CircOAS3 regulates proliferation and psoriatic inflammation by interacting with Hsc70 via JNK/STAT3/NF-κB signaling pathways in keratinocytes

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Abstract

Psoriasis is a chronic inflammatory disease of the skin with highly complex pathogenesis. CircRNAs play an important regulatory role in plenty of diseases as well as psoriasis. In this study, we identified that circOAS3 was significantly upregulated in both psoriatic tissues and M5-induced keratinocytes. Silencing circOAS3 in HaCaT and Ker-CT cells inhibited cell viability, promoted apoptosis, and blocked the cell cycle from G1 to S phase. RNA-pulldown and RNA immunoprecipitation (RIP) analysis identified the direct combination between circOAS3 and Heat shock cognate protein 70 (Hsc70). Silencing circOAS3 negatively influences the Hsc70 in protein level rather than mRNA level. In mechanism, circOAS3 knockdown suppressed the activation of JNK/STAT3/NF-κB signaling pathways and blocked the nuclear accumulation of Hsc70 as well as phosphorylated STAT3/JNK/NF-κB as a result. Phenotypically, circOAS3 or Hsc70 silencing down-regulated the protein levels of IL-6 thus reducing psoriatic inflammation in vitro. In conclusion, interaction between circOAS3 and Hsc70 modulates the proliferation and psoriatic inflammation of HaCaT and Ker-CT cells through the JNK/STAT3/NF-κB signaling pathways, suggesting that circOAS3 or Hsc70 may be a promising therapeutic target for psoriasis.

Introduction

Psoriasis is a chronic inflammatory skin disease characterized by hyperproliferation and reduced apoptosis of keratinocytes, affecting up to 2–3% of the population worldwide[1, 2]. However, the occurrence and development of psoriasis are not completely clear. Abnormal differentiation of keratinocytes and infiltration of inflammatory cells have been proposed. Inflammatory cytokines in psoriatic lesions are also have been implicated[3, 4]. Many known immune-related-gene are closely related to psoriasis[5]. Besides, dysregulation in the epigenetic network, especially the role of non-coding RNAs (ncRNAs), has offered pivotal pathogenic insights regarding psoriasis pathogenesis[6].

circRNAs, a novel member of ncRNAs, transcript covalently closed at the 5’ end of the preceding sequence to the 3’ end of the subsequent sequence as a result of backsplicing, are abundant in mammals and are identified to be cell and tissue specific[7–9]. Progress in research on circRNAs is moving at a rapid pace in recent years. Increasing evidence has suggested that circRNAs regulate gene expression at the transcriptional or post-transcriptional level[10]. circRNAs exert crucial functions in aging, tissue development, cancers, and diseases[11]. In psoriasis, a microarray was firstly performed to investigate the circRNA expression profile and identified differentially expressed circRNAs between psoriatic lesions and normal healthy skin tissues[12]. Further studies showed hsa_circ_0003738 and circ_0061012 function by acting as a sponge for microRNAs (miRNAs) in psoriasis[13, 14]. However, other specific and detailed molecular mechanisms of circRNAs in psoriasis need to be further clarified.

The molecular mechanism of psoriasis is mostly studied by HaCaT or primary human keratinocytes. However, both cell systems have their weaknesses in research. As a new cell line of keratinocytes, Ker-CT cells were originally derived from human foreskin keratinocytes and immortalized by expressing human telomerase and mouse CDK4[15, 16]. They show typical characteristics of basal epidermal keratinocyte

stem cells, including the expression of keratin 5 and p63. When grown in 3D organotypic culture, the epidermis formed by Ker-CT cells is pretty similar to that formed by primary keratinocytes under the same conditions[15]. Research showed Ker-CT cells are well suitable for studies on epidermal cell adhesion and Pemphigus pathomechanisms[17]. Here we make an attempt to use Ker-CT cells as a model of psoriasis as well as HaCaT cells.

In this study, we determined circOAS3 was significantly up-regulated in psoriatic tissues and psoriatic cell lines. We further found that Hsc70 may function as the RNA binding protein (RBP) of circOAS3 to promote disease progression via JNK/STAT3/NF-κB signaling pathways. Therefore, circOAS3 or Hsc70 can serve as a biomarker for prognosis prediction and as a potential therapeutic target in treatment.

Methods

Patients and tissue samples collection

A total of 7 psoriatic samples were collected from patients with vulgaris psoriasis from Qilu Hospital of Shandong University. The patient did not receive systemic and local treatment within 3 months. All of them had typical psoriasis vulgaris clinical characteristics. 10 normal tissues were collected from healthy volunteers. Healthy subjects had 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 patients.

circRNAs microarray

Affymetrix GeneChip Human Gene 2.0 ST Array (Invitrogen) was used for circRNAs expression profiling. Cells were cryopulverized and homogenized using the 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. Quick Amp Labeling Kit (Agilent p/n 5190-0442) was used to label the reaction. Purified the labeled/amplified RNA and then measured the labeled cRNA quality. Hybridization was performed using Agilent Gene Expression Hybridization Kit (Agilent p/n 5188-5242). The results were detected by Agilent microarray scanner (Agilent p/n G2565BA).

Cell isolation and culture

The skin specimens of young children's foreskins were got from Qilu Hospital. The specimens were digested with dispase II (Sigma, lot#BCBR9297V) at 4°C overnight to dissect 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. Cells were grown and maintained in keratinocyte medium (ScienCell, CA, USA). The purified NHEKs were obtained after 2–3 passages, and the third passage of NHEKs was used for the 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. Human keratinocyte cell line hTert (Ker-CT)(ATCC® CRL-4048™) from American Type Culture Collection (ATCC) was cultured in KGM-Gold™ with BulletKit™ (Lonza 00192060). All cells were incubated in a humidified chamber at 37℃ with 5% CO2.

RNA fluorescence in situ hybridization (FISH)

Cy3-labeled probes were designed and synthesized by RiboBio (RiboBio Biotechnology). RNA FISH was conducted using the Fluorescent in situ Hybridization Kit (RiboBio Biotechnology). Briefly, tissue paraffin sections and cell samples were fixed with 4% paraformaldehyde and digested by proteinase K. Blocking by the prehybridizing solution and then hybridized overnight by the Cy3-labeled SPRR2C probe at 4°C. Images were acquired by the 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 was reverse transcribed to cDNA using a Transcriptase Kit (Takara, Otsu, Japan). qRT-PCR was carried out using the TB Green PCR Master Mix (Takara) in CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad). GAPDH and U6 were used as internal controls respectively. The primers used are listed in Supplementary Table 1. The relative gene expression was calculated with 2 −ΔΔCT method. RNAs from the nucleus and cytoplasm of HaCaT and Ker-CT cells were separated by the Cytoplasmic & Nuclear 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 (Geneseed Biotech Co., Ltd., Guangzhou, China), for 30 min. The cDNA and genomic DNA (gDNA) of circOAS3 and GAPDH from HaCaT and Ker-CT cells were amplified by divergent primers and convergent primers, respectively. PCR products were detected with 2% agarose gel electrophoresis at 90 V for 40 min. The bands were observed by UV irradiation. All the experiments were repeated three times.

Construction of psoriatic model in vitro

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

Cell transfection

For gene silencing, small interfering RNA (siRNA) (GenePharma, Shanghai, China) of circOAS3 or Hsc70 was transferred into cells using Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, USA). For gene overexpression, cells were transfected with the eukaryotic expression vector pcDNA3.1 (GenePharma) and (Supplementary Fig. S1) by using Jet PRIME transfection reagent (Polyplus, USA). Cells were collected for further treatment or analysis at different time points after transfection. These transfection methods were performed according to the manufacturers' instructions.

Western blotting

Total proteins of cells were prepared using RIPA lysis buffer (Beyotime, Beijing, China) following the manufacturer's protocols. Equal amounts of protein were loaded on an SDS-PAGE and then transferred electrophoretically to PVDF membrane (Millipore, USA). After blocking with TBST (5% milk), the membranes were incubated overnight with primary antibody (1:1000) at 4 °C. After washing and incubation, the membranes were incubated with secondary antibody (1:2000) in TBST. Protein expression levels were detected by ECL Plus (Millipore, Billerica, MA, USA) with a Bio-Imaging System. The following primary antibodies were used in this study: p-JNK1/2, JNK1/2, p-STAT3, STAT3 (all from Cell Signaling Technology, Boston, USA), Hsc70, IL-6, cyclinD1, Histone3, and GAPDH (all from Abcam, Cambridge, UK). p-NF-κB□NF-κB (all from Santa Cruz Biotechnology, Texas, USA).

CCK-8 assay and EdU assay

Cell counting kit-8 (CCK-8) assay was measured using Cell Counting Kit-8 (CCK8) (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) at 24, 48, and 72 h after being transfected as described above. CCK-8 solution was added and incubated for b1h at 37 °C in the dark. The Optical density (OD) value was measured at 450 nm wavelength by microplate reader (BioTek, U.S.A.). For the EdU assay, 1×10^5 cells were inoculated to 24-well plates using an EdU cell proliferation kit (Ribobio, Guangzhou, China). The percentage of EdU-positive cells was counted in four random fields per well.

Flow cytometry analysis of cell apoptosis and cell cycle

Cellular apoptosis was detected using the Annexin-V-FITC Apoptosis Kit (Solarbio, Beijing, China) following the manufacturer's instructions. The cell cycle was detected using a DNA staining cell cycle kit (Solarbio).

Enzyme-linked immunosorbent assay (ELISA)

After 24 h of transfection as mentioned above, cells were stimulated by M5, and culture supernatants were collected after 24 h. The secretions of IL-6 were measured by using specific ELISA kits (Elabscience, China). The absorbance at a wavelength of 450 nm was detected with microplate reader (BioTek, U.S.A.).

RNA pull-down assay and mass spectrometry

The interaction between circOAS3 and RNA-binding protein was detected by Pierce Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher Scientific, USA) according to the manufacturer's protocols. Biotin-labeled probes targeting the junction site of circOAS3 were synthesized by RiboBio (Guangzhou, China) and a control probe was used as a control. Linear circOAS3 was transcribed with Biotin RNA Labeling Mix (Roche) and T7 RNA polymerase (Thermo Fisher Scientific, USA), circularized using T4 RNA ligase I and digested by RNase R. The cells were lysed and incubated with a biotin-labeled circOAS3 probe. Afterward, cell lysates were subjected to streptavidin agarose magnetic beads at normal temperatures. Finally, interacting proteins were identified by mass spectrometry and Western blot. The sequences of the probe were shown in Supplemental Table 2.

RIP analysis

RIP was executed by the Magna RIP kit (Millipore, Billerica, MA, USA) according to the manufacturer's protocol. HaCaT and Ker-CT cells were lysed with RNA lysis buffer, then cell lysates were incubated with the RIP buffer containing magnetic beads conjugated to anti-Hsc70 (Abcam, #ab51052) or negative control IgG antibody (Millipore, Billerica, MA, USA) for 4 h at 4°C. After washing three times with washing buffer, western blot and qRT-PCR were implemented to detect enriched circOAS3

Statistical analysis

All statistical analyses were performed using GraphPad Prism 8 software (GraphPad Software). Data from at least three independent experiments were presented as the mean +/- standard deviation (SD). Comparisons were performed using Student's t-test, and $p < 0.05$ was considered statistically significant.

Results

Characterization and upregulation of circOAS3 in psoriatic samples

Based on the circRNA microarray, a total of 4956 circRNAs (3016 upregulated and 1940 downregulated; fold change ≥2 and $p<0.05$) were identified as differentially expressed in psoriasis. Among the dysregulated circRNAs performed, circ_0028434 was highly expressed in psoriatic tissues than normal epidermal tissues (Fig. 1a), indicating its effect on the process of psoriasis development. circ_0028434, which was termed circOAS3, is located in the chromosome 12 and 6363 base pairs (bp) in length from the OAS3 genome (Fig. 1b). To verify that circOAS3 was circular rather than products of trans-splicing or genomic rearrangements, the back-spliced junction in PCR products of circOAS3 was confirmed by Sanger sequencing (Figure 1b). We focused on the expression and roles of circOAS3 in psoriatic

progression in this study. To verify the microarray results, circOAS3 overexpression in psoriatic tissues was confirmed by qRT-PCR (Fig.1c), suggesting its potential functions in psoriasis. Then we identified the relative expression of circOAS3 in HaCaT, Ker-CT, and NHEK cells, which showed an increased expression in all cell lines after M5 stimulation (Fig. 1d).

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

circOAS3 silencing inhibits proliferation and apoptosis of psoriatic keratinocytes

To investigate the effects of circOAS3 in vitro, siRNA was transfected to silence the circOAS3, qRT-PCR confirmed the transfection efficiency (Fig. 2a). EdU and CCK-8 assay revealed that the proliferation of HaCaT and Ker-CT cells increased after M5 stimulation, which could be partly inhibited by circOAS3 silencing (Fig. 2b, c). DNA staining demonstrated that circOAS3 silencing leads to an increased proportion of cells in the G1 phase and reduced cells in the S phase, which showed a therapeutic effect on M5 stimulation (Fig. 2d). Through Annexin-V/PI staining, silencing circOAS3 induced a higher proportion of apoptotic cells, which was much lower in psoriatic cells than normal control cells (Fig. 2e). In conclusion, these data suggest that circOAS3 was correlated with the proliferation and apoptosis of keratinocytes.

circOAS3 interacts with Hsc70 protein

We further investigated the mechanism of circOAS3 in regulating proliferation and inflammatory response in keratinocytes. circOAS3 was mainly expressed in the cytoplasm detected by qRT-PCR (Fig. 3a). We certified that circOAS3 binds to proteins by RNA pulldown assay. The results of silver staining showed that circOAS3-sense pull-down group had different protein bands especially at about 70KDa (Fig. 3b). The protein products were further detected and analyzed by protein spectroscopy, which showed that circOAS3 could bind to proteins. Kyoto Encyclopedia of Genes and Genomes analysis showed that these proteins were involved in cell cycle, apoptosis, and inflammatory-related pathways, while Gene Ontology analysis revealed the functions of these proteins (Fig. 3c). Among the detected proteins, we found Hsc70 has an enrichment in the circOAS3 sense group, as shown by secondary mass spectrum and western blotting (Fig. 3d). RIP analysis proves this combination on the other hand (Fig. 3e). We also observed the colocalization between circOAS3 and Hsc70. Under a fluorescence microscope, the nucleus of HaCaT cells was labeled by DAPI show blue, Hsc70 were shown green and circOAS3 were shown red using Cy3-labeled probes. The colocalization of circOAS3 and Hsc70 also confirmed the binding in epidermal keratinocytes (Fig. 3f, g). We determined if circOAS3 could regulate the expression levels of Hsc70 in

transcriptional or post-transcriptional levels. Through Western Blotting and qRT-PCR, Hsc70 was decreased in protein expression (Fig. 3h) rather than mRNA expression (Supplementary Fig. S2) after silencing of circOAS3, suggesting circOAS3 could directly bind with Hsc70 and downregulate the expression level of Hsc70 in post-transcriptional level. Studies showed that Hsc70 could affect the proliferation, apoptosis, and inflammation in plenty of diseases but not psoriasis[18-21], thus we hypothesized circOAS3 could influence the pathogenesis of psoriasis via interacting with Hsc70.

Hsc70 regulates the proliferation and apoptosis of keratinocytes

A previous study showed Hsc70 was expressed almost exclusively in the suprabasal layers which was not detected basally[22]. But the mechanisms of effects of Hsc70 in psoriasis are still unclear. Firstly, Hsc70 showed a higher protein level in psoriatic tissues than normal control by Western Blotting (Fig. 4a). To investigate it in psoriatic keratinocytes, siRNA or pcDNA was transfected to silence or overexpress the Hsc70 in HaCaT and Ker-CT cells, western blotting was used to confirm the transfection efficiency (Fig. 4b). EdU and CCK-8 assay showed that the proliferation of HaCaT and Ker-CT cells could be downregulated by silencing Hsc70, which was promoted in contrast after Hsc70 overexpression (Fig. 4c, d). DNA staining demonstrated that Hsc70 silencing decreased the proportion of cells in the G1 phase and increased cells in the S phase, the contrary results obtained after Hsc70 overexpression in keratinocytes (Fig. 4e). Through Annexin-V and PI staining, silencing Hsc70 strongly increased the proportion of early and late apoptosis in keratinocytes and Hsc70 overexpression protected cells from apoptosis conversely (Fig. 4f). These data suggest that Hsc70 was involved in the proliferation and apoptosis in keratinocytes similar to circOAS3.

Hsc70 and circOAS3 regulated phenotypes via JNK/STAT3/NF-κB signaling pathways

Hsc70 was proved the involvement in the JNK/NF-κB signaling pathways[23]. STAT3, as well as IL-6, has a close relationship with JNK/NF-κB and they were all identified as playing crucial roles in inflammation reactions[24-27]. Based on the direct binding with each other, we speculated that circOAS3 and Hsc70 could involve in JNK/STAT3/NF-κB pathways in psoriatic keratinocytes. Western Blotting showed that phosphorylated levels of JNK/STAT3/NF-κB were decreased after circOAS3 or Hsc70 silencing alone, while total protein expressions were not significantly changed (Fig. 5a, b). In contrast, phosphorylated levels of JNK/STAT3/NF-κB were increased by overexpression of Hsc70, while total protein expressions were still unchanged (Fig. 5c). The protein level of IL-6 was detected by Western Blotting and ELISA assays (Fig. 5a-d). We found that IL-6 induced by M5 stimulation could be partly inhibited by circOAS3 or Hsc70 silencing alone. In contrast, an excess of IL-6 was observed in Hsc70 overexpressed group. The protein level of cyclinD1 was also detected by Western Blotting.

In mechanism, Hsc70 could promote the nuclear translocation of NF-κB and thus facilitate its activity[28]. We confirmed this internal connection in keratinocytes. Protein from the cytoplasm and nucleus extracted separately in HaCaT cells. Western Blotting showed M5 stimulation induced a nucleo-cytoplasmic shuttling that caused a nuclear accumulation of Hsc70, cyclinD1, and phosphorylated JNK/STAT3/NF-κB. After circOAS3 silencing, the nuclear accumulation of these proteins was partly inhibited (Fig. 5e), suggesting circOAS3 influenced the nucleocytoplasmic shuttling of Hsc70 and thus blocked the JNK/STAT3/NF-κB pathways. These data explained the regulation of circOAS3 to proliferation and inflammation in psoriatic keratinocytes.

Hsc70 mediates circOAS3-regulated proliferation and inflammation in vitro

To identify whether Hsc70 was involved in the mediation of circOAS3 in psoriatic keratinocytes. We overexpressed the Hsc70 in the circOAS3 silencing keratinocytes. After Hsc70 overexpression, the downregulation of proliferation in keratinocytes was partly resumed to a higher rate (Fig. 6a, b). DNA staining demonstrated that Hsc70 overexpression increased the proportion of cells in the S phase and decreased cells in the G1 phase in circOAS3 silencing keratinocytes (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 by circOAS3 silencing was aggravated because of the overexpression of Hsc70 in psoriatic keratinocytes (Fig. 5d). These results showed that circOAS3 silencing could inhibit M5-induced hyperproliferation and inflammation in a Hsc70 dependent way. Hsc70 overexpression weakens the protective effects of circOAS3 silencing. circOAS3 could thus interact with Hsc70 to regulate the M5-induced hyperproliferation and inflammatory response in psoriatic keratinocytes.

Discussion

This report provides the first demonstration that circOAS3 regulates proliferation and psoriatic inflammation by interacting with Hsc70 in keratinocytes.

Based on the circRNA microarray that circOAS3 was highly expressed in psoriatic lesions compared with that in normal lesions, we supposed circOAS3 contributes to the occurrence and development of psoriasis. Considering psoriasis is a chronic inflammatory disease accompanied by excessive proliferation and does not undergo apoptosis in keratinocytes[29, 30]. We used siRNA to silence the circOAS3 in vitro and observed the reducing proliferation and inflammation in keratinocytes, identifying a therapeutic effect in psoriasis.

In mechanism, other than ceRNA, emerging evidence has shown that circRNAs play an important role in various diseases via different mechanisms. For instance, CircCwc27 is directly bound to purine-rich element-binding protein A (Pur-a) in the pathogenesis of Alzheimer's disease [31]. circTshz2-2 combined with the YY1 transcriptional complex and suppressed Bdnf transcription to regulate the neuronal cell cycle and spatial memory in the brain[32]. circFAT1 promotes Cancer Stemness and Immune Evasion by binding to STAT3[33]. These evidence promoted us to explore a new mechanism of circOAS3 in psoriasis. Through RNA pulldown and protein spectroscopy, we found that circOAS3 could directly bind to Hsc70, which was proved upregulation in psoriasis that may be 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 [34]. Hsc70 has many essential functions. It maintains protein homeostasis[35], partakes in the process of new protein synthesis [36] and other significant cellular activities. Phenotypically, Hsc70 suppresses apoptosis and promotes proliferation in brain Endothelial Cell and tumor cells [23, 37]. In the immune system, Hsc70 modulates antigen transport within cells to control major histocompatibility complex (MHC) class II presentation during cellular stress [38].

A previous study showed Hsc70 was expressed almost exclusively in the suprabasal layers which was not detected basally[22]. However, it is not known whether Hsc70 is involved in the proliferation and inflammatory responses in keratinocytes. Our studies revealed that silencing of Hsc70 inhibited proliferation and reduce inflammation in keratinocytes. In contrast, overexpression of Hsc70 suppressed apoptosis and induced the cell cycle in keratinocytes, which is consistent with previous research. In addition, Hsc70 overexpression could partly reverse the therapeutic effect of circOAS3 silencing in psoriatic keratinocytes in proliferation and inflammation. Thus, we speculate that Hsc70 may represent a novel target for diagnosis and treatment in psoriasis.

In previous researches, Hsc70 could affect the phosphorylation of MAPK and promote nuclear translocation of NF-κB thus facilitating its activity[21, 37, 39]. We identified that Hsc70 reduction decreased the levels of phosphorylated JNK/STAT3/NF-κB in keratinocytes, which were also the downstream signaling proteins of circOAS3. We proved silencing circOAS3 could partly resume the nucleus translocation of Hsc70 induced by M5 stimulation, additionally influenced the translocation of phosphorylated JNK/STAT3/NF-κB and cyclinD1 as a result.

It was concluded in the present study that circOAS3 regulates proliferation and psoriatic inflammation in keratinocytes. circOAS3 directly binds to Hsc70 and affects its nucleocytoplasmic localization, which finally regulated the activation of JNK/STAT3/NF-κB signaling pathways. These findings provide promising insight into the molecular mechanism and target therapy of psoriasis.

Declarations

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Availability of data and materials

The original data is stored in the first author and the correspondent author, and all experimental raw data can be obtained from any one of them if necessary.

Authors' contributions

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

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 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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Figures

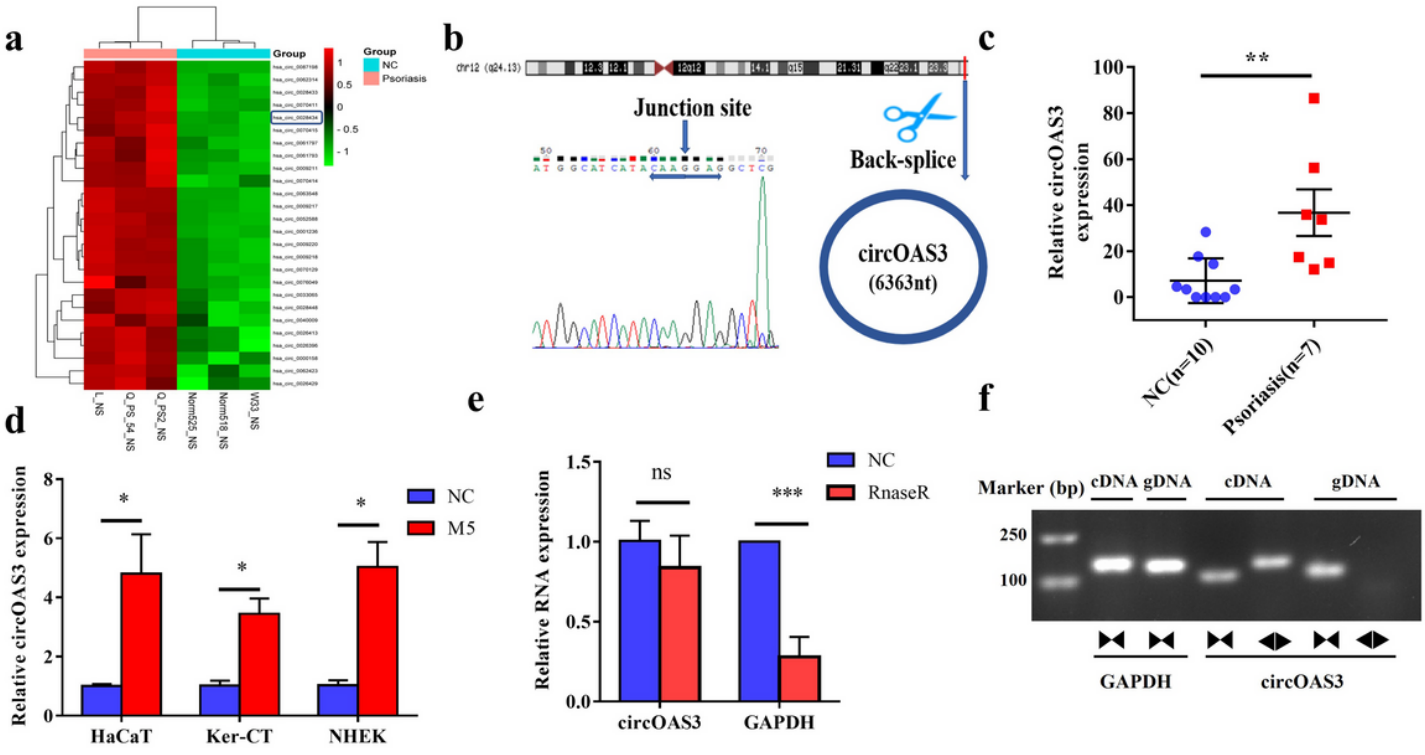

Figure 1

The circOAS3 expression and characterization. (a) Differentially expressed circRNAs in psoriatic and normal epidermal tissues from the circRNA microarray (Accession: GSE181318). (b) Schematic diagram of genomic location and splicing pattern of circOAS3. 'Head-to-tail' splicing sites of circOAS3 according to Sanger sequencing. (c) Total RNAs were isolated for use in qRT-PCR. Psoriatic samples exhibited significantly higher levels of circOAS3 compared to those in the healthy epidermal tissues. (d) Examination of the increased expression of circOAS3 in keratinocytes through qRT-PCR after M5 stimulation. (e Abundances of circOAS3 and GAPDH mRNA (reference gene) in keratinocytes treated with

or without RNase R, detected by qRT-PCR. (f) Divergent primers amplified circOAS3 from cDNA but not from genomic DNA (gDNA). Data are shown as mean ± SD. *p < 0.05, **p < 0.01, ***p□0.001.

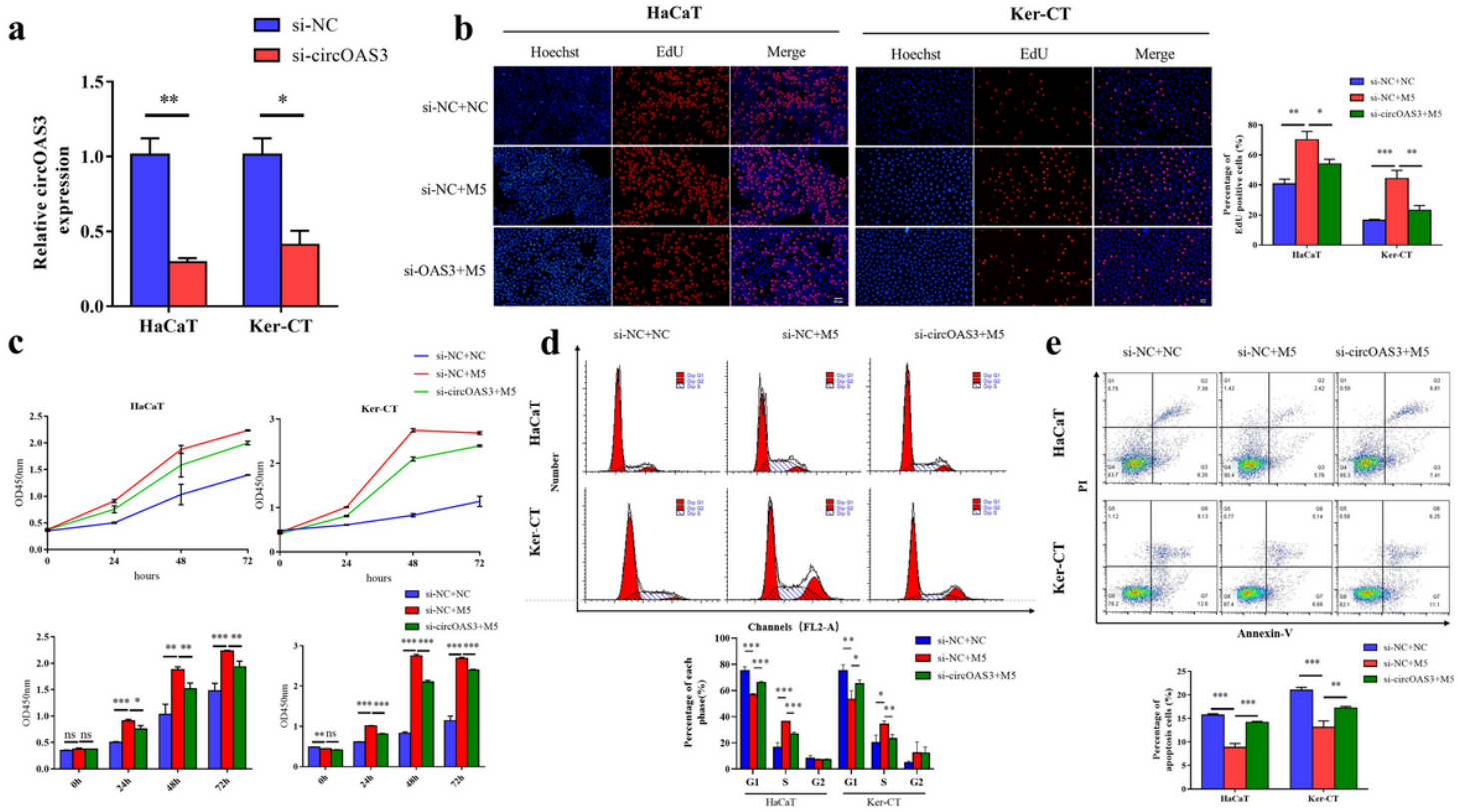

Figure 2

The regulatory function of circOAS3 in M5-induced psoriatic keratinocytes. (a) The efficiency of silencing circOAS3 detected by qRT-PCR. (b) circOAS3 functions in keratinocytes proliferation as detected by EdU assay. Nuclei were stained with Hoechst and a combined reaction involving EdU and Hoechst indicated the proliferating cells. (c) Cell proliferation capacity was detected at the indicated time points by CCK8 assays. (d) Cell-cycle distribution was measured by propidium iodide staining in HaCaT and Ker-CT cells, followed by flow cytometric analysis. (e) The apoptosis rate was detected by flow cytometry after downregulation of circOAS3. Data are shown as mean ± SD. *p < 0.05, **p < 0.01, ***p□0.001.

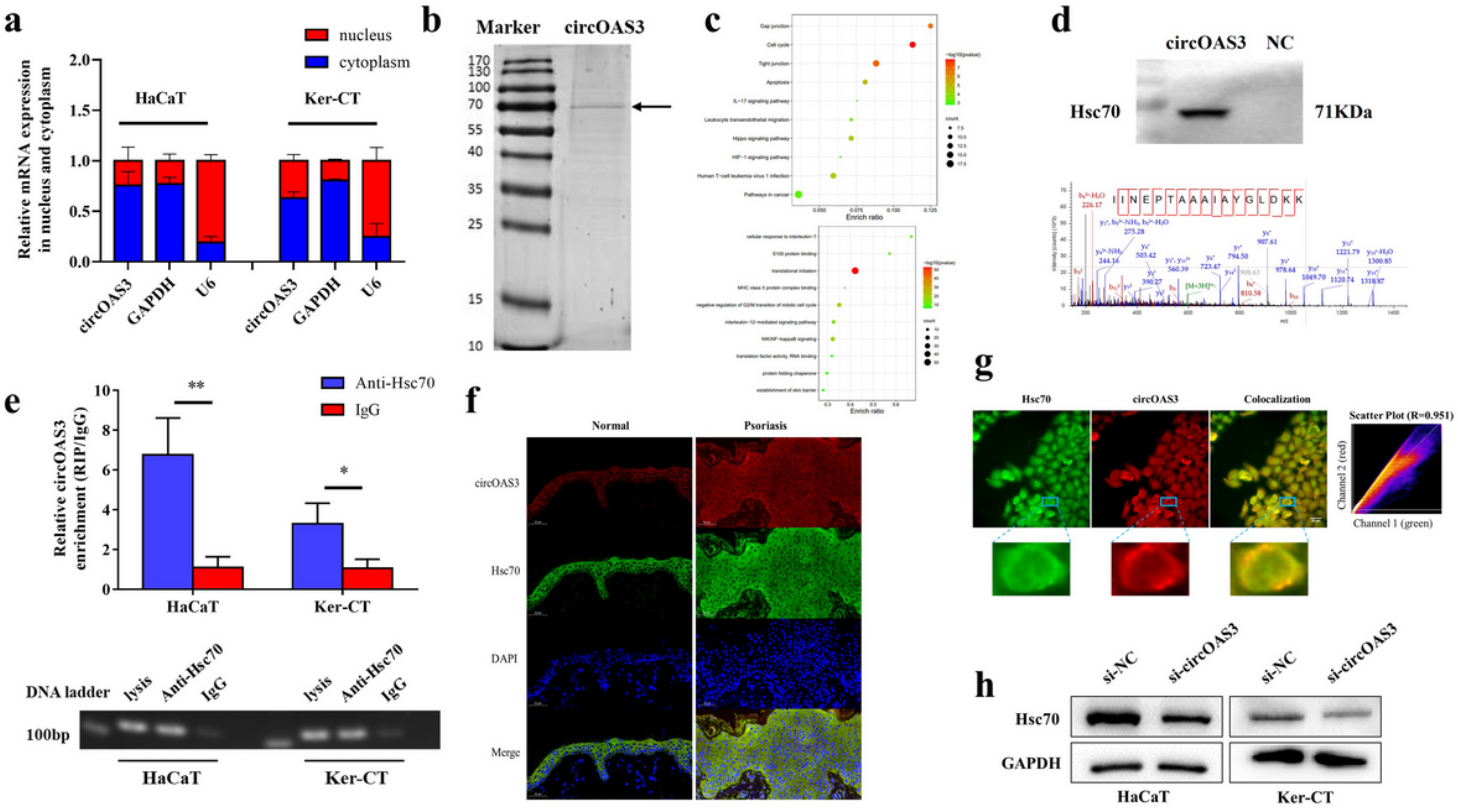

Figure 3

The circOAS3 interacts with Hsc70 protein. (a) circOAS3 mainly located in the cytoplasm of HaCaT and Ker-CT cells, GAPDH and U6 were applied as positive controls in the cytoplasm and nucleus, respectively. (b) Silver staining image of interaction proteins after circOAS3 pulldown experiment. (c) Kyoto Encyclopedia of Genes and Genomes analysis (KEGG) and Gene Ontology analysis (GO) of proteins binding with circOAS3. (d) Protein expression levels of Hsc70 in circOAS3-pulldown products and Mass spectrogram of Hsc70 protein. (e) RIP experiments were performed using an Ab against Hsc70 on extracts from HaCaT and Ker-CT cells, IgG was used as the control group. (f, g) Confocal microscopy was used and showed the colocalization of circOAS3 and Hsc70 in epidermal tissue and HaCaT cells by the circOAS3-Cy3-labeled probe (red) and Hsc70 antibodies (green). DAPI staining (blue) was performed to show the nucleus of cells. (h) Protein expression levels of Hsc70 after silencing circOAS3. Data are shown as mean ± SD. *p < 0.05, **p < 0.01, ***p□0.001.

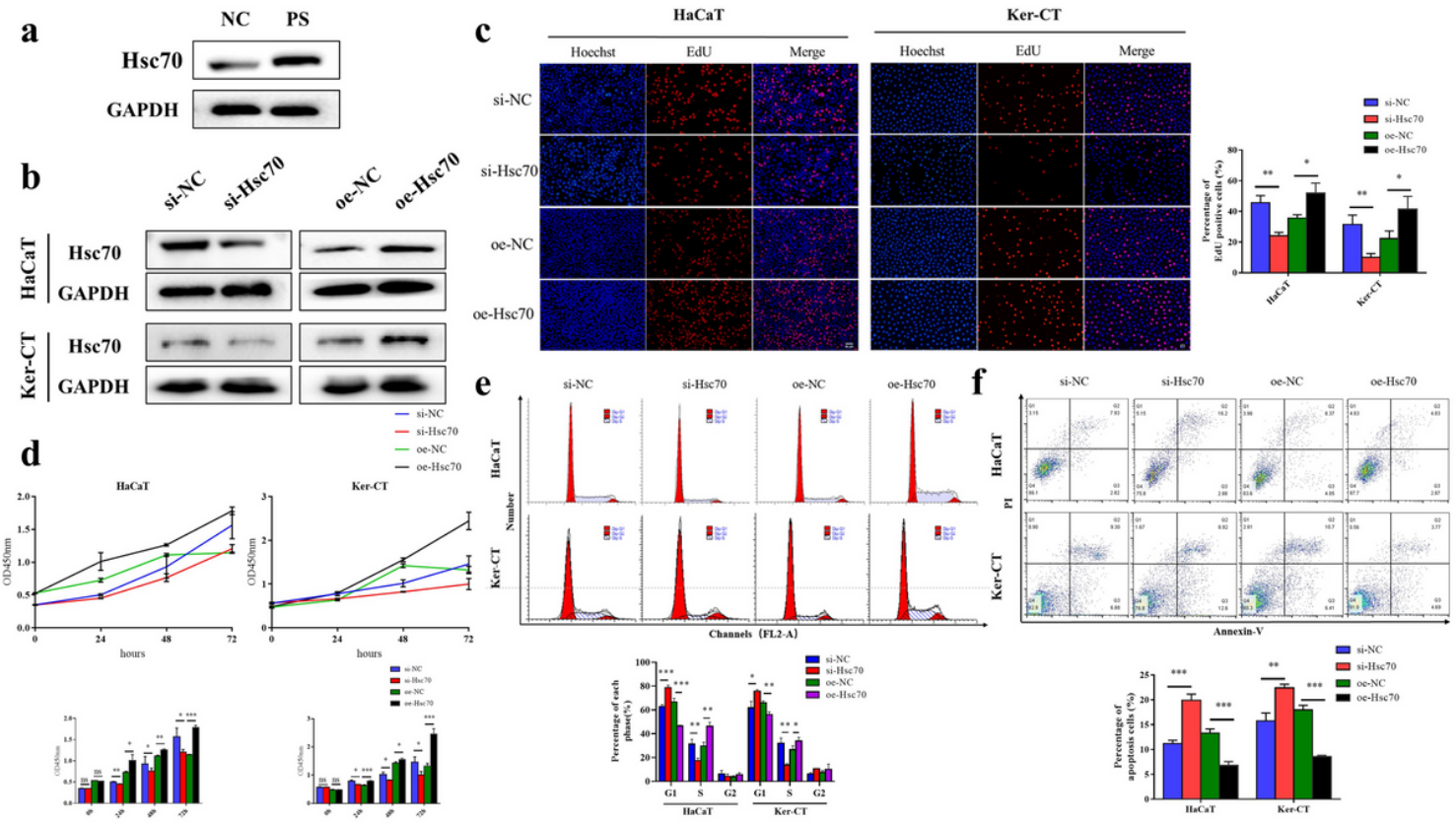

Figure 4

The regulatory function of Hsc70 in psoriatic keratinocytes. (a) Overexpression of protein Hsc70 in psoriatic epidermal tissue compared to healthy control. (b) The efficiency of silencing or overexpression Hsc70 detected by Western Blotting. (c) Hsc70 functions in keratinocytes proliferation as detected by EdU assay. Nuclei were stained with Hoechst and a combined reaction involving EdU and Hoechst indicated the proliferating cells. (d) Cell proliferation capacity was detected at the indicated time points by CCK8 assays. (e) Cell-cycle distribution was measured by propidium iodide staining in HaCaT and Ker-CT cells, followed by flow cytometric analysis. (f) The apoptosis rate was detected by flow cytometry after downregulation of Hsc70. Data are shown as mean ± SD. *p < 0.05, **p < 0.01, ***p□0.001.

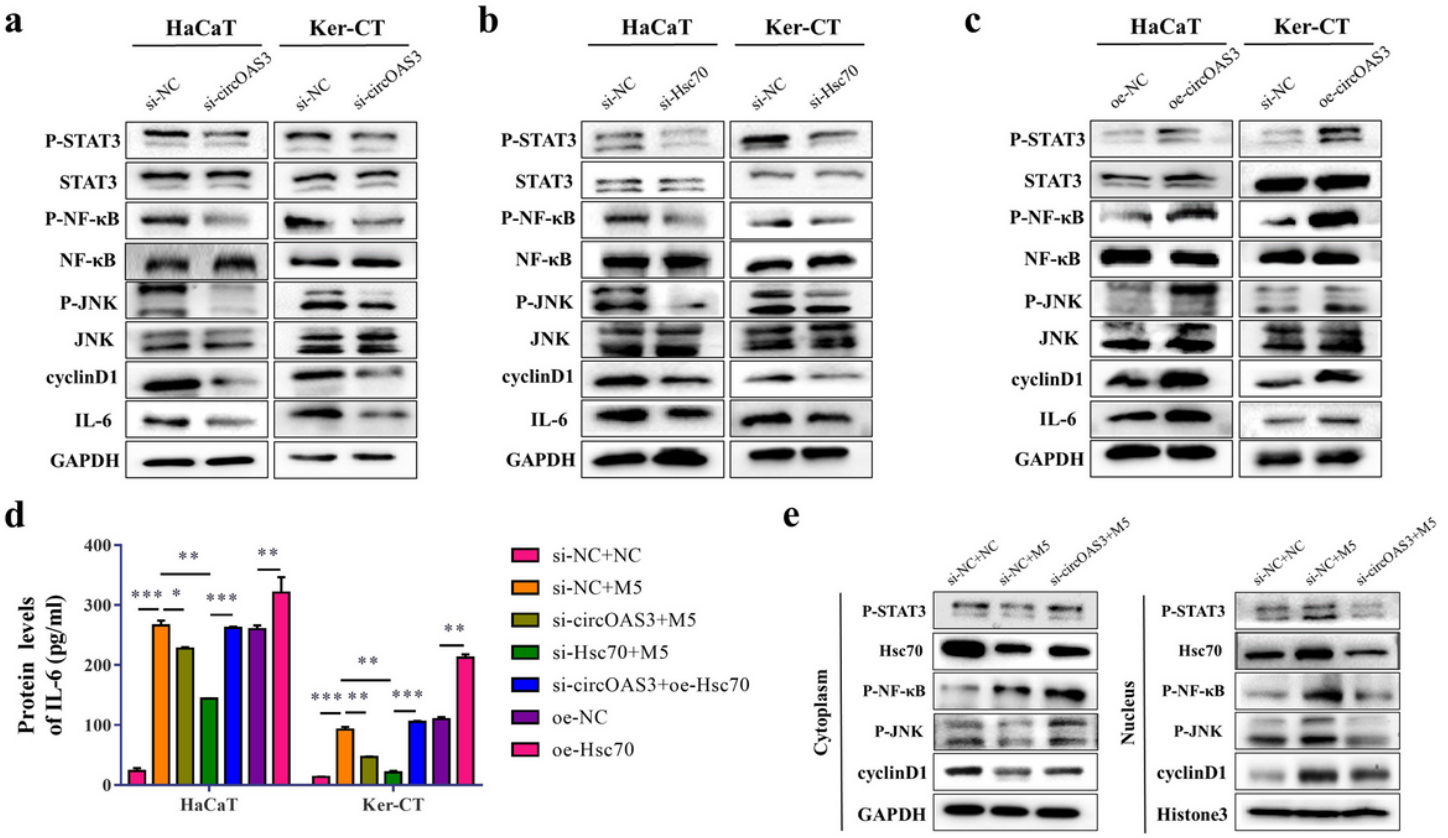

Figure 5

Dynamic effects of circOAS3 and Hsc70 on JNK/STAT3/NF-κB signaling pathway in keratinocytes. (a-c) Protein expression levels of p-STAT3, p-JNK, p-NF-κB, total STAT3, JNK, NF-κB,cyclinD1, and IL-6 in si-circOAS3, si-Hsc70, and oe-Hsc70 keratinocytes. (d) The keratinocytes were treated with siRNA or pcDNA after M5 stimulation for 24h, the supernatants were collected and concentrations of IL-6 were detected by ELISA kit (pg/ml). (e) Proteins in the nucleus and cytoplasm of HaCaT cells were fractionally extracted, and Hsc70, p-STAT3, p-JNK, p-NF-κB, and cyclinD1 expression was determined by western blotting. Data are shown as mean ± SD. *p < 0.05, **p < 0.01, ***p□0.001.

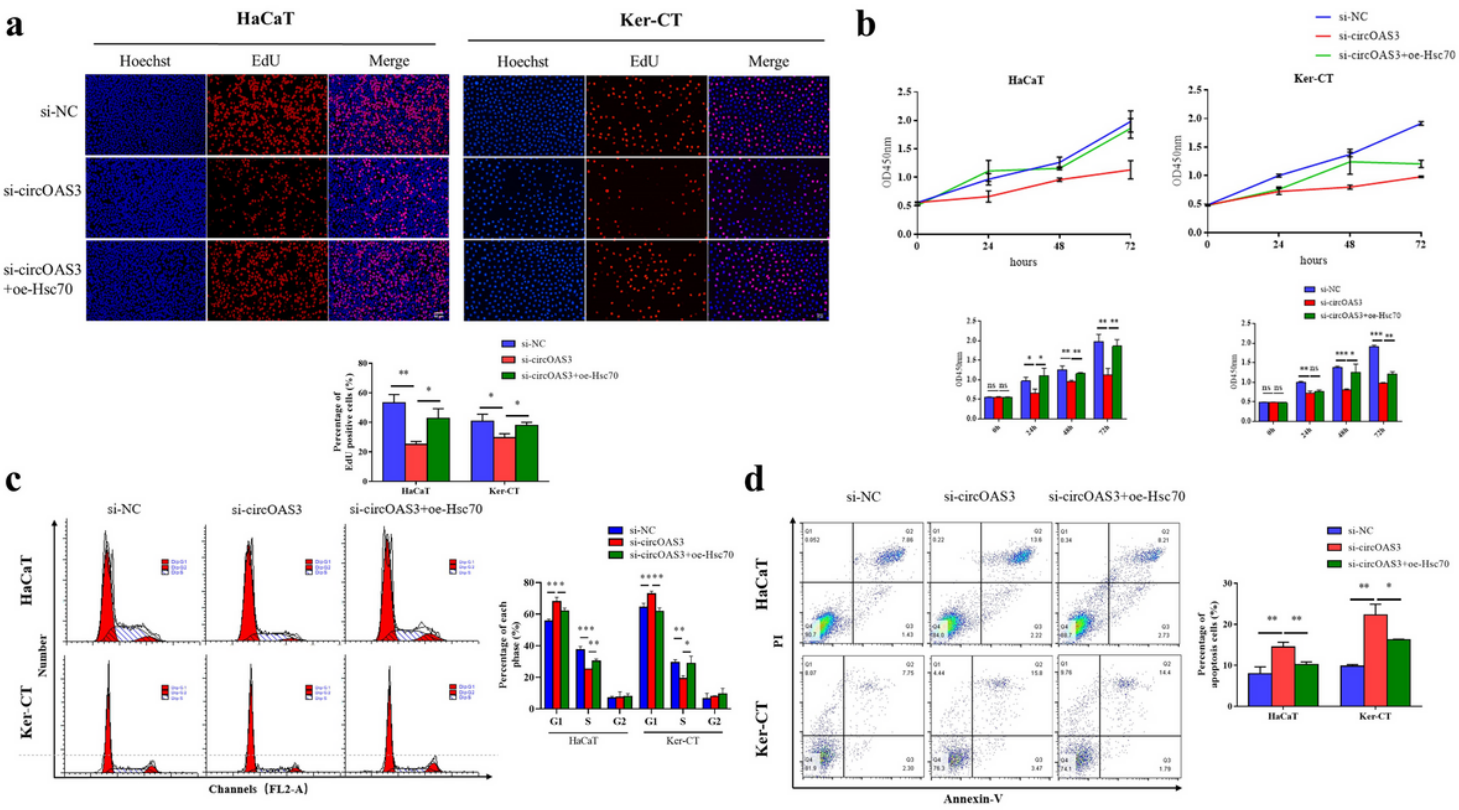

Figure 6

Hsc70 weakens the protective effects of circOAS3 silencing in keratinocytes. (a) Overexpression Hsc70 in circOAS3 silencing keratinocytes, then detected the proliferation rate by EdU assay, a combined reaction involving EdU and Hoechst indicated the proliferating cells. (b) Cell proliferation capacity was detected at the indicated time points by CCK8 assays. (c) Cell-cycle distribution was measured by propidium iodide staining in HaCaT and Ker-CT cells, followed by flow cytometric analysis. (d) The apoptosis rate was detected by flow cytometry after transfection. Data are shown as mean ± SD. *p < 0.05, **p < 0.01, ***p 0.001.

Supplementary Files

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