

# MicroRNA-17-3p is upregulated in psoriasis and regulates keratinocyte hyperproliferation and pro-inflammatory cytokine secretion by targeting *CTR9*

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## ABSTRACT

Psoriasis is a chronic inflammatory skin disease. Although miRNAs are reported to be associated with the pathogenesis of psoriasis, the contribution of individual microRNAs toward psoriasis remains unclear. The miR-17-92 cluster regulates cell growth and immune functions that are associated with psoriasis. miR-17-3p is a member of miR-17-92 cluster; however, its role in dermatological diseases remains unclear. Our study aims at investigating the effects of miR-17-3p and its potential target gene on keratinocytes proliferation and secretion of pro-inflammatory cytokine and their involvement in psoriasis. Initially, we found that miR-17-3p was upregulated in psoriatic skin lesions, and bioinformatic analyses suggested that *CTR9* is likely to be a target gene of miR-17-3p. Quantitative reverse-transcriptase PCR and immunohistochemical analysis revealed that *CTR9* expression was downregulated in psoriatic lesions. Using dual-luciferase reporter assays, we identified *CTR9* as a direct target of miR-17-3p. Further functional experiments demonstrated that miR-17-3p promoted the proliferation and pro-inflammatory cytokine secretion of keratinocytes, whereas *CTR9* exerted the opposite effects. Gain-of-function studies confirmed that *CTR9* suppression partially accounted for the effects of miR-17-3p in keratinocytes. Furthermore, Western blot revealed that miR-17-3p activates the downstream STAT3 signaling pathway while *CTR9* inactivates the STAT3 signaling pathway. Together, these findings indicate that miR-17-3p regulates keratinocyte proliferation and pro-inflammatory cytokine secretion partially by targeting the *CTR9*, which inactivates the downstream STAT3 protein, implying that miR-17-3p might be a novel therapeutic target for psoriasis.

**Key words:** Psoriasis; microRNA-17-3p; *CTR9*; keratinocytes; cell proliferation; pro-inflammatory cytokines.

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## Introduction

Psoriasis, a chronic inflammatory skin disorder, is characterized by erythematous scales or plaques covered with a thick layer of silvery scales.<sup>1</sup> Many studies have suggested that psoriasis is an imbalance in the regulation of the immune system under the interaction of a variety of factors, which ultimately causes abnormal epidermal keratinocyte function including abnormal proliferation and excessive secretion of inflammatory factors by mediating different pathways.<sup>2-4</sup> Previous studies have indicated that the secretion of pro-inflammatory cytokines including IL-6 and IL-1 $\beta$ <sup>5</sup> in the skin can promote chemokine production and induce immune cell infiltration, contributing toward the development of various inflammatory skin diseases, including psoriasis.<sup>2</sup> Nevertheless, the pathogenesis of psoriasis is not yet fully understood.

MicroRNAs (miRNAs) are a class of endogenous small non-coding RNA molecules that have a length ranging from 18 to 22 nt. They can negatively regulate the expression of target genes by binding to the 3'UTR region of target mRNAs, ultimately leading to their degradation or inhibiting their translation.<sup>6</sup> miRNAs have been found to exert considerable effects on many major cellular processes and its dysregulation has recently been confirmed to play a key role in the pathogenesis of psoriasis.<sup>7</sup> Studying the regulation of specific miRNAs and their target genes in psoriasis may therefore provide potential insights into the pathogenesis and could also provide a new perspective on the treatment of psoriasis.

miR-17-92, a well-studied microRNA cluster,<sup>8</sup> is described as the first miRNA oncogene discovered with genomic amplification and high expression in patients with B-cell lymphoma.<sup>9</sup> With a vital function in regulating cell growth and the normal developmental process of the immune system, the miR-17-92 cluster is associated with the development of diverse diseases, including cancers and autoimmune diseases.<sup>10,11</sup> Many members of the cluster have been shown to have critical roles in promoting proliferation, inhibiting apoptosis, and inducing tumor angiogenesis.<sup>12,13</sup> Although multiple studies have focused on the miR-17-92 cluster and its other mature members for a long time, little is known about the miR-17-3p. As an important member of the polycistronic miR-17-92 cluster, miR-17-3p exerts dual functions similar to oncogenes or tumor suppressor genes by regulating target genes which in turn affect downstream key signaling pathways.<sup>14,15</sup> Although the expression of passenger strand miRNA is often lower than that of the guide strand, increasing evidence has suggested that the passenger strand can also exert functions in many cellular processes,<sup>16</sup> making miR-17-3p an interesting avenue for further research.

Interestingly, in our research, upregulation of miR-17-3p was observed in psoriasis tissues, and CTR9 was predicted as a potential target using bioinformatics methods. CTR9, a key subunit of polymerase-associated factor 1 complex (PAF1c), is not only widely involved in oncogenesis by modulating major cellular functions including cell cycle progression,<sup>17</sup> but can also regulate immune response and thus participate in the pathogenesis of autoimmune diseases.

Therefore, we chose to focus on miR-17-3p and its potential targets CTR9 to study the regulation of keratinocytes function and explore the pathogenesis of psoriasis. Together, our results suggest that miR-17-3p regulates proliferation and pro-inflammatory cytokine secretion in keratinocytes partially by targeting CTR9,

providing new ideas and molecular therapeutic targets for the clinical diagnosis and treatment of psoriasis.

## Materials and Methods

### Human skin samples

Lesional skin were obtained from patients with psoriasis who underwent biopsies at the Department of Dermatology, Dermatology Hospital of Southern Medical University. Due to the difficulty in sampling, we failed to obtain non-lesional skin of psoriasis patients as controls. Patients were excluded from our study if they had other autoimmune or systemic diseases, or if they had received systemic therapy, or phototherapy within one month or if they had received topical therapy within 2 weeks of the sample collection period. We collected normal skin specimens at the dermatology department. For further immunohistochemical (IHC) analysis, the skin specimens (1 × 0.5 cm) were partly fixed with 10% formalin for 24 h and sent to the pathology department for further embedding processing. All patients and control subjects were sex- and age-matched, and they all provided written informed consent. Our research was approved by the Research Ethics Committee of Dermatology Hospital of Southern Medical University (Guangzhou, China).

### Cell culture

HaCaT cells were cultured in DMEM (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Sangon Biotech, Shanghai, China) prior to the *in vitro* experiments. HaCaT cells were cultured at 37°C under a 5% CO<sub>2</sub> atmosphere. Normal human epidermal keratinocytes (NHEKs) were extracted from prepuces obtained from healthy individuals who accepted circumcision and were cultured in the serum-free keratinocyte growth medium (Gibco).

### Cell transfection

miR-17-3p expression in HaCaT cells / NHEKs was manipulated by transfection with an miR-17-3p mimic, miR-17-3p inhibitor, or control (Ribobio, Guangzhou, China). CTR9 was overexpressed using the pcDNA3.1-CTR9 plasmid (1  $\mu$ g/mL, BGI), with a pcDNA3.1 empty plasmid (BGI) as the control. CTR9 was knocked down using siRNA-CTR9 (5 nM), with scrambled siRNAs as normal control (Ribobio). Cells were transfected using lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) for the miRNA transfection experiment and Lipofectamine 2000 (Invitrogen) for plasmid transfection. RNA interference experiments were performed following the instructions for use of reagent. Transfection efficiency was measured using reverse-transcriptase quantitative PCR (RT-qPCR) at 48 h after transfection.

### RT-qPCR

Total RNA was extracted from HaCaT cells, NHEKs or skin tissues using TRIzol reagent (Invitrogen) and miRNA was reverse transcribed using an Mir-X miRNA First-Strand Synthesis Kit (Takara, Otsu, Shiga, Japan). miRNA expression was detected using a Mir-X miRNA RT-qPCR TB Green® Kit (Takara) according to the manufacturer's instructions. The CTR9 primer sequences are listed in Table 1. We use GAPDH and RUNU6 (U6) to normalize the expression levels of mRNA and miRNA. We pur-

**Table 1.** mRNA primers used for RT-qPCR.

mRNA	Forward primer (5'–3')	Reverse primer (5'–3')
Homo-CTR9	CCACGTCATATAATCTCGCCAG	AAGTTCCCTTATCTCTAGCCATG

chased the miR-17-3p and U6 primers from Guangzhou Ribobio, but the company did not provide the primer sequences. All real-time PCR assays were performed using a LightCycler Real-Time PCR System (Bio-Rad, Berkeley, CA, USA). Relative miR-17-3p and CTR9 expression were calculated according to the  $2^{-\Delta\Delta CT}$  method.

### Cell counting kit-8 (CCK-8) proliferation assay

The growth rates of HaCaT cells or NHEKs were evaluated by a CCK-8 assay (ApexBio, Houston, TX, USA) according to the manufacturer's instructions. We analyzed the optical absorbance at 450 nm using a plate reader (Bio-Rad). Each sample was analyzed in triplicate. Each experiment has been performed three times, independently, each with technical replicates.

### Luciferase reporter assay

The CTR9 3'UTR DNA sequences containing the miR-17-3p binding site were extended using PCR and established into the psiCHECK<sup>TM</sup>-2 vector to obtain the recombinant psiCHECK<sup>TM</sup>-2-CTR9-WT, which was used to create the recombinant psiCHECK<sup>TM</sup>-2-CTR9-Mut vector *via* site-directed mutagenesis. HaCaT cells were seeded in 96-well plates. psiCHECK<sup>TM</sup>-2-CTR9-WT and psiCHECK<sup>TM</sup>-2-CTR9-Mut (320 ng/well) were transfected into HaCaT cells with the miR-17-3p mimic, inhibitor, and control using Lipofectamine RNAiMAX (Invitrogen). After 48 h, the Renilla and firefly luciferase activity of each group of cells was detected using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) according to the manufacturer's instructions.

### Western blotting analysis

Total protein was extracted after 48 h of cell culture and quantified using the BCA kit. Equal amounts of protein samples were subjected to polyacrylamide gel electrophoresis and then transferred to polyvinylidene fluoride membranes. After blocking with 5% skimmed milk powder for 1 h at room temperature, primary antibodies were added and incubated overnight at 4°C (Table 2) followed by specific secondary antibodies for 1 h at 37°C. Bound antibodies were visualized using an ECL Western blotting detection system (Bio-Rad) and quantified using Image-Pro Plus 8.0. The gray value of target bands was normalized to the GAPDH band.

### IHC analysis

Paraffin-embedded human skin tissues were cut into sections (5 µm). After the sections being deparaffinized and rehydrated, we used citrate solution (pH 6.0) for antigen-retrieval and incubated with CTR9 antibodies for 30 min which were diluted in primary antibody dilution buffer (BH-DB1042), followed by biotinylated goat anti-rabbit secondary antibody for 20 min at 37°C which was diluted in PBS. Signals were detected by staining with DAB, and slides were observed under a bright-field microscope. Human breast cancer tissue was used as a positive control and PBS was used instead of the primary antibody for negative control. The primary antibodies used are listed in Table 2. As we are concerned

about the strength of positive signal in the epidermis, our target section areas to be measured were the epidermis parts of the section and we randomly selected three areas for each section. Then we used the Image-Pro Plus 8.0 to calculate the average optical density (AOD) of the three areas for the semi-quantitative analysis of CTR9.

### ELISA assay

The secretion of the pro-inflammatory cytokines, IL-1β and IL-6, by the transfected HaCaT cells or NHEKs was detected using a human ELISA Kit (Raybiotech, Peachtree Corners, GA, USA). The absorbance at 450 nm was detected with a microplate reader (Bio-Rad).

### Statistical analysis

We used GraphPad Prism (GraphPad Prism 8.0; San Diego, CA, USA) for data analysis. All data are presented as the mean ± standard deviation (SD). Comparisons between two groups were performed using the independent samples Student's *t*-test; *p*-values of <0.05 were considered statistically significant.

## Results

### MiR-17-3p upregulation in psoriatic skin lesions and target prediction

Many reports have demonstrated that miRNA dysregulation plays a critical role in the pathogenesis of psoriasis. We revised public repository for RNAseq data (GSE174763) from psoriasis and performed bio-informatics analysis. The results shown in Supplementary Figure 1 found that miR-17-3p is upregulated in the skin lesions of patients with psoriasis. Consistent with this result, in our study, miR-17-3p was detected to be increased in psoriatic skin lesions (n=7) than in normal tissues (n=6; Figure 1A); Next, we used four bioinformatics software programs (targetscan7.0, DIANA-microT, TmiRDB, and mirDIP) to predict its putative targets. Because CTR9 was identified as a potential target, we measured its expression in psoriatic tissues; our results showed that CTR9 mRNA levels were significantly lower in psoriatic lesion skin samples (n=8) than in normal controls (n=8; Figure 1B). Correspondingly, IHC analysis indicated a marked reduction in CTR9 protein levels in psoriatic skin (n=7) compared to healthy controls (n=10; Figure 1C). Together, these results suggest that miR-17-3p upregulation and CTR9 downregulation are likely functionally involved in the occurrence of psoriasis.

### CTR9 is a direct target of miR-17-3p

To investigate whether CTR9 is a target gene of miR-17-3p, luciferase reporter assays were performed. Briefly, wild-type (WT) 3'UTR target sequences of miR-17-3p were predicted by targetscan (Figure 2A), and we mutated the miR-17-3p seed site to form a mutant (MUT) CTR9 3'UTR (Figure 2B). Then, we constructed the luciferase reporter vectors containing the 3'UTR region of CTR9, including the WT and Mut type. In HaCaT cells

**Table 2. Information for primary antibodies used in described studies.**

Antibodies	Company	Article No.	Application
Rabbit anti-human CTR9 antibody	Cell Signaling Technology Proteintech	#12619 21264-1-AP	WB: 1:1000 IHC: 1:1000
STAT3	Cell Signaling Technology	#12640	WB: 1:1000
Phospho-STAT3	Cell Signaling Technology	#9145	WB: 1:1000

transfected with the recombinant dual-luciferase reporter plasmid psiCHECK™-2-CTR9-WT, the luciferase activity of the miR-17-3p mimic group was decreased, while that of the miR-17-3p inhibitor group was increased. However, in HaCaT cells transfected with the recombinant dual-luciferase reporter plasmid psiCHECK™-2-CTR9-Mut, there were no significant differences in luciferase activity in the miR-17-3p mimic and inhibitor group compared with the control group (Figure 2C). We also evaluated the effects of miR-17-3p on *CTR9* expression; our results showed that miR-17-3p upregulation decreased the mRNA and protein expression of *CTR9* in HaCaT cells transfected with miR-17-3p mimic. Conversely, miR-17-3p downregulation increased the mRNA and protein expression of *CTR9* in the miR-17-3p inhibitor group (Figure 2 D,E). Collectively, we concluded that *CTR9* is a target of miR-17-3p and can negatively regulate *CTR9* expression.

### miR-17-3p and *CTR9* can regulate keratinocyte proliferation

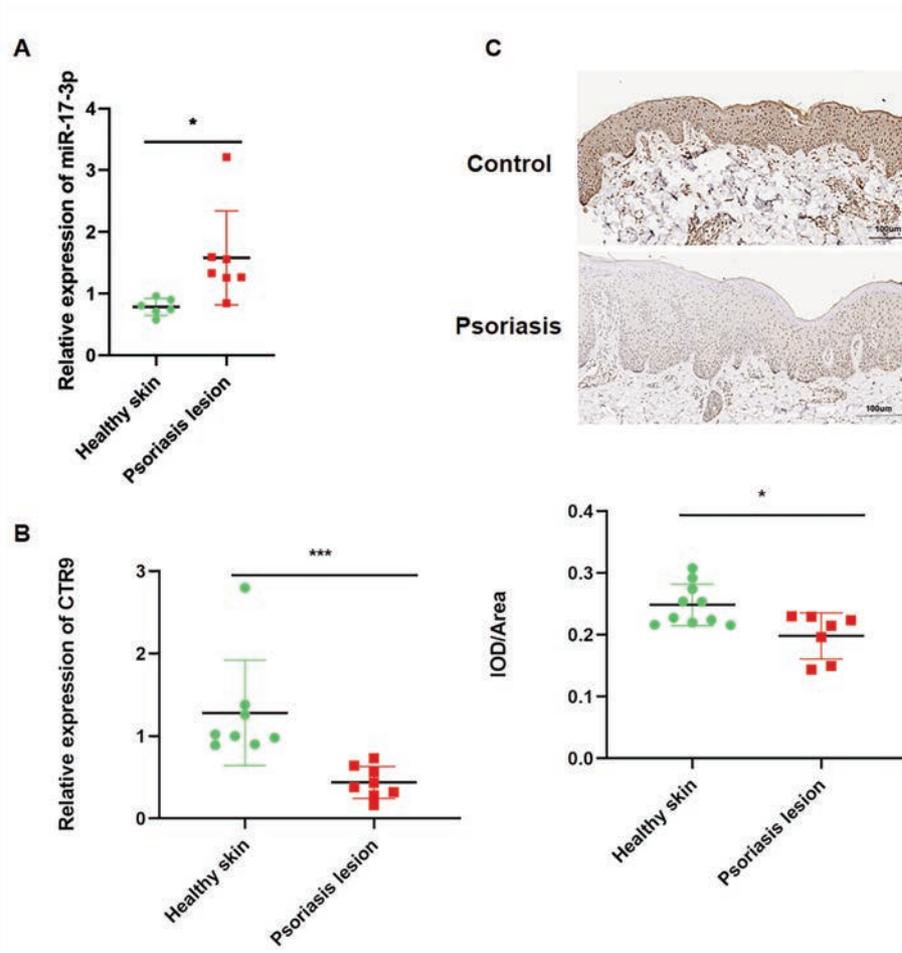
As abnormal keratinocyte proliferation is a key feature in the pathogenesis of psoriasis, we evaluated the potential role of miR-17-3p and *CTR9* in keratinocytes proliferation. After transfecting HaCaT cells with miR-17-3p mimics or inhibitors to increase or decrease miR-17-3p expression, respectively (Figure 3A), we per-

formed CCK-8 assays to measure the cell proliferation ability. Interestingly, the miR-17-3p mimic promoted HaCaT cell proliferation, whereas the miR-17-3p inhibitor suppressed their proliferation (Figure 3B). Furthermore, we conducted those functional experiments in NHEKs and the results were consistent (Supplementary Figure 2A).

To verify the role of *CTR9* in HaCaT cell proliferation, we transfected cells with small interfering RNA (siRNA) or pcDNA 3.1-CTR9 to up- or downregulate endogenous *CTR9* expression. RT-qPCR analysis revealed that *CTR9* expression was successfully silenced by siRNA-CTR9 and overexpressed by pcDNA3.1-CTR9 24 h after transfection (Figure 3C). Similarly, CCK-8 assays demonstrated that silencing *CTR9* promoted HaCaT cell proliferation, whereas *CTR9* overexpression suppressed HaCaT cell proliferation (Figure 3D). Therefore, the effects of *CTR9* knockdown on HaCaT cell proliferation appear to be similar to the effects of miR-17-3p upregulation, whereas *CTR9* overexpression exerts similar effects on miR-17-3p downregulation.

### miR-17-3p and *CTR9* can regulate keratinocyte pro-inflammatory cytokine secretion

We performed ELISA and RT-qPCR to investigate whether miR-17-3p could promote IL-6 and IL-1 $\beta$  production in HaCaT

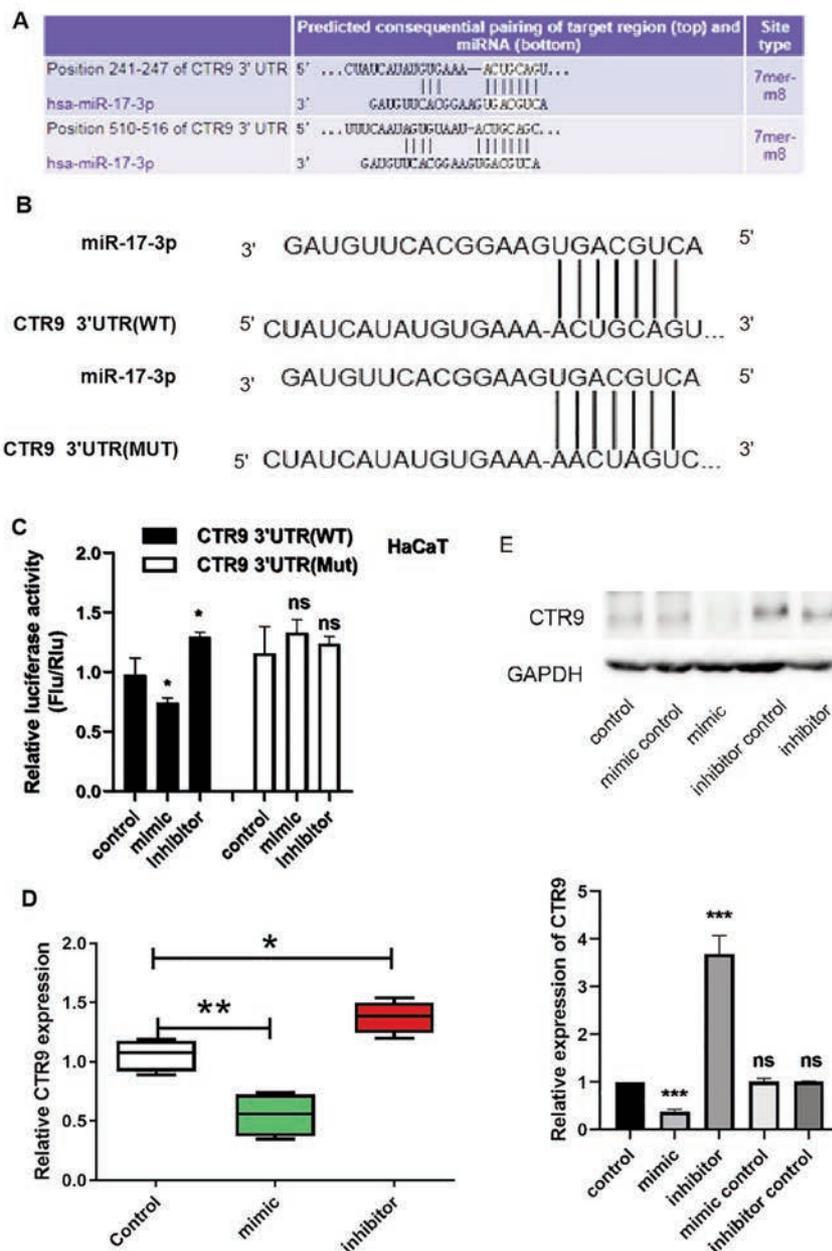


**Figure 1.** Upregulation of miR-17-3p in psoriatic skin and its target prediction. A) miR-17-3p expression was determined in the psoriatic skin (n=7) and in the skin of healthy controls (n=6) by RT-qPCR. B) *CTR9* mRNA expression was determined in the psoriatic skin (n=8) and healthy control skin (n=8) by RT-qPCR. C) *CTR9* protein expression levels are shown with the mean optical density (MOD) in psoriatic skin (n=7) and healthy control skin (n=10) by immunohistochemistry; the expression levels of miR-17-3p and *CTR9* were calculated using the  $2^{-\Delta\Delta C_t}$  method. Data are based on three independently performed experiments and are presented as mean  $\pm$  SD. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

cells / NHEKs. Both the miR-17-3p mimic and siRNA-CTR9 facilitated the production of both pro-inflammatory cytokines in HaCaT cells, whereas the miR-17-3p inhibitor and pcDNA 3.1-CTR9 inhibited the secretion of the cytokines compared to the control group (Figure 4 A,B). Therefore, miR-17-3p upregulation and CTR9 knockdown appear to have similar effects on pro-inflammatory cytokine secretion in HaCaT cells, as well as miR-17-3p downregulation and CTR9 overexpression. Furthermore, we found that miR-17-3p promoted the production of both pro-inflammatory cytokines in NHEKs (Supplementary Figure 2 B,C).

### CTR9 suppression partially accounts for miR-17-3p-induced changes in keratinocytes

To confirm whether CTR9 mediated the regulatory effects of miR-17-3p on proliferation and pro-inflammatory cytokine secretion in keratinocytes, we performed gain-of-function experiments. As shown in Figure 3B, the introduction of CTR9 *via* pcDNA3.1-CTR9 partially abrogated the miR-17-3p mimics-induced increase in cell proliferation, whereas siRNA-CTR9 interference with CTR9 expression partially prevented the miR-17-3p inhibitors-mediated suppression of cell proliferation. Moreover, pcDNA3.1-



**Figure 2.** CTR9 is a direct target of miR-17-3p. A) The potential miR-17-3p seed regions at the 3'UTR of *CTR9* mRNA predicted by TargetScan. B) The vector containing wild-type and mutated-type CTR9 3'UTR sequences was constructed. C) The relative luciferase activity of HaCaT cells co-transfected with psiCHECK<sup>TM</sup>-CTR9-WT + miR-17-3p mimic/inhibitor/control or psiCHECK<sup>TM</sup>-CTR9-Mut + miR-17-3p mimic/inhibitor/control; luciferase activity was normalized by the ratio of Renilla luciferase and firefly signals; the significance of luciferase activity was analyzed using Student's t-test. D) CTR9 mRNA expression was detected following transfection with miR-17-3p mimic/inhibitor/control in HaCaT cells. E) CTR9 protein expression was detected following transfection with miR-17-3p mimic/inhibitor/control in HaCaT cells. Data are based on three independently performed experiments and are presented as mean  $\pm$  SD. \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001.

CTR9 partially abrogated the miR-17-3p mimic-mediated promotion of IL-6 and IL-1 $\beta$  secretion from HaCaT cells, whereas siRNA-CTR9 partially prevented the miR-17-3p inhibitor-mediated suppression of the cytokines (Figure 4A). In summary, CTR9 accounts for some of the effects of miR-17-3p on keratinocytes.

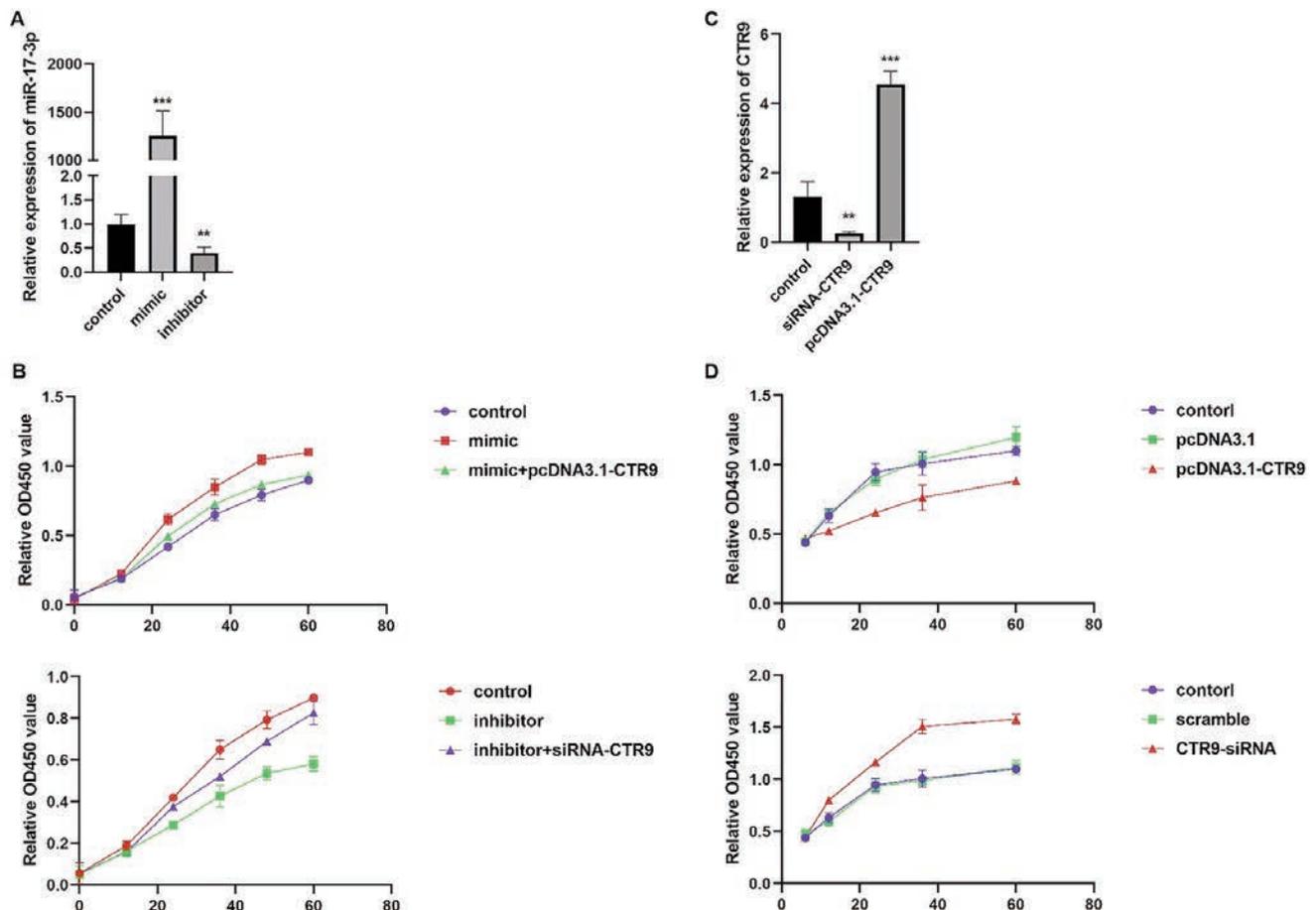
### miR-17-3p likely regulates STAT3 signaling pathway by targeting CTR9 in HaCaT cells

To explore the role of miR-17-3p in the STAT3 signaling pathway, we performed Western blot analyses. In HaCaT cell transfected with the miR-17-3p mimic, phospho-STAT3 (p-STAT3) protein expression was increased, and in HaCaT cell transfected with miR-17-3p inhibitor, p-STAT3 expression levels were decreased (Figure 4C). In addition, we confirmed that STAT3 is a downstream protein of CTR9, as reported previously.<sup>18</sup> As expected, p-STAT3 expression was upregulated following the transfection of HaCaT cells with siRNA-CTR9 but was down-regulated with pcDNA3.1-

CTR9 (Figure 4D). Together, these findings suggest that miR-17-3p likely activates the STAT3 signaling pathway by downregulating CTR9 in HaCaT cells.

### Discussion

Psoriasis is a chronic inflammatory disorder of skin featuring aberrant keratinocyte proliferation and the production of inflammatory mediators, including pro-inflammatory cytokines;<sup>19</sup> however, the exact pathogenesis of psoriasis remains unclear. Recent studies have identified many dysregulated miRNAs in psoriasis,<sup>20,21</sup> and Zhang *et al.*<sup>22</sup> found that the overexpression of the miR-17-92 cluster can promote keratinocyte proliferation and immune function and thus may contribute toward the development of psoriasis. Consistently, in our research, we detected increased miR-17-



**Figure 3.** Effects of miR-17-3p and CTR9 on keratinocyte proliferation *in vitro*. A) miR-17-3p expression levels were measured by RT-qPCR 24 h after transfection with miR-17-3p mimic/inhibitor/control. B) The proliferation curves of HaCaT cells after transfection with miR-17-3p mimic/control or miR-17-3p inhibitor/control at 0, 12, 24, 36, 48, and 60 h obtained by CCK-8 assays. C) CTR9 (mRNA) expression levels were measured by RT-qPCR after transfection with siRNA-CTR9/pcDNA 3.1-CTR9/control. D) Proliferation curves of HaCaT cells after transfection with pcDNA 3.1-CTR9/pcDNA3.1/control or siRNA-CTR9/scramble/control at 6, 12, 24, and 36 h by CCK-8 assays; optical density (OD) values at 450 nm of each point were calculated from three independent experiments. Data are presented as mean $\pm$ SD. \* $p$ <0.05; \*\* $p$ <0.01; \*\*\* $p$ <0.001.

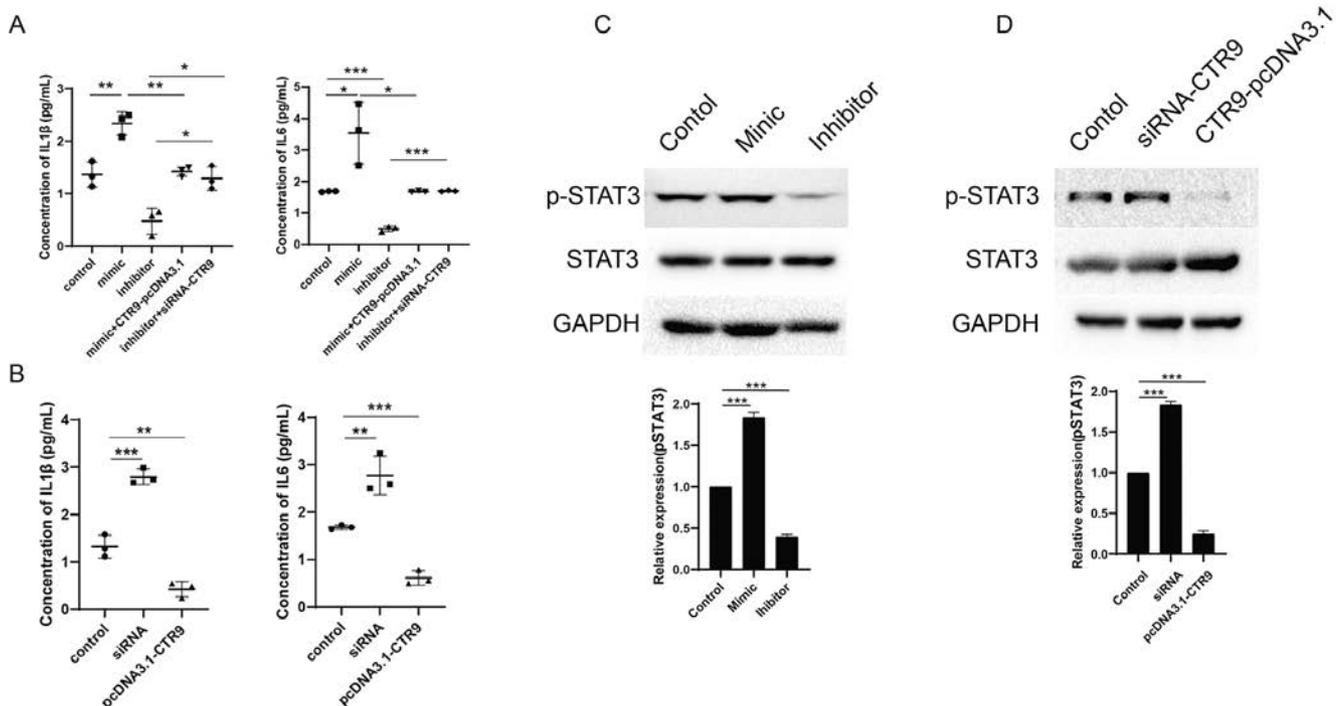
3p expression in the psoriasis skin lesions. In contrast to the present study, miR-17-3p expression levels were downregulated in blood samples of psoriasis patients,<sup>23</sup> this contradictory result may be due to the fact that the majority of patients with psoriasis included in the study by Atalas *et al.* presented milder disease, while the patients had severe diseases in our study, or due to the fact that blood mainly includes immune cells, while epidermis mainly contains keratinocytes, implying that miR-17-3p may have different expression patterns in the two types of cells.

Multiple reports have shown the important function miR-17-3p has in the occurrence of cancers. For instance, miR-17-3p functions as an oncogene in anaplastic thyroid cancer, the inhibition of which causes the complete growth arrest of ATC cells followed by apoptosis *via* caspase activation.<sup>24</sup> Similarly, Lu *et al.*<sup>25</sup> reported that miR-17-3p can regulate colon cancer cells survival by inhibiting Par4 expression, thus contributing toward the tumorigenesis of colorectal cancer. Passenger strand miR-17-3p and its guide strand miR-17-5p were also found to induce hepatocellular carcinoma development by targeting PTEN,<sup>26</sup> while another study demonstrated that miR-17-3p serves as a tumor suppressor gene and suppresses glioblastoma cell proliferation and drug resistance by down-regulating MDM2.<sup>27</sup> Although a study showed that miR-17-3p could promote keratinocyte proliferation and metastasis in cutaneous wound healing,<sup>28</sup> no studies have yet elucidated the precise regulatory networks and mechanisms *via* which miR-17-3p acts in keratinocytes and its role in psoriasis. In our research, we demon-

strated that miR-17-3p regulated the function of keratinocytes *via* CTR9, which had not been reported before.

A previous study by Hanks *et al.* suggested that germline *CTR9* mutations can predispose individuals to Wilms tumor,<sup>29</sup> indicating that *CTR9* may serve as a tumor suppressor gene; however, another study suggested that *CTR9* functions as an oncogene by regulating estrogen signaling during ER $\alpha$ <sup>+</sup> breast tumorigenesis.<sup>30</sup> In addition to playing key roles in human cancer by regulating cell-cycle progression, *CTR9* is known to affect immune responses. For instance, *CTR9* has been reported to regulate the expression levels of downstream genes involved in the IL-6/STAT3 signaling pathway, including IL-17, by interacting with the downstream protein STAT3.<sup>18</sup> Moreover, *CTR9* downregulation has been detected in the anti-glomerular basement membrane glomerulonephritis, in which *CTR9* represses Th17 differentiation.<sup>31,32</sup> Given that *CTR9* critically regulates Th17 cell differentiation *via* the IL-6/STAT3 signaling pathway, which is associated with the pathogenesis of psoriasis,<sup>33</sup> it will be worth to study in future the immunological role of *CTR9* in Th17 cell differentiation in psoriasis.

STAT3 is a critical transcription factor and its activation has been widely reported in psoriasis.<sup>34</sup> Various reports have demonstrated a potential regulatory mechanism of miR-17-3p in human diseases; for instance, miR-17-3p modulates the PI3K/AKT/mTOR signaling pathway during cardiac remodeling in response to physical exercise<sup>35</sup> and regulates Notch1 NF- $\kappa$ B signaling pathways during keratinocyte growth and metastasis.<sup>28</sup>



**Figure 4.** Effects of miR-17-3p and *CTR9* on pro-inflammatory cytokine secretion of keratinocytes; miR-17-3p likely regulates the STAT3 signaling pathway by targeting *CTR9* in HaCaT cell. A) The culture media of HaCaT cells in the miR-17-3p mimic/inhibitor/control group were analyzed by ELISA to measure the secretion levels of IL-1 $\beta$  and IL-6. B) The culture media of HaCaT cells in the siRNA-CTR9/pcDNA 3.1 CTR9/control group were analyzed by ELISA to measure the secretion levels of IL-1 $\beta$  and IL-6. C) HaCaT cells were transfected with miR-17-3p mimics/inhibitor/control; the protein expression levels of p-STAT3 and STAT3 were determined by Western blotting 48 h after transfection. D) HaCaT cells were transfected with siRNA-CTR9/pcDNA 3.1-CTR9/control; the protein expression levels of p-STAT3 and STAT3 were determined by Western blotting 48 h after transfection; *GAPDH* was used as a control. The band intensity values were analyzed using Imagem. Data are based on three independently performed experiments and are presented as mean  $\pm$  SD. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

However, no studies have yet explored the relationship between miR-17-3p and the STAT3 signaling pathway. Interestingly, our research found that miR-17-3p activates the STAT3 signaling pathway in HaCaT cells. Although STAT3 is a downstream protein of *CTR9* in the Th17 cell differentiation, it is still unclear whether the effect on STAT3 was mediated through *CTR9* or by other targets of miR-17-3p in HaCaT cells in the pathogenesis of psoriasis. In the future we will perform more experiments to explore the exact regulatory mechanism.

Despite these important findings, our study has several potential limitations. First, the clinical sample size was small and needs to be expanded in future studies. Second, additional animal studies are required to confirm that targeting miR-17-3p or *CTR9* could have potential therapeutic effects against psoriasis. Third, it has been reported that non-lesional skin in psoriatic patients despite its normal appearance, represents an intermediate state between healthy and lesional skin and exhibits numerous hallmarks of dormant psoriasis.<sup>36</sup> Due to the difficulty in sampling non-lesional skin of psoriasis patients, we failed to conduct relevant experiments to verify it.

In conclusion, our study demonstrates that miR-17-3p regulates the proliferation and pro-inflammatory cytokine secretion of keratinocytes partially by targeting *CTR9* and miR-17-3p likely regulates STAT3 signaling pathway *via* targeting *CTR9*. Collectively, the findings of the present study provide a new perspective on the pathogenesis and treatment of psoriasis, which will provide hope for curing patients with psoriasis.

## References

- Boehncke WH, Schön MP. Psoriasis. *Lancet* 2015;386:983-94.
- Albanesi C, Madonna S, Gisondi P, Girolomoni G. The interplay between keratinocytes and immune cells in the pathogenesis of psoriasis. *Front Immunol* 2018;9:1549.
- Raju SS. Psoriasis and lasting implications. *Expert Rev Clin Immunol* 2014;10:175-7.
- Liang XQ, Ou CX, Zhuang JY, Li JS, Zhang FF, Zhong YQ, et al. Interplay between skin microbiota dysbiosis and the host immune system in psoriasis: Potential pathogenesis. *Front Immunol* 2021;12:764384.
- Di Fusco D, Stolfi C, Di Grazia A. Albendazole negatively regulates keratinocyte proliferation. *Clin Sci* 2020;134:907-20.
- Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009;136:215-33.
- Hawkes JE, Nguyen GH, Fujita M, Florell SR, Callis Duffin K, Krueger GG, et al. microRNAs in Psoriasis. *J. Invest. Dermatol* 2016;136:365-71.
- Mogilyansky E, Rigoutsos I. The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease. *Cell Death Differ* 2013;20:1603-14.
- He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, et al. A microRNA polycistron as a potential human oncogene. *Nature* 2005;435:828-33.
- Kuo G, Wu CY, Yang HY. MiR-17-92 cluster and immunity. *J Formos Med Assoc* 2019;18:2-6.
- Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, et al. A polycistronic MicroRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Res* 2005;65:9628-32.
- Yuan J, Su Z, Gu W, Shen X, Zhao Q, Shi L, et al. MiR-19b and miR-20a suppress apoptosis, promote proliferation and induce tumorigenicity of multiple myeloma cells by targeting PTEN. *Cancer Biomark* 2019;24:279-89.
- Wang W, Zhang A, Hao Y, Wang G, Jia Z. The emerging role of miR-19 in glioma. *J Cell Mol Med* 2018;22:4611-6.
- Xiang P, Yeung YT, Wang JH, Wu Q, Du RJ, Huang CT, et al. miR-17-3p promotes the proliferation of multiple myeloma cells by downregulating P21 expression through LMLN inhibition. *Int J Cancer* 2021;148:3071-85.
- Wu S, Chen H, Han N, Zhang CX, Yan HT. Long noncoding RNA PVT1 silencing prevents the development of uveal melanoma by impairing microRNA-17-3p-dependent MDM2 upregulation. *Invest Ophthalmol Vis Sci* 2019;60:4904-14.
- Suzuki H, Miyazono K. Emerging complexity of microRNA generation cascades. *J Biochem* 2011;149:15-25.
- Marsh DJ, Ma Y, Dickson KA. Histone monoubiquitination in chromatin remodelling: Focus on the Histone H2B interactome and cancer. *Cancers* 2020;12:3462.
- Youn MY, Yoo HS, Kim MJ, Hwang SY, Choi Y, Desiderio SV, et al. hCTR9, a component of Paf1 complex, participates in the transcription of interleukin 6-responsive genes through regulation of STAT3-DNA interactions. *J Biol Chem* 2007;282:34727-34.
- Qiao P, Guo W, Ke Y, Fang H, Zhuang Y, Jiang M, et al. Mechanical stretch exacerbates psoriasis by stimulating keratinocyte proliferation and cytokine production. *J Invest Dermatol* 2019;139:1470-9.
- Sileno S, Beji S, D'Agostino M, Carassiti A, Melillo G, Magenta A. microRNAs involved in psoriasis and cardiovascular diseases. *Vasc Biol* 2021;3:R49-R68.
- Xiuli Y, Honglin W. miRNAs flowing up and down: The concerto of psoriasis. *Front Med (Lausanne)* 2021;8:646796.
- Zhang W, Yi X, An Y, Guo S, Li S, Song P, et al. MicroRNA-17-92 cluster promotes the proliferation and the chemokine production of keratinocytes: implication for the pathogenesis of psoriasis. *Cell Death Dis* 2018;9:567.
- Alatas ET, Kara M, Dogan G, Belli AA. Blood microRNA expressions in patients with mild to moderate psoriasis and the relationship between microRNAs and psoriasis activity. *An Bras Dermatol* 2020;95:702-7.
- Takakura S, Mitsutake N, Nakashima M, Namba H, Saenko VA, Rogounovitch TI, et al. Oncogenic role of miR-17-92 cluster in anaplastic thyroid cancer cells. *Cancer Sci* 2008;99:1147-54.
- Lu D, Tang L, Zhuang Y, Zhao P. miR-17-3P regulates the proliferation and survival of colon cancer cells by targeting Par4. *Mol Med Rep* 2018;17:618-23.
- Shan SW, Fang L, Shatseva T, Rutnam ZJ, Yang X, Du W, et al. Mature miR-17-5p and passenger miR-17-3p induce hepatocellular carcinoma by targeting PTEN, GalNT7 and vimentin in different signal pathways. *J Cell Sci* 2013;126:1517.
- Li H, Yang BB. Stress response of glioblastoma cells mediated by miR-17-5p targeting PTEN and the passenger strand miR-17-3p targeting MDM2. *Oncotarget* 2012;3:1653-68.
- Yan H, Song K, Zhang G. MicroRNA-17-3p promotes keratinocyte cells growth and metastasis via targeting MYOT and regulating Notch1/NF-kappaB pathways. *Pharmazie* 2017;72:543-9.
- Hanks S, Perdeaux ER, Seal S, Ruark E, Mahamdallie SS, Murray A, et al. Germline mutations in the PAF1 complex gene *CTR9* predispose to Wilms tumour. *Nat Commun* 2014;5:4398.
- Zeng H, Xu W. Ctr9, a key subunit of PAFc, affects global estrogen signaling and drives ER $\alpha$ -positive breast tumorigenesis. *Genes Dev* 2015;29:2153-67.
- Yoo HS, Choi Y, Ahn N, Lee S, Kim WU, Jang MS, et al. Transcriptional regulator *CTR9* inhibits Th17 differentiation via repression of IL-17 expression. *J Immunol* 2014;192:1440-8.



32. Lee H, Lee JW, Yoo KD, Yoo JY, Lee JP, Kim DK, et al. Cln 3-requiring 9 is a negative regulator of Th17 pathway-driven inflammation in anti-glomerular basement membrane glomerulonephritis. *Am J Physiol Renal Physiol* 2016;311:F505-19.
33. Li B, Huang L, Lv P, Li X, Liu G, Chen Y, et al. The role of Th17 cells in psoriasis. *Immunol Res* 2020;68:296-309.
34. Sano S, Chan KS, Carbajal S, Clifford J, Peavey M, Kiguchi K, et al. Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. *Nat Med* 2005;11:43-9.
35. Schüttler D, Clauss S, Weckbach LT, Brunner S. Molecular mechanisms of cardiac remodeling and regeneration in physical exercise. *Cells* 2019;8:1128.
36. Nosbaum A, Dahel K, Goujon C, Nicolas JF, Mengeaud V, Vocanson M. Psoriasis is a disease of the entire skin: non-lesional skin displays a prepsoriasis phenotype. *Eur J Dermatol* 2021;31:143-54.

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