LncRNA MIR181A2HG Negatively Regulates Human Keratinocytes Proliferation by Binding SRSF1

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Research Article

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Abstract

Psoriasis is a common chronic inflammatory skin disease. Abnormal proliferation of keratinocytes plays an important role in the pathogenesis of psoriasis. Long non-coding RNAs (lncRNAs) are involved in the regulation of a variety of cell biological processes. The purpose of this study was to investigate the potential role of lncRNA MIR181A2HG in the proliferation of human keratinocytes. qRT-PCR was performed to measure the expression levels of MIR181A2HG, SRSF1, miR-181a, miR-181b, KRT6 and KRT6 in tissue specimens and cells. Cell Counting Kit-8 (CCK-8) was used to measure cell viability. RNA pulldown-mass spectrometry (MS) was applied to identify the proteins interacting with MIR181A2HG. RNA pulldown-Western blotting and RNA immunoprecipitation (RIP)-qRT-PCR were used to confirm the interaction between MIR181A2HG and SRSF1. In this study, we found MIR181A2HG was decreased in psoriatic lesions. Knockdown of MIR181A2HG promoted the proliferation of human keratinocytes, while overexpression of MIR181A2HG inhibited the proliferation of human keratinocytes. 356 proteins were identified to interact with MIR181A2HG potentially. SRSF1 was finally determined to interact with MIR181A2HG. In addition, silencing of SRSF1 inhibited keratinocytes proliferation, which could be reversed with knockdown of MIR181A2HG. In summary, MIR181A2HG negatively regulates keratinocytes proliferation by binding SRSF1, suggesting that MIR181A2HG and SRSF1 may serve as potential targets for the treatment of psoriasis.

Background

Psoriasis is one of the most common inflammatory skin diseases in humans, characterized by excessive proliferation and abnormal differentiation of keratinocytes in lesions and infiltration of inflammatory cells into the dermis and epidermis [1, 2]. So far, the etiological factors and its pathogenesis are not fully clarified yet. However, it was considered to be an autoimmune disease involved by skin keratinocytes and immune cells under the influence of internal and external environmental factors in individuals with genetic susceptibility. The abnormal crosstalk between keratinocytes and immune cells plays important roles in the pathogenesis and development of psoriasis [3–5]. The aberrant proliferation of keratinocytes considered to be amplifiers of the inflammatory response in the psoriatic lesions. The study of keratinocyte proliferation regulation is of great significance for understanding the pathogenesis of psoriasis.

Long non-coding RNAs (lncRNAs) represent a group of non-coding RNAs with a length of more than 200 nucleotides. Increasing evidence has shown that lncRNAs are important regulators in almost all the physical and pathological events, including the occurrence and development of psoriasis [6, 7]. lncRNA PRINS was found to be up-regulated in psoriatic lesions, which could regulate the expression of anti-apoptotic gene G1P3 and directly interact with NPM to promote keratinocyte proliferation [8]. MSX2P1 was induced by IL-22 and served as a ceRNA to regulate proliferation of keratinocytes [9]. RP6-65G23.1 could promote keratinocyte proliferation through activation of AKT signaling pathway [10]. MEG3 was reported to regulate the proliferation and apoptosis of keratinocytes by targeting caspase-8 with the

absorption of miR-21[11]. We also previously found that MIR31HG was up-regulated in the lesions of psoriasis and could affect keratinocyte proliferation by regulating cell cycle [12].

MIR181A2HG, the host gene of miR-181a/b-2, is transcribed into a 617-nt lncRNA (NR_038975.1). Transcriptome sequencing results showed that MIR181A2HG was down-regulated in psoriatic lesions [13], suggesting that it may be involved in the pathogenesis of psoriasis. However, the possible roles of MIR181A2HG in psoriasis are not investigated. In this current study, we found that MIR181A2HG was decreased in psoriatic lesions and functioned as a proliferation-suppressor in keratinocytes by binding serine and arginine rich splicing factor 1 (SRSF1). This study highlighted the important roles of MIR181A2HG in regulating proliferation of keratinocytes, providing insights into the mechanism of abnormal proliferation of keratinocytes in psoriasis.

Methods

Tissue sample collection

Punch biopsies (4 mm) of 10 patients with psoriasis who had not received any treatment for nearly 4 weeks (7 males, 3 females, mean age 53.3 years) were collected. Samples of healthy skin were taken from individuals undergoing plastic surgery. All samples were frozen in liquid nitrogen. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Guilin Medical University (3/2/2018, Guilin, Guangxi, China). Informed consent was obtained from all individuals involved in the study.

Cell culture

HaCaT keratinocytes (Bio-73031) were obtained from Kunming Cell Bank, Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, China), and cultured in DMEM medium containing 10% fetal bovine serum (FBS) at 37°C with 5% CO₂. Primary normal human epidermal keratinocytes (NHEKs, FC-0007) were purchased from Lifeline Cell Technology (Walkersville, MD USA) and cultured in DermaLife basal medium supplemented with DermaLife K LifeFactors Kit (Lifeline Cell Technology) at 37°C with 5% CO₂.

Construction and transfection of plasmid

The full-length MIR181A2HG cDNA was clone to pcDNA3.1 (Invitrogen, Carlsbad, CA, USA). HaCaT keratinocytes were cultured to 80% confluence and transfected with MIR181A2HG overexpression plasmid (pcDNA3.1-MIR181A2HG) and the corresponding empty vector (pcDNA3.1) according to the instructions of Lipo8000™ transfection reagent (Beyotime Biotechnology, China) for 24-96 hours.

siRNAs and transfection

Specific small interfering RNAs (siRNAs) and scramble siRNA (siNC) were designed and synthesized from Gene-Pharma (Shanghai, China), using a final concentration of 50nM. About 24 hours after passage,

keratinocytes with a confluence of 30-50% were transfected with siRNA-Mate transfection reagent (GenePharma) according to the instructions. The sequence of siMIR181A2HG was as follows: 5'-AGGUAGAUUCUGCAUCCACTT-3'. The sequence of siSRF1 was as follows: 5'-GCCCAGAAGUCCAAGUUAUTT-3'.

Lentivirus transduction

MIR181A2HG was synthesized and subcloned into the GV492 vector (Genechem, Shanghai, China). Lentivirus particles were obtained from Genechem. NHEKs with a confluence of 30-50% were infected with the recombinant lentivirus particles according to the instructions for 24-96 hours.

Cell viability assay

CCK8 kit (Dojindo, Kumamoto, Japan) was used to measure cell viability. Keratinocytes were seeded into 96-well plates, transfected with siRNA, plasmid or lentivirus particles for 24-96 hours. 10µl CCK-8 solution was added to each well and incubated for 2 hours at 37°C in the dark. The absorbance value at 450 nm was measured using a Multiskan microplate reader (ThermoFisher).

EdU assay

EdU (5-ethynyl-2'-deoxyuridine) assay was carried out using iClick™ EdU Andy Fluor™ 488 Imaging Kit (GeneCopoeia, Guangzhou, China) according to the manufacturer's protocol. Briefly, Keratinocytes were seeded into 24-well plates, transfected with siRNA, plasmid or lentivirus particles for 48 hours and then incubated with 50 µM EdU for 2 hours at 37°C. Cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.5% Triton X-100 for 20 minutes, then stained with iClick EdU solution and Hoechst 33342. Images were captured using Nikon Eclipse Ti-U microscope.

RNA extraction and quantitative RT-PCR assays (qRT-PCR)

Total RNA was isolated from keratinocytes using TRIzol™ reagent (Invitrogen). cDNA was synthesized using RevertAid First Strand cDNA Synthesis kit (ThermoFisher). The quantitative PCR was carried out with SYBR Green qPCR Master Mix kit (TaKaRa) in CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad). The quantitative PCR reaction procedure was 95°C for 5 minutes, followed by 40 cycles at 95°C for 5 seconds, 60°C for 10 seconds, 72°C for 10 seconds. GAPDH was used as an internal reference. Primers sequences were as follows:

MIR181A2HG, 5'-GTCGTTGCTGCTTTCTCCCA-3' (forward) and

5'-ACGGATCGAGAGCCTGTTAC-3' (reverse);

KRT6, 5'-GGGTTTCAGTGCCAACTCAGCCAGGC-3' (forward) and

5'-CCATACAGACTGCGG-3' (reverse);

KRT16, 5'-TTCCCCAGCTGCATATAAAGGT-3' (forward) and

5'-GCAGTTGGCTGAAAGAAGGAAA-3' (reverse);

SRSF1, 5'-TCAGCCCACATCCTACTGGA-3' (forward) and

5'-GATGCACAGACGTGAGCACT-3' (reverse);

GAPDH, 5'-CACATGGCCTCCAAGGAGTAA-3' (forward) and

5'-TGAGGGTCTCTCTTCTTCTTGT-3' (reverse).

The expression of mature miRNAs was analyzed with Bulge-LoopTM miRNA qRT-PCR Starter Kit (RiboBio, Guangzhou, China). Total RNA was isolated from HaCaT keratinocytes using TRIzolTM reagent (Invitrogen) and reversely transcribed to cDNA with Bulge-LoopTM miRNA RT primers (RiboBio). The quantitative PCR was carried out in CFX96 TouchTM Real-Time PCR Detection System (Bio-Rad) with miR-181a, miR-181b and U6 Bulge-LoopTM primer (RiboBio). The reaction procedure was 95°C for 10 minutes, followed by 40 cycles at 95°C for 2 seconds, 60°C for 20 seconds, 70°C for 10 seconds. U6 served as an internal reference.

The relative expression was calculated by $2^{-\Delta\Delta Ct}$.

RNA pulldown

MIR181A2HG pulldown probe (MIR181A2HG) and antisense strand negative control probe (Antisense) were designed and synthesized by BersinBio (Guangzhou, China). RNA pulldown experiment was carried out with RNA pulldown kit (BersinBio) according to the instructions. The main steps include: RNA secondary structure formation, probe-magnetic bead preparation, total protein extraction of NHEKs, nucleic acid removal and pre-washing of protein samples, pulldown, and protein products collection.

RNA pulldown protein products were collected for SDS-PAGE, silver staining, mass spectrometry (MS) and Western blotting analysis. MS was performed on Q Ex-active mass spectrometer (ThermoFisher) with a routine protocol.

The same amount of RNA pulldown protein products were separated by 10% SDS-PAGE and transferred onto PVDF membranes (Millipore, Billerica, MA, USA). Primary antibodies anti-SRSF1 (ab129108, Abcam), anti-NOP56 (YT5785, Immunoway) and the secondary antibodies were used to detect the interaction protein. Protein bands were visualised in the ChemiDocTM XRS+ System (Bio-Rad).

RNA immunoprecipitation (RIP) assay

RNA immunoprecipitation (RIP) assay was carried out with RIP kit (BersinBio) according to the instructions. The cell lysates were incubated with RIP buffer containing magnetic beads conjugated with human anti-SRSF1 antibody (ab129108, Abcam) or IgG. The magnetic beads were centrifuged and washed with wash buffer, and the RNA was extracted and detected by qRT-PCR.

Bioinformatics analysis

The dataset GSE13355 and GSE145054 were obtained from Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). Genotype-Tissue Expression Project (GTEx) (<https://gtexportal.org/>) was applied to explore the expression level of MIR181A2HG in normal tissues. LncATLAS (<http://lncatlas.crg.eu/>) was used to query the subcellular localization of MIR181A2HG. Gene ontology (GO) and KEGG enrichment analyses were performed by Metascape (<http://metascape.org/>). Search Tool for the Retrieval of Interacting Genes (STRING; <http://string-db.org>) was applied to predict the protein-protein interaction (PPI) network of MS-identified proteins. Cytoscape (Version 3.6.1, <http://www.cytoscape.org>) was used to construct protein interaction network and degree>50 was considered as the key gene.

Statistical analysis

Data are presented as mean $\pm$ standard deviation of at least three independent experiments. Graphpad Prism 6 software was used for statistical analysis. Statistical significance was analyzed by *t*-test. $P < 0.05$ was considered significant.

Results

MIR181A2HG was down-regulated in psoriatic lesions

The expression of MIR181A2HG in psoriatic lesions and normal skins was explored using public available GEO datasets (GSE13355). MIR181A2HG was found to be down-regulated in psoriatic lesions (Figure 1A). qRT-PCR was performed to measure the expression level of MIR181A2HG in clinical specimens. Consistently, the results revealed that MIR181A2HG was lower in psoriatic lesions than in normal skin tissues (Figure 1B). GTEx database result showed that MIR181A2HG was highly expressed in skin tissues (Figure 1C). Taken together, these data suggested that MIR181A2HG may be involved in the pathogenesis of psoriasis.

Enhanced expression of MIR181A2HG inhibited keratinocytes proliferation

In order to determine the possible effects of MIR181A2HG on keratinocyte proliferation, we transfected the overexpression vector of MIR181A2HG into HaCaT keratinocytes. qRT-PCR assay revealed that MIR181A2HG overexpression plasmid transfection significantly increased MIR181A2HG level (Figure 2A). CCK8 assay revealed that MIR181A2HG up-regulation dramatically suppressed HaCaT keratinocytes growth in 72 and 96 hours (Figure 2B). Keratin 6 (KRT6) and keratin 16 (KRT16) were thought to be the hallmarks of psoriatic keratinocytes hyperproliferation [14–16]. qRT-PCR assay indicated that MIR181A2HG up-regulation resulted in significant reduction of the expression of KRT6 and KRT16 (Figure 2C). Consistent with the results of CCK8 assays, EdU incorporation rate was reduced following MIR181A2HG overexpression (Figure 2D). Meanwhile, we enhanced MIR181A2HG expression via a lentiviral vector in primary normal human epidermal keratinocytes (NHEKs) (Figure 2E). CCK8 assay revealed that MIR181A2HG up-regulation inhibited NHEKs growth (Figure 2F) and decreased EdU

incorporation rate (Figure 2H). The expression of KRT6 and KRT16 was also reduced (Figure 2G). Collectively, these results suggested that enhanced expression of MIR181A2HG inhibited proliferation of keratinocytes.

Knockdown of MIR181A2HG promoted keratinocytes proliferation

We further explored the impact of MIR181A2HG knockdown on the proliferation of keratinocytes. qRT-PCR assay showed that MIR181A2HG expression was significantly suppressed by specific siRNA (Figure 3A and 3E). CCK8 assay revealed that MIR181A2HG knockdown could promote HaCaT keratinocytes and NHEKs growth (Figure 3B and 3F) and increased EdU incorporation rate (Figure 3D and 3H). qRT-PCR assay showed that MIR181A2HG knockdown resulted in significant up-regulation of KRT6 and KRT16 expression in HaCaT keratinocytes and NHEKs (Figure 3C and 3G). These results showed that MIR181A2HG knockdown promoted proliferation of keratinocytes.

As the host gene of miR-181a2/b2, the intron region of MIR181A2HG produces miR-181a/b (Figure 4A). Previous studies have shown that miR-181a/b plays an important role in the regulation of cell proliferation [17–20]. We found that miR-181a and miR-181b were down-regulated in psoriatic lesions based on GEO dataset (GSE145054) (Figure 4B). In order to explore whether the role of MIR181A2HG in regulating keratinocytes proliferation depends on miR-181a/b, we knocked down MIR181A2HG and detected the effect on expression of miR-181a/b. The results showed that knockdown of MIR181A2HG could not affect the expression of miR-181a and miR-181b (Figure 4C), suggesting that MIR181A2HG may regulate keratinocytes proliferation in a miR-181a/b-independent manner.

MIR181A2HG could interact with SRSF1 in keratinocytes

LncATLAS database analysis showed that the cellular localization of MIR181A2HG in different cells was not identical and showed cellular specificity (Figure 5A). Nuclear/cytoplasmic separation qRT-PCR result showed that MIR181A2HG transcript was localized both in the nucleus and cytoplasm, and slightly higher in the nucleus (Figure. 5B), implying that MIR181A2HG may play a regulatory role at pre-transcriptional, transcriptional and post-transcriptional level to regulate proliferation of keratinocytes.

As important regulatory RNAs, lncRNAs can play a variety of regulatory roles by binding to proteins. In order to screen the proteins interacted with MIR181A2HG, RNA pulldown-MS was used to identify the proteins that may bind to MIR181A2HG. Compared with the antisense strand probe, MIR181A2HG probe could pull down multiple proteins. 356 proteins were finally identified by MS (Figure 6A). KEGG pathway enrichment analysis showed that these proteins were mainly related to spliceosome, RNA transport, protein processing (Figure 6B). GO biological process analysis showed that these proteins were mainly involved in RNA processing and transport, mRNA metabolism (Figure 6C). These results suggested that MIR181A2HG may interact with RNA-binding proteins (RBPs) to regulate RNA processing, thereby affecting cell proliferation. We used Cytoscape software to construct the interaction network of these proteins (Figure 6D). Among them, 13 genes were considered as key genes (degree>50) (Figure 6E). The detailed information of these genes was shown in Table 1. In order to explore the possible RBPs

interacting with MIR181A2HG, we took the intersection of these 13 key proteins with the interaction proteins predicted in catRAPID and ENCORI databases. The result showed that NOP56 and SRSF1 may be RBPs interacting with MIR181A2HG (Figure 6F).

Table 1
Detailed information of 13 key genes.

Gene symbols	Degrees	Full names	Gene function
NOP56	54	nucleolarprotein 56	Nop56p is required for assembly of the 60S ribosomal subunit and is involved in pre-rRNA processing. The protein encoded by this gene is similar in sequence to Nop56p and is also found in the nucleolus. Expansion of a GGCCTG repeat from 3-8 copies to 1500-2500 copies in an intron of this gene results in spinocerebellar ataxia 36.
SRSF1	53	Serine and arginine rich splicing factor 1	This gene encodes a member of the arginine/serine-rich splicing factor protein family. The encoded protein can either activate or repress splicing, depending on its phosphorylation state and its interaction partners.
SNRPD2	63	Small nuclear ribonucleoprotein D2 polypeptide	The protein encoded by this gene belongs to the small nuclear ribonucleoprotein core protein family. It is required for pre-mRNA splicing and small nuclear ribonucleoprotein biogenesis.
POLR2A	60	RNA polymerase II subunit A	This gene encodes the largest subunit of RNA polymerase II, the polymerase responsible for synthesizing messenger RNA in eukaryotes. This subunit, in combination with several other polymerase subunits, forms the DNA binding domain of the polymerase, a groove in which the DNA template is transcribed into RNA.
FBL	57	Fibrillarin	This gene product is a component of a nucleolar small nuclear ribonucleoprotein (snRNP) particle thought to participate in the first step in processing preribosomal RNA. It is associated with the U3, U8, and U13 small nuclear RNAs and is located in the dense fibrillar component (DFC) of the nucleolus.
DDX39B	56	DEAD-box helicase 39B	This gene encodes a member of the DEAD box family of RNA-dependent ATPases that mediate ATP hydrolysis during pre-mRNA splicing. The encoded protein is an essential splicing factor required for association of U2 small nuclear ribonucleoprotein with pre-mRNA, and it also plays an important role in mRNA export from the nucleus to the cytoplasm.
SNRPD3	55	Small nuclear ribonucleoprotein D3 polypeptide	This gene encodes a core component of the spliceosome, which is a nuclear ribonucleoprotein complex that functions in pre-mRNA splicing.
RPL3	54	Fibosomal protein L3	This gene encodes a ribosomal protein that is a component of the 60S subunit. The protein belongs to the L3P family of ribosomal proteins and it is located in the cytoplasm.
RPL5	53	Fibosomal protein L5	This gene encodes a member of the L18P family of ribosomal proteins and component of the 60S subunit.

Gene symbols	Degrees	Full names	Gene function
GNB2L1	53	Guanine nucleotide binding protein beta polypeptide 2-like 1	Component of the 40S ribosomal subunit involved in translational repression.
EEF1A1	52	Eukaryotic translation elongation factor 1 alpha 1	This gene encodes an isoform of the alpha subunit of the elongation factor-1 complex, which is responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome.
EIF4A1	51	Eukaryotic translation initiation factor 4A1	ATP-dependent RNA helicase which is a subunit of the eIF4F complex involved in cap recognition and is required for mRNA binding to ribosome.
RPS9	50	Ribosomal protein S9	This gene encodes a ribosomal protein that is a component of the 40S subunit. The protein belongs to the S4P family of ribosomal proteins.

In order to confirm whether MIR181A2HG interacts with NOP56 and SRSF1, RNA pulldown-Western blotting assay was performed. The results indicated that MIR181A2HG probe could bind to SRSF1 but not NOP56 (Figure 7A). RIP-qRT-PCR was carried out to further confirm the interaction between MIR181A2HG and SRSF1. The results showed that MIR181A2HG was enriched in SRSF1 but not GAPDH immunoprecipitates in NHEKs (Figure 7B). Notably, the expression level of SRSF1 was found to be higher in the psoriatic lesions than in normal skin tissues in GEO datasets (GSE13355) (Figure 7C). Interestingly, SRSF1 knockdown inhibited the expression of KRT16, which could be reversed by knockdown of MIR181A2HG (Figure 7D). Taken together, these results indicated MIR181A2HG may negatively regulate keratinocytes proliferation, at least in part, by interacting with SRSF1 (Figure 7E).

Discussion

LncRNAs are a kind of important regulatory RNAs, which have been reported to play important regulatory roles at epigenetic, transcriptional and post-transcriptional levels, thereby affecting a variety of biological processes including growth, proliferation and differentiation. The abnormal function of lncRNAs may lead to the occurrence of a variety of diseases [21–23]. In recent years, transcriptome studies have shown that the expression profile of lncRNAs differs between the lesions of patients with psoriasis and normal individuals' skin tissues [13, 24], suggesting that lncRNAs may play a potential role in the pathogenesis of psoriasis. In this study, we found that lncRNA MIR181A2HG was expressed at high level in skin tissues among 53 normal human tissues but was expressed at low level in psoriatic lesions (Figure 1), suggesting that it may be involved in the pathogenesis of psoriasis through regulating skin homeostasis. Previous studies have shown that MIR181A2HG is down-regulated in vascular endothelial cells induced by high glucose and acts as a ceRNA to target AKT2 and inhibit its expression, thereby inhibiting the proliferation and migration of vascular endothelial cells [25]. However, we found that the expression of

MIR181A2HG is negatively associated with proliferative capability of keratinocytes, exhibiting anti-proliferative effects. These results suggested that MIR181A2HG may serve as different proliferation modulator to regulate cell fates in different cells.

Almost 80% of miRNAs are located in intron regions of genes [26, 27]. Most of these miRNAs are regulated by the same promoter with their host genes and exhibit similar expression patterns [28], but their functions are not fixed. They may be functionally related to the host genes [29, 30] or may exhibit opposite functions [31]. Similar to the expression pattern of MIR181A2HG, miR-181a and miR-181b were also found to be down-regulated in psoriatic lesions (Figure 4A). Interestingly, it was found that the knockdown of MIR181A2HG could not affect the expression of miR-181a/b, suggesting that MIR181A2HG may function in a miR181a/b independent manner.

The intracellular localization of lncRNAs provides an important clue for the study of their function [7]. In this study, we found that MIR181A2HG was distributed both inside and outside the nucleus, suggesting that MIR181A2HG may play a regulatory role inside or outside the nucleus. lncRNAs often exert their functions through interacting with proteins. In this study, 356 proteins were identified as potential targets interacting with MIR181A2HG. Enrichment analysis results indicated that these proteins mainly enrich RNA processing process, implying that MIR181A2HG may interact with RBPs to regulate RNA processing. In current study, MIR181A2HG was found to directly interact with SRSF1 to affect proliferation of keratinocytes (Figure 7). Recruiting RBPs appears to be a common mechanism among lncRNAs [32]. lnc 10 binds to QKI-5 regulating germ cell apoptosis via p38 MAPK signaling pathway [33]. lncRNA CCDC26 interacts with CELF2 to enhance myeloid leukemia cell proliferation and invasion [34]. SRSF1 is a classic member of the selective splicing factor SR protein family, which is involved in a variety of physiological processes of precursor mRNA [35]. By regulating the splicing of genes, SRSF1 affects the expression and function of downstream related proteins, and then regulates cell proliferation, autophagy and apoptosis [36–38]. SRSF1 seems to be an important binding target for lncRNAs. It has been found that several lncRNAs including MIR155HG, GASAL1, AGAP2-AS1, HOXA11-AS could interact with SRSF1, thus participating in the regulation of cell proliferation and apoptosis. SRSF1 was found to be up-regulated and function as an oncogene in various cancers, such as hepatocellular carcinoma [39], breast cancer [40] and gastric cancer [41]. Here, we found that the expression level of SRSF1 was higher in psoriatic lesions than in normal skin tissues based on the GEO datasets (GSE13355). The inhibition of SRSF1 attenuated the proliferation of keratinocytes, which could be reversed by knockdown of MIR181A2HG. These results suggested that the down-regulation of MIR181A2HG in psoriatic lesions could not successfully block the function of SRSF1, thereby resulting in the hyper-proliferation of keratinocytes (Figure 7E). However, identification of downstream genes and further investigation of specific mechanisms involved in psoriatic keratinocytes hyper-proliferation should be elucidated.

In summary, the results of present work demonstrated that lncRNA MIR181A2HG was down-regulated in psoriatic lesions and negatively regulated keratinocyte proliferation by binding SRSF1. This study highlighted the roles of MIR181A2HG in regulation of keratinocytes hyper-proliferation in psoriasis and may be an attractive therapeutic target.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Guilin Medical University (3/2/2018, Guilin, Guangxi, China). Informed consent was obtained from all individuals involved in the study.

Consent for publication

Not applicable.

Data Availability

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare no competing interests.

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Authors' contributions

J.G. conceived and designed the study. X.F., P.Y., M.L. and F.C. performed experiments. J.F., X.D. and X.L. performed bioinformatics analysis. X.F. drafted manuscript. S.G., Z.W. and C.W. analyzed data and reviewed manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

The expression of MIR181A2HG in psoriatic lesions and normal tissues. (A) The expression of MIR181A2HG in normal skins and psoriatic lesions was analyzed using public available GEO datasets (GSE13355). *** $P < 0.001$. (B) qRT-PCR was applied to measure the relative expression of MIR181A2HG in the psoriatic lesions and normal skins. GAPDH served as an internal reference. ** $P < 0.01$. (C) GTEx database was used to explore the expression of MIR181A2HG in normal tissues.

Figure 2

Effect of MIR181A2HG up-regulation on proliferation of keratinocytes.

(A) HaCaT keratinocytes were seeded in 12-well plates and transfected with pcDNA3.1 or pcDNA3.1-MIR181A2HG for 48 hours. qRT-PCR was applied to measure the expression of MIR181A2HG. (B) CCK-8 assays were carried out to record the growth curves of HaCaT keratinocytes transfected with pcDNA3.1 or pcDNA3.1-MIR181A2HG for 24-96 hours. ** $P < 0.01$. (C) KRT6 and KRT16 expression was measured by qRT-PCR in HaCaT keratinocytes transfected with pcDNA3.1 or pcDNA3.1-MIR181A2HG for 72 hours. * $P < 0.05$. (D) EdU assays were used to detect the proliferation rate of HaCaT keratinocytes transfected with pcDNA3.1 or pcDNA3.1-MIR181A2HG for 48 h. Scale bars, 50 μ m. (E) NHEKs were seeded in 12-well plates and infected with lentivirus particles LV-NC or LV-MIR181A2HG for 72 hours. qRT-PCR was

performed to measure the expression of MIR181A2HG. (F) CCK-8 assays were carried out to record the growth curves of NHEKs transfected with LV-NC or LV-MIR181A2HG for 24-96 hours. * $P<0.05$, ** $P<0.01$. (G) KRT6 and KRT16 expression was measured by qRT-PCR in NHEKs infected with LV-NC or LV-MIR181A2HG for 72 hours. * $P<0.05$. (H) EdU assays were used to detect the proliferation rate of NHEKs transfected with LV-NC or LV-MIR181A2HG for 48 h. Scale bars, 50 μm .

Figure 3

Effect of knockdown of MIR181A2HG on proliferation of keratinocytes.

(A and E) HaCaT keratinocytes and NHEKs were seeded in 12-well plates and transfected with 50 nM scramble siRNA (siNC) or MI181A2HG siRNA (siMIR181A2HG) for 48 hours. qRT-PCR was performed to

measure the expression of MIR181A2HG. * $P < 0.05$. (B and F) CCK-8 assays were carried out to record the growth curves of HaCaT keratinocytes and NHEKs transfected with 50 nM siNC or siMIR181A2HG for 24-96 hours. * $P < 0.05$, ** $P < 0.01$. (C and G) KRT6 and KRT16 expression was measured by qRT-PCR in HaCaT keratinocytes and NHEKs transfected with 50 nM siNC or siMIR181A2HG for 48 hours. * $P < 0.05$, ** $P < 0.01$. (D and H) EdU assays were used to detect the proliferation rate of HaCaT keratinocytes and NHEKs transfected with 50 nM siNC or siMIR181A2HG for 48 h. Scale bars, 50 μm .

Figure 4

The effect of MIR181A2HG knockdown on the expression of miR-181a/b.

(A) The location relationship among MIR181A2HG, MIR181A2 and MIR181B2. (B) The expression of miR-181a and miR-181b in psoriatic lesions and normal skins was analyzed using public available GEO datasets (GSE145054). * $P<0.05$, ** $P<0.01$. (C) HaCaT keratinocytes were seeded in 12-well plates and transfected with siRNA (siNC) or MIR181A2HG siRNA (siMIR181A2HG) (50nM) for 48 hours. qRT-PCR was used to detect the expression of miR-181a and miR-181b.

Figure 5

Intracellular localization of MIR181A2HG.

(A) LncATLAS was used to explore the localization of MIR181A2HG. (B) Nuclear/cytoplasmic separation qRT-PCR was performed to detect the nuclear and cytoplasmic distribution of MIR181A2HG. GAPDH served as extranuclear RNA control. MALAT1 and U3 served as intranuclear RNA controls.

Figure 6

Screening of MIR181A2HG interaction protein.

(A) The silver staining result of RNA pulldown proteins. (B) Pathway analysis of MIR181A2HG interacting proteins. (C) GO biological process analysis of MIR181A2HG interacting protein. (D) The construction of

protein interaction network of MIR181A2HG interaction protein. (E) The interaction network of 13 key genes. (F) The intersection of 13 key proteins with the proteins predicted by catRAPID and ENCORI.

Figure 7

The validation of MIR181A2HG-interacting protein in vitro.

(A) RNA pulldown-Western blotting assay was used to verify the interaction of MIR181A2HG and NOP56/SRSF1. (B) RIP-qRT-PCR assay was performed to confirm the interaction between MIR181A2HG and SRSF1. (C) The expression of SRSF1 was analyzed using GEO datasets (GSE13355). (D) HaCaT keratinocytes were seeded in 12-well plates and transfected with 50 nM scramble siRNA (siNC), MI181A2HG siRNA (siMIR181A2HG) or SRSF1 siRNA (siSRSF1) for 48 hours. qRT-PCR was applied to measure the expression of KRT16 $*P<0.05$, $**P<0.01$, $***P<0.001$. (E) Schematic representation summarizing data from the present study.