Skin level of microRNA-369-3P in patients with psoriasis and its correlation with disease severity

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Background
Psoriasis is a common autoimmune skin disease characterized by intense proliferation and abnormal differentiation of keratinocytes. Recently, some microRNAs (miRs) have been proven to show an aberrant expression in psoriasis and may play a role in the pathogenesis of the disease.

Objectives
The aim of this study was to detect skin miR-369-3p levels in patients with psoriasis and its correlation with disease severity with measurement of one of its regulated psoriasis-related genes, *SIRT*, and to find the correlation between the studied parameters.

Patients and methods
Skin tissues were collected, and skin miR-369-3p and SIRT1 gene levels were measured. The Psoriasis Area and Severity Index scores of patients and the correlation with skin miR-369-3p levels were evaluated. Correlation between miR-369-3p and SIRT1 gene was also evaluated.

Results
Skin miR-369-3p levels were higher in patients with psoriasis than those in healthy controls ($P = 0.01$). Skin miR-369-3p had an insignificant positive linear relation with Psoriasis Area and Severity Index scores in psoriasis patients ($r = 0.079$, $P = 0.772$). Insignificant negative correlation was found between miR-369-3p and SIRT1 gene levels in skin.

Conclusion
The expression of miR-369-3p is increased in skin tissues from psoriasis patients. Further studies are needed to clarify the role of miR-369-3p in the pathogenesis of psoriasis.

Keywords:
microRNAs, microRNA-369-3p, Psoriasis Area and Severity Index, psoriasis

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Introduction

Psoriasis is a common autoimmune disease of the skin characterized by intense proliferation and aberrant differentiation of keratinocytes, as well as the infiltration of lymphocytes and neutrophils [1]. However, the precise pathogenesis of psoriasis is still not completely clarified. MicroRNAs (miRs) are 22-nt noncoding RNAs that can suppress the expression of protein-coding genes by targeting cognate messenger RNAs for translational repression or, less frequently, degradation [2]. Several psoriasis-associated miRs have already been identified in the skin and are considered to contribute to the development of psoriasis ([3–6]). It was found that miR-369-3p had been predicted to regulate many psoriasis-related genes, including tumor necrosis factor (also called tumor necrosis factor -α), LIMK1, SIRT1, SP3, ADAM10, HES1, and WNT5A ([7–13]). On the basis of these findings, we inferred that miR-369-3p might be involved in the pathogenesis of psoriasis by regulating the expression of those target genes. In this study, we detected the skin level of miR-369-3p, evaluated the correlation between miR-369-3p and clinical severity in psoriasis, and further evaluated the correlation between miR-369-3p and SIRT1 gene.

Patients and methods

Clinical assessment and patient materials

Skin specimens were taken from 25 psoriasis patients and 25 age-matched and sex-matched healthy patients. The disease severity of each patient was assessed using the Psoriasis Area and Severity Index (PASI) score [14]. All the patients enrolled in our study had no other autoimmune or systemic diseases and no systemic treatment, including immunosuppressive drugs and phototherapy, was performed at least 1 month before the PASI score evaluation and sample collection period. Informed consent was obtained from all the patients and healthy controls. The Local Ethics Committee of the Faculty of Medicine at Assiut University approved the study according to the principles of the Declaration of Helsinki.

RNA extraction

Purification of total RNA, including small RNAs from skin samples, was performed using the

miRNeasy Mini Kit (Qiagen, Valencia, California, USA).

Quantitative real-time polymerase chain reaction

To measure the miR-369-3p, reverse transcription into cDNA using miScript II RT Kit (Qiagen) was performed, followed by amplification through real-time PCR using the miScript SYBR Green PCR Kit (Qiagen) with miScript Primer Assay using primers of miR-369-3p and primers of SNORD as a housekeeping gene (Qiagen). To measure the SIRT1, reverse transcription into cDNA using the quantitect reverse transcription kit (Qiagen) was performed, followed by amplification through real-time PCR using the quantitect SYBER Green PCR kit and primers of SIRT with GAPDH primers as a housekeeping gene (Qiagen).

Statistical analysis

Statistical analysis was carried out with the Mann–Whitney *U*-test for the comparison of means. Correlations were assessed by Spearman's rank correlation test. The significant *P*-value was less than 0.05.

Results

Skin microRNA-369-3p level in patients with psoriasis and healthy controls

The miR-369-3p levels of the skin in psoriasis patients were significantly higher than those in the healthy controls ($P = 0.01$) (Fig. 1).

Correlation between skin miR-369-3p levels and Psoriasis Area and Severity Index scores in patients with psoriasis

A positive correlation was observed between relative skin miR-369-3p levels and disease severity, but this correlation was nonsignificant ($r = 0.079$, $P = 0.772$) (Fig. 2).

Measurements of SIRT1 gene

As regards the SIRT1 gene, there was no significant difference between patients and controls when the mean of delta threshold cycle (CT) of both groups was compared ($P = 0.564$), and there was a negative correlation between the miR-369-3p and SIRT1 gene, but this relation did not reach statistical significance ($P > 0.05$).

Discussion

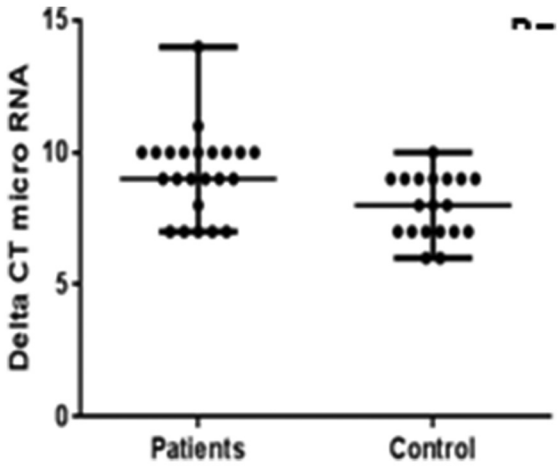
Our study not only investigated the levels of miR-369-3p in skin samples collected from psoriasis patients but also evaluated their correlations with patients' PASI scores, and the SIRT1 gene was measured as a trial to know whether miR-369-3p affects the expression of SIRT1 gene as one of the psoriasis-related genes.

It was first found that miR-369-3p had an increased expression in skin samples collected from patients with psoriasis. The mean fold change of miR-369-3p was 4.5 times higher in patients than in controls.

This agrees with the study of Guo *et al.* [11], who found that skin miR-369-3p had an aberrantly increased expression of 2.3-fold and considered it to be significantly higher in psoriasis patients than in the healthy controls. Therefore, it is reasonable to consider skin miR-369-3p as a potential marker that may assist in the understanding of the pathogenesis of psoriasis.

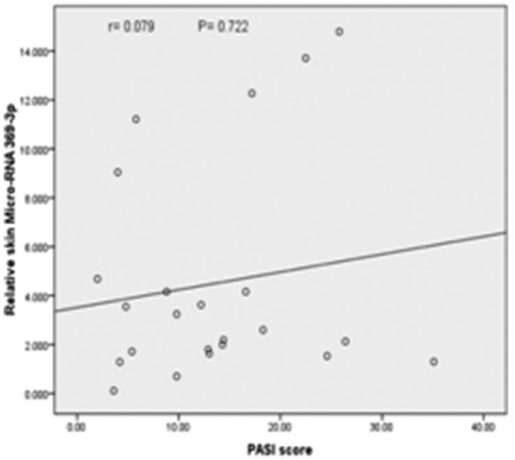
After conducting a correlation analysis, it was found that skin miR-369-3p had a positive correlation with PASI

Figure 1



Delta CT of the studied groups regarding micro-RNA-369-3p.

Figure 2



Correlation between relative skin miRNA-369-3p levels and the disease severity.

scores in psoriasis patients but did not reach statistical significance. This is in contrast with Guo *et al.* [15], whose study showed that the miR-369-3p levels in skin had a statistically significant positive correlation with PASI scores in patients with psoriasis. This could be explained by the difference in disease severity of studied groups in our study (44% had mild psoriasis, 32% of the patients had moderate disease, and 24% of the patients had severe psoriasis), whereas the patients in the study by Guo *et al.* [15] included only severe psoriasis, 60%, and moderate psoriasis, 40%, and no cases with mild psoriasis were included in skin biopsies.

It was expected that SIRT1 gene levels would be downregulated in the psoriatic patients based on the fact that SIRT1 opposes the IL-22-induced STAT3 activity by deacetylating STAT3 and reducing STAT3Tyr705 phosphorylation [9].

The mean fold change of the SIRT1 gene in our study was about 0.6-fold between patients and controls, which indicates nearly similar levels in both. There was no significant difference between patients and controls. These results agree with those of Sestito *et al.* [9], whose study showed that patients with psoriasis and healthy donors showed similar SIRT1 levels, mainly localized in the cytosol, as assessed by western blotting. By immunohistochemistry analysis, basal keratinocytes of lesional skin psoriasis exhibited a reduced and less intense SIRT1 staining when compared with healthy and nonlesional psoriatic skin, especially at the tips of the dermal papillae close to the CD3 T-cell infiltrate. Therefore, the constitutive expression of SIRT1 was similar in psoriatic and healthy cultured keratinocytes, as well as in healthy and nonlesional psoriatic skin.

From the study by Guo *et al.* [15] and our study, it could be said that the impaired SIRT1 expression was the consequence of the microenvironmental cytokine milieu and not an intrinsic defect of the psoriatic epidermis.

There was insignificant negative correlation between the miR-369-3p and SIRT1 gene. We expected to see a statistical significance in this correlation, but our study did not prove this supposition. A larger study sample may be needed to emphasize or to preclude significant correlations between the studied parameters.

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Conflicts of interest

There are no conflicts of interest.

References

1 Nestle FO, Kaplan DH, Barker J Psoriasis. N Engl J Med 2009; 361:496–509.

2 Ambros V. The functions of animal microRNAs. Nature 2004; 431:350–355.

3 Xu N, Meisgen F, Butler LM, Han G, Wang XJ. MicroRNA-31 is overexpressed in psoriasis and modulates inflammatory cytokine and chemokine production in keratinocytes via targeting serine/threonine kinase40. J Immunol 2013; 190:678–688.

4 Xia J, Joyce CE, Bowcock AM, Zhang W. Noncanonical microRNAs and endogenous siRNAs in normal and psoriatic human skin. Hum Mol Genet 2013; 22:737–748.

5 Sonkoly E, Wei T, Janson PC, Sääf A, Lundeberg L. MicroRNAs: novel regulators involved in the pathogenesis of psoriasis? PLoS One 2007; 2: e610.

6 Ichihara A, Jinnin M, Oyama R, Yamane K, Fujisawa A. Increased serum levels of miR-1266 in patients with psoriasis vulgaris. Eur J Dermatol 2012; 22:68–71.

7 Nickoloff BJ. The cytokine network in psoriasis. Arch Dermatol 1991; 127:871–884.

8 Honma M, Benitah SA, Watt FM. Role of LIM kinases in normal and psoriatic human epidermis. Mol Biol Cell 2006; 17:1888–1896.

9 Sestito R, Madonna S, Scarponi C, Cianfarani F, Failla CM, Cavani A, *et al.* STAT3-dependent effects of IL-22 in human keratinocytes are counterregulated by sirtuin 1 through a direct inhibition of STAT3 acetylation. FASEB J 2011; 25:916–927.

10 Oh ST, Schramme A, Stark A, Tilgen W, Gutwein P, Reichrath J. Overexpression of ADAM 10 and ADAM 12 in lesional psoriatic skin. Br J Dermatol 2008; 158:1371–1373.

11 Zhang K, Hou R, Niu X, Zhang J, Yin G, Li X, *et al.* Decreased colony formation of high proliferative potential colony-forming cells and granulocytemacrophag-ecolony- forming units and increased Hes-1 expression in bone marrow mononuclear cell from patients with psoriasis. Br J Dermatol 2010; 163:93–101.

12 Lu X, Du J, Liang J, Zhu X, Yang Y, Xu J. Transcriptional regulatory network for psoriasis. J Dermatol 2013; 40:48–53.

13 Reischl J, Schwenke S, Beekman JM, Mrowietz U, Stürzebecher S, Heubach JF. Increased expression of Wnt5a in psoriatic plaques. J Invest Dermatol 2007; 127:163–169.

14 Kerkhof PC. The Psoriasis Area and Severity Index and alternative approaches for the assessment of severity: persisting areas of confusion. Br J Dermatol 1997; 137:661–662.

15 Guo S, Zhang W, Wei C, Wang L, Zhu G, Shi Q, *et al.* Serum and skin levels of miR-369-3p in patients with psoriasis and their correlation with disease severity. Eur J Dermatol 2013; 23:608–613.