplifiers of psoriatic inflammation during the maintenance phase (6). Due to their high proliferative capacity, once activated by pro-inflammatory cytokines, keratinocytes can produce abundant chemokines and other inflammatory mediators, inducing innate immunity and amplifying inflammation. Moreover, keratinocytes, together with fibroblasts and endothelial cells, accelerate psoriasis plaque formation through the activation and proliferation of endothelial cells and the deposition of extracellular matrix (7,8). Thus, keratinocytes serve as the end-target cells of the local immune response in psoriasis and the abnormal proliferation and differentiation of keratinocytes promote the psoriatic plaques (9).

Hyperproliferation and defective keratinocyte differentiation in psoriasis may impair epidermal barrier function, resulting in the destruction of the protective barrier of the skin (10). The main therapeutic drugs for psoriasis include biological agents, herbal agents and small molecule targeted drugs, which can also cause adverse reactions (11). Therefore,

identifying clinical therapeutic drugs with fewer adverse reactions for the treatment of psoriasis is of great significance.

Peptidyl-prolyl cis/trans isomerase (PPIase), NIMA-interacting 1 (PIN1) belongs to the PPIase family (12). PIN1, which has wide existence in living organisms, specifically recognizes and binds to phosphorylated serine/threonine motifs in proteins, catalyzes cis-trans isomerization of the amide bond therein, and subsequently regulates the bioactivity, stability, phosphorylation level and subcellular localization of the proteins (13,14). PIN1 activates a series of proteins that promote cell proliferation/oncogenesis and also inhibits a series of factors that block cell proliferation/oncogenesis (15). PIN1 has been suggested to be a critical regulator in the differentiation, maintenance and proliferation of numerous types of stem cells, including totipotent stem cells, neural stem cells, dental pulp stem cells and mammary stem cells (16-18). Since the mammary gland also originates from a single epithelial layer of ectoderm during embryonic formation, it is similar to the skin in terms of the molecular mechanisms of advancement and pathogenesis (19). A previous study revealed that the number of $CD24^+CD29^+$ mammary stem cells was reduced, the non-adherent mammospheres formed by cultured stem cells *in vitro* were smaller, and the ability to reconstruct the mammary gland was reduced in PIN1 knockout mice (20). In addition, the PIN1 protein is widely expressed in skin tissues of patients with atopic dermatitis, and PIN1 serves a key role in regulating IL-33 expression in HaCaT cells (21). However, PIN1 expression in psoriasis and its role in the advancement of psoriasis remain unclear. Therefore, the present study was designed to verify the role of PIN1 in five cytokines (M5)-induced HaCaT cells and to clarify the mechanism underlying its effects.

Materials and methods

Cell lines. The HaCaT human immortalized keratinocyte cell line (cat. no. iCell-h066; with STR profiling) provided by Cellverse Bioscience Technology Co., Ltd. was cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS, Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin at 37˚C with 5% CO_2. To induce psoriatic inflammation-like conditions, HaCaT cells were exposed to M5 (IL-17A, TNF-α, IL-1α, IL-22 and Oncostatin-M; final concentration, 10 ng/ml; PeproTech, Inc.), followed by the addition of the mitochondrial autophagy inhibitor mitochondrial division inhibitor-1 (Mdivi-1; 10 μM; MedChemExpress) (22,23) for 1 h at 37˚C, while the control group received no treatment.

Cell transfection. The specific short hairpin RNA (shRNA/sh) targeting PIN1 and the corresponding negative control were constructed by Shanghai GenePharma Co., Ltd. HaCaT cells were transfected with shRNAs (100 nM) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h at 37˚C according to the manufacturer's protocol. The sequences were as follows: sh-NC forward, 5'-AACAAGATG AAGAGCACCAA-3' and reverse, 5'-TTGGTGCTCTTCATC TTGTT-3'; sh-PIN1-1 forward, 5'-GCTACATCCAGAAGA TCAA-3' and reverse, 5'-TTGATCTTCTGGATGTAGC-3'; and sh-PIN1-2 forward, 5'-GCCGAATTGTTTCTAGTTA-3' and reverse, 5'-TAACTAGAAACAATTCGGC-3'. After 48 h transfection, cells were collected for subsequent experiments.

Cell Counting Kit-8 (CCK-8) assay. HaCaT cells were seeded into a 96-well plate at $1x10^3$ cells per well with 100 μl complete medium (Gibco; Thermo Fisher Scientific, Inc.) and cultured at 37˚C, and 10 μl CCK-8 solution (Beyotime Institute of Biotechnology) was added to each well, After 24 h, plates were incubated at 37˚C for 2 h, and then the absorbance at 450 nm was measured with Microplate Reader (Bio-Rad Laboratories, Inc.). All experiments were performed in triplicate.

5-ethynyl-2'-deoxyuridine (EdU) assay. Following inoculation into 6-well plates ($4x10^5$ cells/well), HaCaT cells were cultured at 37˚C overnight. Subsequently, HaCaT cells underwent fixation in 4% polyformaldehyde at room temperature for 1 h and exposure to 0.5% Triton X-100 for 15 min at room temperature. Cells were stained by Cell-Light™ EdU Apollo®488 *in vitro* imaging kit (Thermo Fisher Scientific, Inc.) for 20 min at room temperature, and subsequently counterstained with DAPI (5 μg/ml) for 10 min in darkness at room temperature. The positive cells were counted under a fluorescence microscope (Nikon Corporation).

ELISA. The levels of IL-1β, IL-6, IL-8 and IL-23A in supernatants from HaCaT cells were examined using ELISA kits (cat. no. H002-1-2, H007-1-1, H008-1-1 and H020, respectively; Nanjing Jiancheng Bioengineering Institute) according to the recommended protocols. The optical density value was determined using a BioTek microplate reader (BioTek; Agilent Technologies, Inc.) at 450 nm.

Immunofluorescence colocalization analysis. Cells that were cultured on slides in 6-well plates ($3x10^5$ cells/ml) were cultured with MitoTracker Red (500 nM; Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C for 8 min, followed by fixation in 4% paraformaldehyde for 1 h at room temperature and permeation with 0.1% Triton-X-100 for 15 min at room temperature. Subsequently, cells were successively exposed to LC3B antibody (1:1,000; cat. no. ab232940; Abcam) for 1 h at room temperature and a fluorescent Alexa Fluor® 488-conjugated goat anti-rabbit secondary antibody (1:400; cat. no. ab150077; Abcam). A fluorescence microscope (Nikon Corporation) was used to capture images.

JC-1 staining. For examination of the mitochondrial membrane potential (MMP), JC-1 staining was implemented. Briefly, the collected HaCaT cells were cultured with JC-1 (cat. no. 420200-5MG; MilliporeSigma) for 15 min at 37˚C, and then evaluated using a fluorescence microscope. Red fluorescence represented a potential-dependent aggregation in the mitochondria, reflecting the mitochondrial membrane potential. Green emission of the dye represented the monomeric form of JC-1, appearing in the cytosol after mitochondrial membrane depolarization. Excitation and emission wavelengths of 514 and 529 nm, respectively, were applied for the detection of the monomeric form of JC-1, while excitation and emission wavelengths of 585 and 590 nm, respectively, were used to detect the aggregation of JC-1.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from sample HaCaT cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Reverse transcription of first-strand cDNA was performed using PrimeScript RT Master Mix (Takara Bio, Inc.), followed by qPCR using the SYBR Premix Ex Taq™ II kit (Takara Bio, Inc.) according to the manufacturer's protocol. The PCR program was 95˚C for 3 min and 35 cycles of denaturation at 95˚C for 30 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 1 min, with a final extension step at 72˚C for 7 min. The primer sequences used for qPCR were as follows: PIN1 forward, 5'-CCGCAGCTCAGGCCG-3' and reverse, 5'-GCA AACGAGGCGTCTTCAAA-3'; and GAPDH forward, 5'-GGGAAACTGTGGCGTGAT-3' and reverse, 5'-GAG TGGGTGTCGCTGTTGA-3'. The relative mRNA level was normalized to that of GAPDH using the $2^{-\Delta\Delta Cq}$ method (24).

Western blot analysis. Total protein was isolated from HaCaT cells using RIPA buffer (Auragene Bioscience Co. BCA Protein Assay kit (Beijing Dingguo Changsheng Biotechnology Co., Ltd.) was performed to detect the protein concentration according to the manufacturer's instructions. A total of 30 μg protein per well were resolved by 10% SDS-PAGE (Bio-Rad Laboratories, Inc.) and transferred to PVDF membranes (MilliporeSigma). Subsequently, the membranes were blocked with 5% skim milk for 1 h at 25˚C, and successively incubated with primary antibodies against PIN1 (1:1,000; cat. no. ab192036; Abcam), keratin (KRT)1 (1:1,000; cat. no. ab185628; Abcam), KRT6B (1:1,000; cat. no. ab154313; Abcam), cyclooxygenase-2 (Cox2; 1:1,000; cat. no. ab179800; Abcam), inducible nitric oxide synthase (iNOS; 1:1,000; cat. no. ab178945; Abcam), LC3B (1:1,000; cat. no. ab63817; Abcam), Beclin-1 (1:1,000; cat. no. ab207612; Abcam), PTEN induced kinase 1 (PINK1; 1:1,000; cat. no. ab216144; Abcam), Parkin (1:1,000; cat. no. ab77924; Abcam), p62 (1:1,000; cat. no. ab207305; Abcam), COX IV (1:1,000; cat. no. ab16056; Abcam) or GAPDH (1:1,000; cat. no. ab8245; Abcam) overnight at 4˚C. Then, the membranes were incubated with anti-mouse or anti-rabbit secondary antibodies (cat. nos. sc-2004 or sc-2005; 1:5,000; Santa Cruz Biotechnology, Inc.). An ECL detection system (Amersham; Cytiva) was adopted for the visualization of protein bands in accordance with the recommended specifications, while the band density was semi-quantified using ImageJ software (version 1.49; National Institutes of Health).

Statistical analysis. All experimental data were analyzed using SPSS 23.0 software (IBM Corp.) and are presented as the mean ± SD from at least three independent experiments. For the analysis of differences between two groups, unpaired, two-tailed Student's t-test was employed, while one-way ANOVA with the Bonferroni post hoc test was adopted for comparisons among multiple groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

PIN1 is highly expressed in M5-induced HaCaT cells. To investigate the role of PIN1 in psoriasis, the expression levels of PIN1 in M5-induced HaCaT cells were first detected. RT-qPCR and western blotting showed that PIN1 expression was significantly upregulated in M5-induced HaCaT cells compared with untreated HaCaT cells (Fig. 1A). Subsequently, PIN1 was silenced and the transfection efficiency is demonstrated in Fig. 1B. Of note, sh-PIN1-1 had an improved knockdown effect, and thus, was selected for subsequent assays (referred to as sh-PIN1). In addition, sh-PIN1 also reduced PIN1 expression in HaCaT cells treated with M5 (Fig. 1C).

PIN1 silencing inhibits M5-induced hyperproliferation and inflammation in HaCaT cells. To investigate the biological roles of PIN1 in M5-induced HaCaT cells, cell proliferation was initially examined using a CCK-8 assay. As shown in Fig. 2A, M5 increased the cell viability, while PIN1 silencing repressed the proliferation of M5-induced HaCaT cells. Furthermore, EdU staining revealed that M5 increased the number of positive-green cells, which was then reduced following transfection with sh-PIN1 (Fig. 2B). In addition, M5 reduced KRT1 levels and increased KRT6 levels, which was reversed after silencing of PIN1 (Fig. 2C). ELISA results illustrated that the increased levels of IL-1β, IL-6, IL-8 and IL-23A in HaCaT cells due to M5 stimulation were decreased by sh-PIN1 (Fig. 3A). Western blotting indicated the increase in the levels of Cox2 and iNOS following M5 treatment, while PIN1 silencing had the opposite effect (Fig. 3B).

Knockdown of PIN1 activates M5-induced mitochondrial autophagy in HaCaT cells. As revealed in Fig. 4A, LC3 and MitoTraker levels in double-stained cells were examined using an immunofluorescence assay. The data indicated that M5 stimulation markedly reduced the levels of LC3 and MitoTraker, whereas knockdown of PIN1 reversed the effects of M5 on the suppressive levels of LC3 and MitoTraker in HaCaT cells. Additionally, M5 stimulation decreased the protein levels of LC3II/LC3I, Beclin-1, PINK1 and Parkin (mitochondria), whereas it promoted the production of p62 and Parkin (cytoplasm). However, the trend was reversed by PIN1 silencing (Fig. 4B). Furthermore, JC-1 staining demonstrated that M5 induction increased JC-1 aggregates, whereas it reduced JC-1 monomers, while PIN1 silencing had the opposite effect (Fig. 4C).

PIN1 silencing ameliorates M5-induced hyperproliferation and inflammation of HaCaT cells by activating mitochondrial autophagy. To explore the role of mitochondrial autophagy in PIN1 silencing, the mitochondrial autophagy inhibitor Mdivi-1 was used to treat cells. As illustrated in Fig. 5A, Mdivi-1 significantly increased the reduced cell proliferation following PIN1 silencing. Consistently, EdU staining revealed that the number of positive cells was increased after Mdivi-1 treatment (Fig. 5B). In addition, western blotting indicated that the KRT1 levels were decreased, while the KRT6 levels were increased following treatment with Mdivi-1 (Fig. 5C). Furthermore, Mdivi-1 treatment increased the levels of IL-1β, IL-6, IL-8 and IL-23A in PIN1-silenced HaCaT cells (Fig. 6A), which was consistent with the results of western blotting, which indicated that the levels of Cox2 and iNOS were increased in M5-induced HaCaT cells transfected with sh-PIN1 (Fig. 6B).

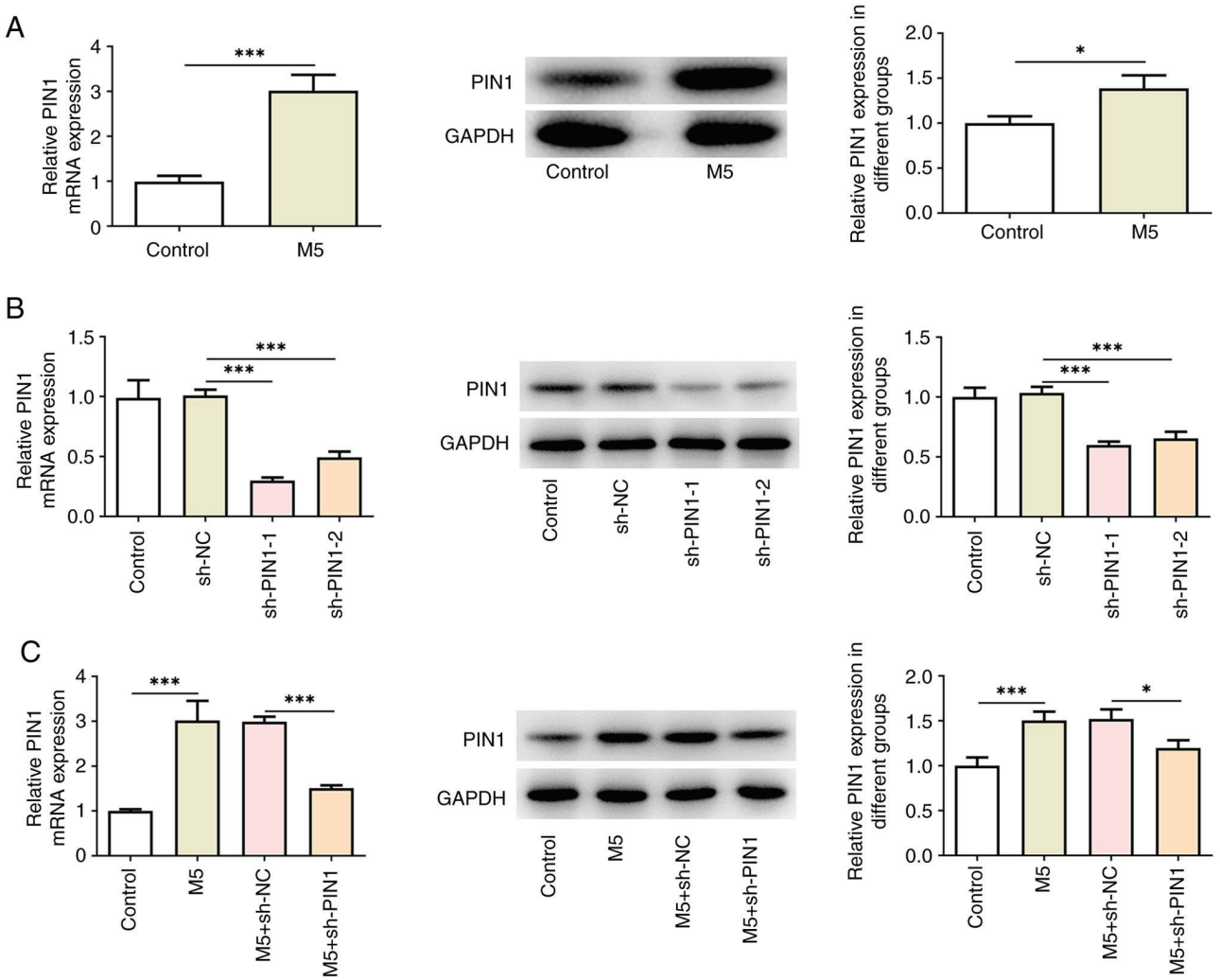

Figure 1. PIN1 is highly expressed in M5-induced HaCaT cells. (A) The mRNA expression and protein levels of PIN1 in M5-induced or untreated HaCaT cells were detected by RT-qPCR and western blot analysis. (B) The mRNA expression and protein levels of PIN1 in HaCaT cells transfected with sh-PIN1 were detected by RT-qPCR and western blot analysis. (C) The mRNA expression and protein levels of PIN1 in M5-induced HaCaT cells after transfection with sh-PIN1 were detected by RT-qPCR and western blot analysis. Results are the mean ± SD. *P<0.05 and ***P<0.001. PIN1, peptidyl-prolyl cis/trans isomerase, NIMA-interacting 1; RT-qPCR, reverse transcription-quantitative PCR; SD, standard deviation; shRNA, short hairpin RNA; NC, negative control.

Discussion

Psoriasis results from a combination of genetic and environmental factors, with environmental triggers including stress, infections, alcohol, tobacco, drugs and obesity (25). Keratinocytes can function as innate immune cells, secreting inflammatory factors, especially chemokines, and also expressing various pattern recognition receptors, such as toll-like receptors, which are capable of initiating an intrinsic immune response, presenting antigens to T cells, and further exacerbating inflammatory responses in the skin (26,27).

PIN1 is a unique enzyme that isomerizes the target protein proline residues (28). It has been reported that PIN1 downregulated serine/threonine kinase 3 (STK3) by promoting its ubiquitination and leads to the dysregulation of Hippo signaling, thereby causing carcinogenic signaling and melanoma. Hippo signaling pathway consists of a core kinase cascade of STK3, LATS1/2, YAP and TAZ. When STK3 was downregulated, the Hippo signaling was repressed (29). Jeong *et al* (30) reported that PIN1 stimulation facilitated the expression of pro-inflammatory proteins by triggering NF-κB, cyclic AMP response element-binding protein (CREB) and CCAAT/enhancer binding protein (C/EBP), suggesting that PIN1 is a prospective therapeutic target for the treatment of rheumatoid arthritis. It has also been reported that PIN1 inhibitor could improve experimental autoimmune encephalomyelitis, and reduce inflammation and demyelination of the central nervous system, indicating the pivotal role of PIN1 in chronic inflammation (31). In the present study, PIN1 expression was revealed to be increased in M5-induced HaCaT cells. Silencing of PIN1 had inhibitory effects on HaCaT cell proliferation and the inflammatory response. A previous study revealed that the natural PIN1 inhibitor Juglone inhibited wound healing by promoting skin cell migration via the Rac1/cell division cycle 42/PAK pathway, and it may be a potential candidate for wound healing and skin regeneration (32). In addition, the inhibition of PIN1 has been reported to suppress the activation of NF-κB, CREB and C/EBP induced by UVA irradiation, which is associated with the malignant transformation of epidermal cells (33).

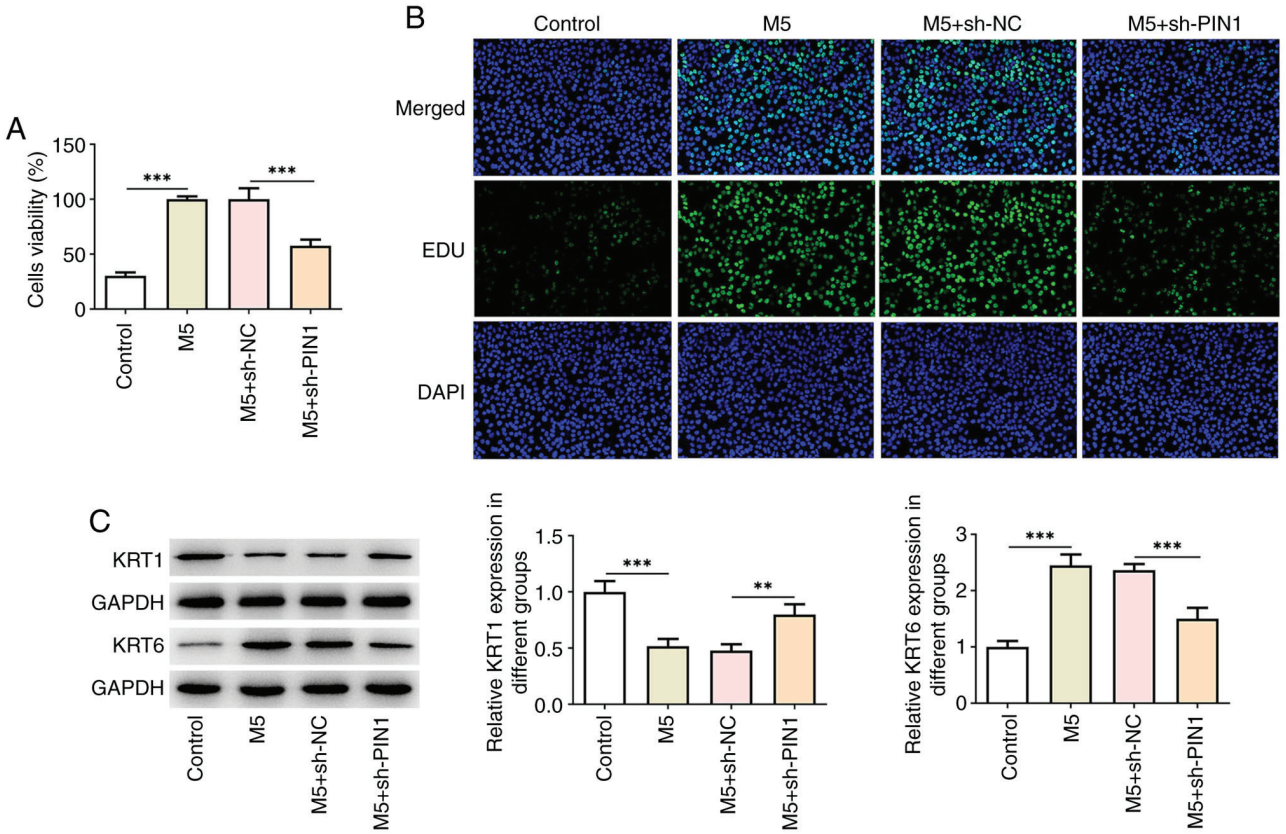

Figure 2. PIN1 silencing inhibits M5-induced hyperproliferation in HaCaT cells. (A) Cell Counting Kit-8 assay was used to assess cell viability. (B) EdU staining was performed to assess cell proliferation (magnification, x200). (C) The protein levels of KRT1 and KRT6 in HaCaT cells transfected with sh-PIN1 were detected by western blot analysis. Results are the mean ± SD. **P<0.01 and ***P<0.001. PIN1, peptidyl-prolyl cis/trans isomerase, NIMA-interacting 1; KRT, keratin; SD, standard deviation; shRNA, short hairpin RNA; NC, negative control.

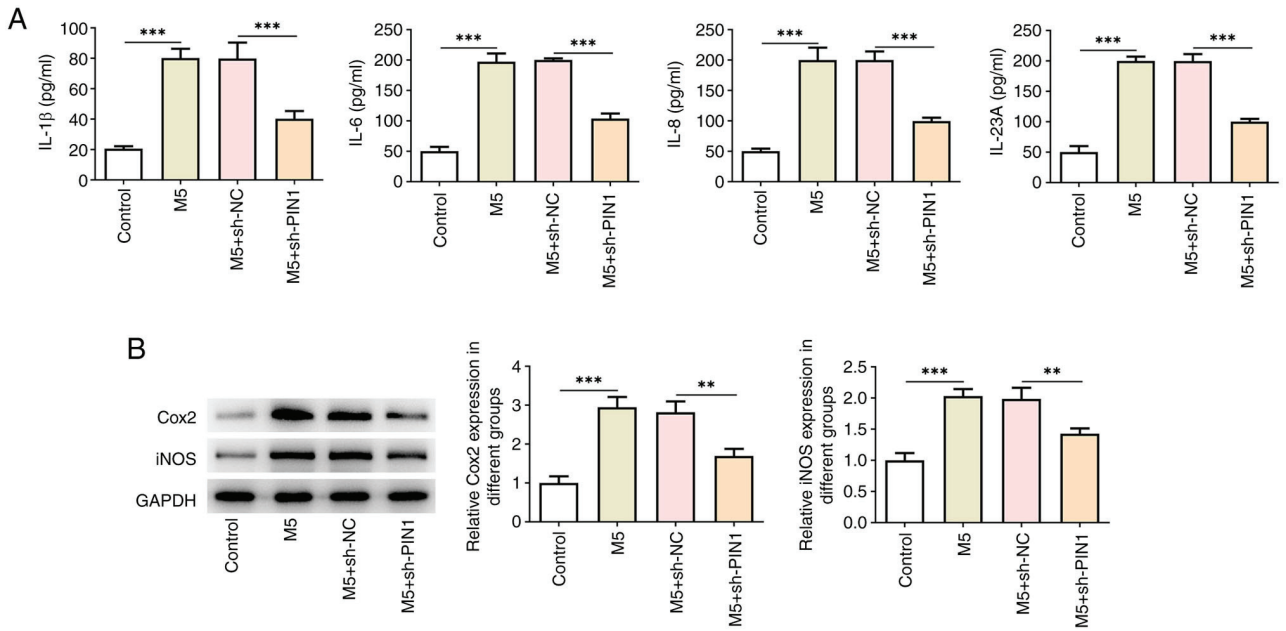

Figure 3. PIN1 silencing inhibits M5-induced inflammation in HaCaT cells. (A) The levels of IL-1β, IL-6, IL-8 and IL-23A were measured by ELISA. (B) The protein levels of Cox2 and iNOS in M5-induced HaCaT cells transfected with sh-PIN1 were detected by western blot analysis. Results are the mean ± SD. **P<0.01 and ***P<0.001. PIN1, peptidyl-prolyl cis/trans isomerase, NIMA-interacting 1; Cox2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase; shRNA, short hairpin RNA; SD, standard deviation; NC, negative control.

Activation of vitamin D receptor has been described to attenuate venous endothelial cell dysfunction by decreasing PIN1-mediated mitochondrial translocation of p66Shc, and thus, reducing mitochondrial reactive oxygen species

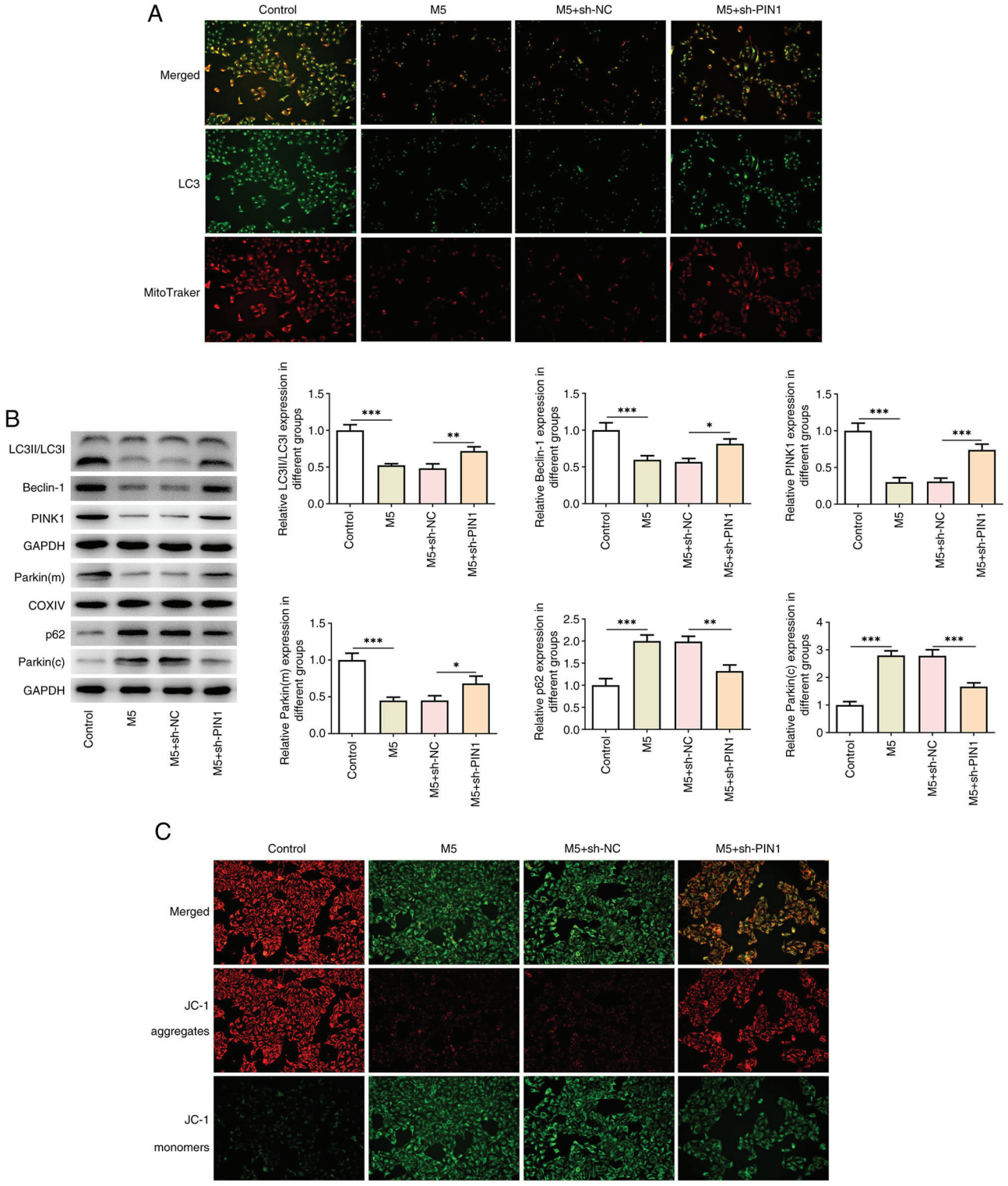

Figure 4. Knockdown of PIN1 activates M5-induced mitochondrial autophagy in HaCaT cells. (A) The levels of LC3 and MitoTraker in double-stained cells were measured by immunofluorescence assay (magnification, x200). (B) The protein levels of LC3II/LC3I, Beclin-1, PINK1, Parkin (mitochondrion), p62 and Parkin (cytoplasm) in M5-induced HaCaT cells transfected with sh-PIN1 were detected by western blot analysis. (C) JC-1 staining was used to assess the mitochondrial membrane potential (magnification, x200). Results are the mean ± SD. *P<0.05, **P<0.01 and ***P<0.001. PIN1, peptidyl-prolyl cis/trans isomerase, NIMA-interacting 1; Cox, cyclooxygenase; shRNA, short hairpin RNA; NC, negative control; SD, standard deviation.

(ROS) (34). Feng *et al* (35) demonstrated that the inhibition of PIN1 expression markedly decreased mitochondrial translocation of p66Shc and subsequent ROS generation and apoptosis, thus mitigating intestinal injury and secondary lung injury by using superior mesenteric artery occlusion-induced rat I/R model and hypoxia/reoxygenation (H/R)-induced Caco-2 cells. Stress factors such as ROS lead to a gradual accumulation of mitochondrial DNA mutations, as well as a reduction in intracellular MMP and depolarization damage, and ultimately lead to cell death (36). Inhibition of PIN1 increases the level

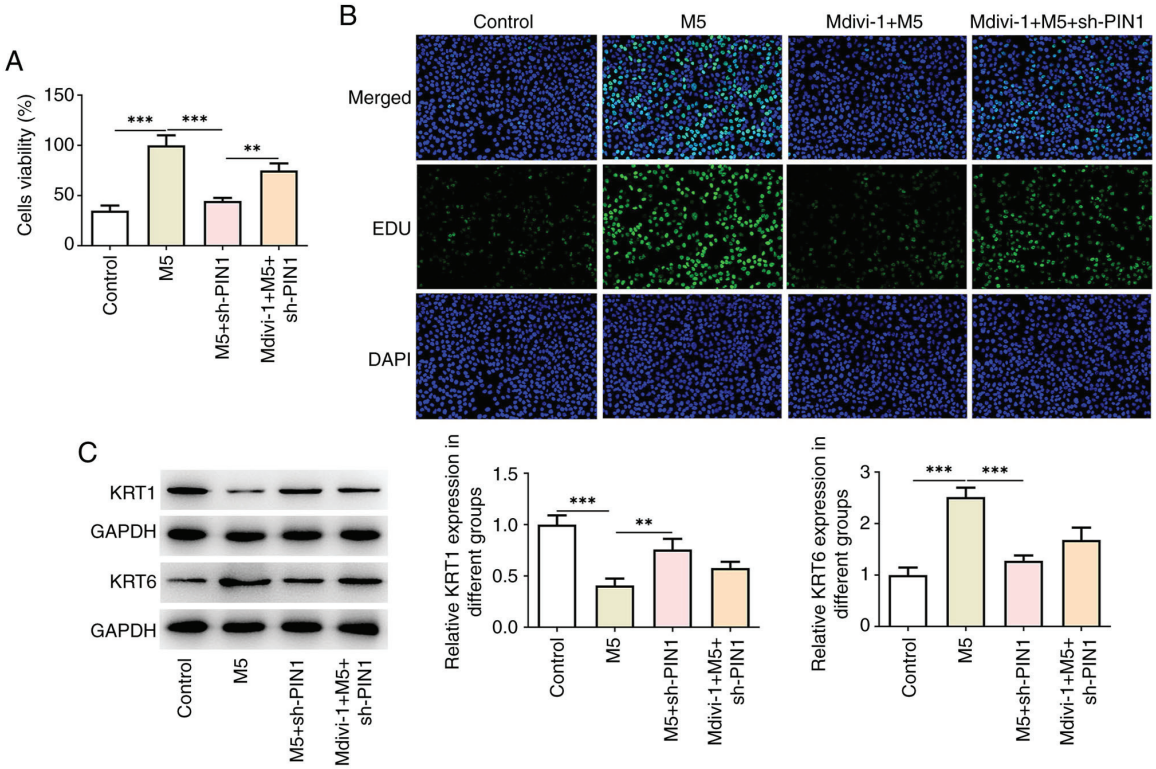

Figure 5. PIN1 silencing ameliorates M5-induced hyperproliferation in HaCaT cells by activating mitochondrial autophagy. (A) Cell Counting Kit-8 assay was used to assess cell viability. (B) EdU staining was performed to assess cell proliferation (magnification, x200). (C) The protein levels of KRT1 and KRT6 in Mdivi-1-treated HaCaT cells transfected with sh-PIN1 were detected by western blotting. Results are the mean ± SD. **P<0.01 and ***P<0.001. PIN1, peptidyl-prolyl cis/trans isomerase, NIMA-interacting 1; KRT, keratin; Mdivi-1, mitochondrial division inhibitor-1; shRNA, short hairpin RNA; SD, standard deviation.

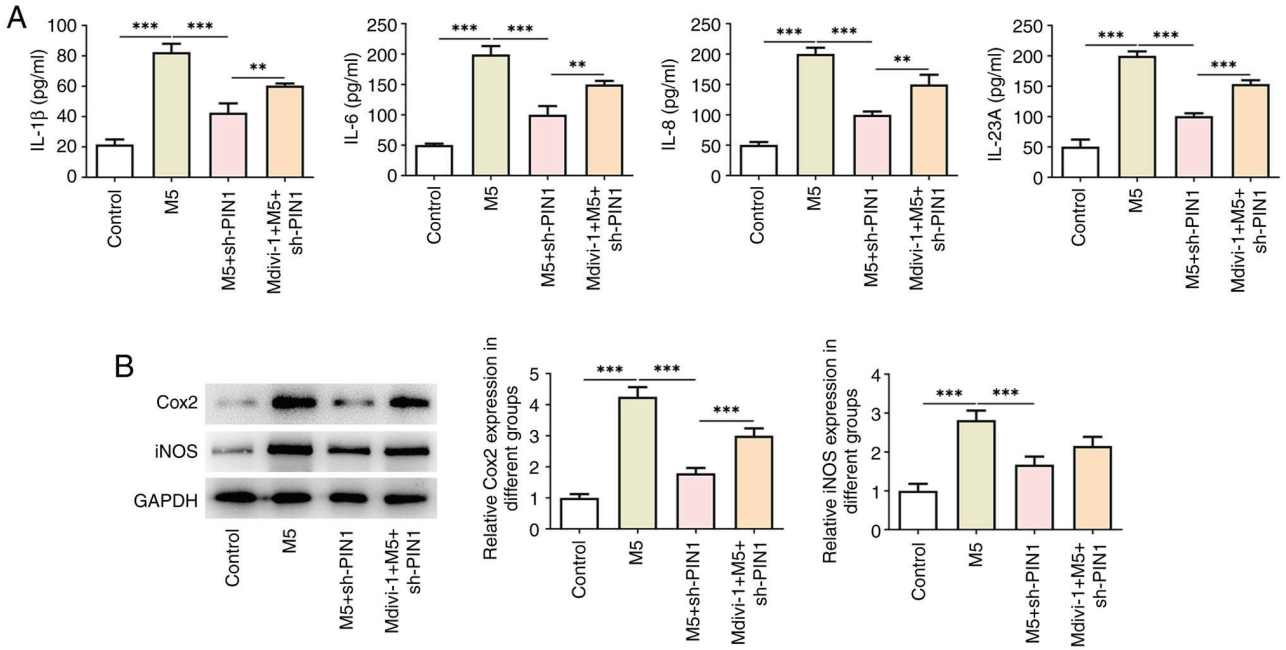

Figure 6. PIN1 silencing ameliorates M5-induced inflammation of HaCaT cells by activating mitochondrial autophagy. (A) The levels of IL-1β, IL-6, IL-8 and IL-23A were measured by ELISA. (B) The protein levels of Cox2 and iNOS in Mdivi-1-treated HaCaT cells transfected with sh-PIN1 were detected by western blot analysis. Results are the mean ± SD. **P<0.01 and ***P<0.001. PIN1, peptidyl-prolyl cis/trans isomerase, NIMA-interacting 1; Cox2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase; Mdivi-1, mitochondrial division inhibitor-1; shRNA, short hairpin RNA; SD, standard deviation.

of autophagy in senescent cells and cochlear hair cells (37). A different study reported that the transfection of SW-48 cells with PIN1 small interfering RNA injured cancer cell proliferation and migration, while it facilitated apoptosis and

autophagy (38). Taken together, the aforementioned studies suggested that PIN1 may regulate mitochondrial autophagy. Additionally, sirtuin 3 triggers mitochondrial autophagy in HaCaT cells by triggering the FOXO3a/Parkin pathway, thus ameliorating TNF-α-induced psoriasis (39). The present study revealed that PIN1 silencing activated M5-induced mitochondrial autophagy in HaCaT cells. To verify the function of mitochondrial autophagy in the regulation of PIN1 silencing in M5-induced HaCaT cells, the mitochondrial autophagy inhibitor Mdivi-1 was applied to treat cells. The data showed that Mdivi-1 treatment reversed the effects of PIN1 silencing on M5-induced hyperproliferation and inflammation, implying the regulatory role of mitochondrial autophagy in M5-induced HaCaT cells. Moreover, the present study did not perform animal and clinical studies, which will be involved in future experiments to confirm the findings of the present study.

In conclusion, the data demonstrated that PIN1 silencing ameliorated the hyperproliferation and inflammation in M5-induced HaCaT cells by triggering mitochondrial autophagy, which revealed the potential of PIN1 for the treatment of psoriasis.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

SX and WY designed the study, drafted and revised the manuscript. JL and HY analyzed the data and searched the literature. All authors performed the experiments. SX and WY confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Rendon A and Schäkel K: Psoriasis pathogenesis and treatment. Int J Mol Sci 20: 1475, 2019.
2. Kamiya K, Kishimoto M, Sugai J, Komine M and Ohtsuki M: Risk factors for the development of psoriasis. Int J Mol Sci 20: 4347, 2019.
3. Tokuyama M and Mabuchi T: New treatment addressing the pathogenesis of psoriasis. Int J Mol Sci 21: 7488, 2020.
4. Tashiro T and Sawada Y: Psoriasis and systemic inflammatory disorders. Int J Mol Sci 23: 4457, 2022.
5. Hao Y, Zhu YJ, Zou S, Zhou P, Hu YW, Zhao QX, Gu LN, Zhang HZ, Wang Z and Li J: Metabolic syndrome and psoriasis: Mechanisms and future directions. Front Immunol 12: 711060, 2021.
6. Lowes MA, Russell CB, Martin DA, Towne JE and Krueger JG: The IL-23/T17 pathogenic axis in psoriasis is amplified by keratinocyte responses. Trends Immunol 34: 174-181, 2013.
7. Hawkes JE, Yan BY, Chan TC and Krueger JG: Discovery of the IL-23/IL-17 signaling pathway and the treatment of psoriasis. J Immunol 201: 1605-1613, 2018.
8. Griffiths CEM, Armstrong AW, Gudjonsson JE and Barker JNWN: Psoriasis. Lancet 397: 1301-1315, 2021.
9. Kamata M and Tada Y: Crosstalk: Keratinocytes and immune cells in psoriasis. Front Immunol 14: 1286344, 2023.
10. Montero-Vilchez T, Segura-Fernández-Nogueras MV, Pérez-Rodríguez I, Soler-Gongora M, Martinez-Lopez A, Fernández-González A, Molina-Leyva A and Arias-Santiago S: Skin barrier function in psoriasis and atopic dermatitis: Transepidermal water loss and temperature as useful tools to assess disease severity. J Clin Med 10: 359, 2021.
11. Korman NJ: Management of psoriasis as a systemic disease: What is the evidence? Br J Dermatol 182: 840-848, 2020.
12. Malter JS: Pin1 and Alzheimer's disease. Transl Res 254: 24-33, 2023.
13. Caligiuri I, Vincenzo C, Asano T, Kumar V and Rizzolio F: The metabolic crosstalk between PIN1 and the tumour microenvironment. Semin Cancer Biol 91: 143-157, 2023.
14. Li J, Mo C, Guo Y, Zhang B, Feng X, Si Q, Wu X, Zhao Z, Gong L, He D and Shao J: Roles of peptidyl-prolyl isomerase Pin1 in disease pathogenesis. Theranostics 11: 3348-3358, 2021.
15. Cheng CW and Tse E: PIN1 in cell cycle control and cancer. Front Pharmacol 9: 1367, 2018.
16. Nishi M, Akutsu H, Masui S, Kondo A, Nagashima Y, Kimura H, Perrem K, Shigeri Y, Toyoda M, Okayama A, *et al*: A distinct role for Pin1 in the induction and maintenance of pluripotency. J Biol Chem 286: 11593-11603, 2011.
17. Nakamura K, Kosugi I, Lee DY, Hafner A, Sinclair DA, Ryo A and Lu KP: Prolyl isomerase Pin1 regulates neuronal differentiation via β-catenin. Mol Cell Biol 32: 2966-2978, 2012.
18. Lee YM, Shin SY, Jue SS, Kwon IK, Cho EH, Cho ES, Park SH and Kim EC: The role of PIN1 on odontogenic and adipogenic differentiation in human dental pulp stem cells. Stem Cells Dev 23: 618-630, 2014.
19. Luo ML, Gong C, Chen CH, Lee DY, Hu H, Huang P, Yao Y, Guo W, Reinhardt F, Wulf G, *et al*: Prolyl isomerase Pin1 acts downstream of miR200c to promote cancer stem-like cell traits in breast cancer. Cancer Res 74: 3603-3616, 2014.
20. Rustighi A, Zannini A, Tiberi L, Sommaggio R, Piazza S, Sorrentino G, Nuzzo S, Tuscano A, Eterno V, Benvenuti F, *et al*: Prolyl-isomerase Pin1 controls normal and cancer stem cells of the breast. EMBO Mol Med 6: 99-119. 2014.
21. Kanamoto M, Takahagi S, Aoyama S, Kido Y, Nakanishi M, Naito M, Kanna M, Yamamotoya T, Tanaka A, Hide M, *et al*: The expression of prolyl isomerase Pin1 is expanded in the skin of patients with atopic dermatitis and facilitates IL-33 expression in HaCaT cells. J Dermatol 50: 462-471, 2023.
22. Xu F, Armstrong R, Urrego D, Qazzaz M, Pehar M, Armstrong JN, Shutt T and Syed N: The mitochondrial division inhibitor Mdivi-1 rescues mammalian neurons from anesthetic-induced cytotoxicity. Mol Brain 9: 35, 2016.
23. So EC, Hsing CH, Liang CH and Wu SN: The actions of mdivi-1, an inhibitor of mitochondrial fission, on rapidly activating delayed-rectifier K^+ current and membrane potential in HL-1 murine atrial cardiomyocytes. Eur J Pharmacol 683: 1-9, 2012.
24. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
25. Raharja A, Mahil SK and Barker JN: Psoriasis: A brief overview. Clin Med (Lond) 21: 170-173, 2021.
26. Zhou X, Chen Y, Cui L, Shi Y and Guo C: Advances in the pathogenesis of psoriasis: From keratinocyte perspective. Cell Death Dis 13: 81, 2022.
27. Chen HL, Lo CH, Huang CC, Lu MP, Hu PY, Chen CS, Chueh DY, Chen P, Lin TN, Lo YH, *et al*: Galectin-7 downregulation in lesional keratinocytes contributes to enhanced IL-17A signaling and skin pathology in psoriasis. J Clin Invest 131: e130740, 2021.

28. Yu JH, Im CY and Min SH: Function of PIN1 in cancer development and its inhibitors as cancer therapeutics. Front Cell Dev Biol 8: 120, 2020.
29. Kim G, Bhattarai PY, Lim SC, Kim JY and Choi HS: PIN1 facilitates ubiquitin-mediated degradation of serine/threonine kinase 3 and promotes melanoma development via TAZ activation. Cancer Lett 499: 164-174, 2021.
30. Jeong HG, Pokharel YR, Lim SC, Hwang YP, Han EH, Yoon JH, Ahn SG, Lee KY and Kang KW: Novel role of Pin1 induction in type II collagen-mediated rheumatoid arthritis. J Immunol 183: 6689-6697, 2009.
31. Ge ZZ, Wu YB, Xue ZY, Zhang K and Zhang RX: The therapeutic effects of the peptidyl-prolyl cis/trans isomerase Pin1 inhibitor juglone on animal-model experimental autoimmune encephalomyelitis. J Physiol Pharmacol: Aug 6, 2021 (Epub ahead of print).
32. Wahedi HM, Park YU, Moon EY and Kim SY: Juglone ameliorates skin wound healing by promoting skin cell migration through Rac1/Cdc42/PAK pathway. Wound Repair Regen 24: 786-794, 2016.
33. Quyen BT, Choi HK and Kang KW: Pin1 is required for ultraviolet A-stimulated cyclooxygenase-2 induction in mouse epidermal cells. Cancer Lett 335: 31-40, 2013.
34. Han YC, Liu YT, Zhang H, Xu Y, Liu J, Chen H, Song N, Qin DL and Yang S: VDR alleviates endothelial cell injury in arteriovenous fistula through inhibition of P66Shc-mediated mitochondrial ROS. Sci Rep 13: 11088, 2023.
35. Feng D, Yao J, Wang G, Li Z, Zu G, Li Y, Luo F, Ning S, Qasim W, Chen Z and Tian X: Inhibition of p66Shc-mediated mitochondrial apoptosis via targeting prolyl-isomerase Pin1 attenuates intestinal ischemia/reperfusion injury in rats. Clin Sci (Lond) 131: 759-773, 2017.
36. Lemasters JJ: Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. Rejuvenation Res 8: 3-5, 2005.
37. Lv Z, Zhang Y, Cao H, Liu Q, Feng X, Yin H and Wang B: PIN1 protects auditory hair cells from senescence via autophagy. PeerJ 10: e14267, 2022.
38. Gholamzadeh Khoei S, Saidijam M, Amini R, Jalali A and Najafi R: Impact of PIN1 inhibition on tumor progression and chemotherapy sensitivity in colorectal cancer. J Gastrointest Cancer 53: 299-310, 2022.
39. Yanli M, Yu W and Yuzhen L: Elevated SIRT3 Parkin-dependently activates cell mitophagy to ameliorate TNF-α-induced psoriasis-related phenotypes in HaCaT cells through deacetylating FOXO3a for its activation. Arch Dermatol Res 315: 847-857, 2023.