MicroRNAs in cutaneous lichen planus

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Summary

Lichen planus (LP) is a chronic, inflammatory, papulosquamous, autoimmune disease. The pathogenesis of LP appears to be complex, with interactions between genetic, environmental and lifestyle factors. MicroRNAs (miRNAs) are short RNAs encoded in both protein coding and noncoding areas of the genome, and have been found to be involved in the pathogenesis of some inflammatory skin diseases. The aim of this study was to map the levels of miRNA (miR-)203 and miR-125b in cutaneous LP to evaluate their possible role in the pathogenesis of the disease. In total, 40 patients with classic cutaneous LP and 40 age- and sex-matched healthy controls (HCs) were enrolled in this study. Punch biopsies (4 mm) were taken from cutaneous LP lesions of patients and from normal skin of HCs. miRNA-203 and miRNA-125b mRNA expression was estimated by reverse transcription PCR. Our analysis revealed a significantly ($P < 0.001$ for both) lower expression of both miR-203 and miR-125b mRNA in the LP than in the HC biopsies. No relationship was found between expression of miR-203 or miR-125b and either age, sex, presence of mucosal lesions or positivity for HCV antibodies. miR-125b and miR-203 could be involved in the pathogenesis of cutaneous LP.

Lichen planus (LP) is an inflammatory mucocutaneous disease with autoimmune pathogenesis.1,2 Some studies suggest that LP lesions result from a cell-mediated autoimmunity directed against keratinocytes of the basal layer, leading to the formation of a subepidermal infiltrate composed initially of CD4+ lymphocytes and subsequently of CD8+ cytotoxic cells.3

MicroRNAs (miRNAs) are short noncoding RNAs that affect a number of physiological and pathophysiological cellular processes, including development, differentiation, proliferation and apoptosis.4,5 and have been shown to play a role in some inflammatory diseases.5–9 While most published studies have concentrated on the pathogenesis of oral lichen planus (OLP), fewer studies have investigated the pathogenesis of cutaneous LP.

Out of the hundreds of novel human miRNAs identified, we chose miRNA (miR)-203 because of its role in regulation of keratinocyte proliferation through targeting P63,10 and miR-125b because of its role in controlling inflammation and apoptosis through negative regulation of tumour necrosis factor.11

Report

The study was approved by the local research ethics committee of the Faculty of Medicine, Beni Suef University, Egypt, and conformed to the ethical guidelines of the 1975 Declaration of Helsinki. The purpose of the study was explained to each patient, and written informed consent was taken before data collection. Patient confidentiality was maintained by code numbers given to each patient, and all personal data were concealed.

In total, 40 patients with classic cutaneous LP referred to the dermatology clinic at Beni Suef University Hospitals and 40 age-and sex-matched healthy controls (HCs) were recruited for the study. Exclusion criteria included presence of malignancies,
autoimmune diseases, skin disease other than LP and receipt of phototherapy.

Venous blood samples were taken from the study subjects for detection of third-generation hepatitis C virus antibodies using ELISA.

Complete clinical examination of the skin and mucosa was conducted by the dermatologists participating in the study. Punch biopsies (4 mm) were taken from skin lesions of patients and the normal skin of HCs. All biopsies were taken from covered areas on the trunk or proximal extremities; none of the patients received any local or systemic treatment 4 weeks prior to the biopsy. One biopsy from each participant was stained with haematoxylin and eosin for histopathological examination to confirm the diagnosis of LP, while the other biopsy was stored at −80 °C until required.

Total miRNA was extracted from tissue (mirVana™ PARIS™ Kit; Ambion Inc., Austin, TX, USA) according to the manufacturer’s instructions. Total miRNA quality and quantity were assessed by spectrophotometry (NanoDrop 1000; Thermo Scientific, Wilmington, DE, USA), then miRNA was reverse-transcribed (MicroRNA Reverse Transcription Kit; Applied Biosystems, Foster City, CA, USA) in a total reaction volume of 15 µL, following the manufacturer’s instructions. Reverse transcription (RT) products were used as PCR template, and PCR for quantifying miR-203, miR-125 and miR-U6 was performed using (TaqMan Universal PCR Master Mix; Applied Biosystems). The forward primers used for amplification are shown in Table 1, and the universal reverse primer (Table 1) was provided with the kit. Quantitative PCR was performed (StepOnePlus Real Time PCR System; Applied Biosystems) with the following cycling conditions: 95 °C for 10 min, 45 cycles at 95 °C for 15 s, and 60 °C for 1 min. The miRNA expression value was expressed relative to that of U6, and the 2−ΔCt method was used for the analysis of PCR data [ΔCt = mean Ct (miRNA of interest)−mean Ct (U6)].

Comparison of quantitative variables between the study groups was carried out using the Mann–Whitney U-test for independent samples. Comparison of sex distribution between the study groups was carried out using the χ2 test, and comparison of age between patients and HCs was performed by t-test.

The correlation between various variables was assessed using the Spearman rank correlation equation for non-normally distributed variables. The significance level was set at P ≤ 0.05. All statistical calculations were carried out using Microsoft Excel (2007: Microsoft Corp. Redmond, WA, USA) and SPSS (v15.0 for Windows; SPSS Inc. Chicago, IL, USA).

The patient group comprised 40 patients [28 men (70.0%), 12 women (30.0%); mean ± SD age 39.28 ± 13.95 years, range 12–65 years]. Mean disease duration was 7.92 ± 11.73 months (range 1–48 months). The HC group comprised 40 healthy individuals [31 men (77.5%), 9 women (22.5%); mean ± SD age 35.03 ± 10.62 years, range 16–52 years]. There was no significant difference between the groups in sex (P = 0.31) or age (P = 0.13).

miR-203 and miR-125b were expressed in all skin biopsies from both patients and HCs. Mean miR-203 and miR-125b level was significantly (P < 0.001 for both) lower in biopsies from patients with LP (0.54 ± 0.29 and 0.50 ± 0.29, respectively) compared those from with HCs (1.10 ± 0.19 and 1.05 ± 0.15, respectively) (Table 2, Fig. 1).

Disease duration positively correlated with the level of both miR-203 (r = 0.59, P < 0.001) and miR-125b (r = 0.44, P < 0.01 respectively).

There was no significant correlation in the patient group between age and expression of either miR-203 (r = 0.30, P = 0.85) and or mir-125b (r = 0.09, P = 0.58).

Similarly, no significant association was detected between levels of miR-203 or miR-125b and either patient sex, presence of mucosal lesions or positivity for HCV (Table 3).

Although there have been previous studies that investigated the role of miRNAs in OLP, this is, to our knowledge, the first study to evaluate the levels of miR-203 and miR-125b in cutaneous LP.

### Table 1 Forward primer sequences.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5′→3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNA-203</td>
<td>GTGAAATGTTAAGACACCTAGAA</td>
</tr>
<tr>
<td>miRNA-125</td>
<td>GGGCACCCTCTGAGCCTAATAC</td>
</tr>
<tr>
<td>U6</td>
<td>CGCTCCGGCACAGCATATAAC</td>
</tr>
<tr>
<td>Reverse primer*</td>
<td>GCGGAGCAAGAAATATACGAC</td>
</tr>
</tbody>
</table>

*Supplied with the kit used (TaqMan Universal PCR Master Mix; Applied Biosystems, Foster City, CA, USA).

### Table 2 Comparison of mRNA levels in patients and controls.

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNA-203</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients (n = 40)</td>
<td>0.54 ± 0.29</td>
<td>0.12–1.02</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Controls (n = 40)</td>
<td>1.10 ± 0.19</td>
<td>0.93–1.90</td>
<td></td>
</tr>
<tr>
<td>miRNA-125b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients (n = 40)</td>
<td>0.50 ± 0.29</td>
<td>0.09–1.03</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Controls (n = 40)</td>
<td>1.05 ± 0.15</td>
<td>0.86–1.80</td>
<td></td>
</tr>
</tbody>
</table>

*Significant (P < 0.05).
In the current study, mean expression level of both miR-203 and miR-125b in LP biopsies was almost half that of the HCs.

In agreement with our study, Danielsson et al.\(^8\) found that expression of miR-125b was low in biopsies from patients with oral LP (OLP) compared with those from HCs, whereas, contrary to our results, miR-203 was found to be significantly overexpressed in OLP lesions.

Previous studies have shown that miR-203 and miR-125b are involved in the pathogenesis of some T cell-mediated inflammatory skin diseases such as psoriasis\(^6,7\) and lupus erythematosus (LE).\(^9\) miR-203, by repressing p63, acts as a switch between keratinocyte proliferation and differentiation in adult epidermis,\(^10,12\) and the expression pattern of miR-203 in the skin was found to be in accordance with involvement in suprabasal keratinocyte differentiation.\(^7\)

Despite the fact that OLP and cutaneous LP share some key pathological features, such as the presence of a subepithelial/subepidermal band-like infiltrate in lymphocytes and the hydropic degeneration of basal cells, other features, such as orthokeratosis, acanthosis and hypergranulosis, which were present in almost all our cases of classic LP, were detected in only 50% of cases of reticular and erosive OLP and 25% of cases of atrophic OLP.\(^13\) These differences in keratinocyte proliferation between OLP and cutaneous LP might explain the contradictory results in miR-203 expression between our study and that of Danielsson et al.,\(^8\) which was conducted on OLP mucosal biopsies.

In contrast to our study also, miR-203 was shown to be unregulated in psoriasis,\(^7\) a well-established papulosquamous disease in which keratinocytes show abnormal states of proliferation and differentiation.\(^14\) Sonkoly et al. suggested that miR-203 might play a minor role in suppressing the pathogenic hyperproliferation found in psoriasis.\(^6,7\)

Likewise, in cell lines of human primary keratinocytes, miR-125b was shown to repress proliferation and promote differentiation,\(^7\) so downregulation of miR-203 and miR-125b might contribute to increased proliferation and decreased differentiation of keratinocytes.

One of the main pathogenetic mechanisms of LP (both cutaneous and OLP forms) is apoptosis of keratinocytes.\(^15\) miR-125b has been identified as a negative regulator of p53 and acts as an oncomiR suppressing p53-induced apoptosis.\(^10\) p53 is a tumour suppressor protein, which functions as a transcription factor involved in induction of cell cycle arrest or apoptosis,\(^10\) and it has been found to be overexpressed in LP lesions.\(^16\)
miR-125b is also a negative regulator of tumour necrosis factor (TNF)-α, which is one of the cytokines that play an inflammatory process and apoptosis in some skin diseases.\(^6\)\(^7\)\(^9\) Increased serum levels of TNF-α have been detected in patients with LP.\(^17\) The low level of miR-125b in LP might abolish its role as a negative regulator of apoptosis mediators, enhancing the process of apoptosis involved in the disease progression.

RANTES (Regulated on activation normal T cell expressed and secreted) is a key chemokine in T-lymphocyte recruitment in inflammatory diseases such as LE and OLP.\(^6\)\(^7\)\(^9\)\(^18\) miR-125 is a negative regulator of RANTES production in activated T cells, thus low levels of miR-125 might lead to RANTES overproduction and recruitment of activated lymphocytes and mast cells, releasing TNF-α in the skin and contributing to the disease pathogenesis.

In conclusion, we found low levels of miR-203 and miR-125b in patients with LP compared with HCs. We propose that the decreased expression of miR-125b and miR -203 in patients with cutaneous LP might play a role in the pathogenesis of LP lesions by increasing proliferation of the epidermal cells, enhancing apoptosis and recruitment of inflammatory cells.

**Learning points**

- LP is a chronic, inflammatory, papulosquamous autoimmune disease that affects the skin and mucous membranes.
- miR-203 levels are increased and miR-125b levels are decreased in patients with oral LP.
- In the current study: Low levels of miR-203 and miR-125b were detected in classic cutaneous LP lesions.
- Both could contribute to the development of LP by increasing apoptosis and inflammation.

**References**