hsa-miR-1-3p and hsa-miR-361-3p as potential biomarkers for onychomycosis: A pilot study

LI MENG*, QING CAI*, HUAIJIAN ZHANG, ZHIQIN GAO and LIANJUAN YANG

Department of Dermatological Mycology, Shanghai Skin Disease Hospital, Tongji University School of Medicine, Shanghai 200443, P.R. China

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Abstract. Non-coding small molecule RNAs are associated with a variety of diseases, including infectious diseases. However, small RNA-related studies in onychomycosis have not been reported. The aim of the present study was to conduct an initial investigation of small RNA in onychomycosis. The present study collected a total of 33 affected nail samples from patients with onychomycosis and 18 normal nail samples from healthy people. Through RNA sequencing, 37 differentially expressed microRNAs (miRNAs or miRs), including 15 upregulated and 22 downregulated miRNAs, were identified in 3 patients with onychomycosis compared with 3 healthy controls. Moreover, three differentially expressed miRNAs were analyzed for further verification by RT-qPCR in other 30 affected nail and 15 healthy nail samples. Among the three verified miRNAs, a significant difference between the downregulated hsa-miR-1-3p and hsa-miR-361-3p was observed ($P<0.05$). A total of 14,511 target genes of 37 differentially expressed miRNAs were predicted by the miRanda and RNAhybrid databases, while the Kyoto Encyclopedia of Genes and Genomes and Gene Ontology analysis showed that these target genes were enriched in multiple signaling pathways. The present study indicated that hsa-miR-1-3p and hsa-miR-361-3p may be potential biomarkers for onychomycosis. Furthermore, the findings of the present study can be used in future research on RNA in onychomycosis.

Introduction

Onychomycosis is the most common nail disease worldwide, accounting for ~90% of all toenail infections (1). Onychomycosis can occur at any age and is mostly common among older adults; the number of affected nails and prevalence rate continue to increase (2). Onychomycosis is a type of disease caused by fungal infection. Although dermatophytes are the most common pathogen of onychomycosis, non-dermatophyte infections are also prevalent. The transmission of onychomycosis can be caused by direct or indirect contact, and this disease can lead to finger/toenail discoloration, thickening, peeling, deformity, and pain or other discomfort in patients. Therefore, it can have a negative impact on the psychological wellbeing and the social interactions of the patient. Currently, the treatment of this disease remains a challenge, considering its high recurrence rate (2). Since onychomycosis is a chronic disease that is difficult to treat, it is important to develop new therapeutic methods for its treatment.

Non-coding small molecule RNAs, including microRNAs (miRNAs or miRs), are the key regulators of the gene expression pathways and systems in various different cells (3). Previous research has shown that miRNAs are associated with various diseases (4-7). Circulating and exosome-derived miRNAs have been considered as biomarkers for a number of diseases, including viral infections, neurological diseases, cardiovascular diseases, and diabetes (7). As key regulatory factors of gene expression, miRNA-related studies in various diseases have been increasing and these molecules are expected to become candidates for the development of certain biomarkers. Moreover, miRNA mimics and miRNA inhibitors have been proven as promising therapeutic drugs in preliminary clinical studies (8). However, it has not been determined whether miRNAs are associated with onychomycosis and whether they can also be used as drug targets for the treatment of onychomycosis.

Therefore, after obtaining study approval from the Ethics Committee of Shanghai Skin Disease Hospital (approval no. 2022-68; Shanghai, China) for the present study, the potential role and relationship of miRNAs in onychomycosis were investigated. A small molecule RNA-related study on patients with onychomycosis was subsequently conducted, for the first time, to the best of the authors' knowledge, which provides insights for future studies.

Patients and methods

Ethics statement and clinical specimens. The present study was approved (approval no. 2022-68) by the Ethics Committee

Correspondence to: Professor Lianjuan Yang, Department of Dermatological Mycology, Shanghai Skin Disease Hospital, Tongji University School of Medicine, 1278 Baode Road, Jingan, Shanghai 200443, P.R. China
E-mail: lianjuanyang@163.com

*Contributed equally

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of Shanghai Skin Disease Hospital (Shanghai, China) on February 15, 2022, and was performed in accordance with the Declaration of Helsinki. All samples including those of healthy controls were obtained from patients at Shanghai Skin Disease Hospital, Tongji University School of Medicine from February 20, 2022 to December 31, 2022 (Shanghai, China). Written informed consent was obtained from all subjects prior to the study. The inclusion and exclusion criteria were as follows: The affected nail samples were obtained from patients with onychomycosis who were diagnosed by clinical features and positive mycological examination. Normal control nail samples were obtained from healthy individuals without onychomycosis and other systemic or immune diseases. All subjects were required to have no history of systematic medication, 3 months prior to commencement of the sample collection, and no history of drugs or other chemical agents applied to the surface of the nail, 1 month prior to commencement of the sample collection. A total of 33 affected nail samples from onychomycosis and 18 normal nail samples from healthy individuals were obtained. Each nail sample was stored in a separate tissue box in a refrigerator at a temperature of -80˚C. The clinical information of the onychomycosis and control groups are shown in Table I. The patient cohort median age was 47 years, and the age range was 21-72 years.

Table I. Clinical features of subjects in the onychomycosis and control groups.

Feature	Affected group (n=33)	Control group (n=18)
Age (years)	46.87±11.53	35.64±10.1
Sex (female/male)	15/18	10/8
Position (fingernail/ toenail)	11/22	8/10
Positive mycological examination	33	-

Values are expressed as the mean ± SD (range) or n. SD, standard deviation.

Nail sample preparation and RNA extraction. The nail samples were obtained by scissors, put into tissue boxes, and stored at a temperature of -80˚C. Moreover, the nail clippings were further pulverized and stored at a temperature of -80˚C. The total RNAs from the pulverized nails were extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), and their quality and quantity were measured using the NanoDrop ND1000 Spectrophotometer (Thermo Fisher Scientific, Inc.).

Small RNA library construction and miRNA sequencing. Purified RNA was sent to Shanghai Yingbai Biotechnology Co., Ltd. for the construction of the small RNA library. Nail-derived RNAs from three affected nails in onychomycosis and three normal nails from the control group were used for miRNA sequencing. The small RNA library was constructed using the NEBNext Multiplex Small RNA Library Prep kit (cat. no. E7560S; New England Biolabs, Inc.). In brief, the total RNA was combined with 3' and 5' adapters, and complementary DNA (cDNA) was synthesized by reverse transcription quantitative PCR. The fragments from 135-150 bp adapters, including 120 bp adapters, were extracted using the QIAquick gel extraction kit (cat. no. 28704; Qiagen China Co., Ltd.). The small RNAs and the purified library were sequenced using the Illumina HiSeq 2500 sequencing system (Illumina, Inc.). The loading concentration of the library was 4.71 pM and concentrations were measured using Qubit™ 3 Fluorometer Invitrogen™ (cat. no. Q33216; Thermo Fisher Scientific, Inc.).

Data analysis for miRNA sequencing. At first, the raw sequencing data needed to be filtered, their low-quality reads and short reads (<15 nt) were removed, and they were subjected to quality control by fast-QC (v0.12.1) (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). All clean reads were aligned to the miRbase database (http://www.mirbase.org/) to determine the known miRNAs. The clean data was screened using EBSeq (9) to identify the differentially expressed miRNAs between the onychomycosis and control groups in accordance with the following criteria: Log_2 fold change >1 and false discovery rate <0.05. miRanda (https://regendbase.org/tools/miranda; selection criteria, score ≥150 and energy <-20) and RNAhybrid (https://omictools.com/rnahybrid-tool; selection criteria, energy <-25) (10) were used to predict the target genes of the screened miRNAs showing significant differences. The overlapping genes between these two databases were considered as the final target genes. Gene Ontology (GO; http://www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg) were used to classify the functions and pathways of the predicted target genes.

Reverse transcription-quantitative PCR (RT-qPCR). In order to further screen miRNAs and further confirm the association between miRNAs and onychomycosis, firstly, six differentially expressed miRNAs, including downregulated hsa-miR-1-3p hsa-miR-34c-5p and hsa-miR-361-3p and upregulated hsa-miR-766-5p, hsa-miR-6767-5p, and hsa-miR-4253, were selected for RT-qPCR in nails from 5 patients with onychomycosis and 5 healthy controls. The identified miRNAs with differences were then further verified in another 10 controls and 25 cases. The RNAs were reverse transcribed into cDNA using the RevertAid First Strand cDNA Synthesis kit (cat no. K1691; Thermo Fisher Scientific, Inc.) in accordance with the manufacturer's instructions. RT-qPCR was conducted using the SYBR Green PCR kit (cat. no. 208054; Qiagen China Co., Ltd.) on an ABI Q6 detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The PCR thermocycling conditions were as follows: 95˚C for 10 min, 45 cycles of 15 sec at 95˚C and 60 sec at 60˚C. The relative calculations and quantification were conducted with the quantitative threshold (Cq) cycle method, $2^{-\Delta\Delta Cq}$ (11). U6 served as the internal control for miRNA.

Principle for the design of stem-loop primers. In the reverse transcription process, a stem-loop primer that can bind to

Table II. Sequences of primers for microRNAs.

Primers	Sequences
hsa-miR-1-3p	R: 5'-GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACATACATA-3'
	F: CGCAGTGGAATGTAAAGAAG-3'
hsa-miR-361-3p	R: 5'-GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAAATCAG-3'
	F: 5'-TCCCCCAGGTGTGATTC-3'
hsa-miR-766-5p	R: 5'-GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAAGACCA-3'
	F: 5'-CAGAGG AGGAATTGGTGCT-3'
hsa-miR-34c-5p	R: 5'-GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGCAATCA-3'
	F: 5'-GCAGAGGCAGTGTAGTTAG-3'
hsa-miR-4253	R: 5'-GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACACCCCCT-3'
	F: 5'-GCAGAGGGCATGTCCAG-3'
hsa-miR-6767-5p	R: 5'-GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTCTCCAT-3'
	F: 5'-CGCAGACAGGGACACA-3'
U6	F: 5'-CGATACAGAGAAGATTAGCATGGC-3'
	R: 5'-AACGCTTCACGAATTTGCGT-3'
IRI (Downstream universal primer)	R: 5'-AGTGCGTGTCGTGGAGTCG-3'

R, reverse transcription; F, forward.

the miRNA sequence and extend the length of miRNA had to be designed. The stem-loop primer is a primer that is ~45 bp in length and can self-loop. Every miRNA has its own specific stem-loop primer, the most important reason for this is that the designed stem-loop primer includes a section that is related to the complementary sequence of the miRNA: Stem-loop reverse transcription primer=5' end stem-loop sequence + 3' end specific complementary sequence of miRNA.

Features of the design of stem-loop primers. Initially, the mature miRNA sequences (5 '-3') were found in the miRBase database, using the substitution function in excel to replace all U in the sequence with T, in a general universal sequence of stem ring primers such as 5'-*CTCAACTG*GTGTCGTGGA GTCGGCAATT*CAGTTGAGC*-3' or 5'-*GTCGTATCCAGT GC*AGGGTCCGAGGTATT*CGCACTGGATACGAC*-3'; the italicized part of the sequence can form its own cyclization. In addition, stem-loop primers were designed for specific miRNA. The reverse complementary sequence of 6-8 bases (6 in general) was added to the -3' end of the universal stem-loop primer, after the U in the mature sequence of miRNA was replaced by T in the -3' end of the universal stem-loop primer, so as to obtain the stem-loop primer of a certain miRNA. The specific primer sequences are shown in Table II.

Statistical analysis. Statistical analysis was performed using the SPSS version 12.0 statistical software (SPSS, Inc.). The quantitative data was presented as the mean ± standard deviation and compared via the two-tailed unpaired Student's t-test. Statistical significance was set at a P-value of <0.05. The experimental data was obtained from at least three independent experiments.

Results

Sequencing of miRNAs from the nails of patients with onychomycosis. A total of 2,136 miRNAs were identified between 3 patients with onychomycosis and 3 controls, of which, 37 differentially expressed miRNAs were considered statistically significant after screening (Fig. 1A), including 15 upregulated and 22 downregulated miRNAs, in the onychomycosis group compared with the control group (Fig. 1B). The detailed differentially expressed miRNAs are revealed in Table III.

Target gene prediction and functional analysis of differentially expressed microRNAs. A total of 14,511 target genes from 37 differentially expressed microRNAs were predicted using the miRanda and RNAhybrid databases. The GO analysis indicated that these target genes were enriched in the 'positive regulation of transcription from the RNA polymerase II promoter', 'transcription, DNA-templated', and 'regulation of transcription, DNA-templated' (Fig. 2A). The KEGG pathway analysis revealed that these target genes were enriched in the 'Rap1 signaling pathway', 'Wnt signaling pathway', 'Ras signaling pathway', 'calcium signaling pathway', and 'MARK signaling pathway' (Fig. 2B).

Validation of differentially expressed miRNAs. To further screen miRNAs, six differentially expressed miRNAs, including downregulated hsa-miR-1-3p hsa-miR-34c-5p and hsa-miR-361-3p, and upregulated hsa-miR-766-5p, hsa-miR-6767-5p and hsa-miR-4253, were subjected to RT-qPCR analysis in nails from 5 patients with onychomycosis and 5 healthy controls. As a result, three of them including hsa-miR-1-3p, hsa-miR-361-3p and hsa-miR-766-5p exhibited significant differences (P=0.032, P=0.027 and P<0.001, respectively; Fig. 3A). In further verification of larger samples

Table III. Differentially expressed miRNAs identified in onychomycosis.

miRNAs	$\log_2$ FC	FDR	Expression	P-value
hsa-miR-1-3p	-6.62285	3.51E-08	Down	7.89×10^{-11}
hsa-miR-4253	6.340101	0.002272	Up	0.0000102
hsa-miR-34c-5p	-5.46533	0.00285	Down	0.0000192
hsa-miR-361-3p	-4.62943	0.004969	Down	0.0000447
hsa-miR-1248	-6.07778	0.006409	Down	0.0000720
hsa-miR-135b-5p	-4.38787	0.006887	Down	0.000101
hsa-miR-6767-5p	5.231031	0.006887	Up	0.000112
hsa-miR-766-5p	5.077687	0.006887	Up	0.000124
hsa-miR-1911-3p	4.635966	0.007694	Up	0.000156
hsa-miR-10395-3p	-4.45961	0.010072	Down	0.000226
hsa-miR-125a-5p	-3.70702	0.017771	Down	0.000488
hsa-miR-26a-5p	-4.28808	0.017771	Down	0.000505
hsa-miR-634	4.863213	0.017771	Up	0.000524
hsa-miR-6843-3p	5.109063	0.017771	Up	0.000602
hsa-miR-4755-5p	6.043341	0.017771	Up	0.000628
hsa-miR-1911-5p	3.672735	0.017771	Up	0.000639
hsa-miR-1246	-3.49335	0.017979	Down	0.000687
hsa-miR-152-3p	-3.9556	0.018725	Down	0.000757
hsa-miR-200a-3p	-3.87371	0.019056	Down	0.000855
hsa-miR-6763-5p	4.195021	0.019056	Up	0.000864
hsa-miR-101-3p	-3.80914	0.019056	Down	0.000946
hsa-miR-99b-5p	-3.64934	0.019056	Down	0.000985
hsa-miR-6823-3p	4.581339	0.019056	Up	0.000985
hsa-miR-7154-3p	4.312293	0.022085	Up	0.001191
hsa-miR-5196-5p	3.954891	0.023612	Up	0.00137
hsa-miR-182-5p	-3.73798	0.023612	Down	0.00138
hsa-miR-27a-5p	-3.23665	0.02792	Down	0.001722
hsa-miR-514a-3p	-3.89711	0.02792	Down	0.001807
hsa-miR-1234-3p	3.816317	0.02792	Up	0.001819
hsa-miR-148a-5p	-3.51467	0.029162	Down	0.002017
hsa-miR-186-5p	-3.46579	0.029162	Down	0.002032
hsa-miR-769-5p	-3.59224	0.033852	Down	0.002494
hsa-miR-873-3p	4.245054	0.033852	Up	0.00251
hsa-miR-28-3p	-3.2411	0.036765	Down	0.002809
hsa-miR-5585-5p	-3.14452	0.044994	Down	0.003539
hsa-miR-6800-5p	3.787112	0.046003	Up	0.003722
hsa-miR-141-3p	-3.33558	0.04909	Down	0.004082

P<0.05 was considered to indicate a statistically significant difference. miRNA or miR; microRNA; FC, fold change; FDR, false discovery rate; Up, upregulated; Down, downregulated.

from 25 patients with onychomycosis and 10 healthy controls, no differentially expressed miRNAs were detected. However, after the removal of these samples, including the three repetitions of the same sample, there were significant variations among the experimental data, with some samples showing excessively large or small deviations, while others remained indeterminate. Notably, downregulated hsa-miR-1-3p and hsa-miR-361-3p exhibited significant differences in expression (P=0.009 and P=0.041, respectively; Fig. 3B). These results indicated that the expression of hsa-miR-1-3p and hsa-miR-361-3p in onychomycosis might be downregulated compared with control group.

Target gene prediction and functional analysis of hsa-miR-1-3p and hsa-miR-361-3p. A total of 544 target genes from hsa-miR-1-3p and 604 target genes from hsa-miR-361-3p were predicted by the miRanda and RNAhybrid databases. The GO and KEGG analyses showed that these target genes were enriched in multiple signaling pathways as shown in Figs. 4 and 5.

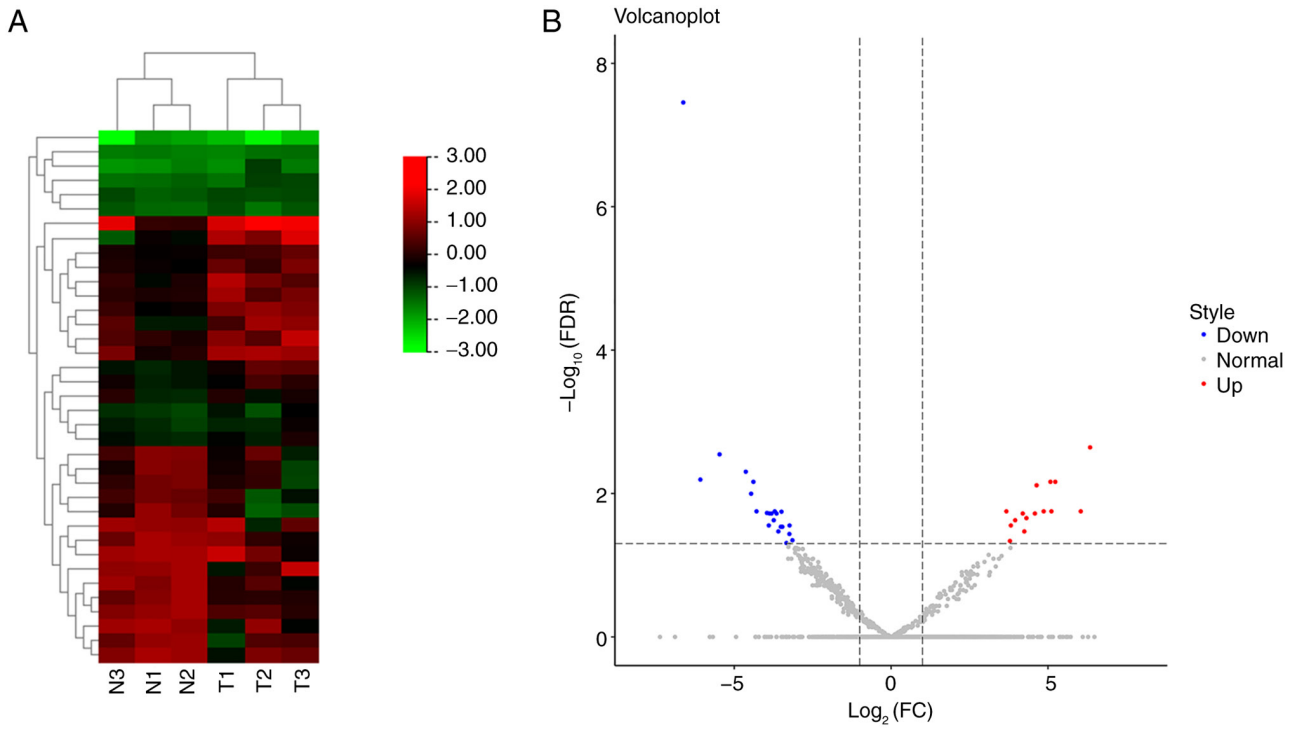

Figure 1. (A) Hierarchical clustering of differentially expressed miRNAs between the onychomycosis (n=3) and control groups (n=3). The color scale represents high (red) to medium (black) to low (green) relative expression. (B) The volcano plot on the differentially expressed miRNAs showed the significantly upregulated miRNAs (red), significantly downregulated miRNAs (blue), and non-significant miRNAs (gray). miRNAs, microRNAs; FC, fold change; FDR, false discovery rate; N, normal control group; T, onychomycosis group.

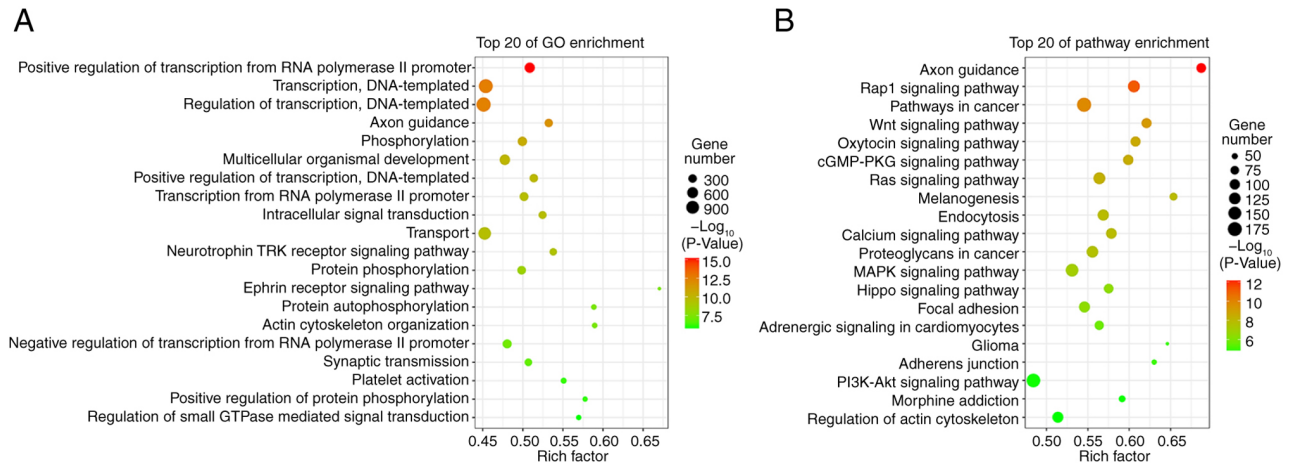

Figure 2. Bubble graphs of (A) GO and (B) KEGG pathway enrichment analyses showing the top 20 identified terms and pathways for the target genes of differentially expressed microRNAs (larger circles represent a larger number of genes). The color scale represents the low (green) to high (red) P-values. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Discussion

Onychomycosis is commonly caused by fungal infections and has a high prevalence. Since its treatment remains a challenge, new treatment methods need to be developed. miRNAs are small non-coding RNAs that usually bind with mRNA targeting their 3-UTR and regulating gene expression at the pre-transcription level. The dysregulation of miRNAs ultimately leads to differentially expressed profiles of other miRNAs and their target genes. Moreover, miRNAs have been suggested to be involved in various functions, such as impact on the immune system, apoptosis, cellular proliferation and differentiation, and the cellular stress response (12). In the last decade, previous research (4-7) has shown that miRNAs might be involved in multiple biological and pathological processes in various diseases, and can be considered as potential biomarkers for a number of diseases and targets in the treatment of various diseases. miRNAs may also be used as a treatment option or can be implemented in the treatment strategies for onychomycosis. Therefore, studies that focus on miRNAs are required to determine their potential contribution to the treatment of onychomycosis.

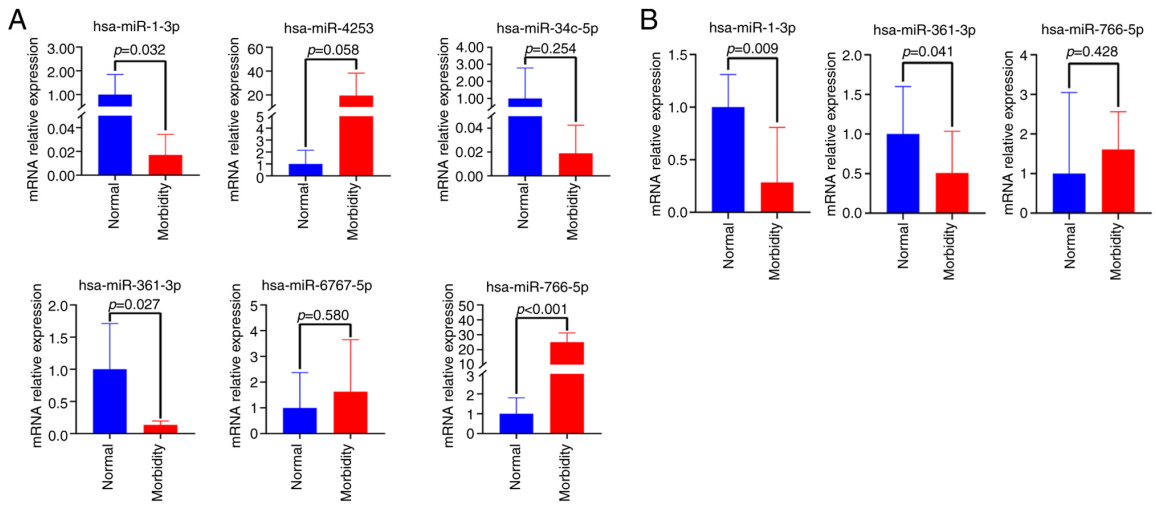

Figure 3. mRNA relative expression of differentially expressed miRNAs in nails of the onychomycosis and control groups, detected using reverse transcription quantitative-PCR. (A) mRNA relative expression of six miRNAs in 5 cases and 5 controls. (B) mRNA relative expression of three miRNAs in another 25 cases and 10 controls. miRNAs, microRNAs.

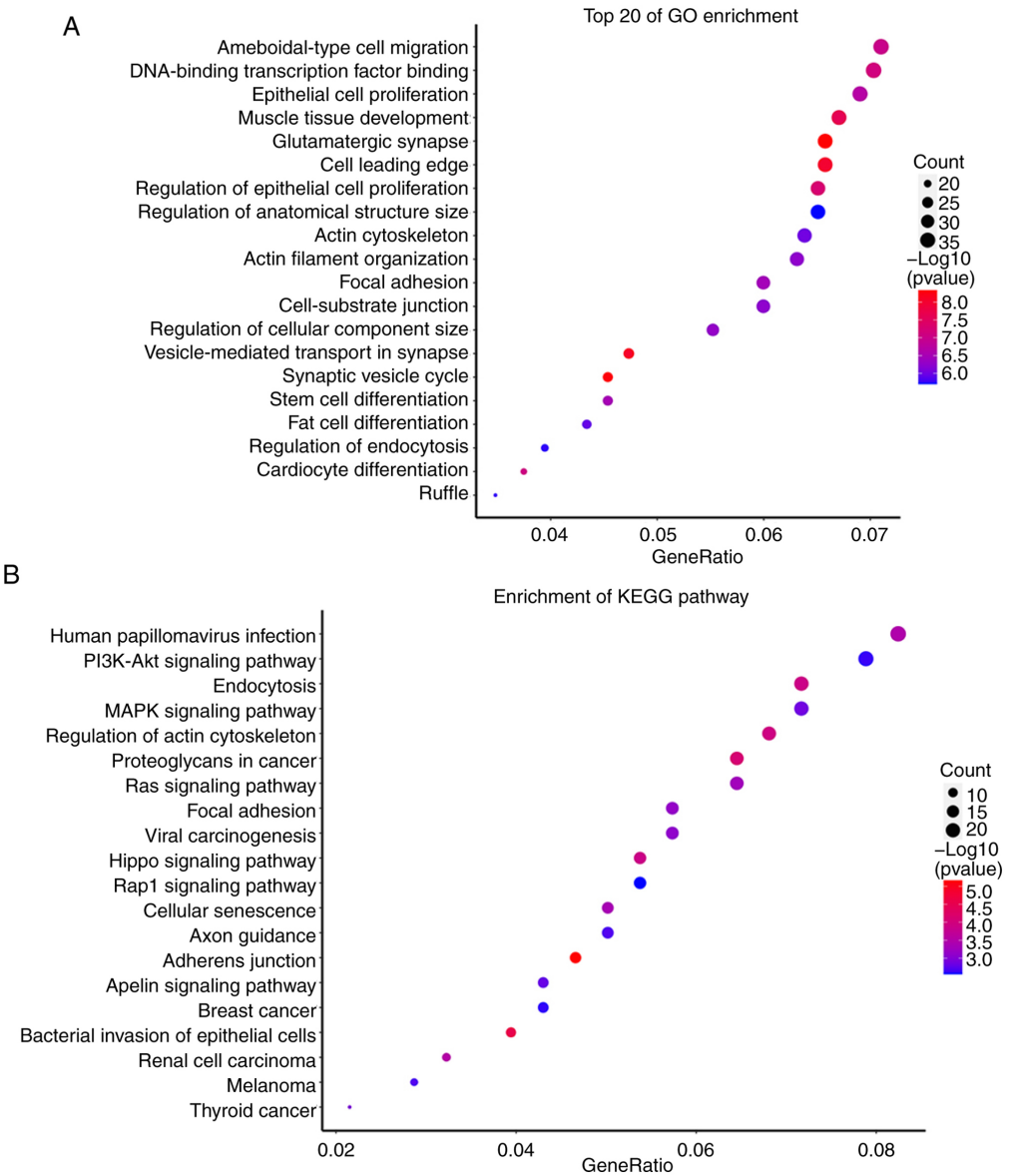

Figure 4. Bubble graphs of (A) GO and (B) KEGG pathway enrichment analyses for the target genes of hsa-miR-1-3p (larger circles represent a larger number of genes). The color scale represents low (green) to high (red) P-values. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

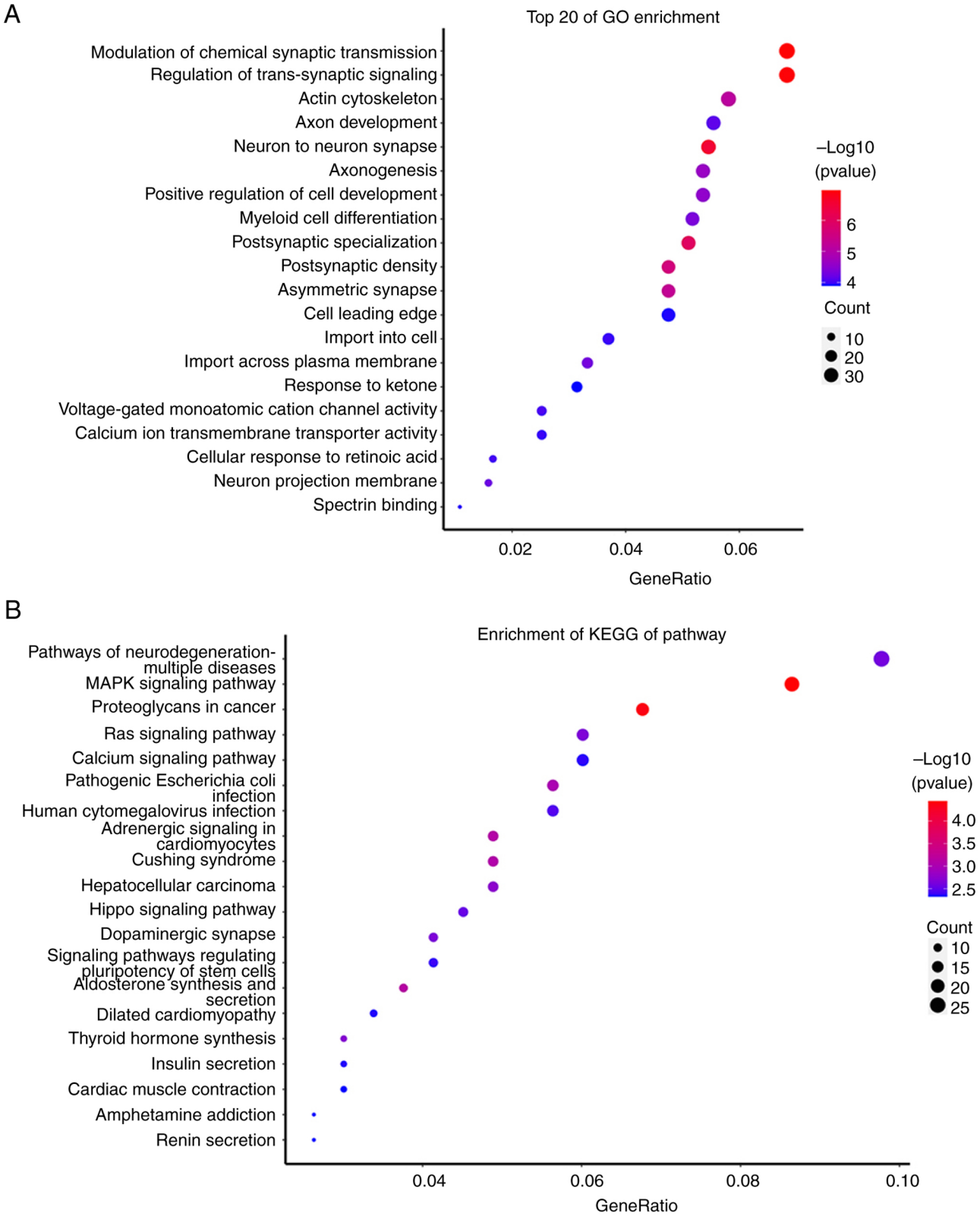

Figure 5. Bubble graphs of (A) GO and (B) KEGG pathway enrichment analyses for the target genes of hsa-miR-361-3p (larger circles represent a larger number of genes). The color scale represents low (green) to high (red) P-values. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

The is the first RNA-related study on patients with onychomycosis that has been conducted, to the best of the authors' knowledge, and 37 differentially expressed miRNAs from nail clippings were identified. Thus, an initial miRNA panel of onychomycosis is presented, which may aid in future research. GO and KEGG analyses revealed that the targets of these 37 miRNAs were enriched in multiple functional pathways, including 'axon guidance', 'Wnt signaling pathway', 'calcium signaling pathway', 'Rapl signaling pathway', and the 'ephrin receptor signaling pathway' (Fig. 2), suggesting the potential

role of miRNAs in the treatment of onychomycosis. Further RT-qPCR verification results showed that the expression of hsa-miR-1-3p and hsa-miR-361-3p in onychomycosis was downregulated compared with the control group. However, further studies with larger sample sizes are needed, to confirm these results.

Research has shown that has-miR-1-3p and T-synthase mRNA could be considered as independent risk factors for the patients with intestinal mucosal barrier dysfunction with severe acute pancreatitis, and can facilitate the diagnosis of intestinal mucosal barrier dysfunction in patients with severe acute pancreatitis (13). In addition, a number of studies have reported the role of has-miR-1-3p in various cancers, such as head and neck squamous cell carcinoma (14), breast cancer (15), and lung adenocarcinoma (16). In the present study, GO and KEGG analyses suggested that the targets of has-miR-1-3p were enriched in 'human papillomavirus infection', 'bacterial invasion of epithelial cells', 'breast cancer', and 'thyroid cancer' (Fig. 4). The results of the present analysis were consistent with those of the previous studies aforementioned and also suggested that has-miR-1-3p may have a potential role in the pathogenesis of the fungal infection of onychomycosis, and might be a potential biomarker for onychomycosis. However, further research is required to confirm the potential role of has-miR-1-3p in onychomycosis.

hsa-miR-361-3p has been revealed to inhibit the progression of lymphoma via the Wnt/β-catenin signaling pathway (17), and to promote colorectal cancer progression by targeting the TRAF3-mediated noncanonical NF-κB pathway (18) as well as human breast cancer cell viability by suppressing the E2F1/P73 signaling pathway (19). Moreover, hsa-miR-361-3p was overexpressed in the oral squamous cell carcinoma tissues, and targeting hsa-miR-361-3p may be a useful therapeutic approach for patients with oral squamous cell carcinoma (20). In the present study, GO and KEGG analyses showed that the targets of has-miR-361-3p were enriched in 'pathogenic *Escherichia coli* infection' and 'human cytomegalovirus infection' (Fig. 5). It is known that onychomycosis is a disease caused by fungal infections. Therefore, the results of the present study indicated that hsa-miR-361-3p may be a potential biomarker for patients with onychomycosis and could be involved in infection-related pathways in onychomycosis. Considering the limitations of the present study, further research is still needed to determine the potential role of hsa-miR-361-3p in patients with onychomycosis.

Of note, the current study has several limitations. Firstly, the sample size was relatively small. Therefore, in the interpretation of the results, the possibility of a beta error may exist and lead to the lack of significance. Thus, future studies with larger sample sizes are needed. Secondly, some differences in the weight of each sample were observed between the patient and the control groups, potentially introducing data bias in the data analysis, although the concentration which was detected, was not influenced by the weight of the sample. Moreover, the samples used in the present study were obtained from the nail clippings of affected nails and healthy nails. For the most part, affected nails tend to be relatively thick, rendering it easy to acquire a large weight of nail clipping sample. However, normal healthy nails are relatively thin, and a low weight of nail clipping was obtained. Thirdly, the nail clippings obtained from individuals were used to only perform RNA sequencing and RT-qPCR. Therefore, more experiments and testing methods are needed to further confirm and clarify the potential role of miRNAs in patients with onychomycosis. Finally, the present study was just an initial investigation, the experimental design was relatively simple, and no other in-depth functional pathway studies were conducted.

Regardless of the limitations, a differential expression miRNA panel of onychomycosis was provided in the present study, and it was revealed that hsa-miR-1-3p and hsa-miR-361-3p were downregulated in the onychomycosis group compared with the control group, which suggested that hsa-miR-1-3p and hsa-miR-361-3p might be potential biomarkers for onychomycosis. The present study was an initial investigation of potential miRNAs in onychomycosis, and a larger sample size and additional functional analyses are required, to further provide a more comprehensive and accurate understanding of this topic.

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Availability of data and materials

The data generated in the present study may be found in the Sequence Read Archive (SRA) under accession nos. SUB14485829 (Submission ID) and PRJNA1117633 (BioProject ID) or at the following URL: http://www.ncbi.nlm.nih.gov/bioproject/1117633.

Authors' contributions

LM and QC were involved with the conception and design of the study, as well as manuscript writing, editing, and acquisition, analysis and interpretation of data. LM and QC confirm the authenticity of all the raw data. HZ and ZG were involved with the collection of clinical data. LY was involved with study conception, supervision and manuscript revision. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Shanghai Skin Disease Hospital (approval no. 2022-68; Shanghai, China), and all subjects have signed the written informed consent.

Patient consent for publication

All subjects have provided written informed consent for the publication of any associated data and accompanying images.

Competing interests

The authors declare that they have no competing interests.

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