Involvement of miR-214-3p/FOXN1 axis during the progression of psoriasis

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Abstract

Psoriasis is a common, chronic, and relapsing skin disease characterized by hyperproliferation of keratinocytes and apoptosis delay. However, the molecular mechanisms underlying the progression of psoriasis remain elusive. MicroRNAs (miRNAs) are single-stranded, small non-coding RNAs that play a crucial role in the development of psoriasis by promoting targeted mRNA degradation or translational inhibition. Here, we report that miR-214-3p, one of the down-regulated miRNAs identified in the skin of psoriatic patients and imiquimod (IMQ)-induced mouse models, can negatively regulate the expression of forkhead box M1 (FOXM1). miR-214-3p inhibition leads to hyperproliferation and increased apoptosis of keratinocytes *in vitro*. Moreover, we show that miR-214-3p inhibition causes an arrest of the cell cycle at the S stage by elevating the expression of NEK2, KIF20A, CENP-A, CENP-F, Cyclin B1, and by reducing the expression of Cyclin D1 in HaCaT cells. *In vivo*, administration of miR-214-3p attenuates the psoriasis-like phenotype in IMQ-induced mice. Collectively, our results suggest that miR-214-3p/FOXM1 axis in keratinocytes could be a novel target in the treatment of psoriasis.

Introduction

Psoriasis is a chronic autoimmune skin disease that varies greatly from person to person. It affects 2~3% of the global population with an unbalanced regional prevalence [1]. The typical histological feature of a skin lesions in patients with psoriasis vulgaris are the plaque covered with inflamed and silvery scales [2]. Psoriasis is characterized by neovascularization, hyperproliferation of keratinocytes, and dermal infiltration of inflammatory cells [3]. Hitherto, the cellular and molecular mechanisms of psoriasis behind its pathogenesis have not been fully investigated.

MicroRNA (miRNA) is a short non-coding RNA which is consisted of 19-25 nucleotides [4]. It can bind to the 3'untranslated region (UTR) of target mRNA transcripts of protein-coding genes and silence the translation or cause mRNA degradation [5]. Recent evidence suggests that miRNAs play a crucial role in post-transcriptional gene regulation of skin development [6-10]. For example, miR-31 is one of the overexpressed miRNAs in the skin of psoriatic patients. It promotes keratinocyte hyperproliferation by directly targeting the 3'UTR of the protein phosphatase 6 (ppp6c) mRNA in keratinocytes. Anti-miR-31 administration could markedly decrease keratinocyte hyperproliferation and dermal cellular infiltration [11]. Another case is miR-197, which has a pronounced decrease in psoriatic lesions. Activation of the expression of miR-197 could inhibit keratinocytes proliferation and migration [12]. These studies strongly suggest that the inhibition of upregulated miRNAs or the supplementation of mimics of downregulated miRNAs in psoriatic skin lesions would offer benefits in the treatment of topical psoriasis. Recently, high-throughput transcriptome analysis of clinical psoriasis showed that miR-214-3p is significantly down-regulated in psoriatic lesions compared to healthy skin (GSE142582) [13]. More importantly, the expression of miR-214-3p was increased by 1.73-fold in the psoriatic patient lesional skin after treated with adalimumab for 14 days [14]. These data suggest that miR-214-3p may play an important role in the progression of psoriasis. However, a systematic understanding of how miR-214-3p contributes to psoriasis is still lacking.

Forkhead box M1 (FOXM1), also known as MPP2, Trident, or HFH-11, is a proliferation-specific transcription factor that belongs to the forkhead family [15]. Elevated expression and activity of FOXM1 are usually accompanied by tumor proliferation and overgrowth, such as prostate carcinomas, melanoma, and breast cancer [16-22]. As an important regulatory factor in the cell cycle, the loss-function of FOXM1 is correlated with cell cycle arrest [23]. However, the role of FOXM1 in epidermal hyperplasia of psoriasis has not been thoroughly examined. Remarkably, previous studies showed that FOXM1 is one of the core transcription factor regulators in psoriasis [24]. These studies further indicated that FOXM1 may contribute to the occurrence of psoriasis.

Here we demonstrated a novel role of miR-214-3p in inhibiting the proliferation of keratinocyte cells by directly targeting to FOXM1. Moreover, we have revealed that FOXM1 is overexpressed in psoriatic lesions and promotes cell-cycle progression to the S phase by increasing the expression levels of cell cycle-related and mitosis-related genes. Taken together, our results suggest that miR-214-3p/FOXM1 axis might be suitable for targeted therapy of psoriasis.

Materials And Methods

Clinical tissue samples

Five adult cases of psoriatic lesion (PS) tissue and the same amount of psoriasis lesions-adjacent normal skin tissue (PN) and healthy control-derived normal skin tissue (NN) were collected from Shanghai Tenth People's Hospital, none of which had underwent medical treatment. The psoriasis severity was evaluated and graded by the Psoriasis Area Severity Index (PASI). Clinical tissue samples were stored at liquid nitrogen immediately after sampling [13].

Mice

All animal experiments were performed on ICR background mice and approved under the guidelines of the Animal Experimental Ethics Committee of Hubei University of Chinese Medicine. Mice were obtained from Vital River Laboratories (Beijing, China) and were maintained under SPF conditions. The IMQ-induced psoriasis mouse model was induced in 8–12 weeks of age mice. The mice were applied to a daily topical dose of 62.5 mg IMQ cream (5%) (MedShine, #120503; China) on the shaved dorsal skin or 25 mg on ears for 6 consecutive days. As negative controls, wild-type (WT) mice were treated with the same dose of Vaseline cream. Erythema, scales, and thickness were scored independently on a scale from 0 to 4: 0, none; 1, slight; 2, moderate; 3, marked; and 4, very marked.

Cell culture and transfection

Human keratinocyte HaCaT cells were seeded into a six-well plate (5×10^5 cells/well) and cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, USA) containing 10% fetal bovine medium with 5% CO₂ in a 37°C incubator. miR-214-3p mimics or inhibitors and their negative controls were designed and synthesized by RiboBio Inc. (Ribobio, China). The sequences of miR-214-3p mimic: sense strand: 5'

CGACGUGGUCGGACGGACGACU 3' and antisense strand: 5' AGUCGUCCGUCCGACCACGUCG 3'; miR-214-3p inhibitor is single-strand: 5' AGUCGUCCGUCCGACCACGUCG 3'. When cells grew to approximately 70% confluent, miR-214-3p mimics or its inhibitors or negative controls was transfected at a final concentration of 100 nM using Lipofectamine 3000 Transfection Reagent (Invitrogen, USA) according to the manufacturer's instructions. The cells were then harvested for the extraction of RNA or protein.

RNA extraction and quantitative real-time PCR (qPCR)

Total RNA was extracted using Trizol reagent (TaKaRa, Japan) and chloroform. The concentration of total RNA was measured using Nanodrop 2000/2000c (Thermo Fisher Scientific). To separate the epidermis from the dermis and following epidermis RNA extraction, an overnight incubation of the dorsal skin at 4°C in dispase II (2.5 U/mL, BD, USA) was performed. Complementary DNA was synthesized by the cDNA RT kit (Applied Biosystems, USA). TaqMan probes were used to quantify the expression of miR-214-3p and specific RT primers were used to quantify the expression of other genes. The qRT-PCR was performed using All-in-One™ miRNA qRT-PCR Detection Kits (GeneCopoeia, Inc., USA). TaqMan probes were obtained from Guangzhou RiboBio company. The other primer sequences were listed as follows: *FOXM1*, forward: 5'- CGTCGGCCACTGATTCTCAA -3' and reverse: 5'- GGCAGGGGATCTCTTAGGTTC -3'; *Foxm1*, forward: 5'- CTGATTCTCAAAGACGGAGGC -3' and reverse: 5'- TTGATAATCTTGATTCCGGCTGG -3'; *NEK2*, forward: 5'- TGCTTCGTGAACTGAAACATCCG -3' and reverse: 5'- CCAGAGTCAACTGAGTCATCACT-3'; *KIF20A*, forward: 5'- TTGAGGGTTAGGCCCTTGTTA-3' and reverse: 5'- GTCCTTGGGTGCTTGTAGAAC -3'; *CENP-A*, forward: 5'- CTCCCATCAACACAGTCGGC-3' and reverse: 5'- GAAGTCCACACCACGAGTGA-3'; *CENP-F*, forward: 5'- ACCTTCACAACGTGTTAGACAG -3' and reverse: 5'- CTGAGGCTCTCATATTCGGCA-3'; *CCNB1*, forward: 5'- AACTTTCGCCTGAGCCTATTTT-3' and reverse: 5'- TTGGTCTGACTGCTTGCTCTT-3'; *CCND1*, forward: 5'- CAATGACCCCGCACGATTTC -3' and reverse: 5'- CATGGAGGGCGGATTGGAA-3'; *U6*, forward: 5'-ATTGGAACGATACAGAGAAGATT-3' and reverse: 5'- AGGAACGCTTCACGAATTTG-3'; *GAPDH*, forward: 5'-AACTTTGGGATTGTGGAAGG-3' and reverse: 5'- ACACATTGGGGGTAGGAACA -3'. *U6* and *GAPDH* were used as endogenous controls. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Cell proliferation, apoptosis, and cell cycle assay

Cell Counting Kit-8 (CCK-8) (Beyotime, China) was used to measure cell proliferation. In brief, the HaCaT cells were seeded in 96-well plates at a density of 5×10^3 cells/well. After transfecting miR-214-3p mimics or inhibitors or controls for 24, 48, and 72 h, CCK-8 solution (10 μ L) was added to each well, and then the plates were incubated for 1 h at 37°C in the incubator. The absorbance was measured at a wavelength of 450 nm using a Microplate Reader (Bio-Rad, USA). For cell cycle assay, transfections were done in 6-well plates (4×10^5 cells/well). Following transfecting with miR-214-3p mimics or inhibitors or controls for 48 h, the cells were collected, washed in ice-cold phosphate-buffered saline (PBS), and fixed with ice-cold 75% ethanol at -20°C overnight. Then, the cells were washed with ice-cold PBS, centrifuged, and incubated with 0.1% RNase A solution for 30 min at 37°C. Subsequently, cells were incubated in 0.4 mL propidium iodide (PI) at 4°C for 30 min in the dark and the cell cycle distribution was analyzed using FACSria™ III

Cells (BD, USA). The results were analyzed using Flowjo software. For apoptosis analysis assay, cells were seeded in 12-well plates (2×10^5 cells per well). After transfected with miR-214-3p mimics or inhibitors or controls for 24 h, cells were stained with Annexin V-FITC/PI Apoptosis Detection kit (Beyotime, China) in the dark for 15 min according to the manufacturer's instructions. The percentage of early and late apoptotic cells were acquired and analyzed using Flowjo software.

Western blot

HaCaT cells were lysed in RIPA buffer containing proteases and phosphatase inhibitors. The total lysate was denatured for 10 min at 95°C in a metal bath. The concentration of protein was quantified using BCA kit (Beyotime, China) and an equal amount of protein was separated on SDS-PAGE gels. Separated protein bands were transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were then blocked with 5% skimmed milk for 1 h at room temperature, incubated with appropriate primary antibodies FOXM1 (1: 1,000; Cell Signaling Technology, 5436S), NEK2 (1: 1,000; Santa Cruz Biotechnology, sc-55601), KIF20A (1: 1,000; Santa Cruz Biotechnology, A300-879A), CENP-A (1: 1,000; Abcam, ab13939), CENP-F (1: 500; Novus Biologicals, NB500-101), Cyclin B1 (1: 1,000; Santa Cruz Biotechnology, sc-245), Cyclin D1 (1: 1,000; Cell Signaling Technology, 2978S), GAPDH (1: 5,000; Cell Signaling Technology, 2118) at 4°C overnight. After three times washing with TBST, the membranes were subsequently incubated with HRP-conjugated secondary antibody (1: 10,000) at room temperature for 1 h. Protein bands were visualized using ECL substrates on Amersham Imager 600 (General Electric Company, USA) and analyzed by Quantity One software (Bio-Rad, USA). GAPDH was used as an endogenous control to obtain the optical density ratio of the detected proteins.

RNA pull-down

Biotinylated antisense-miR-214-3p and miR-214-3p were transcribed *in vitro* and labeled with Biotin RNA Labeling Mix (Roche, USA). 500 µg whole-cell lysates from HaCaT cells were incubated with 1 µg of labeled RNA for 30 min at 25°C. Then biotin-labeled RNA-protein complex was captured by streptavidin agarose beads (Bimake, USA). Eluted RNA-bound protein was detected by western blotting.

H&E staining

The mouse dorsal and ear skin treated with IMQ or Vaseline cream was fixed with 4% paraformaldehyde at 4°C overnight, followed by dehydration in 30% sucrose and embedded in OCT (SAKURA, USA). Frozen sections (5 µm) were stained with hematoxylin and eosin (H&E, Sigma-Aldrich, 51275, 318906) according to standard procedures.

Luciferase reporter assay

The DNA fragments of *FOXM1* 3'-UTR were amplified and cloned into pmirGLO Dual-Luciferase vector (Promega, USA) to generate the WT *FOXM1* 3'-UTR luciferase vector. The mutated *FOXM1* 3'-UTR luciferase vector was generated by site-directed mutated PCR. HaCaT cells were seeded in a 24-well plate

(5×10^4 cells/well) and then co-transfected with miR-214-3p mimics (50 pmol) and luciferase reporter plasmid (100 ng) and hRluc-neo plasmid (500 ng) using Lipofectamine 3000 (Invitrogen, USA). After 24 h of transfection, the firefly and Renilla luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega, USA). The ratio of firefly luciferase to Renilla luciferase was calculated for each well.

RNA-ChIP

Immunoprecipitation of RNA binding protein–RNA complexes (RNA-ChIP) was performed using EZMagna RIP Kit (Millipore, USA) according to the protocol provided by the kit. In brief, epidermal cells were lysed by RIP lysis buffer after treatment with IMQ or Vaseline cream. Then the lysate was incubated with magnetic beads and anti-Argonaute 2 (AGO2, 5 μ g) or control IgG antibody (Abclonal, AC005, 5 μ g) with rotation for 6 h at 4°C. The immunoprecipitate was washed and incubated with Proteinase K and then subjected to qRT-PCR.

In vivo administration of miR-214-3p mimics

To overexpress miR-214-3p in the skin, 7 μ g miR-214-3p mimics or scrambled controls with the transfecting agent (mirVana; Life Technologies, USA) were injected intradermally into the shaved mouse dorsal skin on days 1, 2, and 5 during the application of IMQ. Mice were sacrificed and analyzed on day 6.

Statistical analysis

All experiments were repeated at least three times. Data were presented as mean $\pm$ SEM. The differences between two groups were assessed using the two-tailed Student *t*-test. Multiple comparisons were analyzed by One-way ANOVA with post hoc Tukey test. GraphPad Prism 8.0 was used for the statistical analysis. $p < 0.05$ was considered statistically significant.

Results

Expression of miR-214-3p in the lesional skin of psoriasis mouse models and patients

Recently, a high-throughput transcriptome analysis (GSE142582) was carried out to comprehensively assess differentially expressed genes (DEGs) in adult healthy control-derived normal (NN), psoriasis lesions-adjacent normal (PN), and psoriasis lesional (PS) skin tissues ($n = 5$ each) [13]. Of the differentially expressed miRNAs analyzed, we focused on miR-214-3p, which was remarkably down-regulated in psoriatic tissue and significantly up-regulated in psoriatic skin after treatment with adalimumab [14] (Figure 1A). The expression changes of miR-214-3p in the psoriasis lesions encouraged us to investigate its *in vivo* function. Previous studies suggested that the imiquimod (IMQ)-induced psoriasis mouse model could closely recapitulate the phenotype in psoriasis patients [25]. Therefore, we first assessed the expression of miR-214-3p in IMQ-induced mouse model by quantitative real-time PCR (qPCR). Notably, we found that the mRNA expression level of miR-214-3p was decreased in the dorsal

and ear skin of IMQ-induced mice, compared with WT mice and Vaseline-treated mice (Figures 1B and 1C). These results revealed that miR-214-3p may serve an important function in the pathogenesis of psoriasis.

MiR-214-3p promotes cell apoptosis and inhibits the proliferation of keratinocytes

Because miR-214-3p is reduced in hyperproliferative keratinocytes in IMQ-induced psoriasis-like skin, we next carried out loss- or gain-of-function experiments to investigate whether miR-214-3p was involved in the regulation of keratinocyte proliferation. First, we quantified the mRNA expression level of miR-214-3p after transiently transfected miR-214-3p mimics or inhibitors into HaCaT cells. As expected, the expression of miR-214-3p was increased after miR-214-3p mimics (miR-214-3p mi) treatment while reduced after miR-214-3p inhibitors (miR-214-3p in) treatment compared with negative control-mimics (NC-mi) and negative control-inhibitors (NC-in) groups (Figure 2A). The CCK8 assay indicated that miR-214-3p overexpression or knockdown significantly suppressed or promoted the proliferation of HaCaT cells, respectively (Figure 2B). Next, we further studied the influence of miR-214-3p on cell apoptosis through Annexin V-FITC/PI double staining-based FACS. Our results revealed that the apoptosis cells were increased after overexpressing miR-214-3p in the HaCaT cell line (Figure 2C and 2D). Taken together, our data indicated that miR-214-3p could suppress cell proliferation and induce cell apoptosis.

miR-214-3p promotes cell cycle arrest at S phase

To further determine whether miR-214-3p suppressed cell proliferation through cell cycle arrest, we analyzed the cell cycle distribution of HaCaT cells after transfecting with miR-214-3p mimics or inhibitors by fluorescence-activated cell sorting (FACS). Our data showed that compared with controls, the percentage of cells at the S phase of the cell cycle was remarkably reduced after overexpressing miR-214-3p, and cells at the G1 stage of the cell cycle were significantly increased simultaneously (Figure 3A). These results suggest that miR-214-3p has a vital role in cell cycle regulation. Hence, we determined the expression of several key proteins related to cell cycle regulation. It was observed that miR-214-3p positively regulate the mRNA and protein expression of Nek2, KIF20A, CENP-A, CENP-F, and Cyclin B1, but negatively regulate the expression of Cyclin D1 in HaCaT cells (Figure 3B, 3C, 3D, 3E and 3F). These results revealed that a low abundance of miR-214-3p arrested the cell at the S phase by altering the expression of cell cycle and mitosis related genes.

miR-432-5p directly targets FOXM1

To identify putative target mRNAs of miR-214-3p, we used the public bioinformatics tool (TargetScan) to predict the potential targets of miR-214-3p, and thousands of potential target genes were identified. We compared the mRNA expression profile of the potential targets in normal healthy skin and psoriasis patients' skin (GSE142582). *FOXM1*, the most significantly upregulated gene, was identified as a potential target gene of miR-214-3p (Figures 4A and 4B). In the IMQ-induced mouse model of psoriasis, the expression of FOXM1 was increased more than 4-fold at mRNA level and 2-fold at protein levels in the ear and dorsal skin compared with that of WT and Vaseline-treated controls (Figures 4C to 4E). Moreover,

in contrast to control skin, a significantly increased expression of FOXM1 was revealed in the IMQ-induced mice skin by immunohistochemistry staining (Figures 4F and 4G). Overexpression or inhibition of miR-214-3p in HaCaT cells resulted in a decreased or increased expression of FOXM1, respectively (Supplementary Figures 1A and 1B). These results suggest that FOXM1 is a potential target of miR-214-3p.

To further confirm whether *FOXM1* is a direct target of miR-214-3p, we performed the dual-luciferase reporter assay with a control construct which includes the 3'UTR region of the *FOXM1* mRNA or a mutant-form construct which lacked the target sequence. In contrast to the mutant-form construct, overexpression of miR-214-3p significantly resulted in a reduction of relative luciferase activity when co-transfected with the control construct, which fused to the 3'UTR of *FOXM1* mRNA (Figure 4H). To assess whether miR-214-3p interact with *FOXM1*, the biotin-streptavidin RNA pull-down assay was performed, revealing that miR-214-3p co-precipitated *FOXM1* (Figure 4I). Besides, we also carried out RNA-chromatin immunoprecipitation (RNA-ChIP) to detect miR-214-3p and *Foxm1* mRNA abundance in the Argonaute 2 (Ago2) associated complexes of the epidermis derived from Vaseline- and IMQ-induced mice. The levels of miR-214-3p and *Foxm1* detected in the IMQ-induced psoriatic epidermal immunoprecipitates were reduced and increased respectively, than those in Vaseline-treated controls (Figures 4J and 4K). Furthermore, we determined that *FOXM1* expression was increased in the AGO2 immunoprecipitates after overexpression of miR-214-3p in HaCaT cells (Figure 4L). These data demonstrate that psoriasis patients and imiquimod-induced (IMQ) psoriatic mouse skin have a high abundance of FOXM1, and miR-214-3p directly targets the 3'UTR of *FOXM1* mRNA.

Administration of miR-214-3p attenuates the psoriasis-like phenotype in IMQ-induced mice

Our findings revealed the pivotal role of the miR-214-3p/FOXM1 axis in psoriasis pathogenesis. Hence, to assess the efficacy and potential application of miR-214-3p-based therapy for psoriasis, we administrated miR-214-3p mimics or negative control miRNA (NC-mi) intradermally 6 times from the beginning to the sixth day to evaluate the therapeutic effect on psoriasis progression in the IMQ-induced psoriasis-like mouse model. Compared with the NC-mi group, the miR-214-3p mi group showed decelerated psoriasis-like pathological progression and decreased disease severity (Figures 5A and 5B). Cumulative psoriasis area and severity index (PASI) scores were remarkably alleviated in IMQ-induced mice treated by miR-214-3p mimics (Figure 5C). Besides, the reduced FOXM1 expression in the epidermis after miR-214-3p administration was further confirmed by qPCR and Western blotting (Figures 5D and 5E). Overall, these results provide important insight into the role of miR-214-3p/axis play in psoriasis.

Discussion

In this study, we provided the first evidence that miR-214-3p is significantly downregulated in the skins of IMQ-induced psoriasis-like lesions and its down-regulation promotes keratinocyte proliferation by directly elevating FOXM1 expression. Importantly, we found that overexpressing miR-214-3p by the

administration of miR-214-3p mimics could dramatically ameliorate IMQ-induced psoriasis-like skin lesions.

Accumulating evidence reported that miR-214-3p generally shows an aberrant expression patterns, which could either serve as a tumor suppressor or promoter in a disease-context-dependent manner, but its role in psoriasis progression is still poorly understood. For instance, miR-214-3p is down-regulated in non-small cell lung cancer (NSCLC), promotes lung cell migration and invasion *in vitro*, and acts as tumor suppressor by regulating the expression of a cohort of oncogenes [26]. In most malignant gliomas, the expression of miR-214-3p is also severely reduced compared to healthy brain samples. The poor prognosis of glioblastoma multiforme (GBM) patients is generally associated with low-expression of miR-214-3p. Moreover, overexpression of miR-214-3p could markedly lead to apoptosis and suppress the migration and proliferation of glioblastoma cells *in vitro* and *in vivo* [27]. In contrast, miR-214-3p is over-expressed and has a potential oncogenic role in synovial sarcoma cells [28]. Moreover, miR-214-3p is up-regulated and plays a vital role in the hypoxia response of retinoblastoma cells [29]. Our data identified miR-214-3p as a negative regulator of the proliferation of keratinocytes and highlight the critical role of miR-214-3p in psoriasis as evidenced by the observation that intradermal (i.d.) injection of miR-214-3p mimics in IMQ-induced mouse models proves especially efficacious in the relief of the psoriasis-like symptoms.

Recently, FOXM1 was identified as a psoriasis-activated transcription factor (TF) in psoriasis DEGs, as it is interacting with “psoriasis response elements” (PREs) [30]. However, the function of FOXM1 is not yet well studied in psoriasis. FOXM1 is an oncogenic and proliferative transcription factor that promotes cell cycle progression at the G1/S and G2/M transitions [15]. In our study, the cell cycle was arrested in S phase by up-regulating of FOXM1 stimulating the expression of Nek2, KIF20A, CENP-A, CENP-F, and cyclin B after treatment of miR-214-3p inhibitors in HaCaT cells. Besides, we also found a positive correlation between FOXM1 expression and keratinocytes proliferation. These findings are in agreement with previous studies that the role of FOXM1 in cell cycle regulation and cell proliferation [31, 32]. Of note, although miR-214-3p is capable of inhibiting cell proliferation by directly targeting FOXM1, we found that inhibition of miR-214-3p *in vitro* resulted in less significant changes of FOXM1 expression than in psoriatic skin. It is reasonable to believe that FOXM1 was not regulated only by one miRNA. Therefore, further study of miRNAs involved in regulating the expression of FOXM1 is still needed.

In conclusion, the present study has revealed that the expression of miR-214-3p is diminished in the skin of psoriasis patients and IMQ-induced mouse models. Up-regulation of miR-214-3p decreased epidermal hyperplasia and attenuates the psoriasis-like phenotype in IMQ-induced mouse models. miR-214-3p directly targets FOXM1, a psoriasis-active TF in regulating cell cycle, which is elevated in the lesional skin from patients with psoriasis. Our findings reveal a previously unknown function of miR-214-3p in FOXM1-mediated hyperproliferative keratinocytes and highlights a critical role of the miR-214-3p/FOXM1 axis in psoriasis progression.

Declarations

Funding

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Conflicts of interest

The authors declare that they have no competing financial interests.

Ethics approval

All animal experiments were approved under the guidelines of the Animal Experimental Ethics Committee of Hubei University of Chinese Medicine.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are available upon reasonable request.

Code availability

Not applicable.

Authors' Contributions

Jin Zhao: Conceptualization, Methodology, Software. **Fei Wang:** Conceptualization, Methodology, Software. **Qingjun Tian:** Methodology. **Jing Dong:** Methodology. **Liuqing Chen:** Supervision. **Rongyi Hu:** Writing- Reviewing and Editing. **Jin Zhao** and **Fei Wang** contributed equally to this article; both of them are first authors.

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Figures

Figure 1

Figure 1

The expression of miR-214-3p was reduced in psoriasis skin. (A) Heatmap of top 40 downregulated miRNAs in psoriasis lesion group (PS) compared to healthy control-derived normal skin group (NN), with no significant differences between psoriasis lesions-adjacent normal skin group (PN) and NN (data from GSE142582). Red arrow indicates the expression of miR-214-3p among NN, PN, and PS. Five adult skin samples of each group are plotted individually in the heatmap. Expression of miR-214-3p in the dorsal skin (B) and ear skin (C) of WT, Vaseline-treated (Vaseline), and IMQ-induced (IMQ) mice. Results are presented as the ratio of miR-214-3p to the small nuclear RNA U6. Data are presented as mean $\pm$ s.e.m. ** < 0.01 .

Figure 2

Figure 2

Effects of miR-214-3p on the proliferation capacity and apoptosis of HaCaT cells. (A) miR-214-3p expression in HaCaT cells transfected with miR-214-3p mimics (miR-214-3p mi), miR-214-3p inhibitors (miR-214-3p in), and negative controls (NC-mi and NC-in). Results are presented as the ratio of miR-214-3p to the small nuclear RNA U6. (B) HaCaT cells were transfected with NC-mi, NC-in, miR-214-3p mi, and miR-214-3p in, followed by the determination of cell proliferation ability after 0h, 24h, 48h, 72h, and 96h by CCK-8 assay. (C) Apoptotic rate analysis of HaCaT cells transfected with NC-mi, NC-in, miR-214-3p mi, and miR-214-3p in. (D) Quantification analysis of apoptotic rate of HaCaT cells after transfection with NC-mi, NC-in, miR-214-3p mi, and miR-214-3p in. Data are presented as mean $\pm$ s.e.m. ** $p < 0.01$ *** $p < 0.001$.

Figure 3

Figure 3

Inhibition of miR-214-3p promotes cell cycle arrest at the S phase. (A) Cell cycle analysis of HaCaT cells transfected with NC-mi, NC-in, miR-214-3p mi, and miR-214-3p in. (B and C) RT-qPCR analysis of cell cycle-related and cell mitosis-related genes expression in HaCaT cells transfected with NC-mi, NC-in, miR-214-3p mi, and miR-214-3p in. Western blotting analysis (D) and quantification (E and F) of cell cycle-related and cell mitosis-related proteins expression in HaCaT cells transfected with NC-mi, NC-in, miR-214-3p mi, and miR-214-3p in. Data are presented as mean $\pm$ s.e.m. * $p < 0.05$, ** $p < 0.01$.

Figure 4

Figure 4

miR-214-3p directly targets FOXM1. (A) miR-214-3p target sequences of the 3'-UTR of FOXM1 and corresponding mutant, which were included in luciferase reporter vectors. (B) Fold change of expression of 10 predicted upregulated target genes of miR-214-3p in mRNA expression profile in psoriasis patients' skin compared to normal healthy skin (GSE142582). Expression of Foxm1 in the dorsal skin (C) and ear skin (D) of WT, Vaseline-treated, and IMQ-induced mice. (E) Western blotting of FOXM1 expression in epidermis lysates derived from WT, Vaseline-treated, and IMQ-induced mice dorsal and ear skin. (F) Immunohistochemical staining of FOXM1 with hematoxylin re-staining in skin sections derived from WT, Vaseline-treated, and IMQ-induced mice dorsal and ear skin. Red arrowheads indicate FOXM1+ cells.

Scale bar, 100 μ m. (G) Quantification of FOMX1+ cells in skin sections derived from WT, Vaseline-treated, and IMQ-induced mice dorsal and ear skin. (H) Luciferase activity was determined in HaCaT cells that were transfected with NC-mi and miR-214-3p mi with the indicated wild-type FOMX1 3' UTR reporter construct or with the mutated FOMX1 3' UTR reporter construct. (I) RNA pull-down assay was performed using biotinylated antisense-miR-214-3p and miR-214-3p in HaCaT cells lysates. (J and K) Ago2 was immunoprecipitated from epidermis lysates derived from Vaseline-treated or IMQ-induced mice. Immunoprecipitates were assayed for miR-214-3p or FOXM1. IgG used as negative control. Values were expressed as fold changes relative to Vaseline-treated groups. (L) Ago2 was immunoprecipitated from HaCaT cells transfected with NC-mi and miR-214-3p mi. Immunoprecipitates were assayed for FOXM1. IgG used as negative control. Values were expressed as fold changes relative to NC-mi groups. Data are presented as mean $\pm$ s.e.m. * p < 0.05, ** p < 0.01 ***< 0.001.

Figure 5

Figure 5

Overexpression of miR-214-3p can decrease dermal cellular infiltration in IMQ-induced mice dorsal skin. (A) Phenotypic analysis of dorsal skin from Vaseline-treated or IMQ-induced mice injected with NC-mi or miR-214-3p mi. (B) H&E staining of dorsal skin from Vaseline-treated or IMQ-induced mice injected with NC-mi or miR-214-3p mi. Scale bar, 100 μ m. (C) Daily scoring of erythema, scaling, thickness, and cumulative score (erythema plus scales plus thickness). (D) Western blotting of FOMX1 expression in

epidermis lysates derived from dorsal skin from Vaseline-treated or IMQ-induced mice injected with NC-mi or miR-214-3p mi. (E) RT-qPCR analysis of FOXM1 mRNA expression in dorsal skin from Vaseline-treated or IMQ-induced mice injected with NC-mi or miR-214-3p mi. Data are presented as mean $\pm$ s.e.m. *p < 0.05.

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