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Recombinant human collagen XVII protects skin basement membrane integrity by inhibiting the MAPK and Wnt signaling pathways

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Abstract. Collagen XVII is a key component linking the cytoskeleton to the basement membrane, serving an essential role in maintaining skin integrity. With the advancement of synthetic biology, recombinant human collagen XVII (RHCXVII) has emerged as a promising novel collagen material. The present study aimed to elucidate the efficacy and mechanisms of action of RHCXVII in protecting skin basement membrane integrity. A skin injury model was established using ultraviolet B (UVB) irradiation on human HaCaT keratinocytes treated with RHCXVII. The effects of RHCXVII on cell migration and adhesion were assessed using wound healing assay and hematoxylin and eosin staining, respectively. The expression of key extracellular matrix (ECM) components such as collagen IV, collagen VII, laminin 332 and integrin α6 (ITGA6) were quantified using reverse transcription-quantitative PCR and western blotting. The mechanism of action of RHCXVII in protecting skin basement membrane integrity was investigated using a phosphorylated-antibody array and verified by western blotting. RHCXVII significantly increased the migration and adhesion of UVB-irradiated HaCaT cells ($P<0.01$). Additionally, RHCXVII significantly upregulated expression levels of collagen type IV α1 chain, collagen type VII α1 chain, laminin subunit β3 and ITGA6 in UVB-irradiated HaCaT cells ($P<0.05$). RHCXVII significantly inhibited the phosphorylation of p38 and c-Jun in the MAPK and Wnt signaling pathways ($P<0.01$). In conclusion, RHCXVII protected skin basement membrane integrity by enhancing migration and adhesion of keratinocytes, upregulating key ECM components and inhibiting protein phosphorylation in MAPK and Wnt pathways. The present study enhanced the current understanding of RHCXVII as a protector of skin basement membrane integrity. Furthermore, the present study highlighted clinical implications and the broad therapeutic potential of RHCXVII in both medical and cosmetic application.

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Abbreviations: RHCXVII, recombinant human collagen XVII; ECM, extracellular matrix; UVB, ultraviolet B; EGF, epidermal growth factor; TGF-β1, transforming growth factor β1; H&E, hematoxylin and eosin; COL4A1, collagen type IV α1 chain; LAMB3, laminin subunit β3; ITGA6, integrin α6

Key words: basement membrane, recombinant human collagen XVII, phospho-antibody array, MAPK, Wnt

Introduction

Human skin, a highly complex organ, serves as the primary barrier against external environmental insults, including physical, chemical and microbiological challenges (1). It is primarily composed of three layers: Epidermis, dermis and subcutaneous tissue (2). The stratum basale is the deepest epidermal layer, sustaining epidermal renewal by continuously generating new cells that migrate towards the stratum corneum, replacing aged keratinocytes (3). The dermis lies beneath the stratum basale, interfacing with the epidermis through a collagenous basement membrane (4). Dermal papillae, projecting from the dermis-like fingers, strengthen this junction, with denser folding of these structures indicating increased adhesion (5). The basement membrane, comprising the lamina lucida, lamina densa and lamina reticularis, along with associated structures such as hemidesmosomes and anchoring fibrils, ensures firm attachment of the epidermis to the dermis (6).

The basement membrane is a dense layer of extracellular matrix (ECM) components, which serves multifaceted roles in skin homeostasis and function (7). It is not only involved in epidermal turnover and wound healing but also maintains structural integrity and regulates the cellular microenvironment (8,9). Additionally, the basement membrane serves as a permeability barrier and is involved in signal transduction (10). However, aging leads to alterations not only in skin appearance but also in the structure of the dermoepidermal junction, particularly affecting the basement membrane, alongside modifications in cellular and molecular components (11). Extrinsic factors such as ultraviolet (UV) irradiation activate enzymes including MMPs, urokinase-type plasminogen

activator/plasmin and heparanase (12). These enzymes degrade collagen, elastin and the epidermal basement membrane, compromising skin integrity and leading to loosening, multilayering and potential rupture (13). Thus, a healthy basement membrane is key for skin integrity, synchronizing growth and repair processes in a positive feedback loop with the epidermis and dermis.

Several bioactive molecules have been identified to support the integrity of the basement membrane. For example, the matricellular glycoprotein, exogenous secreted protein acidic and rich in cysteine, has been reported to promote production of type IV and VII collagen and their accumulation in the skin basement membrane (14). Palmitoyl-Arg-Gly-Asp has the ability to enhance the expression of dermal-epidermal junction components in human keratinocyte (HaCaT) cells (15). Additionally, thioredoxin promotes regeneration and binding of elastic fibers and the basement membrane (16).

Furthermore, repairing basement membrane damage by increasing the synthesis of its components or curbing degradative enzyme activity can alleviate skin problems associated with photoaging and other dermatological conditions, such as wrinkles, hyperpigmentation, and loss of skin elasticity. Collagen XVII (also known as BP180 or BPAG2) is a key transmembrane protein in skin hemidesmosomes. It has an N-terminal globular head inside the hemidesmosomal plaque and a C-terminal collagen-like tail extending into the basal lamina, facilitating connection between the cytoskeleton and basement membrane (17). Collagen XVII is implicated in various dermatological disorders, including linear IgA bullous dermatosis, junctional epidermolysis bullosa, basal cell carcinoma and malignant melanoma (18). A previous study reported the role of collagen XVII in healthy skin, highlighting its involvement in skin aging and wound healing (19). Collagen XVII serves as a key niche for epidermal stem cells and its reduction is associated with changes in cell polarity and aging of the epidermis (20). Sustaining collagen XVII expression has shown promise in mitigating skin aging and may serve as a target for anti-aging treatments (21). Nanba *et al* (22) revealed that collagen XVII orchestrates migration of keratinocyte stem cells by integrating actin and keratin networks, thereby promoting epidermal regeneration. This suggests a key role for collagen XVII in skin wound repair through its influence on migration, proliferation and differentiation of stem cells. Notably, advancements in synthetic biology have facilitated production of recombinant human collagen XVII (RHCXVII), a promising therapeutic protein for skin repair and anti-aging treatments (18,23,24). To the best of our knowledge, however, the specific mechanisms of action and effects of RHCXVII in protecting skin basement membrane integrity have yet to be reported.

In the present study, the protective effect of RHCXVII in maintaining the structural integrity of the basement membrane was evaluated through the assessment of gene and protein expression levels of ECM components. Furthermore, phosphorylated (phospho)-antibody array analysis was used to elucidate the underlying mechanisms of RHCXVII. The present study aims to explore the potential roles of RHCXVII as a protector of the skin basement membrane, with potential future medical and cosmetic applications.

Materials and methods

Reagents. RHCXVII, with an average molecular weight of 23.79 kDa, was purchased from Jiangsu Chuangjian Medical Technology Co., Ltd.). Transforming growth factor β1 (TGF-β1) was purchased from PeproTech Inc. Epidermal growth factor (EGF), DMEM, FBS, penicillin, streptomycin and trypsin were purchased from Gibco (Thermo Fisher Scientific, Inc.). The selective PPAR activator WY14643 (pirinixic acid) and MTT were purchased from Merck KGaA. Collagen type IV α1 chain (COL4A1; cat. no. ab214417), COL7A1 (cat. no. ab309143), laminin subunit β3 (LAMB3; cat. no. ab14509), integrin α6 (ITGA6; cat. no. ab181551), MMP2 (cat. no. ab97779) and vinculin (cat. no. ab129002) antibodies were purchased from Abcam. p38 MAPK (cat. no. 9212S), phospho-p38 MAPK (Thr180/Tyr182; D3F9) XP® rabbit mAb (cat. no. 4511S), c-Jun (60A8) rabbit mAb (cat. no. 9165S) and phospho-c-Jun (Ser243; cat. no. 2994) antibodies were obtained from Cell Signaling Technology, Inc. Goat anti-rabbit (cat. no. YK2231) and anti-mouse IgG HRP (cat. no. YK2232) were purchased from Y&K Bio, Inc.

Cell culture. Human epidermal keratinocyte HaCaT cells were procured from iCell Bioscience, Inc. and authenticated through STR profiling. HaCaT cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin at 37˚C and 5% CO_2. At 70-80% confluence, cells were digested with 0.05% trypsin and seeded onto 24- or 96-well plates for subsequent experiments.

Cell viability assay. HaCaT cells were seeded onto 96-well plates at a density of $1x10^4$ cells per well and incubated overnight at 37˚C and 5% CO_2. When cells reached 40-60% confluence, they were treated with RHCXVII (0.08, 0.16, 0.31, 0.63, 1.25, 2.50, 5.00 and 10.00 mg/g) for 24 h at 37˚C. After discarding the supernatant, 0.5 mg/ml MTT solution was added and cells were incubated at 37˚C for 4 h in the dark. Subsequently, 150 μl DMSO was added to each well for dissolution of the formazan product. The absorbance was measured at 490 nm using an Epoch Microplate Spectrophotometer (BioTek Instruments, Inc.).

Cell migration assay. HaCaT cells were seeded into 6-well plates at a density of $2x10^5$ cells per well and incubated overnight at 37˚C. Cells were divided into four groups: Blank control (BC), negative control (NC), EGF (positive control; PC) and RHCXVII. Cells in the RHCXVII group were cultured with 50 μg/g RHCXVII-supplemented medium, while those in the PC group were treated with 1 ng/ml EGF. Cells in the BC and NC groups were cultured with medium only. All groups were incubated for 24 h at 37˚C. Cells were grown to ~90% confluence and then scratched using a 5 ml pipette tip. Cells were washed three times with PBS and replenished with serum-free DMEM. NC and RHCXVII groups were exposed to UVB irradiation at a dose of 300 mJ/cm^2 for 2 min and 6 sec. Cells were returned to the CO_2 incubator for an additional 24 h. Scratch images were captured at 0 and 24 h using a BX53 light microscope (Olympus Corporation; magnification, x4). Cell migration was calculated as follows: migration rate (%)=[original wound area]-[wound area]/[original wound area x100%.

Table I. Primer sequences for reverse transcription-quantitative PCR.

Gene	Forward primer	Reverse primer
COL4A1	5'-AGGTGTCATTGGGTTTCCTG-3'	5'-GGTCCTCTTGTCCCTTTTGTT-3'
COL7A1	5'-ACTGTGATTGCCCTCTACGC-3'	5'-GGCTGTGGTATTCTGGATGG-3'
LAMB3	5'-GAAGATGTCAGACGCACACG-3'	5'-TAGTGGCTGCATCAGTGTCG-3'
ITGA6	5'-TCCCATAACTGCCTCAGTGG-3'	5'-GTCGTCTCCACATCCCTCTT-3'
β-actin	5'-TGGCACCCAGCACAATGAA-3'	5'-CTAAGTCATAGTCCGCCTAGAA GCA-3'

COL4A1, collagen type IV α1 chain; LAMB3, laminin subunit β3; ITGA6, integrin α6.

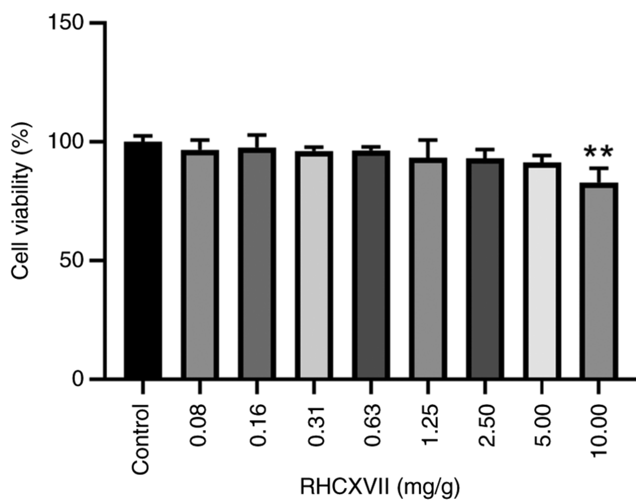

Figure 1. Cytotoxicity of RHCXVII in HaCaT cells. Following 24 h treatment with RHCXVII, cell viability was assessed using an MTT assay. **P<0.01 vs. control. RHCXVII, recombinant human collagen XVII.

Cell adhesion assay. HaCaT cells were seeded into 6-well plates at a density of $2x10^5$ cells per well, incubated overnight at 37˚C and treated as aforementioned. Cells were fixed with 4% paraformaldehyde for 15 min at room temperature, followed by hematoxylin and eosin (H&E) staining at room temperature. Cells were stained with hematoxylin for 10 and eosin for 2 min and finally washed twice with 70% ethanol for 2 min each. Finally, cells were imaged using a BX53 light microscope (magnification, x20) and analyzed using Image-Pro®Plus software (version 6.0; Media Cybernetics, Inc.).

Reverse transcription-quantitative (RT-q)PCR. Cells were divided into four groups: BC, NC, TGF-β1 (PC) and RHCXVII. Cells in the RHCXVII group were cultured 50, 100 or 150 µg/g RHCXVII-supplemented medium, while those in the PC group were treated with 100 ng/ml TGF-β1. Cells in the BC and NC groups were cultured with medium only. All groups were incubated for 24 h at 37˚C. Total RNA was extracted from HaCaT cells at a density of $2x10^5$ cells using RNAiso Plus reagent (Accurate Biology, Inc.), followed by homogenization and lysis by repeated pipetting. cDNA synthesis was performed using the SuperScript VILO cDNA Synthesis kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. RT-qPCR was performed using the Platinum™ SYBR™ Green qPCR SuperMix-UDG (Invitrogen; Thermo Fisher Scientific, Inc.) and CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.). The thermocycling conditions were as follows: Initial denaturation at 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec, 62˚C for 30 sec and 67.5˚C for 5 sec. The $2^{-\Delta\Delta Cq}$ method was used to quantify relative gene expression (25). β-actin was used as an endogenous control. Primer sequences are shown in Table I.

Phospho-antibody array. Total protein was extracted from HaCaT cells ($5x10^6$) by lysing in buffer containing Halt™ Protease and Phosphatase Inhibitor (1:50; Thermo Fisher Scientific, Inc.) with the aid of magnetic beads (Full Moon Biosystems, Inc.), using 5 cycles of vortexing (30 sec) and ice incubation (10 min). After bead removal, samples were centrifuged at 13,200 rpm for 15 min at 4˚C, and the supernatant was collected, stored at -80˚C overnight, and re-centrifuged after thawing. Phospho-Explorer [PEX100; Wayen Biotechnologies (Shanghai) Inc.] was used for phospho-antibody array detection and data analysis. Briefly, protein samples were biotinylated and hybridized to the Phosphorylation ProArray using the Antibody Array kit (Full Moon BioSystems, Inc.; cat #: PEX100). The antibody array consisted of 1,318 antibodies to detect both the phosphorylated and unphosphorylated forms of proteins. Fluorescence intensity was determined using a GenePix 4000B (Axon Instruments) with GenePix Pro (version 6.0) software (Molecular Devices, Inc.). Raw data were processed using Grubb's test in GraphPad Prism (version 8.3.0; Dotmatics) to exclude outliers (26). The phosphorylation rate was calculated as follows: Phosphorylation rate=phosphorylated antibody signal value/unphosphorylated antibody signal value. Proteins that demonstrated phosphorylation change >50% and P<0.05 were included in subsequent analysis. Further analysis of key signaling pathways was conducted using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.kegg.jp/kegg/kegg1.html).

Western blotting. Total protein was extracted from HaCaT cells ($2x10^5$) using RIPA lysis buffer (Thermo Fisher Scientific, Inc.). Protein concentration was quantified using the BCA Protein Assay Kit. The proteins (25 µg/lane) were separated by 8% SDS-PAGE and transferred to a polyvinylidene

Figure 2. RHCXVII increases migration of HaCaT cells. (A) Migration of HaCaT cells. Scale bar, 500 μm. (B) Migration rate of HaCaT cells was quantified. ##P<0.01 vs. BC; **P<0.01 vs. NC. RHCXVII, recombinant human collagen XVII; NC, negative control; EGF, epidermal growth factor; PC, positive control; BC, blank control.

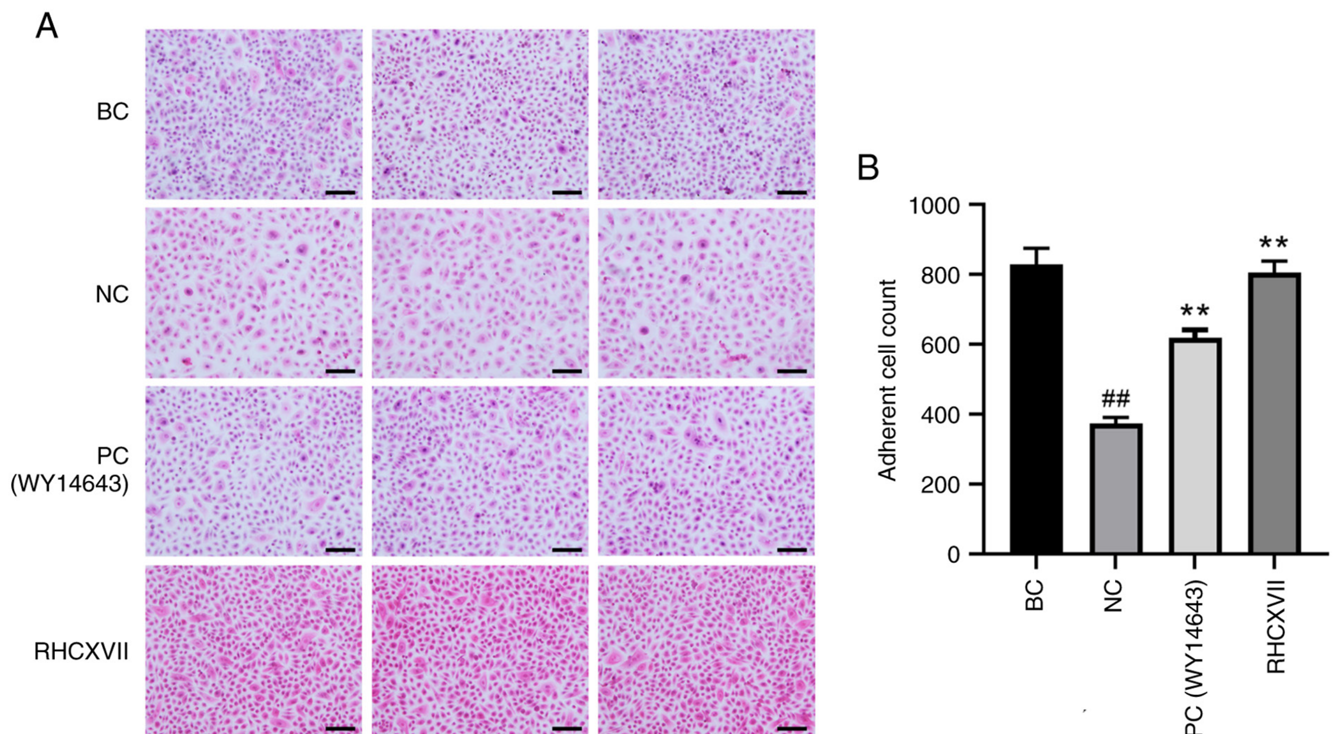

Figure 3. RHCXVII increases adhesion of HaCaT cells. (A) Cell adhesion was observed using hematoxylin and eosin staining. Scale bar, 100 μm. (B) Number of adherent HaCaT cells. ##P<0.01 vs. BC; **P<0.01 vs. NC. RHCXVII, recombinant human collagen XVII; NC, negative control; PC, positive control; BC, blank control.

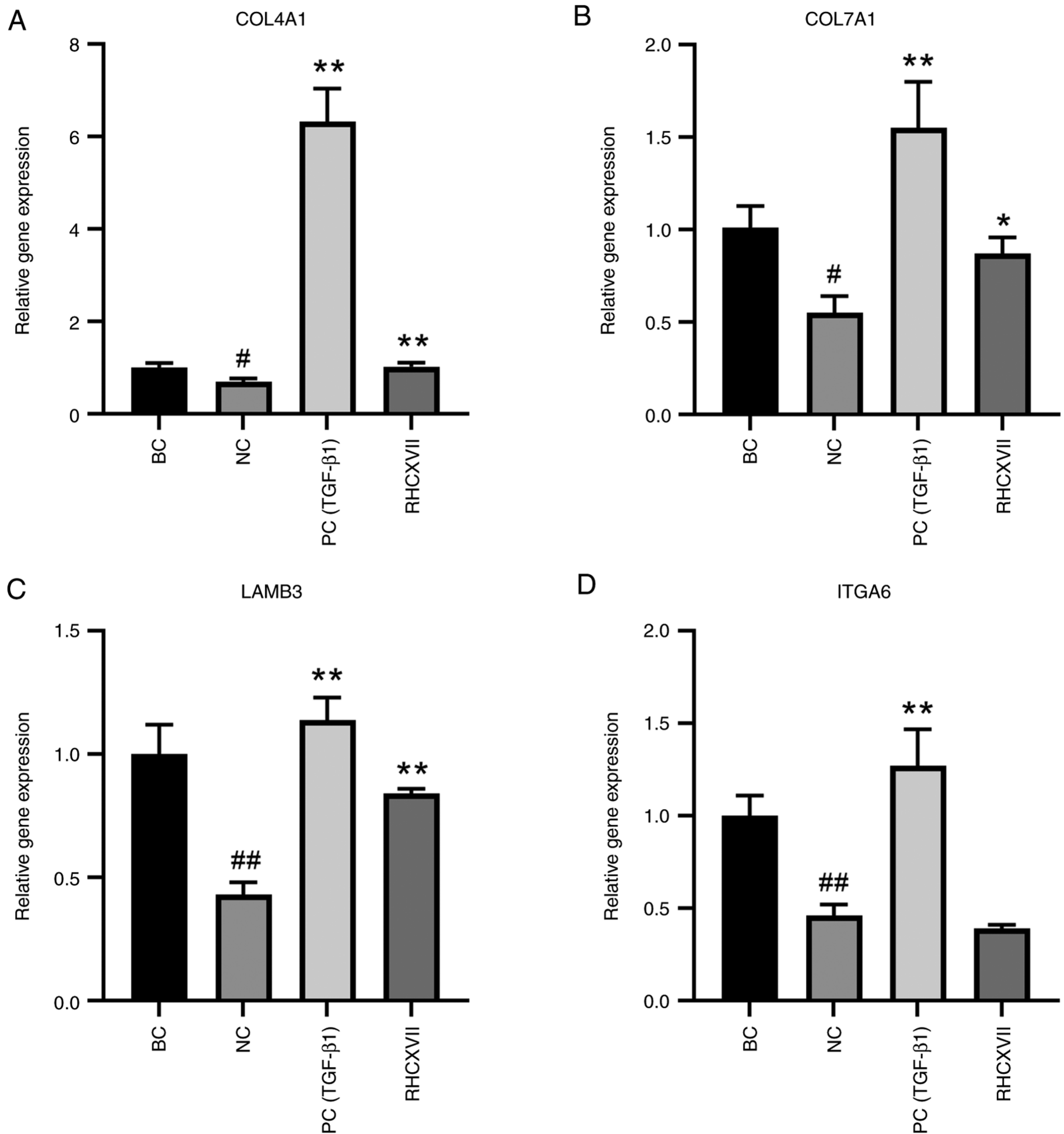

Figure 4. RHCXVII increases mRNA expression of ECM components in HaCaT cells. mRNA expression levels of (A) COL4A1, (B) COL7A1, (C) LAMB3 and (D) ITGA6. #P<0.05, ##P<0.01 vs. BC; *P<0.05, **P<0.01 vs. NC. RHCXVII, recombinant human collagen XVII; ECM, extracellular matrix; COL4A1, collagen type IV α1 chain; LAMB3, laminin subunit β3; ITGA6, integrin α6; NC, negative control; TGF-β1, transforming growth factor β1; PC, positive control; BC, blank control.

fluoride membrane. The membranes were blocked for 2.5 h at room temperature in PBST containing 5% (w/v) skimmed milk to prevent non-specific binding. Membranes were incubated overnight at 4˚C with primary antibodies against COL4A1, COL7A1, LAMB3, ITGA6, p38, phospho-p38 (Tyr182), c-Jun, phospho-c-Jun (Ser243), MMP2 and vinculin (all 1:1,000). Membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (1:10,000) for 1 h at room temperature. Protein bands were visualized using the ECL Detection Reagent (Beyotime) and the Tanon-5200 Multi Gel Imaging Analysis System and analyzed with GIS 1D Analyzing Software (version 4.2; Tanon Science and Technology Co., Ltd.).

Statistical analysis. All cell experiments were performed in triplicate. Statistical analyses were performed using GraphPad Prism (version 8.3.0; Dotmatics) and data are presented as the mean ± SD. Statistical comparisons were performed one-way

ANOVA followed by Tukey's post hoc test for multiple comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

Cytotoxicity of RHCXVII in HaCaT cells. Cytotoxicity and optimal treatment concentration of RHCXVII on HaCaT cells was assessed using MTT assay. RHCXVII did not exhibit significant cytotoxicity to HaCaT cells ≤5 mg/g (Fig. 1). Therefore, for subsequent experiments, RHCXVII was used at concentrations <5 mg/g.

RHCXVII increases migration of HaCaT cells. Following UVB irradiation (NC group), the cell migration rate was significantly decreased compared with the BC group. RHCXVII or EGF (PC group) significantly increased the migration of UVB-irradiated HaCaT cells compared with NC (Fig. 2A and B). These results suggested that RHCXVII enhanced the migration of HaCaT cells following UVB irradiation.

RHCXVII increases adhesion of HaCaT cells. H&E staining demonstrated that the adhesion of HaCaT cells was significantly decreased after UVB irradiation when compared with BC. Treatment with RHCXVII or WY14643 (PC) significantly increased the number of adherent cells compared with NC (Fig. 3A and B). This suggested that RHCXVII enhanced adhesion of HaCaT cells following UVB irradiation.

RHCXVII increases expression of ECM components in HaCaT cells. To determine the most effective concentration of RHCXVII for regulating basement membrane integrity, UV-irradiated HaCaT cells were treated with RHCXVII (50, 100 and 150 μg/g) or TGF-β1 (100 ng/ml); 50 μg/g RHCXVII was more effective in upregulating the expression of collagen IV and VII compared with the higher concentrations (Fig. S1). Therefore, 50 μg/g RHCXVII was selected for subsequent experiments. UVB irradiation caused a significant decrease in mRNA expression of COL4A1, COL7A1, LAMB3 and ITGA6 in HaCaT cells compared with BC (Fig. 4A-D). RHCXVII or TGF-β1 (PC group) significantly increased mRNA expression levels of COL4A1, COL7A1 and LAMB3 and protein expression levels of COL4A1 and ITGA6 in UVB-irradiated HaCaT cells when compared with NC (Figs. 4A-D and 5A-E). Therefore, RHCXVII may increase expression of ECM components in UVB-irradiated HaCaT cells.

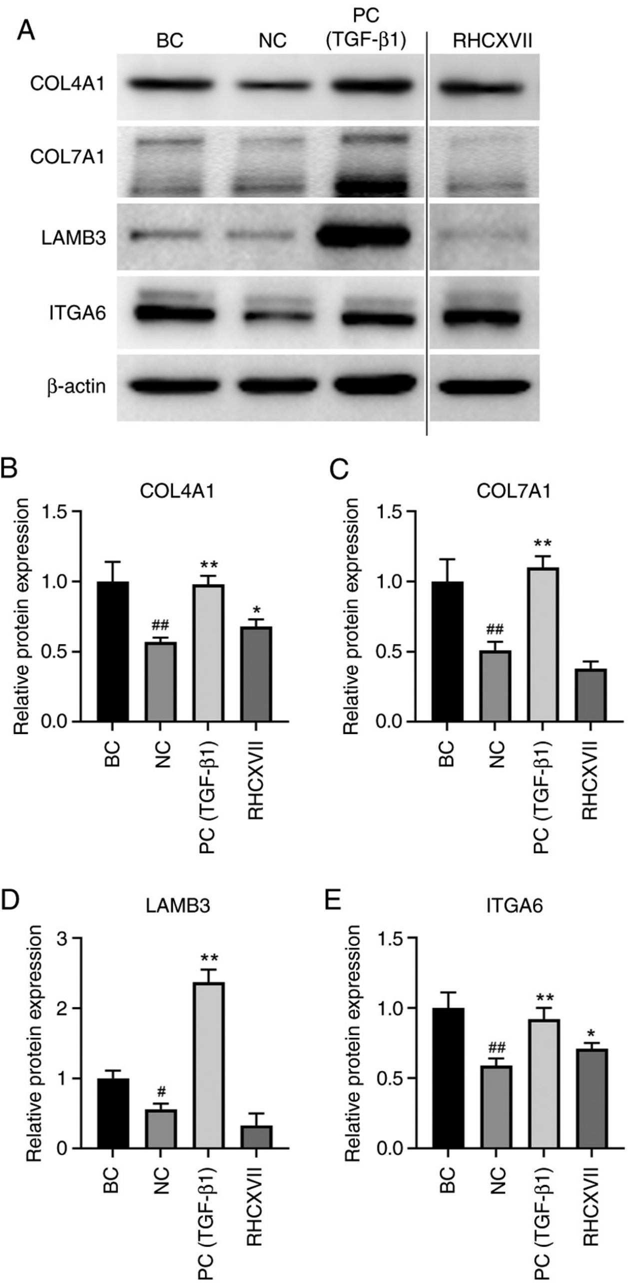

Figure 5. RHCXVII increases protein expression levels of ECM components in HaCaT cells. (A) Representative western blotting. Protein expression levels of (B) COL4A1, (C) COL7A1, (D) LAMB3 and (E) ITGA6. #P<0.05, ##P<0.01 vs. BC; *P<0.05, **P<0.01 vs. NC. RHCXVII, recombinant human collagen XVII; ECM, extracellular matrix; COL4A1, collagen type IV α1 chain; LAMB3, laminin subunit β3; ITGA6, integrin α6; NC, negative control; TGF-β1, transforming growth factor β1; PC, positive control; BC, blank control.

RHCXVII regulates MAPK and Wnt signaling pathways. To investigate the mechanism of RHCXVII in protecting basement membrane integrity, a phospho-antibody array was conducted on HaCaT cells treated with RHCXVII. Compared with NC group, RHCXVII treatment led to a >50% increase in phosphorylation levels for 66 proteins and a >50% decrease for 207 proteins (Fig. 6A). KEGG pathway analysis demonstrated that 79 and 20 differentially phosphorylated proteins were enriched in the MAPK and Wnt signaling pathways, respectively (Fig. 6B-D). These pathways serve key roles in cell migration, adhesion and basement membrane formation (27,28). Therefore, RHCXVII may protect basement membrane integrity by modulating phosphorylation of proteins in the MAPK and Wnt signaling pathways.

RHCXVII inhibits phosphorylation of proteins in the MAPK and Wnt signaling pathways in HaCaT cells. To verify the mechanisms of RHCXVII in regulating phosphorylation of proteins in the MAPK and Wnt signaling pathways, HaCaT cells were treated with UVB irradiation and RHCXVII. The expression of MAPK and Wnt pathway-related proteins [p38,

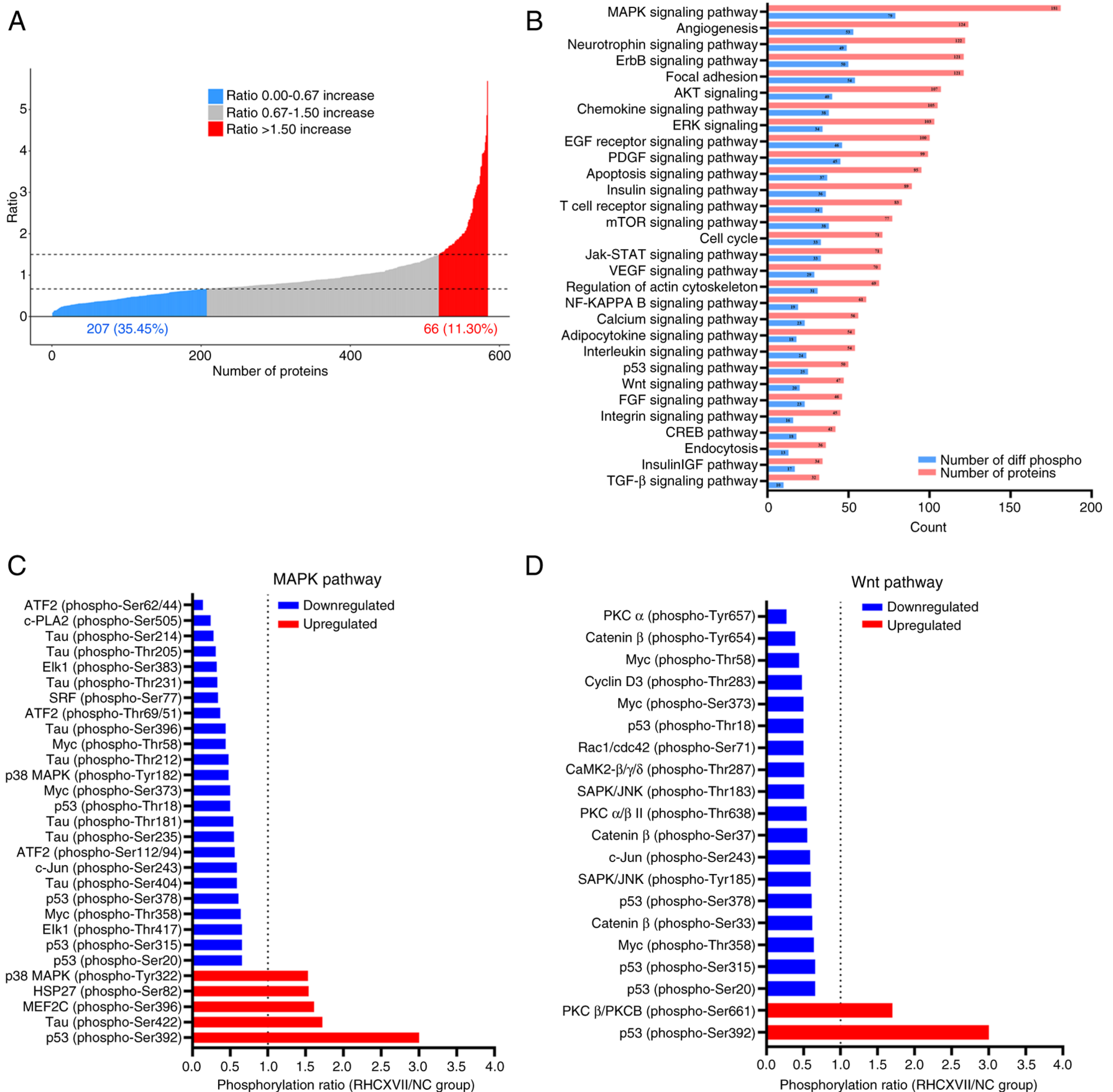

Figure 6. Regulatory effect of RHCXVII on protein phosphorylation. (A) Phospho-antibody array analysis was performed to assess changes in phosphoprotein expression in HaCaT cells with and without RHCXVII treatment. (B) Kyoto Encyclopedia of Genes and Genomes pathway analysis of differentially phosphorylated proteins in RHCXVII-treated HaCaT cells compared with NC. Changes in expression levels of upregulated and downregulated phosphoproteins in (C) MAPK and (D) Wnt pathways in RHCXVII-treated HaCaT cells compared with NC group. RHCXVII, recombinant human collagen XVII; NC, negative control; diff phospho, differentially phosphorylated.

p38 (phospho-Tyr182), c-Jun and c-Jun (phospho-Ser243)] were examined. UVB irradiation significantly increased the expression levels of p38 (phospho-Tyr182)/p38 and c-Jun (phospho-Ser243)/c-Jun in HaCaT cells, whereas RHCXVII or TGF-β1 (PC) treatment significantly reduced their expression levels (Fig. 7A-C). MMP2 is a 72-kDa type IV collagenase, which can be regulated by MAPK and Wnt pathways (29-31). UVB irradiation increased the protein expression of MMP2 in HaCaT cells compared with BC (Fig. 7A and D). RHCXVII or TGF-β1 (PC group) significantly decreased UVB-induced upregulation of MMP2 protein expression in HaCaT cells when compared with NC. These results indicated that RHCXVII may inhibit phosphorylation of proteins in the MAPK and Wnt signaling pathways in keratinocytes, thereby protecting basement membrane integrity.

Discussion

Damage to the basement membrane structure affects signal communication and material exchange between the epidermis and dermis. This can lead to skin dryness, decreased wound healing, impairment of the epidermal barrier function and

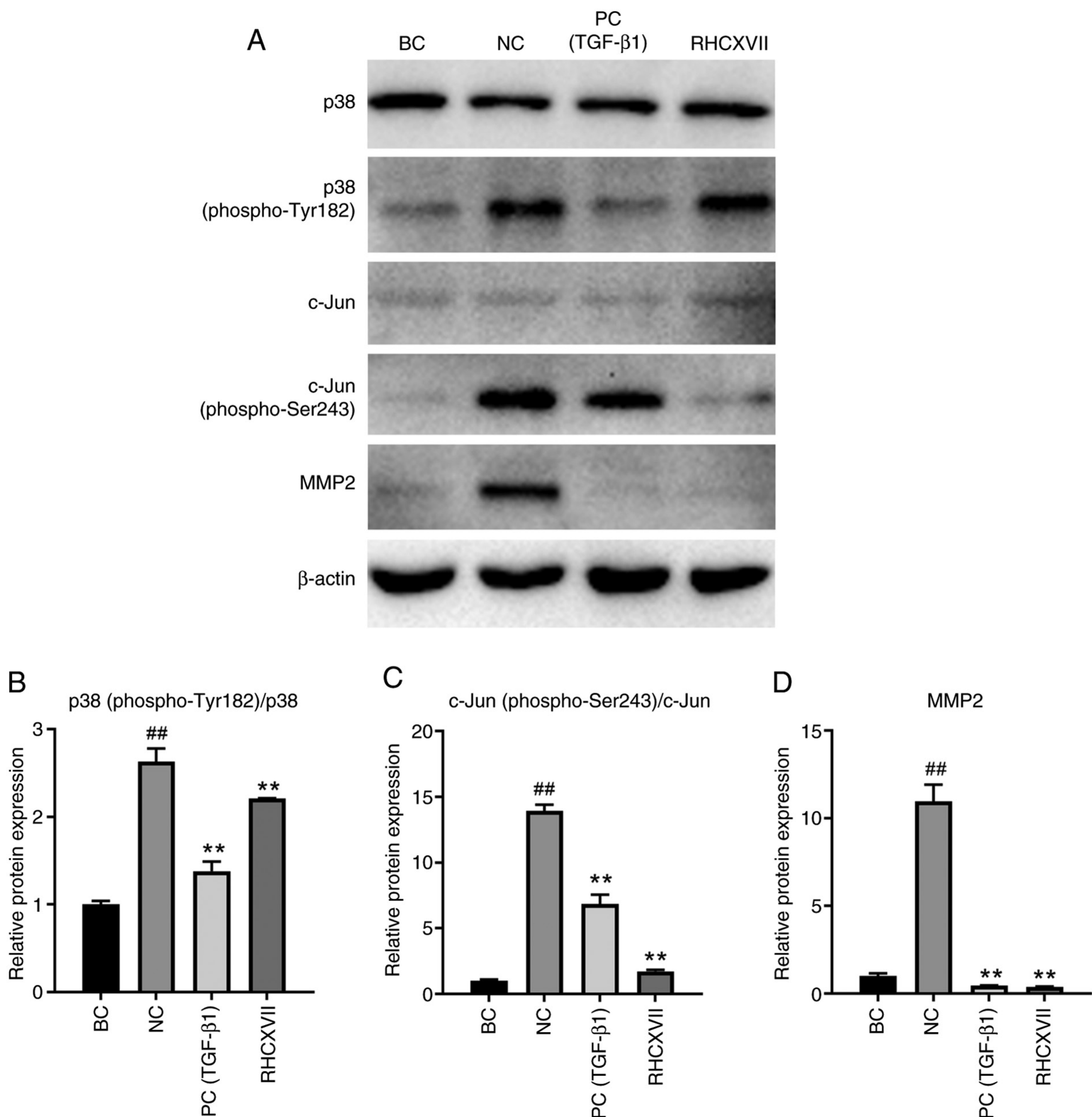

Figure 7. RHCXVII inhibits phosphorylation of proteins in the MAPK and Wnt signaling pathways in HaCaT cells. (A) Representative western blotting. The protein expression levels of (B) p38 (phospho-Tyr182)/p38, (C) c-Jun (phospho-Ser243)/c-Jun and (D) MMP2. ##P<0.01 vs. BC; **P<0.01 vs. NC. RHCXVII, recombinant human collagen XVII; NC, negative control; TGF-β1, transforming growth factor β1; PC, positive control; BC, blank control; phospho, phosphorylated.

pathological skin changes (32-34). Enhancing basement membrane components is a promising strategy to improve epidermal-dermal communication, maintain skin homeostasis and strengthen skin defenses. Collagen XVII, a key basement membrane protein, is essential for maintaining cell-matrix adhesion, facilitating signal transduction and promoting keratinocyte differentiation (32). The present study demonstrated that RHCXVII may enhance the migration and adhesion of keratinocytes and increase expression of ECM components, thereby protecting basement membrane integrity.

Integrins within the epidermal layer of the skin serve as pivotal receptors for basement membrane adhesion, exerting regulatory control over cell adhesion, migration, proliferation and differentiation (35). The present study demonstrated that RHCXVII increased keratinocyte migration and adhesion by increasing ITGA6 protein expression levels, thereby strengthening interactions with the ECM. However, RHCXVII did not significantly influence the mRNA expression of ITGA6, suggesting that it may enhance the post-transcriptional translation efficiency of ITGA6 mRNA. ECM proteins that form the basement membrane primarily include collagen IV, laminins, nidogens and perlecan (36). Collagen IV is key to the lamina densa of the basement membrane and is primarily secreted by keratinocytes in early developmental stages. The aggregation of collagen IV stimulates proliferation of basal keratinocytes and facilitates establishment of the epidermal layer (7). Collagen IV can promote cell adhesion, migration and invasion, particularly in skin tumor cells such as melanoma (37). Increase in collagen

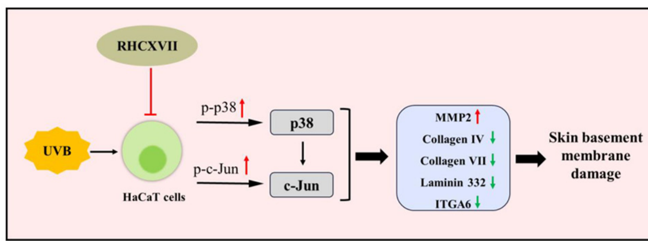

Figure 8. Key signaling pathways affected by RHCXVII. RHCXVII protects the skin basement membrane integrity by increasing expression of key extracellular matrix components such as collagen IV, collagen VII, laminin 332 and ITGA6 and decreasing MMP2 expression. RHCXVII decreases the phosphorylation of p38 and c-Jun in the MAPK and Wnt pathways. RHCXVII, recombinant human collagen XVII; ITGA6, integrin α6; p-, phosphorylated.

IV expression in the ECM may provide a more favorable environment for cell adhesion. The present study demonstrated that RHCXVII led to an upregulation of COL4A1 expression in keratinocytes exposed to UVB irradiation.

The reticular structure formed by collagen IV and laminins is key for the high stability of the basement membrane (36). Laminins are a family of proteins comprising three linked chains, α, β and γ (38). Notably, laminin 332, with its α3β3γ2 chain structure, features a distinctive laminin N-terminal domain at the end of the β3 chain (36). This facilitates interaction with integrin α6β4 receptors expressed by basal keratinocytes. Integrin α6β4 possesses a long β-subunit tail that enables binding to hemidesmosomal lectins linked to keratin filaments. Laminin 332 forms bonds with anchoring fibrils of collagen VII within the basement membrane zone (36). Hence, laminin 332 serves as a key link between cellular hemidesmosomes and anchoring fibrils, ensuring stability and functional unity of the basement membrane. RHCXVII increased the mRNA expression of LAMB3 and COL7A1 in UVB-irradiated keratinocytes. However, RHCXVII did not affect protein levels of LAMB3 and COL7A1. This suggests that the increased mRNA expression may not be efficiently translated into proteins, or that other mechanisms may inhibit the post-transcriptional translation of LAMB3 and COL7A1 mRNA. Further investigation is needed to uncover these underlying mechanisms.

In addition to collagen VII, collagen XVII is also a specific interaction partner for laminin 332. Collagen XVII domains at the hemidesmosomes interact with the intracellular segment of the integrin β4 subunit, forming a key component of the complex. This hemidesmosome complex, along with plectin and bullous pemphigoid antigen 1, forms a stable anchorage point for keratin intermediate filaments, ensuring successful structural linkage between the cell and ECM. Collagen XVII is proposed to serve a key role in accurate positioning of laminin 332 within the basement membrane (17). Its regulatory function is key for maintaining tissue integrity and functionality, particularly when laminin-integrin binding is attenuated (36). In the present study, RHCXVII significantly increased expression levels of COL4A1, COL7A1, LAMB3 and ITGA6 in UVB-irradiated keratinocytes. This suggests a key role for RHCXVII in protecting basement membrane integrity.

Phospho-antibody array demonstrated that RHCXVII significantly modulated phosphorylation levels of key proteins regulating the formation of basement membrane, particularly those affecting the MAPK and Wnt pathways. The MAPK family comprises c-Jun N-terminal kinases, ERK and p38 MAPKs (39). Although the complete role of the MAPK pathway in basement membrane dynamics is not clear, its potential in controlling levels of collagen I, IV and VII in this structure have been reported (14). c-Jun serves as a downstream effector of numerous key signaling cascades, including MAPK and Wnt/β-catenin signaling, serving roles in cell proliferation and differentiation (40). The present study demonstrated that UVB-induced phosphorylation of p38 and c-Jun in keratinocytes was significantly downregulated following treatment with RHCXVII, indicating a potential inhibitory effect of RHCXVII on MAPK and Wnt pathways. Moreover, the transcription factor AP-1, formed by the c-Jun and c-Fos dimer, triggers MMP upregulation, causing collagen degradation and diminished synthesis (41,42). MMP2, expressed in the dermal basement membrane zone, exerts proteolytic activity by cleaving collagen IV and VII, thereby influencing the structural integrity of ECM (43). The present study showed that RHCXVII decreased the protein expression levels of MMP2 in UVB-irradiated keratinocytes. Therefore, it could be hypothesized that RHCXVII suppresses UVB-induced MMP2 expression, potentially by inhibiting the MAPK and Wnt pathways, thus protecting collagen from degradation.

Previous studies have reported that collagen XVII regulates various signaling pathways, including integrin α6β4/PI3K/AKT/mTOR, Ras-related C3 botulinum toxin substrate 1 (RAC1), Notch, TGFβ/Smad and ERK pathways (18,44-46). Consistent with these findings, the present phospho-antibody array showed that RHCXVII was involved in regulation of AKT, mTOR, ERK and TGFβ signaling pathways. However, the present study did not show regulation of RAC1 and Notch signaling, which may be due to off-target effects. Future investigations should validate these signaling pathways regulated by RHCXVII.

In summary, the present study demonstrated that RHCXVII protects skin basement membrane integrity by improving keratinocyte migration and adhesion and increasing expression of key ECM components. RHCXVII may exert its effects by inhibiting protein phosphorylation of p38 and c-Jun within the MAPK and Wnt signaling pathways (Fig. 8). These findings suggest RHCXVII holds promise as a future potent therapeutic agent for stabilizing and protecting skin basement membrane integrity, as well as a potential candidate for the formulation of skincare products designed to combat signs of aging. Further studies should use UVB-induced skin damage in nude mice as an *in vivo* model to investigate the protective effects and mechanisms of RHCXVII on basement membrane integrity. Clinical trials of RHCXVII should evaluate its therapeutic potential for UVB-induced skin damage. Furthermore, co-application of RHCXVII with other bioactive substances may amplify the reparative effects on the basement membrane, offering novel strategies for development of potent skincare formulations.

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Availability of data and materials

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

Authors' contributions

JW and ZY conceived and designed the present study. JW, SL and YW contributed to the acquisition, analysis and interpretation of data. YW drafted manuscript and revised it critically for important intellectual content. JW and SL confirm the authenticity of all the raw data. ZY agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors have 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.

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