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Expression of miR-155 and miR-146a in Skin Tissue, Peripheral Blood Mononuclear Cells and Serum of Patients with Psoriasis and Its Clinical Significance

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Abstract

Objective: To explore the expression and clinical significance of miR-155 and miR-146a in psoriasis patients from different sources. **Methods:** The expression levels of miR-155 and miR-146a in psoriasis vulgaris tissues, peripheral blood mononuclear cells, and serum were detected by the QRT-PCR method in 30 patients with psoriasis vulgaris and 30 healthy subjects as the normal control group, and the correlation between the expression levels of miR-155 and miR-146a in psoriasis samples from different sources was analyzed. And the expression levels of miR-155 and miR-146a in peripheral blood mononuclear cells, and serum after treatment. **Results:** The expression of miR-155 and miR-146a in skin tissue, peripheral blood mononuclear cells and serum of patients with psoriasis was significantly higher than that of normal controls ($P < 0.01$). miR-155 and miR-146a were highly expressed in skin tissue, peripheral blood mononuclear cells and serum of the psoriasis experimental group ($P < 0.01$). After treatment, the expression of miR-155 and miR-146a in peripheral blood mononuclear cells and serum decreased ($P < 0.05$). **Conclusions:** The significantly increased levels of miR-155 and miR-146a in psoriasis samples from different sources may mediate the immune mechanism involved in the activity process of psoriasis.

Keywords

Psoriasis, miR-155, miR-146a

1. Introduction

Psoriasis is a chronic inflammatory skin disease that is prone to recurrence and

has a long course of the disease. Erythema and scales may occur on the skin of the whole body, which seriously affects the quality of life of patients [1]. At present, the prevalence of psoriasis is about 1.5% - 3%, and it shows an increasing trend year by year. The pathogenesis of psoriasis is complex and diverse, related to genetic, environmental, immune, endocrine, neuropsychiatric and other factors [2] MicroRNA (miRNA), as regulatory factors encoded by endogenous genes, can be involved in various processes of cell life activities and is closely related to immune-inflammatory diseases. Using bioinformatics methods, we found significant differences in the expression levels of miR-155 and miR-146a in the serum of patients with psoriasis. As an important member of the miRNA family, miRNA-155 plays an important role in the regulation of T lymphocytes and has been reported to play an important role in immune-related diseases such as atopic dermatitis, human immunodeficiency virus infection, and rheumatoid arthritis. miR-146a is a member of the microRNAs family. Most vertebrates have two genes encoding miR-146, namely miR-146a and miR-146b. Among them, the miR-146a gene is located in Q33.3 and the miR-146b gene is located in 10Q24.32. miR-146a is a microRNA found to be abnormally expressed in peripheral blood and skin lesions of patients with psoriasis in recent years [3]. miR-155 is highly expressed in the skin tissue of patients with psoriasis vulgaris [4], and miR-146a is also highly expressed [5]. However, most research focused on the expression validation in psoriasis skin tissue, peripheral blood mononuclear cells, expression in serum of study is less, in this paper, the systematic study of patients with psoriasis vulgaris skin tissue and peripheral blood mononuclear cells, serum miR-155, miR-146—a mode of expression and correlation, and the change of the relationship between before and after therapy, explore the clinical significance.

2. Objects and Methods

2.1. Subjects

Thirty patients with psoriasis vulgaris treated in the dermatology department of Qionghai People's Hospital of Hainan Province and the dermatology department of Chongqing Hospital of Traditional Chinese Medicine from July 2016 to July 2017 were selected as the experimental group of psoriasis. All patients had typical clinical manifestations and were confirmed by pathology. The diagnosis met the clinical criteria [6]. Samples were not treated with glucocorticoids, retinoids, immunosuppressants, or psoralen, or ultraviolet light (UVA, wavelength 320 - 420 nm) for at least 1 month prior to collection. Before blood collection, liver and kidney function and urine routine were in the normal range. The patients are aged between 20 and 65 years, and the course of the disease is 6 to 9 years. Exclusion criteria: accompanied by other types of skin diseases, especially systemic lupus erythematosus, skin tumors, etc.; patients with other serious diseases such as rheumatoid arthritis and abnormal liver and kidney function; Pregnant and breast-feeding women, menstruating women, with cataract and phototherapy contraindications. Thirty healthy subjects without systemic dis-

eases or other autoimmune diseases during the same period were selected as the normal control group, aged 20 - 65 years. This study was approved by Qionghai People's Hospital Ethics Committee, and all patients and healthy controls from all specimen sources signed informed consent.

2.2. Main Reagents and Instruments

SYBR Perfect Real-Time Kit, M-MLV (Moloney Murine Leukemia Virus) Reverse Transcriptase, M-MLV Reverse Transcriptase and RNAiso for Small RNA extraction reagents were purchased from Beijing Baori Doctor Material Technology Co., LTD. TransScript ii First-strand cDNA Synthesis SuperMix Kit was purchased from Beijing Whole Type Gold, IL-17A Human ELISA Kit was purchased from Thermo Fisher Scientific. Fluorescence quantitative PCR instrument LightCycler 480, Roche Pharmaceuticals, Switzerland; Autoclave sterilizer, Tomi Corporation, Japan; General PCR instrument, Bole Co., USA; Low-temperature high-speed centrifuge, Ebender Germany.

2.3. Experimental Methods

2.3.1. Sample Collection

Skin tissue: 0.5 × 0.5 cm^2 of psoriatic skin tissue and 0.5 × 0.5 cm^2 of peripheral skin tissue were collected and frozen with liquid nitrogen and stored at −80˚C. Peripheral blood mononuclear cells: 10 mL of elbow venous blood was extracted (anticoagulation with sodium citrate) and isometric dilution was performed with normal saline. The 15 mL sterile centrifugal tube purchased by cell culture was used. First, 5 mL lymphocyte separation solution was added inward, and then the diluted blood was slowly added along the wall of the test tube with a sterile dropper. In this process, the interface must be kept clear, and the diluted blood was added to the lymphocyte separation solution twice. The solution was centrifuged horizontally at 2500 r/min for 25 min and divided into three layers. Using a sterile pipette, the monocyte layer located in the middle liquid layer is gently sucked into the new centrifugal tube. Diluted with PBS and washed for 2 - 3 times, the cells were centrifuged again at 3000 r/min for 5 min. At this time, the cells were located at the bottom of the centrifuge tube, and 1 mL RNAiso for Small RNA reagent was added and frozen at −80˚C.

Serum: 5 mL elbow venous blood was extracted, placed at room temperature for 2 h, centrifuged at 800 × g, the supernatant was taken as peripheral blood serum samples, divided into 2 portions, each 500 μL frozen at 80˚C.

2.3.2. RNA Extraction and cDNA Synthesis

0.2 g of tissue was weighed and crushed by the liquid nitrogen grinding method. 1 mL RNAiso for Small RNA reagent was added. After peripheral blood mononuclear cells were isolated, 1 mL RNAiso for a Small RNA reagent was added directly. Take 500 μL of serum, and add 1 mL RNAiso for the Small RNA reagent; the extraction process was carried out in strict accordance with the instructions of RNAiso for the Small RNA reagent. cDNA synthesis: stem-loop method was

used for reverse transcription, and U6 was used as an internal reference gene in the whole process. The primers are shown in Table 1 below. Total system 20 μL, extracted Small RNA 8 μL, stem ring RT primer 2 μL (*i.e.* miR-155 and miR-146a 1 μL respectively), U6 reverse transcription primer 1 μL, RNase inhibitor 1 μL, Reverse Transcriptase (M-MLV) 1 μL, 5 × 1st Strand Synthesis Buffer 5 μL, dNTP 5 μL, instantaneous centrifuge mixing, reaction conditions were 65˚C for 10 min. 25˚C 15 min, 42˚C 60 min, 75˚C 15 min, after the reaction, stored at −20˚C.

2.3.3. Fluorescence Quantitative PCR

The detection of fluorescent quantitative PCR was carried out using the chimeric dye SYBR method. The whole process was carried out in strict accordance with the system and conditions of the SYBR Perfect Real-Time kit. Primer sequences are shown in Table 2 below. With U6 as the reference gene, the results were calculated using the $2^{-\Delta\Delta CT}$ method. In each reaction, each sample was repeated at least 3 times. The fluorescence analysis software was used to automatically quantify the results, and finally, the average value was taken.

2.4. Statistical Analysis

SPSS 22.0 software was used for data statistics, and the data were shown as mean ± standard deviation. Specifically, homogeneity of variance Tests and screening were carried out first. According to the F value table, if F Test value > 0.05, t Test value was obtained by equal variance two-sample Test; if F Test value < 0.05, T Test value was obtained according to heteroscedasticity two-sample Test. Paired T Test was used to analyze the expression of miR-155 and 146a in skin lesions and adjacent tissues, and $P < 0.05$ was considered as a statistically significant difference.

3. Results

3.1. Expression Detection of miR-155 and 146a

The results showed that the amplification curve of miR-155 and 146a was s-shaped,

Table 1. Reverse transcription cervical ring primer sequence table.

Name	miRBase	Reverse transcription cervical ring primer
hsa-miR-155	MIMAT0000646	5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACCCCTAT-3'
hsa-miR-146a	MIMAT0000449	5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACCCATG-3'
U6		5'-AACGCTTCACGAATTTGCGT-3'

Table 2. Primers detected by Real time PCR.

Name	Upstream detection primer	Downstream detection primer
hsa-miR-155	5'-TTAATGCTAATCGTGATA-3'	5'-TGGTGTCGTGGAGTCG-3'
hsa-miR-146a	5'-TGAGAACTGAATTCCATGG-3'	5'-TGGTGTCGTGGAGTCG-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'

and the Tm value of the dissolution curve was single, indicating that the specific fluorescence quantitative PCR detection of miR-155 and 146a was realized from different sample sources, and the Ct value obtained by the amplification could be used for subsequent analysis, as shown in Figure 1.

3.2. Expression of miR-155 and 146a in Skin and Para-Skin Tissues of Psoriasis Experimental Group

The expression of miR-155 in the skin tissue of the experimental group was (3.57 ± 1.05), which was significantly higher than that in the para-skin tissue (1.27 ± 0.28) ($t = 8.324$, $P < 0.01$). The expression level of miR-146a was (2.18 ± 0.59), which was significantly higher than that of para-skin tissues (1.12 ± 0.37), with an extremely significant difference ($t = 5.213$, $P < 0.01$), as shown in Table 3.

3.3. Expression of miR-155 and 146a in Peripheral Blood Skin Tissue and Para-Skin Tissue in Psoriatic Experimental Group

The expression level of miR-155 in peripheral blood mononuclear cells of the experimental group was (5.31 ± 1.23), significantly higher than that of the normal

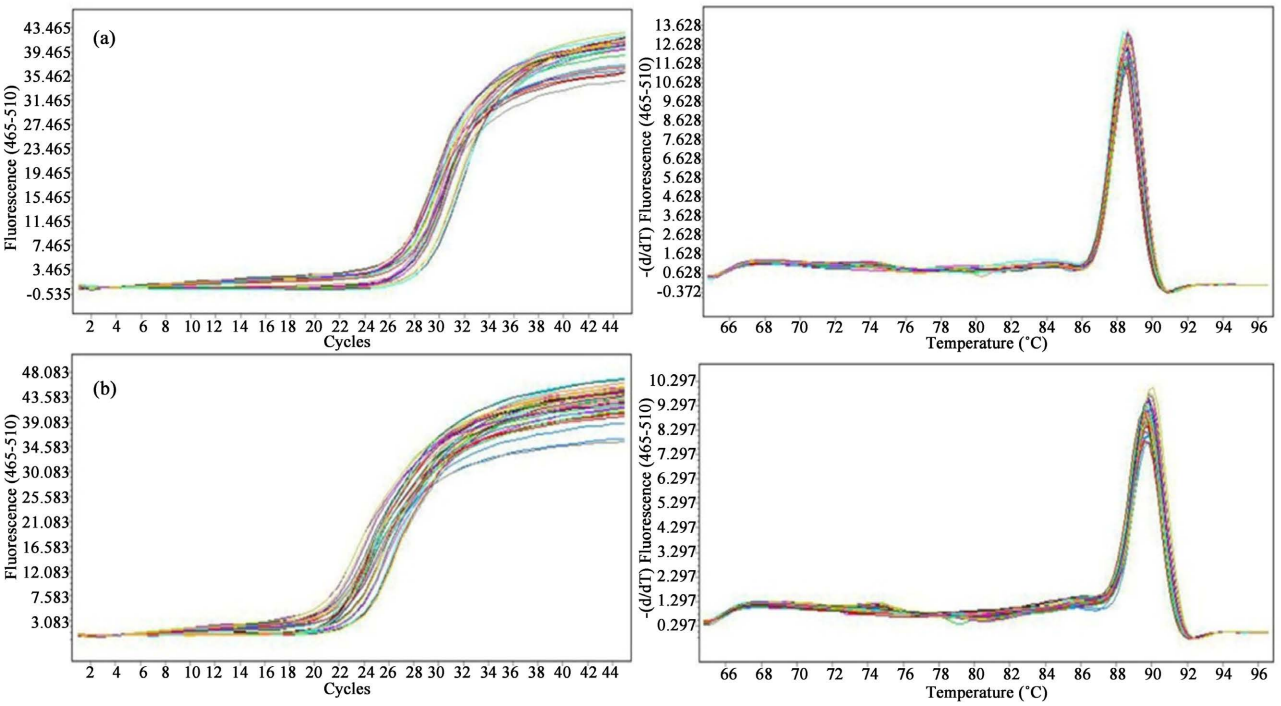

Figure 1. Amplification and dissolution curves of miR-155 and 146a. (a) miR-155; (b) miR-146a.

Table 3. Detection results of serum miR-155/146a levels before and after treatment.

	N	Parataxillary tissue	Skin tissue
miR-155	30	1.27 ± 0.28	3.57 ± 1.05*
miR-146a	30	1.12 ± 0.37	2.18 ± 0.59*

Note: VS para-lesion tissue, *$P < 0.01$.

control group (1.53 ± 0.39) (t = 12.561, P < 0.01), and the expression level of miR-146a was (4.13 ± 1.05). Significantly higher than normal control group (1.41 ± 0.62), the difference was extremely significant (t = 8.647, P < 0.01); The expression level of miR-155 in serum of experimental group was (1.84 ± 0.52), significantly higher than that of normal control group (1.05 ± 0.34) (t = 3.152, P < 0.01), and the expression level of miR-146a was (2.71 ± 0.37), significantly higher than that of normal control group (1.18 ± 0.27). The difference was extremely significant (t = 4.322, P < 0.01), as shown in Table 4.

Table 4. Expression levels of peripheral blood mononuclear cells and serum miR-155 and 146a in each group.

	N	Normal control group		Psoriasis experimental group	
		Mononuclear cell	Serum	Mononuclear cell	Serum
miR-155	30	1.53 ± 0.39	1.05 ± 0.34	5.31 ± 1.23*	1.84 ± 0.52*
miR-146a	30	1.41 ± 0.62	1.18 ± 0.27	4.13 ± 1.05*	2.71 ± 0.37*

Note: VS normal control group, *P < 0.01.

3.4. Correlation of miR-155 and 146a from Different Psoriasis Samples

The expression of miR-155 was up-regulated in skin tissue, peripheral blood mononuclear cells and serum of patients in the psoriatic group at the same time, with a positive correlation (r = 0.636, 0.524, 0.443, P < 0.05). The expression of miR-146a in skin tissue, peripheral blood mononuclear cells and serum were also up-regulated in the psoriatic experimental group, with a positive correlation (r = 0.725, 0.661, 0.527, P < 0.05).

3.5. Expression of Peripheral Blood Mononuclear Cells and Serum miR-155 and 146a in Patients with Psoriasis before and after Treatment

After treatment, the expression level of miR-155 in the peripheral blood mononuclear cells of the experimental group was (2.31 ± 0.41), significantly lower than that before treatment (5.31 ± 1.23) (t = 7.345, P < 0.01), and the expression level of miR-146a was (2.13 ± 0.55). Significantly lower than before treatment (4.13 ± 1.05) (t = 5.334, P < 0.01); The expression level of miR-155 in serum of the experimental group was (1.24 ± 0.29), which was significantly lower than that before treatment (1.84 ± 0.52) (t = 2.18, P < 0.05), and the expression level of miR-146a was (1.69 ± 0.42), which was significantly lower than that before treatment (2.71 ± 0.37). The difference was extremely significant (t = 2.71, P < 0.05), as shown in Table 5.

4. Discussion

Psoriasis has a specific miRNA expression profile, and a large number of miRNAs have been found in the skin. Some of these important miRNAs play a role

Table 5. Expression levels of peripheral blood mononuclear cells and serum miR-155 and 146a in each group.

	N	Psoriasis experimental group before treatment		Psoriasis experimental group after treatment	
		Mononuclear cell	Serum	Mononuclear cell	Serum
miR-155	30	5.31 ± 1.23	1.84 ± 0.52	2.31 ± 0.41*	1.24 ± 0.29#
miR-146a	30	4.13 ± 1.05	2.71 ± 0.37	2.13 ± 0.55*	1.69 ± 0.42#

Note: VS psoriasis group before treatment, #P < 0.05, *P < 0.01.

as a class of post-transcriptional gene regulators in psoriasis. So far, more than 250 miRNAs have been found to be abnormally expressed in peripheral blood or skin lesions of patients with psoriasis [7]. miR-155 is highly expressed in skin lesions and peripheral blood monocytes [8]. miR-146a is highly expressed in skin lesions and peripheral blood monocytes [5] [9]. In this paper, the expressions of miR-155 and miR-146a were analyzed from samples from different sources, and the results showed that the skin tissue, peripheral blood mononuclear cells and serum weight were all highly expressed, which was consistent with the published conclusions.

miR-146a and miR-155 are closely related to immune inflammation and regulate normative inflammatory gene expression in vivo and in vitro by targeting inflammatory mediators to ensure an appropriate level of host inflammatory response [10]. Studies have shown that miR-155 can regulate the differentiation of CD4~ + T cells into Th17 cells in peripheral blood of patients with psoriasis vulgaris, thus participating in the inflammatory response [11]. The uncontrolled regulation of miR-146a on target gene IRAK1 is also the main cause of persistent inflammation in psoriatic lesions [12]. Pearson correlation analysis showed that the expression status of miR-155/146a in skin lesions, peripheral blood mononuclear cells and serum was consistent. Therefore, the high expression of miR-155/146a in peripheral blood mononuclear cells and serum may be the embodiment of playing inflammatory mediators. The high expression of miR-155/146a in skin lesions must be performed by regulatory target genes. The functional study of miR-155/146a in tissues was not explored in this paper, but previous studies have found that miR-155 promotes the proliferation of psoriatic cells and inhibits apoptosis through the PTEN signaling pathway [13]. miR-146a may regulate the expression of VEGF through the target gene Smad4 at the translation level, and the overexpression of VEGF promotes the inflammatory response and angiogenesis of psoriasis, and promotes the occurrence and development of psoriasis [14]. In recent years, serum miRNA levels have been found to be useful biomarkers in the diagnosis, prevention and treatment of various diseases [15] [16].

5. Conclusion

In this paper, peripheral blood mononuclear cells and serum miRNAs from pa-

tients with psoriasis were collected to study miR-155/146a, and it was found that the high expression of peripheral blood mononuclear cells and serum miR-1266-5p in patients with psoriasis decreased significantly after treatment. These results suggest that miR-155/146a is involved in the therapeutic process of psoriasis, which may provide new ideas for the future treatment of psoriasis.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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