

CTNNBIP1 modulates keratinocyte differentiation through promoting the transcription of β -catenin/TCF-complex downstream genes

Chang Wang

the Second Affiliated Hospital of Hunan University of Chinese Medicine

Haizhen Wang

the Second Affiliated Hospital of Hunan University of Chinese Medicine

Youhua Peng

the Second Affiliated Hospital of Hunan University of Chinese Medicine

Bijun Zeng

the Second Affiliated Hospital of Hunan University of Chinese Medicine

Yujin Zhang

the Second Affiliated Hospital of Hunan University of Chinese Medicine

Xueyong Tang

the Second Affiliated Hospital of Hunan University of Chinese Medicine

Lan Mi

the Second Affiliated Hospital of Hunan University of Chinese Medicine

Yi Pan

the Second Affiliated Hospital of Hunan University of Chinese Medicine

Zhibo Yang (✉ dr_yangzhibo@163.com)

the Second Affiliated Hospital of Hunan University of Chinese Medicine

Research

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Abstract

Background: During psoriasis initiation and development, deregulations in signaling pathways and gene expression are observed.

Methods: Herein, we downloaded seven sets of microarray mRNA expression profiles showing differentially-expressed genes in psoriasis lesion skin and non-lesion skin tissues and three sets of RNA-seq data and analyzed these online data attempting to screen for crucial genes related to keratinocyte differentiation and psoriasis development.

Results: The expression of catenin beta interacting protein 1 (CTNNBIP1) was remarkably downregulated within psoriasis lesion skin tissue samples compared to that within non-lesion skin tissues based on both online data and experimental results. In response to a period of different therapies, respectively, CTNNBIP1 expression could be rescued in lesion skin tissues. Within IMQ-induced psoriasis-like dermatitis in mice, CTNNBIP1 silence further aggravated psoriatic phenotypes. In human immortalized keratinocytes, HaCaT cells, CTNNBIP1 silence significantly inhibited cell apoptosis and promoted cell proliferation. Regarding the molecular mechanism, CTNNBIP1 silence in HaCaT cells promoted β -catenin nucleus translocation, enhanced the transcriptional activity of TCF4, and increased β -catenin/TCF-complex downstream c-Myc and cyclin D1 proteins while decreased the late differentiation marker filaggrin. In contrast to CTNNBIP1, the expression of c-Myc and cyclin D1 showed to be dramatically upregulated within psoriasis lesion tissue samples than that within non-lesion tissue samples. Within tissues, c-Myc and cyclin D1 showed to be negatively correlated with CTNNBIP1, respectively.

Conclusions: We identify CTNNBIP1 as an abnormally downregulated gene in psoriasis. CTNNBIP1 silence significantly disturbs the differentiation of keratinocytes through promoting the transcription of β -catenin/TCF-complex downstream genes.

Background

Approximately 2 to 3 percent of the total population have psoriasis, a commonly seen chronic skin condition [1], which is often characterized by raised, well-defined erythemas with adherent silvery scales [2]. These scales are formed by abnormal keratinocyte differentiation and excess proliferation [3, 4]. During psoriasis initiation and development, deregulations in signaling pathways and gene expression are observed [5–7], and potent therapeutic targets might be determined through analyzing these deregulations.

The Wnt signaling exerts a critical effect on cell proliferation, cell fate determination, cell differentiation and many other biological activities in the process of adult homeostasis [8]. Canonical Wnt signaling can activate β -catenin nucleus translocation, which is usually associated with the determination of cell fate. β -Catenin not only interacts with the TCF/LEF family of transcription factors, but also activates the transcription of Wnt target genes, including c-Myc [9] and cyclin D1 [10, 11], and is integral in development and cell proliferation and differentiation [12]. As previously reported, the expression of

membrane β -catenin showed to be dramatically downregulated within control cases compared to that in active psoriatic lesions, while the expression of nucleus β -catenin showed to be remarkably upregulated within psoriatic lesions compared to that in control group [13]. Upon IL-36 γ exposure, β -catenin was upregulated, keratinocyte differentiation was inhibited, and pro-inflammation cytokines secretion was increased [14]. In summary, β -catenin can exert a critical effect on the differentiation of keratinocytes.

Herein, we downloaded seven sets of microarray mRNA expression profiles showing differentially-expressed genes in psoriasis lesion skin and non-lesion skin tissues (GSE13355, GSE14905, GSE30999, GSE34248, GSE41662, GSE50790, and GSE6710) and three sets of RNA-seq data (E-GEOD-54456, GSE114286, and GSE121212) and analyzed these online data attempting to screen for crucial genes related to keratinocyte differentiation and psoriasis development. Among the top ten downregulated genes in psoriasis lesion skin tissues, catenin beta interacting protein 1 (CTNNBIP1) attracted our attention because of its inhibitory role in Wnt signaling. CTNNBIP1 targets two different armadillo regions of β -catenin via its N-terminal and C-terminal domains, disrupting the interaction between β -catenin and TCF [12, 15, 16], therefore serving as a negative regulatory factor of Wnt signaling pathway. Based on the previous findings and online data described above, we speculate that CTNNBIP1 might exert an important effect on keratinocyte differentiation, therefore affecting psoriasis development, possibly through regulating β -catenin-TCF interaction.

To investigate this speculation, we collected clinical psoriasis lesion skin and non-lesion skin tissue samples and validated CTNNBIP1 expression and the correlation between CTNNBIP1 expression and PASI scores in tissue samples. The changes in CTNNBIP1 expression in response to different therapies were also monitored. Next, the in vivo effects of CTNNBIP1 upon psoriatic phenotype in IMQ (imiquimod)-induced psoriasis-like dermatitis in mice and in vitro effects on HaCaT cell proliferation and differentiation were examined. Furthermore, β -catenin protein level and distribution, TCF4 transcriptional activity, and the protein levels of the β -catenin/TCF-complex downstream genes, such as c-Myc [9] and cyclin D1 [10, 11], were detected in response to CTNNBIP1 silence. Finally, c-Myc and cyclin D1 expression within tissue samples and their correlation with CTNNBIP1, respectively, were examined. In summary, we identify CTNNBIP1, a well-known negative regulatory factor of Wnt/ β -catenin signaling might be a promising therapeutic option in psoriasis.

Materials And Methods

Clinical psoriasis lesion and non-lesion tissue samples

Thirty-two cases of psoriasis lesion tissues and same amount of non-lesion tissues were collected from patients with psoriasis underwent treatment in the Second Affiliated Hospital of Hunan University of Chinese Medicine with the informed consents signed. This study was approved by the Research Ethics Committee of the Second Affiliated Hospital of Hunan University of Chinese Medicine. Tissue samples were stored at -80°C immediately after sampling until further experiments. The psoriasis severity was monitored and Psoriasis Area Severity Index (PASI) was graded [17].

Histopathological analyses by hematoxylin and eosin (H&E) staining

Tissue samples were fixed in 10% formalin solution and embedded in paraffin and processed following the standard procedure [18]. Samples were then cut into 5 µm-thick slice and stained with H & E for histopathological analyses.

Immunohistochemistry (IHC) staining

Sample slices were deparaffinized, rehydrated and incubated with proteinase K for 15 min. Non-specific bindings were blocked using 3% BSA blocking solution. After that, the slices were incubated with anti-CTNNBIP1, anti-c-Myc, anti-cyclin D1, or anti-β-catenin primary antibody overnight at 4 °C. The following steps were generated using the PolyExcel HRP/DAB Detection System kit (PathnSitu Biotechnologies, Livermore, CA, USA) according to the manufacturer instructions. Signal visualization was generated using the DAB (3,3'-diaminobenzidine tetrachloride) and the sections were counterstained with hematoxylin for IHC analysis.

Immunofluorescence (IF) staining

For IF analysis, sample slices were processed as described above and incubated with anti-β-catenin primary antibody at 4 °C overnight. Slices were then washed and incubated with Cy5-anti-rabbit antibody for 1 h at room temperature, washed, mounted, and were visualized using a confocal microscope.

Immunoblotting

The protein levels of CTNNBIP1, c-Myc, cyclin D1, filaggrin, and β-catenin were examined by immunoblotting following the methods described before [19] with primary antibodies against CTNNBIP1, c-Myc, cyclin D1, filaggrin, and β-catenin, and the HRP-conjugated secondary antibody. Signals were visualized using enhanced chemiluminescent (ECL) substrates (Millipore, MA, USA) normalizing to GAPDH.

RNA extraction and real-time PCR analysis

Total RNA was extracted from cultured cells using Trizol reagent (Invitrogen). The expression of mRNA was measured using a SYBR Green qPCR assay (Takara, Dalian, China) following the methods described before [19]. The expression of GAPDH served as an endogenous control. The $2^{-\Delta\Delta CT}$ method was applied for data processing.

Imiquimod (IMQ)-induced psoriasis-like dermatitis model in mice

IMQ-induced psoriasis-like dermatitis model was generated in C57 mice following the method described by Van Der Fits et al. [20]. The mice were randomly assigned to four groups: control group injected with Lv-sh-NC (negative control for Lv-sh-CTNNBIP1), control group injected with Lv-sh-CTNNBIP1, IMQ group injected with Lv-sh-NC, and IMQ group injected with Lv-sh-CTNNBIP1. Mice in IMQ groups were applied with IMQ cream on to the shaven back dorsal region of the skin at a dose of 62.5 mg/day per 5 cm²

surface areas for 6 days. For lentivirus injection, 20 μ l of 10^7 TU lentivirus were injected intradermally into the shaved back skin of each mouse every two days for 6 days. The psoriasis severity was monitored and Psoriasis Area Severity Index (PASI) was graded [17]. After seven days mice were sacrificed and skin samples were collected and stored at -80°C until further experiments.

Cell line and cell transfection

Human immortalized keratinocytes, HaCaT cells, were obtained from ProCell (CL-0090, Wuhan, China) and cultured in MEM medium (PM150410, ProCell) supplemented with 15% FBS (164210-500, ProCell) and 1% P/S (PB180120, ProCell). Cells were cultured at 37°C in 5% CO_2 .

CTNNBIP1 silence was generated by the transfection of si-CTNNBIP1 (Genepharma, Shanghai, China). The transfection was performed using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA).

Cell viability determination by MTT assay

A modified MTT assay was used to evaluate cell viability following the methods described before [19]. DMSO was added after the supernatant discarded to dissolve the formazan. OD values were measured at 490 nm. The viability of the non-treated cells (control) was defined as 100%, and the viability of cells from all other groups was calculated separately from that of the control group.

DNA synthesis determined by EdU assays

DNA synthesis is based on the method of incorporating thymidine analogue 5-ethoxy 2-deoxyuridine (EdU) into genomic DNA by using the Click-IT EdU Alexa Fluor 488 kit following the methods described before [21, 22]. Apollo staining and DAPI staining were performed and EdU positive cells were observed with a fluorescence microscope. The incorporation rate of EdU was calculated as the ratio of EdU-positive cells (green cells) to total DAPI-positive cells (blue cells).

Cell apoptosis examined by the Flow cytometer assay

The cell apoptosis was determined using an Annexin V-FITC apoptosis detection kit (Keygen, China) following the methods described previously [23]. Propidium iodide (PI) was used for nuclei staining. The excitation wavelength (Ex) was 488 nm and the emission wavelength (Em) was 530 nm.

Luciferase reporter plasmids and luciferase assays

Cloned TCF4 promoter fragments were inserted into the psiCHECK2 vector (Promega) according to manufacturer's protocol. TCF4 luciferase reporter vector was co-transfected in 293T cells with si-NC (negative control) or si-CTNNBIP1 and the Luciferase reporter assays were performed using the Dual-Glo Luciferase Reporter Assay System (Promega).

Statistical Analysis

Data of results from at least three independent experiments are processed using SPSS17.0 (IBM, Armonk, NY, USA) and presented as the mean \pm S.D. A Student *t*-test was used for statistical comparison between means where applicable. Differences among more than two groups in the above assays were estimated using one-way ANOVA. **P* < 0.05; ***P* < 0.01.

Results

Screening for critical genes related to psoriasis pathogenesis

To screen for differentially-expressed genes between psoriasis lesion and non-lesion tissues that might exert a crucial effect on the pathogenesis of psoriasis, we downloaded and analyzed various online microarray expression profiles and RNA-seq data (Fig.1A). First, we collected 7 sets of mRNA expression microarray chip data reporting the differentially expressed genes between psoriasis lesion skins and normal control tissue (non-involved/non-lesion skin) (GSE13355, GSE14905, GSE30999, GSE34248, GSE41662, GSE50790, GSE6710) from the gene expression omnibus database of the National Center for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov/geo/>) and 3 sets of RNA-seq sequencing data (E-GEOD-54456, GSE114286, GSE121212) from the European Bioinformatics Institute's Expression Atlas database (<https://ebi.ac.uk/gxa/home>). The details of the data involved are shown in Table S1. Then, we use the limma or DE2Seq package in the R program language to normalize chip data and perform differential expression analysis. The RANK value (ie, the FDR value or adjusted p-value) was calculated using R package, and 320 genes with FDR values less than 0.01 and average $|\log_2FC| > 0.56$ were obtained, including 261 genes that were up-regulated and 59 genes that were down-regulated. Heatmap based on seven sets of online microarray expression profiles (GSE13355, GSE14905, GSE30999, GSE34248, GSE41662, GSE50790, and GSE6710) and three sets of RNA-seq data (E-GEOD-54456, GSE114286, and GSE121212) showed the top twenty differentially-expressed genes (ten upregulated and ten downregulated) in psoriasis lesion skin tissues compared to that in non-lesion skin tissues (Fig.1B). To further identify critical genes related to psoriasis severity, we analyzed the correlation between the expression of top ten downregulated genes and the PASI scores in each sample included in GSE85034 (total sample size = 179) and the expression of four genes, namely BTC, BCAR3, CHP2, and CTNNBIP1, was significantly negatively correlated with PASI score in each sample (data not shown). As is well-known, CTNNBIP1 targets two different β -catenin armadillo regions via its N-terminal and C-terminal domains, disrupting the interaction between β -catenin and TCF [12, 15, 16], therefore exerting a negative regulatory effect on Wnt signaling. Based on the essential role of Wnt/ β -catenin signaling pathway within psoriasis pathogenesis [24, 25], we selected CTNNBIP1 for further experiments. According to GSE85034, CTNNBIP1 expression was significantly negatively correlated the PASI score in each sample (Fig.1C).

According to NCBI online database, the sequencing results of 27 tissues from 95 persons demonstrated that CTNNBIP1 expression within skin tissue samples showed to be remarkably increased compared to that within other tissue samples (Fig.S1). Online data we selected, including GSE85034 (Fig.1D), GSE63741 (Fig.1E), GSE30768 (Fig.1F), GSE30999 (Fig.S2A), GSE6710 (Fig.S2B), GSE121212 (Fig.S2C), E-GEOD-54456 (Fig.S2D), GSE13355 (Fig.S2E), all reported that the expression of CTNNBIP1 showed to

be obviously downregulated within psoriasis lesion tissue samples, in comparison with that in non-lesion tissue samples.

CTNNBIP1 expression and protein levels within psoriasis lesion and non-lesion tissue samples

Before investigating the specific effect of CTNNBIP1 on psoriasis, we first evaluated the expression of CTNNBIP1 within tissue samples. H&E staining showed that, compared to the non-lesion skin tissues, there were obvious erythema, plaque, infiltration, and scales in psoriasis lesions (Fig.2A). In the meantime, IHC staining showed that CTNNBIP1 protein was decreased in psoriasis lesion tissue samples than that within non-lesion tissue samples (Fig.2A). Consistently, Immunoblotting revealed that CTNNBIP1 protein levels were significantly reduced in psoriasis lesion than those within non-lesion tissues (Fig.2B), and real-time PCR revealed that the mRNA expression of CTNNBIP1 showed to be remarkably downregulated within psoriasis lesion tissue samples than that in non-lesion tissue samples (Fig.2C). Also similar as online data, Pearson's correlation analysis demonstrated that the expression of CTNNBIP1 had a negative correlation with PASI scores in psoriasis lesion samples (Fig.2D). These data suggest that CTNNBIP1 expression and protein levels are decreased in psoriasis lesion tissues and might play a role in psoriasis pathogenesis.

Changes in CTNNBIP1 expression in response to therapies

To further confirm that CTNNBIP1 is involved in psoriasis pathogenesis, we monitored CTNNBIP1 expression within non-lesion skin and lesion skin tissue samples in response to different therapies. According to GSE85034, the expression of CTNNBIP1 showed to be markedly downregulated within lesion tissue samples than that in non-lesion tissue samples, while continually increased from the 2nd to the 16th week after adalimumab/methotrexate treatment (Fig.3A). Similarly, dynamic observation on five cases of psoriasis patients underwent methotrexate treatment found that CTNNBIP1 expression was downregulated in psoriasis lesion tissues while rescued 20 days after treatment (Fig.3B). According to GSE30768, CTNNBIP1 expression was significantly downregulated in four cases of lesion skin tissues compared to that in non-lesion tissues, while upregulated in non-relapse lesion area upon efalizumab treatment and failed to be rescued by efalizumab treatment in relapse area (Fig.3C). These data suggest that CTNNBIP1 upregulation might be related to psoriasis improvement.

Effects of knocking down CTNNBIP1 upon IMQ-induced psoriasis-like dermatitis in mice

Next, we investigated the specific effects of CTNNBIP1 on psoriasis pathogenesis by establishing IMQ-induced psoriasis-like dermatitis model in mice. Based on the Materials and methods section, we divided mice into 4 groups, and the appearance of mice back skin within four groups was shown in Fig.4A. Knocking down CTNNBIP1 in control mice could induce psoriatic changes on mice skin, while IMQ-induced damages on mice skin were further aggravated by CTNNBIP1 knockdown (Fig.4A). Histopathologic analyses on four groups by H&E staining showed that psoriatic phenotypes, including erythema, plaque, infiltration, and scales, began to appear in CTNNBIP1 knocked-down control mice and IMQ-treated mice while these phenotypes were aggravated in CTNNBIP1 knocked-down IMQ mice

(Fig.4B). We performed real-time PCR (Fig.4C) and Immunoblotting (Fig.4D) to confirm the *in vivo* knockdown of CTNNBIP1.

Effects of knocking down CTNNBIP1 on HaCaT cell apoptosis and proliferation

First, we confirmed the effects of CTNNBIP1 on IMQ-induced psoriasis-like dermatitis in mice. Next, we investigated the *in vitro* effects of CTNNBIP1 upon keratinocytes. We transfected si-CTNNBIP1 to generate CTNNBIP1 silence in HaCaT cells, and performed Immunoblotting to verify the transfection efficiency (Fig.5A-B). Next, the cell viability, the cell apoptosis and the DNA synthesis capacity of CTNNBIP1-silenced HaCaT cells were determined using Flow cytometry, EdU, and MTT assays. CTNNBIP1 silence significantly inhibited HaCaT cell apoptosis (Fig.5C-D), promoted the DNA synthesis capacity (Fig.5E), and promoted the cell viability (Fig.5F). These data indicate that CTNNBIP1 silence could promote the over proliferation of keratinocytes.

CTNNBIP1 silence promotes β -catenin nucleus translocation-mediated TCF transcriptional activity

As for the underlying mechanism, CTNNBIP1 could disrupt β -catenin-TCF interaction and reduce TCF transcriptional activity [12, 15, 16]. Thus, next, we investigated whether CTNNBIP1 silence could affect the nucleus translocation of β -catenin and TCF4 transcription of downstream genes, such as c-Myc [9] and cyclin D1 [10, 11]. We also monitored changes in the late keratinocyte differentiation marker filaggrin [26, 27]. In CTNNBIP1-silenced HaCaT cells, the plasma protein levels of β -catenin showed to be obviously upregulated (Fig.6A-B) while the total cellular protein levels of β -catenin showed no obvious alterations (Fig.6C-D). Meanwhile, IF staining showed that β -catenin protein tended to distribute in the cell nucleus in CTNNBIP1-silenced HaCaT cells (Fig.6E). Following increased β -catenin nucleus translocation, the transcriptional activity of TCF4 was also significantly enhanced (Fig.6F). Consistently, β -catenin/TCF-complex downstream cyclin D1, c-Myc, and Ki-67 proteins were significantly increased (Fig.6G-H). These data indicate that CTNNBIP1 silence promotes β -catenin nucleus translocation and enhances TCF4 transcription of downstream cyclin D1, c-Myc, and Ki-67.

Expression and correlation of c-Myc and cyclin D1 with CTNNBIP1 in tissue samples

As a further confirmation of above-described *in vitro* findings, we examined the protein content and distribution of c-Myc and cyclin D1 within psoriasis lesion and non-lesion tissues. Both these two downstream genes showed to be upregulated within psoriasis lesion tissue samples than those within non-lesion tissues (Fig.7A-B). Consistently, MYC and CCND1 mRNA expression showed to be markedly upregulated within psoriasis lesion tissues than that in non-lesion tissues (Fig.7C and E). CTNNBIP1 mRNA expression had a negative correlation with MYC and CCND1 mRNA expression, respectively, within tissues as analyzed by Pearson's correlation analysis (Fig.7D and F).

Discussion

Herein, we demonstrated that the expression of CTNNBIP1 was remarkably downregulated within psoriasis lesion skin tissue samples compared to that within non-lesion skin tissues based on both online data and experimental results. In response to a period of different therapies, respectively, CTNNBIP1 expression could be rescued in lesion skin tissues. Within IMQ-induced psoriasis-like dermatitis in mice, CTNNBIP1 silence further aggravated psoriatic phenotypes. In human immortalized keratinocytes, HaCaT cells, CTNNBIP1 silence significantly inhibited cell apoptosis and promoted cell proliferation. Regarding the molecular mechanism, CTNNBIP1 silence in HaCaT cells promoted β -catenin nucleus translocation, enhanced the transcriptional activity of TCF4, and increased β -catenin/TCF-complex downstream c-Myc and cyclin D1 proteins while decreased the late differentiation marker filaggrin. In contrast to CTNNBIP1, the expression of c-Myc and cyclin D1 showed to be dramatically upregulated within psoriasis lesion tissue samples than that within non-lesion tissue samples. Within tissues, c-Myc and cyclin D1 showed to be negatively correlated with CTNNBIP1, respectively.

Inhibitor of β -catenin and TCF4 (ICAT), CTNNBIP1, is a 9-kDa polypeptide which binds β -catenin and competes its interaction with transcriptional factor TCF to suppress β -catenin nucleus signaling pathway [12]. CTNNBIP1 not only disrupts β -catenin/TCF/DNA complexes, but also reduces the activation of reporter genes via β -catenin/TCF-4 complex [12, 16]. Studies have shown that the levels of CTNNBIP1 transcript was dramatically increased within human cancers [28], which may be the result of a negative feedback machinery that suppresses β -catenin/TCF signaling pathway, since CTNNBIP1 is forcibly expressed within cells when β -catenin levels show to be increased, thus eliciting G2 arrest and cell death via strongly inhibiting the proliferation of these cells [29]. Interestingly, in the present study, online data and experimental results both showed that the expression of CTNNBIP1 showed to be remarkably downregulated within psoriasis lesion skin than that in non-lesion skin. More importantly, upon different therapies, CTNNBIP1 expression was rescued in lesion tissues. In summary, the aberrant downregulation of CTNNBIP1 within psoriasis might be related to keratinocyte over proliferation and the inhibition of differentiation in keratinocyte.

The investigations during the past decades have demonstrated IMQ-induced psoriasis-like skin inflammation in both mice and humans [30–32]. IMQ, a Toll-like-receptor (TLR), 7/8 ligand and potent immune activator, exerts the immunomodulatory effects upon inducing psoriasis, which is due to TLR7 stimulation upon pDCs (plasmacytoid dendritic cells) [33]. IMQ-induced skin inflammation in mice showed to be immunologically regulated through the IL-23/IL-17 axis and presented psoriasis-like histopathologic changes [34]. Thus, in the study of pathogenesis of psoriasis, IMQ-induced psoriasis-like dermatitis model in mice has been widely used. Consistent with these previous studies, IMQ stimulation induced psoriatic changes in mice skin in the present study, including erythema, plaque, infiltration, and scales. Interestingly, knocking down CTNNBIP1 in non-IMQ treatment group also caused similar psoriatic changes in mice skin, while knocking down CTNNBIP1 in IMQ treatment group even aggravated IMQ-induced damages in mice skin. In summary, the abnormal downregulation of CTNNBIP1 contributes to psoriasis development.

The outer layer of skin, the epidermis, is a translucent layer made of cells that function to protect us from the environment. The regular differentiation of keratinocytes is the key to complete epidermal barrier. Both the dysfunction of epidermal barrier and the abnormal differentiation of keratinocytes contribute to the pathophysiological process of various skin conditions, including psoriasis [35]. In addition to the inflammatory changes, psoriasis lesions also demonstrate significant changes in epidermal differentiation. Premature cell death leads to the interruption of keratinization, resulting in the incomplete keratinization of psoriasis; therefore, the markers of late differentiation, including profilaggrin and loricrin, are abolished in psoriasis lesions [26]. It has been reported that aberrant keratinocyte differentiation results in impaired skin barrier function. Similarly, herein, we confirmed that CTNNBIP1 silence in keratinocytes HaCaT cells significantly promoted cell apoptosis while inhibited cell proliferation. Moreover, CTNNBIP1 silence decreased the protein levels of late differentiation marker filaggrin, indicating that CTNNBIP1 silence in HaCaT cells disturbed the differentiation of keratinocytes.

CTNNBIP1 has been reported to be increased within a population of mature, non-dividing intestinal villus-lined enterocytes while it's absent within the β -catenin/TCF signaling-active crypt region, indicating that its protein levels could possibly have a negative correlation with the activity of β -catenin signaling pathway [36]. Herein, CTNNBIP1 silence in HaCaT cells significantly promoted β -catenin nucleus translocation, increased TCF4 transcriptional activity, and subsequently promoted the protein levels of β -catenin/TCF-complex downstream genes, such as c-Myc [9], cyclin D1 [10, 11], and Ki-67. These data indicate that CTNNBIP1 exerts its effects on keratinocytes through regulating β -catenin-TCF4 interaction, therefore affecting downstream gene expression. As a further confirmation, in contrast to CTNNBIP1, the expression of c-Myc and cyclin D1 showed to be dramatically upregulated within psoriasis lesion skin tissues. Moreover, the expression of c-Myc and cyclin D1 in tissue samples showed to be negatively correlated with CTNNBIP1, respectively.

Conclusions

In conclusion, we identify CTNNBIP1 as an abnormally downregulated gene in psoriasis. CTNNBIP1 silence significantly disturbs the differentiation of keratinocytes through promoting the transcription of β -catenin/TCF-complex downstream genes. However, the further application of CTNNBIP1 as a promising treatment for psoriasis needs in-depth in vivo and clinical investigations.

Declarations

Ethical Approval and Consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Second Affiliated Hospital of Hunan University of Chinese Medicine and with the 1964 Helsinki declaration. Informed consent to participate in the study has been obtained from participants.

Consent for publication

Consent for publication was obtained from the participants.

Availability of supporting data

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

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Authors' contributions

Chang Wang, Haizhen Wang, Zhibo Yang made substantial contribution to the conception and design of the work; Youhua Peng, Bijun Zeng analyzed and interpreted the data; Chang Wang, Yujin Zhang, Xueyong Tang drafted the manuscript; Lan Mi, Yi Pan, Zhibo Yang revised the work critically for important intellectual content; Chang Wang, Zhibo Yang collected grants; All authors read and approved the final manuscript.

Acknowledgment

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Authors' information

First author: Chang Wang, mail: skinwch@vip.163.com; Co-first author: Haizhen Wang, mail: 491476130@qq.com; Youhua Peng, mail: hua85227@126.com; Bijun Zeng, mail: dr_zengbijun@163.com; Yujin Zhang, mail: 346281180@qq.com; Xueyong Tang, mail: tangxueyong19830@163.com; Lan Mi, mail: 522155442@qq.com; Yi Pan, mail: 1021685366@qq.com. Correspondence: Zhibo Yang, Tel: +86-731-84917850; mail: dr_yangzhibo@163.com.

Abbreviations

CTNNBIP1: catenin beta interacting protein 1; DAB: 3,3'-diaminobenzidine tetrachloride; EdU: 5-ethoxy 2-deoxyuridine; Em: emission wavelength; H&E: hematoxylin and eosin; IF: Immunofluorescence; IHC: Immunohistochemistry; IMQ: imiquimod; PASI: Psoriasis Area Severity Index; PI: Propidium iodide.

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Figures

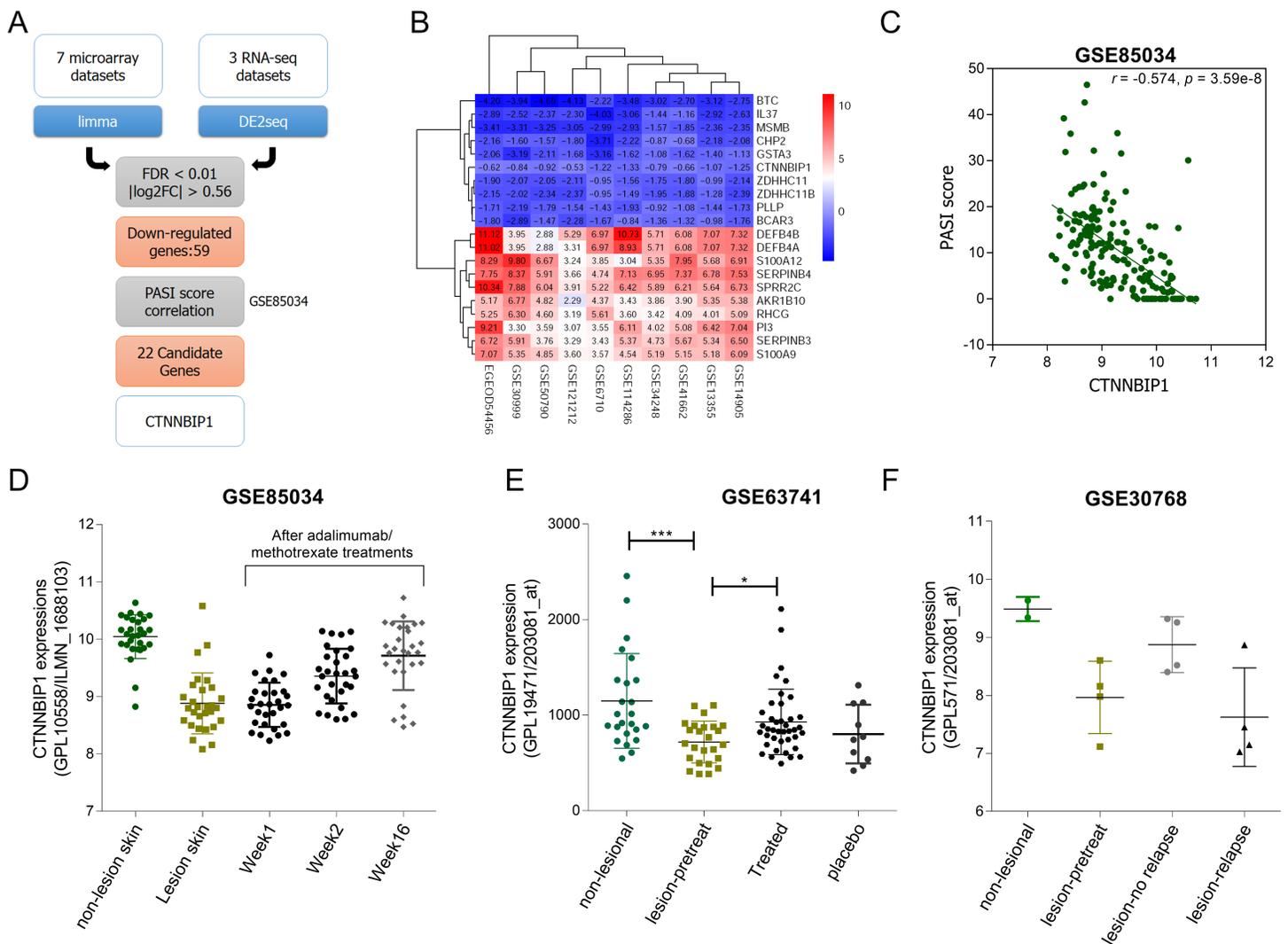


Figure 2

Screening for critical gene related to psoriasis pathogenesis (A) The schematic diagram showing the process of selecting the critical genes related to psoriasis pathogenesis. (B) Heatmap showing the differentially-expressed genes in psoriasis lesion skin tissues and non-lesion skin tissues according to seven sets of online microarray expression profiles (GSE13355, GSE14905, GSE30999, GSE34248, GSE41662, GSE50790, and GSE6710) and three sets of RNA-seq data (E-GEOD-54456, GSE114286, and GSE121212). (C) The correlation of CTTNBIP1 expression and PASI scores in each sample according to GSE85034. (D) CTNNBIP1 expression in non-lesion control tissue samples and psoriasis lesion tissues, with or without methotrexate treatment, according to GSE85034. (E) CTNNBIP1 expression in non-lesion control tissue samples and pretreated, treated, and placebo-treated psoriasis lesion tissues according to GSE63741. (F) CTNNBIP1 expression in non-lesion control tissue samples and pretreated, lesion non-relapse, and lesion with relapse according to GSE30768.

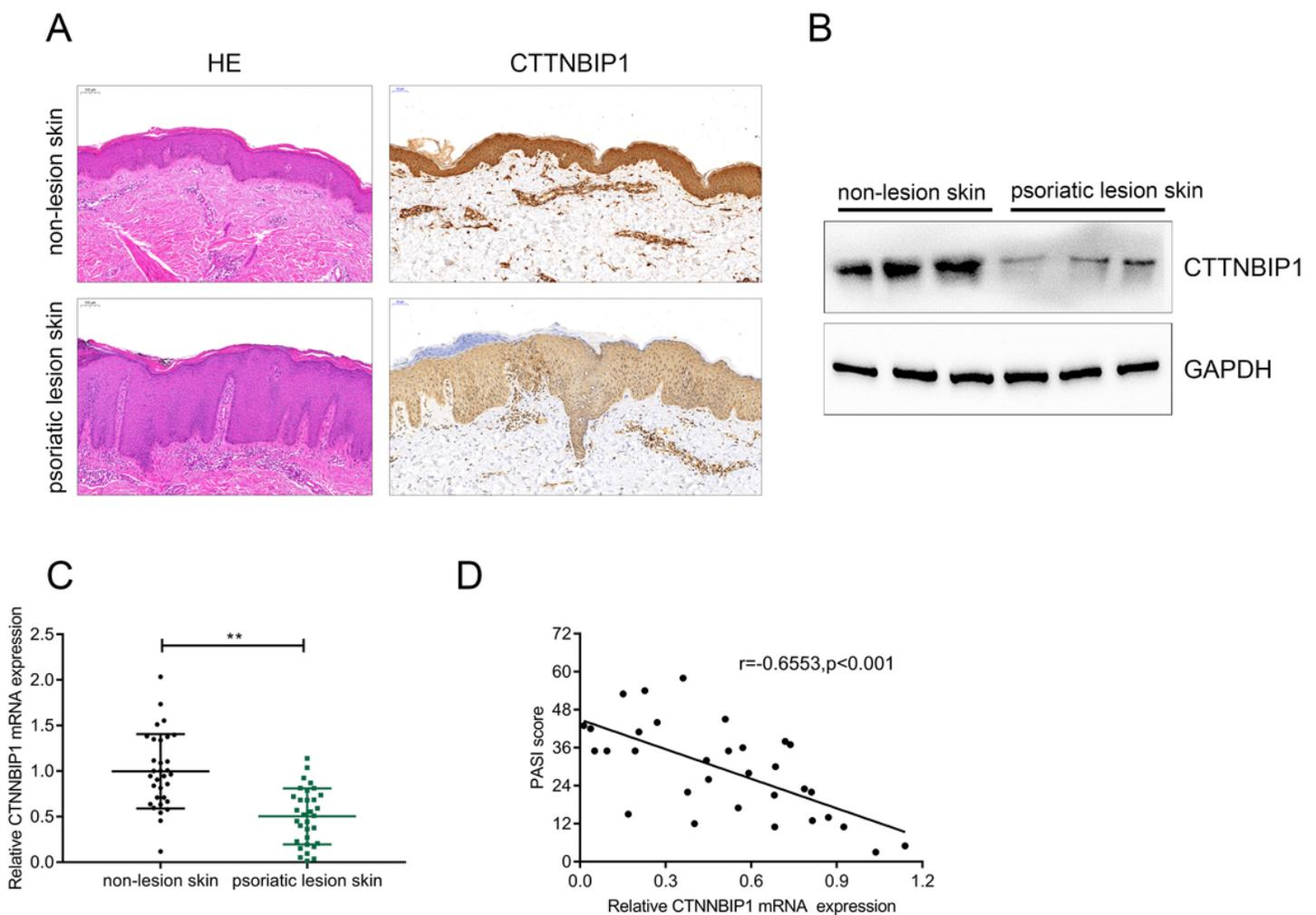


Figure 4

Expression and protein levels of CTNNBIP1 in psoriasis lesion and non-lesion tissues (A) Hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) staining were performed on psoriasis lesion and non-lesion tissue samples for histopathological analysis and CTNNBIP1 protein distribution,

respectively. (B) CTNNBIP1 protein levels in psoriasis lesion and non-lesion tissue samples determined by Immunoblotting. (C) CTNNBIP1 mRNA expression in psoriasis lesion and non-lesion tissue samples determined by real-time PCR. (D) The correlation of CTNNBIP1 expression and PASI scores in psoriasis lesion and non-lesion tissue samples analyzed using Pearson's correlation analysis. **P<0.01.

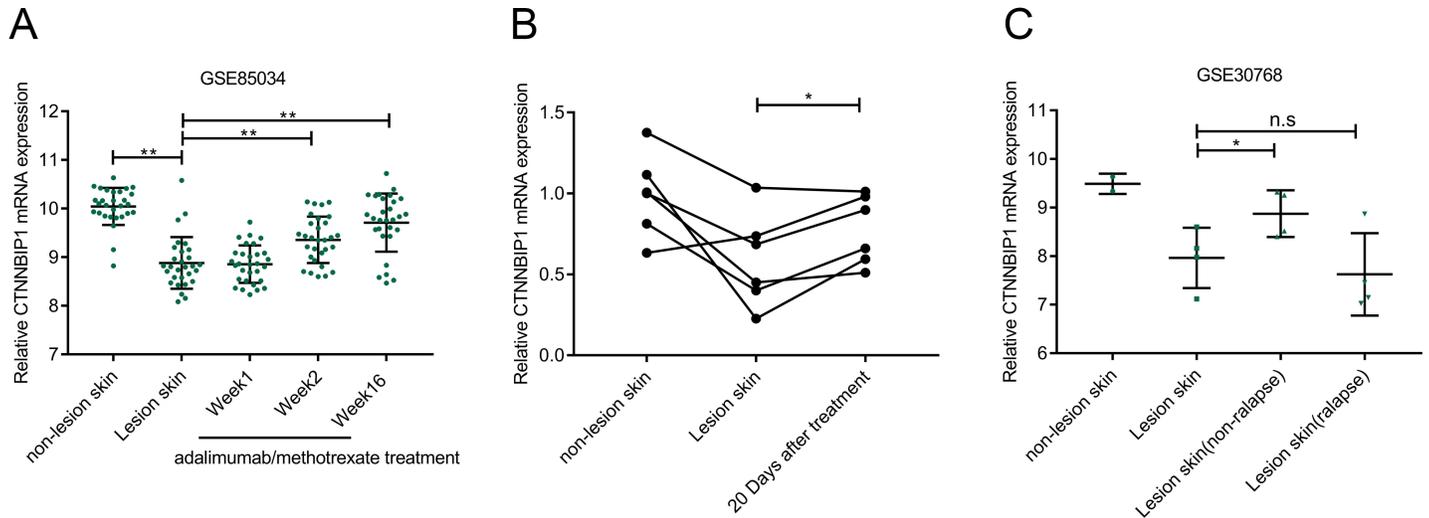


Figure 5

Changes in CTNNBIP1 expression in response to therapies (A) CTNNBIP1 expression in non-lesion skin and lesion skin tissues without adalimumab/methotrexate treatment or 1, 2, and 16 weeks after adalimumab/methotrexate treatment according to GSE85034. (B) CTNNBIP1 expression in five cases of non-lesion skin and lesion skin tissues without methotrexate treatment or 20 days after treatment determined by real-time PCR. (C) CTNNBIP1 expression in four cases of non-lesion skin and lesion skin tissues under efalizumab treatment with or without relapse determined by real-time PCR. *P<0.05, **P<0.01.

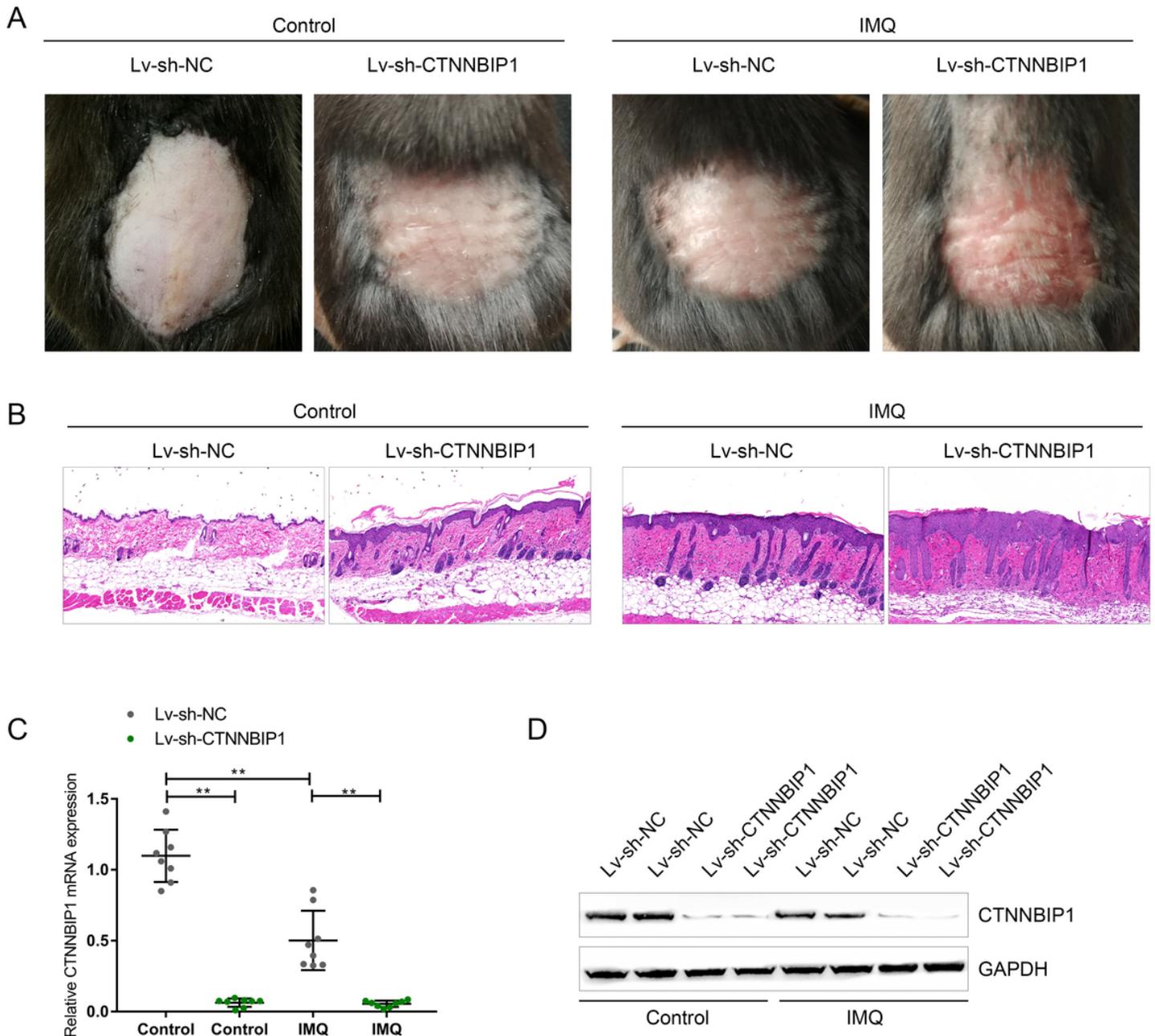


Figure 7

Effects of knocking down CTNNBIP1 on Imiquimod (IMQ)-induced psoriasis-like dermatitis in mice (A) The appearance of mice back skin in control group injected with Lv-sh-NC (negative control for Lv-sh-CTNNBIP1), control group injected with Lv-sh-CTNNBIP1, IMQ group injected with Lv-sh-NC, and IMQ group injected with Lv-sh-CTNNBIP1. (B) Histopathologic analyses on four groups by H&E staining. (C) CTNNBIP1 expression in four groups determined by real-time PCR. (D) CTNNBIP1 protein levels in four groups determined by Immunoblotting. ** $P < 0.01$.

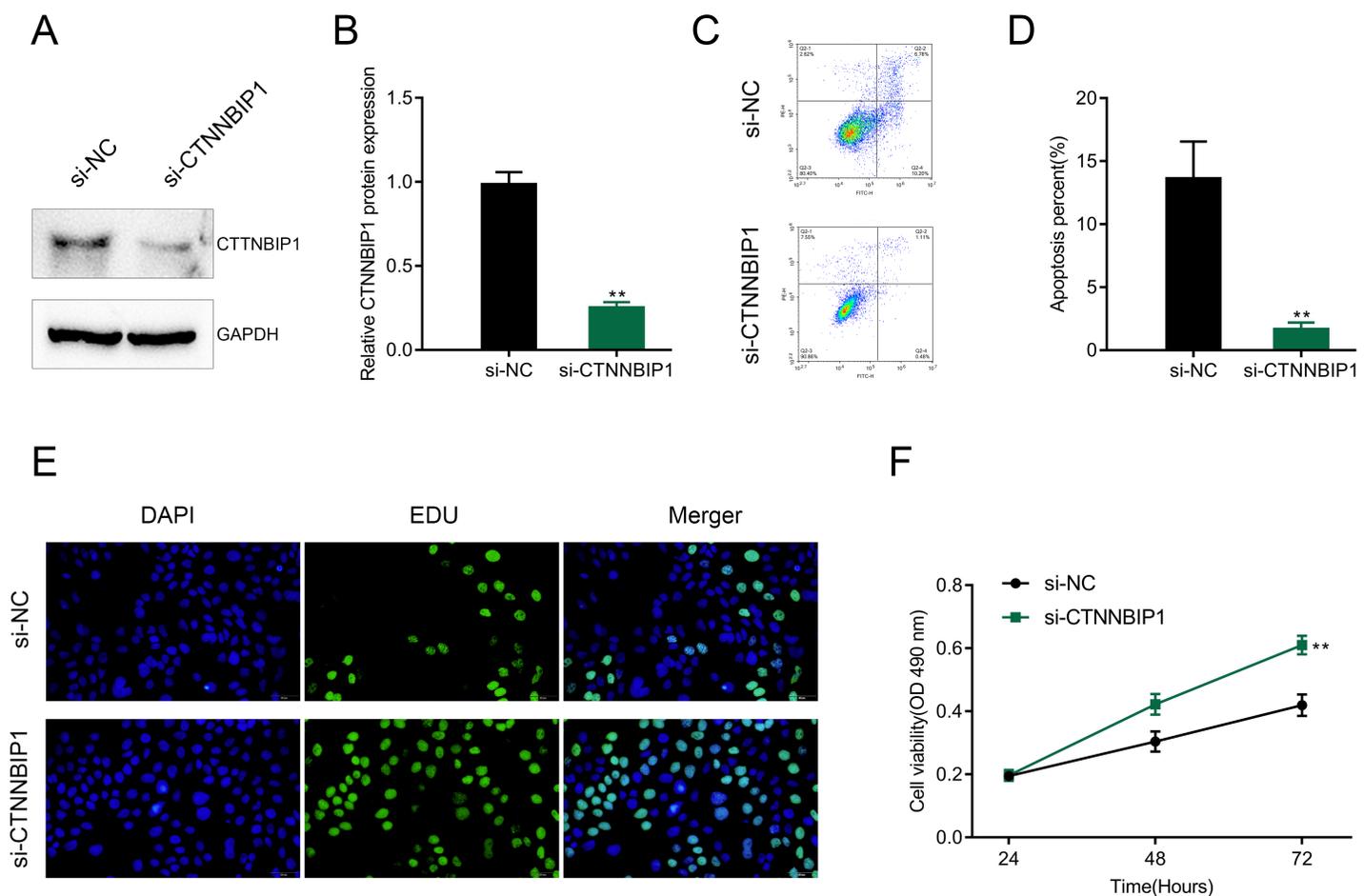


Figure 9

The effects of knocking down CTNNBIP1 on HaCaT cell apoptosis and proliferation (A-B) CTNNBIP1 silence generated in HaCaT cells by transfection of si-CTNNBIP1, as confirmed by Immunoblotting. The cell apoptosis (C-D), the DNA synthesis capacity (E), and the cell viability (F) of CTNNBIP1-silenced HaCaT cells were detected using MTT, EdU, and Flow cytometry assay. **P<0.01.

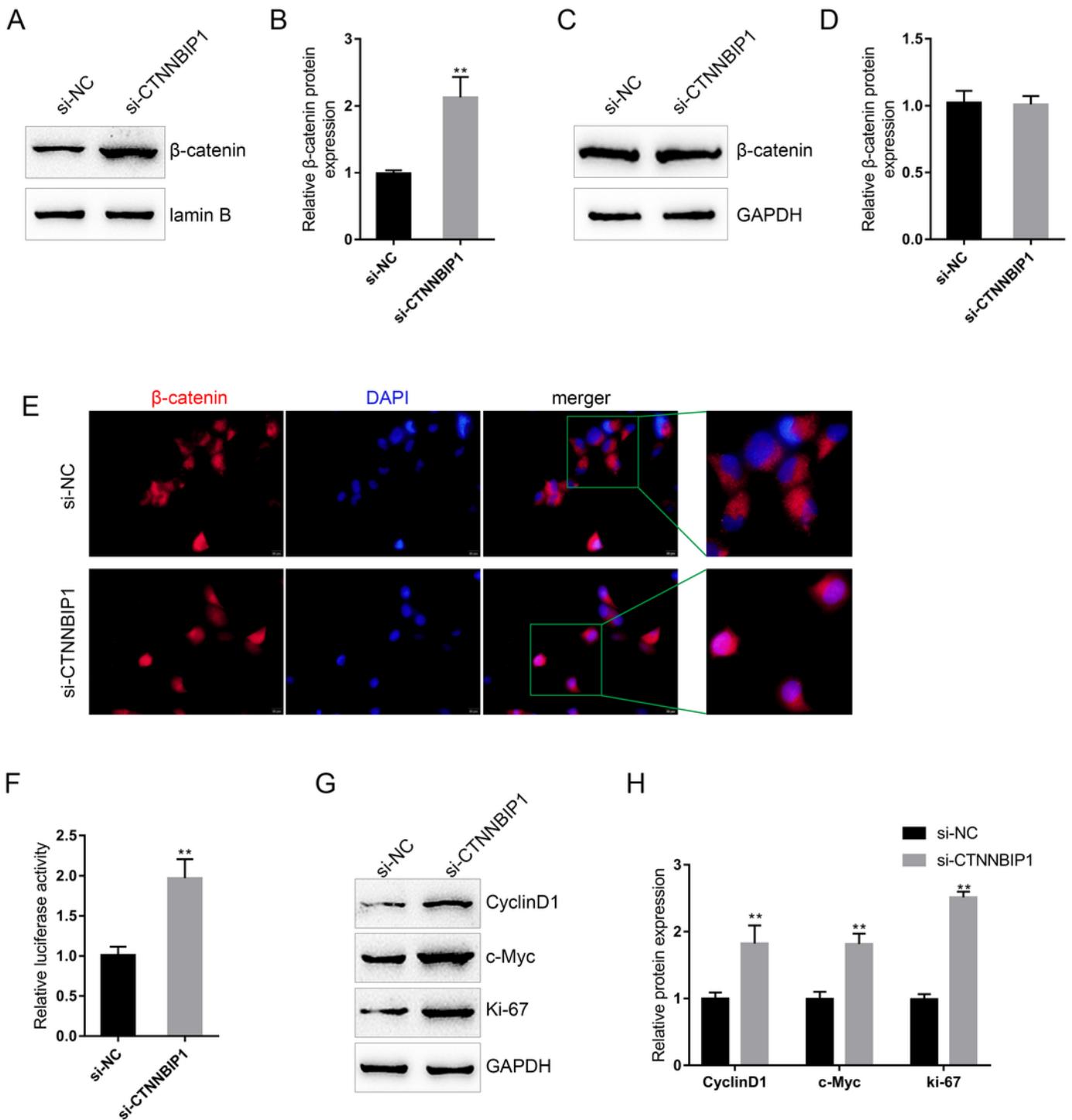


Figure 11

CTNNBIP1 silencing promotes β-catenin nucleus translocation-mediated TCF transcriptional activity in HaCaT cells. HaCaT cells were transfected with si-CTNNBIP1 and examined for (A-B) the plasma protein levels of β-catenin and (C-D) the total cellular protein levels of β-catenin by Immunoblotting; (E) the distribution of β-catenin protein by IF staining; (F) the transcriptional activity of TCF4 by luciferase reporter assay; (G-H) the protein levels of cyclin D1, c-Myc, and Ki-67 by Immunoblotting. **P<0.01.

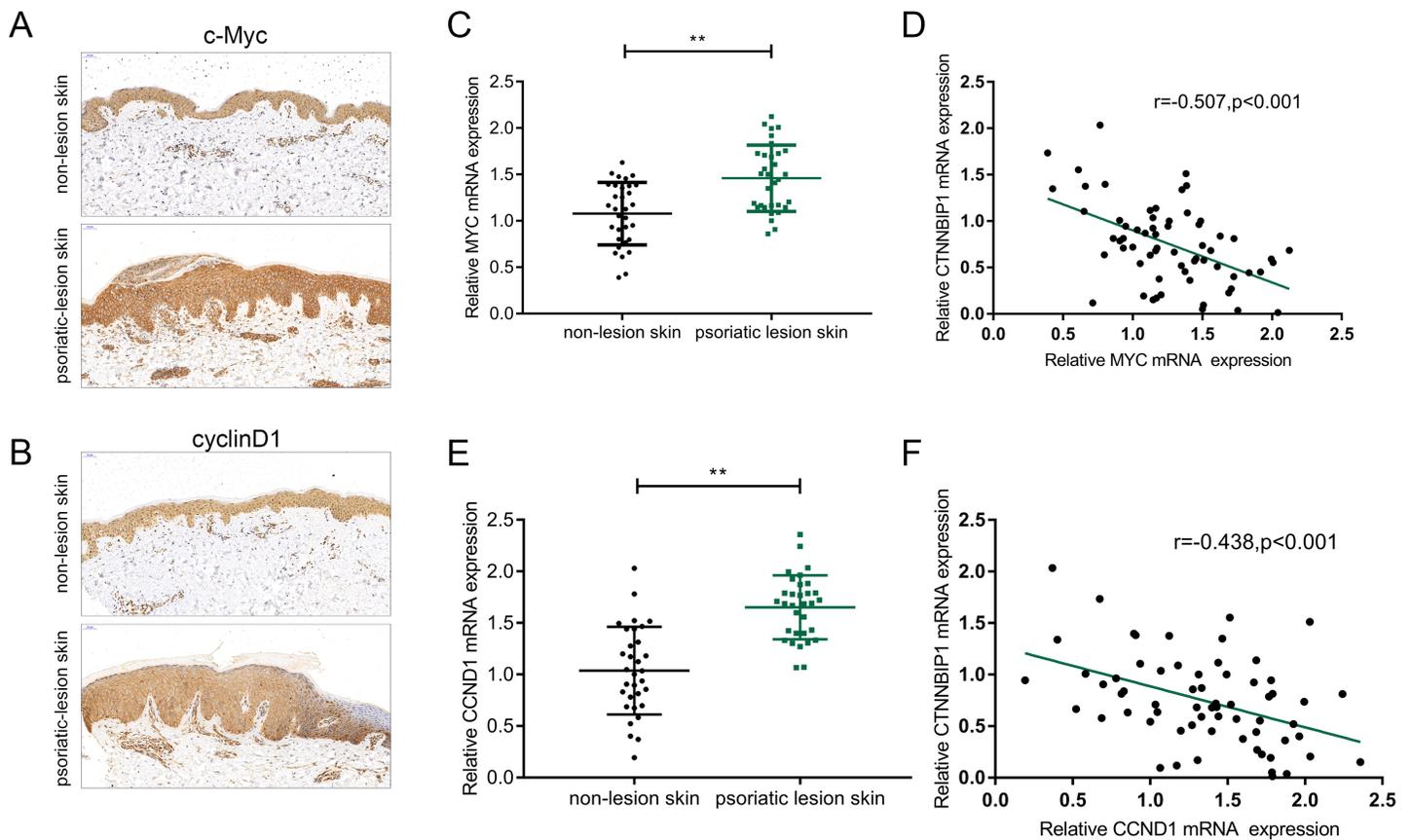


Figure 13

Expression and correlation of c-Myc and cyclin D1 with CTNNBIP1 in tissue samples (A-B) The protein content and distribution of c-Myc and cyclin D1 in psoriasis lesion and non-lesion tissues detected by IHC staining. (C and E) The mRNA expression of MYC and CCND1 in psoriasis lesion and non-lesion tissues determined by real-time PCR. (D and F) The correlation of MYC and CCND1 mRNA expression with CTNNBIP1 mRNA expression in tissue samples analyzed using Pearson's correlation analysis. **P<0.01.

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