CircOAS3 Regulates Keratinocyte Proliferation and Psoriatic Inflammation by Interacting with Hsc70 via the JNK/STAT3/NF-κB Signaling Pathway

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Abstract—Psoriasis is a chronic inflammatory disease of the skin with a very complex pathogenesis. Circular RNAs (circRNAs) play important regulatory roles in many diseases, including psoriasis. In this study, we found that circOAS3 expression was significantly upregulated in both psoriatic tissues and M5-induced keratinocytes. Silencing circOAS3 in HaCaT and Ker-CT cells inhibited their viability, promoted apoptosis, and blocked the cell cycle from the G1 to the S phase. RNA pull-down and RNA immunoprecipitation (RIP) analyses led to the identification of a direct interaction between circOAS3 and heat shock cognate protein 70 (Hsc70). Silencing circOAS3 expression negatively influenced Hsc70 protein expression but not mRNA expression. circOAS3 knockdown suppressed the activation of the JNK/STAT3/NF-κB signaling pathway. circOAS3 or Hsc70 silencing led to downregulated protein IL-6 expression, thus reducing psoriatic inflammation in vitro. In conclusion, the interaction between circOAS3 and Hsc70 mediates the proliferation and psoriatic inflammation of HaCaT and Ker-CT cells through the JNK/STAT3/NF-κB signaling pathway, suggesting that circOAS3 or Hsc70 may be a promising therapeutic target for psoriasis.

KEY WORDS: psoriasis; circOAS3; RNA-binding protein; Hsc70; proliferation; inflammation.

INTRODUCTION

Psoriasis is a chronic inflammatory skin disease characterized by hyperproliferation and inhibited apoptosis of keratinocytes that affects up to 2–3% of the population worldwide [1, 2]. However, the occurrence and development of psoriasis are not completely understood. Abnormal differentiation of keratinocytes and infiltration of inflammatory cells have been proposed mechanisms. Inflammatory cytokines in psoriatic lesions have also been implicated in disease progression [3, 4]. Many known immune-related
genes are closely related to psoriasis [5]. In addition, dysregulation of the epigenetic network, especially of noncoding RNAs (ncRNAs), has led to pivotal pathogenic insights into psoriasis pathogenesis [6].

In circular RNAs (circRNAs), the 5’ end of the transcript sequence is covalently attached to 3’ end of the sequence as a result of backsplicing, and circRNAs are abundant in mammals and known to be cell- and tissue-specific [7–9]. circRNA research has progressed at a rapid pace in recent years. Increasing evidence has suggested that circRNAs regulate gene expression at the transcriptional and/or post-transcriptional level [10]. circRNAs exert crucial functions in aging, tissue development, and cancer, and disease development and/or progression [11]. Some circRNAs, such as circMAPK14 and circCOL6A3_030, have shown the potential of coding novel proteins to affect disease progression [12, 13]. In psoriasis studies, a microarray was first performed to investigate the circRNA expression profile, and differentially expressed circRNAs were found to be expressed between psoriatic lesions and normal healthy skin tissues [14]. Other studies showed that hsa_circ_0003738 and circ_0061012 sponged microRNAs (miRNAs) in psoriasis [15, 16]. However, other specific and detailed molecular mechanisms of circRNAs in psoriasis need to be clarified.

The molecular mechanism of psoriasis has been mostly studied in HaCaT or primary human keratinocytes. However, both of these cell lines have disadvantages when used in research. As a newly isolated keratinocyte line, the Ker-CT cell line was originally derived from human foreskin keratinocytes and immortalized by expressing human telomerase and mouse CDK4 [17, 18]. These cells show typical characteristics of basal epidermal keratinocyte stem cells, including keratin 5 and p63 expression. When grown in 3D organotypic culture, Ker-CT cells form epidermal tissue similar to that formed by primary keratinocytes under the same conditions [17]. Research has shown that Ker-CT cells are suitable for use in studies on epidermal cell adhesion and Pemphigus pathomechanisms [19]. In the present study, we used Ker-CT cells, as well as HaCaT cells, as models of psoriasis.

In this study, we determined that hsa_circ_0028434 (circOAS3) was significantly upregulated in psoriatic tissues and psoriatic cell lines. We also found that Hsc70 may function as an RNA-binding protein (RBP), specifically binding circOAS3 to promote disease progression by activating the JNK/STAT3/NF-κB signaling pathway. Therefore, circOAS3 or Hsc70 may be a useful biomarker for psoriasis prognostics and a potential therapeutic target in psoriasis treatment.

METHODS

Patient and Tissue Sample Collection

Seven psoriatic samples were collected from patients with vulgaris psoriasis at the Qilu Hospital of Shandong University; these patients had not received systemic or local treatment within 3 months of sample collection. All patients exhibited typical psoriasis vulgaris clinical characteristics. Ten normal tissues were collected from healthy volunteers. Healthy subjects showed no family history of psoriasis or any other autoimmune diseases. This study was approved by the Ethics Committee of Shandong University, Qilu Hospital (Jinan, China), and written informed consent was obtained from all participating patients.

circRNA Microarray

An Affymetrix GeneChip Human Gene 2.0 ST Array (Invitrogen) was used for circRNA expression profiling. The cells were cryopulverized and homogenized with a Biopulverizer™ (Biospec) and Mini-Bead-Beater16 (Biospec), respectively. The homogenized samples were separated. RNA was precipitated, washed with 75% ethanol, and dissolved in RNase-free water. A Quick Amp Labeling Kit (Agilent p/n 5190–0442) was used to label the RNA. The labeled/amplified RNA was then purified, and the quality of the labeled cRNA was determined. Hybridization was performed with an Agilent Gene Expression Hybridization Kit (Agilent p/n 5188–5242). The results were obtained with an Agilent microarray scanner (Agilent p/n G2565BA).

Cell Isolation and Culture

Skin specimens of young children’s foreskins were obtained from Qilu Hospital. The specimens were digested with Dispase II (Sigma, lot #BCBR9297V) at 4 °C overnight to separate the epidermis from the dermis. Then, the epidermal specimens were digested with a 0.25% trypsin-0.01% EDTA mixture (37 °C, 10–15 min) to obtain single-cell suspensions. The cells were grown and maintained in keratinocyte medium (ScienCell, CA, USA). Purified normal human epidermal keratinocytes (NHEKs) were obtained after 2–3 passages, and the third passage of NHEKs was used for subsequent experiments. Human immortalized keratinocyte (HaCaT) cells were purchased...
from Procell Life Science & Technology Co., Ltd., and were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco, USA) containing 10% fetal bovine serum (FBS) (Sangon Biotech, China), 100 μg/ml streptomycin, and 100 U/ml penicillin. The Ker-CT human hTert keratinocyte cell line (ATCC® CRL-4048™) obtained from the American Type Culture Collection (ATCC) was cultured in KGM-Gold™ with BulletKit™ medium (Lonza #00,192,060). All cells were incubated in a humidified chamber at 37 °C with 5% CO₂.

RNA Fluorescence In Situ Hybridization (FISH)

Cy3-labeled probes were designed and synthesized by RiboBio (RiboBio Biotechnology). RNA FISH was performed using a FISH Kit (RiboBio Biotechnology). Briefly, sections of tissue embedded paraffin and cell samples were fixed with 4% paraformaldehyde and digested by proteinase K. These samples were blocked with a prehybridization solution and then hybridized overnight with a Cy3-labeled circOAS3 probe at 4 °C. Images were acquired with an A1RþMP confocal laser microscope system (Nikon).

RNA Extraction, qRT–PCR, Nuclear-Cytoplasmic Fractionation, RNase R Treatment, and Nucleic Acid Electrophoresis Assays

Total RNA was extracted from tissues and cells with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reverse transcribed to cDNA with a transcriptase kit (TaKaRa, Otsu, Japan). qRT–PCR was carried out using TB Green PCR Master Mix (TaKaRa) in a CFX96 Touch™ Real-Time PCR Detection System (Bio–Rad). GAPDH and U6 were used as internal controls. The primers used in this study are listed in Supplementary Table 1. The relative gene expression was calculated by the 2 − ΔΔCT method. RNA from the nucleus and cytoplasm of HaCaT and Ker-CT cells was separated with cytoplasmic and nucleic RNA purification kit (Norgen BioTek Corporation, St. Catharines, Ontario, Canada) following the manufacturer’s instructions. RNase R treatment was executed at 37 °C with 4 U/µg of RNase R (Geneseeed Biotech Co., Ltd., Guangzhou, China) for 30 min. The circOAS3 and GAPDH cDNA and genomic DNA (gDNA) in HaCaT and Ker-CT cells were amplified with divergent and convergent primers, respectively. PCR products were detected with 2% agarose gel electrophoresis at 90 V for 40 min. The bands were observed after ultraviolet (UV) irradiation. All experiments were repeated three times.

Construction of the In Vitro Psoriatic Model

When cell confluence reached approximately 60–70%, cells were starved in serum-free DMEM for 12 h. Then, M5, a cocktail of cytokines (interleukin-17A [IL-17A], tumor necrosis factor-α [TNF-α], IL-1α, IL-22, and Oncostatin-M at a final concentration of 10 ng/mL; PeproTech), was used to induce a psoriatic-inflammatory-like condition in NHEKs and HaCaT and Ker-CT cells cultured in serum-free DMEM for 24 h.

Cell Transfection

For gene silencing, small interfering RNA (siRNA) (GenePharma, Shanghai, China) targeting circOAS3 or Hsc70 was transferred into cells with Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, USA). si-NC was used as a natural control. Sequences were shown as follows (5’–3’): si-circOAS3: GCC UCC UUG UAU GAU GCC ATT; si-Hsc70: GUC CUC AUC AAG CGU AAU ATT; si-NC: UUC UCC GAA CGU GUC ACG UTT. For inducing gene overexpression, cells were transfected with the eukaryotic expression vector pcDNA3.1 (GenePharma) with Jet PRIME transfection reagent (Polyplus, USA). Empty vector (oe-NC) was used as a natural control. Cells were collected for further treatment or analysis at different time points after transfection. The transfection methods were performed following the reagent manufacturers’ instructions.

Western Blotting

Total protein of the cells was prepared using RIPA lysis buffer (Beytime, Beijing, China) following the manufacturer’s protocols. Equal amounts of protein were then loaded on an SDS–PAGE gel, and after electrophoretic separation, they were transferred electrophoretically to a PVDF membrane (Millipore, USA). After blocking with 5% milk in TBST, the membrane was incubated overnight with primary antibody (Ab, 1:1000) at 4 °C. After washing and incubation, the membrane was incubated with secondary Ab (1:2000) in TBST. Protein expression levels were detected by enhanced chemiluminescence (ECL) Plus reagent (Millipore, Billerica, MA, USA) in a bio-imaging system. The following primary antibodies were used in this study: anti-phosphorylated (p)-JNK1/2, anti-JNK1/2, anti-p-STAT3, anti-STAT3 (all obtained from Cell Signaling Technology, Boston, USA), anti-Hsc70, anti-IL-6, anti-cyclinD1, anti-Histone3, and anti-GAPDH (all obtained from Abcam,
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Cambridge, UK), anti-p-NF-xB, and anti-NF-xB obtained from Santa Cruz Biotechnology (Texas, USA).

CCK-8 Assay and EdU Assay

A cell counting kit-8 (CCK-8) assay was performed to measure cell viability (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) 24, 48, and 72 h after transfection as described above. The CCK-8 solution was added and incubated for 1 h at 37 °C in the dark. The optical density (OD) value was measured at a wavelength of 450 nm with a microplate reader (BioTek, USA). For the EdU assay, 1 × 10⁵ cells were inoculated into 24-well plates and an EdU cell proliferation kit (RiboBio, Guangzhou, China) was used. The percentage of EdU-positive cells was counted in four random fields per well.

Flow Cytometry Analysis of Cell Apoptosis and Cell Cycle Distribution

Cells undergoing apoptosis were detected with an Annexin-V-FITC apoptosis kit (Solarbio, Beijing, China) following the manufacturer’s instructions. The cell cycle phase of each was detected by DNA staining with a cell cycle kit (Solarbio).

Enzyme-Linked Immunosorbent Assay (ELISA)

After 24 h of transfection as described above, cells were stimulated with M5, and culture supernatants were collected 24 h later. The expression of IL-6 was measured with specific ELISA kits (Elabscience, China). The absorbance measured at a 450 nm wavelength was detected with a microplate reader (BioTek, USA).

RNA Pull-Down Assay and Mass Spectrometry

The interaction between circOAS3 and RNA-binding proteins was detected with a Pierce magnetic RNA–Protein pull-down kit (Thermo Fisher Scientific, USA) according to the manufacturer’s protocols. Biotin-labeled probes targeting a junction site in circOAS3 were synthesized by RiboBio (Guangzhou, China), and a control probe was used. Linear circOAS3 was transcribed with a biotin RNA-labeling mix (Roche) and T7 RNA polymerase (Thermo Fisher Scientific, USA), circularized with T4 RNA ligase I, and digested by RNase R. The cells were lysed and incubated with the biotin-labeled circOAS3 probe. Afterward, cell lysates were subjected to streptavidin agarose magnetic beads at normal temperature. Finally, the interacting proteins were identified by mass spectrometry and Western blotting. The sequences of the probes are shown in Supplementary Table 2.

RIP Analysis

RIP was executed with a Magna RIP kit (Millipore, Billerica, MA, USA) according to the manufacturer’s protocol. HaCaT and Ker-CT cells were lysed with RNA lysis buffer, and the cell lysates were incubated with RIP buffer containing magnetic beads conjugated to an anti-Hsc70 Ab (Abcam, #ab51052) or a negative control IgG Ab (Millipore, Billerica, MA, USA) for 4 h at 4 °C. After washing three times with washing buffer, Western blotting and qRT–PCR were performed to detect circOAS3 enrichment.

Gene Ontology (GO) Term and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis

GO is a database for unification of biology [20]. KEGG is a database to classify the selected gene sets into their respective signaling pathways [21]. In our study, KOBAS was applied to do GO and KEGG pathway enrichment analysis [22]. The p-value was corrected by the method introduced by Benjamini and Hochberg [23]. In this study, enriched GO terms and pathways with p-value < 0.05 were selected. The bioinformatics tools on the free online data analysis platform were used to draw the enrichment dot bubble of KEGG and GO analyses (http://www.bioinformatics.com.cn/).

Statistical Analysis

All statistical analyses were performed with the GraphPad Prism 8 software (GraphPad Software). Data from at least three independent experiments are presented as the means ± standard deviation (SD). Comparisons were performed with Student’s t-test, and p < 0.05 was considered to be statistically significant.

RESULTS

Characterization and Upregulation of circOAS3 in Psoriatic Samples

Based on the circRNA microarray, 4956 circRNAs (3016 upregulated and 1940 downregulated; fold change ≥ ± 2
and \( p < 0.05 \) were found to be differentially expressed in psoriasis. Among the dysregulated circRNAs detected, circOAS3 was overexpressed in psoriatic tissues, compared to its expression in normal epidermal tissues (Fig. 1a), suggesting its potential effect on the process of psoriasis development. circOAS3 is located on chromosome 12 and is 6363 base pairs (bp) in length from the OAS3 gene (Fig. 1b). To verify that circOAS3 was circular and not a product of trans-splicing or genomic rearrangement, the back-splice junction in the circOAS3 PCR products was confirmed by Sanger sequencing (Fig. 1b). In this study, we focused on the expression and roles of circOAS3 in psoriatic progression. To verify the microarray results, circOAS3 overexpression in psoriatic tissues was confirmed by qRT–PCR (Fig. 1c). Then, we measured the relative expression of circOAS3 in HaCaT and Ker-CT cells and NHEKs, and we found increased circOAS3 expression in all cell lines stimulated with M5 (Fig. 1d).

Moreover, circOAS3 resistance to RNase R exonuclease digestion confirmed that circOAS3 was circular (Fig. 1e). cDNA and gDNA of HaCaT and Ker-CT cells were used as templates. circOAS3 was amplified from the cDNA with only divergent primers; no amplification product was observed in the gDNA (Fig. 1f).

**circOAS3 Silencing Inhibits the Proliferation and Apoptosis of Psoriatic Keratinocytes**

To investigate the effects of circOAS3 in vitro, siRNA was transfected into cells to silence circOAS3 expression, and qRT–PCR confirmed the transfection efficiency (Fig. 2a). EdU and CCK-8 assays revealed that the proliferation of HaCaT and Ker-CT cells increased after M5 stimulation, which was subsequently partially inhibited by circOAS3 silencing (Fig. 2b, c). DNA staining demonstrated that circOAS3 silencing led to an increased proportion of cells in the G1 phase and a reduced proportion of cells in the S phase, indicating a therapeutic effect after M5 stimulation (Fig. 2d). As indicated by Annexin-V/propidium iodide (PI) staining, silencing circOAS3 led to a higher proportion of apoptotic cells, with the proportion of apoptotic psoriatic cells much higher than control cells.

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**Fig. 1** circOAS3 expression and characterization. a Differentially expressed circRNAs in psoriatic and normal epidermal tissues in the circRNA microarray (accession number: GSE181318). b Schematic diagram showing the genomic location and splicing pattern of circOAS3. “Head-to-tail” splicing sites in circOAS3 as determined by Sanger sequencing. c Total RNA was isolated for use in qRT–PCR. Psoriatic samples exhibited significantly higher levels of circOAS3 than healthy epidermal tissues. d Examination of the increase in circOAS3 expression after cell in keratinocytes after M5 stimulation as determined through qRT–PCR. Abundances of circOAS3 and GAPDH mRNA (reference gene) in keratinocytes treated with or without RNase R, as detected by qRT–PCR. f Divergent primers amplified circOAS3 from cDNA but not from genomic DNA (gDNA). The data are shown as the means±SD. *\( p < 0.05 \), **\( p < 0.01 \), and ***\( p < 0.001 \).
lower than that of normal control cells (Fig. 2e). In conclusion, these data suggest that circOAS3 level was modulated with the proliferation and apoptosis of keratinocytes.

circOAS3 Interacts with the Hsc70 Protein

We further investigated the mechanism by which circOAS3 regulates the proliferation of keratinocytes and the inflammatory response in keratinocytes. circOAS3 was found to be mainly expressed in the cytoplasm by qRT–PCR (Fig. 3a). We confirmed that circOAS3 binds to proteins by RNA pull-down assay. The results of silver staining showed that the circOAS3-sense pull-down group produced different protein bands, especially at approximately 70 kDa (Fig. 3b). The protein products were then analyzed by protein spectroscopy, which showed that circOAS3 bound to proteins. A Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showed that these proteins were involved in the cell cycle, apoptosis, and inflammatory-related pathways, while a Gene Ontology (GO) analysis revealed the functions of these proteins (Fig. 3c). Among the detected proteins, we found that Hsc70 was enriched in the circOAS3 sense group, as shown by secondary mass spectrometry and Western blotting (Fig. 3d). The RIP analysis results confirmed these findings (Fig. 3e). We also observed colocalization of circOAS3 and Hsc70. The nucleus of the HaCaT cells was labeled blue with DAPI, and the Cy3-labeled probes were used to label Hsc70, which fluoresced green, and circOAS3, which fluoresced red, as determined by fluorescence microscopy. The colocalization of circOAS3 and Hsc70 in epidermal keratinocytes confirmed their binding (Fig. 3f, g). We sought to determine whether circOAS3 regulates Hsc70 expression at the transcriptional or posttranscriptional level. Through Western blotting and qRT–PCR analyses, we found that Hsc70 protein expression was decreased (Fig. 3h) but Hsc70 mRNA expression was not decreased (Supplementary Fig. S1). CircOAS3 was silenced, suggesting that circOAS3 directly binds Hsc70 and downregulates its expression at the posttranscriptional level. Studies have shown that Hsc70 can...
affect cell proliferation, apoptosis, and inflammation in many diseases, but this effect has not been found in psoriasis [24–27]; therefore, we hypothesized that circOAS3 influences the pathogenesis of psoriasis by interacting with Hsc70.

**Hsc70 Regulates the Proliferation and Apoptosis of Keratinocytes**

A previous study has shown that Hsc70 was expressed almost exclusively in the suprabasal layers and that it was not detected at the basal layer [28]. However, the mechanisms of the Hsc70 effects in psoriasis remain unclear. First, we found the Hsc70 protein was expressed at a higher level in psoriatic tissues than in normal control tissues, as determined by Western blotting (Fig. 4a). EdU and CCK‐8 assays showed that the proliferation of HaCaT and Ker-CT cells was inhibited after Hsc70 silencing, and in contrast, their proliferation was promoted after Hsc70 overexpression (Fig. 4c, d). DNA staining demonstrated that Hsc70 silencing decreased the proportion of cells in the G1 phase and increased the proportion of cells in the S phase; the opposite results were obtained after Hsc70 silencing decreased the proportion of cells in the G1 phase and increased the proportion of cells in the S phase; the opposite results were obtained after Hsc70 silencing decreased the proportion of cells in the G1 phase and increased the proportion of cells in the S phase. DNA staining demonstrated that Hsc70 silencing decreased the proportion of cells in the G1 phase and increased the proportion of cells in the S phase, and in contrast, their proliferation was protected after Hsc70 overexpression (Fig. 4c, d). DNA staining demonstrated that Hsc70 silencing decreased the proportion of cells in the G1 phase and increased the proportion of cells in the S phase; the opposite results were obtained after Hsc70 silencing decreased the proportion of cells in the G1 phase and increased the proportion of cells in the S phase; the opposite results were obtained after Hsc70 silencing decreased the proportion of cells in the G1 phase and increased the proportion of cells in the S phase. DNA staining demonstrated that Hsc70 silencing decreased the proportion of cells in the G1 phase and increased the proportion of cells in the S phase; the opposite results were obtained after Hsc70 silencing decreased the proportion of cells in the G1 phase and increased the proportion of cells in the S phase.
Hsc70 and circOAS3-Regulated IL-6 Expression and JNK/STAT3/NF-κB Activation

The KEGG and GO analyses in Fig. 3 suggested the potential relationship between circOAS3 and MAPK/NF-κB pathway. As the binding protein of circOAS3, Hsc70 has been shown to affect the phosphorylation of JNK [29] and promote the nuclear translocation of NF-κB, thus facilitating NF-κB activity [27]. The activation of either JNK [30] or NF-κB [31] signaling could upregulate IL-6 expression in diseases. IL-6 is one of the multifunctional cytokines, which is significantly increased in psoriatic lesions and keratinocytes and is implicated in the pathology of psoriasis [32]. STAT3 is recognized as the main mediator of the function of IL-6 and activated in psoriasis skin [33], which in turn regulates the transcription of target genes including IL-6.

Considering that Hsc70 and circOAS3 direct bind with each other, we speculated that circOAS3 and Hsc70 are involved in the IL-6 expression and JNK/STAT3/NF-κB activation in psoriatic keratinocytes. Western blotting and ELISA showed that IL-6 expression induced by M5 stimulation was partially inhibited by either circOAS3 or Hsc70 silencing. In contrast, increased IL-6 expression was observed in the Hsc70-overexpression group (Fig. 5a–d). The phosphorylation in the JNK/STAT3/NF-κB pathway were decreased after either circOAS3 or Hsc70 expression was silenced, but total protein expression was not significantly changed (Fig. 5a, b). In contrast, the phosphorylation of JNK, STAT3, and NF-κB was increased by overexpressed Hsc70, and total protein expression was unchanged (Fig. 5c). The protein level of cyclin D1 was decreased after either circOAS3 or Hsc70 expression was silenced and increased by overexpressed Hsc70 (Fig. 5a–c).
Mechanistically, Hsc70 promotes the nuclear translocation of NF-κB and thus facilitates its activity [34]. We confirmed this process in keratinocytes. Protein in the cytoplasm and nucleus of HaCaT cells was extracted as separate fractions. Western blotting showed that M5 stimulation induced nucleocytoplasmic shuttling that caused nuclear accumulation of Hsc70, cyclin D1, and phosphorylated JNK, STAT3, and NF-κB. After circOAS3 silencing, the nuclear accumulation of these proteins was partially inhibited (Fig. 5e). These data explain the regulatory effect of circOAS3 on psoriatic keratinocyte proliferation and inflammation.

**Hsc70 Mediates circOAS3-Regulated Keratinocyte Proliferation and Inflammation In Vitro**

To determine whether Hsc70 is involved in the effects of circOAS3 in psoriatic keratinocytes. We overexpressed Hsc70 in circOAS3-silenced keratinocytes. After Hsc70 overexpression, the reduction in keratinocyte proliferation was partially re-established at an increased rate (Fig. 6a, b). DNA staining demonstrated that Hsc70 overexpression increased the proportion of circOAS3-silenced keratinocytes in the S phase and decreased the proportion of these cells in the G1 phase (Fig. 6c). The increasing proportion of apoptotic cells induced by circOAS3 silencing was reduced by Hsc70 overexpression (Fig. 6d). More importantly, the reduced IL-6 secretion induced by circOAS3 silencing was exacerbated because of Hsc70 overexpression in psoriatic keratinocytes. These results showed that circOAS3 silencing inhibited M5-induced hyperproliferation and inflammation in an Hsc70-dependent manner. Hsc70 overexpression weakened the protective effects of circOAS3 silencing. circOAS3 was thus shown to interact with Hsc70 to regulate M5-induced hyperproliferation and the inflammatory response in psoriatic keratinocytes.

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**Fig. 5** Effects of circOAS3 and Hsc70 on the IL-6 expression and JNK/STAT3/NF-κB activation in keratinocytes. **a**–**c** the Protein expression levels of phosphorylated (p)-STAT3, p-JNK, p-NF-κB, total STAT3, JNK, NF-κB, cyclin D1, and IL-6 in silenced (si)-circOAS3, si-Hsc70, and overexpressed (oe)-Hsc70 keratinocytes. **d** The keratinocytes were treated with short interfering RNA (siRNA) or plasmid DNA (pcDNA), and after M5 stimulation for 24 h, the supernatants of these cells were collected, and the concentration of IL-6 in these keratinocytes was detected with an ELISA kit (pg/ml). **e** Proteins in the nucleus and cytoplasm of HaCaT cells were extracted in separate fractions, and Hsc70, p-STAT3, p-JNK, p-NF-κB, and cyclin D1 expression was determined by Western blotting. The data are shown as the means ± SD. *p < 0.05, **p < 0.01, and ***p < 0.001.
This report provides the first demonstration of circOAS3 regulating keratinocyte proliferation and psoriatic inflammation by interacting with Hsc70.

On the basis of the circRNA microarray showing that circOAS3 was highly expressed in psoriatic lesions compared to its level in normal tissues, we hypothesized that circOAS3 contributes to the occurrence and development of psoriasis. Psoriasis is a chronic inflammatory disease accompanied by excessive proliferation of keratinocytes that do not undergo apoptosis [35, 36]. We used siRNA to silence circOAS3 expression in vitro and observed reduced keratinocyte proliferation and inflammation, revealing a therapeutic effect of M5 in psoriatic keratinocytes.

In addition to that showing the effects competitive endogenous RNA (ceRNA), emerging evidence has shown that circRNAs play important roles in various diseases through different mechanisms. For instance, CircCwc27 directly binds to purine-rich element-binding protein A (Pur-a) in Alzheimer’s disease [37]. circTshz2-2 combines with the YY1 transcriptional complex and suppresses Bdnf transcription to regulate the neuronal cell cycle and spatial memory in the brain [38]. circFAT1 promotes cancer cell stemness and immune evasion by binding to STAT3 [39]. This evidence prompted us to explore the mechanisms of circOAS3 action in psoriasis. By performing RNA pull-down assays and protein spectroscopy, we found that circOAS3 directly binds Hsc70, the expression of which had been previously proven to be upregulated in psoriasis, making it a potential target in the circOAS3 regulatory axis.

Hsc70, an Hsp family member, is a cytosolic protein that is abundantly, constitutively, and ubiquitously expressed in most cells [40]. Hsc70 participates in many essential functions. It maintains protein homeostasis [41] and participates in protein synthesis [42] and other significant cellular activities. Phenotypically, Hsc70 suppresses the apoptosis and promotes the proliferation of brain endothelial cells and tumor cells [29, 43]. In the immune system, Hsc70 modulates antigen transport within cells to control major histocompatibility complex (MHC) class II presentation during cell stress [44].
A previous study has shown that Hsc70 was expressed almost exclusively in the suprabasal layers, but it was not detected in the basal layer [28]. However, whether Hsc70 is involved in the proliferation and inflammatory responses of keratinocytes was unknown. Our studies revealed that silencing Hsc70 inhibited the keratinocyte proliferation and reduced inflammation in these cells. In contrast, overexpression of Hsc70 suppressed apoptosis and induced the cell cycle in keratinocytes, consistent with previous research reports. We found that Hsc70 expression reduction led to a decrease in the levels of phosphorylated JNK/STAT3/NF-κB, which are signaling proteins downstream of circOAS3 in keratinocytes as well. Silencing either circOAS3 or Hsc70 contributes to a lower expression of IL-6, which was a proinflammatory cytokine associated with the pathogenesis of psoriasis [45, 46]. In addition, Hsc70 overexpression partially reversed the therapeutic effect of circOAS3 silencing on psoriatic keratinocytes proliferation and inflammation. Thus, we speculate that Hsc70 may represent a novel target for the diagnosis and treatment of psoriasis.

The present study demonstrated that circOAS3 plays an important role in proliferation and inflammation in M5 induced psoriatic keratinocytes. We further revealed that circOAS3 mediates JNK/STAT3/NF-KB pathway activation via interacting with Hsc70 protein and regulates the release of the inflammatory factors IL-6. These results provide promising insight into the molecular mechanism and may lead to targeted therapy of psoriasis.

SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTION

Zhenxian Yang contributed to the conception and design of the work and the analysis and interpretation of the data and drafted the manuscript. Xiran Yin and Cheng Chen contributed to the design of the work and the analysis and interpretation of the data. Shan Huang, Xueqing Li, and Jianjun Yan contributed to the acquisition and interpretation of the data. Qing Sun contributed to the conception and design of the work. All authors read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS

The original data are maintained by the first author and the corresponding author, and all experimental raw data can be obtained from either of these authors when necessary.

DECLARATIONS

Ethics Approval and Consent to Participate This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by Qilu Hospital of Shandong University, which issued an affidavit of approval of animal ethics and welfare (No. KYLL-2017(KS)-152).

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

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