

SCIN Overexpression Attenuates LPS-Induced Apoptosis and Differentially Modulates EGFR Abundance and Phosphorylation in Human Oral Keratinocytes

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Background: Oral lichen planus (OLP) is a chronic inflammatory and immune-mediated oral mucosal disease characterized by epithelial damage, basal cell degeneration, and recurrent lesions. However, the molecular mechanisms responsible for keratinocyte injury and apoptosis during OLP progression remain incompletely understood. This study aimed to investigate the protective role of scinderin (SCIN) and the potential involvement of epidermal growth factor receptor (EGFR) signaling during lipopolysaccharide (LPS)-induced inflammatory stress in human oral keratinocytes (HOKs).

Methods: HOKs underwent transfection and were treated with LPS. Relative 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) metabolic activity and apoptosis were evaluated using an MTT assay and flow cytometry. Apoptosis-related proteins were assessed using Western blotting. Caspase 3 and 9 activities were assessed using a fluorometric assay. SCIN expression was analyzed by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and Western blotting, whereas total EGFR, inhibitor of nuclear factor kappa B alpha (I κ B α), and their phosphorylated forms were evaluated by Western blotting.

Results: LPS stimulation decreased SCIN and total EGFR protein levels and relative MTT metabolic activity while increasing p-EGFR protein levels and p-EGFR/EGFR ratios ($p < 0.001$). LPS also promoted apoptosis, as evidenced by an increased apoptotic rate, elevated BCL-2-associated X protein (BAX) expression, cleaved caspase 3/caspase 3 ratio, and caspase 3 and caspase 9 activities, as well as decreased B-cell lymphoma 2 (BCL-2) expression ($p < 0.05$). SCIN overexpression significantly counteracted these LPS-induced molecular and cellular changes ($p < 0.05$). Compared with the Control group, LPS treatment also increased the phosphorylated (p)-I κ B α /I κ B α ratio ($p < 0.001$). EGFR knockdown further aggravated LPS-induced injury, whereas SCIN overexpression partially alleviated the associated apoptotic changes under EGFR knockdown conditions ($p < 0.05$).

Conclusion: SCIN overexpression alleviated LPS-induced apoptosis in HOKs and was associated with changes in EGFR expression and phosphorylation. These findings highlight SCIN as a potential regulator of keratinocyte responses to inflammatory stress and support its further investigation in OLP-associated epithelial injury.

Keywords: SCIN; oral lichen planus; keratinocytes; apoptosis; EGFR

Introduction

Oral lichen planus (OLP) is an immune-mediated inflammatory disease characterized by multifocal symmetric white striations, papules, plaques, blisters, atrophy, and erosion in the oral mucosa [1]. Patients with OLP may have difficulty tolerating irritating substances (e.g., acidic or spicy foods or toothpaste) due to pain caused by the buccal lesions [2]. These lesions can be asymptomatic, and approximately 1.4% of them can transform into cancer within 7 years, when risk factors, such as ulceration, location on the tongue and female sex, are involved [3].

The development of OLP involves aberrant gene expression [4]. Through a literature review, we focused on scinderin (SCIN), a protein belonging to the calcium-

dependent gelsolin superfamily of actin that regulates filamentous actin (F-actin) organization [5]. Among the immune abnormalities reported in OLP, increased interleukin-23 (IL-23) expression and T helper 17 (Th17)-related responses have been observed in OLP lesions [6,7,8,9]. Nuclear factor kappa B (NF- κ B) signaling also plays a pivotal role in OLP pathogenesis [10]. In human oral keratinocytes (HOKs), lipopolysaccharide (LPS) has been shown to induce inhibitor of nuclear factor kappa B alpha (I κ B α) phosphorylation and NF- κ B activation [11], as well as impaired cellular metabolic activity and increased apoptosis [12]. SCIN overexpression has also been reported to enhance NF- κ B signaling in nasopharyngeal carcinoma cells [13]. However, whether SCIN is associated with NF- κ B signaling and apoptosis in oral keratinocytes under inflammatory stress

remains unclear. We therefore hypothesized that SCIN may modulate the response of oral keratinocytes to inflammatory stress.

Moreover, a previous study has reported that loss of SCIN resulted in significant downregulation of epidermal growth factor receptor (EGFR) in prostate cancer cells [14]. EGFR is a receptor tyrosine kinase playing a fundamental role in cell proliferation and differentiation [15], and its expression often correlates with carcinogenesis, cancer progression and resistance to chemotherapy, in part through inhibition of apoptosis [16,17,18]. Dysregulation of EGFR signaling has been implicated in a variety of pathological conditions, including chronic inflammatory diseases and cancer. Notably, EGFR has been proposed as a potential biomarker for risk stratification and malignant transformation in OLP [3]. Besides, cell apoptosis along with increased inflammation in noncancerous cells is shown to be caused by reduced EGFR expression [19,20,21]. Furthermore, EGFR inhibitors have been extensively reported to activate Caspase 3 and 9, two effector caspases executing apoptosis following the release of pro-apoptotic cytochrome c [22,23]. These findings led us to examine whether the effects of SCIN on oral keratinocyte apoptosis are associated with alterations in EGFR expression and phosphorylation.

In the present study, LPS-treated HOKs were used as a simplified *in vitro* model of inflammatory stress. We evaluated the effects of SCIN overexpression on LPS-induced metabolic impairment and apoptosis and explored their potential associations with EGFR and NF- κ B signaling.

Materials and Methods

Culture of HOKs

HOKs were purchased from ScienCell Research Laboratories (2610, Carlsbad, CA, USA) and grown in oral keratinocyte medium (2611, ScienCell Research Laboratories, CA, USA) with 5% CO₂ at 37 °C. The HOKs were authenticated by short tandem repeat (STR) profiling. HOKs were stimulated with 100 ng/mL LPS (ST1470, Beyotime, Shanghai, China) for 24 h to establish an *in vitro* inflammatory injury model in HOKs, which has been widely used to investigate inflammatory responses in oral keratinocytes [24]. HOKs were tested for mycoplasma contamination prior to experimentation and were confirmed to be free of mycoplasma.

HOKs Transfection

For overexpression experiments, HOKs were transfected with a Myc-DDK-tagged human SCIN ORF clone (RC205801; transcript variant 2, NM_033128; OriGene, MD, USA), constructed in the pCMV6-Entry vector. The corresponding empty pCMV6-Entry vector (PS100001, OriGene, MD, USA) was used as the matched vector control. For gene silencing, an EGFR-targeting short hair-

pin RNA plasmid (shEGFR; TR320326, OriGene Technologies, Rockville, MD, USA) and its matched scrambled shRNA control (TR30012, OriGene, MD, USA) in the same pRS backbone were used. The hairpin sequence of shEGFR was 5'-GCCACAAAGCAGTGAATTTATCTC GAGATAAATTCAGTCTTTGTGGC-3', and shNC was 5'-GCACTACCAGGCTAACTCAGATAGTACT-3'. All plasmids were commercially obtained and sequence-verified by the manufacturer.

HOKs were seeded into appropriate culture plates according to the requirements of each experiment and cultured to approximately 70%–80% confluence before transfection. For each well of a 6-well plate, 2.5 μ g plasmid DNA was mixed with 5 μ L P3000 reagent (L3000015, ThermoFisher, Waltham, MA, USA) in 125 μ L Opti-MEM medium (31985070, ThermoFisher, Waltham, MA, USA). Separately, 7.5 μ L Lipofectamine 3000 reagent was diluted in 125 μ L Opti-MEM medium. The two solutions were combined and incubated for 10 min at room temperature before being added to the cells. Following transfection for 48 h, the efficiency of transfection was assessed using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Cells from independently transfected cultures were then collected for subsequent experiments, including Western blotting, flow cytometry, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and Caspase activity assays.

qRT-PCR

Total RNA from HOKs was extracted with TRIzol reagents (15596026, ThermoFisher, Waltham, MA, USA) and then quantified using a spectrophotometer (NanoDrop 2000, ThermoFisher, Waltham, MA, USA). Reverse transcription kits (K1622, Yaanda Biotechnology, Beijing, China) were used to synthesize first-strand cDNAs from total RNA. For real-time PCR reaction, the cDNAs were then transferred into a PCR detection System (Light-Cycler 96, Roche, Indianapolis, IN, USA) along with Eastep qPCR Master Mix (LS2062, Promega, Madison, WI, USA), and the reaction was initiated at: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The primers used were as follows: (SCIN: forward, 5'-ATGGCTTCGGGAAAGTTTATGT-3', reverse, 5'-CATCCACCATATTGTGCTGGG-3'; EGFR: forward, 5'-AGGCACGAGTAACAAGCTCAC-3', reverse, 5'-ATGAGGACATAACCAGCCACC-3'; GAPDH: forward, 5'-GGAGCGAGATCCCTCCAAAAT-3', reverse, 5'-GGCTGTTGTCATACTTCTCATGG-3'). Relative number of transcripts was analyzed using the 2^{- $\Delta\Delta$ Ct} method [25], and relative gene expression levels were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

HOKs Treatment

HOKs were divided into the following six groups: Control, LPS, LPS + vector, LPS + SCIN, LPS + shEGFR, and LPS + shEGFR + SCIN. Cells in the Control group were cultured under normal conditions. Cells in the LPS group were treated with 100 ng/mL LPS for 24 h. In the LPS + vector, LPS + SCIN, LPS + shEGFR, and LPS + shEGFR + SCIN groups, cells were transfected with the corresponding shRNA or plasmid as previously described and incubated for 48 h, followed by stimulation with 100 ng/mL LPS for an additional 24 h.

Western Blotting

HOKs were lysed using RIPA Lysis Buffer (20-188, Sigma-Aldrich, St. Louis, MO, USA) containing protease inhibitor (P8340, Sigma-Aldrich, St. Louis, MO, USA) to harvest total protein. After the assessment of protein concentration using a BCA kit (A53227, ThermoFisher, Waltham, MA, USA), the total protein was electrophoresed in an SDS-PAGE gel (1615100, BIO-RAD, Hercules, CA, USA) for separation. The separated proteins were blotted onto a polyvinylidene fluoride membrane (1620256, BIO-RAD, Hercules, CA, USA), which were later blocked in 5% bovine serum albumin (BSA; 9998, Cell Signaling Technology, Danvers, MA, USA) for 1 h at room temperature, followed by incubation with primary antibodies for phosphorylated (p)-I κ B α (ab133462, 40 kDa, 1:10,000, Abcam, Cambridge, MA, UK), I κ B α (ab32518, 35 kDa, 1:1000, Abcam, Cambridge, MA, UK), SCIN (ab199723, 80 kDa, 1:1000, Abcam, Cambridge, MA, UK), cleaved caspase 3 (ab32042, 17 kDa, 1:500, Abcam, Cambridge, MA, UK), caspase 3 (ab13847, 35 kDa, 1:500, Abcam, UK), BAX (BCL-2-associated X protein; ab32503, 21 kDa, 1:1000, Abcam, Cambridge, MA, UK), BCL-2 (B-cell lymphoma 2; ab59348, 26 kDa, 1:1000, Abcam, Cambridge, MA, UK), p-EGFR (Y1068, ab223499, 170 kDa, 1:400, Abcam, Cambridge, MA, UK), EGFR (ab52894, 175 kDa, 1:5000, Abcam, Cambridge, MA, UK), and GAPDH (ab245355, 36 kDa, 1:2000, Abcam, Cambridge, MA, UK) overnight at 4 °C. Following two washes with T20 TBS (37571, ThermoFisher, Waltham, MA, USA), the membrane was incubated with Goat anti-Rabbit IgG secondary antibody (ab97051, Abcam, Cambridge, MA, UK) for 2 h at room temperature. Protein signals were visualized with ECL Substrate (1705060, BIO-RAD, Hercules, CA, USA) with a luminescence imager (ChemiDoc, BIO-RAD, Hercules, CA, USA), and densitometric analysis was performed using ImageJ software (version 3.0, National Institutes of Health, Bethesda, MA, USA). The band intensities of SCIN, BAX, BCL-2, total EGFR, and p-EGFR were normalized to GAPDH. The cleaved caspase 3/caspase 3, p-I κ B α /I κ B α , and p-EGFR/EGFR ratios were calculated to assess caspase 3 cleavage and the relative phosphorylation levels of I κ B α and EGFR, respectively.

MTT Assay

MTT Cell Growth Assay kits (CT01-5, Sigma-Aldrich, St. Louis, MO, USA) were used to measure relative MTT metabolic activity. Briefly, HOKs (1×10^4 cells per well) were seeded in 96-well plates and gently tapped. Then, 10 μ L of MTT reagent was added to each well, and the plates were incubated at 37 °C for 4 h, after which 0.1 mL of isopropanol with 0.04 N HCl was used to dissolve the generated formazan. Lastly, absorbance was detected using a microplate reader (PHERAstar FSX, BMG LABTECH, Ortenberg, Germany) at 570 nm.

Flow Cytometry

Cell apoptosis was determined using Annexin V-FITC Early Apoptosis Detection kits (6592, Cell Signaling Technology, Danvers, MA, USA). Briefly, after centrifugation at $500 \times g$ for 5 min, HOKs were harvested, washed with pre-cooled phosphate-buffered saline (PBS; P4474, Sigma-Aldrich, St. Louis, MO, USA), and resuspended in Annexin-V binding buffer at a density of 1×10^6 cells/mL. The cell suspension (96 μ L) was added to Annexin V-FITC solution (1 μ L) and propidium iodide solution (12.5 μ L) and incubated together on ice for 10 min, protected from light. Afterward, the cell suspension was diluted using Annexin-V binding buffer to a final volume of 250 μ L and immediately subjected to apoptosis analysis carried out using a flow cytometer (CytoFLEX, Beckman Coulter, Brea, CA, USA).

Caspase 3 and 9 Activity Assays

HOKs were lysed on ice in lysis buffer containing protease inhibitors for 10 min. After centrifugation at $14,000 \times g$ for 10 min, cell lysates were obtained. Caspase 3 and 9 activities in the cell lysates were tested for 2 h at 37 °C with fluorogenic caspase substrates DEVD-AFC and LEHD-AFC (ab39383 and ab65607, Abcam, Cambridge, MA, UK), respectively, and fluorescence was measured at excitation/emission wavelengths of 400 and 505 nm using a microplate reader.

Statistical Analysis

Results were presented as mean $\pm$ standard deviation (SD) of three independent experiments. Statistical analysis was performed using GraphPad Prism (version 8.0, GraphPad Software Inc., San Diego, CA, USA). Data normality was assessed using the Shapiro-Wilk test. Differences between the two groups were assessed using an independent Student's *t*-test. For differences among more than two groups with a single experimental factor, one-way ANOVA followed by Tukey's post hoc multiple-comparisons test was applied. A statistically significant difference was considered when $p < 0.05$.

Results

SCIN Overexpression Attenuated LPS-Induced Inflammatory Injury and Apoptosis in HOKs

Western blotting suggested that, compared with the Control group, the LPS group decreased expression of SCIN while elevating p-IkBa/IkBa levels in HOKs (Fig. 1A–C, $p < 0.001$). qRT-PCR analysis revealed that SCIN mRNA expression was significantly increased following transfection with the SCIN overexpression plasmids (Fig. 1D, $p < 0.001$). LPS treatment reduced the relative MTT metabolic activity of HOKs (Fig. 1E, $p < 0.001$), which was partially reversed by SCIN overexpression (Fig. 1E, $p < 0.001$), as shown by the MTT assay. Conversely, flow cytometry showed that apoptosis in HOKs was induced following LPS treatment (Fig. 1F,G, $p < 0.001$), whereas SCIN overexpression attenuated the pro-apoptotic effect of LPS in HOKs (Fig. 1F,G, $p < 0.001$).

SCIN Overexpression Alleviated LPS-Induced Activation of Apoptotic Pathways and Reversed Alterations in EGFR Expression and Phosphorylation in HOKs

Western blotting showed that LPS treatment increased the protein levels of pro-apoptotic markers BAX and cleaved caspase3, while decreasing the expression of the anti-apoptotic protein BCL-2. These LPS-induced changes were significantly reversed by SCIN overexpression (Fig. 2A–D, $p < 0.05$). LPS treatment also increased Caspase 3 and 9 activities (Fig. 2E,F, $p < 0.001$). However, Caspase 3 and 9 activities in LPS-treated HOKs were reduced by SCIN overexpression (Fig. 2E,F, $p < 0.001$). Meanwhile, LPS treatment significantly decreased the total EGFR/GAPDH level while increasing the p-EGFR/GAPDH level and the p-EGFR/EGFR ratio. SCIN overexpression significantly reversed these changes, as evidenced by an increase in total EGFR/GAPDH and decreases in p-EGFR/GAPDH and p-EGFR/EGFR compared with the LPS + vector group (Fig. 2G–J, $p < 0.001$). These findings indicate a potential association between the regulation of EGFR expression and phosphorylation and the protective effects of SCIN against LPS-induced apoptosis.

EGFR Knockdown Exacerbated LPS-Induced Apoptosis in HOKs, Whereas SCIN Overexpression Alleviated Apoptosis Under EGFR-Knockdown Conditions

Subsequently, EGFR knockdown experiments were performed to further explore the relationship between EGFR signaling and protective effects of SCIN against LPS-induced apoptosis in HOKs. Transfection with shEGFR achieved knockdown of EGFR in HOKs compared with shNC transfection (Fig. 3A, $p < 0.001$). LPS-induced reduction in MTT metabolic activity was further exacerbated by EGFR knockdown (Fig. 3B, $p < 0.01$). SCIN

overexpression partially increased relative MTT metabolic activity under EGFR-knockdown conditions in LPS-treated HOKs (Fig. 3B, $p < 0.001$). EGFR knockdown potentiated LPS-induced apoptosis in HOKs (Fig. 3C,D, $p < 0.001$), whereas SCIN overexpression significantly attenuated apoptosis under EGFR-knockdown conditions (Fig. 3C,D, $p < 0.001$). Consistently, EGFR knockdown increased the levels of the pro-apoptotic proteins BAX and cleaved caspase-3 and decreased the expression of the anti-apoptotic protein BCL-2 in LPS-treated HOKs. Notably, these changes were partially attenuated by SCIN overexpression (Fig. 3E–H, $p < 0.05$). Similarly, Caspase 3 and 9 activities in HOKs increased following LPS treatment, which was further intensified by EGFR knockdown (Fig. 3I,J, $p < 0.001$). Nevertheless, SCIN overexpression partially attenuated these apoptotic changes under EGFR knockdown conditions (Fig. 3I,J, $p < 0.001$).

Discussion

The pathogenesis of OLP involves antigen presentation by the oral keratinocytes, and this antigenic trigger cooccurs with inflammatory responses [26,27]. The present study developed an *in vitro* LPS-induced model and revealed the molecular mechanism underlying keratinocyte apoptosis in OLP. Activated CD4⁺T cells undergo differentiation into subsets, such as Th1, Th2, Th17 and regulatory T cells (Treg) [27]. These subsets further produce a large number of various cytokines to mediate immune-inflammatory responses, thereby determining the character, extent and duration of OLP [28]. IL-23 is a proinflammatory cytokine that can regulate the immune imbalance in OLP and lead to keratinocyte apoptosis by driving the differentiation and activation of Th17 cells [29,30]. The mRNA level of IL-23 is significantly elevated in OLP lesions [8,9], and IL-23 has been previously demonstrated to activate NF- κ B signaling through the phosphorylation of IkBa [31]. Notably, NF- κ B signaling has been revealed to be activated in OLP and has been associated with the regulation of genes involved in inflammatory responses and cell survival [32]. Although the relationship between IL-23, NF- κ B, and SCIN has not been fully elucidated in OLP, our results demonstrated that LPS stimulation increased IkBa phosphorylation while markedly reducing SCIN expression in HOKs.

Excessive oral keratinocyte apoptosis is a hallmark of OLP and is associated with impaired oral epithelial barrier function [33]. SCIN is a Ca²⁺ actin-binding protein involved in vesicle transport and endocytosis. It is believed to play a dysregulated role in various malignant tumors, including breast, colon, liver, lung, prostate, and gastric cancers [34,35]. Previous studies have shown that SCIN is involved in the regulation of cell survival and apoptosis, suggesting a potential role for SCIN in regulating cell fate [14,36]. In this study, we demonstrated that LPS-induced

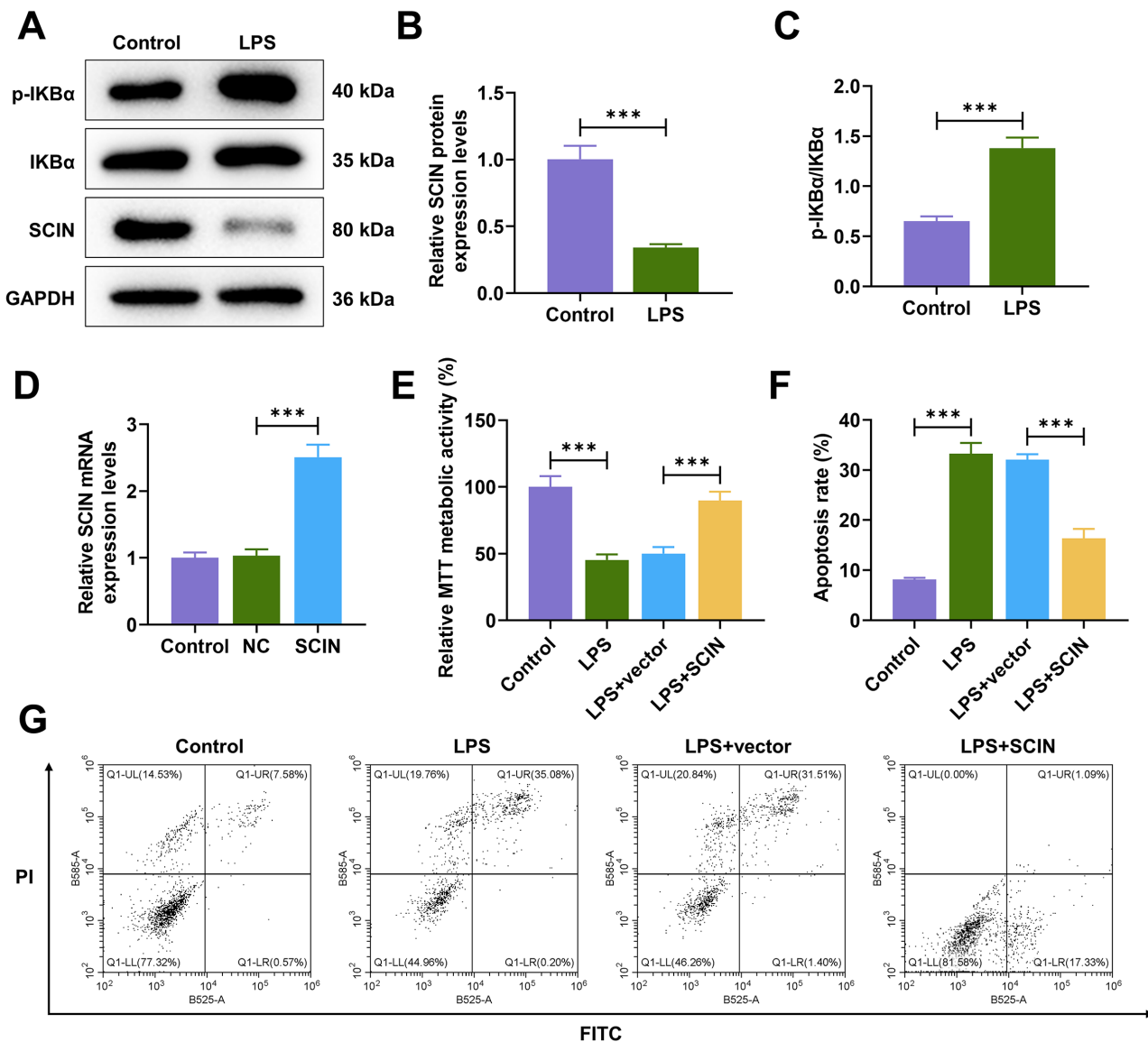


Fig. 1. SCIN overexpression attenuated LPS-induced inflammatory injury and apoptosis in HOKs. (A–C) Protein expression of SCIN and the p-IκBα/IκBα ratio in LPS-treated HOKs were analyzed by Western blotting, with GAPDH serving as the loading control. (D) SCIN mRNA expression was analyzed in untreated HOKs (Control), vector-transfected HOKs, and SCIN-overexpression plasmid-transfected HOKs by qRT-PCR, with GAPDH serving as the reference gene. (E–G) HOKs were transfected with SCIN overexpression plasmids or the pCMV6-Entry vector, followed by treatment with LPS. (E) Relative metabolic activity was measured by MTT assay. (F,G) Apoptosis in HOKs was determined by flow cytometry. Data are presented as the mean ± SD from three independent experiments (n = 3). *** $p < 0.001$. SCIN, scinderin; HOKs, human oral keratinocytes; IκBα, inhibitor of nuclear factor kappa B alpha; p-IκBα, phosphorylated-inhibitor of nuclear factor kappa B alpha; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; vector, empty pCMV6-Entry vector; SD, standard deviation; LPS, lipopolysaccharide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

reductions in relative MTT metabolic activity and increases in apoptosis were partially reversed by overexpression of SCIN in HOKs.

Intrinsic apoptosis is defined as a mitochondrion-centered cell death that is consequent on mitochondrial outer membrane permeabilization (MOMP) [22]. MOMP is regulated by the balance between pro- and anti-apoptotic

members of the BCL-2 family [37] and induces the release of cytochrome c from the mitochondria, after which Caspase 9 is activated and further activates Caspase 3 [38], the dominant executioner caspase responsible for cell death [39]. SCIN knockdown augments the activity of Caspase 3 to prompt breast carcinoma cell apoptosis [40]. SCIN knockdown has been reported to sensitize hepatocellular

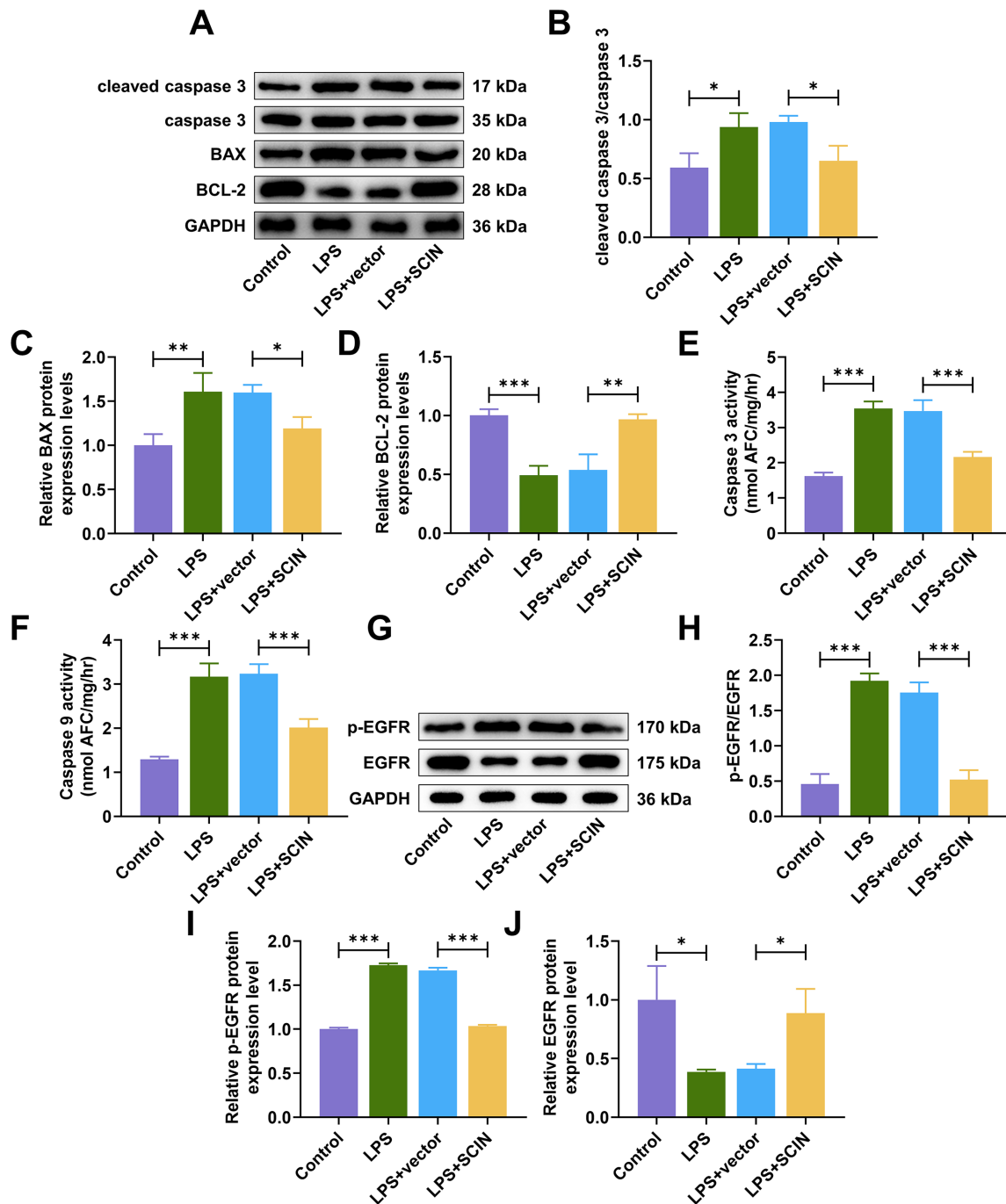


Fig. 2. SCIN overexpression alleviated LPS-induced activation of apoptotic pathways and restored EGFR phosphorylation in HOKs. (A) Representative Western blotting images of apoptosis-related proteins. GAPDH was used as the loading control. (B–D) Quantitative analysis of the cleaved caspase 3/caspase 3 ratio, BAX and BCL-2 protein expression. (E,F) The Caspase 3 and 9 activities in HOKs were assessed with a fluorometric assay. (G) Representative Western blotting images of p-EGFR and total EGFR in HOKs. GAPDH was used as the loading control. (H–J) Quantitative analyses of p-EGFR/EGFR, p-EGFR/GAPDH, and total EGFR/GAPDH, respectively. The p-EGFR/EGFR ratio represents the relative phosphorylation level of EGFR, whereas p-EGFR/GAPDH and EGFR/GAPDH represent the GAPDH-normalized levels of phosphorylated and total EGFR, respectively. Data are presented as the mean $\pm$ SD from three independent experiments ($n = 3$). HOKs were transfected with the SCIN overexpression plasmid or pCMV6-Entry vector, followed by LPS treatment. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. vector, pCMV6-Entry empty vector; BCL-2, B-cell lymphoma 2; BAX, BCL-2-associated X protein.

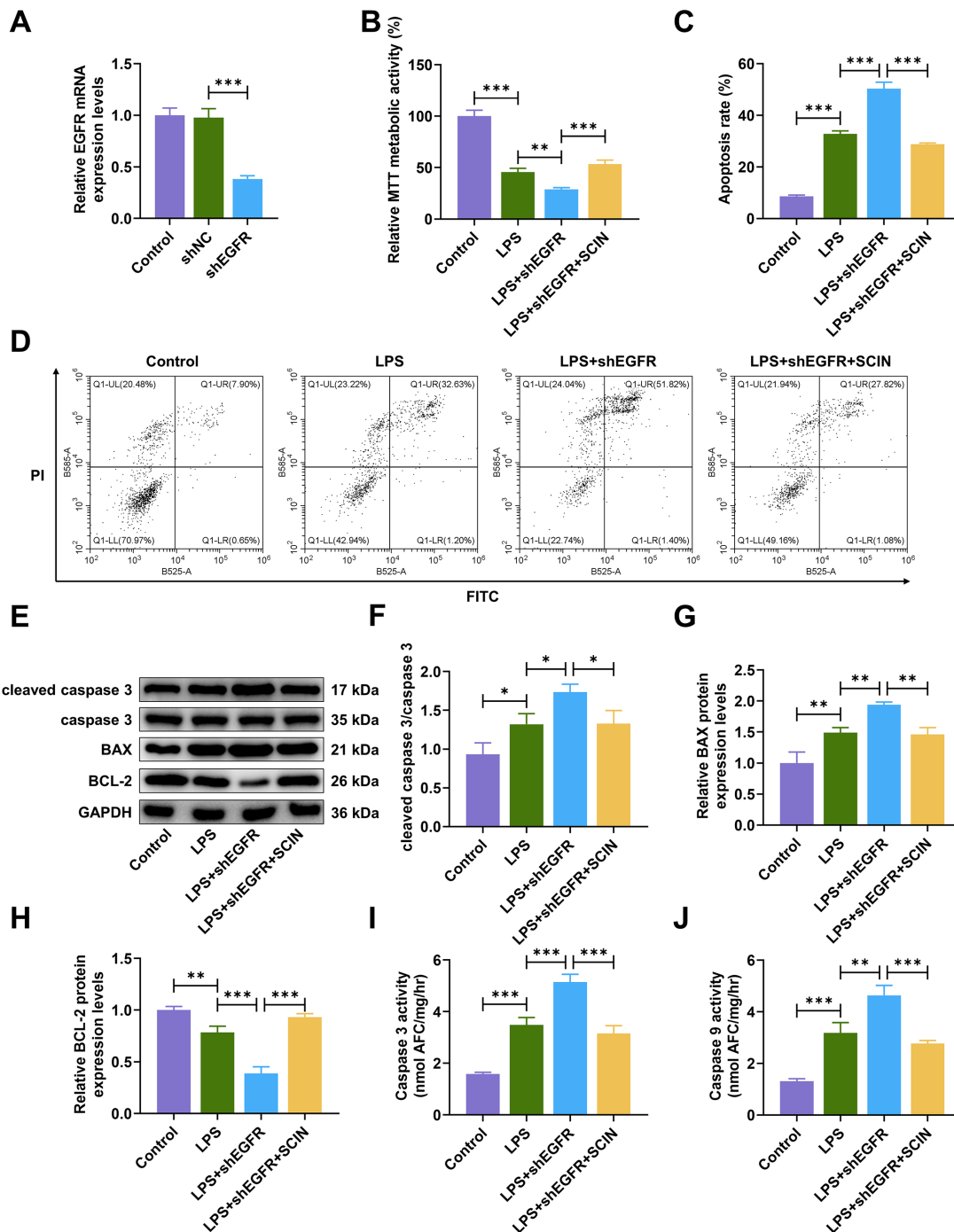


Fig. 3. EGFR knockdown aggravated LPS-induced apoptosis in HOKs, whereas SCIN overexpression alleviated apoptosis under EGFR-knockdown conditions. (A) EGFR mRNA expression in Control (untreated), shNC-transfected, and shEGFR-transfected HOKs was determined by qRT-PCR, with GAPDH serving as the normalizer. (B–J) The functional experiments included the Control, LPS, LPS + shEGFR, and LPS + shEGFR + SCIN groups. HOKs in the latter two groups were transfected with shEGFR alone or together with the SCIN overexpression plasmid, respectively, followed by LPS treatment. (B) Relative metabolic activity was measured by MTT assay. (C,D) Apoptosis in HOKs was determined by flow cytometry. (E) Representative Western blotting images of BAX, BCL-2, cleaved caspase 3, and caspase 3 in HOKs. (F–H) Quantitative analyses of BAX and BCL-2 protein expression and the cleaved caspase 3/caspase 3 ratio. (I,J) The Caspase 3 and 9 activities in HOKs were assessed using a fluorometric assay. Data are presented as the mean $\pm$ SD from three independent experiments ($n = 3$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. shEGFR, short hairpin RNA targeting EGFR; shNC, scrambled short hairpin RNA control.

carcinoma cells to apoptosis, accompanied by Caspase 9 activation [41]. In line with these roles of SCIN, SCIN overexpression reduced BAX and cleaved caspase 3 expression, increased BCL-2 expression, and suppressed Caspase 3 and Caspase 9 activities in LPS-treated HOKs, consistent with suppression of mitochondrial apoptosis-related signaling.

A large body of literature has shown that inhibition of EGFR leads to Caspase 3 and 9 activation, subsequently inducing apoptosis in cancerous or noncancerous cells [42,43]. More pertinently, EGFR downregulation induced by ultraviolet A (UVA) exposure involves Caspase activation and inhibits survival signaling via extracellular signal-regulated kinase (ERK) and protein kinase B (AKT) to induce apoptosis in keratinocytes [44]. In addition, activation of the EGFR pathway works in an apparent effort to counteract the overactivation of keratinocyte pro-inflammatory functions [45]. Collectively, these findings suggest that EGFR exerts a protective effect in keratinocytes. Our results showed that LPS decreased total EGFR protein expression in HOKs, similar to the effect of UVA exposure. Of note, deficiency of SCIN has been previously reported to significantly downregulate EGFR in prostate cancer [14]. In our study, SCIN overexpression partially restored total EGFR protein expression in LPS-treated HOKs. However, LPS increased p-EGFR/GAPDH and the p-EGFR/EGFR ratio, whereas SCIN overexpression reduced both indices. The opposite changes in total EGFR expression and EGFR phosphorylation suggest that EGFR abundance and phosphorylation status were differentially regulated under LPS-induced inflammatory stress. The increase in EGFR phosphorylation following LPS exposure may represent a compensatory or stress-associated response in the remaining EGFR pool, whereas its reduction following SCIN overexpression may accompany the alleviation of LPS-induced cellular stress. Nevertheless, the functional significance of these changes in EGFR phosphorylation remains unclear. These findings suggest a potential association between SCIN and EGFR signaling in HOKs.

Consistent with the reported protective role of EGFR in keratinocytes, EGFR knockdown intensified LPS-induced apoptosis in HOKs, accompanied by increased expression of BAX and cleaved caspase-3, decreased BCL-2 expression, and enhanced Caspase 3 and Caspase 9 activities. Notably, SCIN overexpression partially reversed these apoptotic changes even under EGFR knockdown conditions, indicating that SCIN retained its protective effect despite reduced EGFR expression. Therefore, the present findings support an association between the protective effects of SCIN and changes in EGFR signaling but do not establish EGFR as a necessary mediator of SCIN-mediated protection. The precise relationship between SCIN and EGFR signaling requires further investigation using an appropriate factorial design and additional mechanistic validation.

This study has several limitations. First, LPS-induced oral keratinocytes were used as an *in vitro* inflammatory injury model. Although this model is widely used to mimic inflammatory injury in oral epithelial cells, it represents a simplified system that does not fully recapitulate the complex immune and inflammatory microenvironment of OLP. Second, downstream EGFR signaling pathways, such as AKT and ERK, which are classical mediators of EGFR function, were not investigated. Future studies should employ more physiologically relevant models, including coculture systems and *in vivo* experiments, to further validate and extend the present findings, and should also explore whether AKT, ERK, and other downstream signaling pathways are involved in the functional relationship between SCIN and EGFR during inflammatory injury. Finally, although our data suggest a potential association between SCIN and EGFR signaling, the absence of matched shNC and double-transfection vector controls, together with incomplete verification of EGFR and SCIN expression across the final treatment groups, precluded assessment of their independent and interactive effects. Therefore, future studies using a complete factorial design with matched controls, verification of transfection efficiency, and appropriately designed rescue experiments are needed to clarify the mechanistic role of EGFR in SCIN-mediated protection.

Conclusion

In conclusion, the present study demonstrated that SCIN overexpression alleviated LPS-induced metabolic impairment and apoptosis in HOKs. These protective effects were accompanied by partial restoration of total EGFR expression and attenuation of LPS-induced EGFR phosphorylation, supporting further investigation of the functional relationship between SCIN and EGFR during inflammatory injury of oral keratinocytes.

Availability of Data and Materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Author Contributions

KQS designed the research study. JL performed the research, collected and analyzed the data. KQS has been involved in drafting the manuscript and both authors have been involved in revising it critically for important intellectual content. Both authors gave final approval of the version to be published. Both authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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